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Antimicrobial Susceptibility/Resistance of Streptococcus Pneumoniae

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SUMMARY

Introduction: Pneumococcal infections are a major cause of morbidity and mortality worldwide, whose treatment is threatened with an increase in the number of strains resistant to antibiotic therapy. **Goal:** The main goal of this research was to investigate the presence of antimicrobial susceptibility/resistance of *S. pneumoniae*. **Material and methods:** Taken are swabs of the nose and nasopharynx, eye and ear. In vitro tests that were made in order to study the antimicrobial resistance of pneumococci are: disk diffusion method and E-test. **Results:** The resistance to inhibitors of cell wall synthesis was recorded at 39.17%, protein synthesis inhibitors 19.67%, folate antagonists 47.78% and quinolone in 1.11%. *S. pneumoniae* has shown drug resistance to erythromycin in 45%, clindamycin in 45%, chloramphenicol–0.56%, rifampicin–6.11%, tetracycline–4.67%, penicillin-G in 4.44%, oxacillin in 73.89%, ciprofloxacin in 1.11% and trimethoprim-sulfamethoxazole in 5.34% of cases. **Conclusion:** The highest resistance pneumococcus showed to erythromycin, clindamycin and trimethoprim-sulfamethoxazole and these should be avoided in the treatment. The least resistance pneumococcus showed to tetracycline, rifampicin, chloramphenicol, penicillin-G and ciprofloxacin.

Keywords: pneumococcus, resistance, susceptibility, antibiotics

1. INTRODUCTION

S. pneumoniae is associated with a high degree of morbidity and mortality in many countries around the world and is considered the main cause of death of millions children in the transition countries (1), which account for up to 70% of deaths (2). It is also considered the leading cause of significant mortality and morbidity in children in developed countries, emphasizing the age of less than two years (3), which resulted in placing of this bacteria in the unenviable first place within morbidity hierarchy in transition countries (1).

A growing proportion of *S. pneumoniae* in the etiology of morbidity and mortality especially within vulnerable groups (children under 3 years, adults and immunocompromised patients) is highly correlated with a high incidence of individual (especially to penicillin) and multiple antibiotic resistance. Antimicrobial resistance of *S. pneumoniae* is not only local but also a global problem. Resistance of pneumococci leads to changes in the clinical presentation of diseases which in turn leads to more difficult diagnosis and treatment.

In addition to resistance to an antibiotic, additional treatment problems are caused by the increase of multiple antimicrobial resistance of certain pneumococcus strains, as a consequence of failure to doctrinal positions therapy (therapy *ex juvantibus*) and the implementation of the same without susceptibility testing. Also, great contribution to this is given by the massive, unjustified use of antibiotics. Unfortunately, in our country we do not have relevant data on the status of resistance, as well as the morbidity and mortality caused by it.

The resistance may result from several different mechanisms, such as the inactivation of the antibiotic by bacterial enzymes, low permeability to antibiotics bacteria, changes in the target protein, leading to reduced binding of the antibiotic, or overproduction of the target protein structure and bypassing the metabolic pathways of the target (4). Bacterial antimicrobial resistance can be achieved by intrinsic and acquired mechanisms. The intrinsic mechanisms are determined by naturally occurring genes found in the chromosomes of the host. Acquired mechanisms include gene mutations and horizontal transfer of resistance determinants resulting in plasmids, bacteriophages, transposomima and other mobile genetic material, leading to genetic differences which help the micro-organism to adapt to environmental influences, including antibiotics (5). This type of transfer takes place not only among the members of one strain, but also between evolutionary distant organisms such as Gram-

negative and Gram-positive bacteria. However, the intrinsic mechanisms that are not associated with the mobile elements, such as an efflux pump that removes many antibiotics, are currently recognized as the most significant factors that contribute to bacterial resistance to multiple antibiotics (6).

Pneumococcus has developed various mechanisms of resistance to certain groups of drugs.

The main resistance mechanism of the pneumococci toward protein synthesis inhibitors is based on a modification of the mediated ermB-coding methylase; efflux pump, mutation of 23s rRNA, point mutation of rifampin-binding region of rpoB (6).

The mechanism of resistance to inhibitors of cell wall implies structural changes of penicillin-binding protein 1A, 2X and 2B (7).

Dihydropteroate synthase gene mutation or dihydrofolate reductase is responsible for the occurrence of resistance to folate antagonists (6).

