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Review paper

A REVIEW ON ANTIBIOTIC RESISTANCE: ORIGIN AND MECHANISMS OF BACTERIAL RESISTANCE AS BIOLOGICAL PHENOMENON

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Antimicrobial resistance is a natural biological phenomenon in their struggle for existence and represents a major health care issue, associated with high mortality and morbidity. The first cases of mass emergence of resistant strains were observed in the middle of the 20th century, and since then cases of resistance have been reported all over the world, and in the last two decades even more frequently are reported multiple bacterial resistance. Factors that contribute to the development of bacteria resistance are abuse in the use of antibacterial agents, in humans or livestock, and releases of antibacterial agents into the environment. Moreover, the development of new effective antibiotics is decreasing, contrary to increasing the overall effort for the synthesis of new ones. Identification and reporting of bacterial resistance, as well as monitoring of the use of antibiotics in the outpatient and inpatient setting, today is the obligation of all countries.

Keywords: bacterial resistance, mechanisms, metagenomics, natural products, *P. aerugonosa*, *S. aureus*, *M. tuberculosis*, *Acinetobacter*

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INTRODUCTION

Antibiotic resistance was to occur when a drug loses its ability to inhibit bacterial growth effectively. Bacteria become 'resistant' and continue to multiply in the presence of therapeutic levels of the antibiotics (ZAMAN *et al*., 2017). Microorganisms generally acquire antibiotic resistance by genetic changes, but sometimes they do so by non-genetic mechanisms (MUNITA and ARIES, 2016).

Non-genetic resistance occurs when certain bacterial strains temporarily change to L forms that lack most of their cell walls. For several generations, while the cell wall is lacking, these organisms are resistant to antibiotics that act on the cell walls. However, when they revert to producing cell walls they again become susceptible to the antibiotics (WALSH, 2000).

Genetic resistance to antimicrobial agents develops genetic changes followed by natural selection. The *Mollicutes* of bacteria which genetically lack the cell walls, and is not affected with the main classes of antimicrobial agents, such as β-lactam, glycopeptides, and phosphomycin (CHREMOVA *et al*., 2016).

Genetic resistance might be the chromosomal and extra-chromosomal. Chromosomal resistance can be spontaneous, which is very rare. Further, in most bacteria, mutations occur spontaneously at a rate of about 1 per 10 million to 10 billion organisms. Bacteria reproduce rapidly and among them there will always be a few mutants. If a mutant happens to be resistant to antibiotics in the environment, after a few generations, most survivors will be resistant to this antimicrobial agent. Antibiotics do not induce mutations, but they can create environments which could favor the survival of resistant mutant (WALSH, 2000).

Bacterial resistance occurs due to the existence of extra-chromosomal genetic structures, such as plasmids and transposons, which can be transferred from bacteria to bacteria in the processes known as conjugation, transduction and transformation (COCULESCU, 2009). Bacteria conjugation is one of the main mechanisms whereby bacteria become resistant to antibiotics. Therefore, the search for specific conjugation inhibitors is of interest in the fight against the spread of antibiotic resistances (CABEZON *et al*., 2017). Conjugation is the transfer of DNA through a multi-step process requiring cell to cell contact via cell surface pili or adhesins. It is facilitated by the conjugative machinery which is encoded either by genes on autonomously replicating plasmids or by integrative conjugative elements in the chromosome (WINTERSDORFF *et al*., 2016). ARGs are in many cases associated with conjugative elements such as plasmids or transposons. β-lactam resistance genes are commonly located on plasmids and thus disseminate by inter- and intraspecies conjugation in the *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter* (LERMINIAUX and CAMERON, 2018). The transfer of plasmids in pathogens has led to the worldwide spread of numerous ARGs encoding resistance to β-lactams, quinolones, aminoglycosides, tetracyclines, sulfonamides, and many other drug classes (HUDDLESTON, 2014). The free fragments of DNA, to the environment can be taken up and incorporated into the chromosome of a living bacterium to provide the recipient with new characteristics. This process is called bacterial transformation, and if the incorporated DNA contains genes that encode for resistance to an antibiotic, a previously susceptible bacterium can be "transformed" to now be resistant. In order for transformation to take place, several conditions have to be met. There must be DNA present in the extracellular environment; the recipient bacteria must be in a state of competence; and the translocated DNA must be stabilized, either by integration into the recipient genome, or by recircularisation in the case of plasmid DNA (WINTERSDORFF *et al*., 2016). Several clinically relevant antibiotic resistant pathogens are capable of DNA uptake and natural

transformation, including *Acinetobacter*, *Haemophilus*, *Neisseria*, *Pseudomonas*, *Staphylococcus*, and *Streptococcus* (LERMINIAUX and CAMERON, 2018). Bacteriophages play an important role in shaping the bacterial microbiome in any environment. Through specialized or generalized transduction, bacteriophages can transfer genes that are advantageous to their microbial hosts, in turn promoting their own survival and dissemination (MODI *et al*., 2013). The transferable DNA sequences range from chromosomal DNA to mobile genetic elements, such as plasmids, transposons and genomic islands. Recent studies found that over 70% of hospital fecal samples tested positive for bacteriophages containing ARGs (QUIROS *et al*., 2013), and hospital wastewaters were contaminated with bacteriophages containing ARGs.

In clinical practice, it is important that some bacteria could survive antibiotic therapy in a latent state. For example, some patients with Lyme borreliosis develop a chronic infection because of the ability of *Borrelia* to survive in the latent state. The persistence of *Borrellia* have been confirmed by isolation from clinical material after antibiotic therapy while in some other patients the therapy is successful only after repetition or prolonged use of antibiotics (VEINOVIC *et al.*, 2013; VEINOVIC *et al*., 2015).

