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Antimicrobial Research and One Health in Africa

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We dedicate this book to our beloved parents
Yussuf and Farida Bayat and Debora Abia.
They instilled in us the value of education.

Foreword

For tomorrow belongs to the people who prepare for it today. (African Proverb)

Africa currently faces enormous challenges as it strives to move forward in economic development, educational opportunities, and the modernization of its science infrastructure. The efforts to respond to these challenges fall under the global umbrella of the climate change emergency. Although Africa has 17% of the global population, it has historically accounted for a negligible 3% of the planet's carbon dioxide emissions. However, climate change, extreme weather events, and disease transmission disproportionately affect Africa resulting in severe economic, social, and environmental consequences for its people. Despite these challenges, African countries received only $18.3 billion in climate finance between 2016 and 2019, producing a projected climate finance gap of up to $1288.2 billion annually from 2020 to 2030.

One major challenge driven by climate change in Africa is the need to identify and develop new responses to address the resistance to antibiotics (AMR) and antibiotic resistance genes (ARGs). The antibiotics commonly used to treat microbial diseases in humans and animals used for food are developing new resistance patterns driven by climate change events. Responses must be developed using the One Health model to be effective because it includes the major interactions that affect microbial antibiotic resistance and efforts to deal with that challenge. *Antimicrobial Research and One Health in Africa* consist of 14 chapters that provide an update on different dimensions of the research underway to address the AMR challenges on the diverse continent of Africa. Two chapters describe the importance of context in understanding the social and behavioral aspects of antimicrobial use and resistance in Africa and the value of antibiotic stewardship (AMS) as an essential strategy for reducing and optimizing antibiotic use and preventing the emergence of AMR. Three other chapters discuss the topic of the occurrence and health risks of antibiotic resistance in African aquatic systems, the etiology of early-onset bacterial sepsis and antibiotic resistance in neonates in an Algerian intensive care unit, and the genetics of viral resistance as it relates to the clinical relevance and role in viral disease outbreaks. In addition, four chapters focus on food production and food safety with

information on the distribution and prevalence of antimicrobial resistance of NTS *Salmonella* isolated from farm animals and animal food products in Africa, a description of the current state of antimicrobial use in bovine mastitis in various African countries, a report of the current status of antibiotic-resistant bacteria and resistance genes in African aquaculture, and studies of microbiological safety and antimicrobial resistance in fresh produce production in Africa. Two chapters describe the biosecurity and disinfectant resistance in a post-antibiotic era and the critical linkage between antibiotic and disinfectant resistance noted in recent decades. Finally, the book is completed with three chapters describing the biocidal activity of plant extracts in Algeria, the combined use of African natural products and conventional antimicrobials as alternative tools against antimicrobial resistance, and an African overview of the use of nanomaterials for the elimination of antibiotic-resistant bacteria from water and wastewater.

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Preface

The threat posed by antimicrobial resistance (AMR) to human, animal and environmental health continues to rise, placing a tremendous burden on the global economy. If no solution is found to curb this ill, it is estimated that tens of millions of lives and hundreds of trillions of dollars will be lost in the next few decades. The situation is exacerbated by the massive demand for animal protein that has engendered an ever-rising use of antimicrobials in food-animal production. Furthermore, the indiscriminate use of these therapeutics continues to be reported worldwide. Unfortunately, the bulk of the antimicrobials consumed by humans and animals is excreted into the environment partially or unmetabolised and potent enough to exert selective pressure on environmental microorganisms, further escalating the environmental dimension of AMR. This, therefore, calls for concerted efforts between stakeholders within environmental, animal and human health – One Health.

Although AMR is of global concern, Africa remains the most affected continent. This is driven by a complex network of circumstances, including a high disease burden, favoured by poverty, malnutrition and lack of access to potable water and sanitation facilities. Also, the lack of adequate policies to guide antimicrobial use and the lack of implementation of these policies, when they exist, further places pressure on the already stressed continent.

We have compiled this volume on antimicrobial resistance and One Health in Africa, aiming to stimulate greater discourse on the spread of AMR on the continent. The ultimate goal is to create greater awareness while proposing possible alternatives for use by all sectors to allow better management and prevention of AMR in Africa. In addition, the presented chapters would spur policymakers to set mechanisms enabling more excellent stewardship guided by scientific evidence.

We firmly believe that this book, being the first of its kind on the continent, would open in-depth debates on AMR in Africa, calling on human, animal and environmental health practitioners to come together in a joint effort to create an AMR-free Africa.

If you want to go fast, go alone; if you want to go far, go together. (An African proverb)

Westville, Durban, KwaZulu-Natal, South Africa — Akebe Luther King Abia
Sabiha Yussuf Essack

Contents

Chapter 1
Why Context Matters: Understanding Social and Behavioural Aspects of Antimicrobial Use and Resistance in Africa

Neusa F. Torres, Susan Nayiga, and Lenore Manderson

1.1 Introduction

Behavioural factors influence the prescription, use and disposal of antibiotics for humans and animals. These are recurrently identified as key drivers of antimicrobial resistance, threatening health, food security and national economies. Explanations of resistance have emphasised the roles of health providers, primarily doctors and veterinarians but also pharmacists, nurses and auxiliary veterinary personnel, in prescribing antibiotics without clear evidence of their need and the related actions of individuals in their expectations of care for personal, household and farm use of antibiotics. In addition, dispensing antibiotics without prescription and without exercising stewardship has amplified the overuse and misuse of this class of drugs. The emphasis on the responsibility of individuals is consistently reflected in policy statements and health promotional material and has shaped interventions directed at health professionals and the general public. But how and why antibiotics and other antimicrobial drugs are used is considerably more complicated than this, as we consider in this chapter. Below, we analyse the limits of current behavioural

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interventions to contain antibiotic utilisation. We also address the interplay of human behaviour with the structures and networks that support pharmaceutical use (Tompson et al. 2021). In this context, we argue the need to consider key socioeconomic and political factors contributing to continuing antimicrobial resistance. We illustrate the social and economic factors which underpin how people acquire and use drugs and how they support general approaches to medication and health care, in contrast to the need to discriminate between different drugs. Finally, we highlight the importance of linking local understandings of antibiotic use to wider questions of drug prescription and patient advice.

1.2 Policy Context

In this section, we summarise the concerns of the World Health Organization (WHO 2020) and other international bodies on antimicrobial resistance. We also illustrate how global values around the management and distribution of drugs influence regional and national drug control programmes. Finally, we introduce our research in Uganda (Nagiya), Mozambique (Torres) and South Africa (Manderson), on which we will subsequently draw to highlight the social factors that influence drug use and resistance.

WHO statements emphasise specific personal behaviours that are deemed necessary to limit antimicrobial resistance in human health and food animal production. In support of this, WHO and health promoters argue the need for improved awareness and understanding of antimicrobial resistance through effective communication, education and training. This perspective, with its attention to the direct contributing factors to misuse of drugs and the importance of community education, is consistent with the Global Action Plan on Antimicrobial Resistance, as proposed in 2015 by WHO. Action plans were published around the same time by the Food and Agriculture Organization of the United Nations (FAO) and the World Organisation for Animal Health (OIE), and these directives were endorsed by Heads of State (resolution A/RES/71/3) at the United Nations General Assembly [for overviews and analysis, see Munkholm and Rubin (2020), Prestinaci et al. (2015), Ruckert et al. 2020)]. However, the directives to governments included in the plans are vague – to "improve surveillance" – and more ambiguous still to the healthcare industry ("invest in research and development of new antibiotics") (see the landing page, WHO 2020). Accordingly, governments are urged to support sustainable investment in new medicines, diagnostics and related technologies and increased knowledge through surveillance and research. Human and animal health professionals are supported rather than directed to "talk to patients" to ensure the optimal use of antimicrobial medicines in human and animal health. In contrast, people – as individuals targeted with health education – are encouraged to practice good sanitation, hygiene and infection prevention/biosecurity in food-animal production (through farming and fishing). Objectives are linked to the responsibilities of member states, local public health action and international agencies. The advice to

governments and recommended action required of patients highlight the challenges to the stewardship of drugs, particularly when production, supply and marketing are largely global commercial concerns.

The emphasis on behavioural change as a core area for action is continued in national-level policy statements and guidelines in different African settings. In Uganda, for example, the National Action Plan (NAP) on Antimicrobial Resistance, launched in 2018, closely follows the WHO Global Action Plan in terms of principles and strategic objectives and emphasises behaviour change among prescribers, dispensers and consumers. Consistent with ideas of the "rational" and "irrational" use of drugs, the Uganda National Action Plan highlights that the main factor driving AMR is the "misuse" of antimicrobials, deriving from unrestricted access to medicines and self-medication. The NAP proposes to address this by improving awareness about AMR among healthcare professionals and the general public, limiting access and ensuring appropriate use primarily through increasing the requirements for and enforcing regulations regarding prescription-only drugs. This approach is based on the supposition that education and awareness about AMR, with greater control of the supply of drugs, will change behaviour. Furthermore, the use of drugs once procured rests on the effectiveness of advice of clinicians and pharmacists that the drug is used only as directed by the person prescribed the medication in response to their presenting symptoms. In working towards a sustainable behavioural change, the WHO and other groups, working globally and locally, have prepared an extensive body of resources to be used to undertake local assessments and to train health providers and the public, including school children, with materials that include videos, personal stories, guidelines for assessment and infographics (e.g., WHO 2021; World Health Organization, Regional Office for Europe 2021).

Mozambique's updated law on medicine (Law 12/2017) defines antibiotics as an essential medicine; due to the burden of infectious disease, the same law classifies antibiotics as prescription-only medicine. The Mozambican National Action Plan (NAP) on antibiotic utilisation and resistance was launched in 2019, based on a "One Health" approach, and stresses the need to ensure the rational use of antimicrobials in humans, animals and food (Mozambique 2019). The NAP also stresses the need to reduce the presence of antimicrobials in the environment to prevent the development and spread of resistant organisms to humans and animals to preserve and guarantee the future effectiveness of antimicrobials. The NAP recognises that key sociocultural, economic and infrastructural factors affect the utilisation of antibiotics in the country, and it recommends increasing research and generation of scientific evidence and strategies on antibiotic prescribing, dispensing and utilisation. It also notes the need for strategies to reduce the healthcare costs of antibiotics without adversely affecting the quality of care. In another African context, in addition to tackle the fast growth of antimicrobial resistance in the country and to advise health authorities, a multi-sectoral working group, the Mozambican Global Partnership for Antibiotic Resistance (GARP), was created in 2012 in Mozambique (Global Antibiotic Resistance Partnership 2015). The working group togheter with academic institutions and partners, has been engaged in promoting and creating a critical mass of multidisciplinary researchers to produce evidence on antibiotic

stewardship and conservation and better address stewardship at the policy-making level. Undoubtedely the GARP sparked collaboration of the Ministries of Health and Agriculture, a greater interest among students and researchers to fill information gaps, the beginning of AMR surveillance in three regions (B. Sigauque M. Saide Open Access DOI:https://doi.org/10.1016/j.ijid.2016.02.056.).

The South Africa policy framework, meanwhile, predated WHO initiatives. The first Antimicrobial Resistance National Strategy Framework was published in 2014; a revised plan was published in 2018 under the imprimatur of the Minister of Health and Minister of Agriculture, Forestry and Fisheries. The plan emphasised One Health, and so, like Mozambique, South Africa highlighted a multidisciplinary and intersectoral approach and identified the need for data collection, monitoring, stewardship, control of supply and distribution and use of antibiotics. In framing the work required to realise these objectives, the document drew attention to legislative and policy reform, education and communication strategies and research. Towards this end, a Ministerial Advisory Committee on AMR was established in 2016.

The various frameworks supporting legislation and action plans identify areas for interventions, but as suggested, these are general and do not address the underlying factors and preconditions for the overuse or misuse of antibiotics. In the following, we draw on our social research conducted in Mozambique, South Africa and Uganda, to illustrate the challenges in managing antibiotics and ensuring successful stewardship. First, Susan Nayiga examined how antimicrobials intersect with life, livelihoods and health care in rural and urban Uganda (Nayiga et al. 2020, 2022a, b). Drawing on data from participant observation, health worker interviews and participant feedback meetings conducted between 2018 and 2020, she showed how these are linked to broader social, economic and political trends in modern Uganda. Second, through qualitative research, including interviews and group discussions, Neusa Torres and colleagues identified commonly used non-prescribed antibiotics in urban areas of Maputo city, Mozambique (Torres et al. 2019, 2020a, b, 2021). She described the main patterns of selfmedication practices, how and when patients seek non-prescribed antibiotics, investigated the factors influencing the practices of self-medication with antibiotics and described the practices and reasons for non-prescribed antibiotic dispensing by pharmacists. Finally, Lenore Manderson (2020) examined doctors' and nurses' prescribing practices and patients' expectations of clinical consultations to gain insight into the use of antibiotics for upper respiratory tract infections in urban South Africa; her data derived from interviews with providers, patients and parent/guardians, key informant interviews and observations of therapeutic encounters in community health centres and private doctors' surgeries.

1.3 Legal Context

The present status of the provision of antibiotics is as follows. In Uganda, the National Drug Authority Act places antibiotics in Group I of Class B drugs or controlled drugs; these can only be legally prescribed by registered medical

practitioners, dentists and veterinarians. Authority to dispense is given to licensed pharmacies under the direct supervision of a pharmacist. However, pharmacists, nurses and drug shop vendors prescribe antibiotics. These can be accessed without a prescription in licensed and unlicensed drug shops, community pharmacies, shops selling assorted items, "shift markets", or temporary and mobile markets in rural areas and from drug hawkers. Healthcare providers in primary care settings and drug shops frequently provide amoxicillin to treat common conditions with which patients present (Mbonye et al. 2016; Chandler et al. 2017; Batwala et al. 2011), and amoxicillin was among the top three antibiotics reported as frequently used by participants in rural and urban Uganda (Nayiga et al. 2020). In our conclusion, we return to the relevance of people's familiarity with this drug to its misuse.

Legally, in Mozambique, the authority to prescribe is invested in medical doctors, dentists and nurses. In rural settings with few or no doctors, medical technicians also have the right to prescribe. In practice, antibiotics are also prescribed by other categories of healthcare professionals. Although efforts are slowly taking place by the National Director of Pharmacy and partners to supervise antibiotic commercialisation and dispensing while establishing robust accountability mechanisms, the easy availability of antibiotics in pharmacies, the willingness of pharmacists to administer and the ready accessibility of these drugs to the public highlight the country health authority's inability to enforce this class of drugs as prescription-only medicine. Ready access to the drugs has contributed to the growing practice of self-medication and non-prescribed dispensing, systematically exposing individuals to the risk of antibiotic side effects and developing antibiotic resistance. Consequently, this increases the healthcare system's costs of acquiring more potent antibiotics to fight resistant bacteria and contributes to side effects, complications due to overuse, underuse or incorrect use, other health problems associated with inappropriate use and mortality due to antibiotic-resistant infections (Jit et al. 2020; Llor and Bjerrum 2014; WHO 2020).

In South Africa, again, antibiotics are available by prescription only. The South African (SA) Antibiotic Stewardship Programme (2015) and the Essential Medicines List for SA (2018) recommend amoxicillin or tetracycline as first-line treatment for upper respiratory tract infections to prevent as well as treat bacterial infections such as otitis media and acute sinusitis; these guidelines also recommend ciprofloxacin for community-acquired urinary tract infections. In addition, there are exceptions for people with pre-existing conditions that make them vulnerable to bacterial infections and complications, and there are changes to prescriptions for people who report allergies to penicillin. Moreover, controlling access to antimicrobials through prescription does not address whether the prescription is warranted, nor does it influence how the drug is used once filled (Tadesse et al. 2017; Manderson 2020; Tompson et al. 2021).

1.4 Biomedicine's Power

The overuse and misuse of antibiotics, implicated in the acceleration of drug resistance, derives from the dominance of biomedical practice and its technologies, including medications. On the continent, this dominance was established under colonial rule and through missionaries (Palanco Lopez and Chandler 2020), with ideologies of benign development that minimised opposition to and countered the adverse health effects of the imposed rule, labour migration and disruptions to the environment. The provision of hospitals and schools and the establishment and maintenance of policing and a judiciary provided colonial powers with a way to balance the harshness of their treatment of labour recruited into extractive industries and plantation economies and to minimise disease in concentrated areas of colonist settlement and where local and slave labour were in regular contact with overseers. Biological and chemical developments through the nineteenth century bolstered the healing power of colonial medicine and the reputation of its doctors. Establishing local research centres, including Pasteur Institutes from the 1890s and hospitals and later primary healthcare posts, provided governments and churches with platforms to reach local populations. At the same time, endemic and introduced diseases, including plague, African trypanosomiasis and malaria, provided platforms for colonial doctors and government control programmes to showcase science and promote an ideology of advancement (Vaughan 1991). The co-directional relationship between pharmaceutical companies and medical practitioners in promoting medicine use, and encouraging particular drugs as a panacea, took root in this context.

Historically, pharmaceuticals were important in reducing severe morbidity and mortality, allowing the often swift and successful treatment of a range of ailments central to the care provided by biomedical practitioners to their patients. New drugs came on board through the twentieth century to tackle severe and often fatal infections. Efforts to prevent smallpox date from the earliest colonies and were slowly eradicated; by the late twentieth century, polio had also been virtually eradicated, while yellow fever and leprosy continue to be transmitted, although significantly contained through vaccinations and border control for yellow fever and early case detection and multidrug therapy for leprosy. The incidence of TB has also declined, and HIV has moved from a life-threatening condition to a chronic, manageable condition. Other endemic infections and non-communicable diseases are primarily managed through preventive medicine, mass drug therapy, and early treatment. As stated by van der Geest and Whyte (1989), medications have been considered commodities with very attractive charm in different cultures, enhancing the perception of illness as something tangible which can be manipulated.

The widespread use of antibiotics to manage infections, and the increase of resistance, as a result, must be seen, therefore, in the context of the use of drugs as central in disease control programmes. In addition, antibiotics have been one of the most successful medical resources for controlling infection and preventing death. Their value for health system responses to prevalent conditions, particularly at a community level, led to their rapid commercialisation, and their relatively low cost,

high stability and ease of storage likely encouraged their prescription and use (Do et al. 2021; Tompson et al. 2021).

The most common antibiotics in Uganda, Mozambique, South Africa, and worldwide are widely prescribed (or self-prescribed) for common ailments without evidence of their need or effectiveness. Patients often use the names of pharmaceuticals such as amoxicillin and cotrimoxazole to refer generically to these drugs. In his book on the community use of antibiotics in Vietnam, David Craig (2002) wrote of the techniques of inclusion of antibiotics into everyday life. Without the requirement of a prescription, people presented to a local pharmacy and asked for one or two tablets to quell symptoms, applied ground tablets topically to resolve earache and repeated the rules of use as a mnemonic: "take two tablets twice a day". As common as paracetamol, discretionary antibiotic use is as common in Vietnam as then, particularly for conditions like a sore throat or earache, which are considered inconvenient rather than serious, and unlicensed suppliers sell Amo (amoxicillin) or Cefa (Cefalen) on request (Nguyen et al. 2019). As we have illustrated in our previous work (see references below, all authors), this is equally the case in different African settings, including where pharmacists provide prescription-only drugs at patients' request, where unregulated generics drugs are available and where drugs "leak" from community centres and are sold on to consumers for immediate or later use. However, as Do et al. (2021) note, we lack comprehensive and contextual data that would provide insight into the local complexities of antibiotic use in much of the world, particularly in African settings.

1.5 Self-Medication

The continuing practice of self-medication with antibiotics, systematically exposes individuals and communities to the risk of antibiotic resistance and side effects. Self-medication practices are influenced by various factors, including the healthcare system, participants' health-seeking behaviour and social and economic circumstances. These are reinforced by local understandings that antibiotics heal all illnesses, which determines the social representations of medicines and shapes attitudes and behaviours regarding self-medication. Embedded patterns of self-care and home-based care, the social representations of the healthcare system and the therapeutic itineraries that people adopt all influence how people manage health and illness and when and from whom they seek advice and care (Whyte et al. 2002; Hardon and Sanabria 2017).

Self-medication with antibiotics is a significant factor driving antibiotic resistance (Torres et al. 2021). As we have already argued, first-line antibiotics are as familiar by name and as commonplace as proprietary analgesics such as Aspro© and Panado© and readily available through multiple formal and informal sources. People purchase such medicines over the counter, without health professional advice, because they are cash- and time-poor and stock-outs are common. In South Africa, for example, people were aware of pharmacists willing to provide drugs

such as amoxicillin over the counter and health providers who would routinely prescribe the medications on presentation. However, people consistently reported the general rules of stewardship "store in a safe place", "dispose of carefully" and "always adhere to the prescribed dose" (Manderson 2020). Moreover, social and economic factors underpin how people acquire and use drugs. People's familiarity with antibiotics as a generic cure for conditions such as respiratory infections supported their decision to alter the frequency and to include antibiotics as a home remedy for others with the same symptoms. Antibiotics have, in this sense, moved from being treated as sufficiently toxic only to be used as prescribed, as is the case with standard medication regimes, including for diabetes and HIV, to being used with discretion, as occurs with non-prescription drugs. This fits with advice for people to be responsible for their own (and others') care and not "overuse" health services for trivial conditions.

Likewise, in urban Mozambique, antibiotics are perceived as essential medical resources to manage health and illnesses (Torres et al. 2020a). Similar figures where found in a rural context and non prescrided antibiotics were used to self-medicate and treat signs and symptoms of the disease without clinical diagnosis and understanding of the causes, as also an expression of "self-care" (Olgo et al. 2020; Carla 2020). Disease symptoms led patients to self-diagnose, and on this basis, they would approach a pharmacist, describe their symptoms or health problem and request antibiotic drug to sef-diagnose. Patients might mention a generic name or brand, present with a prescription that had already been used or had expired, or an empty package in hand, or describe the drug by appearance. People commonly referred to antibiotic drugs such as amoxicillin, also known as "two colours", cotrimoxazole and amoxicillin with clavulanic acid. While taking an active role in their health-disease process, people used such drugs to self-medicate and to treat family members. Finally, people shared prescriptions and/or medications routinely, including antibiotics, with family members, friends, and neighbours.

1.6 The Limits to State Health Services

Individuals take responsibility for their personal and household use of antibiotics because of the challenges in the access to and affordability of services and inefficiencies in available services (Gatwiri et al. 2020; Nayiga et al. 2020). In Uganda, as in other African settings, the allocation of resources by the state toward public services such as health care and education has declined. Public services have been privatised, encouraging people to be entrepreneurial and financially independent (Birungi et al. 2002). Leaving responsibility for health care to the individual shifts attention away from structural factors that continue to drive poverty and inequality. Individuals are left to find solutions to the broken health system, which shapes how they use medicines. In Uganda, where the private health sector is dominant, drugs are widely available and poorly regulated. As a result, the use of medications is closely linked to people's everyday social and economic realities. Sometimes

people have to prioritise food over medicine, and in such cases, they cannot afford a full dose of antibiotics. Despite doubts about the quality of medicines provided in drug shops and the quality of care provided in private clinics, people's choices are limited, and they often rely on these sources of medical care.

The pharmacies often serve as the first and the last point of contact for patients in the healthcare-seeking chain, including across Africa (Granado et al. 2009; Mayora et al. 2018; Mburu et al. 2021). The global expansion of the pharmaceutical industry has led to the rapid growth of chemist outlets in urban and peri-urban areas in many resource-constrained countries. In Mozambique, while this expansion provides previously underserved populations with access to professional advice and medicines., sales in those facilities, are primarily driven by the aggressive marketing of pharmaceutical companies who offer local pharmacists (and doctors) attractive incentive schemes, bonuses and gifts for increased sales (Torres et al. 2020b; see also Carpenter et al. 1996).

Pharmacists are ideally positioned as front-line health providers to limit indiscriminate use and promote medicines' safe and effective administration, mainly for antibiotic drugs in this context of ntimicrobial resistance, however. Torres and colleagues' research suggests that dispensing antibiotics without a valid prescription is a very common practice. Pharmacists are therefore positioned in the middle of "two swords" (Torres et al. 2020b): on the one hand, patient's demands for non-prescribed antibiotics and the need to retain customers and generate profit and, on the other, awareness of the legal status of antibiotics (as prescription-only drugs), the public health consequences of inappropriate dispensing practices and their professional and ethical responsibility for upholding the law. These same tensions emerged in Manderson's (2020) interviews with health providers in South Africa, where doctors in private practice spoke of balancing the possibility that patients would procure antibiotics nevertheless, with or without a prescription, and the importance to them of retaining their patients. Such legal and pecuniary concerns were moderated by the fears of providers that a patient might not be able to afford to return for a second consultation should medication be deferred and worry that the patient could not afford to take time off work to recover from an infection with bed rest alone.

In some regions in Africa (see Porter et al. 2021), stock-outs and irregular availability of drugs are additional factors that influence members of the public to keep drugs for later use and share medicines, impacting the decision of health providers to give patients antibiotics or a prescription to be filled at a pharmacy. In Uganda, for example, the National Medical Stores supply primary care health facilities with a limited range of medicines considered "essential" at this level of care (Uganda Ministry of Health 2016). The antibiotics supplied included amoxicillin, nitrofurantoin, metronidazole, doxycycline and ciprofloxacin, and stock-outs of antibiotics (particularly amoxicillin and metronidazole) were common. When medicines were provided to the health centres by the National Medical Stores, up to 40 patients might present for care, compared with a daily average of around 25 patients. Health workers expressed concern that most patients came to the health facility to collect medicines to store at home in anticipation of future stockouts. Although prescribed antibiotics were dispensed for free in Mozambique at public health facilities,

pharmacies attached to public health facilities were often out of stock, leading patients to approach private pharmacies and request antibiotics. Complete courses of over-the-counter antibiotics were often not affordable, making patients request smaller quantities of drugs. In all study sites, too, people reported saving drugs from previous treatment for use if they had a recurrence of similar symptoms or to be shared with others who might be ill among family members, friends and neighbours.

1.7 How Perceptions of Inequality and Access Influence Supply

The concerns of health providers about the inability of patients to meet the costs of return visits for care, or to take leave from income-generating activities to recover from illness, highlight the critical socioeconomic and political factors contributing to continuing antibiotic resistance. In all study settings, patients often delayed presenting for care for practical and financial reasons, and when they did, they expected "something" from their provider. This was often translated into an antibiotic prescription because people were familiar with certain antibiotics as an effective common prescription-only drugs.

The studies we draw were all conducted in urban areas, and access to and quality of professional care reflected other inequalities. For example, Susan Nayiga and colleagues (2021), in their work with precariously employed day-wage workers living in an informal settlement in urban Uganda, illustrate that frequent use of antibiotics was entangled with the realities of living in a politically, economically and environmentally degraded landscape (Nabirye et al. 2021). Similarly, taking antibiotics in rural households relates to social and economic imperatives to "take opportunities" in everyday precarity and discourses of betterment in contemporary Ugandan society (Nayiga et al. 2022a). In a context of scarcity where "care" is characterised by the delivery of medicines, as discussed above, the authors observed that clinical practice, including antibiotic use in primary care facilities in rural Uganda, was determined by the availability of resources and by professional and patient expectations as much as by clinical guidelines.

Delays in treatment took two forms in the Ugandan study settings: delays in patients presenting at the health facility and health workers anticipating delays in patients presenting to a higher-level health centre following their assessment, recommendations and referral. Both forms of delay were managed with an antibiotic prescription. In the first instance, infections were perceived to have progressed to a severe condition that required "strong" medicines like antibiotics; in the second case, health workers gave patients referrals but also provided a course of antibiotics for possible infections because of expected delays in presenting to or being able to be signed in the referred facility (Nayiga et al. 2022b). Such delays illustrate the opportunity costs of care seeking.

In urban Maputo, delays take three forms. The first, like in Uganda, is the delay in patients presenting to a healthcare facility and seeking professional care. Most times, patients consider their health conditions to be minor and able to be managed at home; clinic advice often reinforces this when health workers advise patients that they do not need medication and that they should treat symptoms such as cough and sore throat with home remedies. The second delay relates to the long queues or waiting times patients experience to see a healthcare provider. Finally, the third delay relates to medication availability when public health facility pharmacies advise patients that the prescribed medicine is out of stock and that the patient needs to seek medication at a private pharmacy (Torres et al. 2021; Cambaco et al. 2020).

Health providers' awareness of patients' economic and social vulnerability influences their prescribing practices: they acknowledge patients' need to keep working or to continue to care for others, the difficulties they experience in transport, lack of funds and the difficulties associated with presenting for repeat visits at clinics. This is true in all our settings, as elsewhere on the continent. In South Africa, women might walk several miles at dawn to queue at a health centre and then wait for several hours before perfunctory assessment and advice; the health workers whom they see are often overworked and poorly paid and lack time to spend to advise patients other than to outline the medication regime (Manderson 2020). People living in the inner city or informal urban settlements rely on contract work, day labouring and informal employment and may lose employment if they take a day off work to seek advice about symptoms of illness or to spend a day in bed.

Health providers' concerns about patients' economic vulnerability in primary care settings shape antibiotic prescribing in two forms. In Uganda, healthcare workers in primary care settings with frequent stock-outs made adjustments to prescriptions to suit the ability of patients to pay (or not) for antibiotics elsewhere; they were also mindful of the uncertainty of stock at private pharmacies. In addition, limited laboratory capacity and delays between sending samples (e.g., of sputum) for analysis and receiving results influenced clinical decisions. In Mozambique, doctors make "blind prescriptions" due to the lack of laboratory capacity to identify the specific bacteria causing the infection. In addition, providers made a clinical judgement based on the presenting symptoms of the patient and the possibility of future bacterial infection and prescribed antibiotics on this basis.

At the same time, South African doctors working in private clinics routinely referred to their concerns about patients' financial situation. They also considered other conditions such as access to hygiene and sanitation and other likely risk factors for persistent infection, including comorbidities, to inform their decisions. On this basis, they prescribe antibiotics to treat possible existing conditions and to head off the possibility of opportunistic/secondary bacterial infection (Manderson 2020).

Patient expectations for the prescription or supply of antibiotics during a medical visit were high. In South Africa, although some patients saw consultation as a means of reassurance, not medication, others directly stated that they expected an antibiotic to be prescribed; otherwise, they were "wasting their time". In addition, doctors opted to provide a prescription to influence the choice of drug, strength and duration of medication, knowing that if they did not do so, patients would seek whatever

antibiotic was available from local drug stores. Further, in South Africa, while health providers assumed that patients expect antibiotics, they also emphasised that they lacked the time and skills in communication to discuss the alternative management of health conditions with their patients (Manderson 2020).

Similarly, in Mozambique, pharmacists complained that pharmacy clients directly sought non-prescribed antibiotics for various health conditions, including those that did not necessarily need antibiotic treatment (Torres et al. 2020b). Despite legal measures to contain the uncontrolled distribution of antibiotics, these drugs were widely available informally. There appears to be a higher rate of use of non-prescribed antibiotics among those buying drugs from informal markets than those who sought advice from a formal health provider (Mate et al. 2019).

In primary care settings, nurses' conclusions on possible bacterial infections seemed to be based on patient symptoms. In Uganda, the persistence of patient symptoms following treatment was sometimes interpreted by nurses to indicate possible bacterial infection, leading them to prescribe what they considered a strong antibiotic like erythromycin. Nurses often complained about making diagnoses that they believed to be beyond their scope of training and often gave broad diagnoses that allowed them to prescribe the antibiotics that were available at the health centre.

Tensions between nurse providers and patients often inform treatment seeking and prescribing (Manderson 2020). For example, in South Africa, patients spoke of being yelled at by nurses for delays in treatment seeking without acknowledging circumstantial factors that might cause such delay. At the same time, patients referred to nurses aggressively acting because they presented unnecessarily with minor ailments for health advice. Patients also occasionally suggested that presenting for care was a waste of time if they did not receive a prescription. In turn, nurses were frustrated by unnecessary treatment seeking and delays in presentation. But they were also influenced by pressure for prescriptions from patients; in Manderson's study in South Africa, nurses spoke of physical aggression from frustrated patients who demanded an antibiotic as an outcome of presenting. The hostility and blame that occasionally erupted in health settings and underpinned more civil interactions likely reflect limits to the health system and legacies of past inequalities that continue to be played out in clinical settings (Moll 2021).

In Uganda, as explained above, antibiotics are mainly acquired through the private sector (Nayiga et al. 2020). With the poor performance of the public health system in the 1990s, there was a proliferation of private healthcare services in Uganda, including licensed and unlicensed private clinics and drug shops, with estimates that 60–70% of human healthcare services in Uganda were by the turn of the century provided by the private sector (Birungi et al. 2002). Most of the rural population in Uganda continues to rely on the public health system, where health care is available free of charge. However, residents described the challenges they often encountered in public health centres, including long waiting times, absence of health workers, frequent stock-outs and lack of equipment and supplies. Given the inadequacies of the public health system, local residents turn to lower-level private sources of healthcare such as drug shops and clinics, where medicines were available at the lowest cost possible. However, residents revealed doubts about the

quality of health care and medicines provided at these drug shops and clinics. People believed that the profit motive in drug shops surpassed the goal of providing quality care and medicines. On the other hand, most could not afford to seek care from more expensive private facilities.

In Mozambique, the objective of primary health care is to improve the utilisation and quality of services, particularly in underserved areas. Therefore, access to primary healthcare services is essential in promoting health equity and quality of life (Pfeiffer et al. 2010). Since its inception in 1975 following national independence, the Mozambican National Health Service (NHS) has rapidly expanded primary healthcare (PHC) services through widespread health facility networks. As a result, the NHS is the major provider of formal healthcare services. Not withstanding the efforts to expand PHC coverage, the accessibility to health care is a problem for most of the population in many urban and rural settings. Infrastructural, economic and social challenges impact people's access to primary healthcare facilities, and these factors all contribute to the practice of self-medication, including antibiotics. The "inappropriate" use of antobiotic drugs can be understood through factors related to the supply side, owing to the practice of excessive prescription, uncontrolled, uneven access to PHC services as well as delays and, on the patient's demand for antibiotics (Cambaco et al. 2020; Torres et al. 2021).

1.8 Conclusion

The WHO's concern is increasing with the intersecting fields and practices that precipitate antibiotic resistance. There is also growing concern about the long-term threats to human and animal health unless there are substantial changes to how antimicrobials are used. Regional, national and local government policies and plans, as well as professional public health actors, have taken up these interests, but the multiple factors that influence the supply, cost and use of antibiotics interact and unfold in different ways. For example, drug companies and the capital they invest in research and development, government monitoring and control of drugs and health provider and patient expectations of given illnesses and their likely course vary in different settings and circumstances.

The exacerbation of antibiotic resistance in Africa has been largely attributed to the inappropriate use of antibiotics. They are commonplace and readily available, assumed to be effective for both prevention and treatment of an extensive range of health problems, and like over-the-counter analgesics, regarded as benign. In addition to their widespread use for humans, they are also – not discussed in this chapter – used as a preventive measure and to treat animals in the primary commercial industry and by fishermen and farmers (Van Boeckel et al. 2015, 2017).

The misuse and overuse of antibiotics in communities, for human and non-human use, are also routinely linked to a lack of understanding of the drugs' purpose and the risks of resistance to them by pathogens. In response, proposed interventions include greater control over drug access by ensuring that they are

prescription-only and educational programmes to reiterate to providers the risks of over-prescription. In addition, health education directed to patients emphasise that they only use antibiotics as prescribed, only for themselves, that they do not adjust the prescription or use leftover medication later and that they dispose of unused drugs safely. However, patient expectations of health services and providers, provider concerns about patient health and well-being and weak regulatory and accountability mechanisms help explain how antibiotics are distributed and consumed in communities. We have referred above to the ubiquity of antibiotics in the everyday treatment of various conditions, and people's familiarity with the drugs is reflected in the ready way in which people speak of amoxicillin (or Amo) and Augmentin. We see this as reflecting antibiotics' wide use in recent generations, such that this class of drugs has the everyday familiarity of paracetamol and other non-prescription drugs, tonics, inhalants and ointments. This has contributed to the perception of antibiotics as the solution to various health problems – to treat viral and bacterial infections, prevent complications, cure diseases and prevent the onset of other diseases.

Because of the familiarity of antibiotics for many infections, we suggest that these drugs are regarded differently from other prescription medications. Cures for neglected diseases of poverty, such as antimalarials and anthelminthic medication (for schistosomiasis, for instance), are also often available outside clinics and may be misused, although they are still purpose-specific medicines. Other drugs are tightly regulated in terms of availability and consumption: antiretrovirals for HIV, for example, multidrug therapy regimes for tuberculosis, and various medications prescribed and used daily for common cardio-metabolic conditions (diabetes, hypertension and hyperlipidaemia). Except for drugs to cure tuberculosis, these are all "drugs for life" (Dumit 2012). These drugs, and other prescription-only drugs, are sometimes provided "in case" and may be taken pre-emptively. Medication may be provided to treat-to-prevent diabetes. For instance, medication may be prescribed for heart conditions to prevent more severe and possible lethal conditions. The PrEP (pre-exposure prophylaxis) protocol enables people who are HIV negative to take antiretrovirals to protect them from acquiring HIV infection; likewise, women antenatally are provided with ARVs to protect in utero transmission as well as to maintain their health. Antiparasitic medication is supplied as mass drug therapy for filariasis, soil-transmitted helminths and schistosomiasis and administered regardless of the pathogen; antimalarials are available to prevent and treat. The stewardship of antibiotic medication requires the reverse of this pattern to drug prescription and advice – to not prescribe and to avoid taking antibiotics, if at all possible, to manage the drug for future use, rather than to avert (the possibility) of a severe health condition in the present. Hence, the message regarding the use of antibiotics is somewhat counter-intuitive; current antibiotic use appears consistent with a general understanding of the preventive use of certain prescription drugs and the symptomatic use of non-prescription drugs (Manderson 2020; Manderson and Ross 2020).

Where health system resources are constrained, prescribing practices are mainly empirical and based on signs of illness. Health providers in primary care settings in

the study sites and elsewhere in African settings make decisions for their patients in the context of diagnostic uncertainty, inadequately skilled staff, overcrowded facilities, drug stock-outs, too few laboratories and weak laboratory capacity, ineffective regulations and the profits that might be made, in many cases, from the clinic-based provision of medicines (Kakkar et al. 2020; Porter et al. 2021). At the same time, as we have indicated, individuals and communities learn to interpret signs of illness and make use of the health information given to them by their providers. They self-diagnose through their desire and need to care for themselves, sometimes resulting in the unnecessary and inappropriate use of antibiotics. But social and behavioural factors influence antibiotic use, both by providers and communities, and consideration of these factors is paramount in the global effort to contain antibiotic resistance and enhance antibiotic stewardship and conservation in Africa.

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Chapter 2
Antimicrobial Stewardship in Africa

Mirfin Mpundu, Raphael Chanda, and R. Salman Khan

2.1 Antimicrobial Use and Resistance in Humans

The challenge of antimicrobial resistance (AMR) is universally recognised in developed and developing countries (Laxminarayan et al. 2013). Low- and middle-income countries (LMICs) are hotspots for infectious disease and antimicrobial resistance (Theuretzbacher 2014). Challenges of poorly functioning and inadequate infrastructure underpin inappropriate antimicrobial use. Political instability contributes to weak governance systems, often affecting priorities in healthcare and weakening regulations and their enforcement (Pokharel et al. 2019). In this setting, some of the drivers of AMR include the lack of access to essential antibiotics, inadequate diagnostic capacity and fragmented healthcare systems.

Due to challenges with technical and diagnostic capacity, the true burden of AMR is largely underreported. The 2014 WHO report identified Africa and Southeast Asia as the regions without established AMR surveillance systems. Few African countries can conduct surveillance to inform the national prioritisation of AMR activities and contribute to World Health Organization's (WHO) Global Antimicrobial Resistance Surveillance System (GLASS). As of 2020, out of 47 countries, only 23 African countries enrolled and contributed data (Global Antimicrobial Resistance and Use Surveillance System (GLASS) 2021). Several reviews have summarised AMR in Africa. A systematic review by Leopold et al. (2014) revealed that the median prevalence of resistance to third-generation

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cephalosporins ranged between 0.0% and 46.5% in West Africa, between 6.0% and 15.4% in Central and Southern Africa and between 0.0% and 22.0% in East Africa (Leopold et al. 2014). In 2020, a systematic literature review conducted by WHO shows that in the African region, among gram-negative bacteria, *Klebsiella* spp. remained the most resistant, and *E. coli* isolates showed concerning levels of resistance to the recommended first- and second-line antibiotics: amoxicillin (24.5%), ampicillin (23.5%), trimethoprim/sulfamethoxazole (22.5%), amoxicillin/clavulanic acid (13.2%) and chloramphenicol (12.3%). Resistance to ciprofloxacin was 8.2%. For gram-positive bacteria, *Streptococcus pneumoniae* also showed concerning levels of resistance against the key tested antibiotics: trimethoprim/sulfamethoxazole (64.3%), oxacillin (32.2%), penicillin (23.2%), tetracycline (28.3%), amoxicillin (20.6%), ampicillin (19.3%%), chloramphenicol (19.3%), amoxicillin/clavulanic acid (17.4%), ciprofloxacin (14.8%), gentamicin (13.5%), doxycycline (1.9%) and erythromycin (1.9%) (World Health Organization. Regional Office for Africa 2021) According to the fourth Global AMR Surveillance System (GLASS) report, for the WHO African Region, median resistance rates for the two Sustainable Development Goals (SDG) AMR indicators monitoring the proportion of AMR in bloodstream infections were 40.1% for *E. coli* resistant to third-generation cephalosporins and 10.3% for methicillin-resistant *S. aureus* (MRS), respectively (Africa regional strategy on antimicrobial resistance communications and advocacy 2022). A reported lack of consistency in measuring and reporting susceptibility data among African countries made it difficult to compare findings among different countries and laboratories, sometimes even within one country (Perdigão-Neto et al. 2014; Lee and Chung 2015). To address this issue, standardisation of laboratory practice through accreditation and participation in external quality assessment (EQA) is required.

The lack of regulation or poor enforcement of laws prohibiting the sale of prescription-only drugs presents a challenge in LMIC acting as a driver of inappropriate antibiotic prescription, dispensing and use (Kalungia et al. 2016; Torres et al. 2019). In addition, poor regulatory enforcement in this setting may contribute to challenges of access to antibiotics, especially in remote communities with limited access to healthcare facilities (Charani et al. 2019; Yantzi et al. 2019). Therefore, advocacy for regulatory enforcement should be championed in tandem with improved access as part of the package.

In Africa, the threat posed by AMR is unrecognised due to poor awareness among stakeholders, including the general public and government, hence presenting a problem for priority setting and public engagement. Furthermore, most African countries have a mixed healthcare system with service provision by public and private sectors. Such systems create an environment that allows patients to move between facilities quickly, and because of poorly integrated services, accurate antibiotic history is challenging to obtain (Charani et al. 2019). In addition, these complex systems are difficult to regulate, especially in the private sector.

Despite signing the Abuja Declaration in 2001 and pledging 15% of national budget allocation to healthcare (World Health Organization 2010), African countries are far from attaining universal health coverage, with most healthcare systems

remaining poorly financed. The lack of access to essential medicines creates a drive for black market sale of poor-quality medication, prescription of broad-spectrum antibiotics and increased unlicensed prescribers (Anstey Watkins et al. 2019). Additionally, in an effort to meet sales quarters and satisfy customers, pharmacies as a business rarely view compliance to regulation in their best interest and hence may contribute to inappropriate antibiotic use.

Quantifying antimicrobial use (AMU) in LMIC is also an important area of concern due to illegal antibiotic trade, unlicensed prescribers and, in the healthcare setting, stockout with individuals purchasing their own medication. Using the defined daily dose (DDD) matrix for measuring AMU is thus challenging in that it fails to reflect the true antibiotic consumption. In community settings, common behavioural practices that contribute to AMU include self-prescription for nonbacterial infections (Tanday 2016), i.e. flu, sharing antibiotics with other individuals and inability to complete an antibiotic course (Charani et al. 2019). Poor vaccine uptake for preventable diseases, either due to access or behavioural factors, also contributes to inappropriate AMU and subsequent AMR in Africa (Kwong et al. 2009).

AMR presents a complex problem, highlighting the disparity of access to essential medicines and stressors in healthcare systems in most African countries. In addition, AMR increases the risk of mortality, especially among immunocompromised patients, leads to challenges in managing hospital-associated infection, reduces treatment options for future generations and also increases the operational cost of providing health services (Hocking et al. 2021; Dadgostar 2019).

With the many problems faced by many African countries, AMR is still a foreign concept requiring awareness among government leaders and its citizens for it to be considered a priority. It requires a voice on the African continent, where it is a faceless pandemic. AMR requires contextualisation in the African setting to resonate with the local population's problems, using terms they understand and relate to Cars and Nordberg (2005).

2.2 Antimicrobial Use and Resistance in Animals

The United Nations estimates that Africa's population will reach 2.4 billion by 2050 (Demographic evolution in Africa 2022). The rise in population causes an increase in demand for food products. As a result, more agricultural and livestock products must be produced to meet economic, social and dietary needs (Schmidt 2012). The production of meat in Africa has risen by 64% since 2000 due to the increasing demand for protein by the growing population (Global trends in antimicrobial resistance in animals in low- and middle-income countries). The widespread and expanding use of antimicrobials in livestock due to the rising global demand for animal protein is cause for concern, given the threat of AMR. Antimicrobial agents are administered to various food animal species, most notably poultry. Antimicrobial agents used on farms ranged from 77.6% in Nigeria to 100% in Tanzania, Cameroon,

Zambia, Ghana and Egypt. There were 14 different classes of antimicrobial agents used, the most common being tetracycline, aminoglycosides and penicillin. In Africa, antimicrobial agents such as macrolides were used, which are prohibited for animal use in some developed countries due to their impact on human health (Kimera et al. 2020).

The AMU to maintain animal health and productivity is vital in intensive agriculture and food production systems (Global trends in antimicrobial resistance in animals in low- and middle-income countries). Adverse effects such as the development and spread of resistant pathogenic microorganisms are of great concern. Resistant organisms can be transmitted directly through contaminated animals and their products, crops, soils and ground and surface water (Marshall and Levy 2011). Due to a lack of adherence to hygienic practices, resistant microorganisms can also be transmitted during harvesting, post-harvest handling and distribution (Phares et al. 2020). In Africa, the rise in AMU and AMR is primarily due to weak regulations in human and animal health practices; weak enforcement of existing AMU laws and regulations; poor surveillance systems for AMR and AMU in humans, animals and crops; absence of antimicrobial disposal policies and regulations; and a lack of updated standard treatment guidelines for human and animal diseases (Schar et al. 2018). In most Sub-Saharan African countries, where capital, credit and government support are limited, livestock farming is preferred because it requires minimal investment. Farmers frequently diversify their activities to reduce economic risks rather than specialising in a single product.

Nevertheless, antimicrobial access is still required to treat sick animals and thus maintain overall health. In Africa, the annual cost of infectious animal diseases is estimated to be US $9.35 billion, with losses due to lack of treatment far outweighing losses caused by AMR (Grace 2015). As a result, in the absence of generalised improvements in access to veterinary services, significantly reducing the use of antimicrobials, which is required in the fight against AMR, will almost certainly result in an increase in disease and a decrease in production. Furthermore, this reduction will have a significant economic impact on small farmers and reduce access to affordable meat for the most vulnerable groups, resulting in substantial adverse effects on nutrition (Littmann and Viens 2015). Therefore, it is necessary to strengthen domestic regulations to limit the emergence of AMR by considering the needs of family farmers and the low-income group and appropriately addressing the ethical issues of social value and equal access.

2.3 AMR in the Environment

AMR is prevalent in microorganisms found in the environment. However, using antimicrobial agents such as antibiotics in humans, terrestrial and aquatic animals and plants has been linked to the evolution and amplification of antimicrobial-resistant pathogens and their AMR genes (ARGs) (Africa regional strategy on antimicrobial resistance communications and advocacy 2022). The environment serves

as a way to mix mobile genetic elements, which can then interact and spread to other parts or human and animal hosts (Góchez et al. 2019). Antibiotic discharge occurs through various sources, including municipal and hospital waste, animal husbandry, the manufacturing industry, and runoff from agricultural fields containing livestock manure and landfill leachates (Kümmerer 2016; Antibiotics and antibiotic resistance in agroecosystems 2022). Antibiotics have a half-life of hours to hundreds of days, but antibiotic residues are considered persistent environmental pollutants (Sanderson et al. 2019). Many African communities, including urban areas, lack access to proper hygiene, directly releasing wastes and faeces into surface waters (Ncube et al. 2021). The subsequent contamination of soil, sediments, sludge, groundwater, wastewater, tap and surface water and plants leads to the emergence and spread of MDR organisms in the environment. In addition, uncontrolled quantities of wastes containing antimicrobial substances produced by pharmaceutical manufacturers, hospitals and livestock contribute to the selection of resistomes in the environment, potentially impacting animals and humans (Oyekale and Oyekale 2017; Drug-Resistant Infections | Other papers 2022). The prevalence of MDR *E. coli* ranged from 33.3% in South Africa to 100% in Algeria in environmental samples (Kimera et al. 2020). The environmental aspects of antibiotic resistance (AMR) have been largely ignored. First, greater attention was paid to the human side of the AMR crisis, followed by the animal sector. Adopting an AMR-centric waste management approach and controlling the spread of AMR determinants into the larger environment is required to combat AMR in the environment (Khurana and Sinha 2019).

2.4 One Health Approach and AMR

To address the global threat of AMR and the challenges it presents for the provision of optimal delivery of health services, the WHO at the 68th World Health Assembly developed a Global Action Plan (GAP) on AMR to which countries aligned their AMR National Action Plans (NAPs) (Global action plan on antimicrobial resistance 2015). As part of the strategy to address AMR, optimisation of AMU in both human and animal sectors was identified as a key focus. The targeted area acknowledges the importance of the One Health approach to AMR, which includes interventions in animal, human and environmental sectors. This is essential as AMR is a multi-sectoral issue that needs a cross-sectorial approach. In Africa, One Health remains theoretical mainly due to several challenges, including minimal interagency collaboration and a lack of regulation of AMU across sectors (Destoumieux-Garzón et al. 2018). Political instability, natural disasters and famine increase demand for food security, further impacting AMU in agriculture, specifically the food animal sectors. International trade among African countries also contributes to AMR transmission as trade regulations are poorly adhered to and enforced. Furthermore, infection prevention practices are challenging to implement in most healthcare facilities due to a lack of expertise,

resource allocation and overcrowding arising from increased demand (Barrera-Cancedda et al. 2019). Waste management is another area neglected in most African countries, with medical and pharmaceutical waste contaminating water sources utilised for agriculture (Singer et al. 2016).

2.5 Antimicrobial Stewardship in Africa

Antibiotic stewardship (AMS) as a strategy is aimed at promoting prudent use of antimicrobials, with the ultimate goal of optimising clinical outcomes while minimising the unfavourable consequences, which include the emergence of AMR (Barlam et al. 2016). With LMICs significantly contributing to high antimicrobial consumption and consequently high rates of AMR, AMS programmes are a crucial intervention in this setting to optimise AMU.

The major barrier to AMS programme implementation is the lack of AMS policy at both institutional and national levels. Furthermore, when policies are in place, support to create awareness and implementation is required (Gebretekle et al. 2018). AMS interventions require long-term commitment and continuity as they are only usually measurable after 2–5 years (Mendelson et al. 2020).

In addressing the implementation of AMS programmes in LMICs, several tools have been developed by organisations, including the WHO and Action against AMR (ReAct). However, these tools are not well adapted to address the challenges in the African context, presenting a barrier in assessing functionality and monitoring and evaluating these programmes.

AMS interventions are targeted at various levels of healthcare which include prescribers, patients, drug providers, policymakers and the general public (Barlam et al. 2016). Available evidence supports the effectiveness of AMS interventions at the hospital level, demonstrating an impact with reduced length of stay, shorter treatment duration and improved patient outcomes. In addition, the cost-effective benefit of AMS on healthcare delivery has been demonstrated (Timbrook et al. 2016). However, in many LMICs, it is challenging to establish the cost-benefit of AMS at the facility level due to centralised procurement processes of antimicrobials; hence, institutions do not directly bear the cost (Mathew et al. 2020). Additionally, antibiotic stockouts and out-of-pocket purchases further complicate assessing the economic impact (Asmamaw et al. 2021).

The hospital stewardship programme typically includes an antimicrobial committee, continuous monitoring of AMU through prospective audit and feedback, surveillance of AMR and AMU and evaluation of intervention outcomes and the development of evidence-based local treatment guidelines and drug formularies (Demographic evolution in Africa 2022). In general, most healthcare institutions in LMICs, in addition to being part of a broken system, face considerable constraints, which include infrastructure, significant patient load, high patient-provider ratios and lack of orientation and training towards rational AMU and are all contributing to challenges of establishing AMS programmes (Kakkar et al. 2020). One of the

solutions in addressing skilled personnel challenges is task shifting, with pharmacist-led interventions having been proven effective in closing this gap (Brink et al. 2016).

In LMICs, community AMU is an important driver of AMR, especially with poor regulation of antibiotic use exacerbated by cultural practices. However, fewer studies have been done on interventions targeting prescribers in the outpatient setting. Despite this, available studies have shown the impact of AMS intervention in this setting, demonstrating reduced antibiotic prescriptions and resistance rates (Sumpradit et al. 2012; Shao et al. 2015; Santa-Ana-Tellez et al. 2013).

One of the significant activities to ensure the successful implementation of AMS programmes is the development of evidence-based antibiotic guidelines based on locally generated data (Dellit et al. 2007). The lack of laboratory capacity to perform microbial culture and susceptibility testing and conduct hospital-based surveillance is a limiting factor in most LMICs. The paucity of diagnostics and representative surveillance data makes AMS in LMICs particularly difficult as prescribers frequently lack essential information to guide their clinical decision-making, develop context-specific treatment guidelines and inform policymakers (Kakkar et al. 2020). In addition, acceptability and uptake of guidelines is an important area to address, as it is not enough to develop guidelines if prescribers do not use them. The involvement of local stakeholders can achieve this during the process, hence imparting a sense of ownership to the prescribers. Regular dissemination of guidelines among all the prescribers to ensure compliance with these documents is necessary. In a South African study, only 30% of clinicians knew of *Clostridium difficile* guidelines, and fewer could correctly recall them. Furthermore, many healthcare professionals prescribe antibiotics based on personal experience rather than guidelines (Asante et al. 2017; Pearson and Chandler 2019). Junior physicians prescribe broad-spectrum antibiotics out of fear of negative career evaluation if they use narrow-spectrum antibiotics (Schmidt 2012). This practice also extends to the prolonged use of antibiotics for pre- or postsurgical prophylaxis (Alemkere 2018). The existence of evidence-based and surveillance-informed guidelines and the contribution to establishing a functional AMS programme is an important subject that needs to be emphasised, and their absence is a challenge in most LMIC health facilities.

It has been realised that most routine curricula and learning in medical, pharmacy and nursing training do not adequately cover antibiotic stewardship principles (Pulcini and Gyssens 2013). Thus, specific training in the discipline of AMS is essential before completion of training to build knowledge and create awareness regarding the rational use of antibiotics in the African setting (Ncube et al. 2021). Several studies conducted in diverse settings highlight the inadequacy of knowledge and lack of awareness regarding rational antibiotic use among physicians and medical students in resource-limited settings (Kose and Colak 2021; Akhtar et al. 2020).

2.6 Recommendations

- Increased awareness to ensure political buy-in is required to prioritise human and veterinary AMS interventions at national and facility levels.
- There is a need to develop additional tools for implementing and monitoring AMS programmes adapted to resource-limited settings.
- Improved laboratory capacity is key to a successful AMS programme, and sustainable funding support models must be developed.
- Prioritisation of activities based on the treatment guidelines for humans and terrestrial and aquatic animals.
- The development of AMS training curricula as part of the training programme for health and veterinary professionals is essential.

2.7 Conclusion

Successful AMS programme implementation in Africa requires a systems approach, tailoring the interventions to address problems within the local context. However, countries often lack the solid political commitment to generating the local evidence base necessary for developing and utilising data to develop solutions to address AMR in the national context.

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Chapter 3
Etiology of Early-Onset Bacterial Sepsis and Antibiotic Resistance in Neonates: A Case Study in an Algerian Neonatal Intensive Care Unit

Mairi Assia, Boudrelal Dihia, Ahmane Hassina, Christophe De Champs, and Touati Abdelaziz

3.1 Introduction

Neonatal age represents a critical period in the life of newborns. Globally, an estimated 2.5 million newborns die in the first month of life, approximately 7000 newborn deaths every day in 2018, accounting for 98% of neonatal deaths in developing countries despite recent advances in the health system (World Health Organization [WHO] 2019). The majority of these deaths were caused by infections (35%), prematurity (28%), pregnancy-related complications (24%), and asphyxia (23%) (Alemu et al. 2019).

Neonatal sepsis is an infection involving the bloodstream in infants younger than 28 days old (Singh et al. 2021). It remains one of the greatest causes of morbidity and mortality among neonates, especially in the low-income countries of sub-Saharan Africa, South Asia, and Latin America, with a case fatality risk of 9.8% in the first month of life (Getabelew et al. 2018). According to the onset age of symptoms, neonatal sepsis is divided into early-onset sepsis (EOS) and late-onset sepsis (LOS). EOS refers to sepsis in neonates at or before 72 h of life, and LOS is defined as sepsis occurring after 72 h of life (Singh et al. 2021).

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The causative agents of neonatal sepsis vary across the world; however, according to reports, Gram-negative bacilli (GNB), mainly *Klebsiella* sp. and *Escherichia coli*, are of greater importance in developing countries and group B *Streptococcus* (GBS) in developed countries (Le Doare and Heath 2013).

It is generally assumed that the transmission of pathogens from the female genitourinary system to the newborn or the fetus is the leading cause of EOS. Besides, the infection can also occur in utero or during delivery as they pass through the vaginal tract. Typically, GBS, *E. coli*, coagulase-negative *Staphylococcus*, *Haemophilus influenzae*, and *Listeria monocytogenes* are the primary bacterial pathogens responsible for EOS (Singh et al. 2021; Simonsen et al. 2014). Risk factors for EOS include both maternal and infant factors. These maternal factors include at least dietary intake of contaminated foods, procedures during pregnancy (such as cervical cerclage and amniocentesis), maternal risk factors during labor (prolonged rupture of membranes, vaginal colonization with GBS), and chorioamnionitis. Infant factors include prematurity/low birth weight, congenital anomalies, complicated or instrument-assisted delivery, and low APGAR scores (≤6 at 5 min) (Simonsen et al. 2014).

The WHO guidelines recommend using combination therapy, including gentamicin and benzylpenicillin or ampicillin for at least 7–10 days to manage severe bacterial infection in infants younger than 2 months (WHO 2020). However, these recommendations are based on the common antibiotic susceptibility of the predominant pathogens causing EOS reported in high-income countries (Sivanandan et al. 2011). Understanding the frequency and the drug resistance patterns of bloodstream infections in hospitalized newborns is critical for appropriately directing empiric antimicrobial prescribing and guiding antimicrobial-resistance containment strategies (Larru et al. 2016).

Studies documenting the pathogens causing EOS in African countries are very limited. Thus, local data on the etiology and antibiotic susceptibility of pathogens causing EOS are urgently needed to evaluate the empirical treatment's adequacy and adapt antibiotic regimens to local antibiotic susceptibility patterns. Although various studies have reported the risk factors of neonatal sepsis in different parts of the world, to our knowledge, no study on this topic has been reported in Algeria yet. Thus, we aimed to investigate the risk factors of EOS among neonates admitted to the NICU of the Khalil Amrane University Hospital, Algeria. The study also aimed to identify the pathogens causing EOS and to assess their antibiotic susceptibility patterns.

3.2 Methods

3.2.1 Study Period and Inclusion Criteria

This study was conducted at the NICU of the Khalil Amrane University Hospital, Bejaia, North Algeria. Bejaia city is located on the edge of the Mediterranean Sea with 1,012,274 inhabitants (Algerian census data population statistics projections in

2008). The hospital receives referred patients from all parts of the region, other neighborhood regions, and private clinics.

All neonates aged 72 h or less admitted to the NICU of the Khalil Amrane University Hospital between February and July 2020 were enrolled in this study. This study was approved by the hospital's local ethics committee and conformed to the ethical guidelines of the Declaration of Helsinki. We asked the parents of all neonates to sign a written consent form.

According to the WHO recommendations, the suspicion of EOS is based on the presence of the following signs: incapacity to feed, fever, hypothermia, tachypnea, severe chest indrawing, nasal flaring, grunting, lethargy, reduction of movements, poor capillary refill time, bulging fontanelle, convulsions, jaundice, skin pustules, and/or unconsciousness (World Health Organization 2005; Shane et al. 2017).

The Khalil Amrane NICU uses the standard antibiotic regimen for sepsis in neonates and children. This consists of a combination of aminopenicillins, third-generation cephalosporins (3GC) (cefotaxime or ceftazidime), and gentamicin, according to the WHO guidelines for antibiotic use (WHO 2020; Fuchs et al. 2018). In case there was no clinical improvement, an empirical regimen of imipenem in combination with amikacin or ciprofloxacin was initiated.

A minimum of 0.5 ml of blood samples from all neonates suspected of having EOS were sent to the microbiology laboratory for hemoculture and C-reactive protein (CRP) assessment.

3.2.2 Operational Definitions

Clinically suspected sepsis was defined according to the surveillance guidelines published by the Centers for Disease Control and Prevention (CDC), which required a positive blood culture result with signs or symptoms of systemic illness (i.e., fever >38 °C, hypothermia (<36 °C), apnea or respiratory distress, and feeding intolerance). Laboratory-confirmed sepsis was defined as a recognized pathogen isolated from blood culture that was not related to an infection at another site (Horan et al. 2008).

Neonates that develop sepsis in the first 72 h of life were classified as early-onset (Wynn 2016). Premature rupture of membranes (PROM) refers to a patient beyond 37 weeks' gestation who presented with rupture of membranes (ROM) before the delivery (Premature Rupture of Membranes 2020). Preterm birth was defined as live-born neonates delivered before 37 weeks of gestation (Quinn et al. 2016). Low birth weight (LBW) neonates were those with a birth weight of less than 2500 g (Cutland et al. 2017). Neonatal mortality is defined as the number of neonates who died at the NICU throughout the study period (Owusu et al. 2018). Sepsis-related mortality was defined as death with positive blood culture and sepsis or septic shock diagnosis in the same episode.

3.2.3 Data Collection and Analysis

A structured data collection format derived from the WHO Young Infant Study Group's guidelines was used to obtain sociodemographic data and other relevant information (WHO Young Infants Study Group 1999). We collected data about risk factors for EOS, including PROM, meconium-stained liquor, fever within 7 days before delivery, Apgar score, CRP, the weight of the newborn, gestational age, mode of delivery, nutrition, use of a peripheral catheter, and antibiotic treatment. The length of stay in the NICU and mortality were also recorded to assess the outcome.

Data were analyzed using GraphPad Prism 6 (GraphPad Software, La Jolla, CA). Categorical variables such as the history of antibiotic use, history of admission, and risk factors of and maternal-fetal infections were presented proportionally and compared using the chi-squared test or Fisher's exact test. Continuous variables such as gestation age, length of hospital stay, and weight were described as medians (interquartile range (IQR)). Factors that could predict EOS were investigated using multivariate analysis. All factors with a p-value less than 0.05 were considered statistically significant.

3.3 Results

3.3.1 Demographic and Clinical Characteristics

A total of 412 neonates admitted from February to July 2020 were enrolled. Male neonates formed the majority (258; 62.6%) of study participants.

The demographic and clinical characteristics of the patients are summarized in Table 3.1. A total of 137 (33.2%) neonates had birth weights below 2500 g with a median birth weight of 3.0 kg (IQR: 2.1–3.6).

Also, 396 (96.1%) neonates had a peripheral catheter; the median Apgar score after 5 min of birth was 9 (IQR: 8–9). Sixty-five (15.8%) neonates had positive C-reactive protein (CRP).

Concerning neonate feeding, 202 (49.1%) neonates received formula feed, 125 (30.3%) nutrition by a probe, 26 (6.3%) exclusive breast milk, and 59 (14.3%) both breast milk and formula.

The median gestation age was 38 weeks (IQR: 33–39). A total of 217 (52.7%) neonates were delivered by cesarean section (C/S), and 404 (99%) were delivered at NICU public hospital.

Neonates presented perinatal factors including 188 (45.7%) of risk factors for maternal-fetal infections, 127 (30.9%) of PROM (>18 h), 27 (6.4%) of meconium-stained liquor, 28 (6.8%) of fever within 7 days before delivery, and 70 (17%) of mothers who presented gestational diabetes.

Most neonates (86.9%, 358/412) received antibiotics for a median duration of 7 (3–10) days during their hospitalization. The common antibiotics administrated

Table 3.1 The demographic and clinical characteristics of the patients included in this study

Neonates characteristics	Number	Percent (%)/median
Mean birth weight (kg)	2.84 ± 0.94	3 (IQR: 2.1–3.58)
Birth weight		
<2500 g	137	33.2
≥2500 g	275	66.8
Mean birth size	46.6 ± 5.12	48 (IQR: 43–50)
Sex		
Male	258	62.7
Female	154	37.3
Mean gestational age (weeks)	36.6 ± 3.93	38 (IQR: 33–39)
Gestation age		
Full-term	238	57.8
Premature	174	42.2
Healthcare facility		
NICU public hospital	408	99.0
Private hospital	4	1.0
Perinatal history		
Apgar score (5 min)	8.43 ± 1.21	9 (IQR: 8–9)
Cesarean section	217	52.7
Vaginal delivery	195	47.3
Risk factors of maternal-fetal infections	188	45.7
Premature rupture of membranes (>18 h)	127	30.9
Meconium stained liquor	28	6.8
Fever within 7 days prior to delivery	33	8.0
Gestational diabetes	70	17.0
Nutrition		
Enteral nutrition by a probe	125	30.3
Enteral feeding, exclusive breast milk	26	6.3
Enteral feeding, formula feed only	202	49.1
Enteral feeding, breast milk and formula	59	14.3
Vaccination before admission to the NICU	100	24.2
Use of peripheral catheter	396	96.1
C-reactive protein		
Negative	347	84.2
Positive	65	15.8
Antibiotic treatment in NICU	358	86.9
Type of antibiotics (n = 358)		
Ampicillin (or amoxicillin)	336	93.9
Gentamicin	352	98.3
Amikacin	30	8.3
Third-generation cephalosporins	183	51.1
Imipenem	49	13.7
Ciprofloxacin	14	4.0

(continued)

Table 3.1 (continued)

Neonates characteristics	Number	Percent (%)/median
Vancomycin	5	1.3
Duration of antibiotic therapy	7.87 ± 7.05	7 (IQR: 3–10)
Length of stay in NICU		
<7 days	150	36.4
7–15 days	174	42.2
16–30 days	56	13.6
>30 days	32	7.8
Outcome		
Death	43	10.4
Survival	369	89.6
Causes of death (n = 43)		
Respiratory distress	25	58.1
Prematurity (low birth weight)	6	14.0
Sepsis	5	11.7
Others[a]	7	16.2

[a]Others: renal failure and polymalformative syndrome

were aminopenicillins (ampicillin or amoxicillin) (93.9%, 336/358), gentamicin (98.3%, 352/358), 3GC (51.1%, 183/358), imipenem (13.7%, 49/358), amikacin (8.3%, 30/358), ciprofloxacin (4%, 14/358), and vancomycin (1.3%, 5/358). The combination of aminopenicillins and gentamicin was frequently administered (46.2%), followed by the association of aminopenicillins, gentamicin, and 3GC (33.3%).

A total of 43 (10.4%) neonatal death were recorded resulting from different causes, including respiratory distress (25; 58.1%), prematurity (low birth weight) (6; 14%), sepsis (5; 11.7%), and other causes (renal failure and polymalformative syndrome) (7; 16.2%). Besides mortality, 174 (42.2%), 56 (13.6%), and 32 (7.8%) of neonates had a length of stay of 7–15 days, 16–30 days, and >30 days, respectively.

3.3.2 Profile of Pathogens

Fifty-two bacterial strains were isolated from blood cultures in 49 neonates during the study period giving a culture positivity rate of 11.9% (49/412), of which three neonates presented two different bacterial species per blood culture sample.

The Gram-negative isolates were predominant (78.9%, 41/52) and included *Klebsiella pneumoniae* (*n* = 35, 67.3%), *Enterobacter cloacae* (*n* = 3, 5.8%), *Escherichia coli* (*n* = 1, 2%), *Acinetobacter baumannii* (*n* = 1, 2%), and *Pseudomonas aeruginosa* (*n* = 1, 2%).

The Gram-positive bacteria identified were *Enterococcus* spp. (n = 7, 13.4%), coagulase-negative *Staphylococci* (CoNS) (n = 2, 3.9%), *Staphylococcus aureus* (n = 1, 2%), and group B *Streptococci* (GBS) (n = 1, 2%).

3.4 Antimicrobial Susceptibility

Different antibiotic resistance rates of *Enterobacterales* strains (n = 39) were recorded toward ampicillin (79.4%, 31/39), amoxicillin-clavulanate (79.4%, 31/39), cefotaxime (69.2%, 27/39), gentamicin (53.9%, 21/39), amikacin (28.2%, 11/39), cefoxitin (7.7%, 3/39), and ciprofloxacin (5.1%, 2/39). However, all *Enterobacterales* isolates were susceptible to imipenem.

The *A. baumannii* isolate was resistant to ciprofloxacin and susceptible to imipenem, while the *P. aeruginosa* isolate was resistant to imipenem.

Concerning the Gram-positive bacteria, the *Staphylococcus* spp. isolates were oxacillin-resistant except for one isolate. The seven *Enterococcus* spp. isolates were vancomycin-susceptible, and the GBS isolate was penicillin-resistant.

3.5 Predictors of Early-Onset Bacterial Sepsis

We investigated different factors that could predict EOS using multivariate analysis. Thus, low gestational age, low birth weight (<2500 g) and size, gestational diabetes, enteral nutrition by a probe, and antibiotic use (amikacin, 3GC, imipenem, and ciprofloxacin), were significantly associated with neonatal EOS. Sex, Apgar score, perinatal history, peripheral catheter use, and CRP were not associated with EOS (Table 3.2).

3.6 Discussion

The epidemiology of each NICU is different, and each unit should know its trend. To our knowledge, this study is the first report evaluating the EOS from NICU in Algeria.

In our study, neonates hospitalized in a precarious clinical state often present serious pathologies that may justify the use of invasive procedures. Our study assessed the risk factors influencing EOS and revealed that the probability of a neonate developing EOS increased with low birth weight.

NICU stays are among the most expensive hospitalizations. In this study, EOS significantly extended the length of hospital stay in affected neonates compared to those without EOS. Atif et al. reported that the mean additional NICU stay was 9.2 days, and the mean additional fixed cost was $769. Besides, the mean

Table 3.2 Predictors of early-onset bacterial sepsis

Characteristics	Neonates with EOS ($n = 49$)	Neonates with no-EOS ($n = 363$)	*P*-value
Mean gestational age (weeks)	33.9 ± 4.4	36.9 ± 3.72	<0.001
Mean birth weight (kg)	2.27 ± 1.03	2.92 ± 0.90	<0.001
Birth weight			
<2500 g	31 (63.2%)	106 (29.2%)	<0.001
≥2500 g	18 (36.8%)	257 (70.8%)	
Mean birth size	44.0 ± 5.09	46.9 ± 5.03	<0.001
Sex			
Male	32 (65.3%)	226 (62.2%)	0.68
Female	17 (34.7%)	137 (37.8%)	
Gestation age			
Full-term	15 (30.7%)	223 (61.4%)	<0.001
Premature	34 (69.3%)	140 (38.6%)	
Healthcare facility			
NICU public hospital	49 (100%)	359 (98.9%)	1
Private hospital	0 (0%)	4 (1.1%)	
Perinatal history			
Apgar score (5 min)	8.10 ± 1.21	8.47 ± 1.20	0.049
Cesarean section	22 (44.9%)	195 (53.8%)	0.25
Vaginal delivery	27 (55.1%)	168 (46.2%)	
Risk factors of maternal-fetal infections	24 (6.7%)	164 (45.1%)	0.62
Premature rupture of membranes (>18 h)	19 (38.8%)	108 (29.8%)	0.2
Meconium stained liquor	1 (2%)	27 (7.4%)	0.23
Fever within 7 days before delivery	4 (8.1%)	29 (8.0%)	1
Gestational diabetes	3 (6.1%)	67 (18.4%)	0.031
Nutrition			
Enteral nutrition by a probe	30 (61.3%)	95 (26.1%)	<0.001
Enteral feeding, exclusive breast milk	2 (4%)	24 (6.7%)	
Enteral feeding, formula feed only	15 (30.7%)	187 (51.5%)	
Enteral feeding, breast milk and formula	2 (4%)	57 (15.7%)	
Use of peripheral catheter	49 (100%)	347 (95.6%)	0.24
C-reactive protein			
Negative	38 (77.6%)	309 (85.1%)	0.17
Positive	11 (22.4%)	54 (14.9%)	
Antibiotic treatment in NICU	47 (96.0%)	311 (85.7%)	0.046
Type of antibiotics			
Ampicillin (or amoxicillin)			

(continued)

Table 3.2 (continued)

Characteristics	Neonates with EOS ($n = 49$)	Neonates with no-EOS ($n = 363$)	*P*-value
Yes	44 (89.8%)	292 (80.4%)	0.11
No	5 (10.2%)	71 (19.6%)	
Gentamicin			
Yes	46 (93.9%)	306 (84.2%)	0.074
No	3 (6.1%)	57 (15.8%)	
Amikacin			
Yes	16 (32.7%)	14 (3.9%)	<0.001
No	33 (67.3%)	349 (96.1%)	
Third-generation cephalosporins			
Yes	35 (71.4%)	148 (40.8%)	<0.001
No	14 (28.6%)	215 (59.2%)	
Imipenem			
Yes	27 (55.1%)	22 (6%)	<0.001
No	22 (44.9%)	341 (94%)	
Ciprofloxacin			
Yes	27 (55.1%)	22 (6%)	<0.001
No	22 (44.9%)	341 (94%)	
Vancomycin			
Yes	2 (4%)	3 (0.9%)	0.11
No	47 (96%)	360 (99.1%)	
Duration of antibiotic therapy	15.6 ± 10.2	6.83 ± 5.79	<0.001

[a]Others: renal failure and polymalformative syndrome

cumulative extra cost per patient was $1315 (Atif et al. 2008). Previous studies showed an average extra stay of 5.2 days (Leroyer et al. 1997), 16 days (Abdel-Wahab et al. 2013), and 24 days (Mahieu et al. 2001) for infected neonates.

The mortality rate of neonates at the Khalil Amrane NICU in Algeria was low compared to some studies conducted in other African countries. Thus, the overall percentage of neonatal mortality reported at the neonatal inpatient unit at the Komfo Anokye Teaching Hospital in Ghana was 20.2% (Owusu et al. 2018). In their study, Demisse et al. reported a mortality rate of 14.3% among all admitted neonates in the NICU of the University of Gondar Referral Hospital, Northwest Ethiopia (Demisse et al. 2017). In their systematic review of clinical risk factors for mortality in infants under 12 months of age hospitalized for sepsis or severe infections in low−/middle-income countries, Liang et al. reported very high mortality rates, ranging from 14.6% to 36.0% of admitted neonates. Prematurity and low birth weight were significantly associated with mortality (Liang et al. 2018). These differences could be attributed to many factors, including the healthcare system of the African countries, treatment and care access, the nutritional statute of both mothers and newborns, and the immunity statute of people.

The distribution of the strains per species showed that *K. pneumoniae* accounted for 64.8% ($n = 35$) of all isolates recorded in this study. It is assumed that GBS and

E. coli are the most frequently involved organisms in EOS, accounting for approximately 70% of infections. The remaining pathogens are other *Streptococci*, *S. aureus*, *Enterococcus* spp., *Haemophilus influenzae*, and *Listeria monocytogenes* (Simonsen et al. 2014; Bizzarro et al. 2005; Stoll et al. 1996).

In this study, GNB, especially *K. pneumoniae*, are the leading pathogens causing EOS. Our study constitutes the first report on the microbiological etiology of EOS in Algeria. Our data are in line with some studies published in other African countries. For example, Mulinganya et al. in the Democratic Republic of Congo found that 82% of pathogens isolated from EOS were Gram-negative, of which *E. cloacae* complex (42%), *K. pneumoniae* (18%), and *Serratia marcescens* (12%) were the most prevalent (Mulinganya et al. 2021). Similarly, in the systematic review and meta-analysis conducted by Okomo et al., the authors reported that *Klebsiella* (42%), *E. coli* (19%), and *S. aureus* (14%) were the most prevalent pathogens causing neonatal sepsis (Okomo et al. 2019).

Only one GBS strain was isolated in the current study. This result is in line with the previously cited works of Mulinganya et al. and Okomo et al. They reported one case (1/660) and two cases (2/90), respectively (Mulinganya et al. 2021; Okomo et al. 2019). Our finding is somehow contradictory to what is assumed in the literature, of which GBS is one of the leading neonatal sepsis pathogens globally, which could have an exceptionally high burden of disease in Africa (Sinha et al. 2016; Edmond et al. 2012). WHO recommended using GBS vaccination as a potential control strategy to reduce the GBS EOS in low- and middle-income countries (Giersing et al. 2016). However, in light of our results, we think that GBS vaccination would not be necessary as a strategy to reduce EOS in Bejaia.

