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Determining the frequency of *Streptococcus pneumoniae* carriers and its microbial resistance in children

Langhuan Lei[1], Xingyong Wang[2*]

[1]Department of Critical Care Medicine, Children's Hospital of Chongqing Medical University, Chongqing 400014, China. Ministry of Education Key Laboratory of Child Development and Disorders, Chongqing 400014, China. China International Science and Technology Cooperation Base of Child Development and Critical Disorders, Chongqing 400014, China. Chongqing Key Laboratory of Pediatrics, Chongqing 400014, China

[2]Department of Critical Care Medicine, Children's Hospital of Chongqing Medical University, Chongqing 400014, China

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ABSTRACT

Streptococcus pneumoniae is a common cause of bacterial infections of the respiratory system, middle ear infection, bacteremia, meningitis, and pneumonia, especially in children. Due to the lack of information about the frequency and resistance of *Streptococcus pneumoniae* to antibiotics, the present study was performed to determine the frequency of carriers of *Streptococcus pneumoniae* and its microbial resistance in children. For this purpose, the current descriptive cross-sectional study was conducted from November to March 2020 on 554 children aged 2-12 years in kindergartens and schools. This study collected samples with a sterile swab from the nasopharyngeal region, transported them to the laboratory by a transport medium, and then cultured them on an agar culture medium. After isolation, confirmatory tests and antibiotic susceptibility were performed. The results were analyzed using SPSS16 software and interpreted according to Mann Whitney U and Chi-Square Tests. *Streptococcus pneumoniae* was found in 15% of samples, and the antibiotic resistance of the isolates to the antibiotics azithromycin, amoxicillin, rifampicin, amoxicillin-clavulanic acid, trimethoprim/Sulfamethoxazole, and ceftriaxone were 63.9%, 56.6%, 41%, 37.3%, 37.3%, and 3.6%, respectively. Also, 31.1% of the isolates were not resistant to any antibiotics. According to the results, excessive use of antibiotics has led to high resistance to azithromycin, amoxicillin, amoxicillin/clavulanic acid, and trimethoprim/Sulfamethoxazole, which indicates an increased risk of refractory infectious diseases. For this reason, it is necessary to adequately educate physicians and the general public about the overuse of antibiotics.

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Introduction

Streptococcus pneumoniae is a primary worldwide pathogenic bacterium that often causes severe infections, including invasive pneumonia, a significant cause of morbidity and mortality in young children, and significant causes of bacterial pneumonia are meningitis, otitis, sepsis, and bacteremia (1). *Streptococcus pneumoniae* is a capsular bacterium with many different serotypes based on capsular polysaccharides (2). The clinical spectrum of pneumococcal infection varies from mild to severe and aggressive.

The highest carrier rate for *Streptococcus pneumoniae* is in childhood and gradually decreases (3). On the other hand, the rate of nasopharyngeal carriers is more common among children kept outside the home (4, 5). Other predisposing factors include food poverty, use of solid fuels in the house, overcrowding and crowded places, lack of breastfeeding, lack of maternal education, limited secondary health care, and post-disease care that causes aggressive pneumonia and death (4, 6).

Despite the pneumococcal polysaccharide vaccine and its effect on invasive pneumococcal diseases, this bacterium is still one of the leading causes of illness and death, especially in countries where the source of disease transmission is not restricted (7). Over the past decade, pneumococci have caused an average of 14.5 million serious infections worldwide each year, accounting for 11 percent of deaths in children under five years old (8).

*Corresponding author. E-mail: 137183528@qq.com

On the other hand, the identification of nasopharyngeal carriers of *Streptococcus pneumoniae* in healthy children is of particular importance due to the risk of invasive pneumococcal disease in these children and the risk of transmission to populations that have not received the vaccine (7). However, limited studies have been conducted on the prevalence of pneumococcal diseases in some countries. The results of previous studies indicate that this disease is highly prevalent in many countries (9). On the other hand, the prevalence of invasive pneumococcal diseases in different seasons shows different patterns (10). Therefore, there is a need for extensive research to investigate the prevalence of these infections in these countries (11). In addition, it is vital to study and find the risk factors that contribute to childhood pneumonia and can provide solutions to prevent the disease (6).

Today, antibiotic resistance in *Streptococcus pneumoniae* is a major public health issue, and resistance to macrolides, tetracyclines, and cephalosporins (such as ceftriaxone) has recently become widespread (12). Eleven percent of pneumococcal isolates worldwide are classified as multi-drug resistant. According to previous studies, the serotypes and antibiotic susceptibility patterns in pneumococci obtained from asymptomatic carriers are similar to invasive species (8). Therefore, antibiotic susceptibility can also be detected in invasive species by determining the antibiotic susceptibility pattern in *Streptococcus pneumonias* from carriers like the nasopharynx (13). Currently, in all outpatients and inpatients and in the early stages of diagnosis, treatment is performed experimentally and without antibiograms, the identification of antibiotic susceptibility patterns in the effective treatment of these infections is critical. Also, studying asymptomatic carriers and antibiotic susceptibility patterns to *Streptococcus pneumoniae* is necessary (14). Therefore, this study was performed to determine the prevalence of carriers of *Streptococcus pneumoniae* and to evaluate its microbial resistance in children.