Five mechanisms to resistance have been identified so far, and each of them involves the alteration of a different microbial structure. Resistance to antibiotics can be caused by five general mechanisms: 1) alteration of targets (antibiotics can no longer bind to the target); 2) alternation of membrane permeability; 3) development of enzymes (β-lactamases exist in various bacteria and they are capable of breaking β-lactam ring in penicillins); 4) alteration of enzymes; and 5) alteration of metabolic pathways (MC MANUS, 1997; SCHMIEDER and EDWARDS, 2012). Mechanisms of antibiotic resistance in bacteria are shown in Table 1.

Table 1. Mechanisms of antibiotic resistance in bacteria (SCHMIEDER and EDWARDS, 2012)

Antibiotic	Aminoglycosides Amphenicols Antifolates β-lactams Glycopeptides Rifamycins	Aminoglycosides β-lactams	Aminoglycosides β-lactams Macrolides Quinolones Tetracyclines	Sulfonamides Trimethoprim	Aminoglycosides β-lactams Fluoroquinolones Glycopeptides Macrolides Rifamiycins Tetracyclines
Mode(s) resistance	Antibiotic inactivation	Decreased influx	Increased efflux	Target amplification	Target site alteration

Bacteria can develop resistance to antibiotics by mutations of existing genes (vertical evolution) or by acquiring new genes from other strains or species (horizontal gene transfer). The sharing of genes between bacteria by horizontal gene transfer occurs by many different mechanisms. Mobile genetic elements, including phages, plasmids and transposons mediate this transfer, and in some circumstances, the presence of low levels of the antibiotic in the environment is the key signal that promotes gene transfer (DZIDIC *et al*., 2008).

Culture-independent study of resistance through metagenomics

Many studies have identified the presence of antibiotic resistance genes (ARG) from a wide variety of ecological niches: soil, animals (especially edible animals, are sources of ARG

due to antibiotic use in agriculture), fecal pollution of water, human gut microbiomes. The presence of a resistance gene does not indicate if this is the source or sink of the resistance gene (FITZPATRICK *et al*., 2016). The soil is thought to be a substantial source of ARG, and it is thought that antibiotic producing microorganisms in soil are the source of resistance genes found in clinical pathogens (CANTON and COBO, 2009).

The most of the bacterial species from environment have never been cultivated by conventional microbiology techniques. In this situation, a comprehensive analysis of bacterial communities requires the utilization of non-culture-based methods, which have been named metagenomics (GARMENDIA *et al*., 2012; BEJARANO *et al*., 2018). Functional metagenomics involve cloning and expression of DNA in a surrogate host with coupled activity-based screening. Sequence-based metagenomics involves extracting and random sequencing of deoxyribonucleic acid (DNA) directly from the environment. To date, 16S rDNA sequencing, metagenomics and metatranscriptomics are the three basic sequencing strategies used in the taxonomic identification and characterization of environment-related microbiomes. These sequencing strategies have used different next generation sequencing platforms for DNA and RNA sequence identification (CAO *et al*., 2017).

Next generation sequencing (NGS) allows sequencing of the whole genome of numerous pathogens in one sequence run, either from bacterial isolates of (different) patients, or from multiple species present in patient material from one individual (metagenomics) (DEURENBERG *et al*., 2017). For NGS, there is no need for target specific primers, which are needed for tradicionally 16S rDNA sequencing. In a single run, the whole genome of a pathogen is sequenced at random. This approach provides greater sequence resolution than traditional methods by delivering a definitive catalog of genetic polymorphisms, particularly single-nucleotide polymorphisms (SNPs). WGS also associates epidemiology to genome evolution, genome structure, pathogen biology and gene content; which provides insights to biological markers, such as antibiotic resistance and virulence factors (RAMANATHAN *et al*., 2017).

The metagenomics sequences are then compared to known sequences to identify genes and/or mutations (SCHMIEDER and EDWARDS, 2012). The recent analysis of the human gut resistome by using functional metagenomics techniques demonstrated that human microbiota contains a large number of resistance elements. Several of them are distinct from those acquired by human pathogens through horizontal gene transfer (HGT) and probably constitute the intrinsic resistome of those microorganisms. However, the microbiota harbored as well genes coding for TEM-type, CblA, CfxA and CTX-M b-lactamases, which are classical resistance determinants disseminated by HGT. The presence of these genes suggests that they can be maintained in the gut microbiota in the absence of selection and indicates that human microbiota can be a reservoir for HGT-acquired resistance genes (GARMENDIA *et al*., 2012). The research according NESME *et al*. (2014) reported that soil metagenomes have the most diverse pool of antibiotic resistance gene of determinates. The most common types of resistances found in environmental metagenomes were efflux pumps and genes conferring resistance to vancomycin, tetracycline, or β-lactam antibiotics used in veterinary and human healthcare. The most prevalent resistance classes found in the metagenome environments are: ABC class transporter system: bacitracin (bcr, bcr_mfs), RND class transporter (macab), tetracycline-specific efflux pump (tet efflux), penicilin-binding protein (pbp), class A and class B β-lactamases (blaA, blaB) and vancomycin resistance operon genes (vanA, vanB, vanC, vanD, vanE, vanG).

The conventional methods combining culture with molecular methods are enhancing efforts in resistance profiling. Functional metagenomics has become a vital tool for identifying genes that confer resistance to selected antibiotics in environmental samples (MCLAIN *et al*., 2016).