WHO has provided guidelines for the management of common childhood illnesses. These guidelines recommended supplying ampicillin and gentamicin for at least 2 days in neonates with known risk factors for infection and reassessing them. Treatment should only be continued if there are signs of sepsis (or positive blood culture). It recommends hospitalization and IM or IV antibiotic therapy with a combination of gentamicin and benzylpenicillin or ampicillin for at least 7–10 days in infants aged <2 months who fulfill the case definition of severe bacterial infection (Fuchs et al. 2018).

In this study, the WHO's recommendations were applied to treat newborns before a definitive etiologic diagnosis was available. Empiric antibiotic therapy for suspected sepsis was started with broad-spectrum antibiotics, of which the combination of ampicillin or amoxicillin for 7–10 days and gentamicin or amikacin for 2 days was the more commonly administered. The antibiotics were administered in doses to achieve a bactericidal concentration in the blood according to the newborn weight. Once a pathogen has been identified in the blood culture, antibiotic treatment is restricted and targeted to the isolated bacteria.

We noticed that the *Enterobacterales* strains isolated from blood cultures in our study were highly resistant to penicillin/amoxicillin (85%), gentamicin (62.5%), and cefotaxime (70%). Thus, our results imply revisiting the use of aminopenicillins, gentamicin, and cefotaxime as first-line antibiotics, as high rates of resistance

were reported. Besides, the Gram-positive strains isolated in this study were also resistant. For example, the *Staphylococcus* spp. isolates were resistant to oxacillin.

Extending the duration of antibiotic use is one of the consequences of nosocomial infections. We found a mean extended antibiotic use of 8.4 days in neonates with EOSs compared with neonates without EOS. The excess use of antibiotics has important implications for NICU patients, for whom the risk of acquiring antibiotic-resistant nosocomial pathogens may be further amplified. From our experience, inappropriate and prolonged broad-spectrum antibiotic therapy and non-investigation of neonatal patients treated with antibiotics are very common in Algeria, and this undoubtedly contributes to the emergence and amplification of resistance in hospitals. If the neonate is treated with the inappropriate antibiotic, the sepsis may progress rapidly, and the patient may develop severe sepsis or septic shock. Correct diagnosis and adequate treatment represent the main determinants of patient outcomes (Folgori and Bielicki 2019). In this study, 22 (6%) neonates were treated with imipenem when they did not need to be treated with a carbapenem molecule (no-EOS). Some studies reported that reducing unnecessary antibiotic use in NICU effectively reduced antibiotic exposure without affecting the quality of care, which occurred in parallel with a reduction in length of stay and multidrug-resistant organisms (Cantey et al. 2016; McCarthy et al. 2018; Yin et al. 2021). The frequent use of imipenem could select for carbapenemase producers in the future. In this respect, we reported in a previous study in the same NICU that the prevalence of fecal carriage of *Enterobacterales* strains producing the OXA-48 carbapenemase was 1.6% (7/422) (Mairi et al. 2019). Furthermore, intestinal colonization by multidrug-resistant (MDR) bacteria also plays a role as a risk factor for infection. According to literature data, up to 50% of newborns colonized by MDR bacteria can develop sepsis (Yap et al. 2016; Lukac et al. 2015).

In addition, blood cultures are the gold standard for establishing a diagnosis of neonatal sepsis, despite being positive in a minority of patients with suspected sepsis, particularly EOS. It is important to note that false-negative blood cultures may occur due to a small volume (less than 1 ml) of blood inoculated for culture, low levels of bacteremia, and prenatal antibiotic use (Zea-Vera and Ochoa 2015). However, in the presence of clinical signs and laboratory markers that are consistent with an infection, the antimicrobial treatment is frequently prolonged beyond 48 h. Thus, increased antibiotic use can contribute to developing and spreading resistant pathogens in the NICUs (Oeser et al. 2020).

We are conscious that our study has some limitations regarding sample size and duration. However, this study, which constitutes the first report from Algeria, highlights the importance of determining the etiology of pathogens causing EOS and their antibiotic susceptibility patterns to rapidly address a correct antibiotic treatment regimen. Furthermore, the results obtained from this study may contribute to better management of neonatal sepsis in the first 3 days of life and the development of local policies based on local epidemiological information. Currently, the use of molecular diagnostics such as real-time PCR will provide an opportunity to improve our understanding of the etiology of EOS, thus limiting therapeutic failures in the NICUs in developing countries.

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Chapter 4
Genetics of Viral Resistance: Clinical Relevance and Role in Viral Disease Outbreak

Juliet Adamma Shenge

4.1 Introduction

Globally, antimicrobial resistance is responsible for more than 700,000 deaths annually and may cost close to ten million lives and an estimated annual value of about US$100 trillion by 2050 if concerted efforts are not made to stop its spread (O'Neill 2016; Tadesse et al. 2017).

In Africa and Southeast Asia particularly, substantial gaps have been reported in the lack of antimicrobial resistance surveillance, standard practices, and data sharing (WHO 2014; O'Neill 2016; Tadesse et al. 2017), which have resulted in the emergence of multidrug-resistant microbes that now pose a significant public health threat (WHO 2014; Liu et al. 2016; Xavier et al. 2016). In addition, while a few studies have reported the geographical distribution of antibacterial resistance in Africa (Tadesse et al. 2017), there is a substantial dearth of data on antiviral resistance in the region, which is particularly an important gap to note.

Humans and animal lives are continuously threatened by infectious diseases, especially those caused by viral agents, and the infection may be acute, persistent, or latent in nature. In addition, emerging and re-emerging viruses may undergo certain biological or environmental modifications that may confer resistance to them. This may be the reason for the surge in treatment and vaccine failure (Dudman et al. 2008; Shenge and Opayele 2020).

Viruses are submicroscopic, obligate, intracellular parasites that possess genetic material enclosed in a protein coat or capsid and lack synthetic machinery for reproduction but depend on living cells for their replication (Chambers et al. 2020). The genetic material of a virus may be a deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) and not both (Chambers et al. 2020), determines the viral life cycle, and

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controls most of its activity within living cells, especially eukaryotic cells and response to antiviral therapy (Wolff et al. 2020).

Viral resistance (VR) occurs when a particular viral agent changes its form due to genetic mutations and stops or entirely fails to respond to any form of therapy (whether preventive or curative) and other control measures to curb infection in a population (L'Huillier and Posfay-Barbe 2021). VR is challenging when patients' response to antivirals or gene therapy is poor.

Over time, research has shown that resistance is driven by complex causes or risk factors, including low potent vaccines, long-term exposure to antimicrobials, intermittent use of antivirals which enable the virus to mutate and develop the capacity to evade the immune system, or high viral loads (L'Huillier and Posfay-Barbe 2021). Again, behavioral factors such as nonadherence to the treatment regimen (Zhang et al. 2017) and overprescription or misuse of antimicrobials in clinical and agricultural settings, and those derived from animal or environmental products, have been the major sources of the emergence of drug-resistant pathogens (WHO 2020). The importance of VR cannot be overemphasized due to its environmental, clinical, and therapeutic implications. This has brought to the limelight the importance of human existence in tandem with the increased need for sustainable development in all areas and facets of life, including medicine, agriculture, and industry, shaped by the future of one health, which mainly concerns the interface between human, animal, plant, and environmental health (Shrestha et al. 2018).

4.2 Origin and Mechanism of Viral Resistance

Microbiology, virology, and population genetics provide a unique understanding of the biological concept of viral resistance. Resistance is known to have some evolutionary history that defines its relevance in public health. Evolutionary parameters such as the genetic diversity of most viruses, including HCV and HIV (Shenge et al. 2018) and genetic drifts or shifts in influenza viruses (CDC 2012), which may result in recombination and selection features, when monitored, may reveal the genesis of viral resistance to a noticeable degree in an infected population (Irwin et al. 2016). Some genomic mutations in certain viruses may confer resistance to antiviral agents and, subsequently, an increased incidence of infections (Irwin et al. 2016). Most antiviral agents target specific viral proteins or one stage of the viral life cycle. Depending on the outcome of a viral infection, resistance may occur due to selective pressure, which plays out during viral replication, especially among RNA viruses (Schulz zur Wiesch et al. 2010). DNA viruses have more conserved genomes with proofreading mechanisms that aid in their genetic components and hence, are more stable during genome replication. This is a feature lacking in RNA viruses and the most important reason for their high rate of genetic mutation (Nasir et al. 2017). Major points are the positive-strand RNA viruses, including *Picornaviridae*, *Flaviviridae*, and *Retroviridae*. Positive-strand RNA viruses typically encode RNA-dependent RNA polymerase (RdRp) enzymes which are crucial for genome

replication but lack proofreading capability or tend to be error-prone during replication, coronaviruses (Sexton et al. 2016). Typically, RNA viruses are the primary cause of emerging diseases as they possess high genetic variability, partly responsible for their high mutation and recombination rates (Simon-Loriere and Holmes 2011). For instance, HIV-1 and HCV mutate rapidly, promoting extreme viral diversity in their genomes (Soumana et al. 2016; Matthew et al. 2018).

In general, the more a virus circulates in a population, the more the changes in the genome, and the location of these changes may affect some features, including its transmission or infectivity and disease course or severity (WHO 2021). In addition, such substitutions or mutations may enable these viruses to develop resistance to some specific antiviral cocktails, which may lead to treatment failure or result in difficulty in designing an effective vaccine (Matthew et al. 2018) or inability to control a viral disease outbreak due to several circulating variants. However, such changes may not cause a vaccine to be unproductive, especially if it is pan-genotypic in composition (WHO 2021).

4.3 Molecular Basis of Immune Evasion by Viruses

Typically, virus-host interactions have offered useful insights into studying the pathogenesis of most viruses, including emerging ones. Such interactions may lead to viral clearance or persistence in the host but, most importantly, have shown how viruses take over the entire host's cellular pathway and escape the innate immune cascade while still replicating in the host (Bösl et al. 2019). In an ideal situation, during exposure to a viral agent, the host's immune response is activated to checkmate the activity and spread of the virus to new cells or get rid of virally infected cells (Virgin et al. 2009). The immune system is responsible for launching an organized virus-specific immune response or defense mechanism against invading infectious agents (Iannello et al. 2006). These responses mainly involve the humoral and cellular immune responses, essential components of the immune system. The genetics behind the immune response to infection may vary from host to host, as this may explain some individuals' sensitivity to some pathogens and not others (Velavan et al. 2021). For instance, the role of host genetic factors in treatment response in patients with viral hepatitis has been elucidated by Stattermayer et al. (2014). Studies have shown that cell types such as B or T cells are specific in their roles and involvement in response to infection as they are activated and regulated by different genes, and this specific genetic history or ancestry may influence a host's response to viral infection (Randolph et al. 2021).

Over time, viruses adapt and learn how to evade the immune system by the evolution of some form of defense strategies, mostly through genomic mutation, to avoid being destroyed by the host's immune cells (Vossen et al. 2002). During viral infection, viruses undermine the antiviral ability of interferon (IFN) produced in response to viral infection by activating or downregulating some signaling transduction pathways such as the signal transducer and activator of transduction (STAT) 2

and STAT 1 transcription pathways (Hahm et al. 2005). In addition, certain viral proteins have been implicated in disease progression and oncogenesis. For instance, the role of human papillomavirus (HPV) E5, E6, and E7 oncogenes in cervical tumorigenesis has been elucidated (Gupta et al. 2018). In most cases, a series of processes are induced, including interruption of the cell cycle, deregulation of microRNA, disruption of the antitumor immune response, modification of gene expression, and many more. These induced events can also serve as markers or novel genetic targets for diagnosing and treating HPV-infected and HPV-induced cancers (Gupta et al. 2018).

4.4 Detection and Monitoring of Viral Resistance

4.4.1 Laboratory Investigation

Viral resistance can be detected through clinical and laboratory assays involving circulating viruses to track changes in their genome. Genetic surveillance involves molecular assays such as reverse transcription-polymerase chain reaction (RT-PCR) to detect variations in viral genomes, which may have clinical significance. In addition, important diagnostic tools such as immunochromatographic kits can be used for differential typing to determine if a particular viral agent has developed resistance against certain inhibitors, neuraminidase inhibitors for influenza A and B viruses (Kato et al. 2018). RT-PCR is an important tool for identifying and monitoring circulating or emerging drug-resistant viruses as it can guide the effective treatment of patients who have developed resistance to some antiviral cocktails. The technique is very sensitive and specific in detecting resistant mutations in genome sequences; however, it is limited to changes in viral agents with unidentified genomic alterations (Tamura et al. 2015; Kato et al. 2018).

The emergence of new viral variants, with more than a single substitution in a viral protein, may pose a serious challenge to effective therapy with an inhibitor such as a protease inhibitor (Gane et al. 2016; Ng et al. 2017). To further understand the molecular mechanism of viral resistance to some antiviral agents such as nucleoside and protease inhibitors and equally detect the same, assays including enzyme inhibition and antiviral tests can be used (Gubareva et al. 2002; Matthew et al. 2018; Matthew et al. 2020).

For quick detection and monitoring in clinical settings, rapid tools such as sialidase fluorescent imaging probe and BTP3-Neu5Ac-based filter (Wako Pure Chemical Industries, Ltd., Osaka, Japan) method can be employed in the detection of resistant mutants of influenza viruses. This involves using ultrafiltration membranes, in which a known virus concentration or solution obtained by centrifugation for 1 minute allows viral particles to be trapped on the surface of the membrane (Kurebayashi et al. 2014).

4.4.2 Resistance Testing

This important laboratory test is used as a guide for selecting drugs for HIV and other blood-borne viruses that mutate rapidly. In addition, it could be used when starting therapy for patients or changing regimens such as antiretroviral drugs, especially if virologic failure is suspected, characterized by poor or total failure in response to therapy (Nasir et al. 2017).

The two most common types of resistance testing are genotypic and phenotypic resistance testing. In genotypic resistance tests, mutations are investigated by sequencing a particular gene segment (Nasir et al. 2017), while phenotypic resistance assays measure how susceptible a virus is to drugs at a specific concentration able to inhibit replication of the virus in vitro (Andreoni 2003). One molecular test involves molecular techniques such as PCR to amplify a mutated region of a viral genome to know the exact antiviral cocktail/inhibitor that the virus is resistant to and which to be selected technologies such as Sanger and next-generation sequencing (NGS) can be very instrumental in nucleotide sequencing (Inzaule et al. 2016). Although very expensive, deep sequencing plays an important role in determining novel and rare mutations in hosts and viral pathogens that may be linked to individual's susceptibility to infectious diseases and resistance (Fava et al. 2021). Sanger sequencing technique is also called dideoxy sequencing or chain termination. The application is based on dideoxynucleotides (ddNTPs), which function as DNA chain terminators likened to nucleotides' subunits present in DNA double helix (Alberts et al. 2002; Karger and Guttman 2009). On the other hand, NGS is a more recent molecular tool capable of sequencing several millions of DNA segments in a single run. The ability of NGS to accommodate such big DNA information makes it more cost-effective, readable, and an on-point assay. Its applicability extends to sequencing resistant strains and whole genome sequences, although the need for highly skilled personnel remains a limitation (Nasir et al. 2017).

4.5 Clinical Implications of Viral Resistance

Antiviral therapy may be associated with the emergence of antiviral resistance or drug-resistant viruses due to certain factors, including nonadherence to drug use and change in dosage. This is observed in the long-term treatment of viral infections, as previously observed in HIV-1 treatment using single-drug therapy with reverse transcriptase or protease inhibitors before the introduction of highly active antiretroviral therapy (HAART) (Bonhoeffer et al. 1997). Conversely, using multiple-drug or combination therapy resulted in more reduction in plasma viral load and less resistance by the virus (Bonhoeffer et al. 1997).

The success or failure of a therapeutic agent to treat any viral infection may depend on some important components of that virus. For example, viral resistance is associated with the emergence of viral variants, a serious drawback to effective clinical management of individuals receiving antiviral treatment and to effective

immunization and elimination of viral infections (Shafer and Chou 2015). In essence, proper understanding of the fundamental biological features of an infectious agent and its response to therapeutics could guide in tailoring treatment to individual genetics and needs (Velavan et al. 2021).

The emergence and spread of a particular virus variant may be key to treatment failure and increased transmissibility and virulence of such a viral variant (WHO 2021). A typical scenario exists with the globally distributed SARS-CoV-2 variants, responsible for the present COVID-19 pandemic, with its deadly consequences of new infections, disease severity, and poor prognoses, resulting in numerous deaths (Tavakol et al. 2021). Furthermore, in most cases, mutated forms of viral variants do not respond to antiviral therapy, as seen in the case of HIV (Merigan 1999).

Studies have shown that notable changes in SARS-CoV-2 spike (S) protein influence its infectivity by conferring enhanced virulence and increased transmission, with resultant competence to evade the immune system, as these mutations reduce the ability of SARS-CoV-2 antibodies to fight off infections (Chadha et al. 2021).

4.6 Role of Viral Resistance in Disease Outbreak

Many emerging and re-emerging viruses have been implicated in the outbreak of viral infections. In most cases, this has been made possible by some viruses' ability to jump from animals to humans after some genome transformation, resulting in fresh outbreaks. When this occurs, certain characteristics or modifications in the form of variation in the viral genome may be involved (CDC 2012). Emerging viruses with zoonotic characteristics, such as SARS-CoV, Middle East respiratory syndrome (MERS), and Ebola, may cause a restricted outbreak or one that can lead to a global pandemic (Grubaugh et al. 2019). Several factors, including population growth, globalization through travels and human interactions, conflicts, and climate change, may facilitate the emergence of viral variants and outbreaks (Shenge and Opayele 2020). According to Holmes et al. (2018), a complex interaction exists between virus genetics, ecology, and host factors that control the emergence of a virus; hence, it becomes challenging to envisage the next pandemic and the viral agent that will cause it (Holmes et al. 2018).

Vaccine failure has implications for emerging variants that may be responsible for new or natural reinfection. Another factor is vaccine escape in a population that may result in the inability to achieve herd immunity in such vaccinated populations. As such, new variants may emerge and circulate (WHO 2021). Reinfection after vaccination is frequently reported in several scientific studies on viral infections such as measles (Shenge et al. 2021). However, a newly emerged viral variant may give rise to an entirely new disease in an environment because its mutated gene has conferred the ability to be transmitted and sustained in a population in an entirely different way (Morse 1995; Grubaugh et al. 2019). This points to the importance of deep genome sequencing of known viruses and emerging strains to keep track of changes (Fava et al. 2021) and other epidemic features such as reproductive number (R_0) that may result in new outbreaks (Grubaugh et al. 2019).

4.7 Innovative Approaches to Prevention and Elimination of Viral Resistance

To overcome the challenge of viral resistance and its numerous consequences, several innovative measures will have to be deployed clinically, biotechnologically, and environmentally, considering their level of impact. A useful diagnostic apparatus to be employed when antiviral failure is alleged in patients, though it may be costly in low- and middle-income countries, is susceptibility testing. This proactive method is tailored to individual patient's needs and improves response to therapy. The use of susceptibility testing to reduce the prevalence of viral resistance is practiced in some countries, including Norway, where populations infected by HIV, hepatitis B virus, *Cytomegalovirus* (CMV), and influenza have been diagnosed through susceptibility testing (WHO 2021).

Integrating genomic research into patient care promotes bench-to-bedside translation in medicine. For example, patients are screened for preexisting resistance mutations in addition to treating some medical conditions, using genomic techniques such as next-generation sequencing.

Systematic surveillance of viral resistance is another approach that can be employed to explore experimental protocols or trials that can prove effective over time for the proper treatment of patients. In addition, the spread of resistant strains in clinical settings, especially those causing nosocomial infections, can be monitored through continuous surveillance to either halt infection spread or target the infected population for proper therapy, which may eventually reduce transmission. Resistance testing should be incorporated into patient's care as early as possible, as soon as infection with a viral agent is diagnosed. In low- and middle-income countries such as sub-Saharan Africa, this may be challenging at the individual patient level, but it can be achieved with funding support and insurance from governments and other stakeholders.

Measures to curb infectious disease transmission ab initio, such as frequent hand washing, social and physical distancing, use of personal protective equipment (PPE), and other preventive strategies, depending on the pathogenesis of the infectious agent, may reduce the transmission of emerging variants. In addition, mutants or variants can be tracked through effective global collaborations and networking, which may involve reference laboratories, infectious disease centers of excellence, and institutes in different countries to detect new strains or variants easily in any population through genomic sequencing and data sharing (WHO 2021). For instance, in the case of SARS-CoV-2, the World Health Organization established a Risk Monitoring and Evaluation Framework to identify, monitor, and assess circulating variants of public health importance (WHO 2021). The team was saddled with the tasks of surveillance and research on emerging variants while at the same time assessing the effects of these variants on existing diagnostic tools, therapeutics, and potential vaccines (WHO 2021). This can be replicated for every known viral disease or epidemic at national and global levels.

Recently, applications involving artificial intelligence (AI), in addition to existing viral genomics and bioinformatics that have been used for decades for investigating infectious disease outbreaks, have become handy tools for tracking, detecting, and eradicating viral resistance and future outbreaks (Grubaugh et al. 2019).

4.8 Conclusion

Understanding the basic information bordering on the host and viral gene modifications in response to viral disease is a critical way to tackle the challenge of viral resistance around the globe. Furthermore, since rapidly mutating viruses are bound to evade the immune system or are less likely to respond to most vaccines due to emerging variants, integrating genome-wide association studies (GWAS) and other genomic studies in combination with AI to detect markers of disease expressed in human genes will lead to proactive and effective preparedness for epidemics and future pandemics.

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Chapter 5
Distribution and Prevalence of Antimicrobial Resistance of NTS Salmonella Isolated from Farm Animals and Animal Food Products in Africa

Bellil Zahra, Mairi Assia, and Touati Abdelaziz

5.1 Background

Salmonella enterica is one of the most important zoonotic and foodborne pathogens. It is one of the main bacterial causes of foodborne diseases worldwide. Several food products such as eggs, white or red meat, and dairy products are the main sources of most of these diseases. Food-producing animals such as pigs, cattle, and chickens are the main reservoirs of human *Salmonella*.

Antimicrobial resistance (AMR) poses a major threat to public health, complicating the treatment of infectious diseases and creating additional health-care costs. It has been estimated that 1.27 million deaths worldwide are attributable to bacterial AMR in 2019, with a particularly high burden in sub-Saharan Africa.

Non-typhoidal Salmonella (NTS) has developed resistance to many antimicrobials and poses a serious challenge to public health. Given the limited data available on *Salmonella* resistance in African countries, this study aimed to systematically determine the prevalence of antimicrobial resistance of NTS isolated from farm animals and animal food products in Africa.

5.2 Introduction

The Salmonella genus constitutes a global public health problem and has attracted significant interest regarding foodborne illnesses responsible for thousands of deaths worldwide. This genus is commonly recovered from food-producing animals and water and is considered the major cause of zoonotic infections in humans and animals. Thus, *Salmonella* infections constitute significant public health and safety

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challenges, affecting animals and the food industry worldwide (Jajere 2019). This genus is associated with three major diseases: typhoid fever, bacteremia, and gastroenteritis (manifested as diarrhea, fever, and abdominal aches), with an incubation period of 4–72 h and mortality being rare (Lokken et al. 2016; Antunes et al. 2016). A well-characterized spectrum of human diseases, ranging from asymptomatic carriage to hemorrhagic colitis and fatal typhoidal fever, may be induced by members of the genus *Salmonella*. In the developed world, foodborne acute gastroenteritis and enterocolitis are the most frequent forms of *Salmonella* infection, which lead to high case-fatality rates, representing an estimated 1.2 million annual cases of non-typhoidal salmonellosis occurring in the world, with considerable costs estimated in hospitalizations (Dekker and Frank 2015). Though relatively uncommon in Africa, typhoid, paratyphoid, and enteric fever constitute a severe global public health problem, with 25 million new infections and over 200,000 deaths occurring annually (Abdi et al. 2017).

The Gram-negative bacterial genus *Salmonella* contains as many as six subspecies, more than 50 serogroups, and over 2600 serovars, including numerous human and animal pathogenic serovars (Lou et al. 2019). Non-typhoidal *Salmonella* (NTS, i.e., *Salmonella enterica* that does not induce typhoid or paratyphoid illness) are responsible for more than 94 million cases of disease and 155,000 cases of death annually worldwide, 86% of which are estimated to be foodborne. However, not all *Salmonella* serovars cause infections among humans and animals. Therefore, efforts are being made to develop NTS vaccines, with most approaches eliciting protection against serovars Typhimurium and Enteritidis (serogroups B [O:4] and D [O:9], respectively), as they are the most prevalent (Fuche et al. 2016).

The greater part of non-typhoidal *Salmonella* disease is restricted to uncomplicated gastroenteritis that infrequently requires treatment with antimicrobial drugs. However, invasive infections, like bacteremia, meningitis, and osteomyelitis, may necessitate antimicrobial treatment (Chen et al. 2013). In addition, the clinical manifestation of NTS is discriminated by undiagnosed fever, while diarrhea is commonly absent. Therefore, these infections are referred to in the clinical/epidemiologic literature as "invasive NTS (iNTS) disease" (Lokken et al. 2016).

5.3 *Salmonella* Taxonomic Classification

Salmonella is a genus of Gram-negative rod-shaped, facultatively anaerobic gammaproteobacteria with flagella; they are intracellular pathogens belonging to the *Enterobacteriaceae* family (Mares 2017; Holschbach and Peek 2018). Since *Salmonella*'s first discovery by Daniel Elmer Salmon and Theobald Smith in 1885, it has become among the most studied microorganisms, along with *Escherichia coli*. The main reason for this attention is that *Salmonella* spp. is a worldwide foodborne pathogen and the second most responsible etiologic agent of human gastrointestinal infections after *Campylobacter* spp. (Lamas et al. 2018). Initially, the genus *Salmonella* was distinguishable by its efficiency in utilizing citrate as the sole

carbon source, lysine as a nitrogen source, and hydrogen sulfide; however, the taxonomy of *Salmonella* is notoriously confusing and has changed over the years (Gal-Mor 2018).

The classification of *Salmonella* has become a complicated history, resulting in part from multiple independent investigators using phenotypic, serologic, and genotypic methods to characterize phylogenetic relationships within the genus and in part from disagreements on nomenclature concerning the number of species and subspecies that form the genus (Dekker and Frank 2015). However, currently, most reference centers for *Salmonella* worldwide (e.g., the Centers for Disease Control and Prevention (CDC)) have taken into account the *Salmonella* nomenclatural system approved previously by the World Health Organization (WHO) (Popoff et al. 2003). This nomenclatural system classifies the genus *Salmonella* into two species based on differences in their 16S *rRNA* sequence. It included *S. bongori* and *S. enterica*. This latter was divided into six distinctive subspecies, each subspecies assigned with Roman numeral: *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), and *indica* (VI), of which 99.5% of strains belong to *S. enterica* subspecies *enterica* (Alikhan et al. 2018; Chen et al. 2013). The *S. bongori* (V) consists of 22 less studied serotypes as they are mainly associated with cold-blooded animals, and human infections are very uncommon, primarily affecting children aged 1 month to 3 years (Dekker and Frank 2015).

Further classification of the particular serovars (serotypes) is differentiated by their antigenic properties into serogroups depending on the somatic (O), flagellar (H), and capsular (Vi) antigens; this is known as the Kauffman and White classification system (Holschbach and Peek 2018). The resolution provided by serologic typing methods has proved valuable to epidemiologic tracking of isolates in outbreaks.

5.4 Morphology, Bacteriological Culture, and Isolation Procedures

5.4.1 Collection, Transport, and Storage of Specimens for Salmonella *Detection*

Members of this genus can metabolize nutrients by respiratory and fermentative pathways referred to as chemoorganotrophic. Except for a few serovars, such as *S.* Paratyphi A and *S.* Choleraesuis, most *Salmonella* serovars produce hydrogen sulfide. Besides, most members of the genus do not ferment lactose. This important unique feature has been used to develop different selective and differential media for the cultivation, isolation, and *Salmonella* presumptive identification (Jajere 2019).

Regarding the laboratory diagnosis of gastrointestinal disease, the fecal specimens should be collected at the initial phase of illness, ideally before initiating antibiotic therapy. Whole stools are the preferred specimen for culture, and examination of multiple samples may improve the recovery of *Salmonella*. Most

commonly used pH-buffered stool transport media are compatible with the recovery of *Salmonella*, although many labs prefer the Cary-Blair transport medium because of its compatibility with other common stool pathogens (Carrique-Mas and Davies 2008).

In cases of suspected systemic infection, as with typhoidal *Salmonella*, cultures from other sources (blood, bone marrow, lymph node, and bone biopsy) should be collected and transported according to standard procedures appropriate to these specimen types (Dekker and Frank 2015).

Regarding food samples, the samples would first be transported to the laboratory in a cooler box with ice packs. After collection, the samples would be analyzed immediately (2–3 h). Next, a representative subsample of each sample (approximately 25 g) would be aseptically removed with a pair of scissors and forceps. Next, this subsample would be homogenized with 225 ml of buffered peptone water in a lab blender, representing the pre-enrichment step, followed by incubation for 24 h at 37 °C. Furthermore, after overnight, the enrichment culture method would be performed by inoculating 0.1 ml aliquot of overnight pre-enrichment broth culture into 10 ml of Rappaport-Vassiliadis (RV) broth and then incubation at 42 °C for 18–24 h (Samaxa et al. 2012).

5.4.2 Salmonella *Culture and Isolation*

Typically, *Salmonella* isolation from swabs, food, and other environmental samples utilizing the traditional method involves an initial nonselective pre-enrichment of a specified sample volume, followed by a selective enrichment step by plating the culture on specific agar and subsequent confirmation of the suspected colonies by biochemical and serological tests. Recently, several regulatory agencies have standardized different *Salmonella* enrichment approaches utilizing its unique biochemical and physical properties. The current ISO standard method, ISO 6579: 2002, has been approved by many Reference Centers for *Salmonella* (Jajere 2019).

Stool culture is the usual source from which the serovars of non-typhoidal *Salmonella* are recovered. Non-typhoidal strains of *Salmonella* might also be recovered from blood and tissue (lymph nodes and bone marrow) (Eibach et al. 2016). Thus, because *Salmonella* competes with other Gram-negative bacteria in feces, most diagnostic laboratories use enrichment media, such as tetrathionate or selenite broth, to enhance *Salmonella* growth. These enrichment broths suppress the normal fecal flora and improve the growth of *Salmonella*, thereby enhancing recovery yield. Once isolated, *Salmonella* should be subcultured using standard techniques to obtain colonies for identification and susceptibility testing (Holschbach and Peek 2018).

Regarding other sample types (food and environment samples), the conventional culture isolation method consists of pre-enrichment of samples in buffered peptone water (or lactose broth) to recover sublethally injured *Salmonella* cells while inhibiting the growth of other competing bacterial flora. This is then followed by a selective enrichment in Rappaport-Vassiliadis (soy base) and Muller-Kauffmann

Tetrathionate-Novobiocin containing two or more inhibitory reagents that allow continued growth of *Salmonella* while inhibiting the growth of other bacteria (Jajere 2019). Subsequently, the incubated selective enrichment broth is streaked on various selective media. Different bacteriological media containing lactose and a pH indicator, such as MacConkey agar, have been traditionally used to differentiate the lactose-non-fermenting *Salmonella* from other enteric pathogens like *Escherichia coli*. However, inoculating stool samples onto an additional differential and selective media is required due to other enteric bacteria, such as *Shigella* spp. and *Proteus* spp., which neither ferment lactose (Gal-Mor 2018). Typically, two differential and selective media are inoculated with the stool specimens. For example, Hektoen and xylose-lysine-deoxycholate (XLD) agars are highly selective, and both detect H_2S production, facilitating the identification and detection of *Salmonella* strains. On the other hand, more highly selective agars, embedded *Salmonella-Shigella*, bismuth sulfite, and brilliant green agars could inhibit some *Salmonella* spp.; therefore, they are often used in combination with a less selective agar. For this reason, a less selective differential enteric medium, such as MacConkey, and a nonselective medium, such as 5% sheep blood agar, may be inoculated as part of the stool culture workup, depending on lab preference (Dekker and Frank 2015).

The resulting presumptive colonies obtained on isolation media are then presumptively identified using triple sugar iron (TSI) and lysine iron agar (LIA). Isolates that yield reactions characteristic of *Salmonella* on TSI agar and/or LIA are further confirmed to the genus level using either a manual identification tool, like the API 20E system, or an automated bacterial identification system, such as Vitek2, Phoenix, MicroScan, or matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) (Gal-Mor 2018). Finally, a range of chromogenic media for the characterization of *Salmonella* in stool samples has been developed. These media utilize enzyme substrates that create a colored product following hydrolysis, resulting in colored colonies that can be easily recognized against the background commensal gut flora.

5.4.3 Biochemical Identification

Salmonella is a non-fastidious bacterium and can grow and multiply under various environmental conditions. However, they can grow in the presence of 0.4–4% sodium chloride, which is not required for their growth. Most serotypes can grow at a temperature range of 5–47 °C with optimum growth at 32–35 °C. However, some serotypes can multiply in a large temperature range, as low as 2–4 °C or as high as 54 °C. The pH necessary for the growth of *Salmonella* ranges between 4 and 9, with the most favorable range varying from 6.5 to 7.5. Although *Salmonella* can survive in <0.2 water activity as in parched foods, they require high water activity (0.94) for survival (Jajere 2019).

Although almost all serotypes do not produce indole, hydrolyze urea, and deaminate phenylalanine or tryptophan, most serotypes readily convert nitrate to nitrite and ferment large amounts of carbohydrates with acid production and are negative

for the Voges-Proskauer reaction. Except for *S. enterica* subsp. *diarizonae* and *S*. subsp. *arizona*e, most serotypes use hydrogen sulfide, decarboxylate lysine, arginine, and ornithine. In addition, most serotypes use citrate except for some *S*. Choleraesuis, *S*. Typhi, and *S*. Paratyphi A serovars. Most serovars do not ferment lactose, and all serovars utilize dulcitol except for *S. enterica* subsp. *arizonae* (IIIa) and *S. enterica* subsp. *arizonae* (IIIb) (Jajere 2019; Lamas et al. 2018).

5.4.4 *Serological Identification for* Salmonella

Definitive identification of *Salmonella* is established by combining phenotypic and serologic methods. The widely used Kauffmann-White serologic typing scheme is based on the LPS O antigen, H1 and H2 flagellar antigens, and the Vi antigen. While the O and H1 antigens are detectable in almost all strains of *Salmonella*, the H2 antigens are only present in certain serovars, and the Vi antigen is found predominantly in typhoidal serovars. It should be noted that Vi antigen may also be expressed in *Citrobacter* sp. (Dekker and Frank 2015; Gal-Mor 2018).

5.4.5 *Identification of* Salmonella *by MALDI-TOF MS*

Commercial matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) instruments can provide rapid identifications of *Salmonella*. Both the Bruker Biotyper system and Biomerieux Vitek MS MALDI-TOF MS systems are FDA (US Food and Drug Administration) approved for in vitro diagnostic use to identify cultured isolates of *Salmonella* (genus-level identification). In addition, the Biomerieux Vitex MS system carries a manufacturer's note that confirmatory testing is recommended when *Salmonella* identification is investigated (Gal-Mor 2018; Sparbier et al. 2012).

5.4.6 *Antimicrobial Susceptibility Testing for* Salmonella

Current CLSI (Clinical and Laboratory Standards Institute) guidelines state that routine susceptibility testing is only recommended for serovars of typhoidal *Salmonella* (such as Typhi and Paratyphi A–C) from all sources and non-typhoidal serovars from extraintestinal sources. Fecal isolates should be tested for susceptibility to ampicillin, a fluoroquinolone, and trimethoprim-sulfamethoxazole. A third-generation cephalosporin should be tested and reported for extraintestinal *Salmonella* isolates; chloramphenicol may also be tested and reported if requested. Interestingly, first- and second-generation cephalosporins, cephamycins, and aminoglycosides may appear active in vitro but are ineffective clinically and should not be reported as susceptible (Dekker and Frank 2015).

5.5 Host Specificity and Adaptation

The ability of a particular pathogenic *Salmonella* strain to adapt to an environment may define its specific host. This concept is regulated by many microbial properties, resulting in the clinical manifestation of symptoms in specific hosts. A particular mechanism that makes a serovar virulent for a particular animal has been shown to make the same serovar less or even avirulent for another animal. This phenomenon is called "serovar host specificity" or "serovar host adaptation." For example, salmonellosis in cattle and pigs is generally caused by serovars Dublin and Choleraesuis, respectively (Anderson and Kendall 2017).

Salmonella persistence and asymptomatic carriage are typical in many animals, including food-producing livestock and domestic pets. These animals are often the origin of NTS foodborne outbreaks. Poultry is treated as a significant animal reservoir for *Salmonella enterica* serovars. Chickens can be infected either with host-specific *Salmonella* serovars, like *S*. Pullorum and *S*. Gallinarum, or with wider NTS serotypes, most of which are carried in the animal's intestinal tract asymptomatically (Jajere 2019; Gal-Mor 2018).

The principal reservoir of non-typhoidal *Salmonella* in industrialized countries is the intestinal tract of food-producing animals, which leads to the contamination of diverse foodstuffs. Therefore, despite other sources (e.g., contact with animals/reptiles, environment, or person-to-person), salmonellosis generated by contaminated food is the most relevant source of *Salmonella* infections. It was estimated that NTS cause around 93.8 million illnesses and 155,000 deaths each year worldwide (Gal-Mor 2018; Antunes et al. 2016). *In addition, Salmonella* Enteritidis is frequently linked to poultry, whereas *S*. Typhimurium has a wider species range, including pigs, cattle, and poultry. Therefore, foods originating from animals, particularly contaminated poultry products (poultry meat and eggs), have been recognized as the principal vehicles of *Salmonella* infection (Antunes et al. 2016; Whiley and Ross 2015).

The degree of adaptation to hosts differs among *Salmonella* serotypes and affects the pathogenicity of humans and animals. Serotypes that are highly adapted to animal hosts, such as *S.* Abortusovis (sheep) or *S.* Gallinarum (poultry), can provoke very smooth symptoms in humans (Chen et al. 2013).

5.6 Sources and Modes of *Salmonella* Transmission *as Zoonosis Pathogen*

Nowadays, the main source of human infection globally includes meat products, despite the success of *Salmonella* control measures applied in food-animal production in industrialized countries (Antunes et al. 2016). However, epidemiologic NTS studies in endemic areas of sub-Saharan Africa are very limited. Although the transmission through food contaminated by animal feces must be considered, waterborne transmission or person-to-person transmission has been highly reported. Sub-genomic investigation of invasive NTS (iNTS) strains from children with

severe invasive infections in Africa and those recovered from animals did not suggest any domestic animal sources for spread and transmission; however, family members of cases were established to have more closely related isolates (Kariuki et al. 2015).

Furthermore, antimicrobial resistance in non-typhoid *Salmonella* is also considered the most important public health issue linked to the poultry production chain and poultry meat, which is an additional concern in investigating salmonellosis. This is characterized by the dissemination of the multidrug-resistant *Salmonella* clones and/or mobile genetic elements encoding the corresponding antibiotic resistance genes from poultry to humans (Antunes et al. 2016).

Additionally, it has been established that *Salmonella* could contaminate the internal portions of eggs in the reproductive organs during egg formation. Both *S.* Typhimurium and *S.* Enteritidis have the potential to colonize the reproductive system of chickens; however, *S.* Enteritidis is more commonly isolated from the internal contents of eggs due to its ability to adhere better to the mucosa of the reproductive tract compared to *S.* Typhimurium. Thus, internal contamination affects human health and the egg production industry (Whiley and Ross 2015).

Salmonella transmission could be vertical and horizontal. The former involves the circulation of the bacteria from parents to their progeny. Vertical transmission is especially important in poultry-related *Salmonella* infections caused by the serovar Enteritidis, which has a particular affinity for the reproductive system of chickens. In this case, transmission to progeny occurs by transovarial infection when the parent birds have a systemic disease, leading to ovary infection and egg development in the oviducts (Mares 2017). Another means by which the serovar Enteritidis enters eggs is migration from the cloaca to the reproductive organs. In addition, an accumulating body of evidence suggests that *Salmonella* can be transmitted vertically from mother to fetus in utero in dairy cattle (Hanson et al. 2016).

On the other hand, horizontal transmission occurs either through the fecal-oral or aerogenous routes. Introduction of *Salmonella* into herds can also occur through new purchases and infected pigs; there is evidence of its spread by fomites, contaminated drinking water, contaminated feeds, dirty feeders, asymptomatic carriers, and feces from clinically infected farm animals (Mares 2017). Consequently, chronic or non-symptomatic carriers that continue to shed the bacteria in their feces are created by the increased frequency and colonization of *S.* enterica in the intestinal tracts of food animals. Therefore, these carriers could be considered future reservoirs contaminating and distributing *Salmonella* from contaminated meat, eggs, milk, and other food-animal products to other agricultural products and humans (Jajere 2019).

Poultry populations, especially chickens and turkeys, are regularly colonized with *Salmonella* without observable symptoms (subclinical infections/healthy carriers) by horizontal and vertical transmission at the primary production level. Therefore, the existence of *Salmonella* in healthy chickens is a significant risk, as bacteria, including antibiotic-resistant once, can easily infect humans through table eggs and poultry (Antunes et al. 2016).

Besides, pests such as rodents (mice and rats), flies, and cockroaches play a major role in the spread of *Salmonella* from one farm to another (Mares 2017). Rodents are important vectors and reservoirs of *Salmonella;* they can carry the bacteria in their intestinal tracts asymptomatically without any clinical disease (Gal-Mor 2018). These rodents acquire *Salmonella* through feed, water, and stored grains contaminated by the feces of sick or wild animals on the farm (Mares 2017). Flies act as mechanical vectors, transmitting the bacteria from one farm to another and spreading from cattle to humans (Xu et al. 2018). Farm animals in their slaughterhouse become infected by ingesting *Salmonella*-infected flies (Choo et al. 2011). Wild animals such as wild birds and other wildlife are important reservoirs of *Salmonella* infection (Brobey et al. 2017). They cause the introduction and propagation of the bacteria into livestock farms through contamination of feed, water, or direct environmental contamination (Mares 2017). Human traffic on the farm has been reported to increase the exposure to *Salmonella* infection in pigs and poultry (Mares 2017).

5.7 Manifestations of *Salmonella* Disease and Virulence Genes Involved

The consequence of *Salmonella* illnesses depends on the combination of the virulence and pathogen genotype, host species, and host immune status. Most infections result in self-limiting gastroenteritis with a low mortality rate (Branchu et al. 2018). Infections with *Salmonella* (salmonellosis) generally take two different disease courses, usually based on whether the responsible *Salmonella* strain belongs to a typhoidal or non-typhoidal serovar. Infection with non-typhoidal serovars ordinarily presents as diarrhea associated with fever and abdominal cramps 12–72 h after infection. In most healthy individuals, this infection runs a self-limited course over 4–7 days, but in susceptible hosts, certain non-typhoidal *Salmonella* strains may spread systemically to other sites in the body. Although this is more common in immunocompromised individuals or those with underlying medical conditions (e.g., sickle cell anemia), systemic spread of non-typhoidal *Salmonella* strains might also be seen in healthy individuals (Kurtz et al. 2017).

Many virulence determinants have been shown to play an important role in *Salmonella*'s pathogenesis, from colonization to attachment, and then intestinal defense mechanisms bypass, such as gastric acid; however, the *Salmonella* pathogenicity island (SPI)-1 and SPI-2 and other SPIs encoding for different responsible virulence factors including flagella, capsule, plasmids, adhesion systems, and type 3 secretion systems (T3SS) have been recorded (Branchu et al. 2018; Lou et al. 2019). These factors are distinguished by antigenic variation in long-chain lipopolysaccharides (LPS) and flagella and present a crucial variation in the host range and infection outcomes. However, host adaptation is often accompanied by a more severe illness. It is suspected that such host adaptation patterns arise through

evolution from ancestors associated with gastroenteritis (Branchu et al. 2018). Indeed, SPIs have been acquired by *Salmonella* during their evolution. There are five principal SPIs (1–5) in *Salmonella* related to their pathogenicity, of which SPI-1 and SPI-2 are the most studied. SPI-1 encodes several proteins linked to the invasion of epithelial cells, and SPI-2 contributes to survival and replication inside host cells such as epithelial cells and macrophages (Lamas et al. 2018).

Pathogenic *Salmonella* species occupy non-phagocyte intestinal epithelial cells using a wide set of specialized effectors through a sophisticated machinery comprising the type 3 secretion system (T3SS). *Salmonella* employs two T3SSs encoded by SPI-1 and SPI-2. SPI-1 is a gene cluster consisting of a 40-kb region, which includes 39 genes encoding T3SS-1 and its chaperones, effector proteins, and some transcriptional regulators that regulate the expression of many virulence genes located within and outside SPI-1. T3SS-1 of *Salmonella* may affect the phenotype, polarization, and function of macrophages (Lou et al. 2019). These systems are qualified to inject directly into the eukaryotic host cells several bacterial proteins known as effectors (Ramos-Morales 2012).

Collectively, these virulence factors contribute to invading host cells, interfering with host cellular functions, subverting immunity, establishing an intracellular niche, and promoting pathogen proliferation. Although *Salmonella* passes through the digestive tract, *Salmonella* must counteract the acidic pH of the gut, gallbladder, and antimicrobial peptides and compete with resident microbiota. After invading host cells, *Salmonella* employs the inflammatory pathways of infection and the process of autophagy. Finally, by interacting *Salmonella* with dendritic cells and T and B lymphocytes, the adaptive immune system could be inhibited (Bernal-Bayard and Ramos-Morales 2018).

5.8 Antimicrobial Resistance in NTS Serotypes

Antimicrobial therapy for salmonellosis is controversial. Of almost concern is the potential of creating antibiotic-resistant *Salmonella* strains that may present a risk to humans or animals in the future. Furthermore, although antimicrobial therapy may aid in clinical recovery, it has as well been criticized as failing to limit fecal shedding or to impart a positive effect on the period of fecal shedding (Holschbach and Peek 2018). Unfortunately, the extensive and frequent use or misuse of antimicrobial drugs not only in the treatment of human and animal infections but also as growth-promoting agents in livestock production has led to the evolutionary emergence of resistance to one or more of the antimicrobial molecules applied against the bacterial agents (Fuche et al. 2016).

To face MDR infections, fluoroquinolones, such as ofloxacin and ciprofloxacin, have been widely used for treatment in the last 20 years. These drugs are very effective when isolates are fully susceptible (ciprofloxacin MIC <0.06 μg/mL). However, with the extensive use of these agents, resistance has emerged, initially low-level but later high-level. Low-level resistance, linked to ciprofloxacin MIC of 0.1–0.5 μg/mL, has also been known as nalidixic acid resistance and decreased ciprofloxacin susceptibility

and most recently has been recategorized as intermediate susceptibility to ciprofloxacin. Thus, the last generation of fluoroquinolone, gatifloxacin, is clinically more efficient against diseases with such isolates, probably due to the differences in its topoisomerase target (Kariuki et al. 2015). Previously, quinolone-resistant *Salmonella* was established by point mutations in the *gyr*A gene encoding the A subunit of gyrase. Resistance mutations of *gyrA* arise in the quinolone resistance determining region (QRDR) (Chen et al. 2013). Also, plasmid-mediated quinolone resistance mechanisms, which typically confer reduced susceptibility to ciprofloxacin, have been widely detected in *Salmonella*. Besides, Qnr proteins (e.g., QnrS1, QnrB2, QnrB19) have been frequently reported in different serotypes and geographic locations, along with the aminoglycoside acetyltransferase AAC(6′)-Ib-cr and the efflux pump OqxAB among different *Salmonella* serotypes recovered from Asian poultry and poultry meat. In fact, poultry seems to be a crucial vehicle of NTS carrying plasmid-mediated quinolone resistance genes, highlighting the role of food-producing animals, including the animal/food trade, in its dissemination (Antunes et al. 2016).

Azithromycin is another effective oral option for uncomplicated disease. Unfortunately, resistance to azithromycin has been difficult to monitor because of the shortage of validated breakpoints. The extended-spectrum cephalosporins, such as ceftriaxone and cefotaxime, have remained a reliable reserve antimicrobial, particularly for hospital admitted cases. The response to treatment with ceftriaxone can be slow and relapse a problem, but resistance until recently has been rare. Whence the resistance has been demonstrated, CTX-M has been the most frequent plasmid-borne extended-spectrum cephalosporin (ESBL) gene (Kariuki et al. 2015). Besides, among NTS the resistance to ceftriaxone is usually conferred by the presence of a plasmid-mediated β-lactamase gene, $bla_{\text{CMY-2}}$. For patients with invasive *Salmonella* infections resistant to ciprofloxacin and ceftriaxone, carbapenems may be the last drug of choice (Antunes et al. 2016; Chen et al. 2013). However, few reports of carbapenem-resistant NTS have been published lately.

The genes responsible for the MDR phenotype have typically been borne on transmissible plasmids, particularly of the IncHI1 group (Kariuki et al. 2015). For example, multidrug resistance in *S.* Typhimurium DT104 is mainly associated with genomic island 1 (SGI) of *Salmonella*, an integrative mobile element carrying various gene clusters encoding antibiotic resistance and conjugative R plasmids that provide resistance to multiple antibiotics, including extended-spectrum cephalosporins. Additional resistance to trimethoprim may be encoded by a nonconjugative but mobilizable plasmid (approximately 40 kb in length) which also encodes resistance to sulfonamides, occasionally observed among *S.* Typhimurium DT104 strains (Chen et al. 2013).

Furthermore, resistance genes against antibiotics only used in animals were found soon after their introduction in animal bacteria and the commensal flora of humans in zoonotic pathogens like *Salmonella*. Therefore, not only may the clonal spread of resistant strains occur, but there is also a transfer of resistance genes between human and animal bacteria (Chen et al. 2013; Lamas et al. 2018).

The increasing incidence of resistance to traditional agents (i.e., ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole) has turned invasive salmonellosis therapy into a clinical dilemma. Clinical observation and laboratory

findings suggested that the organism bacteria can adapt easily to antimicrobial selection pressure. Therefore, continuous monitoring of the presence of *Salmonella* in both humans and animals is obligatory and necessary for better control of the infections induced by this organism (Chen et al. 2013).

5.9 Methods

The current study aimed to investigate the antimicrobial susceptibility profiles of *Salmonella* serotypes recovered from different animals and food product samples in Africa. Therefore, a full web search using the corresponding specific keywords like ("*Salmonella*" or "Nontyphoidal *Salmonella*") AND (adding each time "one country of Africa") was performed in English languages in PubMed database search engines. Besides, we further checked the papers cited in the references list of the selected articles to be sure that all eligible articles were selected.

In accord with the inclusion and exclusion criteria, this meta-analysis review selected studies focusing on articles studying the antimicrobial susceptibility pattern of *NTS* serotypes recovered from different farm animals and food product samples in Africa. Initially, titles, abstracts, and keywords were read to include studies establishing the purpose of our research. Then, review articles, notes, e-mails, editorials, letters, papers presented at scientific events, and articles without original data were excluded, along with (i) studies performed on other species than *Salmonella*, (ii) studies that did not identify *Salmonella* serovars, (iii) studies that did not perform the antimicrobial susceptibility testing, (iv) articles which examined *Salmonella* genus isolated in samples other than animal and animal products, (v) studies that performed outside of Africa, and (vi) articles reporting on *Salmonella* Typhi and *Salmonella* Paratyphi.

The first and last literature search on PubMed was conducted on March 22, 2021. The selected articles were analyzed, and the following data were extracted into an excel table: (i) the country in which the study was performed, (ii) the sampling period, (iii) the origin and type of samples, (iv) *Salmonella* serotype, (v) the associated resistance genes and plasmid's Inc. group, (vi) the ST type of the identified strains (clonality), (vii) the antimicrobial resistance profile against the antibiotics that have been tested in each study, and (viii) reference (authorship and year).

5.10 Results

5.10.1 Literature Search

Following the inclusion and exclusion criteria, 80 unique studies reported on *Salmonella* across 19 of 52 African countries. One thousand twenty-four records were retrieved and submitted for screening titles, abstracts, and full texts of eligible

studies presenting data on the antimicrobial resistance of *Salmonella* serotypes recovered from farm animals and animal products in Africa. Briefly, 532 duplicates, non-original and nonrelevant articles, were initially excluded through the evaluation of titles and abstracts. Among 492 remaining studies identified for full-text screening, 412 articles with inadequate data were excluded, 196 articles that did not report antibiotic resistance patterns, and 46 articles performed out of Africa were excluded. Afterward, 69 articles were reported about *Salmonella* Typhi and Paratyphi, and 50 articles that did not identify the serotype of *Salmonella* species were also excluded. The 51 remaining articles were excluded because of the missing antimicrobial susceptibility testing.

5.10.2 NTS per African Regions

From 2003 to 2021, 80 selected studies reported NTS isolates collected in 19 of 52 countries and tested an overall of 3391 *Salmonella* serovar isolates for antibiotic resistance. There were 44.91% (n = 1523) of NTS strains isolated in Northern Africa, 32.91% (n = 1116) in Western Africa, 16.83% (n = 571) in Eastern Africa, 4.15% (n = 141) in Southern Africa, and 1.20% (n = 41) from Central Africa (Table 5.1).

Table 5.1 Distribution of *Salmonella* isolates among African countries

Country	Region	Number	%
Algeria	North	109	3.2
Benin	West	62	1.8
Botswana	South	44	1.3
Burkina Faso	West	368	10.7
Egypt	North	721	21.0
Ethiopia	Eastern	304	8.9
Gambia	West	11	0.3
Ghana	West	85	2.5
Kenya	Eastern	47	1.4
Malawi	South	4	0.1
Morocco	North	349	10.2
Namibia	South	40	1.2
Nigeria	West	223	6.5
Senegal	West	367	10.7
South Africa	South	53	1.5
Sudan	Eastern	37	1.1
Tchad	Central	41	1.2
Tunisia	North	343	10.0
Uganda	Eastern	225	6.6

5.10.3 Distribution of NTS by Origin and Serotypes

An overall of 3391 NTS strains was obtained from farm animals and animal food product samples, including 3244 (95.66%) strains from farm animals, 100 (2.94%) from seafood, and the remaining 47 strains (1.38%) from food of undetermined origin. Concerning farm animals, 2358 strains were isolated from poultry samples [fecal samples (n = 1122), food products (n = 864), intestine samples (n = 1), and unavailable origin sample (n = 371)], 724 strains were recovered from cattle [food (n = 379) and fecal (n = 334)], 145 strains were obtained from swine samples [food (n = 25), fecal (n = 120)], and finally 17 strains were isolated from camels (14 of them were from food origin and 3 from fecal samples).

Overall, the NTS strains were assigned to 229 serotypes. The most serotype was *Salmonella* Enteritidis (n = 457, 13.48%) followed by *S.* Typhimurium (n = 441, 13.01%) and *S.* Kentucky (n = 328, 9.67%) (Table 5.2).

5.10.4 Antibiotic Resistance Pattern of NTS in Different Sources

As the antibiotic list tested by each research group differed, we only reported susceptibility rates for some specific antibiotics. We extracted all antibiotic resistance profiles of NTS reported from different types of farm animals and food sample sources. More than 50% of the *Salmonella* strains in the included studies were MDR strains. Thus, high resistance rates were observed for streptomycin (1236/2589, 47.74%), cefuroxime (185/389, 47.56%), tetracycline (1283/2825, 45.41%), cotrimoxazole (834/2376, 35.10%), nalidixic acid (967/2862, 33.78%), and spectinomycin (148/439, 33.71%) (Fig. 5.1).

5.10.5 Antibiotics Resistance Patterns in S. Enteritidis

S. Enteritidis was resistant to many antibiotics including spectinomycin (24/30, 80%), nalidixic acid (260/372, 69.89%), cotrimoxazole (145/349, 41.55%), ampicillin (168/436, 38.53%), streptomycin (133/413, 32.20%), imipenem (3/8, 37.50%), amoxicillin + clavulanic acid (81/250, 32.40%), tetracycline (90/312, 28.85%), chloramphenicol (81/402, 20.15%), ciprofloxacin (55/420, 13.10%), cefotaxime (8/66, 12.12%), amikacin (2/17, 11.76%), and ceftazidime (1/139, 0.72%) (Fig. 5.2).

Table 5.2 Serotypes distribution in NTS isolated from animals and food

Serotype	Number	%
S. Enteritidis	457	13.48
S. Typhimurium	441	13.01
S. Kentucky	328	9.67
S. Saintpaul	111	3.27
S. Virchow	103	3.04
S. Brandcaster	99	2.92
S. Heidelberg	82	2.42
S. Istanbul	71	2.09
S. Muenster	69	2.03
S. Infantis	67	1.98
S. Derby	65	1.92
S. Anatum	58	1.71
S. Newport	56	1.65
S. Give	47	1.39
S. Mbandaka	47	1.39
S. Chester	46	1.36
S. Haifa	45	1.33
S. Hadar	45	1.33
S. Choleraesuis	43	1.27
S. Hato	34	1.00
S. Muenchen	33	0.97
S. Zanzibar	32	0.94
S. Agona	31	0.91
S. Oakland	29	0.86
S. Dublin	28	0.83
S. Goelzau	28	0.83
S. Poona	27	0.80
S. Drac	26	0.77
S. Corvallis	24	0.71
S. Montevideo	22	0.65
S. Senftenberg	21	0.62
S. Bredeney	21	0.62
S. Indiana	20	0.59
S. Agama	20	0.59
S. Kingston	20	0.59
S. Blockley	18	0.53
S. Eppendorf	18	0.53
S. Molade	17	0.50
S. Urbana	16	0.47
S. Stanleyville	16	0.47

(continued)

Table 5.2 (continued)

Serotype	Number	%
S. Colindale	15	0.44
S. Schwarzengrund	15	0.44
S. Aberdeen	14	0.41
S. Suberu	12	0.35
S. Altona	11	0.32
S. Bovismorbificans	11	0.32
S. Livingstone	11	0.32
S. Menston	11	0.32
S. Farakan	10	0.29
S. Parkroyal	10	0.29
S. Thompson	10	0.29
Others	480	14.15

Legend
Others: *S.* Bolton = 9, *S.* Kiel = 9, *S.* Lagos = 9, *S.* Nima = 9, *S.* Minnesota = 8, *S.* Albany = 8, *S.* Braenderup = 8, *S.* Llandoff = 8, *S.* Newlands = 8, *S.* Rubislaw = 8, *S.* Ruiru = 8, *S.* Uganda = 7, *S.* Eko = 7, *S.* Havana = 7, *S.* Nigeria = 7, *S.* Santiago = 7, *S.* Tamale = 7, *S.* Arizonae = 6, *S.* Bargny = 6, *S.* Bongori = 6, *S.* Cairina = 6, *S.* Inganda = 6, *S.* Kaapstad = 6, *S.* Larochelle = 6, *S.* Legon = 6, *S.* Mikawasima = 6, *S.* Reading = 6, *S.* Salamae = 6, *S.* Amsterdam = 5, *S.* Apeyeme = 5, *S.* Bukuru = 5, *S.* Fresno = 5, *S.* Javiana = 5, *S.* Sangalkam = 5, *S.* Adelaide = 4, *S.* Amager = 4, *S.* Bardo = 4, *S.* Chartres = 4, *S.* Chomedey = 4, *S.* Duisburg = 4, *S.* Eastbourne = 4, *S.* Gallinarum = 4, *S.* Labadi = 4, *S.* Shubra = 4, *S.* Soumbedioune = 4, *S.* Tennessee = 4, *S.* Banana = 3, *S.* Bandia = 3, *S.* Hillingdon = 3, *S.* Kissii = 3, *S.* Kokomlemle = 3, *S.* Kottbus = 3, *S.* Miami = 3, *S.* Mississippi = 3, *S.* Nottingham = 3, *S.* Ohio = 3, *S.* Ouakam = 3, *S.* Ruzizi = 3, *S.* Takoradi = 3, *S.* Uno = 3, *S.* Waycross = 3, *S.* Monschaui = 2, *S.* Abaetetuba = 2, *S.* Abony = 2, *S.* Ajiobo = 2, *S.* Bareilly = 2, *S.* Bessi = 2, *S.* Binningen = 2, *S.* Blegdam = 2, *S.* Brenzany = 2, *S.* Brunei = 2, *S.* Chandans = 2, *S.* Colobane = 2, *S.* Dahra = 2, *S.* Durham = 2, *S.* Eingedi = 2, *S.* Fischerkietz = 2, *S.* Gaillac = 2, *S.* Galiema = 2, *S.* Giza = 2, *S.* Hull = 2, S. Ilala = 2, *S.* Johannesburg = 2, *S.* Kalina = 2, *S.* Kampala = 2, *S.* Koessen = 2, *S.* Korlebu = 2, *S.* Lawndale = 2, *S.* Luke = 2, *S.* Magherafelt = 2, *S.* Maloma = 2, *S.* Nitra = 2, *S.* Onderstepoort = 2, *S.* Renea = 2, *S.* Ried = 2, *S.* Riggill = 2, *S.* Rissen = 2, *S.* Trachau = 2, *S.* Umbadah = 2, *S.* Warnow = 2, *S.* Westhampton = 2, *S.* Ank = 1, *S.* Africana = 1, *S.* Ank = 1, *S.* Africana = 1, *S.* Amina = 1, *S.* Antwepen = 1, *S.* Badagry = 1, *S.* Barranquilla = 1, *S.* Beaudesert = 1, *S.* Benfica = 1, *S.* Birkenhead = 1, *S.* Brandenburg = 1, *S.* Brive = 1, *S.* Carmel = 1, *S.* Carno = 1, *S.* Cerro = 1, *S.* Cardoner = 1, *S.* Chingola = 1, *S.* Crossness = 1, *S.* Dakar = 1, *S.* Dalo = 1, *S.* Elisaberthville = 1, *S.* Essen = 1, *S.* Farsta = 1, *S.* Ferruch = 1, *S.* Fischerhutte = 1, *S.* Freetown = 1, *S.* Frintrop = 1, *S.* Fufu = 1, *S.* Gdansk = 1, *S.* Gera = 1, *S.* Goku = 1, *S.* Houten = 1, *S.* Idikan = 1, *S.* Ikeja = 1, *S.* Israel = 1, *S.* Ituri = 1, *S.* Kalamu = 1, *S.* Kedougou = 1, *S.* Kibi = 1, *S.* Kinondoni = 1, *S.* Kivu = 1, *S.* Leatherhead = 1, *S.* Lerum = 1, *S.* Lexington = 1, *S.* Liverpool = 1, *S.* Llobrega = 1, *S.* Lomita = 1, *S.* London = 1, *S.* Manchester = 1, *S.* Masembe = 1, *S.* Moero = 1, *S.* Neumuenster = 1, *S.* Offa = 1, *S.* Onireke = 1, *S.* Oranienburg = 1, *S.* Orion = 1, *S.* Oskarshamn = 1, *S.* Othmarschen = 1, *S.* Oxford = 1, *S.* Panam = 1, *S.* Papuana = 1, *S.* Phaliron = 1, *S.* Pullorum = 1, *S.* Rechovot = 1, *S.* Salford = 1, *S.* Sandiego = 1, *S.* Shangani = 1, *S.* Telelkebir = 1, *S.* Teshie = 1, *S.* Tornow = 1, *S.* Tsevie = 1, *S.* Umbilo = 1, *S.* Wandsworth = 1, *S.* Weltevreden = 1, *S.* Weybridge = 1, *S.* Yeerongpilly = 1, *S.* Yoruba = 1, *S.* Yovokome = 1

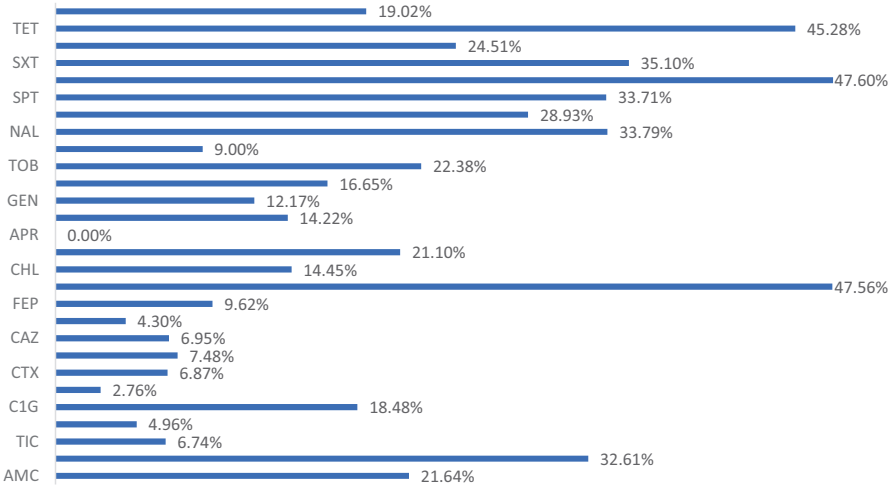

Fig. 5.1 Antibiotic resistance profile of *Salmonella* strains isolated from animals and animal products in Africa

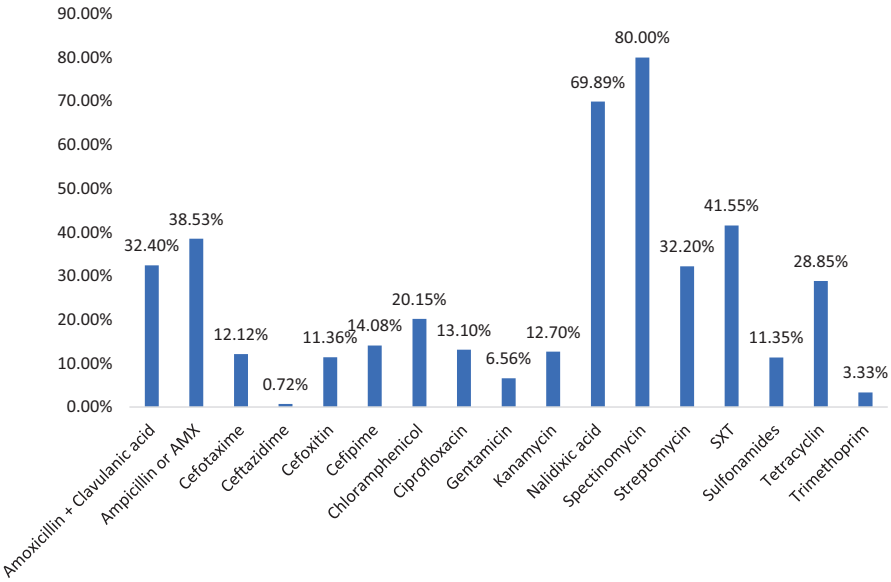

Fig. 5.2 Antibiotic resistance profile of *Salmonella* Enteritidis strains isolated from animals and animal products in Africa

5.10.6 *Antibiotics Resistance Patterns in* S. *Typhimurium*

Different rates of antibiotic resistance were observed in *S*. Typhimurium strains and included resistance to spectinomycin (28/38, 73.68%), tetracycline (209/313, 66.77%), amoxicillin (265/417, 63.55%), streptomycin (223/363, 61.43%), sulfonamide (38/75, 50.67%), trimethoprim-sulfamethoxazole (167/338, 49.41%), chloramphenicol (185/398, 46.48%), nalidixic acid (116/270, 42.96%), trimethoprim (39/91, 42.86%), amoxicillin + clavulanic acid (85/225, 37.28%), ciprofloxacin (84/288, 29.17%), cefotaxime (37/164, 22.56%), ceftazidime (13/89, 14.91%), amikacin (2/69, 2.90%), and imipenem (3/107, 2.80%) (Fig. 5.3).

5.10.7 *Antibiotics Resistance Patterns in* S. *Kentucky*

S. Kentucky strains were resistant to tetracycline (190/255, 74.51%), streptomycin (128/203, 63.05%), spectinomycin (28/46, 60.87%), ciprofloxacin (164/288, 56.94%), nalidixic acid (164/307, 53.42%), ampicillin (131/311, 42.12%), trimethoprim/sulfamethoxazole (93/255, 36.47%), cefotaxime (12/182, 6.56%), and ceftazidime (8/135, 3.70%). Indeed, no imipenem-resistant and amikacin-resistant isolates were detected (Fig. 5.4).

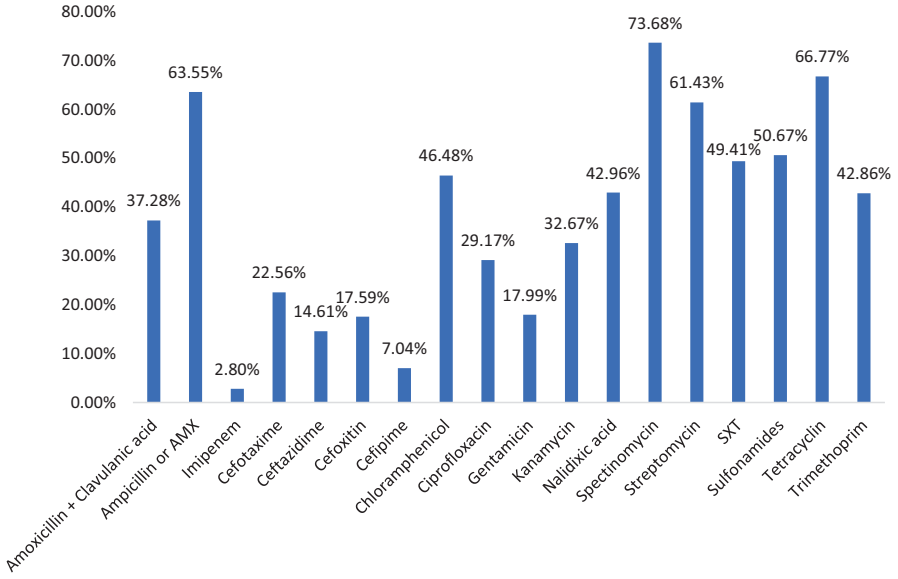

Fig. 5.3 Antibiotic resistance profile of *Salmonella* Typhimurium strains isolated from animals and animal products in Africa

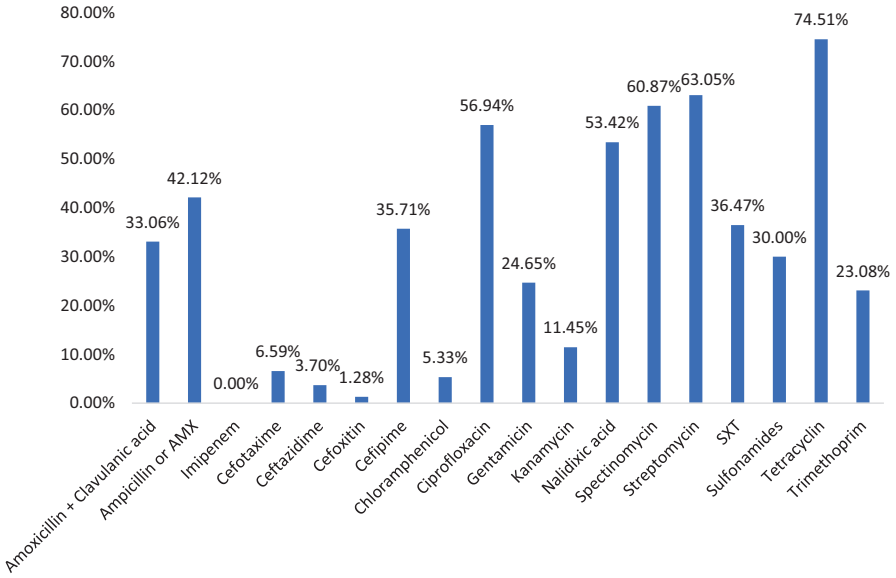

Fig. 5.4 Antibiotic resistance profile of *Salmonella* Kentucky strains isolated from animals and animal products in Africa

5.11 Discussion

Foodborne infections caused by NTS are considered a major public health concern since these pathogens are disseminated through contaminated food products. Thus, monitoring food safety is a key point in preventing and controlling the spreading of *Salmonella*, as well as in providing healthier food products (Bouchrif et al. 2009b). Furthermore, there has been a steady increase over the years in the frequency of *Salmonella* serotypes isolated and the number of animal species involved (Gelaw et al. 2018).

We report 11 years of antimicrobial susceptibility data for the most common *NTS* serovars. Thus, *Salmonella* strains were recovered from scores of sites in Africa and isolated from various farm animals and animal products such as chicken, pork, cattle dairy product, and meat products. Our results demonstrated that *Salmonella* was more frequently encountered in poultry samples (from food products and either from fecal specimens) and exhibited more diversity in serotypes than other farm animals. For instance, approximately 96.3% of all detected *Salmonella* serovars are predominantly associated with domestic species, namely, poultry, cattle, camels, and swine, whereas fish samples including seafood contributed only with 2.9%. However, it has been warned that care must be taken when considering these data due to the possibility of a skewed focus from the targeted sampling of mostly food animals as compared to wild and game animals. Despite this, the data probably give a reasonable assessment of the salmonellosis impact and the *Salmonella* serotypes

involved (Gelaw et al. 2018). It was well noted that despite the presence of a formidable number of different serotypes, only a few are commonly associated with clinical diseases in humans and animals (Gelaw et al. 2018). It is also noted in the current study that despite the large number of serotypes involved (n = 229), the majority of isolates were mainly represented by very few serotypes. For instance, from the overall of 229 serotypes detected among 3391 isolates, the three serotypes *S.* Enteritidis, *S.* Typhimurium, and *S.* Kentucky contributed to 36.15% (1226 isolates) of all isolates, while 226 serotypes collectively accounted for 63.85% (2165 isolates). More clearly, among the overall number of isolates, *S.* Saintpaul (3.27%; n = 111), *S.* Virchow (3.04%; n = 103), *S.* Brandcaster (2.92%; n = 99), *S.* Heidelberg (2.42%; n = 82), *S.* Istanbul (2.09%; n = 71), *S.* Muenster (2.03; n = 69), *S.* Infantis (1.98%; n = 67), *S.* Derby (1.92%; n = 65), *S.* Anatum (1.71%; n = 58), *S.* Newport (1.65%; n = 56), and *S.* Mbandaka (1.39%; n = 47) were distinguished by their medium prevalence compared to the other serotype.

The predominant serovar was *S.* Enteritidis which was mainly isolated from chickens, followed by *S.* Typhimurium and then *S.* Kentucky. To our knowledge, it was well mentioned that the emergence of antibiotic-resistant *S.* Kentucky strains in Egypt and their dissemination to African countries can explain its predominance in Morocco (Amajoud et al. 2017). Le Hello et al.'s hypothesis was that the north and east of Africa could be the origin of infections by *S.* Kentucky in Europe (Le Hello et al. 2011).

Regarding the antibiotic susceptibility evaluation, almost all serotypes were resistant to antibiotics belonging to one or more classes; thus, the antimicrobial-resistant *Salmonella* were found to be more than 50% of the total *Salmonella* isolates tested, which is higher than those reported in previous studies conducted elsewhere in the world. Even though it needs a better understanding of antibiotic use in Africa, as it is already suggested by Gillings et al. that this resistance variation may be due to discriminatory use of antimicrobial agents in animal production without prescription in the animal health sector, which may favor selection pressure that increased the advantage of maintaining resistance genes in bacteria (Gillings et al. 2008). This is also supported by a high prevalence of *Salmonella* reported from humans and different food animals, with the strains having high levels of drug resistance (Tadesse 2015).

In this study, we noticed that most of the identified isolates are resistant to two or more antibiotics, particularly to tetracycline (45.41%), sulfamethoxazole-trimethoprim (35.10%), streptomycin (47.74%), spectinomycin (33.71%), nalidixic acid (33.78%), and amoxicillin (41.38%). All strains (100%) were sensitive to apramycin and fosfomycin. The resistance *of Salmonella* in this finding was higher than in the previous studies done in other countries (Stevens et al. 2006). It was indicated that the remarkable rise in antimicrobial resistance in *Salmonella* for the mentioned antibiotics was probably an indication of their frequent usage in livestock and in African public health sectors (Ejo et al. 2016). Precedent studies of chicken-borne *Salmonella* in 15 different areas in Brazil have shown different rates of resistance to ampicillin (38%), ceftiofur (28%), aztreonam (19.2), cefoxitin (13.2%), and cephalothin (12%) (Cunha-Neto et al. 2018). In Australia, authors demonstrated low

levels of resistance to antimicrobials with less critical ratings such as cefoxitin, trimethoprim/sulfamethoxazole, and tetracycline among *Salmonella* spp. obtained from meat chickens (Abraham et al. 2019).

However, it is noteworthy to know that these antibiotics are commonly used in veterinary medicine, and infections with these resistant *Salmonella* isolates could lower antibiotic therapy's efficiency and potentiality. Similarly, in recent times, the level of antimicrobial drug resistance in *Salmonella* and the number of drugs to which the strains are resistant have increased worldwide due to antimicrobial usage in food production. In addition, precedent reports have also underlined the occurrence of *Salmonella* with decreased susceptibility to fluoroquinolones and other drugs, which is related to treatment failures and poor outcomes in human infections (Ejo et al. 2016).

The present study found low resistance to second-, third-, and fourth-generation cephalosporins, but a medium resistance to cefuroxime (second generation) was detected (47.56%). Indeed, several studies described the increased resistance to cephalosporins in *Salmonella* strains isolated from food products and veterinary and human sources (Abbassi-Ghozzi et al. 2012). In Morocco, for example, in 2008, the authors recorded ceftazidime resistance in *S.* Typhimurium that caused illnesses in 45 people (Bouchrif et al. 2009a). In Algeria, the use of cephalosporins is uncommon in poultry production contaminated by other sources or resulted from the acquisition by avian *Salmonella* strains of ESBL resistance determinants that are usually carried on mobile genetic elements (e.g., plasmids) (Djeffal et al. 2017).

