IT'S GETTING WORSE: ANTIBIOTIC RESISTANCE AMONG OPPORTUNISTIC PATHOGENS

M. Bartoszewicz, U. Czyżewska, S.N. Molendowska, A. Winiewicz

Department of Microbiology and Biotechnology, Faculty of Biology, University of Bialystok, Ciolkowskiego 1J, 15-265 Białystok, Poland

Abstract

The phenomenon of bacterial opportunism has been known for two centuries. It means the occurrence of species that generally remain completely harmless, but in conditions unfavorable for the host may lead to the development of disease symptoms. Until now, opportunistic pathogens have often been marginalized and the number of infections caused by them has been underestimated. In recent years, however, the importance of opportunistic human pathogens in the general pool of bacterial and fungal infections has been increasingly emphasized. Unfortunately, as the frequency of infections increases, so does the percentage of resistant strains among these bacteria and fungi. This is a serious therapeutic challenge, but also clearly demonstrates the need to take measures to prevent the emergence of antibiotic resistance among opportunists. These species, apart from causing infection, may constitute a natural reservoir of resistance for other, often more virulent, microorganisms and contribute to a marked reduction in the effectiveness of treatment with antibiotics and antimycotics.

Keywords: facultative bacteria, horizontal gene transfer, side effects, Bacillus cereus sensu lato

Introduction

Since the discovery of the first antibiotics in the first half of the 20th century, the era of antibiotics has begun. Thanks to the possibility of using effective bactericidal drugs in treatment, it became possible to control the most dangerous infectious diseases, which were the cause of numerous deaths, both among people susceptible to infections (patients with immunodeficiency, children, the elderly, people with comorbidities) as well as immunocompetent individuals.

Unfortunately, with the spread of antibiotic therapy, more and more cases of resistance to selected groups of drugs began to be noted. Already in the first years after the use of penicillin (β-lactam antibiotics class) for treatment, an increasing incidence of infections caused by gram-positive cocci resistant to these antibiotics was noted. Increasing insensitivity to selected aminoglycosides (e.g. streptomycin) and tetracycline was also noted fairly quickly. This resulted in the

need to search for new drugs and revise the treatment procedures used so far (Davies and Davies 2010).

The points where the phenomenon of antibiotic resistance seems to develop most rapidly proved to be an additional threat. These are mainly hospitals, where people carrying different drug-resistant strains contact each other, and the phenomenon of horizontal gene transfer and selection pressure, associated with the widespread use of antibiotics that favors strains showing a high degree of insensitivity to various medicinal preparations, manifest.

Even more worrying is the phenomenon associated with numerous infections caused by species of bacteria that are facultative pathogens. For many years, the greatest risk from staphylococci was associated with *Staphylococcus aureus* infections, while nowadays, serious illnesses caused by coagulase-negative staphylococci (CNS) are more common. Similar observations were made for bacteria from the *Enterobacteriaceae* family, but also for *Bacillaceae*, especially *Bacillus cereus*, and related species (Iredell et al. 2016, Forrester et al. 2018, Bartoszewicz et al. 2019, Bartoszewicz and Czyżewska 2021).

The aim of the present work is to discuss the occurrence of antibiotic resistance among selected species of opportunistic pathogens and point out important sources of these kind of resistant bacteria.

Opportunistic pathogens in humans

Commensal microorganisms have aroused scientific interest since the end of the 19th century. At that time, people wondered about the influence of these microorganisms on human health. Such considerations were taken up, among others, by microbiologist Louis Pasteur after he had observed non-pathogenic bacteria present both in the environment and on the body surfaces of humans and animals. Consequently, the belief that colonization by bacteria is always synonymous with disease has gradually been abandoned. Commensalism refers to microorganisms that do not cause disease symptoms (or cause them only sporadically and only under strictly defined conditions) and thus do not meet Koch's third postulate, which says that a microorganism isolated from an infected person, introduced into another organism, must cause the same disease. The first concept of commensalism appeared in the 1860s. It was formulated by Pierre-Joseph van Beneden, presenting almost 270 examples showing a relationship between two species, in which one of them (commensal) gets benefits (e.g. access to nutrients, shelter), while the other (host) gets no profit or loss (Poreau 2014). Currently, this term is also used in relation to the commensal bacterial biota of humans. However, it is worth mentioning that it is not limited to bacteria only. A number of yeast-like fungi act the same way (mainly *Candida* and *Malassezia* spp). Species naturally related to the human microbiota, as a result of a significant reduction in the level of immunity of the body, cause candidiasis of the mouth, throat or mucous membranes of the gastrointestinal tract (caused mainly by *Candida albicans*) or dermatoses, e.g. atopic dermatitis associated with *Malassezia furfur* (Czyżewska et al. 2018).