Materials and methods

The present study was a descriptive cross-sectional study that was done from November to March 2020. The study samples included 554 children aged 2-12 years from kindergartens and schools in the city. The confidence level was 95%, and the accuracy rate was 5%. Children were selected by random sampling from kindergartens and schools in the town, and kindergartens and schools were also chosen randomly (random cluster sampling). After completing the questionnaire and selecting the samples, the recent illness history and the history of receiving medication in the last month were examined. Children who had received antibiotics at least two weeks to one month before sampling or were receiving antibiotic treatment at the time of sampling were excluded from the study.

No charges were imposed on individuals for carrying out this plan, and the provisions of the Helsinki Declaration were made. Samples were taken with the parents' written permission by an experienced laboratory technician and with the help of sterile swabs orally and from the back of the throat (in the area of the palatine tonsils) (15, 16). The limitation of this study was to obtain the medical history and records of the subjects to confirm the accuracy of information obtained from the patient's parents about the underlying disease or history of antibiotic use.

Swaps were placed on Amies Transport Medium for sending to the microbiology laboratory. Then samples were cultured on an agar medium (Merck, Germany) containing 5% sheep blood. The plates were incubated in a jar containing 5% carbon dioxide at 37°C for 48 hours. Then the plates were examined for the growth of fine gray colonies by alpha hemolysis, and the confirmed colonies were isolated for alpha hemolysis and re-cultured on a blood agar medium. After determining susceptibility to aptoxin (growth inhibition zone ≥14 mm), catalase test, gram staining, microscopic examination, and confirmation of positive samples were taken for *Streptococcus pneumoniae*. Samples were performed for antibiogram on Müller-Hinton agar medium (Merck, Germany) with blood by disk diffusion

under anaerobic conditions with 5% carbon dioxide. Antibiotic discs including ceftriaxone, azithromycin, trimethoprim/sulfamethoxazole, amoxicillin, amoxicillin/clavulanic acid, and rifampicin (HiMedia) were selected, and antibiotic susceptibility of isolated bacteria was determined according to the standard method recommended by the Clinical and Laboratory Standards Institute (CLSI).

The results were analyzed using SPSS16 software and interpreted according to Mann Whitney U and Chi-Square Tests due to the randomness of the collected data.

Results and discussion

The sample consisted of 320 (57.8%) boys and 234 (42.2%) girls with a mean age of 7.4 years; the mean age for boys was 7.8 ± 2 years and 7.1 ± 1.36 years for girls. To evaluate the relationship between gender and the prevalence of the disease, *Streptococcus pneumoniae* was isolated from 83 children (14.98%). Forty-six (14.37%) of boys and 37 (15.81%) of girls were carriers, and there was no statistically significant difference between the number of carriers of boys and girls (P = 0.46) (Figure 1).

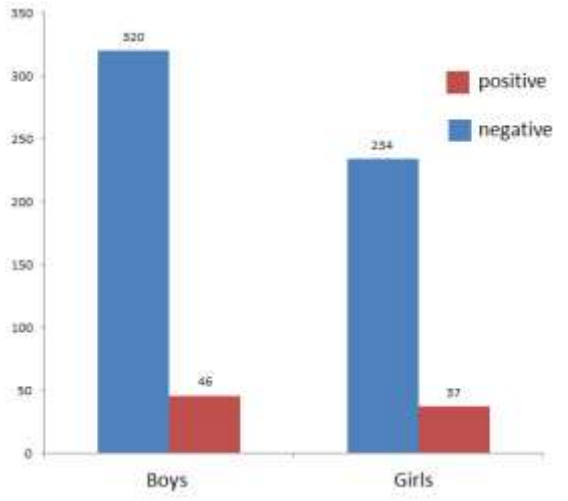

Figure 1. Prevalence of *Streptococcus pneumoniae* in two groups of girls and boys

