# Chapter 1
# Antimicrobial Resistance: A Crisis in the Making

## 1.1 Introduction

Antimicrobial resistance (AMR) is ancient and probably predates the evolution of Humanids to *Homo sapiens* or modern humans [1]. Although antibacterial resistance existed before the discovery of penicillin in 1928, it is largely due to overuse of antimicrobials in humans and animals. The current trend of increasing AMR threatens the antimicrobial effectiveness of increasing sphere of serious life-threatening infections due to bacteria, parasites, viruses, and fungi. Despite guidelines and antibiotic stewardship programs, antibiotic consumption from 2000 to 2015 in 76 countries had increased by 65%, and the global antibiotic consumption is projected to increase by 200% by 2030 [2]. There is an ever-increasing use of antimicrobials in livestock, as growth promoter and prophylaxis, since the practice was introduced in industrialized countries in 1950. In 2013, antimicrobial animal consumption globally was estimated to be 131,109 tons and is projected to reach 200,235 tons in 2030 [3]. Food animal production has plateaued in high-income countries since 2000 but has grown by 40–68% in low- and middle-income countries (LMIC) [4]. This has resulted in a corresponding increase in antimicrobial consumption by livestock in these countries. In Europe, regulations have been implemented to limit antimicrobial use in animal husbandry, while in the US consumer preference may have limited their use. A recent survey has found that AMR in animals is drastically rising in LMICs, with China and India representing the greatest hotspots of resistance and Brazil and Kenya are emerging hotspots [5]. The highest resistance rates are found in antimicrobials most commonly used in animals: tetracyclines, sulfonamides, penicillins, and quinolones. A recent report from the European Union on antimicrobial resistance from zoonotic indicator bacteria from animals and humans in 2016 found that resistance overall in critically important bacteria was generally uncommon, except for specific *Salmonella* serovars which showed very high multidrug resistant levels especially to ciprofloxacin and

**Table 1.1**  Factors associated with antimicrobial resistance [AMR]

| Direct factors | Indirect factors |
| --- | --- |
| Overuse in healthcare | Poor sanitation |
| Overuse in farm animals | Poverty |
| Environmental contamination | Underdeveloped PHS |
| Easy access ["over-the-counter"] | Underuse of vaccines |
| Ineffective drugs | Low diagnostic methods |
| ↑Demand | ↑Prostheses and transplants |

Abbreviation: *PHS* public health system

extended-spectrum β-lactamase (ESBL) producers [6]. It is estimated that >75% of antimicrobials produced are used in food animals.

Low concentration of antimicrobials used in animal feed for growth promotion and mass prophylaxis promote the evolution of resistance, and food animal reservoir is a greater source of resistant genes than in humans. There is increasing evidence that antimicrobial resistance in animals can lead to resistant infections in humans [7–9]. While restricting use of antibiotics in food animals is associated with reduced antibiotic-resistant bacteria in animals and humans in direct contact with food-producing animals, the implication for the general population is less clear [10]. Thus, restricting use of antimicrobials in animals alone will not be sufficient to control AMR in humans [11]. However, the main driver of AMR globally is antibiotic pressure due to a combination of factors (see Table 1.1).

## 1.2   Antimicrobial Resistance: An Evolutionary Process

Soon after the discovery of each class of antibiotics, there would appear resistant bacteria with transmissible genetic elements or *r* genes which were considered a modern phenomenon. However, metagenomic analyses of ancient DNA from 30,000-year-old Beringian permafrost sediments identified diverse number of genes encoding resistance to β-lactams, tetracycline, and glycopeptide antibiotics [1]. Thus, AMR is a natural phenomenon which predates the discovery of antibiotics and is likely an evolutionary selective process for the survival of microbes living adjacent to antibiotic-producing bacteria or fungi (i.e.*, Actinomycetes* and *Streptomycetes*). Antibiotic-producing *Actinomycetes* possess genes encoding resistance to the antimicrobials they generate and *Streptomyces* produce diverse β-lactamases, some of which may be responsible for clinical resistance [12, 13]. An environmental *Kluyvera* species appears to be origin of the CTX-M genes that encode the extended β-lactamase that hydrolyze third-generation cephalosporin [14]. It should not be surprising that antibiotic *r* genes and resistance-encoding integrons were found in the gut flora of isolated indigenous people who live in remote areas away from modern civilization without antibiotic exposure [15].

Antibiotic-resistant genomes are widespread in nature, and analysis of 13,293 genes yielded a core set of 4554 antibiotic resistant proteins/genes [16]. Functional metagenomic analysis of soil for bacterial resistance was reported to yield 2895 antibiotic resistance genes and represented all major resistance mechanisms [17]. However, recently 6000 antibiotic resistance genes were discovered in the bacteria from human gut [18]. Thus, humans harbor more microbial resistance genes than the environment.

## 1.3   Mechanisms of Microbial Resistance

AMR is a natural phenomenon that occurs over time through genetic changes of microbes, but this process is accelerated by high antimicrobial pressure due to overuse and misuse. Antimicrobial-resistant microbes are found worldwide in people, animals, food, and environment (soil and water), and transfer of resistance to humans can occur from any of these sources. Spread of antimicrobial resistance among humans is facilitated by poor infection control, inadequate sanitary conditions, and inappropriate food-handling. The ease of rapid modern transportation (air travel) has also facilitated the spread of antimicrobial-resistant microbes between peoples and animals of different countries across the world.

## 1.4   Bacterial Resistance

AMR is best studied and recognized in bacteria as antibiotics are the most frequently used antimicrobial agents in people and animals. Development and persistence of antibiotic-resistant bacteria are encouraged by the widespread use of antibiotics, broader-spectrum greater than narrow-spectrum agents, and longer-term use facilitate increased resistance more than shorter course. The widespread indiscriminate use of proton pump inhibitors (PPI) in healthcare facilities and by physician in general for gastric acid suppression also appears to be playing a role in intestinal colonization with multiresistant bacteria with possible cross-transmission in healthcare institutions [19].

Presently, there are >16 classes of antibiotics (used in the broad term) discovered, based on their structure and mode of action. Some are synthetic compounds (sulfonamides, quinolones, etc.) and others are natural antibiotics produced by microbes, most commonly from the phylum *Actinobacteria* of the genus *Streptomyces* (penicillin, streptomycin, etc.). The mechanisms of action of various antibiotics are important to review to appreciate the development and means by which bacteria develop AMR. Although there are seven different mechanisms by which antibiotics inhibit or kill bacteria, their actions result in the interruption of the synthesis and function of four main targets or pathways: (i) cell wall (beta-lactams, glycopeptides); (ii) cell membrane (polymixins, lipopeptides); (iii) nucleic acid
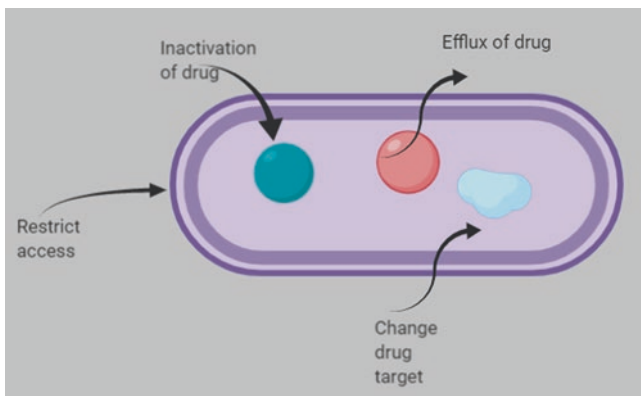
**Fig. 1.1**  Mechanisms of bacterial resistance

synthesis (sulfonamides/pyrimidines [folate synthesis], quinolones [DNA gyrase], rifamycins [RNA polymerase]); and (iv) protein synthesis via 30S ribosomal subunit (aminoglycosides, tetracyclines) and 50S ribosomal subunit (macrolides, lincosamides, oxazolidinones, phenicols, streptogramins, and pleuromutilin) [20, 21].