All microorganisms considered as facultative pathogens have certain features that determine the predisposition to colonize individual parts of the organism, thanks to which they can be part of the natural microbiota. Commensal microorganisms usually do not show the ability to produce toxins typical for obligatory pathogens, or they are less effective due to the low level of gene expression that determines their synthesis. Facultative pathogens possess other factors of pathogenesis like adhesins of CNS used in order to adhere to the host's extracellular matrix proteins. Among them, the key role is played by the SdrG protein, which determines contact with the layers coated with fibrinogen, or the SdrF protein, which is responsible for binding collagen, which is the main component of the skin (Nowicka et al. 2012, Milles et al. 2018). *Clostridioides difficile* (formerly *Clostridium difficile*) can attach to components such as collagen, fibrinogen or fibronectin, by the fibronectin-binding protein (Fbp68), which facilitates colonization of colon enterocytes (Mehlich et al. 2015). In addition, adhesion is provided by extracellular structures, characteristic of gram-negative bacteria, i.e. fimbriae, thanks to which *Klebsiella pneumoniae* can adhere to tracheal or lung cells. *E. coli* binds to the surface of intestinal epithelial cells in the same way. In turn, secretion of mucous enables the formation of aggregates and biofilm which make it easier for bacteria to oppose the host's immune mechanisms. Most facultative microbial pathogens, including microscopic fungi, exhibit the ability to produce various enzymes that act as virulence factors. Studies indicate that 50% of CNS isolated from patients with infectious endocarditis showed the ability to produce proteases and 30% of these strains produced fibrinolysin (Nowicka et al. 2012). Thanks to the ability to secrete urease, *K. pneumoniae* can successfully oppose the antibacterial effect of urea.

In the case of opportunistic fungi of the genus *Malassesia*, the virulent factor is a lipophilic cell wall, consisting of approximately 20% fatty acids, which facilitates the binding of these yeasts to the host cells and protects against phagocytosis (Jagielski et al. 2013, Czyżewska et al. 2018). There are also proteases or phospholipases, synthesized by both *C. albicans* and *M. furfur*. Human skin is a very complex and diverse habitat for microorganisms. It is the first line of defense (included in the physiological and anatomical immune mechanisms. However, in every human being the composition of microorganisms depends on the local microenvironment of an individual body and its parts. Podgórska and Kędzia (2018a, 2018b) indicated that poly-γ-DL-glutamic acid produced by CNS plays a major protective role. In addition, the hyperosmotic nature of the environment hinders the proliferation of other bacteria, thus reducing the pressure from a potential competitive biota. The dermal environment is also favorable for lipophilic fungi of the genus *Malassezia*, which prefer areas with abundant and active sebaceous glands, such as facial skin (Malinowska et al. 2018). Another potential limitation for the growth of microorganisms could be skin pH (4.2-5.9). The microbes also easily adapted to this, both through the ability to produce urease and through the formation of a biofilm, effectively protecting against acidic skin reactions. Interestingly, *S. epidermidis*, as a facultative anaerobic, is able to colonize areas with reduced oxygen content, e.g. the mucous membranes of the pharynx (Podgórska and Kędzia 2018a). It should be

noted that cutaneous staphylococcus also supports the host's defense mechanisms. Literature overview shows that about 96% of strains have the ability to produce proteins – bacteriocins, which effectively inhibit the multiplication of bacteria with a much higher pathogenic potential, such as *S. aureus* (Podgórska and Kędzia 2018a, 2018b).

The digestive tract is colonized by microorganisms in almost every part, although significant differences in the quantitative and species composition of individual parts of the intestine are observed. The reasons for this are different conditions prevailing there. The first limitation is the lack of oxygen availability, as a result of which this environment will be preferred by obligatory anaerobes represented mainly by *C. difficile* or facultative anaerobes such as *E. coli* and *E. faecalis*. The concentration of bacteria in the colon is in the range of 10^{11}-10^{12} cells/ml. An alkaline environment with a pH of ~ 8.4 and a temperature of about 37°C are favorable conditions for these species. The mucus secreted by specialized epithelial cells also plays a protective role, but *C. difficile* is able to penetrate the thick layer of mucus with the help of flagella to reach the target site, i.e. the cells of the colon. In addition, the main component of mucus, a protein named mucin 2 (MUC2), influences the biofilm formation capacity of non-pathogenic *E. coli*, increasing its colonization capacity. The microbes of the colon microbiota confer many benefits to the host (Malinowska et al. 2018). By competing effectively with pathogenic microorganisms for the same ecological niche, they eliminate them from the environment. In addition, they take part in the decomposition of food residues and also support the synthesis of folic acid or vitamins from groups B and K.

