Chapter 2
Nano-antimicrobials: A Viable Approach to Tackle Multidrug-Resistant Pathogens

Bushra Jamil and Muhammad Ali Syed

Abstract Escalating resistance to almost every class of antibiotics is reducing the utility of currently available antimicrobial drugs. A part of this menace is attributed to poor pharmacokinetics and pharmacodynamics of the drug. Improvement in drug delivery is the most challenging task encountered by the pharmaceutical industries; however, nanotechnology can bring a revolution in drug design and delivery. Nano-antimicrobials have their own intrinsic antimicrobial activity (nanoparticles) or augment overall efficacy of enclosed antibiotics (nano-carriers), thus contribute in mitigating or reversing the resistance phenomenon. Nanoparticles (NP) having their own intrinsic antimicrobial activity kill microbes by mimicking natural course of killing by phagocytic cells, i.e., by producing large quantity of reactive oxygen species (ROS) and reactive nitrogen species (RNS). It is believed that NPs kill microbes by simultaneously acting on many essential life processes or metabolic routes of microbes; that as many genetic mutations to develop resistance against them seems to be impossible. Nano-carriers improve the pharmacokinetics of the enclosed drug. Moreover, one of the major techniques by which NAMs can overcome resistance is targeted drug delivery to the site of disease. In this chapter, a comprehensive detail about the mechanism of action of NAMs is presented in context to multidrug-resistance phenomenon.

Keywords Nano-antimicrobial · Nano-carriers · Multi drug resistance
Targeted drug delivery · Extended Spectrum Beta Lactamases (ESBL)

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2.1 Introduction

Antibiotics have brought a revolution to the history of modern medicine and have played a fundamental role in ensuring safe surgical procedures, organ transplants, and chemotherapy (Fabbretti et al. 2011). Due to antibiotics, both the mortality and morbidity rates have significantly declined as the infectious diseases were always considered as the principal cause of death. However, antibiotic's meteoric rise proved to be short lived, because of rapid emergence of resistance to almost every class of antibiotics. World Health Organization (WHO) has already declared antibiotic resistance among the three paramount threats to health. The theme of World Health Day, on April 7, 2011, was "antimicrobial resistance: no action today and no cure tomorrow" (Piddock 2012).

Antimicrobial resistance (AMR) is the ability of a microorganism (like bacteria, viruses, and some parasites) to stop an antimicrobial (such as antibiotics, antivirals, and antimalarials) from working against it. As a result, standard treatments become ineffective, infections persist and may spread to others (http://www.who.int/antimicrobial-resistance/en/). Such multidrug-resistant (MDR) microbes make the treatment more difficult, expensive, and with more side effects. All those diseases that were under control are causing difficulty in their treatment after the advent of MDR bacteria (Alanis 2005; Hajipour et al. 2012; Al-Assil et al. 2013). Because now the higher dose and potent antibiotics are needed to cure them.

The situation continues to be more alarming due to meager efforts put into develop new drugs (Wright 2012). Since 2000, almost 22 new antibiotics had been developed to overcome MDR phenomenon (Butler et al. 2013). Yet, antibiotic resistance still persists. The major contributors to this menace are increased difficulty in isolating novel antibiotics, prolonged development time, immense clinical trials cost, "merger mania" in the industry, and most importantly the emergence of resistance against newly developed compounds.

WHO has placed three pathogens in the newly revised list (2017) of critical priority pathogens. It includes carbapenem-resistant pathogens, i.e., *Acinetobacter baumannii, Pseudomonas aeruginosa,* and all other enterobacteriaceae that display resistance against carbapenems. WHO has urged the need to develop new antibiotics against these three pathogens on priority basis (http://www.who.int/mediacentre/news/releases/2017/bacteria-antibiotics-needed/en/).

Almost a decade back, it was the resistance in Gram-positive microbes that was posing the threat. Nonetheless, with the implementation of control policies and through the development of new medicines, it is considered to be under good control now (Kumarasamy et al. 2010). However, though the situation against Gram-positive microbes has improved the same against Gram-negative has exacerbated to an extent that clinical microbiologists reached to the consensus that multidrug-resistant Gram-negative bacteria are the existent threat to public health. There are many whys and wherefores that can be attributed to this menace (Jamil 2014).

Gram-negative bacilli (GNB) include large number of clinically significant pathogens: including both enterobacteriaceae and non-fermenting GNB

(*P. aeruginosa, A. baumannii,* and *Stenotrophomonas maltophilia*) (Ruppé et al. 2015). Only the enterobacteriaceae comprises of more than 70 genera, it constitutes normal flora of GIT and is the principal causative agents of gastrointestinal (GI) infections. Clinically, significant pathogens belonging to this family are *Escherichia coli (E. coli), Klebsiella species, Salmonella, shigella,* and *Enterobacter* species (Pickering 2004; Paterson 2006; Lupo et al. 2013). Enterobacteriaceae spread easily by hand carriage besides contaminated food and water. It has genetic plasticity that reveals tendency to acquire genetic material through horizontal gene transfer, mediated mostly by plasmids and transposons. This combination is why emerging multidrug resistance in enterobacteriaceae is of the utmost importance for clinical therapy (Paterson 2006).

It was considered to be causative agent of easily curable ailments; however, past several decades have observed the spread of enterobacteriaceae with resistance to broad-spectrum antimicrobials. Especially, the emergence of carbapenem-resistant enterobacteriaceae (CRE) has invalidated almost all the available therapies. It has created the havoc that once easily treatable infections like diarrhea may become untreatable or very difficult to be managed. KPC (*Klebsiella pneumoniae* carbapenemase) and NDM (New Delhi metallo-beta-lactamase) are the recently discovered types of CRE. KPC and NDM are the enzymes that break down carbapenems and make them ineffective (Talukdar et al. 2013; Bologa et al. 2013; Jamil 2014).

It is evident that enterobacteriaceae has developed resistance against Carbapenem, which is considered to be the drug of last resort. Second reason why resistance in enterobacteriaceae is considered to be more notorious is that the rate of spread of resistance; being very fast in Gram-negative than the same in Gram-positive bacteria, the resistance genes in enterobacteriaceae are found on mobile genetic elements that can readily disseminate resistance through horizontal gene transfer to unrelated bacterial populations (Wellington et al. 2013). Moreover, unprecedented human air travel is also considered to be a cause of dissemination of resistance between countries and continents. Thirdly, there is currently no new antibiotic in the developmental pipeline that can specifically target Gram-negative microbes though many against Gram-positive are in the way (Butler et al. 2013).

The aim of this chapter was to discuss the various resistance mechanisms in bacteria and how they can be addressed to prevent the development of further resistance enzymes and dissemination of resistance phenomenon.

2.2 Multidrug-Resistance Prevalence

Multidrug-resistant phenomenon is displaying an escalating trend and represents a great challenge to the health care system. The situation is particularly more drastic for "ESKAPE" pathogens including *Enterococcus* spp., *Staphylococcus aureus, Klebsiella* spp., *A. baumannii, P. aeruginosa,* and *Enterobacter* spp. A few of these pathogens like *A. baumannii* has already become pan-resistant (Lewis 2013). Exact data on MDR prevalence is not available from many countries, particularly from

developing countries. Nonetheless, few studies have reported more than 80% prevalence of ESBL positive *E. coli* (Nahid et al. 2013; Shakya et al. 2017), whereas 32.5% prevalence of bla NDM-1 was observed in *K. pneumoniae* by Dadashi et al. (2017).

2.3 Dissemination of Resistance

The mixing of susceptible to resistant microbes is an important cause of spread of resistance genes. The resistance genes are mostly located on mobile genetic elements, and they can easily transfer the resistance genes between unrelated species upon contact with each other. The most important and significant spot for the transfer of genetic elements is the gut where all types of microbes mix and interact with each other. Consumption of multiple antibiotics is also a very important factor to induce resistance in microbes (Wellington et al. 2013). Another important factor for spread of antibiotic resistance is the use of polluted water for irrigating the crops. As healthy individuals consume these contaminated crops, they acquire resistance genes and further spread it to whole food chain. Numerous studies have already reported the presence of resistance pathogens in potable water, soil, and environment (Wellington et al. 2013; Wang and Sun 2014; Fernando et al. 2016).