Bacteria (and fungi) develop defense strategies to evade antibiotics by mutation or upregulation of existing resistosome broadly by four mechanisms (see Fig. 1.1): (i) restriction of access of these agents at the cell wall and membrane by changing entryways or limiting the number of entryways; (ii) changing the antibiotic target so the drug cannot fit or act at the target site or develop new cell processes that avoid using the antibiotic target; (iii) destroying or breaking down the drugs by enzymes; and (iv) extruding the agents from the cell by using efflux pumps. Table 1.2 shows an incomplete list of antibiotic classes and mechanisms of resistance [20–22].

## 1.4.1   Restriction of Access

Gram-positive bacteria possess a thick complex but very permeable cell wall which readily allows antimicrobials and are easier to kill than gram-negative bacteria. However, resistance due to restricted penetration can occur as demonstrated by vancomycin-intermediate *Staphylococcus aureus* strains (VISA) that produce greatly thickened wall which decrease penetration and activity [23]. Gram-negative bacteria allow drug molecules diffusion through a bilayer of the outer membrane (OM) by porins. Small hydrophilic molecules (β-lactams and fluoroquinolones [FQ]) can cross the OM only through porins. Resistance to these classes of agents can occur through decrease in number of porin channels leading to decreased cell entry of the β-lactams and FQ. *Pseudomonas aeruginosa* acquire resistance to all classes of antibiotics through decreased OM permeability [20].

**Table 1.2** Classes of antibiotics and mechanisms of resistance

| Antibiotic class | Resistance mechanisms | New addition |
|---|---|---|
| Aminoglycoside | Decrease uptake, enzymatic modification, and efflux | Plazomicin |
| Carbapenems | Carbapenemase, changed porin selectivity, efflux | Mero.-Vaborbactam Imipen.-Relebactam |
| Cephalosporins | Cephalosporinase, porin selectivity, efflux | Ceftaz.-Avibactam Cefiderocol |
| Penicillins | Penicillinase, altered PBPs, efflux | |
| Monobactams (Aztreonam) | Efflux, altered PBP? | |
| Cationic peptides (Colistin) | Efflux, altered target | |
| Fidaxomicin (macrocyclic lactone) | Rare, unclear, *rpo* [B] mutation | |
| Fosfomycin | Decrease uptake, altered target, and degradation | |
| Fusidic acid | Decrease uptake, altered target | |
| Glycopeptides | Decrease uptake, modified target. | Oritavancin |
| Lipopeptide [Daptomycin] | Modified net cell surface charge, altered target | |
| Lincosamide [Clindamycin] | Altered target, efflux, nucleotidylation | |
| Macrolides | Altered target [methylation], efflux | |
| Metronidazole | Decrease uptake, efflux | |
| Nitrofurans | Unclear—efflux? altered target? | |
| Oxazolidinones (Linezolid) | Altered target, efflux | Torezolid |
| Phenicols (Chloramphenicol) | Acetylation, altered target, efflux | |
| Pleuromutilin | Unclear [altered target?] | Lefamulin |
| Quinolones | Altered target, efflux, acetylation of drug | |
| Sulfonamides/ Trimethoprim | Altered target, decrease uptake, efflux | |
| Tetracyclines | Decreased uptake, altered target, efflux, | Omadacycline, Eravacycline |

## *1.4.2 Modification of Target*

Modification of target molecules by natural variation or acquired changes in the target sites of antimicrobials is a common mechanism of drug resistance. Spontaneous mutation of a bacterial gene on the chromosome often results in drug target sites modification. Drug interaction with the target molecule is usually very specific and minor alterations can affect antibiotic binding and decrease action. Examples of drug target modifications can be subdivided as follows: (a) Alterations in the 30S subunit can result in resistance to tetracyclines and aminoglycosides (AG) and to the 50S subunit lead to resistance to macrolides, chloramphenicol, lincosamides, and streptogramin B [24]. (b) Modification of the penicillin-binding

proteins (PBP) is a common mechanism used by gram-positive bacteria to reduce affinity to β-lactam drugs. This is demonstrated by mutation in the PBP leading to *Enterococcus faecium* resistance to ampicillin and *Streptococcus pneumoniae* to penicillin. *S. aureus* resistance to methicillin/oxacillin is through mec gene A that encodes PBP2a protein with reduced affinity to the β-lactams leading to methicillin-resistant *S. aureus* (MRSA). The mec A gene is transmitted through a large mobile genetic element, "staphylococcal cassette chromosome mec," that is integrated into the chromosome of MRSA [25]. There is resistance to all β-lactam agents, and cross-resistance to macrolides, clindamycin, aminoglycosides, and less commonly tetracyclines may be seen. (c) Cell wall precursor modification (i.e., D-alanyl-alanine changed to D-alanyl-lactate) will lead to glycopeptide resistance by preventing their binding to D-analyl-D-alanine residues of the peptidoglycan precursors. Van A type resistance leads to high resistance of *E. faecium* and *E. faecalis* to vancomycin and teicoplanin, whereas Van B and Van C type resistance show resistance to vancomycin but sensitive to teicoplanin [26]. (d) Quinolones bind to DNA gyrase A subunit and mutated DNA gyrase and topoisomerase IV leads to FQ resistance. The resistance mechanism involves the modification of two enzymes: DNA gyrase (coded by genes gyr A and gyr B) and topoisomerase IV (coded by genes par C and par E), and mutation in genes gyr A and par C leads to failure of FQ to bind to the target site [27]. (e) Ribosomal protection mechanisms imparting resistance to tetracyclines. (f) RNA polymerase mutation conferring resistance to rifampin [20].

## 1.4.3   Degradation by Enzymes

Antibiotic degradation or modification by bacterial enzymes is one of the most commonly recognized mechanisms of AMR. This mechanism for self-defense by the antibiotic-producing microbe was recognized in 1970 in soil bacteria of the genus *Streptomyces* [28]. This mechanism is frequently used by gram-negative bacilli and to a lesser degree by gram-positive bacteria. The three main groups of enzymes that inactivate antibiotics are (1) β-lactamases, (2) aminoglycoside-modifying enzymes, and (3) chloramphenicol acetyltransferases (AAC).

