

## Article

# Urban and rural disparities in pneumococcal carriage and resistance of Jordanian children, 2015-2019

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**Abstract:** Background: Pneumococcal carriage surveillance study took place in urban and rural areas for Jordanian children in the period 2015-2019. Objectives: Determine urban and rural differences in pneumococcal carriage rate, resistance, and serotypes from healthy Jordanian children of Amman (urban) and eastern Madaba (rural). Methods: Nasopharyngeal swabs (NP) were taken from 682 children aged 1 to 163 months. Pneumococcal identification, serotyping and resistance were done according to standard method. Results: Number of cases tested for Amman 267 and for eastern Madaba 415. Carriage rate for eastern Madaba was 39.5% and for Amman 31.1%. Predominant serotypes for eastern Madaba and Amman were 19F (21.3%; 15.7%), 23F (12.2%; 9.6%), 14 (6.7%; 2.4%), 19A (4.9%; 2.4%), 6A (5.5%; 3.6%). Resistance rates for eastern Madaba and Amman were: penicillin (95.8%; 81.9%), clarithromycin (68.9%; 59.0%), clindamycin (40.8%; 31.3%), and trimethoprim-sulfamethoxazole (73.2%; 61.4%). Coverage of PCV7, PCV13, and the future PCV20 for Amman were 42.2%, 48.2%, and 60.2%; and for eastern Madaba were 50.0%, 62.2%, and 73.2%, respectively. In Amman 25.8% have received 1-3 PCV7 injections compared to 1.9% in eastern Madaba. Conclusions: There was significant differences in carriage, resistance and coverage in both regions. The potential inclusion of PCV vaccination program for rural areas is essential.

**Keywords:** *Streptococcus pneumoniae*, Carriage, Resistance, PCVs, Urban, Rural.

## 1. Introduction

*Streptococcus pneumoniae*, an infectious agent causing invasive and non-invasive sicknesses ranging from meningitis to lung infections especially in low-income countries with low or minimum vaccinations (1-3). It is also known as an opportunistic human-adapted pathogen transmitted by the nasopharynx as an ecological reservoir for this bacterium inhabiting the nasopharynx during the first few months of life (4). This NP-carriage is available in healthy children in rates between 20 to 40%, which is influenced by different risk factors including day care center attendance, size of day care center, living in close contact as siblings, living in urban or rural areas, previous antibiotic consumption within one month before sample collection, cigarette smoking, overcrowdings in households, and socioeconomic factors (5-7). Furthermore, NP-carriage is subject to genomic changes during natural colonization (8), and is acquired at approximately 4-6 months of age (1, 9). Unlike children, carriage in elderly is rarely detected (10). The pneumococcal carriage

prevalence is used to estimate the potential for the use of pneumococcal conjugate vaccines (PCVs) in reducing the vaccine type isolates of *S. pneumoniae* (3), and therefore can indirectly determine the impact of the PCVs on disease (11). Annual death estimates of the World Health Organization from pneumococcal infections is more than 1.2 million of children (12, 13), therefore introduction of PCVs proved to have excellent efficacy on NP-carriage and resistance (14). Furthermore, emergence of penicillin- and cephalosporin-resistant strains made the treatment more difficult and the consequence was the urgent need for the PCVs, which proved to be effective in infants. Disparities in race, age, gender, living in urban or rural districts, or even socioeconomic variations showed significant variations in the carriage, invasive pneumococcal diseases (IPD), resistance and even the coverage of the available pneumococcal conjugate vaccines (15-17). Other studies on the impact of vaccination on racial disparities of the IPD incidence showed that high reduction after the introduction of the PCV7 and PCV13 (18). However, pneumococcal studies published for Jordan were concentrated only on the rural areas, especially where no PCV were available (1, 5, 19). Furthermore, Jordan is a mixture of culture, especially after the Gulf and Syrian wars, in addition to the availability of other nationalities and refugees. This study aims to differentiate between the urban city of Amman with the rural area of eastern Madaba for the rates of pneumococcal carriage, resistance and coverage of the available conjugate vaccines. Taking into consideration that all PCVs are found privately in the market since the year 2000, but not found to date in the National Immunization Program (NIP) of Jordan.

