JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Streptococcus pneumoniae among the children of Aden, Yemen: a cross-sectional report of post-pneumococcal conjugate vaccine

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Abstract

Introduction: *Streptococcus pneumoniae* cause a significant global health challenge. We aimed to determine nasopharyngeal carriage, serotypes distribution, and antimicrobial profile of pneumococci among the children of Aden.

Methodology: A total of 385 children, aged 2-17 years, were included. Asymptomatic samples were randomly collected from children in selected schools and vaccination centers. Symptomatic samples were obtained from selected pediatric clinics. The nasopharyngeal swabs were tested for pneumococci using culture and real time polymerase chain reaction (RT-PCR). Serotyping was done with a pneumotest-latex kit and antimicrobial susceptibility was tested by disc diffusion and Epsilometer test.

Results: The total pneumococcal carriage was 44.4% and 57.1% by culture and RT-PCR, respectively. There was a statistically significant association between carriage rate and living in single room (OR = 7.9; $p = 0.00001$), sharing a sleeping space (OR = 15.1; $p = 0.00001$), and low monthly income (OR = 2.02; $p = 0.007$). The common serotypes were 19, 1, 4, 5, 2, and 23. The proportion of non-pneumococcal conjugate vaccine (non-PCV13) serotypes was 24%. Pneumococci were resistant to penicillin (96.5%), cefepime (15.8%), ceftriaxone (16.4%), and amoxicillin-clavulanate (0%). Erythromycin, azithromycin, and doxycycline had resistance rates of 48%, 31%, and 53.3%, respectively.

Conclusions: A high pneumococcal carriage rate was observed in Yemeni children, particularly in low-income households and shared living conditions. There was significant penicillin resistance at meningitis breakpoint. Furthermore, non-PCV13 serotypes were gradually replacing PCV13 serotypes. The findings underscore the urgent need for enhanced surveillance and stewardship to improve vaccination and antibiotic policies in Yemen.

Key words: pneumococci; Yemen; serotypes; children; vaccine; antimicrobial.

J Infect Dev Ctries 2024; 18(4):579-586. doi:10.3855/jidc.18935

(Received 22 July 2023 – Accepted 16 November 2023)

Introduction

Streptococcus pneumoniae (pneumococci) are upper airway commensals and have a complex relationship with their host [1,2]. Their carriage increases from 9% in infancy to 43% in childhood due to many factors [3,4]. Asymptomatic carriage in the nasopharynx is crucial for transmission and precedes invasive pneumococcal diseases [1,2,5]. In 2019, pneumococci were ranked as the fourth leading bacteria contributing to mortality from antimicrobial resistance (AMR) [6]. Studies indicate that implementing the pneumococcal conjugate vaccine (PCV13) in the Middle East can reduce pneumococcus mortality by approximately 38% [7]. There were considerable variations in the vaccination covering rates of PCV13 among the children of neighboring countries: 66.7% in Bahrain, 37.2% in Oman, and 75-78.3 in Qatar [8,9,10]. Additionally, recent evidence suggests that the PCV13 serotypes 1, 3, 5, 6A, 7F, and 19A did not provide effective protection against invasive pneumococcal disease (IPD) in Kuwait [11]. Although PCV saves lives and reduces the occurrence of AMR strains, it also leads to the emergence of non-PCV strains with AMR [12,13]. In Yemen, 44.3% of the children were reported to have respiratory diseases and it is a common illness [14]. The isolation rate of *Streptococcus pneumoniae* (*S. pneumoniae*) among Yemeni children with bacterial meningitis was 34.9% [15]. Furthermore, pneumococci

accounted for 27% of the isolated Gram-positive bacteria causing otitis media in Yemeni children [16]. Moreover, pneumococcal carriage was reported to be 35% in Middle Eastern countries [17]. Despite global concerns about AMR, there is a high level of antimicrobial misuse in Yemen [18,19]. Pneumococcal disease represents challenges in western Asia, including Yemen, that require a comprehensive approach to combat resistance and ensure low rates of IPD in children [20].

Methodology

Sample collection

A cross-sectional investigation was conducted in Aden City, Yemen from January to end of July 2022, targeting 385 symptomatic and asymptomatic children, aged 2-17 years. The asymptomatic samples were randomly collected from schools and vaccination centers, while the symptomatic children were selected from Al-Sadaqa teaching hospital and Al-Basateen healthcare center. Clinical signs were assessed by physicians based on the British Thoracic Society guidelines [21], and a socioeconomic questionnaire was administered. Children who took antibiotics within two weeks or lived outside Aden City were excluded.

Laboratory procedures

Nasopharyngeal swabs collected in skim milk-tryptone-glucose-glycerin (STGG) transport media were transferred to the National Center of Public Health Laboratories, Aden City. *S. pneumoniae* was identified from swabs following the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) guidelines, and with the use of *S. pneumoniae* ATCC 49619 for quality control [22,23]. The confirmed colonies were cultured in Serum Todd Hewitt broth (HIMEDIA, Mumbai, India) for serotyping using the Pneumotest-Latex Kit (Staten Serum Institute, Copenhagen, Denmark) [24]. The frozen STGG specimens were used for molecular detection by real-time polymerase chain reaction (RT-PCR) (Blackbio Biotech, Bhopal, India). Antimicrobial susceptibility testing was performed using the Kirby-Bauer method on Mueller-Hinton agar (HIMEDIA, Mumbai, India), supplemented with sheep blood for screening. The Epsilometer Test was employed to determine the minimum inhibitory concentration for resistant antibiotics [25,26].