### 1.4.3.1   Beta-lactamases

There are >900 β-lactamases circulating and identified in bacteria to date. β-lactamases hydrolyze nearly all β-lactam agents that have ester and amide bond, i.e., penicillins, cephalosporins, monobactams, and carbapenems. The β-lactamases can be classified into four groups (Ambler structural system): Class A β-lactamases (referred to as penicillinase was the first β-lactamase discovered in 1940) include the penicillinase produced by *S. aureus* and the *Enterobacteriaceae,* termed TEM-1, TEM-2, and SHV-1 which have no activity against the cephalosporins (especially expanded spectrum) [20]. TEM-1 is the most common β-lactamase found in

gram-negative bacteria, accounting for ampicillin resistance *in Escherichia coli*, *Klebsiella pneumoniae, Haemophilus influenzae*, and *Neisseria gonorrhoeae.* Mutations in the *Enterobacteriaceae* gave rise to the extended spectrum β-lactamases (ESBLs) that provide multi-resistance to penicillins, cephalosporins, and cephamycins, but the carbapenems are usually effective. CTX-M β-lactamases also belong to Class A, they are mainly found in *Salmonella enterica* serovar *Typhimurium* and *E. coli*, which acquire plasmid β-lactamase genes normally found on commensal bacteria and produce hydrolysis of cefotaxime more than ceftriaxone, ceftazidime, and cefepime due to structural differences [Wikipedia, beta-lactamases, 3/26/2020]. Class B β-lactamases are the metallo-β-lactamases (MBL), containing zinc ions, that can hydrolyze nearly all β-lactam drugs, and unlike other classes (A, C, and D enzymes), they are resistant to the β-lactamase inhibitors, i.e., clavulanic acid, sulbactam, tazobactam, and avibactam, and carbapenems [29]. These enzymes are divided into three subclasses based on the zinc content, but the most relevant include VIMs (Verona integron-encoded MBL), IMPs (imipenases), and NDMs (New Delhi MBL). Class C β-lactamases (called cephalosporinases) hydrolyze all cephalosporins and other β-lactams except carbapenems; the best known is Amp C β-lactamase which is common in ESBL bacteria [20]. Class D β-lactamases (OXA) are oxacillin-hydrolyzing enzymes (weakly inhibited by clavulanic acid) which are most commonly found in *Pseudomonas aeruginosa* and the *Enterobacteriaceae*. The OXA type can result in the ESBL phenotype, and some of the enzyme can hydrolyze cefotaxime, cefepime, and ceftazidime [Wikipedia].

ESBL-producing gram-negative bacteria infections have been a challenge to treat in hospitalized and chronic care facilities worldwide for the past two decades [30]. ESBLs are transmissible (plasmid mediated) β-lactamases that hydrolyze extended-spectrum cephalosporins with oxyimino side chains, i.e., cefotaxime, ceftriaxone, ceftazidime, and aztreonam [Wikipedia]. The plasmids encoding ESBL frequently carry genes encoding resistance to other drug classes (aminoglycoside, quinolone, etc.). Although the carbapenems are considered treatment of choice for severe infection by ESBL-gram-negative bacilli, carbapenem-resistant (primarily ertapenem-resistant) isolates have been reported. The ESBLs were primarily derived from genes for TEM-1, TEM-2, and SHV-1 by mutations, but subsequently these enzymes include other classes of β-lactamases.

### 1.4.3.2 Aminoglycoside-Modifying Enzymes

Aminoglycoside-modifying enzymes (AME) are the most common mechanism of resistance to this class of antibiotics. There are over 100 AME which can be divided in three subclasses: aminoglycoside [A]-acetyltransferases (AACs), A-nucleotidyltransferases (ANTs), and A-phosphotransferases (APHs) [31]. These enzymes reduce the affinity of modified agents and impair binding to the 30S ribosomal subunit, resulting in resistance to aminoglycosides and quinolones [20]. AME are identified in gram-negative bacilli, *Mycobacterium tuberculosis*, *S. aureus*, *E. faecalis*, and *S. pneumoniae* [20].

### 1.4.3.3 Chloramphenicol Acetyltransferase

Chloramphenicol resistance in gram-positive and gram-negative bacteria, including *H. influenza*, is most common through modification of the antibiotic by acetyltransferases. The modified antibiotic is unable to bind to the ribosomal 50S subunit [26].

### 1.4.3.4 Efflux Pumps

Although efflux pump was first described as a mechanism of tetracycline resistance in *E. coli* in 1980 [32], it is now recognized as an ancient evolutionary protective process that constitutes the most ubiquitous system present in all organisms, including bacteria, eukaryotic pathogens such as *C. albicans* and *P. falciparum,* etc., but also mammals including human cells [33, 34]. Essentially efflux pumps are MDR resistant mechanisms present in all microorganisms. They are nearly always chromosomally encoded, conserved at the genetic and protein level, and most bacterial strains of the same species have the same chromosomally coded efflux pumps [35]. MDR efflux pumps are present in all organisms, but are tightly regulated and low-moderate expression may result in intrinsic resistance (i.e., *Ps. aeruginosa*), but acquired resistance may occur in two ways. In chronic infections, antibiotic pressure may cause overexpression of MDR efflux pumps due to mutations in the genes that control downregulation of their expression; phenotypic resistance occurs transiently from the presence of specific inducers of the efflux pumps expression [35]. The efflux systems can actively extrude a variety of compounds besides antimicrobials, such as heavy metals, toxins, organic solvents, dyes, detergents, and others. Overexpression of a single efflux pump can give resistance to multiple antimicrobials, but simultaneous overexpression of multiple efflux pumps may occur with some organisms [35].

## *1.4.4 Genetic Mechanisms of Bacterial Resistance*

AMR can either be intrinsic, adaptive, or acquired. Intrinsic antibiotic resistance is common in the environmental bacteria, and the mechanisms are normally chromosome-encoded, including nonspecific efflux pumps, inactivating enzymes, and permeability barriers [28]. These mechanisms are fixed in the core genetic makeup of the microbe and often confer low level resistance in the original host. Normal commensal flora and environmental bacteria with intrinsic mechanisms of resistance can become opportunistic pathogens in immunocompromised hosts [31]. Adaptive antibiotic resistance of bacteria occurs as a result of harmful environmental exposure (changes in nutrients or subinhibitory concentration of antibiotics) that results in transient changes in gene and protein expression with tolerance to the antimicrobial [36]. Acquired antibiotic resistance occurs by acquisition of exogenous genes from other bacteria by transduction of free DNA by bacteriophages, or

conjugation via plasmids, or through mutation of existing genes. Dissemination of resistant genes by plasmids is considered the most prevalent means among various bacterial species. Transfer of resistant genes by plasmids between bacteria is most expedient in high-density settings such as the gut of humans or animals, biofilms, hospitals, and conditions with co-infection [37]. This process is facilitated by transposons and integrons incorporated in plasmids or phages for conjugation [28]. Transposon is a DNA sequence than can change its position within a genome ("jumping genes") and can carry resistance genes from plasmid to plasmids or from a DNA chromosome to plasmid or vice versa. Integron is a mobile DNA element that can capture and carry genes (expression or gene cassettes encoding antibiotic resistance), by site-specific recombination. Plasmid is an extrachromosomal self-replicating, double-stranded DNA molecule that carries genes not essential for cell growth, such as antibiotic resistant genes, that can be transferred from cell to cell by conjugation or transduction [Dorland Medical Dictionary].

It is now evident that the environment is an important source for pathogenic bacteria to acquire antibiotic-resistant genes. This process may involve four stages: (i) emergence of novel resistance genes, (ii) mobilization (transposons/integrons), (iii) transfer to pathogens (by plasmids), and (iv) dissemination by horizontal transfer [28]. Novel resistance genes are likely occurring all the time in the environment and the most important factor to promote persistence of the resistance genes is selective pressure. The predominant source of selective pressure is the widespread and indiscriminate use of antibiotics, which leads to dominance of resistant and multiresistant strains of bacteria among human pathogens, in the environment near human activities (i.e., antibiotic manufacturing plants), and in food animal farms. It has been estimated that in the past 50 years, millions of metric tons of antibiotic compounds have been released in the biosphere [15], which is undoubtedly contributing to resistant genes in the environment.

