ANTIBIOTIC RESISTANCE PATTERNS AND RISK FACTORS OF *STREPTOCOCCUS PNEUMONIAE* CARRIAGE AMONG HEALTHY JORDANIAN CHILDREN

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Abstract

***Objectives**: Isolation of Streptococcus pneumoniae from healthy children in Amman, Jordan.*

***Methods**: Nasal swabs were taken from 1002 healthy children. Isolates were analysed for antimicrobial susceptibility, serotypes, macrolide resistance genotypes and phenotypes.*

***Results**: The overall carriage rate was 19.5% (n= 195 isolates). The percentage of resistance was as follows: penicillin (91.8%), cefotaxime (29.2%), trimethoprim-sulfamethoxazole (66.7%), erythromycin (46.7%), clindamycin (19.5%), tetracycline (32.3%), chloramphenicole (6.2%) and ciprofloxacin (6.2%). 67 isolates (34.4%) were multi drug-resistant. MIC_{50} and MIC_{90} were as follows (µg/ml): Penicillin (1, 4), cefotaxime (1, 4), erythromycin (0.5, 32), and clindamycin (0.125, 32). PCR-based serotyping of the serotypes included in the 7v-PCV showed the following percentages: 19F (11.8%), 23F (9.7%), 9V (7.6%), 6B (6.7%), 14 (5.6%), 18C (4.6%) and 4 (2.6%). Among macrolide resistant isolates (n= 91), erm(B) was detected in 20.9% (n= 19), mef(A) in 53.8% (n= 49), both erm(B) and mef(A) 16.5% (n= 15) and 8.8% (n= 8) were negative for both determinants. Among the macrolide resistant isolates MLS_B- and M-phenotypes were 41.8% and 58.2%, respectively.*

***Conclusions**: The overall carriage rate of pneumococci in Jordan is low, but the level of antibiotic resistance is high. The spread of pneumococcal multi-drug resistance is worrisome.*

Summary

A total of 1002 children aged 2 to 6 years from kindergartens and pediatric clinics were subjected to nasopharyngeal swabs for the recovery of Streptococcus pneumoniae as potential infectious agent in the years 2005-2006 in Amman, Jordan. The overall carriage rate was 19.5% (n= 195 isolates). The percentage of resistance was as follows: penicillin (91.8%), cefotaxime (29.2%), trimethoprim-sulfamethoxazole (66.7%), erythromycin (46.7%), clindamycin (19.5%), tetracycline (32.3%), chloramphenicole (6.2%) and ciprofloxacin (6.2%). MIC_{50} and MIC_{90} for antibiotics were as follows (µg/ml): penicillin (1, 4), cefotaxime (1, 4), erythromycin (0.5, 32), and clindamycin (0.125, 32). 67 isolates (34.4%) were multi drug-resistant. PCR-based serotyping of the serotypes included in the 7v-PCV showed the following percentage of serotypes: 19F (11.8%), 23F (9.7%), 9V (7.6%), 6B (6.7%), 14 (5.6%), 18C (4.6%) and 4 (2.6%). Only two macrolide resistant phenotypes were detected: MLS_B (41.8%) and M (58.2%). Among macrolide resistant isolates (n= 91), erm(B) was detected in 20.9% (n= 19), mef(A) in 53.8% (n= 49), both erm(B) and mef(A) 16.5% (n= 15) and 8.8% (n= 8) were negative for both determinants.

Keywords: *Streptococcus pneumoniae*, resistance, colonization, Jordan.

Introduction

Streptococcus pneumoniae is a leading cause of bacterial pneumonia, meningitis, 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* accounts for more than one third of acute bacterial sinusitis and more than one half of community-acquired bacterial pneumonia (3). It remains a major cause of childhood morbidity and mortality; 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 (4).

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, tetracyclines and trimethoprim-sulfamethoxazole were reported (5). However the resistance pattern varied greatly from country to country (6). This emphasises the importance of local data in determining the appropriate antibiotic therapy.

Streptococcus pneumoniae is a common colonizing bacterium in the respiratory tract, mostly symptomless; however it can progress to respiratory or even systemic disease. An important feature is that pneumococcal disease will not occur without preceeding 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 (7). *S. pneumoniae* that is present in healthy children's nasopharynx reflects the strains that are present in the community, and may cause respiratory infections (8). Thus identifying the nasopharynx isolates may provide more useful indications for the treatment of the infections they cause, than studying their sensitivity in hospital patients only (9).

In Jordan, only one study has reported the emergence of antibiotic resistance among isolates of *S. pneumoniae* (10) with 56% penicillin resistance. However, recent data about resistance rates among children are not available. Furthermore, it is essential to determine the distribution of *S. pneumoniae* serotypes among children in order to address the possibility of using available vaccines to minimize the pneumococcal infections (5, 6, 11).

Material and Methods

Study design. One thousand and two children aged 2-6 years from the kindergartens and outpatients of pediatric clinics of the Jordan University Hospital in Amman, Jordan were enrolled in this study. In the recruitment phase, letters explaining the purpose of the study and containing a consent form and a questionnaire regarding potential risk factors for the carriage of *S. pneumoniae* were sent to the parents. Only children whose parents gave consent to participate were enrolled in the study.

Specimen collection and transport: Sterile nasal swabs pre-moistened with sterile water were inserted in the nasal cavity and rotated 180 degrees, then placed in STGG transport medium (skim milk powder 2.0 g, tryptone-soya broth 3.0 g, glucose 0.5 g, glycerol 10 ml and double distilled water 100 ml) (12). The vials were transported in an 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% CO_2. *S. pneumoniae* colonies were selected based on colony morphology, α-haemolysis, susceptibility to optochin, and bile solubility (13). The identification was confirmed by detection of *lyt*A gene using PCR (14). Pneumococcal colonies were purified and stored at -20°C (15).

