African Journal of Microbiology Research Vol. 6(1), pp. 28-33, 9 January, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.271
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Serotype distribution and antimicrobial resistance of invasive *Streptococcus pneumoniae* isolates from children in Zahedan, Iran

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Accepted 5 December, 2011

Streptococcus pneumoniae **is the leading cause of death among children worldwide. This study was conducted to evaluate the antibiotic resistance patterns and seroepidemiology of invasive *S. pneumoniae* isolates in children of Zahedan. Invasive isolates of *S. pneumoniae* were obtained from normal sterile sites (blood, cerebrospinal fluid, pleural fluid, joint aspirate, peritoneal fluid and pericardial fluid) of pediatric patients (aged ≤14 years) from 3 large hospitals in Zahedan, Iran. The serotyping was carried out by latex agglutination test and the minimum inhibitory concentrations (MIC) of penicillin, as well as other commonly used antibiotics were determined by broth dilution method. During February 2008 to November 2010, a total of 1265 children with suspected invasive bacterial disease were recruited, of whom 75 (5.9%) were identified to have *S. pneumoniae* as the causative agent of invasive disease. The leading serotypes were 23F, 6B, 3, 19F and 14 (70.7% of all isolates). Intermediate- and high-level resistance, were observed in 37.3 and 45.4% of isolates respectively. Resistance was also demonstrated against erythromycin (90.7%), cotrimoxazole (80.0%), tetracycline (66.6%), cefuroxime (62.7%), imipenem (17.3%) and amoxicillin/clavulanate (9.2%). All the isolates were susceptible to vancomycin and levofloxacin. Multiple drug resistance was observed mostly in types 19F, 23F, 6B and 14. The application of heptavalent pneumococcal conjugate vaccine (PCV7) covered 68.0% of all IPD cases, 42.7% of the penicillin-nonsusceptible *S. pneumoniae* (PNSSP), and 48.0% of the multiple drug resistant (MDR) isolates. The highest PNSSP prevalence and highest PCV7 coverage were in children aged <2 years. Antibiotic resistance was common among invasive isolates of *S. pneumoniae* in Zahedan. The present study suggests that the introduction of anti-pneumococcal conjugate vaccines into immunization programme would likely lead to a significant reduction of IPD- associated morbidity among children in Zahedan.**

Key words: Antimicrobial resistance, invasive pneumococcal disease, serotype, *Streptococcus pneumoniae*.

INTRODUCTION

Streptococcus pneumoniae is among the most important pathogens in bacterial pneumonia, septicemia, and meningitis worldwide (Imöhl et al., 2010). It continues to be a major cause of morbidity and mortality globally (O'Brient et al., 2009). According to reports by the World Health Organization, 1.6 million persons die of pneumococcal disease each year, of whom 0.8-1 million are children aged <5 years and mostly living in developing countries (Centers for Disease Control and

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Prevention, 2008). The capsular polysaccharide of *S. pneumoniae* is an important factor in the pathogenicity of the organism and association between the capsular serotypes and the severity of invasive pneumococcal disease (IPD) has been described (Imöhl et al., 2010). In spite of extensive use of antibiotic treatment in such invasive disease, the emergence of a progressive multi-drug resistance is a major concern in many parts of the world in recent years (Doern et al., 2005). This is especially true in Iran, where it restricts the therapeutic options available for serious pneumococcal infections (Kohanteb and Sadeghi, 2007).

There are more than 90 known pneumococcal serotypes and these are classified into 46 serogroups, but the majority of invasive and noninvasive diseases are associated with few numbers of serotypes (Hausdorff et al., 2005). The World Health Organization has recommended that the vaccines against S*. pneumoniae* should be a priority for introduction into immunization schedules for all countries (World Health Organization, 2007). A heptavalent pneumococcal conjugate polysaccharide vaccine (PCV7) is now licensed in more than 90 countries and includes serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F (Dagan, 2002). This vaccine has contributed to significant decline in the incidence of IPD (Centers for Disease Control and Prevention 2008; Hausdorff et al., 2005). Few data on vaccine efficacy gathered outside the western have revealed significant differences in serotype distribution, the vaccine coverage, and the levels of antibiotic resistance among the continents and countries (Dinleyici and Yargic, 2009). Countries are encouraged by WHO to perform suitable surveillance of IPD to assess the outcome of vaccine coverage, to set up a baseline measurement for disease, and to govern the impact of vaccination (Centers for Disease Control and Prevention, 2008).