Ethical approval

This study received approval from the Research and Ethics Committee at the University of Aden, Yemen (Research Code REC-98-2021), and informed consents were obtained from the parents of the children prior to collecting samples.

Statistical analysis

Data analysis included calculating carriage rates, serotype distribution, and antimicrobial resistance using IBM SPSS Statistics for Windows, Version 22.0 software and EZ SPSS Tutorials. Univariate analysis using the Chi-square (χ^2) test was used to assess differences in antibiotic resistance. Odds ratios (OR) with 95% confidence interval (CI) were calculated to evaluate the association between carriage rate and associated risk factors, with $p \leq 0.05$ considered statistically significant.

Multiple drug resistance (MDR) was calculated using the formula MDR = a/b, where 'a' represents the number of resistant antibiotics, and 'b' represents the total number of tested antibiotics.

Results

The study targeted 385 symptomatic and asymptomatic children. Females represented 51.7% of the sample and the remaining participants were males. The average age was 8.549 years, with a standard deviation of 4.245 (Table 1). The overall carriage of *S. pneumoniae* was 44.4% by culture and 57.1% by RT-PCR. In the symptomatic group, the rates were 45.9% by culture and 59.5% by RT-PCR; while in the asymptomatic cohort, the rates were 43% by culture and 55% by RT-PCR. The rates varied between the different age groups. Using the culture technique, the rates for

Table 1. Demographic characteristics of study cohort. Age group, mean, and standard deviation for participant age, as well as the nasopharyngeal carriage rate of *S. pneumoniae* grouped by gender, for the entire cohort in the city of Aden.

Age group (Years)	Frequency (%)	Minimum	Maximum	Mean	Standard deviation
2 — 6	142(36.8)				
7—11	135(35.1)	2 years	17 years	8.549 years	4.245 years
12—17	108(28.1)				
Total	385(100)				
Carriage rate of total cohort and gender					
Gender	**Carriage by culture (%)**	***p* value**		**Carriage by RT-PCR (%)**	***p* value**
Female	90(45.2)			115 (58)	
Male	81(43.5)	0.410		105 (56.5)	0.436
Total	171(44.4)			220 (57.1)	

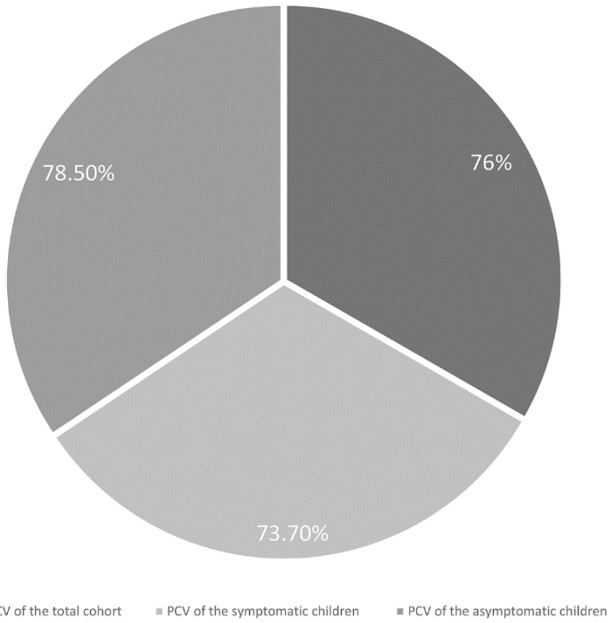
the age groups of 2-6 years, 7-11 years, and 12-17 years were 49.3%, 54.8%, and 29.8%, respectively in the symptomatic cohort. Among the asymptomatic children, the rates were 50.7%, 31.5%, and 48.2%, respectively for corresponding age groups. RT-PCR detected overall carriage rates of *S. pneumoniae* as 59.2%, 67.7%, and 50% for the symptomatic cohort in the respective age groups. Conversely, the rates for the asymptomatic group were 64.8%, 43.8%, and 57.2%, respectively (Table 1, 2 and 3).

The vaccination rate among the children was 76.1% (95% CI, 71.6–80.1%). The vaccination rate was 73.7% in the symptomatic cohort and 78.5% in the asymptomatic group (Figure 1). Common observations in the symptomatic group included fever (91.4%), shortness of breath (82.2%), rapid breathing (82.2%), cough (89.7%), wheezing (18.8%), respiration rate over 20 breaths per minute (82.7%), pulse rate over 120 beats per minute (93.5%), and oxygen saturation below 94% (17.8%) (Figure 2A). In the symptomatic cohort, elevated white blood cell count (> 11,000/μL) was present in 39.9% children, neutrophil count (> 70%) in 33% children, lymphocyte count (> 40%) in 29.7% children, and C-reactive protein was positive in 39.5% children (Figure 2B).

Pneumococcal carriage was not associated with gender or other characteristics in our research. However, carriage rate was significantly linked with children from households with a single room (OR = 7.9, p = 0.00001), children sharing sleeping space with family members (OR = 15.1, p = 0.00001), and those with low monthly wages (OR = 2.02, p = 0.007) (Table 4).