***Susceptibility testing*:** Minimal inhibitory concentration (MIC) testing was performed using the broth microdilution method as recommended by the Clinical Laboratory Standards Institute (CLSI) (16) . *S. pneumoniae* ATCC 49619 was used as a control strain. The disk diffusion method was performed on Mueller-Hinton agar (Oxoid, UK) supplemented with 5% sheep blood.

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´-AGTATCATTAATCACTAGTGC-3´ (57-77) and 5´-TAATAGATGCAATCACAGC-3` (550-532). The macrolide resistance phenotype was determined on the basis of the pattern of susceptibility to MLS_B (macrolide-lincosamide-streptogramin B) (18, 19).

PCR-based serotyping. Determination of the seven pneumococcal capsule types targeted by the currently licensed 7-valent pneumococcal conjugate vaccine (PCV7; 4, 6B, 9V, 14, 18C, 19F, and 23F) were performed by one-step multiplex PCR (20). The capsular polysaccharide loci were used as targets for PCR because they represent the genetic loci of pneumococcal capsular serotypes. The sequences of the primers used for multiplex PCR are shown in Table (1). The reaction mixture was performed in 50 µl which was subjected to 40 cycles of amplification in the programmable DNA (1 min at 94°C, 45 s at 58°C, and 1 min at 72°C; MyCycler; Bio-Rad, USA). Following amplification, all reactions were extended at 72°C for 10 min. PCR products were analyzed by agarose gel electrophoresis (90 min at 70 volt) using 10µl of each PCR sample and 3 µl of loading buffer (blue- orange dye 6x). 2% agarose gel (Promega, USA) was prepared in tris-borate-EDTA (TBE) buffer. DNA marker (100 bp) (Bio Basic Inc., Canada) was used to estimate the approximate size of amplified products.

Data collection and statistical analysis:

A standardized questionnaire was collected about different demographic and clinical conditions. All data were analyzed with SPSS for Windows (version 15.0 ;SPSS Inc. Chicago). Potential risk factors for carriage of penicillin non-susceptible *S. pneumoniae* (PNSSP) were identified by univariate analysis. Odd ratios (ORs) and 95% confidence intervals (95% CIs) were given as approximations of the relative risks.

Interaction between variables was tested by logistic regression using the forward method. A *p*-value of 0.05 was considered to be statistically significant (21)

Results

S. pneumoniae carriage rate

Children whose parents filled the questionnaire were included in the study. 195 out of 1002 children (19.5%) were found to carry *Streptococcus pneumoniae* in the nasal cavity. The median age of children was 57 months and the mean age was 53 months. Although 60% of the carriers were males, and carriage was more common among children less than 5 years old (25.3%) (Table 2), there was no significant statistical difference in the sex (*P*=0.15) or age (*P*=0.74) distribution between carriers and non-carriers (Table 6).

Risk factors associated with nasal carriage of multiresistant *S. pneumoniae*

Table (7) summarizes the factors affecting carriage of multi drug-resistant isolates. Univariate analysis and logistic regression analysis showed that only frequent episodes of influenza (OR= 2.15, *P*<0.03, OR= 4.84, *P*<0.02), and the use of antibiotics in the preceding 3 months (OR= 2.20, P<0.03, OR= 5.21, P<0.008) are risk factors for nasal carriage of multi drug-resistant pneumococci.

Antimicrobial susceptibility

The highest resistance rate of pneumococcal isolates was observed for penicillin G (91.8%), followed by trimethoprim-sulfamethoxazole (66.7%) as indicated in table 3. 67 multi drug-resistant isolates (34.4%) were observed, mostly against the combination of β-lactam, erythromycin, and trimethoprim-sulfamethoxazole.

Macrolide-resistant phenotypes

Resistance to erythromycin reached 46.7% (n= 91). Two different phenotypes of macrolide resistance were found: the MLS_B constitutive phenotype 41.8% (n= 34), and the M-phenotype 58.2% (n= 57). It was observed that penicillin resistant strains had higher MICs towards macrolide antibiotics.

PCR-based serotyping:

A total of 96 strains (49.2%) produced different DNA bands by PCR-based serotyping, The most prevalent serotype was 19F (11.8%) which was found in 23 isolates. the other serotypes identified by PCR reaction, arranged in descending order as follow: 23F (10.3%), 9V (7.7%), 6B (6.7%), 14 (5.6%), 18C (4.6%) and 4 (2.6%) (Table 4 & 5). The other (99) isolates did not produce any bands indicating that these isolates belonged to serotypes that are not included in the PCV7.

The total coverage of the PCV7 among all isolates was (49.2%). Higher coverage of the PCV7 vaccine (68.4%) was observed among children 49-54 months of age (Table 4) .

The study also showed that serotype19F was most frequent among multi drug-resistant isolates (17/23 isolates) followed by 23F (7/20 isolates). Of the 76 isolates with a penicillin MIC > 2 μg/ml found in our study, 5 isolates had an MIC of 8 μg/ml, all belonging to serotype 14; 2 more serotype 14 isolates were penicillin resistant, with an MICof 2 - 4μg/ml. The remaining highly resistant isolates were distributed among other serotypes as follows: 19F (20 isolates), 23F (10 isolates), 9V (7 isolates), 6B (3 isolates), and one isolate belonged to serotype 4 (Table 5). Furthermore, 19F (18 isolates), 23F (13 isolates) were predominant among 7 serotypes detected in penicillin intermediate-resistant strains with an MICs range of 0.12-1 μg/ml. Moreover, the MIC values of erythromycin and clindamycin also were higher among serotype 19F and 23F as compared to other serotypes.

