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Research article

Changes in pneumococcal serotypes distribution and penicillin resistance in healthy children five years after generalization of PCV10

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ABSTRACT

Objective: *Streptococcus pneumoniae* (*S. pneumoniae*) nasopharyngeal carriage has significantly decreased after the generalization of pneumococcal vaccination worldwide. This study sought to investigate changes in *S. pneumoniae* carriage rates, serotype distribution and penicillin non-susceptibility following the generalization of 10-valent pneumococcal conjugate vaccine.
Methods: A prospective study was conducted in Marrakesh, Morocco, between 2017 and 2018, among healthy children attending vaccination centers. We collected nasopharyngeal swabs and questionnaire data for each child. Using univariate logistic regression, we analyzed the association between *S. pneumoniae* carriage and various risk factors. Comparisons of serotype diversity and penicillin resistance between 2017 and 2018 and the period before introduction of vaccination (2008–2009, n = 660) were performed using Simpson index and the chi-squared test, respectively.
Results: During 2017–2018, 515 children aged between 6 and 36 months participated. The *S. pneumoniae* carriage rate was 43.3%. Looking at the distribution serotypes, the rate of PCV10 serotypes rate was only 9.6%. Among non-vaccine serotypes, an increase in serotypes 6C/6D (22; 14%), 19B/19C (17; 10.8%), and 15B/15C (11; 7%) was observed. A particular increase in serotype diversity was also observed after the generalization of PCV10 ($p < 0.001$). *S. pneumoniae* non-susceptible to penicillin decreased, reaching a rate of 26.6% in 2017–2018.
Conclusion: The significant change in *S. pneumoniae* carriage, serotype distribution, and penicillin resistance highlights the effectiveness of the pneumococcal conjugate vaccine among children in Marrakesh, Morocco.

1. Introduction

Streptococcus pneumoniae is a major cause of mortality and morbidity worldwide, among under-five children [1,2]. The nasopharynx serves as route of entry for these bacteria, being important for their acquisition and spread [3]. Despite often being asymptomatic, nasopharyngeal carriage is considered as a determinant in the development of pneumococcal illness [4]. With over 100

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defined *S. pneumoniae* serotypes characterized by their polysaccharide capsule [5], the majority of these serotypes are carried, and only a minority are potentially capable of causing both invasive and non-invasive pneumococcal illnesses [6].

The pneumococcal conjugate vaccine (PCV) has been shown to be effective in preventing vaccine serotypes (VSs), commonly found in invasive pneumococcal disease (IPD) [7,8]. PCV has significantly reduced the prevalence of nasopharyngeal carriage among vaccinated children, providing immunity, as reported in several worldwide studies [9–11]. However, the phenomenon of serotype replacement has occurred, whereby non-vaccine serotypes (NVSs) have expanded to fill the niche left vacant by VSs [12]. Typically, NVSs have low invasive potential [13–15], and the overall incidence of IPD has consistently been lower after the implementation of PCV [16,17]. In addition, serotype replacement remains an issue, as it may relatively negate the effectiveness of PCV due to the emergence of IPD related to NVSs [18,19].

Before the introduction of PCV, we conducted a prospective surveillance study on the asymptomatic nasopharyngeal carriage of *S. pneumoniae* among under-five healthy children in Marrakesh, Morocco [20]. This unique investigation provided valuable data on the baseline proportion of pneumococcal carriage, serotype distribution, and antimicrobial resistance, helping in selecting the appropriate vaccine to introduce into the Moroccan National Immunization Program (NIP). In July 2012, the Moroccan Ministry of Health introduced PCV10 (including serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F) into the NIP using a three-dose schedule administered at 2, 4, and 12 months. Before the introduction of PCV10, the Moroccan NIP had used PCV13 (including serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F), which continues to be accessible in the private sector since 2010 [21].

The main aim of this study was to investigate the epidemiology of asymptomatic nasopharyngeal carriage of *S. pneumoniae* in Marrakesh, Morocco, following the introduction of PCV10. Specifically, we sought to assess whether the use of PCV10 led to changes in serotype distribution and penicillin resistance, thereby evaluating the impact of pneumococcal vaccination in Marrakesh, Morocco, where mass vaccination of infants has been implemented.

