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The Impact of the 7-Valent Pneumococcal Conjugate Vaccine on Nasopharyngeal Carriage of *Streptococcus pneumoniae* in Infants of Ajlun Governorate in Jordan

Adnan Al-Lahham[1,*], Jumana Abu Qayyas[1] and Mark van der Linden[2]

[1]*School of Applied Medical Sciences, German Jordanian University, P.O. Box 35247, 11180 Amman, Jordan;*

[2]*Institute of Medical Microbiology, National Reference Center for Streptococci, University Hospital of Aachen, Germany*

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Abstract

Streptococcus pneumoniae colonization is a serious problem since dissemination to other organs in the body can cause serious infections. This study investigates the impact of the pneumococcal conjugate vaccine (PCV7) on pneumococcal carriage and resistance in healthy infants in the rural areas of Ajlun, Jordan. 415 infants younger than two years of age were selected for this study. The vaccination was given at ages 2, 4 and 10 months between April 2009 and July 2010 (Scheme 2+1). Nasopharyngeal (NP) swabs were taken at the first vaccination (phase one), the third vaccination (phase two), and also three months after the last vaccination (phase three). Pneumococcal isolates were identified according to morphology, bile solubility, and optochin sensitivity. Antimicrobial susceptibility was tested via the micro-broth dilution method and serotyping by the (Neufeld) Quellung reaction. At the first vaccination, carriage in two-month old infants was 26.3%. At the third vaccination, carriage in ten-month–old infants was 29.9%, and at three months after their last vaccinations it was 29.4%. Twenty cases were found positive for all the three nasopharyngeal swabs (5.1%). At the end of the study 241/415 cases (58.1%) were carriers. Resistance rates were as follows: (intermediate and resistant): penicillin (84.0%), cefotaxime (5.6%), clarithromycin (47.2%), clindamycin (25.6%), trimethoprim-sulfamethoxazole (59%), and tetracycline (39%). Multidrug resistance rate was 39.5% for phase one, 48.4% for phase two, and 46.7% for phase three. Frequent serotypes at the time of the first vaccination were: 6A (14.7%), 19F (12.8%), 6B, 23F, and 15B at 6.4% each, and 11A (5.5%). Frequent serotypes after the last vaccination were: 11A (12.3%), 19A (10.7%), 6A (9.0%), and 19F (8.2%). The prevalence of vaccine serotypes (4, 6B, 9V, 14, 19F and 23F) at phase one (0.9%, 6.4%, 1.8%, 3.7%, 12.8%, 6.4%, respectively) was reduced at phase three (0.0%, 2.5%, 0.0%, 3.3%, 8.2%, 0.8%, respectively). Serotype 18C was not identified. An increase of 19A in phase one from 2.8% to 10.7% at phase three was observed. The impact of vaccination was an observed reduction in the resistance to penicillin, cefotaxime, clarithromycin, and clindamycin by 5.9%, 2.4%, 11.4%, and 18.3%, respectively. Coverage of the PCV7 and PCV13 three months after receiving the third injection was 27.8% and 49.4% respectively. High carriage and resistance rates were observed among the infants. Vaccine administration reduced pneumococcal carriage and antimicrobial resistance among the infants.

Keywords: *Streptococcus pneumoniae*, Carriage, Resistance, Pneumococcal Conjugate Vaccines.

1. Introduction

Streptococcus pneumoniae is an infectious agent causing meningitis, pneumonia, and bacteraemia especially for young children, mainly in low-income countries where pneumococcal conjugate vaccines (PCVs) are still underused. Understanding the epidemiology of carriage for *Streptococcus pneumoniae* (*S. pneumoniae*) and other common respiratory bacteria in developing countries is crucial for implementing appropriate vaccination strategies and evaluating their impact (Adegbola *et al*., 2014). Nasopharyngeal colonization with *S. pneumoniae* in infants is generally acquired at approximately 4-6 months of age (Al-Lahham and Van der Linden 2014; Bogaert *et al*., 2004), and is considered a prerequisite for a disease. Unlike children, carriage in the elderly is rarely detected (Krone *et al*., 2015). Pneumococcal disease will not occur without preceding nasopharyngeal colonization with the homologous strain. Therefore, pneumococcal carriage is believed to be an

