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Prevalence of *Streptococcus pneumoniae* Serotypes (Nasopharyngeal Colonization) in Children in North Jordan: Genotypic and Phenotypic Characteristics

[1]Hani A. Masaadeh, [1]Wail A. Hayajneh, [2]Isra'a A. Al Azzam and [1]Ahed J. Alkhatib
[1]Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan
[2]Faculty of Applied Medical Sciences, Jordan University of Science and Technology, Irbid, Jordan

Abstract: Nasopharyngeal swabs were collected from children <5 years who attend King Abdullah Teaching Hospital and the emergency room at Princess Rahma Teaching hospital in North Jordan. *S. pneumoniae* identification was confirmed using colonial morphology, susceptibility to optochin and bile solubility. Sequential multiplex PCR test was used to determine capsular serotypes of *S. pneumoniae* isolates. 37.8% of children were carriers to capsulated *S. pneumoniae*. The most prevalent *S. pneumoniae* serotypes were 6A/B (13.2%), 23F (7%), ST14 (6%), 9V (4.4%), 11A (3.2%), 19F (3%), ST3 (1.8%), ST4 (1.8%), 12F (1.6%), 35B (1.4%), 19A (0.8%), 7F (0.6%). This type of colonization was not statistically related to acute illness. These moderately high colonization rates are expected to help future research to determine streptococcal strains causing invasive disease and subsequently help in making decision regarding the appropriateness of using new conjugate pneumococcal vaccines.

Key words: *Streptococcus pneumoniae*, serotype, nasopharyngeal colonization, children, genotype, phenotype

INTRODUCTION

Pneumococci are often part of nasopharyngeal flora; probably all humans are colonized with this organism at least once early in life. The risk of pneumococcal colonization is high, especially under conditions with crowding such as daycare centers, nursing homes, hospitals and jails. A strong relation between carriage and middle ear infections has been found but the association between colonization and invasive disease has not been confirmed (Bogaert *et al*., 2001).

Streptococcus pneumoniae is one of the leading bacterial pathogens causing illness and death among young children, the elderly and persons with underlying medical conditions. Pneumococcal infections are usually preceded by colonization of the human nasopharynx and this is an important step toward infection. Consequently, pneumococcal colonization is an important risk factor for developing disease. For instance, young children who are frequently colonized with pneumococci more often to develop acute otitis media than children who are not or less frequently colonized. A subset of the 90 pneumococcal serotypes are commonly carried by children and these isolates are also the major causes of disease in children (Dejsirilert *et al*., 1999; Meats *et al*., 2003).

Carriage is a highly dynamic process with pneumococci being acquired, carried for a period of weeks or months and then lost. It has been shown that there are two general patterns of pneumococcal nasopharyngeal colonization: children universally acquire pneumococcus in the first few months of life or more common, the time of first colonization with pneumococcus in the second 6 months of life or beyond (Meats *et al*., 2003; Obrien and Nohynek, 2003).

In developing countries, the rate of pneumococcal carriage in children is extremely high, approaching 100%, while in the United States, the carriage rate in preschool children has been reported to be 35% (Lehmann *et al*., 1992; Lankinen *et al*., 1994).

Certain serotypes of *S. pneumoniae* predominate differently in different areas and have tendencies to cause different clinical infections. The predominance variable patterns are also seen among different age groups (Dejsirilert *et al*., 1999; Zhao *et al*., 2003).

The clinical view of *S. pneumoniae* infections is getting worse by the increasing rate of drug-resistant strains. There is now convincing evidence that certain serotypes might predominate within penicillin-resistant *Streptococcus pneumoniae* strains (Ubukata *et al*., 2004).

Much morbidity and mortality due to Acute Lower Respiratory Infections (ALRI) might be prevented if there is an effective pneumococcal vaccine available. In light of an increased risk of pneumococcal disease with advancing age, the costs associated with infection and the rising rates of drug resistance, vaccination has emerged as a public health priority (Lankinen *et al*., 1994; Artz *et al*., 2003).

Corresponding Author: Hani A. Masaadeh, Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan

The availability of a type-specific pneumococcal vaccine for children is a worldwide problem. Geographical differences in the distribution of serotypes as well as a large number of *S. pneumoniae* type antigens emphasize the need why a study on the distribution of serotypes should be done prior to introduction of a type-specific pneumococcal vaccine in a country (Prymula *et al*., 2004).

Since, pneumococci are generally acquired from carriers and since nasopharyngeal carriage generally precedes infection, much of the recent thinking regarding the development of pneumococcal vaccine is that the optimal vaccines would be those that protected against carriage as well as invasive disease (Briles *et al*., 1998).

The 23-Valent Pneumococcal capsular polysaccharide (PPV23) vaccine was licensed in 1983. It contains 23 purified capsular polysaccharide antigens which account for 85-90% of invasive infections in the USA (Lankinen *et al*., 1994; Ubukata *et al*., 2004).

PPV23 is recommended for children older than 2 years of age and for adults. Unfortunately, this vaccine has no effect on nasopharyngeal carriage and does not prime for an anamnestic response. The newer world wide licensed vaccine is a heptavalent Pneumococcal Conjugate Vaccine (PCV7) that contains purified capsular polysaccharide of seven serotypes (4, 6B, 9V, 14, 18C, 19F and 23F) conjugated to a non-toxic variant of diphtheria toxin (CRM197). It is highly immunogenic in infants and children, inducing a T-cell-dependent response and was licensed in the United States in 2000. Certain serotypes contained in PCV7 produce a cross-reactive protective response to other serotypes not contained in the vaccine. The vaccine has demonstrated an estimated 97% efficacy for preventing invasive pneumococcal infections (meningitis and bacteremia) caused by the serotypes included in the vaccine (Black *et al*., 2000; Jakobsen and Jonsdottir, 2003).

A finish study demonstrated 57% efficacy for preventing acute otitis media infections caused by the serotypes included in the vaccine (Eskola *et al*., 2001).

Several reports have documented the effect of conjugate pneumococcal vaccine on carriage of vaccine serotype and nonvaccine serotype; some studies have shown a reduction in carriage of vaccine serotype pneumococci among those immunized with pneumococcal protein conjugate vaccine (Obrien and Nohynek, 2003).