Facultative pathogens, although in most cases associated with the normal microbiota of the organism, appear more and more frequently in reports of endogenous infections. Most of them are related to the hospital environment. For example, data from the National Institute of Hygiene (PZH) indicate that in 2019 the total number of cases caused by *C. difficile* was 11,310, of which almost 86% required hospitalization. Moreover in 2014, we noted less than 17 cases per 100 thousand people, but now this rate is almost two times higher. As a result of the disturbed balance of microorganisms in the colon, this bacterium can multiply uncontrollably, synthesizing toxins that damage the intestinal epithelial cells leading to fatal cases (Mehlich et al. 2015). Another serious risk is posed by the extra-intestinal pathogenic *E. coli* (ExPEC) strains. They are responsible for approximately 75-85% of primary urinary tract infections. Within this group 80-90% of cases is attributed to uropathogenic coliform strains (UPEC). The frequency of infections is also related to the progress of medicine and the increasingly common use of invasive medical procedures. Despite the low incidence level (1.5-4.95 per 100,000 people), mortality among the elderly is significantly increased, ranging 14-46% (Podgórska and Kedzia 2018a, 2018b). *K. pneumoniae* infections also occur in hospitalized patients with additional severe diseases. In Poland, 2016 saw a sharp increase in the number of cases compared to previous years. The situation is getting worse every year. According to the report of the National Reference Center for Antimicrobial Susceptibility (KORLD), about 2,355 infections (mainly pneumonia and urinary tract infections) were registered

in 2018, which is five times more than in 2015. They were also caused by the strain *K. pneumoniae* NDM-1, which is difficult to treat due to multi-resistance.

Another organism that poses a serious challenge is the bacteria of the *Bacillus cereus* group. Although they generally do not cause health issues, they may lead to mild food poisoning causing diarrhea (due to the production of a number of enterotoxins by bacteria) and vomiting (due to cereulide intoxication), they can also cause serious infections, which can be life threatening. The literature describes cases of inflammation of the eye, periodontitis, but also inflammation of the joints caused by *B. cereus* and *B. cytotoxicus*. Sepsis caused by these microorganisms has also been reported. In their case, however, the environment plays an extremely important role. On the one hand, these bacteria are commonly found in soil, water, air and food, and on the other hand, they are easily persistent in various products as they form spores (Bartoszewicz and Czyżewska, 2017). Interesting observations were made by Bartoszewicz and Czyżewska (2021), who showed significantly higher minimum inhibitory concentrations for selected antibiotics and more frequent occurrence of multiresistant strains among isolates derived from food of animal origin, primarily milk. In addition, recent data also indicates that these bacteria may be a source of resistance to other bacteria (unpublished data). On the other hand, transfer of *B. cereus* and related species can take place both through water, soil, as well as through food and synanthropic species of urban birds (unpublished results). Finally, recent data suggests, that among bacteria we often deal with evolution leading to the formation of distinct ecotypes and one such ecological form could be a multiresistant variant (Bartoszewicz et al. 2019).

Summing up, we must be aware that in recent years numerous infections are caused by distinct opportunistic bacteria, not only by obligatory pathogens. These microbes are not the primary goal for antibiotic therapy and sometimes are omitted by reports, which causes their role to be underestimated. Consequently, we are still convinced that they pose little risk. Nevertheless, the deteriorating immunity among people, the aging of the population, and unhealthy lifestyle increase our susceptibility to infections with opportunistic bacteria. And in a situation where our immunity is not optimal, even the less effective mechanisms of virulence of such bacteria can lead to health disorders that require antibiotic therapy, which is increasingly limited by the common resistance of these bacteria.

Antibiotics against bacteria

Antibiotics constitute a group of therapeutics with antibacterial activity, diverse in terms of origin, structure and mechanisms of action. In lower concentrations, they are often bacteriostatic by blocking metabolic pathways in susceptible cells. Higher concentrations may be bactericidal by disturbing processes of cell wall synthesis, replication and protein biosynthesis. Most of the antibiotics act as inhibitors of cell synthesis (mainly β-lactams), substances that disrupt the activity of the cell membrane (gramicidin), blockers of nucleic acid

synthesis (rifampin) or protein synthesis inhibitors (tetracyclines, macrolides, aminoglycosides).

