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Characteristics and Genotyping for Children With Pneumonia Caused by Streptococcus Pneumonia and Using Antimicrobial Drugs

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ABSTRACT

The prevalence of Streptococcus pneumoniae and resistance to antibiotics have become a public health problem in different countries of the world. This study aimed to investigate the occurrence of Streptococcus pneumoniae nasal carriage and sputum among children, and their antibiotics susceptibility profiles. A nasal swab and sputum was obtained from 463 healthy children aged from 5to12 years in dohuk hospital for kids, Kurdistan region, Iraq. The swabs nasal carriage and sputum were cultured on appropriate culture media to isolate Streptococcus pneumoniae and to examine their susceptibility to antibiotics. The antibiotic susceptibility testing was performed using the standard disk-diffusion method, Streptococcus pneumoniae was isolated from 206 (44.49%) of the specimens; were resistant for penicillin and ampicillin, in addition Cefuroxime and Ceftriaxone, while was sensitive for Clindamycin, Moxifloxacin, Clarithromycin, Ciprofloxacin, Erythromycin and Azithromycin, Genotypes c, e, f and k were detected by PCR. Of the 206(44.49%) samples, 143 (30.89%) isolates corresponded to children that infected were positive for S. pneumoniae genotype c, 21 (4.53%) genotype f, genotype k, and genotype c and k, respectively.

INTRODUCTION

Streptococcus pneumoniae is the most common bacterium that causes community acquired pneumonia (CAP) in children (1-3). The burden caused by S. pneumoniae is strongly related to high morbidity and mortality in children under 5 years of age (4), Every year in developing countries, some 4.5 million persons, most of them under 5 years of age, die of acute respiratory infections (ARI) (5). Most ARI episodes are caused by viral agents and are self-limiting, but the bacterial pneumonias, which occur less frequently, carry a much higher risk of complications and death (6). According to World Health Organization (WHO), pneumonia was a major cause of mortality among children in 2010. (7) Pneumonia accounts for 21% of mortality in children younger than 5 years in African and Eastern Mediterranean regions and 12% of mortality in the Americas and in European regions (8), Pneumococcus is a Gram-positive bacterium and is a leading cause of bacterial pneumonia in children worldwide. In 2000, it was reported that 741,000 children less than 5 years old (accounting for 36% of all-cause pneumonia deaths) succumb to pneumococcal pneumonia, with the majority of them from developing countries (9). Necrotizing pneumococcal pneumonia in children was first reported in 1994 (10), and an increase in its prevalence has been observed since then. However, the detailed mechanism is still unknown. Streptococcus pneumoniae is a common commensal of the upper respiratory tract. Through colonization, it can lead to local or systemic infections. Spread from the nasopharynx can lead to local infections such as sinusitis or acute otitis media. Aspiration can result in direct spread of S. pneumoniae to the lungs, leading to community-acquired pneumonia (CAP), with pleural empyema as a complication occurring in less than 5% of CAP cases. Invasive disease can occur in 10– 15% of patients with CAP. The pneumococcus can also spread via the blood to the brain, causing pneumococcal meningitis. Peritonitis, arthritis and osteomyelitis are rare manifestations of pneumococcal disease (11).

Although S pneumoniae exists in encapsulated and encapsulated forms, only encapsulated strains have been isolated from clinical material. The importance of the capsule in pneumococcal virulence was first established by enzymatic removal of the capsule, (12) and has recently been confirmed using genetically engineered pneumococci which diver only in capsular type. The virulence of the mutants in relation to the parental strains was determined mainly, though not entirely, by the

capsular type (13). In the treatment of streptococcal diseases, the use of antibiotics is one of the most efficient therapeutic strategies. However, some Streptococcus species possess factors that provide them with antibiotic tolerance. Penicillin binding proteins (PBPs) are associated with β-lactam antibiotic resistance in S. pneumoniae (14), and six high molecular-weight PBPs have been identified in this species (PBP1a, 1b, 2a, 2b, 2x, and 3) (14). Moreover, our studies have shown that ClpL binds to PBP2x, which subsequently enhances the antibiotic tolerance in S. pneumoniae by increasing cell wall thickness (15). We also found that VncS/R, a member of the pneumococcal TCS, plays an important role in the regulation of pneumococcal virulence and vancomycin tolerance (unpublished data). Furthermore, ClpP has been shown to protect S. mutans from cell-wall-damaging antibiotics (16).