Table 1. Target genes, primers, primer sequences, amplicon size and reference of primers

Target gene	Primer name	Sequences of primers (5'- 3')	Product size	Reference
Serotype 4	4 F	CTGTTACTTGTTCTGGACTCTCGTTAATTGG	430	(20)
	4 R	GCCCACTCCTGTTAAAATCCTACCCGCATTG		
Serotype 6B	6B F	CGACGTAACAAAGAACTAGGTGCTGAAAC	220	(20)
	6B R	AAGTATATAACCACGCTGTAAAACTCTGAC		
Serotype 9V	9V F	CATGAACAAGAACGATATCAGGC	509	(20)
	9V R	GATATCCCCGGAATAAATGAAG		
Serotype 14	14 F	GTCTGTTTATTCTATATACAAAGAGGCTCC	268	(20)
	14 R	GCATTGCTACAATCGCTATACTAGATATGC		
Serotype 18C	18C F	GCATCTGTACAGTGTGCTAATTGGATTGAAG	354	(20)
	18C R	CTTTAACATCTGACTTTTTCTGTTCCCAAC		
Serotype 19F	19F F	GTTCAACGACTAGGACGC	130	(20)
	19F R	TAGGCACCAATGTTTCACT		
Serotype 23F	23F F	TGGTAGTGACAGCAACGA	177	(20)
	23F R	CAAAGGCTAATTCAGCATC		
lyt A	*lyt* A F	CAACCGTACAGAATGAAGCGG	319	(14)
	lyt A R	TTATTCGTGCAATACTCGTGCG		
erm (B)	*erm* (B) F	CGTACCTTGGATATTCACCG	224	(14)
	erm (B) R	GTAAACAGTTGACGATATTCTCG		
mef (A)	*mef* (A) F	CTGTATGGAGCTACCTGTCTGG	302	(14)
	mef (A) R	CCCAGCTTAGGTATACGTAC		

Table 2: The carriage rate of *Streptococcus pneumoniae* among studied children according to age

Age (Years)	No. of male carriers / Total No. of males (%)	No. of female carriers / Total No. of females (%)	No. of total carriers / Total No. of children (%)
2- <3	20/ 119 (16.8%)	14/ 63 (22.2%)	34/ 182 (18.7%)
3- <4	26/ 98 (26.5%)	20/ 84 (23.8%)	46/ 182 (25.3%)
4- <5	40/ 170 (23.5%)	25/ 145 (17.2%)	65/ 315 (20.6%)
5- <6	30/ 165 (18.2%)	20/ 158 (12.6%)	50/ 323 (15.5%)
Total	116/ 542 (21.4%)	79/ 460 (17.1%)	195/ 1002 (19.5%)

Table 3: Susceptibilities of 195 *Streptococcus pneumoniae* isolates to antimicrobial agents

[a]Antibiotic	S n; (%)	I n; (%)	R n; (%)	% Resistance	MIC_{50}	MIC_{90}	MIC range
PEN	16 (8.2%)	103 (52.8 %)	76 (39%)	91.8%	1	4	0.016- 8
CTX	138 (70.8%)	13 (6.7%)	44 (22.5%)	29.2%	1	4	0.03- 8
ERY	104 (53.3%)	12 (6.2%)	79 (40.5%)	46.7%	0.5	32	0.03- 32
CLI	157 (80.5%)	3 (1.5%)	35 (17.9%)	19.5%	0.5	32	0.03- 32
SXT	65 (33.3%)	20 (10.3%)	110 (56.4%)	66.7%	2/38	16/304	0.25/4.75- 32/608
TET	132 (67.7%)	14 (7.2%)	49 (25.1%)	32.3%	1	32	0.5- 32

[a] Breakpoints (I, R) according to CLSI: Penicillin G (PEN): 0.1–1 µg/ml, ≥2 µg/ml; cefotaxime (CTX) (non-meningitis): 2 µg/ml, ≥ 4 µg/ml; erythromycin A (ERY): 0.5 µg/ml, ≥ 1 µg/ml; clindamycin (CLI): 0.5 µg/ml, ≥ 1 µg/ml; tetracycline (TET): 4 µg/ml ≥ 8 µg/ml; trimethoprim-sulfamethoxazole (SXT): 1/19-2/38 µg/ml, ≥ 4/76 µg/ml; all isolates were susceptible for vancomycin, MIC ≤ 1 µg/ml.

Table 4: Distribution of the serotypes of the 7v-PCV in the children according to age

Age (months)	Serotype								Total	PCV7 coverage n (%)
	4	6B	9V	14	18C	19F	23F	Other serotypes[a]		
24-30	0	1	1	0	5	3	4	12	26	14 (53.8%)
31-36	0	2	4	2	1	2	1	12	24	12 (50%)
37-42	1	2	0	3	1	1	1	7	16	9 (56.2%)
43-48	1	2	1	0	0	3	2	21	30	9 (30%)
49-54	0	3	0	2	0	4	4	6	19	13 (68.4%)
55-60	2	2	6	1	0	3	2	16	32	16 (50%)
61-66	1	1	1	2	1	4	4	11	25	14 (56%)
67-72	0	0	2	1	1	3	1	15	23	8 (34.7%)
Total	5	13	15	11	9	23	19	99	195	96 (49.2%)

[a]Other serotypes not included in the 7 valent pneumococcal conjugate vaccine

Table 5: Resistance patterns according to the serotype

Serotype	No. and % of isolates n (%)	Resistant isolates to antibiotics						Multiresistant isolates n (%)
		PEN n (%)	CTX n (%)	ERY n (%)	CLI n (%)	SXT n (%)	TET n (%)	
4	5 (2.6)	5 (100)	2 (40)	3 (60)	1 (20)	5 (100)	0 (0)	1 (20)
6B	13 (6.7)	13 (100)	4 (30.8)	9 (69.2)	2 (15.4)	9 (69.2)	5 (38.5)	3 (23.1)
9V	15 (7.7)	15 (100)	3 (20)	6 (40)	0 (0)	11 (73.3)	1 (6.7)	5 (33.3)
14	11 (5.6)	11 (100)	8 (72.7)	10 (90.9)	8 (72.7)	9 (81.8)	4 (36.4)	5 (45.5)
18C	9 (4.6)	9 (100)	4 (44.4)	5 (55.6)	2 (22.2)	6 (66.7)	5 (55.6)	1 (11.1)
19F	23 (11.8)	23 (100)	8 (34.8)	16 (69.6)	7 (30.4)	18 (78.3)	7 (30.4)	17 (73.9)
23F	20 (10.3)	20 (100)	8 (40)	14 (70)	6 (30)	14 (70)	10 (50)	7 (35)
[a]Others	99 (50.8)	83 (83.8)	20 (20.2)	28 (28.3)	12 (12.1)	58 (58.6)	31 (31.3)	28 (28.3)
Total	195	179 (91.8)	57 (29.2)	91 (46.7)	38 (19.5)	130 (66.7)	63 (32.3)	67 (34.4)

[a]Other serotypes not included in the 7 valent pneumococcal conjugate vaccine.

