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Representation of Streptococcus Pneumoniae in Outpatient Population of Sarajevo Canton

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ABSTRACT

Introduction: *Streptococcus pneumoniae* in asymptomatic manner colonize the mucous membranes of the nasopharynx of children and adults, but can cause serious illness in the media which are naturally sterile. In 5-40% of healthy population this bacteria colonize the nasopharyngeal mucosa thanks to the surface adhesin protein, which allow the bacteria to attach to the epithelial cells. The normal nasopharyngeal microflora retains pneumococcus in a small number and does not allow it to express its pathogenic potential and cause disease. If this dominance of the normal microflora is violated, after adherence and local duplication, pneumococcus can spread to the middle ear, sinuses or lungs. Colonization is more common in children than in adults. **Goal:** The goal of this study was to determine the prevalence of the carrier state and susceptibility of pneumococcal strains that circulate in the outpatient population of Sarajevo Canton as a potential source of infection. **Material and methods:** In the microbiological laboratory of the Institute of Public Health of Canton Sarajevo in the period from July 1, 2013 until April 15, 2014 were analyzed swabs of the nose and nasopharynx, eye and ear from a total of 4109 outpatients. Swabs were inoculated on blood agar nutrient medium. Then was performed catalase test, preparation by Gram and susceptibility test on Optochin. Isolates positive for *S. pneumoniae* were subjected to in vitro assays to investigate the antimicrobial susceptibility/resistance. **Results:** Out of 4109 analyzed swabs the pneumococcus positive was 180 (4.38%). Of these, 137 (76.11%) nasal and nasopharyngeal swabs, 33 (18.33%) of the eyes and 10 (5.56%) ear. The highest number of positive swabs were isolated in children aged 6 years and less, a total of 168 (93.33%), in children aged 7-13 years were positive 7 (3.89%), while among respondents aged 14-20 years only 5 (2.78%). **Conclusions:** The most common site for isolation of pneumococci is the nose and throat, and the most common carriers of these bacteria are children under 6 years of age. Determining the prevalence of the carrier state contributes to the improvement of preventive measures to reduce the risk of infection and possible sequels.

Keywords: pneumococcus, carriage, prevalence, Optochin

1. INTRODUCTION

Streptococcus pneumoniae (pneumococcus/diplococcus) is a gram positive aerobic bacteria. Although part of the normal microflora of the respiratory tract it is a potential cause of many diseases. Primarily by its invasiveness pneumococcus causes various diseases (1).

It is an obligate parasite in the human body, and it is found in the nasopharynx of healthy people as one of the most important pathogens in humans. In most people, the colonization is asymptomatic and does not cause the disease (2,3). In 5-40% of healthy individuals these bacteria first colonize the mucous membranes of the nasopharynx where the essential role has surface protein adhesins, so that they bind bacteria to the epithelial cells. The normal nasopharyngeal microflora maintains pneumococcus at low levels and it does not enable it to present its pathogenic potential and the development of the disease. In the event of disruption of the normal microflora, after adherence and local duplication, pneumococcus can spread to the middle ear, sinus or lung alveoli which is being phagocytosed and entered in the lung tissue. At these locations it causes severe inflammatory reaction, survives intracellularly and spread from the place of primary infection, enters the bloodstream and causes sepsis and meningitis. The migration of the bacteria into the lower parts of the respiratory tract can be prevented if the bacteria

is surrounded by the mucus of the respiratory tract and removed by the action of ciliated epithelial cells. Bacteria resist wrapping process mucus producing secretory IgA protease pneumolysin. Pneumolysin binds cholesterol host cell membranes to form pores. This activity can destroy ciliary epithelial cells phagocytes. Pneumococci have the ability to survive in phagocytes and capsules due to pneumolysin which inhibit the oxidative processes that is essential for the process of phagocytosis of pathogens (5). In children and adults, especially the elderly it causes pneumonia, in children otitis media, acute purulent meningitis and is a host of other infections in all ages. During the winter and spring up to 70% of children living in closed collectives (kindergartens, child care centers, preschools and schools) are colonized with pneumococcus, and percentage of decreases in adults is only 1-10%. Colonization is more common in children than in adults, but it is more common in adults who live with children (2,4,6).