More conjugated vaccines like 10 and 13-valent conjugate pneumococcal vaccines for serotypes (1, 3, 4, 5, 6A, 6B, 7 F, 19A, 9 V, 14, 18C, 19F and 23F) are expected to license in the near future which may increase the reduction in carriage as soon as possible (Lawrence *et al*., 2003).

Many studies have been published recently which examine the prevalence of different pneumococcal serotypes/serogroups as a cause of invasive and respiratory disease across the different parts of the world (Zhao *et al*., 2003).

In this study, we intend to study the phenotypic and genotypic characteristics of *S. pneumoniae* isolates colonizing the nasopharynx of children in North Jordan. This would include standardized culture, identification, serotyping and genetic fingerprinting correlations between these elements together and with clinical patterns will be sought.

MATERIALS AND METHODS

Specimen collection: Nasopharyngeal swabs were obtained from a total of 500 children <5 years old 271 (54.2%) were males and 229 (45.8%) were females who attended to King Abdullah Teaching Hospital and to the emergency room at Princess Rahma Teaching Hospital.

All relevant demographic data including name, age, sex, phone number, address, family number, going to day care center, type of specimen, signs and symptoms, clinical diagnosis and antibiotics treatment usage was collected. Medical records were considered after ensuring appropriate legal and ethical procedures.

Swab transport media preparation: Oxoid Skim milk-Tryptone-Glucose-Glycerin (STGG) Transport and storage media were used to store the original nasopharyngeal material for at least 12 months at -70°C. Once a swab specimen was collected, it was placed in a tube of STGG transport medium.

Culture techniques: Selective Oxoidtrypticase soy agar-Blood Agar (BA) with sheep blood and 2.5 μg mL^{-1} gentamicin was used. The primary culture was obtained before the specimen was frozen by inoculating the swab onto a selective plate and incubating at 37°C in 3-10% CO_2 overnight. Optochin susceptibility test and bile solubility test were obtained for the diagnosis of *S. pneumoniae*.

Molecular capsular typing DNA extraction: Pellet cells lysing cells protein precipitation DNA precipitation and rehydration were used.

DNA extraction product detection by agarose gel electrophoresis

Oligonucleotide primers: About 29 primer pairs were designed based on the primer design used in a molecular epidemiological study published in 2006 known as equential multiplex PCR approach for determining capsular serotypes of *Streptococcus pneumoniae* serotypes to target serotypes 1, 3, 4, 5, 6 A/B, 7F, 7C, 8, 9V, 10A, 11A,

12F, 14, 15A, 15B/C, 16F, 17F, 18, 19A, 19F, 20, 22F, 23F, 31, 33, 34, 35B, 35F and 38. A primer pair (primers cpsA-f and cpsA-r) was also included as an internal control targeting the cpsA locus found in all pneumococci (Jedrzejas, 2001). The primer designations, sequences and product sizes are listed in Table 1 (Jedrzejas, 2001).

Multiplex PCR scheme: The primers were grouped into seven multiplex reactions as shown in Table 2. These primers were grouped together based on serotype distributions among invasive pneumococci (Jedrzejas, 2001).

Each reaction was designed to include four primer pairs targeting serotype-specific regions of four different serotypes and also included an internal positive control targeting all known pneumococcal capsular polysaccharide (cps). In addition, the compatibility between primers included in two entirely different reactions was also assessed by modifying the multiplex PCRs 1 and 2 to include two PCRs that could detect the most prevalent types in other geographic regions. In the modified reactions, reaction 1 contained primers for serotypes 19A,1 6A/6B and 19F while reaction 2 could detect serotypes 14, 5, 7F/7A (the rare serotype 7A can also be detected by 7F primers) and 23F. These two reactions would detect the eight most predominant serotypes in regions of Asia and Africa as shown in Fig. 1 (Jedrzejas, 2001).

PCRs: The PCRs were performed in 25 μL volumes with each reaction mixture containing 12.5 μL ready 2X PCR Taq mixture (Omega, Inc.), 6 μL nuclease free water, 0.5 μL from each primer as specified in Table 2, 1.5 μL DNA extract (Wizard Genomic DNA Purification Kit, Promega, Inc.)

Thermal cycling was performed in I Cycler BIO-RAD Gene Amp PCR system under the following conditions: Cycle 1 (1X): Step 1: 94°C for 4 min. Cycle 2 (30X): Step 1: 94°C for 45 sec, Step 2: 54°C for 45 sec, Step 3: 65°C for 2.30 min. Cycle 3 (1X): Step 1: 72°C for 5 min, Step 2: 4°C for 8.

Table 1: Frequency and percent of male and femaleof study population

Sex	Frequency	Percent
Male	271	54.2
Female	229	45.8
Total	500	100.0

Table 2: Analysis of *S. pneumoniae* carriage in male and female

	S. pneumoniae carriage	
Sex	Uncapsulated	Capsulated
Male		
Count	14.0	102.0
Total (%)	2.8	20.4
Female		
Count	8.0	87.0
Total (%)	1.6	17.4
Total		
Count	22.0	189.0
Total (%)	4.4	37.8

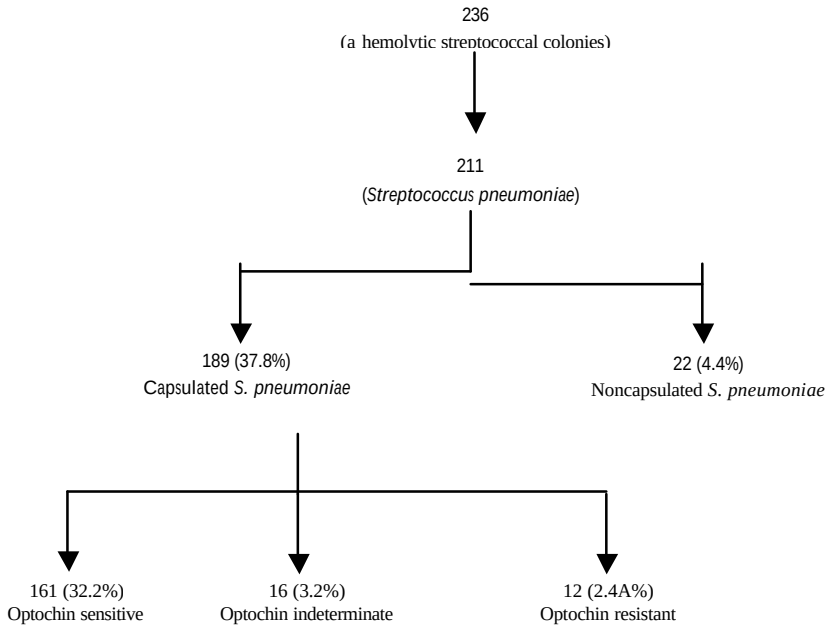

Fig. 1: Scheme for analysis of optochin sensitivity for *S. pneumoniae*

PCR product analysis: The PCR products were analyzed by gel electrophoresis on 2% agarose gels in 1X TBE buffer at 120 V for 45 min. Gels were stained with ethidium bromide and gel images were recorded. The sizes of the PCR products were determined by comparison with the molecular size standard (50 and 100 bp ladder; Promega, Inc).