The total covering rate of PCV13 was reported at 76% in the current investigation. It was found that

Figure 1. Vaccination rate of the cohort, including the rates in the entire cohort, in symptomatic cohort (185 children) and asymptomatic cohort (200 children) PCV, pneumococcal conjugate vaccine



80.2% of serotypes were related to PCV13 from asymptomatic children, compared to 71.8% in the symptomatic group. The prominent serotypes among asymptomatic and symptomatic children were 19 (15.1%, 8.2%), 1 (12.8%, 11.8%), 4 (9.3%, 12.9%), 5 (12.8%, 8.2), 2 (9.2%, 7.1%) 23 (8.1%, 7.1%), 3 (7.1%, 7.1%), and 6 (5.7%, 7.1%) respectively. The non-PCV-13 serotypes accounted for 19.8% of isolates in asymptomatic group, while in symptomatic children, they represented 28.2% of isolates. The prevalent non-PCV-13 serotypes among the total sample were 2, 22, 15, 8, and 20 (Figure 3).

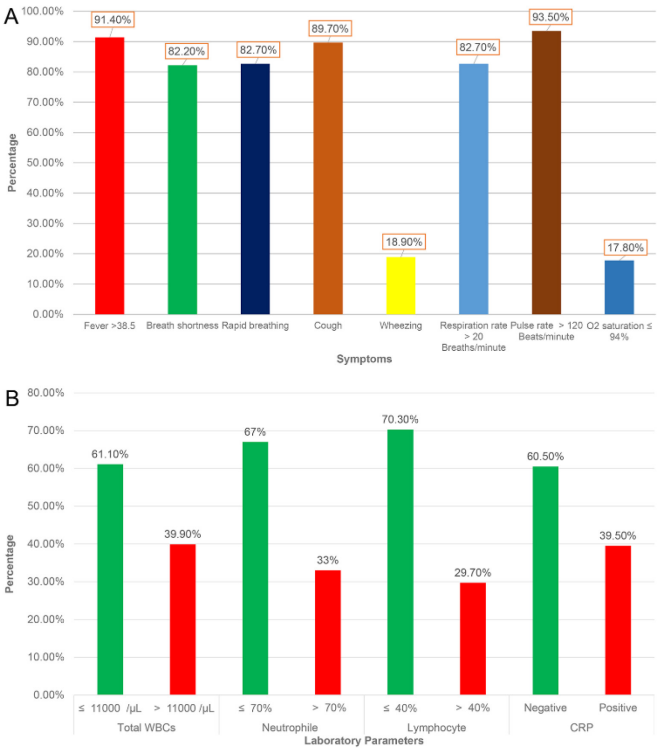
Table 2. Prevalence of *S. pneumoniae* carriage in asymptomatic and symptomatic children of the city of Aden, grouped by age, and tested with the conventional culture method.

Age group (years)	Carriage among symptomatic children			Carriage among asymptomatic children		
	Negative (%)	Positive (%)	Total (%)	Negative (%)	Positive (%)	Total (%)
2— 6	36 (50.7)	35 (49.3)	71 (38.4)	35 (49.3)	36 (50.7)	71 (35.5)
7— 11	28 (45.2)	34 (54.8)	62 (33.5)	50 (68.5)	23 (31.5)	73 (36.5)
12— 17	36 (69.2)	16 (29.8)	52 (28.1)	29 (51.8)	27 (48.2)	56 (28.0)
Total	100 (54.1)	85 (45.9)	185 (100)	114 (57.0)	86 (43.0)	200 (100)

Table 3. Nasopharyngeal carriage rate of *S. pneumoniae* among the different age groups in asymptomatic and symptomatic children of Aden City, determined using the real time polymerase chain reaction (RT-PCR) technique.

Age group (years)	Carriage among symptomatic children			Carriage among asymptomatic children		
	Negative (%)	Positive (%)	Total (%)	Negative (%)	Positive (%)	Total (%)
2— 6	29 (40.8)	42 (59.2)	71 (38.4)	25 (32.2)	46 (64.8)	71 (35.5)
7— 11	20 (32.3)	42 (67.7)	62 (33.5)	41 (56.2)	32 (43.8)	73 (36.5)
12— 17	26 (50)	26 (50)	52 (28.1)	24 (42.9)	32 (57.2)	56 (28.0)
Total	75 (40.5)	110 (59.5)	185 (100)	90 (45)	110 (55)	200 (100)

Figure 2. Clinical picture and laboratory parameters of symptomatic group.



A: Percentage of clinical manifestations of symptomatic children included in the study; **B:** Total white blood cells (WBCs), differential count of neutrophils and lymphocytes, and the results of the C-reactive protein (CRP) screening test among symptomatic children.

Figure 3. The various serotypes of pneumococcus identified from asymptomatic and symptomatic children of Aden city. A total of 171 pneumococcal isolates were identified, and their serotypes were determined using the Pneumotest-Latex technique.

