Antimicrobial Resistance

Vineeta Singh[1]

[1]National Institute of Malaria Research (ICMR), Sector-8, Dwarka, New Delhi-110077, India.

A vast majority of pathogens, the etiologic agent for several diseases have become resistant to one or more microbial agents. It is pertinent to understand the evolution of antibiotic resistance in pathogens for several factors from the drug to pathogen to their genetics to their ecology; as they all interplay together increasing the complexity of this process. The wide range of pathogens which ranges from bacteria causing tuberculosis, the viruses causing influenza, the parasites causing malaria to the fungi causing yeast infections are becoming resistant to the various antimicrobial agents such as antibiotics, antivirals, antimalarials and antifungals used for their treatment. Due to this, the risk of losing the treatment of infectious diseases looms large. In this perspective, we discuss here the emergence of antimicrobial resistance in pathogenic organisms on the treatment of infectious diseases. The first section of the chapter discusses the main mechanisms of resistance – an important aspect of the emerging and re-emerging resistance with serious consequences on the treatment of infectious diseases. The rampant use/misuse of the antibiotics in human medicine is the major contributing factor in this prevailing phenomenon. The next section of the chapter elaborates the measures to be undertaken to combat the multidrug resistant pathogens and their spread worldwide, causing clinical failures adding more woes to the public health crisis. The last section of the chapter talks about the need for discovering new antibiotics/novel drugs and the importance of further research in this field, the importance of partnerships between governments, research institutions and public.

Keywords pathogens; antimicrobial resistance; clinical breakpoints; drug resistance mechanism; resistant strategies.

1. Introduction

The antimicrobial resistance is defined majorly as the microorganism becoming resistant to the antimicrobial medicine though earlier being sensitive to it. The various disease causing pathogens have a tremendous capacity to mutate and acquire resistance genes to develop resistance to antimicrobial drugs. Resistant organisms such as bacteria, viruses, fungi and parasites are able to withstand attack by antimicrobial medicines, like antibiotics, antivirals, antifungals and antiparasitic, so that the conventional treatment procedure turns ineffective leading to persistence and spread of infections [1-5]. Several new resistance phenotypes have emerged highlighting the unpredictable and fluid nature of antimicrobial resistance [6]. This antimicrobial resistance development is largely the consequence of rampant use/misuse of the antimicrobial medicines and is solely attributing for the treatment of infections being compromised worldwide. When an antimicrobial drug is used, the selective pressure exerted by the drug favors the growth of organisms that are resistant to it. The extensive usage of antimicrobial drugs has resulted in drug resistance threatening to reverse the medical advances made in the last seventy years. Once the multidrug resistant pathogens are established they thrive and spread worldwide causing more clinical failures leading to human health crisis.

The ability of a microorganism to survive at a given concentration of an antimicrobial agent at which the normal population of the microorganism would be killed is – Antimicrobial resistance I. This is called the epidemiological breakpoint.

The ability of a microorganism to survive treatment with a clinical concentration of an antimicrobial agent in the body is – Antimicrobial resistance II. This is called the clinical breakpoint. It is important to define resistance using clinical breakpoints so as to determine appropriate anti-microbial therapy for achieving improved clinical outcomes [7]. There are reports where it has been shown that appropriate therapy improved outcomes in hospitals, in bloodstream infections and pneumonia [8]. It has also been observed that infections due to specific pathogens like *Enterobacteriae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* respond much better to appropriate than inappropriate antibiotics [8,9]. The use of wild cut offs as clinical breakpoints are very useful in predicting the clinical outcomes.

2. Mechanism of action of antimicrobial resistance: The drug resistance can arise in the microorganisms due to any of the following mechanisms

2.1. Changes in the cell wall and membrane permeability.

The cell wall present in bacteria is critical for their life and survival. The drug that can target the peptidoglycan layer in the cell walls will thus selectively kill or inhibit the bacteria eg: penicillins, cephalosporins, bacitracin and vancomycin [10]. The glycopeptide (present in antibacterials) peptidoglycan (present in the cell wall of Gram positive bacteria) complex is the cause of inhibition of the cell wall transpeptidase arresting the cell wall biosynthesis, blocking cell division and its growth [11]. Similarly, the eukaryotes and prokaryotes all have cell membranes which serve as

important barriers segregating and regulating the intracellular and extracellular flow of substances into the cell. Drugs like polymixin B and colistin disrupt the cell survival mechanism by disrupting the cell membrane and thus causing cell death [12]. Changes in the cell outer membrane permeability of the microbial organisms is also associated with decreased uptake resulting in increased resistance to drugs like fluoroquinolones [13].