Not only the gut of humans and animals is the reservoir for exchange of genetic materials but in fact, many environmental sites like waste management sites and drainage sites also provide ideal environment for exchange of genetic materials and act as hot spots for horizontal gene transfer (Laxminarayan et al. 2013). The situation is more gruesome because of the very fact that the resistant genes in enterobacteriaceae are usually associated with mobile genetic elements such as transposons and integrons on large plasmids; therefore, they easily acquire and spread resistance phenotypes (Nordmann et al. 2012). Likewise, they also have the tendency to accumulate resistance genes from other species as well, and then these multiple genes of resistance persist for years in them.

In addition, many antibiotics particularly, some synthetic antibiotics are not easily biodegradable and can persist in soils for many years and microbes residing there develop resistance against them naturally (Wellington et al. 2013).

2.4 Resistance Mechanisms in Bacteria

There are numerous ways by which bacteria can acquire resistance to antibacterial agents (Fig. 2.1). However, to comprehend these mechanisms and more importantly, to control resistance, it is imperative to understand the basic chemistry, physiology, and structure of bacteria.

At the very onset, all the Gram-negative pathogens carry an outer membrane and are displaying more complex structure in comparison with Gram-positive

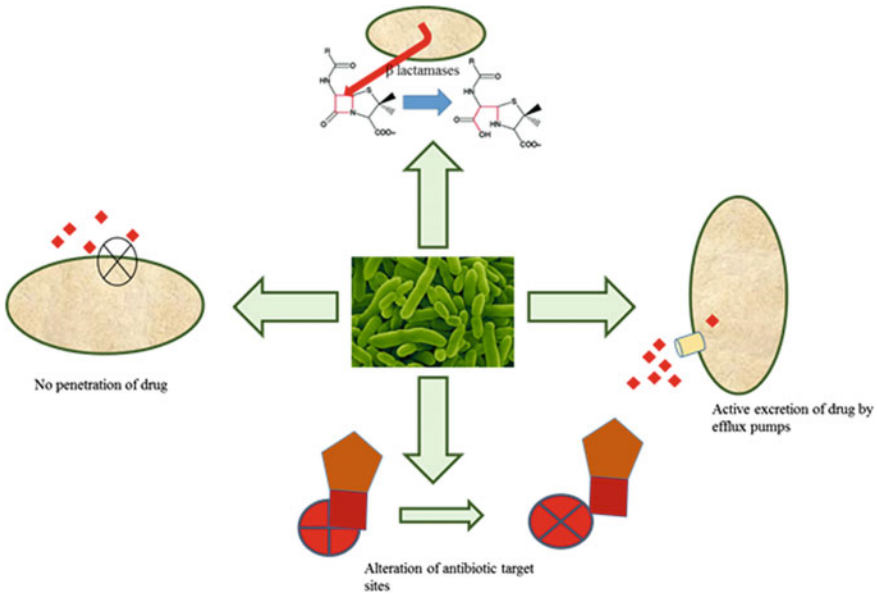


Fig. 2.1 Mechanisms of antibiotic resistance

pathogens. This outer membrane is rich in lipopolysaccharides (LPS). Lipopolysaccharides (LPS) not only gives negative charge but also act as permeability barrier to transport of materials, nutrients, drugs, and chemicals (Bolla et al. 2011). In this section, we will discuss various resistance mechanisms in detail.

2.4.1 Porins Modifications

In bacteria, intra- and extracellular drug concentration is maintained primarily by porins and efflux transporters. Hydrophobic compounds can cross membranes more easily. Their transportation across the membrane is depending upon concentration gradient. Conversely, hydrophilic molecules can only traverse via porins. Lipopolysaccharides (LPS) in Gram-negative pathogens is offering a permeability barrier to them.

Consequently, porin modifications are a significant resistance mechanism adopted by various Gram-negative microbes (Bolla et al. 2011; Iyer et al. 2017; Phan and Ferenci 2017; Shuvo et al. 2017).

2.4.2 Over-Expression of Efflux Pumps

Efflux pumps are the concentration-dependent proteinaceous pumps that can extrude drugs actively from inside the microbes. There are five classes of these

active transports, and antibiotics are their most significant substrate. If these efflux pumps are over expressed, then they can actively pumped out antibiotics and thus are major source of resistance in enterobacteriaceae (Pages and Amaral 2009). In Gram-negative microbes, down regulation of porins along with over-expression of efflux pump establishes as a major source of defense against all bactericidal and bacteriostatic agents. The genetic elements responsible for regulation of efflux pumps may be located on both plasmids and chromosomes. Thus, the resistant may be intrinsic or acquired.

2.4.3 Modification of Antibiotic Target Sites

Third important mechanism of resistance in Gram-negative microbes is the alteration in antibiotic binding site. Most of the β-lactam antibiotics inhibit bacterial cells by inhibiting the process of cell wall synthesis. They actually bind with Penicillin-binding proteins (PBPs) that are crucial for cross-linking the developing peptidoglycan layers. As they occupy PBP, the process of cell wall synthesis gets stop. Alteration in target site, i.e., PBP will end up in losing the β-lactam to bind with PBPs. Many antibiotics also target ribosome as binding site. The antibiotic binding sites are usually clustered at functional centers of the ribosome and the antibiotic binding interfere with the normal protein synthesis. Nature has evolved an effective and elegant way of preventing drug binding to the ribosome by simply adding methyl groups to rRNA at appropriate sites. Strangely, methylation is the only type of RNA modification found to date that provides acquired antibiotic resistance (Vester and Long 2013).

Antibiotic resistance is also conferred by RNA methyltransferases. These MTases act at RNA placed near the binding site of the antibiotic to which they confer resistance.

2.4.4 Beta-Lactamase Production

Beta-lactamases (β-lactamases) are the enzymes produced by microbes that have the capacity to cleave a four-membered ring present in beta-lactam (β-lactam) antibiotics. This four-membered ring is an active moiety in the structure of beta-lactam antibiotics. Once it is cleaved, the whole antibiotic is ineffective.

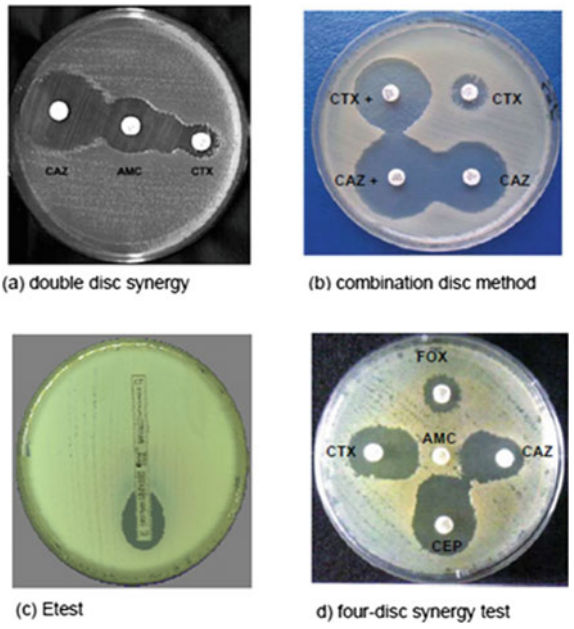
Bacteria have developed a large variety of β-lactamases like penicillinases that cleave penicillin, cephalosporinases that hydrolyses cephalosporin antibiotics, extended-spectrum beta-lactamases (ESBL), carbenicillinases, oxacillinases, carbapenemases etc., that are classified according to two schemes of classifications viz. Bush-Jacoby-Medeiros classification system and Ambler classification (Bush and Jacoby 2010).

All those enzymes that have the capacity to hydrolyze penicillins (ampicillin and piperacillin), third- and fourth-generation cephalosporins and monobactams (aztreonam), are termed ESBL. Cephamycins (i.e., cefoxitin and cefotetan) and carbapenems (i.e., imipenem, meropenem, doripenem, and ertapenem) are susceptible to their action. ESBL enzymes are readily inhibited by lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam.

As ESBLs are inhibited by beta-lactamase inhibitors, this unique property serves as an important phenotypic test that is conveniently exploited to identify ESBLs in bacteria (Perez et al. 2007) (Fig. 2.2).

The majority of ESBLs belongs to the class A (TEM, SHV, CTX-M) and D (OXA-type ESBLs) in Ambler classification and from groups 2be (TEM, SHV, CTX-M) and 2d (OXA-type ESBLs) in Bush's classification (Pitout and Laupland 2008). Pertinent to mention is that OXA-type ESBLs have been found mainly in *P. aeruginosa* and only rarely in enterobacteriaceae (Paterson 2006).