Discovered as the first, but still frequently applied: β-lactams

The most common and preferred antibiotics for treating numerous infections are β-lactams. In the natural state, they are most often produced by fungi of the genus *Aspergillus* and *Penicillum*, but also by some bacteria belonging to the genus *Streptomyces* and *Nocardia*. Their name is related to their structure, namely the presence of the β-lactam ring in the antibiotic molecule, as showed in Figure 1. This class includes the first isolated antibiotic, penicillin, and related substances – carbapenems, cephalosporins and monobactams with higher effectiveness, lower side effects and more resistant to β-lactamases (Bush and Bradford 2016, Tooke et al. 2018).

The mechanism of their action is based on the inhibition of the last stage of peptidoglycan biosynthesis, which is the main component of the cell wall. This is because the antibiotic blocks the enzyme transpeptidase (penicillin-binding protein, PBP), which catalyzes the proper cross-linking of murein (main component of prokaryotic cell wall). Ultimately, the cell lyses due to osmotic stress.

In response to the action of β-lactams, many bacteria, including commensal and pathogenic isolates, have developed a number of resistance mechanisms that protect them from the adverse effects of these substances. One of the best known mechanisms, both among gram-negative bacteria (represented by *E. coli* and other enterobacteria) and gram-positive bacteria (staphylococci), is the production of β-lactamases, which are designed to hydrolyze the amide bond in the β-lactam ring. What is worse, as a result of mutations in the genes responsible for coding β-lactamases, a significant number of microorganisms began to produce enzymes with a much broader substrate spectrum. Metallo-β-lactamases (MBL) produced by *Enterobacteriaceae*, especially by *K. pneumoniae*, pose a particular risk (Khan et al. 2017). They are not only capable of hydrolyzing all β-lactams (except of monobactams), but also the genes encoding the enzyme are located on mobile genetic elements, which facilitates their transfer between bacteria (HGT, horizontal gene transfer). Moreover, hospital-origin strains of *S. epidermidis* resistant to methicillin (MRSE) possess the *mecA* gene on mobile SCCmec genetic cassettes (Podgórska and Kędzia 2018a, 2018b). It conditions the production of additional or modified PBP proteins (PBP2a), which is more insensitive, enabling bacteria to synthesize the cell wall even in the presence of high drug concentrations. On the other hand, in gram-negative bacteria, e.g. *E.* coli, resistance may be the result of limited outer membrane penetration due to loss or alteration of the porin channels conformation. Interestingly, the β-lactam antibiotics also include atypical therapeutics, e.g. clavulanic acid, sulbactam or tazobactam, which, unlike the rest of the class, do not show a therapeutic effect by inhibiting murein synthesis, but act as β-lactamase inhibitors. Therefore, the combined use of an inhibitor (clavulanic acid) with an antibiotic (e.g. amoxicillin)

enhances the bactericidal effectiveness of the described preparations (Bush and Bradford 2016). Nevertheless, we must still bear in mind that resistance to this class of antibiotics can spread rapidly by horizontal gene transfer. In the case of infections caused by opportunistic bacteria, therapy should be continued until the infection is completely eliminated, to prevent the survival of strains with increased resistance, which may later become a source of re-infection.

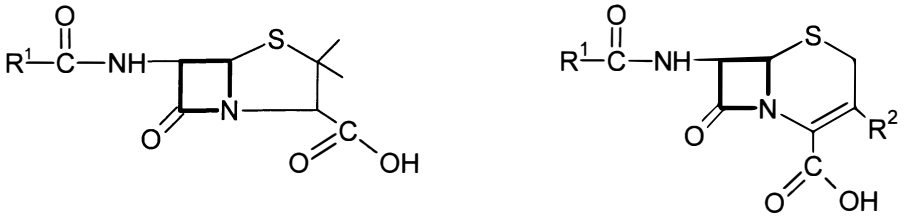

Figure 1. Chemical structure of penicillin (a) and cephalosporin (b), including the β-lactam ring (bold). Figure made in ChemSketch 14.0.1.

