Assessment of Bacteria Resistance According to Antibiotic Chemical Structure

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Modern medicine has a vast set of antibiotics frequently prescribed in therapeutic practice. Beta-lactam antibiotics are often indicated in prophylaxis and treatment of bacterial infections caused by susceptible microorganisms. This work concerned on analysis of antibiotic structure influence on antibiotic resistance knowing that a wide variety of bacteria developed different mechanism that make bacteria resistant to some or to nearly all antibiotics. The emergence of antibiotic-resistant pathogens is a relevant area of study in medical practice. Furthermore, multi-drug resistance is a worldwide healthcare issue tightly connected to hospital acquired infections.

Keywords: antibiotics, chemical structure, SEM, infection, child, pediatrics, pediatric cardiology, valvular prosthesis.

One of the greatest achievements in medicine is the discovery of antibiotics. These drugs improved clinical outcomes from infections by reduction of morbidity and mortality in transplant, surgical and critical care patients. Long-term administration of broad-spectrum antibiotics determined antibiotic resistance to be a worldwide threat. Many hospital acquired infections are caused by multidrug-resistant pathogens, making antibiotic therapy progressively difficult.

Bacteria resistance refers to opposition of microorganisms to an antimicrobial agent to which they were firstly sensitive. The evolution of this phenomenon was increased by the misuse of antimicrobial medicines and the global spread of antimicrobial resistance affecting unhealthy patients thus giving rise to superbugs. Different antibiotics faced with the resistance in last few years, a resistance that may be generated and transmitted in various ways.

The main cause of antimicrobial resistance is the lack of public knowledge about antibiotics and their overuse. Therefore, self-medication affects the effective therapy and the correct diagnosis would avoid the administration of last-line antimicrobials [1-3].

The misapplication of antibiotics has placed a selective pressure on bacteria favoring the accelerated evolutionary process.

Procedures on resistance control were successful in many cases in spite of great understanding of antibiotic resistance mechanism. Several antimicrobial-resistant organisms are mainly spread among patients and healthcare profession. Five of most frequently mechanisms of resistance bacteria showing high prevalence in clinical isolates are penicillin binding protein modification, protein mutations, enzymatic inhibition, efflux pumps and target changes [4-19].

Two of the most commonly used classes of antibiotics (presented in fig. 1) are penicillins and cephalosporins. Both groups contain a β-lactam nucleus but are different in that penicillins contain a thiazolidine β-lactam ring complex while cephalosporins contain a dihydrothiazine β-lactam ring complex. Cephalosporin present lower toxicity and broader spectrum compared to penicillin based on the chemical structure and structure activity relationship.

The antibacterial effect of these antimicrobial agents depends on the capacity of the drug to penetrate cell membrane, the stability of the antibiotic against bacterial degradation as much as the affinity of the antibiotic for its target proteins. Final step of cell-wall synthesis is
transpeptidation implying the transpeptidase-catalized cross-linking of peptidoglycan chains. From figure 2 it can be observed that transpeptidase-mediated hydrolysis of β-lactam bond (CO-N) consist of inhibition of transpeptidation by the covalent bond of β-lactam drug to bacterial transpeptidase.

The mechanism action of β-lactam antibiotics involves inhibition of bacterial cell-wall synthesis. Cell-wall synthesis inhibition by β-lactam antibiotics does not modify the activity of autolytic enzymes, the most common cause of antibiotic resistance being the enzyme-mediated antibiotic degradation. These medicines are indicated for prophylaxis and treatment of bacterial infections by susceptible microorganisms. Prophylactic antibiotic therapy is administered routinely in the peri-operative period in order to prevent surgical infections. Accomplishment of an effective prophylaxis is needed for children undergoing cardiac surgery, especially in view of worldwide antibiotic misuse and the promotion of drug resistance. [20-25].

The major aim of this study was to investigate the connection between antibiotic chemical structure and the bacteria resistance to those types of drugs. SEM micrographs focused on investigation the effect of ampicillin on E.Coli biofilm formed on silicone and glass surface materials.