Breakpoints (I, R) according to CLSI: Penicillin G (PEN): 0.1–1 µg/ml, ≥2 µg/ml; cefotaxime (CTX) (non-meningitis): 2 µg/ml, ≥ 4 µg/ml; erythromycin A (ERY): 0.5 µg/ml, ≥ 1 µg/ml; clindamycin (CLI): 0.5 µg/ml, ≥ 1 µg/ml; tetracycline (TET): 4 µg/ml ≥ 8 µg/ml; trimethoprim-sulfamethoxazole (SXT): 1/19-2/38 µg/ml, ≥ 4/76 µg/ml; all isolates were susceptible for vancomycin, MIC ≤ 1 µg/ml.

Table (6): Risk factors for *Streptococcus pneumoniae* carriage

Variable	Children colonized with *S. pneumoniae* (*n*=195)	Univariate analysis		Logistic regression analysis	
		(OR)[1] 95% CI[2]	*P*	(OR)[1] 95% CI[2]	*P*
Sex					
Male=552	116 (21.0%)	0.80	0.17		
Female=450	79 (17.6%)	0.58-1.10			
Age					
≤ 36 months=182	34 (18.7%)	1.063	0.77	0.902	
>36 months=820	161 (19.6%)	0.70-1.60		0.361-2.253	0.83
Education father – university or higher					
Yes=577	106 (18.4%)	0.85	0.31		
No=425	89 (20.9%)	0.62-1.16			
Education mother - university or higher					
Yes=470	101 (21.5%)	1.275	0.13		
No=532	94 (17.7%)	0.93- 1.74			
Working mother					
Yes=265	60 (18.5%)	1.30	0.13		
No=737	135 (22.6%)	0.92-1.83			

No. of rooms in the house ≤ 3 room= 544 > 3 room= 458	 109 (20.0%) 86 (18.8%)	 0.923 0.67-1.26	 0.62		
Quality of drinking water Filtered water=665 Tap water=337	 126 (18.7%) 69 (20.8%)	 0.908 0.65-1.26	 0.56		
Kind of heating Gas=791 Other=211	 161 (20.4%) 34 (16.1%)	 0.752 0.5-1.13	 0.17		
No. of siblings ≤ 5= 911 > 5=91	 177 (19.4%) 18 (19.8%)	 1.023 0.6-1.75	 0.94	 1.071 0.463-2.477	 0.87
Parental smoking Yes=517 No=485	 115 (22.2%) 80 (16.5%)	 1.448 1.055-1.988	 0.022*	 0.678 0.418-1.101	 0.12
Breast feeding Yes=750 No=252	 143 (18.9%) 53 (24.9%)	 0.877 0.62- 1.25	 0.45		
Kindergarten attendance Yes=782 No=220	 151 (19.3%) 44 (20%)	 1.045 0.72-1.52	 0.82	 0.97 0.649-0.459	 0.89
Good appetite for food Yes=411 No=591	 79 (19.2%) 116 (19.6)%	 0.974 0.71-1.34	 0.84	 1.065 0.824-1.375	 0.63

Drinking milk everyday Yes=480 No=522	 85 (17.7%) 110 (21.1%)	 0.806 0.59-1.10	 0.18	 0.984 0.767-1.264	 0.9
Eating fresh fruit everyday Yes=600 No=402	 124 (20.7%) 70 (17.7%)	 1.21 0.88-1.679	 0.24	 1.068 0.836-1.364	 0.59
Eating yogurt everyday Yes=509 No=493	 99 (19.4%) 96 (19.5%)	 0.999 0.73-1.37	 0.99	 0.933 0.721-1.208	 0.6
Eating potato chips everyday Yes=858 No=144	 174 (20.3%) 21 (14.6%)	 0.671 0.41-1.097	 0.11	 0.998 0.695-1.431	 0.99
Taking vitamin supplements Yes=50 No=552	 6 (12%) 93 (16.8%)	 0.673 0.28- 1.63	 0.38	 0.901 0.555-1.463	 0.67

Frequent episode of **:**					
Influenza					
Yes=605	131 (21.7%)	1.438*	0.03*	0.789	0.36
No=397	64 (16.1%)	1.034-2		o.473-1.316	
Pharyngitis					
Yes=368	77 (20.9%)	1.157	0.37	0.926	0.77
No=634	118 (18.6%)	0.84-1.59		0.547-1.567	
Otitis media					
Yes=214	55 (25.7%)	1.60*	0.009*	1.072	0.81
No=788	140 (17.8%)	1.12-2.29		0.612-1.880	
Lower respiratory tract infection	49 (26.1%)	1.613*	0.01*	1.957	0.05*
Yes=188	146 (17.9%)	1.11-2.33		1.006-3.804	
No=814					
Allergy	26 (19.8%)	1.029	0.91	0.704	0.327
Yes=131	169 (19.4%)	0.64-1.63		0.349-1.421	
No=871					
Any other chronic disease	37 (18.7%)	0.947	0.79	0.629	0.40*
Yes=197	158 (19.6%)	0.64- 1.41		0.213-1.855	
No=805					
Taking medication without prescription	55 (24.4%)	1.472*	0.03*	2.629	0.0001*
Yes=225	140 (18%)	1.032-2.099		1.644-4.206	
No=777					
Physician visits per year	109 (16.7%)	2.81*	0.0001*	1.879	0.05*
≤ 6=652	62 (36%)	1.93-4.07		1.011-3.493	
> 6=172					
Use of antibiotics in preceding 3 months					
Yes=691	146 (21.1%)	1.473*	0.04*	1.874	0.03*
No=286	44 (15.4%)	1.018-2.132		1.085-3.239	