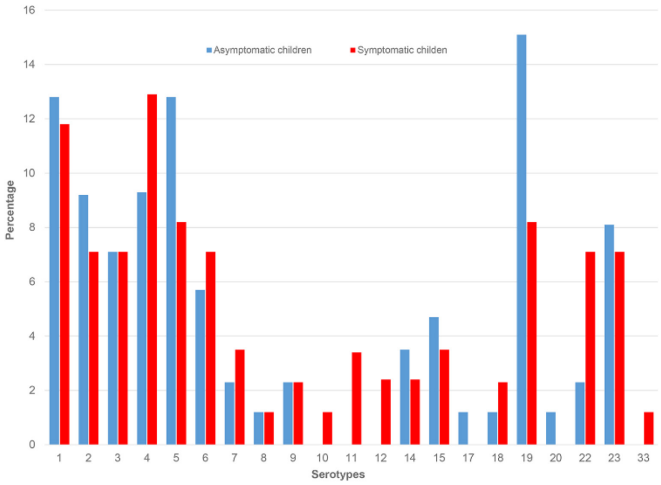


Table 4. Factors associated with nasopharyngeal carriage of *S. pneumoniae* among the total children cohort of the city of Aden.

Associated Risk factors		Frequency (%)	*S. Pneumonia* carriage (culture)		*p* value	OR (95% CI)
			Negative (%)	Positive (%)		
Gender	Male	186 (48.3)	105 (56.5)	81 (43.5)	0.74	0.93 (0.62–1.4)
	Female	199 (51.7)	109 (54.8)	90 (45.2)		
Age	≤ 9	227 (59)	116 (51.1)	111 (49.9)	0.03	1.56 (1.03– 2.4)
	≥ 10	158 (41)	98 (62)	60 (38)		
No. of siblings < 6 years	0	78 (20.3)	28 (36)	50 (64)	0.088	1.6 (0.9–2.6)
	≥ 1	307 (79.7)	143 (46.6)	164 (53.4)		
No. of rooms per family	1	134 (34.8)	33 (24.6)	101 (75.4)	0.00001	7.9 (4.9–12.8)
	≥ 2	251 (61.1)	181 (75.2)	70 (24.8)		
Sleeping in one room	Yes	286 (74.3)	123 (43)	163 (57)	0.00001	15.1 (7.1–32.2)
	No	99 (25.7)	91 (91.9)	8 (8.1)		
Domestic animals	Yes	40 (10.4)	22 (55)	18 (45)	0.93	1.03 (0.5–1.9)
	No	345 (89.6)	192 (55.7)	153 (44.3)		
Smoker in family	Yes	159 (41.3)	81 (50.9)	78 (49.1)	0.12	1.4 (0.9–2.1)
	No	226 (58.7)	133 (44.3)	93 (55.7)		
Using incense	Yes	168 (43.6)	93 (55.4)	75 (44.6)	0.93	1.02 (0.68–1.5)
	No	217 (56.4)	121 (55.8)	96 (44.2)		
Using mosquito coil	Yes	171 (44.4)	95 (55.6)	76 (44.4)	0.99	1.0 (0.67–1.5)
	No	214 (56.6)	119 (55.6)	95 (44.4)		
Monthly income ≥ $100	No	302 (78.4)	157 (52)	145 (48)	0.007	2.02 (1.2–3.4)
	Yes	83 (21.6)	57 (68.7)	26 (31.3)		

Table 5. Antimicrobial susceptibility patterns of *S. pneumoniae* isolated from symptomatic and asymptomatic children's cohorts of the city of Aden, as determined by the disc diffusion method, and the differences in antibiotic resistance between the two study groups.

Antimicrobial agents	Zone diameter breakpoints in mm	Symptomatic	Asymptomatic	Significant difference in χ^2	*p* value
		Frequency (%)	Frequency (%)		
Oxacillin	≥ 20mm (Sensitive)	2 (2.4)	4 (4.7)	0.667	0.41
	≤ 20mm (Resistant)	83 (97.6)	82 (95.3)		
Erythromycin	≥ 21mm (Sensitive)	25 (29.4)	22 (25.6)		
	16-20mm (intermediate)	16 (18.8)	26 (30.2)	1.27	0.25
	≤ 20mm (Resistant)	44 (51.8)	38 (44.2)		
Azithromycin	≥ 18mm (Sensitive)	43 (50.6)	49 (57.0)		
	14-17mm (intermediate)	12 (14.1)	14 (16.3)	1.46	0.23
	≤ 13mm (Resistant)	30 (35.3)	23 (26.7)		
Doxycycline	≥ 28mm (Sensitive)	17 (20)	21 (24.4)		
	25-27 (intermediate)	20 (23.5)	22 (25.6)	0.72	0.40
	≤ 24mm (Resistant)	48 (56.5)	43 (50.0)		

Low susceptibility rates (SRs) were observed in isolated pneumococci. The SRs were 2.4% and 4.7% for oxacillin, 29.4% and 25.6% for erythromycin, 50.6% and 57.0% for azithromycin, and 20% and 24.4% for doxycycline, respectively in the symptomatic and asymptomatic groups. There was no significant difference in antimicrobial resistance between the isolates from both cohorts (Table 5). Penicillin resistance (meningitis breakpoint) among the entire cohort was 96.5%, whereas it was 95.3% among asymptomatic children and 97.6% in the symptomatic cohort. In contrast the penicillin-resistance (non-meningitis breakpoint) among the total cohort was 13.3%. Moreover, cefepime resistance (meningitis breakpoint) was 15.8% of the total cohort, while it was zero for the non-meningitis breakpoint. Likewise, ceftriaxone resistance (meningitis breakpoint) was 16.4% of the total cohort, while it was zero at the non-meningitis breakpoint among the asymptomatic group and 1.2% of the symptomatic children. Moreover, the sensitivity of pneumococci against amoxiclav was 100%. There was no significant difference in antimicrobial resistance during investigations among groups of study. Nonetheless, a significant difference ($\chi^2 = 3.9$; $p = 0.04$) was found between the resistance to penicillin (non-meningitis break points) in the two groups of children (Table 6). MDR was at 85.7% for the seven tested antibiotics (meningitis breakpoint) and 71.4% for the non-meningitis breakpoint.