2. Materials and methods

2.1. Study design and population

A prospective study of *S. pneumoniae* nasopharyngeal carriage was conducted among 515 healthy children aged between 6 and 36 months visiting vaccination centers in Marrakesh, Morocco, between 2017 and 2018, following the generalization of PCV10. All children were invited to participate, except for those with a fever, respiratory symptoms, or who had recently consumed antibiotics (<7 days). Parents or guardians were provided with comprehensive information about the study and requested to sign an informed consent form before their child's participation. Data of each child, including demographic characteristics (age, gender), risk factors related to *S. pneumoniae* carriage (number of siblings, recent antibiotic therapy), and vaccination status (number of doses received), were collected.

2.2. Specimen collection

Nasopharyngeal specimens were collected following the recommendations of the World Health Organization (WHO). For each child, a sterilized flocked nylon swab (COPAN swab collection, 482CE) was used to collect a nasopharyngeal specimen, which was then placed in a medium containing skim milk, tryptone, glucose, and glycerol. All specimens were then transported to the laboratory of Microbiology, virology, and Molecular biology of Avicenna Military Hospital in Marrakesh, Morocco. Upon arrival at the laboratory, the specimens were examined to detect the presence of *S. pneumoniae*.

2.3. Identification of S. pneumoniae strains

The specimens were streaked onto colistin nalidixic acid (CNA) agar (Biolife, Milano, Italia), supplemented with 5% of sheep blood, and were incubated overnight at 37^{O}C with 5% CO2. After overnight incubation, the plates were examined for the presence of α-hemolytic colonies. Identification of *S. pneumoniae* was performed by optochin susceptibility [22], bile solubility [23], and an agglutination test (Slidexpneumo-Kit Bio Mérieux, Marcy-l'Etoile, France).

2.4. S. pneumoniae serotyping

The serogroups of *S. pneumoniae* strains were determined by the latex agglutination method (Pneumotest-Latex kit, Statens Serum Institute antisera, Copenhagen, Denmark). Strains that exhibited no agglutination were defined as non-typeable. The serotypes of *S. pneumoniae* were identified by real-time PCR (RT-PCR) assay. Strains belonging to serotypes: 2, 3, 4, 8, 14, and 20 were exempted from the serotyping process. DNA extraction was performed using QIAamp DNA Kits (Qiagen, Germantown, MA, USA) according to the manufacturer's instructions. PCR reactions were carried out in a final volume of 25 μl using Invitrogen-Platinum qPCR SuperMix-UDG (Thermo Fisher Scientific, Waltman, MA, USA). The details of all primers (forward and reverse) and probe (Eurogentec, Seraing, Belgium) used for serotyping *S. pneumoniae* strains are summarized in (Table 1). The primers and probe concentrations were set at 10 mM for each one. Amplification was operated in 96 CFX BioRad RT-PCR machine (BioRad, Hercules, Californie, USA) under the following conditions according to manufacturer's guidelines: 50^{O}C for 2 min, 95^{O}C for 2 min, followed by 40 cycles of 95^{O}C for 3s and 60^{O}C for 30s. None serotyped *S. pneumoniae* strains, due to budget constraints, were defined according to their serogroups.

2.5. Antimicrobial susceptibility

Antimicrobial susceptibility testing of *S. pneumoniae* strains was accomplished on Mueller-Hinton agar (Biokar, Allone, France) supplemented with 5% of sheep blood. The disk (Bioanalyse, Ankara, Turkey) diffusion method was employed for erythromycin (15 μg), clindamycin (2 μg), tetracycline (30 μg), cotrimoxazole (1.25/23.75 μg), and chloramphenicol (30 μg), while E-tests (Bio Mérieux, Marcy-l'Etoile, France) method was used for penicillin G, amoxicillin and cefotaxime. Interpretations of antibiotic diffusion diameters and minimal inhibitory concentrations (MICs) were made according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2018) guidelines.

2.6. Statistical analyses

Statistical analyses were performed using SPSS/PC 23.0 program (SPSS Inc, Chicago, IL, USA). Univariate logistic regression analysis was done to determine risk factors associated with *S. pneumoniae* nasopharyngeal carriage between 2017 and 2018.

Simpson's Diversity Index was determined to assess the effect of PCV10 introduction in *S. pneumoniae* serotype distribution between the results of this study (period after vaccination) and our previous published paper [20] (period before vaccination). The previous study was conducted between 2008 and 2009, included 660 healthy children, aged less than two years, visiting dispensaries for routine immunization in Marrakesh, Morocco. The Simpson's Diversity Index was calculated using the following equation:

$$D = 1 - \left(\frac{\sum n(n-1)}{N(N-1)}\right)$$

n: the total number of strains of particular serotype.