ESBL genes (blaESBL) are mostly encoded by large plasmids (up to 100 kb and even more) that are transferable from strain to strain and between different bacterial species through conjugation; these are not chromosomally mediated (Stürenburg and Mack 2003). Thus, ESBL genes can be transmitted between different Gram-negative bacteria. In addition, their association with mobile genetic elements (e.g., integrons and transposons) facilitates dissemination of resistance pattern many folds. This is not the only resistance gene presents on plasmid rather the situation is more gruesome because of the very fact that ESBL –producing bacteria show cross-resistance to other classes of antibiotics such as aminoglycosides, fluoroquinolones, cotrimoxazole, and tetracycline (Pitout and Laupland 2008). There are



(a) double disc synergy (b) combination disc method

(c) Etest d) four-disc synergy test

Fig. 2.2 Detection of extended-spectrum β-lactamases

more than 700 types of ESBLs that have been detected all over the world (Al-Assil et al. 2013).

Carbapenemases are β-lactamases able to hydrolyze β-lactam antibiotics, including carbapenems and cephamycins which are not hydrolyzed by ESBL. However, they cannot catalyze the hydrolysis of Aztreonam. These are termed metallo-β-lactamases because metal ion is necessary for their activity. They are inhibited by metal chelators, for instance, EDTA but not by beta-lactamase inhibitors.

Pertinent to mention here is that carbapenems (imipenem, ertapenem, meropenem, and doripenem) are the latest developed molecules that possess the broadest spectrum of activity and are considered to be as last resort therapy. CRE has become notorious and deadly with a death toll up to 50%.

Class A carbapenemases (Ambler Group and 2f from Bush's grouping) can be chromosomally encoded (SME) or plasmid encoded (KPC). The KPC-types are the most clinically common carbapenemases found in Ent and are responsible for hospital outbreaks. Class B carbapenemases (Ambler Group and 3a from Bush's grouping) are called as metallo-β-lactamases (MBLs). They are usually of VIM and IMP types, but the recently emerged NDM-types are becoming the most threatening carbapenemases and have spread rapidly among Ent in all continents (Jabes 2011; Piddock 2012). In Ent, class D carbapenemases (Ambler Group 2df from Bush's grouping) are mainly represented by the OXA-48-like enzymes (e.g., OXA-48, -162, and -181). These genes are extensively reported among *E. coli* and *K. pneumoniae* isolates in the European and African Mediterranean countries. However, very recently, OXA-48 producers have been reported in North America (Nordmann et al. 2012).

Carbapenem-Resistant enterobacteriaceae (CRE) develops a form of infection that is hard-to-treat, and it is displaying an escalating trend among patients in medical facilities. CRE have become resistant to nearly all the antibiotics. Particularly, CRE with NDM-1 carbapenemase are a particular intimidation, as the blaNDM-1 gene is highly promiscuous and is readily transmitted between species and genera, with concomitant transfer of up to 14 antibiotic resistance genes. There is much evidence that CPE with NDM-1 carbapenemase are widespread in the population of the Indian subcontinent (Kumarasamy et al. 2010; Bushnell et al. 2013).

This poses a significant challenge to effective antimicrobial therapy of patients in this region, and there is a clear danger of global dissemination of NDM-1 via international travel (Day et al. 2013).

NDM-1 has been disseminated in different countries and is being reported globally. Because of its association with Indian subcontinent, a number of studies have included samples from Pakistan and India to evaluate the prevalence and spread of this enzyme. A study conducted on stool samples from patients at Military hospitals in Pakistan revealed an overall prevalence of 18.5% NDM-1 positive enterobacteriaceae with 27.1% from inpatients while 13.8% were from out patients (Perry et al. 2011). Out of 356 clinical isolates, 160 showed carbapenem resistance. Of these 160 isolates, 131 displayed MBLs production as accessed by combined disk method. In MBLs producing organisms, PCR amplification confirmed 31 (23.6%) isolates harboring blaNDM-1 gene, 33 (25.1%) isolates having blaVIM

gene, and 2 (1.5%) isolates displaying blaIMP gene. Plasmid profile analysis of NDM-1 positive organisms showed variable number of plasmids which were stable during serial passages in antibiotic-free media. The prevalence of ESBL producing organisms was recorded to be 87.5% (Nahid et al. 2013).

2.5 Counter Strategies to Combat Resistance

2.5.1 Overcoming Beta-Lactamases

The very first counter strategy to combat resistance is to overcome β-lactamases enzyme production. This could be done through finding β-lactamases inhibitor or by discovering new antibiotics that are not target for β-lactamases. β-lactamases inhibitor has structural similarity to the antibiotic molecules, but they themselves are not active. They actually occupy the β-lactamases and thus, the actual antibiotic would be free from their catalytic activity (Babic et al. 2006) (Fig. 2.3).

2.5.2 Increasing Antibiotic Influx

Second most significant resistance mechanism in Gram-negative microbes is the inability of hydrophilic drugs to cross the outer membrane barrier due to alteration

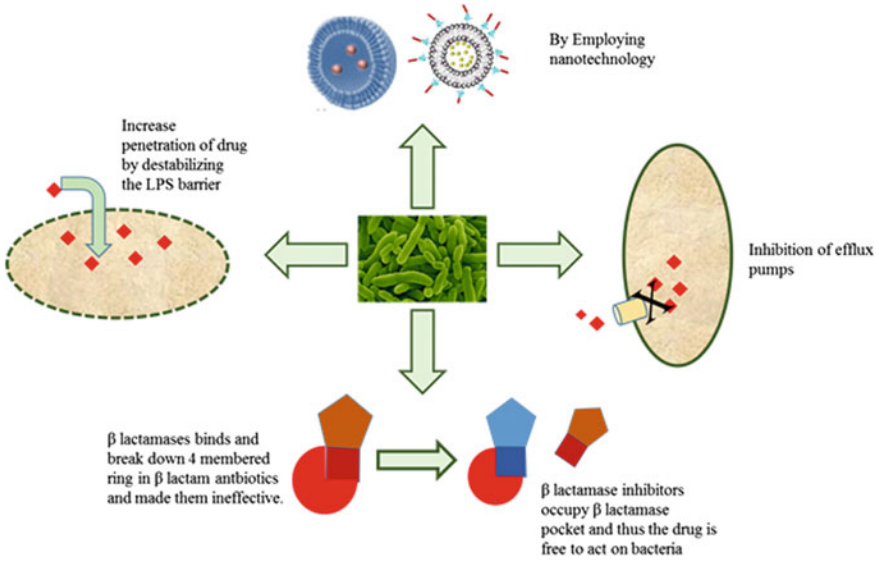


Fig. 2.3 Counter strategies to inhibit antibiotic resistance

in porins. On the other hand, hydrophobic drugs cannot trespass barrier due to modifications in LPS. To combat resistance in Gram-negative microbes because of limited entry, there is a need to enhance penetration of antibiotics. Certain chemical facilitators and chemosensitizers, such as detergents, surfactants, chaotropic agents, polymyxins, and antimicrobial peptides can be employed for this purpose.

2.5.3 Destabilization of LPS Barrier

Lipopolysaccharides (LPS) presence in Gram-negative pathogens is a natural mechanism to hinder the diffusion of hydrophilic molecules. If this barrier is destabilized by any mean, then it could be another possible solution to evade this resistance mechanism. This barrier could be damaged by using chaotropic agents or detergents like Tris/EDTA. Usage of Tris/EDTA will cause the release of LPS in the medium, and it will be replaced by glycerophospholipids. This replacement of LPS by glycerophospholipids will create pores in outer membranes barrier and make it more permeable.

2.5.4 Blocking the Efflux

Among two most important mechanisms of resistance in microbes are that firstly, antibiotics cannot penetrate bacterial cell membrane and secondly, if some of the fraction trespass the barrier, then it would be eventually pumped out by means of efflux pumps. Efflux pump inhibitors in combination with conventional antibiotic therapy are now considered as an attractive target for the development of a combinational therapy (Pages and Amaral 2009; Kourtesi et al. 2013).