In Iran, the pneumococcal vaccination is not currently a component of the childhood immunization schedule. Moreover, there is insufficient information about the incidence of IPD, circulating serotypes, antibiotic-resistance rates, and predicted vaccine efficacy. Thus, the aim of this present study was to evaluate the serotype distribution and antibacterial susceptibility of *S. pneumoniae* isolates causing invasive disease among children in Zahedan, South-East Iran.

MATERIALS AND METHODS

Recruitment

Children recruited for study were those aged ≤14 years with fever and a possible clinical diagnosis for pneumonia, septicemia or meningitis, admitted to the pediatric wards. A case of IPD was defined by the isolation of *S. pneumoniae* from a normally sterile site. Invasive isolates were obtained from blood, cerebrospinal fluid, pleural fluid, joint aspirate, peritoneal fluid and pericardial fluid in pediatric patients (aged ≤14 years) from 3 large hospitals in Zahedan, Iran. Only one isolate from each IPD case was included in the study.

Microbiological identification

Alpha-hemolysis, optochin sensitivity, and bile solubility tests were employed to identify *S. pneumoniae* isolates.

Susceptibility tests

Kirby-Bauer disk diffusion method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) M2-A6 guidelines were used to determine antimicrobial susceptibility of oxacillin (1 µg). After 24 h of incubation at 35°C in the presence of 5% CO_2, the diameter of inhibition zone was measured. The inhibition zones of ≥ 20 and ≤19 mm were considered as penicillin-susceptible and penicillin-resistant, respectively.

Minimal inhibitory concentrations (MICs) for penicillin, amoxicillin/clavulanic acid, ceftriaxone, cefuroxime, chloramphenicol, erythromycin, imipenem, levofloxacin, tetracycline, cotrimoxazole (1.25-23.15) and vancomycin were determined by micro broth dilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2010). The quality control strain was *S. pneumoniae* ATCC 49619. The results were interpreted according to current CLSI guidelines for breakpoints.

Serotyping

Pneumococcal serogroups and serotypes were determined by latex agglutination test and the Neufeld's Quellung reaction method using the polyclonal rabbit antisera and selected factor sera (Pneumotest-Latex kit; Statens Serum Institute, Copenhagen, Denmark), respectively. Isolates reacting negatively with the omni serum were defined as nontypable. The coverage rates of the 7-valent pneumococcal conjugate vaccine (PCV7) were estimated, theoretically.

Statistical analysis

Data analysis by the chi-square test and Fisher's exact test were performed in SPSS 14.0 for Windows. *P* values of <0.05 were considered significant.

RESULTS AND DISCUSSION

Detection of *S. pneumoniae* in patients' specimens

A total of 75 isolates were collected from February 2008 to November 2010. The patients' age varied from 1 month to 14 years. Of the 75 strains, 31 (41.3%) were isolated from blood, 25 (33.3%) from cerebrospinal fluid, 10 (13.3%) from pleural fluid, and 12% from other sterile material such as peritoneal (n = 3) and pericardial (n = 2) fluids. The definite diagnoses were septicemia (n = 39), pneumonia (n = 23), meningitis (n = 9), and arthritis (n = 4). Of all the isolates, 16 (21.3%) strains were isolated from children aged <2 years, 39 (52.0%) from children aged 2 to 4 years, and 20 (26.7%) from children aged 5 to 14 years.

Antimicrobial susceptibility

Table 1 shows a summary of antibacterial activity of

Table 1. Sensitivity of 75 invasive isolates of *S. pneumoniae* to 11 antimicrobial agents, Zahedan, Iran.