* Corresponding author. e-mail: adnan.lahham@gju.edu.jo.

important source of a horizontal spread of this pathogen within the community (Bogaert *et al*., 2004). Increased prevalence of *S. pneumoniae* in the nasopharynx of healthy paediatrics reflects a potential risk to the development of more frequent respiratory infections in the community (Saha *et al*., 2003; Volonakis *et al*., 2006). *S. pneumoniae* was given the name of the forgotten killer in children by the WHO (Wardlow, 2006). As stated by the WHO in 2007 at least 1.2 million children die of pneumococcal infections each year, with 70% of them being from Africa and southeast Asia; mostly from developing countries (Williams, 2002). It accounts for more than one third of acute bacterial sinusitis and more than one half of community-acquired bacterial pneumonia (File, 2006). Resistance of *S. pneumoniae* to antimicrobials makes the treatment more difficult. On the other hand, the emergence of penicillin- and cephalosporin-resistant strains has created an urgent need for pneumococcal vaccines that are effective in treating infants (Jacobs and Dagan, 2004; Pallares *et al*., 2003). Pneumococcal vaccines containing capsular polysaccharides of five (6B, 14, 19F, 18C, 23F) and seven (4, 6B, 9V, 14, 19F, 18C, 23F) serotypes have been proven safe and immunogenic in children (Wardlow, 2006).

Pneumococcal infections are particularly common in younger children and in older adults. They can be divided broadly into invasive and non-invasive diseases (Ludwig, 2013; Ludwig *et al*., 2012). Pneumonia is one of the most common clinical presentations of pneumococcal infections, and may itself be invasive or non-invasive (Amodio *et al*., 2014; Said *et al*., 2013).

Although there are differences in the prevalence and rank order of the serotypes obtained from NP specimens and from those with invasive diseases, the pneumococcal nasopharyngeal isolates may reflect the strains circulating in the community. They can be used as a marker to predict the serotype prevalence of invasive diseases and resistance patterns. Monitoring serotype distribution is essential for the appropriate application of the vaccines. Vaccine use in infants has proved highly efficacious in the prevention of the Invasive Pneumococcal Disease as well as in decreasing the carriage of vaccine serotypes in the nasopharynx of infants, which significantly affects, in the long run, the occurrence of otitis media and helps decrease infection rates among contacts of the vaccinated infants. Information concerning the pneumococcal strains found in Jordanian children and the NP carriage in infants is limited, and does not include children living in rural areas. A population-based study was undertaken to determine the impact of the vaccination of healthy infants with the PCV7 in Ajlun, the first district in Jordan where vaccine (PCV7) was used. The aims of this study were to determine the frequency of *S. pneumoniae* NP carriage and serotypes circulating among infants aged 2-18 months in the rural areas of Ajlun, to identify the antimicrobial susceptibility of these isolates, and to study the impact of the pneumococcal conjugate vaccine on carriage and resistance.

2. Material and Methods

2.1. Study Design

A 15-month population based surveillance study of pneumococcal NP carriage and the antimicrobial resistance of *S. pneumoniae* in healthy Jordanian infants was conducted in Ajlun on babies born between March and April of 2009. The first NP-swab was taken by a medical doctor at the time of the first vaccination at 2-3 months of age (phase one). The second NP-swab was taken at the time of the second vaccination at ages 4-5 months (phase two). The third vaccination was at age of 10-11 months (phase three). The third NP-swab was taken three months after the last vaccination, i.e. at age of 13-14 months. Infants were included in the study after obtaining a signed parental consent. Vaccination was free of charge and took place at the 12 main Day Care Centers (DCCs) in the governorate of Ajlun. PCV7 and PCV10 are pneumococcal conjugate vaccines available for use only in the private sector. PCV7 was the only administrated pneumococcal vaccine for this study. Doses were donated by Wyeth Pharmaceutical Company (now called Pfizer) to the Ministry of Health in Jordon in 2008. All the 415 infants, included in the study, received the three vaccine doses. Special questionnaires and forms were completed for each sample. Swabs were analysed at a reference laboratory of the German Jordanian University and at the National Reference Center for Streptococci in Aachen, Germany. The protocol was approved by the Ministry of Health (MOH) and the ethical committee of the MOH, before the study was undertaken.