## 2. Materials and Methods

**Ethical clearance statement:** The research project was approved by the Independent Ethical Committee (IEC) from the Ministry of Health (MOH) in Jordan followed by the approval of the MOH, and approvals of the directorates of each Day Care Center (DCC) taking part in this research project. Informed written consent from parents as a written permission for participating the children with NP-swabs, and for any relevant data to be used for protocoling. The parents were educated on the benefits of the future vaccination with the available Pneumococcal Conjugate Vaccines. Questionnaires with names, date of birth, gender, number of household, address, and history of PCV vaccination were registered at the time of sample collection. All NP-samples were collected from trained medical doctors of each DCC. Positive results of carriage with resistance analysis and serotyping were sent to the medical doctors of each DCC to be registered on files of the participating children.

**Study population and Design:** Nasopharyngeal swabs were taken from outpatients of private clinics of Amman and from governmental Day Care Centers of east Madaba during routine check-ups. 682 NP-swabs were tested including 267 samples from the urban area of the capital Amman, and 415 samples from the rural area of eastern Madaba in the period from April 2015 to April 2019.

**Laboratory procedures:** NP-swabs were processed by cultivation on Columbia Agar plates supplemented with 5% sheep blood for identification as previously described (20). The plates were incubated at 35°C with 5% CO<sub>2</sub> overnight. Identification was performed by conventional microbiological methods like colony morphology, susceptibility to optochin (bioMérieux) and bile solubility. Confirmed *S. pneumoniae* isolates were tested for the Minimal Inhibitory Concentration (MIC) using the micro broth dilution method as recommended by the Clinical Laboratory Standards Institute (CLSI) (21) using the VITEK2 compact system with vitek cards (AST03) and E-test provided by bioMérieux. Antibiotics used were penicillin G (PEN), amoxicillin (AMOX), cefotaxime (CETA), cefuroxim (CEFU), cefpodoxim (CEPO), clarithromycin (CLA), clindamycin (CLI), tetracycline (TET), levofloxacin (LEV), moxifloxacin (MOX), telithromycin (TEL), trimethoprim/sulfamethoxazole (SXT), chloramphenicol (CHA), and vancomycin (VAN). Breakpoints and interpretation of susceptibility was according to the latest CLSI standards. *S. pneumoniae* ATCC

49619 was used as a control strain. Serotyping of the pneumococcal isolates was performed by the Neufeld's Quellung reaction method using type and factor sera provided by the Statens Serum Institute (SSI), Copenhagen, Denmark.

**Statistical analysis:** Student t-test was considered for significant differences using 2-tailed values with the level of significance at  $p < 0.05$ . Other analysis include rate of carriage, vaccine and non-vaccine serotype coverage, resistance rates to antibiotics.

### 3. Results

A total of 682 children aged 1 to 163 months old were enrolled this study. The study includes the urban area of Amman as the capital city ( $n=267$ ), and the rural area of eastern Madaba with 415 children. Table 1 describes the gender distribution of all enrolled children with total carriage rate in both cities of 36.2%. Total carriage rate for Amman was 31.1%, so that male carriage rate was 30.3% and female carriage rate was 32.1%. As a comparison, carriage rate for eastern Madaba was 39.5% with male carriage rate 42.9% and female carriage rates 35.6%. Significant difference ( $P < 0.05$ ) was noticed between carriage rates between both locations.