Environmental sampling studies have revealed multiresistant *r* genes to 7–8 antibiotics, which has been labeled environmental antibiotic "resistome" [38]. Moreover, many environmental bacteria can subsist and grow on 18 different antibiotics as the sole source of carbon and nitrogen, called "subsistome," including aminoglycoside, FQ, and others [39]. Most of strains identified were proteobacteria, >40% are *Burkholderia* spp., and pseudomonads were also represented.

The origin of antimicrobial resistance and generation of *r* genes for horizontal spread is through the process of "natural selection" in which evolutionary change occurs through genetic mutation. In vitro resistant mutants can be generated spontaneously to virtually any antibiotics, but the frequencies vary markedly depending on the agent and microbial species, with most frequencies usually $\leq 10^{-6}$ [40]. Resistant mutants may be less fit than wild-type organisms, but compensatory mutations may occur so the resistant mutants become equally fit as the wild-type organisms and some strains even maintain the resistant mutation in the absence of the antibiotic selective pressure [41]. Bacterial resistance to some classes of antibiotics occurs primarily by genetic mutation rather than by acquisition of *r* genes by horizontal transfer, i.e., by plasmids. Resistant mutations readily occur to rifamycins, fusidic acid, and streptomycin when used as monotherapy and less readily to FQ

and oxazolidinones (linezolid) [40]. Unlike rifampin, resistance to linezolid is extremely rare clinically and in vitro to generate, as a single mutation in one gene is insufficient to confer phenotypic resistance. In some bacterial species, resistance occurs primarily or solely by genetic mutation, such as *Mycobacterium tuberculosis* and *Helicobacter pylori. M. tuberculosis* develops resistance to all anti-tuberculosis agents by mutation, thus the need for multidrug therapy. Similarly, *H. pylori* requires at least two antibiotics and PPI to avoid resistant mutation, as chromosomal mutation is responsible for resistance to clarithromycin (in 23S rRNA), amoxicillin (changes in penicillin-binding protein 1), metronidazole (multiple genes), and tetracycline (in 16S rRNA and other genes) [40]. In addition, many *Enterobacteriaceae* carry chromosomally encoded cephalosporinases resulting in resistance to broadspectrum β-lactam agents. *Ps. aeruginosa* which is intrinsically resistant to many antibiotics, in certain environment (cystic fibrosis, bronchiectasis), is difficult to eradicate because of biofilm existence and mutations that result in overexpression of many intrinsic efflux pumps [42]. In this setting, the pseudomonas represent a hypermutator strain of bacteria, which have increased mutation rates in genes relating to DNA repair and replication constancy [42]. In the *Enterobacteriaceae* members that produce ESBL (150 TEM variants and 90 SHV variants), some variants have undergone mutations which render the enzymes capable of hydrolyzing extended spectrum cephalosporins or able to resist the action of β-lactamase inhibitors [43]. Moreover, mutation is necessary for the development and acquisition of new resistant genes in the TEM family of β-lactamases.

Conjugation, transfer of DNA via cell surface pili or adhesions, is considered the most important means for bacteria to disseminate antibiotic-resistant genes usually utilizing transposons, integrons, and plasmids. The worldwide spread of resistant genes to many drug classes is attributed to the transfer of plasmids in pathogens with antibiotic-resistant genes encoding resistance to β-lactams, tetracyclines, sulfonamides, quinolones, aminoglycosides, and many others [44]. The increasing reports of pathogens harboring plasmids for carbapenem resistance and the spread of plasmid-encoding colistin resistance to many continents are of major concern [45]. Plasmids are equivalent to a carriage basket, as multiple antibiotic-resistant genes can be co-localized on the same plasmid allowing for the spread of multidrug resistance. The spread of pan-resistant *Enterobacteriaceae* is now a reality.

The horizontal spread of resistance genes by transformation and transduction are considered less important than conjugation in dissemination, but understanding all the means of gene transfer to pathogens and mechanisms of spread of antibiotic resistance is necessary for their control. Transformation is the process by which certain bacteria (first demonstrated with *S. pneumoniae*) are capable of uptake, integration, and functional expression of naked fragments of extracellular DNA [46]. Bacteria could use this mechanism to evade antibiotics by exchanging resistant genes. Intra- and inter-species of DNA could be transferred by this means under certain conditions: presence of extracellular DNA in the environment; the recipient bacteria must be in a state of competence; and the translocated DNA must be stabilized into the recipient genome. Exposure to antibiotics can induce competence in many species of bacteria and enhance transformation of resistant genes. In addition,

it has been shown that natural transformation facilitates the transfer of transposons, integrons, and gene cassettes (which may contain antibiotic-resistant genes) between bacterial species [47].

Transduction is the process by which bacteriophages transfer genes that are advantageous to the microbial host but also promotes their survival and dissemination. The genetic materials that can be transferred include chromosomal DNA, plasmids, transposons, and genomic islands [48]. Bacteriophages have been documented to transfer antibiotic resistance genes for many antibiotics (erythromycin, tetracycline, β-lactams, and aminoglycoside) by various bacterial species: streptococci, enterococci, *E. coli*, *Salmonella*, and *S. aureus* (MRSA) [46]. It is now evident from recent studies using metagenomic methods on various environmental samples (including wastewater from hospitals), patient samples (feces, respiratory secretion), and animals (feces, meat) that bacteriophages are significant reservoirs of many antibiotic resistant genes and are capable of transducing resistance genes to diversified bacterial communities.

## 1.5   The Toll of Antimicrobial Resistance

AMR presents an urgent threat to the public health systems of the world. The consequences can be measured by its effects on human lives and function, the healthcare system, and the economic burden. With respect to patient outcome, there is increasing evidence that AMR infections increase the mortality and morbidity of affected subjects. Compared to patients with infection due to non-resistant bacteria, those infected with AMR bacteria have double the risk of serious complication and triple the risk of death [49]. Presently, it is estimated that about 700,000 people lose their lives as a result of AMR infection worldwide each year [49], which may escalate to ten million by 2050 (see Table 1.3). In the United States (US) in 2013, the Centers for Disease Control and Prevention (CDC) estimated that two million persons were infected with AMR pathogens each year and 23,000 patients died as result annually [50]. About two thirds of those deaths were associated with infections caused by multidrug-resistant (MDR) pathogens. MRSA alone appears to be

**Table 1.3** Projected mortality rates for various conditions by 2050 worldwide

| Conditions | Deaths annually |
|---|---|
| Cancer | 8.2 million |
| Cholera | 100,000–120,000 |
| Diabetes | 1.5 million |
| Diarrheal disease | 1.4 million |
| Measles | 130,000 |
| Tetanus | 60,000 |
| Road traffic accidents | 1.2 million |
| Antimicrobial resistance | 10 million (presently 700,000) |

causing nearly 50,000 deaths yearly in the US and Europe combined [49]. Recently, the trend of MDR bacterial infections in US hospitalized patients from 2012 to 2017 has been reported by the CDC [51]. These patients accounted for 41.6 million hospitalization (>20% annually). In 2017, MDR bacteria accounted for 622,390 infections of which (surprisingly) 83% had their onset in the community. Although between 2012 and 2017, the incidence decreased for MRSA, vancomycin-resistant enterococcus (VRE), and MDR *Ps. aeruginosa* infection, the incidence of carbapenem-resistant *Enterobacteriaceae* did not change and the incidence of ESBL infection increased by 53% (mainly from community-onset cases) [51].