Mechanisms of resistence to antimicrobial agents in different bacteria

Since the discovery and subsequent widespread use of antibiotics, a variety of Gram positive and Gram negative bacteria of human and animal origin have developed numerous mechanisms of antibiotic resistance (DZIDIC *et al*., 2008). In this review we are demonstrated mechanisms some of bacteria.

Staphylococci, together with pneumococci and streptococci, are members of a group of invasive Gram-positive pathogens, known as the pyogenic cocci, which cause various suppurative or pus-forming diseases in humans and animals.

When antibiotic resistance was first encountered among bacteria, including *Staphylococcus aureus*, it was believed to arise solely by mutation and selection. Spontaneous bacterial mutants resistant to certain antibiotics can be generated at frequencies of 10^{-6} to 10^{-8} per cell in the laboratory, and it was assumed that analogous events had occurred in natural populations to produce resistant organisms. Indeed, resistance within the staphylococci to several therapeutically useful antibiotics, including streptomycin, rifampin, fusidic acid, and novobiocin, is thought to be derived by chromosomal mutation. A number of resistance determinants have been mapped on the *S. aureus* chromosome. In some cases, however, chromosomal point mutations, which lead to antibiotic resistance, can be deleterious to the organism, resulting in the creation of less virulent forms. The acquisition of new characters, without affecting the fitness of the bacteria to survive in their natural environment, would therefore be expected to occur over a substantial time span. In an evolutionary sense, then, the accumulation of chromosomal mutations would seem to be unsatisfactory as the sole explanation for the rapid emergence of multidrug resistant bacteria. The relatively minor role played by spontaneous mutation in the stridden appearance of antibiotic-resistant microorganisms was confirmed by the discovery of gene transfer and the demonstration that bacteria can acquire additional genetic material in the form of extrachromosomal or plasmid DNA. The existence of plasmid DNA molecules was suggested by the transfer of discrete genetic units of resistance between bacterial strains and the irreversible loss of such units from cells at relatively high frequencies. Resistance to antimicrobial agents in the staphylococci, as in the gram-negative bacteria, is due to the presence of plasmids that carry the genetic determinants of resistance (LYON and SKURRAY, 1987).

Following the discovery of penicillin, it seemed that *S. aureus* infections were controllable; however, the resistance was short-lived. The discovery and introduction of methicillin in 1959 were thought to be a sure defense against the penicillinases, but the appearance of methicillin resistant *S. aureus* (MRSA) within just 3 years led inexorably to other multidrug resistant variants, and the acronym now denotes multidrug resistant *S. aureus*. MRSA infections can be very serious and are among the most frequently occurring of all antibiotic-resistant threats. MRSA is resistant to penicillin-like β-lactam antibiotics (SENGUPTA *et al*., 2013).

A number of drugs still retain activity against MRSA, including glycopeptides (e.g., vancomycin and teicoplanin), linezolid, tigecycline, daptomycin, and even some new β-lactams, such as ceftaroline and ceftobiprole (PEREZ *et al*., 2008). MRSA has shown outstanding

versatility within emerging and spreading in different epidemiological settings over time (in hospitals, the community, and, more recently, in animals). This connects the epidemiology of MRSA infections and creates a challenge for infection-control systems that focus only on health care–associated infections (HAIs) (ROSSOLINI *et al*., 2014; VENTOLA, 2015).

Although MRSA is often associated with infection in hospitals, community-associated MRSA (CA-MRSA) often cause infections in healthy people living in the community, who have not been in hospital or had any medical procedures. CA-MRSA has most of the properties of MRSA, albeit with different *mec* gene clusters, and has acquired new pathogenicity genes, such as the gene encoding the cytotoxic Panton-Valentine leukocidin (DAVIES and DAVIES, 2010; DE LEO and CHAMBERS, 2009). These are regulated by defined signaling systems (PIDDOCK, 2006).

Acinetobacter is a Gram-negative bacteria that causes pneumonia or bloodstream infections, especially in critically ill patients on mechanical ventilation. Some *Acinetobacter* species have become resistant to all or nearly all antibiotics, including carbapenems, which are often considered to be the drug of last resort (VENTOLA, 2015). Mechanisms of antibiotic resistance of this organism include acquirement of β-lactamases, up-regulation of multidrug efflux pumps, modification of aminoglycosides, permeability defects, and alteration of target sites (LEE *et al*., 2017). Inactivation of β-lactams by β-lactamases is a major antibiotic resistance mechanism in *Acinetobacter baumannii.* Recent studies according TRAGLIA *et al*., (2014) have shown that *A. baumannii* has natural competence to incorporate exogenous DNA and its genome has foreign DNA at high frequencies, implying frequent horizontal gene transfer in this pathogen (LEE *et al*., 2017). Some genes involved in cell division, including *blhA, zipA, zapA, and ftsK*, are associated with intrinsic β-lactam resistance in *A. baumannii* (KNIGHT *et al*., 2016). The very high genetic plasticity of *A. baumannii* allows an accumulation of resistance determinants that give rise to multidrug resistance at an alarming rate. The multidrug resistance in *A. baumannii* seems to result from both the accumulation of multiple mutations and the acquisition of resistance genes from other bacterial genera, with the latter occurring by a variety of mechanisms, including the transfer of plasmids, transposons, and integrons, sometimes leading to the formation of clusters of resistance genes termed resistance islands (POIREL *et al*., 2011).