N: the total number of strains of all serotypes.

The Rate Ratio (RR) was calculated to estimate changes in serotype distribution before and after the introduction of PCV10 vaccination. The PCV-10 effectiveness on carriage of VSs was calculated using to the following equation: VE = (1-RR)100.

Penicillin resistant rates were analyzed by chi-squared test. 95% confidence intervals (95%CI) were determined. The differences were considered significant if the p-value was less than 0.05.

Table 1
Primers and probe used in RT-PCR essay for *S. pneumoniae* serotyping.

Serotype	Primers/Probe	Nucleotide sequence (5′ to 3′)
6A/6D	6A/6D-f	GTTTGCACTAGAGTATGGGAAGG
	6A/6D-r	TAGCCTTTCTGAAAACATTTAGCG
	6A/6D-p	TGTTCTGCCCAGAGCAACTGGTCTTGTATC
6C/6D	6C/6D-f	TTGGGATGATTGGTCGTATTAG
	6C/6D-r	CTCTTCAATTAGTTCTTCAGTTCG
	6C/6D-p	CCACGCAATTCGCCATC
9V	9V-f	TCCTCAGTCAATTTTAACAAGAAAC
	9V-r	AGAGAATATACCCCGAAATCATG
	9V-p	CCAGCACAACACCAATAAC
11A	11A-f	CATTGTGTATGCTACCATTCTTC
	11A-r	GTGCTAACTGTAAAACTTGATTATG
	11A-p	TCTCCCAATTTCTGCCACG
11A/11D	11A/11D-f	AAATGGTTTGGATATGGTTTGTTTGG
	11A/11D-r	AGTGCTAACTGTAAAACTTGATTATGAG
	11A/11D-p	ATTCCAACTTCTCCCAATTTCTGCCACGG
15A/15F	15A/15F-f	AATTGCCTATAAACTCATTGAGATAG
	15A/15F-r	CCATAGGAAGGAAATAGTATTTGTTC
	15A/15F-p	CCCGCAAACTCTGTCCT
19A	19A-f	CGCCTAGTCTAAATACCA
	19A-r	GAGGTCAACTATAATAGTAAGAG
	19A-p	TATCAATGAGCGGATCCGTCACTT
19F	19F-f	TGAGGTTAAGATTGCTGATCG
	19F-r	CACGAATGAGAACTCGAATAAAAG
	19F-p	CGCACTGTCAATTCACCTTC
23A	23A-f	CTCCCCTCCATTACCCATTTGG
	23A-r	TGAAGAAAGTGCTGTTTGTGAACC
	23A-p	AGCTAGAACTCCCACACTCCCTACTCCCA
23F	23F-f	GACAGCAACGACAATAGTCATCTC
	23F-r	TCCATCCCAACCTAACACACTTC
	23F-p	ATTGTGTCCATAACCCTTCGTATTTCCAAAG
33A/33F/37	33A/33F/37-f	GGAACTGGTTCAGCAACTATACG
	33A/33F/37-r	GGTTCTAAGACCGTCTGAAATACC
	33A/33F/37-p	CCCCAAATAGGAC"T"TTTCTGCCATGCCAAA

2.7. Ethics statement

The study received the agreement of the ethics committee of the Mohammed VI University Hospital Center, Marrakesh, Morocco (19/07/20,223). A consent form was signed by the parents or guardians of the children included in the study, mentioning the respect of anonymity.

3. Results

The baseline characteristics of the 515 healthy children included in this nasopharyngeal carriage study are presented in (Table 2). The average age of the participants was 11.7 ± 5.9 months, with a sex ratio of 0.93 (249 males and 266 females). Of the participants, 182 (35.3%) were fully vaccinated.

3.1. Nasopharyngeal carriage rate and risk factors

From 2017 to 2018, a total of 223 *S. pneumoniae* isolates were collected, resulting in a nasopharyngeal carriage rate of 43.3% (223/515). Of the carriers, 118(52.9%) were male and 119 (53.4%) aged less than 12 months old. The mean age of carriers was 11.9 months (range: 6–30 months), with a sex ratio of 1.02.

Univariate logistic regression analysis revealed that age (6–12 months) and complete vaccination were significant risk factors of *S. pneumoniae* nasopharyngeal carriage ($p < 0.05$). Conversely, previous antibiotic therapy exhibited a significant protective effect against *S. pneumoniae* nasopharyngeal carriage. Notably, male gender and the presence of siblings did not demonstrate a statistically significant effect on the rate of *S. pneumoniae* carriage among healthy children (Table 3).