Discussion

In the current study, RT-PCR detected *S. pneumoniae* more frequently (57.1%) than culture (44.4%) in nasopharyngeal samples of symptomatic and asymptomatic children in the city of Aden. This was consistent with findings of previous studies [27,28]. The nasopharyngeal carriage of pneumococci among children of Aden was higher compared to the city of Sana'a [29]. Additionally, this finding was higher than the documented result in Saudi Arabia (13%) [30]. It was consistent with results reported from low- and middle-income Middle Eastern countries, and higher than that found in developed countries [12,17,27,31,32]. We observed a high possibility of cross-infection among children who shared a room for sleeping or belonged to households that had only one room per family, and had a low income, as documented in previous research [4,33,34].

The vaccination rate among the study population was disappointing; likely due to the ongoing war and may be linked to an increase in the prevalence of acute pneumococcal meningitis [35,36]. Furthermore, our study showed a high prevalence of PCV13 serotypes in children 12 years after its introduction. This was in line with previous research findings [28,37]. Serotypes 19, 1, 4, 5 and 2 were the common serotypes in the study sample. This result was aligned with findings of a previous study and data from Gulf Cooperation Council (GCC) [8,9,29]. There was slight replacement of PCV13 serotypes in our sample with non-PCV13

Table 6. Pneumococcal resistance and differences in antibiotic non-susceptibility rates using the Epsilometer test in the two study groups of children in the city of Aden.

Antibiotics		Non-susceptibility rate among 85 isolates (Symptomatic children) (%)	Non-susceptibility rate among 86 isolates from (Asymptomatic children) (%)	Significance of difference in χ^2	*p* value
Penicillin	(Meningitis Break Points)	83 (97.6)	82(95.3)	0.667	0.41
	(Non-Meningitis Break Points)	7(8.2)	16(18.6)	3.94	0.04
Cefepime	(Meningitis Break Points)	10(11.8)	17(19.8)	2.05	0.15
	(Non- Meningitis Break Points)	0	0	-	-
Ceftriaxone	(Meningitis Break Points)	10(11.8)	18(20.9)	2.62	0.11
	(Non- Meningitis Break Points)	1(1.2)	0	-	-
Amoxiclav		0	0	-	-

serotypes, as seen elsewhere [32,38]. This replacement should be given serious consideration in light of regional reports about the role of non-PCV13 serotypes in IPD, and the partial failure of certain PCV13 serotypes to provide full protection [11,13]. The PCV13 coverage rate in Aden city (76%) was consistent with data from GCC and other low- and middle-income countries [9,39].

Our study revealed significant AMR, indicating widespread antibiotic misuse in this community [18,19,40]. This finding underscores the role of such misuse in the development of pneumococcal resistance, as demonstrated in recent pneumococcal research [41]. A higher penicillin resistance (meningitis breakpoint) was found in the current study (96.5%), and it was slightly higher than that reported in the previous study [29]. This finding was in line with recently reported data from Jordan (94.4-95.8%) and Iran (95.3%) [12,42,43]. On other hand, the rate was higher than that found in GCC [44]. The penicillin (non-meningitis) MIC was consistent (13.5%) with the results of a previous study (15%) conducted in Yemen on isolates from children with otitis media [16]. The rates of non-susceptibility to cefepime and ceftriaxone in the entire children cohort were consistent with those reported in GCC and India [39,45]. However, the sensitivity of pneumococcal isolates to cefepime and ceftriaxone, using non-meningitis breakpoint, differed from previous findings for otitis isolates [16]. Additionally, the sensitivity of pneumococcal isolates to amoxiclav was similar to a previous Yemeni study [46]. The resistance to macrolides was higher in our study than previous Yemeni reports [29, 46], but consistent with findings in the Middle East and other developing countries [17, 47, 48]. The resistance profile of pneumococci against doxycycline was consistent with that established in low and middle-income countries [47].

Conclusions

The current study revealed a high pneumococcal carriage rate among children, especially in those sharing a single room, living in one-bedroom households, or with low income due to war-related socioeconomic consequences. Approximately three quarters of pneumococcal isolates were included under the coverage of PCV13. However, we observed a significantly higher rate of penicillin resistance, as well as alarming MDR and slight replacement with non-PVC 13 serotypes. These findings underscore the need for strict antimicrobial dispensing systems to reduce resistance rates, update prescribing guidelines for invasive pneumococcal infections. In addition, more research attention is needed in this field and the urgent need for national surveillance to evaluate the impact of immunization on pneumococcal serotypes, and antimicrobial susceptibility patterns should be highlighted.

Acknowledgements

We express our sincere gratitude to the Faculty of Medicine and Health Sciences, University of Aden, Yemen, for granting us permission to conduct this research study at the National Center of Public Health Laboratories -Aden, Yemen, in collaboration with Lovely Professional University, India.