MDR-TB is a global security risk and public health crisis. In 2018, the WHO estimated there were 484,000 new cases with rifampin resistance, of which 78% had MDR-TB and 6.2% of these were extensively drug-resistant (XDR-TB) [WHO, Tuberculosis, 2020]. Moreover, only 56% of MDR-TB patients are presently treated successfully. Although in the past decade, there has been a decline in the global incidence of malaria, 228 million cases with 405,000 deaths from the infection were reported in 2018 [WHO, Malaria Report 2019]. The trend of increasing artemisinin resistance *in Plasmodium falciparum* in Southeast Asia (SEA) is causing high treatment failures with artemisinin combination therapy (ACT) in Cambodia, Vietnam, Thailand, Laos, and Myanmar [52]. In addition, there is evidence of increasing *Plasmodium vivax* resistance to chloroquine in SEA, up to 10% in Indonesia [WHO, Malaria Report 2019]. Hence, the prospect of global elimination of malaria in this century appears bleak. The WHO has listed microbes or infections which are of concern for AMR that need close surveillance and coordinated global action (see Fig. 1.2 for priority areas for development of new agents).



**Fig. 1.2** Priority infections for research and development of new antimicrobials. Abbreviations: *AR* artemisinin resistant, *GC* gonorrhea, *MDR* multidrug-resistant, *HIV* human immunodeficiency virus, *NR* neuraminase-resistant, *R & D* research and development, *TB* tuberculosis
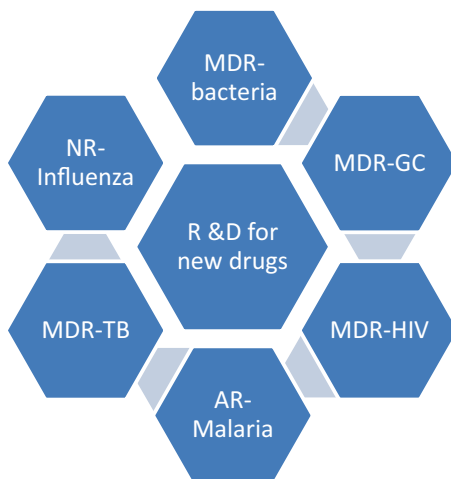
### 1.5.1   Effect on Healthcare

With respect to the healthcare perspective, AMR is having enormous effect on healthcare costs and public health, the burden being felt more in low- and middle-income than high-income countries. Calculation of the cost to healthcare systems is complex and multifaceted including expensive second-line drugs (often with increased side effects), cost of isolation/containment, additional diagnostics, more intensive care (ICU) utilization, cost of surveillance, cancelling of elective surgeries, closure of some units (i.e., dialysis, chemotherapy, etc.), longer hospital stay and turnover, and decreased revenues. The additional cost varies in studies but could be more than $2 billion every year, and by 2050 the annual cost globally has been projected to vary from $300 billion to more than $1 trillion [53, 54]. Just enumerating the economic cost of five drug-resistant pathogens (*S. aureus* [MRSA], *E. coli*, *K. pneumonia*, *A. baumannii*, and *Ps. aeruginosa*), narrowly defined as incremental cost and indirect productivity losses, the annual cost is estimated to be $0.5 billion and $2.9 billion in Thailand and the US, respectively [55]. Despite measures to contain overuse of antibiotics, including antibiotic stewardship programs in hospital in the US, treatment of AMR infections had doubled since 2002, exceeding $2 billion annually [56]. Treatment of a patient with a MDR infection cost the hospital (on average) an additional $10,000–40,000 (US) compared to one with a sensitive organism causing infection. The human toll from AMR infections is quite substantial. In the US alone, antibiotic-resistant bacterial hospital infections result in 99,000 deaths yearly, and by 2050 it is projected that without a solution to the current trend in AMR, up to 444 million people worldwide would die from infections, resulting in rapid decline in birth rates [57].

### 1.5.2   Economic Effect

On a broader economic scale, the World Bank research indicate that AMR is a threat to our future and would increase the rate of poverty with greater impact on low-income countries than others [58]. Previous estimates by CDC from 2013 indicated that AMR cost $55 billion per year in the US, $20 billion for healthcare and nearly $35 billion from loss of productivity [59]. More recent studies show that the annual global gross domestic product (GDP) could shrink by about 1% with loss of 5–7% in developing countries by 2050 ($100–210 trillion) [60–62]. MDR-TB alone could cost the world $16.7 trillion by 2050 according to some estimates [61]. The economic impact of AMR is more complex than shrinkage of the GDP. Labor shortage from sickness and premature deaths is predicted to occur in ten years at the current level of AMR, resulting in decrease in global exports from labor-intensive sectors especially from Eurasia by 2050 (according to the World Bank). The impact of AMR will be realized as well by the livestock industry due to sickness and mortality of food animals, resulting in shortages of meat and dairy products, with persistent trend of AMR producing a 11% loss in livestock by 2050 [62]. The loss in food animal production will affect export and trade with decline in gross national product, decrease employment, average income, and economic stagnation or decline [63].

## 1.6   Global Response to Antimicrobial Resistance

The Global Action Plan on Antimicrobial Resistance was developed in 2015 by the WHO, the Food and Agriculture Organization of the Unite Nations (FAO), and the World Organization for Animal Health (WOAH), recognizing the high level of antimicrobial resistance from inappropriate use of these drugs in humans, animals, food, agriculture, and aquaculture farms. World leaders from 193 countries agreed to address the spread of AMR at a high-level meeting at the 71st UN General Assembly in September 2016 [Global Health, JAMA 2016; 316: 1936]. The "Global action plan on antimicrobial resistance" 5 strategic objectives were to (i) improve awareness and understanding of antimicrobial resistance; (ii) strengthen surveillance and research; (iii) reduce incidence of infection; (iv) optimize the use of antimicrobial drugs; and (v) ensure sustainable investment in countering antimicrobial resistance. To address these issues, the WHO initiated a broad, coordinated approach with the UN Member States to correct the root causes of AMR across multiple sectors, in particular human health, animal health, and agriculture. The WHO provided support to Member States to develop national action plans on AMR based on the global action plan. These initiatives included the following: World Antibiotic Awareness Week (a global, multiyear campaign); the Global AMR and Use Surveillance System (GLASS), the WHO supports a standardized approach to the collection, analysis, and sharing of data related to AMR at a global level to inform decision-making and actuate local, national, and regional action; Global Antibiotic Research and Development Partnership (GARDP), to encourage research and development through public-private partnerships for producing novel and new antimicrobials; and Interagency Coordination Group on AMR (IACG), to improve coordination between international organizations and to ensure effective global action against the threat of AMR [WHO, Antibiotic resistance, February 2018]. Figure 1.3 outlines the key factors in tackling AMR.