Phylogenic and whole genome sequencing analyses have indicated that Streptococcus has 4,514 genotypes (https://www. patricbrc.org), including S. pneumoniae (3,404 genotypes), S. agalactiae (300 genotypes), S. pyogenes (230 genotypes), S. mutans (165 genotypes), and others (415 genotypes). Based on their hemolytic activity, Streptococcus species can be divided into two major groups: β-hemolytic (e.g. S. pyogenes, S. agalactiae) and non-β-hemolytic (e.g. S. pneumoniae, S. mutans) (17). In pneumococci, antibiotic resistance has been associated with diseases such as COPD that receive multiple courses of antibiotics for exacerbations (18,19) In previous studies, we described changes in antimicrobial susceptibility and in the serotype and genotype distributions of pneumococci responsible for AE-COPD over a 12-year period, including the early PCV13 period (19).

This study aimed to investigate the occurrence of S. pneumoniae by taken nasal carriage swab and sputum samples for children, and their antibiotics susceptibility profiles. And its genotypes in children (boys and girls) aged 1-12years.

MATERIAL AND METHODS

Study was carried out during the period from January 2019 to December 2019. A nasal swab was obtained from each child with a cotton swab pre-moistened with sterile normal saline, then inserted and rotated in each of the anterior nares. (20). The studied population consisted of healthy children aged 5–12 years in Dohuk hospital for kids, Kurdistan region, Iraq. Sputum specimens are routinely collected from to children determine the etiology of lower respiratory tract infections. In addition, the sputum was cultured on media, after which a swab was taken from the media and examined with a microscope. Microbiologic examination of sputum is central to the diagnosis of pneumonia. Although the sensitivity of sputum examination has been reported, endings can be affected by antibiotic use prior to specimen collection, and sputum culture results can be difficult to interpret because of difficulties discriminating between infecting and colonizing bacteria. success of the procedure was defined as (i) obtain at least 1 ml of sputum, (ii) obtaining good quality sputum.

The isolates were identified as S. pneumoniae by colony morphology hemolysis on blood agar, and Gram stain. The identification was confirmed by optochin sensitivity and bile solubility test.23 Antibiotic susceptibilities to Penicillin G, Ampicillin, Cefuroxime, Ceftriaxone, Ciprofloxacin, Moxifloxacin, Erythromycin, Azithromycin, Clarithromycin, and Clindamycin.

Genotypes c, e and f of isolates identified as S. mutans were detected using the Primers used in the PCR assay were SC-F, SC-R, SE-F, SE-R, SF- multiplex PCR (21) F, SF-R. Each 50 µL reaction mixture contained 16 mM (NH4)2SO4, 67 mM Tris-HCl (pH 8.8), 0.01% Tween-20, 2 mM de MgCl2, 0.2 mM of each dNTP, 0.5 µM of each oligonucleotide primer, 1U Taq DNA polymerase and 50-100ng DNA. Samples were amplified in a thermocycler iCycler (Bio-Rad). PCR conditions were described previously (30). S. mutans genotype k was identified by conventional PCR using a set of specific primers CEFK-F and KR based on the sequence of genes rgp F and rgpE and the PCR conditions previously described.

Statistical Analysis Statistical analysis was carried out using to analyze the data statistically and determine the statistical differences on the base SPSS program.

RESULTS

A total of 463 children of whom 266 were boys, and 197 girls the majority of the children were living in urban environments (Table 1). According to age groups, the high percentage of S. pneumoniae was (61 children out of 206) among children in the age group 5 to 6 years (Table 1). The rate of S. pneumoniae was 24.19% among boys, and it was 20.30% among girls. The high percentage of antibiotics resistance was 55.83% to penicillin and 55.34% to ampicillin, followed by cefuroxime (45.64 %), Ceftriaxone (44.18%), All S. pneumoniae were sensitive to Azithromycin, Erythromycin, Ciprofloxacin, Clarithromycin, Clindamycin and Moxifloxacin (Table 2).

Table 1: Characteristics of studied children and Distribution of children with S. pneumonia infected according to age and gender.