2.2. Active efflux of the antimicrobial from the cell.

One of the important mechanisms underlying the antibiotic resistance constitutes the multidrug efflux pumps responsible for export or expulsion of various drugs from the cell. The drug is pumped out faster by the efflux pumps than it can diffuse in the cell so that drug levels in the cell can be kept low, ineffectual and the protein synthesis continues without any interference. Efflux pumps are found both in Gram positive and Gram negative bacterial species, having a major role in antibiotic resistance causing high minimum inhibition concentrations (MICs-test used to determine the lowest effective dose that can be used to treat the microbe) of drugs [14]. The efflux pumps based on proteins, belong to several families *viz* superfamily ABC (ATP binding casette), families SMR (small multidrug resistance), MF (major facilitator), MATE (multi drug and toxic compound extrusion) and RND (resistance nodulation division) [15]. These variants of membrane pumps are possessed by all bacteria to move lipophilic or amphiphathic molecules in and out of the cells.

2.3. Mutation in the active target sites.

Modification of the biomolecular target for antimicrobials occurs either by a spontaneous mutation of the gene encoding the target or by substitution of the target function by an exogenous gene. This mode of drug resistance and multidrug resistance is acquired through chromosomal mutations or exchange of extrachromosomal elements from other bacteria through either transformation (*ie* incorporation of free DNA segments into their chromosomes), or transduction (*ie* via bacteriophages) or conjugation (*ie* plasmids and conjugative transposons) [16]. A principal mechanism for the rapid spread of antibiotic-resistance genes occurs via plasmids and transposons. These genes on extrachromosomal DNA are replicated within and passed between bacterial cells and species independently thus moving and spreading their antibiotic-resistance conferring genes with increased frequency [17].

2.4. Enzymatic degradation or modification of the antimicrobials.

This is another strategy by which the pathogens evade the action of antimicrobials on them. Enzymes modifying the antibacterial drugs fall broadly into two categories:
   a.   β lactamases – antimicrobial degrading enzymes. Resistance to the β lactams is usually due to the hydrolysis of the drug by the β lactamase enzyme or the cellular permeability [18].
   b.   Antimicrobial modifying enzymes that perform chemical transformations of drugs by acetylation, phosphorylation, adenylation, glycosylation, hydroxylation such as macrolide and aminoglycoside modifying enzymes or flavin-dependent mono-oxygenase modifying tetracycline [19]. This modification interefers with the target 16S rRNA in ribosome action causing inhibition of protein synthesis [20, 21].

2.5. Acquisition of the alternative metabolic pathways by microbes to evade drug inhibition.

This strategy is also known as target bypass as the microbial agent acquires novel metabolic pathways to produce an alternate target molecule bypassing the primary target necessary for survival of the organism. This scenario is well depicted by methicillin-resistant *S.aureus* (MRSA) which produces alternative penicillin binding protein (PBP2a) alongwith the normal protein. This PBP2a is resistant to flucloxacillin leading to peptidoglycan production and normal cell wall synthesis [22]. Another example of this strategy is seen in *Vancomycin-Resistant Enterococci* (*VRE*) which encodes a new pathway of enzymes that educes pyruvate to D-lactate (*van* H) adds D-alanine and D-lactate together to produce D-Ala-D-Lac (*van* A) and then hydrolyses the normal metabolite D-Ala-D-Ala though sparing D-Ala-D-Lac (*van* X). The peptidoglycan ending with D-Ala-D-Lac lowers the binding affinity of vancomycin by 1000-fold resulting in VRE growth at 1000-fold higher [23].

   In the unfavourable situations the pathogens develop any of the above mentioned strategies for survival leading to development of resistance strains (Fig 1). Since the microbial agents have developed different strategies to evade the effect of antimicrobials, the knowledge and identification of the resistance would help in the discovery and designing of novel drugs.