2.2. Culture and Identification

The NP samples were inoculated on Columbia Agar plates with 5% sheep blood. The plates were incubated overnight at 35°C in 5% CO_2. Identification was performed by conventional microbiological methods; colony morphology, susceptibility to optochin (bioMérieux), and bile solubility.

2.3. Susceptibility Testing

Minimal inhibitory concentration (MIC) testing was performed using the micro broth dilution method as recommended by the Clinical Laboratory Standards Institute (CLSI) (CLSI, 2017). Antimicrobial agents used were penicillin G, amoxicillin, cefotaxime, cefuroxime, cefpodoxime, clarithromycin, clindamycin, tetracycline, levofloxacin, moxifloxacin, telithromycin, trimethoprim/ sulfamethoxazole, chloramphenicol, and vancomycin. *S. pneumoniae* ATCC 49619 was used as a control strain. Multidrug resistance phenotype was recorded when an isolate had resistance to three or more classes of antimicrobial agents.

2.4. Analysis of Resistance Determinants (genotyping and phenotyping)

PCR of macrolide resistance determinants was performed as described previously by (Reinert *et al*., 2004; Reinert *et al*., 2005; Reinert *et al*., 2003). For the classical detection of *erm*(B) and *mef* (A) the following primers were used: *erm*(B) 5´-CGAGTGAAAA AGTACTCAACC-3´ (362-382) and 5´-GGCGTGTTT CATTGCTTGATG-3´ (978-958), *mef* (A) 5´-AGTAT CATTAATCACTAGTGC-3´ (57-77) and 5´-TAATAG ATGCAATCACAGC-3` (550-532). Results were confirmed by a Light Cycler protocol with the following primers: *erm*(B) 5´-TTTTGAAAGCCATGCGTCTGA-3´, and 5´-ATCTGTGGTATG GCGGGTAAGTT-3´, and *mef*(A) 5´-TATGGGCAGGGCAAGCAGTATC-3´ and 5´-TCRGCACCAATCATTATCTTCTTC-3´ (Farrell *et*

al., 2001). The macrolide resistance phenotype was determined on the basis of the pattern of susceptibility to MLS_B (macrolide-lincosamide-streptogramin B) (Montanari *et al*., 2001).

2.5. Serotyping

Capsular typing of the pneumococcal isolates was performed by the Neufeld's Quellung reaction using type and factor sera provided by the Statens Serum Institute, Copenhagen, Denmark.

2.6. Primary and Secondary Endpoints

Endpoints included the frequency of NP carriage, serotype distribution, and antimicrobial resistance patterns of the strains in infants younger than two years old in rural areas. They were attending DCCs for normal check-ups and for vaccination through the National Immunization Program. Assessment of the reduction in vaccine-type pneumococcal carriage as a result of vaccination was determined in order to model a routine schedule of vaccination with PCV7.