Characteristics	Number of children examined	children that infected S. pneumonia	
Age (years)		No.	%
5-6	144	61	29.61
7-8	121	55	26.69
9-10	102	48	23.30
11-12	96	42	20.38
Total	463	206	44.49
Gender	Number of children examined	children that infected S. pneumonia	
		No	%
Girls	197	94	20.30
Boys	266	112	24.19
Total	463	206	44.49

Table2. Antibiotics susceptibility of S. pneumonia isolated from sputum and nasal carriage.

Antibiotics (concentrations)	Total number of isolated tested=206			
	. Sensitive		Resistance	
	No.	%	No.	%
Azithromycin (10 µg)	188	91.26	18	8.74
Erythromycin (15 µg)	184	89.32	22	10.68
Ciprofloxacin (10 µg)	187	90.77	19	9.23
Clarithromycin (15 µg)	197	95.63	9	4.37
Penicillin G (10 units)	91	44.17	115	55.83
Cefuroxime (15 µg)	112	54.36	94	45.64
Ampicillin (10 µg)	92	44.66	114	55.34
Clindamycin (5 µg)	190	92.23	16	7.77
Moxifloxacin (5 µg)	192	93.20	14	6.80
Ceftriaxone (10 µg)	115	55.82	91	44.18

Table 3. Number and percentage of children that infected and non-infected Streptococcus pneumonia and genotypes

Groups	No.(%)
children no infected S. pneumonia	257)%55.51)
children that infected S. pneumonia	206)%44.49)
Genotype c	143 (30.89%)
Genotype f	21 (4.53%)
Genotype k	21 (4.53%)
Genotype c and k	21 (4.53%)

genotypes in children (boys and girls) that infected by S. pneumoniae, Overall, prevalence of S. pneumoniae was 44.49 % (Table 3). children that no infected S. pneumonia was not detected. Prevalence of S. pneumoniae genotype c 143 (30.89%) genotype f and genotype k 21 (4. 53%).Genotype c and k 21 (4.53%), sputum and nasal examination of one patient with genotype c and k showed the percentage of infected by S. pneumoniae was very high.

DISCUSSION

In the present study, the high rate of S. pneumoniae was observed among high rate of S. pneumoniae was observed among children aged 5 to 6years than other age groups. The immunological developments of cellular and humoral responses to S. pneumoniae capsular polysaccharide contribute towards a much lower incidence of pneumococcal disease in older children than in young children. (22,23,24). the study was observed that the prevalence of S. pneumoniae more common in

boys compared to the girls; this may be due to the boys in our culture is more exposed to the external environment. The prevalence of antibiotics resistance has been studied in different countries in the world. In the present study, the high rate of penicillin and ampicillin resistance was observed S. pneumoniae of nasal and sputum isolates that lower than reported in Taiwan, Korea, Sri Lanka, Vietnam, and Saudi Arabia (50%). (25). Therefore, use of new antibiotics such as azithromycin, clarithromycin, clindamycin, and vancomycin for treatment of S. pneumoniae, the study reported a low level of resistance to these antibiotics. The rate of urban children to penicillin and ampicillin resistance of S. pneumoniae isolates was significantly lower than the rate obtained in children of the rural area; this was in agreement with a study has been reported in Southern Vietnam of which the use of the antibiotic in rural children was lower than in urban children. studies have shown that variations of antibiotic consumption are well correlated to S. pneumoniae rates at the country level.

prevalence of S. pneumoniae genotype c 143 (30.89%)genotype f and genotype k 21 (4.53%) was lower, this study reports for the first time the presence of genotype k in Dohuk. Prevalence of genotype k 42 (9.06%) was, similar to reports from Japan and Thailand (1-5%) (26, 27,28,30,31) but lower than reported in México (16.9%) (32). Streptococcus pneumoniae serotype k has been found in saliva and blood isolates (26,29 33,34). and its ability to survive for longer periods in blood, due to resistance to phagocytosis (35). Also, this new serotype has been related with bacteremia, systemic inflammation and was described as one of the risk factors of infective endocarditis and hemorrhagic stroke (36). Thus, future studies in Colombia are required for understanding the role of S. pneumoniae genotype k in the development of cardiovascular infective.

CONCLUSION

Streptococcus pneumoniae isolated from Nasal and sputum among children that could serve as reservoirs for the transmission to the community and cause disease, the high rate of S. pneumoniae antibiotics susceptibility was observed for 8 antibiotics while showed resistance for ampicillin and penicillin Thus, future studies are required for understanding the role of S. pneumoniae genotype k in the development of cardiovascular infective.

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