Resistance to chloramphenicol was at a medium level (14.5%). This antibiotic is forbidden in veterinary practice in some African countries (Amajoud et al. 2017). Indeed, in every analyzed study, the resistance to different antimicrobial molecules was predominantly seen in Enteritidis, Typhimurium, Kentucky, Saintpaul, and Virchow serotypes. Likewise, the resistance profile of these serotypes was different from each other. It is noteworthy that the results of the analyzed study did not show any relation between the resistance profile of the strains and the origin of the isolate as well as the country of the isolation.

In the present study, a high level of resistance was observed for *Salmonella* strains isolated from chickens compared with those isolated from other products since most of the strains from chickens were *S.* Enteritidis, *S.* Kentucky, and *S.* Typhimurium, which demonstrated multidrug resistance. Therefore, the results of this study could be the starting point for monitoring *Salmonella* serovars and their emerging resistance profiles in animal-derived food in Africa (Amajoud et al. 2017).

5.12 Conclusion

Salmonella is an established pathogen, and the presence of multidrug resistance in these pathogens presents a healthcare challenge. The occurrence of MDR *Salmonella* (with a high level of antibiotic resistance) isolated from animals (cattle, swine, and poultry) and animal food products (meat and milk) in African countries revealed a

high prevalence and high contamination risk of food with these strains. Thus, this suggests a clonal spread of these MDR strains and the need for strict pathogen control strategies to hamper the further spread of *Salmonella*. The information on the prevalence and the correlation between the serotype and antimicrobial resistance can help identify trends and assess the effectiveness of current initiatives within the food industry to improve food hygiene strategies aimed at reducing bacterial loads in primary production and at the various stages of the food production chain. Besides, as most of the isolates in the analyzed studies were isolated from healthy animals at the farm level, the data presented here may not represent the global status. Therefore, further studies on extended-spectrum beta-lactamase and other antibiotic resistance mechanisms in *Salmonella* are recommended.

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Chapter 6
The Current Status of Antibiotic-Resistant Bacteria and Resistance Genes in African Aquaculture

S. M. Limbu

6.1 Introduction

Aquaculture is currently the fastest-growing food production sector, supplying half of the fish consumed by humans globally (Limbu et al. 2021). The global human per capita fish consumption is estimated at 20.2 kg annually, of which aquaculture contributes 49% of the total production and 56% of human fish consumption (FAO 2022). The world aquaculture grew at an average rate of 6.7% per annum between 1990 to 2020, attaining a record high production of 122.6 million tonnes in response to the ever-increasing population (FAO 2022). Aquaculture has been forecasted to grow to 106 million tons at the end of 2030, supplying most of the consumed fish and seafood products (Reverter et al. 2020; FAO 2022). The increased demand for animal protein in developing countries has prompted fish rearing in intensive systems. Fish cultured in intensive systems have reduced immunity response leading to diseases eruption and promoting antibiotic use in aquaculture (Reverter et al. 2020; Limbu et al. 2021). Consequently, there has been an increase in antibiotic-resistant bacteria (ARB) and their corresponding antibiotic resistance genes (ARGs) in aquaculture. Currently, ARB and ARGs represent one of the challenges facing aquaculture production, requiring immediate actions.

Antibiotic resistance threatening global public health also originates from antibiotics used for treating bacterial diseases in livestock, agriculture, and humans (Watts et al. 2017). Therefore, aquaculture has been reported as a major contributor to ARB and ARGs (Nallaiah et al. 2021) because it is an environmental gateway (Cabello et al. 2016) and at the crossroads of the development of bacteria resistance (Reverter et al. 2020). Thus, ARB and ARGs have been reported as a crisis for

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concern (Preena et al. 2020a) due to their prominence in the aquaculture industry (Preena et al. 2020b). Although the development of ARB and ARGs is ancient (Watts et al. 2017), the current increase is mainly due to the rampant prophylactic use of antibiotics in global agriculture, including aquaculture (Nallaiah et al. 2021). Similar to the global situation, African aquaculture has also been considered "a genetic reactor" or "hotspot" for ARB and ARG development (Iwu et al. 2020). This is due to an increase in reliance on aquaculture to supply safe, reliable, and economical food, responding to the fast population growth in the continent (FAO 2018). As a result, the rest of African aquaculture has increased its production by 14.5% between 2019 to 2020, when the analysis excludes data from Egypt and Nigeria (FAO 2022). This entails using intensive systems inheritably characterised by antibiotics use for treating bacterial diseases (Bouelet Ntsama et al. 2018).

The African continent has distinctive characteristics concerning antibiotic use. For example, many African countries have a high prevalence of communicable diseases and weak healthcare systems (Mathew et al. 2020). Moreover, most African countries have either inefficient regulatory agencies or absent/weak regulations concerning antibiotics usage (Byarugaba 2004) and inappropriate prescription and self-medication of readily available antibiotics (Sanou et al. 2018). Furthermore, most African countries have inadequate systems for surveillance and monitoring of used antibiotics. Accordingly, antibiotics are primarily given as over-the-counter drugs at community pharmacies (Mukonzo et al. 2013); this has been strongly linked to ARB and ARGs in low-income and middle-income countries (Alsan et al. 2015). Therefore, it is not surprising that the African aquaculture industry is also characterised by abuse, overuse and misuse of antibiotics leading to the natural selection and spread of resistant bacteria and the development of ARB and ARGs. This is an alarming situation because the types of ARB and ARGs in aquaculture can be transferred from animals to humans through various routes, causing devastating socioeconomic consequences. However, the precise sources, factors responsible, types and effects of ARB and ARGs in African aquaculture are currently unknown.

This chapter fills such a knowledge gap by discussing the sources of ARB and ARGs in aquaculture, the possible factors contributing to their increase, the types of ARB and ARGs found in the industry and the socioeconomic consequences to human health. The information generated informs the need for a "One Health" approach to combat ARB and ARGs while formulating stringent measures, legislations and regulations for antibiotics use in aquaculture, agriculture and sick patients to safeguard human health.

6.2 The Sources of ARB and ARGs in Aquaculture

The ARB and ARGs in the aquaculture environment originate from various sources (Fig. 6.1). These sources are described in this section.

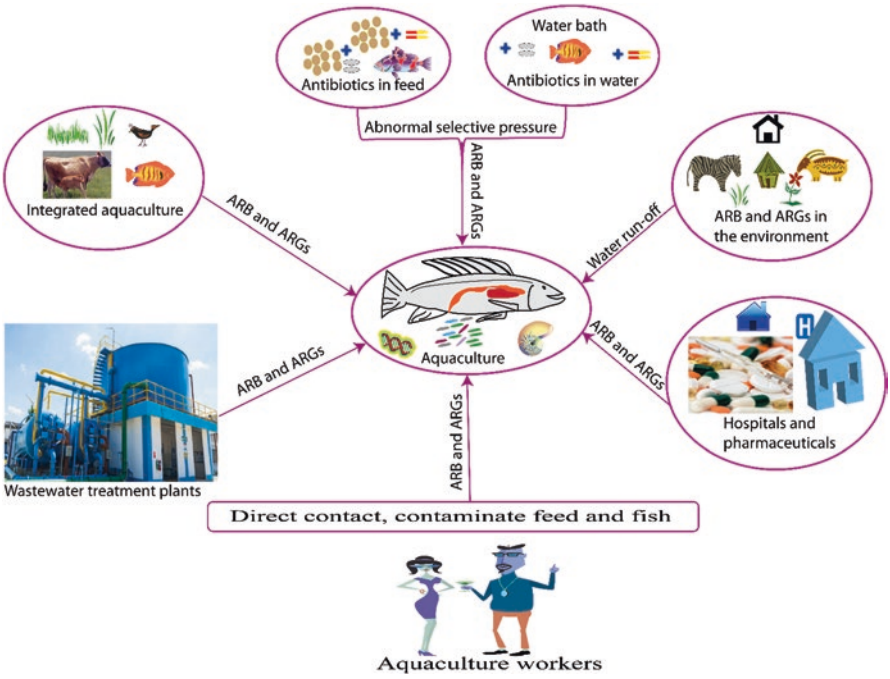

Fig. 6.1 The potential sources of antibiotic-resistant bacteria and resistance genes in aquaculture

6.2.1 Application of Antibiotics in Aquaculture

Using antibiotics for fish health management is an integral practice to intensive aquaculture systems (Limbu 2020; Limbu et al. 2021). Antibiotics are used in aquaculture feeds, either mixed during manufacturing or surface-coated onto pellets and as immersion medication (Musefiu and Olasunkanmi 2015; Preena et al. 2020a). Although the available information suggests minimal use of antibiotics in African aquaculture (Minich et al. 2018; Limbu 2020), the industry represents one of the sources of ARB and ARGSs (Cabello et al. 2016). Antibiotics applied in aquaculture are a source of ARB and ARGs due to three scenarios.

First, antibiotics lead to ARB and ARGs due to their application. Antibiotics are administered abusively for longer periods due to producers' inadequate knowledge about their purpose and proper application (Pham et al. 2015). Moreover, antibiotics are abusively used due to a lack of legislation, enforcement and inspection by regulatory agencies (Limbu et al. 2021). Commonly, antibiotics are used in cultured fish daily and in the absence of disease problems to health, carrier and diseased fish (Limbu et al. 2021). Second, antibiotics applied in aquaculture are not completely metabolised in the body of cultured organisms. Therefore, residues remain in the tissues and organs of cultivated species (Chuah et al. 2016) because they have high-water solubility and degradation resistance (Limbu et al. 2018). The third scenario for increased ARB and ARGs in aquaculture is through antibiotic-supplemented

feeds. The antibiotics-contaminated feeds retain antibiotic residues depending on biodegradability, initial concentration and physical and chemical characteristics of antibiotics (Preena et al. 2020a). Consequently, bacteria in the tissues and organs of cultured species, water or culture structure and facilities used in aquaculture are exposed to antibiotics cocktail for a prolonged time, which generates abnormal selective pressure (Samreen et al. 2021). Accordingly, antibiotics cause resistant strains development (Chuah et al. 2016; Kathleen et al. 2016) through mutation or acquisition of new resistance genes by genetic exchange mechanisms (Samreen et al. 2021) depending on antibiotics types. The resistance determinants could be retained within the microbial population even without the respective antibiotics (Martinez 2009). Thus, genes are transferred widely to susceptible microbes giving rise to resistant types (Nallaiah et al. 2021).

Evidently, ARB and ARGs have been isolated in cultured Nile tilapia in Egypt (El-Gohary et al. 2020), Tanzania (Aron 2017) and Ivory Coast (Koudou et al. 2020); tilapia and catfish in Malawi (Minich et al. 2018) and Uganda (Wamala et al. 2018); tilapia, trout and koi aquaculture systems in South Africa (Jacobs and Chenia 2007); and aquaculture abattoir environments in South Africa (Igbinosa et al. 2017a, b). Moreover, ARG and ARGs have been isolated in aquaculture systems (Mukwabi et al. 2019) and farmed fish (Wanja et al. 2020) in Kenya and a marine fish (*Argyrosomus japonicus*) and water samples at aquaculture farms in South Africa (Fri et al. 2018). In general, the application of antibiotics in aquaculture acts as a source of ARB and ARGs.

6.2.2 Animal Manure and Integrated Aquaculture as ARB and ARGs Sources

Animal wastes are used as fertilisers for fish cultured in African aquaculture ponds (Limbu et al. 2016; Shoko et al. 2019). Manures from livestock such as cattle (Rapatsa and Moyo 2013; Limbu et al. 2016; Minich et al. 2018), chicken (Rapatsa and Moyo 2013; Minich et al. 2018; Shoko et al. 2019), pigs (Rapatsa and Moyo 2013; Bouelet Ntsama et al. 2018; Minich et al. 2018), rabbit (Rukera Tabaro et al. 2012) and goat (Lundeba et al. 2022) production are administered to fish ponds mainly to release nutrients to support the photosynthetic organisms. Apart from manure fertilisation, livestock animals and plants are reared together or concurrently with fish in integrated farming systems. Such systems include fish-poultry (Bouelet Ntsama et al. 2018; Shoko et al. 2019), fish-piggery (Bouelet Ntsama et al. 2018), fish-rabbit (Tabaro et al. 2012) and fish-crop farming (Limbu et al. 2017; Bouelet Ntsama et al. 2018). In these systems, the livestock animals and plants are reared intensively, necessitating antibiotics use for prophylactic and therapeutic purposes (Aly and Albutti 2014). Antibiotics are poorly absorbed in the animals' guts after medication, remaining in manures, either unchanged or modified into metabolites (Chuah et al. 2016; Limbu et al. 2018; Limbu 2020), which consequently act as ARB and ARGs sources. Apparently, animal manures contaminated with antibiotics served as ARB and ARGs sources in fish with no history of

antibiotic use in Tanzania (Shah et al. 2012). Moreover, manure has been reported as ARB and ARGs source in fish in Uganda (Bagumire et al. 2009) and Malawi (Minich et al. 2018). Similarly, integrated fish farming has also been reported to spread ARB and ARGs in Nigeria (Adeyemi et al. 2022). Elsewhere, integrated aquaculture systems exhibited a higher prevalence of antibiotic resistance than traditional aquaculture systems in China (Zhang et al. 2013; Klase et al. 2019) and for a report on global catfish aquaculture (Chuah et al. 2016). These studies indicate that livestock manure and integrated aquaculture systems contribute for developing bacterial resistance and disseminating ARGs in African aquaculture.

6.2.3 Aquatic Environment as a Source of ARB and ARGs

The antibiotics used in aquaculture are also administered in agriculture and human medicine (Done et al. 2015). Recent data suggest that over 70% of all antibiotics sold globally are used in animals raised for food (Van Boeckel et al. 2019). Tanzania alone consumed 12,147,491 kg of antibiotics from 2010 to 2017 (Sangeda et al. 2021). Antibiotics applied in various compartments are poorly absorbed in the intestine and subsequently are released into the aquatic environment (water and sediment) at low concentrations (Chuah et al. 2016; Limbu et al. 2018; Zhou et al. 2018), through effluent discharges, sewage and river runoff where they selectively cause ARB and ARGs (Fu et al. 2017; Nallaiah et al. 2021). The extensive use of antibiotics for treating human infections, livestock and agriculture acts as a source of ARB and ARGs in the water used for aquaculture (Miranda and Zemelman 2001). The ARB from these systems enter aquaculture, spreading their genes into water-indigenous microbes, which also contain resistance genes (Baquero et al. 2008). Accordingly, river surface water was reported as a possible source of ARB in South Africa (Mariano et al. 2009) and Egypt (Azzam et al. 2017) and ARGs in Ghana (Chester et al. 2022). The water has also been reported to contribute to ARB and ARGs in South Africa (Adegoke et al. 2018) and Nigeria (Adesiyan et al. 2019). Moreover, river bed sediments in South Africa (Abia et al. 2015) and Ghana (Chester et al. 2022), river and estuarine water (Suzuki et al. 2015) and coastal water (Adeniji et al. 2021) were reported as ARB and ARGs sources in South Africa. In a nutshell, the widespread use of antibiotics in different environmental compartments, including fish, agriculture and human health, contributes to the development of ARB and ARGs existing in aquaculture.

6.2.4 Wastewater Treatment Plants as ARB and ARGs Sources

The antibiotics or their residues from manufacturing industries or any other activities contained in influent or effluent water are supposed to be treated using wastewater treatment plants (WWTPs). However, most WWTPs were originally not designed to treat antibiotic wastes or their residues (Iwu et al. 2020). Accordingly,

most WWTPs are inefficient in eliminating antibiotic wastes or their residues, thus discharging them into the aquatic milieu (Igwaran et al. 2018; Hamiwe et al. 2019; Molale-Tom and Bezuidenhout 2020). Antibiotics residues have been reported at various concentrations after the WWTPs in South Africa (Faleye et al. 2019), Kenya (Ngigi et al. 2019; Kairigo et al. 2020; Muriuki et al. 2020) and Nigeria (Ajibola et al. 2021). The antibiotics in WWTPs represent a source of ARB and ARGs (Faleye et al. 2019) due to the use of the recipient water for aquaculture activities. Several ARB strains have been isolated in WWTPs in South Africa (Okoh and Igbinosa 2010; Igbinosa et al. 2011; Igwaran et al. 2018; King et al. 2020; Molale-Tom and Bezuidenhout 2020; Mbanga et al. 2021). Moreover, ARGs were prevalent in WWTPs in South Africa (Suzuki et al. 2015) and Nigeria (Obayiuwana and Ibekwe 2020). Furthermore, WWTPs contributed as ARB and ARGs sources in South Africa (Hamiwe et al. 2019; Nzima et al. 2020) and Nigeria (Adesoji et al. 2020). ARGs were detected in a pond receiving municipal wastewater, an important fishing ground in Nigeria (Abu and Wondikom 2018). These studies indicate the potential of WWTPs acting as ARB and ARG sources when the water is used in aquaculture activities.

6.2.5 Hospitals and Pharmaceutical Industries as ARB and ARGs Sources

Hospitals are crucial hotspots for disseminating ARB and ARGs (King et al. 2020) due to the extensive antibiotics use for treating bacterial diseases in patients. However, the antibiotics consumed or injected into patients are not entirely absorbed. Ultimately, they are released in urine and faeces into the WWTPs, which inadequately treat the antibiotics and their residues. Moreover, antibiotic residues from pharmaceutical industries also circulate in water environments following poor treatment by WWTPs (Baquero et al. 2008). Therefore, it is unsurprising that ARB were isolated from hospital effluent (King et al. 2020) and high-risk effluent hospital water (Eze et al. 2021) in South Africa. Moreover, ARB and ARGs were detected from effluents in various hospitals in the Democratic Republic of the Congo (Laffite et al. 2016) and South Africa (Perovic et al. 2020; Mbhele et al. 2021; Ramsamy et al. 2022). Regardless of the source, antibiotics and their residues stimulate ARB and ARG development once the water is used for aquaculture. The current studies emphasise the source of ARB and ARGs from hospitals and pharmaceutical industries entering into African aquaculture industry.

6.2.6 Aquaculture Workers as ARB and ARGs Sources

Aquaculture workers contaminated with ARB and ARGs are a potential source of antibiotic resistance in an aquaculture setting (Chuah et al. 2016). The transfer of ARB and ARGs from aquaculture workers to aquaculture may occur via various

pathways. First, ARB and ARGs may be transferred to aquaculture when wounded farmworkers are in contact with fish, water or aquaculture facilities (Jacobs and Chenia 2007). Second, ARB and ARGs colonising aquaculture workers can enter the aquaculture during fish feed production and hand-dispersing (Madubuike and Kennedy 2016). Third, contaminated fish-farm workers may also act as a source of ARB and ARGs during fish handling for food preparation or consumption (Chenia and Jacobs 2017). Fourth, ARB and ARGs in contaminated workers may be transferred into aquaculture systems through faeces especially in rural areas with limited toilets or when vectors such as insects transfer them to aquaculture systems (Klase et al. 2019). Accordingly, Hamza et al. (2020) found that half of the workers in the integrated agriculture-aquaculture system in Egypt carried *Enterobacteriaceae* strains, which were also present in the fish-rearing water and the fish. Elsewhere, aquaculture workers, especially in intensive culture systems, acted as ARB and ARGs sources in the aquaculture systems (Pruden et al. 2013). These studies highlight that occupational exposure to antibiotics may also contribute to increased resistance rates.

In summary, ARB and ARGs occurring in aquaculture systems originate from several sources (Fig. 6.1). The ARB and ARGs caused by antibiotics application in aquaculture combine with those used in other activities such as livestock, humans and plants and find their way into the aquatic environment following inefficiency treatment in WWTPs. Moreover, ARB and ARGs also originate from hospitals and pharmaceutical industry effluents and contaminated workers at farms. Consequently, aquaculture retains ARB and ARGs, acting as a hotspot for their dissemination.

6.3 Factors Causing ARB and ARGs in Aquaculture

Antibiotic resistance is currently one of the major public health crises worldwide. It is usually known that antibiotic resistance evolves naturally via natural selection through random mutation or evolution in a stressed population (Iwu et al. 2020). Therefore, the evolution of resistance in bacteria is deemed a natural process (Watts et al. 2017; Samreen et al. 2021). However, the recent increase in the number of ARB and ARGs is contributed by several factors as discussed in this section.

6.3.1 Antibiotics Overuse and Misuse

The discovery of antibiotics by Fleming (1944) marked an important milestone in treating bacterial diseases. Since then, antibiotics have been commonly used to treat diseases caused by bacteria in humans and economically important livestock animals and plants. Antibiotics are also used to treat bacterial diseases in aquaculture for therapeutic and prophylaxis purposes. Unfortunately, antibiotics are used abusively in aquaculture and other organisms due to overprescription (Iwu et al. 2020),

inadequate knowledge about their purpose and proper application by producers (Pham et al. 2015) and inappropriate socio-ecological behaviours (Iskandar et al. 2020). Misuse of antibiotics in aquaculture in developing countries causes ARB and ARGs development in different cultured organisms (Limbu 2020). The overprescribed or abusively used antibiotics kill or inhibit drug-sensitive bacteria, leaving resistant strains to proliferate through natural selection (Limbu et al. 2021). As such, the tolerant bacterial strains develop resistance to further application of antibiotics. The resistant bacteria mutate, altering the target sites of the antibiotics through various factors (Iwu et al. 2020), thus developing ARGs. Consequently, ARB and ARGs develop in Africa's various components of aquaculture systems due to antibiotics overuse and misuse in the sector.

6.3.2 Regulatory Barriers

It is globally advised that all antibiotics legally used in aquaculture must be approved by the government agency responsible for veterinary medicine (Limbu et al. 2021). However, many regulatory agencies on antibiotic use in African aquaculture are absent or weak. In some countries, regulations may exist but are not effectively enforced due to loose rules (Iskandar et al. 2020), causing farmers to use any antibiotics available in the markets as in countries with no regulations (Musefiu and Olasunkanmi 2015). In addition, in some countries, regulations on antibiotics used in aquaculture are hampered by ineffective communication channels and fluctuations in licensing, administrative and regulatory rules (Iwu et al. 2020). Accordingly, antibiotics use for treating cultured species in African aquaculture is largely unregulated (Aly and Albutti 2014), which causes antibiotics overuse, leading to the development of ARB and ARGs.

Moreover, the ARB and ARGs in African aquaculture are caused by inadequate surveillance and monitoring systems (Iskandar et al. 2020). Lack of surveillance and monitoring systems has been reported to exacerbate the magnitude of ARB and ARGs in African aquaculture in South Africa (Ekwanzala et al. 2018), Ivory Coast (Koudou et al. 2020) and Tanzania (Mzula et al. 2021). In addition, most surveillance and monitoring systems for antibiotics in African aquaculture have inadequate funds (Mzula et al. 2021), poor coordination and lack adequately trained staff (Mathew et al. 2020). In general, ARB and ARGs in African aquaculture are caused by lack of regulations, ineffective enforcement, loose rules and lack of surveillance and monitoring systems.

6.3.3 Availability, Accessibility and Affordability of Antibiotics

The lack of/weak regulations for antibiotics use in African aquaculture have increased their availability to practitioners. Most antibiotics used in African aquaculture are those intended to treat bacterial diseases in humans and other animals,

which are readily available due to diverse distribution systems caused by inadequate designated healthcare systems (Byarugaba 2004). Accordingly, antibiotics intended for aquaculture can be purchased in community pharmacies (Mukonzo et al. 2013), general stores and even market stalls (Aly and Albutti 2014). Antibiotics in Africa are given as over-the-counter drugs at community pharmacies. This practice increases the accessibility of the antibiotics required for treating diseased animals in aquaculture. Moreover, the common antibiotics used in African aquacultures, such as tetracyclines (Bouelet Ntsama et al. 2018), are cheap and thus affordable to most farmers (Iwu et al. 2020). Selling antibiotics over-the-counter has been strongly correlated with ARB and ARGs in low-income and middle-income countries (Alsan et al. 2015). Therefore, the injudicious sale of antibiotics over-the-counter increases their availability and accessibility. The easy availability and accessibility, coupled with the affordability of antibiotics, increase the development and spread of ARB and ARGs in African aquaculture.

6.3.4 Incorrect Antibiotic Prescriptions

The African aquaculture is characterised by inadequate expertise in fish disease diagnosis and treatment (Mathew et al. 2020; Mzula et al. 2021). Consequently, aquaculture practitioners use antibiotics without correct prescriptions, similar to common self-medication for human disease treatment (Sanou et al. 2018). Inappropriate prescription of antibiotics in African aquaculture might also be caused by increasing intensification to improve fish production (Mathew et al. 2020). Incorrect prescriptions of antibiotics in African aquaculture might lead to ARB and ARGs in produced cultured animals (Minich et al. 2018). In addition, incorrectly prescribed antibiotics may also lead to ARB and ARGs by promoting gene modifications such as mutagenesis and deviations in gene expression (Iwu et al. 2020). Incorrect prescriptions of antibiotics in African aquaculture include improper choice of antibiotics and treatment period, which is one of the factors for the existence of ARB and ARGs in the industry.

6.3.5 Prolonged Use of the Same Antibiotics

Globally, the aquaculture industry mainly uses eight groups of common antibiotics (Limbu et al. 2021). Similarly, the African aquaculture also uses common antibiotics such as tetracycline, oxytetracycline, florfenicol, and chloramphenicol, usually for prolonged periods (Limbu 2020). The continued use of similar antibiotics creates selective pressure on sensitive bacteria strains leading to the proliferation of resistant ones (Iwu et al. 2020). In addition, specific antibiotics have been linked to selective pressure on distinct bacteria strains leading to antibiotic resistance elsewhere (Kolář et al. 2001). Therefore, the continued use of similar antibiotics in African aquaculture might be one of the factors causing ARB and ARGs in the sector.

In summary, overuse and misuse of antibiotics in the sector, lack of regulations, ineffective enforcement, loose rules and lack of surveillance and monitoring systems coupled with easy availability, accessibility and affordability of antibiotics increase the development and spread of ARB and ARGs in African aquaculture. Moreover, incorrect prescriptions of antibiotics and continued use of similar antibiotics in African aquaculture are responsible factors for the increased ARB and ARGs in the sector.

6.4 Types of ARB and ARG in Aquaculture

Aquaculture has been considered as one of the "hotspot environments" for ARB and ARGs transfer (Cabello et al. 2016; Watts et al. 2017; Reverter et al. 2020; Hossain et al. 2022). The ARB and ARGs in aquaculture are similar due to the use of related antibiotics, level of aquaculture production and inadequate laws, regulations and enforcement (Limbu et al. 2021). Unfortunately, aquaculture practices from low- and middle-income countries, which characterise most African nations, contribute high ARB and ARG levels (Reverter et al. 2020). This chapter recorded 27 bacterial species causing ARB in African aquaculture, as depicted in Table 6.1. The ARB were dominated by *Citrobacter* spp., *Aeromonas* spp., *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Vibrio* spp., *Pseudomonas* spp., *Proteus* spp., and *Enterococcus* spp., with rare species such *Edwardsiella tarda*, *Salmonella typhi*, *Plesiomonas* spp., *Exiguobacterium* spp., *Providencia* spp. and *Staphylococcus* spp. (Table 6.1).

Antibiotic resistance in these bacteria is alarming because they cause various human diseases. For instance, *Citrobacter* spp. and *Salmonella typhi* cause intestinal diseases such as typhoid (Johnson et al. 2018); *Aeromonas* spp., *Vibrio* spp., *Plesiomonas* spp., *Enterococcus* spp. and *Edwardsiella tarda* cause foodborne illnesses (Bhunia 2018); and *Escherichia coli*., *Staphylococcus* spp., *Exiguobacterium* spp. and *Providencia* spp. are causative agents for diarrheal disease (Schuetz 2019). Moreover, *Pseudomonas* spp., *Proteus* spp. and *Klebsiella* spp. are responsible for the common urinary tract infection (UTI) (Marques et al. 2019a) in African people. Furthermore, *Enterobacter* spp. is a causative agent for nosocomial infections (Ramirez and Giron 2022), while *Kurthia* spp. is responsible for sexually transmitted diseases (Marques et al. 2019b), and *Vagococcus* spp. cause bloodstream and decubitus ulcer infection (Matsuo et al. 2021).

The bacteria studied were resistant to a total of 53 antibiotics, mainly dominated by tetracycline (TCN), gentamycin (GEN), sulfamethoxazole plus trimethoprim (clotrimazole, SXT), ampicillin (AMP), chloramphenicol (CAP), ciprofloxacin (CIP) and erythromycin (ERM). Other main antibiotics were nalidixic acid (NAL), streptomycin (STP), aztreonam (AZT), imipenem (IMP), cefuroxime (CEF), ceftazidime (CAZ), amoxicillin (AMX), ceftriaxone (CFX), trimethoprim (TMP), kanamycin (KAN), cefotaxime (CFT) and amoxicillin/clavulanic acid (AMC) (Table 6.1). Similar to ARB, several ARGs were also detected dominated by *tet*, *dfr*,

Table 6.1 The types of antibiotic-resistant bacteria and resistance genes isolated in African aquaculture

Source of sample	Type of ARB and ARGs	Resistant to antibiotics	MAR	Country	Reference
Fish	*Proteus mirabilis*, *Citrobacter* spp., *Klebsiella* spp., *Escherichia coli*, *Enterobacter aerogenes* and *Staphylococcus aureus*	LIN, AMP, PCN, ERM, TCN, NEC, CLX, KAN and SMZ	0.2–0.7	Zimbabwe	Gufe et al. (2019)
Tilapia, trout and koi aquaculture systems	*Aeromonas* spp.	AMP, OXA, AMX, AUG, CEF, CFX, IMP, CAP, SXT, ERM, GEN, AMK, NAL, OFX, CIP, TCN and AZT	0.12–0.59	South Africa	Jacobs and Chenia (2007)
	*Tet*A and class 1 integron such as *ant(3″)Ia*, *aac(6′)Ia*, *dhfr1*, *oxa2a* and/or *pse1* genes	TCN, GEN, STP, CAP, TMP and β-lactam	NA		
Fish pond water	*Vibrio* spp.	AMP, ERM, NAL, SMZ, TMP, SXT, TCN, OTC and CAP	0.365	Nigeria	Igbinosa (2016)
Nile tilapia and African catfish	*Aeromonas* spp. and *Plesiomonas shigelloides*	SXT, TCN, GEN, STP, IMP, ERM, AMP, CXT, CFT, CAP, AZT NAL and ENF	NR	Uganda	Wamala et al. (2018)
Water, sediments and fish pond	*Aeromonas* spp., *Acinetobacter* spp., *Exiguobacterium* spp., *Kurthia gibsonii*, *Empedobacter brevis*, *Klebsiella oxytoca*, *Proteus* spp., *Planococcus* spp., *Providencia alcalifaciens* and *Vagococcus fluvialis*	TCN, TMP, SFM, AMX, OXA, STP, CAP, FFO and ERM	0.2–1	Tanzania	Shah et al. (2012)
	*tet*A, *tetG*, *dfrA1*, *dfrA5*, *dfrA7*, *dfrA12*, *dfrA15*, bla_{TEM}, *strA*, *strB*, *cat-1*, *mefA*, *int1* genes	TCN, TMP, AMX, STP, CAP and ERM			

(continued)

Table 6.1 (continued)

Source of sample	Type of ARB and ARGs	Resistant to antibiotics	MAR	Country	Reference
Nile tilapia and water samples	*Escherichia coli*, *Citrobacter freundii*, *Morganella morganii*, *Enterococcus faecalis*, *Enterobacter hormaechei* and *Pseudomonas aeruginosa*	AMC, TCC, TZP, PIL, TIC, CAZ, CEF, AZT, NAL, TCG, FSF and SXT	Multidrug resistance for three classes of antibiotics	Ivory Coast	Koudou et al. (2020)
Aquaculture and abattoir environments	*Pseudomonas aeruginosa*	GEN, KAN, AZT, MRP, TCN, CAZ and CEF	0.4–0.8	Nigeria	Igbinosa et al. (2017a)
Aquaculture and abattoir environments	*Aeromonas* spp.	PCN, CFP, CEF, GEN, KAN, AZT, ETP, MRP, CIP and TCN	0.3–0.7	Nigeria	Igbinosa et al. (2017b)
	pse, bla_{TEM} and class 1 integron	Carries two integrons and encodes multiple drug resistance and β-lactam	NA		
Mullet and tilapia from fish farms	*Aeromonas hydrophila*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Escherichia coli*, *Citrobacter koseri* and *Vibrio alginolyticus*	AMC, AMP, AZT, CAZ, CAP, CIP, CPD, CFX, CTT, CFT, CEF, GEN, IMP, KAN, NAL, NOR, OXC, STP, SXT and TCN	Multiple resistance	Egypt	Ishida et al. (2010)
	*tet*A, *tet*E, *tet*C, *tet*D, *tet*B, *dfrA1*, *dfrA2*, *dfrA5*, *dfrA7*, *dfrA12*, *dfrA17*, *aadA1*, *aadA2*, *aadA5*, *aadA7*, *aac(3)-Id*, *catB3*, bla_{TEM}, $bla_{TEM-104}$, bla_{TEM-1}, bla_{OXA-30}, $bla_{CTX-M-15}$, bla_{SHV-89}, *qnrS*, *qnrA*, *qnrB*, *aac(6′)-Ib-cr* and *floR* genes	TCN, TMP, STP, SPT, GEN, SSM, CAP, *β*-lactam, QNL and FFO	NA		

(continued)

Table 6.1 (continued)

Source of sample	Type of ARB and ARGs	Resistant to antibiotics	MAR	Country	Reference
Tilapia aquaculture system	*Aeromonas* spp., *Salmonella* spp., *Shewanella* spp., *Citrobacter* spp., *Myroides* spp., *Chryseobacterium* spp., *Enterobacter* spp., *Serratia* spp., *Bordetella* spp., *Klebsiella* spp., *Pseudomonas* spp., *Vibrio* spp.	AMX, AUG, CFX, CEF, IMP, SMZ, SHR, TMP, SXT, NAL, CIP, AMK, GEN, STP, AZT, ERM, TCN, CAP, and COL	0.14–0.67	South Africa	Chenia and Jacobs (2017)
	aac(6')Ia, *pse1*, *oxa2a*, *catB3* and *sulI* genes	GEN, STP, *β*-lactam, CBP, CAP and SMZ	NA		
Commercial dusky kob aquaculture farms	*Vibrio* spp.	GEN, NOR, FFO, TCN, CAP, STP, IMP, SXT, and NAL	0.0–0.67	South Africa	Fri et al. (2018)
	ampC, *blaOXA*, *tetA*, *tetM*, *dfr1*, *sul1*, *sul2*, *ermB*, *nptII*, *strA*, *SXT*, *ermB*	*β*-lactam, TCN, SFM, SMZ, STP, ERM, SXT	NA		
Nile tilapia and African catfish	*Edwardsiella tarda*	AMX, AMP, CFT, ERM, STP, GEN, ENF, CIP, COL, TCN and SXT	Multidrug resistance to six antibiotics	Egypt	Algammal et al. (2022)
	bla_{TEM}, *sul1*, *tet*A, $bla_{CTX\text{-}M}$, *aad*A1, *qnr*S, and *qnr*A	*β*-lactam, SMZ, TCN, QNL and AMG	NA		
Freshwater aquaculture systems	*Aeromonas* spp.	NAL and OFX	NR	South Africa	Chenia (2016)
	qnrB and *qnrS* genes	QNL	NA		
African catfish	*Escherichia coli*	AMX, GEN, SXT, CIP, CEF, CFX, CAZ, AMC and TZP	> 0.2	Nigeria	Akande and Onyedibe (2019)
	AmpC	AMP	NA		

(continued)

Table 6.1 (continued)

Source of sample	Type of ARB and ARGs	Resistant to antibiotics	MAR	Country	Reference
Infected African catfish from a pond	*Pseudomonas putida*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus* spp.	CAZ, CRO, CTX, CPX, TCN, GEN and CIP	NR	Nigeria	Ogbonna and Inana (2018)
Coastal waters	*Enterococcus* spp.	RFP, ERM, TCN, VCM, ERM, AMP and CIP	0.3–0.9	South Africa	Adeniji et al. (2021)
	*erm*B, *tet*M, *tet*L, *gyr*A, *amp*C and *Van* C2/3 genes	ERM, TCN, CIP, AMP and VCM	NA		
Aquaculture pond water	*Vibrio* spp.	AMP, AMX, AMC, CAZ, IMP, CIP, SXT, CAP and TCN	NR	Nigeria	Adekanmbi et al. (2021)
River and estuarine water	*sul*1, *sul*2, *sul*3 and *tet*M genes	SMZ and TCN	NA	South Africa	Suzuki et al. (2015)
African catfish, Nile tilapia and water samples	*Serratia marcescens*, *Citrobacter freundii*, *Klebsiella oxytoca*, *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Edwardsiella tarda, Salmonella typhi*, *Proteus mirabilis*, *Citrobacter amalonaticus*, *Shigella sonnei* and *Citrobacter diversus*	SXT, GEN, CEF, CAP, CFX, CFT, AMP and TCN	0.00–0.88	Ghana	Adinortey et al. (2020)

ARB antibiotic-resistant bacteria, *ARGs* antibiotic resistance genes, *MAR* multiple antibiotic resistance, *LIN* lincomycin, *AMP* ampicillin, *PCN* penicillin, *ERM* erythromycin, *TCN* tetracycline, *NEC* neomycin, *CLX* cloxacillin, *KAN* kanamycin, *SMZ* sulfamethoxazole, *OXA* oxacillin, *AMX* amoxicillin, *AUG* augmentin, *CFX* ceftriaxone, *IMP* imipenem, *CAP* chloramphenicol, *GEN* gentamicin, *AMK* amikacin, *NAL* nalidixic acid, *OFX* ofloxacin, *CIP* ciprofloxacin, *AZT* azithromycin, *TMP* trimethoprim, *SXT* sulfamethoxazole plus trimethoprim, *OTC* oxytetracycline, *OXA* oxolinic acid, *STP* streptomycin, *FFO* florfenicol, *SFM* sulfonamides, *CFT* cefotaxime, *AZT* aztreonam, *ENF* enrofloxacin, *AMG* aminoglycoside, *AMC* amoxicillin/acid clavulanic, *TCC* ticarcilline/clavulanic acid, *TZP* piperacillin/tazobactam, *PIL* piperacillin, *TIC* ticarcillin, *CAZ* ceftazidime, *CEF* cefoxitin, *TCG* tigecycline, *FSF* fosfomycin, *MRP* meropenem, *CEF* cefuroxime, *CFP* cefepime, *ETP* ertapenem, *CPD* cefpodoxime, *CTT* cefotetan, *NOR* norfloxacin, *OXC* oxacillin, *SPT* spectinomycin, *SSM* sisomicin, *QNL* quinolone, *SHR* sulphatriad, *COL* colistin sulphate, *CBP* carbapenem, *CAZ* ceftazidime, *CRO* ceftriaxone, *CTX* cefotaxime, *CPX* cephalexin, *RFP* rifampicin and *VCM* vancomycin

bla, *sul*, *qnr* and *aadA* coffering resistance to TCN, TMP, β-lactam, sulfamethoxazole (SMZ and other sulfonamides), a quinolone (QNL) and aminoglycoside (AMG), respectively (Table 6.1). It is possible that the ARGs detected in cultured fish might be transferred to humans due to the existence of class 1 integrons responsible for the horizontal transfer of ARGs (Jacobs and Chenia 2007; Shah et al. 2012; Igbinosa et al. 2017b). Notably, the bacteria and genes identified in African aquaculture were resistant to common antibiotics used for human bacterial diseases treatment. The socioeconomic consequences are further enhanced by the fact that most of the bacteria isolated from African aquaculture had multiple antibiotic resistance (MAR) (Jacobs and Chenia 2007; Ishida et al. 2010; Shah et al. 2012; Igbinosa 2016; Chenia and Jacobs 2017; Igbinosa et al. 2017a, b; Fri et al. 2018; Akande and Onyedibe 2019; Gufe et al. 2019; Adinortey et al. 2020; Koudou et al. 2020; Adeniji et al. 2021; Algammal et al. 2022). The identifications of ARB and ARGs in aquaculture and their potential transfer to people manifest damaging health and socioeconomic effects because the main antibiotics to which bacteria and genes were resistant are often used to treat common bacterial diseases in humans.

6.5 The Socioeconomic Consequences of ARB and ARGs in Aquaculture

The possible transfer of ARB and ARGs indicates devastating human socioeconomic effects and is discussed in this section. The socioeconomic effects in humans vary depending on distinct settings.

6.5.1 The Transfer of ARB and ARGs from Aquaculture to Humans

The ARB and ARGs in aquaculture and other environmental components can be transferred from animals to humans through various routes (Fig. 6.2). First, ARB and ARGs may be transmitted to humans via the food chain through contaminated aquaculture products while handling and consuming raw or undercooked food (Ramsamy et al. 2022). Aquaculture animals harbour deposits of ARB and ARGs in their edible tissues (Iwu et al. 2020). Furthermore, the ARB and ARGs in aquaculture products are transferred from host to host after contamination with biological substances such as blood, urine, faeces, milk, saliva and semen or direct human contact (Founou et al. 2016). Occupationally exposed workers such as veterinarians, farmers, abattoir workers, food handlers and those directly in contact with them are at a high risk of being colonised or infected with ARB and ARGs (Igbinosa et al. 2017a, b). Moreover, the human population may be exposed indirectly to ARB and ARGs via contact with or consumption of other contaminated food products such as

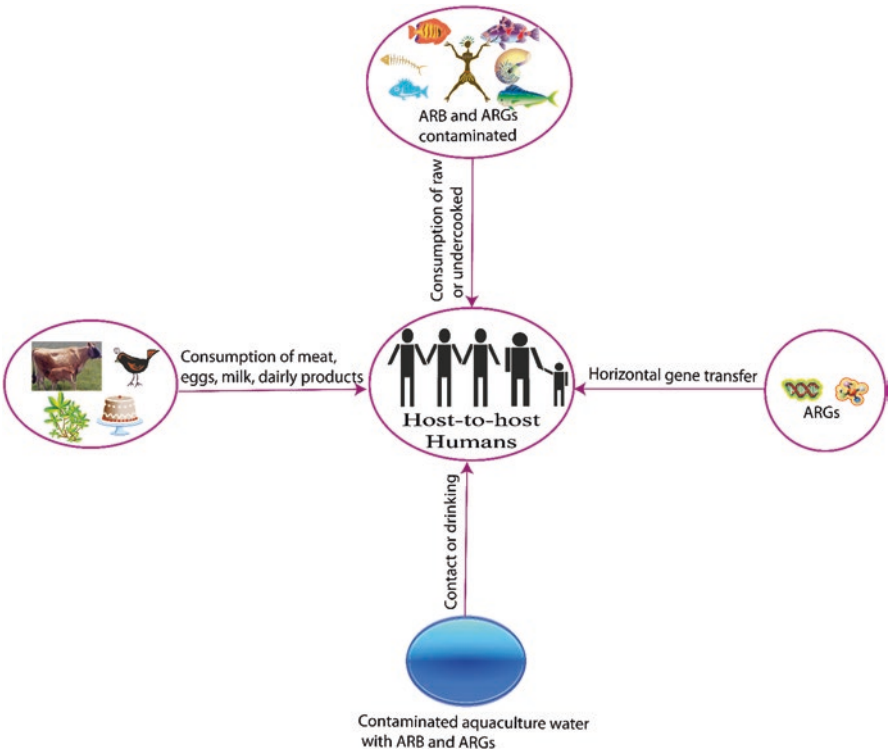

Fig. 6.2 The possible transmission routes of antibiotic-resistant bacteria and genes from aquaculture to humans

meat, eggs, milk and dairy products (Founou et al. 2016). In fact, food animals have been suggested to possess substantial ARB and pose a serious threat for food safety and security in Africa (Badi et al. 2018; Founou et al. 2018).

The second means of ARB and ARG transfer from aquaculture products to human health is through horizontal and vertical gene transfer (Iwu et al. 2020). Horizontal gene transfer can occur from bacteria to other bacteria through mutation, recombination, conjugation, transformation and transduction (Chuah et al. 2016). Horizontal gene transfer promotes genetic communication, changing bacterial pathogenicity, which increases ARB and ARGs (Iwu et al. 2020). The third route for the spread of ARB and ARGs from aquaculture to humans may be through direct contact with water or drinking contaminated water (Aly and Albutti 2014). Drinking waters from rivers in South Africa (Abia et al. 2015) and Nigeria (Adelowo et al. 2018) and well water in Guinea-Bissau (Machado and Bordalo 2014) contaminated with ARB and ARGs posed severe health risks for users. The ARB and ARGs transmitted through the above routes cause various socioeconomic effects, as detailed in the next section.

6.5.2 Human Health Risk

The results in Table 6.1 indicate a fearful situation on the number of ARB and ARGs and the common antibiotics used for treating human diseases to which bacteria are resistant. The ARB and ARGs from aquaculture have been detected across the African continent in South Africa (Jacobs and Chenia 2007; Chenia 2016; Chenia and Jacobs 2017; Fri et al. 2018; Adeniji et al. 2021), Nigeria (Igbinosa 2016; Igbinosa et al. 2017a, b; Ogbonna and Inana 2018; Akande and Onyedibe 2019; Adekanmbi et al. 2021), Egypt (Ishida et al. 2010; Algammal et al. 2022), Tanzania (Shah et al. 2012), Uganda (Wamala et al. 2018), Zimbabwe (Gufe et al. 2019), Ivory Coast (Koudou et al. 2020) and Ghana (Adinortey et al. 2020). The detection of ARB and ARGs from aquaculture poses public health hazards because they are transmittable to humans. A previous study reported that aquaculture workers are at an increased risk of developing occupational diseases such as leptospirosis and skin problems such as dermatitis, skin abrasions and skin infections (Ngajilo and Jeebhay 2019). Moreover, the ARB were reported as media for potentially transferring waterborne bacteria or ARGs to aquaculture workers in South Africa (Jacobs and Chenia 2007).

Indeed, half of the examined workers at an integrated agriculture-aquaculture farm in Egypt carried resistant *Enterobacteriaceae* strains, harbouring a wide range of multidrug resistance (Hamza et al. 2020). In China, workers and community members in contact with waste-fed aquaculture water were reported to have an increased risk of acquiring diarrhoea and skin diseases (Klase et al. 2019). The consequences of ARB and ARGs causing human infections include increased infections, frequency of treatment failures, severe infection, prolonged duration of illness and increased frequency of bloodstream infections and hospitalisation (Aly and Albutti 2014). Furthermore, the spread of ARB and ARGs and increasing resistance to the commonly prescribed antibiotics make primary healthcare redundant (Mathew et al. 2020), thereby significantly reducing the available medical treatment options for previously curable infections (Watts et al. 2017). Accordingly, Mathew et al. (2020) reported frightening ARB and ARGs situation in Tanzania, Uganda, Ethiopia, Kenya and Egypt. In a nutshell, the ARB and ARGs from aquaculture are worsening the already limited health facilities for treating bacterial diseases in Africans.

6.5.3 Economic Consequences

The economic consequences of ARB and ARGs are twofold. On the one hand, the widespread ARB and ARGs in cultured species in Africa causes repeated applications of antibiotics, weakening the immunity of fish (Limbu et al. 2018; Zhou et al. 2018; Limbu 2020; Limbu et al. 2021) and further prompting continued use of antibiotics. Consequently, aquaculture production costs are increased (Musefiu and Olasunkanmi 2015) because of money spent on purchasing higher antibiotics

(Mathew et al. 2020). On the other hand, the ARB and ARGs acquired by humans confer resistance to prescribed antibiotics. Consequently, healthcare costs are increased due to poor outcomes, the need for more patients to undergo more diagnostic tests and extended hospitalisation periods (Nyasulu et al. 2012). Human healthcare costs are also increased because patients need to travel great distances to access higher medical centres. Thus, they spend more money on advanced bacterial diagnostic tools to identify appropriate cures and purchase more potent antibiotics for treatment (Mathew et al. 2020). Therefore, the ARB and ARGs from aquaculture increase the cost to society for treating cultured species and humans.

6.5.4 Increased Human Deaths

The presence of ARB and ARGs in aquaculture contributes to the increased severity of infection (Aly and Albutti 2014) and reduces the available medical treatment options for previously curable infections (Watts et al. 2017). Consequently, ARB and ARGs have led to increased mortality of hospitalised patients (Aly and Albutti 2014) and deaths from infections, which were treatable formerly (Watts et al. 2017). Regrettably, the number of deaths due to ARB and associated ARGs is not consistent and only available in certain areas. Reverter et al. (2020) estimated that over 35,000 human deaths occurring annually in the USA, 33,000 in the European Economic Area and 58,000 in India are caused by ARB and ARGs. Mathew et al. (2020) estimated that around 50,000 deaths happen every year in Europe and USA due to ARB and ARGs. Samreen et al. (2021) reported that infections with multidrug-resistant microbial pathogens cause approximately 700,000 annual deaths globally.

Despite the inconsistency in data, the deaths reported are already alarming. The numbers may dramatically worsen if the unavailable data from other parts of the world, such as low-middle income countries, become available or improved data quality (Mathew et al. 2020). Future projections indicate that more than ten million human deaths will occur every year globally due to ARB and ARGs by 2050, assuming the current resistance increase by 40% and the mortality risk per infection remains unchanged (O'Neill 2014). Currently, the direct contribution of aquaculture to human deaths caused by ARB and ARGs remains challenging to unravel. Other global weather patterns also contribute to the difficulty in realising the data due to climate change (Reverter et al. 2020) and morbidity from other diseases.

In summary, African aquaculture might be contributing to ARB and the associated ARGs, increasing the severity of infections and reducing available medical treatment options for previously curable diseases, thus increasing human deaths. However, the precise number of deaths attributed to antimicrobial resistance from African aquaculture is difficult to estimate, an aspect that requires further research.

6.6 Conclusion

The African aquaculture represents a hotspot reservoir of ARB and ARGs. However, the ARB and ARGs existing in African aquaculture are complex and not solely derived from the industry. They originate from multiple sources, including antibiotics application in aquaculture, livestock manure and integrated aquaculture systems, agriculture and human health. The ARB and ARGs also originate from WWTPs, hospitals and pharmaceutical industries. The ARB and ARGs in aquaculture are caused by overuse and misuse of antibiotics in the sector, lack of regulations, ineffective enforcement, loose rules and inadequate surveillance and monitoring systems, which increase the availability and accessibility of antibiotics. Moreover, incorrect prescription, easy affordability and prolonged use of the same antibiotics also contribute to increased ARB and ARGs in the African aquaculture industry.

The African aquaculture sector is currently dominated by multiple ARB and their genes, resistant to antibiotics commonly used to treat human diseases. The ARB and ARGs in aquaculture are transferred to humans via aquaculture product contamination during handling and consumption of raw or undercooked food, horizontal and vertical gene transfers and direct contact with water or drinking contaminated water. The consequences of ARB and ARGs from aquaculture include increased infections, frequency of treatment failures, severe infections, prolonged duration of illness and increased frequency of bloodstream infections and hospitalisation. These make primary healthcare redundant for previously curable diseases, thereby increasing costs to the society on treating cultured species and humans, leading to animal and human deaths.

6.7 Recommendations

Combating ARB and ARGs requires collective effort in all sectors using a “One Health” approach. Therefore, stringent measures, legislations and regulations for antibiotics use in aquaculture, agriculture, livestock and humans should be developed and implemented. In addition, research should be conducted to design proper treatment for ARG and ARGs in hospital sewage wastes and pharmaceutical industrial effluents. Furthermore, the African continent should institute coordinated, continuous monitoring programmes following appropriate legislation and regulations. The African continent should also emphasise alternative treatment options for bacterial diseases, considering the existence of diverse medicinal plants.

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Chapter 7
Occurrence and Health Risks of Antibiotic Resistance in African Aquatic Systems

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7.1 Introduction

Global estimates indicate that by 2050, antibiotic resistance will cause approximately ten million deaths yearly (de Kraker et al. 2016). In 2020, the World Health Organization (WHO) declared antibiotic resistance as a global human health concern that warrants significant research and policy attention (WHO 2020). To date, antimicrobial resistance has attracted the attention of the public, research community, and decision- and policymakers. The latest studies reveal that the global burden of mortalities due to antimicrobial resistance is now highest in Sub-Saharan Africa compared to other regions (Murray et al. 2022). Antimicrobial resistance is a broad term referring to the ability of microorganisms, including bacteria, fungi, protozoa/helminths, and viruses, to develop mechanisms to resist or inactivate antimicrobial agents (WHO 2014, 2020).

The present chapter focuses on antibiotic resistance because this is the most documented form of antimicrobial resistance, partly because antibiotics are more

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commonly used than other antimicrobial agents. Among the most common antibiotic-resistant bacteria of human health concern are the ESKAPE pathogens: (i) *Enterococcus faecium*, (ii) *Staphylococcus aureus*, (iii) *Klebsiella pneumoniae*, (iii) *Acinetobacter baumannii*, (iv) *Pseudomonas aeruginosa*, and (v) *Enterobacter species* (Ma et al. 2020; Gwenzi et al. 2022). Recently, *Clostridium difficile*, *Escherichia coli*, and *Proteus* have also been included among the antibiotic-resistant bacteria of human health concern (De Oliveira et al. 2020).

A substantial body of literature, including reviews, exists on antibiotic resistance in various environmental compartments, including terrestrial and aquatic systems. For example, several reviews of global evidence exist on the sources, occurrence, behavior, fate, and health risks of antibiotic resistance in the environment (Michael et al. 2013; Rizzo et al. 2013; Smyth et al. 2020; Sanganyado and Gwenzi 2019; Gwenzi et al. 2022). In summary, evidence shows that antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) are ubiquitous. Hence, antibiotic resistance has been detected in soils, livestock production systems, urban environments, wildlife, humans, the funeral industry, human foods, drinking water systems, insects, rodents, and aquatic systems (Gwenzi et al. 2018, 2022; Gwenzi 2020; Sanganyado and Gwenzi 2019).

Several studies conducted in Africa have detected antibiotic resistance in aquatic environments. In summary, the available evidence on antibiotic resistance in African aquatic systems have focused on the following aquatic environments: (1) surface water and groundwater (Oluyege et al. 2009; Ayandiran et al. 2014; Carstens et al. 2014; Mulamattathil et al. 2014), wastewaters (Azuonwu and Ogbonna 2019; Fars et al. 2005; Igbinosa et al. 2011), drinking water supply (Obi et al. 2004; Abera et al. 2016; Adesoji and Ogunjobi 2016; Akoachere et al. 2013; Antai 1987; El-Zanfaly et al. 1987; Lyimo et al. 2016; Machado and Bornalo 2014; Dekker et al. 2016), and rainwater harvesting systems (Anyadike and Obeta 2012; Chidamba and Korsten 2015). Humans can be exposed to ARB and ARGs in drinking, washing, bathing, and domestic water sources (WHO 2014). However, a comprehensive review of the evidence on antibiotic resistance in African aquatic systems is still lacking. Therefore, the present chapter seeks to examine and present a critical review of the existing evidence on antibiotic resistance in African aquatic systems.

The specific objectives of the present chapter are (1) to examine the nature, sources, occurrence, and behavior of antibiotic resistance in African aquatic systems, (2) to discuss human exposure pathways and health risks of antibiotic resistance, (3) to present mitigation measures including methods for the removal of antibiotic resistance in aquatic systems, and (4) to propose future research directions and strategies to overcome research challenges.

The chapter is structured as follows: First, ARB and ARGs detected in various aquatic systems are presented. Second, the behavior, dissemination, and fate of antibiotic resistance in aquatic systems are discussed. Third, human exposure pathways via drinking water, risk drivers, and potential human health risks are presented. Lastly, future perspectives and research directions are proposed, including key knowledge gaps, research challenges, and possible solutions.

7.2 Occurrence in Aquatic Systems

7.2.1 Sources and Drivers

Various bacterial species, including opportunistic pathogens such as *Aeromonas* and *Pseudomonas* spp., which are often resistant to several classes of antibiotics, can also be found in aquatic systems (Mulamattathil et al. 2000). Therefore, ARB and ARGs in aquatic environments such as groundwater, surface water, wastewater treatment plant (WWTP) effluents, and municipal potable water pose human health risks (WHO 14/7).

The widespread use and misuse of antibiotics in animal and human healthcare, animal husbandry, and aquaculture drive the production of antibiotics and other antimicrobial agents by pharmaceutical industries (Gwenzi and Chaukura 2018). However, several other factors also influence the use and misuse of antibiotics and the emergence and persistence of antibiotic resistance in Africa. These include (1) high human and animal disease burden associated with tropical environments which necessitates the frequent use of antibiotics and other co-selecting antimicrobial agents and (2) weak and poorly enforced animal and public health and environmental regulations. For instance, due to COVID-19, there has been an upsurge in the use and misuse of antibiotics such as azithromycin and related drugs (Butler et al. 2021), (3) the existence of informal and black markets for antibiotics and (4) severe environmental pollution including that of aquatic systems (Gwenzi and Chaukura 2018; Gwenzi et al. 2020). Besides their therapeutic uses, antibiotics are widely used in livestock and aquaculture feeds as additives and growth promoters (Amable et al. 2022). Although the use of antibiotics in livestock and aquaculture feeds has been banned in most developed countries, no evidence of such bans exists in Africa except in countries such as Namibia, where antibiotics were banned in animal production as early as 1990 (Selaledi et al. 2020). Nevertheless, the total quantities of antibiotics used in animal and human healthcare and livestock and aquaculture feed in Africa are uncertain.

Wastewaters from industrial processes such as those from the production of pharmaceuticals are often enriched in antibiotics and their metabolites (Gwenzi 2020). These antibiotics and other metabolites may exert selection pressure and promote the emergence of antibiotic resistance. Hence, in African countries with pharmaceutical industries, solid wastes and wastewater from such industries are potential hotspot reservoirs of antibiotic resistance. Several studies from other regions show that wastewaters from pharmaceutical industries harbor ARB and ARGs (Rizzo et al. 2013; Michael et al. 2013). However, compared to other regions, besides a few studies on municipal wastewater (Table 7.1), data reporting antibiotic resistance in industrial wastewater/effluents are still limited in Africa. Furthermore, it is noteworthy that, besides antibiotics per se, several other chemical agents, including trace metals, disinfectants, and even emerging chemical contaminants, may also co-select for antibiotic resistance.

Table 7.1 A summary of the antibiotic-resistant bacteria (ARB) and their antibiotic resistance genes (ARGs) detected in African aquatic systems

Location/country	Aquatic media	Target/detected ARB and corresponding antibiotics	Detected ARGs	References
A: Surface aquatic and drinking water systems				
Durban, South Africa	Effluent receiving river water	*Listeria and Aeromonas* spp. For *Listeria* spp. penicillin, erythromycin, and nalidixic acid Among *Aeromonas* spp., ampicillin, penicillin, vancomycin, clindamycin and fusidic acid, cephalosporin, and erythromycin), nalidixic acid, and trimethoprim	*act*A, *plc*A, and *iap* genes Aerolysin (*aer*) virulence-associated gene, lipase (*lip*) virulence	Olaniran et al. (2015)
Ghana	Dam water	*Salmonella* isolates resistant to erythromycin vancomycin ceftriaxone gentamicin tetracycline chloramphenicol ciprofloxacin and amoxicillin. *Salmonella* isolates also exhibited six different antibiotic resistant patterns	Erythromycin, vancomycin	Adzitey et al. (2015)
Northwest province of South Africa	Drinking water distribution system	β-lactam antibiotics and trimethoprim	*str*A, *str*B, *dfr*B 13, *aad*A 11, $bla_{CTX\text{-}M}$, and *tet*A	Ateba et al. (2020)
Nigeria	Stream	*E. coli*, *Staphylococcus*, *Klebsiella*, *Shigella and Enterobacter* spp., *Pseudomonas* spp., *Salmonella Streptococcus* spp. Sulfamethoxazole, cephalothin, tetracycline penicillin G, oxytetracycline, cefotaxime, nalidixic acid, and cefuroxime sodium		Onuoha (2017)
Gaborone, Botswana	River	*Staphylococcus* spp., *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas* spp., *Brucella* spp., *Salmonella* spp., *Listeria* spp., *Campylobacter* spp.	*Tet*A, *mph*A, *Str*B, *Sul*1, *dfr* and *Int*1	Tapela and Rahube (2019)

(continued)

Table 7.1 (continued)

Location/country	Aquatic media	Target/detected ARB and corresponding antibiotics	Detected ARGs	References
B: Rainwater harvesting systems				
South Africa	Rainwater tanks	*E. coli* strains resistant to ampicillin, cephalothin, colistin sulphate, gentamicin, streptomycin, tetracycline, cotrimoxazole, ciprofloxacin, Augmentin, trimethoprim, and nitrofurantoin	No data reported on ARGs	Malema et al. (2018)
South Africa	Rainwater tanks	*E. coli* strains resistant to gentamicin, ampicillin, amikacin, tetracycline, and amoxicillin	No data reported on any ARGs or virulent genes	Chidamba and Korsten (2015)
C: Wastewaters/effluents				
South Africa, Alice, Dimbaza, and East London	Wastewater treatment plants (WWTPs) in	Multiple antibiotic-resistant (MAR) *Pseudomonas* species resistant to penicillins (90–100%), rifampin (90%), sulfamethoxazole (90%), and cephems (70%) Viable counts: 1.20 × 104 (cfu/100 mL) Alice, 1.08 × 104 (cfu/100 mL) Dimbaza, and 2.66 × 104 (cfu/100 mL) East London		Odjadjare et al. (2012)
Eastern Cape, South Africa	Wastewater reatment plants (WWTPs)	*Escherichia coli* strains	*Str*A (88.2%), *aad*A (52.9%), *cat* I (15%), *cml*A1 (4.6%), *bla*TEM (56.4%), *tet*A (30.4%), *tet*B (28.4%), *tet*C (42.2%), *tet*D (50%), *tet*K (11.8%), and *tet*M (68.6%)	Adefisoye and Okoh (2016)
North West Province, South Africa	Three WWTPs' final effluent and receiving waters	*Enterococcus* spp.	No specific data have been reported	Molale-Tom et al. (2020)

(continued)

Table 7.1 (continued)

Location/country	Aquatic media	Target/detected ARB and corresponding antibiotics	Detected ARGs	References
uMgungundlovu District, KwaZulu-Natal, South Africa	Hospital effluent and proximate wastewater treatment plants	Antibiotic-resistant *Klebsiella* spp. Resistance was observed to all antibiotic classes tested (16 antibiotics were utilized) Viable counts hospital effluents (1.38×10^2 to 1.03×10^4), WWTP (1.76×10^3 to 5.10×10^3 CFU/ml)	No data was reported	King et al. (2020)
Eastern Cape province, South Africa	Wastewater and freshwater milieus in the freshwater and wastewater treatment plant	*Pseudomonas* species were 100% resistant to penicillin, oxacillin, clindamycin, and rifampicin and 100% susceptible to ciprofloxacin and gentamicin with varied percentages resistances to cephalothin, nalidixic acid, tetracycline, and ampicillin *Aeromonas* species	bla_{TEM} (12.5% of *P. putida*, 57.14% of *P. fluorescens*, 100% *P. aeruginosa*, and 40% in other *Pseudomonas* spp. Integrons (12.5% of *P. putida*, 57.14% of *P. fluorescens*, 100% of *P. aeruginosa*, and 40% of other *Pseudomonas* spp.) Fresh water (*lip* (67%), *aer* (43%), *alt* (33%), *fla* (62%), *ast* (10%), and *hly*A (86%)), in wastewater samples the occurrence were as follows: *lip* (92%), *aer* (21%), *alt* (54%), *fla* (83%), *ast* (29%), and *hly*A (88%)	Igbinosa et al. (2012, 2013)

The animal and human microflora is a reservoir for ARGs (Salyers et al. 2004), and the bacteria that passes through the gastrointestinal intestinal tract can acquire antibiotic resistance through one of the microbial gene transfer methods and end up in human and animal excreta (Anderson et al. 2006; Salyers et al. 2004). The use of antibiotics can provide selective pressure inside the gut, leading to antibiotic resistance in enteric bacteria. Antibiotic resistance can also occur in the gut of animals exposed to stock-feed containing antibiotics, and animal manure collected from farms that use antibiotics may contain ARB (Langford et al. 2003; Yang et al. 2014).

Human healthcare facilities such as hospitals, clinics, and veterinary facilities administer large quantities of antibiotics. In addition, several over-the-counter antibiotics are also used in homes and other institutions. Only a certain fraction of

antibiotics administered to humans and animals undergo metabolism in their bodies, while the remainder persists as parent compounds (Kuroda et al. 2021; O'Flynn et al. 2021). Thus, both parent compounds and metabolites of antibiotics are excreted via feces and urine into wastewater and on-site sanitation systems. Accordingly, on-site sanitation systems such as septic tanks, pit latrines, and wastewater treatment systems are hotspots of antibiotics and their metabolites and antibiotic resistance. Similarly, solid wastes and wastewater from animal husbandry systems and slaughterhouses/abattoirs harbor ARB and ARGs (Gwenzi 2020; Gwenzi 2021). Once in solid waste and wastewater systems, antibiotic resistance undergoes wider dissemination into other environmental compartments.

Compared to developed countries such as the USA and Europe and other developing regions such as South Asia and South America, there is a paucity of data on antibiotic resistance in industrial wastewaters, including those associated with pharmaceutical industries. The lack of data could reflect restricted access to such industrial facilities and strict confidentiality associated with industrial operations in Africa. The lack of access to industrial operations in Africa is common and has been reported in the case of mining and mineral processing operations (Gwenzi 2020). For example, in Zimbabwe, it is difficult to obtain reliable information on solid waste and wastewater management practices for individual industrial operations, while in most cases accessing industrial premises to sample waste and wastewaters is not even permissible. In a few cases where access is granted, the publication of the research findings is often not permissible. The restricted access points to potential mistrust between companies and the research community and fear of public exposure. Therefore, it is difficult to determine the extent and contribution of various industrial processes to antibiotic resistance in Africa. Most African countries' environmental surveillance systems also focus on traditionally regulated contaminants (toxic metals, BOD, pH, nutrients) while excluding novel or emerging contaminants such as antibiotics and antibiotic resistance. Two reasons may explain this: (1) lack of analytical equipment and expertise to monitor antibiotic resistance and (2) as an emerging contaminant, the lack of regulatory guidelines and standards associated with antibiotic resistance, making enforcement problematic. Therefore, developing analytical capacity and expertise in antibiotic resistance surveillance is essential in Africa.

7.2.2 Wastewater Systems

Human excretions, especially feces and urine and wastewater systems, are commonly known as hotspots for antibiotic agents, their metabolites, ARB and their ARGs because of the widespread and extensive use of antibiotic agents by the human population (Ateba et al. 2020; Shamsizadeh et al. 2021). Wastewater systems mainly include urban municipal WWTPs and those associated with industrial operations, including animal husbandry, handling, and processing facilities (e.g., abattoirs, slaughterhouses) (Ebomah and Okoh 2020a). WWTPs receive different

types of domestic and industrial wastewaters contaminated with self-prescribed and prescription drugs such as antibiotics and antivirals, pharmaceutical products, personal care products, and pesticides, which co-select and promote the emergence of antibiotic resistance. Thus, the release of antibiotics and other co-selecting chemicals into aquatic systems such as wastewater systems is linked to the development of ARGs (Nappier et al. 2020). Urban wastewaters may undergo treatment in some developing African countries, but ARB and ARG removal are likely low and undocumented. This is because the wastewater treatment systems use conventional methods and are often dilapidated, overloaded, and thus inefficient (Gwenzi et al. 2018).

Several African studies have detected ARB and ARGs in wastewater (Mann et al., 2019; Table 7.1). In a study undertaken in Durban, South Africa, to determine the antibiotic resistance profile and virulence signatures of *Aeromonas* spp. and *Pseudomonas* spp., isolates from surface water and wastewater showed some antibiotic resistance properties (Govender et al. 2021). The different bacterial species in the water systems were characterized using polymerase chain reaction (PCR) and MALDI-TOF. Results showed that 71% of *Aeromonas* and 94% of *Pseudomonas* isolates displayed resistance to three or more antibiotics. High bacteria counts of *Aeromonas* spp. (2.5 (± 0.8) − 3.3 (± 0.4) $\log_{10}$ CFU mL^{-1}) and *Pseudomonas* spp. (0.6 (± 1.0) − 1.8 (± 1.0) $\log_{10}$ CFU mL^{-1}) were detected in the same study. *Aeromonas* spp. isolates displayed high resistance to ampicillin and had higher multiple antibiotic resistance (MAR) indices for samples close to a hospital since hospitals are usually sources of effluent that contain significant levels of antibiotics and their metabolites. The *Pseudomonas* spp. isolates exhibited low resistance to carbapenems but very high resistance to the third-generation cephalosporins and cefixime. Hence, both species of bacteria in wastewater systems exhibited antibiotic resistance. Of great interest is the fact that the study detected the virulence gene *aer* in *Aeromonas*, which was significantly positively associated with the antibiotic-resistant gene, bla_{OXA} ($\chi^2 = 6.657$, $p < 0.05$) and the antibiotic ceftazidime ($\chi^2 = 7.537$, $p < 0.05$).

Another study in Durban, South Africa, investigated the antibiogram and virulence gene profiles of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates recovered from treated wastewater effluent and receiving surface waters (Ramessar and Olaniran 2019). MRSA is a harmful bacterium that causes pneumonia, septicemia, and skin and soft tissue infections. The study obtained 80 MRSA isolates from treated effluent and receiving rivers of two municipal WWTPs. High antibiotic resistance was observed for lincomycin (100%), oxacillin (98.75%), cefoxitin and penicillin (97.50%), and ampicillin (96.25%). The bacteria also showed 72.50%, 66.25%, 52.50%, 40%, and 33.75% resistance against cefazolin, azithromycin, amoxicillin/clavulanic acid, erythromycin, and vancomycin, respectively. In the same study, many ARGs were detected from the isolates, namely, *aac* (6′)/*aph* (2″) (56.25%), *erm*C (62.50%), *msr*A (22.50%), and *bla*Z and *tet*K (70%). The frequency of various virulence genes was reported as follows: *hla* and *sea* was 57.50%, *hld* was 1.25%, while *luk*S P/V was 0%. A related study conducted in Durban, South Africa, investigated antibiotic resistance in disease-causing bacteria such as *Listeria* and *Aeromonas* species obtained from treated effluents of two WWTPs and

receiving rivers (Olaniran et al. 2015). All the 78 *Listeria* spp. showed high resistance to penicillin, erythromycin, and nalidixic acid, which was very concerning because these were obtained from samples of treated WWTPs effluent. The *Listeria* spp. also showed antibiotic resistance to other drugs such as ampicillin (83.3%), trimethoprim (68.0%), nitrofurantoin (64.1%), and cephalosporin (60.3%). Another curious finding from the study was that 26.92% of the *Listeria* spp. contained different virulence genes, with 14.1%, 5.1%, and 21% harboring the *act*A, *plc*A, and *iap* genes, respectively, even after treating the water. All the *Aeromonas* spp. had the highest resistance (100%) against ampicillin, penicillin, vancomycin, clindamycin, and fusidic acid. These species also showed slightly reduced resistance to cephalosporins (82%) and erythromycin (58%), and 56% of the isolates were resistant to nalidixic acid and trimethoprim. Out of all the *Aeromonas* spp. tested, 52% contained the aerolysin (*aer*) virulence-associated gene, while the lipase (*lip*) virulence-associated gene was also detected in 68% of this type of bacterial species. A study in Alice and Fort Beaufort, Eastern Cape Province, South Africa, investigated the presence of antibiotic-resistant *Aeromonas* species in WWTPs (Igbinosa et al. 2012). As with recent studies, the *Aeromonas* isolates from both locations were 100% resistant to penicillin, oxacillin, ampicillin, and vancomycin. Among others, different susceptibilities were observed against ciprofloxacin, chloramphenicol, nalidixic acid, gentamicin, and minocycline. Another important result from the study was that higher phenotypic resistance was observed in isolates from Fort Beaufort than in Alice Town. This might have been due to lesser pollution and pollutants in Alice town since it is smaller with lower industrial activity than Fort Beaufort. About 20.8% of the isolates had Class A *pse*1 β-lactamase and a lower detection rate of 8.3% for bla_{TEM} gene. Again, Class 1 integron was present in 20.8% of *Aeromonas* isolates, while class 2 integron and *tet*C gene were not detected in any *Aeromonas* isolate. The study suggested that wastewater is a potential hotspot for environmental antibiotic resistance determinants.

Hamiwe et al. (2019) investigated the presence of *Enterococci* harboring genes for resistance to four antibiotics classes in water samples collected from WWTPs, surface water, and hospital sewage. After screening, *Enterococci* isolates containing ARGs were detected in raw influent and treated wastewater effluent from WWTPs and hospital sewage water. Plasmid and transposon encoded *erm*B (macrolide), *tet*M and *tet*L (tetracycline), as well as *aph(3′)-IIIa* (aminoglycosides) genes, which were frequently detected among the isolates, especially in *E. faecalis*. One of the surprising discoveries from the study was the presence of enterococci clonal complexes (CCs), namely, *E. faecium* CC17 (ST18), in wastewater, which was frequently associated with hospital outbreaks and a novel *E. faecalis* sequence type (ST), ST780.

In Pietermaritzburg, KwaZulu-Natal in South Africa, antibiotic-resistant *Escherichia coli* from wastewater systems were characterized (Mbanga et al. 2021). Twelve MDR diarrheagenic *E. coli* isolates were obtained from the final effluent of a WWTP. A total of 31 virulence genes were identified across the study isolate. The presence of many different microorganisms in water systems has increased waterborne diseases and waterborne disease-related deaths (ref). Several organisms,

including different bacterial species, viruses, and protozoa, have been detected in influents and effluents from municipal WWTPs, some of which have mutated to become ARB (Naidoo and Olaniran 2014).

A study on sediments from two rivers receiving animal husbandry wastewaters under tropical conditions in Kinshasa, Democratic Republic of the Congo, investigated the presence of ARB (Al Salah et al. 2019). To fully understand the pathway and trace the host, human and pig host-specific markers were exploited to examine the sources of contamination. From the study, the antibiotic resistance profile showed that both pigs and anthropogenic activities contributed to this problem. Furthermore, the most abundant and most commonly detected ARGs were *sul*1 and *sul*2. More so, using a quantitative (q) PCR, total bacterial load correlated with relevant bacteria and $bla_{\text{OXA-48}}$, *sul*3, and *tet(*B*)* ($p < 0.01$) genes. *E. coli* strongly correlated with 16 s rDNA, *Enterococcus*, *Pseudomonas* spp., $bla_{\text{OXA-48}}$, *sul*3, and *tet(*B*)* ($p < 0.01$) and with $bla_{\text{CTX-M}}$, *sul1*, and *sul2* at a lower magnitude ($p < 0.05$). Hence, even in Congo, ARB were found in wastewater systems. Another study investigated the levels of selected antibiotics in four WWTPs and the receiving water bodies in Kenya (Kairigo et al. 2020a, b). The actual concentration of doxycycline, amoxicillin, sulfamethoxazole, trimethoprim, ciprofloxacin, and norfloxacin within the influents, effluents, surface waters, and river sediments ranged between 0.2 and 49.3 μgL^{-1}, 0.1 to 21.4 μgL^{-1}; ˂ 0.1 and 56.6 μgL^{-1}; and 1.8 and 47.4 μgkg^{-1}, respectively. The risk quotient for bacterial resistance selection in effluent and surface water ranged between less than 0.1 and 53, hence a medium to high risk of antibiotic resistance developing within the study areas. Again, this further supported wastewater systems as hotspots for ARB. In a separate study, the antibiotic resistance and virulence profiles of some common *Enterococcus* spp., which are associated with human infections, recovered from Victoria Hospital wastewater and the final effluent of the receiving WWTP in Alice, Eastern Cape, were determined (Iweriebor et al. 2015). Sixty-six isolates were collected; 62 were confirmed to belong to the *Enterococcus* genus, of which 30 were identified as *E. faecalis* and 15 *E. durans*. The primers used in the screening study did not identify the remaining isolates. The study targeted six virulence genes, and only three of them, *ace*, *efa*A, and *gel*E, were detected. Faleye et al. (2019) also noted the presence of antibodies in the effluent from WWTP, which promote antibiotic-resistant bacteria.

In another investigation, a study was conducted to understand the potential of raw sewage used for urban agriculture in harboring antibiotic-resistant bacteria in two cities of different sizes in Cameroon (Central Africa) and compare the outcome with data obtained in Burkina Faso (West Africa) (Bougnom et al. 2019). The study identified 136 ARGs commonly associated with MDR plasmid carriage in both cities. Similar *Enterobacteriaceae* plasmid replicons and ARGs were found in water samples from both cities. Of interest is that an abundance of *Enterobacteriaceae*, plasmid replicons, and ARGs were found in Yaounde, the city with the greater population. Hence, this further agrees with other authors' findings that suggest increased pollution and pollutants from human activities promote ARB. Amine (2013) detected ARB in both the influent and effluent waters of wastewater in Alexandria,

Egypt. They investigated the quantity of extended-spectrum β-lactamases (ESBLs) producing Gram-negative bacteria in wastewater. The ESBLs compromised 69.8% in influent sewage and 57.7% in effluent sewage of the total Gram-negative bacteria. There was a slight decrease in the number of bacteria after water treatment. The most frequently detected gene among *E. coli* isolates was bla_{TEM}, while the most common among *K. pneumoniae* isolates was bla_{SHV}. These are a threat to human life and health. In a similar study, Akpan et al. (2020) investigated the antibiotic resistance and ESBL production profiles of Gram-negative bacteria isolated from effluent collected from Lafenwa municipal abattoir and receiving surface water, Ogun River, in Abeokuta, Ogun State, Nigeria. Fifty-four Gram-negative bacteria were isolated from the study, including *Salmonella* spp. (9), *Escherichia coli* (15), *Klebsiella* spp. (7), *Shigella* spp. (5), *Pseudomonas* spp. (12), and *Enterobacter* spp. (6). The isolated bacteria were tested against five antibiotics: ceftazidime, cefpodoxime, cefotaxime, ertapenem, and amoxicillin-clavulanate. The results showed that both *Enterobacteriaceae* and *Pseudomonas* isolates (31% and 66.6%, respectively) were resistant to all selected antibiotics except ertapenem (98% susceptibility). In the study, 77% of the isolates had multiple antibiotic resistance index (MARI) values, but no evidence of ESBL production was shown. Water samples from the influent and effluent of the Legon sewage treatment plant (STP) in Accra, Ghana, were investigated for their antibiotic resistance patterns and bacterial counts by Adomako et al. (2021). After water treatment, the mean bacterial counts (colony-forming units/100 mL) in the STP effluents had reduced *E. coli* (99.9% reduction; 102,266,667 to 710), *A. hydrophila* (98.8%; 376,333 to 9603), and *P. aeruginosa* (99.5%; 5,666,667 to 1550). Proper water treatment reduced antibiotic resistance for tetracycline, ciprofloxacin, cefuroxime, and ceftazidime. However, there was increased antibiotic resistance for drugs like gentamicin, amoxicillin/clavulanate, and imipenem. The highest antibiotic resistance levels were experienced for amoxicillin/clavulanate (50–97%) and aztreonam (33%). There was also a progressive increase in antibiotic resistance from upstream, outfall, and downstream. The highest antibiotic resistance levels were experienced for amoxicillin/clavulanate (80–83%), cefuroxime (47–73%), aztreonam (53%), and ciprofloxacin (40%). The study concluded that STP efficiently reduces bacterial counts and thus reduces environmental contamination. However, the recipient stream was contaminated with antibiotic resistance confirming that wastewater systems are ARB and ARG sources. Hence, similar to data from other regions, studies in African countries confirmed wastewater systems as hotspots for ARB and ARGs.

