

Antibiotic Resistance: Molecular Mechanisms of Resistance

Sandip Zine^{1,*}, Parth Mehta², Ankita Rai², Sayli Sawant²

Abstract

Antibiotics are called as 'Wonder Drugs', the miracles of modern sciences. They help combat bacteria and prevent their growth. But due to unmonitored and uncontrolled consumption of these antibiotics, there is an emergence of antibiotic resistance. This has led to a series of downfall in the therapeutic potency and efficacy of many drugs. More and more bacteria are becoming Multi-Drug Resistant (MDR); such highly resistant strains of certain bacteria are called Superbugs. The misuse and overconsumption of many antibiotics, has led to a steep rise in the number of antibiotic-resistant strains at a global level, thus elevating global mortality and morbidity rates. This prompted World health Organisation in 2011 to coin a slogan "No action today, no cure tomorrow". This review gives a piece of detailed information on the origin, molecular mechanisms of antibiotic resistance, supported by factual data and case studies.

Keywords: Superbugs, antibiotics, antibiotic resistance, antivirulence therapy, MDR

INTRODUCTION

Antibiotics are mediators that destroy or slow down the growth of bacteria. They generally interfere with either the formation of the bacterial cell or its contents [1]. They are of two types: Broad Spectrum and Narrow Spectrum.

Broad spectrum antibiotics act on both gram-positive and gram-negative. Narrow spectrum antibiotics are preferred over broad-spectrum as they target a certain type of bacteria, mainly gram-negative bacteria, as they are found to be more resistant due to impenetrable cell wall and efflux pumps.

Antibiotic resistance is the ability of a micro-organism to withstand the effect of an antibiotic. Antibiotic resistance is a subset of Anti-microbial resistance (AMR), as it applies only to bacteria becoming resistant to antibiotics [2]. This occurs when bacteria mutates or modifies itself in such a way that it reduces or nullifies the effectiveness and therapeutic properties of antibiotics [3]. This generally occurs by the unmonitored and casual use of antibiotics. Alexander Fleming was the first person who cautioned about the emergence of penicillin resistant strains [4].

*Author for Correspondence

Sandip Zine

¹Assistant Professor, Department of Pharmaceutical Chemistry, Vivekanand Education Society's College of Pharmacy, Collector Colony, Chembur, Mumbai, Maharashtra, India

²Student, Department of Pharmaceutical Chemistry, Vivekanand Education Society's College of Pharmacy, Collector Colony, Chembur, Mumbai, Maharashtra, India

Received Date: November 17, 2020

Accepted Date: November 19, 2020

Published Date: April 19, 2021

Citation: Sandip Zine, Parth Mehta, Ankita Rai, Sayli Sawant. Antibiotic Resistance: Molecular Mechanisms of Resistance. Research & Reviews: A Journal of Microbiology & Virology. 2021; 11(1): 1–8p.

Antimicrobial agents, sulfonamides were introduced in 1937; they are a class of very effective microbial agents. Development of specific mechanisms of resistance has to lead to a steep decrease in their therapeutic effectiveness. Sulfonamides resistance was reported first in the late 1930s and the mechanisms persist till today

[4]. Penicillin was discovered by Alexander Fleming in 1928 and even before its introduction, resistant strains were found, containing bacterial penicillinase. Once the antibiotic was used widely, their resistance became widely prevalent around the globe [3]. Antibiotics are considered as miracle of modern sciences. They have significantly reduced mortality rate and improved quality of life across the globe. But due to their casual and unmonitored use, there is an emergence of multi-drug resistant bacteria, leading to difficulties in treating infections, thus leading to death. The World Economic Forum Global Risks reports have indicated antibiotic resistance as one of the biggest threats to human kind [5–7].

MOLECULAR MECHANISMS OF ANTIBIOTIC RESISTANCE

There are various mechanisms of resistance, but they fall into three main categories:

1. Those that minimize the intracellular concentration of the antibiotics as a result of poor penetration into the bacterium or of antibiotic efflux (Prevention of access to target).
2. Those that modify the antibiotic target by genetic mutation or post-translational modifications of the target.
3. Those that inactivate the antibiotic by hydrolysis or modifications.

