Streptococcus pneumoniae carriage, resistance and serotypes among Jordanian children from Wadi Al Seer District, Jordan

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Abstract

Objectives: Detection of carriage rate of Streptococcus pneumoniae from children in Wadi Al Seer district, Jordan.

Methods: Nasopharyngeal were collected from 118 children aged between one to 50 months. S. pneumoniae isolates were analysed for resistance, serotyping and macrolide resistant genotypes and phenotypes.

Results: Carriage rate was 55.1% (n= 65/118). Resistance rate was as follows: Penicillin (80%), trimethoprim-sulfamethoxazole (73.8%), erythromycin (61.5%), tetracycline (53.8%) and clindamycin (33.8%). Multidrug resistant isolates were 56.9%. (MIC50 & MIC90 µg/ml) were as follows: Penicillin (0.5,2), erythromycin (2, >=32), clindamycin (0.06, >=32), trimethoprim-sulfamethoxazole (4, >=32). M-Phenotypes (45%), iMLSB (2.5%) and cMLSB (52.5%) with genotypes erm(B) 55%, and mef(A) 45%. Common serotypes were: 19F (18.5%), 6B (16.9%), 23F (12.3%), 35B (6.2%). Coverage of PCV7, PCV10 and PCV13 was 52.3%, 52.3% and 58.5%, respectively.

Conclusions: High rates of S. pneumoniae carriage and drug resistance is a potential serious risk to increase pneumococcal invasive and non invasive infections in Jordanian children.

Keywords: Streptococcus pneumoniae, Resistance, Colonization, Jordan.
Introduction

*Streptococcus pneumoniae* is a leading cause of bacterial pneumonia, meningitis, bacteraemia, otitis media, and sinusitis and continues to be a significant cause of morbidity and mortality in humans [1]. The worldwide increase in antibiotic resistance in these species has become a serious problem within the last twenty years [2]. *S. pneumoniae* was given the name as the forgotten killer in children in 2008 by the WHO [3], which accounts for more than one third of acute bacterial sinusitis and more than one half of community-acquired bacterial pneumonia [4]. It remains a major cause of childhood morbidity and mortality, where at least 1.2 million children die of pneumococcal infections each year as stated by the WHO in 2007 and 70% of them in Africa and southeast Asia; mostly in developing countries [5].

Antibiotic treatment of invasive disease has been widely countered by the increasing emergence of resistance in many parts of the world in the recent years. Resistance to beta-lactam, macrolides, tetracycline and trimethoprim-sulfamethoxazole were reported [6]. However, the resistance pattern varied greatly from country to country [7]. This emphasises the importance of local data in determining the appropriate antibiotic therapy.

*S. pneumoniae* is a common colonizing bacterium in the respiratory tract, mostly symptom less; however it can progress to respiratory or even systemic disease. An important feature is that pneumococcal disease will not occur without preceding nasopharyngeal colonization with the homologous strain, so pneumococcal carriage is believed to be an important source of horizontal spread of this pathogen within the community [8]. Increased prevalence of *S. pneumoniae* in healthy children’s nasopharynx reflects a potential risk to develop more frequently respiratory infections in the community [9,10].

There is only one old Jordanian study published in 2000 which has reported on the antimicrobial susceptibility pattern of *S. pneumoniae* isolates from various clinical specimens, and this study showed that 56% of the isolates were penicillin resistant [11], however, recent data about prevalence of antimicrobial susceptibility pattern of this organism in children population are not available. Furthermore, it is essential to determine the distribution of *S. pneumoniae* serotypes among children in each country in order to address the actual value of using available commercial vaccines to minimize the pneumococcal infections [12].

Material and Methods

Study design

One hundred and eighteen children aged 1 month to 50 months were chosen randomly from the Maternity Health Center of Wadi Al Seer district in Amman to be included in this during 2009. The study has involved 7 smaller neighborhoods. The recruitment phase started by sending letters explaining the purpose of the study and containing consent form and questionnaire regarding potential risk factors for the carriage of *S. pneumoniae* were sent to parents. Only children whose parents consented to participate were enrolled in the study. Also, at the time of the study, all investigated children were admitted to the Maternity Health Care Center for the periodic checkups, and these were not vaccinated with the 7 valent pneumococcal conjugate vaccine (PCV7, Pfizer Inc., USA).