2.5.5 Natural Antibiotics

The most significant reason for the development of resistance is the injudicious use of antibiotics. So there is a need to limit the overuse of antibiotics. The quest for novel effective antimicrobial agents from natural products has attracted much attention in recent years as complementary and alternative therapy (Calo et al. 2015). Essential oils (EOs) are complex mixtures of biologically active substances and offer potential novel template molecules and mixtures of bioactive compounds that came into existence after thousands of years of evolution. They have been confirmed to possess antibacterial, antifungal and anti-inflammatory, antioxidant, anticancer, and antiviral activities (Aumeeruddy-Elalfi et al. 2015; Sharifi-Rad et al. 2015). The efficiency of the essential oils depends on its chemical composition, genotypes, environmental, and agronomic conditions (Mohamed et al. 2014).

Numerous studies have highlighted antimicrobial effects of EOs even against multidrug-resistant bacteria (Coelho and Pereira 2013). EOs have already been used as an aerosol to clean hospital surfaces, environment, and equipment. Because of their fresh fragrance, they also provide a soothing and refreshing environment.

Furthermore, use of EOs as food preservatives has also been described by many others (Calo et al. 2015). Because of their complex chemical composition, often composed of more than 100 different terpenic compounds and thousands of years of evolution EOs have broad spectrum of activities. In the pharmaceutical field, EOs have been included in the composition of many dosage forms (capsules, ointments, creams, syrups, suppositories, aerosols, and sprays) (Mohamed et al. 2014) and the number of pharmaceutical preparations containing EOs is constantly growing.

Despite the excellent antimicrobial activity of EOs against pathogenic microorganisms, their utilization is very limited owing to their low water solubility and less stability to environmental factors like heat, moisture, oxygen. To improve water dispersion and protect EOs from degradation, nano-sized formulations emerge as a viable solution to the problem (El Asbahani et al. 2015). Nanoencapsulation has already proved to improve the antibacterial activity of several antibiotics (Jamil et al. 2016a, b).

2.5.6 Nano-Antibiotics

One of the recent struggles in addressing the challenge of resistance lies in exploring antimicrobial nanomaterials to which microbial pathogens may not be able to develop resistance, and novel nano-sized platforms for efficient antibiotics delivery (Pelgrift and Friedman 2013).

All those nanomaterials, which possess their intrinsic antimicrobial activity or augment the overall efficacy and safety of enclosed or adsorbed antibiotics, are termed "nano-antibiotics" (Huh and Kwon 2011). This definition includes nano-carriers as well. Nano-carriers are drug vectors which retain and transport drug; deliver it within or in the vicinity of target. In general, nano-carriers may protect a drug from degradation, enhance drug absorption, modify pharmacokinetic and pharmacodynamics, and improve intracellular penetration (Thorley and Tetley 2013).

Bio-based nano-carrier systems (including liposomes, chitosan, etc.,) as drug delivery vehicle are replacing metallic NPs because of various advantages rendered by them being biodegradable, biocompatible, economical, easy to manufacture with minimal or no side effects (Kumari et al. 2010).

There is a general consensus that nano-antimicrobials (NAMs) could be an effective alternative to conventional antibiotics and helpful in combating drug resistance. Recent studies have reported improved efficacy of antibiotics after encapsulation in nano-carrier systems (NCS) or by directly using bactericidal NPs with intrinsic antimicrobial potential (Hajipour et al. 2012; Jamil et al. 2017a).

2.6 Advantages of Nano-Antibiotics Over Conventional Antibiotics

The key advantages of nano-antibiotics over conventional antibiotics are that they can improve bioavailability by enhancing solubility, protecting the drug from premature degradation, both in vivo and during storage (Huh and Kwon 2011). Thus, the desired therapeutic effect could be achieved by improving bioavailability at low dose. Ultimately, it will reduce the dose-dependent side and toxic effect of drug, and patient compliance will be improved indirectly. By targeted drug delivery, drug will be released at site of action only. The infected site will get the maximum quantity of drug and antimicrobial effect would be optimum. These nano-formulations can ensure sustained and controlled release of the drug, which help to reduce therapeutic dose and its frequency. Another advantage offered by nano-antibiotics is their cost effectiveness and stability during manufacturing and shipping. Most important problem associated with conventional antibiotic therapy is antibiotic resistance, which could also be overcome through nano-antibiotics (Pelgrift and Friedman 2013).

2.7 Mechanism of Action of Nano-Antimicrobials

There are many hypotheses regarding antimicrobial mechanisms of action (MOA) of NAMs though exact MOA is still unknown (Fig. 2.4). There is a general consensus that the NAMs could prove to be a good alternative to conventional antibiotic therapy, and they could also serve as a potential agent to combat MDR microbes. As they kill microorganisms by simultaneously acting on many different essential life processes and/or metabolic routes, that so many genetic mutations in microbes are theoretically not possible to make them resistant against all modes (Hajipour et al. 2012; Pelgrift and Friedman 2013). NAMs show good antibacterial properties owing to their large surface to volume ratio so provide a more direct contact to bacterial surface. Particularly, cationic NPs can attach firmly by electrostatic interaction to the negatively charged outer membrane of bacteria, which causes the leakage of cell contents by disrupting its integrity. NAMs have also been reported to combat multi-resistant pathogens by enhanced cellular internalization and by decreased efflux of drug from bacterial pathogens. NAMs can prevent or overcome biofilm formation. There are many pathogens that are intracellular and are difficult to target by ordinary antibiotics, but NAMs can be manipulated to specifically combat intracellular bacteria. NAMs can target antimicrobial agents to the site of infection so that only the infected area will get the maximum dose of drug (Pelgrift and Friedman 2013) and the rest of the body will be safe from the toxic effects of the drug.

All the multi-cellular organisms are endured with a very strong immune system. Intact immune system protects the body against infectious agents and foreign toxic

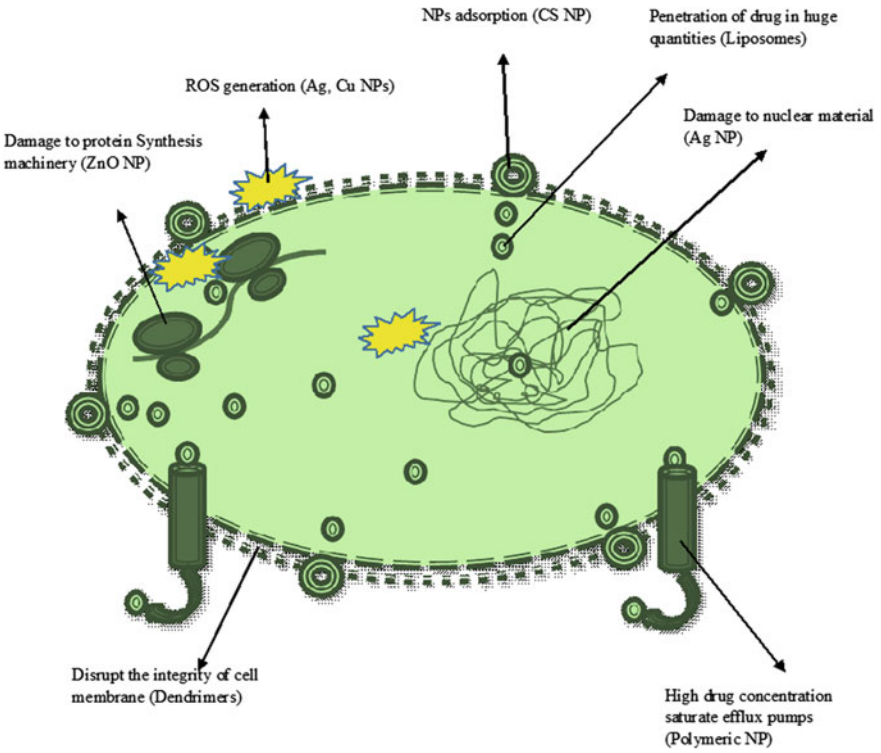


Fig. 2.4 Possible mechanisms of action of Nano-antimicrobials (NAMS) on bacteria (Jamil et al. 2017a)

substances. Main component of the innate immune system is phagocytic cells; they engulf and destroy any intruder. They mainly kill microbes and other foreign toxic substances by producing large quantity of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Bogdan et al. 2000). NPs particularly metallic NPs exert their toxic effect by mimicking natural immune response or augment the natural immunity by generating large quantity of ROS or RNS that is more than the physiological concentration. Others may exert direct killing effect by directly targeting cellular DNA, proteins, or lipids.