Prevention of Access to the Target

Reduced Permeability

Compared to Gram-positive, Gram-negative bacteria are less permeable to many antibiotics, due to the presence of an additional outer membrane which acts as a permeability barrier [8, 9]. Being lipophilic in nature it prevents the entry of hydrophilic antibiotics. Hydrophilic antibiotics enter through membrane porin proteins present on the outer membrane. They are generally non-specific and have many drug binding sites.

One of the important mechanisms of resistance, shown by *Enterobacteriaceae*, *pseudomonas spp*, etc. is a significant reduction in porin expression. This generally occurs by mutation. Mutations can reduce porin production or produce mutant porin alleles that may be produced, overall preventing entry of the drug into the cell. The classical example of this is the resistance of carbapenems in species of *Enterobacteriaceae* which do not produce carbapenemase (enzymes that degrade carbapenems), resistance occurs due to mutation of porin genes and genes that regulate the expression of porins. The mutation occurs due to selective pressure exerted by carbapenems [10, 11].

Increased Efflux

Bacterial efflux pumps actively transport antibiotics out of the cell and are major contributors to intrinsic resistance. They are more prevalent in gram-negative bacteria than gram-positive ones. When they are overexpressed, they can lead to high levels of resistance. They are of two types, few are substrate-specific like Tet pumps, and others transport a wide variety of molecules out of the cell thus called Multi-Drug Resistant (MDR) efflux pumps. Most MDRs occur due to increased expression of efflux pumps and repression of porin proteins. Increased expression of efflux genes occurs due to mutation of the regulatory network controlling the expression of the proteins. Mutations can occur in the local repressor, transcription factors, or intergenic sites thus altering expressions of efflux genes. For example, in *Enterobacteriaceae* increased expression of transcription factors, including MarA, SoxS, RamA, or Rob increase production of efflux proteins. Mutations can also lead to improved promoters showing stronger activity [12, 13].

Environmental factors can also lead to the stimulation of genes, thus causing resistance as shown in Figure 1. For example, the *acrAB* genes in *E. coli* and *Salmonella* spp. can be induced by small molecules like indole and bile, which can be found in the body [14–16].

Changes in the Antibiotic Target by Mutation

Antibiotics work by binding to certain targets; this binding causes a cascade of events thus leading to overall cell lysis. Mutation can induce changes in the target structure thus preventing efficient

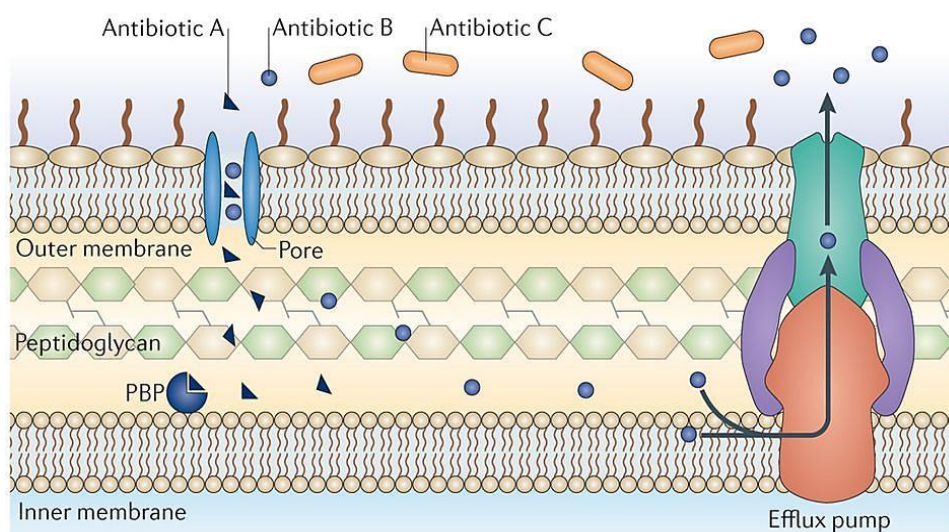


Figure 1. Intrinsic mechanisms of resistance [8].

binding of antibiotics to the target, thus leading to resistance. These mutations are generally single point and occur in the genetic code. Due to natural selection, the mutant bacteria thrive and reproduce and in due course of time, the entire population becomes resistant to the antibiotic.