What progress has been made since 2016 in tackling drug-resistant infections globally? A recent review of the progress made by the Global Health Program on Antimicrobial Resistance was reported in Oct. 2019 [Charles Clift, Chatham House]. The findings of the review are summarized as follows:

- Little progress has been made in transforming research and development incentives for antibiotics, vaccines, and diagnostics.
- Significant advances in reducing antibiotic use in agriculture in high-income countries, but much more needed to convince low- and middle-income countries [LMICs] to reduce antibiotics in this area.
- Although there has been greater investment in awareness raising, questions remain on the impact and effectiveness in changing behavior.
- Restriction of over-the-counter antibiotics is yet to be implemented in LMICs, which is hampered by poor living conditions and access to healthcare.
- Limitations in LMICs that affect infection control and antibiotic overuse awareness messages are high rates of unhygienic conditions in the community and healthcare facilities.
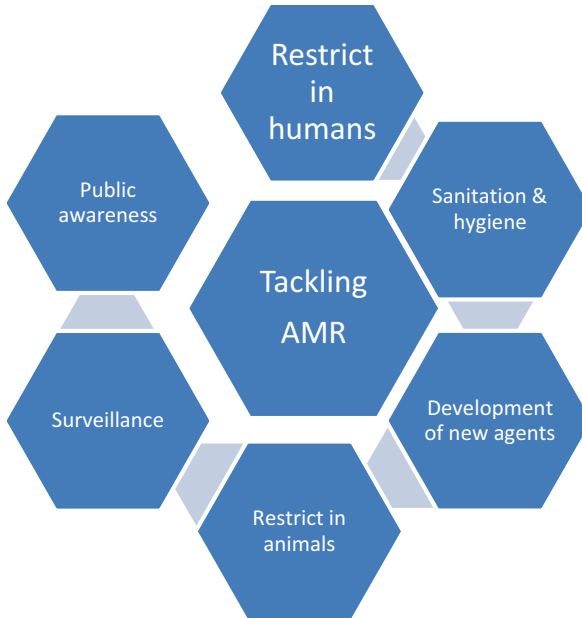
**Fig. 1.3** Outline of the key factors in tackling antimicrobial resistance (AMR). Other elements not shown include vaccines and alternatives, rapid diagnostics, Global Innovation Fund, and International Coalition for Action

- Providing quality healthcare to all and moving toward universal health coverage in LMICs are crucial in addressing the problems of both adequate access to antibiotics and restriction of over-the-counter sales.
- Funding agencies (World Bank, International Monetary Fund [IMF]) and governments should put greater emphasis in investments in providing clean water, sanitation, and housing to reduce the reliance on antibiotics in the long term in LMICs.
- Although investments have been made in improving surveillance of antibiotic use and resistance, particularly for humans, more effort is required to create surveillance systems that provide data sufficiently accurate to influence policy and actions. This applies also to antibiotics and resistant genes circulating in the environment.

## 1.6.1   Comments on Global Response

The approach to tackling the AMR crisis is a difficult one and should be different for LMICs compared to high-income countries where the root causes may be different, but overlap exists. The funding agencies and governments of high-income countries should invest and assist lower-income countries to improve their public health systems and healthcare facilities. Proper housing and clean running water

with proper hygienic conditions are much needed for thousands of communities worldwide, and these measures with basic childhood vaccines (measles, mumps, pertussis, conjugate *S. pneumoniae, H. influenzae,* and rotavirus) could save hundreds of millions of lives and unnecessary antibiotics with reduction of antibiotic-resistant bacteria [64]. Some middle-income countries need assistance in these areas, but they more likely need incentives to decrease environmental pollution with antimicrobials (China, India, etc.) and discourage the use of antibiotics in farming and agriculture (China, India, Brazil, and Kenya).

The forces driving AMR in high-income countries are different and are two-fold, healthcare associated and animal health and farming associated, including aquacare. The use of antibiotics in healthcare are in outpatient settings, nursing homes, and hospitals, and the reasons for overuse and means of control may be different. The vast majority of antibiotic use occurs in the outpatient setting ($\approx$80%) and > 250 million outpatient prescriptions are written every year in the US [65]. These outpatient settings include clinics, doctors' offices, dental offices, and emergency rooms, and the CDC estimates that 30% of all antibiotics prescribed in the outpatient clinics are unnecessary [CDC, 2017, Antibiotic use in the United States]. However, overprescribing of antibiotics may be even worse than this estimate. In a recent unpublished study presented at IDWeek 2018, October 5 [Infectious Diseases Society of America] [JA Linder], of >500,000 antibiotic prescriptions, nearly half of the time antibiotics were prescribed without an infection-related diagnosis and one in five prescriptions were provided without an in-person visit. Antibiotic stewardship programs for outpatient management may be of value for ERs and outpatient clinics but would be difficult to implement for doctors' offices outside hospitals, especially primary care physicians. Most inappropriate outpatient antibiotic prescribing is for viral respiratory infections, i.e., viral bronchitis, otitis, and sinusitis, and secondly for unnecessary broad-spectrum agents. In a recent study, clinical education on antibiotic use resulted in >50% reduction in inappropriate antibiotic prescribing for acute respiratory infections at 1 year, but behavior interventions did not have sustained effect except for monthly emails with peer comparison practices [66].

Patients in nursing homes are commonly prescribed antibiotics for urinary tract, respiratory tract, and skin and soft tissue (pressure ulcers) infections. Some studies have reported that two thirds of patients in nursing homes are prescribed antibiotics each year and up to 75% may be inappropriate [67, 68]. In a small study of 9 nursing homes, the CDC found 11% of residents were receiving antibiotics and 40% lacked prescribing information [CDC, 2017]. Hence, a larger study was implemented.

Antibiotic overuse and overprescribing in acute care hospitals were recognized as major factors leading to multiresistant bacterial infections associated with hospital-acquired infections, resulting in increased demand for broader, more expensive, or more toxic agents which propagated the spiral or vicious circle of greater microbial resistance. The CDC estimated that 70% of the two million infections acquired in US hospitals each year are resistant to at least one commonly used antibiotic, and 20–50% of antibiotics prescribed in acute care hospitals are unnecessary or inappropriate [69]. Antibiotic stewardship programs were introduced in hospitals just over 30 years ago to improve inappropriate antibiotic prescribing and decrease antibiotic resistance rates [70, 71]. These programs are now widely

implemented in hospitals of Europe and North America, and recent reviews of randomized and non-randomized studies have confirmed their value [72, 73]. Two types of antibiotic prescribing interventions are usually employed: restrictive, limit on which antibiotics can be prescribed, and enablement technique, education, verbal and written reminders, evaluation, audit, and feedback of individual physicians prescribing habits, but sometimes the combination of the two interventions. Antibiotic overuse or inappropriate use are usually considered when not clinically indicated, use of broad-spectrum when narrow-spectrum agents are more suitable, failure to transition from parenteral to oral therapy, and excessive duration than necessary. In a recent multihospital cohort study from Michigan, assessing excessive duration of antibiotic for pneumonia in hospitalized patients, two thirds (67.8%) were prescribed excess antibiotic therapy (mainly prescribed at discharge) and each excess day of treatment was associated with 5% increase in adverse events [74].

