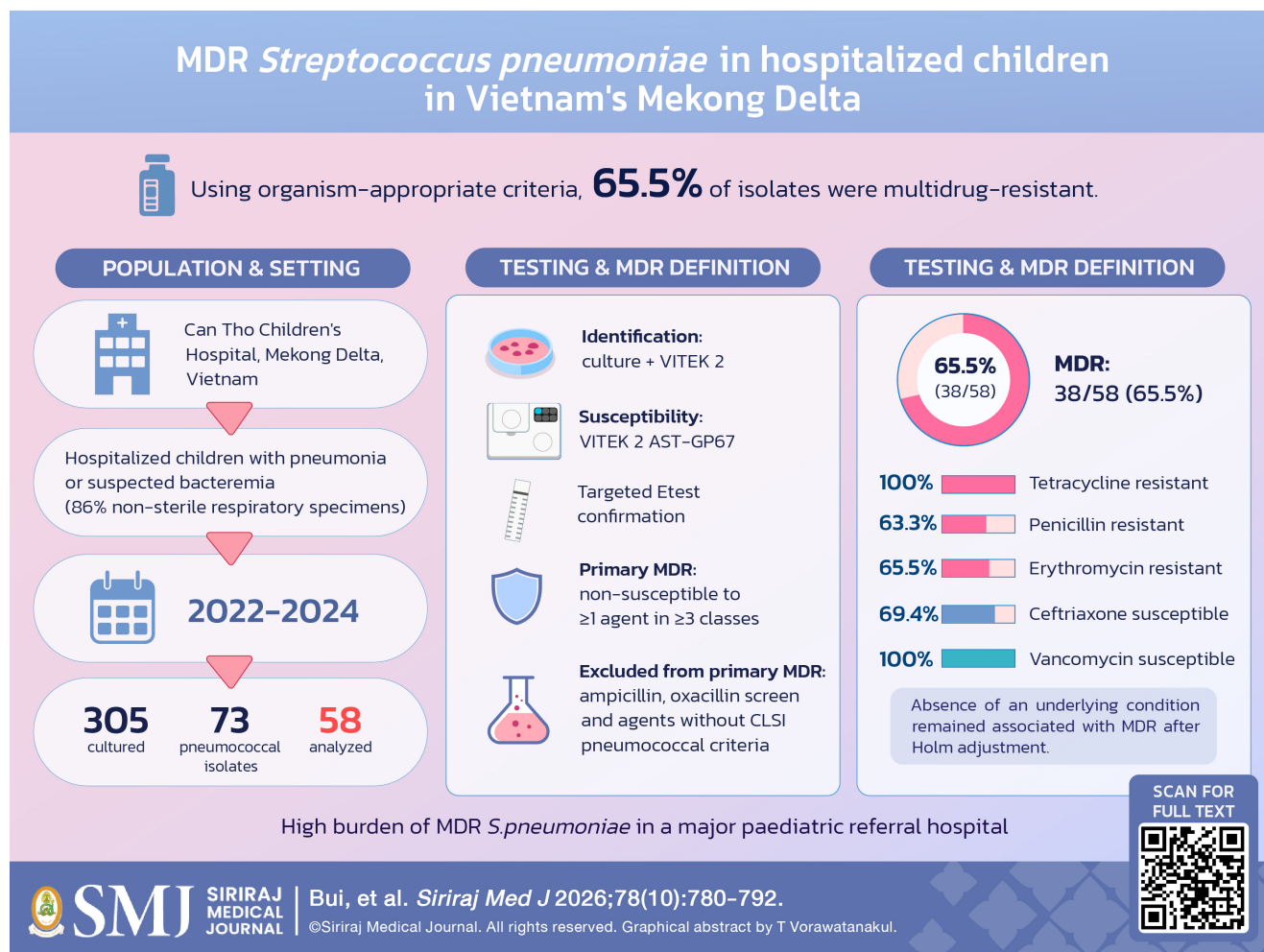


Antimicrobial Susceptibility and Multidrug Resistance among *Streptococcus pneumoniae* Isolates from Predominantly Non-Sterile Respiratory Specimens in Children Hospitalized with Pneumonia or Suspected Bacteremia in the Mekong Delta, Vietnam, 2022–2024: A Hospital-Based Cross-Sectional Study