For example, linezolid, a new drug (introduced 10 years ago) which binds to 23s rDNA ribosomal unit of many Gram-positive bacteria like *S. aureus* or *S. pneumoniae*, thus preventing their proliferation. Mutation and recombination between homologous alleles has led to the emergence of highly resistant strains. Transformation can also lead to resistance. It involves uptake of DNA from the environment. This may cause target protein modification by the formation of mosaic genes. For example, resistant strains of *S. pneumoniae* formed by mosaic penicillin-binding proteins (pbp) genes, which code for penicillin insensitive enzymes [17–19].

Modification (and Protection) of Targets

Protection by modification of target is the key pathway for antibiotic resistance. This does not involve mutation of genes encoding for the target. This is a relevant mechanism for antibiotic resistance for many important antibiotics. For example, the erythromycin ribosome methylase(erm) family of genes methylates 16S rRNA thus changes the drug's target binding site, as shown in Figure 2. This overall makes the bacteria resistant to various antibiotics like macrolides etc. [20]. The qnr genes, present in plasmids encode pentapeptide repeat proteins (PRPs), which bind and protect to Topoisomerase IV and DNA gyrase from quinolones type antibiotics [21].

Daptomycin specifically targets the anionic phospholipid membrane of Gram-positive bacteria like *S. aureus*. It inserts into the membrane in the presence of calcium ions and further depolarises the membrane, causing leakage of contents. mprF gene mutation may encode for a protein, which may lead to remodelling of the membrane, by adding L-Lys into the membrane. This may change the surface charge and composition of the membrane, thus leading to resistance [22, 23].

Direct Modification of Antibiotics

Inactivation by Hydrolysis

This involves enzyme-catalysed destruction on antibiotics. This method is of major relevance after the discovery of penicillin (antibiotic containing β -lactam ring). It was found that many bacteria developed β -lactamases enzyme (first discovered in 1940) which hydrolyses the β -lactam ring in penicillin, as shown in Figure 3(a–b). In due course of time, many β -lactamases have emerged which degrade different antibiotics in the same class as cephalosporins, carbapenems, etc. [24–26]. Early β -lactamases were only active against first-generation β -lactams; they were followed by extended-spectrum

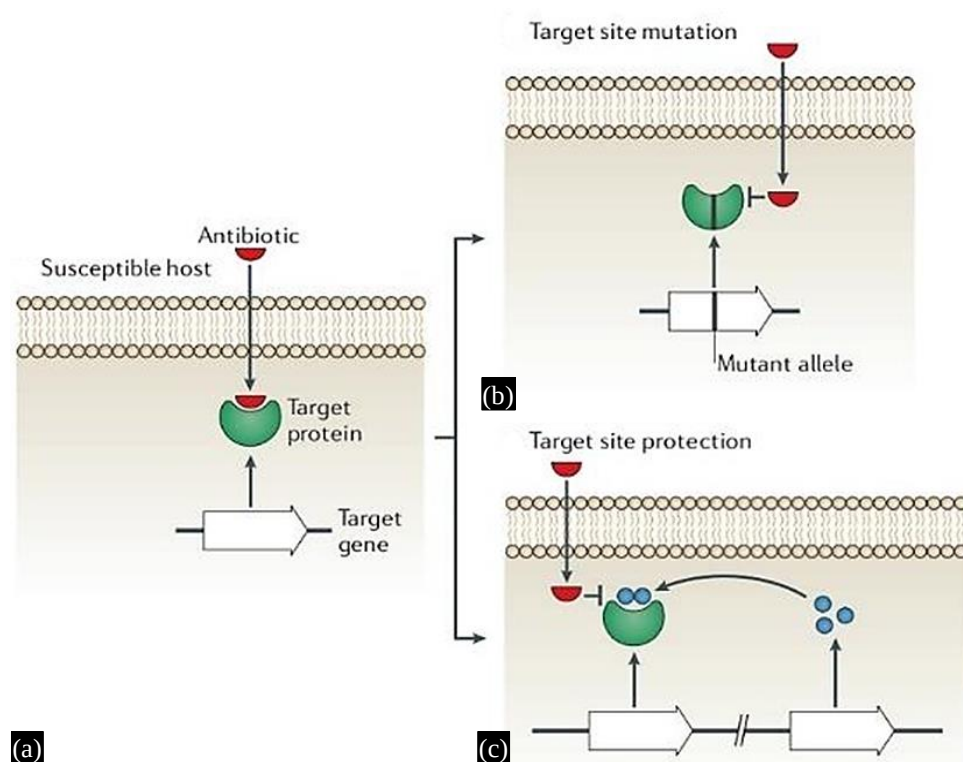


Figure 2. Target site changes. (a) A host in which an antibiotic is able to bind strongly to its target and exert an inhibitory effect, (b) Mutation of the target site, (c) Modification of the target by addition of a chemical functional group [20].

