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Short Communication

Nasopharyngeal carriage, serotype, antimicrobial susceptibility, and genotype of pneumococci in young healthy children attending daycare centres in Klang Valley, Malaysia

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ABSTRACT

Objective: Implementation of pneumococcal conjugate vaccines (PCV) targeting selected serotypes has led to a decrease in invasive pneumococcal diseases. In Malaysia, PCV10 was recently included in the national immunisation programme. Therefore, monitoring serotype distribution and antimicrobial resistance of pneumococci in high-density areas is crucial. This study investigated the serotype distribution, antimicrobial resistance pattern, and genotype of pneumococci in healthy children below 5 years old attending daycare centres in Klang Valley, Malaysia, while indirectly assessing transmission dynamics through nasopharyngeal carriage.

Methods: 168 children across 25 daycare centres provided parental consent. Nasopharyngeal swabs collected between January 2023 and May 2024, were analysed for pneumococcal carriage, and serotyped by PCR. Antimicrobial susceptibility was determined using E-test assay. Multilocus sequence typing was performed on isolates with reduced susceptibility.

Results: The pneumococcal carriage rate was 22.6%. Serotype 15C was the most prevalent (15.8%), followed by 23A and 11A. In the E-test assay, 47.4% isolates were resistant to erythromycin, 28.9% to tetracycline, and 5.3% to penicillin. One isolate was resistant to ceftriaxone, whereas none demonstrated resistance to cefotaxime; reduced susceptibility to the two antibiotics was observed in one isolate, while two others had intermediate susceptibility to either ceftriaxone or cefotaxime alone. Sequence type distribution was diverse, with ST338 being the most common (n = 3).

Conclusions: The predominance of nonvaccine serotypes, particularly 15C and 23A, alongside antibiotic resistance, indicates a potential epidemiological shift in the post-PCV era. These results emphasise the need for continuous surveillance to monitor serotype dynamics and antimicrobial resistance pattern in the community.

1. Introduction

Streptococcus pneumoniae is a Gram-positive, encapsulated diplococcus found in the human respiratory tract. It is detected in the nasopharynx of 5%–90% of healthy people, particularly in children at daycare settings [1]. Colonisation precedes disease onset, and young children under 5 years of age and elderly people are usually at higher risk [2].

There are 101 pneumococcal serotypes that have been reported, but not all are equally associated with disease [3]. The

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bacterial polysaccharide capsules serve as a virulence factor by crucially avoiding the host immune system. The implementation of pneumococcal conjugate vaccines (PCVs) that focus on common disease-causing serotypes has resulted in a marked decrease in invasive pneumococcal disease [3]. PCVs have undergone several expansions since their first introduction in 2000, with PCV7 targeting serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F; PCV10 (introduced 9 years later) targeting serotypes 1, 5, and 7F; PCV13 (introduced in 2010) targeting serotypes 3, 6A and 19A; PCV15 (introduced in 2021) targeting serotypes 22F and 33F; and finally PCV20 (also introduced in 2021) targeting serotypes 8, 10A, 11A, 12F and 15B [4]. Malaysia has already integrated PCV10 into its National Immunisation Programme (NIP) for newborns as of December 2020 [5]. Nevertheless, the adoption of PCVs into regular immunisation schedules across Southeast Asia varies. Four countries in the Southeast Asia region have introduced PCVs: Singapore (PCV13 in 2009), Lao People's Democratic Republic (PCV13 in 2013), Cambodia (PCV13 in 2015), and Myanmar (PCV10 in 2016). Meanwhile, despite the gradual introduction of PCV10 and PCV13 in the Philippines in 2013 and the pilot demonstration of PCV13 in numerous provinces in Indonesia in 2018, no decision has yet been made to integrate these vaccines into routine childhood immunisation programmes in these two countries [6].

Malaysia's implementation of PCV10 underscores the critical necessity for thorough monitoring of pneumococcal carriage patterns among young children in the community. Limited data on serotype distribution as well as antimicrobial resistance patterns and circulating genetic lineages in Malaysian settings have hindered assessment of vaccine effectiveness and the development of public health strategies [1]. Klang Valley, Malaysia's most developed area with the highest population density, serves as an ideal location to address these knowledge gaps. The aim of this study was to determine the serotype distribution, antibiotic susceptibility patterns, and genetic lineages by multilocus sequence typing (MLST) to indirectly assess the transmission dynamics of pneumococcal nasopharyngeal carriage in healthy children under 5 years of age attending daycare centres in the proposed area.