Nghia Quang Bui, M.D., Ph.D.¹, Khai Quang Tran, M.D., Ph.D.¹, Le Thi Nhu Tran, M.D., Ph.D.², Long Duy Phun, M.D., M.Sc.¹, Duy Khanh Nguyen³, Duong Thai Bach³, No Thi Tran³, Tram Ngoc Ly³, Thinh Nguyen³, Hien Thi Nguyen, M.D., Ph.D.^{4,*}

¹Department of Pediatrics, Faculty of Medicine, Can Tho University of Medicine and Pharmacy, Can Tho 94000, Vietnam, ²Department of Microbiology, Faculty of Medicine, Can Tho University of Medicine and Pharmacy, Can Tho 94000, Vietnam, ³Medical Student, Can Tho University of Medicine and Pharmacy, Can Tho 94000, Vietnam, ⁴Department of Nutrition and Food Safety, Faculty of Public Health, Can Tho University of Medicine and Pharmacy, Can Tho 94000, Vietnam.



*Corresponding author: Hien Thi Nguyen

E-mail: nthien@ctump.edu.vn

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ORCID ID: <http://orcid.org/0000-0002-3997-5943>

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ABSTRACT

Objective: To characterize antimicrobial susceptibility and multidrug resistance (MDR) among *Streptococcus pneumoniae* isolates from children hospitalized with pneumonia or suspected bacteremia in Vietnam's Mekong Delta and explore factors associated with MDR.

Materials and Methods: This cross-sectional study included children aged 2 months–15 years with culture-confirmed *S. pneumoniae* and susceptibility results at a tertiary pediatric hospital during 2022–2024. Identification used conventional methods and VITEK 2 Compact; susceptibility testing used VITEK 2 AST-GP67, Etest when indicated, and contemporaneous CLSI M100 criteria. MDR was defined as non-susceptibility to ≥ 1 agent in ≥ 3 classes with an independent pneumococcal CLSI criterion; screening tests and non-independent results (including surrogate ampicillin) were excluded. Associations were explored using Fisher's exact test with Holm adjustment.

Results: Among 58 children, 38 (65.5%, 95% CI 52.7–76.4) had MDR isolates. Resistance was 100% to tetracycline, 65.5% to erythromycin, 63.3% to penicillin, and 52.2% to cefepime. Ceftriaxone was susceptible in 25/36 isolates (69.4%); all 58 isolates were vancomycin-susceptible. MDR occurred in 5/8 blood isolates and 33/50 nasopharyngeal aspirate isolates, reported descriptively. Absence of an underlying condition was associated with MDR after Holm adjustment (adjusted $P=0.020$); vaccination was not (adjusted $P=0.367$). Risk-factor analyses were exploratory and underpowered (20 non-MDR isolates).

Conclusion: MDR *S. pneumoniae* was common in this referral population. Because most isolates came from non-sterile respiratory specimens and testing was clinically directed, the 65.5% estimate should be interpreted as institution-level surveillance conditional on the observed testing panel rather than an unbiased population prevalence.

Keywords: *Streptococcus pneumoniae*; Drug resistance, multiple, bacterial; Child, hospitalized; Pneumococcal vaccines; Vietnam (MeSH terms) (Siriraj Med J 2026;78(10):780-792)

INTRODUCTION

Streptococcus pneumoniae remains one of the leading bacterial causes of pneumonia, bacteremia, and meningitis in children worldwide, and it was the leading pathogen attributed to lower respiratory infection deaths in children younger than 5 years in the Global Burden of Disease Study 2021.¹ Antimicrobial resistance in this organism has narrowed the range of reliable empirical options and is recognized by the World Health Organization as a medium-priority pathogen for research and development and surveillance in the 2024 Bacterial Priority Pathogens List.²

Because pneumococcal resistance is driven by locally circulating clones, local antimicrobial pressure, and the composition of the referral population, resistance profiles differ substantially between countries, between hospitals within a country, and between time periods.³ Institution-level surveillance is therefore not a duplication of national data but a prerequisite for rational empirical prescribing at the bedside. Vietnamese national hospital-based surveillance through VINARES likewise documented high and heterogeneous antimicrobial resistance across 13 hospitals in 2016–2017, including pneumococcal resistance indicators.⁴ These national data predate the present study period and do not substitute for contemporary

institution-level pediatric *S. pneumoniae* surveillance in the Mekong Delta.