* indicated the significance of variable as risk factor

1 (OR) Odds ratio; 2 (CI) 95% confidence intervals

Table (7): Risk factors for carriage of multi-resistant *Streptococcus pneumoniae*

Variable	**Multi-resistant *S. pneumoniae*** **(*n*=67)**	**Univariate analysis**		**Logistic regression analysis**	
		(OR)[1] 95% CI[2]	***P***	**(OR)[1] 95% CI[2]**	***P***
Sex Male=116 Female=79	37(31.9%) 30(38.0%)	1.307 0.72-2.38	0.38		
Age ≤ 36 months=34 >36 months=161	10(24.4%) 57(35.4%)	1.315 0.588-2.943	0.51	0.620 0.081-4.730	0.644
Education father -university or higher Yes=106 No=89	34(32.1%) 33(37.1%)	0.801 0.443-1.450	0.47		
Education mother -university or higher Yes=101 No=94	39 (38.6%) 28 (29.8%)	1.483 0.817-2.692	0.2		

Working mother Yes=60 No=135	 20(33.3%) 47(34.8%)	 0.936 0.492-1.781	 0.84		
No. of rooms in the house ≤ 3 room= 109 ➢ 3 room =86	 42 (38.5 %) 25 (29.1%)	 0.654 0.357-1.197	 0.17		
Quality of drinking water Filtered water=126 Tap water=69	 48 (38.1%) 19 (27.5%)	 1.619 0.855-3.068	 0.14		
Kind of heating Gas=161 0thers= 34	 51 (31.7%) 16 (47.1%)	 1.917 0.905-4.062	 0.09		
No. of siblings ≤ 5= 177 > 5= 18	 61 (34.5%) 6 (33.3%)	 1.951 0.340- 2.658	 0.787	1.339 0.234-7.653	0.74
Parental smoking Yes= 115 No= 80	 41(35.7%) 26 (32.5%)	 0.869 0.475- 1.589	 0.65	0.418 0.138-1.265	0.12
Breast feeding Yes=142 No=53	 50 (35.2%) 17 (32.1%)	 1.151 0.588-2.253	 0.68		
Kindergarten attendance Yes= 151 No=44	 57 (37.7%) 10 (22.7%)	 0.485 0.223- 1.056	 0.07		

Good appetite for food Yes= 79 No=116	 29 (36.7%) 38 (32.8%)	 1.191 0.654-2.169	 0.57	0.707 0.395-1.268	0.25
Drinking milk everyday Yes=85 No=110	 33 (38.8%) 34 (30.9%)	 1.419 0.783-2.71	 0.25	0.979 0.583-1.645	0.94
Eating fresh fruit everyday Yes=124 No=71	 44 (35.5%) 23 (32.4%)	 1.148 0.618-2.130	 0.66	0.969 0.594-1.579	0.90
Eating yogurt everyday Yes=99 No=96	 33 (33.3%) 34 (35.4%)	 0.912 0.505-1.647	 0.76	 0.775 0.428-1.404	0.40
Eating potato chips everyday Yes= 21 No=174	 6 (28.6%) 61 (35.1%)	 0.741 0.274-2.00	 0.58	1.186 0.539-2.608	0.67
Taking vitamins supplements Yes=6 No=93	 3 (50%) 36(38.7%)	 1.583 0.303- 8.276	 0.59	1.451 0.517-4.074	0.48
Frequent episodes of : **Influenza** Yes=131 No=64 **Pharyngitis** Yes= 77 No=118	 52 (39.7%) 15 (23.4) 28 (36.4%) 39 (33.1%)	 2.150* 1.094-4.227 1.158 0.634-2.114	 0.03* 0.64	 4.847 1.503-15-625	 0.008*

Otitis media					
Yes= 55	13 (23.6%)	0.493	0.06	2.826	0.42
No=140	54 (38.6%)	0.243-1.002		0.231-34.655	
Lower respiratory tract infection					
Yes=49	14 (28.6%)	0.702	0.33	0.290	0.08
No= 146	53 (36.3%)	0.347-1.421		0.071-1.88	
Allergy					
Yes=26	9 (34.6%)	1.013	0.98	1.325	0.72
No=169	58 (34.3%)	0.425-2.414		0.291-6.042	
Any other chronic disease					
Yes=37	15 (40.5%)	1.390	0.38	1.176	0.89
No=158	52 (32.9%)	0.666-2.90		0.132-10.50	
Taking medication without prescription	23 (41.8%)	0.638	0.71	1.159	0.8
Yes= 55	44 (31.4%)	0.335-1.214		0.375-3.584	
No=140					
Physician visits per year	54 (36.7%)	0.640	0.22	0.78	0.76
≤ 6=147	13 (27.1%)	0.312-1.314		0.161-3.789	
> 6=48					
Use of antibiotic in preceding 3 month	43 (30.1%)	2.209*	0.03*	5.219	0.02*
Yes= 143	19 (48.7%)	1.073-4.550		1.347-20.22	
No=39					

* indicated the significance of variable as risk factor

1 (OR) Odds ratio; 2 (CI) 95% confidence intervals

[b] Resistance phenotypes: p, penicillin G-intermediate; P, penicillin G-resistant; M, macrolide-resistant; L, lincosamide-resistant; T, tetracycline-resistant; c, cotrotrimoxazole-intermediate; C, cotrotrimoxazole-resistant; F, fluoroquinolone-resistant.