2.7.1 Generation of Reactive Oxygen Species (ROS)

The main mechanism of action of metallic NPs is through the generation of reactive oxygen species (Avalos et al. 2014; Fu et al. 2014; Onodera et al. 2015; You et al. 2016; Saravanakumar et al. 2017)

ROS is a general term for all those substances that can act as an oxidizing agent and may have the potential to generate free radicals. However, it comprises both radicals (O_2-) and non-radicals (H_2O_2). This term includes hypochlorous acid (HOCl), hypobromous acid (HOBr), ozone (O_3), peroxynitrite (ONOO), and hydrogen peroxide (H_2O_2). Most of these ROS are very short lived with a half-life of microseconds or nanoseconds and so act only locally with the exception of H_2O_2 which has the half-life of few minutes (Manke et al. 2013) and is the most notorious of all. The sources of ROS may be both endogenous and exogenous. Endogenous sources include transient metals, myeloperoxidase, NADPH oxidase, mitochondria, peroxisomes, endoplasmic reticulum, and lysosomes. The exogenous sources include UV radiations, smoking, certain chemicals, and drugs. ROS have many physiological functions and serve both beneficial and deleterious roles. Its beneficial role includes defense against pathogens, cell signaling molecule, anti-tumorigenic potential, and most importantly aid in maintaining redox homeostasis (Valko et al. 2007).

ROS are also normal physiological by-product of oxidative phosphorylation. At the end of respiratory chain, terminal electron acceptor is oxygen. Molecular oxygen is minimally reactive due to spin restriction and is usually converted to inert molecule, i.e., water. However, occasionally, a very small portion of oxygen instead of being converted to water is reduced to generate superoxide radical. Then the sequential reduction of superoxide forms a number of ROS including hydrogen peroxide, hydroxyl radical, and hydroxyl ion. ROS despite being the normal physiological product (s) can become toxic if produced in large quantity so can be voided by natural scavenger mechanisms to prevent it from becoming toxic (Buonocore et al. 2010). There is a balance between generation and neutralization of ROS. During the course of an infection, the phagocytic cells produce ROS. This process requires an increased consumption of oxygen by these cells by the action of NADPH oxidase. As the immune cells come across pathogens, there is "respiratory burst," i.e., rapid release of ROS to kill the invaders. ROS generation is generally self-limiting because of their very short life span and because of detoxification mechanism of the body. But at times, there may be oxidative stress due to over production of ROS or less mitigation of produced ROS (Buonocore et al. 2010).

ROS exert its effect on all vital cell constituents, i.e., nucleic acid, proteins, and lipids (Cabiscol et al. 2000; Manke et al. 2013). Lipids are the foremost important targets. Free radicals induce lipid peroxidation of polyunsaturated fatty acids present in the cell membranes. The cell membranes are their main target, as a result there will be increase in membrane rigidity, and ultimately, membrane properties will be altered. There will also be disruption of membrane-bound proteins. Lipid peroxidation of polyunsaturated fatty acids will further yield more toxic products such as aldehyde, which will expand the damage by targeting proteins as well. Generally, free radicals are very short lived; however, aldehydes are very reactive and long lived as well. That is why their damage is also more diffused. They act as "second toxic messengers" of the complex chain reactions (Cabiscol et al. 2000).

ROS target DNA by attacking the DNA backbone and cause the single- and double-strand breaks. It leads to the generation of DNA adduct (a piece of DNA

covalently bonded to a carcinogenic chemical), and generation of AP sites (apurinic/apyrimidinic site) also recognized as an abasic site (location in DNA or RNA that has neither a purine nor a pyrimidine base, either spontaneously or due to DNA damage). All these lesions are lethal to the cell and will block replication (Cabiscol et al. 2000).

ROS bring about oxidation of proteins, and as a result, several modules of damage are recognized. Disulfide bond is critical for protein folding and stability.

Cytosolic proteins have cysteine's residues in the reduced environment of cytosol, whereas many secreted proteins have disulfide bond to increase the stability of secretory proteins. Disulfide bond is not stable under the reduced environment of cytosol. ROS change the reduced environment of cytosol, and thus, surplus disulfide bonds are produced. ROS also alter the protein structure by inducing the modifications of amino acid side chains. Consequently, the protein structure is altered. As the structural changes lead to functional changes, the whole cellular metabolism gets disturbed (Cabiscol et al. 2000).

All the above-mentioned changes in protein structure were reversible changes. ROS also bring about certain irreversible changes by metal ion-catalyzed oxidation (MCO) reactions. This system converts oxygen to hydrogen peroxide and ferric to ferrous ions. These H_2O_2 and ferrous ions will bind at metal binding sites, and other free radicals will be produced which will bring about alterations in amino acids side chains. As a result of these modifications, these proteins will be subjected to degradation (Cabiscol et al. 2000; Butler et al. 2013; Manke et al. 2013).

2.7.2 Generation of Reactive Nitrogen Species (RNSs)

Reactive nitrogen species (RNS) are derived from the combination of nitric oxide (NO) and superoxide (O_2^-) radicals (Fig. 2.5). Nitric oxide is generated by phagocytic cells of immune system. It kills all types of microbes both Gram-positive and Gram-negative. These phagocytes have inducible nitric oxide synthase (iNOS). Phagocytes, particularly macrophages, express iNOS after induction by cytokines like interferon-gamma (IFN-γ) and after exposure to microbial products such as lipopolysaccharide (LPS). RNS usually act in combination with ROS (Manke et al. 2013).

Nitric oxide (NO) forms peroxynitrite ($ONOO^-$) after combination with superoxide (O_2^-). The peroxynitrite so produced is also highly reactive and sequentially generate other RNS like nitrogen dioxide (NO_2) and dinitrogen trioxide (N_2O_3) (Pelgrift and Friedman 2013). Induction of RNS kills pathogens by simultaneously reacting with various amino acids, protein, lipids, and DNA (Pelgrift and Friedman 2013). It also reacts with nonprotein fraction of a protein called prosthetic groups such as heme in hemoglobin. As RNS binds with heme, it is removed from proteins. All heme containing bacterial proteins such as nitric oxide synthetase, cytochrome P450, and guanylate cyclase will be degraded. All these effects act simultaneously to kill the pathogens, so the development of

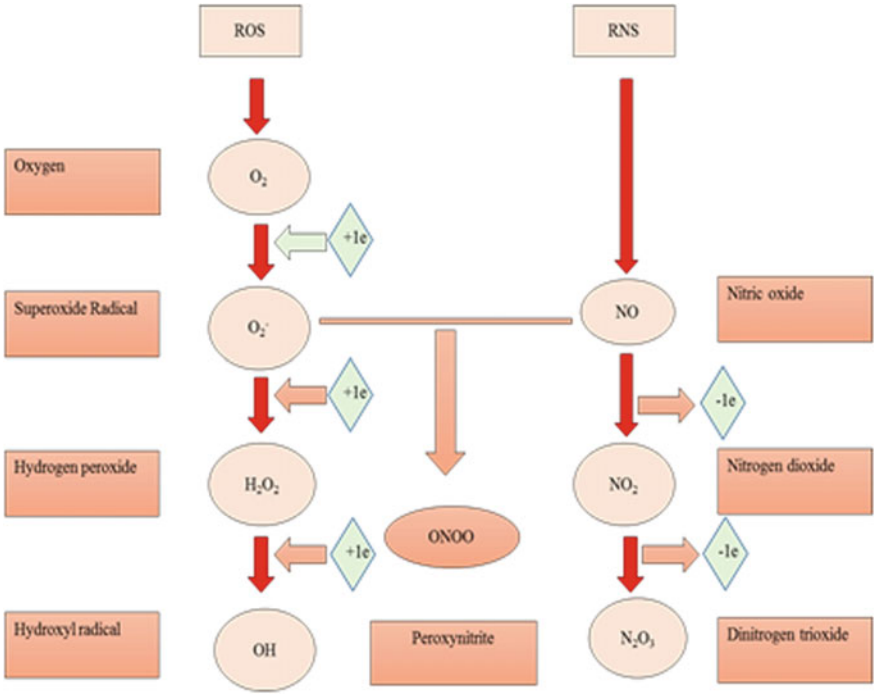


Fig. 2.5 Generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (adapted from Jamil et al. 2017a)

resistance to both ROS and RNS is very low. Finally, it also improves wound healing after inflammation (Hetrick et al. 2009).