3. Results

Of the 415 infants included in the study, the overall carriage rate at the end of the study was 58.1% (i.e., 241 infants were carriers at any phase of the study). 171 male infants had a carriage rate of 55.3%, and 244 females had a carriage rate of 62.0%. A total of twelve DCCs were included in the study, each having 5-110 infant participants (Table 1). At phase one, 26.3% of the infants were tested positive, at phase two, 29.6% were found positive, and at phase three 29.4% of the cases were pneumococci positive. Among the 12 centers, the highest carriage rate (70%) was recorded in Halawa at phase one, which was reduced at phase two to 50%, and then to 40% at phase three. Rajeb center had the highest rate of carriage (80%) at phase three. The resistance ranges in the three phases were: penicillin G (82%-82.5%); cefotaxime (4.9%-7.3%); clarithromycin (40.4%-55.7%); clindamycin (18.0%-36.3%); tetracycline (33.6%-46.8%); and trimethoprim-sulfamethoxazole (56%-62.3%). No resistance was shown for vancomycin, levofloxacin, moxifloxacin, and telithromycin (Table 2). The most commonly detected serotypes in phase one were 6A (14.7%), 19F (12.8%), 6B (6.4%), 15B (6.4%), 23F (6.4%), 11A (5.5%) and 24F (5.5%); at phase two: 19F (18.6%), 6A (12.9%), 19A (8.1%), 6B (8.1%), 11A (5.7%) and 35B (4.8%), and at phase three: 11A (12.3%), 19A (10.7%), 6A (9.0%), 19F (8.2%), 15B (6.6%), 15A (4.9%), 23A (4.9%) and 35B (4.9%) (Table 3). Serotypes 1, 5 and 18C were not recovered during the study. Vaccine coverage (PCV7, PCV10 and PCV13) for the three phases was as follows: phase one (32.1%, 32.1%, 50.5%), phase two (33.9%, 33.9%, 48.4%) and phase three (14.8%, 15.6%, 36.9%). The carriage rate was 26.3% at phase one, 29.6% at phase two, and 29.4% at phase three. However, there was a reduction in the prevalence of PCV7 serotypes from 32.1% at phase one to 14.8% at phase three, and for PCV13 from 50.5% at phase one to 36.9% at phase three (Table 2). An increase of the 19A from 2.8% in phase one to 10.7% at phase 3 was noticed (Table 3). The effectiveness of the vaccination was also observed in the reduction of the isolate's resistance to penicillin, cefotaxime, clarithromycin, and clindamycin by 6%, 2.4%, 11.4%, and 17.7%, respectively (Table 2). Table 4 shows the carriage rate at each phase with vaccine and non-vaccine serotypes, and whether carriers showed positive *S. pneumoniae* more than one time during the study. On the whole, twenty infants (5.1%) were positive for the three NP swabs taken during the study period. For all DCCs, the rate of non-vaccine serotypes isolated from infants at phase one was 49.5% and 63.1% at phase three (Table 5). Macrolide resistance for all study isolates was 49%, where $cMLS_B$ phenotype was the most prevalent at 50.8%, and *erm*B was most prevalent genotype at 50% (Table 6). There were 75 infants carrying *S. pneumoniae* PCV7 serotypes at the end of the second vaccination from all the twelve DCCs, while 76% of these infants showed no PCV7 serotypes at the end of the study (Table 7). Among these 75 infants, the number of carrier infants with possible serotype shifts from 19F to 19A, and from 6B to 6A at the end of the study was seven (9.3%) (Table 7). Multidrug resistance (MDR) rates among the pneumococcal isolate were 39.5% at phase one, 48.4% at phase two, and 46.7% at phase three (Table 8).

Table 1. Distribution of participants by center in Ajlun.

Day Care Center	Number of recruited infants
Ajlun City	35
Alhashmia	08
Anjara	97
Arjan	22
Baaoon	09
Ebben	35
Ein Janna	22
Eshtafena	11
Halawa	10
Kafranjeh	110
Rajeb	05
Sakhra	51
Total	415

Table 2. Antimicrobial resistance and coverage of pneumococcal conjugate vaccines for the three phases.

	Resistance rate % (intermediate and resistant)						Vaccine serotypes (%)		
Phase	Pen	Ceta	Cla	Cli	Tet	Sxt	PCV7	PCV10	PCV13
I (n=109)	82.5	7.3	40.4	22.0	35.8	56.0	32.1	32.1	50.5
II (n=124)	87.9	4.8	55.7	36.3	46.8	58.1	33.9	33.9	55.7
III (n=122)	82.0	4.9	44.3	18.0	33.6	62.3	14.8	15.6	36.9
*Total	84.0	5.6	47.2	25.6	39.0	59.0	27.0	27.3	47.8

Abbreviations: Pen, penicillin G; Ceta, cefotaxime; Cla, clarithromycin; Cli, clindamycin; Tet, tetracycline; Sxt, trimethoprim/sulfamethoxazole. Breakpoints (I, R) according to CLSI 2017: penicillin G; 0.1–1 µg/mL, ≥2 µg/mL; cefotaxime; (non-meningitis): 2 µg/mL, ≥4 µg/mL; clarithromycin; 0.5 µg/mL, ≥1 µg/mL; clindamycin: 0.5 µg/mL, ≥1 µg/mL; trimethoprim/ sulfamethoxazole; 1/19-2/38 µg/mL, ≥4/76 µg/mL.