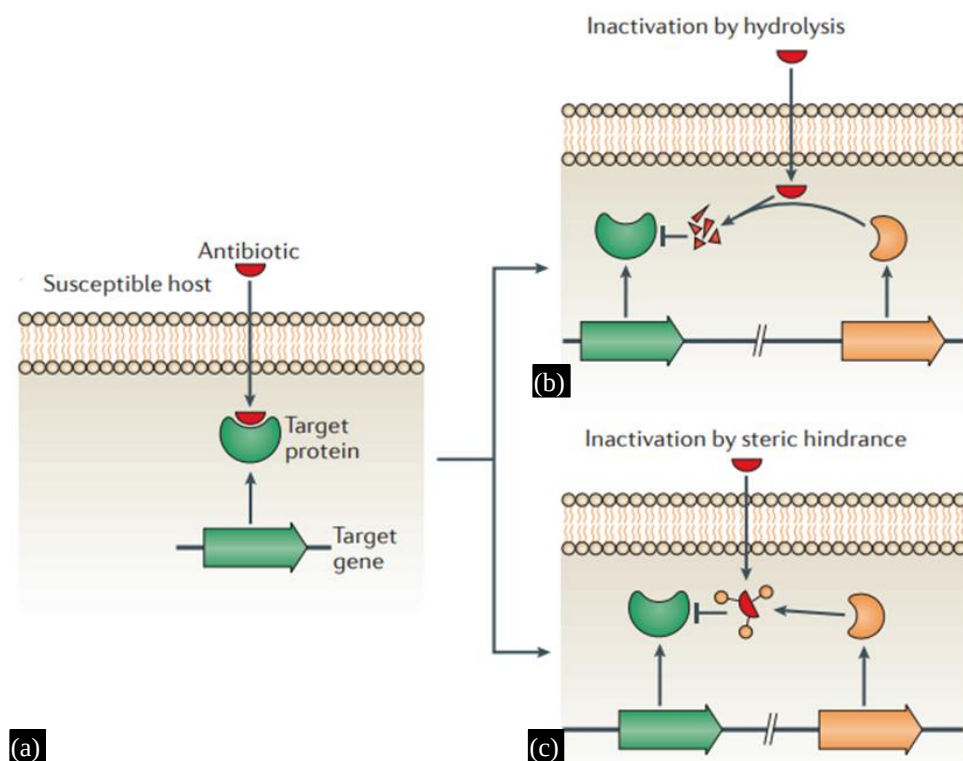


Figure 3. Direct interactions with antibiotics (a) A susceptible host with a target that is efficiently inhibited by an antibiotic, (b) Acquisition and production of an enzyme that destroys the antibiotic, (c) acquisition and production of an enzyme that modifies the structure of the antibiotic [23].

β -lactamases (ESBLs) that have an action against a wide range of drugs like oxyimino-cephalosporins, etc. These enzymes are prominent in various bacterial species like *K. pneumoniae*, *E. coli*, *P. aeruginosa*, and *A. baumannii*, thus making it difficult to treat serious diseases caused by these bacteria [26–28].

Inactivation of Antibiotics by Transfer of a Chemical Group

Certain bacterial enzymes inactivate the antibiotic by adding chemical groups to certain sites on the antibiotic molecule. This in turn leads to changes in the molecule, which thus prevents it from binding to its target protein as a result of steric hindrance; thus leading to antibiotic resistance, as shown in Figure 3c. Various groups that can be added include acyl, phosphate, nucleotidyl, and ribityl, etc. [29].