Authors' contributions

YMM, AMAH, SS; proposed the study; AK, HAS, YMM: edited the manuscript; SS: performed intensive editing and reframing; AS: conducted statistical analysis and reviewed the study; SS: finalized and validated the manuscript.

References

1. Al-Lahham A (2021) Prevalence of pneumococcal carriage among Jordanian infants in the first 6 months of age, 2008-2016. Vaccines 9: 1283. doi: 10.3390/vaccines9111283.
2. Weiser JN, Ferreira DM, Paton JC (2018) *Streptococcus pneumoniae*: transmission, colonization and invasion. Nat Rev Microbiol 16: 355-367. doi: 10.1038/s41579-018-0001-8.
3. Syrjänen RK, Kilpi TM, Kaijalainen TH, Herva EE, Takala AK (2001) Nasopharyngeal carriage of *Streptococcus pneumoniae* in Finnish children younger than 2 years old. J Infect Dis 184: 451-459. doi: 10.1086/322048.
4. Abateneh DD, Shano AK, Dedo TW (2020) Nasopharyngeal carriage of *Streptococcus pneumoniae* and associated factors among children in Southwest Ethiopia. Open Microbiol J 14: 171-178. doi: 10.2174/1874285802014010171.
5. Simell B, Auranen K, Käyhty H, Goldblatt D, Dagan R, O'Brien KL (2012) The fundamental link between pneumococcal carriage and disease. Expert Rev Vaccines 11: 841-855. doi: 10.1586/erv.12.53.
6. Antimicrobial Resistance Collaborators (2022) Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 399: 629-655. doi: 10.1016/S0140-6736(21)02724-0.
7. Ezoji K, Yaghoubi M, Nojomi M, Mahmoody S, Zahraie SM, Moradi-Lakeh M, Tabatabaei SR, Karimi (2019) Cost-effectiveness of introducing the pneumococcal conjugate vaccine for children under 5 years in the Islamic Republic of Iran. East Mediterr Heal J 25: 686-697. doi: 10.26719/emhj.19.039.
8. Haifa Al-Muhtaresh A, Bindayna KM (2020) The prevalence of antimicrobial resistance and serotypes of *Streptococcus pneumoniae* in the Kingdom of Bahrain. J Pure Appl Microbiol 14: 133-140. doi: 10.22207/JPAM.14.1.14.
9. Al-Jardani A, Al Rashdi A, Al Jaaidi A, Al Buluishi M, Al Mahrooqi S, Al Abri S, Al Maani A, Kumar R (2019) Serotype distribution and antibiotic resistance among invasive *Streptococcus pneumoniae* from Oman post 13-valent vaccine