Tuberculosis (TB) infections are treatable and curable with available of first-line drugs, such as isoniazid or rifampicin; however, in some cases, *Mycobacterium tuberculosis* can be resistant to one or more of these first-line drugs. The careless use of antibiotics has created a selective pressure pushing a rapid evolution of *M. tuberculosis*, marching from mono-drug resistant to the multidrug resistant (MDR), extensively drug resistant (XDR), and eventually totally drug resistant (TDR), through sequential accumulation of resistance mutations (FONSEKA *et al*., 2015). Because the treatment of drug-resistant TB can be a complex, requiring longer treatment periods and more expensive drugs that often have more side effects. Extensively of resistant TB (XDR-TB) is resistant to most TB drugs, including isoniazid and rifampicin, any fluoroquinolones, and any of the three second-line injectable drugs (i.e., amikacin, kanamycin, and capreomycin); Therefore, fewer treatment options are available for patients with XDR-TB, and the drugs that are available are much less effective (GOLKAR *et al*., 2014).

The treatment of MDR-TB patients commonly lasts for 2 years or longer and relies on the use of second-line drugs (such as fluoroquinolones and injectable aminoglycosides) that are less effective, more toxic, and far more costly. The prognosis of patients infected with XDR-TB is extremely poor and the spread of these strains raises the possibility of the return to a pre-

antibiotic era (GAGNEUX *et al*., 2003). The *de novo* emergence of drug resistance in an individual patient can occur as a result of low adherence to treatment, inadequacy of the drug regimen (e.g., wrong antibiotic choices or dosages, poor drug quality), and patient-dependent pharmacodynamic and pharmacokinetic properties of the drugs administered. In recent years, several determinants of acquired resistance to the drugs commonly used in the treatment of TB have been elucidated (FONSECA *et al*., 2015; GANDHI *et al*., 2010).

The mechanisms and pathways that result in the emergence and subsequent fixation of resistant strains of *M. tuberculosis* are not fully understood and recent studies suggest that they are much more complex than initially thought (FONSECA *et al*., 2015). In *M. tuberculosis*, horizontal transfer of drug resistance genes has not been reported; but resistance mostly arises from gene mutations. The genes involved in acquired drug resistance in *M. tuberculosis* are: *katG* (encoded catalase peroxidase), *ndh* (NADH dehidrogenase), *ethA* (flavin monooxygenase), *tlyA* (rRNA methyltransferase), *folC* (dihydrofolate synthase) and others. The mutations in these genes lead to increased gene expression or to enzymatic activities which rescue mycobacteria cells from the activity of antimicrobial agents (NGUYEN, 2016).

Furthermore, there are indications that the acquisition of clinically significant resistance to certain drugs might be a stepwise process that often involves an initial low-level mutation that acts as a gateway for high-level resistance. The clinical implications of these findings should be investigated (FONSECA *et al*., 2015).

Pseudomonas aeruginosa is a common cause of community-acquired and nosocomial acquired pneumonia and is responsible for 10% of all hospital-acquired infections worldwide (MATTA *et al*., 2018). The development of resistance of *P. aeruginosa* to antibiotics is increasing globally due to the overuse of antibiotics (YAYAN *et al*., 2015). Epidemiological outcome studies have shown that infections caused by drug-resistant *P. aeruginosa* are associated with significant increases in morbidity, mortality, need for surgical intervention, length of hospital stay and chronic care, and overall cost of treating the infection (GASINK *et al*., 2006; LISTER *et al*., 2009). *P. aeruginosa* can develop resistance to antibacterials either through the acquisition of resistance genes on mobile genetic elements (i.e., plasmids) or through mutational processes that alter the expression and/or function of chromosomally encoded mechanisms (LISTER *et al*., 2009).

The resistance of *P. aeruginosa* to fuoroquinolone (FQs) is mainly due to: (1) the point mutations in the DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*) genes, (2) the presence of transferable plasmid-mediated quinolone resistance (PMQR) determinants, and (3) mutations in genes regulating the expression of efflux pumps and decreased expression of outer membrane porins (YANG *et al*., 2015). Imported resistance of *P. aeruginosa* to aminoglycosides most commonly includes enzymatic inactivation of the drug molecule through chemical modification. In addition to the variety of aminoglycoside-modifying enzymes, high-level resistance to multiple aminoglycosides can be associated with methylation of the 16S rRNA. This mechanism was first reported for *P. aeruginosa* in 1993, and the methylase encoding gene was designated as *rmtA*. There are currently five characterized ribosomal methyltransferase enzymes (RmtA, RmtB, RmtC, RmtD, and ArmA) that have been found worldwide among clinical isolates of *P. aeruginosa* and *Enterobacteriaceae* (LISTER *et al*., 2009). The *P. aeruginosa* strains possess the *ampC* gene for the inducible chromosomal β–lactamase. However, the spontaneous mutations in regulatory gene *ampR*, can increase the expression of chromosomal β-lactamase genes. These mutants will be selected under the pressure of antibiotic usage, especially where monotherapy is employed (RAMANATHAN *et al*., 2017).