7.2.3 Surface Aquatic Systems

Surface aquatic environments are the most common transmission routes and antibiotic resistance reservoirs (Karkman et al. 2018; Suzuki et al. 2017; Wellington et al. 2013). These aquatic environments provide an ideal setting for the acquisition and

spread of antibiotic resistance, and human exposure to ARB and ARGs in aquatic environments poses a serious health risk (Amarasiri et al. 2020).

Studies have shown that most drinking and wastewater treatment processes are incapable of completely removing ARGs (Ben et al. 2017; Li et al. 2015; Rodriguez-Mozaz et al. 2015). Thus, WWTP effluents containing residual ARGs eventually end up in aquatic environments like rivers, dams, and lakes (Amarasiri et al. 2020). In addition, using reclaimed wastewater in agricultural irrigation, recreational activities and other beneficial purposes can introduce ARB and ARGs to the surface aquatic environment (Pruden et al. 2013; Rodriguez-Mozaz et al. 2015; Ben et al. 2017; Shamsizadeh et al. 2021). Humans can thus be exposed to ARB and ARGs through drinking water, bathing, aquatic sports, agricultural irrigation, and consumption of agricultural produce irrigated with reclaimed water (Leonard et al. 2018; Amarasiri et al. 2020; Shamsizadeh et al. 2021).

Surface water systems receive wastewaters containing antibiotics, ARB, ARGs, and other co-selecting chemical substances (Karkman et al. 2018; Ben et al. 2017; Amarasiri et al. 2020). However, in Africa, a large population still depends on directly using raw water for household uses. Moreover, most of the population does not have access to proper sanitation facilities (Gadgil and Derby 2003). In addition, most rural and informal settlements, which form most of the African population, rely primarily on shallow wells, springs, streams, rivers, and lakes as primary and direct water sources for drinking and other domestic purposes (Oluyege et al. 2009). The pollution and contamination of these water sources with pathogenic microorganisms in developing countries is thus a potential threat to human health (Baquero et al. 2009).

Most African countries are also faced with poor waste management, poor drainage systems, and the closeness of dumpsites and latrines to water sources, all of which negatively impact the quality of surface aquatic systems through seepage and leaching (Odeyemi et al. 2021). Furthermore, improper livestock farming coupled with surface run-off during rainfall and the presence of dangerous pharmaceutical substances from dumpsites are among the pollution sources to water bodies, and gastrointestinal tract infections like typhoid, diarrhea, dysentery, cholera, and enteric viruses are transmitted through water (Odeyemi et al. 2015). Therefore, investigations into antibiotic resistance in Africa are of great significance and urgent because of the serious environmental and human health risks.

Several studies have documented the contamination of surface waters by pharmaceuticals worldwide, and several reviews on this area have been published (Michael et al. 2013; Rizzo et al. 2013; Gwenzi 2020). In Africa, such studies still lag, although the number of articles and reviews published past few years has increased (Madikizela et al. 2020). Although it is widely accepted and documented that pharmaceuticals heavily contaminate surface aquatic systems in Africa (Fayiga et al. 2018; Archer et al. 2017; Fekadu et al. 2019), studies are still relatively limited on the occurrence of antibiotic resistance in African surface aquatic systems that could occur as a result of the presence these contaminants in African countries. Table 7.1 summarizes the studies documenting antibiotic resistance in surface water in several African regions.

To date, antibiotic resistance in surface aquatic systems has been detected in the following African regions:

1. Southern Africa: (i) South Africa (Mann et al. 2019; Molale-Tom and Bezuidenhout 2020; Ebomah and Okoh 2020b; Gentle et al. 2020; Mbanga et al. 2021), (ii) Zimbabwe (Mugadza et al. 2021), (iii) Botswana (Tapela and Rahube 2019).
2. West Africa: (i) Nigeria (Ekhosuehi et al. 2018; Akande and Onyedibe 2019; Akpan et al. 2020; Ateba et al. 2020), (ii) Ghana (Osei et al. 2021; Adomako et al. 2021; Adzitey et al. 2016), (iii) Senegal (Aslam et al. 2018), (iv) Ivory Coast (Ollo et al. 2021), (v) Guinea Bissau (Machado and Bordalo 2014), and (v) Burkina Fasso (Bougnom et al. 2019).
3. East Africa: (i) Kenya (Boga et al. 2007; Wambugu 2016, Wambugu et al. 2018; Ngumba et al. 2016; Kairigo et al. 2020a, b; Muriuki et al. 2020) and (ii) Uganda (Dalameh et al. 2019; Wamala et al. Soge et al. 2009).
4. North Africa: (i) Egypt (Abo-state et al. 2012; Diab et al. 2021), (ii) Libya (Burmadian), and (iii) Morocco (Hafiane et al. 2019; Nassri et al. 2021).

A closer examination showed that most of these studies had been conducted in South African surface aquatic systems, with over 40 peer-reviewed journal articles dating back to 2001 (Bezuidenhout et al. 2001; Lin et al. 2004; Lin and Biyela 2005; Mann et al. 2019). Nigeria ranks second, followed by Kenya and then Ghana in terms of the number of studies conducted on antibiotic resistance in aquatic surface systems. In other African countries, studies have been limited, with some countries only having a single peer-reviewed article documenting the occurrence of antibiotic resistance in aquatic surface systems (e.g., Zimbabwe, Mugadza et al. 2021). Overall, there has been a progression in scientific studies on antibiotic resistance in surface waters since 2017.

In South Africa, early studies on antibiotic resistance were conducted in the Mhlatuze River in KwaZulu-Natal (Bezuidenhout et al. 2001). *E. coli*, *Pseudomonas*, *Enterobacter*, *Salmonella*, *Shigella*, and *E. coli* 0157:H7 were isolated, and more than 90% of these isolates were resistant to ampicillin and penicillin (Bezuidenhout et al. 2001). This study demonstrates that the challenge of antibiotic resistance in South African surface aquatic systems dates over two decades and has been attributed to medical, veterinary, domestic, and agricultural use of antibiotics. Lin et al. (2004) determined the antibiotic resistance profiles of the environmental samples from the same river and demonstrated that 94.7% of all identified isolates were resistant to at least one class of antibiotics used and were multidrug resistant. The resistance levels exhibited by isolates to specific antibiotics were as follows: penicillin, 72.6%; rifampicin, 69.2%; novobiocin, 52.1%; ampicillin, 43.6% and cephalothin, 28.2%. The antibiotic resistance patterns for both *E. coli* and non-*E. coli* groups were comparable, and enteric bacteria isolated from a downstream urban area were more resistant to several antibiotics than those from an upstream rural area. This suggested that anthropogenic activities impact resistance levels (Lin et al. 2004).

The prevalence, antibiotic resistance profiles, and associated ARGs of *Pseudomonas* species isolated from freshwater and mixed liquor environments in the Eastern Cape Province of South Africa were assessed (Igbinosa et al. 2012). The occurrence of *Pseudomonas* spp. in freshwater and mixed liquor was as follows: 71.42% and 37.5% (*P. putida*), 14.28% and 31.25% (*P. fluorescens*), 7.14% and 6.25% (*P. aeruginosa*), and 7.14% and 25% for other *Pseudomonas* species. The antibiogram of the *Pseudomonas* isolates from the two locations showed 100% resistance to penicillin, oxacillin, clindamycin, rifampicin, and 100% susceptibility to ciprofloxacin and gentamicin with varied percentage resistances to cephalothin, nalidixic acid, tetracycline, and ampicillin (Igbinosa et al. 2012). Furthermore, this study identified the bla_{TEM} gene in 12.5% of *P. putida*, 57.14% of *P. fluorescens*, 100% of *P. aeruginosa*, and 40% of other *Pseudomonas* species; this poses a severe health risk as they are mostly multidrug-resistant (MDR) and extremely drug-resistant (XDR). It is thus crucial that the determination of antibiotic susceptibility/resistance patterns becomes part of the microbial monitoring process of water.

Environmental bacteria from various raw water sources and the drinking water distribution system in Mafikeng, South Africa, were isolated, and their antibiotic resistance profiles were determined (Mulamathathil et al. 2014). Fecal indicator bacteria as well as *Aeromonas* and *Pseudomonas* species were isolated with *Pseudomonas* spp. being the most prevalent microbe. Antibiotic susceptibility tests were conducted for the following antibiotics: ampicillin (AMP), cephalothin (CEF), streptomycin (STR), erythromycin (ERY), chloramphenicol (CHL) (C), neomycin (NEO), amoxycillin (AMX), ciprofloxacin (CIP), trimethoprim (TMP), kanamycin (KAN), and oxytetracycline (OTC). The most prevalent multiple antibiotic resistance phenotypes observed were CEF-AMP-CHL-ERY-OTC-KAN-TMP-AMX. All isolates evaluated were resistant to erythromycin, trimethoprim, and amoxicillin. In addition, all isolates were susceptible to ciprofloxacin; fecal coliforms and *Pseudomonas* spp. were susceptible to neomycin and streptomycin. The *gyr*B, *ecf*X, and *hyl*H *gene* fragments were present in the isolates, indicating that the microbiological quality of drinking water in Mafikeng was poor and the water was a reservoir of ARGs.

Antimicrobial-resistant heterotrophic bacteria and genes in raw and treated water in the North West province of South Africa were also investigated for two water sources, A and B, over four seasons (Ateba et al. 2020). Most isolates were resistant to β-lactam antibiotics and trimethoprim but were susceptible to ciprofloxacin, erythromycin, and neomycin. More than 50% of isolates were high risk, demonstrating that they originated from extensive antibiotic-use sources. The study further showed that 35% of the isolates were multidrug-resistant, and most of them (53.5%, $n = 38$) possessed the *str*A gene, *str*B 21 (29.6%), *dfr*B 13 (18.3%), *aad*A 11 (15.5%), $bla_{\text{CTX-M}}$ 5 (7.0%), and *tet*A 3 (4.2%). The 16S rRNA gene sequences of the isolates showed that they belong to the eight clinically important bacterial families.

In Nigeria, surface water samples were collected from three streams for bacteriological analysis (Onuoha 2017). Of all the isolates obtained, *E. coli* and *Staphylococcus* species had the highest percentage occurrence (23.1%). *Klebsiella*,

Shigella, and *Enterobacter* spp. had 11.5% each, *Pseudomonas* spp. had 7.7%, while *Salmonella* and *Streptococcus* spp. had the least percentage occurrence of 3.8%. A large proportion of isolates were resistant to sulfamethoxazole (SUL), cephalothin (CEP), tetracycline (TET), penicillin G (PEN), oxytetracycline (OXY), cefotaxime (CEF), nalidixic acid (NAL), and cefuroxime sodium (CXM). The most effective antibiotic against all the isolates was azithromycin, followed by imipenem. The occurrence of multidrug-resistant strains in water could thus facilitate the dissemination of antibiotic resistance in the environment posing a human health risk (Onuoha 2017).

Antibiogram profiles of *Escherichia coli* ($n = 300$) isolated from selected rivers in Osun State, Nigeria, were also evaluated (Titilawo et al. 2015), and all the *E. coli* isolates were susceptible to imipenem, meropenem, amikacin, and gatifloxacin. In addition, the isolates were variously susceptible to the other antibiotics as follows: ciprofloxacin (96%), kanamycin (95%), neomycin (92%), streptomycin (84%), chloramphenicol (73%), nalidixic acid (66%), nitrofurantoin (64%), gentamycin (63%), doxycycline (58%), cefepime (57%), tetracycline (49%), and cephalothin (42%). These findings signify an increase in the incidence of antibiotic resistance of *E. coli* toward conventionally used antibiotics. Proper surveillance programs are thus essential to monitor antibiotic resistance determinants in water bodies. ARB and ARGs detected in surface aquatic systems are summarized in Table 7.1.

7.2.4 Rainwater Harvesting Systems

Besides their application in agriculture (Hatibu et al. 2006; Biazin et al. 2012), rainwater harvesting systems such as rooftop water harvesting are commonly used for direct drinking and domestic purposes (Kahinda and Taigbenu 2011). Relative to surface water in rivers, dams, and groundwater, rainwater has fewer contaminants and is thus a safer source of drinking water (Gwenzi et al. 2015). However, when rainwater comes into contact with contaminated media, it accumulates many pollutants. Consequently, harvested rainwater may be contaminated with algae, bacteria, chemicals, sediments, and microbes deposited on the catchment surface by insects, birds, rodents, and human activities (Dobrowksy et al. 2014b). In addition, pigeons are common vectors for bacteria, fungi, protozoa, and viruses, and the microorganisms in their fecal matter may be carried by rain into harvested rainwater storage tanks, causing contamination (Waso et al. 2018). A study in Uganda showed that most harvested rainwater samples had microbiological contamination, despite meeting the physiochemical quality standards (Nalwanga et al. 2018). A study undertaken in South Africa detected a range of pathogens, including *Shigella* spp., *Pseudomonas* spp., *Aeromonas* spp., *Legionella* spp., *Klebsiella* spp., *Salmonella* spp., *Giardia* spp., and *Yersinia* spp. in harvested rainwater (Dobrowsky et al. 2014a). Overall, the quality of the harvested rainwater depends on the weather,

topography, closeness to sources of pollution, the nature of the catchment area, the nature of the storage facility, and the water handling processes (Kahinda et al. 2007).

There is limited information on antibiotic resistance in harvested rainwater in Africa. Nevertheless, antibiotic resistance contamination in rainwater harvesting systems could accumulate during collection or storage emanating from insect vectors, animal droppings, airborne contaminants, and dead animal residues. The occurrence of antibiotic resistance in rooftop-harvested rainwater in South Africa was demonstrated by *E. coli* resistance to several antibiotics (Malema et al. 2018). Because cleaning roof surfaces is impracticable, pollutants can best be prevented from entering storage tanks by flushing or diverting the first wash of rain (Kahinda et al. 2007).

7.2.5 Groundwater Systems

Groundwater is the primary public water supply source worldwide, including in Africa. Although the incidence of a range of antimicrobial compounds in various surface aquatic systems in many low-income countries has been investigated, there is limited data on their presence in groundwater (Magwira et al. 2019). Nevertheless, various pollutants from agricultural and industrial activities can contaminate aquifers and threaten groundwater quality (Zainab et al. 2020). Antibiotics may contaminate groundwater via hospital effluent, industrial effluent, landfill leachate, surface run-off from aquaculture and agricultural land treated with sludge, sewerage leaks, and wastewater effluent (Zainab et al. 2020). The effluent from pharmaceutical industries contains antibiotic residues, which can be flushed as surface run-off by rain into the surface water systems or permeate into groundwater (Adegoke et al. 2020). Livestock farming is another important source of the spread of antibiotic resistance in groundwater. A previous study has detected ARGs in water from a cattle feedlot catch basin and manure (Sura et al. 2015). In addition, aquaculture farms have been reported to be reservoirs for antibiotics and antibiotic resistance, ultimately contaminating groundwater (Zainab et al. 2020). The prevalence of sulfonamides in groundwater is attributable to their poor biodegradability, weak sorption behavior to the soil, and fast mobility, which facilitates leaching into groundwater (Zainab et al. 2020). Due to differences in antibiotic usage, topography, and hydrological factors, groundwater's antibiotics and antibiotic resistance load vary between regions (Zainab et al. 2020).

Approximately 35–60 million cases of acute gastrointestinal infection are associated with groundwater consumption annually (Murphy et al. 2017). Aggravated by an already high disease burden, the likely effects of antibiotic resistance in groundwater threaten human health (Murphy et al. 2017). The situation is potentially worse in low-income countries, where a high proportion of the population lives in rural or peri-urban settlements, and inadequate sanitation, unrestricted access to antibiotics, and limited access to safe drinking water exacerbate the risk of disease transmission (Mathew et al. 2020). Some of these communities rely on shallow wells as a source

of drinking water (Machado and Bordalo 2014; Montwedi et al. 2018). For instance, in South Africa, most rural communities have limited access to safe drinking water and depend on untreated groundwater (Ateba and Maribeng 2011). A study on shallow wells in Guinea-Bissau reported the presence of *Acinetobacter*, *Burkholderia*, *Chromobacterium*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, or *Salmonella* genera, which were resistant to chloramphenicol, doxycycline, a combination of ampicillin, clavulanic acid and amoxicillin, and gentamicin (Machado and Bordalo 2014). Several studies have been undertaken to determine the antibiotic susceptibilities of a variety of microbial species in groundwater in South Africa. One study isolated and determined the antibiotic susceptibility profiles of three *Enterococcus* species: *Enterococcus avium*, *Enterococcus faecalis*, and *Enterococcus faecium,* at 5.8%, 23.0%, and 71.2%, respectively (Ateba and Maribeng 2011). At least 75% of the isolates exhibited resistance to amoxicillin, chloramphenicol, erythromycin, oxytetracycline, penicillin G, sulfamethoxazole, and vancomycin. A similar study on groundwater in South Africa reported that a significant proportion (51.5–78.8%) of *E. coli* isolates showed resistance to ampicillin, erythromycin, and vancomycin (Phokella et al. 2011). In addition, a higher proportion (90.0–100%) of *Salmonella* exhibited resistance to vancomycin (VAN), erythromycin (ERY), and ampicillin (AMP), while others were resistant to tetracyclines (TET). The AMP-ERY-VAN and AMP-ERY-TET-VAN were the common phenotypes among the isolated *Salmonella* species. A more recent study reported the presence of *Enterococcus faecalis* in groundwater in South Africa, at least 62% of which showed resistance to amoxicillin, ampicillin, erythromycin, penicillin, tetracycline, and vancomycin and exhibited multiple antibiotic-resistant phenotypes (Montwedi et al. 2018).

A study in Uganda determined antibiotic resistance trends and specific ARGs in *Proteus* spp., *Klebsiella* spp., *Serratia odorifera*, *Salmonella* spp., *Pseudomonas* spp., *Citrobacter* spp., *Providencia rettgeri*, *E. coli*, *Enterobacter* spp., and *Morganella morganii* from rural groundwater sources. Approximately 48% of the isolates were ampicillin-resistant, and about 23% showed resistance to tetracycline (Soge et al. 2009). The ARGs reported include the *bla*TEM, *sul*1, *int*1, *tet*, macrolide resistance *mef*(A), and the macrolide-lincosamide-streptogramin B resistance *erm*(B) genes. A study on antibiotic resistance in Nigeria detected *Enterobacter aerogenes*, *Escherichia coli*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Proteus vulgaris*, *Salmonella typhi*, and *Staphylococcus aureus* in groundwater (Oluwasusi et al. 2019). These were resistant to amoxicillin, ampiclox, Augmentin, chloramphenicol, ciprofloxacin, erythromycin, gentamycin, pefloxacin, rocephin, sparfloxacin, septrin, streptomycin, tarivid, and zinacef. Unfortunately, the number of studies on antibiotic resistance in Africa is few, and reports on ARGs are even fewer. Therefore, further studies should investigate the ARG types associated with antibiotic resistance.

Besides the unregulated usage of antimicrobial products, overcrowding and poor sanitation also play a role in the transmission and spread of antibiotic resistance (Gemeda et al. 2021). For example, in South Africa, approximately 21–40% of the population uses pit latrines, and these often do not have appropriate physical barriers between fecal sludge and soil, potentially leading to the leaching of bacterial

contaminants such as *Enterobacteriaceae* into groundwater (Beukes et al. 2017). The existence of bacteria in pit latrine excreta can contribute to the spread of antibiotic resistance; thus, these pose a potential human health risk in rural and peri-urban settlements (Beukes et al. 2017; Montwedi et al. 2018). Furthermore, there is war and conflict in some African countries, such as the Democratic Republic of Congo (Bunduki et al. 2020), which affect the supply of medicines and disturb habitats and sanitation facilities. Thus, providing proper waste disposal and modern toilet facilities is problematic. This is exacerbated by poor or nonexistent microbiological surveillance. There is, therefore, a need to understand the dissemination, transmission routes, and spread of ARB and ARGs in aquatic systems to reduce the potential risk to human health.

Due to rapid population growth, inadequate wastewater management, poor sanitation, urbanization, and unregulated use of antimicrobials, a rise in ARB and ARGs in groundwater is predictable. The risk of groundwater contamination with ARB and ARGs could be exceptionally high in the following cases: (1) shallow groundwater systems receiving wastewaters enriched in ARB and ARGs, and (2) groundwater sources in coarse-textured aquifer material with high hydraulic conductivity and closely located to on-site sanitation systems. Combined, these conditions ensure strong connectivity and short travel times between contaminated sources and groundwater systems. Therefore, a comprehensive global perspective is essential to assess the extent of groundwater contamination with antibiotic resistance in Africa and estimate the risks associated with consuming contaminated groundwater. However, this is confounded by the dearth of appropriate methodologies and vital germane data in research studies, especially in low-income countries. Furthermore, considering the widespread dependence on groundwater sources for domestic consumption, the occurrence and transfer of ARB in groundwater systems deserve further research.

7.3 Behavior, Dissemination, and Fate

Discharges of wastewater, groundwater recharge, infiltration, leachates, and surface run-off transport and distribute antibiotic resistance from various hotspots into aquatic environments (Fig. 7.1). Apart from household, hospital, and industrial effluents, urban surface run-off waters are also partly generated through anthropological activities. Following a rainfall event, surface run-off originating from farmland, industrial land, forestry, or urban areas either flows through the natural watershed or is transported through sanitary sewers from where it is discharged into freshwater systems (Almakki et al. 2019; Gwenzi 2020). Antibiotic resistance from these sources is transported to municipal wastewater systems, which act as sinks of antibiotic resistance, where ARGs can further multiply and spread (Gwenzi 2020). The water cycle is gradually becoming artificial due to rapid urbanization with the accompanying increase in impervious surfaces and sewer systems, mobilizing chemical pollutants, nutrients, sediments, and bacteria through surface run-off. For

instance, veterinary antimicrobials ultimately enter surface and groundwater via surface run-off and leaching (Sura et al. 2015). Therefore, along with wastewater, urban surface run-off can potentially create conducive conditions for the occurrence and spread of ARM (Almakki et al. 2019; Gwenzi 2020). Heavy rainfall events and flooding frequently occur in some regions, transporting pollutants and disseminating pathogens, ARB, and ARGs in the environment (Yang et al. 2021). Additionally, interactions between surface and groundwater through inter-flow and base-flow allow pollutant exchange between surface water and groundwater. In groundwater environments, sediments, and soils, ARB may be adsorbed onto solid media (Gwenzi 2020). Aerosols and airborne particulates such as dust have been reported to transmit antibiotic resistance.

Land application of compost, WWTP effluent, manure, and sludge introduces residual antibiotics and their metabolites, bacteria, and ARGs into the soil, and these are progressively transferred to deeper layers of the soil and, ultimately, groundwater (Cerqueira et al. 2019; Liu et al. 2022). In addition, a major rainfall event after the application or the overflow of sewage reticulation systems in urban areas can cause vertical migration of bacteria and antibiotic resistance into the soil profile along with off-site migration as a result of surface run-off (Chee-Sanford et al. 2009).

The dissemination and transfer of antibiotic resistance into aquatic systems are governed by environmental factors such as pH, soil properties, organic matter, salinity, temperature, redox potential, and the depth of the water table, among other physiological characteristics (Sanganyado and Gwenzi 2019; Zainab et al. 2020). Once in the municipal wastewater systems, antibiotics may undergo different biotic or chemical transformation processes (Magwira et al. 2019). In the case of antibiotic resistance, the processes that affect their spread in the environment are more complex, being natural and human-dominated (Gwenzi 2020). Several mechanisms, comprising vectors and hydrological processes, promote the further spread of antibiotic resistance from hotspot reservoirs into environmental compartments (Gwenzi 2020). The factors include precipitation and temperature (Andrade et al. 2020).

Bacteria may acquire ARGs via random chromosomal mutation or horizontal gene transfer (HGT), resulting in increased antibiotic resistance in the aquatic systems. The transfer of ARGs between bacteria can occur via bacterial conjugation, which may transfer ARGs existing on mobile gene elements (MGEs) (integrons, plasmids, or transposons) through acquiring free DNA from their environment and through bacteriophages (Gwenzi 2020). Thus, the HGT of ARGs among bacteria can induce antibiotic resistance in pathogens. The spread of antibiotic resistance is connected to MGEs (Almakki et al. 2019), and the environmental transfer of ARGs in MGEs and bacteria occurring in humans, poultry, and animals can occur via wastes such as manures (Gwenzi 2020). The link between ARGs and MGEs has resulted in the transfer and transformation of antibiotic resistance in the environment, increasing the environmental and human health risks (Liu et al. 2022). In addition, HGT facilitated by MGEs spreads antibiotic resistance into resistomes, which are resistance pools (Gwenzi 2020; Gwenzi et al. 2022).

Apart from human-human, human-animal, and animal-animal contact, significant antibiotic resistance transmission arises from animal-environment or

human-environment interactions (Booton et al. 2021). The pathways responsible for transferring ARGs in aquatic systems to humans include dermal contact, inhalation, the ingestion of contaminated water and food, and through vectors (Gwenzi 2020). Antibiotics and ARGs can be up-taken by aquatic plants and accumulate in leaves and fruits, or they can bioaccumulate in aquatic organisms such as microorganisms, fish, and plants (Cerqueira et al. 2019; Adegoke et al. 2020). The use of antibiotic-resistant-contaminated water for irrigation exposes the organisms to antibiotic resistance occurring in the water (Adegoke et al. 2020; Shamsizadeh et al. 2021).

7.3.1 Hydrologically Driven Dissemination

Groundwater recharge, infiltration, surface run-off, and rainfall are critical hydrological processes that mobilize and transfer ARGs from different hotspots into aquatic environments (Gwenzi 2020). The geological characteristics, hydrological sources of aquifers, and river flow contribute to recharge and antibiotic resistance load. For example, rainfall recharge permeates manure and sludge, or wastewater effluent applied as fertilizers to farmland, resulting in ARGs entering the unsaturated zone and subsequently the aquifer (Fig. 7.1). Following a rainfall event, partially saturated zones temporarily retain ARGs, which are gradually leached to groundwater through gravity (Zainab et al. 2020). Thus, rainfall is a critical element in mobilizing antibiotic resistance in groundwater. In contrast to surface water, the longer residence times in subsurface systems result in prolonged bacterial exposure to low concentration antimicrobial residues, ARB, and ARGs within a restricted and

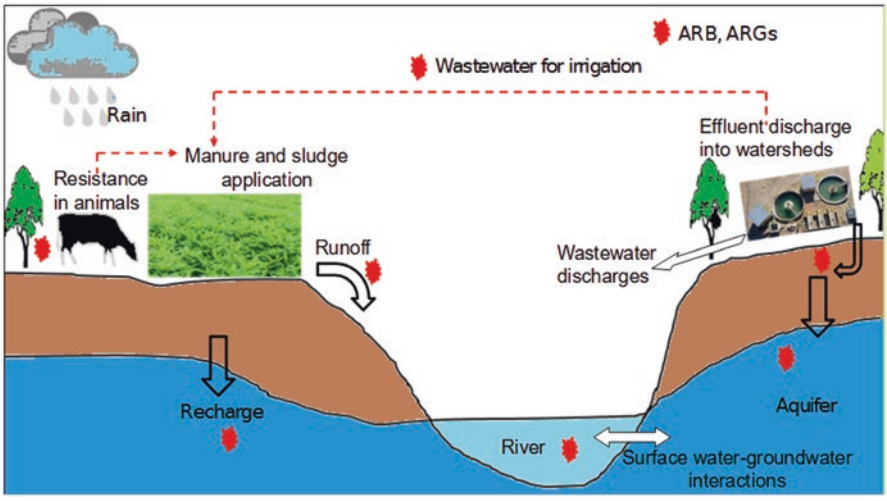

Fig. 7.1 A schematic diagram of the dissemination of antibiotic resistance from various sources into aquatic systems

usually controlled environment (Andrade et al. 2020). One study reported that groundwater wells near swine production facilities had high tetracycline and attendant ARG load compared to wells located farther from the facilities (Chee-Sanford et al. 2009; Zainab et al. 2020). In addition, the tetracycline ARG load in deep wells exceeded that in shallow wells, suggesting that, depending on the hydrology, the ARGs may be vertically dispersed.

Studies on antibiotic resistance in LICs are few. In Sub-Saharan Africa, the climate is different from other regions, with lower temperatures and more consistent rainfall. The types of groundwater sources are therefore expected to be different. In addition, anthropological practices such as informal settlements and inadequate sanitation facilities are common in low-income countries. Thus, there is a need for strengthening empirical data on the influence of hydrological processes on ARG dissemination in aquatic environments in low-income countries.

7.3.2 Horizontal Gene Transfer and the Role of Mobile Genetic Elements

Water environments, such as urban aquatic ecosystems (Wang et al. 2021), including wastewater and WWTPs (Schages et al. 2021; Osińska et al. 2020; Karkman et al. 2018), and aquacultures (Abe et al. 2020) have been reported to represent huge ARG reservoirs into which clinical and terrestrial bacteria flow. In addition, ARG and ARB have already been detected in groundwater and drinking water (Schages et al. 2021). ARB and ARGs can disseminate and persist within such environments (Schages et al. 2021) even without selective pressures (Abe et al. 2020). For example, tetracycline-resistant genes and sulfonamide-resistant genes, *tet*(M) and *tet*(S) and *sul*1–*sul*3, respectively, have been found in various water environments. Similarly, diverse ARB and ARGs (tetracycline-, aminoglycoside-, β-lactam-, penicillin-, macrolide-lincosamide-streptogramin-, and chloramphenicol-resistant genes) were detected in pharmaceutical wastewaters in Nigeria (Obayiuwana and Ibekwe 2020), making pharmaceutical wastewater reservoirs of ARGs. As such, like clinical settings, these environments should be a focus of attention targeting the control of ARB and ARGs (Abe et al. 2020).

HGT is one of the processes responsible for the dissemination of ARGs in aquatic environments between nonrelated microorganisms (Wang et al. 2021), wastewater and WWTPs being reported as hotspots for the gene transfer (Osińska et al. 2020; Karkman et al. 2018). The dissemination of ARGs occurs in the environment mainly through MGEs, which are segments of DNA encoding a variety of enzymes and proteins that mediate their movement within the host genome (intracellular mobility) or between bacterial cells (intercellular mobility) (Balcázar et al. 2015). These include plasmids, transposons, integrons (Osińska et al. 2020; Schages et al. 2021), gene cassettes, integrative and conjugative elements (ICE), and insertion sequence common regions between bacterial species (Mbanga et al. 2021). As such, HGT

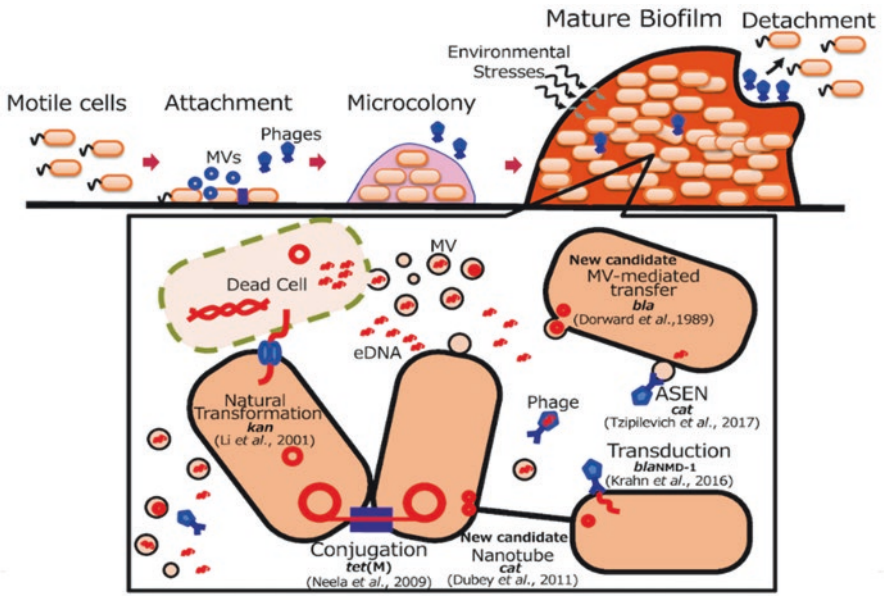

Fig. 7.2 Depiction of the horizontal gene transfer and its relationship with the biofilm life cycle. The figure is taken without modification from Abe et al. (2020). This is an open access article published and distributed by Oxford University Press under the Creative Commons CC BY license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

allows microbial species to acquire new genetic material from outside their clonal lineage (Lerminiaux and Cameron 2019) and is mediated by three main mechanisms: (1) conjugation, (2) transduction, and (3) transformation (Fig. 7.2) (Abe et al. 2020; Rios Miguel et al. 2020; Mbanga et al. 2021). In addition, membrane vesicles (MVs), abundant DNA reservoirs in aquatic environments, have been reported to potentially transfer genes between bacteria horizontally (Abe et al. 2020).

A few studies have investigated ARGs and HGT in African aquatic systems (Mlandu 2017; Adelowo et al. 2018; Coertze and Bezuidenhout 2018; Bougnom et al. 2019; Gufe et al. 2019; Fadare et al. 2020; Mugadza et al. 2021). However, in a recent study, *Amp*C β-lactamase and integrase genes were detected in genomic and plasmid DNA from bacterial populations in sampled water in three rivers in the North West province of South Africa. Unfortunately, detecting such genes on plasmids suggests a high risk of HGT and the potential dissemination of these and other ARGs surrounding immediate aquatic environments (Coertze and Bezuidenhout 2018). Similarly, the presence of different antibiotic resistance determinants in freshwaters (Tsomo and Tyhume rivers) within the Eastern Cape Province in South Africa was reported, suggesting ARG dissemination in bacterial communities in such environments (Fadare et al. 2020). Similarly, in Burkina Faso, many ARGs transmissible by MGEs were identified in wastewater used for urban agriculture in Ouagadougou. These included seven ESBL genes encoding the synthesis of four

enzyme families, including two metallo-β-lactamases ($bla_{\text{AIM-1}}$ and $bla_{\text{GES-21}}$) (Bougnom et al. 2019).

In Zimbabwe, several multidrug-resistant bacteria were isolated from fish obtained from natural aquatic systems in and around Harare (Gufe et al. 2019) and from different drinking water sources in Gweru urban (Mugadza et al. 2021). All the isolated bacterial species were resistant to at least three antibiotics used in the study, showing the dissemination of ARGs among the bacterial communities in the aquatic systems, presumably by HGT. HGT occurs via conjugation, natural transformation, and transduction.

7.3.2.1 Conjugation

Bacterial conjugation, presumably the principal route of HGT in bacterial communities (Abe et al. 2020), is the contact-dependent transmission of genetic information from a donor bacterium to a recipient cell. MGEs, such as chromosomally integrated transposons or conjugative plasmids, are transferred to the recipient bacteria through a proteinaceous apparatus, the conjugation pilus, which serves as a physical link between the donor and recipient cells (Woodall 2003; Balcázar et al. 2015; Partridge et al. 2018; Rios Miguel et al. 2020). This accelerates the spread of ARGs among bacterial communities (Leungtongkam et al. 2018), especially with the transfer of conjugative plasmids since they are reported to harbor multiple ARGs (Abe et al. 2020), and they can be exchanged among both related and phylogenetically distant bacteria (Balcázar et al. 2015). The transfer of the conjugative plasmids from F^+ cells (donor cell) to F^- cells (recipient cell) is rapid, so the F plasmid can spread tremendously throughout a population from strain to strain.

Several studies have shown increased conjugation efficiencies in biofilms compared to motile planktonic cells since the transfer of genes requires direct contact between the donor and recipient cells (Balcázar et al. 2015; Abe et al. 2020). Bacterial biofilms are complex surface-attached bacteria communities held together by self-produced polymer matrices mainly composed of polysaccharides, secreted proteins, and extracellular DNAs (Muhammad et al. 2020). This results in a stabilized cell-to-cell contact between bacterial communities. Literature reported that this relative spatial stability of bacteria makes biofilms hotspots for HGT, especially by conjugation, as they permit plasmid transfer at high frequencies (Hausner and Wuertz 1999; Stalder and Top 2016). Several authors have reported that about 40–80% of bacterial cells in nature live in biofilms on surfaces or at interfaces, including in water environments (Ilyina et al. 2004; Jefferson 2004; Flemming and Wuertz 2019), and this makes conjugation playing a vital role in HGT (Hausner and Wuertz 1999). Furthermore, biofilms are the main ARG reservoirs to proliferate ARGs and ARB in aquatic environments. Therefore, ARB that detach themselves from biofilms can spread in the environment and threaten human health (Abe et al. 2020).

Previous research showed conjugation elements carrying multiple ARGs. For example, in Nigeria, *P. aeruginosa*, with the multiple antibiotic-resistant indices of the individual isolates ranging from 0.4 to 0.8, was isolated from aquaculture and abattoir environments in urban communities (Igbinosa et al. 2017). The report concurs with the findings of a similar study, where a 204-kb conjugative plasmid was isolated from coastal aquaculture carrying *tet*(M), *tet*(B), *sul*2, *flo*R, a β-lactamase ($bla_{\text{CARB-9}}$-like), and macrolide resistance *mph*(G) and *mef*(C) genes (Nonaka et al. 2012). Similarly, several integrons have been reported to act as vehicles of gene exchange between the environmental resistome and bacterial species through conjugation, spreading multidrug resistance. For example, class 1 integrase genes (*int*I*1* and *int*I2) and class 1 integron gene cassettes were detected in four urban wetlands in Nigeria (Adelowo et al. 2018). Furthermore, pB10, a 64-kb broad-host-range conjugative plasmid, isolated from a WWTP, harbored *tet*A, *sul*1, an amoxicillin-resistant gene (*oxa*2), streptomycin-resistant genes (*str*A and *str*B), and mercury-resistant genes (*mer*A*, mer*D*, mer*E*, mer*P, and *mer*T) (Schluter et al. 2003).

7.3.2.2 Natural Transformation

Natural transformation is a genetic alteration mediated by the uptake of exogenous DNA through the competence machinery consisting of a transformation pilus and a DNA transporter. Earlier studies have considered transformation a powerful mechanism of HGT in natural bacterial populations from all trophic and taxonomic groups (Lorenz and Wackernagel 1994). Naturally transformable bacteria can enter a genetically programmed and often transient physiological state called competence, in which they can take up exogenous DNA and integrate it into their chromosome (Juan et al. 2015). Like conjugation, transformation is a key process that facilitates the dissemination of antibiotic resistance and virulence determinants throughout bacterial populations (Nolan et al. 2020).

Literature shows that the exogenous DNA (free DNA released by cell lysis) is found in significant quantities in diverse environments and can be a reservoir of genetic material sampled by bacteria competent of natural transformation (Ibáñez de Aldecoa et al. 2017). Dubnau and Blokesch (2019) highlighted that type IV pili (T4P) are required for natural transformation in many naturally competent bacterial species. There is no consensus among researchers on the exact role of T4P in natural transformation. However, recent studies have accepted a model suggesting that transfer DNA (T-DNA) binds to the pilus structure, which retracts and pulls the DNA to the cell surface (Hahn et al. 2021; Nolan et al. 2020). In the periplasm, the T-DNA encounters the DNA binding protein ComEA, which mediates DNA uptake, possibly by a ratchet mechanism. In bacterial species that do not produce T4P, natural transformation involves many proteins with homology to T4P proteins, which are thought to form a pseudopilus structure that spans the cell wall and is coupled to the DNA translocation complex at the cytoplasmic membrane. Once the T-DNA enters the cytosol, it associates with DNA-binding proteins and recombines with homologous DNA sequences to yield a transformant (Hahn et al. 2021) or be

maintained as an independent replicon if plasmid DNA is taken up by an appropriate host.

Older evidence for bacterial transformation in aquatic biofilms has been reported (Abe et al. 2020). For example, *Streptococcus mutans* cells were naturally transformed by adding a plasmid (pDL289) encoding a kanamycin-resistant gene. Similarly, *Acinetobacter* spp. BD413 biofilms formed in LB medium are transformable with an exogenous plasmid (pGAR1) that carries a tetracycline resistance gene, using flow cell system, and *Acinetobacter* BD413 cells in river biofilms were also transformable with pQM17, a mercury resistance plasmid.

7.3.2.3 Transduction

Transduction is a genetic exchange mechanism mediated by independently replicating bacterial viruses called bacteriophages or phages (Balcazar 2014). Phages are the most abundant gene transfer particles in nature, and some MGEs have evolved to use that to their advantage. When phages propagate, they can sometimes encapsidate host bacterial DNA to form transducing particles. Transducing particles are ostensibly like mature phage particles and only inject bacterial DNA instead of a viral genome when they infect other cells. The DNA can then recombine into the chromosome or replicate as a plasmid in the new host cell (Chiang et al. 2019). In addition, phages may act as vectors for genetic exchange when they transfer any portion of the donor genome to the recipient cell by either a lytic or lysogenic (temperate) phage or as in temperate phages only, in which a few specific donor genes can be transferred to the recipient cell. Apart from mere transferring genes, phages have been reported to contribute to the emergence and spread of ARGs in diverse environments (Balcazar 2014).

Previous experimental evidence has demonstrated the contribution of phages to the spread of ARGs into the environment. For example, HGT and phage infections were reported in the South African deep subsurface bacterial population. Using metagenomic sequencing analysis of fracture water from a diamond mine showed phage-related genes and bacterial communities in biofilm structures. In addition, the same study reported the presence of genes thought to be part of HGT events involving nitrogen fixation, cobalamin synthesis and sulfide reduction pathways, and motility and sporulation (Mlandu 2017). In other studies, metagenomic analyses have detected various ARGs in phage fractions isolated from environmental water samples, including genes encoding resistance to aminoglycoside, β-lactam, macrolide, quinolone and sulfonamide, and tetracycline antibiotics from sewages, river water, seawater, and wastewater (Abe et al. 2020). Furthermore, ARG-like genes, which confer resistance to tetracycline, ampicillin, and bleomycin, were discovered in the virome of activated sludge, using both sequence- and function-based metagenomic approaches. Similarly, a study using real-time quantitative PCR assays revealed the presence of two genes (bla_{TEM} and $bla_{\text{CTX-M}}$) encoding β-lactamases and one gene (*mec*A) encoding a penicillin-binding protein in phage DNA from urban sewage and river water samples (Balcazar 2014).

7.3.3 Fate of Antibiotic Resistance in Aquatic Systems

Aquatic systems are among the most prominent environments and routes for ARB propagation, thereby enhancing the distribution of ARGs between environments, humans, and plants (Manaia et al. 2016). According to work reviewed by Faleye et al. (2019), the occurrence of ARB and ARGs has been reported in African aquatic ecosystems. This is consistent with work conducted in Port Harcourt metropolis, Rivers State in Nigeria, where ARB, including *Salmonella* spp., *Escherichia coli*, and *Vibrio* spp. were identified (Onuoha 2018). Water is a critical component in the alteration and transportation of ARB and ARGs (Amarasiri et al. 2020; Manaia et al. 2016). In South Africa, research was conducted to screen for antibiotic-resistant *E. coli* isolates from sediment and aquatic environments (Genthe et al. 2020). According to this study, antibiotic-resistant *E. coli* isolates were isolated in WWTPs influents, effluents, and sediments. Therefore, the fate of ARB and ARGs in African aquatic systems can be understood by following the urban water cycle (Manaia et al. 2016). In line with the urban water cycle, ARB and ARGs are collected in WWTPs and drinking treatment plants, transported within sludge from WWTPs, collected in sediments, remain within the water column, get into drinking water, are absorbed by plants, are consumed by animals, and are released back into the aquatic systems through excretion and egestion.

After their release into wastewater, ARGs and ARB accumulate in WWTPs (Thakali et al. 2020). According to a research conducted in Africa, specifically Tunisia, ARGs such as *sul*1, *int*l1, *ern*B, and bla_{TEM} were identified in WWTPs (Rafraf et al. 2016). This is so since untreated wastewater is a rich source of ARB and ARGs, as indicated by studies conducted in Nigeria by Onuoha et al. (2016; Onuoha 2018). These studies concluded that slaughterhouses and meat processing industries contribute significantly to ARB and ARG enrichment of surface water. These findings are in line with other reports which indicate that ARB and ARGs from aquatic systems accumulate in WWTPs and wastewater (Zhuang et al. 2021). Furthermore, it was reported that ARB die and ARGs are hydrolyzed in WWTPs (Zhuang et al. 2021), reducing the frequencies of ARB and ARGs in effluents. In support of this phenomenon, ARB reduction in sewage treatment plant (STP) effluents have been noted in a cross-sectional study conducted in Accra, Ghana (Adomako et al. 2021). Some studies outside Africa reported a decrease in ARGs and ARB after wastewater treatment (Felis et al. 2020; Pärnänen et al. 2019; Kulkarni et al. 2017). However, an increase in ARB and ARGs in WWTPs was reported in African aquatic systems. According to a study conducted in Botswana, high levels of pathogenic ARB such as *Staphylococcus* species and ARGs such as *dfr* and *int*1 were detected in WWTPs (Tapela and Rahube 2019).

WWTPs in some parts of Africa have been implicated as sources of untreated wastewater which pollute African aquatic systems (Tapela and Rahube 2019). Effluents from WWTPs are usually drained into aquatic systems, which can be drinking water sources for people (Thakali et al. 2020). According to Tapela and Rahube (2019), there is evidence of ARB and ARG accumulation in WWTPs, and

these resistance determinants are subsequently released into WWTPs effluents. Further, the presence of ARB in influent (97.5%), effluent (94.4%), and downstream WWTPs (85.1%) was reported (Tapela and Rahube 2019). Conventional water treatment strategies such as sedimentation and sand-aided filtration rarely remove ARGs (Huang et al. 2019). Effluent from WWTPs is finally disposed into aquatic environments; therefore, the ARB and ARGs associated with this discharge may end up in rivers and lakes (Amarasiri et al. 2020). For instance, Legon WWTPs in Accra, Ghana, are known for treating wastewater from several institutions, such as the Achimota Hospital; effluents from this plant are released into the Onyasia stream. Effluents from Legon WWTPs have been implicated as ARG vehicles and ARB transmission in aquatic systems (African Development Fund 2005). ARB and associated ARGs increase rapidly in treated wastewater since treated wastewater is generally enriched with nutrients and antimicrobials, increasing cell-to-cell interactions (Felis et al. 2020; Barbosa et al. 2021). In addition, chlorine-based water disinfection has sometimes increased ARGs in drinking water (Huang et al. 2019). Aquatic ARGs confined in cells and free in water can be transferred to aquatic bacteria (He et al. 2020). The ARGs and ARB from WWTPs interact with microbes in various environmental conditions and contribute to antibiotic resistance in the microbes in these environments (Amarasiri et al. 2020). ARGs in aquatic systems can be transferred to endemic bacteria (Goulas et al. 2018).

When sludge is used as fertilizer, ARGs and ARB immobilized in sludge can finally be found in farmlands. If sludge-derived ARGs and ARB are not removed from the farmland, they are discharged back into aquatic systems (Wang and Chen 2022). The retained ARB and ARGs may be deposited into sewage sludge (a most important waste product of wastewater treatment processes) which contains considerable quantities of ARB and ARGs (Waseem et al. 2017). Investigations have concluded that ARB and ARGs could remain persistent either in the influent or effluent (Karkman et al. 2016; Caucci et al. 2016). Notably, ARB can survive and reproduce in an aquatic environment irrespective of the existence of antimicrobial agents. Usually, ARB and ARGs in aquatic ecosystems are finally deposited into WWTPs, where some are removed. These ARB contribute significantly to the presence of ARGs in aquatic systems (Felis et al. 2020).

Wastewater has been reclaimed and utilized for agricultural purposes, bathing, and recreational activities, providing an important route for human exposure to ARB and ARGs (Amarasiri et al. 2020). In response to climate change issues, wastewater reuse is encouraged in Africa, and unfortunately, it is at the heart of ARB and ARG transmission in African aquatic systems. Based on research conducted in South Africa, antibiotic-resistant *E. coli* isolates were identified from water sources used for irrigation, and disturbingly these antibiotic-resistant *E. coli* isolates were associated with lettuce grown with water from one of these sources (Njage and Buys 2015). In addition, effluent from Legon WWTPs in Ghana is used for vegetable irrigation purposes, and this has been identified as an important source of ARB transferred to humans through vegetable consumption (Andoh 2014). Moreover, effluent from Legon WWTPs has been used for domestic purposes, increasing the chances of ARB being transmitted to human and other animal

populations. Generally, using ARB-containing wastewater for irrigation is a critical route for ARB transfer in the water and sludge into crops (Shamsizadeh et al. 2021).

Sediments can act as sinks for ARG antibiotic resistance bacteria and thus contain many microbes possessing ARGs (Huang et al. 2019). However, when the water flow is sluggish, the confined ARGs and ARB are presumably released into the water. There is, nevertheless, a dearth of information on this phenomenon (Xie et al. 2019). In South Africa, research was conducted to screen for antibiotics-resistant *E. coli* isolates from sediment and aquatic environments (Genthe et al. 2020), and according to the findings, antibiotic-resistant *Escherichia coli* were isolated in WWTPs influents, effluents, and sediments. The prevalence of ARGs in sediments concurs with a study conducted in Gauteng Province, South Africa, where ARB, especially those associated with fecal contamination, were identified in Apies River sediments (Abia et al. 2015). In case of floods and resuspension, ARB and ARGs in sediments are released into the surface and deep-water columns. Once in aquatic environments, ARGs can collect in sediments again due to alluviation and adsorption. These ARGs can be transferred to bacteria in the intestines of fish (He et al. 2020). The presence of ARGs and ARB in fish species concurs with the report of ARB in *Oreochromis niloticus* and *Clarias gariepinus* collected in aquatic systems in Uganda. The identified ARB in this study included *Plesiomonas shigelloide*s, *Serratia marcescens*, and *Aeromonas hydrophil*a, and ARB resistant to ampicillin, penicillin, and oxacillin (Wamala et al. 2018).

Tap and treated water samples were reported to be associated with increased ARG quantities ranging from 6- to 109-fold (Felis et al. 2020; Waseem et al. 2017). Contamination of African treated and tap water with ARB and ARGs has been studied and noted. In research conducted in Tunisia, ARGs such as *sul1*, *intl*1, *ernB*, and bla_{TEM} were identified in WWTPs and drinking water (Rafraf et al. 2016). Studies conducted in South Africa's North West province revealed the existence of opportunistic pathogenic bacteria and ARB in drinking water and water used for domestic purposes (Ateba et al. 2020; Kinge et al. 2010; Ateba and Mbewe 2011; Mulamattathil et al. 2014). In another study conducted in Khartoum State, Sudan, ARGs were isolated from *E. coli* identified in drinking water, and 40% of the *E. coli* isolates had ARG $bla_{\text{CTX-M-1}}$ (Altayb et al. 2020). Additionally, multidrug-resistant bacteria were isolated from South African drinking water treatment plant effluent. However, a significant ARB decrease was noted in effluents from these drinking water treatment plants compared to influents (Kinge et al. 2010). Bacteria isolated from treated wastewater and municipal tap water in Southwestern Nigeria were associated with ESBL resistance genes such as bla_{TEM} and bla_{CTX} (Adesoji and Ogunjobi 2016). Treatment techniques applied to drinking water and wastewater cannot completely remove ARGs (Amarasiri et al. 2020). This is in line with a previous study, which isolated 203 bacteria from South African drinking water and a greater percentage of these isolates were resistant to Trimethoprim and β-lactam antibiotics (Ateba et al. 2020). ARGs might be transferred from treated water to aquatic organisms such as fish (Amarasiri et al. 2020). When bacteria with ARGs gain access to humans, the ARGs can be transferred to human microflora through HGT (Stanton et al. 2020). In addition to these findings, antibiotic-resistant *E. coli* and *Salmonella* spp. were

isolated from drinking water sources in Gweru urban, Zimbabwe (Mugadza et al. 2021).

7.4 Human Health Risks, Mitigation, and Policy Perspectives

The ubiquity of antibiotic resistance means that the prevalence of this phenomenon has now warranted attention in Africa. Data show that up to 90% of drugs that pass through the human system end up in the water cycle from incomplete disposal methods and waste products (Ngqwala and Muchesa 2020). Research also reveals that about 60% of antibiotics produced are used in animal husbandry, which becomes the avenue through which these chemicals are introduced into the environment, such as aquatic systems (Agyare et al. 2018). Due to the WWTP, small dosages of antimicrobials persist in local rivers, lakes, and wetlands (Bezuidenhout et al. 2019). Water samples from raw and drinking water in South Africa were found to have fecal indicator bacteria and showed antibiotic resistance implicating the WWTP as an inefficient tool for removing this pollution (Mulamattathil et al. 2014). The WWTP remains one of the important routes through which water is contaminated with antimicrobials (Adefisoye and Okoh 2016), thus requiring significant investment for surveillance and reticulation at this level (Adefisoye and Okoh 2016).

The One Health phenomenon posits human exposure to resistant strains through thehuman-animal-environment nexus (Ateba et al. 2020). Globally, about ten million people are estimated to die because of antibiotic resistance by 2050 (Amarasiri et al. 2020), which is as many as those dying from cancer. According to the Africa Centres for Disease Control and Prevention, which has set up a surveillance network to monitor the exposure to antibiotic resistance, problems have already been experienced in treating malaria, tuberculosis, typhoid, cholera, meningitis, gonorrhea, and dysentery. At the same time, currently 700,000 succumb to this scourge (Africa Center for Disease Control 2018). Antibiotic resistance from pathogenic bacteria by its very nature means people exposed to these more virulent microbes are at serious risk of exposure to more infective and severe forms of diseases posed by these bacteria. Several cases of antibiotic resistance have been experienced in the African region with several other diseases such as meningitis, cholera, dysentery, typhoid, meningitis, gonorrhea, TB, malaria, and AIDS, increasing the burden in Africa (Ndihokubwayo et al. 2013). The risk in Africa is worsened by an intrinsic lack of capacity to monitor antibiotic resistance, while there is a greater risk of various infections compounding the situation (Williams et al. 2018). One study showed that antibiotic resistance in developing countries is a complex function of the lack of best practices by the health professionals in dispensing medication, lack of knowledge by patients, and lack of technical capacity, among other important variables (Ayukekbong et al. 2017).

The agricultural ecosystem is one vehicle through which antibiotics are introduced into the aquatic system in Africa (Iwu et al. 2020). Crop irrigation necessarily involves the exchange and transfer of antibiotics and resistant genes into crops using

contaminated water (Genthe et al. 2020). Conversely, farmers in Africa also use antibiotics on crops, directly and indirectly introducing antibiotics into the production chain (Taylor and Reeder 2020). As such, the agricultural food-processing industry is often cited as one of the critical avenues through which antibiotic resistance is introduced into the human domain, with research revealing that antibiotic resistance is statistically significant in the African food chain, thereby impacting public health (Founou et al. 2018). Studies on poultry products have shown isolates of bacterial strains to be less susceptible to tetracycline, ampicillin nalidixic acid tetracycline, and trimethoprim even in free-range chickens (Agyare et al. 2018). One hundred percent use of antibiotics has been observed on farms in countries such as Tanzania, Cameroon, Zambia, Ghana, and Egypt, while additionally, 100% antibiotic resistance has been witnessed in South Africa, Zimbabwe, and Tunisia (Kimera et al. 2020).

Like the rest of the world, Africa is vulnerable to the risk associated with exposure and danger of antibiotic contamination and pollution. Several meta-analyses of literature on antibiotics have been performed for the African continent. For example, in Zimbabwe, antibiotic-resistant strains have been isolated in fish from a multistream environment and sold in informal markets in an urban setup, meaning that these isolates are eventually destined for the WWTPs (Gufe et al. 2019). Antibiotic use has been registered in livestock in Uganda (Mikecz et al. 2020) and inadvertently passed onto the shared water systems. Agriculture, and especially animal rearing, has always been a huge vehicle through which water systems are exposed to antibiotics which eventually affect humans along the chain. For instance, by 2016, researchers in South Africa observed about 64 registered antibiotics, eventually finding their way into feeding animals to enhance production by directly dissolving drinking water (Liphadzi and Bhagwan 2016).

Large hospitals such as the United Teaching Hospital in Zambia dispense antibiotics to noncritically ill patients inappropriately, increasing the burden of these chemicals on the shared environment (Masich et al. 2020). While some studies in rural Mozambique have expressed little knowledge about antibiotic resistance in the community (Cambaco et al. 2020), research conducted on the capital of Maputo has shown high levels of fecal matter impregnated not only with microbes such as *Escherichia coli* but ARB introduced into municipal waters via the sewage systems (Salamandane et al. 2021). Although antibiotic resistance has been detected in various African aquatic systems, including drinking water supply, evidence is emerging of the dangers associated with human exposure and health risks. In Sub-Saharan Africa, nonsusceptibility to treatment by antibiotics for sepsis caused by Gram-positive bacteria has been witnessed in neonatal children (Williams et al. 2018). However, the origins of the resistance were not determined; hence, it is unclear whether it was associated with antibiotic resistance in aquatic systems.

A recent study in Kenya showed the presence of several common antimicrobials in a wide spectrum of water systems ranging from the effluent of WWTP in the aqueous phase, the suspended matter of the effluent, in river waters and their sediments, and water treatment lagoons which were identified as vehicles through which these chemicals are transported back into the water cycle (Kairigo et al. 2020a, b).

Findings in parts of rural Kenya, where 99.8% of the local populations use groundwater for various purposes, also revealed that the groundwater gets contaminated because of the improper disposal of antimicrobials into pit latrines and composts (Karimi et al. 2020). The global population's reliance on aquaculture for food has resulted in a 44% increase in production in 40 years to 2002. As a result, producers have resorted to antibiotics to combat the pollution from waste associated with the production process. However, this has become a direct conduit for contaminating water systems (Watts et al. 2017). Rather worryingly, bacteria obtained from boreholes and hand-dug wells were significantly resistant to 19 antibiotics in some urban councils of Cameroon, indicating the insidious nature of the problem (Tangwa et al. 2019).

ARB, particularly pathogenic ones, cause infections that have higher mortality and morbidity rates and more extended hospital stays since they transfer their resistance to humans. The World Health Organization recommends the need for surveillance on the emergence of pathogenic antibiotic resistance globally, and studies have been done to document such efforts in Africa (Sanganyado and Gwenzi 2019). However, there is a level of opaqueness in the African region in establishing the true status of ABR because of a lack of up-to-date information, monitoring, and substantive policy on AMR (Elton et al. 2020). This is because research and knowledge on ABR and AMR in African environmental systems are limited and poorly developed. This also applied to groundwater, surface water, WWTP effluent, and municipal potable water (Faleye et al. 2018). Researchers have recognized the need for high-quality research and monitoring to minimize risk (García-Vello et al. 2020). This means putting policy and infrastructure in place for robust monitoring and surveillance of the value chain associated with antibiotics from the manufacture, use, and disposal.

The risk assessment for antibiotic resistance includes hazard identification, exposure assessment, dose-response assessment, and risk characterization (Ben et al. 2019). One study on antibiotic resistance in Africa reveals that 42.6% of information on antibiotic resistance is absent, probably signifying a lack of prioritization of this important area (Tadesse et al. 2017). Sadly, the health of the population on the continent has been compromised, as evidenced by more prevalence of urinary tract infections (UTI), bloodstream infections (BSI), meningitis, diarrhea, and pneumonia in West Africa (Bernabe et al. 2017). As of 2016, only South Africa and Kenya had made significant efforts to promulgate a national policy addressing antibiotic resistance (Essack et al. 2017). The lack of surveillance due to diminished human, technical, and financial capacity in Africa is often referenced as the reason for the increase and subsequent impact of antibiotic resistance (Wangai et al. 2019). Civil society organizations are considered important in the fight against the scourge of antibiotic resistance. An assessment in 37 countries shows that while surveillance activities are in all four parts of the continent, only 14% of the organizations show monitoring metrics (Fraser et al. 2021). While this has been slow, African countries have begun to work toward instituting surveillance systems geared toward mitigating the effects of antibiotic resistance. The Africa Centres for Disease Control and Prevention has drafted a guiding Framework for Antimicrobial Resistance

2018–2023 upon which most African countries are pivoting to install their monitoring system (Africa Center for Disease Control 2018). Additionally, the One Health approach is being promulgated in Africa to conscientize the public on the cross-sectoral nature of AMR and ABR. For example, some authors in Nigeria have discussed the establishment of databases that would help establish the extent of multiple drug resistance in humans, animals, and the environment (Akinsuyi et al. 2021).

ARB and ARGs in African aquatic systems, including wastewater, surface water, groundwater, and drinking water, point to potential human exposure and health risks. Human exposure may occur in occupational settings such as WWTPs via inhalation of airborne ARB and ARGs in bioaerosols (Gwenzi et al. 2022). Non-occupational exposure may occur via (1) ingestion of contaminated raw drinking water and (2) bathing and recreational uses of contaminated water. However, limited studies have investigated human exposure and health risks associated with antibiotic resistance in African aquatic systems. This is partly because human exposure and health risk assessment of antibiotic resistance in environmental media is a non-trivial task (Gwenzi et al. 2022). Such risk assessment requires the application of quantitative risk estimation tools such as quantitative microbial risk assessment (QMRA) (Gwenzi 2020, 2021; Schoen et al. 2021) and disability-adjusted life years (DALYs) (Zheng et al. 2020; Gwenzi and Siyawamwaya 2022).

QMRA and DALYs require more comprehensive information on antibiotic resistance than is currently available in Africa. For example, QMRA requires the following data (Schoen et al. 2021; Gwenzi and Siyawamwaya 2022): (1) multiple exposure pathways, (2) dose-response models including threshold infectious doses, and (3) estimation of likelihood or probability of occurrence of adverse human health outcomes following exposure to antibiotic resistance. The lack of such information coupled with limited expertise in QMRA and DALYs may account for the lack of studies estimating human exposure and health risks in Africa. Hence, quantitative human exposure and health risk assessment of antibiotic resistance in the African aquatic system requires further research under environmentally relevant exposure conditions. Such research should prioritize the following: (1) occupational workers in the wastewater treatment industry and (2) highly congested settings such as informal settlements (e.g., squatter camps, refugee camps, slums), where communities rely on unsafe drinking water sources closely located to points of fecal contamination such as on-site sanitation systems (pit latrines, septic tanks) (Gwenzi 2020, 2021).

QMRA has been suggested as one methodological tool which can be used to assess human exposure and health risks (World Health Organization 2016). As a result, this approach has been adopted by some African scholars in South Africa (Mbanga et al. 2020), Côte d'Ivoire (Kouamé et al. 2017), Ghana (Machdar et al. 2013), and Uganda (Butte et al. 2021) among other jurisdictions. However, subsequent research shows that the method has not been extensively applied in Sub-Saharan Africa, indicating a lack of knowledge in assessing the threat of antibiotic resistance (Van Abel and Taylor 2018).

Countries such as Tanzania initiated a program in 2017 with the tentative installation of governance structures, community awareness, surveillance systems, and promulgation of guidelines to ensure antibiotic stewardship (Frumence et al. 2021). Several African countries have responded by instituting policies and programs geared toward antibiotic resistance. Among the early adopters, the Kenyan government launched a template for combating antibiotic resistance in 2017, recognizing antibiotic resistance as a threat that requires collaborative efforts among stakeholders. Monitoring efforts have been performed in the Malawian chain of the consumption of antibiotics, signifying a level of awareness of the problem (Kumwenda et al. 2021; Lester et al. 2020). Sufficient investment in Africa has to be undertaken in diagnostic and analytical tools, technologies, and equipment (Mulamattathil et al. 2014), such as whole genome sequencing, which enables accurate detection of antimicrobial genes, especially as this relates to the WWTP and hospitals as important sources causing the contamination of water systems.

It is noteworthy that generally, in Africa, there is a limited regulatory framework and surveillance toward antibiotic resistance, implying that an existential crisis is still present and getting worse (Kimera et al. 2020). Direct input of antimicrobials into the shared environment has been proven to be the major driver of antibiotic resistance, and while controls can be put to avoid input from agricultural sources, researchers recommend mitigation from human source pollution (Sanderson et al. 2018). The extent of antibiotic resistance in Africa and the limitations and lack of appropriate ability to detect and control exposure have recently been recognized (Osei Sekyere and Reta 2020). The One Health principles have already been proposed and are being adopted on the African continent as some of the tools to assess and mitigate risks (Iwu-Jaja et al. 2021).

7.5 Removal of Antibiotic Resistance in Aquatic Systems

The removal and reduction of ARGs involve applying biological, physical, and chemical processes (Table 7.2) (Waseem et al. 2017). Among other strategies, the removal of ARGs employs methods such as ozonation, storage, dilution, and aerobic and anaerobic treatments (Felis et al. 2020; Goulas et al. 2018). However, literature on efficacy and techniques used in Africa to remove aquatic ARB and ARGs is limited, although there is no specific data, and ARB and ARGs are normally removed using conventional methods (such as boiling, bio-sand filtration, chlorination, solar disinfection) and drinking water and wastewater treatment techniques (Sanganyado and Gwenzi 2019). These traditional (conventional) wastewater and drinking water treatment techniques are less efficient and low cost (Sanganyado and Gwenzi 2019). Removal of ARB and ARGs through wastewater treatment techniques is underpinned by two critical and broad processes: interception and exposure to reactive oxygen species. Interception involves coagulation and membrane separation strategies, while reactive oxygen species exposure exploits techniques such as advanced oxidation processes (Wang and Chen 2022).

Table 7.2 Summary of methods for the removal of antibiotic resistance in aquatic systems

Removal method	Application of strategy	References
Anaerobic digestion	This treatment technique removes ARGs in biological sludge from wastewater treatment plants Efficiency of this method lies in solid retention time and digester operational temperature In this strategy, a two-phase (acidophilic or methanogenic) mesophilic or thermophilic digestion can be applied Generally, two-phase thermophilic digestion is more efficient In anaerobic batch digesters, microbial diversity of influent sludge can influence the ARGs quantity	Wang and Chen (2022), Manaia et al. (2016), Waseem et al. (2017), Xie et al. (2019), Zhuang et al. (2015), Thakali et al. (2020)
Activated sludge treatment	Two tanks are involved: an aerated biological reactions tank and a settling tank Though in some studies an increase in ARGs in treated wastewater was reported, in a conventional activated sludge treatment, ARGs removal is of approximately 1–2 log reductions	Wang and Chen (2022), Xie et al. (2019), Zhuang et al. (2015), Amarasiri et al. (2020)
Constructed wetlands	Many physicochemical and biological processes play a significant role in removing wastewater The processes include aquatic plant sequestration, photolysis, biodegradation, filtration, and adsorption Although these processes remove ARGs, the best techniques are adsorption and biodegradation It is important to note that the sediments generated in constructed wetlands are beneficial in harboring ARGs in these environments	Wang and Chen (2022), Waseem et al. (2017)
Chlorination	Damages cells and their organelles, resulting in leakage of cellular contents, biomolecules, e.g., DNA Important in oxidizing nucleic acids and intracellular components, as chlorine decomposes into oxidizing agent OCl^- and HOCl in water Due to its ability to produce strong oxidizing agents in water, chlorine is an excellent ARGs inactivation The efficacy of chlorination varies significantly with the quality of water, chlorine dosage, contact time, microbial ecology, nature of ARGs, and spices of ARB Optimization of chlorination is key since in some cases chlorination increases ARGs and selection pressure on ARB Is currently applied in conjunction with other methods, e.g., UV irradiation	Manaia et al. (2016), Waseem et al. (2017), Wang and Chen (2022), Zhuang et al. (2015), Thakali et al. (2020), Huang et al. (2019)

(continued)

Table 7.2 (continued)

Removal method	Application of strategy	References
UV irradiation	Highly effective technique for direct and indirect ARG removal as it disrupts intracellular nucleic acids of ARB UV irradiation (315–400 nm) normally generates reactive oxygen species (ROS), which interferes with DNA, resulting in permanent oxidative DNA damage In addition, UV irradiation can lead to lipid peroxidation, mutagenesis, and bacterial cell death Relatively higher UV irradiation is directly proportional to the efficiency of ARG removal, and, therefore, the UV dose for ARGs removal is significantly higher than that for killing ARB carrying these ARGs The success of UV irradiation in removing ARGs depends on several factors, including ARB and ARGs types, irradiation duration, microbial community, UV dose size, and water temperature UV irradiation is efficient when combined with other methods such as UV-C/H_2O_2, Fenton treatment, chlorination	Wang and Chen (2022), Waseem et al. (2017), Zhuang et al. (2015)

There is a dearth of empirical evidence on removing ARGs with conventional methods in African aquatic systems. Therefore, most of the work presented here was reported outside Africa. African and developing countries mainly use passive wastewater treatment methods, which provide a low-cost alternative. Passive wastewater treatment techniques in South Africa include constructed wetlands and algal integrated wastewater pond systems (AIWPS) (Genthe et al. 2020). These techniques have reduced the antibiotic-resistant *E. coli* in South African aquatic ecosystems. In one study conducted in the UK by Destiani and Templeton (2019), a combination of chlorine and UV was identified as an efficient method of completely removing ARGs from drinking water. Notably, 30 mg/min/L of chlorine reduced *tet*(A), bla_{TEM1}, *sul*1, and *mph*(A) by 1.7-log, and UV 200 mJ/cm^2 fluence inactivated the same genes by approximately 1.2-log. This study concluded that UV disinfection and chlorination have a synergistic effect as indicated by a reduction of tested ARGs by about 0.01- and 0.62-log when these techniques are applied in combination. Results indicated that multiple antimicrobial-resistant *E. coli* in aquatic systems can be inactivated by higher doses of UV (8 mJ/cm^2) and are resistant to low doses of UV.

Moreover, it was concluded that antimicrobial-sensitive *E. coli* requires low doses (8 mJ/cm^2) for inactivation (Zhang et al. 2017). Chlorination has been found to reduce microbial species biodiversity and ARG richness. This is in line with qPCR results, which indicated ARG richness from 150 to 120 and 160 to 120 in separate DWTPs (Wan et al. 2020). Although there is evidence that UV reduces

ARGs and ARB in aquatic environments, UV has been implicated in conferring resistance in bacteria toward several antibiotics, including sulfadiazine, rifampicin, and chloramphenicol. The same phenomenon has been reported for chlorination, where chlorine concentrations greater than 20 mg/L contributed significantly to the decrease of ARGs, including *tet*G and *tet*X (Zhang et al. 2017; Zhang et al. 2015), yet promoted the emergence of ARB. In another study, chlorine application failed to remove ARGs in aquatic systems, but ARB were significantly reduced and inactivated (Furukawa et al. 2017).

According to Wan et al. (2020), sand filtration and sedimentation can substantially remove ARGs. Biosand filtration exploits biofilm formation to remove bacteria through entrapment and adsorption (O'Connell et al. 2018). Accordingly, qPCR findings suggested that sand filtration directly reduces 97% of the identified ARGs (Wan et al. 2020). Slow sand filtration has shown great potential in reducing wastewater micro-pollutants, including antibiotics. According to a laboratory-based experiment conducted by Zearley and Summers (2012), 4.2% and 92% of sulfamethoxazole and trimethoprim were efficiently removed (Xu 2020).

Solar disinfection (SODIS) is based on the bactericidal activity of sunlight and its efficacy in inactivating ARB in drinking water. The use of SODIS in the inactivation of aquatic microorganisms was reported in Zimbabwe (Murinda and Kraemer 2008). Based on the findings by Fisher (2011), it was reported that solar disinfection inactivates microorganisms in aquatic systems. Although there were no reports of ARG reduction in this study, the presence of hydrogen peroxide-producing components accelerated solar-derived inactivation of MS2 bacteriophages *E. coli* and *Enterococcus. S*unlight wavelengths (UVA and UVB) 320–400 nm and 280–320 nm inactivated PRD1 bacteriophage and MS2, respectively. Furthermore, three strains of wastewater *E. coli* were inactivated in a laboratory setup by both UVA and UVB. SODIS naturally results in 99.9% inactivation of bacteriophage MS2 in 30 hours (Fisher et al. 2012; Ryberg et al. 2020). The methods summarized here are fully reviewed in earlier papers (Wang and Chen 2022; Waseem et al. 2017).

7.6 Future Perspectives and Research Directions

7.6.1 Knowledge Gaps

Compared to other continents, research on the occurrence, behavior, fate, and health risks of antibiotic resistance in aquatic systems and different environmental compartments in Africa is still in its infancy. The present chapter reviews the limited early results on antibiotic resistance in aquatic systems. Most of the studies were limited to the mere occurrence of ARB and ARGs in aquatic systems. Data on the behavior, fate, human exposure, and health risks are still missing. To increase the evidence base on antibiotic resistance in aquatic systems, further research is required to address the following knowledge gaps:

1. The available data is limited to a few countries out of 56 countries in Africa. Hence, further work is required to determine antibiotics' nature and occurrence in other countries' aquatic systems.
2. The consumption of contaminated drinking water is one of the human exposure routes to antibiotic resistance. Yet comprehensive studies investigating the occurrence, fate, and human exposure risks along the drinking water-human life cycle continuum are still lacking in Africa. Studies on human health risk assessment based on quantitative microbial risk assessment (QMRA) are needed to address this gap. Such studies should also determine the nature and occurrence of antibiotic resistance at the point of water consumption and the effects of the various water treatment methods on the removal and fate of antibiotic resistance.
3. The few available studies on antibiotic resistance are scattered among the various individual aquatic compartments, including surface water, rainwater harvesting systems, wastewaters, and groundwater systems. The limited evidence base makes it difficult to conduct systematic quantitative reviews using meta-analysis and bibliometric analysis. Therefore, there is a need to increase the evidence base on antibiotic resistance in the individual aquatic compartments.
4. Limited data exists on the effects of various conventional and low-cost wastewater and water treatment methods on the removal and fate of antibiotic resistance. Hence, comparative studies are required to determine the impact of different treatment methods on removal efficiencies and the fate of antibiotic resistance in aquatic systems. Such information is critical in the selection of appropriate remediation methods to safeguard environmental and human health.
5. Human exposure to antibiotic resistance may arise via multiple routes, including clinical administration of antibiotics and environmental exposure. However, studies investigating the human health burden of antibiotic resistance in aquatic systems in terms of morbidity and mortality are still lacking. Hence, estimating the human health burden of antibiotic resistance in aquatic systems is necessary.
6. Most studies on antibiotic resistance are based on conventional culturing methods, while those using advanced and novel techniques, including genomics, Big Data analytics, and in-silico computational modeling, are still limited. Hence, future research should integrate both conventional and emerging research tools to better understand the occurrence, behavior, fate, and health risks of antibiotic resistance in aquatic systems.

7.6.2 Challenges and Proposed Solutions

Research to address these knowledge gaps in antibiotic resistance face several challenges in Africa. Here, the challenges and proposed solutions are discussed.

7.6.2.1 Lack of Accredited and Well-Equipped Analytical Laboratories

Barring just a few, most African research institutions, including universities, lack well-equipped and accredited analytical laboratories to research antibiotic resistance. This is particularly the case concerning advanced analytical techniques such as genomic tools. Hence, national governments and funding agencies should prioritize establishing research infrastructure, including equipping and accrediting research laboratories.

7.6.2.2 Lack of Research Funding

In most African countries, research on antibiotic resistance and other disciplines is constrained by a severe lack of research funding. This lack of funding may explain Africa's limited research on antibiotic resistance. Individual governments and regional and international agencies should prioritize funding research on antibiotic resistance, given its potential impacts on human, livestock and environmental health.

7.6.2.3 Lack of Research and Technical Expertise

Research outputs on antibiotic resistance remain low in Africa compared to other regions, including developing ones. Research is limited to a few research groups in a few countries, while other countries have no evidence of any study on antibiotic resistance. This potentially points to the lack of research and technical expertise and a lack of funding and research infrastructure. Hence, capacity-building is needed to develop research and technical knowledge by training academic staff, postgraduate students, and laboratory technicians. Moreover, there is also a need for more collaborative research among the various groups working on antibiotic resistance in individual African countries.