introduction. Int J Infect Dis 85: 135-140. doi: 10.1016/j.ijid.2019.05.027.
10. Taj-Aldeen SJ, Shamseldin Elshafie S (2016) Emerging resistant serotypes of invasive *Streptococcus pneumoniae*. Infect Drug Resist 9: 153-160. doi: 10.2147/IDR.S102410.
11. Mokaddas E, Syed S, Albert MJ (2021) The 13-valent pneumococcal conjugate vaccine (PCV13) does not appear to provide much protection on combined invasive disease due to the six PCV13 non-PCV7 serotypes 1, 3, 5, 6A, 7F, and 19A in Kuwait during 2010-2019. Hum Vaccines Immunother 17: 4661-4666. doi: 10.1080/21645515.2021.1968216.
12. Al-Lahham A (2020) Multicenter study of pneumococcal carriage in children 2 to 4 years of age in the winter seasons of 2017-2019 in Irbid and Madaba governorates of Jordan. PLoS One 15: 1-14. doi: 10.1371/journal.pone.0237247.
13. Reslan L, Finianos M, Bitar I, Moumneh MB, Araj GF, Zaghlout A, Boutros C, Jisr T, Nabulsi M, Kara yaccoub G, Hamze M, Osman M, Raad EB, Hrabak J, Ghassan M. Matar GM, Dbaibo G (2021) The emergence of invasive *Streptococcus pneumoniae* serotype 24F in Lebanon: complete genome sequencing reveals high virulence and antimicrobial resistance characteristics. Front Microbiol 12: 1-10. doi: 10.3389/fmicb.2021.637813.
14. Al-Shamahy HA, Ishak AA (2021) Trends and causes of morbidity in part of children in the city of Sana'a, Yemen 1978-2018: findings of single children's health center. Univers J Pharm Res 5: 1-5. doi: 10.22270/ujpr.v5i6.504.
15. Hamood Alshehari A (2018) Epidemiology and outcome of acute bacterial meningitis among children in Saudi Haospital Hajjah, Northwest Territories of Yemen. Am J Pediatr 4: 56. doi: 10.11648/j.ajp.20180403.13.
16. Al-Ofairi BA, Nagi NA, Nagi SA, Al-Tawil TM, Saif A (2017) Otitis media in children: identification and antibiotics sensitivity of bacterial pathogens in Ibb city, Yemen. PSM Microbiol 2: 51-58.
17. Karimaei S, Tohidinik HR, Afshar D, Pourmand MR, Ghahfarokhi SH, Goodarzi NN, Azarsa M (2021) Antimicrobial susceptibility pattern and serotype distribution of *Streptococcus pneumoniae* in the middle east region: a systematic review and meta-analysis. Acta Med Iran 59: 64-78. doi: 10.18502/acta.v59i2.5572.
18. Halboup A, Abdi A, Ahmed M, Al-Qadasi F, Othman GQ (2020) Access to antibiotics without prescription in community pharmacies in Yemen during the political conflict. Public Health 183: 30-35. doi: 10.1016/j.puhe.2020.03.003.
19. Orubu ESF, Al-Dheeb N, Ching C, Bu Jawdeh S, Anderson J, Sheikh R, Hariri F, Basaleem H, Zaman MH. (2021) Assessing antimicrobial resistance, utilization, and stewardship in Yemen: an exploratory mixed-methods study. Am J Trop Med Hyg 105: 1404-1412. doi: 10.4269/ajtmh.21-0101.
20. Matran YM, Al-Haddad AM, Sharma D, Kalia NP, Sharma S, Kumar M, Sharma S (2023) Prevalence and resistance patterns of *Streptococcus pneumoniae* recovered from children in Western Asia. Curr Infect Dis Rep 25: 169-180. doi: 10.1007/s11908-023-00807-7.
21. Harris M, Clark J, Coote N, Fletcher P, Harnden A, McKean M, Thomson A (2011) British Thoracic Society guidelines for the management of community acquired pneumonia in children: update 2011. Thorax 66 Suppl 2: ii1-ii23. doi: 10.1136/thoraxjnl-2011-200598.
22. Carvalho M DG, Pimenta FC, Jackson D, Roundtree, A, Ahmad Y, Millar EV, O'Brien, KL, Whitney CG, Cohen AL, Beall BW (2010) Revisiting pneumococcal carriage by use of broth enrichment and PCR techniques for enhanced detection of carriage and serotypes. J Clin Microbiol 48: 1611-1618. doi: 10.1128/JCM.02243-09.
23. Satzke C, Turner P, Virolainen-Julkunen A, Adrian PV, Antonio M, Hare KM, Henao-Restrepo AM, Leach AJ, Klugman KP, Porter BD, Sá-Leão R (2014) Standard method for detecting upper respiratory carriage of *Streptococcus pneumoniae*: updated recommendations from the World Health Organization Pneumococcal Carriage Working Group. Vaccine 32: 165-179. doi: 10.1016/j.vaccine.2013.08.062.
24. Sanz JC, Culebras E, Ríos E, Rodríguez-Avial I, Wilhelmi I, Ramos B, Ordobás M, Picazo JJ (2010) Direct serogrouping of *Streptococcus pneumoniae* strains in clinical samples by use of a latex agglutination test. J Clin Microbiol 48: 593-595. doi: 10.1128/JCM.01651-09.
25. Clinical and Laboratory Standards Institute (2021) Performance standards for antimicrobial susceptibility testing, M100 Ed 31. USA. CLSI. 92-97.
26. Sader HS, Pignatari ACC1 (1994) E test: a novel technique for antimicrobial susceptibility testing. Sao Paulo Med J 112: 635-638. doi: 10.1590/S1516-31801994000400003.
27. El-Kholy A, Badawy M, Gad M, Soliman M (2020) Serotypes and antimicrobial susceptibility of nasopharyngeal isolates of *Streptococcus pneumoniae* from children less than 5 years old in Egypt. Infect Drug Resist 13: 3669-3677. doi: 10.2147/IDR.S250315.
28. Ricketson LJ, Lidder R, Thorington R, Martin I, Vanderkooi OG, Sadarangani M, Kellner JD (2021) PCR and culture analysis of *Streptococcus pneumoniae* nasopharyngeal carriage in healthy children. Microorganisms 9: 2116. doi: 10.3390/microorganisms9102116.
29. Al-Shamahy, H.A. Jabbar AR, Al Nabhi B, ALBadry A, Al Robasi A (2008) The prevalence of *Streptococcus pneumoniae* carriage among healthy children in Yemen. Emirates Med J 26: 25-29.
30. Walid J, Moshref L (2016) Antibiotic resistance pattern in healthy children diagnosed with community acquired respiratory tract infections in King Abdulaziz University Hospital. J Med Microbiol Diagnosis 5: 1-7. doi: 10.4172/2161-0703.1000232.
31. Alfayate Miguélez S, Yague Guirao G, Menasalvas Ruíz AI, Sanchez-Solís M, Domenech Lucas M, González Camacho F, Ortíz Romero MM, Espejo Garcia P, Guerrero Gómez C, Iofrio de Arce A, Moreno Parrado L, Sánchez Andrada RM, Alcoleaec EC, García SL, Reyes PP, Barceló AC, López Yepes M, López Yepes Vigueras Abellán J, Mateo GS, Murcian Pneumococcal Study Group (2021) Impact of pneumococcal vaccination in the nasopharyngeal carriage of *Streptococcus pneumoniae* in healthy children of the Murcia region in Spain. Vaccines 9: 1-13. doi: 10.3390/vaccines9010014.
32. Kim GR, Kim EY, Kim SH, Lee HK, Lee J, Shin JH, Kim YR, Am Song S, Jeong J, Uh Y, Kim YK (2019) Serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* causing invasive pneumococcal disease in Korea Between 2017 and after introduction of the 13-valent pneumococcal conjugate vaccine. Ann Lab Med 43: 45-54. doi: 10.3343/alm.2023.43.1.45.
33. Koliou MG, Andreou K, Lamnisos D, Lavranos G, Iakovides P, Economou C (2018) Risk factors for carriage of *Streptococcus pneumoniae* in children. BMC Pediatr 18: 144. doi: 10.1186/s12887-018-1119-6.
34. Abaye G, Fekadu H, Haji K, Alemu D, Anjulo AA, Yadate DT (2019) Prevalence and risk factors of pneumococcal