Bacterial multidrag resistence proteins

Lactococcus lactis is a lactic acid bacteria (LAB) widely used as a starter culture in dairy industry. Although lactococci have acquired the „Generally Regarded As Safe"(GRAS) status, many investigators have speculated that lactococci as well as other LAB may act as reservoirs of antibiotic resistance genes. In order to prevent the spread of these genes, the studies on the antibiotic resistance mechanisms and multidrug resistance transporters within them, are of the great importance for lactococci intended for use in the food industry (BOLHUIS *et al*., 1997). Bacterial multidrug resistance (MDR) proteins play an important role in the resistance of bacterial cells to various cytotoxic structurally unrelated compounds such as heavy metals, organic solvents, dyes, disinfectants, and antibiotics. The MDR transporters have been broadly classified into two major classes: the ATP-binding cassette (ABC) primary transporters, which use the hydrolysis of ATP as energy source for transport and the secondary transporters, driven by a proton or sodium motive force (pmf or smf). Studies on bacterial MDR transporters are becoming highly relevant during the last years since the MDR activities have been associated with the ongoing emergence of antibiotic resistance in pathogenic bacteria. Although *L. lactis* is considered to be non-pathogenic and safe to use in starter cultures for cheese production, previous results showed the broad antibiotic specificity of LmrP, LmrA, LmrCD and the most recently described CmbT MDR transporters present in *L. lactis*. MDR transporter could be associated with the mobile genetic elements implicating the possible transfer of the related genes to other bacteria present in food or the gastrointestinal tract, and as the final consequence, a serious threat to the efficacy of valuable antibiotics. Both the pmf-driven and the ABC transporters in *L. lactis* mediate resistance to toxic hydrophobic compounds, mainly cations and antibiotics, although several lactic acid bacteria also possess MDR transporters that mediate the extrusion of anionic antimicrobial compounds possibly conferring resistance to cholate. CmbT multidrug resistance protein of *L. lactis* contributes to extruding the structurally, chemically, and pharmacologically diverse range of substrates out of bacterial cells. This function of CmbT may result in the failure of antibiotic therapy. Homology model of CmbT protein was constructed and further optimized. The 3D-QSAR (Quantitative structure-activity relationships) model predictive potential was proved by use of leave-one-out cross validation of the training set (Q2: 0.69, R2Observed vs: Predicted: 0:918; RMSEE: 0.193) and verification set (R2Observed vs: Predicted: 0:704; RMSEP: 0.289) (LUBELSKI *et al*., 2006). The results showed that high CmbT affinities to ethidium, sulbactam, and sulfathiazole could be related to the absence of significant unfavorable interactions. In contrast, the presence of specific unfavorable interaction between two hydrogen bond donor groups in bacitracin, apramycin, novobiocin, vancomycin, kanamycin, gentamycin, and tobramycin is found to be the main reason for their lower CmbT affinities. In addition, membrane position of the CmbT binding site and positive correlation between substrates lipophilicity (log DpH 5.0) and CmbT affinity strongly indicates that CmbT recognizes its substrates within the membrane (FILIPIC *et al*., 2014; FILIPIC *et al*., 2013).

Natural products from aromatic and medical plants as antimicrobial agens

In the last 10 years, knowledge and expertise in biosynthetic capacity and secondary metabolites of medicinal and aromatic plants has been acquired in Europe and the world. According to World Health Organization (WHO), medicinal plants would be the best source in bioactive compounds to obtain a variety of drugs and alternative antibiotics. The therapeutic effect of essential oils from medical and aromatic plants is related to the content of many

biologically active compounds, including naphthoquinones, flavonoids, terpenoids and phenols (SHARMA *et al*., 2009). These constituents have a wide range of pharmaceutical activities, including anti-inflammatory, antimutagenic, antiviral and antibacterial activities (VUKOVIC GACIC *et al.*, 2006). Also, recent investigations have demonstrated that nanoparticles with essential oils have significant antimicrobial potential against multidrug resistant pathogens (CHOUHAN *et al*., 2017). The significant antimicrobial activity and chemical constituents of essential oils from *Lamiaceae* species: *Mentha piperita* (DAMJANOVIC-VRATNICA *et al*., 2016), *Thymus vulgaris* (DAMJANOVIC-VRATNICA *et al*., 2015) and *Satureja montana* (DAMJANOVIC-VRATNICA *et al*., 2011; DAMJANOVIC-VRATNICA *et al*.,2015a) was also reported. According to the general literature *Lavandula angustifolia* has been used as an antibiotic and in combination with *Pelargonium graveolens* and *Melaleuca alternifolia* demonstrated activity against MRSA (DE RAPPER *et al*., 2013). Further essential oils from *Eucalyptus globulus* and *Melaleuca alternifolia* have effects against *Pseudomonas aeruginosa* (SAMBYA *et al*., 2017). The volatile oil extracts from *Cinnamomum verum* (*Lauraceae*), *Syzygium aromaticum* (*Myrtaceae*), *Ocimum gratissimum* and *Ocimum basilicum* (*Lamiaceae*) have significant antimicrobial effects against *Acinetobacter baumannii*, one of the most important drug resistant pathogens worldwide (INTORASOOT *et al*., 2017). The recent investigation demonstrated that essential oil isolated from leaves of *Tetradenia riparia* and the pure compound 6,7-dehydroroyleanone display good activity against *Mycobacterium tuberculosis* clinical isolates, including MDR isolates, with low cytotoxicity to murine macrophages. The 6,7-dehydroroyleanone compound is a potential candidate for anti-TB drug (BALDIN *et al*., 2018).

The mechanisms of action of essential oils against pathogen microorganisms are different: induced leakage of the cell, loss of membrane integrity, disruption of membrane, cell wall damage (CHOUHAN *et al*., 2017). Essential oils from plant would be promising antimicrobial agents for further treatment of human pathogens, including multidrug resistant bacteria.

CONCLUSIONS

Bacterial resistance to antibiotics has been reported in all parts of the world and it is relatively prevalent. Despite the measures of the WHO and the management of public health institutions, the use of antibiotics in agriculture, veterinary medicine and health care is still relatively high. Treatments for bacterial infections become more intense every day, leading to an increased resistance. Demand for new antibiotics, bioactive molecules derived from aromatic and medical plants increase the chances of creating new antibacterial therapies.