**Table 1.** Source of NP-samples according to the gender and total carriage, 2015-2019.

City	No. Samples	Male n (%)	Male carrier n (%)	Female n (%)	Female Carrier n (%)	Total Carriage n (%)
Amman	267	155 (58.1)	47 (30.3)	112 (41.9)	36 (32.1)	83 (31.1)
East Madaba	415	224 (54.0)	96 (42.9)	191 (46.0)	68 (35.6)	164 (39.5)
Total	682	379 (55.6)	143 (37.7)	303 (44.4)	104 (34.3)	247 (36.2)

Table 2 shows the number of PCV7 injections taken by the children from both areas, taking into consideration that all nasopharyngeal samples were taken at least 3 months post PCV injections of all cases from both urban and rural areas. The number of cases taken from Amman with no history of PCV injections was 198 and 69 (25.8%) have a history of 1 to 3 previous injections with PCV7. Only 11 from the 69 (15.9%) vaccinated cases from Amman showed carriage with non-PCV7 serotypes. The number of cases taken from Amman with no history of PCV7 injections was 198, where 72 cases were carriers, so that 35/72 (48.6%) could be covered by the PCV7; 39/72 (54.2%) could be covered by the PCV13, and 47/72 (65.3%) could be covered by the future vaccine PCV20. The number of cases carried the pneumococcus after the first, second and third injection with the PCV7 was 1/69, 3/69, and 7/69, respectively. All serotypes recovered from these cases after previous injections were 6A, 9N, 11A, 23A and others not included in PCV serotypes. In the rural area of east Madaba, only 8 cases had a previous history of 2 PCV7 injections, where 3 out of the 8 (37.5%) were carriers after the second injections with the serotypes 6B, 19A and others not included in the PCV7 serotypes. Whereas the number of cases with no history of PCV injection in eastern Madaba was 407 cases, so that 161/407 (39.6%) cases were carriers. 81/161 (50.3%) could be covered by the PCV7; 97/161 (60.2%) could be covered by the PCV13, and 111/161 (68.9%) could be covered by the future PCV20.

**Table 2.** Carriers from Amman and East Madaba with history of PCV7 injections.

City	# PCV7 injections	Carriage n (%)	*Serotype or VTs and none VTs recovered
Amman $n=267$	1 injection ( $n=11$ )	1/11 (9.1%)	6A
	2 injections ( $n=9$ )	3/9 (33.3%)	9N; Others
	3 injections ( $n=49$ )	7/49 (14.3%)	11A; 23A; 9N; Others
	0 injections ( $n=198$ )	72/198 (36.4%)	VTs 35 (48.6%), and none VTs 37 (51.4%)

Eastern Madaba n= 415	1 injection (n=0)	0/0 (0.0%)	none
	2 injections (n=8)	3/8 (37.5%)	6B; 19A; Others
	3 injections (n=0)	0/0 (0.0%)	none
	0 injection (n=407)	161/407 (39.6%)	VTs 84 (52.2%), and none VTs 77 (47.8%)

\*VTs and none VTs= Vaccine types and none vaccine types related to PCV7.

Table 3 shows the antibiotic resistance profile for 14 antibiotics for isolates from both areas. No resistance detected for moxifloxacin, levofloxacin, telithromycin and vancomycin. Resistance to penicillin, clarithromycin and trimethoprim-sulfamethoxazole was high in both areas. Nevertheless, significant differences ( $P < 0.05$ ) in the resistance of penicillin, clarithromycin, clindamycin, and trimethoprim-sulfamethoxazole were higher in the rural area of eastern Madaba than isolates from the urban area of Amman.