7.7 Conclusions

The present chapter discusses the early results on antibiotic resistance occurrence, dissemination, behavior, and health risks in African aquatic systems. Although the evidence remains limited, early results show that ARB and ARGs have been detected in wastewaters, surface water, groundwater, rainwater harvesting systems, and drinking water supply systems. The potential sources of antibiotic resistance include municipal wastewaters and effluents from pharmaceutical industries, animal production systems, healthcare facilities, biosolids/manures, solid waste repositories, and the funeral industry, including cemeteries. The dissemination and circulation of antibiotic resistance occur via hydrologically driven processes such as wastewater discharges, surface run-off/erosion, groundwater recharge, infiltration, and surface

groundwater interactions. In addition, horizontal or lateral gene transfer aquatic compartments involving MGEs such as transposons circulate antibiotic resistance among various aquatic systems and other environmental resistomes. The risk factors for potential human exposure and health risks to African communities relying on untreated drinking water were highlighted. The human health risks are potentially high in cases where fecal contamination of drinking water sources occurs. Methods for removing antibiotic resistance in aquatic systems, including their advantages and limitations, were presented to safeguard human health. Finally, the chapter highlights the knowledge gaps and challenges constraining antibiotic resistance in Africa, including lack of funding, research infrastructure, and expertise. The need to apply advanced and emerging analytical tools such as genomics, Big Data analytics, and in silico computational methods were also discussed. Addressing the knowledge gaps and challenges is critical to advancing the current antibiotic resistance knowledge in African aquatic systems.

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Chapter 8
The Current State of Antimicrobial Use in Bovine Mastitis in Various African Countries

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8.1 Introduction

A significant economic burden known as bovine mastitis threatens the global dairy industry (Bradley 2002; Wellenberg et al. 2002; Petrovski et al. 2006; Margarita and Elena 2012; Man'ombe 2014). Specifically, mastitis infections remain a significant concern in African countries (Motaung et al. 2017). This disease results from prokaryotic and eukaryotic species invading the udder tissue, causing inflammation of the mammary glands (Bradley 2002). Mastitis infections cause harm to infected cows and, on a larger scale, have devastating economic effects on dairy farming (Motaung et al. 2017). Mastitis infections in dairy cows lead to poor quality milk and a reduction in milk yield (Petrovski et al. 2006; Halasa et al. 2009). Furthermore, the degree of infection differs with each causative agent, and the economic losses associated with the specific pathogen also vary (Gröhn et al. 2004). On many occasions, farmers do not realise the financial losses they suffer, as they are concealed in premature culling, decreased quality milk, expenditure on prevention and health problems (Du Preez 2000; Gruet et al. 2001; Bradley 2002; Petrovski et al. 2006).

Bovine mastitis is caused by over 140 pathogenic bacteria (including *Mycoplasma*), fungi, algae and viruses (Watts 1988; Petrovski et al. 2011). A significant number of species responsible for a single disease may alter veterinarians' interpretation and determination of the epidemiology of the disease (Motaung et al. 2017). The infectious pathways of the pathogenic species should thus be studied in a particular way (Motaung et al. 2017).

Mastitis presents two major types: environmental and contagious (Bradley 2002). Both types severely damage infected subjects' udder tissue (Motaung et al. 2017).

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In most African countries, the aetiologies of different pathogens, incidences, prevalence and management of mastitis infections are yet to be comprehensively documented (Motaung et al. 2017). These pitfalls hinder prevention, detection and treatment in African countries and negatively affect the profitability of dairy farming (Motaung et al. 2017). Currently, no universal treatment or management approach exists for mastitis infections (Du Preez 2000), but phage therapy is being investigated in South Africa as a possible alternative treatment option (Basdew and Laing 2011; Basdew 2012). Phage therapy includes many benefits such as host specificity, ease of isolation and propagation, reduced toxicity, prolonged shelf life and presence in the same environment as their bacterial hosts (Motaung et al. 2017). This will allow dairy farmers to avoid problems such as drug resistance, high costs and the constant need to develop host-specific antimicrobials (Adamu et al. 2020). Unfortunately, bacteria may develop resistance mechanisms to bacteriophages by secreting enzymes that target the phages' cell wall surface receptors or altering the specificity (Adamu et al. 2020). *Staphylococcus aureus* is the dominant mastitis-causing pathogen (Basdew and Laing 2011; Basdew 2012), and bacteriophage therapy has been tailored specifically for this bacterium, but there are several other antibiotic-resistant bacteria as well, and phage therapy should be expanded to accommodate the other pathogens (Adamu et al. 2020).

The mainstream treatment option for bovine mastitis is antibiotic therapy, including penicillin, ampicillin, tetracycline, gentamycin and many more (Hossain et al. 2017). It has been established that the ideal antibiotic treatment should be long enough to treat subclinical mastitis and short enough not to cause antibiotic resistance once the cow has calved (Cheng and Han 2020). However, antibiotic treatment has brought several problems to the dairy industry (Cheng and Han 2020). Despite the cost, antibiotics are being misused and overused, and antibiotic residues found in milk have given rise to a significant public health concern (Cheng and Han 2020). Treating cows with antibiotics during the dry cow period is the best option since there is no milk production during this time, and the risk associated with incorporating antibiotic residues into the milk is significantly reduced (Biggs 2017). Thus, long-acting antibiotic treatment is not recommended for mastitis infections during lactation as the farmer's primary concern is getting the cow back to milking as soon as possible (Cheng and Han 2020). Unfortunately, treating mastitis infections with antibiotics includes increasing resistance, leading to low cure rates and sensitive reactions and alterations in the gut microbiota in the general public (Kassa et al. 1999). In addition, antibiotic-resistant bacteria survive intracellularly within the udder and cause extremely difficult sores to treat (Ajose et al. 2022). For this reason, zoonotic transmission is now possible from bovines to people via milk and meat, making disease control even more challenging (Maity and Ambatipudi 2021). Therefore, the World Health Organization has released guidelines for limiting antibiotic use in livestock farming (WHO 1998, 2017).

Dairy production in Africa, in terms of licensed herds, is weak compared to developed countries like the USA and the European Union (EU); however, the average herd size in South Africa is more significant (USAD 2010; Lacto Data 2015). Therefore, milk has become an important food commodity in African farming enterprises and a source of income for commercial farmers (Motaung et al. 2017).

Dairy farms, unfortunately, experience continuous losses due to heavy penalties charged for any antibiotic residues found in milk (Cheng and Han 2020), discarding milk with antibiotic residues as the consumer cannot consume it due to the risk of allergies and possible drug resistance (Gomes and Henriques 2016). Antibiotics also do not directly protect the mammary glands from irreversible damage even though they might eliminate infection, leading to a continuous decrease in lifetime milk productivity (Zhao and Lacasse 2008).

8.2 Associated Causative Agents (Mastitis and Contributory Microbes)

The pathology of mastitis infections can be distinguished as environmental or contagious (host-adapted), depending on their transmission mode (Gomes and Henriques 2016). Infectious pathogens are usually introduced to the cows during the milking process via the milking equipment or by the milkers' hands (Motaung et al. 2017). *S. aureus* and *Streptococcus agalactiae* are the primary contagious pathogens, predominantly found in the mammary gland of infected cows (Gomes and Henriques 2016). The primary environmental pathogens include those that subside in the cows' immediate environment, i.e. *Streptococcus uberis*, *Escherichia coli* and *Klebsiella* species (Bogni et al. 2011). Despite the specific bacterial species mentioned above, numerous pathogens may be responsible for mastitis infections. Staphylococci, streptococci and enterobacteria are the most common groups of species found upon detection and diagnosis (Gomes and Henriques 2016).

Mycoplasma species play a significant role in the contagious pathogen group (Motaung et al. 2017). They do not only show differences in their transmission, but due to their lack of cell wall, they evade antimicrobial treatment that targets or disrupts the cell wall, such as penicillins (Motaung et al. 2017). Routine assessment tests performed for diagnosis rarely include *Mycoplasma* species, making an accurate *Mycoplasma* mastitis infection diagnosis uncommon (Motaung et al. 2017). Consequently, rapid detection and effective control measures are stunted, leading to unforeseen and substantial economic losses (Motaung et al. 2017). The species involved in *Mycoplasma* mastitis include but are not limited to *Mycoplasma bovis*, *Mycoplasma bovigenitalium*, *Mycoplasma californicum*, *Mycoplasma canadense* and *Mycoplasma alkalescens* (Motaung et al. 2017). The most common isolated pathogen in *Mycoplasma* mastitis is *M. bovis*; it may cause arthritis, and respiratory and reproductive diseases (Pfützner and Sachse 1996), which may exacerbate or be exacerbated by mastitis (Motaung et al. 2017). *Mycoplasma* mastitis may be facilitated by cattle harbouring *M. bovis* in their respiratory tracts, thus acting as mutual reservoirs for the pathogen, resulting in *M. bovis* spreading across the herd (Pfützner and Sachse 1996). *Mycoplasma* mastitis infections have regularly been reported in the USA (Fox et al. 2003, 2008; Olde Riekerink et al. 2005; Roy et al. 2008; Punyapornwithaya et al. 2012), but in countries like Asia and Africa, less frequent reports occur due to lacking or ineffective detection systems (Motaung et al. 2017). *Mycoplasma* mastitis outbreaks remain highly likely in African countries, making

early detection, research and laboratory diagnostics a critical starting point (Motaung et al. 2017), highlighting research needs.

A less common cause of mastitis, but no less costly and devastating, is mycotic mastitis caused by various fungal species, including *Aspergillus fumigatus*, *Aspergillus nidulans*, *Candida* species, *Pichia* species and *Trichosporon* species (Motaung et al. 2017). Given the evidence that mycotic mastitis has been detected in some parts of the world, i.e. Brazil, Poland, New Zealand and Tanzania (Williamson and di Menna 2007; Mdegela et al. 2009; Wawron et al. 2010; Dworecka-Kaszak et al. 2012), the probability that mycotic mastitis may develop into a costly disease should never be overlooked (Motaung et al. 2017). In mycotic mastitis, few studies have been conducted to characterise fungal species predominantly responsible for mycotic mastitis. Still, given that mycoses are common in mammals, the possibility always remains for mycotic mastitis to take a turn for the worst (Motaung et al. 2017).

Some members of the algae genus *Prototheca* are known to cause incurable acute or chronic mastitis in dairy cows (Motaung et al. 2017). *Prototheca zopfii* and *Prototheca wickerhamii* have been isolated in numerous clinical case studies (Ranjan et al. 2006; Pieper et al. 2012; Sobukawa et al. 2012; Krukowski et al. 2013). Protothecal mastitis infections commonly occur due to predisposing factors, including age and prolonged antibiotic use in quarters with a history of clinical mastitis (Ranjan et al. 2006). In addition, wet and humid environments like muddy pastures and pens tend to harbour *Prototheca* species, and infection is likely to happen when the teat is exposed to high pathogen loads (Motaung et al. 2017). Despite the lack of an effective detection system for protothecal mastitis and rarely reported infections, molecular typing tools have been applied in some countries to detect these infections (Motaung et al. 2017). The available data makes it possible to formulate a guide for identifying such diseases in African countries (Motaung et al. 2017).

Bacterial, algal and fungal species do not account for 100% of mastitis causative agents responsible for infection (Motaung et al. 2017). Although viruses are not regarded as common aetiological factors, they have been isolated from cows suffering from mastitis (Motaung et al. 2017). Despite lacking evidence that viruses are solely responsible for mastitis infections, viruses like bovine herpesvirus (BHV), BHV4, foot-and-mouth disease virus and parainfluenza have been associated with bovine mastitis, even without the isolation of bacterial pathogens (Wellenberg et al. 2002).

8.3 Diagnosis and Treatment

The farmer's diagnosis of clinical mastitis is less complex and observable with the eye since a clinical mastitis infection will go along with swollen quarters or udders, depression and poor milk quality (Mahmmod 2015). On the other hand, subclinical mastitis is less evident to the naked eye and thus requires specific diagnostic techniques (Motaung et al. 2017). In the field, mastitis infections can be detected by using traditional diagnostic tests, for example, the California mastitis test (CMT) or

the somatic cell count (SCC) (Deb et al. 2013; Duarte et al. 2015). The CMT is commonly used to determine the somatic cell count in the milk of possibly infected subjects (Motaung et al. 2017). Somatic cells usually comprise macrophages, lymphocytes, erythrocytes and epithelial cells (Dohoo and Meek 1982; Pillai et al. 2001; Sharma et al. 2011). The proportion of these cells is an excellent indicator of infection and the disease status in the udder gland (Motaung et al. 2017). White blood cells constitute one-third of total blood cells in healthy udders; however, during infection, white blood cells might increase to proportions reaching 99% (Motaung et al. 2017). The somatic cell counts indicate the presence and severity of udder tissue damage caused by contagious or environmental pathogens (Motaung et al. 2017).

Several other tests are used for the detection of a mastitis infection, i.e. electrical conductivity, pH, the white side test (NaOH), the measurement of *N*-acetyl-b-D-glucosaminidase, lactate dehydrogenase, the bacterial culture of milk, milk enzyme-linked immunosorbent assays (ELISA) and the most robust, polymerase chain reaction (PCR) (Mahmmod 2015). Several PCR assays have been developed to detect mastitis-causing pathogens such as *Staphylococcus* species, *E. coli*, *M. bovis*, *S. agalactiae* and *Enterococcus* species, including multiplex, conventional and qPCR (Koskinen et al. 2009; Taponen et al. 2009; Shome et al. 2011; Hiitiö et al. 2015; Barbier et al. 2016). In addition, another nucleic acid amplification technique, known as loop-mediated isothermal amplification (Tomita et al. 2008), is used for the detection of *Staphylococcus* species, *E. coli*, *M. bovis*, *S. agalactiae* and *Enterococcus* species (Kato et al. 2007; Zhang et al. 2011; Wang et al. 2015; Bosward et al. 2016). Fortunately, various diagnosis methods can be utilised as the first line of detection from milk samples in African countries to fight future mastitis infections and outbreaks (Motaung et al. 2017).

Currently, there is no universally used method to treat mastitis infections. Developing an effective treatment therapy remains challenging due to the significant number of pathogens contributing to the disease (Motaung et al. 2017). du Preez (2000) reported that *S. aureus* is the most prevalent species causing mastitis due to the pathogen's resistance mechanism. The resistance mechanism is either displayed as the formation of abscesses in the udder or the evasion of antibiotics by residing in the macrophages and avoiding antibiotics circulating in the bloodstream (du Preez 2000). Furthermore, some *S. aureus* strains exist as latent bacteria in a capsule and later reactivate growth when the conditions are favourable (du Preez 2000). These pathogens' antibiotic resistance impacts the possible treatments and associated costs, especially in African countries (Motaung et al. 2017).

8.4 Antimicrobial Resistance

Despite the efforts of dairy farmers to control and prevent mastitis infections, antibiotic intervention is often required when cows get infected with mastitis (Oliver and Murinda 2012). When an outbreak occurs on a farm, and antibiotic treatment

needs to be initiated, it is necessary to follow standard operation procedures (Oliver and Murinda 2012). The infected quarter is identified, the required treatment is activated, the complete series of the recommended treatment is administered, a meticulous treatment record is kept, the treated cows are identified and kept apart from the healthy herd and measures are set to ensure that the milk is free from any antibiotic residues before it is added to the bulk tank (Oliver and Murinda 2012).

In animal production, antibiotics are used extensively to combat disease and improve animal performance (Oliver and Murinda 2012). A wide variety of antibiotics are being used on dairy farms, including penicillin, cephalosporin, streptomycin and tetracycline, to treat and prevent future mastitis infections and outbreaks on dairy farms caused by a variety of gram-negative and gram-positive bacteria (Oliver and Murinda 2012). In animal production, antibiotics are administered routinely to entire herds and not just infected subjects as a preventative tool during the nonlactating period of the cows (Oliver and Murinda 2012). Antibiotic treatment aims to ensure healthier and more productive cows, lower disease incidence, decreased morbidity and mortality, a reduced pathogen load and high-quality milk for human consumption (Oliver and Murinda 2012). Unfortunately, the agricultural administration of antibiotics is mainly responsible for the emergence and intensifying of antibiotic resistance, which has a ripple effect on human diseases, requiring frequent antibiotic intervention (Oliver and Murinda 2012).

The environment serves as an essential reservoir and a transfer medium for the emergence and spread of antibiotic resistance (Van et al. 2020). Animal sources such as manure introduce antibiotic drugs, resistance genes and resistant bacteria to the environment (Finley et al. 2013; Cabello et al. 2013; Ghosh and Lapara 2007). Some antibiotic drugs used in livestock production are poorly absorbed by the gut resulting in the parent compounds or metabolites of the antibiotic medicines being excreted in the faeces and urine (Campbell et al. 1983; Chiu et al. 2000; Donoho 1987; Magnussen et al. 1991; Sommer and Nielsen 1992) (Stout et al. 1991). Many farm animals are housed indoors, and large quantities of manure are produced and disposed of in the environment (ADAS 1997, 1998) (Montforts 1999). Besides veterinary medicines contaminating the soil when excreted or disposed of, it is also possible for the medication to leak into shallow groundwater from manured fields or reach surface water bodies by surface runoff (Hamscher 2000a, b, c; Hirsch et al. 1999; Meyer et al. 2000; Nessel et al. 2000). Antibiotic use in animal production accelerates the rate and emergence of antibiotic resistance traits in bacteria (Van et al. 2020). Unfortunately, the environment holds antibiotic residues from food and animal excretions (Liu et al. 2016; Tasho and Cho 2016). These antibiotic residues linger in the background and remain unchanged, later found in sewerage installations next to farmlands being treated with animal manure (Taylor et al. 2011). Lateral gene exchange takes place in these sewage installation plants. Although the antibiotic concentrations in these plants are far below the minimum inhibitory concentrations (MIC), specific antibiotic-resistant strains are favoured (Andersson and Hughes 2014). The slightest presence of an antibiotic is enough to inhibit the growth of bacteria entirely or partially, causing selective pressure and increasing the prevalence of resistance in bacteria (Tello et al. 2012).

Furthermore, cows treated with antibiotics in the fight against mastitis infection deliver a higher incidence of antibiotic residues in the milk, which is a public health and food safety concern as well as an economic factor as the producer may be penalised for producing adulterated milk, jeopardising the manufacturing industry worldwide (Oliver and Murinda 2012).

The discovery and usage of antimicrobial agents in the twentieth century have been the most outstanding achievements of all times in medical science; however, little did we realise how quick and efficient bacteria would be able to build up resistance to antimicrobial agents (Roberts 2002). There is a link between antimicrobial resistance in humans and farm animals. Despite reduction attempts, many antibiotics are still being used on farm animals, establishing a reservoir of resistance genes (Woolhouse et al. 2015).

Coagulase-negative *Staphylococcus* (CoNS) species are the most frequently isolated bacterium from dairy cows suffering from mastitis infections (Pitkälä et al. 2004; Foster 1996; Schukken et al. 2014) and have been emerging as opportunistic pathogens in South Africa (Petzer et al. 2009) and globally (Kudinha and Simango 2002; Fry et al. 2014; Taponen et al. 2007; Sampimon et al. 2009; Becker et al. 2014; El-Jakee et al. 2013). The most commonly isolated CoNS from clinical and subclinical mastitis are *Staphylococcus chromogenes*, *Staphylococcus epidermidis*, *Staphylococcus simulans*, *Staphylococcus haemolyticus* and *Staphylococcus xylosus* (Foster 1996; Xu et al. 2015; Bexiga et al. 2014). Penicillins were reported to be effective against CoNS infections (Becker et al. 2014; Bhattacharyya et al. 2016; Koksal et al. 2009) until the rise of antibiotic resistance, where CoNS species are now showing resistance (Raspanti et al. 2016; Schmidt et al. 2015; Beuron et al. 2014) to antimicrobials like penicillin, tetracycline, lincomycin and streptomycin (Taponen et al. 2006; Microbiology and Advance 2016). Studies have shown that the increase in resistance in CoNS species may be due to the injudicious use of antimicrobials (Fair and Tor 2014), the presence of penicillin-binding protein 2a (PBP2a) (Koksal et al. 2009; Brakstad and Mæland 1997; Silva et al. 2014), the *mecA*-mediated oxacillin resistance (Jain et al. 2004; De Silva et al. 2002; Szweda et al. 2014; Mahato et al. 2017), the ability of CoNS species to form biofilms and facilitate persistent infections (Becker et al. 2014; Yu et al. 2017; Cepas et al. 2019) and the decreased susceptibility to commonly used antimicrobials (Tremblay et al. 2014).

8.5 Occurrence of Mastitis in African Regions

Considering the relevance of mastitis infections in African regions, reports from Botswana, Ethiopia, Nigeria, Sudan and Zambia were assessed (Motaung et al. 2017). Results indicated a variable prevalence of pathogens in different regions and the highest variability, where Kenya and Niger led with approximately six pathogens isolated from mastitis-infected milk. In contrast, South Africa, Tanzania, Uganda and Zimbabwe showed less than six pathogens (Motaung et al. 2017).

In addition, identification studies on milk samples collected across Africa showed that the most prevalent pathogen was *S. aureus* (Motaung et al. 2017).

Udder health has a direct and indirect economic burden on dairy farms worldwide; however, the initial impact may be observed only at the farm level. It can escalate to a continental scale if not detected and addressed promptly (Motaung et al. 2017). One of the disadvantages of sampling from African countries is the lack of collaboration between veterinarians and scientists, withholding mastitis data and making the sample size more variable (Motaung et al. 2017). Therefore, less than 1000 cows were surveyed in reports, with Nigeria, Tanzania and Uganda having the lowest sampling number of 200 cows (Motaung et al. 2017). Most of the data were obtained from the Middle-Eastern and North African regions (Fadlelmula et al. 2009; Alekish et al. 2013; Boujenane et al. 2015). This data represents how Africa is progressing slower in surveying bovine mastitis (Motaung et al. 2017).

In contrast to developed countries with frequent reports of incidences and prevalence of clinical and subclinical mastitis (Bradley 2002; Petrovski et al. 2006; Sharma et al. 2011), mastitis infection is only well documented in 30% of African countries (Motaung et al. 2017). Ethiopia, Kenya, South Africa and Uganda show the highest prevalence of subclinical infections, between 60 and 80%. Still, significant reports in Ethiopia showed that more than 3400 cows had been tested for mastitis in the last decade (Dego and Tareke 2003; Sori et al. 2005; Lakew et al. 2009; Abebe et al. 2010; Bitew et al. 2010; Mekibib et al. 2010; Moges et al. 2011; Almaw et al. 2012; Daka et al. 2012; Girma et al. 2012; Haftu et al. 2012; Tadesse and Chanie 2012; Abera et al. 2013; Belayneh et al. 2013; Yohannis and Molla 2013; Zeryehun et al. 2013; Benti and Zewdie 2014; Hailemeskel et al. 2014; Zenebe et al. 2014). Given studies conducted between 2011 and 2014, several scientists reported that the adverse effects associated with mastitis might be overwhelming to the African economy and devastating (Motaung et al. 2017). Until 2014, several surveys from large and small dairy farms in sub-Saharan Africa, the Middle East and North Africa showed that efforts implemented for mastitis treatment may be fast-tracked (Motaung et al. 2017).

A general view of mastitis occurrence in African countries can be inferred from the data provided. The prevalence of subclinical mastitis was around 40% and below clinical mastitis until 2014 since clinical signs are easily detected and could be dealt with as soon as they appear (Motaung et al. 2017). In Ethiopia specifically, subclinical mastitis infections are higher than clinical mastitis in most other parts of Africa, which could be a consequence of the time of year the samples were collected and whether the sampling locations used typical feeding schemes (Motaung et al. 2017).

To determine the baseline of mastitis infections in African countries, conditions in the quarters of dairy cows were monitored (Motaung et al. 2017). According to these reports, most African countries displayed a 30–60% prevalence of mastitis infections, whereas Niger and Sudan displayed less than 13% infection prevalence (Motaung et al. 2017). Saudi Arabia collected a large sample size (north of Africa) and showed a substantial >70% prevalence of mastitis infections (Fadlelmula et al. 2009). Evidence-based information on most mastitis infections in Africa can be well maintained if different locations are sampled at least two to three times a year (Motaung et al. 2017).

A study to evaluate the economic value of somatic cell counts (SCC) was conducted in South Africa on Holstein and Jersey cattle (Motaung et al. 2017). It was crucial to incorporate SCC into breeding objectives (Motaung et al. 2017). Current estimates show that a single reduction in milk yields causes a profit reduction between ZAR 491.48 to ZAR 1795.57 per cow per year, depending on the breed, production and payment system (Banga et al. 2014). Furthermore, these reductions were estimated to be derived from healthy cows, indicating that the profit loss associated with mastitis cows could be devastating (Motaung et al. 2017). Using SCC as a detection method is thus critical to providing a more reliable estimate of milk production losses (Motaung et al. 2017).

8.6 Antimicrobial Resistance and Mastitis in Africa

Over the years, livestock production increased significantly in Africa (Ssajjakambwe et al. 2017). In Uganda, 1.7 million households keep cattle as a source of income for feeding purposes and employment (Ssajjakambwe et al. 2017). The total cattle population in Uganda stands at 11.4 million, and although their annual milk production stands at 1.5 metric tonnes, livestock production comes with significant economic implications (Hogeveen 2005; Balikowa 2011). In sub-Saharan Africa, veterinary service provision is fragmented, and professional advice is rarely available and adhered to (March 2004). Cattle farmers in Africa tend to manage mastitis outbreaks without the support and guidance of professionals (Ssajjakambwe et al. 2017). With the focus on livestock production in rural Southwestern Uganda, a study found that the most common antibiotics used by farmers include tetracycline and penicillin (Ssajjakambwe et al. 2017). These are broad-spectrum antibiotics bought over the counter without veterinary supervision (Ssajjakambwe et al. 2017). Surprisingly, these antibiotics show no inhibition of some mastitis-causing pathogens, which should have been responsive (Ssajjakambwe et al. 2017). Optimally, antibiotic treatment is recommended for clinical cases rather than subclinical infections (Ssajjakambwe et al. 2017). The correct antibiotic dosage is not always met, and veterinary services are not readily available, forcing farmers to self-medicate the infected cows (Ssajjakambwe et al. 2017). As a result, the pathogens adapt mechanisms at a molecular level to evade the drug target pathways, ultimately leading to resistance (Saini 2012). Soriano et al. showed tetracycline to inhibit *Corynebacterium* spp. (Soriano et al. 1995), whereas the study from Uganda showed some resistance to the antibiotic (Ssajjakambwe et al. 2017).

A study in Kenya focused on the two leading mastitis pathogens in the country, *S. aureus* and CoNS and their antimicrobial resistance patterns (Mbindyo et al. 2021). It was reported that the highest resistance was shown towards ampicillin, followed by tetracycline (Mbindyo et al. 2021). Lower resistance was seen against fluoroquinolones and gentamicin. *S. aureus*, in contrast to CoNS, shows significantly higher resistance to cefoxitin and ampicillin (Mbindyo et al. 2021). *S. aureus* isolates showed 75.8% resistance to at least one antimicrobial tested in the study

(Mbindyo et al. 2021). Ampicillin was the most frequent phenotype, followed by erythromycin, and the least resistance was shown towards fluoroquinolones (Mbindyo et al. 2021). Based on a cefoxitin disc diffusion test, 25% of the *S. aureus* isolates were methicillin-resistant *S. aureus* (MRSA) (Mbindyo et al. 2021). Furthermore, all MRSA isolates showed 100% resistance to ampicillin, erythromycin, tetracycline, streptomycin and trimethoprim-sulfamethoxazole (Mbindyo et al. 2021). The CoNS isolates showed 68.5% resistance to at least one of the antimicrobials tested, with ampicillin being the highest at 57.6% (Mbindyo et al. 2021). The following ampicillin was tetracycline (22.8%) and fluoroquinolones (3%) (Mbindyo et al. 2021). Cefoxitin resistance was recorded in this study at 10.8%, and the isolates were afterwards termed methicillin-resistant coagulase-negative staphylococci (MRCoNS) (Mbindyo et al. 2021). Besides being resistant to a single antimicrobial agent, some pathogens show resistance to three or more classes of antibiotics, i.e. multi-drug resistance (Mbindyo et al. 2021). In this study, MDR was detected in 22.9% of staphylococci isolates, 29.7% of *S. aureus* isolates and 16.3% of CoNS (Mbindyo et al. 2021). The continued development of multi-drug-resistant bacterial strains does not bode well for mastitis treatment in the future, which will create serious problems, particularly for small-scale farmers.

In Egypt, three mastitis-causing pathogens, namely, *S. aureus*, *E. coli* and *P. aeruginosa*, were tested against antimicrobials and all isolates included antibiotic-resistant strains (Ameen et al. 2019). *S. aureus* showed 90% resistance against penicillin, but only 10% resistance was observed against oxacillin (Ameen et al. 2019). MRSA isolates also showed high resistance to penicillin and other β-lactam antibiotics (Erskine et al. 2002, 2003; Sabour et al. 2004; Guven et al. 2009; Akindolire et al. 2018). MSRA strains have become a severe problem due to their resistance to β-lactams and tetracyclines, aminoglycosides, macrolides and lincosamides (Ameen et al. 2019). In Egypt, *S. aureus* still seems to be sensitive to several antibiotics (Ameen et al. 2019). *P. aeruginosa* showed broad-spectrum resistance to several antibiotics, making this pathogen a significant concern in the dairy industry (Ameen et al. 2019). Although it is not a common mastitis-causing pathogen, *P. aeruginosa* shows resistance to several commercial antibiotics (Ameen et al. 2019). The only effective antibiotic in this study was imipenem (Ameen et al. 2019).

South Africa has a small selection of intra-mammary antibiotics available and mainly consists of ampicillin and cloxacillin products (Karzis et al. 2021). Once again, due to the emergence and maintenance of antibiotic-resistant strains in pathogens, dairy cattle producers are negatively affected due to the impact-resistant strains have on milk production and the economy (Bean et al. 2004). A study on antimicrobial resistance in South Africa focused on MRSA and *S. aureus*, the country's most frequent causes of mastitis (Karzis et al. 2021). MRSA bacteria were found to carry a gene rendering them resistant to methicillin and other β-lactam antibiotics (Perovic et al. 2006). MRSA is a growing and serious concern in veterinary medicine and has lapped over to human medicine (García-Álvarez et al. 2011). Milk samples from South African dairy cattle tested negative for the *mecA* gene, conferring methicillin resistance, following a PCR reaction (Karzis et al. 2021). Still, it tested positive for phenotypic MRSA using the cefoxitin disc (Badenhorst et al. 2014). We now know that this could have been because of an *S. aureus* strain

which carries a homologue of the *mecA* gene, the *mecC* gene (García-Álvarez et al. 2011). Due to the increase in antibiotic resistance, the European Medicines Agency (EMA) and the European Food Safety Authority (EFSA) have published a joint opinion that the use of antimicrobials in animals needs to be reduced and rethought and replaced (International Dairy Federation 2017). A study conducted in KwaZulu-Natal in 2015 found that 48% of *S. aureus* isolates were resistant to β-lactams (Schmidt et al. 2015). In most South African provinces, the prevalence of antibiotic resistance was above 50%, except for cefuroxime (Karzis et al. 2021). *S. aureus* isolates from South Africa showed to have the highest complete or partial resistance to spiramycin (100%) and erythromycin (36.4%) (Monistero et al. 2020). Out of 11 isolates, three isolates showed resistance to ampicillin and penicillin, while only one isolate showed β-lactamase activity, and another showed intermediate resistance towards lincomycin (Karzis et al. 2021).

Resistance to antimicrobials is an increasing threat to animal disease control and public health (Karzis et al. 2021). The focus in South Africa and the rest of the world is now on the well-judged use of antibiotics (Karzis et al. 2021). More detailed research and information on antibiotic resistance in Africa will be valuable to veterinarians and farmers when they use antibiotics to treat cattle infections (Karzis et al. 2021). This should improve the antibiotics' effectiveness and reduce the likelihood of any consequent resistance (Karzis et al. 2021).

8.7 Prevention and Control

Following the diagnosis of mastitis infections, the first treatment method that comes to mind is antibiotics (El-Sayed and Kamel 2021); however, already available antibiotics are drastically limited by increasing antibiotic resistance and public health restrictions (Khazaie and Ahmadi 2021). We find ourselves moving towards a post-antibiotic era where the development of new antibiotics is hugely time-consuming and costly, shifting the paradigm to alternative preventative and control measures (El-Sayed and Kamel 2021). Unfortunately, mastitis can never be entirely eradicated from a herd, but it is possible to keep infections at the lowest possible frequency (Zigo et al. 2021).

The establishment of the dairy herd improvement (DHI) system greatly enlarges the genetic selection and genome prediction for mastitis resistance (Martin et al. 2018). The penultimate goal of the system is for better prevention of mastitis infections (Ashraf and Imran 2020). Fang et al. (2017) reported that genomic markers such as single nucleotide polymorphism (SNP) could be used for genomic feature best linear unbiased prediction model, which can improve the genomic prediction for mastitis resistance traits (Fang et al. 2017). Identifying specific high-density SNPs associated with mastitis traits and genomic breeding values is expected to increase resistance to mastitis over consecutive generations of breeding (Deb et al. 2012; Mustafa 2018). Future improvements in genomic selection technology will lead to genetic modification in mastitis-resistant animals (Vukasinovic et al. 2017).

Control strategies for mastitis infections should rely less on antibiotics and significant losses in culling and focus more on meticulous screening and inspection of dairy farms, the evaluation of welfare plans and the use of prognostic diagnostic tests (Ashraf and Imran 2020). These strategies are crucial for identifying infected subjects and cows at the most considerable risk for infection. In doing so, the spread of mastitis infections in herds can be controlled and decreased (Ashraf and Imran 2020). Research has focused on alternative measures to maintain and prevent mastitis infections. Trevisi et al. (2014) reported that by using immuno-modulators, the immuno-competence and disease resistance in cows might significantly improve (Trevisi et al. 2014). Vaccines have been used to induce immunity against infection; for example, ultraviolet-killed *E. coli* bacterin was injected for intra-mammary immunisation in the nonlactating periods of cows and provided partial protection against *E. coli*-induced mastitis (Ashraf and Imran 2020). Gurjar et al. (2013) compared the *E. coli* bacterin injection with the J-5 bacterin vaccine. They found that the *E. coli* injection improved protection and increased milk production (Gurjar et al. 2013). Given that *S. aureus* is another major mastitis-causing pathogen, the pathogen's and host's virulence factors have been explored to study the immune response (Scali et al. 2015). Thus, the development of new vaccines may aid in minimising antibiotic use and control against *S. aureus* infections (Festa et al. 2013).

S. uberis is also responsible for mastitis infection worldwide, and vaccine antigens from the protein of *S. uberis* were evaluated as a potential target and tested in murine models (Collado et al. 2016). Unfortunately, major mastitis-causing pathogens can form biofilms, making them more resistant and difficult to control since they can cause recurrent infection (Gomes et al. 2016). Therefore, more in-depth knowledge of biofilm formation and the control measures would lead to better disease control (Gomes et al. 2016). In addition, comparative studies on conventional and organic farms could be used to identify factors involved in the control of mastitis and lead to better management and control measures (Levison et al. 2016).

The well-known saying "prevention is better than cure" is still applicable today in the dairy farming industry. Despite the other tools available for animal production, biosecurity and strict hygiene measures remain crucial for decreasing the pathogen load in the environment and on the udder. Unfortunately, resistance to disinfectants could also be an increasing problem due to the agricultural use of antibiotics. Future research may focus on the existing link between antibiotic and disinfectant resistance in the field. However, the significance of cleaning and disinfecting the milking machinery and equipment should not be overlooked, as mastitis-causing pathogens can be introduced in various ways.

8.8 Conclusion

Mastitis infections have always been a significant concern in African countries and worldwide. The economic losses associated with mastitis may be devastating, and farmers may not realise the full extent of loss by mastitis infections. More than 140 species are causing mastitis infections, presenting themselves in clinical or

subclinical mastitis. In African countries, more work is required to diagnose and treat mastitis infections more reliably and accurately to determine the economic losses. Subclinical mastitis infections are rising in African countries like Ethiopia, Kenya, South Africa and Uganda, about 50–80%. This is highly concerning as subclinical infections give rise to antimicrobial resistance and are the costliest disease, as early detection is difficult. In Africa, subclinical mastitis infections are widespread, resulting in an elevated SCC, emphasising the need for proper communication regarding the acceptable SCC for a healthy udder. African countries report about 30% of mastitis infections compared to developed countries, leaving 70% behind. The economic losses associated with bovine mastitis are poorly documented, suggesting poor collaborations between the dairy industry, scientists and economists. To assess direct and indirect losses better, dairy farmers are advised to screen their cattle for clinical and subclinical mastitis regularly.

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Chapter 9
Microbiological Safety and Antimicrobial Resistance in Fresh Produce Production in Africa

Yinka M. Somorin, Gabriel B. Akanni, and Amarachukwu Anyogu

9.1 Introduction

Fresh produce, consisting of fruits and vegetables, is increasingly recognised and promoted as an essential component of a healthy diet. There is increasing evidence that regular consumption of fresh produce reduces the incidence of chronic diseases, including cardiovascular disease, type 2 diabetes, obesity, dementia, and the risk of cancer (Boeing et al. 2012; Boffetta et al. 2010; Gonzalez et al. 2012; Hodder et al. 2020; Hu et al. 2014; Oyebode et al. 2014). These health benefits have been attributed to these foods' high amounts of vitamins, minerals, fibres, and antioxidants (Pennington and Fisher 2010).

The World Health Organization (WHO) recommends consuming at least five portions (400 g) of fruits and vegetables per day for a healthy diet (WHO 2004, 2020). However, fresh produce consumption relies on availability, access, and consumer socio-demographic characteristics such as age, gender, income, and educational level (Gustat et al. 2015). Several studies have noted that considerable numbers of the global population, particularly in low-income settings, have yet to achieve the WHO recommendation (Kabwama et al. 2019; Lutfiyya et al. 2012; Miller et al. 2016). However, global production and consumption of fresh produce are increasing (Balali et al. 2020). For example, a recent modelling study by Mason-D'Croz et al. (2019) estimated that between 1965 and 2015, the global population achieving the WHO recommendation had increased from 17% to 55%.

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At the same time, the role of fresh produce as a vehicle for the transmission of pathogenic and antimicrobial-resistant bacteria has become well established (Allen et al. 2013; Callejón et al. 2015; Hölzel et al. 2018; Willis et al. 2020; Zhang et al. 2020). Some contributory factors include the increased demand for fruit and vegetables as ready-to-eat (RTE) products which require minimal or no processing before consumption, thus removing a significant hurdle for eliminating or inactivating foodborne pathogens (Olaimat and Holley 2012). In addition, to ensure the availability of fresh produce year-round, supply chains have become increasingly global and complex (Carstens et al. 2019). As a result, the international food trade is also becoming a route for transmitting pathogenic bacteria (Dada et al. 2021; Somorin et al. 2021). Fresh produce trade has been established through geographical routes culminating in the import or export of varieties of tropical fruits and vegetables across the globe. Sub-Saharan Africa is the hub for the cultivation and processing of tropical fruits and vegetables, with many countries harnessing the opportunities for fresh produce export. The highlight of fresh produce export is providing off-season fresh fruits and vegetables to countries with temperate climates, principally the European Union, as one of their most important export markets (Huang 2004).

Fruit and vegetables are increasingly implicated in foodborne outbreaks globally. For example, in the United States, 37% (85) of the multistate foodborne outbreaks between 2010 and 2017 were linked to consuming contaminated fresh produce. These led to 4501 cases of illness, of which 1117 required hospitalisation and 55 deaths (Carstens et al. 2019). Some notable examples include the 2017 multistate *Salmonella* infection outbreak linked to contaminated papaya. Two hundred and twenty (220) cases across 23 states were recorded, leading to 68 hospitalisations and 1 fatality (CDC 2017). More recently, 40 people across 19 states were infected with *Escherichia coli* 0157:H7 after consuming leafy greens (CDC 2020).

A similar trend has also been observed in Europe. For example, between 2006 and 2016, the proportion of foodborne outbreaks associated with non-animal-based foods increased from 5% to 13% (Machado-Moreira et al. 2019). In 2011, one of the largest outbreaks of enterohaemorrhagic *Escherichia coli* (EHEC) serotype O107:H4 occurred in Germany (Robert Koch Institute 2011). There were over 4000 cases of illness and 55 deaths, including visitors from 15 other countries, and a smaller outbreak in France. The vehicle implicated in the outbreak was contaminated fenugreek sprouts produced from imported seeds (Köckerling et al. 2017; Machado-Moreira et al. 2019). Recently, a multi-national listeriosis outbreak linked to frozen sweetcorn involved 54 cases across the United Kingdom, Australia, Finland, Sweden, Denmark, and Austria (McLauchlin et al. 2021). These examples demonstrate that fresh produce can be considered a major vehicle for transmitting pathogenic bacteria, which remains a significant challenge even in high-resource settings.

In Africa, fruit and vegetable consumption has increased significantly over the last 30 years (Mensah et al. 2021). However, there is limited reliable information about the prevalence of pathogenic bacteria in fresh produce and epidemiology studies linking these to foodborne illness outbreaks on the continent (Aworh 2021;

Imathiu 2018). Consuming food contaminated with microbial hazards remains a significant health threat in Africa (WHO 2015). Contributory factors include inadequate physical infrastructure (e.g. clean water, storage facilities, transport networks), poor awareness of food safety issues and good manufacturing practices among relevant stakeholders (farmers, distributors, manufacturers, handlers, and consumers), and limited capacity for developing and enforcing food safety regulations (Anyogu et al. 2021; Jaffee et al. 2019).

In addition, the presence of antimicrobial-resistant (AMR) pathogenic microorganisms in food is a risk to public health. Antibiotic-resistant bacteria have been observed worldwide in surface water, often used as irrigation water for fresh produce (Blaak et al. 2014), making fresh produce a frequent vehicle of AMR bacteria, including multidrug-resistant ones. There are reports on the prevalence of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* (third-generation-resistant *Enterobacteriaceae*) in retail vegetables produced in the Netherlands, the United States, and Denmark (Van Hoek et al. 2015). According to Cantón et al. (2008), the prevalence of ESBL producers in Europe is higher than in the United States but lower in South America and Asia. A study by Nüesch-Inderbinen et al. (2015) reported that ESBL producers are mostly found in the irrigation water used for fresh produce rather than on the fresh produce itself.

Thus, this chapter provides a comprehensive overview of current information about pathogenic and antimicrobial-resistant bacteria in fresh produce in Africa. It examines relevant factors contributing to the microbial contamination of fresh produce, identifies data gaps, and discusses recommendations to support stakeholders in taking appropriate steps to improve the safety of the fresh produce chain in Africa.

9.2 Microorganisms of Public Health Significance in Fresh Produce in Africa

Pathogenic bacteria have multiple entry routes into the fresh produce supply chain. These have been identified as soil, faeces, irrigation water, unhygienic processing plants, and human handling (Iwu and Okoh 2019; Olaimat and Holley 2012). In addition, studies investigating the presence of contaminants along the farm-to-fork fresh produce continuum in Africa have identified bacteria of public health concern. For example, Jongman and Korsten (2016) established a link between *Escherichia coli* in irrigation water sources and vegetables grown in home gardens and commercial farms. However, contrary observations were reported by van Dyk et al. (2016) as there was no significant overlap of microbial species in irrigation water samples, the processing environment, or the tomatoes at retail sale. Despite this, they also reported a high coliform count in tomatoes at the market.

The most commonly reported pathogens associated with fresh produce supply chains in Africa include *Salmonella* spp., *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* (Table 9.1). This observation is not surprising and

Table 9.1 Studies on the significant indicator and pathogenic bacteria in fresh produce in Africa

Microorganisms	Number of positive samples/ number of samples (prevalence)	Food product	Sample collection stage	Country	References
Enterobacteriaceae	117/160	Mixed vegetables (baby corn, beans, carrots, chilli Fresno, mangetout, peas, and patty pan) and green beans	Processing plant	Zambia	Nguz et al. (2005)
Listeria monocytogenes	16/80				
Salmonella spp.	37/160				
Staphylococcus aureus	54/80				
Listeria monocytogenes	63/120	Leafy vegetables	Markets	Nigeria	Nwachukwu et al. (2010)
Listeria monocytogenes	104/160	Leafy vegetables	Markets	Nigeria	David and Odeyemi (2007)
Listeria spp.	17/20	Cabbage, lettuce, carrots, green peas	Markets	Nigeria	Ikeh et al. (2010)
Campylobacter spp.	7/128	Leafy vegetables	Farms	Benin	Kougblénou et al. (2019)
Salmonella spp.	16/86	Ready-to-eat vegetable salad	Not mentioned	Kenya	Imathiu (2018)
Staphylococcus aureus	63/86				
Listeria monocytogenes	56/86				
Salmonella spp., *Enterococcus* spp., Faecal coliforms	ND	Leafy greens	Farms and markets	Ghana	Quansah et al. (2018)
Staphylococcus aureus, *Salmonella* spp.	ND	Lettuce, cabbage, cucumber	Markets	Nigeria	Abdullahi and Abdulkareem (2010)
Escherichia coli	64/96	Lettuce, strawberries	Farm, markets	Egypt	Uyttendaele et al. (2014)
Salmonella spp.	34/96				
Listeria monocytogenes	1/99	Vegetables and fruits	Farm market	Rwanda	Ssemanda et al. (2018a)
Thermotolerant *Campylobacter* spp.	3/99				
Salmonella spp.	5/99				
Pathogenic *Escherichia coli*	6/99				

Bacillus cereus, Clostridium perfringens, *Staphylococcus* spp., *Salmonella* spp.	ND	Spinach, kenaf, lettuce, pepper, okra	Farms	Ghana	Adetunde et al. (2015)
Salmonella spp.	10/20	Lettuce	Garden	Burkina Faso	Traoré et al. (2015)
Staphylococcus aureus	36/150	Lettuce, cabbage, tomato, carrot	Farms	Ethiopia	Weldezgina and Muleta (2016)
Salmonella spp.	31/150				
Enterococcus spp.	73/100	Vegetables	Farms	Tunisia	Ben Said et al. (2016)
Escherichia coli O157:H7	4/180	Vegetables	Shops, supermarkets, open-air markets	South Africa	Abong'O et al. (2008)
Bacillus cereus, *Staphylococcus aureus*, *Listeria monocytogenes*	ND	Frozen vegetables	Supermarkets	Botswana	Manani et al. (2006)
Escherichia coli	ND	Tomatoes	Fields, markets	Nigeria	Shenge et al. (2015)
Escherichia coli O157:H7	4/180	Vegetables	Shops, supermarkets, open-air markets	South Africa	Abong'O et al. (2008)
Escherichia coli 0157	28/486	Leafy vegetables	Markets and supermarkets	Egypt	Khalil et al. (2015)
Shiga-toxin-producing *Escherichia coli*	6/486				
Escherichia coli	81/545	Vegetables	Street traders, trolley vendors, farmer's market	South Africa	Richter et al. (2021)
Salmonella spp.	10/106	Lettuce	Farms and markets	Senegal	Ndiaye et al. (2011b)

ND: Not determined

correlates with investigations from other regions (Ramirez-Hernandez et al. 2020; Townsend et al. 2021; Vital et al. 2014). However, there is sparse data on the prevalence of pathogenic microbes, especially antimicrobial-resistant bacteria, in fruits and vegetables along the supply chain compared to other parts of the world. In addition, in most studies, identifying indicator and pathogenic bacteria has been undertaken using phenotypic methods, which are limited in providing reliable information. The microbiological quality of fruits and vegetables has been of increasing interest to researchers (Aiyedun et al. 2021; Machado-Moreira et al. 2019). However, more studies are required to understand better how current food systems influence the microbial profile of fresh produce as it relates to safety. In addition, the role of fresh produce as a vehicle for antimicrobial resistance requires consideration.

9.3 Antimicrobial Resistance (AMR) in Fresh Produce in Africa

Gram-negative bacteria are predominantly implicated in many outbreaks linked with fresh produce consumption (Blaak et al. 2014; Vital et al. 2017). Furthermore, some of these Gram-negative bacteria have been reported to increasingly become resistant to multiple antibiotics, making the choice of antimicrobial therapy difficult (Oliphant and Eroschenko 2015a). Fresh produce is minimally processed and consumed raw for the most part. Hence, humans may be infected with antimicrobial-resistant bacteria through fresh produce consumption (Blaak et al. 2014; Van Hoek et al. 2015).

Antimicrobial resistance genes can be disseminated into the environment through faecal matters, contaminating surface waters and soils (Said et al. 2015; Thanner et al. 2016). The resistance genes may then be transferred to the bacteria found in the environment via contaminated irrigation water, inadequately treated manure, and soils. Resistant microorganisms transmitted to crops proliferate or survive in the growing stems and may remain on crops until consumed (Schwaiger et al. 2011; Van Hoek et al. 2015; Holzel et al. 2018).

The increasing occurrence of antibiotic-resistant bacteria, particularly *Enterobacteriaceae*, in healthcare systems, the environment, and fresh produce is of serious concern globally. Microorganisms can develop resistance to certain classes of antibiotics through chromosomal genes mutation (Munita and Arias 2016; Partridge 2015). In *Enterobacteriaceae*, resistance mainly occurs due to resistance genes carried on various mobile genetic elements (Munita and Arias 2016; Partridge 2015). Resistance genes on plasmids can be transferred between cells of different bacteria and species (horizontal transfer of genes) and can also be transferred during cell division – vertical transfer (Munita and Arias 2016; Partridge 2015).

Some antimicrobial classes in healthcare include penicillins, cephalosporins, carbapenems, aminoglycosides, vancomycin, tetracyclines, fluoroquinolones, and sulphonamides (Oliphant and Eroschenko 2015b). These antimicrobial agents have different mechanisms through which they act against bacteria. For instance, some agents like penicillins and cephalosporins are β-lactams that counteract

bacterial infections by inhibiting bacterial cell wall synthesis (Oliphant and Eroschenko 2015b).

However, bacteria like the *Enterobacteriaceae* produce the β-lactamases, which render β-lactams inactive (Oliphant and Eroschenko 2015a). Members of the *Enterobacteriaceae* family have emerged with resistance to penicillin and the broad-spectrum cephalosporins, resulting from the extended-spectrum β-lactamases (ESBLs) production becoming a threat globally (Pitout and Laupland 2008; Blaak et al. 2014; Zurfluh et al. 2015). Contaminated fresh produce may represent a route of human exposure to the ESBL-producing bacteria (Van Hoek et al. 2015). The dissemination of resistant bacteria between irrigation water and fresh produce can be related to a study done in South Africa by Du Plessis et al. (2015), which reported the transfer of bacteria from river water used for irrigation to irrigated onions.

In Switzerland, ESBL-producing *Enterobacteriaceae* have been isolated from vegetables imported from the Dominican Republic, India, Thailand, and Vietnam, with 25% of the isolates being ESBL-producing *Enterobacteriaceae* and 78% of them were identified with multidrug resistance (Blaak et al. 2014; Zurfluh et al. 2015). In Nigeria, there have been reported cases of ceftazidime- and cefuroxime-resistant bacteria from street-vended fruits and vegetables (Adekanle et al. 2015). Furthermore, third-generation cephalosporin (3GC)-resistant *Enterobacteriaceae* have been reported on some vegetables bought from Dutch stores (Van Hoek et al. 2015). *Escherichia coli* and *Klebsiella pneumonia* isolates have been reported as the predominant ESBL-producing organisms globally (Pitout and Laupland 2008; Van Hoek et al. 2015). However, other *Enterobacteriaceae* species such as *Citrobacter* spp., *Enterobacter* spp., *Kluyvera*, *Serratia*, and *Rahnella* also carry the ESBL genes and are found in agricultural soils, animal manure, and faecal contaminated water (Blaak et al. 2014; Van Hoek et al. 2015) thereby standing a chance of transferring the ESBL genes to the fresh produce. In Africa, AMR in fresh produce has been under-reported with limited data. Reports on the incidence of AMR bacteria from produce from various African regions are summarised in Table 9.2.

Microbial drug resistance is one of humanity's most pressing public health issues, but its significance in public health is underappreciated in many countries. Bacteria resistant to various antibiotics have been discovered in hospital and community settings; thus, there is no longer any localisation of drug resistance gene reservoirs to a specific location. Migratory birds, domestic animals, travellers, and the global movement of commercial food are among the natural forces responsible for spreading bacteria with multidrug-resistance properties (Islam et al. 2021).

Multiple drug resistance in bacterial isolates causing diarrhoea has severe implications for empiric therapy against pathogenic isolates and the possible co-selection of antimicrobial-resistant plasmids. Bacteria from clinical settings are known to harbour plasmids of various molecular sizes; it has also been widely reported that bacteria harbour antibiotic-resistant genes that can be horizontally transferred to other bacteria. In addition, antibiotic resistance among enteric pathogens has been reported to be increasing in developing countries, which could be due to environmental factors, geographic differences, or different antibiotic usage patterns (Ayukekbong et al. 2017).

Table 9.2 AMR in fresh produce from African regions

Country	Produce	Microorganisms	AM type resistance	Genome/ plasmid-mediated resistance	AMR genes	References
Southern Africa region						
South Africa	Variable fruits	*Salmonella* spp.	Chloramphenicol, kanamycin, trimethoprim, sulfamethoxazole, streptomycin, ampicillin, amikacin, amoxicillin-clavulanic acid			Gomba et al. (2016)
	Spinach Lettuce Cucumber Tomato Green beans	*E. coli*	Neomycin, gentamicin, ampicillin, amoxicillin, augmentin, cotrimoxazole, tetracycline, chloramphenicol, cefepime, imipenem			Richter et al. (2021)
	Lettuce	*E. coli*	Ceftazidime, cefotaxime, ceftriaxone, cefpodoxime, aztreonam	Plasmid		Njage and Buys (2015)
	Spinach	*Serratia fonticola*, *Escherichia coli* *Klebsiella pneumoniae*, *Rahnella aquatilis*	Amoxicillin, ampicillin, augmentin, cefoxitin, imipenem, cotrimoxazole, tetracycline			Richter et al. (2020)
	Spinach cabbage	*Escherichia coli*	Neomycin, kanamycin, ampicillin, trimethoprim-sulfamethoxazole, gentamicin, streptomycin, florfenicol, amoxicillin-clavulanate, aztreonam, chloramphenicol, cefoxitin, ciprofloxacin, cefepime, tetracycline, enrofloxacin, nalidixic acid			Du Plessis et al. (2017)
	Spinach Tomatoes Cucumbers Lettuce Green beans	*E. coli*, *Enterobacter cloacae* *Serratia fonticola*	Cefoxitin, cefoxitin, ceftazidime, Cefotaxime, cefoxitin		bla_{TEM}, bla_{SHV}, bla_{CTX-M}, $bla_{OXA,}$ bla_{ACC}, bla_{FOX}, bla_{MOX}, bla_{DHA}, bla_{CIT}, bla_{EBC}	Richter et al. (2019)

Eastern and Central African region						
Kenya	Vegetable salad	*Pseudomonas* spp. *Citrobacter freundii*	Streptomycin, gentamicin, amoxicillin, tetracycline, nalidixic acid, chloramphenicol, cefotaxime, trimethoprim + sulphamethoxazole			Muriuki et al. (2020)
Ethiopia	Lettuce Cabbage Tomato Carrot	*S. aureus* *Salmonella* spp.	Ampicillin, gentamicin, penicillin, chloramphenicol, tetracycline, erythromycin, norfloxacin, cotrimoxazole, cefuroxime-sodium			Weldezgina and Muleta (2016)
	Avocado potato	*E. coli* *S. aureus* *Salmonella* spp. *Proteus* spp. *Klebsiella* spp.	Ampicillin, clindamycin, cloxacillin, erythromycin, oxacillin, penicillin G, vancomycin, cefotaxime, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, doxycycline, nalidixic acid, norfloxacin, trimethoprim, sulphamethoxazole			Eromo et al. (2016)
	Lettuce Garlic Carrot	*Bacillus* spp. *E. coli* *Streptococcus* spp. *Corynebacterium* spp. *Neisseria* spp. *Salmonella* spp. *S. aureus* *Lactobacillus* spp.	Vancomycin, penicillin, ampicillin, chloramphenicol, perfloxacilin, erythromycin			Belay et al. (2020)

(continued)

Table 9.2 (continued)

Country	Produce	Microorganisms	AM type resistance	Genome/ plasmid-mediated resistance	AMR genes	References
Northern African region						
Algeria	Cucumber Tomato Watermelon Celery Mint Parsley Lettuce Chili Nectarine Pear Carrot Grape Peach Apple	*Enterobacter cloacae* *Enterobacter asburiae* *Klebsiella pneumonia* *Citrobacter murliniae*	Ampicillin, ticarcillin, cefamandole, cefuroxime, cephalothin, amoxicillin-clavulanic acid, cefoxitin, cefotaxime, ceftazidime, ceftriaxone, cefepime, aztreonam, ertapenem, gentamicin, kanamycin, streptomycin, tetracycline, tigecycline, ciprofloxacin, pefloxacin, nalidixic acid, trimethoprim-sulfamethoxazole, trimethoprim, sulphonamides; chloramphenicol, temocillin			Mesbah Zekar et al. (2017)
	Lettuce Carrot Onions Tomato Potato	*Escherichia coli* *Klebsiella pneumoniae*	Streptomycin, kanamycin, gentamicin, tobramycin, chloramphenicol, florfenicol, tetracyclines, sulphonamides, trimethoprim, nalidixic acid, enrofloxacin		$bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}14}$, $bla_{CTX\text{-}M\text{-}2}$, $bla_{SHV\text{-}2}$	Yaici et al. (2017)
Tunisia	Barley Tomato Parsley Fennel Radish Apricot	*E. coli* *Enterobacter hormaechei* *Citrobacter freundii* *Klebsiella pneumoniae*	Cefotaxime, chloramphenicol, ciprofloxacin, cefoxitin, gentamicin, nalidixic acid, streptomycin, sulphonamides, tobramycin, trimethoprim-sulfamethoxazole, tetracycline	Plasmid-mediated resistance	$bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}14}$, $bla_{SHV\text{-}12}$	Ben Said et al. (2016)

Western African region						
Ghana	Lettuce Cabbage	*E. coli*	Ofloxacin, ampicillin, erythromycin			Adzitey (2018)
		Salmonella enterica	Ofloxacin, erythromycin			
Nigeria	Lettuce Cabbage	*E. coli* O157 serogroups	Tetracycline, cephalothin	Plasmid	Quinolone resistance-determinant (*gyrA*)	Abakpa et al. (2015)
	Cucumber Guava Pineapple Tomatoes Tangerine Lemon Eggplant leaf African spinach *Worowo* Jute leaf	*Citrobacter freundii* *Citrobacter braakii* *Citrobacter youngae*	Ceftriaxone, amoxicillin, piperacillin, cefotaxime, gentamicin, augmentin, tetracycline, cotrimoxazole, nitrofurantoin		bla_{CTX}, bla_{SHV}, *aac6*	Adegun et al. (2019)
	Amaranth Fluted pumpkin leaves Scarlet eggplant leaf Water leaf	*E. coli*	Cefuroxime, norfloxacin, chloramphenicol, ciprofloxacin			Chigor et al. (2020a)
	Pineapples Watermelon	*E. coli* *Shigella flexneri* *Enterobacter hormaechei* *Enterobacter sichuanensis*	Tetracycline, ampicillin, sulbactam, gentamicin			Oyedele et al. (2020)

Multidrug-resistant *E. coli* isolates from spinach, apples, carrots, cabbage, and tomatoes from both the formal and informal fresh produce sector of South Africa have been reported to show resistance to aminoglycosides, cephalosporins, penicillins, and amphenicol antibiotic classes (Baloyi et al. 2021).

9.4 Sources of Antimicrobial-Resistant Pathogen Contamination

Antimicrobial-resistant bacteria contaminating fresh produce enter the produce value chain at various stages of production, from farm to the fork. Therefore, the sources of contamination in fresh produce vary depending on the production system and scale of cultivation. The sources of contamination of fresh produce in Africa by antimicrobial-resistant pathogens could be categorised as on-farm/field, processing, distribution, retail, and food preparation sources.

9.4.1 On-Farm Sources of Contamination

9.4.1.1 Soil

This is the primary resource for growing crops in Africa, including fruit and vegetables. However, the soil is a complex environment harbouring indigenous populations and pathogenic bacteria. Some foodborne pathogens, such as *Listeria monocytogenes* and *Bacillus cereus*, are natural contaminants of soil (Weis and Seeliger 1975; Dowe et al. 1997; Vilain et al. 2006; Stenfors Arnesen et al. 2008; Locatelli et al. 2013). Furthermore, some others that are not natural soil contaminants have been reported to survive for extended periods in the soil. They include *E. coli* (Mukherjee et al. 2006; Ibekwe et al. 2007; Somorin et al. 2016); *Salmonella* (Uyttendaele et al. 2014), and *Campylobacter* (Donnison and Ross 2009). In addition, many African soils used for cultivating fresh produce are often contaminated by irrigation water and manure, animal grazing, municipal solid wastes, and other effluents (Santamaria and Toranzos 2003; Amoah et al. 2005). When crops are grown in such contaminated soils, pathogens could be transmitted through the seeds or roots to other parts of the plant, where they could become internalised and persist in vegetables (Ibenyassine et al. 2006; Ávila-Quezada et al. 2010; Solomon et al. 2002; Wright et al. 2013).

9.4.1.2 Seeds

Contaminated seeds constitute an important contamination route for some fresh produce, particularly edible sprouts (Proctor et al. 2001), as the pathogens could become internalised during germination (Liu et al. 2017a, b). The internalisation of pathogens promotes the transmission of the pathogens within the plant, which

makes the pathogens challenging to eliminate (Wright et al. 2013). For example, contaminated fenugreek seeds from Egypt were implicated in a large multi-country *E. coli* STEC O104:H4 outbreak in Europe and North America in 2011 (EFSA 2011).

9.4.1.3 Organic Manure/Soil Amendment

Using raw or poorly treated manure and compost is a significant source of direct contamination of fresh produce with *E. coli* O157:H7 and *Salmonella enterica* (Beuchat 2002). In addition, it has been shown that the application of manure increases the population of antimicrobial-resistant bacteria in soil (Fatoba et al. 2021, 2022; Udikovic-Kolic et al. 2014). Nevertheless, smallholder farmers commonly use poultry manure as a fertiliser in Africa because it is relatively cheap and readily available (Amoah et al. 2007; Orji et al. 2005). For example, it is used by over 70% of producers of irrigated lettuce in Ghanaian cities (Amoah et al. 2007) and in maize and pepper farms in Nigeria (Omeike et al. 2021).

Live chicken, cattle, and other animals in many African countries harbour antimicrobial-resistant pathogens, including *E. coli* O157 (Abdalla et al. 2021; Abakpa et al. 2015); extended-spectrum beta-lactamase (ESBL)- and carbapenemase-producing (CPE) *E. coli* (Abdallah et al. 2015); ESBL *Salmonella* (Abdel-Maksoud et al. 2015; Abd-Elghany et al. 2015; Abdellah et al. 2009); *Aeromonas caviae* (Abu-Elala et al. 2015); *Enterococcus* (Molechan et al. 2019); *Campylobacter* (Sithole et al. 2021); and *Listeria monocytogenes*, which they shed in their faeces (Arrus et al. 2006; Abakpa et al. 2015; Delahoy et al. 2018).

These pathogens can survive in manure slurry (Arrus et al. 2006), and if the faecal materials are not adequately composted before use as organic manure/fertiliser, they could transmit pathogens to the developing fruit or vegetable. In addition, pathogenic bacteria deposited into the soil by organic fertilisers and soil amendments could be transferred to fresh produce by water droplets splashing from rain and irrigation (Girardin et al. 2005; Monaghan and Hutchison 2012; Cevallos-Cevallos et al. 2012; Ndiaye et al. 2011a).

9.4.2 Exposure to Contaminated Water

9.4.2.1 Irrigation

Using contaminated irrigation water could be a source of contamination of fresh produce. Shallow groundwater, wastewater, and well water are often used for irrigating fresh produce in Africa (Ndiaye et al. 2011a). Of the types of water used for irrigation, wastewater and surface waters have poor microbial quality. Surface water sources are often polluted by water runoff, animal faecal material, or sewage effluent. Irrigation water could be contaminated by faecal coliforms and pathogens like cephalothin-resistant *E. coli* O157 and *Salmonella* spp. (Ndiaye et al. 2011b; Abakpa et al. 2013, 2015; Uyttendaele et al. 2014).

The occurrence of plasmid-mediated multidrug-resistant *E. coli* O157 in surface waters used for irrigating fresh produce has been reported (Chigor et al. 2020b). Furthermore, groundwater and rainwater are important sources of irrigation water, which have better microbial quality than surface water. For example, groundwater is used for 78% of irrigation in South Africa (Water Research Commission 2019). However, urbanisation and increasing population size have further put pressure on the scarce land and water resources, and most farmers have limited land for farming in African countries. This situation increases the risk of contamination of fresh produce by pathogenic organisms (Jongman and Korsten 2018). In addition, many other countries depend on rainwater for irrigation. However, climate change is causing reduced rainfall in many parts of Africa (World Meteorological Organization 2020), thus leading to greater dependence on other water sources, which may be of poor microbiological quality, for irrigation. This consequently results in more frequent contamination of fresh produce.

The irrigation method also plays a vital role in contaminating fresh produce. For example, irrigation using sprinkling water over the plants with watering cans resulted in higher crop contamination than when drip irrigation was used (Ndiaye et al. 2011a; Keraita et al. 2007). However, when furrow irrigation was used, there was lower contamination with *E. coli* in the irrigated vegetables even when there was a high *E. coli* concentration in the irrigation water (Ensink et al. 2007; Ndiaye et al. 2011a). On the other hand, irrigation using watering cans results in higher contamination of vegetables due to splashes causing deposition of pathogens from animal manure in the soil.

9.4.2.2 Sewage Sludge/Runoff Water

Home gardens and smallholder farms situated close to sewage pits may experience leakage in their sewage system, leading to seepage of sewage into nearby soils and contamination with faecal pathogens. Domestic sewage and runoff are used to water vegetables such as lettuce, cabbage, and cucumber in Nigeria (Abdullahi and Abdulkareem 2010), and the frequent use of wastewater is mainly due to water scarcity (Ndiaye et al. 2011a).

9.4.3 Faecal Contamination from Livestock and Wild Animals

Livestock and wild animals could be natural habitats for antimicrobial-resistant pathogens. Many animals harbour enteric pathogens, which are shed along with faeces (Moriya et al. 1999; Corrente et al. 2004; Foster et al. 2006; Kaufmann et al. 2006; Sproston et al. 2006). For example, norfloxacin-resistant *E. coli* O157 was found in the faeces of sheep and goats (Abreham et al. 2019). Similarly, multidrug-resistant *Listeria monocytogenes* was reported in cow faeces (David and Odeyemi 2007), while methicillin-resistant *Staphylococcus aureus* (MRSA) harbouring the

mecA gene and *SCCmec* mobile genetic element was detected in faeces of healthy chicken (Amoako et al. 2019). Besides the direct contact of faecal materials with the soil upon which fresh vegetables are produced, insect pests could also transmit antimicrobial-resistant pathogens from faecal materials to fresh produce (Ignasiak and Maxwell 2017).

9.4.3.1 Farm Workers

Individuals involved in the primary production of fruits and vegetables could also be a source of contamination. Unsanitary practices by farm workers, such as open defaecation and not washing hands properly after defaecation, could lead to the transmission of enteric pathogens to fresh produce. Farm workers could harbour methicillin-resistant *Staphylococcus aureus* (Amoako et al. 2019), and this could be transmitted to leafy vegetables when they sneeze without using face covering. This becomes particularly worrying during harvest when farm workers sneeze into their palms and use the same hands (without washing) to harvest fresh produce manually.

9.4.3.2 Farm Implements

Farm tools, such as hand trowels and rakes that have come in contact with contaminated soil or manure, could be vehicles for transmitting antimicrobial-resistant pathogens to fruits and leafy and root vegetables during crop husbandry, such as transplanting and harvesting (Amoako et al. 2020). In addition, contaminated knives and other tools used to harvest leafy vegetables and buckets, baskets, and sacks used to transport the produce from the farm could contaminate the fresh produce.

The on-farm sources of contamination of fresh produce are summarised in Fig. 9.1.

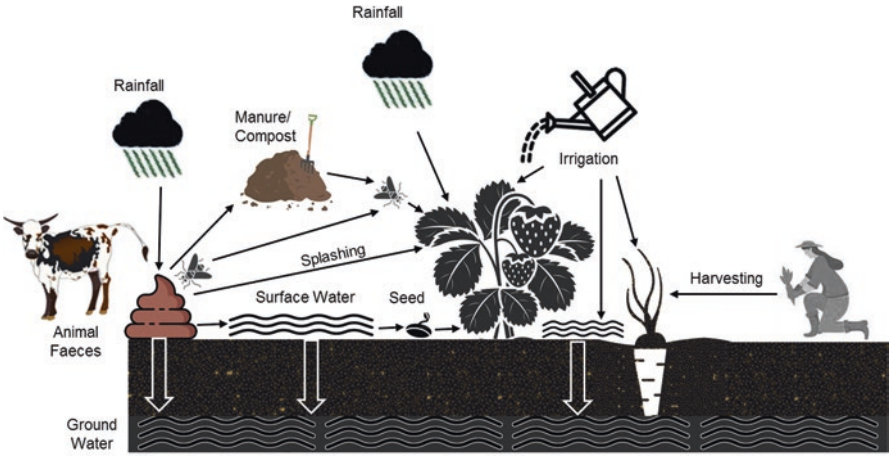

Fig. 9.1 On-farm sources of antimicrobial-resistant pathogen contamination in fresh produce production in Africa

9.5 Processing/Postharvest Sources of Contamination

9.5.1 Washing

Vegetables are sometimes washed with water on/around the farm to remove dirt before being transported to distribution centres or retail markets (Fig. 9.2). However, washing fruits and vegetables with contaminated water could transmit antimicrobial-resistant pathogens to the fruit and vegetables. For example, washing vegetables in the field was crucial in contaminating lettuce with *Salmonella* (Ndiaye et al. 2011a).

9.5.2 Handling During Processing

Poor hygiene, lack of handwashing, and other unsanitary practices have been reported among individuals handling fresh produce (Akoachere et al. 2018). In addition, handling fresh produce without proper personal protection equipment could expose the fresh produce to pathogens from the handler's saliva and mucus. Cracks on surfaces in food processing facilities could also be a source of persistent pathogen contamination during processing (Leong et al. 2017).

9.5.3 Transport, Distribution, and Storage Equipment

Transportation of fruits and vegetables to market centres and other retail points in open and non-refrigeration conditions (Fig. 9.2) exposes the produce to contaminants and environmental conditions that promote the proliferation of pathogens

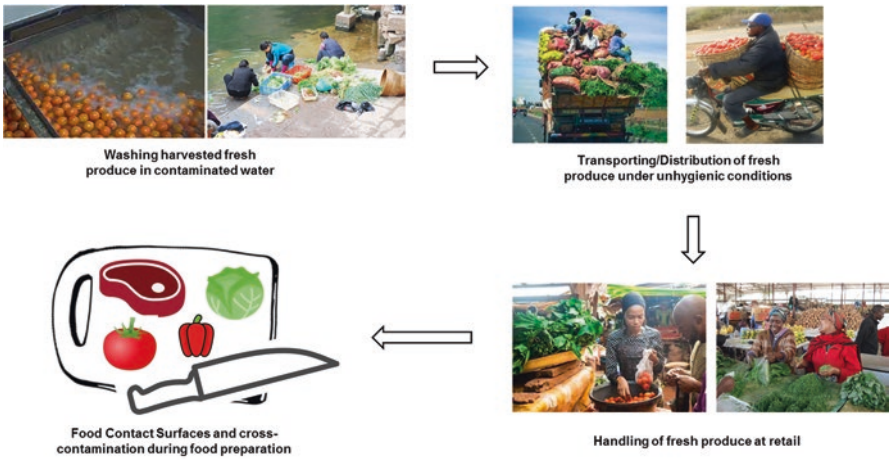

Fig. 9.2 Postharvest sources of antimicrobial-resistance pathogen contamination in fresh produce production

(Akoachere et al. 2018). Fresh produce transported under these conditions is further exposed to pathogen-harbouring insects and faecal materials from animals or humans sitting on them. Transport vehicles and crates have been reported to be sources of *S. aureus* contamination (Amoako et al. 2020), while bags/sacks, previously used for collecting poultry waste, have been reported to be used to transport fruits and vegetables (Omeike et al. 2021).

9.5.4 Handling During Retail

In Africa, fresh produce is sold mostly in open markets with limited sanitary facilities. As a result, fruits and vegetables are not protected from environmental contaminants and are often touched with bare hands (Fig. 9.2). Retail fresh produce has been reported to be more contaminated than those collected directly from primary production/farms (Uyttendaele et al. 2014; Quansah et al. 2018), thus suggesting postharvest handling contributes significantly to microbial contamination of fresh produce. Retailers often cut vegetables to make them easier for consumers to use. However, chopping boards are rough and cannot be thoroughly cleaned or washed, and the knives used are not cleaned and/or disinfected. As a result, *E. coli* O157:H7 has been found on cutting boards in retail shops (Abdissa et al. 2017). Furthermore, cut vegetables are sometimes placed in dirty bags during sales, and unsold vegetables are kept in the market or left uncovered in the backyard of houses (Akoachere et al. 2018), where they are exposed to further contamination.

9.5.5 Cross-Contamination During Food Preparation

Cross-contamination of fruit and vegetables from contaminated foodstuff during preparation could be another source of contamination. In addition, food processing surfaces and other foodstuffs which are not adequately washed or separated during food preparation may be a source of contamination (Abayneh et al. 2019).

Foods often prepared alongside fresh produce have been reported to be contaminated by antimicrobial-resistant pathogens. For example, several meat products (mutton, beef, chevon, guinea fowl, and chicken) in different parts of Africa have been reported to carry antimicrobial-resistant *E. coli*, *Salmonella* spp., *Staphylococcus aureus*, and *Shigella* spp. (Adzitey 2020; Adzitey et al. 2020; Ahmed et al. 2016; Amoako et al. 2020; Afnabi et al. 2019; Ahmed and Shimamoto 2014; Al-Gallas et al. 2013). Antimicrobial-resistant *Vibrio* spp. were found in shrimps, crabs, and cuttlefish, with 18% of the isolates resistant to all (10) antibiotics tested (Adeleye et al. 2008). *V. parahaemolyticus* and *V. cholerae* were found in crab and shrimp (Ahmed et al. 2018a), while antimicrobial-resistant *Salmonella* was detected in seafood (Al-Gallas et al. 2013) and *Aeromonas hydrophila* in fish (Ahmed et al. 2018b; Algammal et al. 2020). Dairy products, such as cheese, often

used to make salads and vegetables, could be contaminated with MRSA (Ahmed et al. 2019). Antimicrobial-resistant *Staphylococcus aureus* and MRSA have also been detected in African indigenous cheeses, such as Damietta and Kareish cheese (Al-Ashmawy et al. 2016).

Since some of the pathogens found in these food items harbour integrons and gene cassettes that could transfer multiple-drug resistance to other pathogens (Ammar et al. 2016), their presence in foods and food preparation environments could become a source of antimicrobial resistance in fresh produce.

9.6 Strategies for Controlling Antimicrobial-Resistant Pathogens in Fresh Produce

To minimise contamination with antimicrobial-resistant foodborne pathogens in fruits and vegetables, it is important to create awareness of the practices that promote bacterial contamination throughout the supply chain and take necessary actions to reduce or possibly avoid them. Therefore, all actors within the fresh produce value chain (from the farmers/farm workers through the processors and retailers to the consumers) must be continuously educated on good agricultural, handling, and storage practices, some of which include:

(a) *Disinfecting seeds before planting*: Disinfection of seeds with 5% commercial bleach (hypochlorite) and dry heating at 45 °C can remove bacterial infections without affecting the germinability of the seeds (Taški-Ajdukovic and Vasic 2005). Other treatment options for reducing or removing bacterial pathogens from vegetable seed surfaces include chlorine solutions, acid, and hot water treatment (Li et al. 2014; Saunders and Everis 2014).

(b) *Organic soil amendments*: Using organic amendments has been shown to reduce the population of soilborne pathogens (Noble 2011). Organic manure must be stored and treated in such a way as to ensure proper composting before application to soil. The heat and microbial community generated during the composting process inactivate foodborne pathogens and reduce the risk of fresh produce contamination (Gurtler et al. 2018).

(c) *Irrigation*: Groundwater and rainwater should be used to irrigate fresh produce as they are of better microbial quality than ponds, lakes, and rivers. Irrigating fresh produce with river and marshland water should be avoided as they have a high prevalence of pathogens (Ssemanda et al. 2018a). Furthermore, irrigation using sprinklers and watering cans should be avoided because of their higher likelihood of depositing pathogens on fresh produce crops through splashing. Alternatively, drip and furrow irrigation should be encouraged (Ndiaye et al. 2011a).

(d) *Avoiding faecal contamination from animals*: Vegetable farms should not be located near poultry farms as faecal materials from these farms could be carried by runoff to the vegetable farms. Also, mixed farming should be discouraged,

where livestock is reared or allowed to roam around vegetable farms. This is because the animals' faecal droppings could carry pathogens, which could be readily transferred to the vegetables. Barriers should be erected around vegetable farms to prevent access by livestock and wild animals.

(e) *Handwashing and hygienic practices:* Adequate sanitation facilities must be provided for employees on the farm, processing plants, distribution points, and retailers. Good hygienic practices such as handwashing with soap should be regularly practised in farms and produce handling environments. Hand sanitisers should be provided where soap and water are unavailable (de Aceituno et al. 2015).

(f) *Farm implement*: Tools used in fresh produce cultivation should be regularly disinfected to avoid the transmission of pathogens. Tools, such as boots, shovels, and spades, used on raw sewage or manure could transfer enteric pathogens to fresh produce. Hence, separate tools should be used for other farming practices. Where not possible, such tools should be thoroughly cleaned and disinfected before use in other farm operations.