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REFERENCES

BALDIN, V., R.B. DE LIMA SCODRO, M.A. LOPEZ-ORTZ, A.L. DEALMEIDA, Z.C. GAZIM, L. FERARRESE, *et al*. (2018): Anti-*Mycobacterium tuberculosis* activity of essential oil and 6,7-dehydroroyleanone isolated from leaves of *Tetradenia riparia* (Hochst.) Codd (Lamiaceae). Phytomedicine, *47*: 34-39.

BEJARANO, O., Z. PEREZ, C.S. MORA (2018): Microbial resistance from one metagenomic perspective. NOVA, *16* (29): 91-100.

OLHIS, H., W. HENDRIK, H.W. VAN VEEN, B. POOLMAN, A.J.M. DRIESSEN, W.N. KONINGS (1997): Mechanisms of multidrug transporters. FEMS Microbiol. Rev., *21*(1): 55–84.

CABEZON, E., F. CRUZ, I. ARECHAGA (2017): Conjugation inhibitors and their potential use to prevent dissemination of antibiotic resistance genes in bacteria. Frontiers in Microbiology, *8*: 2329.

CANTON, R. and J. COBO (2009): Hospital antibiotic use and resistance: a relationship that is difficult to measure and complex to interpret. Enferm. Infecc. Microbiol. Clin., *27*(8):437-40.

CAO, Y., S. FANNING, S. PROOS, K. JORDAN, S. SRIKUMAR (2017): A Review on the applications of next generation sequencing technologies as applied to food-related microbiome studies. Frontiers in Microbiology, *8*: 1829.

CHERNOVA, O.A., E.S. MEDVEDEVA, A.A. MOUZYKANTOV, N.B. BARANOVA, V.M. CHERNOV (2016): Mycoplasmas and Their Antibiotic Resistance: The Problems and Prospects in Controlling Infections. Acta Naturae, *8*(2):24-34.

CHOUHAN, S., K. SHARMA, S. GULERIA (2017): Antimicrobial activity of some essential oils present status and future perspectives. Medicines, *4*(58): DOI:10.3390/medicines 4030058

COCULESCU, B.I. (2009): Antimicrobial resistance induced by genetic changes. J. Med. Life., *2*(2):114-123.

DAMJANOVIC VRATNICA, B., A. PEROVIC, D. SUKOVIC, S. PEROVIC (2011): Effect of vegetation cycle on chemical content and antibacterial activity of *Satureja montana* L. Arch. Biol. Sci., *63*(4): 1173-1179

DAMJANOVIC VRATNICA, B., S. PEROVIC, D. CAKOVIC (2015): Composition and antimicrobial studies of essential oil of *Thymus vulgaris* from Montenegro. Biologica Nyssana, *6*(2): 67-73.

DAMJANOVIC VRATNICA, B., S. PEROVIC, T. LU, R. SANTOS (2015a): Effects of matrix pretreatmant on the supercritical CO_2 extraction of *Saturaja montana*. Chem. Ind. Chem. Eng. Q., *22*:34-34.

DAMJANOVIC VRATNICA, B.D., D. SUKOVIC, S. PEROVIC (2016): Essential oil components and antimicrobial activity of peppermint (*Mentha piperita*) from Montenegro. Agricult. Forest., *62*(1): 259-268.

DAVIES, J. and D. DAVIES (2010): Origins and Evolution of Antibiotic Resistance. Microbiol. Mol. Biol. Rev., *74*(3):417–433.

DE LEO, F.R. and H.F. CHAMBERS (2009): Reemergence of antibiotic-resistant *Staphylococcus aureus* in the genomics era. J. Clin. Invest., *119*:2464–2474.

DE RAPPER, S., G. KAMATOU, A. VILJOEN, S. VAN VUUREN (2013): The *In Vitro* Antimicrobial Activity of *Lavandula angustifolia* Essential Oil in Combination with Other Aroma-Therapeutic Oils. Evid. Based. Complement. Alternat. Med, Volume 2013, Article ID 852049.

DEURENBER, R.H., E. BATHOORN, M.A. CHLEBOWIEZ, N. COUTO, M. FERDOUS, S. GARCIA-COBOS, M.D. KOOISTRA-SMID, E.C. RAANGS, S. ROSEMA, A. VELOO, K. ZHOU, A.W. FRIEDRICH, J. ROSSEN (2017): Application of next generation sequencing in clinical microbiologyand infection prevention. J. Biotech., *243*: 16-24.

DZIDIC, S., J. SULJKOVIC, B. KOS (2008): Antibiotic Resistance Mechanisms in Bacteria:Biochemical and Genetic Aspects Antibiotic Resistance in Bacteria, Food Technol. Biotech., *4*(1):11–21.

FILIPIC, B., N. GOLIC, B. JOVCIC, M. TOLINACKI, D.C. BAY, R.Y. TURNER, J. ANTIC STANKOVIC, M. KOJIC, LJ. TOPISIROVIC (2013): The cmbT gene encodes a novel major facilitator multidrug resistance transporter in Lactococcus lactis. Res. Microbiol., *164*(1):46-54.

FILIPIC, B., K. NIKOLIC, S. FILIPIC, B. JOVCIC, D. AGBABA, J. ANTIC STANKOVIC, M. KOJIC, N. GOLIC (2014): Identifying the CmbT substrates specificity by using a quantitative structure–activity relationship (QSAR) study. J. Taiwan. Inst. Chem. Eng., *45:764–*771.

FITZPATRICK, D. and F. WALSH (2016): Antibiotic resistance genes across a wide variety of metagenomes. FEEMS Microbiol. Ecolol., *92*(2): 1-8.

FONSEKA, J.D., G.M. KNIGHT, T.D. MCHUGH (2015): The complex evolution of antibiotic resistance in *Mycobacterium tuberculosis*. Int. J. Infect. Dis., *32*:94-100.