**Table 3.** Comparison of antibiotic resistance in both Amman and eastern Madaba.

Antibiotic	Amman (n=83/267)				Eastern Madaba (n=164/415)			
	%S	%I+R	MIC <sub>50</sub>	MIC <sub>90</sub>	% S	% I+R	MIC <sub>50</sub>	MIC <sub>90</sub>
Penicillin	18.1	81.9	1	4	4.3	95.8	1	2
Amoxicillin	85.5	14.5	1	>4	92.7	7.3	0.5	2
Cefotaxime	96.4	3.6	0.5	1	93.9	6.1	0.5	1
Cefuroxime	41.0	59.0	4	>4	39.0	61.0	2	>4
Cefpodoxime	39.8	60.2	1	2	35.4	64.6	1	2
Clarithromycin	41.0	59.0	2	>32	31.1	68.9	8	>32
Clindamycin	68.7	31.3	≤0.125	>32	59.2	40.8	0.06	>32
Moxifloxacin	100	0.0	0.125	0.25	100	0.0	0.125	0.25
Levofloxacin	100	0.0	1	2	100	0.0	1	2
Trimethoprim-Sulfamethoxazole	38.6	61.4	2/38	>8/152	26.8	73.2	4/76	>8/152
Tetracycline	48.2	51.8	8	16	48.8	51.2	4	32
Chloramphenicol	92.8	7.2	≤4	≤4	96.3	3.7	≤4	≤4
Telithromycin	100	0.0	0.03	0.125	100	0.0	0.016	0.06
Vancomycin	100	0.0	0.5	0.5	100	0.0	0.5	0.5

Table 4 shows the number of serotypes detected in each area with the rate of occurrence of each serotype. Four serotypes of the PCV7 isolated from eastern Madaba (14, 18C, 19F, 23F) showed significantly higher ( $P < 0.05$ ) rates of isolation in the rural area of eastern Madaba as in the urban area of Amman, with the exception of the serotype 6B, which was higher in Amman than eastern Madaba even with less number of isolates. One strain was isolated from each area with the serotype 9V, and no serotype 4 was detected in both areas that belong to the PCV7.

**Table 4.** Serotype distribution of isolates from Amman and eastern Madaba.

Serotypes (No. of isolates)	Amman (n=83) n (%)	Eastern Madaba (n=164) n (%)
3 (n=3)	0 (0.0%)	3 (1.8%)
6A (n=12)	3 (3.6%)	9 (5.5%)
6B (n=26)	11 (13.3%)	15 (9.2%)
6C (n=2)	1 (1.2%)	1 (0.6%)
7B (n=2)	1 (1.2%)	1 (0.6%)
9N (n=5)	3 (3.6%)	2 (1.2%)
9V (n=2)	1 (1.2%)	1 (0.6%)
10A (n=3)	0 (0.0%)	3 (1.8%)
11A (n=13)	6 (7.2%)	7 (4.3%)

14 (n=13)	2 (2.4%)	11 (6.7%)
15A (n=5)	4 (4.8%)	1 (0.6%)
15B (n=4)	1 (1.2%)	3 (1.8%)
15C (n=1)	0 (0.0%)	1 (0.6%)
15F (n=1)	1 (1.2%)	0 (0.0%)
16A (n=2)	0 (0.0%)	2 (1.2%)
16F (n=2)	1 (1.2%)	1 (0.6%)
17F (n=5)	1 (1.2%)	4 (2.4%)
18A (n=1)	1 (1.2%)	0 (0.0%)
18C (n=4)	0 (0.0%)	4 (2.4%)
19A (n=10)	2 (2.4%)	8 (4.9%)
19F (n=48)	13 (15.7%)	35 (21.3%)
22A (n=2)	0 (0.0%)	2 (1.2%)
22F (n=2)	2 (2.4%)	0 (0.0%)
23A (n=9)	3 (3.6%)	6 (3.7%)
23B (n=1)	0 (0.0%)	1 (0.6%)
23F (n=24)	8 (9.6%)	20 (12.2%)
28 (n=3)	0 (0.0%)	3 (1.8%)
33A (n=1)	1 (1.2%)	0 (0.0%)
33F (n=1)	1 (1.2%)	0 (0.0%)
34 (n=2)	2 (2.4%)	0 (0.0%)
35A (n=2)	1 (1.2%)	1 (0.6%)
35B (n=4)	4 (4.8%)	0 (0.0%)
Pool C (n=1)	0 (0.0%)	1 (0.6%)
Pool G (n=5)	0 (0.0%)	5 (3.1%)
Pool I (n=1)	0 (0.0%)	1 (0.6%)
NT (n=6)	1 (1.2%)	5 (3.1%)
Others (n=15)	8 (9.6%)	7 (4.3%)

Coverage rates of the pneumococcal isolates from both areas is presented in table 5. Coverage for PCV7, PCV10, PCV13, and the future PCV20 for Amman were 42.2%, 42.2%, 48.2%, and 60.2%, respectively. Significantly higher coverage ( $P<0.05$ ) was detected for the eastern area of Madaba with rates of PCV7 (50%), PCV10 (50%), PCV13 (62.2%), and the future PCV20 (73.2%).