2. Materials and methods

2.1. Sampling and pneumococcal identification

Study infographics and invitations were circulated through a local daycare association to reach registered facilities around Klang Valley, Malaysia. Pneumococcal isolates were collected from healthy children aged 5 years and below through nasopharyngeal swabs in liquid Amies medium (Copan Eswab, Italy). A post hoc power analysis was conducted using G*Power to address the sample size and statistical power of the study [7]. Sampling was conducted between January 2023 and May 2024 at all facilities that provided parental consent. Demographic data were collected for race, gender, age, and vaccination status using a questionnaire. Children who were hospitalised or on antibiotics within 14 days prior to sampling were excluded. Samples were transported in an icebox to the microbiology laboratory at Universiti Putra Malaysia for bacterial cultivation within 6 h. Swabs were streaked on 5% Columbia Blood Agar plates and incubated at 35–37°C with 5% CO_2 for 18–24 h. Pneumococcal isolates were identified using standard microbiological methods, including observing alpha haemolysis, colony morphology, Gram stain, bile solubility, and optochin susceptibility.

2.2. Confirmation of pneumococci by PCR and capsular typing

Genomic DNA was extracted using the GeneAll Exgene Cell SV (GeneAll Biotechnology Co. Ltd., South Korea) according to the manufacturer's instructions. PCR was used to amplify pneumococcal virulence genes *ply* and *lytA* using established primers and conditions. Serotyping was performed using a sequential multiplex PCR method employing five primer sets from Shakrin et al. [8]. Isolates that tested negative for all targeted serotypes, including the control gene *cpsA*, were labelled as 'nontypable' (NT). PCR results were analysed through electrophoresis on an agarose gel, and DNA bands were visualised and captured with a UV illuminator. For further identification, these NT isolates were subjected to *cpsB* sequetyping. The *cpsB* gene was amplified using specific primers and sequenced, with results compared with the GenBank database to identify the serotype based on the highest BLAST bit score (>98% identity) [9]. If necessary, amplification, sequencing, and BLAST steps were repeated to minimise errors. Isolates with no *cpsB* gene detection were classified as NT. Those with a negative result for all targeted serotypes but positive *cpsA* gene detection in multiplex PCR, along with no *cpsB* gene detection, were categorised as 'inconclusive' (INC).

2.3. Antimicrobial susceptibility testing

An E-test was used to determine the MICs of several antibiotics (erythromycin, tetracycline, penicillin, ceftriaxone, cefotaxime, and trimethoprim-sulfamethoxazole) on Mueller-Hinton agar with 5% sheep blood. The procedure followed Clinical and Laboratory Standards Institute guidelines, taking into consideration different MIC criteria for meningitis and nonmeningitis isolates [10]. Isolates resistant to three or more antibiotics were classified as multidrug-resistant.

2.4. MLST and phylogenetic analysis

MLST was performed on isolates with reduced antibiotic susceptibility. PCR amplified the segments of seven housekeeping genes (*aroE, gdh, gki, recP, spi, xpt, ddl*) following a standard method [11], and sequences were uploaded to the MLST database to determine allele profiles and sequence types (STs). MLST-based phylogenetic analysis was carried out using MEGA7 software with the maximum likelihood method based on the Tamura-Nei model and 1000 bootstrap. Relevant reference sequences from the MLST database were included for comparison.

2.5. Statistical analysis

Demographic features, antimicrobial sensitivity profiles, and PCV serotype coverage were compared using χ^2 and Fisher's exact tests whenever appropriate. Significance was denoted by $P < 0.05$.

3. Results

In total, 25 daycare centres participated in this study, with a potential enrolment of approximately 600 children. Of these, parental consent was acquired for 168 children; 1–15 subjects were present per centre, with more than 5 participants at 21 respective daycares. Based on a sample size of 168 individuals at an effect size (Cohen's w) of 0.3, the post hoc power analysis showed a high statistical power of 97.3%, indicating that the study was adequately powered to detect meaningful effects. Among the 168 sampled subjects, a total of 38 pneumococcal isolates were detected, reflecting an overall carriage rate of 22.6%; 86 were male (51.2%) and 82 were female (48.8%), whereas 133 (79.8%) were aged 2 years or older. Age data were unreported for 23 participants (13.7%), and the denominator was adjusted in the subsequent analysis whenever appropriate to analyse this in relation to the 38 carriages. Race was excluded as most subjects were Malay, while vaccination status was not considered as not all subjects responded to this item in the questionnaire.