2.6. Antimicrobial drugs and their mode of resistance.

Drug class with examples	Mode of resistance	Effective against
Polypeptides eg: Bacitracin	Inhibition of cell wall synthesis	Gram positive bacteria
β lactams eg: Penicillins Cephamycin	Inhibition of cell wall synthesis, Efflux, Altered target	Gram negative bacteria
Cyclic peptides eg: Gramicidins	Alter cytoplasmic membranes	Gram positive bacteria
Polypeptides eg: Polymixin		Gram positive bacteria particularly *Pseudomonas*
Tetracyclines eg: Doxycycline Tetracycline	Efflux pump	Broad spectrum effective against Gram positive and negative bacteria and also Mycoplasma
Phenicols eg: Chloramphenicol	Mutation in active target sites, Efflux	Broad spectrum bacteria
Aminoglycosides eg: Gentamicin Kanamycin Neomycin		
Macrolides eg: Azithromycin Erythromycin Telithromycin	Target modification due to enzymatic degradation or modification, Efflux	Broad spectrum bacteria
Pyrimidines eg: Trimethoprim	Target bypass, Efflux	Broad spectrum bacteria, some protozoa and fungi.

3. Impact on clinical outcome due to antimicrobial resistance:

The increase in the antimicrobial resistance is occuring at an alarming rate both in the community and hospital based infections causing immense concern to the physicians. To name a few clinically important pathogens that have rapidly developed antimicrobial resistance include bacteria causing pneumonia, ear infections and meningitis (*Streptococcus pneumoniae*); skin, bone, lung and bloodstream infections (*S.aureus*); urinary tract infections (*Escherichia coli*); foodborne infections (*E.coli* or *Salmonella*) and infections transmitted in healthcare settings (*Enterococci* and *Acinetobacter baumanii*, *P.aeruginosa* and *Klebisella* spp). The resistance to third-generation cephalosporin and fluoroquinolone has already emerged whereas the public health burden of methicillin-resistant *S.aureus* is staggering [24]. Chloroquine resistance in malaria strains is well established but the recent emergence of artemisinin resistance, one of the most effective antimalarial drugs is certainly frightening showing how troublesome the antimicrobial resistance (AMR) is turning out to be [5]. The treatment failures due to drug resistance can lead to serious complications especially in critically ill patients [25].

3.1. Super-bugs

The microbes with enhanced morbidity and mortality and having high levels of resistance to the various antimicrobials drugs are termed as superbugs. Several pathogens which have evolved out of this phenomenon include hospital based infections like *Mycobacterium tuberculosis*, *A.baumannii*, *E.coli*, *Klebsiella pneumoniae*, *P.areuginosa*, *S.aureus*, *S. pneumoniae* etc [26, 27]. Tuberculosis is a major disease prevalent in both developing and industrialized countries infecting about one-third of world population. *M. tuberculosis* strains resistant to more than four front-line therapies have appeared and spread rapidly in the last decade [28]. The diseases caused by *E.coli*, *S.enterica* and *K.pnemoniae* is on the rise due to the resistance development in these organisms [29]. Among the nosocomial infections; the most notorious superbug is the Gram positive *S.aureus*. The apperance of MRSA has emerged as the major hospital-linked infection though now it has turned into a major community-acquired pathogen [24]. The superbugs seem to be present

everywhere and are not only microbial threats but are most difficult to manage with increased mortality and morbidity. From the medical prespective AMR can cause serious infections increasing the risk of life-threatening infections post-surgery. The success of organ transplantation, cancer chemotherapy and major surgery would be compromised without effective antimicrobials for care and for prevention of infections.

4. Control

The emergence of the antimicrobial resistance is a complex problem which needs to be addressed thoroughly as our ability to predict and combat the emergence of resistance in pathogenic organisms is limited. The knowledge of international patterns of antibiotic resistance is an important element in resistant control programmes. But it is difficult to establish efficient resistance correlations among countries as each region practises different sampling strategies and have different parameters for defining a strain as resistant. There is a high regional variablity of drug resistance to specific antibiotics. Research should be done together with the clinical studies and pharmacokinetic/ pharmoacodynamic studies to predict the antibacterial effects and for optimizing patient outcome. There is a need to have well constructed and well executed antimicrobial surveillance programmes determining the burden of antibiotic resistance in relevant clinical situations. The surveillance should be done on a regular basis to ensure and identify the occurrence and pattern of antimicrobial reistance in the pathogens.