**Table 5.** Coverage of *Streptococcus pneumoniae* to pneumococcal conjugate vaccines.

Coverage in Amman & eastern Madaba	7v PCV n (%)	10v PCV n (%)	13v PCV n (%)	20v PCV n (%)
Amman (n=83)	35 (42.2)	35 (42.2)	40 (48.2)	50 (60.2)
Eastern Madaba (n=164)	82 (50.0)	82 (50.0)	102 (62.2)	120 (73.2)

Serotype related antibiotic resistance for the urban area of Amman in table 6 clearly shows the highest resistance rates among the serotypes available in the pneumococcal conjugate vaccines, so that the resistance rates for penicillin in serotypes 19F, 6B, 23F, 14 and 9V are 92.3%, 90.9%, 100%, 100%, and 100%, respectively. Similarly, in table 7 for the rural area of eastern Madaba, 100% resistance rates were detected for penicillin in serotypes 19F, 6B, 23F, 14, 9V, and 18C.

**Table 6.** Vaccine and non-vaccine serotype related antibiotic resistance for isolates from Amman.

Serotypes from Amman	% Pen R	% Cla R	% Cli R	% Lev R	% Sxt R	% Tet R	% Cha R
6A (n=3)	100%	66.7%	66.7%	0.0%	33.3%	66.7%	0.0%
6B (n=11)	90.9%	72.7%	72.7%	0.0%	90.9%	63.6%	18.2%

6C (n=1)	100%	100%	100%	0.0%	0.0%	100%	0.0%
7B (n=1)	100%	100%	0.0%	0.0%	100%	100%	0.0%
9N (n=3)	100%	0.0%	0.0%	0.0%	33.3%	100%	0.0%
9V (n=1)	100%	100%	0.0%	0.0%	100%	100%	0.0%
11A (n=6)	100%	83.3%	16.7%	0.0%	100%	66.7%	0.0%
14 (n=2)	100%	100%	100%	0.0%	100%	100%	0.0%
15A (n=4)	25%	100%	75.0%	0.0%	100%	100%	0.0%
15B (n=1)	100%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
15F (n=1)	100%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
16F (n=1)	100%	100%	0.0%	0.0%	0.0%	0.0%	0.0%
17F (n=1)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
18A (n=1)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
19A (n=2)	100%	100%	50%	0.0%	50%	50%	0.0%
19F (n=13)	92.3%	84.6%	46.2%	0.0%	92.3%	76.9%	0.0%
22F (n=2)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
23A (n=3)	66.7%	33.3%	33.3%	0.0%	33.3%	33.3%	0.0%
23F (n=8)	100%	50%	0.0%	0.0%	100%	50%	0.0%
33A (n=1)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	50.0%
33F (n=1)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
34 (n=2)	50%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
35B (n=4)	100%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
35A (n=1)	0.0%	0.0%	0.0%	0.0%	100%	0.0%	0.0%
NT (n=1)	100%	100%	0.0%	0.0%	100%	100%	0.0%
Others (n=8)	87.5%	62.5%	12.5%	0.0%	12.5%	12.5%	0.0%