Statistical analysis: All results of optochin sensitivity testing and molecular serotyping test were analyzed for their significance ($p<0.05$) by using SPSS 10 Software in order to uni-variant analysis and multi-variant analysis for pneumococcal carriage. And to do analysis for relation between risk factors and carriage of serotypes contained in 7, 10 and 13-PCV.

RESULTS AND DISCUSSION

Nasopharyngeal swabs were obtained from a total of 500 children <5 years old, 271 (54.2%) of children were males and 229 (45.8%) of children were females who attended to King Abdullah Teaching Hospital and to the emergency room at Princess Rahma Teaching Hospital (Table 2). The 10.4% of carriage were in children >2 years while 27% of carriage were in children equal to 2 years (Table 3-5).

In reaction No. 1 multiplex PCR, 41.8% of capsulated *S. pneumoniae* isolates were detected (Fig. 1-5). The 2.1% of them were 19A *S. pneumoniae*, 34.9% were 6 A/B *S. pneumoniae*, 4.8% were ST3 *S. pneumoniae* (Table 6).

In reaction No. 2 multiplex PCR, 36.5% of capsulated *S. pneumoniae* isolates were detected (Fig. 7-15). The 4.8% of them were ST4 *S. pneumoniae*, 15.9% were ST14 *S. pneumoniae*, 4.2% were 12F *S. pneumoniae* and 11.6% were 9V *S. pneumoniae* (Table 6).

In reaction No. 3 multiplex PCR 28.6% of capsulated *S. pneumoniae* isolates were detected (Fig. 7-15). The 18.5% of them were 23F *S. pneumoniae*, 1.6% were 7F *S. pneumoniae* and 8.5% were 11A *S. pneumoniae* (Table 6).

In reaction No. 4 multiplex PCR 11.6% of capsulated *S. pneumoniae* isolates were detected (Fig. 7-15). The 7.9% of them were 19F *S. pneumoniae* and 3.7% of them were 35B *S. pneumoniae* (Table 6).

Jordan is one of the developing countries where are certain serotypes of *S. pneumoniae* predominate differently and have tendencies to cause clinical infections. But, until now there is little data about the distribution of serotypes in Jordan while neighboring countries such as Saudi Arabia and Kuwait started work on this problem earlier (Brito *et al*., 2003; Mokaddas *et al*., 2003).

The importance of studying serotypes distribution is to know the efficacy of 7-valent conjugate vaccine and the future 10 and 13 valiant conjugate vaccines on children <5 years old prior to introduction of these vaccines in our country and to help the health policy makers in making sound decisions regarding the appropriateness the conjugate pneumococcal vaccine in Jordan.

From May 2007 to August 2007, 500 nasopharyngeal swabs were collected from children younger than 5 years in North Jordan. The 42.2% of children were carrier to *S. pneumoniae*, 5% of children were carrier to species other than *S. pneumoniae*. While 52.8% were not colonized.

The majority of *S. pneumoniae* carrier (27.2%) were children equal to 2 years ($p = 0.041$). While 10.6% of carrier were children >2 years. Carriage is higher in children younger than 2 years; because the immune system in this age group is immature, it can identify protein ag's but not polysaccharide ag's on

Table 3: Analysis of the relation between *S. pneumoniae* carriage and age

Age	*S. pneumoniae* carriage
<2 years	
Count	52.0
Total (%)	10.4
≤2 years	
Count	137.0
Total (%)	27.4
Total	
Count	189.0
Total (%)	37.8

Table 4: Uni-variant analysis for pneumococcal carriage with risk factors

				95.0% CI	
Risk factor	Carriage (%)	p-values	Odd ratio	Lower	Upper
2 years	27.20	0.041	1.444	0.973	2.143
Nursery	4.00	0.197	1.375	0.741	2.551
Fever	12.60	0.016	0.652	0.448	0.950
Breathing difficulty	0.52	0.234	1.270	0.738	2.187
Nasal discharge	5.60	0.336	1.157	0.689	1.943
Cough	5.40	0.469	0.946	0.567	1.578
Vomiting	5.80	0.070	0.680	0.421	1.099
Rash	4.20	0.428	1.100	0.614	1.971
Otalgia	0.60	0.406	1.670	0.334	8.361
Eye discharge	0.60	0.683	0.829	0.075	9.204
Diarrhea	9.80	0.435	1.058	0.699	1.601
Wheezing	4.00	0.322	1.207	0.660	2.211
Sore throat	3.80	0.001	0.427	0.247	0.739

Table 5:Multi-variant analysis for pneumococcal carriage

			95.0% CI	
Risk factors	p-values	Odd ratio	Lower	Upper
Sex	0.853	1.037	0.708	1.517
2 years	0.103	1.426	0.931	2.183
Nursery	0.186	1.545	0.811	2.942
Fever	0.121	0.720	0.475	1.090
Breathing difficulty	0.285	1.469	0.726	2.971
Nasal discharge	0.343	1.349	0.727	2.505
Cough	0.570	0.838	0.454	1.544
Vomiting	0.112	0.618	0.342	1.118
Rash	0.893	1.044	0.558	1.953
Otalgia	0.433	2.012	0.350	11.557
Eye discharge	0.586	0.501	0.042	6.037
Diarrhea	0.287	1.335	0.784	2.273
Wheezing	0.968	0.985	0.464	2.092
Sore throat	0.014	0.480	0.268	0.860