Antibiotic	Total number of cases (n=75)			Penicillin sensitivity									MIC range (µg/ml)
				Sensitive (n = 13)			Intermediate (n = 28)			Resistant (n = 34)			
	S	I	R	S	I	R	S	I	R	S	I	R	
Penicillin	13* (17.3)	28 (37.3)	34 (45.4)										0.006 - 4
Amoxicillin/ clavulanic acid	68 (90.7)	5 (6.7)	2 (2.6)	10 (77.0)	2 (15.4)	1 (7.7)	24 (85.7)	2 (7.1)	2 (7.1)	28 (82.3)	1 (2.9)	5 (14.7)	0.06 - 8
Ceftriaxone	63 (84.0)	12 (16.0)	0 (0)	8 (61.5)	3 (23.1)	2 (15.4)	23 (82.1)	3 (10.7)	2 (7.1)	27 (79.4)	2 (5.9)	5 (14.7)	0.008 - 4
Cefuroxime	28 (37.3)	5 (6.7)	42 (56.0)	2 (15.4)	5 (38.5)	6 (46.1)	10 (35.7)	5 (17.8)	13 (46.4)	12 (35.3)	6 (17.6)	16 (47.0)	0.016 - 32
Chloramphenicol	63 (84.0)	0 (0)	12 (16.0)	10 (77.0)	1 (7.7)	2 (15.4)	23 (82.1)	2 (7.1)	3 (10.7)	26 (76.5)	2 (5.9)	6 (17.6)	0.12 - 16
Erythromycin	7 (9.3)	2 (2.7)	66 (88.0)	6 (46.1)	4 (30.8)	3 (23.1)	2 (7.1)	6 (21.4)	20 (71.4)	7 (20.6)	9 (26.5)	18 (52.9)	0.03 - 512
Imipenem	62 (82.7)	13 (17.3)	0 (0)	9 (69.2)	3 (23.1)	1 (7.7)	25 (89.3)	1 (3.6)	2 (7.1)	28 (82.3)	3 (8.8)	3 (8.8)	0.016 - 1
Levofloxacin	75 (100.0)	0 (0)	0 (0)	13 (100.0)	0 (0)	0 (0)	28 (100)	0 (0)	0 (0)	34 (100)	0 (0)	0 (0)	0.25 - 1
Tetracycline	25 (33.3)	7 (9.3)	43 (57.3)	7 (53.8)	4 (30.8)	2 (15.4)	2 (7.1)	5 (17.8)	21 (75.0)	3 (8.8)	8 (23.5)	23 (67.6)	0.5 - 32
Cotrimoxazole	15 (20.0)	13 (17.3)	47 (62.7)	6 (46.1)	4 (30.8)	3 (23.1)	1 (3.6)	7 (25.0)	20 (71.4)	2 (5.9)	9 (26.5)	23 (67.6)	0.12 - 64
Vancomycin	75 (100.0)	0 (0)	0 (0)	13 (100)	0 (0)	0 (0)	28 (100)	0 (0)	0 (0)	34 (100)	0 (0)	0 (0)	0.25 - 1.5

Figures in parentheses are percentages. S = Sensitive; I = intermediate; R = resistant.

several antibiotics tested against the 75 invasive streptococcal isolates categorized by penicillin susceptibility. Of the 75 *S. pneumoniae* isolates, only 13 (17.3%) isolates were penicillin-sensitive, the remaining 62 (82.7%) penicillin-non-susceptible, while 28 isolates (37.3%) intermediately resistant and 34 (45.4%) fully resistant. Resistance patterns to other antimicrobial agents were erythromycin (90.7%), cotrimoxazole (80.0%), tetracycline (66.6%), cefuroxime (62.7%), imipenem (17.3%), and amoxicillin/ clavulanic acid (9.3%). All isolates were susceptible to vancomycin and levofloxacin. It was found that 78.7, 76.0 and 70.7% of the cotrimoxazole, tetracycline and erythromycin-resistant isolates were also PNSSP, respectively. Based on our results, 43 isolates (57.3%) were multidrug-resistant. In addition, children less than 2 years showed the highest age-group-related rate (87.4%) of penicillin non-susceptibility (Table 2).