**Table 7.** Vaccine and non-vaccine serotype related antibiotic resistance for isolates from eastern Madaba.

<b>Serotypes from eastern Madaba</b>	<b>% Pen R</b>	<b>% Cla R</b>	<b>% Cli R</b>	<b>% Lev R</b>	<b>% Sxt R</b>	<b>% Tet R</b>	<b>% Cha R</b>
3 (n=3)	33.3%	0.0%	0.0%	0.0%	33.3%	33.3%	0.0%
6A (n=9)	100%	100%	66.7%	0.0%	44.4%	66.7%	11.1%
6B (n=15)	100%	73.3%	60.0%	0.0%	86.7%	73.3%	13.3%
6C (n=1)	100%	100%	100%	0.0%	0.0%	100%	0.0%
7B (n=1)	100%	100%	100%	0.0%	0.0%	100%	0.0%
9N (n=2)	100%	0.0%	0.0%	0.0%	50.0%	100%	0.0%
9V (n=1)	100%	0.0%	0.0%	0.0%	100%	0.0%	0.0%
10A (n=3)	66.7%	0.0%	0.0%	0.0%	66.7%	0.0%	0.0%
11A (n=7)	100%	42.9%	14.3%	0.0%	71.4%	28.6%	0.0%
14 (n=12)	100%	100%	100%	0.0%	90.9%	63.6%	0.0%
15A (n=1)	100%	100%	0.0%	0.0%	100%	100%	0.0%
15B (n=3)	100%	100%	0.0%	0.0%	66.7%	100%	0.0%
15C (n=1)	100%	100%	0.0%	0.0%	100%	100%	0.0%
16A (n=2)	50%	0.0%	0.0%	0.0%	50%	0.0%	0.0%
16F (n=1)	100%	0.0%	0.0%	0.0%	100%	0.0%	0.0%
17F (n=4)	100%	0.0%	0.0%	0.0%	25.0%	0.0%	0.0%
18C (n=4)	100%	0.0%	0.0%	0.0%	75%	25.0%	0.0%
19A (n=8)	100%	100%	25.0%	0.0%	87.5%	25.0%	0.0%
19F (n=35)	100%	97.1%	82.9%	0.0%	94.3%	91.4%	0.0%
22A (n=2)	100%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%



23A (n=6)	100%	66.7%	16.7%	0.0%	50.0%	33.3%	0.0%
23B (n=1)	100%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
23F (n=20)	100%	43.8%	6.3%	0.0%	81.3%	37.5%	12.5%
28A (n=3)	100%	100%	66.7%	0.0%	100%	0.0%	0.0%
35A (n=1)	100%	100%	0.0%	0.0%	100%	0.0%	0.0%
Pool C (n=1)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Pool G (n=5)	100%	100%	80.0%	0.0%	80.0%	20.0%	20.0%
Pool I (n=1)	100%	100%	0.0%	0.0%	100%	100%	0.0%
NT (n=5)	60.0%	80.0%	0.0%	0.0%	100%	20.0%	0.0%
Others (n=7)	100%	28.6%	28.6%	0.0%	14.3%	28.6%	0.0%