DISCUSSION

Surveillance for *S. pneumoniae* susceptibility patterns becomes increasingly important, because of the emergence of antibiotic-resistant and multi drug-resistant strains world wide (2, 22-24). Current information on capsular types causing disease is required in young children in order to guide conjugate vaccine recommendations. The nasopharynx is the usual source of pneumococci for studying the carriage rate (25). In this study, it was difficult to obtain nasopharyngeal samples. However, a nasal swab fulfills the purpose with acceptable results (26). The carriage rate of *S. pneumoniae* in Jordanian children in the Amman area was 19.5%. This rate was relatively low compared to carriage rates reported in Asia - Philippines (32.6%), China (37.5%), and Thailand (40.6%) (27). Higher rates were reported in other parts of the world as well, 49.8% in Russia (28), 55% in Brazil (29), 59.1% in Guatemala, (30), 22% in Israel (31) and 62% in Kampala, Uganda (32). The differences in carriage rates worldwide were related to genetic variables and 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 (33). Furthermore, methodological factors as quality of the sampling, and culture techniques may also have affected the observed differences in reported colonization rates (33). The low carriage rates obtained from our study could be due to the isolation procedure, though anterior nasal swabs are easier to perform in younger children, and it is thought to be as sensitive as the nasopharyngeal method (34). Yet, Lee *et al*. (2001) suggested that this method might be correlated with lower carriage rates (27).

In our study, we report the following risk factors: parental smoking (OR=1.44, P= 0.022) frequent episodes of influenza (OR=1.43 P= 0.03), otitis media (OR=1.60, P= 0.009), lower respiratory tract infection (OR=1.61, P= 0.01); taking medication without prescription (OR=1.47, P= 0.03); number of visits to physician per year (OR=2.81, P= 0.0001), and the use of antibiotics in the preceding 3 months to sampling (OR=1.47, P= 0.04). Although several previous studies reported the attendance of day care as a main factor causing the increase in the carriage rate of *S. pneumoniae* (35-37), this study could not confirm this correlation (OR= 1.045, CI= 0.72-1.52, *P*= 0.82), probably due to limited number of participating children who did not attend kindergarten school. Our results are consistent with reports of Marchisio *et al*. (2002) (38) and Petrosillo *et al*., (2002) (39) as they reported attending kindergarten as risk factor (OR= 1, CI=0.1-1.5) and (OR= 0.88, CI= 0.55-1.42, P= 0.67), respectively.

In this study, the number of children in the family was not a significant risk factor for carriage of pneumococci, an idea reported earlier by Chui et al.(40). The frequent visit to physicians was identified as a risk factor, not only for carriage of *S. pneumoniae* but also for carriage of multi-resistant isolates of *S. pneumoniae*. This may be attributed to frequent consumption of antibiotics, as most of these consultations resulted in antibiotic usage in this group of children, leading to the selection of resistant stains. Otoom et al. (2002) reported that physicians in Jordan tend to over estimate the severity of illness to justify antibiotic prescription (41). Indeed, taking medication without prescription appeared as a risk factor (OR= 1.472, CI= 1.032, P= 0.03), and reflected the misuse of antibiotics (42). This study also confirmed that prior antibiotic use is not only an important risk factor for increased *S. pneumoniae* carriage but also an important risk factor for carriage of multiple-resistant strains of pneumococci. The antibiotic susceptibility of *S. pneumoniae* isolated from children's nostrils reflected alarming rates of resistance to penicillin, erythromycin and multi-resistant isolates among Jordanian children. This may be attributed to the isolation site, age and nature of population study. Rates of resistance in carriage isolates were higher than of clinical isolates in Taiwan, Singapore, and Sri Lanka (27). It is also possible that pneumococci isolated from the anterior nares may have susceptibility patterns different from those in the posterior nasopharynx. Lehmann et al. reported that pneumococci from nasal swabs tended to lead to an overestimation of the levels of resistance in invasive pneumococcal isolates (43). However, our resistance rates resemble the rates that were reported in some Asian countries (26, 40, 44). The high rates of resistance to different antibiotics in *S. pneumoniae* found in

this study are presumably a consequence of antimicrobial consumption and abuse within the Jordanian population (45). Otoom et al., (2002) reported that 60.9% of antibiotic prescriptions at different health centers in Jordan ranged from 46.7% to 83.3%; they added that this value is very high compared with that observed in other parts of the world (41). Serotype distribution is usually monitored by culturing of the organism followed by serological determination of the capsular type by the capsular Quellung reaction. The high cost of antisera, subjectivity in interpretation, and technical expertise requirements are serious drawbacks of the system. The development of PCR-based serotyping systems has the potential to overcome some of the difficulties associated with serologic testing and is proved to be useful for serotyping pneumococci as was recently shown by Lawrence et al. (2003) (46), Brito et al 2003 (47), and Pai et al. (2006) (48). Our results of PCR-based serotyping showed that the most prevalent serotype was 19F (11.8%) followed by 23F (9.7%) and 9V (7.6%). This is similar to the serotypes prevalent among children in Kuwait (49), Italy (38), and Belgium (50). Prevenar, the seven-valent pneumococcal conjugate vaccine (PCV7) that is licensed in more than 70 countries and is used routinely in several industrialized nations, covers 49.2% of serotypes among children according to our study. Around the world, the highest serotype 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 lower percentages were obtained for coverage of this vaccine (65% and 50% respectively), (51). Finally, our study was performed in the capital Amman only. We are aware of the fact that the carriage patterns may vary considerable between various areas 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.

Acknowledgements
The study was supported by the deanship of scientific research at the University of Jordan.