To investigate the relationship between aging and the prevalence of the disease, the subjects were divided into two groups: 3-7 years old and 8-12 years old, of which 45 (54.2%) were 3-7 years old, and 38 (45.8%) were in the age range of 8-12 years that there was no statistically significant difference between the two age groups in terms of prevalence (P = 0.27). Antibiotic susceptibility testing was performed for 83 *Streptococcus pneumoniae* isolates, and the antibiotic resistance to azithromycin and amoxicillin was 63.9% and 56.6%, respectively, and the lowest antibiotic resistance ceftriaxone (3.6%) was observed and 25 cases (31.1%) of the isolates were not resistant to any of the studied antibiotics. 27.7% of isolates were resistant to less than three antibiotics, and 40.9% of isolates were multi-drug resistant. None of the isolates showed resistance to all six antibiotics. There was no statistically significant difference between the two genders in drug resistance to ceftriaxone, azithromycin, trimethoprim/sulfamethoxazole, amoxicillin, and amoxicillin/clavulanic acid. Still, the rate of rifampicin resistance was 45.7% in boys and 35.1% in girls, and it was statistically significant (P = 0.03). There were no statistical differences observed between ceftriaxone, azithromycin, trimethoprim/sulfamethoxazole, amoxicillin, and amoxicillin/clavulanic acid in the age groups of 3-7 and 8-12 years (p = 0.39). While in the case of rifampicin, the rate of drug resistance was 51.1% in the age group of 3-7 years and 28.9% in the age group of 8-12 years, which is a statistically significant difference (P = 0.04) (Table1).

Table 1. Frequency of susceptibility and resistance of *Streptococcus pneumoniae* isolates to the studied antibiotics from the nasopharynx of healthy children

Antibiotic	susceptibility		Relative susceptibility		Resistance	
	number	percent	number	percent	number	percent
Amoxicillin	36	43.3	0	0	47	56.6
Amoxicillin/Clavulanic Acid	52	62.7	0	0	31	37.3
Azithromycin	26	31.3	4	4.8	53	63.9
Ceftriaxone	80	96.4	0	0	3	3.6
Trimethoprim/Sulfamethoxazole	52	62.7	0	0	31	37.3
Rifampicin	49	59	0	0	34	41

The carriers of *Streptococcus pneumoniae* in the community cause pneumonia, otitis, meningitis, and mortality, especially in children (17). In this study, the prevalence of pneumococcal carriers compared with similar studies shows a significant difference. For example, in a study, the pharyngeal prevalence of

this bacterium in primary school children was 10.9% (18). In another study in kindergartens, the prevalence was reported to be 8.78%, which was lower than in our study (19). This difference can be justified according to the region's temperature and sampling in the cold months of the year. But in another study, the prevalence of carriers was reported to be 37.5% (20). In another research, the prevalence of carriers of *Streptococcus pneumoniae* was 44.1%, which was much higher than in the present study (21). This high rate may be due to congestion or a low level of hygiene in kindergartens in these areas. Also, in the last two studies, samples were collected in winter, when the prevalence of carriers of *Streptococcus pneumoniae* was higher, while, in our study, sampling was in autumn and winter. Sampling techniques are also effective in reporting the prevalence. In Turkey, the prevalence of carriers of *Streptococcus pneumoniae* in healthy children aged 6-13 years was reported to be 13.9% (22), and in a study in Poland, the prevalence in children aged 3-5 years in kindergarten was 35.7% (23). In a study in Belgium, *Streptococcus pneumoniae* was 69% (8).

Also, this study showed that the rate of asymptomatic carriers of *Streptococcus pneumoniae* is low compared to other countries. In addition to geographical differences, this information is a sign of good health care conditions.

In the present study, the resistance rate to the six studied antibiotics showed a different pattern than in similar studies in previous years. According to a study conducted in Turkey, the resistance to trimethoprim/sulfamethoxazole was consistent with the results of this study (22). Still, the resistance to azithromycin in our study was much higher than the result reported in Turkey, which could be due to differences in common antibiotics in the two countries. In our study, 30.1% of the isolates were not resistant to any of the six antibiotics. In contrast, in a study in Spain, 95% of the isolated *Streptococcus pneumoniae* were resistant to amoxicillin-clavulanic acid due to overuse of this antibiotic for the last few decades (24). Also, in a study in Taiwan, the rate of resistance to ceftriaxone was significantly higher than the rate obtained in this study (25). This difference in the level of resistance and the type of resistant antibiotics in different parts of the world is due to the different types of antibiotics. Treatment of *S. pneumoniae* infections should be based on the site of infection and the underlying disease, and the high resistance to trimethoprim/sulfamethoxazole and rifampicin in our study showed that these two antibiotics do not help treat these infections. One of the main reasons for the high resistance of *Streptococcus pneumoniae* and other bacterial species to these antibiotics is their excessive use in lung infections, sinusitis, and middle ear infections, as well as over-the-counter sales in pharmacies (26). It may also be due to the high prevalence of brucellosis and tuberculosis and the over-use of antibiotics in treating these two diseases, especially at a young age.

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None.

Conflict interest

None.

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