GAGNEUX, S., C.D. LONG ,P.M. SMALL, T. VAN, G.K. SCHOOLNIK, B.J. BOHANNAN (2003): The competitive cost of antibiotic resistance in *Mycobacterium tuberculosis*. Science, *312*:1944–1946.

GANDHI, N.R., P. NUNN, K. DHEDA, H.S. SCAAF, M. ZIGNOL, D. VAN SOOLINGEN (2010): Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. Lancet, *375*:1830–1843.

GARMENDIA, L., A HERNANDEZ, M.B. SANCHEZ, LJ. MARTINEZ (2012): Metagenomics and antibiotics. Clin. Microbiol. Infect., *18* (4): 27–31.

HUDDLESTON, J.R. (2014): Horizontal gene transfer in the human gastrointestinal tract: potential spread of antibiotic resistance genes. Infect. Drug Resist., *7*: 167-176.

GASINK L.B., N.O. FISHMAN, M.G. WEINER, I. NACHAMKIN, W.B. BILKER, E. LAUTENBACH (2006). Fluoroquinolone-resistant *Pseudomonas aeruginosa*: assessment of risk factors and clinical impact. Am. J. Med., *119*: 52619–52625.

GOLKAR, Z., O. BAGAZRA, D.G. PACE (2014): Bacteriophage therapy: a potential solution for the antibiotic resistance crisis. J. Infect. Dev. Ctries., *8*(2):129–136.

INTORASOOT, A.P., S. CHORNCHOEM, S. SOOKHHEE, S. INTORASOOT (2017): Bactericidal activity of herbal volatile oil extracts against multidrug-resistant *Acinetobacter baumannii*. J. Intercult. Ethnoph., *6*(2): 218-222.

KNIGHT, D., D. DIMITROVA, D.D. RUDIN, S. D. BONOMO, P.N. RATHER (2016): Mutations decreasing intrinsic β-lactam resistance are linked to cell division in the nosocomial pathogen *Acinetobacter baumannii*. Antimicrob. Agents Chemother., *60*: 3751–3758.

LEE, C.R., J.H. LEE, M. PARK, K.S., PARK, I.K. BAE, Y.B. KIM, C.J. CHA, B.C. JEONG, S.H. LEE (2017): Biology of *Acinetobacter baumannii*: Pathogenesis, Antibiotic Resistance Mechanisms, and Prospective Treatment Options. Front. Cell. Infect. Microbiol., *7*: 1-35.

LERMINIAUX, N.A. and A.D.S. CAMERON (2018): Horizontal transfer of antibiotic resistance genes in clinical environments. Canadian Journal of Microbiology, https://doi.org/10.1139/cjm-2018-0275.

LISTER, P.D., D.J. WOLTER, N.D. HANSON (2009): Antibacterial-resistant *Pseudomonas aeruginosa:* clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin. Microbiol. Rev., *4*:582-610.

LUBELSKI, J., A. DE JONG, R.V. MERKERK, H. AGUSTANDARI, O.P. KUIPERS, J. KOK, A.J.M. DRIESSEN (2006): LmrCD is a major multi drug resistance transporter in *Lactococcus lactis.* Mol. Microbiol., *61*(3):771–781.

LYON, B.R. and R. SKURRAY (1987): Antimicrobial Resistance of *Staphylococcus aureus*: Genetic Basis. Microbiol. Rev., *51*:88–134.

MATTA, R., S. HALLIT, R. HALLIT, W. BAWAB, A.M. ROGUES, P. SALAMEH (2018): Epidemiology and microbiological profile comparison between community and hospital acquired infections: A multicenter retrospective study in Lebanon. Journal of Infection and Public Health, *11*: 405-411.

MCLAIN, J., E. CYTRYN, L. DURSO, S. YOUNG (2016): Culture-based Methods for Detection of Antibiotic Resistance in Agroecosystems: Advantages, Challenges, and Gaps in Knowledge. J. Environ. Qual., *45*:432–440.

MCMANUS, M.C. (1997): Mechanisms of bacterial resistance to antimicrobial agents. Am. J. Health Syst. Pharm., *54*:1420–1433.

MODI, S.R., H. LEE, C. SPINA, J. COLLINS (2013): Antibiotic treatment expands the resistance reservoir and ecological network of the phage metagenome. Nature, *499*: 219-222.

MUNITA, J.M., C.A. ARIAS (2016): Mechanisms of Antibiotic Resistance. Microbiol Spectr., *4*(2):10.1128/microbiolspec.VMBF-0016-2015.

NESME, J., S. CECILLON, T. DELMONT, J.M. MONIER, T. VOGEL, P. SIMONET (2014). Large-Scale Metagenomic-Based Study of Antibiotic Resistance in the Environment. Curr. Biol., *24*:1096–1100.

NGUYEN, L. (2016): Antibiotic resistance mechanisms in *M. tuberculosis*: an update. Arch. Toxicol., *90*(7): 1585–1604.

PEREZ, F., R.A. SALATA, R.A. BONOMO (2008): Current and novel antibiotica against resistant Gram-positive bacteria. Infection and Drug Resistance, *1*: 27-44.

PIDDOCK, L.J. (2006): Multidrug-resistance efflux pumps—not just for resistance. Nat. Rev. Microbiol., *4*:629–636.

POIREL, L., A. BONNIN, P. NORDMANN (2011): Genetic Basis of Antibiotic Resistance in Pathogenic *Acinetobacter* Species. IUBMB Life, *63*(12):1061–1067.