Aminoglycosides antibiotics are extremely susceptible to such resistance. Aminoglycosides are large molecules with many exposed hydroxyl and amide groups, which can act as active sites for attachment of chemical groups. This is done by a special class of enzymes called aminoglycoside-modifying enzymes. They are of three sub-types: acetyltransferases, phosphotransferases, and nucleotidyltransferases. These classes are evolutionarily diverse and vary in the aminoglycosides that they can modify and in the part of the molecule that they modify [30].

Molecular Mechanism of Antibiotic Resistance in *Staphylococcus aureus*

Staphylococcus aureus is a common colonizer in skin and nares; it is a pathogenic organism responsible for both trivial and life-threatening infections [31]. *Staphylococcus aureus* is of two types: methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA). Characteristically MRSA strains are more often multi-drug resistant; they are resistant to a wide range of antibiotic classes such as macrolides, aminoglycosides, and fluoroquinolones. They are quick to adapt to the new environment and develop quick mutations, thus leading to a high degree of resistance [32, 33].

In the beginning, the MRSA spreading was slow but in the 1980s, many highly resistant strains were found among hospital patients and soon it became a major nosocomial pathogen, throughout the world. Since 1999 a new strain with unusual propensity was found among children and adults, causing serious infections [34–36]. *S. aureus* has two major mechanisms for mutation, one being hydrolysis of antibiotic caused by enzymes and the other is mutation. It has an extracellular enzyme called penicillinase (β -lactamase), which hydrolyses the amide bond in penicillin, thus destroying the antibiotic and causing resistance. The production of the enzyme is coded by structural gene *blaZ* and is controlled by two regulatory genes *blaR1* and *blaI*. The presence of penicillin leads to a cascade of events thus contributes to enhanced penicillinase expression, thus causing resistance. These genes are also encoded in plasmids; thus, this can lead to horizontal gene transfer in the bacterial population [37].

Mutation can also lead to resistance. The bacteria contain various penicillin-binding proteins (PBP), which aid in the synthesis of peptidoglycan chains constituting the bacterial cell walls. Penicillin binds to these PBPs thus disrupting the cell wall biosynthesis and killing the bacteria. Mutation has caused to form an additional penicillin-binding protein (PBP), designated PBP2a, with reduced affinity for penicillin and β -lactams in general. Thus, these antibiotics cannot inhibit PBP2a; thus the biosynthesis of peptidoglycan is not fully inhibited, thus overall making the antibiotic ineffective and leading to resistance. PBP2a is the product of the gene *mecA* and its regulatory genes *mecI* and *mecR1*. Both the sequences and the organization of these genes are homologous to those of the *bla* gene complex [38, 39].

CONCLUSION

Frequent and uncontrollable use of antibiotics across the globe for medicine in human medicine, horticulture, animal medicine etc. has led to extensive rise in antibiotic resistance. Overuse of these drugs has led to evolutionary pressure causing strong emergence of antibiotic resistance among many strains of bacteria. Studies of resistance development and mechanisms of resistance are crucial during

early drug development. Knowing these mechanisms, we can rapidly evaluate emergence of resistance to new drugs. Knowledge of various mechanisms of resistance helps to rule out development of new agents which are highly susceptible to existing mechanisms of resistance, thus may fail in clinic. Long term advantages of studying resistance is that newer synthetic drug can be made which are unaffected by the existing mechanisms of resistance. Even in the short run, knowledge of resistance can help develop special dosing regimens consisting a synergistic combination of various drugs, thus overall minimizing emergence and effects of resistance.