Experimental part
Materials and methods
Purchased E.Coli bacterial suspension was prepared inoculating 500 µL of a glycerol stock in a total volume of 200 µL of inoculation medium. Culture medium consisted of 5.5 g/L glucose, 2.5 g/L peptone and 1.25 g/L yeast extract in phosphate buffer was grown on a 1 L shake-flask incubated at 37°C under agitation. Cell were then harvested by centrifugation and suspended in broth for removing of all traces of medium and again harvested by centrifugation and suspended in broth for obtaining an inoculum containing about 1x10^7 cell/mL.

Glass and silicone surfaces were prepared by washing and drying them for further determination.

A β-lactam antibiotic, ampicillin, was used in this study which acts by blocking a specific cross-linking step in the cell wall synthesis, this process creating weak bacterial cell walls inducing thus cell lysis.

Results and discussions
For quantification of E.Coli biofilm formation and antibiotic sensibility on glass and silicone surfaces was used epifluorescence microscopy. After 24 h of biofilm development silicone surfaces exhibits enhance biofilm formation compared to glass. Viable cells number remained constant during the first 3 hours for both materials (fig. 3) and then the viability of biofilms formed on glass markedly decreased and a 7-log reduction was achieved after 7.5 h of treatment. E.Coli biofilm formed on silicone was more resistant to antibiotic than those formed on glass and had a reduction of 1-log in the amount of viable bacteria.

SEM images analyzed the morphological changes on the sessile cells exposed to antibiotic (fig.4). The micrographs showed that the size of protrusions varies with features up to 10 µm and yet most of them exceed the E.Coli cells size (fig. 4C). Bacteria cells adhered to silicone appeared to be extracellular polymeric substances compared to the cells observed on the glass surfaces (fig. 4A) which are distributed in aggregates or as individualized cells without adhesive material in their vicinity.

After 6 h ampicillin exposure, the amount of E.Coli biofilm cells adhered to silicone and glass decreased (fig. 4B and fig. 4D respectively). The antibiotic-treated cells are more elongated on both materials compared to untreated cells and in the case of E.Coli biofilm developed on glass (fig. 4B), the treated cells are longer than on silicone (fig. 4D). In both tested materials, the cell wall of sessile cells showed no severe damage after 6 h of ampicillin treatment (fig. 4B and 4D).

Cell length determined from SEM micrographs consisted of histograms revealing the size distribution of biofilms cells exposed (fig. 5B) and not exposed (fig. 5A) to ampicillin. Treated cells adhered to glass were more elongated than those present on silicone surfaces (fig. 5B).

Moreover, after antibiotic treatment, cells adhered to silicone measured between 1.2 and 6.5 µm while cell lengths between 3.5 and 9.2 µm were determined for glass. Additionally, a narrower size distribution was found for the untreated cells for both materials tested.
Conclusions

In last few years, bacteria have developed resistance to all classes of antimicrobial agents and the novel mechanism of multi-drug resistance caused considerable problems in the treatment of infections caused by some pathogen bacteria. Researchers planned new strategies to combat antibiotic resistance that induced the altering of antibiotics already in use concomitantly with the administration of nonantibiotic drugs that inhibit antibiotic biodegradation mediated by bacterial enzymes. Despite these discoveries, antibiotic resistance continues to be a major clinical issue.

Some methods were developed to lower the risk of infections with antibiotic-resistant bacteria including the choice of an antibiotic with a narrow spectrum when the pathogen is known, shortening the duration of antibiotic prophylaxis and restricting topical and oral therapy with drugs of parenterally use. Although the use of antibiotics increases the occurrence of drug-resistant pathogen agents, these drugs need to be used carefully in order to prolong their efficacy.

References

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Fig. 5. Cell length distribution of 24-hour biofilms not exposed to ampicillin (A) and after 6 h of exposure to ampicillin.