Pneumococcus has a capsule that is made up of a complex polysaccharide that determines serologic type and contributes to virulence and pathogenicity. Serotyping of antigens identified over 90 different serotypes, of which the most difficult types of infections are caused by 4, 6, 9, 14, 18, 19 and 23. Initial Pneumococcal colonization occurs in six months, after which the duration of the carrier state reduces the acquisition of each of the following serotypes partly because they evolve serospecific immunity. The children carry this pathogen in the nasopharynx and are asymptomatic for about 4-6 weeks, often several serotypes at the same time. New serotypes are acquired approximately every two months (17).

The process of colonization with a new serotype lasts for years and the incidence of the carrier state and possible disease is greatest during the cold months. Pneumococcal strains causing disease are the same as those that lead to colonization. Although the infection spreads by droplets, epidemics develop very rarely (4). The biggest risk for pneumococcal infection is children between 6 months and 4 years of age and persons over 60 years. Studies have shown that during the first year more than 60% of children diagnosed with at least one of the acute form of otitis media (AOM–acuta otitis media) had pneumococcal etiology. In some cases, more than 95% of children can be colonized with up to 6 serotypes to their second year of life. Some serotypes together colonize the nasopharynx where they have a suitable temperature conditions for the invasion. On the other hand, some serotypes causing Parasitic Diseases with each episode of colonization (7). Pneumococcus is the leading cause of bacterial pneumonia.

In the treatment of infections caused by pneumococcus penicillin has quickly become the drug of choice and in patients allergic to penicillin, applied are cephalosporins, erythromycin and chloramphenicol. In the seventies of the last century are beginning to emerge pneumococcal strains resistant to antibiotics. Today, more than a third of the isolates are resistant to penicillin. The increased number of resistant strains of pneumococcus leads to an increased risk of adverse outcomes of disease. Therefore, in order to prevent and control diseases of great importance is the vaccination of children and adults (17).

2. MATERIAL AND METHODS

The study was conducted at 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 (swabs of the nose, nasopharynx, eye and ear) from outpatients with severe symptoms, as well as healthy subjects necessary for entry into kindergarten/school. All samples were taken with sterile swabs and adapted for this purpose. In the microbiological laboratory samples obtained were planted on blood agar culture medium, and then incubated for 24 h at 37 °C with an elevated concentration of CO_2. The isolates were identified by typical colony appearance–small and transparent with a prominent zone of alpha hemolysis. Colonies were initially prominent but in the older culture are becoming flat and eventually leads to their autolysis which is manifested in the collapse of the central part where autolysis begins. Identification of pneumococci was confirmed by Gram-preparation (gram positive diplococcus commonly found in pairs, individual cocci in pairs take the form of a candle flame or a spear, popular are adjacent flat end), catalase reactions (pneumococcus is catalase negative), and test susceptibility Optochin (suspect colonies were seeded on the sector with disc Optochin, after 24h incubation at 37 °C with 5% CO_2 for positive isolates with Optochin disc is formed of zones of inhibition of growth). Around Optochin disc 1 mg inhibition zone is 8 mm. The final confirmation of pneumococcal isolates was done using the agglutination with specific antiserum. For pneumococcal strains isolated at the end was performed routine test for susceptibility/resistance, or disk-diffusion method of susceptibility testing. The culture medium was used on the blood agar inoculum which was prepared in accordance with NCCLS standards (by suspending a colony in the 2 mm infusion broth) to obtain a suspension equivalent to 0.5 McFarland standard. After inoculation of the test bacterial strain, was administered the antibiotic disks with the usual scheme (penicillin, erythromycin, tetracycline, chloramphenicol, trimethoprim, ciprofloxacin, clindamycin, rifampicin and oxacillin). Seeded plates were incubated at 37 °C with 5% CO_2, and evaluated after 24 hours.

The results obtained were processed statistically using the SPSS software version 19. The results are presented in tables and charts. The accepted level of statistical significance was $p < 0.05$.