Specimen collection and transport

Sterile nasopharyngeal and throat swabs pre-moistened with sterile water were taken by the medical doctor from each child [13]. The swabs were transported in ice box to the laboratory within 4 hours for culture.
Bacterial identification

In the laboratory, each swab specimen was mixed thoroughly using a vortex mixer before inoculation onto Colombia blood agar base (Oxoid, UK) supplemented with 5% sheep blood (Oxoid, UK). Plates were incubated at 37°C for 24h-48h with 5% CO₂. *S. pneumoniae* colonies were selected based on colony morphology, α-haemolysis, susceptibility to optochin, and bile solubility, then pneumococcal colonies were purified and stored at -20°C for further work [14].

Susceptibility testing

Minimal inhibitory concentration (MIC) testing was performed using the broth microdilution method as recommended by the Clinical Laboratory Standards Institute (CLSI) [15]. *S. pneumoniae* ATCC 49619 was used as a control strain.

Serotyping

Serotypes were done by the Neufeld Quellung reaction method with the available antisera from Statens Serum Institute of Copenhagen-Denmark [16]. This part of work was performed at the National Reference Center for Streptococci in Germany.

Analysis of resistance determinants

PCR of macrolide resistance determinants was performed as described previously [17]. For the classical detection of *erm*(B) and *mef*(A) the following primers were used: *erm*(B) 5’-CGAGTGAAAAAGTACTCAACC-3’ (362-382) and 5’-GGCGTGTTTCATTGCTTGATG-3’ (978-958), *mef*(A) 5’-AGTATCATTAATCAGATGGC-3’ (57-77) and 5’-TAATAGATGCAATCAGAGC-3’ (550-532). The macrolide resistance phenotype was determined on the basis of the pattern of susceptibility to MLSB (macrolide-lincosamide-streptogramin B) [18].

Results

*S. pneumoniae* carriage rate

A total of 65/118 children (55.1%) were found to carry *S. pneumoniae* in the nasopharynx (Table 1).

| Age (Years)            | No. of carriers**/ Total No. of children (%) | coverage 7v PCV (%) | coverage 10v PCV (%) | coverage 13v PCV (%) |
|------------------------|---------------------------------------------|---------------------|----------------------|----------------------|
| <= 1 year              | 42/ 65 (64.6%)                              | 20/42 (47.6)        | 20/42 (47.6)         | 22/42 (52.4)         |
| >1- 2                  | 16/ 31 (51.6%)                              | 10/ 16 (62.5)       | 10/ 16 (62.5)        | 11/ 16 (68.8)        |
| >2- 3                  | 4/ 13 (30.8%)                               | 2/ 4 (50)           | 2/ 4 (50)            | 3/ 4 (75)            |
| >3 years- 50 months   | 3/ 9 (33.3%)                                | 2/ 3 (66.6)         | 2/ 3 (66.6)          | 2/ 3 (66.6)          |
| Birth - 2 years       | 58/ 96 (60.4%)                              | 30/58 (51.7)        | 30/58 (51.7)         | 33/58 (56.9)         |
| (Total) 0- 50 months  | 65/118 (55.1%)                              | 34/65 (52.3)        | 34/65 (52.3)         | 38/65 (58.5)         |

* A total of 51.2% the examined children showed upper respiratory tract infection, and 27.1% were previously treated with antibiotics
** S. pneumoniae was isolated at the same time from 12% of both throat and nasopharynx swab specimens.
The mean age of children was 13.4 months, and the range of age was 1 to 50 months. The children included 51.7% males and 48.3% females. A total of 31/118 (27.1%) have been treated previously with antibiotics.