*Total = 356 isolates were isolated from all recruited infants (415). Each of the 415 infants in Ajlun governorate provided 3 nasopharyngeal swabs during the study. Some infants were positive in one swab others were positive 2 or 3 times (Table 4).

Table 3. Number and rate (%) of serotypes detected at each phase.

	Phase I		Phase II		Phase III	
Serotype	Nr.	%	Nr.	%	Nr.	%
6A	16	14.7	16	12.9	11	9.0
19F	14	12.8	23	18.6	10	8.2
6B	7	6.4	10	8.1	3	2.5
15B	7	6.4	5	4.0	8	6.6
23F	7	6.4	4	3.2	1	0.8
11A	6	5.5	7	5.7	15	12.3
24F	6	5.5	2	1.6	1	0.8
14	4	3.7	4	3.2	4	3.3
15A	4	3.7	3	2.4	6	4.9
23A	4	3.7	4	3.2	6	4.9
16F	4	3.7	4	3.2	4	3.3
35B	4	3.7	6	4.8	6	4.9
NT	4	3.7	2	1.6	4	3.3
15C	3	2.8	2	1.6	2	1.6
17F	3	2.8	2	1.6	2	1.6
19A	3	2.8	10	8.1	13	10.7
10A	2	1.8	1	0.8	2	1.6
33F	2	1.8	4	3.2	3	2.5
9V	2	1.8	1	0.8	0	0
3	1	0.9	1	0.8	2	1.6
21	1	0.9	1	0.8	0	0
42	1	0.9	1	0.8	0	0
7C	1	0.9	1	0.8	0	0
9N	1	0.9	2	1.6	5	4.1
4	1	0.9	0	0	0	0
28A	1	0.9	0	0	0	0
16B	0	0	1	0.8	0	0
35C	0	0	1	0.8	0	0
35F	0	0	1	0.8	1	0.8
33A	0	0	2	1.6	1	0.8
13	0	0	1	0.8	3	2.5
34	0	0	1	0.8	2	1.6
10F	0	0	1	0.8	2	1.6
7B	0	0	0	0	2	1.6
18A	0	0	0	0	1	0.8
7F	0	0	0	0	1	0.8
31	0	0	0	0	1	0.8
Total	109	100%	124	100%	122	100%

Nr., number

Table 4. Carriage rate of *S. pneumoniae* with vaccine and non-vaccine serotypes.

	Carriage rate	Vaccine serotypes, n (%)			Non-vaccine serotypes
	n (%)	PCV7	PCV10	PCV13	n (%)
Phase I (n=415)	109 (26.3)	35 (32.1)	35 (32.1)	55 (50.4)	54 (49.6)
Phase II (n=415)	124 (29.9)	42 (33.9)	42 (33.9)	60 (55.7)	64 (51.6)
Phase III (n=415)	122 (29.4)	18 (14.8)	19 (15.6)	45 (36.9)	77 (63.1)
[1]Positive only in Phase I	49 (11.8)	14 (28.6)	14 (28.6)	25 (51)	24 (49)
[2]Positive only in Phase II	42 (10.1)	14 (33.3)	14 (33.3)	22 (52.4)	20 (47.6)
[3]Positive only in Phase III	56 (13.5)	8 (14.3)	9 (16.1)	23 (41.1)	33 (58.9)
[4]Positive in Phase I & II	48 (11.6)	16 (33.3)	16 (33.3)	23 (47.9)	25 (52)
[5]Positive in Phase I & III	35 (8.4)	13 (37.1)	13 (37.1)	22 (62.9)	13 (37.1)
[6]Positive in Phase II & III	34 (8.2)	13 (38.2)	13 (38.2)	26 (76.4)	13 (38.2)
[7]Positive in the 3 Phases	20 (5.1)	10 (50)	10 (50)	17 (85)	3 (15)
[8] Total Carriers	241 (58.1)	77 (32)	78 (32.4)	133 (32)	108 (26)