Vietnam carries a heavy pediatric pneumonia burden, and throughout the 2022–2024 observation period the pneumococcal conjugate vaccine (PCV) was not part of the national Expanded Program on Immunization; PCV10 and PCV13 were obtainable only through fee-based private immunization services from 2014 and 2019, respectively, resulting in low and socially patterned coverage.⁵ Phased public-sector introduction has been initiated only after the period covered by this study, so our findings describe a wholly PCV-naïve programmatic context. Contemporary hospital-level susceptibility data from the Mekong Delta, a region of approximately 17 million people served by a single tertiary pediatric referral center, remain scarce. The most recent dataset from this region was restricted to children with severe pneumonia⁶ and Vietnamese studies that formally quantify multidrug-resistant (MDR) *S. pneumoniae* and examine its correlates are few.^{7,8}

We therefore conducted a hospital-based cross-sectional study with two objectives: (i) to describe the antimicrobial susceptibility profile and the prevalence of MDR among *S. pneumoniae* isolated from children hospitalized at the study hospital between 2022 and 2024;

and (ii) to explore, in a hypothesis-generating manner, demographic and clinical characteristics associated with MDR status.

MATERIALS AND METHODS

This study was approved by the Biomedical Research Ethics Committee of Can Tho University of Medicine and Pharmacy (approval no. 23.101.SV/PCT-HĐĐĐ, 25 December 2023; study code 23.KY.042) and was authorized by the Director of Can Tho Children's Hospital. It was conducted at Can Tho Children's Hospital, the tertiary pediatric referral center for the Mekong Delta region of southern Vietnam. Case ascertainment combined a retrospective phase (1 January 2022 to 31 December 2023), based on review of laboratory and medical records, and a prospective phase (1 January to 31 December 2024), during which eligible children were enrolled at the time of culture confirmation. The ethics approval explicitly covered retrospective review of medical and laboratory records and of stored isolate data for the 2022–2023 period; no retrospective record was accessed before the date of approval. The requirement for individual informed consent was waived for the retrospective component on the grounds that the data were de-identified and the research posed no more than minimal risk. For the prospective component, written informed consent was obtained from a parent or legal guardian of every child, and age-appropriate assent was obtained from children aged 7 years and older. The study is reported in accordance with the STROBE statement for cross-sectional studies⁹, supplemented, where applicable, by the STROBE-AMS extension for epidemiological studies of antimicrobial resistance¹⁰; a completed STROBE-AMS checklist is provided as Supplementary Material.

During 2022–2024, no pneumococcal outbreak, pneumococcus-specific infection-control change, formal hospital-wide antimicrobial stewardship program, antimicrobial restriction policy, or formulary change was identified; routine standard precautions remained unchanged throughout the study period.

Participants

Using total population sampling, we included every hospitalized child aged 2 months to 15 years with (i) a clinical diagnosis of pneumonia or suspected bacteremia assigned by the treating pediatric team at admission, (ii) a culture-confirmed *S. pneumoniae* isolate from any specimen source, and (iii) an available antimicrobial susceptibility result. Diagnostic syndrome and microbiological specimen source were recorded independently. Suspected bacteremia denoted the clinical syndrome prompting blood-culture

collection and did not require a subsequently positive culture; only 4 of the 12 children with suspected bacteremia had *S. pneumoniae* recovered from blood, whereas the remaining 8 had the organism recovered only from a respiratory specimen. Children with a documented primary or secondary immunodeficiency, and those receiving immunosuppressive therapy, were excluded. This exclusion was prespecified because immunosuppression is an established risk factor for carriage of resistant pneumococci and we sought to characterize resistance in the general pediatric inpatient population; we acknowledge that it reduces the representativeness of a tertiary referral series and is likely to bias the MDR estimate downward. Only the first isolate per child was analyzed.

Both diagnoses were assigned by attending pediatric specialists (Specialist Level I or II, or Master of Pediatrics). Pneumonia was diagnosed according to Vietnam Ministry of Health pediatric guidance (Decision 4239/QĐ-BYT) together with WHO clinical-radiographic criteria³, requiring acute respiratory symptoms, age-specific tachypnea or chest indrawing, crackles on auscultation, and chest-radiographic parenchymal infiltrate or consolidation. Suspected bacteremia was defined under the hospital systemic-infection work-up protocol by fever $\geq 38.5^{\circ}\text{C}$ or hypothermia $< 36.0^{\circ}\text{C}$ together with systemic inflammatory or impaired-perfusion signs (including capillary refill > 2 s), lethargy, unusual irritability, or poor feeding, accompanied by elevated C-reactive protein or procalcitonin; meeting these criteria prompted blood-culture collection and did not itself establish culture-confirmed bacteremia.

No a priori sample-size calculation governed recruitment. All eligible isolates identified during the study period were included, and statistical precision is reported post hoc as 95% confidence intervals for all proportions.

