

Distribution and antibiotic susceptibility of *Streptococcus pneumoniae* isolated from hospitalized and outpatient's children in educational hospitals of Ahvaz, Iran

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Abstract

Streptococcus pneumoniae is known as the leading cause of bacterial pneumonia and major cause of death in children under five years annually. Its growing resistance to a variety of antibiotics became contentious. We performed this study to evaluate the prevalence and antimicrobial susceptibility in *S. pneumoniae* in educational hospitals of Ahvaz. Thirty-two *S. pneumoniae* isolated from clinical samples of children, referring to educational hospital of Ahvaz, Iran. All samples were investigated by common microbiology tests including Gram staining, blood agar, bile esculin and optochin disk. For antibiotic resistance evaluation, the disk diffusion method is performed using Mueller-Hinton agar. Isolates were tested by penicillin, erythromycin, oxacillin, vancomycin, levofloxacin, trimethoprim-sulfamethoxazole and clindamycin disks. All of the 32 samples were confirmed as positive for *S. pneumoniae*. Twenty-two of samples (64.70%) were penicillin-susceptible while 8 (23.52%) were intermediately-resistant and 4 (11.76%) were resistant. Also, 20 (62.5%), 25 (78.12%) and 16 (50%) of the isolates were resistant to erythromycin, trimethoprim-sulfamethoxazole and clindamycin, respectively. Near all of the samples (29 of the isolates) were susceptible to levofloxacin. All of the samples were susceptible to vancomycin. Due to the results, the most of the isolates were susceptible to common antibiotics except erythromycin, trimethoprim-sulfamethoxazole and clindamycin. Our results showed encouraging susceptibility for vancomycin.

Keywords: *Streptococcus pneumoniae*, Antibiotic resistance, Ahvaz, Iran

1. Introduction

Streptococcus pneumoniae, a Gram-positive bacterium, is seen in pairs or chains below the microscope. Other principal characteristics of *S. pneumoniae* are alpha hemolysis, lack of catalase, susceptibility to optochin disk, and solubility in bile salts. The bacterium is known as one of the superior respiratory system's normal flora, which possesses 93 serotypes based on its polysaccharide capsule. Among these serotypes, ten top serotypes are the leading cause of the infection. Serotype distribution varies due to

age, type of disease, or geography. *S. pneumoniae* is one of the most commonly investigated causes of pneumonia, meningitis, sepsis, bacteremia, otitis media and sinusitis [1-3].

S. pneumoniae can be introduced as the primary cause of bacterial pneumonia and the leading factor of morbidity and mortality in young children, such that it causes 100-500 thousand deaths from meningitis per year. Most death cases are usually seen in children under five years of age and are more common in developing countries [4, 5]. Children, significantly less

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than two years, have a higher risk regarding their lack of antibodies. Other complications such as immunodeficiency, serotype's pathogenicity level and environmental factors can affect its pathogenesis [6, 7]. To name some other underlying factors that can complicate the condition: food poverty, usage of solid fuel, presence in crowded places, lack of breastfeeding, lack of training for mothers and secondary health care limitation can be mentioned [8]. For an extended period, almost all of the *Streptococcus pneumoniae* were susceptible to penicillin and cotrimoxazole so that pneumococcal infections were effectively treated with penicillin. Unfortunately, this sensitivity reduces rapidly over time, and penicillin-resistant isolates increase in most areas. Although some of them indicate intermediate susceptibility [4]. Nowadays, antibiotic resistance of *S. pneumoniae* turned into a vital issue in public health, and recently resistance to macrolides, tetracycline and cephalosporines became prevalent. Fifteen to thirty percent of pneumococcal isolates are classified in the multidrug-resistant category [8]. Antibiotic resistance is more common in children due to their repeated courses of antibiotic treatment [1].

The emergence of multidrug-resistant species complicated the empirical pneumococcal treatment; hence, prevention of multidrug resistance occurrence has to be a crucial concept in therapeutic protocols [9]. Based on our knowledge, few studies have been published in Ahvaz on the importance of *S. pneumoniae* and its effects on children's health. Therefore, this study aimed to check out the frequency and antibiotic susceptibility of *S. pneumoniae* in children in educational hospitals of Ahvaz. We hope that this survey helps evaluate this bacterium's prevalence and antibiotic resistance profile to find an effective cure for pneumococcal infections and reduce costs associated with the healthcare system.

2. Materials and Methods

2.1 Study population and data collection

This retrospective study composes 32 children's specimens which examined and defined as positive for *S. pneumoniae*, collected at educational hospitals of Ahvaz from 21st March 2019 to 20th March 2021. *S. pneumoniae* isolates were obtained from clinical samples such as blood, cerebrospinal fluid (CSF), eye, and ear samples. The survey was approved by the research ethics committee of Ahvaz Jundishapur

University of Medical Sciences (IR.AJUMS.REC.1399.166).

2.2 Bacterial identification

Clinical Samples was sent to the hospital laboratory, then considered positive for *S. pneumoniae* with the conventional culture method. Gram staining, hemolysis on blood agar, presence of catalase and optochin susceptibility disk was applied to distinguish the bacterium by hospital laboratory technician as well.

2.3 Antibiogram test

Antibiotic susceptibility tests were performed on Mueller-Hinton agar using disk diffusion method according to the Clinical Laboratory Standard Institute (CLSI, 2022) principals. Penicillin, erythromycin, oxacillin, vancomycin, levofloxacin, trimethoprim-sulfamethoxazole and clindamycin disks were used [10].