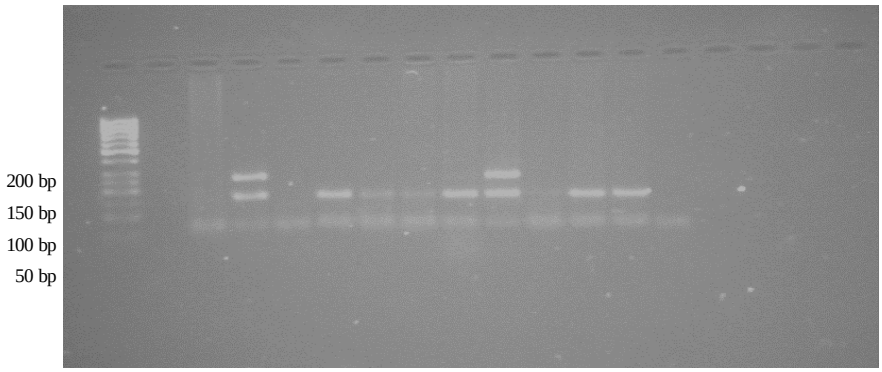

Fig. 2: Representative reaction No. 1 sequential multiplex PCR results strains isolated results are shown above their respective lane; Ladder (50 bp); 6A/B; +ve cpsA; -ve cpsA

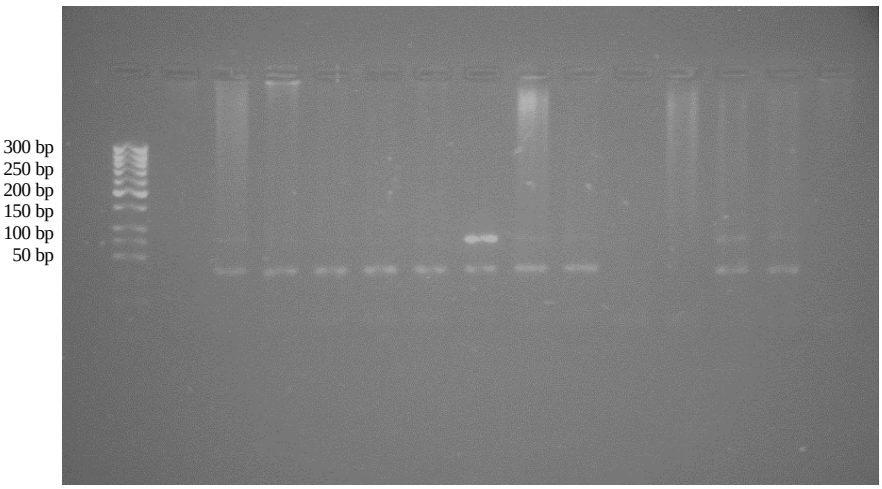

Fig. 3: Representative reaction No. 1 sequential multiplex PCR results strains isolated results are shown above their respective lane; Ladder (50 bp); 6A/B

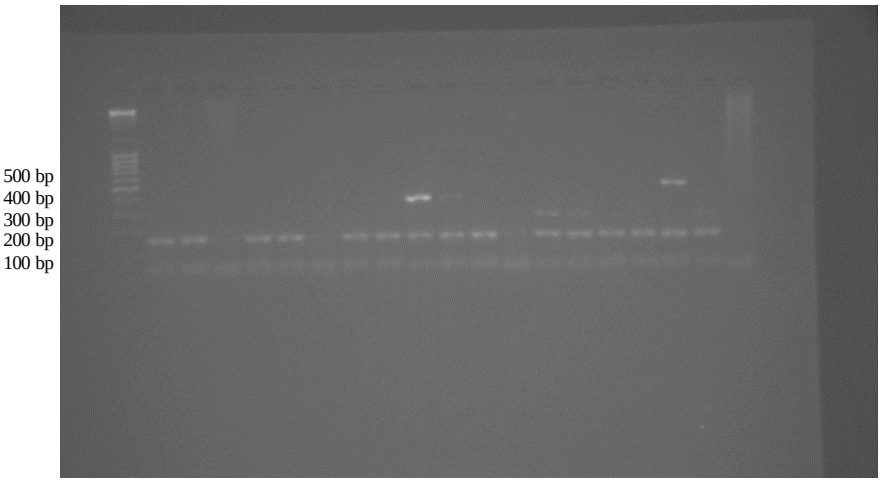

Fig. 4: Representative reaction No. 1 sequential multiplex PCR results strains isolated results are shown above their respective lane; Ladder (100 bp); ST3; 6A/B 19A

Table 6: Optochin susceptibility results and serotyping results deduced by sequential multiplex PCR for 189 *S. Pneumoniae* isolated from children <5 years ago

Serotypes results from multiplex PCR	Optochin susceptibility results	Actual conventional serotyping results	Total (%) (n = 500)	p-values
19F	Sensitive	13	2.6	0.375
	Indeterminate	-	-	-
	Resistant	2	0.4	0.149
	Total	15	3.0	
35B	Sensitive	5	1.0	0.145
	Indeterminate	1	0.2	0.450
	Resistant	1	0.2	0.605
	Total	7	1.4	
23F	Sensitive	27	5.4	0.212
	Indeterminate	3	0.6	0.145
	Resistant	5	1.0	0.332
	Total	35	7.0	
7F	Sensitive	3	0.6	0.099
	Indeterminate	-	-	-
	Resistant	-	-	-
	Total	3	0.6	
11A	Sensitive	13	2.6	0.203
	Indeterminate	1	0.2	0.550
	Resistant	2	0.4	0.640
	Total	16	3.2	
ST4	Sensitive	8	1.6	0.288
	Indeterminate	1	0.2	0.450
	Resistant	-	-	-
	Total	9	1.8	
ST14	Sensitive	27	5.4	0.325
	Indeterminate	1	0.2	0.450
	Sensitive	2	0.4	0.360
	Total	30	6.0	
12F	Sensitive	8	1.6	0.188
	Indeterminate	-	-	-
	Resistant	-	-	-
	Total	8	1.6	
9V	Sensitive	17	3.4	0.094
	Indeterminate	4	0.8	0.625
	Resistant	1	0.2	0.605
	Total	22	4.4	
19A	Sensitive	4	0.8	0.637
	Indeterminate	-	-	-
	Resistant	-	-	-
	Total	4	0.8	
6A/B	Sensitive	59	11.8	0.261
	Indeterminate	6	1.2	0.038
	Resistant	1	0.2	0.605
	Total	66	13.2	
ST3	Sensitive	8	1.6	0.563
	Indeterminate	1	0.2	0.550
	Resistant	-	-	-
	Total	9	1.8	