Point mutation of topoisomerase IV (ParC2ParE2) and DNA gyrase (GyrA2GyrB2) is the basis for the development of pneumococcal resistance to quinolones (8).

The goal of this study was to determine the prevalence of susceptibility/resistance of *S. pneumoniae* isolates to antibiotics that act on the cell wall synthesis, protein synthesis, folate antagonists and quinolones, in order to achieve proper treatment of pneumococcal disease and reduce morbidity and mortality.

2. MATERIAL AND METHODS

This study is of prospective-retrospective and analytical nature conducted by the Institute of Public Health of Canton Sarajevo in the period from July 1, 2013 to April 15, 2014. The sample consisted of 4109 different samples of outpatients in the Sarajevo Canton. Samples were swabs of the nose, nasopharynx, eye and ear of outpatients with severe symptoms, but without the same when taking control swabs during enrollment of children in kindergarten or school. The swabs were immediately seeded on blood agar, then incubated for 24h at 37 °C with 5% CO_2. The isolates were identified by typical appearance of colonies, alpha hemolysis and inhibitory zone around Optochin, and the final confirmation of pneumococcal isolates was performed by specific serum agglutination.

If the laboratory finding was confirmed that the tested isolate is *S. pneumoniae*, susceptibility was made. Routinely it is made by disk diffusion method of susceptibility testing, and for invasive isolates was also determined the value of the MIC for penicillin.

The obtained data were statistically analyzed using SPSS software version 19.

3. RESULTS

In 74.9% of isolated Streptococcus pneumonia susceptibility was reported to the tested antimicrobials, while resistance was present in 25.06%

Figure 1 provides percentage display of susceptibility and resistance of *S. pneumoniae* to the groups of antibiotics. *S. pneumoniae* showed the highest frequency of susceptibility to inhibitors of protein synthesis in 44.63%, followed by cell wall synthesis inhibitors and quinolones, with representation of the susceptibility of 13.52% and 10.99%, respectively. The lowest frequency of the susceptibility *S. pneumoniae* showed to folate antagonists in 5.80%.

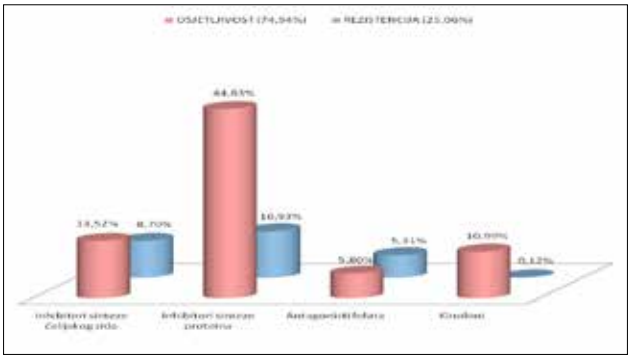

Figure 1. The susceptibility and resistance of S. pneumoniae to tested groups of antibiotics.

Resistance of *S. pneumoniae* is the most prominent to inhibitors of protein synthesis (10.93%), little bit less to the cell wall synthesis inhibitors (8.70%), and folate antagonists (5.31%), and at least to the quinolone (0.12%).

To calculate Yates correction factor, in order to correlate the individual antibiotics groups, compared are data on the susceptibility and resistance to inhibitors of cell wall synthesis with data on the susceptibility and resistance to inhibitors of protein synthesis. The resulting value of Yates factors for data on susceptibility and resistance to inhibitors of cell wall synthesis and data on susceptibility and resistance to inhibitors of protein synthesis, is 45.3853 (df = 1, p = 0.0001), based on which we can conclude that there is an statistical correlation.

Two other groups of antibiotics that are subordinated are folate antagonists and quinolones. The compared data are also obtained on the susceptibility and resistance to folate antagonists, the data obtained on the susceptibility and resistance to quinolones, and Yates correction factor amounted 103.6112 (df = 1, p = 0.0001 (s)); on what basis it was concluded that there is an extreme statistical correlation.