[1]positive only in phase I, negative in phase II and III; [2]positive only in phase II, negative in phase I and III; [3]positive only in phase III, negative in phase I and II; [4]positive in phase I and II, negative in phase III; [5]positive in phase I and III, negative in phase II; [6]positive in phase II and III, negative in phase I; [7]positive in the 3 phases, cases carried *S. pneumoniae* in each phase; [8]at least carried *S. pneumoniae* once during the whole study period

Table 5. Vaccine and non-vaccine serotypes in each phase for centers of the Ajlun governorate.

Centers	Vaccine serotypes coverage at phase I				Vaccine serotypes coverage at phase II				Vaccine serotypes coverage at phase III			
	7v PCV (n; %)	10v PCV (n; %)	13v PCV (n; %)	Non-vaccine serotypes (n; %)	7v PCV (n; %)	10v PCV (n; %)	13v PCV (n; %)	Non-vaccine serotypes (n; %)	7v PCV (n; %)	10v PCV (n; %)	13v PCV (n; %)	Non-vaccine serotypes (n; %)
All centers	35;32.1	35;32.1	54;50.5	54;49.5	42;33.9	42;33.9	60 48.4	64; 51.6	18;14.8	19;15.6	45;36.9	77; 63.1
Ajlun center	2;33.3	2;33.3	4;66.7	2;33.3	3; 42.9	3; 42.9	4; 57.1	3; 42.9	0; 0	0; 0	2; 25	6; 75
Alhashmia	1;33.3	1;33.3	2;66.7	1;33.3	1; 33.3	1; 33.3	2; 66.7	1; 33.3	1;33.3	1; 33.3	2; 66.7	1; 33.3
Anjara	8;30.8	8;30.8	8;30.8	18;69.2	11;29.7	11;29.7	18;48.6	19; 51.4	4;17.4	4;17.4	7; 30.4	16; 69.6
Arjan	3;37.5	3;37.5	5;30.8	3;37.5	3;60	3; 60	5; 100	0; 0	2; 40	2; 40	3; 60	2; 40
Baaoon	1;25	1;25	2;50	2;50	2;66.7	2; 66.7	3; 100	0; 0	1; 25	1; 25	1; 25	3; 75
Ebben	3;30	3;30	5;50	5;50	2;20	2; 20	4; 40	6; 60	2;13.3	2; 13.3	6; 40	9; 60
Ein Janna	4;50	4;50	6;75	2;25	3;42.9	3; 42.9	5; 71.4	2; 28.6	1; 10	1; 10	3; 30	7; 70
Eshtafena	0;0	0;0	0;0	2;100	1;20	1; 20	2; 40	3; 60	1; 50	1; 50	1; 50	1; 50
Halawa	0;0	0;0	2;28.6	5;72.4	1;20	1; 20	3; 60	2; 20	0; 0	0; 0	2; 50	2; 50
Kafranjeh	10;37	10;37	17;63	10;37	9;34.6	9; 34.6	13; 50	13; 50	3;11.5	4; 15.4	9; 34.6	17; 65.4
Rajeb	1;100	1;100	1;100	0;0	0;0	0; 0	1; 50	1; 50	2; 50	2; 50	2; 50	2; 50
Sakhra	2;28.6	2;28.6	2;28.6	5;72.4	6; 42.9	6; 42.9	9; 64.3	5; 35.7	1; 5.6	1; 5.6	5;27.8	13; 72.2

Table 6. Macrolide resistant phenotypes and genotypes for all cumulative study isolates.