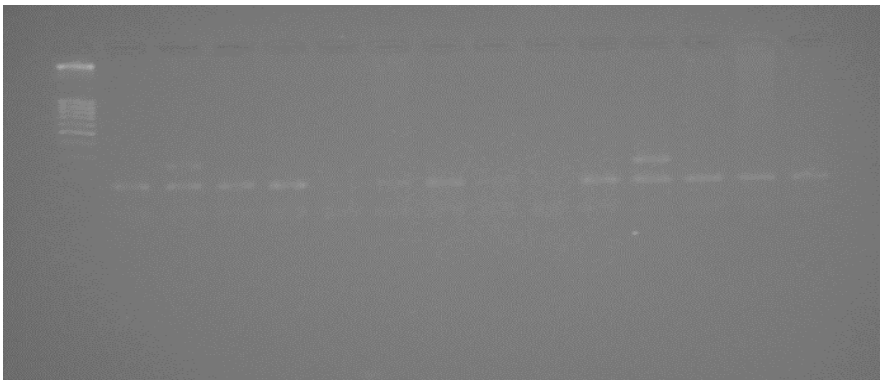

Fig. 5: Representative reaction No. 1 sequential multiplex PCR results strains isolated results are shown above their respective lane; Ladder (100 bp); +ve cpsA; 6A/B

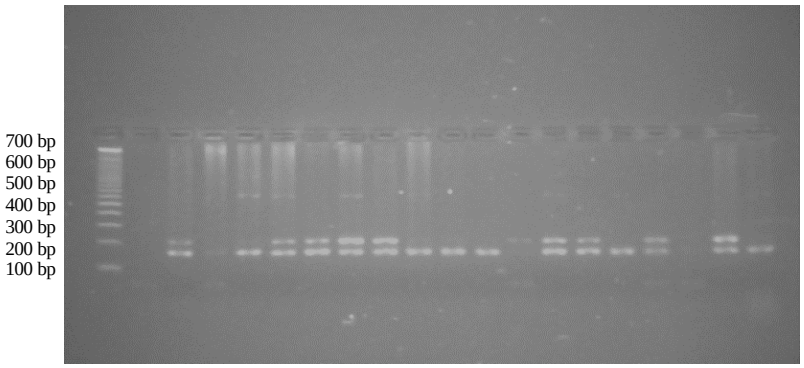

Fig. 6: Representative reaction No. 2 sequential multiplex PCR results strains isolated results are shown above their respective lane; Ladder (100 bp); 9V; +ve cpsA; ST14; -ve cpsA

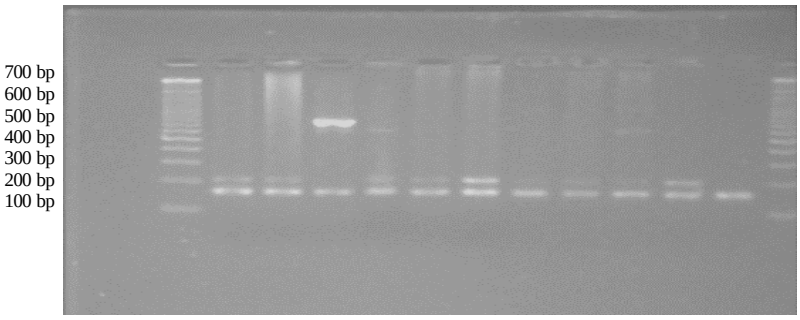

Fig. 7: Representative reaction No. 2 sequential multiplex PCR results strains isolated results are shown above their respective lane; Ladder 100 bp; ST14; 9V; +vecpsA

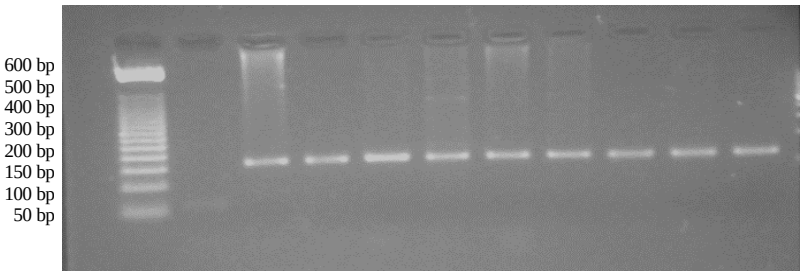

Fig. 8: Representative reaction No. 2 sequential multiplex PCR results strains isolated results are shown above their respective lane; Ladder 50 bp; +ve cps A; 9V

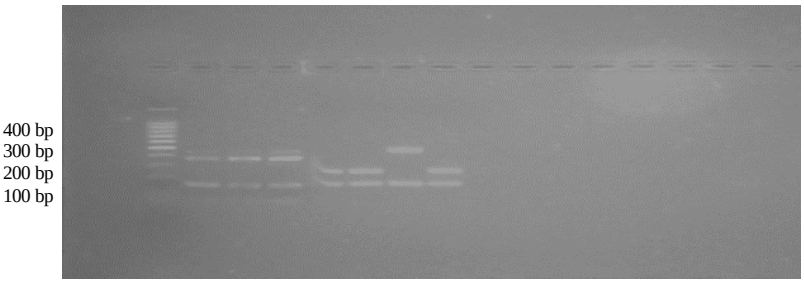

Fig. 9: Representative reaction No. 3 sequential multiplex PCR results strains isolated results are shown above their respective lane; Ladder 100 bp; 23F; 11A; 7F