3.2. Distribution of S. pneumoniae serotypes

Only 157 *S. pneumoniae* isolates were serotyped; 43 were nonviable after conservation and 23 were non-typeable. Nineteen serogroups/types were detected (Fig. 1). The rate of PCV10 serotype was 9.6% (15/157). The percentages of VSs 14, 6B, 4, and 23F were 4.5% (7/157), 3.2% (5/157), 1.3% (2/157), and 0.6% (1/157), respectively. Moreover, the study brought attention to the prevalence of NVSs in carriage among healthy children, accounting for 87.9% (138/157). Serotypes 6C/6D, 19B/19C, and 15B/15C were the most frequent NVSs detected, constituting 14% (22/157), 10.8% (17/157), and 7% (11/157), respectively. The PCV13 serotypes, excluding the PCV10 serotypes, were also identified, accounting for 2.5% (4/157). Among the PCV13 serotypes, 3, 6A, and 19A were recognized, comprising 1.3% (2/157), 0.6% (1/157), and 0.6% (1/157), respectively.

The serotype distribution of *S. pneumoniae* carriage isolates according to age groups is presented in (Table 4). Of the total of *S. pneumonia* strains serotyped (N = 157), 50.6% (84) were detected in the age range of 6–12 months, 48.2% (80) in the range of 12–24 months, and 1.2% (2) in the range of 25–36 months. Serotype 6C/6D (10/84; 11.9%) was the most frequent serotype among children younger than 12 months of age, followed by serotypes 11A/11D and 23B (each 6/84; 7.1%). Among children aged 12–24, serotype 6C/6D (11/80; 13.8%), 19B/19C (11/80; 13.8%), and 15B/15C (7/80; 8.8%) were the most prevalent. Serotypes 4 (1/2; 50%) and 15B/15C (1/2; 50%) were the only serotypes detected.

In general, no statistical differences were found in serotype distribution between age groups.

Table 2
Baseline characteristics of healthy children included in this study.

Characteristics	N (%)
Mean age (Months)	11.7 ± 5.9
Gender	
Male	249 (48.3)
Female	266 (51.6)
Sex ratio	0.93
Mode of care	
Home	472 (91.6)
Preschool	43 (8.3)
Siblings	
0	207 (40.2)
1	185 (35.9)
2	102 (19.8)
3	21 (4.1)
Antibioc therapy during the last 3 months	
Yes	158 (30.6)
No	357 (69.3)
Vaccinated by PCV10	
1 Dose	7 (1.4)
2 Doses	326 (63.3)
3 Doses	182 (35.3)

Table 3
Univariatelogistic regression analysis of risk factors associated with *S. pneumoniae* carriage among healthy children in Marrakesh, Morocco.

Risk factors	Carrier N = 223	Not carrier N = 292	OR (95% CI)	*P*-value
Age (6–12 months) (N = 227)	119 (53.4%)	108 (37%)	1.949 (1.367–2.778)	**<0.001**
Male gender (N = 249)	118 (52.9%)	131 (44.9%)	1.381 (0.973–1.959)	0.07
Siblings ≥1 (N = 308)	140 (62.8%)	168 (57.5%)	1.245 (0.871–1.779)	0.229
Previous antibiotic therapy (N = 158)	56 (25.1%)	102 (34.9%)	0.624 (0.424–0.919)	**<0.05**
Fully vaccinated (N = 182)	62 (27.8%)	120 (41.9%)	0.552(0.379–0.802)	0.001