QUIROS, P., C. LLUCH, M. CASTILLO, A. MIRO, E. ARGENTE, M. JOFRE, J. *et al*. (2013): Antibiotic resistance genes in the bacteriophage DNA fraction of human fecal samples. Antimicrob. Agents Chemother., *58*: 606–609.

RAMANATHAN, B., H.M. JINDAL, C.F. LE, R. GUDIMELLA, A. ANWAR, R. RAZALI, J. POOLE-JOHNSON, R. MANIKAM, S.D. SEKARAN (2017): Next generation sequencing reveals the antibiotic resistant variants in the genome of *Pseudomonas aeruginosa*. PLOS One, https://doi.org/10.1371/journal.pone.0182524

ROSSOLINI, G.M., F. ARENA, P. PECILE, S. POLLINI (2014): Update on the antibiotic resistance crisis. Clin. Opin. Pharmacol., *18*:56–60.

SAMBYA, S.S., P. SHARMA, D. SHRIVASTAVA (2017): Anti-biofilm Activity of Selected Plant Essential Oils against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Int. J Curr. Microbiol. App. Sci., *6*(3): 444-450.

SCHMIEDER, R. and R. EDWARDS (2012): Insights into antibiotic resistance through metagenomic approaches. Future Microbiol., *7(1)*:73-89.

SENGUPTA, S., M.K. CHATTOPADHYAY, H.P. GROSSART (2013): The multifaceted roles of antibiotics and antibiotic resistance in nature. Front. Microbiol., *4*:1-13.

SHARMA, R.A., SINGH B., SINGH D., CHANDRAWAT P. (2009): Ethnomedicinal, pharmacological properties and chemistry of some medicinal plants of Boraginaceae in India. J. Med. Plants. Res., *13*:1153–1175.

TRAGLIA, G.M., K. CHUA, D. CENTRON, M.E. TOLMASKY, M.S. RAMIREZ (2014): Whole-genome sequence analysis of the naturally competent *Acinetobacter baumannii* clinical isolate A118. Genome Biol. Evol., *6*: 2235–2239.

VEINOVIC, G., T. CERAR, F. STRLE, S. LOTRIČ FURLAN, V. MARASPIN, J. CIMPERMAN, E. RUZIC-SABJIC (2013): In vitro susceptibility of European human *Borrelia burgdorfer*i sensu stricto strains to antimicrobial agents. Int. J. Antimicrob. Agents., *41*(3): 288–291.

VEINOVIC, G., Z. STOJIC VUKANIC, J. ANTIC STANKOVIC (2015): Lyme borreliosis- epidemiology, clinical manifestations and treatment (Lajmska borelioza-epidemiologija, klinička slika i terapija). Arhiv za farmaciju, *65*(2):129-146.

VENTOLA, C.L. (2015): The Antibiotic Resistance Crisis. Part 1: Causes and Threats. P&T., *40*: 277–283.

VUKOVIC-GACIC, B., S. NIKČEVIĆ, T. BERIĆ BJEDOV, J. KNEŽEVIĆ VUČKOVIĆ, D. SIMIĆ (2006): Antimutagenic effect of essential oil of sage (*Salvia officinalis* L.) and its monoterpenes against UV-induced mutations in Escherichia coli and Saccharomyces cerevisiae. Food Chem.Tox., *4*:1730-1738.

WALSH, C. (2000): Molecular mechanisms that confer antibacterial drug resistance. Nature, *406*: 775-801.

WINTERSDORFF, C., J. PENDERS, J.M. NIEKERK, N.D. MILLS, S. MAJUMDER, L.B. ALPHEN, P.H.M. SAVELKOUL, P. WOLFFS (2016): Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. Frontiers in Microbiology, *7:* 173.

YANG, Y., B. XING, C. LIANGL, Z. YE, Y. ZHANG (2015): Prevalence and fluoroquinolone resistance of *Pseudomonas aeruginosa* in a hospital of South China. Int. J.Clin .Exp. Med., *8*(1):1386-1390.

YAYAN, Y., B. GHEBREMEDHIN, K. RASCHE (2015): Antibiotic Resistance of *Pseudomonas aeruginosa* in Pneumonia at a Single University Hospital Center in Germany over a 10-Year Period. Plos One, *10*(10): 1-20.

ZAMAN, S.B., M. HUSSAIN, R. NYE, V. MEHTA, K.T. MAMUN, N. HOSSAIN (2017): A Review on Antibiotic Resistance: Alarm Bells are Ringing. Cureus., *9* (6): e1403.

PREGLED O ANTIBIOTSKOJ REZISTENCIJI: POREKLO I MEHANIZMI BAKTERIJSKE REZISTENCIJE

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Izvod

Antibiotska rezistencija je prirodni fenomen i izazov povezan sa visokom stopom morbiditeta i mortaliteta širom sveta. U poslednjih deset godina pojava multirezistentnih bakterija predstavlja ozbiljan problem u javnom zdravstvu. Osnovni faktori koji doprinose pojavi antimikrobne rezistencije su, zloupotreba u korišćenju antibakterijskih agenasa u medicini i poljoprivredi, a takođe, ispuštanje antimikrobnih agenasa u životnu sredinu. Takođe, procesi razvoja novih efikasnih antibiotika nisu bili uspešni u meri u kojoj se očekivalo. Ovaj rad razmatra mehanizme otpornosti na antibiotike i metode za njihovu detekciju, kroz primere rezistencije bakterija, kao što su *Staphylococcus aureus*, *Micobacterium tuberculosis*, *Pseudomonas aeruginosa* i *Acinetobacter*.Takođe, dat je kratak prikaz o bioaktivnim komponentama poreklom iz medicinskih i aromatičnih biljaka, kao nove linije antimikrobne terapije.

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