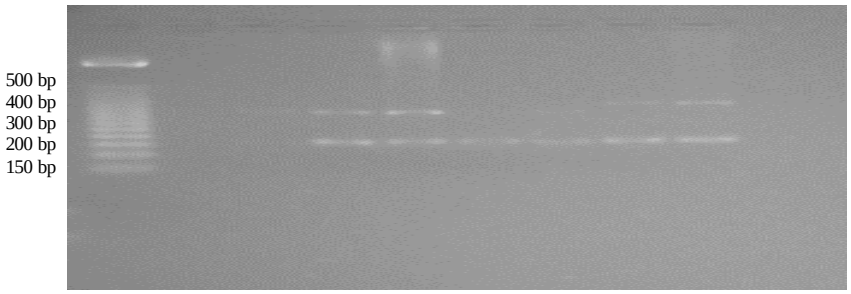

Fig. 10: Representative reaction No. 3 sequential multiplex PCR results strains isolated results are shown above their respective lane; Ladder 50 bp; 23F (384 bp); 11A (463 bp)

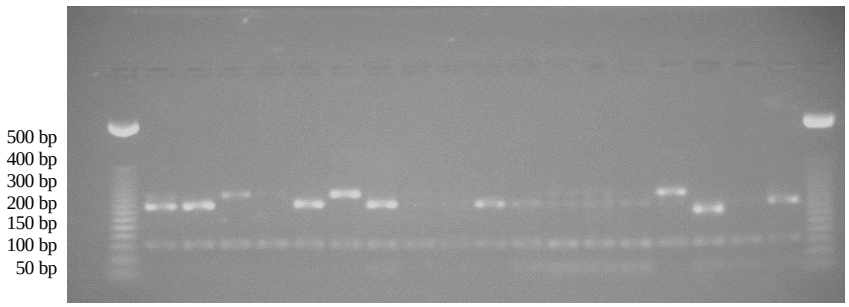

Fig. 11: Representative reaction No. 3 sequential multiplex PCR results strains isolated results are shown above their respective lane; Ladder 50 bp (463bp); 11A (384bp); 23F (338 bp); 7F

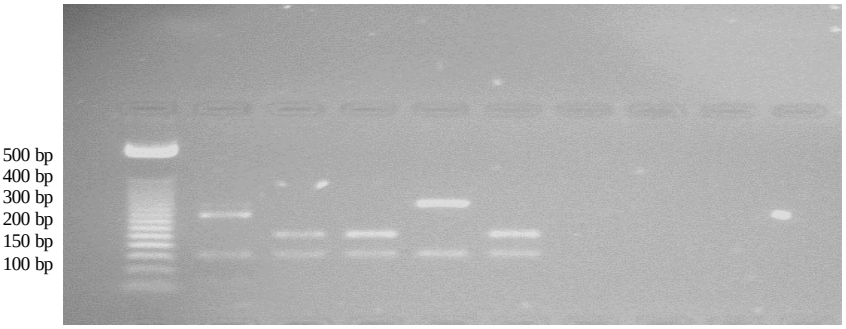

Fig. 12: Representative reaction No. 3 sequential multiplex PCR results strains isolated results are shown above their respective lane; Ladder 50 bp; 23F (384 bp); 7F (338 bp); 11A (463 bp)

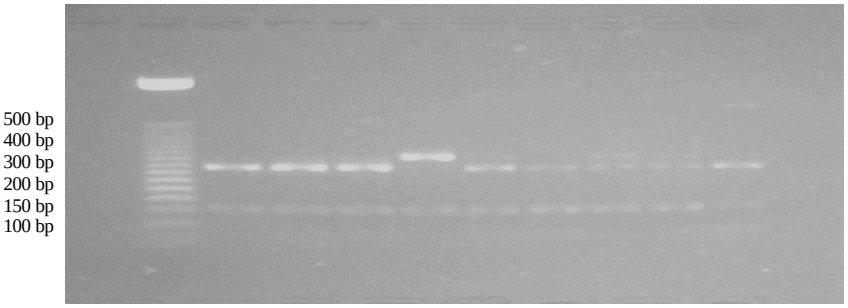

Fig. 13: Representative reaction No. 3 sequential multiplex PCR results strains isolated results are shown above their respective lane; Ladder 50 bp; 23F; 11A

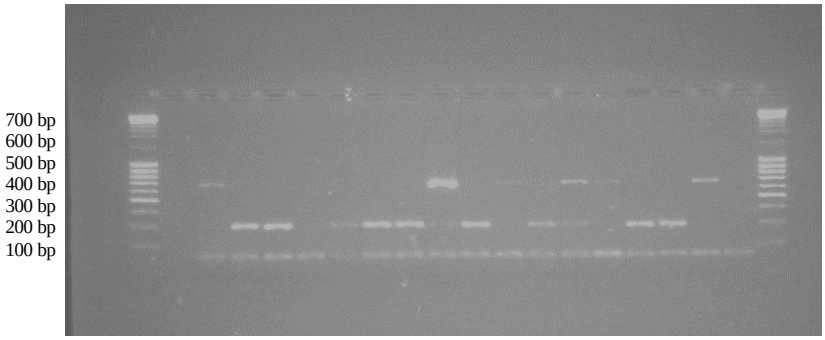

Fig. 14: Representative reaction No. 4 sequential multiplex PCR results strains isolated results are shown above their respective lane; Ladder 100 bp; 19F (304 bp); 35F (677 bp)

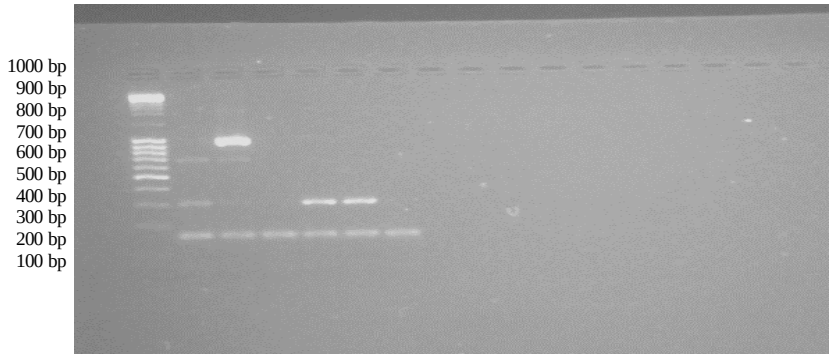

Fig. 15: Representative reaction No. 4 sequential multiplex PCR results strains isolated results are shown above their respective lane; Ladder; 16F 100 bp; 19F and 35B

S. pneumoniae capsule. Accordingly, this pathogen can easily live and infect this age group. As the children grow up, the immune system will develop and become able to identify polysaccharide ag's. Therefore, the percent of *S. pneumoniae* carriage will decline (Brito *et al*., 2003).