**Antimicrobial susceptibility**

The percentage of resistance among *S. pneumoniae* was as follows: Penicillin G (80%), trimethoprim-sulfamethoxazole (73.8%), tetracycline (53.8%), erythromycin (61.5%) and clindamycin (33.8%). Vancomycin, amoxicillin, cefotaxime, levofloxacin and telithromycin showed no resistance. Minimal inhibitory concentrations of *S. pneumoniae* isolates (MIC$_{50}$, MIC$_{90}$ µg/ml) were as follows: Penicillin (0.5, 2), erythromycin (2, >=32), clindamycin (0.06, >=32), trimethoprim-sulfamethoxazole (4, >=32), tetracycline (16, 32), and levofloxacin (0.5, 1) (Table 2). A total of 37 isolates (56.9%) were Multidrug resistant and were belonged mostly to serotypes available in the pneumococcal conjugate vaccines (6B, 6A, 14, 19A, 19F and 23F) (Table 4).

**Macrolide resistant pheno- and genotypes**

Resistance to erythromycin A accounted to 61.5% (n= 40) of *S. pneumoniae* isolates. Of these two different phenotypes of macrolide resistance were found. MLS$_B$ constitutive phenotype accounted to 52.5% of isolates (n= 21), and 45% (18 isolates) had the M-phenotype, and 2.5% (1 isolate) was inducible MLS$_B$ (Table 3). It was observed that penicillin resistant strains had higher MICs towards macrolide drug. Macrolide resistant genotypes were divided in ermB (55%; 22 isolates) and mefA (45%; 18 isolates) (Table 3).

**Serotyping**

The most common serotypes indicated were the following: 19F (18.5%), 6B (16.9%), 23F (12.3%),

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**Table 2. Ranges of MIC$_{50}$, MIC$_{90}$, and antibiotic resistance patterns of 65 isolates of *S. pneumoniae***

| Antibiotic                | MIC range (n=65) | (n) % resistance (I, R) | % Sensitive | MIC$_{50}$ | MIC$_{90}$ |
|---------------------------|------------------|-------------------------|-------------|------------|------------|
| Penicillin G              | 0.016- 8         | (52) 80                 | 20          | 0.5        | 2          |
| Erythromycin A            | 0.06- >=32       | (40) 61.5               | 38.5        | 2          | >=32       |
| Clindamycin               | 0.06- >=32       | (22) 33.8               | 66.8        | 0.06       | >=32       |
| Sulfameth. Trimeth.       | 0.06- >=32       | (48) 73.8               | 26.2        | 4          | >=32       |
| Tetracycline              | 0.25-32          | (35) 53.8               | 46.2        | 16         | 32         |
| Vancomycin                | <=2              | (0) 0                   | 100         | <=2        | <=2        |
| Levofloxacin              | 0.5-2            | (0) 0                   | 100         | 0.5        | 1          |
| Amoxicillin               | 0.016- 1         | (0) 0                   | 100         | 0.03       | 0.06       |
| Cefotaxime                | 0.016- 0.5       | (0) 0                   | 100         | 0.016      | 0.03       |
| Telithromycin             | 0.016- 0.5       | (0) 0                   | 100         | 0.016      | 0.03       |

* Breakpoints (I, R) according to CLSI: Penicillin G: (0.1–1 µg/ml, ≥2 µg/ml); cefotaxime: (2 µg/ml, ≥4 µg/ml); erythromycin A (0.5 µg/ml, ≥ 1 µg/ml); clindamycin : (0.5 µg/ml, ≥ 1 µg/ml); tetracycline: (4 µg/ml, ≥ 8 µg/ml); trimethoprim-sulfamethoxazole: (1/19-2/38 µg/ml, ≥ 4/76 µg/ml); and all isolates were susceptible for vancomycin at MIC ≤ 1 µg/ml.
35B (6.2%), 11A, NT and 15A (4.6%) each isolated, followed by serotypes 14, 34, 23A, 6A, and 19A with 3.1% each isolated (Table 3).

**Coverage of the pneumococcal conjugate vaccines**

The total coverage of the PCV7 among all children with positive isolates was (55.1%). Coverage of PCV7, PCV10 and PCV13 was 52.3%, 52.3% and 58.5%, respectively. Although the highest coverage of the 7v-PCV (66.6%) was observed among infants aged between 2 to 3 years of age but the total number of these children was 13 in the study. In children below one year of age (n=65), coverage of the PCV7 was 64.6% (Table 1).