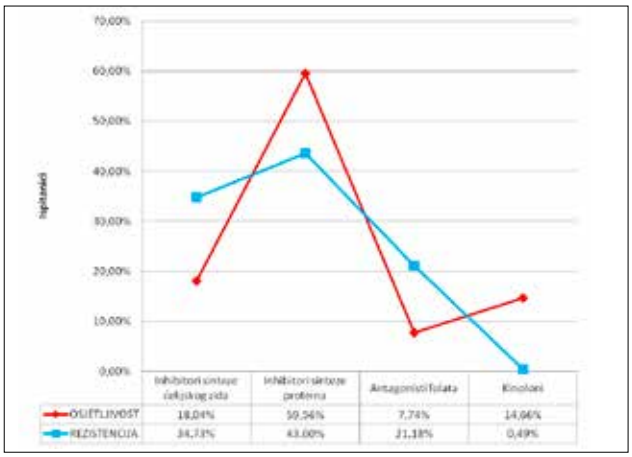

Figure 2. The susceptibility/resistance to groups of antibiotics within the total S/R.

From Figure 2 it can be seen that from the overall susceptibility of S. pneumoniae to all tested antibiotics, inhibitors of cell wall synthesis had participation of 18.04%, while the inhibitors of protein synthesis at the same time had the highest participation in susceptibility or 59.56%. Susceptibility to folate antagonists has been represented in the lowest percentage or 7.74%, while quinolones had a share of 14.66%. Of the total resistance to all tested antibiotics, inhibitors of cell wall synthesis had participation of 34.73%, while the protein synthesis inhibitors also

had a major presence in the resistance or 43.60%. Resistance to antagonists of folate was present in 31.18% of the sample, while quinolones had the lowest participation of only 0.49%.

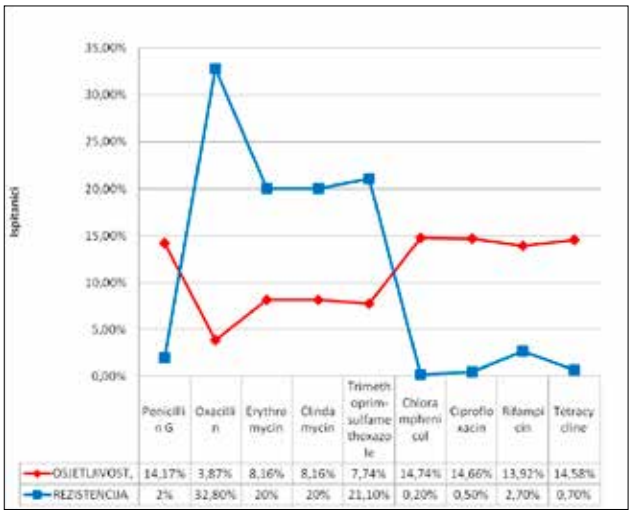

Figure 3. The ratio of susceptibility/resistance of certain antibiotics as compared to total susceptibility/resistance.

Figure 3 shows the relationship of one antibiotic susceptibility representation within the overall susceptibility, according to the representation of resistance within the overall resistance. This relationship provides data on the effectiveness of the tested antibiotics.

The representation of the susceptibility of penicillin-G, oxacillin, erythromycin, clindamycin, trimethoprim-sulfamethoxazole, chloramphenicol, ciprofloxacin, rifampin and tetracycline amounted to: 14.17%, 3.87%, 8.16%, 8.16%, 7.74%, 14.74%, 14.66%, 13.92%, 14.58%, respectively.

Representation of resistance of penicillin-G, oxacillin, erythromycin, clindamycin, trimethoprim-sulfamethoxazole, chloramphenicol, ciprofloxacin, rifampicin and tetracycline amounted to 2%, 32.80%, 20%, 20%, 21.20%, 0.20%, 0.50%, 2.70%, 0.70%, respectively.

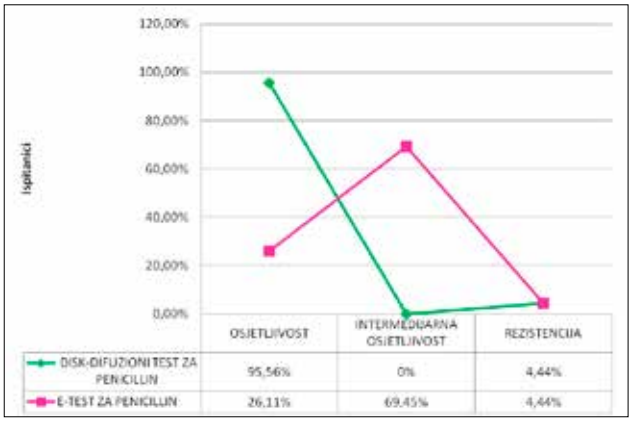

Figure 4. The relationship of the results obtained by disk-diffusion method and E-test for penicillin G

Figure 4 shows the relationship of susceptibility, intermediate susceptibility and resistance of *S. pneumoniae* to penicillin G whose values are obtained by disk diffusion and E-test. Disk diffusion test showed resistance of *S. pneumoniae* to penicillin-G in 4.44% of cases, with the susceptibility of 95.56%. E-test determined resistance in the 4.44%, intermediate susceptibility at 26.11% and 69.44% susceptibility. Intermediate susceptibility to penicillin-G is important in therapeutic treatment for patients because the strains with mentioned type of susceptibility require higher doses and penicillin-G in comparison to the usual ones.