Still relevant, but less frequently used: aminoglycosides

Aminoglycosides have a broad spectrum of activity, including both gram-negative and gram-positive bacteria. In addition, streptomycin is an effective weapon against mycobacteria, including *Mycobacterium tuberculosis*, the tuberculosis causative factor. Most natural aminoglycosides are produced by actinomycetes belonging to the genus *Streptomyces*. A characteristic feature of all of them is the presence in the antibiotic molecule of amino sugars linked by a glycosidic bond with a ring – streptamine, as is the case in neomycin and kanamycin molecules or streptidin in streptomycin (Zaffiri et al. 2012, Becker and Cooper 2013), as shown in Figure 2. The mechanism of action of aminoglycosides is related to the inhibition of translation by binding to the minor subunit of the bacterial ribosome. This leads to a breakdown in interaction of the mRNA codon with the tRNA anticodon and the incorporation of incorrect amino acids into the forming polypeptide (Hobson et al. 2021). Due to the diverse chemical structure of this class of antibiotics, bacteria have developed a variety of resistance mechanisms against them.

Resistant bacteria most often produce enzymes that modify the hydroxyl or amino groups of the antibiotic molecule by acetylation, phosphorylation or adenylation. Some strains of the genus *Enterococcus* (mainly *E. faecalis*) and *Staphylococcus* spp. (especially *S. epidermidis*) have several genes, the products of which display N-acetyltransferase and O-phosphotransferase activity, responsible for inactivating gentamicin or kanamycin (Hobson et al. 2021). From the medical point of view, this property is problematic mainly due to its location on the

transposon (mobile genetic element), the presence of which has been demonstrated in numerous opportunistic and obligatory pathogenic bacterial species. On the other hand, other facultative pathogens can methylate the target site, namely the 16S rRNA, which is part of the 30S subunit. Such a mechanism, conditioned by the *rmtA* and *rmtB* plasmid genes, has been reported in *K. pneumoniae* NDM-1 and *E. coli* (Hobson et al. 2021).

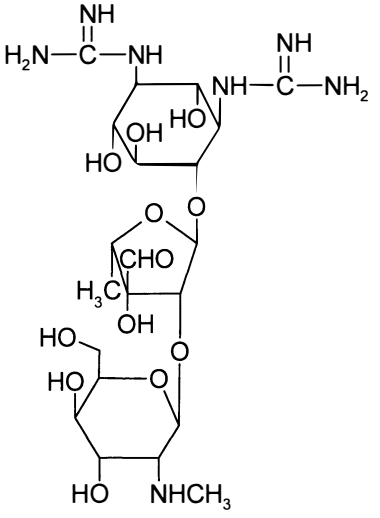

Figure 2. Chemical structure of streptomycin. Figure made in ChemSketch 14.0.1.

Enterobacteria with the aforementioned resistance mechanisms, due to their commonness, are becoming a more and more serious problem. *K. pneumoniae* in particular is indicated as an extremely important etiological factor of difficult-to-treat infections and the spread of resistance genes among other, also pathogenic, strains increase the threat. Hence, an emphasis on a high level of hygiene is important, also in health care facilities, in order to limit the transmission of these resistant bacteria between patients.

Antibiotics with wide spectrum of activity: tetracyclines

Tetracyclines are a relatively early described class of natural and semi-synthetic antibiotics. Its first representative, discovered at the turn of the 1940s and 1950s, was chlortetracycline produced by *Streptomyces aureofaciens* (Zaffiri et al. 2012, Hobson et al. 2021). Unfortunately, quite soon after the introduction of chlortetracycline to therapy, numerous resistant strains appeared and this phenomenon was probably exacerbated by HTG with the participation of commensal strains and opportunistic pathogens. Currently, therapy is dominated by semi-synthetic drugs, including doxycycline, metacycline and minocycline. Their spectrum of activity includes a number of gram-negative and

gram-positive bacteria, as well as some protozoa. The compounds have four characteristic six-membered rings joined together (Figure 3). In addition, they are also characterized by a relatively high molecular weight (>400 Da).

The target structure, exposed to tetracyclines, is mainly the 30S ribosome subunit. Within it, the antibiotic strongly binds to proteins' S4, S18 and 16S rRNA. As a result, it is impossible to bind the tRNA carrying the amino acid at the acceptor site A of the mRNA-ribosome complex and elongate the polypeptide. It should be emphasized that this process is reversible, therefore regular use of the drug is so important in tetracycline therapy.

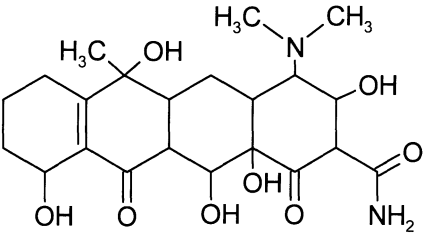

Figure 3. Chemical structure of tetracycline. Figure made in ChemSketch.