The health care institutions must have strict infection control measures for patients with screening programmes to identify high-risk patients. The guidelines must be developed and implemented with drug utilization reviews to ensure that the antimicrobial drug use is optimized. The health care professionals, government, industry and community all have essential roles to play in combating the drug resistance prevalent all over the world. Also individuals must be supported by a greater infrastructure to effect significant and lasting change in collaboration with governments and institutions. Constant vigilance and adhering to an action plan to combat antimicrobial resistance should be practised routinely by all health care professionals, government, institutions to minimize the public health risks due to infectious organisms. Everyone should contribute to help control of antimicrobial resistant pathogens. Education about antimicrobial use for health care professionals, patients and families must be made a part of awareness programme to increase the community contribution in the control programmes. A holistic approach involving an interplay of relevant policies, adequate laws, awareness drive around rationalisation of antimicrobial drugs usage in health care professionals alongwith clinicians and researchers and accelerated research for novel and more effective drugs is needed to combat AMR.

5. Novel antimicrobial compounds

As the antimicrobial resistance to systemic antibiotics continues to persist causing the problem in treating infections recently, it becomes more important to find suitable alternatives for systemic antibiotics. The insight about the possible mechanisms of drug resistance can lead to a rational design of new antimicrobial drugs urgently needed to treat infectious diseases. Since the evolution of microbes is a continous phenomenon due to mutation, it is imperative to prevent the pipelines of new drugs from running dry by constant research and development. The antimicrobial research has two major hurdles:
   a.   Widespread increase in multi antimicrobial resistance.
   b.   Lack of research for identifying novel/new classes of antimicrobial compounds.
   Inspite of the existing issues the quest for new antimicrobials is an absolute necessity today and so scientists are looking in new directions for drug development resulting in arrival of some new classes of antimicrobials which seem promising.

5.1. Antimicrobial peptides (AMPs).

AMPs are the new class of antimicrobials emerging as promising alternatives to conventional antibiotic therapies. AMPs are widespread in nature and produced naturally by animals, plants, fungi and bacteria [30]. AMPs include a cathelicidin family member LL-37 and defensins. The cationic AMPs bind to microbial pathogens disrupting their cell walls. The properties of AMPs termed rightly as nature's antibiotics with their multiple functions in host defense systems of the multicellular organisms support the rationale of developing them as novel peptide-based therapies [31]. Their action against microbial pathogens are of great interest and can serve as apt candidate for novel therapeutic agents in infectious diseases [32]. There is no doubt that numerous AMP based drugs for humans, animals and crops will appear in the next decade.

5.2. Nanoparticles.

These particles could serve as the alternative antimicrobial drug delivery strategies. Several antimicrobial drugs are difficult to administer to the patients because of their low water-solubility, cytotoxicity to healthy tissues and rapid

degradation and clearance from blood stream. The antimicrobial activities of the drugs against intracellular microbes are also affected due to poor membrane transport ability. Recent research has shown that nanoparticles such as liposomes, polymeric nanoparticles, solid lipid nanoparticles and dendrimers are also able to overcome these problems and help antimicrobial delivery to the infection sites [33]. There is no doubt that nanoparticle-based drug delivery systems will improve not only antimicrobial therapies but also life-threatening diseases due to tuberculosis and Staph infections.

6. Conclusions

The versatility of the pathogens to adapt to unfavorable enviournments for their survival by adopting any of the resistance strategy clearly indicates the antimicrobial resistance is an inevitable biological phenomenon that is likely to continue as chronic medical problem. In the absence of new efficient antimicrobials successful management of current antimicrobials and other issues such as continued development of new drugs, education programs based on enhanced hygiene and decrease of the misuse/abuse of drugs is vital to protecting human and animal health against pathogens. It is not a wise option for us to rely totally on the arrival of new drugs to re-establish dominance over disease. Vigilance and dedication following an action plan to combat the AMR is a must. Unless we realise that AMR is a serious problem which affects all people regardless of their age, gender or socio-economic background, we face the prospect of life-threatening illnesses non-treatable by any antibiotic.