#### 4. Discussion

Disparities in pneumococcal infections and carriage were studied worldwide for populations of different socioeconomic, ethnic and races (22). Other studies done on carriage before or after the introduction of the PCVs (23). Differences in pneumococcal carriage rates, pneumococcal conjugate vaccine coverage and resistance rates worldwide depend on the use of vaccination, correct use of antibiotics, socio-economic factors, age and urban or rural residency of people (24). Furthermore, gender and racial disparities in carriage were studied in different parts of the world showing either significant changes or no significant differences (16, 25, 26). On the other hand, research done on both urban and rural differences of the pneumococcal disease or colonization with significant differences was rarely found (27-29). This study research showed a total carriage rate for both areas of 36.2%, but it showed higher and significant rate of carriage in eastern Madaba ( $P<0.05$ ) compared to the urban area of Amman. In a study done in the urban and rural areas of Pakistan, carriage rates were more than 70% for the children enrolled and there were no significant differences between both areas (28). Both carriage rates in both areas of eastern Madaba and Amman were relatively higher than carriage rate obtained from Saudi Arabia or from Taiwan (30). Other study done on the pneumococcal carriage of urban and rural Vietnamese school children on carriage showed no difference between both urban and rural (suburban) areas with 21-22% of carriage rate, and that the non-susceptibility to penicillin and erythromycin in both areas were equally distributed (29). These differences in carriage rates worldwide were related to certain socio-economic conditions including housing, access to health care, poor hygiene, family size, overcrowded living conditions, day-care contact, and number of siblings (31). The pneumococcal drug resistance for Jordan has reached very high levels especially for penicillin, trimethoprim-sulfamethoxazole, and erythromycin for eastern Madaba with rates of 95.8%, 73.2% and 68.9%, respectively. Although these high levels of resistance were significantly higher for eastern Madaba than in the urban area of Amman, resistance rates of these antibiotics detected for the urban area of Amman were also relatively high. Despite the fact that the pneumococcal conjugate vaccines (PCVs) are not yet available in Jordan in the National Immunization Program (NIP), but they are available in the private market. In this research study, in the urban area of Amman 69 from 267 (25.8%) children have received from 1-3 injections of PCV7 privately, out of which only 11 (15.9%) cases from the vaccinated children were carriers with serotypes not available in the PCV7. As a comparison to the non-vaccinated children for the urban area of Amman, carriage rate was 36.4%. For the rural area of Madaba, only 8 cases from 415 (1.9%) have received two injections of the PCV7, three of them were carriers and one case was carrier of 6B included in the PCV7. Main reasons related to these differences are family income and awareness of vaccination benefits. Internationally, PCVs proved to be highly efficient in preventing serious disease caused by serotypes in the vaccine, and it prevents symptomless colonization of the nasopharynx

(19, 32). Prevention of NP-colonization in the infection cycle reduces chances of spread of the infection and indirectly protects from disease. Through these indirect effects, the protection afforded by the vaccine extends to the whole population, including those not vaccinated (herd protection). As mentioned before, the pneumococcal conjugate vaccines are not yet as part of the National Immunization Program (NIP) of Jordan, although an urgent need for the PCVs was discussed and approved at the MOH of Jordan. The corona crisis worldwide has delayed taking decisions to include the PCV in the NIP of Jordan, and most probably has even delayed the introduction of the new PCV20 from Pfizer because of mass need and production of the COVID19 vaccination. The resistance situation of the pneumococcal strains isolated from children colonizers was studied in other rural areas of Jordan previously. These rural areas were Ajlun in 2009-2010 with penicillin resistance rate of 84.0% (19), then was found to be 80.0% in 2014 for Wadi Alseer (1) as a rural area in the peripheries of Amman, then was found in the rural area of eastern Irbid with a rate of 86.3% in 2019 (5). Resistance rates found in this study for penicillin was found to be 95.8% as significantly higher ( $P < 0.05$ ) than all rural areas tested before in Jordan. Same result for clarithromycin, clindamycin and trimethoprim-sulfamethoxazole were significantly higher ( $P < 0.05$ ) for eastern Madaba than in the urban area of Amman, and was the highest in eastern Madaba compared to other rural areas studied in Jordan. Main reason for the increase of antibiotic resistance is presumably high intake and consumption of antibiotics even without prescription of medical doctor (33). These resistance rates were variable among DCCs and in each season. High consumption of antibiotics in the country, and a history of antibiotic consumption prior to their visits to the DCC could be the reason or contribute to increased resistant strains (34, 35). In Jordan, there is a shortage of data and publications related to *Streptococcus pneumoniae*, especially on invasive pneumococcal diseases, and therefore no available serotypes of invasive or non-invasive diseases caused by the pneumococcus. For this reason, monitoring the changes in serotypes of pneumococcal carriage is a practical way for assessing the vaccine impact. In this study, serotypes available in the PCVs were significantly more ( $P < 0.05$ ) in the rural area of eastern Madaba than in the urban area of Amman as the case for serotypes 3, 6A, 9V, 14, 18C, 19A, 19F, and 23F with the exception to 6B. This is due to the much higher vaccinated cases from Amman than in eastern Madaba, and that the exceptional increase of 6B in Amman was mainly from the non-vaccinated cases. These data are consequent with the low coverage data for all PCVs in Amman compared to eastern Madaba. The proportion of circulating pneumococci with serotypes covered by the vaccine may vary among regions. Methodological differences in carriage studies and variations of carriage estimates may result from differences in climate, season or crowding which influence transmission (36). The rate of antibiotic resistance for vaccine and non-vaccine serotypes for both areas showed higher resistance rates for the rural area than Amman. For instance, 6 serotypes showed no resistance to penicillin for Amman, whereas only one of the serotypes isolated from the rural area of eastern Madaba showed no resistance. These results were consistent to the findings published in Vietnam urban and rural areas among Vietnamese school-children (29).