Table 1
Antimicrobial susceptibility profiles of carriage pneumococcal isolates.

Antimicrobial agent	CLSI[a] MIC breakpoint (ug/ml)	Susceptible (S), n (%)	Intermediate (I), n (%)	Resistant (R), n (%)
ERY	S: ≤0.25 I: 0.5 R: ≥1	17 (44.7)	3 (7.9)	18 (47.4)
TET	S: ≤1 I: 2 R: ≥4	27 (71.1)	0 (0)	11 (28.9)
SXT	S: ≤0.25 I: 0.5 R: ≥1	28 (73.7)	4 (10.5)	6 (15.8)
PEN	S: ≤0.06 I: 0.12–1 R: ≥2	22 (57.9)	14 (36.8)	2 (5.3)
CRO	S: ≤1 I: 2 R: ≥4	35 (92.1)	2[b] (5.3)	1 (2.6)
CTX	S: ≤0.5/9.5 I: 1/19–2/38 R: ≥4/76	36 (94.7)	2[b] (5.3)	0 (0)

[a] Data were analysed according to CLSI 2023 guidelines.
[b] Two isolates showed intermediate susceptibility to CRO and CTX each; one of these was a same isolate with intermediate susceptibility to both antibiotics.CRO: Ceftriaxone; CTX: Cefotaxime; ERY: Erythromycin; PEN: Penicillin; SXT: Trimethoprim-sulfamethoxazole; TET: Tetracycline.

Table 2
Combined antimicrobial resistance pattern among carriage pneumococcal isolates in relation to serotype.

Antimicrobial agent	Resistant isolates (n)	Serotype (n)
ERY, TET, SXT, PEN	1	NT[a]
ERY, TET, SXT	2	19F, 23F
ERY, TET, PEN	1	6A
ERY, TET	7	23A (4), 11F (1), NT[a] (2)
ERY, SXT	3	35B (2), NT[a] (1)
Single antimicrobial agent (ERY)	4	15C (2), 11A (1), NT [a] (1)
Single antimicrobial agent (CRO)	1	6A

[a] NT: Nontypable serotype with multiplex PCR and *cpsB* sequetyping (no gene detection). CRO: Ceftriaxone; ERY: Erythromycin; PEN: Penicillin; SXT: trimethoprim-sulfamethoxazole; TET: Tetracycline.

Among the 38 carriages, the most common serotype was 15C (15.8%), followed by 23A and 11A (each 13.2%). Serotypes 15C and 23A are nonvaccine types (NVTs), while 11A is included in PCV20 but is not available in Malaysia. Other serotypes - 6A (7.9%), 11A/D, 34, and 35B – accounted for 5.3% each, with serotypes 6B, 7C, 11F, 19F, 22F, and 23F making up 2.6% each. In total, 15.8% (6/38) of isolates were NT, with 1 isolate INC. Regarding vaccine coverage, 7.9% of isolates were covered by PCV10, 15.8% by PCV13, 18.4% by PCV15, and 36.8% by PCV20 (Supplementary Fig. 1).

This study found a varying level of antibiotic resistance (Table 1). Of the 38 isolates, 47.4% were resistant to erythromycin, 28.9% to tetracycline, and smaller percentages to sulfamethoxazole-trimethoprim (15.8%), penicillin (5.3%), and ceftriaxone (2.6%). Although 57.9% of the isolates exhibited susceptibility to penicillin, a large percentage (36.8%) belonged to the intermediate classification. Ceftriaxone and cefotaxime showed the best effectiveness, with over 90% susceptibility; only one resistant isolate was observed for ceftriaxone, whereas none demonstrated resistance to cefotaxime. Two isolates showed reduced susceptibility to ceftriaxone and cefotaxime each; one of them was a single isolate with intermediate susceptibility to both cephalosporins. Combined antibiotic resistance patterns (Table 2) revealed that one NT isolate was resistant to erythromycin, tetracycline, sulfamethoxazole-trimethoprim, and penicillin. Additionally, two isolates (serotypes 19F and 23F) were resistant to erythromycin, tetracycline, and sulfamethoxazole-trimethoprim. Co-resistance to erythromycin and tetracycline was most common, with eight isolates showing this pattern across various serotypes, including NT. Resistance to a single antimicrobial agent was observed in five isolates: four resistant only to erythromycin (serotypes 15C, 11A, and NT) and one (serotype 6A) resistant only to ceftriaxone.