Nitric oxide-releasing NPs (NO NPs) act by the same mechanisms and augment natural defense system by releasing mass amount of RNS (Hajipour et al. 2012). Bacteria have certain enzymes that protect it from nitrosative damage induced by phagocytes. When nitrosative damage is overcome by pathogens, infection takes place. However, exogenous NO NPs releases NO at infection site only and in such a great concentration that microbes do not find out the prospect to develop resistance, they are destroyed prior to developing resistance (Pelgrift and Friedman 2013).

2.7.3 Augmenting Uptake and Diminishing Efflux of Drugs by Bacteria

Another mechanism of action of NAMs is that they can increase the uptake and decrease the efflux of enclosed/loaded drug. Many microbes have developed resistance to many conventional formulations including β-lactams, vancomycin, aminoglycosides, tetracyclines, and quinolones by reducing their uptake or by

pumping out the drug by efflux pumps before it reaches the target site. Nano-carriers, particularly liposomes and dendrimers, can contribute to overturn these resistance mechanisms. Construction of liposomes is such that it resembles bacterial plasma membrane. As it comes in contact with microbes immediately fuses with them and empties its content inside the cell. Likewise, now the drug concentration inside the cell is so high that it saturates the bacterial efflux pumps. So the active drug will reach to its site of action and will kill the microbe (Pelgrift and Friedman 2013).

2.7.4 *Removing Biofilms and Their Resurgence*

NAMs can also overcome resistance by outstripping biofilm formation and prevent its further growth (Fig. 2.6). Biofilm formation occurs both in vivo and in vitro and on any type of surface. Biofilm formation also poses a complication in biomaterial-associated infections, sanitation surfaces, in food processing areas, and in oral infections (Allaker and Memarzadeh 2014).

Numerous NAMs have displayed their anti-biofilm approach to combat resistance. Liposomes aid in thrashing biofilms by promoting adsorption which is

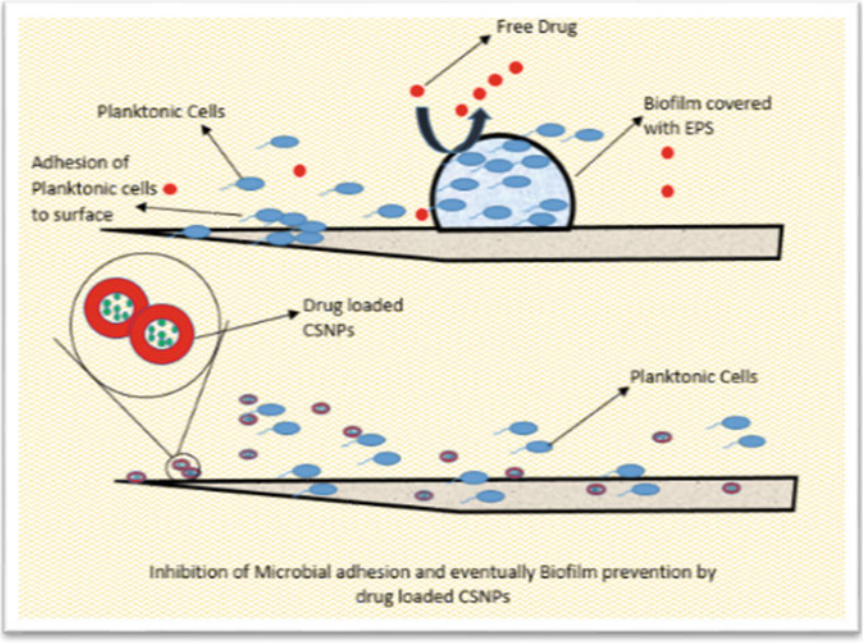


Fig. 2.6 Inhibition of biofilm formation by Nanoparticles

usually difficult because the outer surfaces of biofilms are highly hydrophobic in nature. Silica NPs act by releasing NO, and it is found to be active in deformation of already formed biofilms (Hetrick et al. 2009).

Analogously, ZnO NPs, TiO_2 NP, magnesium fluoride NPs (MgF_2 NPs), and super-paramagnetic iron oxide NPs (SPIONs) inhibit biofilm formation to surfaces including glass surfaces and catheters caused by *S. aureus* and *E. coli* (Pelgrift and Friedman 2013). Moreover, textile dressings coated with magnetite NPs (Fe_3O_4) inhibit biofilm development by *Candida albicans*. Cationic liposomes containing benzyl penicillin inhibit biofilm formation by *S. aureus* (Pelgrift and Friedman 2013). The majority biofilm matrices are negatively charged that promote attachment of positively charged NP (Hajipour et al. 2012).

2.7.5 *Overcoming Intracellular Bacteria*

NAMs are used to confront intracellular bacteria which are difficult to treat with conventional antibiotics (Fig. 2.7). Particularly, liposomes are used for this purpose. Liposomes being hydrophobic in nature are rapidly taken up by MPS by endocytosis. Once inside the host cell, these liposomes can release drugs to combat intracellular microbes (Pelgrift and Friedman 2013). Likewise, certain ligands (like mannose) could be attached to make them more targeted to alveolar macrophages only. In this way, liposomes could be used by pulmonary route to cure pulmonary infections like pneumonia caused by intracellular microbes. Many authors have proved the enhanced efficacy of nano-antibiotics in the cure of intracellular pathogens (Andrade et al. 2013). Liposomal amikacin, kanamycin, streptomycin, gentamicin, vancomycin were found to be highly effective for the eradication of *M. tuberculosis, K. pneumoniae,* and Methicillin-resistant *S. aureus* (MRSA) (Andrade et al. 2013).

2.7.6 *Ensuring Targeted Drug Delivery to Site of Action*

After conventional administration of therapeutic agents, with the exception of parenteral route, first drug has to be absorbed in the systemic circulation. Bioavailability is measured by only that portion of drug that reaches systemic circulation.

Bioavailability after oral route is a big concern to be dealt with. Once the drug reaches systemic circulation, it follows the general course of circulation and is distributed throughout the body. Only a minute fraction of drug reaches the target site. And in few cases, where perfusion is very low no fraction of drug reaches and the result will be resistance. But it will not be acquired resistance rather due to below therapeutic dose owing to low blood circulation.

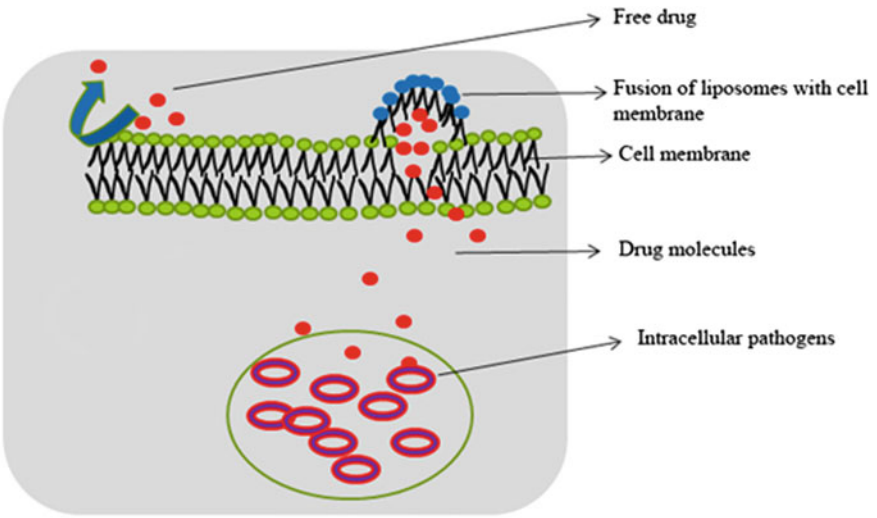


Fig. 2.7 Fusion of liposomes with cell membrane (Jamil et al. 2017a)

Another effective way by which NAMs can tackle antibiotic resistance is to target antibiotics to the site of infection only. As a consequence, maximum effect will be achieved at low drug concentration. Bioavailability will be 100% at infection site, and the whole body will be prevented from the side effects of the drug. The high dose at the site of infection will kill the pathogens prior to the development of resistance (Pelgrift and Friedman 2013; Jamil and Imran 2017).