## 5. Conclusions

The disparity of pneumococcal carriage, resistance and even the coverage of pneumococcal conjugate vaccines between urban and rural areas for Jordanian children was significant. In contrast to theoretical discourses and debates all over the world that show little disparity between rural and urban isolates, significant and profound value of comparing rural with urban had a unique impact in local communities in Jordan. As rural local communities have very different life styles than the urban ones, patterns of carriages relate vastly to the origin of the carriers. Socio-economic status along with social habits and norms have proven to be of valid effect on increasing carriages in rural areas with great disparity to the urban ones. As a precedent research topic in Jordan, with rear reference of similar surveys, this research has pinpointed the aspect of envisioning typologies of isolates within local communities to show case local oriented differentiations in regard



to rural/urban impacts. Introduction of the pneumococcal conjugate vaccine in the National Immunization Program of the country would have a substantial change of such differences. These data together prove the significant disparities of carriage, resistance, serotype distribution and coverage of the PCVs between urban and rural areas of Jordan, which might be indicative for better solutions in the future.

**Author Contributions:** “Conceptualization, Adnan Al-Lahham and Nashat Khanfar; methodology, Noor Albataina; validation and formal analysis, Rana Al Shwayat, Rawsan Altwal, Talal Abulfeilat; data curation, Ghaith Alawneh, Mohammad Khurd and Abdelsalam Alqadi Altamimi. original draft preparation, Adnan Al-Lahham; writing—review and editing, Adnan Al-Lahham and Nashat Khanfar; visualization, Adnan Al-Lahham.; supervision, Adnan Al-Lahham and Noor Albataina; project administration, Adnan Al-Lahham; funding acquisition, Adnan Al-Lahham. All authors have read and agreed to the published version of the manuscript.

**Funding:** “This research was funded by the deanship of scientific research at the GERMAN JORDANIAN UNIVERSITY under the research grant numbers SAMS 22/2015 and SAMS 45/2017.

**Institutional Review Board Statement:** The Institutional Review Board and the ethical Committee of the Ministry of Health of Jordan agree on performing the research proposal of Dr Adnan Al-Lahham by collecting non-invasive samples from the Nasopharynx of children attending Day Care Centers with the condition of agreement of the parents and not causing any harm, then provide the MOH with the results of the research after completion. Approval number by the MOH with letter 8/75/2/ 2257. This study does not involve testing on human or animals.

**Informed Consent Statement:** “Informed consent was obtained from all subjects involved in the study, especially collecting the NP-swabs from children and parents approvals was obtained before collecting the NP-swabs. “Written informed consent has been obtained from the patient(s)/ or parents to publish this paper”.

**Data Availability Statement:** Excluded

**Acknowledgments:** The author(s) would like to thank the Ministry of Health, ethical committee, IRB, and all health directories and DCCs to facilitate this study. The author(s) also acknowledge the financial support of the deanship of scientific research at the German Jordanian University under the research grant numbers SAMS 22/2015 and SAMS 45/2017. Special thanks to all the parents permitting us having the samples and making this research successful.

**Conflicts of Interest:** “The authors declare no conflict of interest in any personal circumstances or interest that may be perceived as inappropriately influencing the representation or interpretation of reported research results. Any role of the funders in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results”.

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