### Table 3. Distribution of *S. pneumoniae* serotypes and macrolide resistant pheno-genotypes among examined children

| Serotype And Pheno-genotype | 19F | 6B | 23F | 35B | 11A | 15A | NT | 14 | 34 | 6A | 23A | 19A | Others | M-Ph | cMLSB | iMLSB | ermB | mefA |
|-----------------------------|-----|----|-----|-----|-----|-----|----|----|----|----|-----|-----|--------|------|-------|-------|------|-----|
| Number                      | 12  | 11 | 8   | 4   | 3   | 3   | 3  | 2  | 2  | 2  | 2   | 2   | 11     | 18   | 21    | 1     | 22   | 18  |
| Percentage                  | 18.5| 16.9| 12.3| 6.1 | 4.6 | 4.6 | 4.6| 3.1| 3.1| 3.1| 3.1 | 3.1 | 16.9   | 45   | 52.5  | 2.5   | 55   | 45  |

* Other serotypes; 33F, 15B, 15F, 16F, 17F, 18A, 22F, 33A, 35A, 7B, and 9V each of these serotypes was detected one time with (1.5%)

### Table 4. Resistance patterns according to the serotype of *S. pneumoniae* isolates

| Serotype | No. (%) of isolates | PEN (n (%)) | FUR (n (%)) | ERY (n (%)) | CLI (n (%)) | SXT (n (%)) | TET (n (%)) | Multiresistant isolates (n (%)) |
|----------|---------------------|-------------|-------------|-------------|-------------|-------------|-------------|---------------------------------|
| 6B       | 11 (16.9)           | 7 (63.6)    | 8 (72.7)    | 8 (72.7)    | 10 (90.9)   | 7 (63.6)    | 7 (63.6)    |                                 |
| 9V       | 1 (1.5)             | 1 (100)     | 1 (100)     | 0 (0)       | 1 (100)     | 1 (100)     | 1 (100)     |                                 |
| 14       | 2 (3.1)             | 2 (100)     | 2 (100)     | 2 (100)     | 2 (100)     | 2 (100)     | 2 (100)     |                                 |
| 19F      | 12 (18.5)           | 11 (91.7)   | 10 (15.4)   | 5 (7.7)     | 12 (100)    | 9 (75)      | 5 (7.7)     |                                 |
| 23F      | 8 (12.3)            | 8 (100)     | 4 (50)      | 0 (0)       | 4 (100)     | 4 (50)      | 4 (50)      |                                 |
| 19A      | 2 (3.1)             | 2 (100)     | 1 (50%)     | 2 (100)     | 1 (50%)     | 0 (0)       | 2 (100)     |                                 |
| 6A       | 2 (3.1)             | 2 (100)     | 1 (50%)     | 2 (100)     | 1 (50%)     | 2 (100)     | 2 (100)     |                                 |
| Others   | 27 (41.5)           | 9 (33.3)    | 11 (40.7)   | 4 (14.8)    | 13 (48.1)   | 9 (33.3)    | 14 (41.8)   |                                 |
| Total    | 65                  | 52 (80)     | 39 (60)     | 40 (61.5)   | 22 (33.8)   | 48 (73.8)   | 34 (52.3)   | 37 (56.9)                      |

* Others: Serotypes not included in the 7 valent pneumococcal conjugate vaccine. Serotypes 4 and 18C were not found in this study.

Abbreviations: PEN, Penicillin; FUR, Cefuroxime; ERY, Erythromycin; CLI, Clindamycin; SXT, Sulfamethoxazole-Trimethoprim; TET, Tetracyclin
DISCUSSION