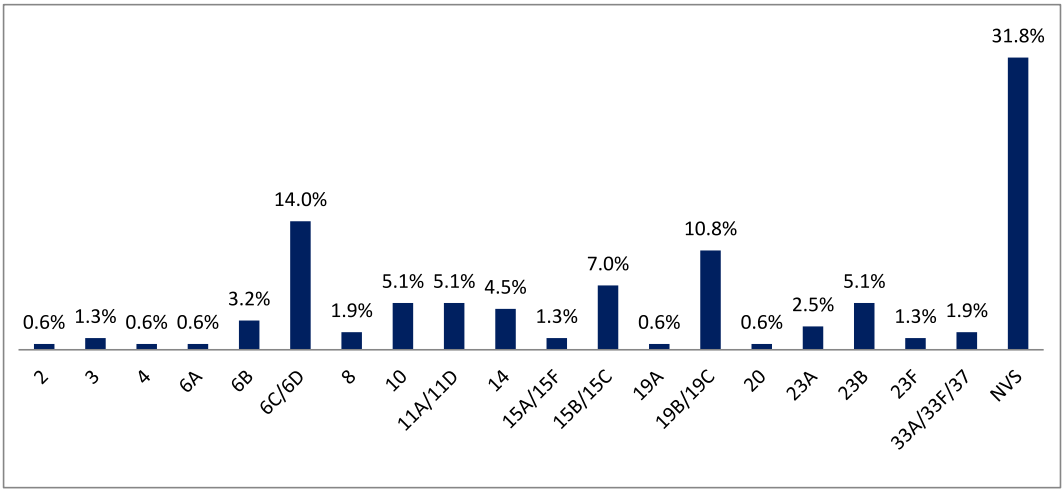

Fig. 1. Serotype distribution of *S. pneumoniae* strains isolated from nasopharyngeal carriage in healthy children after the introduction of PCV10 in Marrakesh, Morocco. *NVS: non-vaccine serotypes, excluding serotypes 2, 3, 6A, 6C/6D, 8, 10, 11A/11D, 15A/15F, 15B/15C, 19B/19C, 20, 23A, 23B, 33A/33F/37.

Table 4
Serotype distribution of 157 isolated S. pneumoniae strains according to age.

Serotypes	All isolates		<12 months		12–24 months		p-value	25–36 months		p-value
	N	%	N	%	N	%		N	%	
2	1	0.6%	0	0%	1	1.4%	0.47	0	0%	0.09
3	2	1.3%	1	1.2%	1	1.4%	0.97	0	0%	0.16
4	1	0.6%	0	0%	0	0%	0.98	1	50%	≤0.001
6A	1	0.6%	0	0%	1	1.4%	0.47	0	0%	0.09
6B	5	3.2%	2	2,4%	3	4.1%	0.61	0	0%	0.26
6C/6D	22	14.0%	10	12.3%	11	15.1%	0.72	0	0%	0.82
8	3	1.9%	0	0%	3	4.1%	0.18	0	0%	0.09
10	8	5.1%	3	3.7%	5	6.8%	0.43	0	0%	0.34
11A/11D	8	5.1%	6	7.4%	2	2.7%	0.18	0	0%	0.58
14	7	4.5%	4	4.9%	3	4.1%	0.65	0	0%	0.43
15A/15F	2	1.3%	0	0%	2	2.7%	0.27	0	0%	0.09
15B/15C	11	7.0%	4	4.9%	7	9.6%	0.31	0	0%	0.43
19A	1	0.6%	1	1.2%	0	0%	0.51	0	0%	0.16
19B/19C	17	10.8%	5	6.2%	11	15.1%	0.10	1	50%	0.11
20	1	0.6%	0	0%	1	1.4%	0.47	0	0%	0.09
23A	4	2.5%	4	4.9%	0	0%	0.14	0	0%	0.43
23B	8	5.1%	6	7.4%	2	2.7%	0.18	0	0%	0.58
23F	2	1.3%	1	1.2%	1	1.4%	0.97	0	0%	0.16
33A/33F/37	3	1.9%	3	3.7%	0	0%	0.20	0	0%	0.34
NVS	50	31.8%	31	38.3%	19	26%	0.28	0	0%	0.43
10-valent	15	9.6%	7	8.6%	7	9.6%	0.92	1	50%	0.18
13-valent	19	12.1%	9	11,1%	9	12.3%	0.93	1	50%	0.26

3.3. Effect of PCV10 on the S. pneumoniae serotypes rate

A Comparison of *S. pneumoniae* serotype distribution between 2008 and 2009 (before vaccination) and 2017–2018 (After vaccination) showed a significant decrease in the rate of *S. pneumoniae* serotypes included in the PCV10 ($p < 0.0001$) and PCV13 ($p < 0.0001$). Conversely, there was a significant rise in NVSs ($p < 0.0001$) (Fig. 2).