Measures to combat antimicrobial resistance:

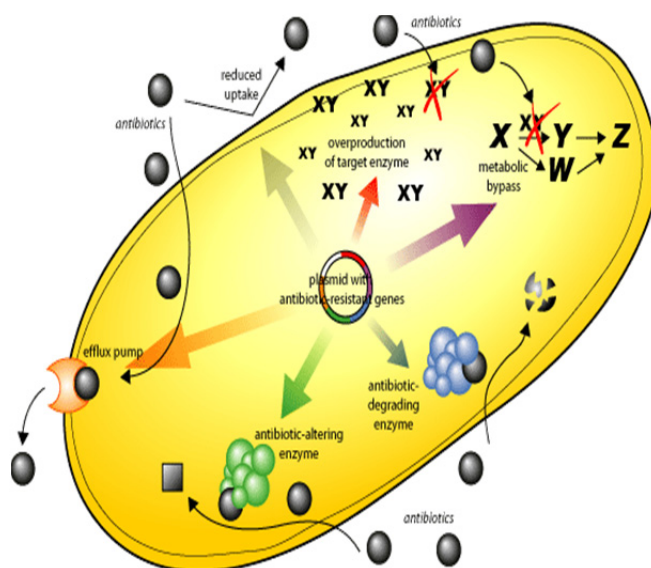| Do s	Dont s
Total compliance – Antibiotics to be taken strictly as prescribed.	Do not prescribe antibiotics irrationally.
Awareness – Clinicians should treat the diseases with the specific antimicrobials.	Save – Do not save antibiotics for next bout of illness.
Need – Antibiotics to be prescribed only when necessary.	Do not prescribe antibiotics for infections like cold, flu etc. Antivirals should be used for treating viral infections.
Education – Awareness programmes for the community about the appropriate use of antibiotics.	Usage – Do not use leftover antibiotics or share them.
Law – enforced legislation should be introduced to stop antibiotic sale without prescription.	Do not indulge in self-medication. Do not use antibiotics often.
Research – to be encouraged for new drug development and innovations.	Do not leave the antibiotic course in between even if the patient is better.

Fig. 1 The different drug resistance strategies acquired by the microbes are depicted in the above figure. (Source: http://www.scq.ubc.ca/cationic-peptides/)

References

[1] Groom AV, Wolsey DH, Naimi TS, Smith K, Johnson S, Boxrud D, Moore KA, Cheek JE. Community-acquired methicillin-resistant Staphylococcus aureus in a rural American Indian community. JAMA. 2001; 286(10):1201-1205.

[2] Garau J. Treatment of drug-resistant pneumococcal pneumonia. The Lancet Infectious Diseases. 2002;2:404-415.

[3] Kimberlin DW, Whitley RJ. Antiviral resistance: mechanisms, clinical significance, and future implications. J Antimicrob Chemo. 1996; 37:403-421.

[4] Loeffler J, Stevens DA. Antifungal drug resistance. Clin Infect Dis. 2003; 36: S31-S41.doi: 10.1086/344658.

[5] Hyde JE. Drug-resistant malaria - an insight. FEBS J. 2007; 274: 4688–4698. doi:10.1111/j.1742-4658.2007.05999.x

[6] Duckworth G. Clinical implications of antimicrobial resistance: how big is the problem? Int J Infect Dis.2002;6:S1-S7.

[7] Silley P. Susceptibility testing methods, resistance and breakpoints: what do these terms really mean? Rev Sci Tech. 2012; 31:33-41.

[8] Kelesidis T, Karageorgopoulos DE, Kelesidis I, Falagas ME. Tigecycline for the treatment of multidrug-resistant Enterobacteriaceae: a systematic review of the evidence from microbiological and clinical studies. J Antimicrob Chemother. 2008; 62:895-904. doi: 10.1093/jac/dkn311

[9] Jeyaratnam D, Whitty CJM, Phillips K, Liu D, Orezzi C, Ajoku U, French GL. Impact of rapid screening tests on acquisition of meticillin resistant Staphylococcus aureus: cluster randomised crossover trial. BMJ. 2008;336:927-930. doi:10.1136/bmj.39525.579063

[10] Barna JC, Williams DH.The structure and mode of action of glycopeptide antibiotics of the vancomycin group. Annu Rev Microbiol. 1984;38:339-537.

[11] Meroueh SO, Bencze KZ, Hesek D, Lee M, Fisher JF, Stemmler TL, Mobashery S. Three-dimensional structure of the bacterial cell wall peptidoglycan. Proc Natl Acad Sci USA. 2006;21:4404-4409.

[12] Falagas ME, Kasiakou SK. Colistin: The Revival of Polymyxins for the Management of Multidrug-Resistant Gram-Negative Bacterial Infections. Clin Infect Dis. 2005;40:1333-1341. doi: 10.1086/429323.