NAMs can be targeted to sites of infection passively or actively. By passive targeting, NAMs will be accumulated at the site of infection by extravasations (Pelgrift and Friedman 2013). While actively targeted NAMs contain ligands that can bind to their receptors selectively. NAMs can be conjugated with antibodies against a given antigen on the surface of the target microbe (Pelgrift and Friedman 2013); likewise, lectins, lipoproteins, hormones, charged molecules, and polysaccharides can be used as targeting moieties. While executing a targeted release system, the properties of medicinal substance, its side effects, and the route of administration, the target site, and the type of disease must be considered to have a successful DDS.

2.7.7 Causing Direct Damage

NAMs may cause certain direct damages to pathogens by directly interacting with cell components.

2.7.7.1 Interacting and Damaging Cell Membranes

NAMs can directly interact with cellular membranes and disrupt its integrity. In particular, Ag NPs interact steadfastly with the bacterial membranes. This effect depends upon several factors. According to McShan et al., adsorption of nano-silver on the bacterial surface depends on pH, zeta potential, and NaCl concentration (McShan et al. 2014). Conferring to them, nano-silver after reacting with SH group of surface proteins interferes with the activity of many potential enzymes. Silver ions also obstruct respiratory chain, affect membrane permeability, ion transportation, and detach cell membrane from cell wall (McShan et al. 2014). Similarly, ZnO NPs also interact directly with bacterial cell membranes and deteriorate its integrity (Hajipour et al. 2012).

2.7.7.2 Binding and Damaging Cellular DNA and RNA

NAMs elicit genotoxic effects through direct interaction with DNA or indirectly via ROS-induced oxidative stress (Manke et al. 2013). DNA being negatively charged interacts with positively charged metallic NPs. They inhibit its replication and transcription. Chitosan also has a positive charge and after binding to DNA inhibit its transcription and translation (Jamil et al. 2017).

2.7.7.3 Damaging Proteins and Lipids

Nanoparticles have the propensity to inhibit the function of proteins and amino acids directly as well as through ROS. This interaction is considered to be the most critical one to induce toxicity to microbial pathogens. Ag NPs can directly inhibit proteins by binding to thiol groups, and it also participates in catalytic oxidation reactions that lead to the generation of disulfide bonds. This disulfide bond ultimately changes the shape and function of protein (Guo et al. 2013).

2.8 Conclusion

The persistent and universal existence of antibiotic resistance should not represent an excuse for discontinuing antibiotic research but should be a stimulus to suppress it. For combating these MDR pathogens, a comprehensive knowledge about resistance phenomenon is required. More effort should be done in product discovery so that many antibiotics with diverse mechanism of action can be developed. Moreover, existing antibiotics could be modified both pharmacokinetically and pharmacodynamically to make them more effective against MDR microbes instead of discarding them while keeping new drugs for emerging resistant microbes.

Technological advancement has led us the possibility of using engineered NPs to combat MDR pathogens. To warrant optimal use of nanomaterials for medical applications, more extensive efforts should be practiced to study the interaction between nanomaterials and the biological systems. Consequently, safety standard of NPs on human health with their fate is desirable. However, it is expected that there will be tremendous increase in the field of drug discovery due to the advancement in NAMs against which development of resistance seems to be impossible. Targeted drug delivery is an ideal approach, and there is a dire need to implement it for getting optimum results against pathogens while safeguarding normal flora. It is most likely the single most effective strategy to control the development of resistance.

References

Alanis AJ (2005) Resistance to antibiotics: are we in the post-antibiotic era? Arch Med Res 36:697–705

Al-Assil B, Mahfoud M, Hamzeh AR (2013) Resistance trends and risk factors of extended spectrum β-lactamases in Escherichia coli infections in Aleppo, Syria. Am J Infect Control 41:597–600

Allaker RP, Memarzadeh K (2014) Nanoparticles and the control of oral infections. Int J Antimicrob Agents 43:95–104

Andrade F, Rafael D, Videira M, Ferreira D, Sosnik A, Sarmento B (2013) Nanotechnology and pulmonary delivery to overcome resistance in infectious diseases. Adv Drug Deliv Rev 65:1816–1827

Aumeeruddy-Elalfi Z, Gurib-Fakim A, Mahomoodally F (2015) Antimicrobial, antibiotic potentiating activity and phytochemical profile of essential oils from exotic and endemic medicinal plants of Mauritius. Ind Crops Prod 71:197–204

Avalos A, Haza AI, Mateo D, Morales P (2014) Cytotoxicity and ROS production of manufactured silver nanoparticles of different sizes in hepatoma and leukemia cells. J Appl Toxicol 34:413–423

Babic M, Hujer AM, Bonomo RA (2006) What's new in antibiotic resistance? Focus on beta-lactamases. Drug Resist Updates 9:142–156

Bogdan C, Röllinghoff M, Diefenbach A (2000) Reactive oxygen and reactive nitrogen intermediates in innate and specific immunity. Curr Opin Immunol 12:64–76

Bolla JM, Alibert-Franco S, Handzlik J, Chevalier J, Mahamoud A, Boyer G, Kieć-Kononowicz K, Pagès JM (2011) Strategies for bypassing the membrane barrier in multidrug resistant Gram-negative bacteria. FEBS Lett 585:1682–1690

Bologa CG, Ursu O, Oprea TI, Melançon CE, Tegos GP (2013) Emerging trends in the discovery of natural product antibacterials. Curr Opin Pharmacol 13:678–687

Buonocore G, Perrone S, Tataranno ML (2010) Oxygen toxicity: chemistry and biology of reactive oxygen species. Semin Fetal & Neonatal Med 15:186–190

Bush K, Jacoby GA (2010) Updated functional classification of β-lactamases. Antimicrob Agents Chemother 54:969–976

Bushnell G, Mitrani-Gold F, Mundy LM (2013) Emergence of New Delhi metallo-β-lactamase type 1-producing Enterobacteriaceae and non-Enterobacteriaceae: global case detection and bacterial surveillance. Int J Infect Dis 17(5):e325–333

Butler MS, Blaskovich MA, Cooper MA (2013) Antibiotics in the clinical pipeline in 2013. J Antibiot 66:571

Cabiscol E, Tamarit J, Ros J (2000) Oxidative stress in bacteria and protein damage by reactive oxygen species. Int Microbiol 3:3–8

Calo JR, Crandall PG, O'Bryan CA, Ricke SC (2015) Essential oils as antimicrobials in food systems–A review. Food Control 54:111–119

Coelho FA, Pereira MO (2013) Exploring new treatment strategies for Pseudomonas aeruginosa biofilm infections based on plant essential oils. Microb Pathog Strat Combating them: Sci Technol Educ 1:83–89

Dadashi M, Fallah F, Hashemi A, Hajikhani B, Owlia P, Bostanghadiri N, Farahani N, Mirpour M (2017) Prevalence of bla NDM-1-producing Klebsiella pneumoniae in Asia: a systematic review and meta-analysis. J des Anti-infectieux

Day KM, Ali S, Mirza IA, Sidjabat HE, Silvey A, Lanyon CV, Cummings SP, Abbasi SA, Raza MW, Paterson DL, Perry JD (2013) Prevalence and molecular characterization of Enterobacteriaceae producing NDM-1 carbapenemase at a military hospital in Pakistan and evaluation of two chromogenic media. Diagn Microbiol Infect Dis 75:187–191

El Asbahani A, Miladi K, Badri W, Sala M, Addi EA, Casabianca H, Elaissari A (2015) Essential oils: from extraction to encapsulation. Int J Pharm 483:220–243

Fabbretti A, Gualerzi CO, Brandi L (2011) How to cope with the quest for new antibiotics. FEBS Lett 585:1673–1681

Fernando DM, Tun HM, Poole J, Patidar R, Li R, Mi R, Amarawansha GE, Fernando WD, Khafipour E, Farenhorst A, Kumar A (2016) Detection of antibiotic resistance genes in source and drinking water samples from a First Nations Community in Canada. Appl Environ Microbiol 82:4767–4775

Fu PP, Xia Q, Hwang HM, Ray PC, Yu H (2014) Mechanisms of nanotoxicity: generation of reactive oxygen species. J Food and Drug Anal 22:64–75

Guo L, Yuan W, Lu Z, Li CM (2013) Polymer/nanosilver composite coatings for antibacterial applications. Colloids Surf, A 439:69–83