Chi square test results are significantly higher than the critical value which indicates the existence of a statistical difference in the results obtained by the disk-diffusion and E-test.

4. DISCUSSION

Not very low representation of resistance (25.06%) that was observed in this study may be due to inadequate prescription and consumption of medicines, ex juvantibus therapy, predisposed specific serotypes of *S. pneumoniae* in the development of resistance to antibiotics and their different geographical distribution. When we talk about groups of drugs, the highest susceptibility demonstrated protein synthesis inhibitors with 44.63%, and the lowest folate antagonists. *S. pneumoniae* showed the resistance to inhibitors of protein synthesis in the highest percentage (10.93%) and the lowest to the quinolone (0.12%). These results could be used in the selection of empirical therapy if it is not possible to perform susceptibility testing.

Results of our study showed extremely low resistance of *S. pneumoniae* to penicillin G, and high on-sulfamethoxazole trimethoprim, clindamycin and erythromycin. This may be a result of the existence of barriers to the use of penicillin G in the treatment of pneumococcal disease, due to fear of the possible dramatic allergic reactions including anaphylactic shock and greater recourse to treatment trimethoprim-sulfamethoxazole infection, erythromycin and clindamycin.

The accumulation in the bones, deformity and temporary inhibition of bone growth, permanent damage to the teeth and hepatotoxicity are serious side effects of tetracycline, which are reason for their lower application especially in children under 12 years, pregnant and nursing women, the results of this study suggest that low-level resistance of the aforementioned drug are not surprising (9). Furthermore, the observed very small resistance frequency of *S. pneumoniae* to chloramphenicol, the aforesaid and is probably the result of a very low rate of application of the same in the treatment of pneumococcal disease, highlighting the child age, due to significant side effects of heavy use of the drug as well as bone marrow depression (9) .

By E-test is observed a difference in response to treatment of *S. pneumoniae* with various doses of penicillin-G. This means that strains exhibiting resistance to standard doses of penicillin-G become susceptible to the same drug increased dose in the non-toxic amount of the drug.

Comparing the results of this study with the results of research Zdilar and associates in 2005 (Canton Sarajevo), were observed variations in the prevalence of susceptibility and resistance of *S. pneumoniae* to certain drugs. There has been a significant decline in resistance to penicillin G (from 58% to 4.44%), trimethoprim-sulfamethoxazole (from 71% to 47.78%), tetracycline (37% to 1.67%), chloramphenicol (with 25% to 0.56%). Notable are not such a big increase in resistance to erythromycin (from 37% to 45%) and a significant increase in the clindamycin (from 11% to 45%). Study results showed 98.89% susceptibility of *S. pneumoniae* to ciprofloxacin, which shows no significant deviation (in the earlier study susceptibility was in 100% of cases).

Results of this study show some deviations from the results of the study by PROTEKT US conducted on patients in the

United States. The same has shown growth of intermediate susceptibility from 12.5% in 2000-2001 to 20% in 2003-2004 to penicillin, which is significantly less than the rate of intermediate susceptibility obtained in this study, which is 69.44%. PROTEKT US study showed a drop in penicillin resistance in the United States from 26.3% in 2000-2001 to 16.5% in 2003-2004. According to this study the largest representation of resistance to penicillin was in South Africa (87.4%), Far East (63%) and Middle East (54%). In Southern Europe the incidence of PNSP is higher than in Northern Europe. The largest representation was found in France, Greece and Spain. In seven countries in Latin America PNSP rates reached a global level which is 30% (10)

Significantly lower incidence of resistance to penicillin G have shown a study conducted in the period from March 2008 to December 2009 in 60 hospitals of 11 Asian countries with the rate of resistance to penicillin of 0.7% in tested material (11) and studies in Germany, with an incidence of resistance of 2 % (12). A study in Italy showed intermediate susceptibility of 30.4% and 0.54% of total resistance (13).