2.4 Statistical analysis

IBM SPSS Statistics version 26 was used in order to statistical analysis. The results are presented as descriptive statistics in terms of relative frequency. Values were expressed as percentages of the groups.

3. Results

During twenty-four months study, there were 32 confirmed positive for *S. pneumoniae* in educational hospitals of Ahvaz. Among these cases, 18 (56.25%) of them were female and 14 (43.75%) were male. This study includes 12 (37.5%), 8 (25%), 9 (28.1%) and 3 (9.4%) of blood, CSF, eye and ear samples, respectively.

In terms of antibiotic resistance, Table 1 gives information about antibiogram results. Based on the information above in Table 1, there were 22 (64.70%) penicillin-susceptible isolates, 4 (11.76%) were resistant to penicillin, and 8 (23.52%) of isolates showed intermediate-resistant to penicillin. Five (15.62%) of bacteria in this study showed susceptibility to erythromycin, and 20 (62.5%) of them were erythromycin-resistant. Results indicates, 25 (78.12%) isolates susceptible, 3 (9.37%) resistant, and 4 (12.5%) intermediated-resistant to oxacillin. All of the isolates showed susceptibility to vancomycin.

Table 1. Results of antibiotic susceptibility testing

Antibiotic	Susceptible No. (%)	Intermediate No. (%)	Resistant No. (%)
Penicillin	22 (64.7%)	8 (23.52%)	4 (11.76%)
Erythromycin	5 (15.62%)	7 (21.88%)	20 (62.5%)
Oxacillin	25 (78.12%)	4 (12.5%)	3 (9.38%)
Vancomycin	32 (100%)	0	0
Levofloxacin	29 (93.54%)	1 (3.22%)	1 (3.22%)
Trimethoprim-sulfamethoxazole	6 (18.75%)	1 (3.12%)	25 (78.12%)
Clindamycin	11 (34.38%)	5 (15.62%)	16 (50%)

4. Discussion

The prevalence and antibiotic resistance of *S. pneumoniae* among children in Ahvaz were investigated in this study, and *S. pneumoniae* was studied in 32 samples. In our study, 11.76 percent of pneumococcal isolates were penicillin-resistant, while 23.52 percent were intermediately-resistant indicating a high rate of penicillin non-susceptible pneumococcal strains (PNSP). PNSP turned into a universal issue. Resistance to penicillin occurs after a considerable drop in the affinity of the penicillin-binding protein (PBP) structure [11]. This problem can be caused by several circumstances. However, improper antibiotic consumption is the leading cause of this occurrence [12]. In a study conducted by Kohanteb et al. in 2006 (Shiraz, Iran), 33.9% of penicillin non-susceptible isolates were observed [13]. In another research, Bakhshae et al. claimed that the rate of penicillin-resistant strain is high in Mashhad, Iran [14]. Following the studies above, penicillin-resistant isolates are on the rise in Iran same as the neighboring countries. For instance, a study in turkey shows that the rate of penicillin resistance is remarkably increasing in some centers. Although, it claims that this rate varies in different centers [15].

In addition, 62.5% of isolates tested positive for erythromycin-resistance. This high rate of erythromycin-resistance isolates is noted in Talebi et al. research as well [16]. Moreover, Ahmadi et al. discovered a high prevalence of erythromycin-resistant in Iran [17]. According to these findings, the rising prevalence of erythromycin resistance is endangering the health condition of Iranian youngsters.

Trimethoprim-sulfamethoxazole resistance was found in 78.12% of our cases. In Beheshti et al. study 2020, all of their penicillin-resistant isolates showed

resistance to erythromycin and trimethoprim-sulfamethoxazole [18]. According to a study in Taiwan, a significant percentage of patients were resistant to trimethoprim-sulfamethoxazole [19]. Despite an increased trend in trimethoprim-sulfamethoxazole resistance, to our knowledge, no independent research has been conducted in Iran to demonstrate this susceptibility.

Vancomycin non-susceptible strains can emerge via uncontrolled vancomycin self-treatment, according to Nazari Nazari Alam et al. study [20]. Our findings, in contrast to theirs, reveal complete susceptibility to vancomycin, which could be promising for children's therapy in Ahvaz. Antimicrobial resistance is increasing globally, according to World Health Organization data, independent of a country's financial level. According to their findings, antibiotic resistance of *Streptococcus pneumoniae* has become contentious, and despite its threat to children, it has received little attention. Based on the emergence of multidrug-resistant serotypes, the health care system must make every effort such as antimicrobial stewardship, public surveillance, vaccination and national awareness in order to prevent resistance and decrease the number of deaths in children annually [21, 22].

The main limitation of study was limited sample size, however, any step towards improving antibiotic stewardship program can be helpful.

As previously stated, more research into *S. pneumoniae* antibiotic resistance is required. According to our findings, the most of the isolates were susceptible to common antibiotics except erythromycin, trimethoprim-sulfamethoxazole and clindamycin. Our results showed encouraging susceptibility for vancomycin.

Authors' contributions

Conception or design of the work: AY; Data collection: MM; Data analysis and interpretation SN; Drafting the article: MM, SN ; Critical revision of the article: AY. All authors read and approved the final version of manuscript.

Conflict of interests

The authors declare that they have no conflict of interest to report.

Ethical declarations

The study was approved by the Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (No. IR.AJUMS.REC.1399.166).

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