3.4. Serotype diversity in carriage and vaccine effectiveness

To assess the effect of PCV10 on the serotype diversity of pneumococcal isolates circulating among healthy children, Simpson's Diversity Index was measured before (2008–2009) and after (2017–2018) the introduction of PCV10 indicating 0.844 (IC95%: 0.712–0.976) and 0.938 (IC95%: 0.791–1.985), respectively. The serotype diversity for carriage was significantly higher in 2017–2018 than it was in 2008–2009 ($p < 0.001$). The RR of carrying VSs was 0.140, indicating a low probability of carrying these serotypes after the introduction of PCV10. This result affirms that vaccination has reduced the prevalence of rate of VSs, demonstrating an effectiveness of 89.6%. (Table 5).

3.5. Antibiotic resistance of S. pneumoniae isolates

The antibiotic resistance profile was checked for only 64 *S. pneumoniae* isolates due to budget constraints. Among the 64 *S. pneumoniae* strains subjected to antimicrobial susceptibility testing, 17 (26.6%) were found to be pneumococci non-susceptible to penicillin G (PNSP), with 13 (20.3%) classified as intermediate and 4 (6.3%) as resistant. Additionally, 6 out of 64 (9.4%) and 8 out of 64 (12.5%) were resistant to amoxicillin and cefotaxime, respectively. An important prevalence of resistance was also found for erythromycin, clindamycin, tetracycline, and cotrimoxazole, as follow: 18 out of 64 (28.1%), 9 out of 64 (14.1%), 8 out of 64 (12.5%), and 8 out of 64 (12.5 %). However, a low prevalence of resistance was observed against chloramphenicol, accounting for 3.1% (2 out of 64) (Fig. 3).

The distribution of *S. pneumoniae* serotypes by penicillin G resistance is presented in Fig. 4. The PNSP strains were associated with serotypes 14, 23F, 6C/6D, 6B and 19B/19C.

3.6. Effect of PCV-10 on S. pneumoniae penicillin G resistance

In general, there was a significant statistical difference in the frequency of PNSP strains before and after the generalization of PCV10 in carriage isolates (38.7% before PCV10 versus 26.6% after PCV10) ($p = 0.02$) (Table 5).

4. Discussion

In this study concerning the nasopharyngeal carriage of *S. pneumoniae*, we assessed changes in serotype distribution and penicillin resistance rates among healthy children aged 6–36 months in Marrakesh, Morocco, following the generalization of PCV10. Unlike previous studies that included children with both invasive and non-invasive pneumococcal diseases [24,25], this study exclusively focused on healthy children.

Several studies have reported a significant decrease in the nasopharyngeal carriage rate of *S. pneumoniae* among children after the generalization of the pneumococcal vaccine [26]. However, there was no available data on the rate of *S. pneumoniae* carriage in healthy children in Morocco after the generalization of PCV10. In this study, we found that the carriage rate in healthy children ≤3 years was 43.3%, which falls between the *S. pneumoniae* carriage rates of 9.4% and 83.6% reported in different countries worldwide [27,28].

Regarding the carriage rate according to age, our study revealed that children aged less than 12 months are more likely to carry

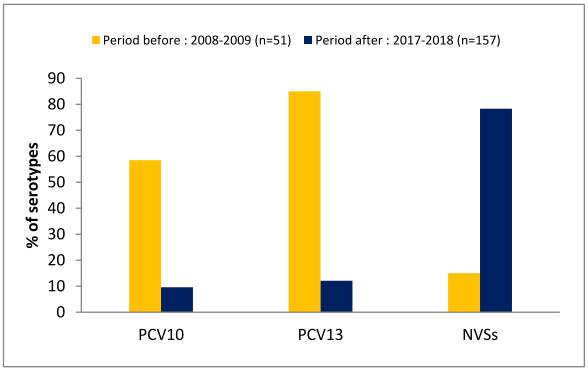

Fig. 2. *S. pneumoniae* serotypes rate (PCV10, PCV13, and NVSs) before (2008–2009) and after (2017–2018) the generalization of PCV10 in Marrakesh, Morocco.

Table 5
Distribution of PNSP strains before and after PCV10 generalization in Marrakesh, Morocco.