Macrolide Resistance phenotypes and genotypes	n (%)
M-phenotype	57 (48.3)
$cMLS_B$ phenotype	60 (50.8)
$iMLS_B$ phenotype	1 (0.9)
erm(B) genotype	59 (50.0)
mef(A) genotypes	53 (44.9)
erm(B) & *mef*(A) genotypes	2 (1.7)
Others	4 (3.4)

Table 7. Impact of vaccination with PCV7 on carriers with vaccine serotypes and the possible serotype shifts 3 months after last vaccination.

Center	Number of carriers having PCV7 serotypes	Number and rate of carriers no longer harboring PCV7 serotypes after last vaccination	Number of carriers with shifts from 19F to 19A and from 6B to 6A
Ajlun	4	4 (100%)	0
Alhashmia	3	2 (66%)	2
Anjara	21	17 (81%)	2
Arjan	5	3 (60%)	0
Baaoon	3	2 (66.7%)	0
Ebben	6	4 (66.7%)	1
Ein Janna	5	4 (80%)	1
Eshtafena	2	1 (50%)	0
Halawa	1	1 (100%)	0
Kafranjeh	17	14 (82.4%)	0
Rajeb	3	1 (33.3%)	0
Sakhra	5	4 (80%)	1
Total	75	57 (76%)	7

Table 8. Multidrug resistant (*MDR) isolates detected in carriers at the 3 phases of the study.

Phase	Number (%)
Phase I (n=109)	43 (39.5%)
Phase II (n= 124)	60 (48.4%)
Phase III (n= 122)	57 (46.7%)

*****MDR**=** isolates resistant to 3 or more classes of antimicrobial agents

4. Discussion

Nasopharynx is the usual source of pneumococci for studying the carriage rate (Malfroot *et al*., 2004). To our knowledge, this is the first study in Jordan to investigate the impact of vaccination with PCV7 on pneumococcal carriage and resistance among infants in Jordan. Two previous studies described pneumococcal carriage in Jordan; the first showed a carriage rate of 55.1% from Wadi Al Seer (Amman, Jordan), with a PCV7 and PCV13 coverage rates of 52.3% and 58.5%, respectively (Al-Lahham and Van der Linden, 2014). The second study showed a carriage rate of 33.8% from children below five years of age attending the pediatric clinic of a major hospital in northern Jordan (Swedan *et al*., 2016). This study demonstrated that the total carriage rate of *S. pneumoniae* among infants in the governorate of Ajlun was 58.1%. Lee *et al*., (2001) investigated carriage rate of pneumococci in 4963 Asian children aged below five years from eleven countries (Lee *et al*., 2001). The results of the study showed the following rates: Philippines (32.6%), China (37.5%), India (43.2%), Thailand (40.6%), Taiwan (15.3%), and Saudi Arabia (9.0%). Similar carriage rates were obtained from Brazil (55%) (Marchese and Schito, 2007), Guatemala (59.1%) (Marchisio *et al*., 2002), and in Kampala Uganda (62%) (Mera, 2005). The observed high rate of pneumococcal colonization in Ajlun can be attributed to history of the sicknesses, low age, viral infections, history of the consumption of antimicrobials before DCC attendance, and low income. 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 the number of siblings (García-Rodríguez, 2002). Previous studies reported that attendance of day care is the main factor causing the increase in the *S. pneumoniae carriage rate* (Huang and Fang, 2004). The continuous surveillance of the antimicrobial susceptibility patterns of *S. pneumoniae* has become increasingly important, because of the rapid emergence of drug-resistant strains worldwide (Goyal *et al*., 2007). Consumption of antimicrobials prior to DCC visits could have contributed to the selection of resistant strains (Montanari *et al*., 2003). Antimicrobial susceptibility testing of the *S. pneumoniae* isolates revealed alarming rates of resistance to penicillin, erythromycin, and occurrence of multidrug-resistant (MDR) isolates. Rates of resistant *S. pneumoniae* isolates in Ajlun were higher than those for clinical isolates from Singapore, Sri Lanka, and Taiwan (Lee *et al*., 2001). The high rates of resistance to different classes of antimicrobial agents in *S. pneumoniae* in this study are presumably a consequence of the unregulated consumption and misuse of antimicrobials by the Jordanian population (Al-Bakri, 2005). Otoom *et al*., (2002) reported that antimicrobial agent prescriptions in Jordan at different health centers ranged between 46.7% to 83.3%; these rates are very high compared to rates in many other parts of the world (Otoom, 2002). Local information on capsular types of *S. pneumoniae* causing diseases in young children is highly important to guide the production of effective conjugate vaccines. Our results showed that 241 out of 245 infants were carriers over the whole year, The most prevalent serotypes among the carriers were 6A and 19F at 12.0% each. Similar serotypes have been reported in children of Kuwait, where 19F accounted for 9.8% of total serotypes (Ahmed *et al*., 2000). A study by Marchisio *et al*. (2002) in Italy, found an *S. pneumoniae* carriage rate of 8.6% with the following serotypes (3, 19F, 23F, 19A, 6B, and 14), and that most of pneumococcal isolates (69.4%) were resistant to one or more antimicrobial classes (Marchisio *et al*., 2002). Children at day-care centers in Belgium, aged 3-36 months, had a 21% *S. pneumoniae* carriage rate with the main serotypes being 19F (27.3%), 6B (20.2%), 23F (19.2%), 19A (10.1%), 6A (7.1%), and 14 (5.1%) (Malfroot *et al*., 2004). Prevenar, the 7-valent pneumococcal conjugate vaccine (PCV7) and the new 13-valent pneumococcal conjugate vaccine (PCV13) are used routinely in the National Immunization Program of at least seventy countries worldwide. The results of this study show that 241 out of 415 infants were carriers during at least one of the study phases, with a coverage of PCV7 serotypes of 27.8%, and a coverage of PCV13 serotypes of 49.8%. Around the world, the highest coverage for PCV7 has been reported in 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 (Hausdorff *et al*., 2005). A retrospective review study in the USA was conducted by Walls *et al*. (2015) using the pediatric reports of 31,738 kids aged between 1-4 years and collected from the National Inpatient Database with complications of meningitis, mastoiditis, periorbital cellulitis, and Bezold abscesses due to *S. pneumoniae* diagnoses. A significant decrease in the incidence of several complications was noticed after the introduction of the PCV7 vaccine, and also when comparing these findings to the predicted incidence calculations if the vaccine was not administered. These findings showed a significant increase in the cost to provide care for each of the described conditions ($P < 0.05$), and resulted in a measurable reduction in the head and neck complications associated with *S. pneumoniae* (Walls *et al*., 2015).