Hajipour MJ, Fromm KM, Ashkarran AA, de Aberasturi DJ, de Larramendi IR, Rojo T, Serpooshan V, Parak WJ, Mahmoudi M (2012) Antibacterial properties of nanoparticles. Trends Biotechnol 30:499–511

Hetrick EM, Shin JH, Paul HS, Schoenfisch MH (2009) Anti-biofilm efficacy of nitric oxide-releasing silica nanoparticles. Biomaterials 30:2782–2789

Huh AJ, Kwon YJ (2011) "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. J Controlled Release 156:128–145

Iyer R, Sylvester MA, Velez-Vega C, Tommasi R, Durand-Reville TF, Miller AA (2017) Whole-cell-based assay to evaluate structure permeation relationships for Carbapenem Passage through the Pseudomonas aeruginosa Porin OprD. ACS Infect Dis 3:310–319

Jabes D (2011) The antibiotic R&D pipeline: an update. Curr Opin Microbiol 14(5):564–569

Jamil B (2014) Enterobacteriaceae: At the verge of treatment. Int J Innov Appl Stud 9:1736–1745

Jamil B, Habib H, Abbasi S, Nasir H, Rahman A, Rehman A, Bokhari H, Imran M (2016a) Cefazolin loaded chitosan nanoparticles to cure multi drug resistant Gram-negative pathogens. Carbohyd Polym 136:682–691

Jamil B, Habib H, Abbasi SA, Ihsan A, Nasir H, Imran M. (2016b) Development of cefotaxime impregnated chitosan as nano-antibiotics: De novo strategy to combat biofilm forming multi-drug resistant pathogens. Front Microbiol 7

Jamil B, Bokhari H, Imran M (2017) Mechanism of action: how nano-antimicrobials act? Curr Drug Targets 18:363–373

Jamil B, Imran M (2017) Factors pivotal for designing of nanoantimicrobials: an exposition. Crit Rev Microbiol 18:1–6

Kourtesi C, Ball AR, Huang YY, Jachak SM, Vera DM, Khondkar P, Gibbons S, Hamblin MR, Tegos GP (2013) Suppl 1: Microbial efflux systems and inhibitors: approaches to drug discovery and the challenge of clinical implementation. Open Microbiol J 7:34–52

Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S, Krishnan P (2010) Emergence of a new antibiotic resistance

mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis 10:597–602
Kumari A, Yadav SK, Yadav SC (2010) Biodegradable polymeric nanoparticles based drug delivery systems. Colloids Surf B 75:1–18
Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, Greko C (2013) Antibiotic resistance—the need for global solutions. Lancet Infect Dis 13:1057–1098
Lewis K (2013) Platforms for antibiotic discovery. Nat Rev Drug Discovery 12:371–387
Lupo A, Papp-Wallace KM, Sendi P, Bonomo RA, Endimiani A (2013) Non-phenotypic tests to detect and characterize antibiotic resistance mechanisms in Enterobacteriaceae. Diagn Microbiol Infect Dis 77:179–194
Manke A, Wang L, Rojanasakul Y (2013) Mechanisms of nanoparticle-induced oxidative stress and toxicity. BioMed Res Int 1–15
McShan D, Ray PC, Yu H (2014) Molecular toxicity mechanism of nanosilver. J Food and Drug Anal 22:116–127
Mohamed AA, Ali SI, EL-Baz FK, Hegazy AK, Kord MA (2014) Chemical composition of essential oil and in vitro antioxidant and antimicrobial activities of crude extracts of Commiphora myrrha resin. Ind Crops Prod 57:10–16
Nahid F, Khan AA, Rehman S, Zahra R (2013) Prevalence of metallo-β-lactamase NDM-1-producing multi-drug resistant bacteria at two Pakistani hospitals and implications for public health. J Infect Public Health 6:487–493
Nordmann P, Dortet L, Poirel L (2012) Carbapenem resistance in Enterobacteriaceae: here is the storm! Trends Mol Med 18:263–272
Onodera A, Nishiumi F, Kakiguchi K, Tanaka A, Tanabe N, Honma A, Yayama K, Yoshioka Y, Nakahira K, Yonemura S, Yanagihara I (2015) Short-term changes in intracellular ROS localisation after the silver nanoparticles exposure depending on particle size. Toxicol Rep 2:574–579
Pagès JM, Amaral L (2009) Mechanisms of drug efflux and strategies to combat them: challenging the efflux pump of Gram-negative bacteria. (BBA)-Proteins Proteom 1794:826–833
Paterson DL (2006) Resistance in gram-negative bacteria: Enterobacteriaceae. Am J Med 119: S20–28
Pelgrift RY, Friedman AJ (2013) Nanotechnology as a therapeutic tool to combat microbial resistance. Adv Drug Deliv Rev 65:1803–1815
Perez F, Endimiani A, Hujer KM, Bonomo RA (2007) The continuing challenge of ESBLs. Curr Opin Pharmacol 7:459–469
Perry JD, Naqvi SH, Mirza IA, Alizai SA, Hussain A, Ghirardi S, Orenga S, Wilkinson K, Woodford N, Zhang J, Livermore DM (2011) Prevalence of faecal carriage of Enterobacteriaceae with NDM-1 carbapenemase at military hospitals in Pakistan, and evaluation of two chromogenic media. J Antimicrob Chemother 66:2288–2294
Phan K, Ferenci T (2017) The fitness costs and trade-off shapes associated with the exclusion of nine antibiotics by OmpF porin channels. The ISME J 11:1472–1482
Pickering LK (2004) Antimicrobial resistance among enteric pathogens. In Seminars in pediatric infectious diseases 15: 71–77. WB Saunders
Piddock LJ (2012) The crisis of no new antibiotics—what is the way forward? Lancet Infect Dis 12:249–253
Pitout JD, Laupland KB (2008) Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis 8:159–166
Ruppé É, Woerther PL, Barbier F (2015) Mechanisms of antimicrobial resistance in Gram-negative bacilli. Ann Intensive Care 5:21
Saravanakumar G, Kim J and Kim WJ (2017) Reactive-oxygen-species-responsive drug delivery systems: promises and challenges. Adv Sci 4
Shakya P, Shrestha D, Maharjan E, Sharma VK, Paudyal R (2017) ESBL production among E. coli and Klebsiella spp. Causing urinary tract infection: a hospital based study. Open Microbiol J 11:23

Sharifi-Rad J, Hoseini-Alfatemi SM, Sharifi-Rad M, Da Silva JA (2015) Antibacterial, antioxidant, antifungal and anti-inflammatory activities of crude extract from Nitraria schoberi fruits. 3. Biotech 5:677–684

Shuvo SR, Kovaltchouk U, Zubaer A, Kumar A, Summers WA, Donald LJ, Hausner G, Court DA (2017) Functional characterization of an N-terminally-truncated mitochondrial porin expressed in Neurospora crassa. Can J Microbiol 63:1–9

Stürenburg E, Mack D (2003) Extended-spectrum β-lactamases: implications for the clinical microbiology laboratory, therapy, and infection control. J Infect 47:273–295

Talukdar PK, Rahman M, Rahman M, Nabi A, Islam Z, Hoque MM, Endtz HP, Islam MA (2013) Antimicrobial resistance, virulence factors and genetic diversity of Escherichia coli isolates from household water supply in Dhaka, Bangladesh. Plos One 8:e61090

Thorley AJ, Tetley TD (2013) New perspectives in nanomedicine. Pharmacol Ther 140:176–185

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84

Vester B, Long KS (2013) Antibiotic Resistance in Bacteria Caused by Modified Nucleosides in 23S Ribosomal RNA

Wang B, Sun D (2014) Detection of NDM-1 carbapenemase-producing Acinetobacter calcoaceticus and Acinetobacter junii in environmental samples from livestock farms. J Antimicrob Chemother 70:611–613

Wellington EM, Boxall AB, Cross P, Feil EJ, Gaze WH, Hawkey PM, Johnson-Rollings AS, Jones DL, Lee NM, Otten W, Thomas CM (2013) The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. Lancet Infect Dis 13:155–165

Wright GD (2012) Antibiotics: a new hope. Chem Biol 19:3–10

You DG, Deepagan VG, Um W, Jeon S, Son S, Chang H, Yoon HI, Cho YW, Swierczewska M, Lee S, Pomper MG (2016) ROS-generating TiO_2 nanoparticles for non-invasive sonodynamic therapy of cancer. Sci Rep 6:23200