Period	Penicillin resistance			P
	Overall	Resistant	Intermediate	
Pre-vaccination	58/150 (38.7%)	7/58 (12.1%)	51/58 (87.9%)	0.02
Post-vaccination	17/64 (26.6%)	4/17 (23.5%)	13/17 (76.5%)	

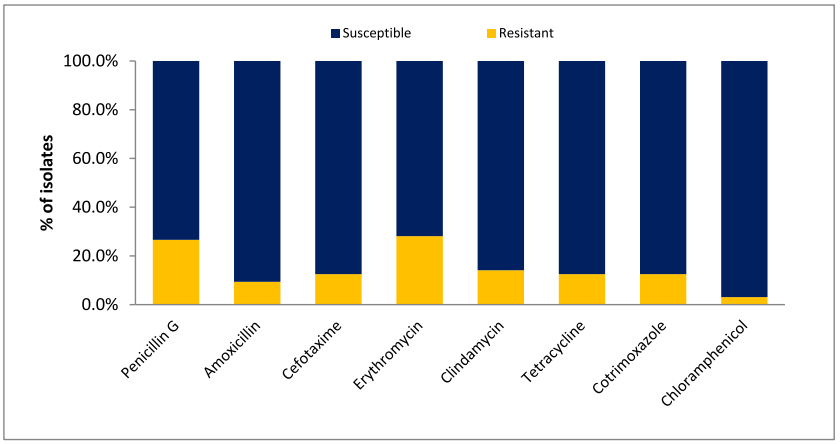

Fig. 3. Antibiotic resistance of 64 *S. pneumoniae* strains isolated among healthy children five years after the introduction of PCV10. *Resistant: Intermediate + Resistant.

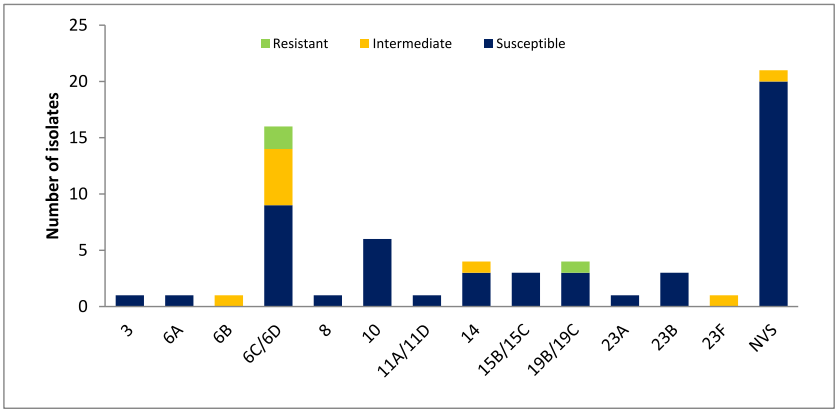

Fig. 4. Distribution of *S. pneumoniae* serotypes according topenicillin resistance.

S. pneumoniae than older age groups. This aligns with the well-established fact that the acquisition of *S. pneumoniae* is most common and at its peak during the first months of life. Generally, the first pneumococcal acquisition in childhood occurs at around 6 months of age [29,30].

On the other hand, we found a low prevalence of VSs following the generalization of PCV10. This low rate of pneumococcal VSsis consistent with the findings of previous studies conducted after the generalization of pneumococcal vaccination, which confirms the effectiveness of the PCV [10,27,31]. In Ethiopia, Sime et al. reported that the prevalence of VSs was 7.1% after PCV10 [32]. High-income countries with robust serotype surveillance, such as the United States, the United Kingdom, and Australia, have reported nearly complete elimination of pneumococcal carriage due to VSs across most population groups following PCV introduction. However, in African settings, some residual VS carriage has been observed post-vaccination, despite high vaccine coverage [33].

When comparing carriage rates before and after the introduction of PCV10 in Marrakesh, Morocco, we observed a decline in the carriage rate of PCV10 serotypes among healthy children at 6–36 months by 86%. This remarkable decline surpasses the 67.2% reduction reported in Fiji [34] reflecting the effectiveness of pneumococcal carriage among healthy children. Regarding the distribution of *S. pneumoniae* serotypes, four PCV10 serotypes were detected, including serotypes: 4, 6B, 14, and 23F. It's worth noting that in the period before the introduction of PCV10 (2007–2008), serogroups 1, 4, 6, 9, 14, 18, 19A, 19F, and 23 were detected in

Marrakesh, Morocco [20]. This suggests that even after the generalization of PCV10 on a 2 + 1 schedule, the carriage of these serotypes, even at lower rates, remains possible among healthy children in Marrakesh, Morocco.