Serotype distribution and antimicrobial resistance

Seventy-five isolates were available for further analysis; these included 13 serotypes and six non-typeable isolates. The leading serotypes detected were 23F (18.7%), 6B (17.3%), 3 (16.0%), 19F (10.7%) and 14 (8.0%). These serotypes accounted for 70.7% of all isolates (Table 3) and the majority of penicillin-resistant isolates. The youngest pediatric age group revealed relatively higher PCV7 coverage rate (81.2% [<2 years] versus 69.2% [2 to <5 years] and 55.0% [5 to 14 years]) (χ^2 test, p= 0.081), and it was 68.0% among all age groups (Table 3). The PCV7 serotypes included a total of 42.7% of the PNSSP. The MDR isolates per serotype are shown in Table 4, with serotypes 23F, 6B, 19F and 14 contributing the majority (81.4%) of the MDRSP. A sum of 48.0% MDRSP was covered by PCV7 serotypes. Based on our results, 5.9% of children had *S. pneumoniae* identified as the etiological agent of invasive disease. The incidence of IPD, circulating serotypes, antibiotic-resistance rates, and predicted vaccine efficacy are poorly defined in Iran (Kohanteb and Sadeghi, 2007; Haghighat et al., 2006). Studies of invasive pneumococcal infection in children have shown geographic variations, making the acquisition of accurate regional data on disease burden a key component in directing public health priorities (Peacock and Newton, 2008).

In the present study of children in Zahedan, 82.7% of the pneumococcal isolates were not susceptible to penicillin. The surveillance of alteration in antibiotic susceptibilities due to time plays an important role in recognizing the potential hazards

Table 2. Penicillin susceptibility stratified by age group.

Age (year)	Total no. of Isolates (%)	No. of isolates (%)		
		Susceptible	Intermediate	Resistant
<2	16 (21.3)	2 (12.5)	11 (68.7)	3 (18.7)
2 to <5	39 (52.0)	11 (28.2)	21 (53.8)	7 (18.0)
5-14	20 (26.7)	9 (45.0)	7 (35.0)	4 (20.0)
Total	75 (100.0)	22 (29.3)	39 (52.0)	14 (18.7)

Table 3. Serotype distribution of invasive *S. pneumoniae* isolates by age in Zahedan, Iran (1999 to 2004).

Serotype	No. of isolates (%)			
	<2 years	2 to <5 years	5 to 14 years	Total
23F	4 (25.0)	8 (20.5)	2 (10.0)	14 (18.7)
6B	4 (25.0)	7 (17.9)	2 (10.0)	13 (17.3)
3	1 (6.2)	8 (20.5)	3 (15.0)	12 (16.0)
19F	1 (6.2)	5 (12.8)	2 (10.0)	8 (10.7)
14	1 (6.2)	3 (7.7)	2 (10.0)	6 (8.0)
18C	2 (12.5)	1 (2.6)	2 (10.0)	5 (6.7)
4	0	2 (5.1)	1 (5.0)	3 (4.0)
9V	1 (6.2)	1 (2.6)	0	2 (2.7)
5	1 (6.2)	0	1 (5.0)	2 (2.7)
Others	0	1 (2.6)	3 (15.0)	4 (5.3)
Nontypable	1 (6.2)	3 (7.7)	2 (10.0)	6 (8.0)
Total	16 (100.0)	39 (100.0)	20 (100.0)	75 (100.0)
Theoretical PCV7 coverage	13 (81.2)	27 (69.2)	11 (55.0)	51 (68.0)

Table 4. Susceptibility of penicillin and MDR isolates from invasive *S. pneumoniae* by serotype in Zahedan, Iran (1999 to 2004).