Our study indicates that cpsA can be targeted to efficiently and reliably determine pneumococcal serotypes. The primers cpsA-f and cpsA-r were included in all reactions as an internal control targeting the cpsA gene, a highly conserved gene present in all pneumococci. However, 10.4% of *S. pneumoniae* isolates yielded no product, even with these primers. The lack of any amplification may result from absence of cps locus or cps sequence alterations.

From our data the most prevalent *S. pneumoniae* serotypes in North Jordan are 19F, 35B, 23F, 7F, 11A, ST4, ST14, 12F, 9V, 19A, 6A/B, ST3 which are included in 7-valent conjugate vaccine, 10-valent conjugate vaccine and13-valent conjugate vaccine. Except 11A, 12F and 35B which are not included in the pneumococcal vaccines.

This serotype distribution is similar to the distribution reported by TwumDanaso in Riyadh, Saudi Arabia for the period 2000-2001 (Brito *et al*., 2003). In this study, three major hospitals, representing the three major provinces of the kingdom, participated in providing 116 *S. pneumoniae* isolates from inpatients and outpatients with *S. pneumoniae* related illnesses. These hospitals were in Jeddah, Western province; Riyadh, Central province and Damman, Eastern province. Identification was confirmed using colonial morphology, susceptibility to optochin and bile solubility. Capsular typing was performed by using a modified latex agglutination method. To determine most serotypes included in the 23-valent pneumococcal vaccine. The results were Twenty-four different serotypes were identified in the 116 isolates tested. The most prevalent in descending order were 4, 3, 19F, 9V, 6A and 19A. Eleven strains were untypable (Brito *et al*., 2003).

Also, there is a similarity in the prevalence of *S. pneumoniae* serotypes in Jordan and Palastine. In this study 437 blood or cerebrospinal fluid from patients <13 years of age from whom *S. pneumoniae* was isolated was sent to the Central Streptococcal Reference Laboratory of the Ministry of Health (CSRL-MOH), located in Jerusalem between 1 January 1998 and 31 December 1999. *S. pneumoniae* isolates were

grown on Trypticase soy agar plates with 5% sheep blood and incubated at 35°C in a 5% CO_2. Isolates were identified as *S. pneumoniae* by their colonial morphology, inhibition by optochin and bile solubility (Mokaddas *et al*., 2008).

Serotyping was performed on the bases of capsular swelling (Quellung reaction) with antisera provided by Statens Serum institut, Copenhagen, Denmark. All isolates were initially analyzed by RAPD analysis and clones were determined. All unique strains and representative isolates of each clone were further analyzed by PFGE. Primer 213 (5'-3' sequence, CAGCGAACTA) was used for RAPD analysis. Each reaction mixture (25 μL) contained 15 ng of genomic DNA and the PCR was performed. For PFGE analysis genomic DNA was prepared *in situ* in agarose blocks and was digested with SmaI. PFGE was carried out with a contour-clamped homogeneous electric field apparatus (CHEF DR-III; Bio-Rad). The following parameters were used for electrophoresis: run time at 5.3 V/cm, 23 h; initial pulse time, 5 sec and final time, 35 sec. The most common prevalent *S. pneumoniae*were 9V, 14, 23F, 19F, 19A and 6B which are similar to serotypes in our country (Mokaddas *et al*., 2008).

Similar results were reported in Kuwait. In this study, a total of 404 *S. pneumoniae* isolates from various infections, such as pneumonia, otitis media, sinusitis, conjunctivitis, meningitis and septicemia from patients attending treatment centers during a period of 2 years, between January 2004 and December 2005 were available for the study. The isolates from blood and Cerebro Spinal Fluid (CSF) were considered invasive and isolates from upper respiratory tract specimens (antral wash and middle ear fluid), lower respiratory tract specimens (sputum and endotracheal aspirates) and eye were considered noninvasive. There were a large number of isolates from conjunctivitis cases referred to the laboratories. All isolates were sent in skim milk transport medium to the *S. pneumoniae* Reference Laboratory, Microbiology Department, Faculty of Medicine, Kuwait University, Kuwait for study. The demographic data for all patients, such as age, sex and whether inpatient or outpatient and the sources of the isolates were carefully recorded. In the reference laboratory, the isolates were confirmed as *S. pneumoniae* by positive tests for alpha-hemolysis on blood agar, optochin susceptibility and bile solubility. Serogrouping and serotyping of the isolates were done by the Quellung reaction with a complete set of specific rabbit pneumococcal antisera in the Danish checkerboard typing system (Statens Serum Institute, Copenhagen, Denmark). The male-to-female ratio was 1.5:1 and the majority of the patients were children <5 years old (30%) and elderly persons equal to 65 years old (28%). The distributions of the predominant serotypes among age were similar and the serotypes were 23F, 19F, 6A, 6B, 14 and 19A. For all these serotypes, the numbers of invasive isolates were smaller than those of noninvasive isolates. For the total number of patients, the most common serotypes among the invasive isolates in descending order were 14, 23F, 19A and 9V whereas those among the noninvasive isolates were 23F, 19F, 6A, 6B, 19A, 14, 9A, 9V and 11A. Among the penicillin-resistant strains, the most common serotypes were 23F, 19F, 6B, 14 and 9A (Mokaddas *et al*., 2003).

The predominate serotypes in Kuwait are essentially similar to serotypes in Jordan. The most common prevalent serotypes in Kuwait are 23F, 19F, 6A, 19A and 14. In Kuwait the 7-valent conjugate vaccine was introduced recently. However, the 23-valent polysaccharide vaccine is currently available in Kuwait for high-risk groups of patients only (Mokaddas *et al*., 2003).

In another study, 685 clinical *S. pneumoniae* isolates were prospectively collected from patients with community-acquired pneumococcal diseases a 14 study centers in 11 countries in Asia and the Middle East from January 2000 to June 2001 (Song *et al*., 2004).