The reduction in VSs carriage has led to an increase in NVSs carriage among healthy children. In this study, the most commonly detected NVSs were serotypes: 6C/6D, 19B/19C, and 15B/15C. The prevalence of these serotypes follows the changes reported in studies conducted in Bangladesh [35], the Netherlands [36], and Nigeria [37] following the introduction of PCV10. Additionally, the carriage of serotype 15 is known to be more prevalent in the PCV era [38]. Some of the NVSs detected in our study are covered by the PCV13, PCV15, and PCV20. The phenomenon of serotype replacement is highly complex as it is highly dynamic and varies geographically [39]. This phenomenon could potentially reduce the effectiveness of current vaccine strategies and must be considered in the development of future alternatives.

The rate of PNSP strains during the period between 2017 and 2018 was 26.6%, marking a significant decrease compared to the rate of 38.7% reported during the period between 2008 and 2009. A meta-analysis assessing the impact of PCV10 and PCV13 on PNSP reported a notable reduction in the rate of PNSP among under-five children in various countries [40]. In our study, we revealed that the rates of PNSP were 100%, 44%, 25%, and 25% for serotypes 6B, 6C/6D, 14, and 19B/19C, respectively. Notably, in China, PNSP was commonly detected among serotypes 23F (81.4%), 19F (70.7%), 19A (97.6%), 14 (77.8%), and 6A (66.7%) isolated from invasive infections [41]. The persistence of PNSP in healthy carriers following the introduction of PCV10 is likely associated with high usage of antibiotics, which may exert significant pressure driving *S. pneumoniae* strains to acquire antibiotic resistance [42].

This study identified important resistance rates of *S. pneumoniae* against amoxicillin, cefotaxime, erythromycin, clindamycin, tetracycline, cotrimoxazole, and chloramphenicol, accounting for 9.4%, 12.5%, 28.1%, 14.1%, 12.5%, 12.5%, and 3.1%, respectively. Notably, these rates are lower than those reported in other parts of the world. In a study conducted in Korea between 2017 and 2019, the amoxicillin resistance rate was 27.7% [43]. Similarly, in China, pneumococcal resistance rates were reported as 96%, 95.7%, and 91.7% for erythromycin, clindamycin, and chloramphenicol, respectively [44]. Additionally, in a South African birth cohort, 41% of *S. pneumoniae* strains isolated from community children were resistant to cotrimoxazole [45]. In contrast, none of our pneumococcal isolates showed resistance to levofloxacin (0%), while in Canada, the rate was 1% [46].

5. Conclusion

It is crucial to note that even though the resistance rates reported in this study are lower than in other countries, the emergence of antibiotic-resistant pneumococcal strains remains a growing public health concern. Antibiotic resistance can significantly limit treatment options and increase the risk of severe and prolonged illness. Therefore, promoting appropriate antibiotic use and stewardship is of paramount importance to slow the spread of antibiotic-resistant strains and preserve the effectiveness of antibiotics for future generations. This study provides information on the impact of PCV10 on nasopharyngeal carriage among healthy Moroccan children. Our findings reveal a notable shift in the distribution of *S. pneumoniae* serotypes, characterized by a decrease in VSs and an increase in NVSs. Additionally, a reduction in the prevalence of PNSP was also observed. These findings underscore the need for ongoing nasopharyngeal carriage surveillance to assess vaccine effectiveness on serotype distribution and penicillin resistance among healthy children.

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Ethical approval and consent to participate

This study was ethically approved by the ethics committee of Mohammed VI University Hospital Center, Marrakesh, Morocco (19/07/20,223). Furthermore, written informed consent was obtained and signed by all participants. The study was done anonymously. All methods were performed in accordance with the relevant guidelines and regulations.

Data availability statement

Data will be made available upon request to the corresponding author.

Additional files

Not applicable.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Karima Warda: Writing – original draft, Investigation, Data curation. **Sara Amari:** Investigation, Formal analysis. **Majda**

Bourredane: Investigation. **Youssef Elkamouni:** Methodology. **Lamiae Arsalane:** Methodology. **Said Zouhair:** Supervision, Conceptualization. **Mohamed Bouskraoui:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

CI	Confidence interval
CNA	Colistin nalidixic acid
EUCAST	European committee on antimicrobial susceptibility testing
IPD	Invasive pneumococcal disease
MIC	Minimum inhibitory concentration
NIP	National immunization program
NVS	Non-vaccine serotype
S. pneumoniae	*Streptococcus pneumoniae*
PCR	Polymerase chain reaction
PCV	Pneumococcal conjugate vaccine
PNSP	Penicillin-non-susceptible pneumococci
RR	Rate Ratio
VS	Vaccine serotype

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