For demographic, serotype, and antimicrobial susceptibility analyses among the 38 carriages, Table 3 shows that age and gender did not significantly affect the detection of PCV serotypes or NVT for all serotypes ($P > 0.05$). This suggests that demographic factors were not major determinants in the presence of these serotypes within our sampled population. Regarding antimicrobial susceptibility, there are significant differences in resistance profile for certain antibiotics in relation to PCV coverage, with erythromycin susceptibility showing no notable variation between most PCV serotypes. For ceftriaxone and cefotaxime, significant associations have been found with specific PCV serotypes, such as PCV13 and PCV15. Additionally, penicillin susceptibility varies significantly between NVT and vaccine serotypes.

Meanwhile, ST was aligned with reduced susceptibility profiles in a phylogenetic analysis of 21 isolates (Supplementary Fig. 2). The isolates (designated P followed by numbers) and 13 reference sequences from the MLST database (designated REF followed by an identity number; IDs 140 467, 183 489, 16 684, 24 499, 24 160, 28 282, 11 620, 10 564, 128 097, 129 326, 179 075, 246 600, and 10 313) were used in the phylogenetic analysis of the nucleotide sequences of seven housekeeping genes for the corresponding isolates. From the analysis, it was seen that the most frequently observed ST among the carriage isolates with reduced antimicrobial susceptibility was ST338 (three isolates, 14.3%), followed by ST18063, ST1106, ST11584, ST62, and ST5447, each consisting of two isolates (9.5%). Isolate P5–5 indicates a potentially new variant due to the lack of a matching reference sequence in the MLST database. Nevertheless, P5–5 closely diverged from ST18063 harbouring serotype 15C on the tree, indicating their potential genetic relatedness. With the exception of P5–5, all reference sequences of the different STs were clustered according to their respective similar STs of the isolates in this study. Overall, ST distribution was diverse without any obvious dominant clustering patterns, reflecting the distinct circulating lineages in our study setting.

4. Discussion

The pneumococcal carriage rate among healthy children in our study reached 22.6%, which remains substantially below the pooled prevalence rate of 36.0% in Southeast Asia, as reported by Daningrat et al. [6]. Previous studies in Malaysia showcased notable variations in pneumococcal carriage rates of 7.6% in 2001 and a higher rate of 35.4% in 2012 [6]. The most recent local study of pneumococcal prevalence in children under 5 years of age was reported in 2013 at 35.4% [12]. Another related study in 2016 showed the carriage and antimicrobial resistance profiles for upper respiratory tract pathogens, including pneumococci, in a rural area in Borneo [13]. The lack of current data highlights the necessity of ongoing monitoring.

The isolates detected in the current study included NVTs, which made up 63.2% of the total, with serotypes 15C and 23A being particularly notable. The same trend was also reported in a study of clinical cases in Taiwan, where a significant increase in non-PCV13 serotypes, particularly 15A, 15B, and 23A, was observed [14]. This is in contrast to findings prior to the introduction of pneumococcal vaccination programmes in Southeast Asian countries, where serotypes 6A/B, 23F, and 19F had the highest prevalence [6]. This may indicate a notable shift in predominant serotypes. Elsewhere, the introduction of PCVs has led to a rise in NVTs such as 19A,

Table 3
Demographic features and antimicrobial sensitivity profile of carriage pneumococci in relation to pneumococcal conjugate vaccine serotype coverage (n = 38).