nasopharyngeal carriage in healthy children attending kindergarten, in district of Arsi Zone, South East Ethiopia. BMC Res Notes 12: 1-6. doi: 10.1186/s13104-019-4283-3.
35. Al-Samhari GA, Al-Mushiki GM, Tamrakar R, Lin YD, Al-Shaebi F, Akroot MA, Al-Nahari SA, Li GJ, Tang XY (2023) Prevalence, aetiology, vaccination coverage and spatio-temporal pattern among patients admitted with acute bacterial meningitis to the sentinel hospital surveillance network in Yemen, 2014-20, before and during the civil war. Int J Epidemiol 52: 1175-1186. doi: 10.1093/ije/dyad047.
36. Al-Tarbi MA. Ghouth AB (2020) Vaccination coverage in Tarim District, Yemen, 2017. Am J Epidemiol Public Heal 4: 10-15.
37. Heath CJ, Nayakwadi-Singer M, King CH, Malhotra I, Mutuku F, Mukoko D, LaBeaud AD (2018) Nasopharyngeal carriage of *Streptococcus pneumoniae* in children in Coastal Kenya. Am J Trop Med Hyg 98: 1046-1050. doi: 10.4269/ajtmh.16-0813.
38. Negash AA, Asrat D, Abebe W, Hailemariam T, Gebre M, Verhaegen J, Aseffa A (2019) Pneumococcal carriage, serotype distribution, and risk factors in children with community-acquired pneumonia, 5 years after introduction of the 10-valent pneumococcal conjugate vaccine in Ethiopia. Open Forum Infect Dis 6: 1-8. doi: 10.1093/ofid/ofz259.
39. Arjun R, Ratheesh RS, Mohan V, Uduman S, Jalaludeen S, Prabhakaran A, Sasidharan A, Niyas VK (2020) Susceptibility and serotypes of *Streptococcus pneumoniae* isolates in invasive pneumococcal disease: a study from Kerala, South India. Infez Med 28: 558-564.
40. Belkina T, Al Warafi A, Hussein Eltom E, Tadjieva N, Kubena A, Vlcek J (2014) Antibiotic use and knowledge in the community of Yemen, Saudi Arabia, and Uzbekistan. J Infect Dev Ctries 8: 424-429. doi: 10.3855/jidc.3866.
41. Čižman M, Mioč V, Bajec T, Paragi M, Kastrin T, Gonçalves J (2021) Correlation between antibiotic consumption and resistance of invasive *Streptococcus pneumoniae*. Antibiotics 10: 758. doi: 10.3390/antibiotics10070758.
42. Al-Lahham A, Khanfar N, Albataina N, Al Shwayat R, Altwal R, Abulfeilat T, Alawneh G, Khurd M, Alqadi Altamimi A (2021) Urban and rural disparities in pneumococcal carriage and resistance in Jordanian children, 2015-2019. Vaccines 9: 789. doi: 10.3390/vaccines9070789.
43. Gharibani KM, Azami A, Parvizi M, Khademi F, Mousavi SF, Arzanlou M (2019) High frequency of macrolide-resistant *Streptococcus pneumoniae* colonization in respiratory tract of healthy children in Ardabil, Iran. Tanaffos 18: 118-125.
44. Jamsheer A, Rafay AM, Daoud Z, Morrissey I, Torumkuney D (2016) Results from the survey of antibiotic resistance (SOAR) 2011-13 in the Gulf states. J Antimicrob Chemother 71 Suppl 1: i45-i61. doi: 10.1093/jac/dkw064.
45. Al-Sherikh YA, Gowda LK, Ali MMM, John J, Mohammed DKH, Shashidhar PC (2014) Distribution of serotypes and antibiotic susceptibility patterns among invasive pneumococcal diseases in Saudi Arabia. Ann Lab Med 34: 210-215. doi: 10.3343/alm.2014.34.3.210.
46. Mohanna MA, Bahannan AA (2016) Bacterial profile and antibiogram of otitis media among children in Yemen. J Ayub Med Coll Abbottabad 28: 480-483.
47. Vidanapathirana G, Angulmaduwa S, Munasinghe T, Ekanayake A, Kudagammana T, Dissanayaka N, Liyanapathirana V (2020) Pneumococcal colonization among healthy and hospitalized vaccine-naive Sri Lankan children. Vaccine 38: 7308-7315. doi: 10.1016/j.vaccine.2020.09.040.
48. Habibi Ghahfarokhi S, Mosadegh M, Ahmadi A, Pourmand MR, Azarsa M, Rahbar M, Nikmanesh B (2020) Serotype distribution and antibiotic susceptibility of *Streptococcus pneumoniae* isolates in Tehran, Iran: a surveillance study. Infect Drug Resist 13: 333-340. doi: 10.2147/IDR.S234295.

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Conflict of interests: No conflict of interests is declared.