To our knowledge, this is the first data from Jordan which describe the pneumococcal serotypes colonized in the pediatric population of one district in Jordan. Nasopharynx is the usual source of pneumococci for studying the carriage rate [19]. This study has demonstrated that carriage rate of \( S. pneumoniae \) among children in Jordan is high (55.1%) compared to the study reported by Lee et al., for carriage rate of pneumococci in 4963 Asian children aged below 5 years from 11 countries [20]. The results of the study investigated showed the following rates: Philippines (32.6%), China (37.5%), India (43.2%) and Thailand (40.6%), but lower rates in Taiwan (15.3%) and Saudi Arabia (9.0%). Similar carriage rates were obtained from Brazil (55%) [12], Guatemala (59.1%) [21], and in Kampala Uganda (62%) [22]. The high rate of pneumococci colonization can be due to different factors such as history of sicknesses, immune deficiency, viral infections and history of antibiotic consumption before attending the Daily Care Center (DCC) as the case with our investigated children. Since 51.2% of our examined children had suffered of upper respiratory tract infections before admission to the DCC, and data taken from children’s medical records of the DCC showed that 27.1% had a history of antibiotic consumption prior to their visits to the DCC, which could be contributed for selection of resistant strains [18]. The differences in carriage rates worldwide were related to certain socio-economic conditions including housing, access to health care, poor hygiene, family size, overcrowded living conditions, day-care contact, and number of siblings [23]. Previous studies reported the attendance of day care as a main factor causing the increase of the carriage rate of \( S. pneumoniae \) [24]. Continuous surveillance of the susceptibility patterns of \( S. pneumoniae \) becomes increasingly important, because of the increasing emergence of antibiotic-resistant strains worldwide [25].

This study suggests that prior antibiotic use is not only an important risk factor for an increased \( S. pneumoniae \) carriage but may be also considered a risk factor for carriage of multiple-resistant strains of pneumococci. The antibiotics susceptibility of the \( S. pneumoniae \) isolated from our children’s nasopharynx reflected alarming rates of resistance to penicillin, erythromycin and occurrence of multidrug-resistant isolates. Rates of antibiotic resistant of \( S. pneumoniae \) isolates among our children were higher than those clinical isolates from Singapore and Sri Lanka but similar to Taiwan [20]. However, our resistance rates resemble the rates reported in some other Asian countries [26]. The high rates of resistance to different classes of antibiotics in \( S. pneumoniae \) in this study are presumably a consequence of antimicrobial consumption and misuse within the Jordanian population [27]. Otoom et al., (2002) reported that antibiotic prescriptions in Jordan at different health centers ranged between 46.7% to 83.3%; and these rates are very high compared to many other parts of the world [28]. Local information on capsular types of \( S. pneumoniae \) causing disease in young children is highly important to guide production of effective conjugate vaccine. Our results of \( S. pneumoniae \) serotyping showed that most prevalent serotype was 19F (18.5%) followed by 6B (16.9%) then 23F (12.3%). Similar serotyping results has been reported from Kuwait among \( S. pneumoniae \) isolates from children (6B and 23F had a prevalence of 9%, and 19F accounted for 9.8%) [29]. A study by Marchisio et al. (2002)in Italy, has found that \( S. pneumoniae \) carrier rate was 8.6% and included the following serotypes (3, 19F, 23F, 19A, 6B, and 14), and that most of pneumococci isolates (69.4%) were resistant to one or more antimicrobial classes [21]. Whereas children aged 3-36 months attending day care centers in Belgium, had 21% \( S. pneumoniae \) carriage rate and the main serotypes were 19F (27.3%), 6B (20.2%), 23F (19.2%), 19A (10.1%), 6A (7.1%), 14 (5.1%) [19]. Prevenar, the 7-valent pneumococcal conjugate vac-
cine (PCV7) and the new 13-valent pneumococcal conjugate vaccine (PCV13) are used routinely in the National Immunization Program of several industrialized nations. This study shows that both vaccines are covering 52.3% and 58.5% of S. pneumoniae serotypes distributed among our children, respectively. Around the world, the highest coverage for PCV7 has been reported for the USA, Canada, and Australia (80–90%), followed by Europe and Africa (70–75%), whereas in Latin America and Asia the coverage rates were 65% and 50%, respectively [30]. Finally, our study was only performed in a small group of children and one district of the capital Amman. We are aware that the carriage patterns may vary between various communities and it is possible that the serotype distribution and resistance patterns described here may not be representative to the overall population of children in Jordan.

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