3. RESULTS

In the period from July 1, 2013 to April 15, 2014 were processed samples of 4109 nasal swabs, nasopharyngeal, eye and ear from outpatients Sarajevo Canton. All subjects were divided by age into three age groups. The first included the patients up to 6 years, another group from

Place of isolation	No. of positive
Nose and nasopharynx	137 (76.11%)
Eye	33 (18.33%)
Ear	10 (5.56%)
TOTAL	180 (100%)

Table 1. Distribution of *S. pneumoniae* in positive samples of respondents

7 to 13 years, and third respondents from 14 to 20 years of age.

From a total of 4109 tested swabs to *Streptococcus pneumoniae* 180 were positive and 3929 negative.

From Table 1 we can see that from 180 to *S. pneumoniae* positive swabs, 137 had positive nasal and nasopharyngeal swabs (76.11%), 33 positive eyes swabs (18.33%) and 10 positive ear swabs (5.56 %).

Patient age:	No. of positive isolates:
≤6 years	168
7-13 years	7
4-20 years	5
Total:	180

Table 2. Age distribution of the patients with isolated *S. pneumoniae*

From Table 2, which shows the age distribution of patients who have been isolated *S. pneumoniae*, it is clear that the respondents in the age group of ≤ 6 years positive were 168 respondents, in the age group 6-12 years positive was 7, and in the age group of patients aged 12-18 years positive only were 5. Finally it can be concluded that the number of positive samples fell drastically with age of the respondents, and in the oldest age group was evidently the smallest number.

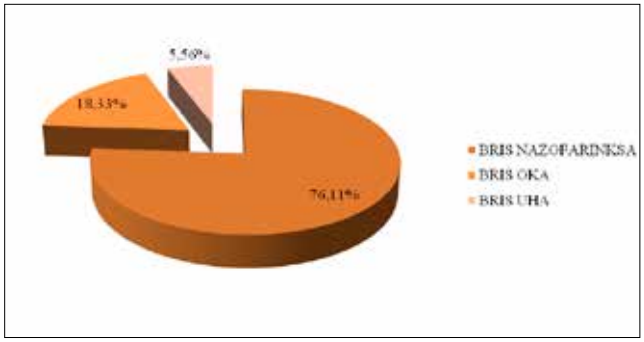

Figure 1. Percentage of the total number of positive isolates in the reporting period

On Figure 2 is given display of the percentage of positive isolates of pneumococci according to the place of isolation. From total 76.11% positive isolates were from nasopharyngeal swab, 18.33% in the smear of the eye, and the lowest percentage of representation was detected from ear and amounted to 5.56%.

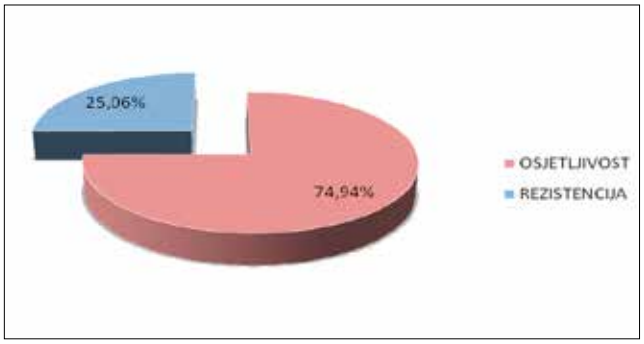

Figure 2. Frequency of susceptibility/resistance of S. pneumoniae from the test sample

On Figure 2 is given the display of the percentage of the overall susceptibility and resistance of isolated bacterial strains, it is clear that the *S. pneumoniae* showed susceptibility to antibiotics tested in 74.94% of cases and resistance at 25.06% of the cases.

4. DISCUSSION

Streptococcus pneumoniae colonizes the upper respiratory tract in children and adults. Home colonization occurs in the sixth month of life, after which children can be transiently colonized with other bacterial serotypes. The duration of the carrier state is reduced by gaining every other serotype, probably due to the development of serum-specific immunity. In people with weakened immune systems, chronic respiratory diseases, asplenia and children less than 5 years of age often causes infection. It can cause mild forms of the disease such as lobar pneumonia, ear infections and sinus. However, may lead to invasive bacterial infections, meningitis and sepsis.