(g) *Washing harvested produce:* Washing fresh produce in clean water with other disinfectants reduces pathogen contamination and cross-contamination of fresh produce through the wash water. Appropriate addition of disinfectants to water used for washing fresh produce is an important strategy for controlling pathogens in fresh produce. Chlorine is widely used for washing fresh produce and has been demonstrated to significantly reduce *E. coli* O157:H7 cross-contamination in fresh produce through wash water (Luo et al. 2011; Tomas-Callejas et al. 2012). Washing fresh produce in atmospheric cold plasma (ACP)-treated water was found to reduce bacteria contamination on fresh produce and inactivate bacteria in wash water (Patange et al. 2019). Plasma treatment of wash water reduces the survival of *E. coli* compared to tap water (untreated water) (Fridman et al. 2021). This allows reusing processing water for washing and reduces contamination of subsequent batches of fresh produce from washing water. UV is also effective in reducing *E. coli* O157:H7 on spinach leaves surfaces and in fresh produce wash water (Cossu et al. 2016) and reducing *Salmonella* on blueberry, tomato, and lettuce (Huang and Chen 2020).

(h) *Safe transportation, distribution, and storage:* Fresh produce should be transported, distributed, and stored under sanitary conditions that prevent microbiological contamination. Vehicles that transport waste and manure should not be used to transport fresh produce. Transport vehicles should have clean floors and walls, and fresh produce should not be sat upon. Furthermore, fresh produce should be maintained at lower (refrigerated) storage temperatures during transportation, distribution, and display for retail to ensure microbiological safety (Zhang et al. 2020; Rapusas and Rolle 2009). Reusable plastic containers (RPCs) for transporting and handling fresh produce have been shown to reduce postharvest losses, although some pathogens, such as *Salmonella* spp., can survive on some RPC materials, and there is a risk of cross-contamination (López-Gálvez et al. 2021). Hence, RPCs and bags, sacks, and other containers used for transporting and storing fruit and vegetables should be cleaned and sanitised

after each use to prevent cross-contamination with pathogenic microorganisms.

(i) *Handling during processing:* Proper hygiene and handwashing are important for reducing microbial contamination of fresh produce from farm workers/handlers. Toilet and handwashing facilities should be provided for use by farm workers/handlers. Using a two-step alcohol-based hand sanitiser significantly reduces soil and bacterial contamination (coliforms, *Escherichia coli*, and *Enterococcus* spp.) on the hands of farmworkers (de Aceituno et al. 2015, 2016). This is especially useful in farms and handling environments where soap and water are unavailable, as is prevalent in many agricultural contexts in Africa. Regular education and training on basic sanitation and hygienic practices should be provided to postharvest and processing operations personnel. This would improve their knowledge and practice on safe fresh produce handling. The use of personal protective equipment (PPE) should be ensured to prevent contamination of fresh produce by handlers. These include suitable aprons, coveralls, hair nets, beard guards, hand gloves, face masks, and footwear covers. Training in the appropriate use of these PPE should also be ensured.

(j) *Handling at retail and household levels:* Good handling practices at postharvest, retail, and consumers are important to minimise pathogen contamination. Retailers and customers buying fresh produce should avoid touching the fresh produce displayed for sale. Washing and rinsing vegetables with clean water are important to reduce microbial contamination (James 2006), although this might be a challenge in countries with limited access to potable water. Washing fresh produce with river and marshland water should be avoided as they have a higher prevalence of pathogens than groundwater (Ssemanda et al. 2018a). Washing vegetables with sanitisers, organic acids, lemon juice, and hot water reduces the load of *Listeria* spp., *E. coli*, and aerobic plate counts (Ssemanda et al. 2018b). Furthermore, cross-contamination should be avoided during food preparation. For example, raw meat, chicken products, and vegetables should not be cut on the same chopping board with the same knife, as pathogens could be transmitted through these food preparation surfaces (Gorman et al. 2002; Redmond et al. 2004).

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Chapter 10
Biosecurity and Disinfectant Resistance in a Post-antibiotic Era

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10.1 Introduction

The use of antibiotics significantly impacts animal production, from the treatment of lethal bacterial infections to mass use as growth promoters. The agricultural industry relies heavily on the efficacy of antibiotics, which is why the development of antibiotic resistance is a significant threat to animal production worldwide. Antibiotic resistance is equally a threat to infection control in the medical industry. Antibiotic-resistant infections are becoming more prevalent as multiple courses of antibiotics are needed to treat infections in individuals. In addition, the emergence of antibiotic-resistant strains in hospitals is a major concern as the number of deaths due to nosocomial infections increases annually (CDC 2021).

As the prevalence of resistant microorganisms increases, a coupled phenomenon has been identified: simultaneous resistance to other antimicrobials (Kim et al. 2018a, b). Microorganisms with decreased susceptibility to several antimicrobials are labelled multi-drug-resistant (MDR) and can breach biosecurity measures resulting in persistent infections and mass loss of livestock (Gibbens et al. 2001; Laanen et al. 2013).

The increased prevalence of MDR microorganisms may lead us into a post-antibiotic era when commonly used antibiotics are less effective and bacterial infections can persist until lethal. Effective biosecurity measures are currently the best defence against MDR bacteria (Bragg et al. 2018). Biosecurity has proven to be an effective preventative treatment to control microbial growth and reduce the need for antibiotics (Laanen et al. 2013; Postma et al. 2016). Biosecurity refers to the

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approaches to protect humans, livestock, and plants against biological agents, such as microorganisms and the toxins they may produce (Waage and Mumford 2008). Biosecurity relies heavily on effective disinfection, and the rise of disinfectant resistance is a major threat to controlling microbial growth.

The lesser-known problem of microbial resistance to disinfectants and antiseptics is emerging at an alarming rate (Sidhu et al. 2002; Bjorland et al. 2005). The development of microbial resistance is primarily due to abuse and improper use of antimicrobials (Bragg et al. 2014). In addition, disinfectant resistance may emerge from insufficient or ineffective disinfection programmes due to inexperience of farm, healthcare, or cleaning workers or improper disinfection protocols. This creates selective pressure and allows microorganisms time to develop resistance mechanisms. The most common resistance mechanism is due to efflux pump proteins acquired by horizontal gene transfer (Ma et al. 1994; Chitsaz and Brown 2017). Multidrug efflux pumps allow for the simultaneous development of antibiotic resistance and disinfectants in a microbial population (Jonas et al. 2001; Chitsaz and Brown 2017; Kim et al. 2018b). A new resistance mechanism is disinfectants' metabolism and subsequent use as an energy source (Dean-Raymond and Alexander 1977; Nishihara et al. 2000; Patrauchan and Oriel 2003; Bassey and Grigson 2011; Oh et al. 2013). The large amounts of disinfectants used in industry result in significant runoff into water systems and environmental accumulation. This constant exposure of environmental isolates to disinfectants encourages the development of survival mechanisms such as the metabolic use of these antimicrobial compounds. The ability of microorganisms to metabolise disinfectants may have devastating effects on the agricultural industry if we do not use current disinfectants responsibly and only when necessary.

Advances in sequencing technology have elucidated microbial resistance mechanisms and added to the understanding of how antimicrobial resistance develops (Kim et al. 2018a; Marcelino et al. 2019). Bioinformatics and genomics can also be used in antimicrobial surveillance and global tracking (Grundmann 2014; Wang et al. 2018; Dewey-Mattia et al. 2018). DNA and RNA sequencing may target MDR bacteria by screening for resistance and susceptibility to various antimicrobials (Khaledi et al. 2016; Blanco et al. 2018; Qin et al. 2018). Sequencing coupled with bioinformatics may also allow tracking of resistance genes and shed light on preventing the dissemination of MDR bacteria (Hendriksen et al. 2019). This technology can further provide insight into how to hinder the development of known resistance mechanisms and identify novel resistance mechanisms (Bikard et al. 2014).

10.2 Alternatives to Antibiotics

Since their discovery, antibiotics have been unregulated and, due to a lack of understanding, sometimes at sub-therapeutic levels (Gerba 2015; Bragg et al. 2018). The most effective alternative to antibiotic use is effective biosecurity procedures and

the proper use of disinfectants to control microbial growth (Bragg et al. 2018). Alternatively, vaccines, bacteriophage therapy, and even herbal products have shown antimicrobial properties and are capable of biological control. However, all these alternatives have limitations and are still under development (Bragg et al. 2018).

Antibiotic resistance is a major threat to public health. Continuous use of antibiotics increases selective pressure for less susceptible bacterial strains (Aarestrup 1999; Acar et al. 2012; Woolhouse and Ward 2013; WHO 2014; Wall et al. 2016; Hoelzer et al. 2017; Morrison and Zembower 2020). To minimise this risk, antibiotics should only be used when necessary and responsibly, i.e. proper antibiotic drug selection regarding treatment (Aarestrup 1999; Woolhouse and Ward 2013; Wall et al. 2016; Hoelzer et al. 2017). Due to the increased cost of developing novel antibiotics and the prevalence of antibiotic resistance, research has shifted to non-antibiotic alternatives (Hoelzer et al. 2018). Alternative treatment methods will reduce the need for antibiotics as they will aid in preventing and controlling diseases in animal production (Hoelzer et al. 2018).

10.2.1 Vaccines

Current veterinary vaccines have severe limitations (Hoelzer et al. 2018). The ideal vaccine is safe, protects against a broad spectrum of pathogens, is easily administered, and is cost-effective. However, all these requirements are challenging to achieve in the field (Hoelzer et al. 2018). In addition, current live vaccines are limited in efficacy and duration of the immunity they produce and can have severe side effects (Jores et al. 2013). Unfortunately, there is no simple solution to developing safer and more effective vaccines. This process is made more difficult by the limited knowledge of host-pathogen interactions (pathophysiological and immunological processes), complications with data interpretations, and the additional regulatory requirements necessary for licensing genetically modified live vaccines (Jores et al. 2013).

Amongst pigs in the swine industry, infection and economic losses increase yearly due to antibiotic-resistant infections and a lack of vaccines for viral infections. African swine fever virus (ASFV) is a devastating and economically significant disease, and there is currently no vaccine available for ASFV (Gaudreault and Richt 2019). The current control methods for ASFV are culling infected animals and quarantine (Gaudreault and Richt 2019). ASFV, being endemic to Africa, poses a significant threat to swine around the world and the development of a vaccine is of high priority (Costard et al. 2009; Cwynar et al. 2019; Gaudreault and Richt 2019). However, the search for a vaccine has been largely unsuccessful, and this is because the virus is highly complex and little is known about the virulence factors and the correlation to protection (Chapman et al. 2008; Costard et al. 2009; de Villiers et al. 2010; Dixon et al. 2013; Alonso et al. 2018).

Inactivated vaccines were proven to be ineffective against ASFV (Stone and Hess 1967; Blome et al. 2014), but more success was seen with naturally attenuated

isolates (King et al. 2011; Gallardo et al. 2018) and modified live viruses (O'Donnell et al. 2015a, b, 2016; Reis et al. 2016, 2017; Monteagudo et al. 2017; Gallardo et al. 2018). Live attenuated vaccines have various limitations, including severe side effects and protection only against homologous strains of the same genotype (Leitão et al. 2001; Reis et al. 2007; King et al. 2011; Abrams et al. 2013; Mulumba-Mfumu et al. 2016). In addition, this type of vaccine may cause chronic infections, revert to a virulent state and lack a stable cell line for production (Gaudreault and Richt 2019). As an alternative, subunit and vectored vaccines have been explored (Gaudreault and Richt 2019). A targeted approach is given by subunit and DNA-based vaccines with fewer side effects and are safer than live and inactivated vaccines (Gaudreault and Richt 2019). Recent studies showed that a vaccine capable of stimulating a T-cell-mediated response is required in addition to antibodies to protect against ASFV (Gaudreault and Richt 2019). In addition, DNA vaccines induce a cell-mediated CTL immune response, which plays a vital role in protecting the host against ASFV (Oura et al. 2005). Another promising option is viral vectors, as they can be used to stimulate both humoral and cell-mediated immune responses (Gaudreault and Richt 2019). The virulence genes of the respective virus are removed or replaced by immunogens, or the virus vector replication is made incompetent (Gaudreault and Richt 2019). An advantage of this method is that the virus vector encoding immunogens serve as vaccine markers (Gaudreault and Richt 2019). Despite this, little success has been achieved with vaccine development and efficacy against ASFV (Gaudreault and Richt 2019).

In the poultry industry, the Newcastle disease (ND) has significant economic implications (Alexander 2001). Over the last 60 years, both live attenuated and inactivated vaccines have been used to fight against ND, with live vaccines being the most efficient, despite their limitations (Perelman and Goldman 2013; Bello et al. 2018). Vaccination is essential to protect birds from Newcastle disease virus (NDV) as vaccines stimulate the bird's mucosal and systemic immune response (Bello et al. 2018). However, new vaccination procedures are required since conventional vaccination methods are not cost-effective (high vaccination cost per bird) and virus shedding is a major problem (Rehmani et al. 2015). Furthermore, live attenuated vaccines may revert to a virulent state and cause disease in the vaccinated bird. In addition, some postvaccination respiratory reactions may occur, particularly in young birds, which makes them susceptible to secondary bacterial infections (Winterfield et al. 1980).

Inactivated vaccines remain the earliest strategy in poultry vaccination (Bello et al. 2018). However, they have one major limitation, no means of mass application. In addition, these virions within the vaccine cannot replicate, and therefore the process is expensive and laborious due to individual administration (Bello et al. 2018).

DNA vaccines are also a promising alternative as they are efficient and induce CD4+ and CD8+ immune responses (Bello et al. 2018). However, many limitations exist, such as poor immunogenicity, high production costs, and no means of mass administration. In addition, the vaccines must also be delivered under specific

conditions to counteract being degraded by nucleases before reaching their final destination (Bello et al. 2018).

Virus-like particles (VLPs) are another alternative to prevent infection and poultry disease. The ratio of proteins in the VLPs is similar to that of the wild-type virus (Pantua et al. 2006). However, it might be challenging to produce large amounts of this vaccine in any other platform than baculovirus as an expression system (Park et al. 2014). VLPs also need to be individually administered because they cannot replicate in the chicken in large quantities. Therefore, VLPs are best administered with adjuvants to elicit an effective immune response (Morrison 2010).

Reverse genetics-based vaccines are the latest strategy of vaccine development which is the recovery of a recombinant virus from its cloned cDNA (Pfaller et al. 2015). However, these vaccines may be expensive to develop due to sequencing and molecular biology costs, although the costs are expected to decrease (Bello et al. 2018).

Various other limitations lead to the limited efficacy of veterinary vaccines, such as quick evolving pathogens and limited cross-protection amongst bacterial and viral strains (Hoelzer et al. 2018). Additionally, booster vaccines may be required as protection by vaccination can be short-lived (Hoelzer et al. 2018). In the case of inactivated and subunit vaccines, protective immune responses have generally not been generated at all so far (Hoelzer et al. 2018). These vaccines do not actively replicate in the host cells by their nature. Thus, only a humoral immune response is induced. For effective protection against many pathogens, cellular immune responses are required (Hoelzer et al. 2018). Vaccine administration can be a major problem because to develop a protective immune response, precision is needed for each bird to account for the lag period (Hoelzer et al. 2018).

The above discussion on viral vaccines in animal production highlights the importance of a good biosecurity programme to control these diseases.

10.2.2 Bacteriophages

Bacteriophages are another possible alternative to antibiotic treatment to control disease in animal production, but few studies have been done on this treatment option (Gomes and Henriques 2016). Bacteriophages are viruses that can infect and kill bacteria (Chibani-Chennoufi et al. 2004). Previous studies used phage K as an alternative treatment against *S. aureus* infections (Gomes and Henriques 2016). However, to be used as a therapeutic measurement against mastitis, the phages must be active in the mammary gland (Gomes and Henriques 2016). O'Flaherty et al. (2005) showed that phage K was inhibited by natural milk and udder secretions. In addition, it was found that phage K can undergo degradation or inactivation by the milk proteins and the cow's immune system (Gill et al. 2006). Despite bacteriophage therapy showing promise as an additional alternative to antibiotics, research is lacking in pharmacokinetics and phage administration (Basdew and Laing 2011).

Additional alternatives exist, such as predatory bacteria, bacteriocins, and even essential oils (Gomes and Henriques 2016). However, all these alternatives have shortfalls and are still in development. Therefore, the only viable option to control bacterial growth is good biosecurity using disinfectants (Bragg et al. 2018).

10.3 Biosecurity in Animal Production

10.3.1 Bovine Mastitis

According to Breen (2019), disinfecting the teats of cows pre- and post-milking forms a crucial part of the milking process. However, disinfectants alone will not control and prevent mastitis infections. Disinfection of the teats acts as a control method for mastitis infections by limiting the transmission of pathogens between cows and preventing the introduction of new pathogens into the teat during the milking process (Breen 2019). Various disinfectants are commercially available, but data are limited on the mechanism of their ability to inhibit bacterial growth (Fitzpatrick et al. 2019). Disinfectants often have multiple components as their primary purpose is maximum cleaning by disinfectant and minimum skin irritation (Oura et al. 2002; Nickerson 2009).

In dairy cows, biosecurity and hygiene play a crucial role, particularly during the expansion of a farm (Barkema et al. 2009). During the expansion of a farm, new dairy cows may be acquired from outside the current herd and may introduce new pathogens, compromising herd immunity (Barkema et al. 2009). The infections may not always be treated successfully, but through disinfection protocols, the transmission of the responsible pathogens may be limited and reduced (Barkema et al. 2006; Zadoks 2007). Contagious mastitis pathogens are spread from one cow to another during milking (Fox and Gay 1993). Therefore, it is highly recommended that the milking machines be adequately disinfected before starting each milking cycle (Fox and Gay 1993). After each milking process, the milking machinery is disinfected by a wash-up routine, which prevents or reduces the transmission of mastitis pathogens between cows (Wilson et al. 1995).

10.3.2 Poultry

Campylobacter is the most prevalent bacterial species responsible for infection in a chicken flock in poultry farming. Battersby et al. (2016) found that basic biosecurity decreased the prevalence of *Campylobacter* infections within the experimental group, but the broilers were significantly heavier at first, thinning by about 400 g.

Gibbens et al. (2001) reported that by implementing biosecurity and disinfection protocols, *Campylobacter* prevalence drastically decreased from 80% to less than

40% in boilers. Despite the advantages, the most stringent biosecurity protocols cannot eliminate *Campylobacter* and most other pathogens (Russa et al. 2005). Biosecurity controls are highly dependent upon the personnel following correct protocols consistently, which is very seldom the case (Sibanda et al. 2018).

Colibacillosis is any infection in poultry with avian pathogenic *Escherichia coli* (APEC) as the causative organism. The systemic or localised infections can lead to a wide array of symptoms that resemble other opportunistic pathogens; hence lesions alone are not enough to confirm colibacillosis (Nolan et al. 2020). Identification of APEC must be done by molecular or culture methods.

Although it is difficult to determine precisely, there is a general agreement that colibacillosis is the leading avian disease caused by bacteria (Nolan et al. 2020). This supports the idea that colibacillosis infection significantly contributes to industry-related disease-related losses through direct mortality condemnation at antemortem and post-mortem and impaired growth (Nolan et al. 2020).

In a recent study, Newman et al. (2021) found more supporting evidence for the established notion that APEC virulence is most commonly associated with the ColV plasmids. In this study, up to 90% of isolates had ColV-associated genes. A concern was the detection of only 34% of all isolates being susceptible to all antimicrobials tested. Similarly, Barbieri et al. (2013) found resistance to tetracycline (69.4% of samples) and sulphonamides (59.7% of samples) in high frequency. However, they observed resistance in less than 30% of isolates for all other antimicrobials. Johnson et al. (2012) also saw significant increases in resistance to several antimicrobials between commensal and pathogenic avian *E. coli*. In addition, they found a widespread distribution of multidrug resistance (MDR) in APEC isolates and evidence of widespread distribution of mobile genetic elements. The study also showed a wide prevalence of ColV and R plasmids encoding MDR, suggesting co-transfer of these elements is common in APEC. They concluded that there is clear support for a correlation between virulence factors and resistance (Johnson et al. 2012). The concern is that there may be selective pressures in poultry production, promoting the development and spread of APEC virulence factors and resistance genes due to conjugative plasmids. Even more concerning is the possibility that the similarities of human pathogenic ExPEC and APEC could transmit these mobile genetic elements and the subsequent emergence of new zoonotic pathogens (Johnson et al. 2008, 2012).

10.4 Swine

A biosecurity programme is seen as a link in a chain, and the programme is only as strong as the weakest link. Disinfectants reduce the percentage of pathogenic viruses so that the disinfected surface is no longer a source of infection (Juszkiewicz et al. 2019). ASFV is a debilitating disease in the swine industry with up to 100% mortality (Juszkiewicz et al. 2020). However, biosecurity measures and disinfectant use have been shown to reduce infections and effectively inactivate ASFV, achieving titre reductions of over five logs in some cases (Juszkiewicz et al. 2020). Tests

showed that quaternary ammonium compounds (QACs) are very effective against enveloped viruses, including ASFV, at a concentration of just 0.003% (Juszkiewicz et al. 2019). However, despite QACs showing low toxicity, prolonged exposure may cause skin and respiratory irritation (Juszkiewicz et al. 2019). QACs attack the envelope of viruses and have a much stronger effect against ASFV than any other disinfectant (Shirai et al. 2000).

10.5 Biosecurity in Human Health

Antimicrobial resistance has become a public health threat, and it has become a priority to limit and treat the further spread and emergence of this resistance (Donaghy et al. 2019). Both antibiotics and disinfectants have been vital in healthcare services and food and animal production. Many factors have contributed to the rise in antimicrobial resistance in bacteria and other microorganisms capable of infecting humans. Antibiotic use in clinical settings such as hospitals and animals for food production has been identified as the primary contributor (Donaghy et al. 2019). Concerns about the misuse and overuse of antimicrobial agents in clinical and livestock treatments have contributed significantly to the rise and development of antibiotic and disinfectant-resistant bacteria (Kumar and Pal 2018). While antimicrobial use in animal production often selects for resistance in commensal and zoonotic bacteria, animal and human waste from homes and hospitals may enter groundwater and soils, leading to resistant bacteria contamination in crops exposed to these sources (Hora et al. 2020). Interactions between hosts and resistant bacteria can spread to humans either through the food supply, animals, or environmental pathways such as contaminated crops and water or vectors such as insects and rodents (Argudín et al. 2017).

The origins of the microorganisms in these hospital settings remain unclear. It has been suggested that patients and healthcare workers may spread diseases that did not originate from hospital environments (Kumar and Pal 2018). This is particularly interesting to patients who have had contact with animal production industries. For example, Shah et al. (1988) described an outbreak of tinea corporis caused by the zoophilic dermatophyte *Microsporum canis* fungi typically found in domestic animals such as cats and dogs. A single infected patient, an elderly farmer, introduced this dermatophyte into the healthcare facility. It resulted in an outbreak infecting a nurse with whom he was in contact and further staff members and other patients within the facility (Shah et al. 1988). This suggests that outpatients from farms may introduce nosocomial infections into hospital environments through contact with staff who move between patients. A growing view for nosocomial infections is the persistent asymptomatic carriage of resistant bacteria through patients, outpatients, staff, and visitors (Smith et al. 2005).

Like SARS and Ebola, other infectious diseases showing antibiotic resistance may have a zoonotic origin (Taylor et al. 2001). While evidence has suggested that antibiotics in agriculture have contributed to antibiotic resistance in potentially

pathogenic human bacteria, there are still great uncertainties in the chain from cause to effect. Antibiotic-resistant bacteria have reportedly been found in soil surrounding farms, surface and groundwater, wastewater from the hospital and water treatment facilities, and meat and poultry in retail (Hamscher et al. 2003; Iversen et al. 2004; Kumar and Pal 2018). In addition, it has been suggested that antibiotic-resistant bacteria can be transferred from contaminated meat or poultry into kitchens, cross-contaminate other foods due to unsafe handling practices, and colonise the human gut after ingestion (Sørensen et al. 2001; Gorman et al. 2002; Smith et al. 2005). Well-known foodborne pathogens of zoonotic origin include *Salmonella*, *Campylobacter*, *Yersinia*, and *Listeria*; thus, the main reservoir for these bacteria are food-producing animals and meat products (Aarestrup 2005).

The indiscriminate use of antibiotics in animal production may have dire consequences for human health. It may result in selecting a reservoir of opportunistic pathogens carrying antibiotic resistance determinants. Enterococci have become a serious cause of nosocomial infections as they readily acquire resistance genes such as *vanA* and *vanB* for vancomycin resistance (Iweriebor et al. 2016). While they occasionally cause animal disease, humans may be exposed to these resistant bacteria from animals or animal products and further spread in the community through person-to-person transmission (Iweriebor et al. 2016). Enterococci from dairy cattle may be spread to humans through direct contact with the animals, manure, or manure used in crop production, dairy products and milk, and agricultural runoff into water bodies (Iweriebor et al. 2016). Additionally, antimicrobial resistance genes can be transferred to other bacteria of the same or different species (Iweriebor et al. 2016). Iweriebor et al. (2016) examined faecal samples of dairy cattle exposed to tylosin and isolated *Enterococcus* spp. with antimicrobial-resistant determinates to erythromycin, streptomycin, vancomycin, aminoglycoside, and macrolides. Other members have also shown resistance to cephalosporins and glycopeptide antibiotics used to treat nosocomial infections. As members of this genus have the inherent ability to acquire and distribute antimicrobial resistant determinants, they have become an important opportunistic pathogen in dairy animals (Iweriebor et al. 2016).

Those in direct contact with infected animals, such as farmers, farm workers, and veterinarians, risk being colonised or infected with resistant bacteria. These workers act as a link for the entry of resistance genes into the community and hospital environments, where microbes' dissemination continues (Davies and Wales 2019). Levy et al. (1976) first reported the spread of resistant bacteria from animals to people through direct contact. Tetracycline-resistant *E. coli* strains were found within the gut microbiota of chicken caretakers and chickens receiving tetracycline-laced feed. This extended to the family members of the farmers and persisted for months after using this tetracycline-supplemented feed (Levy et al. 1976). The resistance was attributed to the pSL222-6 plasmid, encoding resistance to tetracycline, chloramphenicol, sulphonamides, nalidixic acid, and streptomycin transferable via conjugation (Levy et al. 1976).

Evidence shows that human consumption of food carrying antibiotic-resistant bacteria can, directly and indirectly, acquire antibiotic-resistant infections (Bezanson

et al. 1983; Mølbak et al. 1999). Such was described by Mølbak et al. (1999), who traced an outbreak of multidrug and quinolone-resistant *Salmonella enterica* serovar Typhimurium DT104 to pork consumption. The infected included a slaughterhouse worker and a nurse exposed to infected patients through occupational infection. Other patients included those who had ingested pork from the slaughterhouse and those who had contact with the infected, resulting in nosocomial infections (Mølbak et al. 1999).

The emergence of SARS-CoV-2 (COVID-19) has led to increased disinfectant use in households, healthcare, workplace, and industry settings (Hora et al. 2020). Many of these disinfectants contain QACs as their active ingredients due to their efficiency in inactivating enveloped viruses (Tuladhar et al. 2012). SARS-CoV-2 was presumed to have originated from bats and crossed the species barrier due to its rich genetic diversity and frequent recombination events, which may have increased its potential for cross-species transmission (Lu et al. 2020). The rise in QAC usage during the COVID-19 pandemic may enhance antibiotic resistance in mixed microbial communities in the environment, animal- and food-related industries, and hospitals. Increased usage of disinfectants will promote positive selection for microbial isolates that may be cross-resistant to both antibiotics and disinfectants. Effective biosecurity and proper disinfectant usage are required to ensure that the fight against the virus does not cause increased infections from antibiotic-resistant bacteria (Hora et al. 2020).

Alcohol-based disinfectants have also increased substantially due to the COVID-19 pandemic. However, this raises the question of whether resistance to ethanol will be more prevalent in the years to come from the widespread use of hand sanitisers during the COVID-19 era.

10.6 Development of Disinfectant Resistance

Microorganisms respond differently to antimicrobial agents due to their various cellular structures, composition, and physiology (Poursat et al. 2019). Microorganisms may develop mechanisms of resistance and metabolism when exposed to environmental pollutants. The prolonged use of antimicrobial agents can lead to resistance in microorganisms previously sensitive to these agents (Giedraitienė et al. 2011). Resistance may be a natural property of the organism (intrinsic) or as a result of mutations and the acquiring of mobile recombinational elements such as plasmids and transposons as well as horizontal gene transfer (HGT) (Tezel and Pavlostathis 2012).

Disinfectants play a critical role in maintaining hygiene standards and protocols in healthcare, livestock, and food-related industries. Many commercially used disinfectants contain QACs due to their low toxicity and adaptability (Langsrud and Sundheim 1997; Gerba 2015). However, sub-optimal application of disinfectants and the exposure of microorganisms to sub-inhibitory concentrations result in decreased susceptibility, increased tolerance, and resistance development in

microbes (Tezel and Pavlostathis 2015). In several cases, QAC-resistant bacteria have been found in wastewater or activated sludge, as there is the release of QACs into the environment through runoff, thereby creating QAC gradients and allowing microbial adaptation to these sub-inhibitory levels (Dean-Raymond and Alexander 1977; Nishihara et al. 2000; Tezel and Pavlostathis 2012).

Intrinsic resistance is a natural and chromosomally controlled characteristic of bacteria that allows them to counteract the action of antimicrobial agents (Poursat et al. 2019). This typically includes properties such as the cell membrane structure and composition, which influences the ability of the antimicrobial to penetrate the cell and reach its target site. Gram-negative bacteria generally exhibit higher resistance to most antimicrobials than Gram-positive bacteria due to lower membrane permeability (McDonnell and Russell 1999). In addition, induced differential gene expression (such as upregulation of chromosomally encoded efflux pump genes) and the formation of biofilms have been shown to decrease the penetration of antimicrobials into the cell (Langsrud et al. 2003; Moen et al. 2012; Oh et al. 2014; Palmer et al. 2018). The microenvironment of a biofilm can facilitate genetic exchange amongst cells establishing a resistant population (Poursat et al. 2019). In some cases, a reduced growth rate induced by stress can affect microbial susceptibility to disinfectants by improving tolerance to these compounds, as seen with fluoroquinolones and β-lactams (Brauner et al. 2016).

Acquired resistance includes mutations in the antimicrobial target, such as substitution mutation in the *gyrA* gene. This alters the target site in DNA gyrase, which confers resistance to quinolones or mutations in genes that may alter gene function and lead to resistance via biodegradation of the disinfectant (Weigel et al. 1998; Poursat et al. 2019). Acquiring genetic material through plasmids, transposons, integrons, or bacteriophages may significantly increase disinfectant resistance (McDonnell and Russell 1999). Disinfectant resistance genes can be found on the same genetic elements as antibiotic resistance genes. This includes class-1 integrons, plasmids, and genomic islands, which enable the development of co-resistance between these antimicrobial agents, as the QAC resistance pathways and mechanisms of action are similar to those seen in antibiotic resistance and often transfer via conjugation, transduction, and transformation (McDonnell and Russell 1999; Tezel and Pavlostathis 2015; Bragg et al. 2018; Donaghy et al. 2019; Mc Carlie et al. 2020). Many plasmids contain genes encoding resistance genes, such as β-lactamases, capable of degrading antibiotics. The IncP plasmid group is involved in various resistant metabolic functions. It may be responsible for transferring catabolic and other resistance genes between bacteria (Poursat et al. 2019). Resistance plasmids may contain genes encoding for efflux pumps (Iguchi et al. 2014).

Similarly to antibiotics, large-scale disinfectant use has increased the evolution and spread of resistance (Bragg et al. 2018). For disinfectants such as QACs, when used above the minimum inhibitory concentration (MIC), they disrupt bacterial cell membranes and act through the interference of the ionic and physical stability of these membranes, causing leakage and degradation of intracellular molecules and inhibition of membrane-based processes such as respiration (Denyer and Stewart 1998; Tezel and Pavlostathis 2012). However, when QACs are used at sub-MICs

levels, adaptation to these concentrations often includes structural changes of the cell membrane, formation of biofilms that may further aid gene transfer, the overexpression or acquiring of efflux pump genes through mobile genetic elements during stress-induced mutagenesis, conjugation, or HGT (Moen et al. 2012). These efflux pumps allow the penetrated antimicrobial agent to transfer from the inside of the cell to the outside via an energy-dependent mechanism (Tezel and Pavlostathis 2012).

As bacteria can respond to oxidative stress caused by the QACs through SOS-responses, they may promote their survival through DNA repair, which in some cases may result in beneficial mutations, enhancing resistance to antimicrobials, such as the alteration of the antimicrobial target (Tezel and Pavlostathis 2015; Poursat et al. 2019). Furthermore, bacteria can respond to environmental changes, including exposure to antibiotics and disinfectants, which may induce alternative metabolic pathways and the expression of other resistance genes, such as those for biofilm formation and efflux pumps. Therefore, resistance develops due to exposure to antimicrobial compounds and the resultant selective pressure. Disinfectant resistance brought about by the metabolism of antimicrobial compounds has been found and may be an emerging resistance mechanism (Dean-Raymond and Alexander 1977; van Ginkel et al. 1992; Nishihara et al. 2000; Nishiyama and Nishihara 2002; Patrauchan and Oriel 2003; Takenaka et al. 2007; Bassey and Grigson 2011; Tezel et al. 2012; Oh et al. 2013; Ertekin et al. 2016).

Several resistance mechanisms have been investigated, the most common being efflux pumps. However, little is known about the metabolic activities that microorganisms undergo in the presence of QAC-based disinfectants, which must be further explored. The elucidation of the alternative metabolic pathways that use disinfectants as growth substrates may allow the examination of methods to mitigate this resistance, as demonstrated by efflux pump inhibitors (Chitsaz and Brown 2017).

10.7 Metabolic Changes in the Presence of Disinfectants

Evidence is growing of a multistep process of biodegradation by bacteria as a mechanism of disinfectant resistance. This process requires the antimicrobial compound to be recognised and enzyme-substrate binding to occur, influenced by the compound's structure and chemical characteristics (Brycki et al. 2014). The length of the alkyl chains, number of non-methyl groups, and types of groups in QAC-based disinfectants play an essential role in their degradation. An increase in alkyl chain length and non-methyl group number decreases QAC biodegradability and solubility, with the optimal chain lengths for degradation ranging from C_{12} to C_{16} (Nishiyama and Nishihara 2002; Jennings et al. 2015).

Limited information exists on the exact metabolic pathway; however, various pathways show similarities in the primary reactions during QAC exposure. Three strategies have been suggested for QAC metabolism under aerobic conditions, which vary in terms of the initial hydroxylation of the alkyl chain. One of these strategies includes the ω-hydroxylation of the alkyl chain at the C-terminal carbon

(Tezel and Pavlostathis 2015), followed by β-oxidation of the alkyl chain to acetyl-CoA, which progresses towards the hydrophilic nitrogen group (van Ginkel 1996) consistent with that found by Dean-Raymond and Alexander (1977). This suggests the Hexa- and decyltrimethylammonium bromide could be used due to collaboration between a *Xanthomonas* sp. and *Pseudomonas* sp. via ω-hydroxylation. This produced 9-carboxynonyl- and 7-carboxyheptyltrimethylammonium salts, suggesting oxidation of the long alkyl chain and cleavage of acetyl units through β-oxidation (Dean-Raymond and Alexander 1977). It was suggested that many of these alkyl chain metabolising bacteria could not degrade methylamines. Van Ginkel (1996) further proposed that these methylamines are often degraded by methylotrophs in many specific environments, mainly agricultural areas, including rice paddies and runoff from industries.

A second mechanism includes the α-hydroxylation of the alkyl group adjacent to the quaternary nitrogen and cleavage of the C_{alkyl}-N bond (van Ginkel et al. 1992). This pathway was observed by Patrauchan and Oriel (2003), who found that *Aeromonas hydrophila* sp. K could grow with BAC as the only carbon and nitrogen source. Benzyldimethylamine (BDMA) was the first detected product in this BAC metabolism, suggesting that the initial reaction was the cleavage of the Calkyl-N bond with the formation of BDMA, the corresponding alkane, further degraded through β-oxidation. The BDMA was then individually degraded via successive N-demethylation reactions as there was the subsequent formation of benzylmethylamine (BMA) and benzylamine (BA) (Patrauchan and Oriel 2003). Ammonium ions and benzoic acids, the products of BA deamination to benzaldehyde, were also used for growth (Patrauchan and Oriel 2003). Alternatively, Tezel et al. (2012) found that BAC degradation resulted in BDMA formation; however, this was then degraded to dimethylamine (DMA) and benzoic acid via debenzylation. The DMA was further metabolised to ammonium ions and CO2 via mineralisation (Tezel et al. 2012).

Nishihara et al. (2000) found that *Pseudomonas fluorescens* TN4 was able to grow using didecyldimethylammonium chloride (DDAC) as the only carbon source. Furthermore, the DDAC underwent biotransformation to decyldimethylamine (DDA) and DMA following consecutive N-dealkylation processes and β-oxidation of the decanoic acid with DMA transformation, further releasing CO_2, H_2O, and NH_4^+ (Nishihara et al. 2000). A study on *Pseudomonas fluorescens* F7 and F2 by Nishiyama and Nishihara (2002) also found that dodecyltrimethylammonium bromide (DTAB) could be transformed by the bacterium to TMA and DMA following dealkylation and demethylation reactions, respectively, by the F7 strain.

The final mechanism involves the hydroxylation of the attached methyl group carbon and subsequent demethylation, in addition to the cleavage of the C_{alkyl}-N bond. This was observed by Takenaka et al. (2007), who determined that *Pseudomonas* sp. strain 7–6 could cleave the C_{methyl}-N bond, producing methanol and dodecyl-dimethylamine. From these initially observed transformations, the primary mechanism for the biotransformation and metabolism of QAC-based

disinfectants includes the biological hydrolysis of carbon-nitrogen bonds, which yields alkanols and quaternary ammonium salts (Takenaka et al. 2007; Brycki et al. 2014).

While the biodegradation pathways were described in various studies, genetic analyses had yet to reveal the genes enhanced during QAC exposure. Therefore, Oh et al. (2013) performed metagenomic analyses on a community of BAC-degrading microbes identifying essential genes. These BAC-adapted communities were enriched in monooxygenases, dehydrogenases (alcohol/aldehyde-, acyl-CoA-, and formate-dehydrogenase), isocitrate lyase, and amine oxidases, all deemed essential for BAC-biodegradation (Oh et al. 2013). In addition, aromatic-ring opening dioxygenases were found which oxidise the benzyl group of BACs and are associated with energy generation, often through secondary metabolism (van Ginkel et al. 1992; Nishihara et al. 2000; Patrauchan and Oriel 2003; Oh et al. 2013). Furthermore, genes conferring antimicrobial resistance, such as those encoding for efflux pumps, cell wall synthesis, cell envelope modifications, chaperone proteins, and oxidative stress, were also identified (Oh et al. 2013).

These proposed QAC transformation pathways have been shown to differ regarding the initial attack on the alkyl chain. In many cases, α-hydroxylation and the cleavage of the Calkyl-N bond by a monooxygenase are most commonly seen. However, the mechanisms of QAC metabolism share similarities regardless of the QAC used with the degradation of the produced alkanols by β-oxidation being observed along with the amines, which are usually utilised by other microbes in a community with methyl monooxygenase activity (van Ginkel 1996; Tezel et al. 2012).

Microbial adaptation to QAC-based disinfectants may ultimately result in their biotransformation by the microbial communities. Through adaptation during the exposure to QACs, microbes may undergo various processes, finally leading to altered gene expression and biochemical changes allowing them to metabolise antimicrobial agents. The increased resistance and adaptation in some communities may have developed as a result of the selection of a degrading species from a particular community that has had previous exposure or by recombination events including HGT (Oh et al. 2013), mobile recombinational elements including plasmid transfer or integrons, and mutations (Tezel and Pavlostathis 2015). These modifications may alter gene functions and expression and may lead to tolerance or biodegradation in some cases (Poursat et al. 2019). These metabolic pathways, which result in the degradation of antimicrobial compounds, provide insight into the survival of resistant pathogens involved in nosocomial and agricultural infections.

10.8 Methods to Discover Novel Disinfectant Resistance Mechanisms

Identifying the genes responsible for bringing about resistance is essential. Current research is putting a spotlight on the genetic cause of resistance. New advances in sequencing technology and bioinformatics have elucidated antibiotic and disinfectant resistance mechanisms (Qin et al. 2018). Whole-genome sequencing and PCR reactions can identify resistance genes and elucidate what is present within the microbial genome (Lieberman et al. 2011). However, simply because a gene is present does not mean it plays a role in the resistance phenotype. Factors such as inhibitors, promoters, co-expression, regulation changes, induction, and inhibition must be considered (Matsuo et al. 2004; Qin et al. 2018). Knowing what genes are present is one-half of the answer to antimicrobial resistance. Their expression profiles and metabolic interactions provide a complete picture of what is happening on a molecular level with these resistance genes and proteins (Bagge et al. 2002; Matsuo et al. 2004). Therefore, whole-genome sequencing must be coupled with transcriptomic analysis to fully understand resistance mechanisms (Khaledi et al. 2016; Qin et al. 2018).

Transcriptomics can also be used in the fight against disinfectant resistance and go a step further by identifying genetic markers to monitor resistance. A transcriptomics study by Kim et al. (2018b) looked at the changes in gene expression when *Pseudomonas aeruginosa* is cultivated in subinhibitory concentrations of benzalkonium chloride (BAC). It was found that the growth rate was reduced during adaption, efflux pump, and spermidine synthase were upregulated and porin proteins related to QAC transport were downregulated. In addition, mutations in the *pmrB* gene conferring polymyxin resistance were detected (Kim et al. 2018a). This confirms known mechanisms of antimicrobial resistance as diminished susceptibility was brought about by decreasing the intracellular concentration of BAC (upregulated efflux pumps, downregulated porins) and reducing the negative charge on the cell membrane (*pmr*B mutation and spermidine synthesis) as BAC and other QAC-based disinfectants are cationic surfactants (Gunn et al. 1998; McDonnell and Russell 1999; Ishikawa et al. 2002; Kim et al. 2018a). This study revealed that although transcriptomic changes in *P. aeruginosa* were numerous, only a single mutation found at a DNA level correlated to decreased susceptibility to BAC (Kim et al. 2018b). The mutation identified in *pmr*B was unique to all populations adapted to cultivation in BAC and was absent from those without BAC exposure. Kim et al. (2018a) suggest that this may be used as a biomarker for antimicrobial resistance to aid in the identification and surveillance of resistant microorganisms. In a clinical setting, screening infectious organisms for biomarkers of resistance may be vital to direct day-to-day treatment options. Biomarkers of resistance can provide insight into which antibiotics may not be effective and indicate which antibiotics should be prescribed rather than fighting infection.

The study of gene expression through transcriptomics is not a novel field. This includes techniques such as microarrays, real-time PCR, and, more recently,

RNA-sequencing. Qin et al. (2018) used RNA-sequencing to conduct a comparative study of gene expression profiles of *Acinetobacter baumannii* isolates during exposure to antibiotics (amikacin, imipenem, and meropenem). By conducting a transcriptomic study using RNA-sequencing, gene expression profiles were not only compared in response to different antibiotics but also allowed for the analyses of co-transcribed genes. Therefore, entire metabolic pathways and gene interactions were tracked, largely unknown with whole-genome sequence data alone. It was found that different *Acinetobacter baumannii* isolates responded differently to the two different classes of antibiotics. Under treatment of amikacin, which targets translational proteins, 149 genes were upregulated and involved in protein folding and lysis. In addition, many genes found to be differentially expressed were hypothetical or unannotated without GO terms, suggesting that there may be unknown resistance genes and unknown mechanisms of resistance to carbapenem antibiotics. This work identified these unknown and hypothetical proteins and can lead to discovering novel antibiotic resistance genes and mechanisms. This study also noted that both multidrug-resistant and sensitive strains harboured many antibiotic resistance genes and confirmed that the presence of resistance genes does not necessarily mean there will be a resistance phenotype (Qin et al. 2018).

The study by Qin et al. (2018) also highlighted the role of mobile genetic elements in antimicrobial resistance. It was found that transposons may have shaped gene expression patterns in isolates while under stress. It has been reported that transposable elements can induce resistance islands development in *Acinetobacter baumannii*, creating active promoters and inducing the expression of genes downstream of their insertion site. Qin et al. (2018) found that under antibiotic exposure, generally, a higher differential expression was seen for transposable elements compared to non-transposable-associated genes. This study also noted a tremendous knowledge gap regarding antimicrobial resistance genes and the molecular response to antimicrobials. This is mainly due to a lack of transcriptomics, RNA-sequencing data, and limited methodologies for comparing differential gene expression and interactions (Qin et al. 2018).

Once mechanisms of antimicrobial resistance have been identified, this knowledge can be used to prevent the development of multidrug-resistant microorganisms and, in some cases, reverse the resistance phenotype. Efflux pumps are the most common mechanism of antibiotic and disinfectant resistance and function by lowering intracellular concentrations of antimicrobial within the cell. Several efflux pump inhibitors have been proposed to counteract resistance and restore cell susceptibility to antibiotics and other antimicrobials (Lomovskaya et al. 2001; Zhang and Ma 2010; Wang et al. 2017; Lowrence et al. 2019). Wang et al. (2017) described compounds synthesised based on a 2-naphthamide pharmacore structure, designed to inhibit efflux pumps in Gram-negative bacteria and negate resistance brought about by these efflux pumps. One example is synthesised compound 4-(isopentyloxy)-2-naphthamide (A3), which acts through the AcrB multidrug efflux pump, found commonly in *E. coli* and other Gram-negative bacteria (Wang et al. 2017). Compound A3 reduced bacterial MIC values of erythromycin and chloramphenicol to those of the antibiotic-sensitive *E. coli* strain that lacked an efflux pump (Wang

et al. 2017). This restored antibiotic sensitivity to a previously resistant *E. coli* strain by inhibiting the AcrB multidrug efflux pump (Wang et al. 2017).

The more we understand antimicrobial resistance, the more we can hinder the development of multidrug-resistant strains and sometimes reverse resistance altogether.

10.9 Disinfectant Resistance Going Forward

In the past, the concept of resistance to disinfectants was considered irrelevant. However, a growing body of evidence highlights the significance of disinfectant resistance. It has also been stated that many of the other alternatives to antibiotic treatment have limitations, and the most effective disease control option will be good biosecurity and disinfection (Bragg et al. 2018). The rapid development of resistance to disinfectants, with growing evidence of a potential link to antibiotic resistance, will pose a grave risk to agriculture and human health.

There is an urgent need for better levels of control on disinfectant use, not only in the agricultural settings and healthcare environments but also in the domestic use of disinfectants.

In agriculture, there is a need to train people using disinfectants, or we will soon deplete disinfectants' efficacy, which could be our last line of defence. Therefore, selecting efficient disinfectants for use in agriculture, where many thousands of litres of disinfectants are used, is essential. There is also an urgent need to establish disinfection rotation programmes in agriculture to prevent the build-up of resistance to a particular disinfectant.

In Africa, the control and registration of disinfectants are generally lacking. This opens the possibility of sub-standard disinfectants being flooded into the African market. This situation could rapidly advance resistance to disinfectants, which will have very dire consequences for humankind.

10.10 Conclusion

Antibiotic resistance is one of the biggest threats to public health in our time. The lack of new antibiotics and limited alternative options has left the healthcare and agricultural industries scrambling for alternatives. The only viable alternative is good biosecurity and an effective disinfection programme. However, the emergence of disinfectant resistance threatens this last line of defence, and therefore research into disinfectant resistance is urgently needed to safeguard our current disinfectants.

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Chapter 11
The Linkage Between Antibiotic and Disinfectant Resistance

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11.1 Introduction

The rapid emergence of antibiotic resistance is alarming researchers worldwide. It has far-reaching effects in many industries, including animal production and medical environments. The widespread prevalence of multidrug-resistant (MDR) bacteria may lead us into a post-antibiotic era, where many antibiotics are no longer effective at therapeutic doses. Alternatives to control bacterial growth are numerous. This includes vaccines, bacterial peptides, and phage therapy, to name a few; however, these alternatives are still in development and have numerous associated problems (Bragg et al. 2018; Rello et al. 2019). Disinfectants and biosecurity could provide a promising alternative to control microorganisms through continual disinfection programmes (Bragg and Plumstead 2003). Nonetheless, these antimicrobial agents are not a wholesome solution for failing antibiotic treatment. Furthermore, antibiotic resistance is not isolated among microorganisms, as resistance to disinfectants and antiseptics has been identified and is rapidly becoming more prevalent among various pathogens (Sidhu et al. 2002a; Tabata et al. 2003; Jiang et al. 2019). Disinfectant resistance is a relatively novel field of study as the number of publications on this subject is substantially less than that of antibiotic resistance, although this phenomenon could be equally life-threatening. Therefore, caution and great care must be taken to introduce appropriate disinfection regulations to prevent further resistance to disinfectants.

Disinfectants and antiseptics are used excessively in almost every industry, including the agricultural, food and beverage industry, and medical environments

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(Wirtanen and Salo 2003; Weber et al. 2013; de Oliveira et al. 2017; Maertens et al. 2020). Biosecurity is the prevention and control of pests and diseases from an agricultural and medical viewpoint (Waage and Mumford 2008). Our current way of life depends heavily on controlling microbial growth, and disinfectants/antiseptics and biosecurity are vital to achieving this. Together with the foreshadowing of antibiotic resistance, this makes the sudden emergence of disinfectant resistance troubling. Disinfectant resistance can evolve due to selective pressure exerted on the microbial population.

Resistance to quaternary ammonium compounds (QACs), a class of disinfectants, and antibiotics has been demonstrated to occur in bacteria such as *Pseudomonas aeruginosa* after long-term exposure (Oh et al. 2013). Resistance genes can be intrinsic, thus situated on the bacterial chromosome allowing circumvention of disinfectant/antiseptic mode of action, or resistance genes can be acquired through mutation or extrachromosomal transferring events, such as the transmission of plasmids/transposons (McDonnell and Russell 1999). Several mechanisms of bacterial disinfectant resistance exist, including phenotypic changes, inactivation of the disinfectant, target alteration, horizontal gene transfer (HGT), metabolism, and efflux pumps, among others (McDonnell and Russell 1999; Oh et al. 2013; Osman et al. 2018). Multidrug efflux pumps are one avenue of bacterial resistance that could provide resistance to multiple compounds. Multidrug efflux pumps can lead to decreased susceptibility/cross-resistance to antibiotics and disinfectants in the same organism simultaneously (Costa et al. 2013; Kim et al. 2018). Multidrug efflux pumps can be found on mobile genetic elements (MGEs), frequently forming part of resistance plasmids. Also, resistance plasmids can conjugate between bacterial species and allow the development of a resistant bacterial population. Multiple resistance genes can be present within a plasmid, simultaneously conferring resistance to several antimicrobials (Sidhu et al. 2002a). These MGEs conferring resistance are not affected by borders and can circulate globally through HGT, possibly acquiring additional resistance and, in rare cases, virulence genes along the way (Martínez-Vázquez et al. 2018; Wang et al. 2018; Turton et al. 2019).

The SARS-CoV-2 pandemic has highlighted our need for effective systems to combat life-threatening microbial infections. Our reliance on disinfectant products is expected to increase in the future, and as a result, the global market is predicted to rise (Pereira and Tagkopoulos 2019). Disinfectant resistance can have far-reaching effects on animal production, human health, and environmental ecosystems (Fernández Márquez et al. 2017; Youn et al. 2017; Kumar and Pal 2018; Hanczvikkel et al. 2019). Outbreaks of diseases in agriculture such as colibacillosis in poultry and bovine mastitis, will rise, more livestock will be lost to disease, contamination of animal products will increase, and the economic loss may be more significant (Nolan et al. 2013; Heikkilä et al. 2018). Due to the nature of animal production, with tens of thousands of animals in close contact, a single outbreak of disease can have devastating effects due to rapid spread among animals. Animal production is a growing industry; thus, effective disinfectants and biosecurity will

be vital to preventing massive economic losses due to disease. With the threat of many MDR bacterial isolates present in hospitals and clinics, such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii*, *P. aeruginosa*, and glycopeptide-resistant *Enterococcus* (GRE), controlling these populations will be essential (Chaoui et al. 2019). Medical environments must adhere to disinfection protocols involving constant monitoring of bacterial populations and rotational disinfection programmes. The approach used to control bacterial populations in each industry is similar, reinforcing the need for effective detection of resistance in bacterial populations and efficient means to control the populations by exploiting the correct combination of antimicrobials.

11.2 The Link Between Antibiotic and Disinfectant Resistance

Antibiotic resistance in bacteria is seen when an antibiotic no longer inhibits growth or is no longer effective at therapeutic doses. Resistant bacterial populations will be able to multiply continually in the presence of antibiotics, even at therapeutic concentrations (Zaman et al. 2017). An extensive collection of bacteria archived between 1917 and 1954 was used in a study to assess the presence of antibiotic resistance genes before the present antibiotic era (Hughes and Datta 1983). The study results suggested that antibiotic resistance genes were present at significantly lower frequencies (Hughes and Datta 1983). Similarly, another study showed that antibiotic resistance was reported before antibiotic usage for infection control (Abraham and Chain 1940). Natural selection processes allowed bacteria that produce antibiotics to develop antibiotic resistance to be competitive colonisers within a specific environment (Alonso et al. 2001). However, unregulated and excessive use of antibiotics could be responsible for resistance development to antibiotics and possibly disinfectants. For example, after discovering and producing aminoglycosides, the frequency of aminoglycoside-resistant *S. aureus* strains increased to levels that required attention (Davies and Courvalin 1977). Similar events are responsible for methicillin-, vancomycin-, and fluoroquinolone-resistant strains; of particular clinical importance are MRSA and vancomycin-resistant *S. aureus* (VRSA) strains (Lowy 2003; Appelbaum 2006). These studies demonstrate elevated antimicrobial use's propensity to promote the development of resistance against the specific agent. The combined effects of increased antibiotics and disinfectants used in many environments increase the likelihood of developing resistance against these antibacterial compounds. Thus, if bacteria can develop resistance mechanisms to counteract these classes of antimicrobials simultaneously, there could be the possibility of linking the resistance between disinfectants and antibiotics.

11.3 The State of Antibiotic Resistance

The global spread and acquisition of antibiotic resistance genes by clinically relevant microorganisms are linked to the mortality and hospitalisation of patients afflicted by these organisms (Berendonk et al. 2013; World Health Organisation 2014). Antibiotic resistance is regarded as a global health problem, and the World Health Organization warns that humanity could face a global post-antibiotic era (World Health Organization 2015a). In the United States (US), a national estimate of 622,390 incidents of MDR pathogen infections occurred in 2017 (Jernigan et al. 2020). Furthermore, 25,000 patients are estimated to die annually from MDR bacterial infections in 30 European countries surveyed (Freire-Moran et al. 2011; Abat et al. 2018). The burden of healthcare-associated infections (HCAIs) in developed countries costs roughly US$ 6.5 billion in the US and € 7 billion in Europe (World Health Organization 2015b). Although the data is sparse, the estimated occurrence and burden of healthcare-associated infections is a greater economic burden in developing countries. The pooled data suggest that 15.5 per 100 patients with surgical infection sites are the lead cause of HCAIs in developing countries. These HCAIs are often caused by Gram-negative organisms and MDR organisms. However, the real influence of HCAIs in Africa is unclear because of the extensive resource requirements for adequate surveillance and diagnoses. The lack of appropriate surveillance and combat measures to tackle HCAIs caused by MDR organisms and often intrinsically resistant Gram-negative organisms permits the continual proliferation of these organisms, allowing MDR populations to survive for extended periods and possibly disseminate resistance genes through HGT. While the focus of the WHO has been on reducing antimicrobial resistance (AMR) globally, there have been few coordinated endeavours in Africa (World Health Organization 2009). The effects of MDR pathogens on healthcare costs and reduced productivity of healthcare systems are serious, but these effects are further exacerbated by many African countries‘inability to combat MDR HCAIs adequately. The failure stems from a lower percentage of total health spending in Africa linked to reduced availability of funds for research and innovation (Mendelson et al. 2016; University of Washington Center for Health Trends and Forecasts 2017). However, this narrative is evolving with increasing global attention towards AMR; as a result, funding support for AMR control in Africa is impending from many international organisations (Gelband et al. 2016; Mendelson et al. 2016).

Currently, the compounded effects of antibiotic usage in veterinary and human settings are excessive, and irresponsible methods are a cause for concern. The release of antibiotic-resistant bacteria from anthropogenic sources raises concern about the environmental status of resistant bacterial populations and acquired infections by released bacteria (Laxminarayan and Brown 2001; Smith et al. 2002; Baquero et al. 2008; World Health Organisation 2014). Many antibiotics used in agriculture share similar structural components or are identical to clinically applied antibiotics (McEwen and Fedorka-Cray 2002). This over-usage of select antibiotics could be a substantial resistance-promoting factor (Endtz et al. 1991; Witte 1998;

Corre et al. 1999; Chiu et al. 2002; Hein et al. 2003). In Africa, many farms use antimicrobials because of increased demand for animal-derived food and trade opportunities (Schar et al. 2018). Some of the antimicrobials used, such as macrolides, are often restricted in other parts of the world because of associated risks.

Moreover, most antibiotics used are cheaper, such as tetracyclines, aminoglycosides, and penicillin. The coupled effects of excessive antibiotic use and unaware repercussions of AMR can worsen levels of MDR bacterial populations in the environment (Alhaji and Isola 2018). The ban on specific growth-promoting antibiotics in Europe was implemented because of a concern that resistance selected in animals might be transmitted to humans. The ban resulted in the successful removal of growth-promoting compounds in agricultural sectors. Removing these growth promoters had financial implications for farmers to utilise alternative antibiotic treatments and improve other essential aspects of animal agriculture (Casewell et al. 2003). This initiative has not yet been taken in Africa, largely due to the financial impact that livestock agriculture has on the socioeconomic conditions of many people. In African countries, antimicrobial use is unregulated, and imposing bans on Africa would require better stewardship of antibiotics and place further financial burdens on the people (Mensah et al. 2014).

11.4 Development of Antibiotic Resistance

Antibiotic-producing bacteria need to prevent toxicity towards the antibiotic they produce; thus, many encode resistance genes specific to that antibiotic. Often the genes for synthesis of, and resistance to, the antibiotic are located on the same gene cluster (Martin and Liras 1989; Hopwood 2007). In non-antibiotic-producing organisms, the development of resistance relies on changes at the genetic level. Bacteria can transfer genetic martial, such as plasmids, between different or similar species (Laxminarayan and Brown 2001). MGEs tend to be variably present within a population; therefore, they rarely carry genes essential for cell function. In many cases, MGE mobilisation is associated with a stress response, such as exposure to antibiotics or other environmental pollutants. Generally, plasmids are limited in their host range, but certain types, such as IncW plasmids, have a broad host range disseminating within environmental bacteria (Wellington et al. 2013). Conjugative plasmids can transfer DNA to adjacent bacteria via sex pili during conjugation, while other transfer means include transduction or transformation (McManus 1997).

In general, antibiotic treatment can hinder most bacteria in a population. However, under stress, bacterial populations with low availability of (or accessibility to) exogenous DNA must find a solution by utilising their resources. Mutation and recombination could lead to stress-induced survival through increased mutation rates (Blázquez et al. 2002). A genetically mutated colony might exist that is less susceptible to antibiotic treatment, leading to greater survivability of progeny. The cells that have undergone genetic alteration are now better equipped to withstand

environments with the antibiotic and will outcompete cells with no genetic adaptations. This correlates to subinhibitory antibiotic levels continually selecting for resistant progeny, eventually leading to a resistant bacterial population in the given environment (Kohanski et al. 2010). Resident microbial populations and/or foreign infectious bacteria can encounter subinhibitory concentrations within the human host environment when patients are negligent with antibiotic prescriptions (Kohanski et al. 2010; Zaman et al. 2017). Such circumstances can also occur when antibiotics are used in feed and water as growth promoters in agriculture and when industrial wastewater is discharged into the environment (McEwen and Fedorka-Cray 2002; Sarmah et al. 2006; Fick et al. 2009; Graham et al. 2011). The accumulated effects of all the mentioned uses of antibiotics in an unregulated and imprudent fashion lead to less effective antibiotic treatments in settings where AMR is most significant. This phenomenon can be potentiated by various other antimicrobial compounds and/or contaminants, such as disinfectants and heavy metals, which can promote the proliferation of antibiotic-resistant bacteria (Baker-Austin et al. 2006; Davies 2009; Hölzel et al. 2012; Ji et al. 2012; Seiler and Berendonk 2012; Tello et al. 2012).

The evolution and spread of resistance determinants can be promoted by antibiotic use, irrespective of origin, emphasising the need for good antibiotic stewardship in all sectors where antimicrobials are used. Proper antibiotic stewardship links with surveillance of AMR, which is achievable in developed countries, but cannot be as easily implemented in developing countries. In low- and middle-income countries, achieving comprehensive surveillance is challenging because of weak laboratory and communications infrastructure, lack of laboratory personnel, increased prevalence of falsified antibiotics, and substandard diagnostics (Okeke et al. 2005a; Almuzaini et al. 2013). However, a nationwide laboratory-based surveillance initiative was conducted in Ghana to assess antimicrobial resistance. However, the data origin was very skewed, where two-thirds came from the southern sector of the country, suggesting that improvement is required for capacity and infrastructure in other sectors of the country to ensure adequate coverage (Opintan et al. 2015). This study highlights the ability of African countries to undertake local or national AMR surveillance initiatives; however, the discrepancy in the data origin requires improvement to ensure accurate representation of AMR in the country.

11.5 Dissemination of Antibiotic Resistance

The primary route of transfer of antibiotic-resistant bacteria between humans and animals is the food chain (Witte 1998). Not only is the medical setting filled with high-level AMR, but environments surrounding certain human industrial activities, such as food production, animal husbandry, wastewater treatment (WWT), and pharmaceutical manufacturing facilities, are also rife with AMR due to the

anthropogenic effects (Soonthornchaikul et al. 2006; Silbergeld et al. 2008; Rizzo et al. 2013; Hora et al. 2020). Environments that experience frequent antibiotic discharge can select, multiply, and distribute new resistance determinants, inevitably resulting in human host-specific pathogenic and commensal bacteria that have received disseminated resistance, reiterating the importance of these sites as critical points for resistance control (Endtz et al. 1991; Witte 1998; Corre et al. 1999; Rhodes et al. 2000; Chiu et al. 2002; Hein et al. 2003; Li et al. 2009; Kristiansson et al. 2011). The over-lapping nature of antibiotic use in so many sectors requires that adequate antibiotic stewardship and AMR surveillance must become integral parts of the greater system of antibiotic use to ensure that AMR is curbed and not further promoted.

11.5.1 Dissemination of Antibiotic Resistance in Wild Animals

Evidence suggests a positive correlation between increased antibiotic use and a higher prevalence of resistant microorganisms. Antibiotic-resistant populations are more likely present in patients/animals treated with antibiotics (Laxminarayan and Brown 2001). Similarly, data suggests that the bacterial gut profile of wild animals is influenced by what degree of vicinity they share with human populations. Ninety percent of the bacterial isolates from rodents captured in parts of rural England were resistant to β-lactam antibiotics (Gilliver et al. 1999). Similar findings were associated with apes and African baboons that encountered humans. The primates with contact had a greater prevalence of antibiotic-resistant enteric bacteria than those without contact (Rolland et al. 1985; Rwego et al. 2008). Wild birds and migratory waterfowl are prone to being reservoirs of antibiotic resistance capable of long-distance dissemination. These birds can inhabit various environments, from agricultural dams to remote mountainous waterbodies, possibly being exposed to resistant bacterial populations at one water source and spreading resistance along their migratory path (Mohsin et al. 2016). Wild forest birds were captured from a wildlife-protected area in Ghana to assess the prevalence of antibiotic-resistant bacteria. Although the human-wildlife interaction is limited in the protected area, resistance to colistin was still detected in bacteria isolated from these birds (Modupe et al. 2021). Some clinical studies in Ghana have reported colistin resistance, suggesting that human settlements surrounding the protected area could be the source of the colistin-resistant bacteria resulting from consuming meat with some antibiotic residue (Agyepong et al. 2019). A better understanding of resistance profiles in wild animals and how resistance is spread from one location to another will contribute to managing zoonotic disease outbreaks resulting from AMR bacteria associated with wild animals.

11.5.2 Dissemination of Antibiotic Resistance in Agriculture

Due to the proximity that large numbers of animals share at intensive farming facilities, such as poultry, swine, and cattle, the potential for disease spreading is greatly accentuated. Thus, the viability of these facilities depends heavily on routine pharmaceutical use, such as antibiotics and heavy metals. Antibiotics use greatly influences livestock welfare and management, where approximately fourfold greater amounts are used in agriculture than in humans (Kanfer et al. 1998; Maron et al. 2013). Therapeutic doses of antibiotics are administered to treat diseases, and subtherapeutic doses are administered as growth promoters and/or for improved feeding efficacy (McEwen and Fedorka-Cray 2002; Sarmah et al. 2006). Many classes of antibiotics used in agricultural production are mainly similar to those used in medicine, raising concern that resistant bacteria could develop in one sector and cause infection in the other (World Health Organisation 2014). The lack of regulations on antimicrobial use in Africa creates a conducive environment for AMR emergence and spread (Okeke et al. 2005b). Additionally, most African farmers lack the financial freedom to afford more expensive antimicrobials that AMR has not hindered in the region. Many of these farmers lack awareness of proper antimicrobial use and AMR in the region resulting from low/absent AMR surveillance systems.

Many antibiotics administered to livestock are often discharged as unmetabolised substances or bioactive metabolites in manure and slurry (Boxall et al. 2004; Sarmah et al. 2006). Many antibiotics are poorly absorbed, resulting in 30–90% of the parent compound being expelled (Elmund et al. 1971; Lamshöft et al. 2007; Heuer et al. 2008; Sukul et al. 2009). Furthermore, antibiotic metabolites can be bioactive and revert to their parent form after excretion from livestock (Heuer et al. 2008; Lamshöft et al. 2010). These antibiotic and bioactive metabolites can enter the agricultural system either through the dung and urine of grazing livestock or as fertiliser, often in a raw form, contaminating soil and water systems (Goh et al. 2002; Gullberg et al. 2011; Du and Liu 2012). MGEs or translocative elements, such as integrons, transposons, plasmids, and insertion sequence common region (IS*CR*) elements, are the main way resistance genes are carried and transported from manure-isolated bacteria to soil bacteria (Heuer et al. 2011). The application of manure to agricultural soil increases the incidence of various transposable elements, and importantly, some of these elements were detected only when sampled from manured soil (Marti et al. 2013).

Interestingly, excrement from untreated livestock has also been shown to promote antibiotic resistance genes in soil, suggesting that the varying nutrient requirements of livestock animals impact the gut microbiota and may play a pivotal role in the soil environment (Kyselková et al. 2013). Additionally, the presence of naturally intrinsic antibiotic-resistant bacteria in the gastrointestinal tracts of some livestock species is possible. However, the naturally occurring intrinsic antibiotic-resistant bacteria may have lower resistance levels than resistant populations selected based on antibiotic exposure (Heuer and Smalla 2007; Stanton et al. 2011). They suggest

that antibiotic use can lead to more resistant populations released into the environment. Africa is not free from antibiotic contamination as river and lake systems contain AMR bacterial populations resulting from agricultural and community sources (Moremi et al. 2016; Tafoukt et al. 2017). The reality of the poor antibiotic stewardship and lack of AMR awareness in Africa can lead to substantial levels of resistance development. Often African countries lack the infrastructure and control required to regulate proper veterinary antimicrobial use. As a result, the compounds are easily accessible and possibly misused (World Health Organization 2001).

Some animal production facilities employ various systems for decontaminating liquid and solid waste. However, these treatments do not remove antibiotic resistance bacteria and could instead promote their proliferation and the proliferation of ARGs and MGEs (Jongloed and Lenis 1998; Union of Concerned Scientists 2001; Moura et al. 2007; Pei et al. 2007). In most instances, these decontaminating systems are absent in African agricultural facilities, allowing the release of these chemical and biological waste products into surrounding environments. Human contact with resistant bacteria via the agricultural environment, such as farm workers, or consumption of uncooked fruits and vegetables promotes the likelihood of exchange of resistant determinants to the human microbiome, contributing to resistant bacterial infections in humans (Chee-Sanford et al. 2001; Marti et al. 2013).

11.5.3 Dissemination of Antibiotic Resistance in African Communities

Inadequate water treatment has devastating impacts on public health. Roughly 1.2 billion people around the globe are plagued by poor sanitation and a lack of access to safe drinking water (Olaniran et al. 2009). These effects are exacerbated in African countries resulting from the lack of infrastructure support related to sewage and sanitation. The result is that faecal matter is more likely to be introduced into natural water systems, such as rivers or lakes, during rains or floods. In rural conditions, many people utilise rivers and lakes for recreational purposes and as a bathing and drinking water source. However, these water systems interact extensively with other people and animals, providing suitable conditions for transferring waterborne diseases and AMR (Moremi et al. 2017). Methods to address these apparent gaps in water treatment need to be addressed; however, these methods need to cater to the financial capacity of developing countries by being low-cost and requiring minimal maintenance (Bitton 2014).

With the increasing prominence of antibiotic resistance, the need to control bacterial populations by other means has become an essential factor to consider. Research has been conducted on alternative bacterial control methods, such as bacteriophage therapy and antimicrobial peptides, but these technologies are still in their infancy, and precautionary use in the clinical setting is restricted to very specific cases (Czaplewski et al. 2016; Ghosh et al. 2019). Disinfectants and biocides

could provide the means to control bacterial populations when antibiotics fail, but the effects these compounds have on resistance development need to be evaluated to avoid similar resistance trends as seen in antibiotics. In addition, the approach to disinfectants in well-regulated biosecurity protocols needs to be accepted and standardised.

11.6 Disinfectants as Antimicrobial Agents

The definition of disinfection is the destruction of microorganisms, most times excluding bacterial spores. Disinfection does not kill all organisms but reduces the microbial load to an acceptable standard for a defined purpose. An example of a defined standard would be a level of microbial load, which is neither harmful to health nor the quality of perishable goods (Mohapatra 2017). Many disinfectant classes are available, which can be ordered into two groups, oxidising and non-oxidising. Oxidising disinfectants contain halogens, chlorine, iodine, bromine, chlorine dioxide, peracetic acid, and hydrogen peroxide. Non-oxidising disinfectants include QACs, amphoterics, biguanides, and acid anionics (Fisher 2003). Disinfectants may be composed of formulations containing adjuvants that enhance the effects of the disinfectant solution, single or multiple active biocidal agents that target microbial cell structures. The indiscriminate activity of disinfectants differentiates these compounds from antibiotics with specific cellular targets.

Disinfectants are widely used in domestic, industrial, and healthcare environments (Maillard 2005). The increased use of disinfectant compounds at different concentrations during public and routine disinfection protocols in numerous sectors leads to greater volumes of antimicrobial agents present in the industrialised environment. Inevitably, more disinfectants are entering wastewater treatment facilities and the natural environment. Thus, a concern arises as to how this will impact the survival of bacteria and spread resistance (Gillings et al. 2009a, b; Wesgate et al. 2016). Within Africa, surveillance of antibiotic resistance is sparse; however, disinfectant resistance surveillance is even less. The lack of disinfectant resistance surveillance prevents the determination of which disinfectants are used. Thus, little literature is available to provide a wholesome investigation into disinfectant resistance in Africa.

11.6.1 Disinfectant Use in Africa

Currently, little knowledge is available on the general use of disinfectants in African agriculture, medical, or industrial settings. However, a few investigations have been conducted in a few African countries to provide insight into the probable nature of disinfectant utilisation and resistance. Testing of disinfectant formulations against resistant bacterial isolates from hospitals has been conducted in parts of Nigeria.

Disinfectants such as Dettol®, Savlon®, Jik®, and Germicide® were tested against clinical and nosocomial isolates (Alabi and Sanusi 2012; Durowaiye and Ayebo 2015). Some of the disinfectants tested exhibited no activity against MDR isolates at the manufacturer's in-use concentrations (Alabi and Sanusi 2012; Durowaiye and Ayebo 2015), suggesting that during routine disinfection protocols in hospitals, there is a chance that resistant isolates can survive and be placed under selective pressure to become further resistant. The main isolates tested were *S. aureus*, *P. aeruginosa*, *Klebsiella* spp., and *Proteus* spp., key organisms noted in nosocomial infections.

Moreover, investigations in African countries, such as Angola, Cape Verde, and São Tomé and Príncipe, highlighted that biocide resistance genes are highly prevalent among MRSA clonal lineages in these countries (Conceição et al. 2016). The burden of MDR nosocomial infections causes not only health-related implications but also a financial liability by increasing the cost of healthcare, prolonging hospitalisation, and requiring additional antimicrobial treatment (Wenzel 1995). Any form of increased economic liability is not sustainable in African countries, where financial healthcare support is below the recommended requirement already. Thus, disinfection regimens must successfully control MDR bacterial populations in the hospital context to avoid nosocomial infections and further economic burdens. First-time disinfection must be sufficient to reduce the bacterial load to adequate levels and prevent subsequent proliferation. To achieve this, disinfectant resistance surveillance will be required in conjunction with susceptibility testing of isolated MDR bacteria to provide insight into the correct concentrations and formulations of disinfectants to use.

Community-acquired infections are also a means for disseminating resistance, especially in people living near one another. Locations within settlements or cities with communal functionality are conducive to spreading potentially infectious agents. Environments such as grocery stores, beauty salons, and liquor stores are important in terms of the service they provide and on a social level as a place to gather and interact with other community members. Clients and service providers of beauty salons are at risk of skin infections and physical injuries due to bacteria present on inanimate objects, such as tools and equipment. Studies in South Africa and Nigeria on manicure, pedicure, hairdressing, and barbering tools showed contamination of *Mycobacterium fortuitum*, *M. mageritense*, *S. aureus*, *Streptococcus* spp., *Staphylococcus epidermidis*, *Enterococcus* spp., and *Enterobacter* spp. (Gira et al. 2004; Enemuor et al. 2013; Chindu Stanley et al. 2014). This is alarming to find such pathogens in areas many community members frequently visit without considering health-related implications. Proper hygiene protocols using disinfectants would help to control and prevent the spread of these organisms from the tools; however, the resistance of some isolates can nullify disinfection procedures. Exploring the effectiveness of commonly used disinfectants against frequently isolated pathogens and the resistance genes associated with disinfectant resistance can shed light on how to control the spread of pathogens and AMR in the community setting (Gahongayire et al. 2020).

11.7 The Relationship Between Antibiotic and Disinfectant Susceptibility

A growing concern is that reduced susceptibility to disinfectants is associated with increased antibiotic tolerance (SCENIHR 2009). This impacts frequently monitored healthcare environments and the broader scope of disinfectant application sites, such as domestic cleaning sites, common hygiene products, and, of major concern, the livestock food chain. If bacteria can become resistant to two antimicrobial agents simultaneously exerting selective pressure on the organism by a common mechanism, this would provide an energy-efficient means to continue replicating. Theoretically, organisms utilising less energy to survive in an environment that induces cell death will have greater competitive fitness, allowing their survival.

11.7.1 Potential Co-selective Nature of Disinfectants and Antibiotics

Antibiotics have highly selective toxicity allowing them to exhibit high antimicrobial potency. This characteristic allows for their use as anti-infective agents within body tissues of humans and animals (Chopra et al. 2002). The high specificity of action against microbial targets induces an inhibitory effect rather than a lethal one at standard therapeutic concentrations. This allows the host's immune system to function synergistically to combat microbial invasion more effectively. Innate/intrinsic resistance of microbes can reduce susceptibility to the antimicrobial agent using the membrane cell envelope or metabolic pathways, either energy metabolism or an alternate pathway. Adaptive/acquired resistance relies on single- or multi-step mutations that can alter the target site of a specific antibiotic. Additionally, extracellular genetic information can be acquired via MGEs and/or HGT providing mechanisms, such as enzymatic inactivation, to the microbial resistance repertoire (Poole 2002).

Disinfectants are often applied in environments where they must exhibit antimicrobial activity against microbes in a protected state, such as biofilms leading to high organic matter content, nutrient- or moisture-depleted environments, and suboptimal temperature or pH conditions (Condell et al. 2012). These compounds do not benefit from the synergistic effects of a host immune system; consequently, the intended application dose should be sufficient to reduce most of the microbial load after only a single application. The "in-use" concentrations of disinfectants are higher than laboratory-determined minimum inhibitory concentrations (MICs) and, as such, are designed to be rapidly lethal (Tezel and Pavlostathis 2015). The selection of insusceptible strains requires that a fraction of the bacterial population remain viable at the application site. This viability can be the result of either intrinsic or acquired resistance mechanisms. Initial consideration of the general differences between antibiotic and disinfectant target sites, modes of action, and intended

application concentrations would suggest little common ground for reduced susceptibilities against both agents. However, some phenomena can confer reduced susceptibility to both compounds as part of the intrinsic/innate repertoire or acquired/adaptive repertoire.

11.7.2 Resistance Repertoires and Resistance Mechanisms

Intrinsic resistance can be specific for bacterial groupings, such as mycobacteria, spore-forming *Bacilli* spp., or Gram-negative *Pseudomonas* spp. bearing extensively impermeable outer membranes. Additionally, specific environmental conditions can result in phenotypic alterations, such as biofilm formation, or nutrient depletion responses, such as persister cell formation (Brown and Gilbert 1993). Further, active efflux of a broad range of substances by multidrug efflux systems and/or reduced porin activity can be considered intrinsic resistance mechanisms when found in select organisms, such as *P. aeruginosa* (Gilbert and McBain 2003). Some instances of intrinsic adaptation may reduce the susceptibility without long-term change and, therefore, can be considered transient tolerance induced by an effector, reversible once the stress is removed (Jennings et al. 2015). This adaptation process allows a proportion of the bacterial population to display tolerance to the antibacterial compound but contrasts with actual resistance, which results from genetic alterations that allow bacteria to grow at elevated antimicrobial concentrations (Jennings et al. 2015).