Even in industrialized countries, restriction of antimicrobials in agriculture and farming for growth promotion or prophylaxis is not uniform. The European Union ban antimicrobial growth promoters in 2006, while Australia and New Zealand instituted partial ban, and the US restraint is voluntary [75]. In 2014, the Canadian government restricted the use of growth-promoting antibiotics in livestock, but farmers were able to bypass this ban by importing and stocking large supplies of antimicrobials without a prescription. This loophole was closed in December 2018 when farmers were required to obtain a veterinary prescription for antibiotics in livestock [Nicole Williams/Canadian Broadcasting Corporation]. Hence, in high-income countries, much improvement is still needed to limit the overuse of antimicrobials in humans and farm animals.
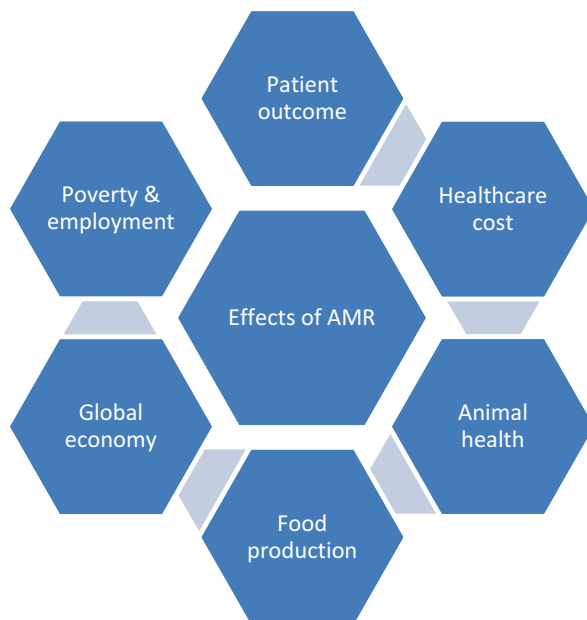
# References

1. D'Costa VM, King CE, Kalan L, et al. Antibiotic resistance is ancient. Nature. 2011;477:457–61.
2. Klein EY, Van Boeckel TP, Martinez EM, et al. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proc Natl Acad Sci USA. 2018;115:E3463–70.
3. Van Boeckel TP, Browser C, Gilbert M, et al. Global trends in antimicrobial use in food animal. Proc Natl Acad Sci U S A. 2015;112:5649–54.
4. Food and Agriculture Organization of the United Nation. (2017). http://www.fao.org/faostat/en/#home
5. Van Boeckel TP, Pires J, Silvester R, et al. Global trends in antimicrobial resistance in animals in low-and middle-income countries. Science. 2019;365:eaaw1944.
6. European Food Safety Authority and European Centre for Disease Prevention and Control. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2016. EFSA J. 2018; https://doi.org/10.2903/j.efsa.2018.5182.
7. Ward MJ, et al. Time-scaled evolutionary analysis of the transmission and antibiotic resistance dynamics of *Staphylococcus aureus* clonal complex 398. Appl Environ Microbiol. 2014;80:7275–82.
8. De Vries SPW, et al. Phylogenetic analysis and antimicrobial resistance profiles of *Campylobacte*r spp. from diarrheal patients and chickens in Botswana. PLoS One. 2018;13:e0194481.
9. Liu YY, et al. Emergence of plasmid –mediated colistin resistance mechanisms MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infecty Dis. 2016;16:161–8.
10. Tang KL, Caffrey NP, Nobrega DB, et al. Restricting the use of antibiotics in food-producing animals and its association with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. Lancet Planet Health. 2017;1:e316–27.
11. Van Bunnik B, Woolhouse M. Modeling the impact of curtailing antibiotic usage in food animals on antibiotic resistance in humans. R Soc Open Sci. 2017;4:161067.
12. Forsman M, Haggerstrom B, Lindgren L, Jaurin B. Molecular analysis of β-lactamases from four species of *Streptomyces:* comparison of amino acid sequences with those of other β-lactamases. Microbiology. 1990;136:589–98.
13. Ogawara H, Kawamura N, Kudo T, Suzuki K-L, Nakase T. Distribution of β-lactamases in actinomycetes. Antimicrob Agents Chemother. 1999;43:3014–7.
14. Livermore D, Canton R, Gniadkowski M, et al. CXT-M changing the face of ESBLs in Europe. J Antimicrob Chemother. 2007;59:165–74.
15. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Molecular Biol Rev. 2010;74:417–33.
16. Liu B, Pop M. ARDB—antibiotic resistance genes database. Nucleic Acid Res. 2009;37:D443–7.
17. Forsberg KJ, Patel S, Gibson MK, Lauber CL, Knight R, Fierer N, Daniels G. Bacterial phylogeny structure soil r5esistomes across habitats. Nature. 2014;509:612–6.
18. Etienne R, Ghozlane A, Tap J, et al. Prediction of the intestinal resistome by a three-dimensional structure-based method. Nat Microbiol. 2018; https://doi.org/10.1038/s41564-018-0292-6.
19. Willems RPJ, van Dijk K, Ket JCF, Vanderdroucke-Grauls CMJE. Evaluation of the association between gastric acid suppression and risk of intestinal colonization with multi-resistant microorganisms. A systematic review and meta-analysis. JAMA Intern Med. 2020; https://doi.org/10.1001/jamaiternmed.2020.0009.
20. Kapoor G, Saigal S, Elongavan A. Action and resistance mechanisms of antibiotics: a guide for clinicians. J Anaesthesiol Clin Pharmacol. 2017;33:300–5.
21. Eyal Z, Matzov D, Krupkin M, et al. A novel pleuromutilin antibacterial compound, its binding mode and selectivity mechanisms. Sci Rep. 2016;6:1–8.

22. Zaman SB, Hussain MA, Nye R, Mehta V, Mamun KT, Hossain N. A review on antibiotic resistance: alarm bells are ringing. Cureus. 2017;9:e1403.

23. Lambert PA. Cellular impermeability and uptake of biocides and antibiotics in gram-positive bacteria and mycobacteria. J Appl Microbiol. 2002;92(Suppl):46S. 54S.

24. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. Am J Med. 2006;119(6 Suppl 1):S3–10.

25. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of methicillin-resistant *Staphylococcus aureus* as a public health threat. Lancet. 2006;368:874–85.

26. Giedraitiene A, Vikauskiene A, Naginiene R, Pavilonis A. Antibiotic resistance mechanisms of clinically important bacteria. Medicina (Kaunas). 2011;47:137–46.

27. Higgins PG, Fluit AC, Schmitz FJ. Fluoroquinolones: structure and target sites. Curr Drug Targets. 2003;4:181–90.

28. Peterson E, Kaur P. Antibiotic resistance mechanisms in bacteria: relationship between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. Front Microbiol. 2018; https://doi.org/10.3389/fmicb.2018.02928.

29. Mojica MF, Bonomo RA, Fasi W. B1-metallo-beta lactamases: where do we stand? Curr Drug Targets. 2016;17:1029–50.

30. Perez F, Endimiani A, Hujer KM, Bonomo RA. The continuing challenge of ESBLs. Curr Opin Pharmacol. 2007;7:459–69.

31. Wright GD. Q & A: antibiotic resistance: where does it come from and what can we do about it? BMC Biol. 2010;8:123.

32. McMurry L, Petrucci RE Jr, Levy SB. Active efflux of tetracycline encoded by four genetically different tetracycline resistance determinants *in Escherichia coli*. Proc Natl Acad Sci USA. 1980;77:3974–7.

33. Nikaido H, Takatsuka Y. Mechanisms of RND multidrug efflux pumps. Biochem Biophys Acta. 2009;1794:769–81.

34. Van Bambeke F, Michot JM, Tulkens PM. Antibiotic efflux pumps in eukaryotic cells: occurrence and impact on antibiotic cellular pharmacokinetics, pharmacodynamics and toxicodynamics. J Antimicrob Chemother. 2003;51:1067–77.