Results of studies conducted in China, Taiwan, Vietnam and Moscow have shown different rates of resistance to erythromycin represented in the following order: 96.4%, 84.95%, 80.7% (11), and were significantly higher than those obtained in this study, which amounts to 45%. Similar values as our results were observed in the study developed in Italy, where the resistance to erythromycin was represented in 42.3% of the tested material (13).

In the period from 2004 to 2005, the prevalence of quinolone resistance was the following: in the Netherlands 4.4%, Poland 4.4%, Finland 6.6% and Italy 7.2% (14), which is significantly higher prevalence of resistance from that found in our study (1.11%)

The study conducted in Asian countries during the period from March 2008 to December 2009 showed existence of resistance to ciprofloxacin in 1.5% of cases, which is similar to the values obtained in this study (11).

Studies conducted in China, Italy and America showed increased presence of pneumococcal resistance compared to our results, by tetracycline in the amount of: 94.3% (15) 36.9% (16) and 15.6% (17).

Resistance to trimethoprim-sulfamethoxazole was observed in 55.1% in China (15) and the same does not differ significantly from our results, while the much lower rate of resistance was observed in America 25,45% (16).

In comparison with other studies are not noted greater deviations of *S. pneumoniae* resistance to chloramphenicol, in relation to this study, suggesting or insufficiently developed mechanisms for achieving resistance to the drug or decreasing the use of the same.

Previously presented results of studies conducted in developed and developing countries indicate that the antimicrobial resistance of *S. pneumoniae* is global problem. There have been countless variations in the occurrence of serious pneumococcal disease, geographical distribution of invasive serotypes, the representation of resistance of *S. pneumoniae* to antibiotics and efficacy of therapeutic treatment. Studies done in other countries point to a complex relationship between antibiotic use and prevalence of resistance and suggest that serotype distribution which causes invasive infections varies depending on the age, the socio-economic standards and timing of antibiotics administration. Unfortunately, there are no relevant data that would pointed to the prevalence and severity of antimicrobial resistance of *S. pneumoniae* in Bosnia and Herzegovina and that would allow the proper approach to solving this problem. However, these data indicate that the presence of *S. pneumoniae* strains resistant to antibiotics of different groups according to the mechanism of action, isolated from samples of nose and nasopharynx, eye and ear of outpatients is in direct correlation with the same resistance in the region.

5. CONCLUSION

The highest frequency of resistance in isolated pneumococci is recorded with erythromycin (20%), clindamycin (20%) and trimethoprim-sulphametoxazole (21.10%), while the lowest incidence of isolated resistant pneumococci is recorded at chloramphenicol (0.20%), ciprofloxacin (0.50%), tetracycline (0.70%), penicillin-G and (2%) and rifampicin-a (2.70%).

E-test differentiated large number of resistant pneumococci as intermediately sensitive, which has implications for further treatment. Comparing representation of antibiotics groups in the overall susceptibility and their representation in the total resistance which show therapeutic adequacy of the same. This point was of great importance, because it approximately can serve as a guide in the selection of a therapeutic agent in the case of impossibility of making susceptibility testing or therapeutic coverage waiting period. For example, inhibitors of cell wall synthesis and folate antagonists have significantly greater representation in the overall resistance then representation in the overall susceptibility and are therapeutically inadequate. It is evident that the presence of inhibitors of protein synthesis in the overall susceptibility is very high, but that also has a much significant share in the overall resistance.

In order to reduce mortality, morbidity and the presence of pneumococcal antimicrobial resistance in Bosnia and Herzegovina, it should be made typing of invasive and resistant strains of *S. pneumoniae* and according to the results make vaccines appropriate for our region. The success of the vaccination program will not only lead to a reduction in the incidence of pneumococcal disease, but will also result in reduced use of antibiotics, used as a primary treatment. It will also reduce the occurrence of antimicrobial resistance to penicillin, β-lactams and macrolides, which are commonly used antibiotics in the last thirty years.

The results obtained in this study indicate the existence of a great need for rational use of antibiotics and establishing adequate monitoring patterns of pneumococcal resistance. We should determine the serotypes and the spread of resistant strains. Physicians should keep local and regional patterns of resistance at the consideration during the selection of empirical therapy for diseases caused by this agent.

For further control of the resistance development necessary is a multidisciplinary approach that includes the clinicians, epidemiologists, microbiologists and pharmacists.

CONFLICT OF INTEREST: NONE DECLARED.

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