Mutations and/or altered expression of endogenous genes or acquisition of exogenous genetic material result in acquired/adaptive resistance. Examples of acquired/adaptive resistance mechanisms associated with co-resistance and cross-resistance include biofilm formation, multidrug efflux pump systems, altered cell permeability or stabilisation, target-site mutation, and overexpression of protein characteristics involved in resistance. For example, biofilm formation provides an extracellular matrix hindering the diffusion of antibacterial substances and enhances resident bacterial signalling and genetic exchange (Pagedar et al. 2012).

Active efflux of molecules to the periplasmic region or the extracellular environment via multidrug efflux pumps is well documented. Efflux activity can be either intrinsic or acquired depending on the microorganism species. For example, Gram-negative bacteria are known to encode some broad substrate-specific efflux pumps chromosomally, and selected Gram-positive bacteria, such as *S. aureus*, can encode some multidrug pumps of chromosomal origin (Chen et al. 2003; Li et al. 2003; Morita et al. 2003; Huang et al. 2004; Randall et al. 2007). Furthermore, efflux pumps of MGE/plasmid origin can be represented among Gram-positive and Gram-negative bacteria, such as *qacA*, *qacB*, *qacE*, *smr*, and *qacI* (Bjorland et al. 2003; Plante et al. 2003; Chang et al. 2007; Huet et al. 2008; Maseda et al. 2009). Finally, mutations in the expression of efflux pump proteins or amino acid substitutions of a specific efflux pump can increase transport efficiency (Hegstad et al. 2010).

11.7.3 The Link Between Disinfectant and Antibiotic Susceptibilities

Many investigations into AMR have mainly focused on *S. aureus* in the medical industry involving hospitals or clinics. The β-lactam-insusceptible MRSA strains that carry *qac* genes and are resistant to benzalkonium chloride (BAC) have been intensively investigated (Al-Masaudi et al. 1988; Cookson 2005). MRSA mutants resistant to BAC presented higher resistance to β-lactam antibiotics, particularly cloxacillin, moxalactam, flomoxef, cefmetazole, and oxacillin (Akimitsu et al. 1999). It has been elucidated that laboratory *S. aureus* strains that are chromosomal efflux mutants were exposed to low concentrations of disinfectant, such as BAC, resulting in increased expression of MDR efflux pumps, such as *mepA* and *norA*. These mutants also displayed reduced susceptibility towards fluoroquinolone antibiotics (Huet et al. 2008). Although data on the linkage of antibiotic and disinfectant resistance is sparse in Africa, some studies have assessed the susceptibility of both antimicrobial agents. Strains of MDR nosocomial pathogens in Nigeria were tested against antibiotics and disinfectants, displaying resistance towards most antibiotics and disinfectants tested (Alabi and Sanusi 2012). However, the resistance mechanism was not elucidated, and thus linkage between the resistances cannot be made. However, investigating the prevalence of biocide resistance determinants in MRSA isolates from Angola, Cape Verde, and São Tomé and Príncipe can hint towards a probable answer. The *qac*AB and *smr* resistance genes located on MGEs displayed resistance towards several antibiotics, such as β-lactams, aminoglycosides, rifampin, trimethoprim-sulfamethoxazole, and chloramphenicol but also for disinfectants such as chlorhexidine. This suggests that these genes located on MGEs could be a means for MRSA and other bacteria carrying these plasmids to associate antibiotic and disinfectant resistance (Conceição et al. 2016).

Stepwise adaptation of *Escherichia coli* O157 and *Salmonella* serovars against BAC, chlorhexidine, and triclosan has shown susceptibility reductions to various antibiotics and other non-tested disinfectants. In addition, the MIC values for the antibiotics increased between 2- and 500-fold (Braoudaki and Hilton 2004). Similarly, field isolates of veterinary origin and a laboratory strain of *E. coli* were exposed stepwise to three QAC compounds. This partially yielded MIC increases above the clinical resistance breakpoints for some antibiotics, such as phenicol, tetracycline, fluoroquinolone, β-lactams, and trimethoprim (Soumet et al. 2012). Another study involved the stepwise adaptation of *E. coli* strains with BAC and chloramphenicol (CHL). The adaptations resulted in mutual increases in MIC, about 3-fold for the BAC after CHL adaptation and more than 20-fold for CHL after BAC adaptation (Langsrud et al. 2004). These findings suggest that both adaptations to antibiotics and QACs can increase susceptibility to the adapted agent and other unrelated antimicrobial agents. In such cases, cross-resistance mechanisms can be inferred to characterise the reduced susceptibility, such as multidrug efflux pumps. These studies represent adaptation outside an African context, but the trend of

altered resistance profiles can be applied to infer what antimicrobial treatments can induce in a general sense.

In many instances, cross-resistance to both disinfectants and other chemical agents, such as antibiotics or dyes, are mediated by the efflux pumps (Paulsen et al. 1996; Masuda et al. 2000; Huang et al. 2004; Romanova et al. 2006; Hansen et al. 2007; Maseda et al. 2009). However, co-resistance of disinfectant formulations and other antimicrobial agents can be found on the same genetic unit, such as on plasmids, transposons, or integrons but conferred by different mechanisms, as discussed in the following section. It is common for *qac* genes to be found on multi-resistance plasmids isolated from clinical strains of *Staphylococcus*, particularly *S. aureus* (Archer et al. 1986; Paulsen et al. 1996). The *qac*A, *qac*B, and a β-lactam resistance gene co-reside on large plasmids isolated from clinical, animal, and food-processing staphylococci (Sidhu et al. 2001, 2002a; Anthonisen et al. 2002; Bjorland et al. 2005). Such multi-resistance plasmids can readily be introduced into plasmid-free *Staphylococcus* indicating the high degree of transferability of these MGEs (Sidhu et al. 2001). The *qac* genes are not restricted to *Staphylococcus*, as *qac*H has been identified in clinical isolates of *Stenotrophomonas maltophilia* encoding aminoglycoside and CHL resistance (Chang et al. 2007). Additionally, *qac*F has been identified in *Serratia marcescens*, providing resistance towards β-lactams (Yum et al. 2002). The *qac* genes are not only responsible for expressing proteins that provide resistance towards QAC-based disinfectants but also other classes of disinfectants (Gahongayire et al. 2020). Disinfectants have the potential to alter the spread of antibiotic-resistant profiles in an environment, depending on the concentrations and conditions. The variation in the observable links between alterations of disinfectant and antibiotic susceptibility suggests that no single chain of events can lead to a uniform outcome of resistance profiles. Thus, additional research is needed to elucidate which conditions best select for resistance development for specific disinfectants and antibiotics.

11.8 Resistance Acquisition and Transfer

Plasmids can use specific mechanisms to move between different bacterial cells and transport any resistance genes they carry (Bennett 2008). Other classes of MGEs like gene cassettes, transposons, and IS*CR*-mediated gene mobilisation allow genes to move from one genetic location to another inside the same cell. These movements, such as transposition, allow plasmids to acquire resistance genes (Bennett 2008). Transposons are highly mobile and can jump from one plasmid to another. Transposons can carry resistance genes between plasmids, such as Tn402, which often harbours class 1 integrons containing QAC resistance genes (*qacEΔ1*), and antibiotic resistance genes (*sul1*) (Gillings et al. 2009b; Toleman and Walsh 2011). Multidrug resistance can be encoded due to a single transposon carrying multiple resistance genes.

Plasmids can be conjugative, meaning they move from one cell to another through conjugation, which encodes all the necessary machinery. Conjugation occurs through a membrane-associated mating pore complex that forms a mating channel through which the mobile elements can be disseminated throughout the bacterial population (Smillie et al. 2010). Mobilisable plasmids that do not encode transfer genes also exist and depend on co-resident conjugative plasmids that encode transfer genes. Despite not encoding the machinery, mobilisable plasmids can encode other functions involved in transferring their DNA (Bennett 2008).

11.8.1 Cross-Resistance to Antibiotics

Mutations that follow adaptation may develop multiple resistance mechanisms with broad specificity, contributing to multi-compound resistance and cross-resistance (Langsrud et al. 2003). Further, resistance genes can cluster on plasmids, which means that resistance to multiple compounds may be obtained by acquiring only a single plasmid. These compounds can range from various antibiotics and/or disinfectants and antiseptics. Plasmids isolated from pathogenic *E. coli* have been found to harbour multiple resistance genes encoding resistance to aminoglycosides, tetracyclines, and QACs (Barlow 2009). The *qac*A/B and *Bla*Z genes in *Staphylococcus*, for example, are genes that encode for resistance against QACs and β-lactam antibiotics, respectively. It has been found that these genes can be harboured together with plasmids of differing sizes (Sidhu et al. 2001; Langsrud et al. 2003).

11.8.2 Plasmid Maintenance

For plasmids to encode a resistance phenotype, they must be maintained within the host (Field and Summers 2012; Carattoli 2013). Stable plasmids are generally well adapted to different hosts, bearing efficient conjugation systems, have a broad host range, and are mobilisable by other resident plasmids (Carattoli 2013). In addition, plasmids often encode specialised mechanisms that facilitate their stability and thus the spread of their potential resistance genes through bacterial populations (Field and Summers 2012). One such mechanism is a restriction−/anti-restriction mechanism where the usual degradation of foreign DNA (plasmid DNA) by bacterial restriction endonucleases will be prevented (Carattoli 2013). Other systems involve toxin-/anti-toxin factors where a plasmid will encode specific toxins, positively selecting for daughter cells that inherited the specific plasmid while killing those that did not. This mechanism will allow the plasmid and thus its resistance genes to continue in the cell line as an integral part of the bacterial genome (Carattoli 2013). In addition to carrying disinfectant resistance genes, some plasmids encode genes that enhance their virulence. Some IncI1 plasmids, for example, carry β-lactam resistance genes along with a cluster of genes that encode for pili, facilitating the

adhesion and invasion process of organisms like Shiga toxigenic *E. coli* (Carattoli 2013). When virulence and resistance are combined, it may facilitate disseminating the microorganism carrying these determinants and further spread resistance.

11.8.3 Inactivation and Degradation

Bacteria can acquire plasmids that encode novel biochemical pathways, allowing them to degrade chemicals in their environment. Studies investigating genes involved in the degradation of xenobiotic compounds found that both naturally occurring and synthetic compounds are connected to MGEs, further supporting the idea that disinfectant resistance may be plasmid-mediated. Furthermore, by the movement of MGEs, different parts of a catabolic pathway may be recruited from different hosts within a population into one single host, giving it the ability to catabolise the disinfectant (Top and Springael 2003).

11.8.4 Cell Surface Alterations and Decreased Compound Uptake

When disinfectants are present at levels below their MIC, adaptation to these compounds involves modification of the cell's fatty acids, phospholipids, and outer membrane lipopolysaccharides. These alterations cause the cell surface to be more hydrophobic and anionic, inhibiting the diffusion of the disinfectant through the cell membrane (Tezel and Pavlostathis 2015). For example, *S. marcescens* and *E. coli* have demonstrated plasmid-encoded changes in membrane proteins that resulted in decreased formaldehyde uptake (Kaulfers et al. 1987).

11.8.5 Efflux

The expulsion of chemicals seems to be the primary mechanism of disinfectant resistance. Without any alteration to the disinfectant, a bacterium equipped with efflux pumps will lower the disinfectant concentration inside the cell by removing the substrate and expelling it to the external medium (Bragg et al. 2014). These efflux pumps can be substrate-specific or effective against multiple compounds, including various disinfectants and antibiotics (Piddock 2006b). The overexpression of specific efflux pumps or acquisition of new efflux pumps could promote cross-resistance. The first-ever efflux pump discovered in bacteria was a plasmid-encoded tetracycline-specific transporter, and currently, numerous plasmid-encoded efflux pumps are seen in bacteria providing resistance to both antibiotics and disinfectants (Li et al. 2016).

11.9 Efflux Pumps as Joint Resistance Mechanisms

The increasing global threat of antimicrobial resistance, and MDR bacteria, has increased the need to understand the various bacterial resistance mechanisms better. Drug efflux is the recognition and expulsion of a toxic chemical from inside the cell to the external medium before reaching its target. Bacterial insusceptibility to disinfectants has been described since the 1950s and 1960s and is increasing over time. The interaction between the organism and the chemical agent determines the nature of the response generated. Furthermore, the environmental factors involved during the reaction can have major implications on the activity of the antimicrobial agent. The effects of temperature at contact, environmental pH, and organic matter alter the antimicrobial agent's activity (Buffet-Bataillon et al. 2012; Handzlik et al. 2013; Mardanova et al. 2013). Efflux was first described for the antibiotic tetracycline; since then, many integral membrane and membrane-associated proteins have been elucidated to be involved in the efflux of antibiotics, disinfectants, and other substances (Ortega Morente et al. 2013). The best-characterised method with which some bacteria, such as *S. epidermidis*, can be tolerant to QACs is through the overexpression of efflux pump systems (Ribi et al. 2020). Efflux systems actively prevent the accumulation of toxic compounds within the bacterial cell. The transport is conducted in an energy-dependent manner which does not involve alteration or degradation of the chemical agents transported (White and McDermott 2001; Fraise 2002; Chapman 2003; Gnanadhas et al. 2013).

11.9.1 Classification of Efflux Pumps

Generally, efflux systems are divided into two classes depending on the energy source required for transport. The ATP-binding cassette (ABC) transporter utilises energy from ATP hydrolysis, and the secondary multidrug transporters use the proton motive force (PMF) (Grkovic et al. 2002; Stavri et al. 2007; Fernández and Hancock 2012). The secondary multidrug transporters can be further sub-divided into four superfamilies depending on structural and amino acid homology (Grkovic et al. 2002). The superfamilies are the major facilitator superfamily (MFS), the small multidrug resistance (SMR) family, the multidrug and toxic compound extrusion (MATE) family, and the resistance-nodulation-cell division (RND) family (Grkovic et al. 2002; Poole 2004). Recently, a new family of transporters has been introduced – the proteobacterial antimicrobial compound efflux (PACE) family (Hassan et al. 2015).

The ABC family is capable of both uptake and efflux, where the substrates for these transporters include a wide variety of compounds, such as sugars, drugs, amino acids, polysaccharides, ions, and proteins. The MFS family has members that can function as uniport (transport without coupled ion movement), symport (coupled ion movement in the same direction), and antiport systems (coupled ion movement in the opposite direction). All MFS transporters that export antimicrobials are

drug/proton antiporters. The MATE family shares topology with the MFS family; however, these proteins can utilise sodium (Na^+) gradients or the PMF to export hazardous compounds (Piddock 2006a; Fernández and Hancock 2012; Chitsaz and Brown 2017). Finally, RND pumps are drug/proton antiporters, meaning there is an exchange of a hydrogen ion (H^+) for a drug molecule (Piddock 2006a; Mardanova et al. 2013; Chitsaz and Brown 2017).

11.9.2 Regulation of Efflux Pumps

Transcriptional regulators, either locally or globally, control the regulation of efflux pump expression (Hernando-Amado et al. 2016; Chitsaz and Brown 2017). Local regulator genes are usually encoded upstream of structural genes (Hernando-Amado et al. 2016). These regulatory systems can involve modulators or two-component systems and different regulatory cascades of small RNA molecules (Chitsaz and Brown 2017). Generally, under laboratory conditions, the expression of efflux pumps is low; however, physiologically high-level expression requires the addition of an effector (Hernando-Amado et al. 2016). However, mutational events can result in high-level non-physiological efflux pump expression, and such mutants can be positively selected using antimicrobial therapy conditions promoting the acquisition of resistance (Hernando-Amado et al. 2016). Generally, these efflux genes are plasmid-borne, but there are exceptions, such as the chromosomally located *Nor*A genes of *S. aureus* (Poole 2002).

11.9.3 Activity of Efflux Pumps

11.9.3.1 Qac Proteins Involved in Efflux

Reduced susceptibility to disinfectants in *Staphylococcus*, particularly *S. aureus*, is often caused by secondary energy-dependent transporters. Common encoded efflux pumps include *qac*A, *qac*B, *qac*C/D, *qac*H, and *qac*G (Littlejohn et al. 1991; Grkovic et al. 2002; Poole 2005; Liu et al. 2009). The *qac*A and *qac*B genes are usually harboured on plasmids that frequently harbour antibiotic resistance genes that can result in co-selection of antibiotic-resistant bacteria (Sidhu et al. 2002b; Poole 2005; Liu et al. 2009). PCR results have shown that *qac*A/B are the most prevalent *qac* genes within QAC tolerant *S. aureus* (Liu et al. 2009). These *qac*A/B genes code for proton-dependent multidrug efflux transporters QacA and QacB, which form part of the MFS family (Nikaido 2009; Jennings et al. 2015; Chitsaz and Brown 2017). These pumps have been shown to actively extrude mono-cationic biocides and dyes, such as BAC and chlorhexidine (Littlejohn et al. 1991; Nikaido 2009; Horner et al. 2012). Within Gram-negative bacteria, there are occurrences of *qac*E and its deletion mutant homolog *qacEΔ1*, which were first described on integrons. These genes have been reported in *Enterobacter* spp., *Pseudomonas* spp.,

Vibrio spp., and *Klebsiella pneumonia* (Poole 2002; Jennings et al. 2015). These *qac*E and *qaE*Δ*1* genes are associated with increased MICs against dyes such as ethidium bromide and QACs such as BAC (Jennings et al. 2015).

11.9.3.2 RND Efflux Systems

The RND-type efflux transporter systems, such as AcrAB-TolC of *E. coli* and MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM of *P. aeruginosa*, are three-component systems that have been extensively studied (Pagès et al. 2005; Piddock 2006a; Chitsaz and Brown 2017). These tripartite systems include an integral membrane protein and an outer membrane porin connected by a fusion protein (Fernández and Hancock 2012). The three-component/tripartite systems are mainly found within Gram-negative bacteria because of their double-membrane cell envelope structure (Fernández and Hancock 2012). The unique structure allows the excretion of toxic chemicals from the cytoplasm directly into the external medium (Nikaido 2009; Zechini and Versace 2009). In addition, the highly impermeable outer membrane of Gram-Negative bacteria slows down the re-entry of the drug into the cell, producing increased resistance levels (Nikaido 2009).

The RND family is the most relevant class of efflux pump within the clinical setting (Mardanova et al. 2013). These proteins are best characterised within Gram-negative bacteria and are also present within Gram-positive bacteria (Blair et al. 2014; Hernando-Amado et al. 2016). These efflux pumps display a large binding profile (poly-selectivity), which includes antibiotics, detergents, toxic fatty acids, disinfectants, aromatic hydrocarbons, dyes, and bile salts (Nikaido 2009; Fernández and Hancock 2012; Chitsaz and Brown 2017). This large substrate specificity of RND pumps forms part of a MDR profile and can promote the acquisition of additional resistance mechanisms to form part of the resistance phenotype (Nikaido 2001; Nikaido and Pagès 2012). The ubiquitous nature of RND pumps suggests a role other than generalised use to export antimicrobials. Some RND pump genes have been found in strains isolated before the antibiotic era, suggesting that these pumps have not appeared in response to widespread antibiotic use (Poole 2005; Nikaido 2009).

Multidrug resistance RND efflux pumps and members of the other families might have a role in the transport of natural substances, bacterial products, or defensive characteristics to different toxic compounds that could usually be encountered in the natural environment (Thanassi et al. 1997; Nikaido 2009). It has been suggested that these multidrug resistance RND-type pumps could facilitate or promote the release of molecules involved in virulence, as seen by the study conducted on the MexAB-OprM efflux system of *P. aeruginosa* (Hirakata et al. 2002; Poole 2004). The AcrAB system within *E. coli* has activity in toxic fatty acid transport, and its action in the export of bile salts has been observed (Ma et al. 1995; Thanassi et al. 1997). This activity has been supported as the role of AcrAB in protecting the cell from the activity of gut agents present within the environment that *E. coli* inhabit (Ma et al. 1995; Thanassi et al. 1997). Bacteria cannot predict the nature of hazardous compounds within the environment; thus, having an efflux system with broad specificity will be most favourable for survival (Mahamoud et al. 2007).

11.9.4 Efflux Pump Adaptations

Environmental exposure of microbes to antibacterial compounds can impact the expression of certain resistance systems. The differential expression of genes encoding efflux pumps resulting from observed changes in the extracellular environment supports the importance of adaptative systems within a microorganism (Fernández and Hancock 2012). This impact is exuberated by the SARS-CoV-2 pandemic where the increased use of disinfectants, particularly QACs, increased the exposure risk of bacterial pathogens to an unwanted low-level stimulus (Hora et al. 2020). This characteristic is particularly true for pathogenic microorganisms where efflux pumps are the most common resistance mechanism against therapeutic agents and play a role in survival within the host. This can be seen with the *Nor*A pump of *S. aureus*, which is involved in iron availability, but the pump can also have a binding affinity for antimicrobials in the environment (Hegstad et al. 2010; Deng et al. 2012; Fernández and Hancock 2012).

11.9.4.1 Constitutive and Transient Expression

Antimicrobials play a vital role in inducing adaptive resistance by overexpression of efflux systems (Levy 2002; Sanchez et al. 2005, 2015). The overexpression can be transient or constitutive, depending on antimicrobial and bacterial cell reactions. If the overexpression is transient, then the antimicrobial acts as an effector of the transcriptional regulator of the efflux pump (Sánchez et al. 2005, 2015). Constitutive overexpression occurs when the antimicrobial selects mutants that overexpress the efflux pump (Sánchez et al. 2015). An example involves the expression of efflux pump SmeDEF in *S. maltophilia*. The biocide triclosan induced the expression of *smeDEF* by binding to the transcriptional repressor of the system, SmeT (Sánchez et al. 2002, 2015; Hernández et al. 2011). As a result, biocide-mediated expression of the efflux pump was induced, allowing extrusion of triclosan and several unrelated antibiotics that were substrates for SmeDEF. This showed that the acquisition of transient triclosan resistance simultaneously provided low-level antibiotic resistance via SmeDEF (Sánchez et al. 2015). Similarly, *P. aeruginosa* grown in continuous culture exposed to subinhibitory concentrations of BAC could gain antibiotic resistance. However, the resistance alterations depend on the nature of the antibiotic, the disinfectant, and the organism (Mc Cay et al. 2010). Repeated sublethal exposure levels of disinfectant might not result in non-susceptibility but rather reduced susceptibility in exposed strains (Moore et al. 2008). This can be extended to the overuse of disinfectants, leading to the acquisition of acquired resistance mechanisms (Jennings et al. 2015; Tamburro et al. 2015). The phenomenon of resistance shows one possible explanation of how an organism can become both cross-resistant to antibiotic and disinfectant treatments when continually exposed to sub-MIC levels (El Garch et al. 2010; Hernández et al. 2011; Guo et al. 2014).

11.10 Concluding Remarks

Current literature does not provide a clear way in which resistance is uniformly developed because of a single route of events. Sub-inhibitory concentrations of antibacterial compounds can cause resistance development after a specified time. Similarly, extrachromosomal MGEs can also enable resistance development, where both mutations and MGE-associated resistance can be possible. The use of antibiotics is present in all settings where optimal growth and disease treatment is required, but the unregulated and overuse of antibiotics could be a driver of bacterial multidrug resistance. Particularly, the agricultural sector is prone to excessive antibiotic use at sub-therapeutic and therapeutic levels. However, the medical sector is not without blemishes. Physicians who casually prescribe antibiotics need to be more strictly regulated. Any patients who are prescribed antibiotics need to complete the full antibiotic prescription course. In attempts to prevent a similar trend as in antibiotics, disinfection needs to be managed appropriately. Disinfectant exposure has varied effects on antibiotic resistance development, where in some cases, positive selection is prevalent and in others, negative selection is seen. Therefore, little is known about the exact biochemical processes that occur during specific disinfection regimes. Additionally, there is a lack of comprehensive research on disinfectant types used in Africa and how they interact with specific bacterial species under real-world disinfection conditions. Implementing a national or local rotational plan of different disinfectant products with varying mechanisms of action will be a possible way to circumvent resistance development in many industries and medical environments. This comparatively low-cost solution can also be applied to several industries regionally and nationally across African countries. However, with MDR efflux pumps having broad substrate specificity, monitoring the resistance profile of intrinsic bacterial populations will be key. Therefore, this strategy must be coupled with surveillance of disinfectant resistance to tackle the problem sufficiently. HGT also plays a crucial role in resistant maintenance and development, but knowledge of these mechanisms under stress conditions is also limited. Understanding the exact chemical and physiological events during disinfection can provide critical knowledge to better control resistance spread.

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Chapter 12
The Use of Nanomaterials for the Elimination of Antibiotic-Resistant Bacteria from Water and Wastewater: An African Overview

Mohamed Azab El-Liethy, Mohamed Mahmoud, Akebe Luther King Abia, and Khalid Z. Elwakeel

12.1 Introduction

As of late, the broad utilisation of antimicrobials, including antibiotics, for the treatment of people and animals has impacted the recurrence and spread of antibiotic-resistant bacteria (ARB) into the environments (El-Shatoury et al. 2015; Abia et al. 2015). Antibiotic resistance is the capacity of bacteria to resist antibiotics to which they were previously sensitive. Microbes become antimicrobial-resistant by two systems, either intrinsically or by gaining antibiotic resistance genes (ARGs) through horizontal gene transfer (HGT) (Munita and Arias 2016; Amarasiri et al. 2020). Recently, antibiotic resistance has spread faster than expected (Singh et al. 2019). The spread of multiple antibiotic-resistant bacteria has been recognised as a severe global human and animal health problem (WHO 2018a). Water environments are considered one of ARB's most significant reservoirs and transmission routes (Karkman et al. 2018). ARB and ARGs can enter water environments such as rivers and lakes through wastewater treatment plant (WWTP) effluents because conventional wastewater treatment processes cannot completely remove ARB and ARGs (Rodriguez-Mozaz et al. 2015). Therefore, reusing treated wastewater for agricultural and recreational purposes can introduce ARB and ARGs to the

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environment (Ben et al. 2017; Rodriguez-Mozaz et al. 2015; Amarasiri et al. 2020). This chapter summarises the data reported in recent studies on ARB and ARGs in African water and wastewater and highlights conventional and advanced wastewater treatment technologies for ARB and ARGs removal. The chapter further suggests prospects and challenges regarding using nanomaterial to remove ARB from African wastewater.

12.2 Antibiotic Resistance Mechanisms in Bacteria

Microbial resistance to antimicrobial agents is considered one of the primary causes of high morbidity and mortality worldwide. Nowadays, most microbial pathogens can develop resistance to at least one or more antimicrobial agents (Reygaert 2018). Bacteria develop antibiotic resistance either through genetic mutations or by gaining ARGs (Amarasiri et al. 2020). Bacteria use many antibiotic resistance strategies (Figs. 12.1 and 12.2). Active efflux is one of the resistance mechanisms where bacteria use pumps in their cell wall to expel antibiotics out of the bacterial cell. In some cases, mutations in the bacterial DNA can increase the bacteria's resistance by producing more pumps (Aparna et al. 2014).

Another resistance mechanism is decreasing the permeability of the bacterial cell membrane to prevent antibiotics' entrance. Bacterial cell permeability is reduced through water-filled diffusion channels and/or active transport systems (Kapoor et al. 2017). Furthermore, bacteria can produce enzymes that destroy antibiotics. For example, bacteria produce β-lactamases that destroy the β-lactam ring of penicillins. Bacteria that produce extended-spectrum β-lactamase (ESBL) enzymes are

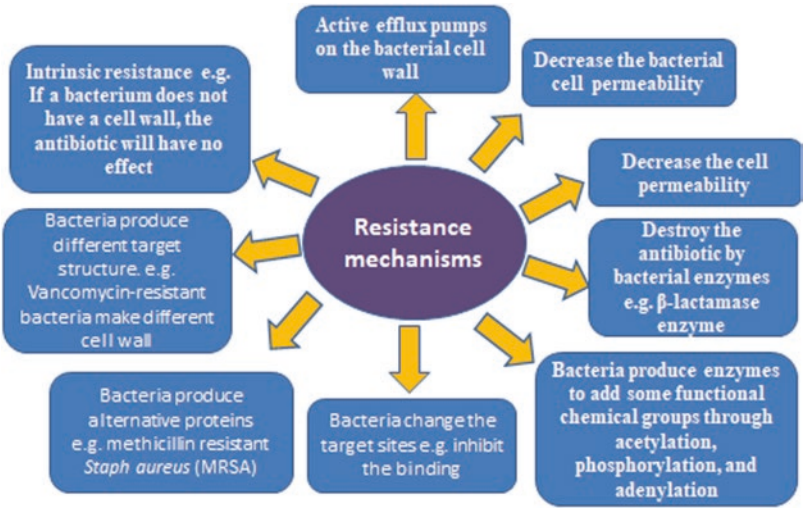

Fig. 12.1 Different antibiotic resistance strategies in bacteria

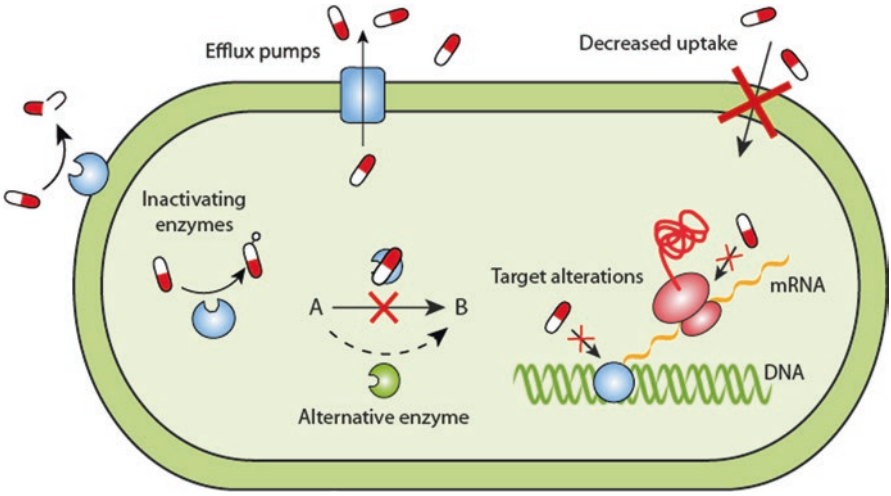

Fig. 12.2 Antibiotic resistance mechanisms in bacterial cells. (Source: Wistrand-Yuen et al. (2018))

called ESBL-producing bacteria (Poole 2004). Occasionally, bacteria can produce enzymes to add functional chemical groups through acetylation, phosphorylation, and adenylation, inhibiting the binding between antibiotics and bacterial cells (Martinez and Baquero 2014). In addition, changes in the creation or construction of the target site in the bacterium (because of transformations in the bacterial DNA) can prevent the antimicrobial from attaching to the target sites. Also, the microorganisms can add diverse synthetic groups to the target site, protecting it from antibiotics (Martinez and Baquero 2014).

A few microorganisms produce other proteins that can be utilised rather than the ones repressed by the antibiotics. For instance, *Staphylococcus aureus* can gain the resistance gene *mecA* and produce another penicillin-binding protein. These proteins are required for bacterial cell synthesis and produce β-lactam antibiotics. The new penicillin-binding protein has a low affinity for β-lactam antibiotics and is subsequently impervious to the medications, and the microorganisms endure treatment. This mechanism is the main one seen in MRSA (methicillin-resistant *Staphylococcus aureus*) (Walsh 2000). At times, microorganisms can modify the site targeted by the drug. For instance, vancomycin-resistant bacteria make an alternate cell structure absent in susceptible strains, preventing the antibiotic from connecting to such cell walls (Munita and Arias 2016). Intrinsic resistance is another resistance phenomenon in which antibiotics targeting the bacterial cell wall do not affect bacteria lacking a cell wall (Reygaert 2018).

12.3 Antimicrobial Resistance Transmission in the Environment

Antimicrobial resistance (AMR) is an important public health challenge in the twenty-first century, and in 2050 it is estimated that ten million people will die yearly if satisfactory measures are not implemented (O'Neill 2014; Prestinaci et al. 2015).

AMR reaches the environment from varied sources like hospitals, industries, and households. These sources are considered potential point sources for the environmental distribution of ARB and promote bacterial proliferation and genetic exchange of antibiotic resistance genes (ARGs) (Zhang et al. 2009; Pruden et al. 2012). The extensive use of antimicrobials to treat people and domestic animals has affected the recurrence and spread of antibiotic-resistant bacteria (ARB) in environmental sources (El-Shatoury et al. 2015). Figure 12.3 illustrates the transmission of ARB and ARGs during various anthropogenic activities. The ARGs commonly spread in the aquatic environment through horizontal gene transfer aided by mobile genetic elements (MGEs) such as plasmids, transposons, integrons, or chromosomes (Fig. 12.3). The ARGs transfer from donor microorganisms, bacteriophages, or even dead microbial cells to other viable microbial cells. Additionally, many resistance genes may be transferred through the same mobile genetic elements (Stokes

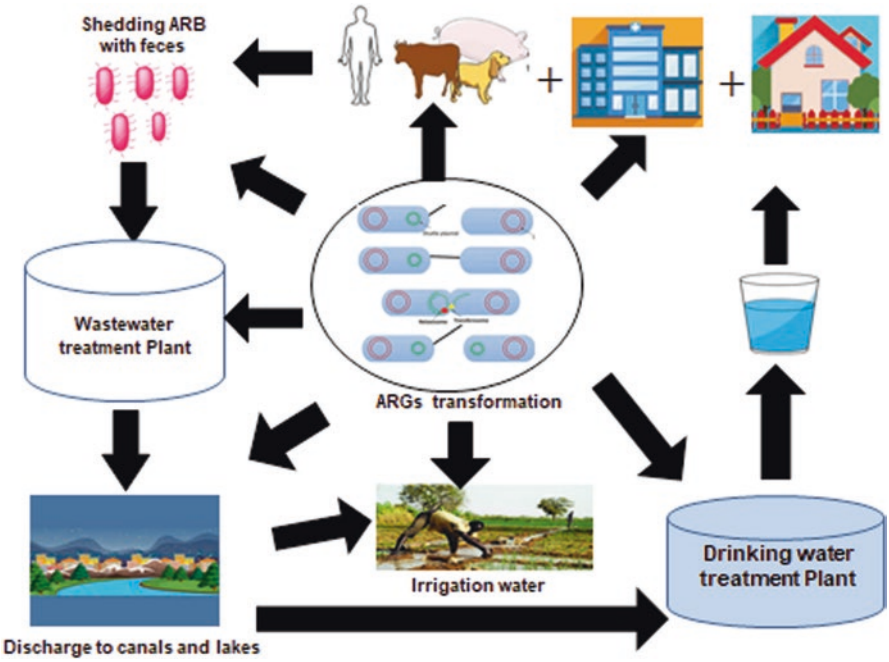

Fig. 12.3 Antibiotic resistance genes transformation in various environments

and Gillings 2011; Partridge et al. 2018). For example, wastewater treatment plants collect sewage from different sources, including hospitals and households.

Hospital wastewater is one of the important sources of antibiotics and ARB (Devarajan et al. 2016). For instance, in Egypt, penicillin concentration as an antibiotic residue in wastewater reached 289,400 μg/L (Abou-Elela and El-Khateeb 2015). Conventional wastewater treatment processes are not intended to eliminate ARB and ARG, so WWTPs usually harbour antimicrobials and different stressors known to co-select for antibiotic resistance. These ARB and ARGs may spread to the workers or people close to the WWTP. Therefore, wastewater and WWTPs are known for spreading antibiotic resistance microbes and promoting horizontal gene transfer (Karkman et al. 2018).

ARB and ARGs may be transferred to groundwater and other natural water bodies through discharged WWTP effluents (Fig. 12.3). In addition, these ARGs could be transferred to microbial communities in agricultural soil via irrigation water (Fahrenfeld et al. 2013; Luo et al. 2014; Szekeres et al. 2018).

12.4 Antibiotic-Resistant Bacteria (ARB) and Antibiotic Resistance Genes (ARGs) in African Water

Most African countries are characterised by limited financial resources, malnutrition, poor and insufficient healthcare structures (Bain et al. 2013), inappropriate prescription, self-medication, and free sale of antibiotics (Sanou et al. 2018). Furthermore, most of these countries have no regulatory agencies controlling antibiotics use, with antibiotics sold over the counter in pharmacies; all of these lead to ARB and ARGs spreading in the environment.

The combination of PCR and phenotypic approaches has been used in several studies to describe the resistance profiles and ARGs associated with AMR bacteria. Other studies have used PCR followed by Sanger sequencing to demonstrate AMR genotypes. Recently, many studies have extensively used whole-genome sequencing and metagenomic analyses to detect the microbial and AMR determinants in contaminated water.

12.4.1 Wastewater

Human and animal microbiota is considered the main reservoir for ARGs. Microorganisms transmitted through the intestinal tract can acquire ARGs via conjugation and reach sewage through faeces (Salyers et al. 2004; Anderson et al. 2013). Thus, treated wastewater is essential to environmental hygiene in urban and suburban regions. Nevertheless, WWTPs gather inorganic and organic chemical pollutants and microbes, including potential enteric pathogens, ARB and ARGs,

from numerous sources, which are released into surface water through their effluents (Numberger et al. 2019). Wastewater contains bacterial communities of different taxonomic groups such as N_2-fixation, nitrifying, and denitrifying bacteria groups and anaerobic, aerobic, phototrophic, and heterotrophic communities; some of these bacteria provide functional privilege for water purification and act as nutrients for scavengers. Conversely, these bacterial communities also contain bacteria of human and animal origin which may negatively interact with the bacterial communities of open waters (e.g. rivers and lakes), for instance, transferring ARGs (Numberger et al. 2019). In Burkina Faso, metagenomics was used to investigate the microbial population, ARGs, and plasmids of medical concern in wastewater used for urban agriculture in Ouagadougou. One hundred and twenty-nine ARGs were detected in the wastewater samples, of which 81 were determined transmissible by mobile genetic elements through HGT. The 81 transmissible ARGs confer resistance to 11 major classes of antibiotics and trimethoprim. The most prevalent resistance genes encoded resistance to aminoglycosides (20 genes), tetracycline (19 genes), ß-lactams (13 genes), and macrolides (9 genes) (Bougnoma et al. 2019).

In Tunisia, Rafraf et al. (2016) investigated the occurrence of seven ARGs before and after treatment in five WWTPs located in different areas of the Monastir Governorate. It was found that all ARGs were detected in both influent and effluent wastewater. On the other hand, in Nigeria, the ARGs of bacterial isolates obtained from wastewater samples from 14 pharmaceutical facilities were detected in Lagos and Ogun States. In addition to this, WWTP effluent in Ogun and river water samples were also examined. The results showed that the most detected ARGs were *cat*A1, *sul*I, *tet*E, *aac*(3)-IV, *erm*C, bla_{TEM}, $bla_{\text{CTX-M}}$, and $bla_{\text{NDM-1}}$, conferring resistance to chloramphenicol, sulfonamide, tetracycline, aminoglycoside, macrolide, streptogramin, ß-lactam, and penicillin, respectively (Obayiuwana and Ibekwe 2020).

Many African studies have emphasised the role of wastewater as a primary environmental reservoir for antibiotics residues, ARB, and ARGs. Some antibiotic residues such as ampicillin, amoxicillin, sulfamethoxazole, and chloramphenicol were determined in Kenya. Kenya is a new emerging hotspot of antibiotic resistance found in animals in low-income countries (Kimosop et al. 2016; Van Boeckel et al. 2019). On the other hand, chloramphenicol, neomycin, and kanamycin B residues have been detected in Tunisia (Tahrani et al. 2015).

Notably, South African researchers have published numerous studies on antibiotics residues, ARB, and ARGs in wastewater, river water, and sediments. Common antibiotics residues such as clarithromycin, erythromycin, azithromycin, roxithromycin, sulfamethoxazole, trimethoprim, ofloxacin, ciprofloxacin, and norfloxacin were determined in wastewater treatment plants (Faleye et al. 2019; Nyamukamba et al. 2019). The prevalence of *E. coli* and *Vibrio* species in the effluents of two WWTPs, in Eastern Cape, South Africa, was studied. Antibiotic susceptibility test of the PCR confirmed *Vibrio* isolates was done by standard disc diffusion method against 18 antibiotics (Table 12.1). Moreover, ten ARGs, including bla_{TEM}, bla_{SHV}, bla_{Z}, $bla_{\text{CTX-M}}$, *aad*A, *str*A, *tet*A, *tet*B, *tet*K, and *tet*M, were tested using PCR. The results showed that the PCR confirmed *Vibrio* isolates were resistant to 18 antibiotics, with resistance frequencies ranging from 0.5% (imipenem) to 96.1% (penicillin G).

Table 12.1 Incidence of ARB and ARGs in wastewater in some African countries

African country	Matrix	Antibiotic/ antibiotic group	Resistant bacteria	Resistance genes/ method	References
Burkina Faso	Wastewater	Trimethoprim, β-lactams, aminoglycosides, sulfonamides, tetracyclines and macrolides	*Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, *Cyanobacteria*, *Deinococcus-Thermus*, *Verrucomicrobia*, *Spirochaetes*, fusobacteria	aad_{A15}, aad_{A13}, *sul*1, *aad*A, and *tet*B, *sul*1, *sul*2, *aad*A13, *aad*A, *Rbp*A, *sul*1, *sul*2, *aad*A13, *aad*A, *aad*A15, $bla_{OXA-226}$, $bla_{OXA-256}$, $bla_{OXA-347}$, bla_{OXA-46}, $bla_{SHV-100}$, bla_{GES-21}, and bla_{AIM-1} $bla_{SHV-100}$, bla_{AIM-1}, $bla_{OXA-226}$, $bla_{OXA-256}$	Bougnoma et al. (2019)
Nigeria	Pharmaceutical wastewater and household wastewater	Chloramphenicol, sulfonamides, tetracycline, aminoglycoside, macrolide-lincosamide-streptogramin, and ß-lactams and penicillins	*Acinetobacter* sp.	*cat*A1, *sul*I, *tet*(E), *aac*(3)-IV, *erm*C, *bla*TEM, *bla*CTX-M, *bla*NDM-1	Obayiuwana and Ibekwe (2020)

(continued)

Table 12.1 (continued)

African country	Matrix	Antibiotic/ antibiotic group	Resistant bacteria	Resistance genes/ method	References
South Africa	Activated sludge in two wastewater treatment plants (Eastern Cape)	Ampicillin, amikacin, imipenem, meropenem, streptomycin, chloramphenicol, ciprofloxacin, nalidixic, tetracycline, trimethoprim, norfloxacin, sulfamethoxazole, gentamycin, neomycin, penicillin G, nitrofurantoin, polymyxin, and cefuroxime	*E. coli* and *Vibrio* spp.	*str*A, *aad*A, *cat* I, *cml*A1, bla_{TEM}, *tet*A, *tet*B, *tet*C, *tet*D, *tet*K, *tet*M	Adefisoye and Okoh (2017)
South Africa	Wastewater effluent (Durban)	β-Lactams, aminoglycosides, carbapenems, cephalosporin, glycopeptides, lincosamides, macrolides, nitrofurans, penicillins, polypeptides, quinolones, sulfonamides, tetracyclines, streptomycin, chloramphenicol, fosfomycin, fusidic acid	*Listeria* and *Aeromonas* spp.	Disc diffusion	Olaniran et al. (2015)
South Africa	Wastewater effluent (Eastern Cape)	Trimethoprim, chloramphenicol, tetracycline, streptomycin, sulfamethoxazole	*Vibrio* spp.	*dfr*18, *dfr*A1, *flo*R, *tet*A, *str*B, *sul*2	Okoh and Igbinosa (2010)

(continued)

Table 12.1 (continued)

African country	Matrix	Antibiotic/ antibiotic group	Resistant bacteria	Resistance genes/ method	References
South Africa	Alice and Fort Beaufort WWTPs, Eastern Cape	Ciprofloxacin, trimethoprim, chloramphenicol, penicillins, clindamycin, ofloxacin, ampicillin-sulbactam, oxacillin,. Ampicillin, gentamicin, nalidixic acid, cefotaxime, nitrofurantoin, sulfamethoxazole, cephalothin, erythromycin, tetracycline, minocycline, vancomycin, rifamycin	*Aeromonas* spp.	*pse*1, $bla_{TEM,}$ *Tet*C, class 1 integron, class 2 integron	Igbinosa and Okoh (2012)
South Africa	WWTP effluents, Eastern Cape	Ampicillin, co-trimoxazole, amikacin, imipenem, erythromycin, meropenem, streptomycin, chloramphenicol, ciprofloxacin, cephalothin, nalidixic acid, tetracycline, trimethoprim, norfloxacin, sulfamethoxazole, gentamicin, neomycin, penicillin G, nitrofurantoin, polymyxin B, cefuroxime	*Vibrio* spp. (including *V. parahaemolyticus*, *V. fluvialis*, and *vulnificus*)	*bla*P1 ß-lactamase cassette and *SX*T integrase, *sul*II, *dfr*A1, *str*B, *flo*R, *dfr*18, and *tet*A	Igbinosa et al. (2011)

(continued)

Table 12.1 (continued)

African country	Matrix	Antibiotic/ antibiotic group	Resistant bacteria	Resistance genes/ method	References
South Africa	Hospital wastewater and effluent of WWTP in Alice, Eastern Cape Province	Erythromycin, imipenem, tetracycline, cefotaxime, gentamicin, clindamycin, kanamycin, vancomycin, ciprofloxacin, penicillin	*Enterococcus* spp.	*van*A, *van*B, *van*C1, *van*C2/3	Iweriebor et al. (2015)
South Africa	Two final effluent of WWTP in eastern Cape Province	Amikacin, ampicillin, cefuroxime, cefotaxime, cephalexin, chloramphenicol, ciprofloxacin, colistin sulphate, gentamycin, imipenem, meropenem, nalidixic acid, nitrofurantoin, norfloxacin, polymyxin B, streptomycin, tetracycline	Pathogenic *E. coli*	*str*A, *aad*A, *cat*I,*cat*II, *cmI*A1, *amp*C, *bla*Z, *bla*TEM, *tet*A, *tet*B, *tet*C, *tet*D, *tet*K, *tet*M	Adefisoye and Okoh (2016)
South Africa	Final effluent of WWTP and discharging point, upstream and downstream, eastern cape	Amikacin, ciprofloxacin, aztreonam, linezolid, chloramphenicol, imipenem, ceftriaxone, meropenem, cephalothin, ertapenem, erythromycin, gatifloxacin, gentamicin, moxifloxacin, ampicillin, streptomycin, penicillin, tetracycline, trimethoprim, sulfamethoxazole	*Listeria* isolates	*sul*I, *sul*II, *erm*A, *erm*B, *era*B, and *amp*C	Odjadjare et al. (2010)

(continued)

Table 12.1 (continued)

African country	Matrix	Antibiotic/ antibiotic group	Resistant bacteria	Resistance genes/ method	References
Tunisia	Influent and effluent of activated sludge WWTP (Monastir)	Lincosamide, macrolides, streptogramin	*S. aureus* and *E. coli*	bla_{CTX-M}, bla_{TEM}, *qnrA*, *qnrS*, *sul* I, *ermB,* and *intI1*	Rafraf et al. (2016)

Approximately 81% (166 out of 205 *Vibrio* isolates) exhibited multidrug resistance, while nine ARGs were detected by PCR (Adefisoye and Okoh 2017). Also, the same authors, in 2016, tested 223 PCR-confirmed pathogenic *E. coli* isolates against 17 different antibiotics (Table 12.1) using the disc diffusion method and evaluated 14 different ARGs by PCR. Pathogenic *E. coli* were isolated from final effluents of two activated sludge WWTPs in Amathole, Eastern Cape. The results showed that *str*A, *aad*A, *cat* I, *cmlA*1, *bla*TEM, *tet*A, *tet*B, *tet*C, *tet*D, *tet*K, and *tet*M were detected in the isolates. They further confirmed that municipal wastewater effluents were significant reservoirs for the distribution of pathogenic *E. coli* and ARGs in the aquatic environment of the Eastern Cape (Adefisoye and Okoh 2016). Iweriebor et al. (2015) determined the antibiotic resistance and ARG profiles of *Enterococcus* isolated from Victoria Hospital wastewater and the final effluent of a WWTP in Alice, Eastern Cape, South Africa. The results showed that all the *Enterococcus* isolates were resistant to vancomycin and erythromycin. Also, 91% of hospital wastewater and WWTP effluent isolates were vancomycin-resistant. Vancomycin resistance (*van*B, *van*C1, and *van*C2/3) and erythromycin resistance genes (*erm*B) were detected in most of the isolates. The authors concluded that *Enterococcus* isolates from the hospital wastewater and effluent of WWTP had similar resistance patterns.

Also, in another study, Olaniran et al. (2015) assessed the antimicrobial resistance, and ARGs in *Listeria* and *Aeromonas* isolates recovered from treated effluents of two WWTPs and receiving rivers in Durban. They found that *Listeria* isolates were resistant to penicillin, erythromycin, and nalidixic acid (100%), followed by ampicillin (83.3%), trimethoprim (67.9%), nitrofurantoin (64.1%), and cephalosporin (60.2%). *Aeromonas* isolates were completely resistant to ampicillin, penicillin, vancomycin, clindamycin, and fusidic acid, followed by cephalosporin (82%), erythromycin (58%), nalidixic acid (56%), and trimethoprim (56%). In another investigation, Igbinosa and Okah (2012) studied the prevalence of antibiotic-resistant *Aeromonas* species from Alice and Fort Beaufort WWTPs, Eastern Cape, South Africa. Approximately 20 different antibiotics were tested using the disc diffusion method. Moreover, ARGs *pse*1 (Class A β-lactamase), bla_{TEM}, *Tet*C, class 1 integron, and class 2 integron were detected by PCR. The results showed that all *Aeromonas* isolates were 100% resistant to penicillin, oxacillin, ampicillin, and vancomycin. Fort Beaufort isolates displayed greater phenotypic resistance than Alice WWTP isolates. Class A *pse*1 β-lactamase was present in 20.8% of the total isolates with a lower detection rate of 8.3% for the bla_{TEM} gene. Class 1 integron was

present in 20.8% of *Aeromonas* isolates, while class 2 integron and *Tet*C gene were not detected in any isolate.

Furthermore, in Eastern Cape, South Africa, Igbinosa et al. (2011) tested *Vibrio* species isolated from WWTP effluents against 21 antibiotics. All *Vibrio* isolates were susceptible to imipenem and meropenem but resistant to chloramphenicol and erythromycin. Moreover, 50% of the *V. vulnificus* strains were also resistant to tetracycline. Likewise, most of *V. fluvialis*, *V. vulnificus*, and *V. parahaemolyticus* (70–80%) were susceptible to neomycin, gentamicin, and amikacin. More than 90% of isolates showed resistance to cefuroxime, nitrofurantoin, norfloxacin, and cephalothin; over 80% were resistant to nalidixic acid and ciprofloxacin. All *V. fluvialis* isolates were 80–90% resistant to the ß-lactams antibiotics, penicillin G, and ampicillin. More than 90% of the *Vibrio* isolates showed resistance to streptomycin, trimethoprim, sulfamethoxazole, and co-trimoxazole. *V. vulnificus* and *V. parahaemolyticus* strains (60%) were susceptible to streptomycin, trimethoprim, sulfamethoxazole, and co-trimoxazole. Most of the *V. vulnificus* and *V. parahaemolyticus* isolates were resistant to penicillin G and ampicillin, and all isolates showed resistance to at least one antibiotic. The *Vibrio* strains displayed a wide range of multiple antibiotics resistances against 7–10 antibiotics. Also, in the same study, eight ARGs, *bla*P1 ß-lactamase cassette and SXT integrase, *sul*2, *dfr*A1, *str*B, *flo*R, *dfr*18, and *tet*A (tetracycline), were investigated in the confirmed *Vibrio* spp. It was found that *flo*R was the most detected in all three species, while *tet*(A) was the least detected.

In another study in Eastern Cape, South Africa, ARGs (*dfr*18, *dfr*A1, *flo*R, *tet*A, *str*B, *sul*2*)* of some *Vibrio* strains isolated from wastewater effluents in a rural community were evaluated. It was found that final effluents from wastewater treatment plants were potential reservoirs of various ARGs (Okoh and Igbinosa 2010). Moreover, Odjadjare et al. (2010) isolated *Listeria* from the final effluent of WWTP and discharging points upstream and downstream in Eastern Cape. The confirmed *Listeria* isolates were tested against 20 different antibiotics and ARGs: *sul*I, *sul*II, *erm*A, *erm*B, *era*B, and *amp*C. It was found that the confirmed *Listeria* isolates were sensitive to 3/20 antibiotics tested and showed varying (4.5–91%) levels of resistance against 17 antibiotics. In addition, only the *sul*II gene was detected in five *Listeria* isolates (Odjadjare et al. 2010). Table 12.1 summarises some studies on ARB and ARGs in African WWTPs.

12.4.2 River Water

The presence of antibiotics residues, ARB, and ARGs has been observed in various rivers in African countries. The deterioration of water quality in African rivers is due to the discharge of untreated, semi-treated, and/or treated effluents from conventional WWTPs into surface water, including the rivers (Sibanda et al. 2015). Thus, the risk estimation of antibiotic resistance is problematic as the continuous addition of new antibiotics complicates the antibiotic aquatic resistome. A study by Adesiyan

et al. (2019) isolated pathogenic *Plesiomonas shigelloides* from some Nigerian rivers (Asejire River, Dandaru River, Ona River, and Erinle River). The PCR-confirmed *P. shigelloides* showed high antibiotic resistance to sulfamethoxazole (100%), followed by erythromycin (93%), ampicillin (90%), cephalothin (82%), streptomycin (64%), chloramphenicol (58%), amoxicillin (53%), cefotaxime (50%), tetracycline (49%), neomycin (38%), and trimethoprim and sulfamethoxazole (38%). Furthermore, the most frequent ARGs were sulfonamide-resistant genes, *sul*1 (18%), *sul*2 (20%), and *dfr*1 (70%); tetracycline resistant genes, *tet*A (78%) and *tet*E (57%); ß-lactam resistant gene, *amp*C (37%); and aminoglycoside-resistant genes, *aph*A2 (36%) and *str*A (67%) (Adesiyan et al. 2019). In another investigation, 300 PCR-confirmed *E. coli* strains isolated from river water sources in Osun State, Nigeria, were examined against 20 antibiotics using the disc diffusion method. The results showed the highest resistance rate was against sulfonamides, β-lactams, and tetracyclines group more than phenicols and aminoglycosides. The occurrence rate of ARGs in *E. coli* strains was *sul*I (8%) and *sul*II (41%) (sulfonamides); *amp*C (22%), *bla*TEM (21%), and *bla*Z (18%) (β-lactams); *tet*A (24%), *tet*B (23%), *tet*C (18%), *tet*D (78%), *tet*K (15%), and *tet*M (10%) (tetracyclines); *cat*I (37%), *cat*II (28%), and *cm*IA1 (19%) (phenicols); and *aac*C2 (8%), *aph*A1 (80%), *aph*A2 (80%), *aad*A (79%), and *str*A (38%) (aminoglycosides) (Titilawo et al. 2015).

Furthermore, the most frequent bacterial strains isolated from polluted rivers in Addis Ababa, Ethiopia, were *E. coli* (26%), *K. pneumoniae* (20%), and *k. oxytoca* (19%). *E. coli* showed a high resistance rate to ampicillin (91.3%) and 70% to cefalotin, cefuroxime, ceftriaxone, and cefepime, whereas *K. pneumoniae* (94%) and *k. oxytoca* (95%) showed high resistance to ampicillin (Belachew et al. 2018).

Suzuki et al. (2015) studied the occurrence of ARGs (*sul*I, *sul*II, *sul*III, and *tet*M) for sulfonamide and tetracycline resistance in the total and culturable-bacterial assemblages detected in river, estuarine water, and WWTP in South Africa. The results showed that the samples were heavily contaminated with sulfonamide (*sul*I, *sul*II, and *sul*III) and tetracycline (*tet*M) resistance genes. Antibiotic resistance genes for tetracycline (*tet*M) were found in natural bacteria with 10^3 copies/16S rRNA in WWTP but were not detected in colony-forming bacteria. They noted that there was no correlation between antibiotic concentrations and ARGs.

Two hundred confirmed *Salmonella* spp. were isolated from Umgeni and Aller rivers and the WWTP effluent in Durban, South Africa. Confirmed *Salmonella* isolates were tested against 20 antibiotics, and they were resistant to sulfamethoxazole, nalidixic acid, and streptomycin and susceptible to quinolones and β-lactam (Odjadjare and Olaniran 2015). Furthermore, antibiotic-resistant *E. coli* was assessed in river water and bed sediments of the Apies River, Gauteng, South Africa. The isolates were most resistant to nitrofurantoin (sediments) and ampicillin (water). Over 80% of all resistant isolates were multidrug resistant. The highest prevalence of multiple antibiotic-resistant (MAR) strains of *E. coli* was observed in the sediment (Abia et al. 2015). *Aeromonas* species were isolated from Kat and Tyume rivers in the Eastern Cape Province, South Africa. *Aeromonas* isolates were resistant to penicillin, oxacillin, vancomycin, clindamycin, and cephalothin (Igbinosa et al. 2013).

In Egypt, using the disc diffusion method, ARB in Nile River at the Rosetta branch was investigated by Azzam et al. (2017). The highest resistance was recorded against cephalothin and clindamycin, while the lowest was to piperacillin, ofloxacin, and norfloxacin. *P. aeruginosa* were resistant to nearly 100% of the tested antibiotics, followed by *Proteus vulgaris* (87.5%), *Salmonella* sp. (85%), *E. faecalis* (82.5%), *S. aureus* (77.5%), *Escherichia coli* (75%), and *C. freundii* (45%). In addition, methicillin-resistant *S. aureus* (MRSA) was reported in 4 isolates from drains and 13 isolates from Rosetta branch.

Also, El-Shatoury et al. (2015) evaluated the antibiotic resistance of *E. coli* O157:H7 isolated from Nile River (Rosetta branch), El-Rahawy drain water, and hospital wastewater in Egypt against amoxicillin, cefixime, ciprofloxacin, tetracycline, clarithromycin, and streptomycin. The results showed that the isolates were more resistant to amoxicillin and 77% were resistant to clarithromycin. In a separate investigation, *E. coli*, *Proteus vulgaris*, *Salmonella* Typhi, and *Citrobacter freundii* were isolated from Nile River (Rosetta branch) and agricultural drains in Egypt. The confirmed bacterial isolates were tested against 20 antibiotics, and all the isolates were resistant to ampicillin, carbenicillin, methicillin, vancomycin, erythromycin, clindamycin, trimethoprim/sulfamethoxazole, and tetracycline (Abo-State et al. 2012). A summary of some African studies on ARB in surface waters is given in Table 12.2.

12.4.3 Drinking Water

In developing countries, drinking water is frequently abstracted from rivers and reservoirs. These sources might be contaminated with antibiotics, ARB, and ARGs (Table 12.3) (Gwenzi et al. 2017). Two main factors can favour and induce the spread of ARB and ARGs in drinking water. One is abiotic factors such as disinfectants, chemical co-pollutants (including metals and biocides), and physicochemical conditions. Another is biotic factors such as bacterial adaptation and stress response (Wu et al. 2018).

Ceftazidime−/cefpodoxime-resistant bacteria were isolated from different water sources, including groundwater (hand-dug wells and boreholes) in Nigeria. The isolates were screened for extended-spectrumβ-lactamase (ESBL) genes (bla_{SHV}, $bla_{\text{CTX-M-15}}$, and bla_{TEM}) by PCR. It was established that $bla_{\text{CTX-M-15}}$ was detected in hand-dug wells and boreholes used as sources of drinking water (Adelowo et al. 2018). Another Nigerian study examined the occurrence of resistant bacteria isolated from surface and underground water in six Ekiti State rural settlements. Gram-negative bacterial isolates included *E. coli* (22.7%), *Enterobacter aerogenes* (2.5%), *Salmonella* spp. (13.3%), *Shigella* spp. (19.3%), *Proteus* spp. (18.5%), *Klebsiella* spp. (19.3%), and *Pseudomonas aeruginosa* (4.2%). The organisms exhibited multiple antibiotic resistances to tetracycline (75.0%), amoxicillin (93.8%), co-trimoxazole (81.3%), nitrofurantoin (81.3%), gentamicin (43.8%), nalidixic acid (81.3%), and ofloxacin (18.5%) (n = 16). Over 10% of the bacteria were resistant to at least

Table 12.2 Incidence of ARB and ARGs in surface water in some African countries

African country	Matrix	Antibiotic/antibiotic group	Resistant bacteria	Resistance genes/ method	References
Egypt	Nile River Rosetta branch	Penicillins, cephalosporins, glycopeptides, aminoglycosides, tetracyclines, macrolides, lincosamides, quinolones, sulfa drugs, nitrofurans, and chloramphenicol	*E. coli*, *salmonella* spp., *Proteus* spp., Citrobacter spp., *P. aeruginosa*, *S. aureus*, *E. faecalis*	Disc diffusion	Azzam et al. (2017)
Egypt	Nile River (Rosetta branch) and El-Rahawy drain and hospital wastewater	Amoxicillin, cefixime, ciprofloxacin, tetracycline, clarithromycin, and streptomycin	*E. coli* O157:H7	Disc diffusion	El-Shatoury et al. (2015)
Egypt	Nile River (Rosetta branch) and drains	Amoxicillin/clavulanic acid, ampicillin, carbenicillin, methicillin, piperacillin, cephalothin, cefotaxime, ceftriaxone, vancomycin, amikacin, tobramycin, kanamycin, tetracycline, erythromycin, clindamycin, norfloxacin, ofloxacin, trimethoprim/ sulfamethoxazole, nitrofurantoin, chloramphenicol	*E. coli*, *Proteus vulgaris*, *Salmonella* Typhi, *Citrobacter freundii*	Disc diffusion	Abo-State et al. (2012)
Ethiopia	Polluted rivers in Addis Ababa	Ampicillin, amoxicillin/ clavulanic acid, piperacillin/tazobactam, cefalothin, cefazolin, cefuroxime, cefuroxime axetil, cefoxitin, cefpodoxime, ceftazidime, ceftriaxone, cefepime, gentamicin, tobramycin, ciprofloxacin, levofloxacin, tetracycline, nitrofurantoin, trimethoprim/ sulfamethoxazole	*E. coli*, *K. pneumoniae*, *K. oxytoca*, *Citrobacter freundii*	Vitek 2	Belachew et al. (2018)

(continued)

Table 12.2 (continued)

African country	Matrix	Antibiotic/antibiotic group	Resistant bacteria	Resistance genes/ method	References
Nigeria	Asejire River, Dandaru River, Ona River, and Erinle River	Amikacin, neomycin, streptomycin, trimethoprim, gentamycin, netilmicin, cephalothin, cefotaxime, ciprofloxacin, meropenem, imipenem, ceftazidime, neomycin, sulfamethoxazole, erythromycin, chloramphenicol, tetracycline, trimethoprim	Pathogenic *Plesiomonas shigelloides*	*tet*A, *tet*E, *cat*II, *cml*A1, *str*B, *sul*1, *sul*II, *dfr*1, *dfr* (18), *aph*A1, *amp*C,	Adesiyan et al. (2019)
Nigeria	Rivers at different locations in Osun State, South-Western Nigeria	Amikacin, streptomycin, kanamycin, neomycin, gentamycin, cefepime, cephalothin, cefuroxime, carbapenems, meropenem, imipenem, ciprofloxacin, gatifloxacin, nalidixic acid, sulfamethoxazole, nitrofurantoin, chloramphenicol, tetracycline, doxycycline, amoxicillin, ampicillin	*E. coli*	*sul*I, *sul*II, *amp*C, *bla*TEM, *bla*Z, *tet*A, *tet*B, *tet*C, *tet*D, *tet*K, *tet*M, *cat*I, *cat*II, *cm*IA1, *aac*C2, *aph*A1, *aph*A2, *aad*A, and *str*A	Titilawo et al. (2015)
South Africa	Umgeni and Aller rivers, effluent of WWTP in Durban	Cephalothin, imipenem, cefoxitin, cefuroxime, piperacillin, ampicillin, cefixime, ceftazidime, aztreonam, gentamycin, amikacin, streptomycin, chloramphenicol, tetracycline, ciprofloxacin, norfloxacin, nalidixic acid, nitrofurantoin, trimethoprim/ sulfamethoxazole, sulfamethoxazole	*Salmonella* species	Disc diffusion	Odjadjare and Olaniran (2015)
South Africa	Rivers, estuarine water	Sulfamethoxazole-resistant (SMXr) and oxytetracycline-resistant (OTCr) bacterial numbers were enumerated on LB agar media	Total bacterial counts	*sul*I, *sul*II, *sul*II, and *tet*M	Suzuki et al. (2015)

(continued)

Table 12.2 (continued)

African country	Matrix	Antibiotic/antibiotic group	Resistant bacteria	Resistance genes/ method	References
South Africa	Kat and Tyume rivers in the eastern cape province of South Africa	Ciprofloxacin, trimethoprim, chloramphenicol, penicillins, clindamycin, ofloxacin, ampicillin-sulbactam, oxacillin, ampicillin, gentamicin, nalidixic acid, cefotaxime, nitrofurantoin, sulfamethoxazole, cephalothin, erythromycin, tetracycline, minocycline, vancomycin, rifampicin	*Aeromonas* spp.	Disc diffusion	Igbinosa et al. (2013)
South Africa	River water and sediment bed, Gauteng	Nitrofurantoin, ampicillin, augmentin, ciprofloxacin, nalidixic acid, trimethoprim, gentamicin, chloramphenicol	*E. coli*	Disc diffusion	Abia et al. (2015)

four or more antibiotics. Antibiotic resistance was highest in members of *Enterobacter*, *Pseudomonas*, and *Proteus* (Oluyege et al. 2006). Similarly, Lyimo et al. (2016) estimated the occurrence of antibiotic-resistant *E. coli* from select drinking water (rivers, lakes, streams, ponds, tap water, and wells) in northern Tanzania. They reported that 46.9% of *E. coli* isolates were resistant to ampicillin, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim. Also, using standard culturing, *Enterobacteriaceae* were isolated from clinical materials and tap water from Bahir Dar City, Ethiopia, and 17% of the bacteria were isolated from drinking water. ESBL-producing *Enterobacteriaceae* was detected using the double-disk method by E-test cefotaxime+clavulanic acid and ceftazidime-clavulanic acid. The occurrence of ESBL-producing *Enterobacteriaceae* in drinking water samples was 9.4%. The predominant ESBL producers were *K. pneumoniae* (34; 69.4%) and *E. coli* (71; 58.2%), with high resistance rates observed against chloramphenicol, ciprofloxacin, and co-trimoxazole (Abera et al. 2016).

Furthermore, a study in Cameroon by Akoachere et al. (2013) investigated antibiotic resistances of 23 confirmed *Vibrio cholerae* O1 isolated from taps, dug wells, and streams in New Bell-Douala. The results showed that *V. cholerae* O1 isolates were resistant to ampicillin (92%), amoxicillin (88%), tetracycline (68%), and co-trimoxazole (64%). The authors concluded that antibiotic-resistant *V. cholerae* O1 in drinking water represented a high risk for human health.

In a study conducted in Congo, the researchers investigated the presence of ESBL-producing *Enterobacteriaceae* (*E. coli*, *Klebsiella* spp., and *Citrobacter*

Table 12.3 Incidence of ARB and ARGs in drinking water in some African countries

African country	Matrix	Antibiotic	Resistant bacteria	Resistance genes/ method	References
Cameroon	Taps, dug wells, and streams	Tetracycline, doxycycline, amoxicillin, ampicillin, trimethoprim-sulfamethoxazole (co-trimoxazole), ciprofloxacin, chloramphenicol	*Vibrio cholerae* O1	Disc diffusion	Akoachere et al. (2013)
Congo	Sachet water	Amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, moxifloxacin, norfloxacin, trimethoprim/sulfamethoxazole	*E. coli*, *K. pneumoniae*, *Enterobacter* spp., and *Citrobacter* spp.	bla_{SHV} and $bla_{CTX\text{-}M;}$ disc diffusion	De Boeck et al. (2012)
Ethiopia	Clinical and drinking water	Cefotaxime + clavulanic acid and ceftazidime + clavulanic acid	*K. pneumoniae* and *E. coli*	ESBL genes	Abera et al. (2016)
Nigeria	Wells, springs, streams, rivers, and lakes	Tetracycline, amoxicillin, co-trimoxazole, nitrofurantoin, gentamicin, nalidixic acid, ofloxacin	*E. coli*, *Enterobacter aerogenes*, *Salmonella* spp., *Shigella* spp., *Proteus* spp., *Klebsiella* spp., and *Pseudomonas aeruginosa*	Disc diffusion	Oluyege et al. (2006)
Nigeria	Surface and groundwater	Ceftazidime or cefpodoxime	*E. coli*	bla_{SHV}, $bla_{CTX\text{-}M\text{-}15,}$ and bla_{TEM}	Adelowo et al. (2018)
South Africa	Rivers and fountains	Ampicillin, tetracycline, chloramphenicol, co-trimoxazole, erythromycin, ciprofloxacin, nalidixic acid, gentamicin, ceftriaxone, and amikacin	*Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Aeromonas* spp., *Vibrio cholera*, *Enterobacter* spp., *Plesiomonas* spp.	Disc diffusion	Obi et al. (2004)
Tanzania	Rivers, lakes, streams, wells, taps, and ponds	Amoxicillin/clavulanate potassium, ampicillin, ceftazidime, chloramphenicol, ciprofloxacin, kanamycin, streptomycin, sulfamethoxazole, tetracycline, trimethoprim	*E. coli*	bla_{TEM1},$bla_{SHV\text{-}1}$, $bla_{CTX\text{-}M}$, *tet*(A), and *tet*(B)	Lyimo et al. (2016)

spp.) in packaged water bags sold as drinking water in the streets of Kinshasa. It was found that *Enterobacter cloacae* and *Klebsiella pneumoniae* were the major antibiotic-resistant bacteria with high resistance towards aminoglycoside and fluoroquinolone (De Boeck et al. 2012).

Drinking water samples were collected from Ngwedi, Mutale, Tshinane, Mutshindudi, and Mudaswali rivers and Makonde, Mudaswali, and Thamathama fountains from rural communities in Limpopo Province, South Africa. The results showed multiple antibiotic resistances of *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Aeromonas* spp., *Vibrio cholerae*, *Enterobacter* spp., and *Plesiomonas* spp. to ampicillin, tetracycline, chloramphenicol, co-trimoxazole, and erythromycin (Obi et al. 2004). In Egypt, tap water collected from a drinking water distribution system (n = 169) that had been treated by flocculation, coagulation, sandbed filtration, and chlorination was monitored for ARB using the disc diffusion method. Although the microbial content was within the maximum permissible limit, 89.7, 78.1, and 56.9% of the isolates 172 exhibited ampicillin, sulfaguanidine, and streptomycin resistance (El-Zanfaly et al. 1987).

12.5 Current Treatment Technologies of Water/Wastewater

As shown in previous sections, African water environments are highly polluted with microbial pathogens, including antibiotic resistance ones and their associated resistance genes. Unfortunately, conventional domestic wastewater and drinking water treatment plants are not efficient enough to remove ARB and ARGs, spreading them in the environment and food chain (CDC 2019; Aruguete et al. 2013). For instance, Guo et al. (2014) observed an increase in *tet*C and *tet*G genes' relative abundance after sand filtration in a conventional drinking water treatment plant. However, Garner et al. (2018) revealed that the traditional water treatment plants were inefficient at removing ARGs since the relative abundance of several ARGs in treated water streams did not significantly alter compared to the influents. Thus, finding a sustainable, effective means to control and remove ARB and ARGs from water bodies would substantially reduce their associated harmful health influences. In this context, several treatment techniques, including UV irradiation, chlorination, ozonation, Fenton oxidation process, and bioremediation, have been applied to prevent the dissemination of ARB and ARGs (Tobechukwu et al. 2020; Liu et al. 2019; Owoseni et al. 2017; Bharath et al. 2017; Ojemaye et al. 2017; Sharma and Malaviya 2016). However, despite their high efficiency in removing most organic and inorganic contaminants, they exhibited poor efficiency in removing ARB and ARGs, facilitating the proliferation of ARB and ARGs in the treated effluent.

12.5.1 The Application of NPs for ARB and ARGs

In the past few decades, there has been tremendous progress in using nanoparticles (NPs) to remove harmful contaminants, including ARB, ARGs, organic matter, and heavy metals, from water and wastewater (Dutt et al. 2020; Lee et al. 2016; Wang et al. 2012). Compared to other powerful treatment technologies (e.g. advanced oxidation processes), nanoparticle-based technologies have high efficiency and relatively low cost, owing to their high specificity and selectivity to efficiently target a specific type of contaminant, especially when the structure of the NPs is optimised, including size, shape, electrical conductivity, and the electronic structure. Several evaluated NPs, such as silver nanoparticles (AgNPs) and silica oxide nanoparticles (SiO_2NPs), have been successfully used to remove microbial contaminants, most likely due to their capability to establish surface electrostatic interactions with microbial DNA, resulting in damage of microbial contaminants (e.g. bacteria) (Kamali et al. 2019; Gao et al. 2009). These unique features of NPs would open up new opportunities for the widespread use of NPs in combating contaminants of emerging concern, which are responsible for infectious diseases, as well as treating a wide range of (waste)waters.

12.5.2 NPs-Microbes Interactions and Antimicrobial Mechanism

The use of NPs for water and wastewater purification has gained interest over the past few decades, mainly due to their affinity to non-selectively target and remove toxic contaminants (e.g. organic, inorganic, and biological compounds) (Lan et al. 2019; Motahari et al. 2015; Das et al. 2013). The bacterial cell damage using NPs depends on several factors (such as electrostatic interactions and van der Waals forces), involving NPs-microbe interactions. However, the structure of the microbial cell wall has a vital role in defining how NPs would interact with the microbes. For example, Gram-negative bacteria, which have hydrophobic lipopolysaccharides bilayer, are less likely to be penetrated by hydrophobic antibacterial agents (such as detergents) compared to Gram-positive bacteria (Fig. 12.4a) (Gupta et al. 2016). Berry et al. (2005) revealed that gold (Au) nanorods and nanospheres modified with positively charged cetrimonium bromide (CTAB), which is a cationic quaternary ammonium surfactant, are well dispersed on the outermost surface of Gram-positive bacterium (*B. cereus*) with negatively charged teichoic acid brushes, resulting in the formation of a percolating cluster with relatively low contact resistance. In another study, gold nanoparticles modified with mannose were firmly bound to the *E. coli* pili, which have a high content of lectin (i.e. sugar-binding proteins), leading to strong binding to the modified Au NPs (Lin et al. 2002). Hayden et al. (2012) observed that positively charged gold NPs (AuNPs) of 2-nm core diameter were strongly bound to the bacterial membrane, yielding well-formed spatial aggregates

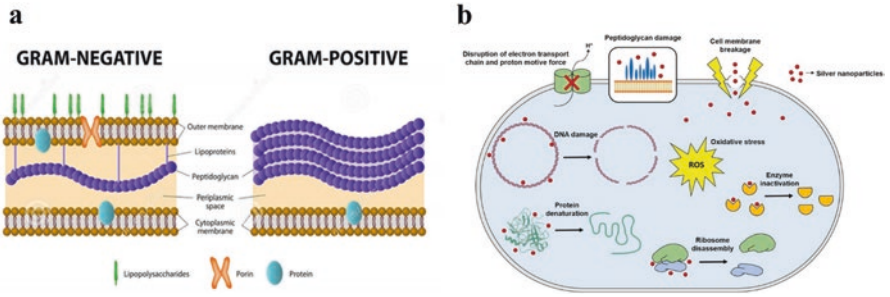

Fig. 12.4 (**a**) Schematic diagram of cell wall structures for Gram-positive and Gram-negative bacteria (Source: www.dreamstime.com) and (**b**) the main antimicrobial mechanisms of NPs. (Source: Roy et al. (2018))

and membrane defects. However, the toxicity effect widely varied due to the bacterial cell structure (i.e. low toxicity against Gram-negative *E. coli* while rapidly damaging Gram-positive *B. subtilis*).

Although antibiotics can inhibit bacterial cell wall synthesis by damaging the DNA replication machinery and the possibility to express essential proteins, bacteria can develop different strategies to overcome these obstacles to develop bacterial resistance to antibiotics (Neu 1992). Due to the unique chemical, electronic, and physical properties of NPs, NPs can relieve antibiotic resistance by electronically binding to and disrupting the cell membrane, resulting in the cytoplasmic component loss (Gupta et al. 2016). Following the membrane permeation, NPs bind to the bacterial intracellular components (e.g. DNA, ribosomes, and enzymes), disrupting the cellular machinery (Fig. 12.4b). Consequently, this disruption of the bacterial cellular machinery leads to enzyme inhibition, electrolyte imbalance, and cell damage/death (Wang et al. 2017). The exact mechanism for cell damage is mainly dependent on the physicochemical properties of NPs (e.g. composition, size, surface chemistry, and shape).