Unfortunately, the issue of tetracycline resistance is also a serious problem. This resistance can be of two types: non-specific (limited drug influx through porin channels) or specific for a given bacterial species. In clinical terms, specific resistance is assigned much more importance. Its most important mechanism is related to the active pumping by pumps belonging to the MSF group (major facilitator subfamily), anchored in the cytoplasmic membrane. In gram-negative bacteria of the genus *Escherichia*, the presence of *tetA* genes was confirmed on plasmids (including the RP1 plasmid) and *tetB* genes were noted on the Tn10 transposon (Coleman et al. 1982). Another well-known mechanism of resistance, confirmed in *C. difficile*, is the production of ribosome protective proteins (RPP) responsible for introducing conformational changes to the ribosome, which in turn leads to displacement of the antibiotic (Fyfe et al. 2016). As in the case of the previous mechanism, this type of resistance is also determined by both plasmid genes (the *tetO* gene) and those located on transposons (the *tetM* gene). In *Bacteroides* spp. in turn, the ability to synthesize flavin-dependent monooxygenase, encoded by the *tetX* gene also, located on the Tn4351 transposon, inactivates the drug. However, due to the location of this gene and the ease of its spread, its presence was noted among the *Enterobacteriaceae* (Fyfe et al. 2016).

Distinct antibiotics with similar mode of action: MLS_b antibiotics

Medicinal substances included in this group constitute a group that is extremely diverse in terms of their chemical structure, although they all have

a common mechanism of action, which also translates into a similar mechanism of resistance. These antibiotics are produced by actinomycetes of the genus *Streptomyces*. However, their spectrum of activity is different. In the case of macrolides, it includes a number of gram-positive bacteria and gram-negative cocci. As for lincosamides, they are also gram-positive bacteria (staphylococci, streptococci), as well as anaerobic non-sporulating bacteria and some protozoa. On the other hand, streptogramins are particularly effective against multi-drug-resistant strains representing mainly gram-positive bacteria. As for their chemical structure, substances classified as macrolides usually consist of 14-, 15- or 16-element lactone rings (without a nitrogen atom), conjugated with sugar residues. One of the primary and most famous representatives of this class is erythromycin, which was isolated in 1952 (Zaffiri et al. 2012). Clarithromycin and azithromycin are among the present representatives of the new generation of macrolides. Lincosamides antibiotics have a simpler chemical structure. They consist of an amino sugar (lincosamine) linked by an amide bond with L-proline. Representatives of this class are lincomycin and its derivative, clindamycin. Another group that is structurally different are streptogramins. Quinupristin (streptogramin B) is a cyclic hexadepsipeptide, and dalfopristin (streptogramin A) is a macrolactone with multiple bonds (Figure 4) (Wolstenholme and Kaplan 2012, Marosevic et al. 2017).

All these therapeutics bind to the 50S subunit of the prokaryotic ribosome, preventing further elongation of the peptide. It happens as a result of blocking the proper operation of peptidyl transferase responsible for the formation of peptide bonds between adjacent amino acids. In addition, macrolides prevent the attachment of peptidyl-tRNA at the P site and lincosamides inhibit the binding of aminoacyl-tRNA at the A site (Wolstenholme and Kaplan 2012, Kozińska and Sitkiewicz 2017). The synergistic effect of streptogramin antibiotics should also be mentioned. On the one hand, this consists in modifying the active site of the enzyme by streptogramin A and on the other hand, in hindering the translocation of tRNA with an attached polypeptide chain by streptogramin B. Ultimately, the mechanisms of both of these antibiotics result in a much stronger antibacterial effect.

Unfortunately, the problem of resistance to these therapeutics is particularly serious. One of the main mechanisms by which bacteria are insensitive to macrolides is the modification of the ribosome by adenine-N6-methyltransferase. This enzyme catalyzes the methylation of adenine, a component of 23S rRNA. As it turns out, in the case of the other two classes of antibiotics, we are dealing with a similar mechanism. This is due to the fact that the binding site of streptogramin B partially coincides with the binding site of other antibiotics. This phenomenon presents a therapeutic challenge as resistance to at least one antibiotic will often equate to resistance to other drugs, which is termed cross-resistance (MLS_b cross-resistance). The *ermB* genes responsible for this type of resistance, included in genomes of *E. coli* or *S. epidermidis*, can be located both on the chromosome and on plasmids, which is why it is transferred horizontally to other bacterial species, contributing to the increase in drug resistance. In addition to modifying the ribosome target site, many facultative pathogens

can also produce enzymes that inactivate antibiotic molecules. The literature review shows that most of the bacteria belonging to the *Enterobacteriaceae* family (*E. coli, K. pneumoniae*) have the *ereA* and *ereB* genes encoding the erythromycin lactone ring-cleaving esterases (Fyfe et al. 2016). The same gene is also found in coagulase-negative staphylococci, e.g. *S. epidermidis*, and also the *msrA* gene encoding the ATP-dependent ABC transporter, responsible for active pumping of the drug out of the bacterial cell (Fyfe et al. 2016, Szemraj et al. 2019).