Variable		PCV 10 n (%)	Non-PCV 10 n (%)	PCV 13 n (%)	Non-PCV 13 n (%)	PCV 15 n (%)	Non-PCV 15 n (%)	PCV 20 n (%)	Non-PCV 20 n (%)
Age category	<2 years (n = 12)	1 (8.3)	11 (91.7)	1 (8.3)	11 (91.7)	2 (16.7)	10 (83.3)	5 (41.7)	7 (58.3)
	≥2 years (n = 24)	2 (8.3)	22 (91.7)	5 (20.8)	19 (79.2)	5 (20.8)	19 (79.2)	9 (37.5)	15 (62.5)
	Unreported[a] (n = 2)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)
	p value	0.913[b]		0.513[b]		0.752[b]		0.524[b]	
Gender	Male (n = 21)	2 (9.5)	19 (90.5)	5 (23.8)	16 (76.2)	5 (23.8)	16 (76.2)	8 (38.1)	13 (61.9)
	Female (n = 17)	1 (5.9)	16 (94.1)	1 (5.9)	16 (94.1)	2 (11.8)	15 (88.2)	6 (35.3)	11 (64.7)
	p value	0.679[b]		0.132[b]		0.341[b]		0.859	
Antimicrobial susceptibility profile (reduced susceptibility)	ERY (n = 21)	2 (9.5)	19 (90.5)	4 (19.0)	17 (81.0)	4 (19.0)	17 (81.0)	6 (28.6)	15 (71.4)
	p value	0.679[b]		0.540[b]		0.912[b]		0.240[b]	
	TET (n = 11)	2 (18.2)	9 (81.8)	3 (27.3)	8 (72.7)	3 (27.3)	8 (72.7)	3 (27.3)	8 (72.7)
	p value	0.133[b]		0.215[b]		0.369[b]		0.435[b]	
	SXT (n = 10)	2 (20.0)	8 (80.0)	3 (30.0)	7 (70.0)	3 (30.0)	7 (70.0)	3 (30.0)	7 (70.0)
	p value	0.098[b]		0.151[b]		0.271[b]		0.601[b]	
	PEN (n = 16)	2 (12.5)	14 (87.5)	3 (18.8)	13 (81.3)	3 (18.8)	13 (81.3)	3 (18.8)	13 (81.3)
	p value	0.369[b]		0.670[b]		0.964		0.049[c]	
	CRO (n = 3)	1 (33.3)	2 (66.7)	2 (66.7)	1 (33.3)	2 (66.7)	1 (33.3)	2 (66.7)	1 (33.3)
	p value	0.089[b]		0.012[b,c]		0.025[c]		0.264	
	CTX (n = 2)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)
	p value	0.023[b,c]		0.173		0.237		0.692	

For the antibiotics, isolates with reduced susceptibility were compared against susceptible isolates of each respective antibiotic in relation to the PCV. Reduced susceptibility comprises both resistant and intermediate isolates.
[a] Unreported' was excluded from the analysis.
[b] Fisher's exact test, otherwise χ^2 test was used.
[c] $P < 0.05$ = statistically significant.CRO: Ceftriaxone; CTX: Cefotaxime; ERY: Erythromycin; PEN: Penicillin; SXT: Trimethoprim-sulfamethoxazole; TET: Tetracycline.

15A/B/C, and 23A across multiple regions including North America and Europe [14,15]

Malaysia has seen substantial changes in its vaccination history throughout recent decades, with PCV7 becoming available in 2005 followed by PCV10 in 2009 and PCV13 in 2010 [1,12]. However, these vaccines only first became available through private health care. The addition of PCV10 in NIP in late 2020 enabled the accessibility of pneumococcal vaccine to all newborn babies in Malaysia, but targeting selected serotypes is likely to impact serotype distribution over time. Intake of the NIP vaccination package was high among the subjects in this study but, unfortunately, inadequate reporting from participants and an absence of differentiation between PCV10 and other PCVs in our questionnaire constituted a limitation for the precise evaluation of vaccination coverage in relation to serotype distribution in our study. Nevertheless, PCV10 serotypes made up only 7.9% of the carriage isolates in this analysis. Compared with a prior study by Yatim et al. in 2013, which reported that 40.6% of serotypes were covered by PCV7 and PCV10 [12], these current findings report a lower percentage, which could be a consequence of the impact of PCV10 introduction.