REFERENCES

1. Levy SB, Marshall B. Antibacterial Resistance Worldwide: Causes, Challenges and Responses. *Nat Med.* 2004; 10(12Suppl): 122–129p.
2. Overview of Bacteria. 2017. <http://www.merckmanuals.com/home/infections/bacterial-infections/overview-of-bacteria>
3. Chopra R, Alderborn G, Podczek F, et al. The Influence of Pellet Shape and Surface Properties on the Drug Release from Uncoated and Coated Pellets. *Int J Pharm.* 2002; 239(1–2): 171–178p.
4. Aminov RI. A Brief History of the Antibiotic Era: Lessons Learned and Challenges for the Future. *Front Microbiol.* 2010; 1: 134p.
5. Walker D, Fowler T. Annual Report of the Chief Medical Officer. Vol. 2. Infections and the Rise of Antimicrobial Resistance. Department of Health; 2011.
6. World Economic Forum. Global Risks 2013. 8th Edn. 2013. <http://www.weforum.org/reports/global-risks2013-eighth-edition>.
7. World Economic Forum. Global Risks 2014. 2014. Report <http://www.weforum.org/reports/global-risks-2014-report>.
8. Kojima S, Nikaido H. Permeation Rates of Penicillins Indicate that *Escherichia coli* Porins Function Principally as Nonspecific Channels. *Proc Natl Acad Sci USA.* 2013; 110(28): E2629–E2634p.
9. Vargiu AV, Nikaido H. Multidrug Binding Properties of the AcrB Efflux Pump Characterized by Molecular Dynamics Simulations. *Proc Natl Acad Sci USA.* 2012; 109(50): 20637–20642p.
10. Baroud M, et al. Underlying Mechanisms of Carbapenem Resistance in Extended-Spectrum β -Lactamase-Producing *Klebsiella pneumoniae* and *Escherichia coli* Isolates at a Tertiary Care Centre in Lebanon: Role of OXA-48 and NDM-1 Carbapenemases. *Int J Antimicrob Agents.* 2013; 41(1): 75–79p.
11. Wozniak A, et al. Porin Alterations Present in Noncarbapenemase-Producing Enterobacteriaceae with High and Intermediate Levels of Carbapenem Resistance in Chile. *J Med Microbiol.* 2012; 61(9): 1270–1279p.
12. Bailey AM, et al. RamA, a Member of the AraC/XylS Family, Influences both Virulence and Efflux in *Salmonella enterica* serovar Typhimurium. *J Bacteriol.* 2010; 192(6): 1607–1616p.
13. Pomposiello PJ, Bennik MH, Demple B. Genome-Wide Transcriptional Profiling of the *Escherichia coli* Responses to Superoxide Stress and Sodium Salicylate. *J Bacteriol.* 2001; 183(13): 3890–3902p.
14. Baucheron S, Nishino K, Monchaux I, et al. Bile-Mediated Activation of the *acrAB* and *toIC* Multidrug Efflux Genes occurs Mainly through Transcriptional Derepression of *ramA* in *Salmonella enterica* serovar Typhimurium. *J Antimicrob Chemother.* 2014; 69(9): 2400–6p. doi: 10.1093/jac/dku140. Epub 2014 May 9. PMID: 24816212.
15. Hirakawa H, Inazumi Y, Masaki T, Hirata T, Yamaguchi A. Indole Induces the Expression of Multidrug Exporter Genes in *Escherichia coli*. *Mol Microbiol.* 2005; 55(4): 1113–1126p.
16. Nikaido E, Giraud E, Baucheron S, et al. Effects of Indole on Drug Resistance and Virulence of *Salmonella enterica* serovar Typhimurium Revealed by Genome-Wide Analyses. *Gut Pathogens.* 2012; 4(1): 5p. <https://doi.org/10.1186/1757-4749-4-5>.
17. Billal DS, Feng J, Leprohon P, et al. Whole Genome Analysis of Linezolid Resistance in *Streptococcus pneumoniae* Reveals Resistance and Compensatory Mutations. *BMC Genomics.* 2011; 12(1): 512p.