References

1. Musher DM. Infections caused by *Streptococcus pneumoniae*: clinical spectrum, pathogenesis, immunity, and treatment. *Clin Infect Dis*. 1992;14:801-807.
2. Appelbaum PC. Resistance among *Streptococcus pneumoniae*: implications for drug selection. *Clin Infect Dis*. 2002;34:1613-1620.
3. File TM, Jr. Clinical implications and treatment of multiresistant Streptococcus pneumoniae pneumonia. *Clin Microbiol Infect*. 2006;12 Suppl 3:31-41.
4. Williams JD. Streptococcus pneumoniae still going strong. *Int J Antimicrob Agents*. 2002;20:75.
5. Pallares R, Fenoll A, Linares J. The epidemiology of antibiotic resistance in Streptococcus pneumoniae and the clinical relevance of resistance to cephalosporins, macrolides and quinolones. *Int J Antimicrob Ag*. 2003;22 Suppl 1:S15-24; discussion S25-16.
6. Jacobs MR, Dagan R. Antimicrobial resistance among pediatric respiratory tract infections: clinical challenges. *Semin Pediatr Infect Dis*. 2004;15:5-20.
7. Bogaert D, De Groot R, Hermans PW. Streptococcus pneumoniae colonisation: the key to pneumococcal disease. *Lancet Infect Dis*. 2004;4:144-154.
8. Volonakis K, Souli M, Kapaskelis A, et al. Evolution of resistance patterns and identification of risk factors for Streptococcus pneumoniae colonisation in daycare centre attendees in Athens, Greece. *Int J Antimicrob Agents*. 2006;28:297-301.

9. Saha SK, Baqui AH, Darmstadt GL, et al. Comparison of antibiotic resistance and serotype composition of carriage and invasive pneumococci among Bangladeshi children: implications for treatment policy and vaccine formulation. *J Clin Microbiol*. 2003;41:5582-5587.
10. Mahafzah A, Shehabi A. Antimicrobial susceptibility among clinical isolates of Streptococcus pneumoniae at the Jordan university hospital over an eighteen-year period. *Jordan Medical Journal* 2000;34:109-112.
11. Marchese A, Schito GC. Recent findings from multinational resistance surveys: are we 'PROTEKTed' from resistance? *Int J Antimicrob Agents*. 2007;29 Suppl 1:S2-5.
12. O'Brien KL, Bronsdon MA, Dagan R, et al. Evaluation of a medium (STGG) for transport and optimal recovery of Streptococcus pneumoniae from nasopharyngeal secretions collected during field studies. *J Clin Microbiol*. 2001;39:1021-1024.
13. Kellogg JA, Bankert DA, Elder CJ, Gibbs JL, Smith MC. Identification of Streptococcus pneumoniae revisited. *J Clin Microbiol*. 2001;39:3373-3375.
14. Nagai K, Shibasaki Y, Hasegawa K, et al. Evaluation of PCR primers to screen for Streptococcus pneumoniae isolates and beta-lactam resistance, and to detect common macrolide resistance determinants. *J Antimicrob Chemother*. 2001;48:915-918.
15. O'Brien KL, Nohynek H. Report from a WHO Working Group: standard method for detecting upper respiratory carriage of Streptococcus pneumoniae. *Pediatr Infect Dis J*. 2003;22:e1-11.
16. Clinical Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing; Nineteenth Informational Supplement M100-S19*. Wayne, PA; 2009.
17. Farrell DJ, Jenkins SG, Reinert RR. Global distribution of Streptococcus pneumoniae serotypes isolated from paediatric patients during 1999-2000 and the in vitro efficacy of telithromycin and comparators. *J Med Microbiol*. 2004;53:1109-1117.
18. Montanari MP, Mingoia M, Cochetti I, Varaldo PE. Phenotypes and genotypes of erythromycin-resistant pneumococci in Italy. *J Clin Microbiol*. 2003;41:428-431.
19. Montanari MP, Cochetti I, Mingoia M, Varaldo PE. Phenotypic and molecular characterization of tetracycline- and erythromycin-resistant strains of *Streptococcus pneumoniae*. *Antimicrob Agents Chemother*. 2003;47:2236-2241.
20. O'Halloran D M, Cafferkey MT. Multiplex PCR for identification of seven Streptococcus pneumoniae serotypes targeted by a 7-valent conjugate vaccine. *J Clin Microbiol*. 2005;43:3487-3490.
21. Petrie A SC. *Medical Statistics at a Glance*: Blackwell Science Ltd, Australia; 2000.
22. Goyal R, Singh NP, Kaur M, Talwar V. Antimicrobial resistance in invasive and colonising Streptococcus pneumoniae in North India. *Indian J Med Microbiol*. 2007;25:256-259.
23. Lin WJ, Lo WT, Chou CY, et al. Antimicrobial resistance patterns and serotype distribution of invasive Streptococcus pneumoniae isolates from children in Taiwan from 1999 to 2004. *Diagn Microbiol Infect Dis*. 2006;56:189-196.
24. Mera RM, Miller LA, Daniels JJ, Weil JG, White AR. Increasing prevalence of multidrug-resistant Streptococcus pneumoniae in the United States over a 10-year period: Alexander Project. *Diagn Microbiol Infect Dis*. 2005;51:195-200.
25. Sulikowska A, Grzesiowski P, Sadowy E, Fiett J, Hryniewicz W. Characteristics of Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis isolated from the nasopharynges of asymptomatic children and molecular analysis of S. pneumoniae and H. influenzae strain replacement in the nasopharynx. *J Clin Microbiol*. 2004;42:3942-3949.
26. Parry CM, Diep TS, Wain J, et al. Nasal carriage in Vietnamese children of Streptococcus pneumoniae resistant to multiple antimicrobial agents. *Antimicrob Agents Chemother*. 2000;44:484-488.