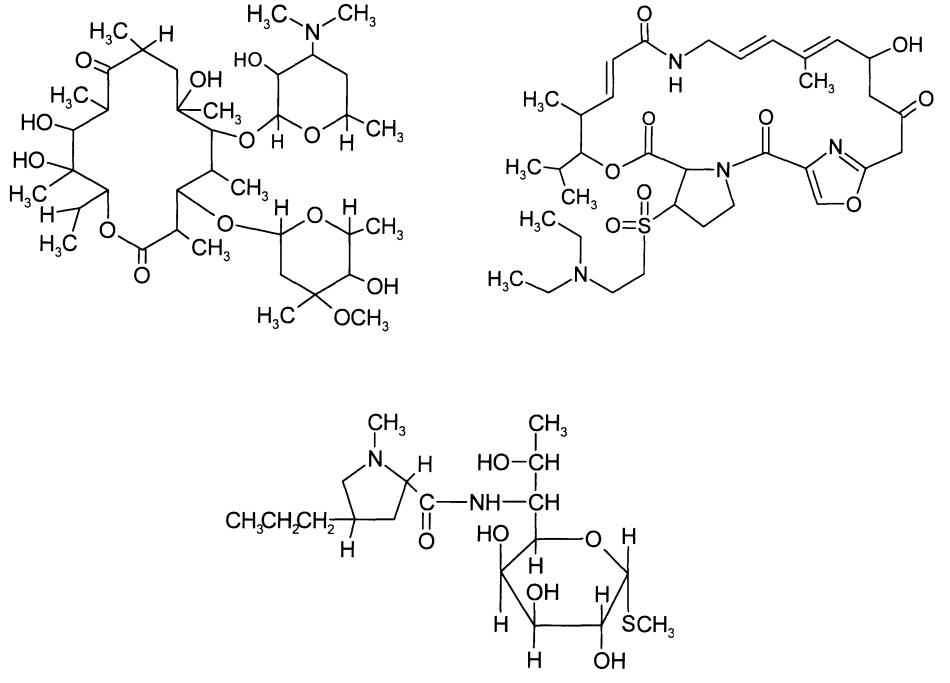

Figure 4. Chemical structure of erythromycin (a), dalfopristin (b) and lincomycin (c).

The MLS_b group of antibiotics is very commonly used in therapy. However, due to the variety of resistance mechanisms present among pathogens, all these limitations of effective therapy of infections should be considered. Moreover, even in the case of the effectiveness of treatment with MLS_b antibiotics, one should be aware of the risks associated with the selection of opportunistic bacteria resistant to these drugs in a particular patient. Such a phenomenon may cause therapeutic difficulties in subsequent infections, especially endogenous ones.

Powerful, but with important side effects: chloramphenicol

Therapeutics classified into this class are characterized by a fairly strong antibacterial effect. The beginnings of therapy with chloramphenicol date back to 1949, when chloramphenicol (also known as chloromycetin or detreomycetin), produced naturally by *Streptomyces venezueale* (Yunis 1988), was introduced into treatment. Currently, due to their ease of production, both chloramphenicol and its derivative thiamphenicol are obtained synthetically. Both of them have a wide range of action, especially against the common anaerobic bacteria, including of the genus *Bacteroides*, but also pathogenic and relatively pathogenic gram-negative and gram-positive bacteria. Recently it has been reported that chloramphenicol could induce invasion of solid tumor cells and cause mitochondrial stress (Li et al. 2010). In the chemical structure, we note two chlorine atoms in its molecule, with chloramphenicol having a nitro group on the benzene ring, and thiamphenicol having a sulfomethyl group (Figure 5).