Antimicrobial susceptibility analysis demonstrated a diverse resistance pattern among the carriage isolates. Resistance to erythromycin was found in 47.4% of the isolates, as was similarly reported in a previous study involving clinical isolates in Malaysia (42.9%) [16]. Tetracycline resistance occurred at a lower incidence (28.9%), wherein every isolate resistant to tetracycline also demonstrated concurrent erythromycin resistance. This pattern was also observed in a study on macrolide resistance in pneumococci from several Asian countries [17]. A study in Vietnam in 2022 also found very high resistance rates for erythromycin (99.2%) and tetracycline (96%) [18]. Nevertheless, the penicillin and trimethoprim-sulfamethoxazole resistance rate in this study was lower compared with the rate reported in a prior study conducted in Malaysia by Yatim et al. in 2013 [12]. The prevalence of penicillin resistance in this study was modest, at 5.3%. However, penicillin displayed intermediate resistance in 14 (36.8%) out of the 38 isolates, which shows reduced susceptibility. A study in Taiwan identified a growing reduced penicillin susceptibility rate in primary non-PCV13 serotypes including 15A, 15B, and 23A [14]. While the effectiveness of ceftriaxone (92.1%) and cefotaxime (94.7%) against the isolates remains high, as also shown by similar findings from studies in Malaysia [16] and Singapore [19], only one isolate was resistant to ceftriaxone in this study, whereas one isolate exhibited reduced susceptibility to both of the cephalosporins and the two isolates were intermediate to either ceftriaxone or cefotaxime alone. This finding suggests that these cephalosporins remain a reliable choice for treating pneumococcal infection [17] but with prudent use to avoid the emergence of resistance in the future.

Additionally, this study identified six isolates as NT, and four of them showed resistance to multiple antibiotics. A clinical study in Taiwan reported a gradual increase in infections associated with NT pneumococci, particularly in the context of the PCV era, with significant resistance rates to commonly used antibiotics such as trimethoprim-sulfamethoxazole and erythromycin [20]. This reflects that the status of NT isolates in our community setting study should not be underestimated.

In this study, the molecular investigation of those with reduced susceptibility identified multiple genetic lineages – ST338, ST1106, ST11584, and ST5246 – all of which have intermediate penicillin susceptibility and are resistant to at least two antibiotics. ST338 presents significant concern due to its association with serotype 23A; this lineage and serotype were previously isolated from invasive pneumococcal disease in Columbia [21]. Strains with similar features have also been detected in clinical settings in Taiwan [14]. ST338 was reported to likely emerge before the introduction of PCV7 through capsular switch events or structural changes from the ST338 Colombia 23F–26 strain [15,21]. Certain serotypes and STs in this study that were not covered by PCV10 and PCV13, such as isolates P13–4 and P13–5 (both were ST62, serotype 11A), had reduced susceptibilities to erythromycin only. The ST62–11A strain was typically associated with asymptomatic carriage, as reported by Brueggemann et al. [22]. However, a more recent study involving ST62–11A isolates from a patient with meningitis reported that this strain was able to cause invasive disease [23].

ST7786 and ST1106 isolates in their respective clusters did not match typical serotypes and were labelled as NT or INC; isolate P5–5 also represents a potential new variant. These isolates highlight the constant evolutionary changes and diversification within

the pneumococcal population, and necessitate further analysis by whole-genome sequencing. Analysis of MLST phylogenetic patterns benefits from the reference isolates gathered in Malaysia, Singapore, Japan, and China to reveal global transmission patterns among specific clones, with the genetic relatedness of isolates across multiple countries confirming that resistant bacterial clones travel between regions [2]. In this study, both ST18063–15C isolates demonstrated a connection with local reference isolate REF183489 (the only ST18063 isolate reported in PubMLST in 2023) as they all exhibited reduced susceptibility to erythromycin, while REF183489 was derived from a clinical sample from Malaysia in 2019.

In conclusion, this study shows that the pneumococcal population in this study setting could be undergoing an epidemiological shift considering the emergence of nonvaccine serotypes such as 15C and 23A, along with the widespread resistance to antibiotics such as erythromycin and tetracycline. The appearance of multidrug-resistant clones such as ST338 together with NT strains reveal how pneumococcal population dynamics have continued to evolve since the introduction of PCV. Therefore, continuous surveillance is highly warranted at national and regional levels to track changes in serotype distribution, antimicrobial resistance patterns, and genetic lineages. Coordinated efforts will play a critical role in guiding vaccination strategies, reducing public health risks from pneumococcal infections.

Declarations

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Declaration of competing interest: None declared.

Ethical approval: Ethical approval to conduct this study was obtained from the Ethics Committee for Research Involving Human Subjects Universiti Putra Malaysia (approval no. JKEUPM-2020–183). All of the daycares that participated in this study were registered under either the Department of Social Welfare Malaysia (JKM) or the Community Development Department Malaysia (KEMAS) that granted authority for sample collection.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2025.05.017.

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