Different preparation methods for synthesising NPs, such as co-precipitation, solid-state reaction, polymerisation, sol-gel, and combustion methods, can produce NPs with different properties based on the potential application (Larayetan et al. 2019; Sharma et al. 2009). For example, Ag NPs are oxidised to release free silver ions (Ag^{+}), which act as active oxidative agents, causing membrane disruption and DNA damage (Zhao et al. 2010). However, copper ions (Cu^{2+}) released from Cu NPs have the ability to produce reactive oxygen species (ROS), which are oxidative agents with a high capability to deactivate the bacterial synthesis of amino acids and DNA. Similarly, other NPs (such as ZnO and TiO_2) have a similar mechanism for cell damage by generating ROS (Miller et al. 2015; Hajipour et al. 2012). In addition to the inhibitory impact of NPs on bacterial cells, using chaotropic salt would disrupt the structure of DNA by mediating the DNA transferring to the NP surface (Kamali et al. 2019; Aruguete et al. 2013). Taken together, it is obvious that the surface chemistry and the physicochemical properties of NPs are of great importance to fine-tuning the electrostatic interaction with bacteria and their selectivity

and sensitivity for cell damage while simultaneously minimising their associated toxicity for mammalian cells. Generally, the use of NPs, such as AgNPs and SiO_2 NPs, and nanoscale iron-based NPs, as a cost-effective, environmentally friendly means for inactivating ARB and the ARGs removal from (waste)water, would eliminate the environmental issues related to the spreading ARB and ARGs in (waste) water, owing to their affinity to inactivate a wide range of microorganisms (Stark et al. 2015; Tajkarimi et al. 2014; Zhang et al. 2013).

12.6 Some African Studies on the Use of NPs for Water and Wastewater Treatment

Research involving nanomaterials has increased over the past few decades. These studies have reported on the manufacture and use of different nanomaterials for different purposes, including water treatment. For example, Maxwell et al. (2021) modified locally collected materials with nano-sodium silicate and silver nitrate for treating water at the point of use in Nigeria. The materials used included red clay, kaolin-formed soil, sawdust, and grog, collected from various parts of Nigeria and moulded into ceramic filters. The manufactured filters were used to test rivers, boreholes, and well water. Following water treatment, removal efficiency for *E. coli* and total coliforms reached 99–100% for the various water samples. Furthermore, Cu/Zn-doped delaminated photocatalytic composite was synthesised from locally acquired clay and pawpaw seeds in Nigeria and used to remove multidrug-resistant bacteria from drinking water (Ugwuja et al. 2021). After 36 h of exposure, the authors observed a 6-log reduction of multidrug-resistant *E. coli* and associated sulfonamide resistance genes from polluted water. Furthermore, although fluoroquinolone resistance genes remained in the treated water after the first treatment step, these genes reduced significantly after the second treatment round. Most importantly, no regrowth was observed in the samples even after 7 days in darkness and light.

In Egypt, Dosoky et al. (2015) evaluated how efficiently silver nanoparticles could remove microbial pollutants from surface water and groundwater. The authors tested different particle concentrations (0.1, 0.05, and 0.01 ppm) with exposure set at 5, 15, and 30 min, 1 h and 2 h. The authors recorded high mean microbial counts in their raw water, especially in river water. However, removal efficiencies of up to 92% were obtained in some instances, indicating that these materials could treat raw environmental water meant for human use. In another Egyptian study, chitosan-silver nanoparticles were used to treat drainage water for use in irrigation and aquaculture. The authors tested the materials against water from a drain in Egypt containing total coliform, faecal coliform, *Staphylococcus aureus*, faecal streptococci, and *Pseudomonas aeruginosa*. Following 40 min of exposure of the water to 2 g/L of the nanomaterials, the authors obtained a 100% microbial removal efficiency. However, when they compared their nanomaterials to immobilised bacteria,

they only got a maximum removal efficiency of 76.1% for faecal coliform; lower removal efficiencies of 38% were obtained for total coliform and 54% for *P. aeruginosa*. These results demonstrated the superiority of using nanoparticles over conventional treatment methods to treat water for human use.

In South Africa, researchers tested quaternary imidazolium salts (QIS) of different lengths against *E. coli* inoculated into distilled, borehole, and river water (Kleyi et al. 2016). They observed that QIS carrying the octyl chains was more potent in all water types tested. In addition, electron microscopy revealed cell membrane damage of the bacterium upon contact with the nanomaterials. Interestingly, no regrowth occurred in the water samples after 48 h of storage. Also, there was minimal leaching of the material, indicating its safe use for drinking water treatment in households.

A concern with using nanomaterials for water and wastewater disinfection is that these materials could also be pollutants with adverse effects on humans and the environment (Bradford et al. 2009; Syafiuddin et al. 2018). Silver nanoparticles, for example, were reported to increase the antimicrobial resistance gene pool in the environment (Yonathan et al. 2022). Thus, these concerns spurred the need to develop methods to remove these contaminants from the environment. Two South African studies successfully removed silver nanoparticles from wastewater and used the adsorbent-nanomaterial to treat microbiologically polluted water (Das et al. 2017; Mahlangu et al. 2019).

12.7 Conclusion

The studies reviewed in this chapter reveal that research involving nanomaterials in increasing in Africa. It was also observed that many of the materials used to synthesis the nanomaterials were obtained locally, indicating that Africa has the necessary raw materials to advance nano research on the continent. However, most of the studies were performed at a laboratory scale, sometimes using unrealistic samples not mimicking true environmental conditions. This shows that upscaling is necessary to fully benefit from using nanomaterials for water and wastewater disinfection in Africa. Also, most studies have been carried out with antimicrobial-susceptible isolates, and little has been done using resistant bacteria specifically. Further research in this area would elucidate the impact of nanomaterials on antimicrobial-resistant microorganisms and their associated resistance genes in African waters.

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Chapter 13
Biocidal Activity of Plant Extracts: The Case of Algeria

Leila Bendifallah

13.1 Introduction

The world flora is estimated at 1,800,000 species, but this inventory is far from complete because extrapolations, based on credible data, estimate that there must be between 5 and 10 million taxa (Dajoz 2008). The Mediterranean basin is the second largest hotspot in the world and the largest of the five Mediterranean climate regions (Médail and Myers 2004). Also, the Mediterranean region is one of the world's great centres of plant diversity; 10% of higher plants can be found on only 1.6% of the earth's surface (Myers 1990; Médail and Quézel 1999). Furthermore, the entire Mediterranean basin contains nearly 50% specific endemism of all its flora (Médail and Quézel 1997). The two main factors determining the richness of the Mediterranean basin is its location at the crossroads of two continental masses, Eurasia and Africa, and the great topographic diversity of its environments (Dernegi 2010).

Among the countries of the Mediterranean basin, Algeria is subject to the combined influence of the sea, the relief and the altitude. Its geographical location straddles two floral empires, Holarctis and Paleotropis, and its six ecological regions give it a much-diversified flora. The climate is the temperate Mediterranean. It is characterised by a long summer drought varying from 3 to 4 months on the coast, 5 to 6 months at the level of the High Plains and more than 6 months at the level of the Saharan Atlas (Seltzer 1946; Stewart 1969). Annual temperatures and rainfall differ from region to region. The average annual temperatures are between 11 and 20 °C, and the annual rainfall is between 500 and 1300 mm. Dobignard and Chatelain (2010–2013) underline a floristic richness of 4450 taxa (3950 natives, 6.5%

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endemic). In addition, about 464 taxa are national endemics, giving traditional pharmacopoeia an inestimable richness.

Through the centuries, human traditions have developed the knowledge and use of medicinal plants. According to estimates by the World Health Organization (2002), over 80% of the population in Africa uses traditional medicine to meet their healthcare needs. The valorisation of aromatic plants with biocidal effects is gaining increasing importance in research programmes worldwide, particularly in Africa. These plants are exploited in several forms to limit losses post-harvest, either whole or in the form of plant powder, essential oils or plant extracts.

Algeria has a long medical tradition and traditional know-how based on medicinal plants known for their antifungal and antimicrobial properties. In valorising the Algerian flora, interest was placed on the flora of mountains and the South, particularly the endemic or patrimonial species (Fig. 13.1).

13.2 The Biocidal Activity of Algerian Plants

Plant biocidal activity is due to their raw, aqueous, organic extracts and essential oils. Algerian plants' bacterial and fungicidal activity is presented here according to their botanical family. The most used in traditional medicine in Algeria include oregano *Origanum vulgare* L. (Lamiaceae), *Myrtus communis* L. (Myrtaceae) and *Artemisia campestris* ssp. *campestris* L. (Asteraceae).

13.2.1 Asteraceae

Asteraceae family is the largest in the plant kingdom, with 1700 genera and around 24,000 species of shrubs and trees distributed worldwide. It includes many species used in ethnomedicine found in arid and semi-arid regions of subtropical and low temperate latitudes (Bitsindou and Lejoly 1993). For example, the Algerian soil has about 109 genera and more than 408 species (Quezel and Santa 1963).

In the family of Asteraceae (Compositae), the *Artemisia* genus has been considered one of the most dispersed and important. It is heterogeneous, consisting of over 500 diverse species, which are highly important in botany, pharmacy and the food industry (Bora and Sharma 2010). The genus is a perennial herb. It is a common medicinal and aromatic plant worldwide, especially in North Africa. It grows on rocky soils on hills and mountains. In Algeria, there are ten species of *Artemisia*, according to Ouyahya (1987), with *Artemisia campestris* L. primarily located in southern Algeria (Sainz 2005; Quezel and Santa 1963). The species *A. campestris* L. is widely used in traditional medicine, especially as a decoction for its antivenom, anti-inflammatory, antirheumatic and antimicrobial properties (Akrout 2005; Dob et al. 2005; Ferchichi et al. 2006). In addition, the essential oil of this plant is used against several pathogenic microorganisms such as *Escherichia coli*,

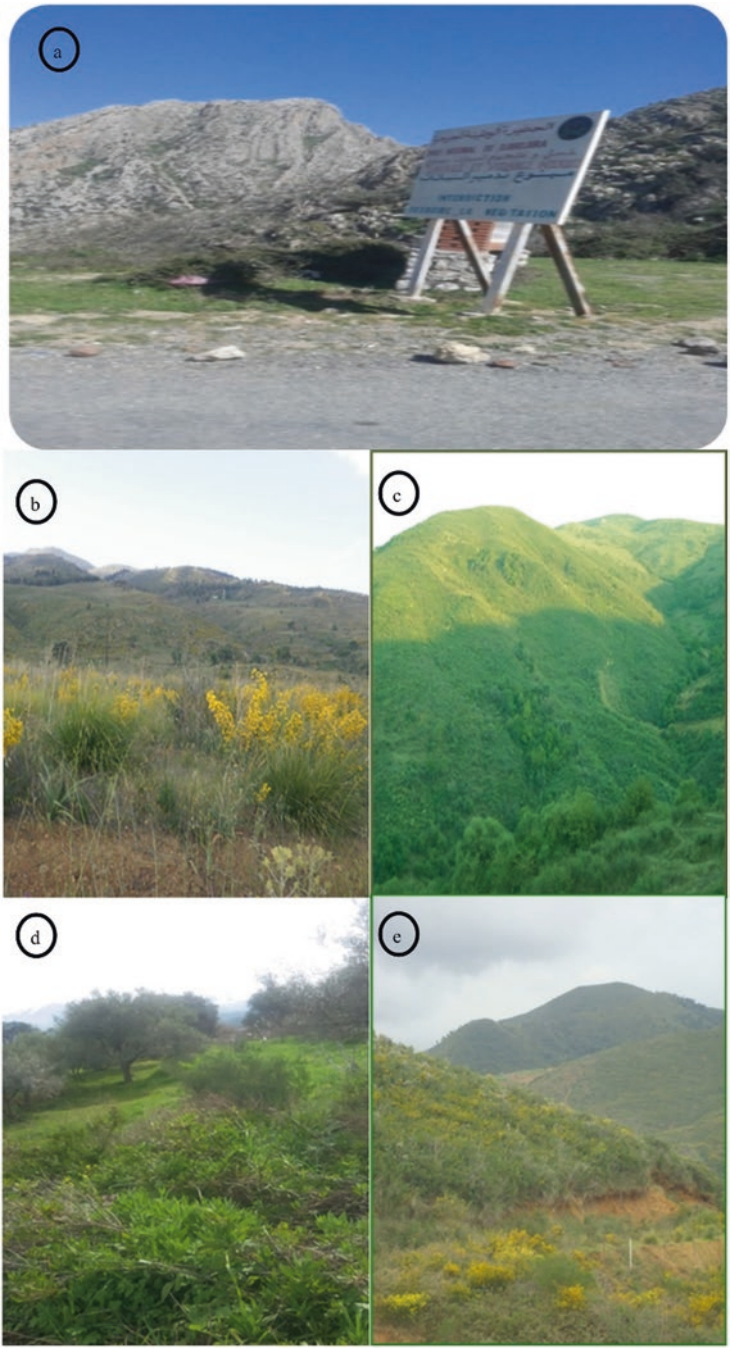

Fig. 13.1 Study sites. (**a**) Djurdjura mountain National Park; (**b**) Bouira Mountain; (**c**) Lakhdaria Boumerdes Mountain; (**d**) Tizi Ouzou Mountain; (**e**) Bejaia Mountain

Staphylococcus aureus, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. The essential oil of *Artemisia judaica* L. ssp. *sahariensis* harvested at Oued de Talanteneche (Tamanraset) in southern Algeria has potent antibacterial activity against 20 multi-resistant bacteria of clinical origin such as *S. aureus*, coagulase-negative *Staphylococcus* and *Enterococcus faecium*, *E. coli*, *K. pneumoniae*, *Klebsiella terrigena*, *Citrobacter freundii*, *Proteus mirabilis*, *Proteus vulgaris* and *Salmonella typhimurium* (Benmansour et al. 2016). In addition, this plant has antifungal activity against pathogenic fungi in humans and plants (Saleh et al. 2006).

Several researchers have studied the bactericidal activity of the essential oil of *A. judaica* and *A. campestris* (Gherib 2009; Mohammedi 2013; Hellali et al. 2019; Zeragui et al. 2019). Essential oils are found in tiny glands in different parts of the aromatic plant: leaves, flowers, fruits, seeds, bark and, for some, plants in the roots. More than 2000 plant species are rich in essential oils; they are spread over 60 families, the main ones being Lauraceae, Labiatae, Apiaceae, Rutaceae, Compositae, Myrtaceae and Pinaceae. For example, the essential oil of *Carthamus caeruleus* and *Calendula arvensis* (Asteraceae) showed interesting antifungal activity against orange disease caused by *Penicillium digitatum*. Furthermore, essential oil and hydrosol extracts of *C. arvensis* can reduce the growth of *Penicillium expansum* and *Aspergillus niger* (Belabbes et al. 2017).

The essential oil from the leaves of *Achillea santolinoides* (Asteraceae) from the mountains of Djelfa (located at the foot of the Saharan Atlas, 1140 m altitude) has good bactericidal activity against *S. aureus* (Lamamra 2018). The endemic species in Algeria *Matricaria pubescens* (Desf.), well-known in North Africa and found particularly throughout the northern and central Sahara and is common throughout the Sahara (Ozenda 2004), has shown good antifungal activity compared to rosemary. *Penicillium expansum* is the most susceptible strain. The antibacterial activity of *R. officinalis* L essential oil is better than that of *M. pubescens* (Makhloufi 2019).

The essential oil of aerial parts of *Phagnalon sordidum* (L.) Rchb. showed good efficacy against *S. aureus*, *K. pneumoniae* and *S. typhimurium* (Chikhi et al. 2019). The same effect was observed with the essential oil of the wild-growing medicinal species *Brocchia cinerea* (Asteraceae) from Brezina (Algerian Sahara), with antibacterial activity against all tested Gram-positive bacteria (*Enterococcus faecalis* and *S. aureus)* and Gram-negative bacteria *(E. coli*, *K. pneumoniae* and *Pseudomonas aeruginosa*) (Boukhobza et al. 2020).

Several plant extracts have been tested and proven effective against plant pathogenic fungi and bacteria in crop plants. Therefore, these extracts could be considered antifungal and antibacterial available to develop novel types of natural fungicides and bactericides to control several plant pathogenic fungi and bacteria. For example, the extracts derived from the flower and leaves of *Anvillea radiata* (Asteraceae) and *Bubonium graveolens* (Asteraceae) collected from south-western Algeria (Bechar) are very effective against the plant pathogenic fungus *Fusarium oxysporum* f. sp. *albedinis* (Foa) (causing vascular wilt of date palm). Furthermore, they had the most potent inhibitory effects on Foa's spore germination and soil population density (Mebarki et al. 2013, 2015).

The aqueous extracts of four indigenous plants, *Artemisia herba alba* (Asteraceae), *Cotula cinerea* (Asteraceae), *Asphodelus tenuifolius* (Asphodelaceae) and *Euphorbia guyoniana* (Euphorbiaceae), that grow spontaneously in the Northern Sahara of Algeria are used in controlling two fungal species that belong to *Fusarium* genus against the cereal pathogens *Fusarium graminearum* and *Fusarium sporotrichioides*. The essential oils of these plants have already been characterised for their chemical composition and biological effects, mainly against human and animal pathogens (Mohamed et al. 2010; Eddine et al. 2015; Djellouli et al. 2013).

13.2.2 Anacardiaceae

Among 15 species of pistachios, only 3 endemic species grow in Algeria, including Atlas pistachio *Pistacia atlantica* Desf., terebinth *Pistacia terebinthus* L. and mastic *Pistacia lentiscus* L. (Boudy 1952). The tannin and polyphenol extracts of *Pistacia lentiscus* L. (Anacardiaceae) leaves collected from the Boumerdes mountainous region have potent antimicrobial activity against the clinical bacteria *S. aureus*, *E. coli*, *K. pneumoniae* and the yeast strain *Candida albicans* (Bendifallah et al. 2014).

Essential oil of *Asteriscus graveolens* (Forssk.) Less. and *Pulicaria incisa* (Lam.) DC. Wild herbs growing in the Hoggar region (southern Algeria) have good antibacterial activity against a common nosocomial pathogen, *Acinetobacter baumannii* (Chaib et al. 2017).

13.2.3 Apiaceae

A dozen essential herbal medicinal products from the botanical family Apiaceae are described in several pharmacopoeias, having antiseptic, expectorant, diuretic, carminative, vasodilator or spasmolytic actions (Tavares et al. 2008). It is considered one of the families rich in essential oils (Amimar et al. 2001). *Daucus* is a genus belonging to this family, comprising about 300–455 genera and 3000–3750 species worldwide (Tabanca et al. 2006). In Algeria, this family is represented by 55 genera, 130 species and 27 subspecies. *Daucus aristidis* Coss. (Battandier and Trabut 1902) is an endemic plant to Algeria and has been locally known as “noukhia”, an annual plant.

The carrot *Daucus carota* subsp. *sativus* peel could be used to enhance the activities of the hydrocarbon-degrading bacteria during bioremediation of crude petroleum-oil polluted soil (Hamoudi-Belarbi et al. 2018).

In addition to contributing flavour to foods, many aromatic plants and their essential oils exhibit antimicrobial activity and could inhibit the growth of spoilage and pathogenic microorganisms, thereby improving food safety and health (Hadizadeh et al. 2009; Souza et al. 2009; Foda et al. 2010; Ghasemi et al. 2010) such as oregano, rosemary, sage and thyme.

The essential oil of fennel seeds *Foeniculum vulgare* Mill has shown good bactericidal activity against pathogenic microbes involved in food poisonings such as *Bacillus cereus* and *Listeria monocytogenes* (bacteria) and *Alternaria*, *Aspergillus fumigatus*, *Fusarium* and *Penicillium* (fungi) (Bouguerra 2012).

13.2.4 Chenopodiaceae

Frequently used in the local traditional medicine in the Bechar region (southwest Algeria), the hydromethanolic extract of *Rhus tripartita* (Anacardiaceae) and *Haloxylon scoparia* red (Chenopodiaceae), and the aqueous extract of *Traganum nudatum* (Chenopodiaceae), were the best to suppress the growth of *Aspergillus nidulans* (Fatehi et al. 2021).

13.2.5 Fabaceae

Native to the Middle East, the carob tree *Ceratonia siliqua* is a Mediterranean tree of indisputable ecological, industrial and ornamental importance (Hariri et al. 2009). It is found in its natural state, mainly in Algeria. Carob essential oil (leaf, seed and pod) has antimicrobial, cytotoxic and pharmaceutical properties (Ben Hsouna et al. 2011). The extracts of its leaves show antibacterial activity against *E. coli*; however, *P. aeruginosa*, *S. aureus*, *K. pneumoniae* and *C. albicans* are resistant to them (Hadjidj 2018). In addition, the aqueous extracts are active against *P. digitatum* (Fadel et al. 2011). Fungi generally show superior sensitivity compared to bacteria; among bacteria, Gram-negatives are more resistant than Gram-positives to essential oils (Cox et al. 2000).

Also, the carob *Ceratonia siliqua* is used in the environment to degrade total petroleum hydrocarbon in contaminated soil (Hamoudi-Belarbi et al. 2018).

The most important antibacterial activity of the essential oil of *Calicotome villosa* subsp. *intermedia* growing in the northwestern region of Algeria was expressed against *E. faecalis*, *S. aureus*, *K. pneumoniae* and *S.* Typhimurium (Chikhi et al. 2014).

13.2.6 Lamiaceae

The genus *Origanum* has been studied by Ietswaart (1980). It comprises 3 groups, 10 sections, 38 species, 6 subspecies, 3 varieties and 16 hybrids. *Origanum vulgare* L. is widespread in the Tell of North Africa (Baba 2006), especially in Algeria's mountainous regions. The extracts from the leaves of *Origanum vulgare* have

inhibitory action against the bacterial species *E. coli*, *S. aureus*, *K. pneumoniae* and *Pseudomonas* sp. and yeasts *C. albicans* and *Aspergillus* sp. (Bendifallah et al. 2015).

The essential oil of *Rosemary officinalis* L. has been the subject of several works for their activities on bacteria, yeasts and mould. It showed activity against most bacterial strains, except *P. aeruginosa* (Makhloufi 2019).

The antimicrobial activities of the essential oils of three species of the Lamiaceae family gowning in Algeria: *Thymus vulgaris* L., *Thymus algeriensis* Boiss. & Reut. and *Mentha pulegium* L. are important. *T. vulgaris* is the most active against bacteria *S. aureus*, *E. coli*, *S. typhi*, *K. pneumoniae* and yeast *C. albicans* (Benabed et al. 2017).

13.2.7 Lauraceae

Laurus nobilis essential oil has demonstrated bacteriostatic activities on *E. coli* and *K. pneumoniae* and fungistatic activity on *S. cerevisiae* and *C. albicans* (Miliani et al. 2017).

13.2.8 Myrtaceae

The tannins and the polyphenols extracts of the *Myrtus communis* L. (Myrtaceae) and *Syzygium aromaticum* L. collected from the mountain of Boumerdes L. have potent antimicrobial activity against *S. aureus* and *E. coli*, *K. pneumoniae* and *C. albicans* (Bendifallah 2015).

13.2.9 Pinaceae

The essential oils of three roots of the genus *Pinus* (*P. halepensis*, *P. pinea* and *P. pinaster*) growing in Algeria showed good antimicrobial activity, including against *S. aureus* and *C. albicans* (Fekih et al. 2016).

13.2.10 Rutaceae

Lemon seeds collected from the lemon orchards of the Boumerdes region in the Lakhdaria area (eastern Algeria) have been the subject of an antifungal study. *Citrus limon* seed powder extracts have an inhibitory effect on the mycelial growth of *Fusarium* spp. (Bendifallah et al. 2016).

13.3 Conclusion

Antimicrobial resistance remains a significant menace to human, animal and environmental health. With limited options available to manage the growing resistance threat, new approaches are needed to treat infections, particularly in Africa, where the disease burden is high. Thus, the rich plant biodiversity within the continent is a rich source of natural remedies that still needs to be explored.

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Chapter 14
The Combined Use of African Natural Products and Conventional Antimicrobials: An Alternative Tool Against Antimicrobial Resistance

Z. Booth and S. F. van Vuuren

14.1 Introduction

14.1.1 Natural Product Classification

Natural products are groups of non-synthetic molecules exhibiting biological activity and hence often used for their medicinal properties. Many products feature under this broad classification of "natural products". Traditional medicines, herbal or supplementary products, are often derived from medicinal plants in various forms (essential oils, extracts, tinctures, teas and isolated phytochemicals), along with commonly consumed foodstuff (most often culinary herbs, spices, seeds, fruit and bee products), containing medicinal compounds (Cragg and Newman 2013). These natural products used for medicinal purposes are often referred to as traditional, complementary and/or alternative medicines (TCAMs). The TCAM framework describes all medicines or healing practices that fall outside the standard biomedical healthcare models (James et al. 2018).

Traditional medicine comprises of local herbal medicines or products, indigenous healthcare practices and local or imported complementary and alternative medicine products and practices (James et al. 2018). Traditional medicine is defined as "the use of theories, beliefs, and native experiences from different cultures, in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness" (WHO 2019). Complementary and alternative medicines both refer to a wide range of healthcare products and techniques that are not part of a country's traditional medicine framework and are not fully incorporated into the mainstream (conventional) healthcare system (WHO 2019). The

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TCAM practices are geographically varied, giving rise to predominantly known African traditional medicine (ATM), traditional Chinese medicine (TCM) and Ayurvedic medicine or traditional Indian medicine (TIM). Although some forms of traditional medicine and practices are specific to certain countries, many well-known types of traditional remedies are being adopted worldwide (Mahomoodally 2013). For this chapter, a strong focus is given to ATM.

In many countries, natural products are not classified as drugs requiring regulatory authority registration and approval before entering the market. This is another contentious subject. More than half of the conventional drugs currently approved and used in modern healthcare facilities were initially derived from or inspired by natural products (Newman and Cragg 2016), thereby emphasising the medicinal potential of natural products and inadvertently, similarly with conventional drugs, the potential for harmful effects if not used correctly. The World Health Organization (WHO) has set out guidelines for advancing the acceptance and integration of TCAM with conventional health systems worldwide to legitimise TCAM and facilitate the drive towards scientifically validating and profiling these products for the safety of patients (WHO 2017).

14.1.2 Global Natural Product Use and Traditional Health Systems

Naturally, derived products have become increasingly popular worldwide (James et al. 2018). An estimated one-third of the US population, 20–50% of the European population and 80% of the African and Asian population use natural products. Reasons for the high prevalence of use pertain to natural product accessibility, availability and affordability being more favourable than conventional medicine (WHO 2019), particularly for those accessing healthcare in the African public sector.

The WHO global report on TCAM (2019) specifies that medicinal plants and herbal medicines constitute the main forms of traditional medicine practices in the American region. However, other forms of traditional, alternative and complementary medicines, like acupuncture and homeopathy, are also notably prevalent, with many clinics offering these services throughout the American region (Ong et al. 2005). Furthermore, Mordeniz (2019) mentions that in 2005, the National Institute of Health (NIH) estimated their expenditure on herbal medicine in America to be approximately 33 million dollars, reflecting the vast use of this form of healthcare in the region.

Asian countries like China, Korea, Japan and India have been known for their well-developed traditional medicine systems, with East Asian medicines originating approximately 3000 years ago. Over the years, however, each region in Asia has developed its form of TCAM that is uniquely practised, such as TCM, traditional Korean medicine (TKM), traditional Japanese medicine (TJM) and TIM (Mordeniz 2019). Another commonly known form of Asian traditional medicine is Ayurveda. Ayurveda is a healthcare system founded by ancient Hindu healers and practised

throughout India. Ayurveda has even been recognised by the Indian government as a complete form of healthcare, together with western medicine (Mordeniz 2019).

Europe has a large and respectable history of natural product use; however, the most dominant healthcare system remains allopathic (Firenzuoli and Gori 2007). In Germany, an estimated 600–700 plant-derived and plant-based medicines are accessible to the public and are prescribed by nearly 70% of German physicians (Mordeniz 2019). Germany also classifies herbal medicines as an element of naturopathy, a medical system that encompasses healthcare and traditional practices, popular in Europe in the nineteenth century (NCCIH 2017). Jacobsen et al. (2015) determined through a study undertaken at 80 hospitals in Norway that 64% of hospital staff offered their patients natural products or alternative therapies.

14.1.3 Natural Product Use in the African Healthcare System

It is widely documented that the use of TCAM is widespread throughout sub-Saharan Africa, with a considerable population relying on it to maintain their health or prevent and treat communicable and non-communicable diseases (James et al. 2018). In addition, African traditional healers in rural Africa are far more accessible and affordable as healthcare practitioners, resulting in local communities consulting these healers first before reaching out to formalised healthcare facilities, such as public clinics and hospitals. As a result, African TCAM use is perhaps one of the oldest and most varied (Mahomoodally 2013).

The WHO estimates that 80% of the African population uses traditional natural products as their primary source of health (WHO 2017). However, a literature review by James et al. (2018) reported a varied prevalence rate for natural product use. James et al. (2018) focused solely on literature documenting natural product use in sub-Saharan Africa from 1 January 2006 to 28 February 2017 and found prevalence rates ranging from 4.3% to 69.4% (mean = 30.5%) in the various developing countries in sub-Saharan Africa. This discrepancy warrants further validation; hence, a dearth of research is still necessary to further explore these statistics and patterns of natural product use.

In 2017, the WHO stated that the most common type of traditional medicine is natural, herbal products of plant origin. This is certainly true in Africa, with medicinal plants forming the largest portion of treatment options available from traditional healers or "muthi" markets (WHO 2017). In southern Africa, approximately 3400 indigenous plant species have been documented as being used traditionally. Of these plants, 2062 plant species are traded in South Africa alone for medicinal purposes. The most prevalent trading of medicinal plants for traditional medicine use occurs in KwaZulu-Natal, Gauteng, Eastern Cape, Mpumalanga and Limpopo (James et al. 2018).

Medicinal plants that are most popularly traded in South Africa include *Agathosma betulina* (buchu), *Aloe ferox* (bitter aloe), *Artemisia afra* (African wormwood), *Aspalathus linearis* (rooibos), *Bulbine frutescens* (burn jelly plant), *Cyclopia*

genistoides (honeybush), *Harpagophytum procumbens* (devil's claw), *Hoodia gordonii* (hoodia), *Hypoxis hemerocallidea* (African potato), *Lippia javanica* (fever tea), *Mesembryanthemum tortuosum* (kanna), *Pelargonium sidoides* (African geranium), *Siphonochilus aethiopicus* (African ginger), *Sutherlandia frutescens* (cancer bush), *Warburgia salutaris* (pepperbark tree) and *Xysmalobium undulatum* (milk bush). Many of these commonly used medicinal plants have been commercialised in the form of standardised formulations rather than the crude, unprocessed plant material purchased at traditional "muthi" markets. Commercialising these natural products further emphasises their efficacy, even with limited regulatory and scientific backing (Van Wyk 2011).

14.1.4 Natural Products with Antimicrobial Properties

Before the development of conventional antimicrobials, natural products, most commonly traditional medicinal plant preparations (commonly referred to as teas, herbs and spices), were consumed to fight infection. It has also been noted that these natural remedies resulted in minimal side effects. Van Vuuren and Holl (2017), Khameneh et al. (2019), Chassagne et al. (2021) and Stan et al. (2021) are just some reviews conducted on medicinal plants (indigenous, invasive, naturalised or commercialised species), as natural products exhibiting noteworthy antimicrobial activity against a range of pathogens.

Mahomoodally (2013) highlighted important medicinal plants in Africa that have short and long-term potential to manage and treat infectious or chronic conditions and the possibility for further development as phytopharmaceuticals. The African medicinal plants most commonly identified in the reviewed literature by Mahomoodally (2013) for their anti-infective properties were *Acacia senegal* (gum arabic), *Aloe ferox* (bitter aloe or Cape aloe), *Artemisia afra* (wormwood), *Aspalathus linearis* (rooibos), *Centella asiatica* (centella), *Catharanthus roseus* (Madagascar periwinkle), *Cyclopia genistoides* (honeybush), *Harpagophytum procumbens* (devil's claw) and *Pelargonium sidoides* (African geranium).

Culinary herbs and spices such as *Rosmarinus officinalis* (rosemary), *Piper nigrum* (black pepper) and *Cinnamomum zeylanicum* (cinnamon) have also demonstrated antimicrobial potential (Abascal and Yarnell 2013). In the review by Abascal and Yarnell (2013), it was claimed that the antimicrobial action of *Allium sativum* (garlic) is comparable to that of standard antibiotics. Liu et al. (2017) reviewed the antimicrobial activity of common household herbs and spices and reported that many exhibited notable antimicrobial activity against various pathogens. Those with the most noteworthy antimicrobial activity were *Eugenia caryophyllata* (clove), *Origanum vulgare* (oregano), *Thymus vulgaris* (thyme), *C. zeylanicum* and *Cuminum cyminum* (cumin), against various bacterial (*Bacillus subtilis*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Salmonella typhimurium*) and fungal *(Aspergillus niger* and *Aspergillus flavus*) microorganisms. Interestingly, these herbs and spices also exhibited activity against

resistant microorganisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Liu et al. 2017).

Some gallic acid-rich culinary herbs, such as *Eupatorium perfoliatum* (boneset) and *Marrubium vulgare* (horehound), exhibit antimicrobial properties (Livestrong 2020). In addition, certain foods that are consumed are also rich in gallic acids, such as *Allium sativum* (garlic) and *Grifola frondosa* (maitake mushrooms) (Abascal and Yarnell 2013).

Many natural products have demonstrated promising antimicrobial and immunomodulatory properties against highly infectious diseases, such as influenza, tuberculosis and malaria (Pandey et al. 2020), and also addressed the role of traditional medicine in the coronavirus disease (COVID-19) pandemic. Furthermore, based on the situation's urgency, immune-boosting approaches using natural products have been promoted widely to reduce the number of SARS-CoV-2 infections worldwide (Ang et al. 2020).

14.2 Exploring the Combination of Natural Products with Conventional Antimicrobial Agents to Address Antimicrobial Resistance (AMR)

With the high incidence of infectious diseases in Africa, AMR is a major threat to the country's public health, further exacerbating the insurmountable burden already placed on the healthcare system (Dalal et al. 2011; Avert 2020).

New conventional antimicrobial discovery has proven a challenge in pharmaceutical research and development. With the rapid increase in AMR and many existing antimicrobials rendered ineffective, the slow research and development process is worrisome. Therefore, researchers must adapt approaches and consider alternative means of enhancing existing antimicrobials potency to ward off AMR.

One such approach would be the use of combination therapies, particularly with natural products that have demonstrated effective antimicrobial activity. Combination therapies using existing conventional antimicrobials and natural products could be a simpler, faster and more cost-effective way of overcoming AMR instead of formulating new antimicrobial compounds (Cock et al. 2017). In 2016, the WHO announced a favourable response towards using combination therapies instead of monotherapy, particularly for treating potentially fatal infectious diseases, such as HIV/AIDS, malaria and tuberculosis. This was based on evidence that combination therapy can limit resistance and allows for multiple targets of the causative organism and/or its host to be targeted holistically (WHO 2017).

It is proposed that the term "natural" confers a sense of safety, so an identified effective natural product combination with existing conventional antimicrobials may be safer in addressing drug-resistant infections (Barbosa et al. 2020). With Ma et al. (2019) stating that combination therapy with traditional and western medicine can lead to synergy and improved outcomes, this approach appears even more favourable. In addition, natural products have commonly been found to enhance or

potentiate conventional antimicrobial activity, even if the product itself does not possess antimicrobial activity (Sibanda and Okoh 2007; Adwan et al. 2010).

Mahomoodally (2013) emphasises that medicinal plants, as natural products, contain different secondary metabolites that have the potential to interact in a variety of ways. It is described that with the complexity of the constituency of medicinal plants, several chemicals may work synergistically to improve health outcomes, such as enhanced antimicrobial potency to fight off infection, but may also present further advantages. Secondary metabolites of plant origin have demonstrated the potential to speed up or slow down pharmacokinetic activities, resulting in fewer undesirable adverse effects or toxicity of conventional drugs when consumed concurrently (Mahomoodally 2013; Kahraman et al. 2020). Furthermore, it has been noted that the complex constituents within plant materials may very well offer more than one mechanism of action and assist in treating the infection and providing symptomatic relief through anti-inflammatory or antipyretic properties. This is why Mahomoodally (2013) suggests the preference for research on crude or standardised plant extracts as opposed to isolated plant compounds for medical use.

Viljoen and Vermaak (2013) also highlight an additional concern related to natural products of plant origin: the challenge associated with standardisation and quality control of plant products. The phytochemical complexity and species variability depend on cultivation methods, geographical harvesting and soil and temperature differences. These are all factors that impact on standardisation of any plant product.

Mahomoodally (2013) and Eman (2014) stress that the vast number of compounds in medicinal plants creates much uncertainty in predicting potential interactive profiles with conventional drugs within the human body and could also prove to impact conventional medicine negatively through antagonism. Many natural products target drug transporters or metabolising enzymes, with another commonly identified effect being drug protein-binding alterations, affecting the metabolism or excretion of conventional drugs. The most documented interaction results from natural products inhibiting or promoting cytochrome P450 enzymes, liver enzymes responsible for metabolising most conventional medicines in the human body. More than 78 natural products have demonstrated the potential to affect cytochrome P450 system activity (Eman 2014). Hence, much is still to be investigated before any implementation or formulation studies can be undertaken.

14.2.1 Interactive Antimicrobial Profiling of Combinations

Natural product combinations with conventional antimicrobials may interact on a pharmacodynamic level, allowing for four interactive antimicrobial profile classifications: synergistic, antagonistic, additive or non-interactive effects. Pharmacodynamic interactions, therefore, relate to natural product potential to either potentiate or reduce the pharmacological activity of a conventional antimicrobial (Eman 2014).

Interactive antimicrobial profiles for natural product-conventional antimicrobial combinations can be determined through in vitro minimum inhibitory concentration

(MIC) assays for each combination component individually and, after that, in combination. The antimicrobial interaction can then be evaluated through a calculation to determine the sum of the fractional inhibitory concentration (ΣFIC). Interactive antimicrobial activities of combinations are classified as either being synergistic (ΣFIC values less than and equal to 0.5), additive (ΣFIC values above 0.5 but less than or equal to 1.0), indifferent (ΣFIC values greater than 1.0 but less than or equal to 4.0) and antagonistic (ΣFIC values greater than 4.0) (Van Vuuren and Viljoen 2011).

14.2.1.1 Synergistic Interactions

Synergy is derived from the Greek word, *synergos*, which means to work together. A synergistic interaction can be classified as an interaction between two components, producing a final effect greater than each of the individual components. Concerning antimicrobial activity, a synergistic effect signifies increased potency in antimicrobial activity of that combination, thereby allowing for a dose reduction and possibly fewer side effects (Abreu et al. 2012). Although most studies consider the ΣFIC of less than or equal to 0.5 as a synergistic interaction, some studies have extended synergism from ΣFIC values to 0.75, classifying them as partial synergistic interactions (Salem et al. 2018).

Synergistic interactions between natural products and conventional antimicrobials could potentially increase conventional agents' solubility or membrane transport (Gurley 2012). This would increase microorganisms' susceptibility to conventional antimicrobials (Stefanovic et al. 2011; Ovais et al. 2018), presenting a further avenue to addressing the surge in AMR (Sanhueza et al. 2017). In addition, it has been documented that antimicrobial resistance may be overcome through synergistic interactions posed by natural products on conventional antimicrobials (Abascal and Yarnell 2013).

14.2.1.2 Antagonistic Interactions

Antagonism refers to the result when two agents in combination oppose one another's effect, producing a final effect that is less than that of the individual agents. For example, in the case of antimicrobial interactions, a reduced antimicrobial efficacy or potency against a microorganism will be observed in combination.

Antagonistic interactions between natural products and conventional antimicrobials could reduce antimicrobial efficacy, thereby increasing the burden placed on healthcare systems with returning patients (Eman 2014). The reduction in efficacy could also translate further into exacerbating the healthcare system's AMR issues. If patients do not disclose the use of natural products to their healthcare professionals, they may assume that a treatment failure may be due to insufficient dosages. The cascade would filter down to higher-dose administration and an increased risk for toxic or adverse effects from conventional drugs (Adwan et al. 2010; Chanda and Rakholiya 2011).

14.2.1.3 Additive and Non-interactive Interactions

Having less impact are additive and non-interactive effects. Additive interactions provide an effect equivalent to the combined effect of the individual agents, which is no less nor greater in combination than when tested separately. Therefore, there is no advantage or disadvantage to the combination. Furthermore, additive interactions usually occur between agents with similar mechanisms or sites of action (Van Vuuren and Viljoen 2011).

With the lack of advantages or disadvantages arising from non-interactive profiles and additive interactions, these combinations do not form the centre of discussion in most literature, as they are not considered clinically relevant (Eman 2014). However, a non-interactive or additive profile identification could confirm that certain combinations would not cause detrimental adverse therapeutic outcomes for the patient. For this chapter, synergistic and antagonistic interactions will be reflected in supporting the efficacy of such combinations to curb antimicrobial resistance.

14.2.2 Conventional Antimicrobials in Synergistic Combinations with Essential Oils

Essential oils are natural products derived via distillation from aromatic plants. Essential oil use has existed for decades, from ancient healing practices to the most frequent aromatherapeutic use today (Langeveld 2013). Research is primarily focused on scientifically evaluating the antimicrobial activity of essential oils or identifying phytochemicals from the essential oils and testing the antimicrobial activity of those alone and then in combination with conventional antimicrobials to identify any potential synergism (Sibanda and Okoh 2007; Adwan et al. 2010).

Essential oils, derived from various plant species, have been found to enhance conventional antimicrobials synergistically. Interestingly, some essential oils, when tested individually, do not exhibit antimicrobial activity; however, they still possess the ability to improve the potency of conventional antimicrobials when tested in combination.

Langeveld (2013) conducted a comprehensive review on the synergistic interactions between various essential oils distilled from commonly consumed culinary herbs (e.g. coriander, cinnamon, lemongrass, oregano, peppermint, rosemary and thyme) in combination with common conventional antimicrobials (e.g. ciprofloxacin, gentamicin, ceftriaxone, norfloxacin, erythromycin, ampicillin and vancomycin). These combinations were tested against Gram-positive and Gram-negative pathogens, and synergistic interactions were evident. Langeveld (2013) also studied some phytochemicals derived from these essential oils in combination with the antimicrobials above and tested these against some pathogens. Again, synergistic interactions were evident. Further evidence of synergistic interactions between conventional antimicrobials and essential oils derived from medicinal plants predominantly focusing on an African context is given in Table 14.1.

Table 14.1 Synergistic interactions between conventional antimicrobials and African medicinal plant (indigenous/invasive/naturalised/commercialised) essential oils (ΣFIC below or equal to 0.5 is considered a synergistic interaction)

Conventional antimicrobial	Natural product	Test microorganism	ΣFIC	References
Amphotericin B	*Thymus vulgaris*	*Candida albicans* (clinical isolate)	0.38	Jafri and Ahmad (2020)
		Candida tropicalis (clinical isolate)	0.25	
	Cymbopogon citratus	*Candida albicans* (clinical isolate 1)	0.16	Khan et al. (2012)
		Candida albicans (clinical isolate 2)	0.38	
	Coriandrum sativum	*Candida albicans* (ATCC 90028)	0.38	Silva et al. (2011)
		Candida albicans (ATCC 24433)	0.19	
	Myrtus communis	*Aspergillus niger* (ATCC 16404)	0.26	Mahboubi and Bidgoli (2010)
		Aspergillus parasiticus (ATCC 15517)	0.26	
		Candida albicans (ATCC 10231)	0.25	
	Citrus aurantium	*Candida albicans* (ATCC 90028)	0.24	Nidhi et al. (2020)
		Candida albicans (MTCC 277)	0.05	
Cefixime	*Thymus broussonetii*	*Salmonella typhimurium* (laboratory isolate $CCMMB_{17}$)	0.18	Fadli et al. (2012)
	Thymus maroccanus	*Staphylococcus aureus* (laboratory isolate $CCMMB_3$)	0.18	
		Salmonella typhimurium (laboratory isolate $CCMMB_{17}$)	0.18	
Ciprofloxacin	*Artemisia afra*	*Escherichia coli* (ATCC 25922)	0.27	Hubsch et al. (2014a)
	Lippia javanica	*Escherichia coli* (ATCC 25922)	0.14	
		Enterococcus faecalis (ATCC 29212)	0.44	
	Thymus broussonetii	*Bacillus cereus* (ATCC 14579)	0.15	Fadli et al. (2012)
		Bacillus subtilis (ATCC 9524)	0.26	
		Escherichia coli (laboratory isolate $K_{12}W_{113}$)	0.37	
		Micrococcus luteus (ATCC 10240)	0.26	
		Pseudomonas aeruginosa (clinical isolate)	0.14	
		Vibrio cholera (environmental isolate)	0.14	

(continued)

Table 14.1 (continued)

Conventional antimicrobial	Natural product	Test microorganism	ΣFIC	References
Ciprofloxacin	*Thymus maroccanus*	*Bacillus cereus* (ATCC 14579)	0.15	Fadli et al. (2012)
		Bacillus subtilis (ATCC 9524)	0.09	
		Enterobacter cloacae (clinical isolate)	0.37	
		Escherichia coli (laboratory isolate $K_{12}W_{113}$)	0.12	
		Klebsiella pneumoniae (clinical isolate)	0.37	
		Micrococcus luteus (ATCC 10240)	0.28	
		Pseudomonas aeruginosa (clinical isolate)	0.15	
		Salmonella typhimurium (laboratory isolate $CCMMB_{17}$)	0.37	
		Staphylococcus aureus (laboratory isolate $CCMMB_3$)	0.26	
		Vibrio cholera (environmental isolate)	0.18	
	Calamintha sylvatica	*Escherichia coli* (ATCC 10536)	0.25	Sienkiewicz et al. (2017)
		Salmonella abony (ATCC 6017)	0.25	
	Calamintha vardarensis	*Escherichia coli* (ATCC 10536)	0.25	
		Salmonella abony (ATCC 6017)	0.25	
	Calamintha nepeta	*Pseudomonas aeruginosa* (ATCC 9027)	0.50	
		Salmonella abony (ATCC 6017)	0.25	
	Plectranthus amboinicus	*Staphylococcus aureus* (ATCC 25923)	0.09	Jugreet and Mahomoodally (2020)
		Methicillin-resistant *Staphylococcus aureus* (clinical isolate)	0.50	
		Bacillus spizizenii (ATCC 6633)	0.31	
Cefixime	*Thymus broussonetii*	*Salmonella typhimurium* (Laboratory isolate $CCMMB_{17}$)	0.18	Fadli et al. (2012)
	Thymus maroccanus	*Staphylococcus aureus* (Laboratory isolate $CCMMB_3$)	0.18	
		Salmonella typhimurium (Laboratory isolate $CCMMB_{17}$)	0.18	
Clotrimazole	*Curcuma longa*	*Candida albicans* (clinical isolate)	0.50	Ogidi et al. (2021)
Fluconazole	*Thymus vulgaris*	*Candida albicans* (clinical isolate)	0.28	Jafri and Ahmad (2020)
		Candida tropicalis (clinical isolate)	0.14	
	Matricaria recutita	*Candida albicans* (clinical isolate)	0.31	Goger et al. (2018)

Fluconazole	*Cinnamomum verum*	*Candida albicans* (clinical isolate)	0.31	Khan et al. (2012)
	Syzygium aromaticum		0.38	
	Cymbopogon citratus		0.38	
	Citrus aurantium	*Candida albicans* (ATCC 90028)	0.24	Nidhi et al. (2020)
		Candida albicans (MTCC 277)	0.36	
Gentamicin	*Thymus broussonetii*	*Bacillus cereus* (ATCC 14579)	0.12	Fadli et al. (2012)
		Bacillus subtilis (ATCC 9524)	0.09	
		Escherichia coli (laboratory isolate $K_{12}W113$)	0.37	
		Micrococcus luteus (ATCC 10240)	0.12	
		Pseudomonas aeruginosa (clinical isolate)	0.28	
		Staphylococcus aureus (laboratory isolate $CCMMB_3$)	0.50	
		Vibrio cholera (environmental isolate)	0.28	
	Thymus maroccanus	*Bacillus cereus* (ATCC 14579)	0.25	
		Bacillus subtilis (ATCC 9524)	0.50	
		Enterobacter cloacae (clinical isolate)	0.19	
		Escherichia coli (laboratory isolate $K_{12}W113$)	0.28	
		Klebsiella pneumoniae (clinical isolate)	0.50	
		Pseudomonas aeruginosa (clinical isolate)	0.18	
		Salmonella typhimurium (laboratory isolate $CCMMB_{17}$)	0.62	
		Staphylococcus aureus (laboratory isolate $CCMMB_3$)	0.50	
	Cinnamomum zeylanicum	*Enterococcus faecalis* (ATCC 29212)	0.08	Rosato et al. (2020)
	Mentha piperita		0.08	
	Origanum vulgare		0.08	
	Thymus vulgaris		0.08	

(continued)

Table 14.1 (continued)

Conventional antimicrobial	Natural product	Test microorganism	ΣFIC	References
Gentamicin	*Cinnamomum zeylanicum*	*Staphylococcus aureus* (ATCC 29213)	0.08	Rosato et al. (2020)
	Mentha piperita		0.08	
	Origanum vulgare		0.11	
	Thymus vulgaris		0.08	
	Calamintha sylvatica	*Salmonella abony* (ATCC 6017)	0.50	Milenkovic et al. (2018)
	Calamintha vardarensis		0.50	
	Calamintha nepeta	*Staphylococcus aureus* (ATCC 6538)	0.25	
		Salmonella abony (ATCC 6017)	0.50	
	Calamintha glandulosa	*Staphylococcus aureus* (ATCC 6538)	0.25	
		Escherichia coli (laboratory isolate K_{12}W113)	0.28	
		Pseudomonas aeruginosa (clinical isolate)	0.50	
		Salmonella typhimurium (laboratory isolate $CCMMB_{17}$)	0.18	
Ketoconazole	*Melaleuca alternifolia*	*Trichophyton rubrum* (clinical isolate)	0.37	Roana et al. (2021)

Norfloxacin	*Cinnamomum zeylanicum*	*Enterococcus faecalis* (ATCC 29212)	0.08	Rosato et al. (2020)
	Mentha piperita		0.11	
	Origanum vulgare		0.08	
	Thymus vulgaris		0.08	
	Cinnamomum zeylanicum	*Staphylococcus aureus* (ATCC 29213)	0.08	
	Mentha piperita		0.08	
	Origanum vulgare		0.18	
	Thymus vulgaris		0.08	
Oxacillin	*Cinnamomum zeylanicum*	*Enterococcus faecalis* (ATCC 29212)	0.08	
	Mentha piperita		0.11	
	Origanum vulgare		0.08	
	Thymus vulgaris		0.08	
	Cinnamomum zeylanicum	*Staphylococcus aureus* (ATCC 29213)	0.08	
	Mentha piperita		0.11	
	Origanum vulgare		0.11	
Oxacillin	*Thymus vulgaris*	*Staphylococcus aureus* (ATCC 29213)	0.17	Rosato et al. (2020)
Terbinafine	*Curcuma longa*	*Aspergillus niger* (clinical isolate)	0.50	Ogidi et al. (2021)
		Candida albicans (clinical isolate)	0.40	
Tetracycline	*Agathosma betulina*	*Bacillus cereus* (ATCC 11778)	0.46	Hubsch et al. (2014a)
	Artemisia afra		0.48	
	Lippia javanica		0.32	
	Salvia fruticosa	*Staphylococcus epidermidis* (clinical isolates)	0.12–0.37	Chovanova et al. (2015)
	Salvia officinalis		0.31–0.49	
	Salvia sclarea		0.12–0.49	

14.2.3 Conventional Antimicrobials in Synergistic Combinations with Plant Extracts

Adwan et al. (2010) and van Vuuren and Viljoen (2011) proposed that the potentiating effect of plant extracts on conventional antimicrobials has been neglected and requires further investigation. Hubsch et al. (2014a) studied various commercially available traditional medicinal plant extracts, combined with commonly used conventional antimicrobials, against a range of pathogens. Even though most interactions were found to be non-interactive, a few notable synergistic interactions were identified. For example, it was found that both the aqueous and organic extract of *Agathosma betulina* (buchu), combined with ciprofloxacin against *E. coli*, provided synergistic interactions. A further notable synergistic interaction was identified between *Sutherlandia frutescens* (cancer bush) organic extract (a 1:1 mixture of dichloromethane/methanol) and ciprofloxacin against *E. coli* (ΣFIC of 0.28). Urinary tract infection, where *E. coli* is the most common causative pathogen, has shown resistance to ciprofloxacin. With *S. frutescens* used traditionally in treating urinary tract infections, the combination with ciprofloxacin demonstrating synergy against *E. coli* could very well be an avenue of addressing the ciprofloxacin resistance (Hubsch et al. 2014a). It was also found that *P. sidoides* extracts demonstrated the highest synergistic interaction compared to other medicinal plants. This finding could provide clinical benefit in that *P. sidoides* is found in many natural remedies to boost the immune system and fight off infection.

A South African study conducted on the combination of the ethanol extract of *Ziziphus mucronata* (buffalo thorn) with conventional antibiotics (tetracycline, chloramphenicol, amoxicillin and ciprofloxacin) found that more synergistic interactions (54.17%) occurred between the combinations than antagonism (1.39%). This study evaluated clinically relevant bacteria (*Bacillus cereus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *E. coli*) (Olajuyigbe and Afolayan 2013). Some of the most noteworthy synergistic interactions identified in combination studies with conventional antimicrobials and plant extracts have been documented in Table 14.2.

14.2.4 Conventional Antimicrobials in Synergistic Combinations with Culinary Herbs, Spices and Other Plant-Based Foods

Culinary herbs and spices, usually in the form of essential oils, extracts or isolated phytochemical compounds, have also demonstrated antimicrobial activity. Liu et al. (2017) even reported that household herbs and spices might be considered alternative antimicrobial treatments for infectious diseases due to their proven antimicrobial efficacy against various microorganisms. However, it has been found that most often, these natural products interact synergistically with antibiotics and conventional antifungals. Although fungal infections are not as prevalent as those caused

Table 14.2 Synergistic interactions between conventional antimicrobials and African medicinal plant (indigenous/invasive/naturalised/commercialised) extracts (ΣFIC of below or equal to 0.5 is considered a synergistic interaction)

Conventional antimicrobial	Natural product	Extract type	Test microorganism	ΣFIC	References
Amoxicillin	*Acacia mearnsii*	Methanol	*Enterococcus faecalis* (ATCC 29212)	0.07	Olajuyigbe and Afolayan (2013)
			Escherichia coli (ATCC 25922)	0.50	
			Enterococcus faecalis (clinical isolate)	0.31	
			Shigella sonnei (ATCC 29930)	0.02	
	Salvia officinalis	Acetone	*Escherichia coli* (ATCC 25922)	0.38	
			Staphylococcus aureus (ATCC 25923)	0.35	Stefanovic et al. (2012)
			Pseudomonas aeruginosa (ATCC 27853)	0.35	
			Bacillus subtilis (clinical isolate)	0.50	
			Enterobacter cloacae (clinical isolate)	0.35	
			Klebsiella pneumoniae (clinical isolate)	0.35	
			Staphylococcus aureus (clinical isolate)	0.35	
			Proteus mirabilis (clinical isolate)	0.49	
	Salvia officinalis	Ethyl acetate	*Escherichia coli* (ATCC 25922)	0.42	
			Bacillus subtilis (clinical isolate)	0.35	
			Enterobacter cloacae (clinical isolate)	0.49	
			Klebsiella pneumoniae (clinical isolate)	0.35	
			Staphylococcus aureus (clinical isolate)	0.49	
Amikacin	*Thymbra spicata*	Aqueous	*Staphylococcus aureus* (clinical isolate)	0.26	Haroun and Al-Kayali (2016)
		Ethanol	*Staphylococcus aureus* (clinical isolate)	0.50	
			Klebsiella pneumoniae (clinical isolate)	0.50	
		Petroleum	*Staphylococcus aureus* (clinical isolate)	0.38	
Ampicillin	*Vitis vinifera*	Ethanol	*Escherichia coli* (clinical isolate)	0.28	Sanhueza et al. (2017)
Cefotaxime	*Thymbra spicata*	Aqueous	*Staphylococcus aureus* (clinical isolate)	0.26	Haroun and Al-Kayali (2016)
		Ethanol	*Staphylococcus aureus* (clinical isolate)	0.19	
		Petroleum	*Staphylococcus aureus* (clinical isolate)	0.26	

(continued)

Table 14.2 (continued)

Conventional antimicrobial	Natural product	Extract type	Test microorganism	ΣFIC	References
Cephalexin	*Clinopodium vulgare*	Ethanol	*Bacillus subtilis* (clinical isolate)	0.44	Stefanovic et al. (2011)
		Ethyl acetate	*Bacillus subtilis* (clinical isolate)	0.44	
		Acetone	*Klebsiella pneumoniae* (clinical isolate)	0.50	
Chloramphenicol	*Acacia mearnsii*	Methanol	*Escherichia coli* (ATCC 25922)	0.38	Olajuyigbe and Afolayan (2012)
Chloramphenicol	*Acacia mearnsii*	Methanol	*Bacillus subtilis* (clinical isolate)	0.14	Olajuyigbe and Afolayan (2012)
			Shigella sonnei (ATCC 29930)	0.38	
			Proteus vulgaris (ATCC 6830)	0.50	
	Salvia officinalis	Ethyl acetate	*Bacillus subtilis* (clinical isolate)	0.40	Stefanovic et al. (2012)
	Vitis vinifera	Ethanol	Methicillin-resistant *Staphylococcus aureus* (clinical isolate)	0.06	Sanhueza et al. (2017)
			Escherichia coli (clinical isolate)	0.05	
Ciprofloxacin	*Acacia mearnsii*	Methanol	*Proteus vulgaris* (clinical isolate)	0.50	Olajuyigbe and Afolayan (2012)
	Vitis vinifera	Ethanol	Methicillin-resistant *Staphylococcus aureus* (clinical isolate)	0.06	Sanhueza et al. (2017)
			Escherichia coli (clinical isolate)	0.03	
Clarithromycin	*Hibiscus sabdariffa*	Aqueous	*Helicobacter pylori* (clinical isolate HP06)	0.21	Hassan et al. (2016)
Erythromycin	*Acacia mearnsii*	Methanol	*Shigella sonnei* (ATCC 29930)	0.08	Olajuyigbe and Afolayan (2012)
	Aloe ferox	Dichloromethane methanol	*Bacillus cereus* (ATCC 11778)	0.34	Hubsch et al. (2014a)
	Pelargonium sidoides	Dichloromethane methanol		0.32	

Metronidazole	*Acacia mearnsii*	Methanol	*Enterococcus faecalis* (ATCC 29212)	0.25	Olajuyigbe and Afolayan (2012)
			Escherichia coli (ATCC 25922)	0.31	
Nalidixic acid	*Vitis vinifera*	Ethanol	Methicillin-resistant *Staphylococcus aureus* (clinical isolate)	0.05	Sanhueza et al. (2017)
			Escherichia coli (clinical isolate)	0.05	
Norfloxacin			Methicillin-resistant *Staphylococcus aureus* (clinical isolate)	0.05	
			Escherichia coli (clinical isolate)	0.06	
Oxacillin	*Daphne genkwa*		Methicillin-resistant *Staphylococcus aureus* (clinical isolate)	0.38	Kuok et al. (2017)
	Vitis vinifera		Methicillin-resistant *Staphylococcus aureus* (clinical isolate)	0.05	Sanhueza et al. (2017)
Penicillin G	*Pelargonium sidoides*	Dichloromethane methanol	*Enterococcus faecalis* (ATCC 29212)	0.24	Hubsch et al. (2014a)
	Aloe ferox		*Bacillus cereus* (ATCC 11778)	0.35	
	Pelargonium sidoides		*Enterococcus faecalis* (ATCC 29212)	0.24	
	Sutherlandia frutescens			0.38	
	Pelargonium sidoides		*Staphylococcus aureus* (ATCC 25923)	0.20	
Tetracycline	*Pelargonium sidoides*	Aqueous	Methicillin-resistant *Staphylococcus aureus* (clinical isolate)	0.06	Sanhueza et al. (2017)
			Escherichia coli (clinical isolate)	0.09	
			Staphylococcus aureus (ATCC 25923)	0.48	Hubsch et al. (2014a)

by bacteria or viruses, physicians have very limited antifungal agents at their disposal. Therefore, it has been noted that one of the approaches to developing more antifungal treatments is to consider the combination of phytochemicals derived from culinary herbs and spices with existing conventional antimicrobial agents (Movahed et al. 2016).

Palaniappan and Holley (2010) investigated isolated compounds thymol, cinnamaldehyde and carvacrol, derived from culinary herbs and spices, with antibiotics (ampicillin, penicillin, tetracycline, erythromycin, bacitracin and novobiocin). Thymol was synergistic with all antibiotics tested, except for erythromycin. Similarly, carvacrol was found to be synergistic with most antibiotics, except for ampicillin and erythromycin. Cinnamaldehyde interacted synergistically with all the antibiotics tested, except for penicillin.

Various plant-based foods can also be medicinal in nature. For example, fruits and other plant-based foods often contain berberine, a compound well-known for exhibiting antimicrobial activity. It has been noted that the mechanism of action of berberine on pathogens could very much lend itself to potentiating conventional antimicrobial activity (Bhandari et al. 2000). Cock et al. (2017) explored a further compound, a-mangosteen, isolated from the mangosteen fruit and found it to interact synergistically when combined with beta-lactam antibiotics.

14.2.5 Conventional Antimicrobials in Synergistic Combinations with Herbal Teas

Another interesting natural product that may be considered for its potentiating antimicrobial properties includes various teas (black, white, green tea, oolong and rooibos). Many teas are prepared from household herbs and spices, which are then classified according to the plant of origin. Examples include, but are not limited to, chamomile, ginseng, ginger, peppermint, cinnamon and rosemary tea (Malongane et al. 2017). Malongane et al. (2017) review the various teas, with the classification, derived plant material and preparation thereof. Teas are well-known for containing phytochemicals that exhibit antimicrobial, anti-cancer and anti-diabetic characteristics. There are many teas derived from indigenous South African herbal plants, such as *Athrixia phylicoides* (bush tea), *Aspalathus linearis* (rooibos tea), *Cyclopia* species (honeybush tea) and *Monsonia burkeana* (special tea), exhibiting medicinal properties, inclusive of antimicrobial activity (Malongane et al. 2017). Tea has also been noted to be effective in treating dermal infections (Pazyar et al. 2012; Malongane et al. 2017).

Chan et al. (2011) investigated the antimicrobial activity of green, black and other herbal teas against multiple pathogens. It was found that the tea samples exhibited antimicrobial activity against Gram-positive *S. aureus*, *B. cereus* and *Micrococcus luteus*. Interestingly, these tea samples did not demonstrate the same antimicrobial efficacy against Gram-negative bacteria, such as *E. coli*, *P. aeruginosa* and *Salmonella enterica*. It was stated that plant extracts, in this case being in

the form of tea, are less effective against fighting Gram-negative microorganisms due to the bacteria having an outer membrane, which limits access of these compounds into the organism.

The catechins in green tea have been found to inhibit bacterial growth in mice infected with *E. coli*. When green tea was combined with the antibiotic levofloxacin, the mice receiving both were protected from organ damage (Setiawan et al. 2001). Tea constituents have abundantly been documented to be useful adjunctive agents to conventional antimicrobial agents, such as beta-lactams, carbapenems, norfloxacin and tetracycline.

A study by Hubsch et al. (2014b) demonstrated that the popular beverage, rooibos tea, derived from *Aspalathus linearis*, showed synergistic effects with conventional antimicrobials, particularly penicillin. Furthermore, Chovanova et al. (2013) reported an excellent synergistic effect displayed by certain species of *Salvia* (sage), as well as *Matricaria recutita* (German chamomile), when used in combination with the conventional antibiotic, oxacillin. Furthermore, the medicinal plant samples were found to damage the cytoplasmic membrane of *S. epidermidis*, causing the loss of intracellular components and allowing the entrance of the antibiotic, resulting in increased efficacy of oxacillin in eradicating bacteremia in patients infected with methicillin-resistant *S. epidermidis* (Chovanova et al. 2013).

Da Silva et al. (2014) studied isolated tea compounds in combination with the antifungal fluconazole against resistant isolates of *Candida tropicalis*, where synergistic interactions were demonstrated through the enhanced antimicrobial activity of fluconazole. In addition, several studies (Tiwari et al. 2005; Erbil and Digrak 2013; Haghjoo et al. 2013; Koech et al. 2014; Mbuthia et al. 2014; Ning et al. 2015) also investigated compounds extracted from teas in combination with conventional antimicrobials, such as doxycycline, erythromycin, fluconazole, miconazole and penicillin G, and promisingly, most often synergistic interactions were identified. A few of the notable synergistic interactions identified in studies of conventional antimicrobials in combination with teas have been summarised in Table 14.3.

14.2.6 Conventional Antimicrobials in Synergistic Combinations with Bee Products

Honey has been used for centuries to treat skin wounds and respiratory and gastrointestinal diseases (Visavadia et al. 2006). Anyanwu (2012), Moussa et al. (2012) and Shenoy et al. (2012), amongst others, investigated the in vitro antimicrobial activity of honey. In particular, a study of 42 South African honey samples demonstrated efficacy that, in some cases, was better than the gold standard Manuka honey (Khan et al. 2014). There are also clinical studies that have demonstrated that honey and other bee products exhibit the potential to treat infections. Manuka honey has received attention concerning its effective antimicrobial treatment of skin infections. It has even demonstrated activity against MRSA (Mandal et al. 2010). A few synergistic interactions identified in studies on bee products in combination with conventional antimicrobials have been documented in Table 14.4.

Table 14.3 Synergistic interactions between conventional antimicrobials and common herbal teas available in Africa (ΣFIC of below or equal to 0.5 is considered a synergistic interaction)

Conventional antimicrobial	Natural product	Extract type	Test microorganism	ΣFIC	References
Ampicillin	Pomegranate blossom tea	Aqueous	*Staphylococcus aureus* (clinical isolate)	0.30	Hacioglu et al. (2017)
	Thyme tea			0.50	
	Wormwood tea			0.50	
	Rosehip tea			0.60	
Fluconazole	Epigallocatechin 3-o-gallate (compound from green tea)	Pure compound	*Candida dubliniensis* (clinical isolate 2)	0.38	Behbehani et al. (2019)
			Candida albicans (clinical isolate 1)	0.38	
			Candida glabrata (clinical isolate)	0.50	
			Candida dubliniensis (clinical isolate 2)	0.50	
			Candida albicans (ATCC 24433)	0.31	
			Candida glabrata (ATCC 15126)	0.50	
	Flavonoid catechin		*Candida tropicalis* (clinical isolate)	0.38	Da Silva et al. (2014)
	Quercetin		*Candida tropicalis* (clinical isolate)	0.25	
	Epigallocatechin 3-o-gallate (compound from green tea)		*Candida tropicalis* (clinical isolate)	0.25	
Gentamicin	*Aspalathus linearis* (rooibos tea)	Dichloromethane methanol	*Staphylococcus aureus* (ATCC 25923)	0.50	Hubsch et al. (2014b)

Ketoconazole	Epigallocatechin 3-o-gallate (compound from green tea)	Pure compound	*Candida albicans* (clinical isolates)	0.38–0.50	Behbehani et al. (2019)
			Candida glabrata (ATCC 15126)	0.38	
			Candida dubliniensis (clinical isolate)	0.38	
			Candida albicans (ATCC 24433)	0.50	
	Flavonoid catechin		*Candida tropicalis* (clinical isolate)	0.25	
	Quercetin			0.25	
Nystatin	Black tea	Aqueous	*Candida albicans* (clinical isolate)	0.50	Hacioglu et al. (2017)
	Green tea		*Candida albicans* (clinical isolate)	0.30	
Penicillin G	*Aspalathus linearis* (rooibos tea)	Dichloromethane methanol	*Bacillus cereus* (ATCC 11778)	0.08	Hubsch et al. (2014b)
		Aqueous	*Enterococcus faecalis* (ATCC 29212)	0.46	
Tetracycline		Dichloromethane methanol	*Staphylococcus aureus* (ATCC 25923)	0.01	
			Bacillus cereus (ATCC 11778)	0.48	

Table 14.4 Synergistic interactions between conventional antimicrobials and African bee products (ΣFIC of below or equal to 0.5 is considered a synergistic interaction)

Conventional antimicrobial	Natural product	Test microorganism	ΣFIC	References
Ciprofloxacin	South African Honey 18-(MIXEDGUM/FS0)	*Pseudomonas aeruginosa* (ATCC 9027)	0.38	Khan et al. (2014)
	South African Honey 19-(CITYMIX/FS)		0.50	
Clindamycin	Melittin (pure compound) from bee venom	*Staphylococcus aureus* (ATCC 33591)	0.38	Mahmoudi et al. (2020)
		Methicillin-resistant *Staphylococcus aureus* (clinical isolate 1)	0.38	
		Methicillin-resistant *Staphylococcus aureus* (clinical isolate 2)	0.19	
		Methicillin-resistant *Staphylococcus aureus* (clinical isolate 3)	0.28	
		Methicillin-resistant *Staphylococcus aureus* (clinical isolate 4)	0.26	
		Methicillin-resistant *Staphylococcus aureus* (clinical isolate 5)	0.08	
		Methicillin-resistant *Staphylococcus aureus* (clinical isolate 6)	0.26	
		Enterococcus faecalis (ATCC 51299)	0.40	
		Streptococcus pyogenes (ATCC 12344)	0.50	
Gentamicin	South African Honey 16-(FYNBOS/WC)	*Staphylococcus aureus* (ATCC 25923)	0.27	Khan et al. (2014)
	South African Honey 18-(MIXEDGUM/FS0)		0.27	
	South African Honey 19-(CITYMIX/FS)		0.27	
	South African Honey 26-(FYNBOS/WC)		0.27	
	South African Honey 41-(INDIGENOUS/WC)		0.27	
	South African Honey 16-(FYNBOS/WC)	*Pseudomonas aeruginosa* (ATCC 9027)	0.31	
	South African Honey 19-(CITYMIX/FS)		0.47	
Nystatin	South African Honey 16-(FYNBOS/WC)	*Candida albicans* (clinical isolate)	0.31	

14.3 Conventional Antimicrobials in Antagonistic Interactions with Natural Products

While synergistic interactions are frequently reported, less attention has been given to antagonistic interactions amongst researchers, which is equally important, especially when considering clinical combination therapy. An interesting common thread resonating through many of the combination studies presented in this chapter is the lack of reporting on antagonistic interactions. Demonstrated in this section are some of the studies that have documented antagonism.

Melaleuca alternifolia (tea tree), *T. vulgaris*, *Mentha piperita* (peppermint) and *R. officinalis* (rosemary) essential oils have demonstrated antagonistic interactions with ciprofloxacin against *S. aureus* and *K. pneumoniae* and with amphotericin B against *C. albicans* (Van Vuuren 2008). Olajuyigbe and Afolayan (2013) and Hubsch et al. (2014a) conducted studies on various combinations of natural products with conventional antimicrobials, demonstrating antagonistic profiles. Hubsch et al. (2014a, b) further found that *A. afra* extract, combined with ciprofloxacin provided a notable antagonistic interaction (ΣFIC of 8.55) when tested against *E. coli*. Furthermore, conventional antimicrobials were found to demonstrate antagonistic interactions more often when in combination with *A. ferox*.

Peng et al. (2010) studied the antagonistic effects of the green tea extracts from *C. sinensis* on modulating the resistance mechanism of MRSA towards beta-lactam antibiotics, namely, amoxicillin, ampicillin, and oxacillin. Under investigation, in mice, it was found that the green tea extract, alone or in combination with amoxicillin, does not have protective benefits in MRSA-infected mice. Therefore, the study suggested that tea drinking is cautioned in combination with amoxicillin treatment. A few natural products demonstrating meaningful antagonistic interactions with conventional antimicrobials have been indicated in Table 14.5.

14.4 Discussion and Conclusion

With the rise in AMR to existing conventional antimicrobial therapies and the limited conventional antimicrobial drugs at the disposal of healthcare professionals in Africa, it is critical that alternative avenues to enhance the antimicrobial activity of existing agents are rapidly discovered to avoid a modern healthcare system being deemed incapable of treating infectious diseases (Bhardwaj et al. 2016).

Findings identified in the interactive scientific studies considered in this chapter indicate synergistic interactions are more frequently reported compared to antagonistic interactions. This could indicate that natural products may show more synergistic potential in addressing AMR in combination with conventional antimicrobials. However, this warrants further exploration to determine whether the increased synergistic interaction reporting is a true reflection or merely because antagonistic identification is less of a research focus. Even though fewer antagonistic

Table 14.5 Antagonistic interactions between conventional antimicrobials and African natural products (indigenous/invasive/naturalised/ commercialised) (ΣFIC value greater than 4.0 is considered antagonistic).

Conventional antimicrobial	Natural product	Extract type	Test microorganism	ΣFIC	References
Amphotericin B	*Artemisia afra*	Dichloromethane methanol	*Candida albicans* (ATCC 10231)	6.76	Hubsch et al. (2014a)
		Aqueous		5.34	
Chloramphenicol	*Acacia mearnsii*	Methanol	*Enterobacter cloacae* (clinical isolate)	4.50	Olajuyigbe and Afolayan (2012)
Ciprofloxacin	*Artemisia afra*	Aqueous	*Escherichia coli* (ATCC 25922)	8.55	Hubsch et al. (2014a)
	Pomegranate blossom tea		*Staphylococcus aureus* (Methicillin-resistant ATCC 43300)	9.00	Hacioglu et al. (2017)
	Rosehip tea		*Staphylococcus aureus* (ATCC 29213)	5.00	
	Pomegranate blossom tea			5.00	
	Rosehip tea		*Staphylococcus aureus* (Methicillin-resistant ATCC 43300)	8.00	
	Rosehip tea		*Enterococcus faecalis* (ATCC 29212)	5.00	
	Pomegranate blossom tea			9.00	
	Acacia mearnsii	Methanol	*Staphylococcus aureus* (ATCC 6538)	4.25	Olajuyigbe and Afolayan (2012)
			Bacillus subtilis (clinical isolate)	4.06	
			Shigella sonnei (ATCC 29930)	18.00	
Erythromycin	Pomegranate blossom tea	Aqueous	*Staphylococcus aureus* (ATCC 29213)	5.00	Hacioglu et al. (2017)
	Rosehip tea			5.00	
Kanamycin	*Acacia mearnsii*	Methanol	*Enterococcus faecalis* (clinical isolate)	8.13	Olajuyigbe and Afolayan (2012)
Penicillin G	*Artemisia afra*	Dichloromethane methanol	*Staphylococcus aureus* (ATCC 25923)	4.25	Hubsch et al. (2014a)
			Bacillus cereus (ATCC 11778)	4.40	

interactions are documented, this should not provide a false sense of complacency on the potential of medicinal plants reducing the efficacy of conventional antimicrobials. Nevertheless, any antagonistic potential reported, even if it be a single combination occurrence, should be a cause for concern.

This chapter has highlighted that many natural products exhibit considerable antimicrobial activity. This is also evident through the extensive history of using natural products, not just in Africa, but globally, in treating various infections. There still, however, remains a dearth of research necessary to further understand the interactive profiles of combination therapies; however, natural product combinations with conventional antimicrobials are arguably a potential avenue of research to assist in discovering new medicines that prove effective against antimicrobial resistance.

One advantage of combination therapy is decreased development costs and reduced time to achieve clinical use in treating infections (Cock et al. 2017). Developing and marketing new antimicrobials involves extensive research, clinical trials and evaluation, whereas combination therapy would require a less extensive process, subsequently costing less. With the vast availability to natural products in Africa, harnessing the medicinal properties of these products to enhance the efficacy of conventional drugs should not be underestimated. With the drive to integrate the two forms of healthcare, with regulation amendments and stricter control of natural products, this is a definite alternative to be considered in the fight against AMR.

Although natural product combinations with conventional antimicrobials provide a promising alternative approach to addressing AMR, it is important to note that to exploit synergistic combinations, it is vital that favourable ratios and doses of antimicrobials and natural product combinations are determined (Bhardwaj et al. 2016), together with the necessary quality control and standardisation of natural products to ensure uniformity in therapy (Viljoen and Vermaak 2013).

With the high estimate of TCAM/natural product use in Africa, policymakers, researchers and healthcare professionals must recognise that these products are legitimate forms of healthcare. Furthermore, these combinations still require an immense amount of research and further in-depth analysis, with development based on scientific research and regulatory mechanisms, on par with allopathic medicines (Pandey et al. 2020). Natural product integration in modern healthcare has long been under contention due to the lack of scientific validation and the scepticism about the efficacy of natural products, which needs to be addressed.

The ever-increasing occurrence of AMR in Africa may very well be due to not only the misuse and abuse of conventional antimicrobials but also as a result of antagonistic interactions between conventional antimicrobials and certain natural products being consumed concurrently. This may be going undetected due to the lack of the aspects mentioned above still not being definitively outlined and understood. The enquiry of natural product use in modern healthcare facilities before prescribing or dispensing has also been noted as a challenge since many patients do not consider the need to disclose TCAM use to other healthcare professionals. Government legislation and regulations regarding natural products and traditional

remedies are thus necessary to avoid future adverse interactions and exacerbation of AMR. These regulations may include more comprehensive enquiry in medical history taking practices, being more inclusive of TCAM use or including more labelling of natural product remedies to include warnings of interactions with conventional drugs or informing and educating traditional healers on these interactions so that the information can be relayed to the patients. Furthermore, healthcare professionals in conventional health settings in Africa need to be trained on TCAM and its potential to interact with conventional medicines (James et al. 2018).

Africa, with its rich diversity in flora, offers the ideal opportunity for researchers to identify and develop innovative products and advantageous combination therapies to treat infections (Van Wyk 2011). Certainly, the options for future integration of natural products with conventional antimicrobials demonstrate promise and are a viable option for combatting AMR's global onslaught.

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Index

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