With the exception of lower respiratory tract specimens, all isolates were recovered from clinical specimens representative of normally sterile body sites such as blood, cerebrospinal fluid, ascitic fluid, pleural fluid, synovial fluid, sinus aspirates and middle ear aspirates. Pneumococcal isolates from lower respiratory tract specimens were included only if *S. pneumoniae* was cultured from adequate respiratory specimens from patients with clinical and radiographic findings of pneumonia. Pneumococcal isolates from throat swab, nasal swab, or nasopharyngeal aspirate specimens were excluded from the study. Nosocomial infections caused by *S. pneumoniae* were not included in the study (Song *et al*., 2004).

Isolates from study centers were transported to a central laboratory (Samsung Medical Center, Seoul, Korea) in a transport tube containing 12 mL of semisolid Ames transport medium with charcoal (Becton Dickinson, Sparks, Md.). Following storage at -70°C, the isolates were thawed and subcultured onto blood agar twice before susceptibility testing was performed (Song *et al*., 2004).

CONCLUSION

All isolates were serotyped by the capsular Quellung method with commercial antisera (Statens Serum institut, Copenhagen, Denmark) as recommended by the

manufacturer. Serotyping was performed only for serogroups 23, 19 and 6. The most prevalent serogroups among the clinical pathogens from Asia were 19, 23, 6, 14, and 9 which accounted for 65.6% of all isolates. Of the 271 isolates from children younger than age 5 years, serotypes 19F (21.8%), 23F (14.7%), 14 (10%), 6B (6.6%), 9 (4.5%), 6A (3.2%) and 19A (3%) were the most common ones (Song *et al*., 2004).

REFERENCES

Artz, A.S., W.B. Ershler and D.L. Longo, 2003. Pneumococcal vaccination and revaccination of older adults. Clin. Microbiol. Rev., 16: 308-318.

Black, S., H. Shinefield, B. Fireman, E. Lewis and P. Ray *et al*., 2000. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Pediatr. Infect. Dis. J., 19: 187-195.

Bogaert, D., M.N. Engelen, R.A.J. Timmers, K.P. Elzenaar and P.G. Peerbooms *et al*., 2001. Pneumococcal carriage in children in the Netherlands: A molecular epidemiological study. J. Clin. Microbiol., 39: 3316-3320.

Briles, D.E., R.C. Tart, E. Swiatlo, J.P. Dillard and P. Smith *et al*., 1998. Pneumococcal diversity: Considerations for new vaccine strategies with emphasis on pneumococcal surface protein A (PspA). Clin. Microbiol. Rev., 11: 645-657.

Brito, D.A., M. Ramirez and D.H. Lencastre, 2003. Serotyping streptococcus pneumoniae by multiplex PCR. J. Clin. Microbiol., 41: 2378-2384.

Dejsirilert, S., K. Overweg, M. Sluijter, L. Saengsuk and M. Gratten *et al*., 1999. Nasopharyngeal carriage of penicillin-resistant streptococcus pneumoniae among children with acute respiratory Tract infections in Thailand: A molecular epidemiological survey. J. Clin. Microbiol., 37: 1832-1838.

Eskola, J., T. Kilpi, A. Palmu, J. Jokinen and M. Eerola *et al*., 2001. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. N. Engl. J. Med., 344: 403-409.

Jakobsen, H. and I. Jonsdottir, 2003. Mucosal vaccination against encapsulated respiratory bacteria-new potentials for conjugate vaccines?. Scand. J. Immunol., 58: 119-128.

Jedrzejas, M.J., 2001. Pneumococcal virulence factors: Structure and function. Microbiol. Mol. Biol. Rev., 65: 187-207.

Lankinen, K.S., M. Leinonen, T.E. Tupasi, R. Haikala and P. Ruutu, 1994. Pneumococci in nasopharyngeal samples from filipino children with acute respiratory infections. J. Clin. Microbiol., 32: 2948-2952.

Lawrence, E.R., D.B. Griffiths, S.A. Martin, R.C. George and L.M. Hall, 2003. Evaluation of semiautomated multiplex PCR assay for determination of streptococcus pneumoniae serotypes and serogroups. J. Clin. Microbiol., 41: 601-607.

Lehmann, D., 1992. Epidemiology of acute respiratory tract infections, especially those due to Haemophilus influenzae, in Papua new guinean children. J. Infect. Dis., 165: S20-S25.

Meats, E., A.B. Brueggemann, M.C. Enright, K. Sleeman and D.T. Griffiths *et al*., 2003. Stability of serotypes during nasopharyngeal carriage of streptococcus pneumoniae. J. Clin. Microbiol., 41: 386-392.

Mokaddas, E.M., V.O. Rotimi and M.J. Albert, 2003. Implications of streptococcus pneumoniae clones in Israel. J. Clin. Microbiol., 41: 5541-5545.

Mokaddas, E.M., V.O. Rotimi and M.J. Albert, 2008. Implications of streptococcus pneumoniae penicillin resistance and serotype distribution in Kuwait for disease treatment and prevention. Clin. Vaccine Immunol., 15: 203-207.

Obrien, K.L. and H. Nohynek, 2003. Report from a WHO working group: Standard method for detecting upper respiratory carriage of streptococcus pneumoniae. Pediatr. Infect. Dis. J., 22: E1-E11.

Prymula, R., J. Motlova and P. Kriz, 2004. Comparison of streptococcus pneumoniae serotypes causing acute otitis media and invasive disease in young children in the Czech Republic. Indian J. Med. Res., 119: 168-170.

Song, J.H., S.I. Jung, K.S. Ko, N.Y. Kim and J.S. Son *et al*., 2004. High prevalence of antimicrobial resistance among clinical streptococcus pneumoniae isolates in Asia (an ANSORP study). Antimicrob. Agents Chemother., 48: 2101-2107.

Ubukata, K., N. Chiba, K. Hasegawa, R. Kobayashi and S. Iwata *et al*., 2004. Antibiotic susceptibility in relation to penicillin-binding protein genes and serotype distribution of streptococcus pneumoniae strains responsible for meningitis in Japan, 1999 to 2002. Antimicrob. Agents Chemother., 48: 1488-1494.

Zhao, G.M., S. Black, H. Shinefield, C.Q. Wang and Y.H. Zhang *et al*., 2003. Serotype distribution and antimicrobial resistance patterns in streptococcus pneumoniae isolates from hospitalized pediatric patients with respiratory infections in Shanghai, China. Pediatr. Infect. Dis. J., 22: 739-742.