Serotype (no.)	No. of isolates (%)			
	Susceptible	Intermediate	Resistant	MDR[a]
23F (14)	3 (21.4)	7 (50.0)	4 (28.6)	12 (85.7)
6B (13)	5 (38.5)	7 (53.8)	1 (7.7)	11 (84.6)
3 (12)	8 (66.7)	3 (25.0)	1 (8.3)	3 (25.0)
19F (8)	1 (12.5)	4 (50.0)	3 (37.5)	7 (87.5)
14 (6)	2 (33.3)	3 (50.0)	1 (16.7)	5 (83.3)
18C (5)	4 (80.0)	1 (20.0)	0	0
4 (3)	3 (100.0)	0	0	0
9V (2)	1 (50.0)	1 (50.0)	0	1 (50.0)
5 (2)	2 (100.0)	0	0	0
Others (4)	2 (50.0)	1 (25.0)	1 (25.0)	1 (25.0)
Nontypable (6)	1 (16.7)	3 (50.0)	2 (33.3)	3 (50.0)
Total (75)	32 (42.7)	30 (40.0)	13 (17.3)	43 (57.3)
Theoretical PCV7 coverage	19 (25.3)	23 (30.7)	9 (12.0)	36 (48)

[a]Defined as resistance to ≥3 classes of antibiotic.

associated with *S. pneumoniae* infections. The overall prevalence of penicillin resistant *S. pneumoniae* has been reported as high as 15.6% in a study carried out in Shiraz, Iran (Kohanteb and Sadeghi, 2007). Owing to persistent spread of PNSSP strains, clinicians are facing therapeutic dilemma and fail to provide antimicrobial

treatment (Feikin and Klugman, 2002). In our study, the highest PNSSP prevalence in children was less than 5 years. The increasing number of antibiotic-resistant pneumococcal infections can lead to higher risks of treatment failure. Globally, the highest level of antimicrobial resistance is reported in Asians with the greatest prevalence of high-level penicillin resistance in Vietnam (71.4%), followed by Korea (54.8%), Hong Kong (43.2%), and Taiwan (38.6%) (Song et al., 2004).

From our results, a predominance of high resistance to erythromycin (88.0%) was noted. Erythromycin is generally suggested as an alternative therapy for pneumococcal infection and penicillin-sensitive individuals. Results obtained herein are inconsistent with such suggestion, since 85.5% of PNSSP strains isolates were also nonsusceptible to erythromycin. Imöhl et al. (2010) have reported a significant decrease in macrolide nonsusceptibility among children in Germany since 2005. In contrast, recent conducted surveillance study data revealed an increase in the prevalence of macrolide-resistant *S. pneumoniae* in Iran and many parts of the world (Kohanteb and Sadeghi, 2007; Felminham et al., 2000). Excessive administration, inappropriate and overdose use of erythromycin and other macrolides for treatment of pneumococcal infections may be the major contributory factors for the elevated prevalence of macrolide resistance in our country and elsewhere.

Also, 62.7 and 57.3% of the isolates showed high resistance to cotrimoxazole and tetracycline, respectively. In a study by Shah et al. (2009) in Nepal, the cotrimoxazole resistance was seen in 68% of isolates, which is similar to the rate in our study. This high resistance to cotrimoxazole and tetracycline is possibly due to dose convenience, frequent prescribing, cost-effectiveness and easy availability over the counter. These results should serve as a warning to pediatricians in Iran, because the most common drugs used for treatment are at an extremely high *in vitro* resistance. Restricted antibiotic use and continued surveillance for antibiotic resistance are essential factors to solve the problem of high prevalence of antimicrobial resistance in Iran. However, none of our isolates showed high resistance to ceftriaxone, although 84% of the isolates were sensitive to this antibiotic. Based on Haghighat et al.'s (2006) report, all *S. pneumoniae* isolates used in their study were sensitive to this antibiotic and they emphasized that a third generation of cephalosporin such as ceftriaxone should be considered for empirical therapy of children with pneumococcal disease in Iran. Moreover, all the isolates in our study were sensitive to levofloxacin and vancomycin. Except for some specific indications, fluoroquinolones are not currently approved for children, therefore such restrictions may probably contribute to lower pediatric resistance rates (Bowlware and Terrence, 2004). Based on our results, 43 isolates (57.3%) were multidrug-resistant *S. pneumoniae* isolates.