[13] Ferraro MJ. The rise of fluoroquinolone resistance: fact or fiction. J Chemother. 2002;14:31-41.

[14] Borges-Walmsley MI, McKeegan KS, Walmsley AR. Structure and function of efflux pumps that confer resistance to drugs. Biochem J. 2003;376:313–338 doi: 10.1042/BJ20020957.

[15] Nikaido H. Antibiotic resistance caused by gram-negative multidrug efflux pumps. Clin Infect Dis.1998;27:S32-41.

[16] Tenover FC. Mechanisms of antimicrobial resistance in bacteria. Am J Med. 2006;119:S3-10; discussion S62-70.

[17] Woo PC, To AP, Lau SK, Yuen KY. Facilitation of horizontal transfer of antimicrobial resistance by transformation of antibiotic-induced cell-wall-deficient bacteria. Med Hypotheses.2003;61:503-508.

[18] Paterson DL, Bonomo RA. Extended-Spectrum β-Lactamases: a Clinical Update. Clin Microbiol Rev. 2005;18:657-686.

[19] Wright GD. Aminoglycoside-modifying enzymes. Curr Opin Microbiol. 1999. 2:499-503.

[20] Doi Y, Arakawa Y. 16S Ribosomal RNA Methylation: Emerging Resistance Mechanism against A minoglycosides. Clin Infect Dis. 2007; 45:88–94.

[21] Llano-Sotelo B. Aminoglycosides modified by resistance enzymes display diminished binding to the bacterial ribosomal aminoacyl-tRNA site. Chem Biol. 2002;9:455-463.

[22] Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH, Lynfield R, Dumyti G, Townes JM, Craig AS, Zell ER, Fosheim GE, McDougal LK, Carey RB, Fridkin SK. Invasive methicillin-resistant Staphylococcus aureus infections in the United States. JAMA. 2007;298:1763–1771.

[23] Cui L, Iwamoto A, Lian JQ, Neoh HM, Maruyama T, Horikawa Y, Hiramatsu K. Novel mechanism of antibiotic resistance originating in vancomycin-intermediate Staphylococcus aureus. Antimicrob Agents Chemother. 2006;50:428–438.

[24] Weber SG, Gold HS, Hooper DC, Karchmer AW, Carmeli Y. Fluoroquinolones and the Risk for Methicillin-resistant Staphylococcus aureus in Hospitalized Patient. Emerg Infect Dis.2003;11:1415–1422.

[25] Opal SM, Calandra T. Antibiotic Usage and Resistance: Gaining or Losing Ground on Infections in Critically Ill Patients? JAMA. 2009;302:2367-2368. doi:10.1001/jama.2009.1774.

[26] Brusselaers N, Vogelaers D, Blot S. The rising problem of antimicrobial resistance in the intensive care unit. Ann Intensive Care. 2011;1:47. doi:10.1186/2110-5820-1-47.

[27] Smith DL, Dushoff J, Perencevich EN, Harris AD, Simon AL. Persistent colonization and the spread of antibiotic resistance in nosocomial pathogens: Resistance is a regional problem. Proc Natl Acad Sci USA. 2004;10:3709-3714.

[28] Sotgiu G, Ferrara G, Matteelli A, Richardson MD, Centis R, Ruesch-Gerdes S, Toungoussova O, Zellweger J-P, Spanevello A, Cirillo D, Lange C, Migllori GB. Epidemiology and clinical management of XDR-TB: a systematic review by TBNET. Eur Respir J.2009; 33: 871–881.

[29] Oteo J, Delgado-Iribarren A, Vega D, Bautista V, Rodriguez MC, Velasco M, Saavedra JM, Perez-Vazquez M, Garcia-Cobos S, Martinez-Martinez L, Campos J. Emergence of imipenem resistance in clinical Escherichia coli during therapy. Int J Antimicrob Agents. 2008;32:534–537.

[30] Zasloff M. Antimicrobial peptides of multicellular organisms. Nature. 2002;415:389-395. doi:10.1038/415389a

[31] Upton M, Cotter P, Tagg J. Antimicrobial peptides as therapeutic agents. Int J Microbiol. 2012; doi:10.1155/2012/326503.

[32] Hancock RE, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol.2006;24:1551-1557.

[33] Zhang L, Pornpattananangku D, Hu CM, Huang CM. Development of nanoparticles for antimicrobial drug delivery. Curr Med Chem. 2010;17:585-594.