A 15-year retrospective study was conducted for the years 2000 to 2014 by Soto-Noguerón, *et al*. (2015) on Mexican infants aged ≤60 days and having invasive and non-invasive pneumococcal infections. It showed that 40.5% of the Mexican infants had pneumonia followed by meningitis (29.4%), septicemia (16.7%), and other clinical presentations, including otitis media and conjunctivitis (13.5%). The study also showed that serotypes 15A/B had increased after the introduction of PCV7, and that serotype 19A was isolated most frequently with pneumonia and meningitis cases only after the introduction of PCV7, and that it displayed a high resistance to penicillin (Soto-Noguerón *et al*., 2015). In the current study, PCV was introduced in the private sector but not in the National Immunization Programme of Jordan. Furthermore, most of the families that vaccinated their children voluntarily were found to be of good income, and are more likely to be residing in Amman, the capital of Jordan. Vaccination with PCV7 in this study was effective in eradicating 76% of vaccine serotypes three months after the last vaccination. Finally, the researchers are aware that the carriage patterns may vary between communities and that it is possible that the serotype distribution and resistance patterns described here may not be representative of all the infant population of Jordan. An obstacle to the eradication of pneumococcal diseases in children is the inability to include more antigens in the conjugated formulations from the 92 serotypes of *S. pneumoniae*. Knowledge about the regional distribution of the pneumococcal capsular types, antimicrobial susceptibility, continuous prevalence studies, and incidence rates of both pneumococcal meningitis and bacteremia in children, is very essential for the future development of effective vaccine strategies and treatment protocols.

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