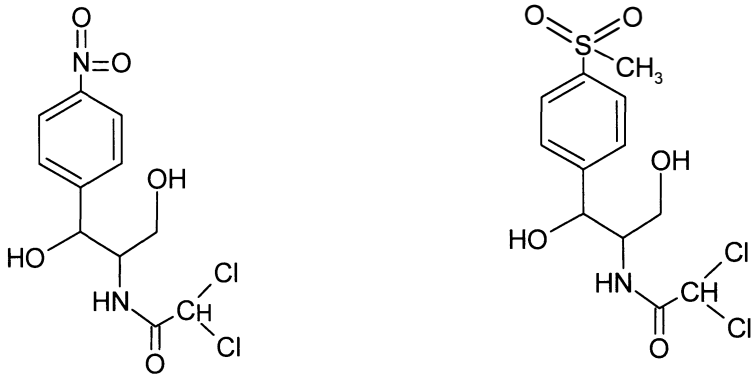

Figure 5. Chemical structure of chloramphenicol (a) and thiamphenicol (b). Figure created in ChemSketch.

The effect of both of these substances is due to their ability to attach to the 50S ribosome subunit, close to the active site of the peptidyltransferase. As a result, there is no binding of the aminoacyl-tRNA at the aminoacyl site of the ribosome. In addition, the formation of a peptide bond between amino acids is disturbed, and the resulting peptide is unable to dissociate from the translation complex, which blocks protein biosynthesis and stops bacterial cell division.

Resistance to this class of antibiotics is most often determined by the presence of chloramphenicol acetyltransferase (CAT), an enzyme responsible for translocation of acetyl residues from acetyl-CoA to the chloramphenicol hydroxyl group. The CAT enzyme is most often encoded in plasmids by the *catA* gene, although its presence was also found on transposons in *E. coli* (Potrykus and Węgrzyn, 2001). Moreover, many other gram-negative bacteria have other types of acetyltransferases encoded by plasmid genes with relatively high homology indicating their common genesis. The second mechanism of resistance

occurs in some gram-negative bacteria, including a fairly common component of the warm-blooded microbiota of the species *Pseudomonas aeruginosa* and is associated with reduced outer membrane penetration due to loss of integral protein. Unfortunately, in this case, the genes are located on transposons, which makes them very easily spread between bacteria, both typically pathogenic and opportunistic pathogens of humans and animals.

Chloramphenicol is a highly effective antibiotic, but its use is severely limited due to its serious side effects. Nowadays, apart from exceptional cases, it is used in the treatment of skin infections as an ingredient of external ointments. Unfortunately, skin infections are often caused by opportunistic species, which show a fairly high level of resistance to this drug and are a reservoir of resistance genes for other, potentially more dangerous species.

With regard to opportunistic pathogens, the phenomenon of resistance to numerous other antibiotics could be discussed, but this is beyond the scope of the present study. Therefore, our goal was to demonstrate examples of the mechanisms of resistance to selected antibiotics and to show their importance not only in terms of the most common medical terms related to obligatory pathogens, but also in relation to species whose pathogenicity is generally negligible.

Conclusions

In the era of constant stress, intense work, accompanied by an unhealthy lifestyle and the related decrease in immunity affecting a significant part of society, the phenomenon of increasing antibiotic resistance among seemingly non-pathogenic bacteria must raise concerns and increased vigilance. These microorganisms are an important element of the microbiota of every human being, and therefore they are often underestimated by us. As a rule, we do not realize that in the presence of immunodeficiency we are exposed to opportunistic infections and their treatment becomes more and more difficult, because facultative pathogens are, like obligatory pathogens, commonly resistant to antibiotics. The presence of various resistance mechanisms and the ability to transfer them horizontally due to genes placed on mobile genetic elements only increase this threat. As opportunistic bacteria are often not the target of antimicrobial therapy, their presence is not monitored during treatment, but the effect of a strong selective pressure leads to the accumulation of various resistance genes, so that in the future they may not only be a factor of etiologically difficult to treat infections, but also act as a reservoir of resistance genes from which obligatory pathogens can also draw. The more frequently described hospital and multidrug resistant opportunistic bacteria pose a huge challenge for modern medicine and epidemiology. They also require an enormous investment of time and resources to develop new, alternative treatments for bacterial infections and to search for other substances with antimicrobial effect. In addition, great care is needed for the effective treatment of diseases caused by strains resistant to certain antibiotics, to eliminate them from the human microbiota, limiting the risk of the formation of bacterial isolates that are even more difficult to eradicate,

as well as the implementation, especially in medical facilities, of the principles of proper hygiene and isolation to reduce potential contact of patients infected with different bacteria with different immune mechanisms. It is also necessary to implement new and continue existing educational and preventive programs, such as the National Antibiotic Protection Program.

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