Results of our study showed that the prevalence of colonization with *S. pneumoniae* isolates in outpatients with severe symptoms and patients without symptoms was in the amount of 4.4%. The most common place of isolation of pneumococci was the nose and nasopharynx (76.11%), from which in healthy carriers the aforementioned agent can be isolated within the normal microflora. The remaining 23.89% of positive samples, were isolated from the eye and the ear, or the area in which *S. pneumoniae* cannot be isolated as part of the normal microflora. The aforementioned places are considered complications of primary places colonization, i.e. the area of the nasopharynx. The highest rate of representation of *S. pneumoniae* was by the age distribution was observed in children aged up to 6 years. Similar results were reported in study by Mayanskiy and associates in Russia (8). They in the five-year study of 863 pneumococcus positive isolates found 617 (71.2%) positive nasopharyngeal swabs. Mills and colleagues conducted a similar survey in Ghana. Their results showed that the prevalence of pneumococcal colonization was 48.9% among children aged 43-48 months (9). Countries like Israel and Finland reported on nasopharyngeal colonization, which ranged from 43 to 90% (10,11). Studies conducted in different regions of India have revealed similar results where the prevalence of nasopharyngeal colonization ranged from 6.5 to 83% (12,13,14). Ravi and associates in the period from 2010 to 2011 in India conducted a study on the 190 respondents, 53 (27.9%) were positive for pneumococcus. There are many factors that independently determine nasopharyngeal colonization such as nationality, age, the influence of the environment, the season and the use of antibiotics (15).

Previous studies indicate the possibility of *S. pneumoniae* occurrence after the fourth day of birth, the same usually starts at the age of six months, reaching its peak in preschool period. As the reason is stated the stay of children in kindergartens and care centers for pre-school children, which are subject to the same environmental factors that contribute to the carriers of pneumococci, and the rapid transfer of tolerance, especially between the ages of 25 to 48 months (15,16).

Test for susceptibility/resistance in pneumococci on appropriate antibiotics showed that the isolated strains of *S. pneumoniae* showed susceptibility in 74.94% of cases, and

resistance at 25.06% of cases. Not so low rate of resistance that was observed in this study may be a consequence of inadequate antibiotic prescriptions, empirical therapy and predisposition to certain serotypes of *S. pneumoniae* in the development of resistance to antibiotics and their different geographical distribution. Antibiotic treatment of infections of the respiratory tract is usually empirical and guided by the expected pathogens and their expected susceptibility to antibiotics. Pneumococcal resistance leads to changes in clinical manifestations of the disease, which inevitably leads to difficulty in their treatment. Therefore, the problem of antimicrobial resistance beyond the local level is becoming a global problem, and inadequate treatment of pneumococcal infections leads to the death of millions of children worldwide annually. This place pneumococcal infection in the unenviable first place within the hierarchy of morbidity in transition countries (15).

5. CONCLUSION

This study showed that the most common place for *S. pneumoniae* isolation were in the area of the nose and nasopharynx, then in children aged 6 years or less commonly virus carriers and sources of resistant pneumococcus strains. The most frequent pneumococcal colonization is among the small children, and this group is the most important reservoir for horizontal dissemination of the pathogen in the community. The process of colonization with new serotypes takes years, and the incidence of the carrier state and development potential of infection is highest during the cold months. The strains of pneumococcus that cause disease are practically the same as those leading to colonization. Therefore, strategies to prevent pneumococcal disease would be to focus on prevention of nasopharyngeal colonization, especially in children. The increasing role of pneumococcus in the etiology of morbidity and mortality especially in vulnerable groups (children under 3 years of age, the elderly and immunocompromised patients) is in close connection with the high frequency of individual (especially penicillin) and multiple antibiotic resistances.

CONFLICTS OF INTEREST: NONE DECLARED.

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