27. Lee NY, Song JH, Kim S, et al. Carriage of antibiotic-resistant pneumococci among Asian children: a multinational surveillance by the Asian Network for Surveillance of Resistant Pathogens (ANSORP). *Clin Infect Dis*. 2001;32:1463-1469.
28. Stratchounski LS, Kozlov RS, Appelbaum PC, Kretchikova OI, Kosowska-Shick K. Antimicrobial resistance of nasopharyngeal pneumococci from children from day-care centres and orphanages in Russia: results of a unique prospective multicentre study. *Clin Microbiol Infect*. 2006;12:853-866.
29. Rey LC, Wolf B, Moreira JL, Verhoef J, Farhat CK. [Nasopharyngeal isolates of S. pneumoniae from healthy carriers and children with pneumonia: colonization rates and antimicrobial susceptibility]. *J Pediatr (Rio J)*. 2002;78:105-112.
30. Dueger EL, Asturias EJ, Matheu J, et al. Increasing penicillin and trimethoprim-sulfamethoxazole resistance in nasopharyngeal Streptococcus pneumoniae isolates from Guatemalan children, 2001--2006. *Int J Infect Dis*. 2008;12:289-297.
31. Dagan R. Impact of pneumococcal conjugate vaccine on infections caused by antibiotic-resistant Streptococcus pneumoniae. *Clin Microbiol Infect*. 2009;15 Suppl 3:16-20.
32. Joloba ML, Windau A, Bajaksouzian S, et al. Pneumococcal Conjugate Vaccine Serotypes of Streptococcus pneumoniae Isolates and the Antimicrobial Susceptibility of Such Isolates in Children with Otitis Media. *Clin Infect Dis*. 2001;33:1489-1494.
33. Garcia-Rodriguez JA, Fresnadillo Martinez MJ. Dynamics of nasopharyngeal colonization by potential respiratory pathogens. *J Antimicrob Chemother*. 2002;50 Suppl S2:59-73.
34. Rapola S, Salo E, Kiiski P, Leinonen M, Takala AK. Comparison of four different sampling methods for detecting pharyngeal carriage of Streptococcus pneumoniae and Haemophilus influenzae in children. *J Clin Microbiol*. 1997;35:1077-1079.
35. Huang WH, Fang SY. High prevalence of antibiotic resistance in isolates from the middle meatus of children and adults with acute rhinosinusitis. *Am J Rhinol*. 2004;18:387-391.
36. Regev-Yochay G, Raz M, Shainberg B, et al. Independent risk factors for carriage of penicillin-non-susceptible Streptococcus pneumoniae. *Scand J Infect Dis*. 2003;35:219-222.
37. Dunais B, Pradier C, Carsenti H, et al. Influence of child care on nasopharyngeal carriage of Streptococcus pneumoniae and Haemophilus influenzae. *Pediatr Infect Dis J*. 2003;22:589-592.
38. Marchisio P, Esposito S, Schito GC, et al. Nasopharyngeal Carriage of Streptococcus pneumoniae in Healthy Children: Implications for the Use of Heptavalent Pneumococcal Conjugate Vaccine. *Emerg Infect Dis*. 2002;8:479-484.
39. Petrosillo N, Pantosti A, Bordi E, et al. Prevalence, determinants, and molecular epidemiology of Streptococcus pneumoniae isolates colonizing the nasopharynx of healthy children in Rome. *Eur J Clin Microbiol Infect Dis*. 2002;21:181-188.
40. Chiu SS, Ho PL, Chow FK, Yuen KY, Lau YL. Nasopharyngeal carriage of antimicrobial-resistant Streptococcus pneumoniae among young children attending 79 kindergartens and day care centers in Hong Kong. *Antimicrob Agents Chemother*. 2001;45:2765-2770.
41. Otoom S, Batieha A, Hadidi H, Hasan M, Al-Saudi K. Evaluation of drug use in Jordan using WHO prescribing indicators. *East Mediterr Health J*. 2002;8:537-543.
42. Barkai G, Greenberg D, Givon-Lavi N, et al. Community prescribing and resistant Streptococcus pneumoniae. *Emerg Infect Dis*. 2005;11:829-837.
43. Lehmann D, Gratten M, Montgomery J. Susceptibility of pneumococcal carriage isolates to penicillin provides a conservative estimate of susceptibility of invasive pneumococci. *Pediatr Infect Dis J*. 1997;16:297-305.
44. Chen CJ, Huang YC, Su LH, Lin TY. Nasal carriage of Streptococcus pneumoniae in healthy children and adults in northern Taiwan. *Diagn Microbiol Infect Dis*. 2007;59:265-269.

45. Al-Bakri AG, Bustanji Y, Yousef AM. Community consumption of antibacterial drugs within the Jordanian population: sources, patterns and appropriateness. *Int J Antimicrob Agents*. 2005;26:389-395.
46. Lawrence ER, Griffiths DB, Martin SA, George RC, Hall LM. Evaluation of Semiautomated Multiplex PCR Assay for Determination of Streptococcus pneumoniae Serotypes and Serogroups. *J Clin Microbiol*. 2003;41:601-607.
47. Brito DA, Ramirez M, de Lencastre H. Serotyping *Streptococcus pneumoniae* by multiplex PCR. *J Clin Microbiol*. 2003;41:2378-2384.
48. Pai R, Gertz RE, Beall B. Sequential multiplex PCR approach for determining capsular serotypes of Streptococcus pneumoniae isolates. *J Clin Microbiol*. 2006;44:124-131.
49. Ahmed K, Martinez G, Wilson S, et al. The prevalence and clonal diversity of penicillin-resistant Streptococcus pneumoniae in Kuwait. *Epidemiol Infect*. 2000;125:573-581.
50. Malfroot A, Verhaegen J, Dubru JM, Van Kerschaver E, Leyman S. A cross-sectional survey of the prevalence of Streptococcus pneumoniae nasopharyngeal carriage in Belgian infants attending day care centres. *Clin Microbiol Infect*. 2004;10:797-803.
51. Hausdorff WP, Feikin DR, Klugman KP. Epidemiological differences among pneumococcal serotypes. *Lancet Infect Dis*. 2005;5:83-93.