18. Gao W. et al. Two Novel Point Mutations in Clinical *Staphylococcus aureus* Reduce Linezolid Susceptibility and Switch on the Stringent Response to Promote Persistent Infection. *PLoS Pathog.* 2010; 6(6): e1000944p.
19. Leclercq R. Mechanisms of Resistance to Macrolides and Lincosamides: Nature of the Resistance Elements and Their Clinical Implications. *Clin Infect Dis.* 2002; 34(4): 482–492p.
20. Kumar N, et al. Crystal Structure of the Transcriptional Regulator Rv1219c of *Mycobacterium Tuberculosis*. *Protein Sci.* 2014; 23(4): 423–432p.
21. Vetting MW, et al. Structure of QnrB1, a Plasmidmediated Fluoroquinolone Resistance Factor. *J Biol Chem.* 2011; 286(28): 25265–25273p.
22. Randall CP, Mariner KR, Chopra I, O'Neill AJ. The Target of Daptomycin is Absent from *Escherichia coli* and Other Gram-Negative Pathogens. *Antimicrob Agents Chemother.* 2013; 57(1): 637–639p.
23. Blake KL, O'Neill AJ. Transposon Library Screening for Identification of Genetic Loci Participating in Intrinsic Susceptibility and Acquired Resistance to Anti-Staphylococcal Agents. *J Antimicrob Chemother.* 2013; 68(1): 12–16p.
24. Livermore DM. Defining an Extended-Spectrum Betalactamase. *Clin Microbiol Infect.* 2008; 14(Suppl. 1): 3–10p.
25. Nordmann P, Poirel L, Walsh TR, Livermore DM. The Emerging NDM Carbapenemases. *Trends Microbiol.* 2011; 19(12): 588–595p.
26. Voulgari E, Poulou A, Koumaki V, Tsakris A. Carbapenemase-Producing Enterobacteriaceae: Now that the Storm is Finally Here, How will Timely Detection Help Us Fight Back? *Future Microbiol.* 2013; 8(1): 27–39p.
27. Johnson AP, Woodford N. Global Spread of Antibiotic Resistance: The Example of New Delhi Metallo- β -Lactamase (NDM)-Mediated Carbapenem Resistance. *J Med Microbiol.* 2013; 62(Pt 4): 499–513p.
28. Lynch III, JP Clark NM, Zhanel GG. Evolution of antimicrobial resistance among Enterobacteriaceae (focus on extended spectrum β -lactamases and carbapenemases). *Expert Opinion on Pharmacotherapy.* 2013;14(2),199–210p.
29. Wright GD. Bacterial Resistance to Antibiotics: Enzymatic Degradation and Modification. *Adv Drug Delivery Rev.* 2005; 57(10): 1451–1470p.
30. Norris AL, Serpersu EH. Ligand Promiscuity through the Eyes of the Aminoglycoside N3 acetyltransferase*ii*a. *Protein Sci.* 2013; 22(7): 916–928p.
31. Levy SB, Marshall B. Antibacterial Resistance Worldwide: Causes, Challenges and Responses. *Nat Med.* 2004; 10(Suppl. 12): S122–S129p.
32. Jones M, Karlowsky J, Draghi D, Thornsberry C, Sahm D, Nathwani D. Epidemiology and Antibiotic Susceptibility of Bacteria Causing Skin and Soft Tissue Infections in the USA and Europe: A Guide to Appropriate Antimicrobial Therapy. *Int J Antimicrob Agents.* 2003; 22(4): 406–419p.
33. Jones M, Draghi D, Thornsberry C, Karlowsky J, Sahm D, Wenzel R. Emerging Resistance among Bacterial Pathogens in the Intensive Care Unit. A European and North American Surveillance Study (2000–2002). *Ann Clin Microbiol Antimicrob.* 2004; 3: 14p.
34. Panlilio A, Culver D, Gaynes R et al. Methicillin-Resistant *Staphylococcus aureus* in US Hospitals, 1975–1991. *Infect Control Hosp Epidemiol.* 1992; 13(10): 582–586p.
35. Oliveira D, Tomasz A, de Lencastre H. Secrets of Success of a Human Pathogen: Molecular Evolution of Pandemic Clones of Methicillin-Resistant *Staphylococcus aureus*. *Lancet Infect Dis.* 2002; 2(3): 180–189p.
36. Stefani S, Varaldo P. Epidemiology of Methicillin-Resistant *Staphylococci* in Italy. *Clin Microbiol Infect.* 2003; 9(12): 1179–1186p.
37. Zhang H, Hackbarth C, Chansky K, Chambers H. A Proteolytic Transmembrane Signaling Pathway and Resistance to β -Lactams in *Staphylococci*. *Science.* 2001; 291(5510): 1962–1965p.
38. Pinho M, Filipe S, de Lencastre H, Tomasz A. Complementation of the Essential Peptidoglycan Transpeptidase Function of Penicillin-Binding Protein 2 (PBP2) by the Drug Resistance Protein PBP2A in *Staphylococcus aureus*. *J Bacteriol.* 2001; 183(22): 6525–6531p.

-
39. Pinho MG, de Lencastre H, Tomasz A. An Acquired and a Native Penicillin-Binding Protein Cooperate in Building the Cell Wall of Drug-Resistant Staphylococci. *Proc Natl Acad Sci USA*. 2001; 98(19): 10886–10891p.