35. Blanco P, Hernando-Amado S, Reales-Calderon J, et al. Bacterial multidrug efflux pumps: much more than antibiotic resistance determinants. Microorganisms. 2016;4:14. https://doi.org/10.3390/microorganisms4010014.

36. Fernandez L, Breidenstein EB, Hancock RE. Creping baselines and adaptive resistance to antibiotics. Drug Resis Updates. 2011;14:1–21.

37. Anderson D, Hughes D. Selection and transmission of antibiotic resistant bacteria. Microbiol Spectr. 2017;5:1–17.

38. D'Costa VM, McGrann KM, Hughes DW, Wright GD. Sampling the antibiotic resistome. Science. 2006;311:374–7.

39. Dantas G, Summer MOA, Oluwasegun RD, Church GM. Bacteria subsisting on antibiotic. Science. 2008;320:100–3.

40. Woodford N, Ellington MJ. The emergence of antibiotic resistant by mutation. Clin Microbiol Infect. 2007;13:5–18.

41. Schrag SJ, Perrot V, Levin BR. Adaptation to the fitness costs of antibiotic resistance in *Escherichia coli*. Proc R Soc Lond B Biol Sci. 1997;264:1287–91.

42. Poole K. Multidrug efflux pumps and antimicrobial resistance in *Pseudomonas aeruginosa* and related organisms. J Med Microbiol Biotechnol. 2001;3:255–64.

43. Heritage J, M'Zali FH, Gascoyne-Binzi D, HawKey PM. Evolution and spread of SHV extended-spectrum β-lactamases in gram-negative bacteria. J Antimicrob Chemother. 1999;44:309–18.

44. Huddleston JR. Horizontal gene transfer in the human gastrointestinal tract: potential spread of antibiotic genes. Infect Drug Resis. 2014;7:167–76.

45. Arcilla MS, van Hattem JM, Matamoros S, et al. Dissemination of themer-1colistin resistance gene. Lancet Infect Dis. 2015;16:147–9.

46. von Wintersdorff CJH, Penders J, van Niekerk JM, et al. Dissemination of antimicrobial resistance in microbial ecosystem through horizontal gene transfer. Front Microbiol. 2016; https://doi.org/10.3389/fmicb.2016.00173.

47. Domingues S, Harms K, Fricke WF, Johnsen PJ, da Silva GJ, Nielsen KM. Natural transformation facilitates transfer of transposons, integrons and gene cassettes between bacterial species. PLoS Pathog. 2012;8:e1002837. https://doi.org/10.1371/journal.ppat.1002837.

48. Friedman ND, Temkin E, Carmeli Y. The negative impact of antibiotic resistance. Clin Microbiol Infect. 2016;22:416–22.

49. O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. Review Antimicrob. Resistance 2016; https://amr-review.org/. Accessed 17 Apr 2020.

50. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States. 2013; https://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508-pdf

51. Jernigan JA, Kelly M, Hatfield MSPH, et al. Multidrug–resistant bacterial infections in US hospitalized patients, 2012–2017. N Engl J Med. 2020;382:1309–19.

52. Antony HA, Parija SC. Antimalarial drug resistance: an overview. Trop Parasitol. 2016;6:30–41.

53. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health. 2015;109:309.

54. Chokshi A, Sifri Z, Cennimo D, Horng H. Global contributors to antibiotic resistance. J Glob Infect Dis. 2019;11:36–42.

55. Shrestha P, Cooper BS, Coast J, et al. Enumerating the economic cost of antimicrobial resistance per antibiotic consumed to inform the evaluation of interventions affecting their use. Antimicrob Resist Infect Control. 2018;7:98. https://doi.org/10.1186/s13756-018-0384-3.

56. Thorpe KE, Joski P, Johnston KJ. Antibiotic-resistant infection treatment costs have doubled since 2002, now exceeding $2 billion annually. Health Aff. 2018;37:662–9.

57. Asiam B, Wang W, Arshad MI, et al. Antibiotic resistance: a rundown of a global crisis. Infect Drug Resist. 2018;11:1645–58.

58. Drug-resistant infections a threat to our economic future; 2017. Available from: www.worldbank.org. Accessed 21 Apr 2020.

59. Antibiotic resistance threats in the United States, 2013. https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf. Accessed 21 Apr 2020.

60. Anderson M, Clift C, Schulze K, et al. Health systems and policy analysis- averting the AMR crisis: what avenues for policy. Eur Obs Heal Sys Policies. 2019; [Google Scholar]

61. Utt E, Wells C. The global response to the threat of antimicrobial resistance and the important role of vaccines. Pharm Policy Law. 2016;18:179–97. https://doi.org/10.3233/PPL-160442.

62. Dadgostar P. Antimicrobial resistance: implications and costs. Infect Drug Resist. 2019;12:3903–10.

63. Animal production I antimicrobial resistance I food and agriculture organization of the United Nations. http://www.fao.org/antimicrobial-resistance/key-sectors/animal-production/en/

64. Buchy P, Ascioglu S, Buisson Y, et al. Impact of vaccines on antimicrobial resistance. Int J Infect Dis. 2020;90:188–96.

65. Hicks LA, Bartoces MG, Roberts RM, et al. US outpatient antibiotic prescribing variation according to geography, patient population, and provider speciality in 2011. Clin Infect Dis. 2015;60:1308–16.

66. Linder JA, Meeker D, Fox CR, Friedberg MW, Persell SD, Goldstein NJ, Doctor JN. Effects of behavioral interventions on inappropriate antibiotic prescribing in primary care 12 months after stopping the interventions. JAMA. 2017;318:1391.

67. Daneman N, Gruneir A, Newman A, et al. Antibiotic use in long-term care facilities. J Antimicrob Chemother. 2011;66:2856–63.

68. Rhee SM, Stone ND. Antimicrobial stewardship in long-term care facilities. Infect Dis Clin N Am. 2014;28:237–46.

69. US Food and Drug Administration. Battle of the bugs: fighting the antibiotic resistance. Updated May 4, 2016.; https://www.fda.gov//drugs/resourcesforyou/consumers/ucm43568.htm

70. Briceland LL, Nightingale CH, Quintiliani R, et al. Antibiotic streamlining from combination therapy to monotherapy utilizing an interdisciplinary approach. Arch Intern Med. 1988;148:2019–22.
71. Owens RC. Antimicrobial stewardship: concepts and strategies in the 21st century. Diag Microbiol Infect Dis. 2008;61:110–28.
72. Davey P, Marwick CA, Scott CL, et al. Interventions to improve antibiotic practices for hospital inpatients. Cochrane Database Syst Rev. 2017;2:CD003543. https://doi.org/10.1002/14651858. CD003543.pub4.
73. Nathwani D, Varghese D, Stephens J, Ansari W, Martin S, Charbonneau C. Value of hospital antimicrobial stewardship programs [ASPs]: a systemic review. Antimicrob Resist Infect Control. 2019;8:35. https://doi.org/10.1186/s13756-019-0471-0.
74. Vaughn VM, Flanders SA, Snyder A, et al. Excess antibiotic treatment duration and adverse events in patients hospitalized with pneumonia. Ann Intern Med. 2019;171:153–63.
75. Manyi-Loh C, Mamphwell S, Meyer E, Okoh A. Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. Molecules. 2018;23:795. https://doi.org/10.3390/molecules23040795.