Resistance to 3 or more classes of drug used to treat *S. pneumoniae* infections is defined as multiple drug-resistant *S. pneumoniae* (MDRSP) (Jen Lin et al., 2006). Multidrug resistant *S. pneumoniae* is increasingly being reported from many parts of the globe (Lalitha et al., 2002). Strains with reduced susceptibility to penicillin usually show cross-resistance to other antibiotics and such cross-resistance was observed with erythromycin, tetracycline, chloramphenicol, and ciprofloxacin. The increasing multidrug resistance of *S. pneumoniae* bears important clinical implications, worldwide. In our study, 22 (29.3%) of children had received antibacterial therapy before collecting clinical samples for bacteriological analysis. Antibiotics are freely available within urban areas of Iran, and self-medication is common during a febrile illness. Based on some reports, prehospitalization antibiotic use affects isolation of *S. pneumoniae* in culture, which results in underestimation of disease burden (Shah et al., 2009; Moore et al., 2010).

Current information on capsular types causing disease is required in young children to guide conjugate vaccine recommendations. The 5 most common serotypes in our investigation were 23F, 6B, 3, 19F and 14. The serotypes commonly defined as "pediatric serotypes" are 6B, 9V, 14, 19F, and 23F (Feikin et al., 2005). There are some studies about pneumococcal diseases in our country, but the serotypes responsible and the outcome from invasive strains have not been reported (Kohanteb and Sadeghi, 2007). In a study from Germany, the proportion of serotypes 19F, 14, and 6B has been reported to be higher among invasive isolates from children which is comparable to our results (Imöhl et al., 2010). In Asian countries, serogroups 19F, 23F, 14, 6 and 9 predominate in children (Song et al., 2004). The prevalence of invasive pneumococcal serotypes shows geographic variability around the world. In Thailand for example, the serotypes 23F, 19F and 6B were predominant in northern regions (Baggett et al., 2009), whereas serotypes 14, 3 and 19A were reported as most common serotypes in northeastern regions (Watanabe et al., 2003).

From our study, the heptavalent pneumococcal conjugate vaccine (PCV7) covered 68.0% of the serotypes from all the IPD isolates. These findings are similar to those reported in western countries (65 to 80%) before the introduction of PCV7 and suggest that the PCV7 would have a preventive effect on IPD in Iran (Hausdorff et al., 2005). The greatest PCV7 coverage in the present study (81.2%) was in children less than 2 years. It has been reported that the PCV-7 serotypes cover 60 to 90% of serotypes associated with IPD in young children in developed world (Moore et al., 2010). On the other hand, in many developing countries such as those in Asia, the PCV7 coverage is lower (Hausdorff et al., 2000).

Conclusion

Considering the above findings, we can conclude that

antibiotic resistance is common among invasive isolates of *S. pneumoniae* in Zahedan, Iran. Most of the *S. pneumoniae* isolates belonged to few serotypes and covered by PCV7 vaccine. Therefore, introduction of anti-pneumococcal conjugate vaccines into immunization programme would likely lead to a significant reduction of IPD-associated morbidity among children in Zahedan. On the other hand, further studies with larger number of isolates are needed to determine whether our findings are reproducible elsewhere in Iran and to define the most appropriate vaccine for the prevention of pneumococcal disease in Iran. Meanwhile, the data obtained in this article and related publications emphasize the hasty need to control the proper use of antibiotics to decrease the antibiotic resistance in IPD.

ACKNOWLEDGEMENT

This work was partly supported by Research Center for Infectious Diseases and Tropical Medicine, Zahedan University of Medical Sciences.

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