Microbiological methods

Respiratory specimens were nasopharyngeal aspirates (NPA); the term “nasotracheal aspirate” used in the original submission was incorrect and has been corrected after verification with the Microbiology Department. NPA was obtained by passing a sterile flexible suction catheter (6–8 Fr) through the nostril to the posterior nasopharynx, using the nostril-to-earlobe distance as the insertion guide, at a suction pressure of approximately 80–120 mmHg with gentle catheter rotation, and collecting the aspirate into a sterile specimen trap. Blood was collected by sterile venipuncture. Sampling was prioritized before the first antimicrobial dose administered at the study hospital; antimicrobial treatment received at a referring facility was documented in the admission

record. Specimen adequacy was assessed by direct Gram stain at low-power examination (10× objective; 100× total magnification) using modified Bartlett/Murray–Washington criteria. A specimen was accepted for culture only when it contained <10 squamous epithelial cells and >25 polymorphonuclear neutrophils per low-power field. Specimens failing either criterion were rejected before culture and repeat sampling was requested; therefore, all respiratory isolates included in the study derived from specimens meeting these criteria. Because rejection occurred before culture and study eligibility, the number of rejected specimens was not captured and cannot be reconstructed. Pneumococcal growth was considered clinically relevant when *S. pneumoniae* was recovered in pure culture or as clearly predominant growth and was concordant with Gram-positive diplococci or short chains and inflammatory cells on the original smear. Where investigated, viral co-pathogens (respiratory syncytial virus, influenza, adenovirus, rhinovirus) and bacterial co-pathogens (*Haemophilus influenzae*, *Moraxella catarrhalis*) were recorded separately using multiplex real-time PCR and/or parallel culture.

Isolates were identified by Gram stain, colony morphology on 5% sheep blood agar, optochin susceptibility, and the VITEK 2 Compact system (bioMérieux, Marcy-l'Étoile, France). Routine antimicrobial susceptibility testing was performed with the VITEK 2 AST-GP67 card (bioMérieux). Quantitative minimum inhibitory concentration (MIC) confirmation by gradient diffusion (Etest, bioMérieux) was performed for penicillin and for cefotaxime or ceftriaxone when the oxacillin 1 µg screening zone diameter was <20 mm, and for other clinically important isolates requiring confirmatory MIC assessment. Oxacillin 1 µg disk diffusion was used solely as a screen for penicillin non-susceptibility: a zone diameter <20 mm was recorded as a positive screen requiring confirmatory penicillin MIC testing and was never interpreted as oxacillin resistance. Results were interpreted using the Clinical and Laboratory Standards Institute (CLSI) M100 edition current in each study year, 32nd edition for 2022, 33rd edition for 2023, and 34th edition for 2024¹¹, applying parenteral non-meningitis criteria for penicillin (susceptible ≤2, intermediate 4, resistant ≥8 µg/mL) and for cefotaxime and ceftriaxone (susceptible ≤1, intermediate 2, resistant ≥4 µg/mL), because no child had meningitis and no cerebrospinal fluid isolate was included; oral penicillin V criteria were not applied to any isolate. This is also the convention recommended by WHO for pneumococcal surveillance reporting, in which non-meningitis breakpoints are applied when isolates are interpreted without a meningitis

designation.³ *Streptococcus pneumoniae* ATCC 49619 served as the quality-control strain. Quality control for disk and gradient diffusion was performed weekly once satisfactory performance had been documented on 20–30 consecutive days, and each new VITEK 2 card lot underwent lot-to-lot verification. Quality-control records for 2022–2024 are retained by the laboratory. The CLSI M100 Table 2G criterion, measurement, breakpoint, and study-year edition applied to each reportable agent are documented in [Supplementary Table S4](#). The original quantitative measurements, however, could not be retrieved for this analysis: neither numerical MIC values nor inhibition-zone diameters were transferred to the study database, and only the categorical interpretations issued by the clinical laboratory at the time of testing — susceptible, intermediate or resistant, and screen-positive or screen-negative for oxacillin — were available. These categorical data are therefore treated as legacy laboratory reports rather than a re-verifiable primary-data susceptibility dataset: they derive from contemporaneous interpretations made under the CLSI edition in force in each study year, and retrospective re-interpretation or independent re-derivation from raw measurements was not possible. [Supplementary Table S2](#) summarizes the testing workflow, interpretive standard, and quality-control procedures by study year.

The panel comprised 16 agents and was selected by the treating team according to clinical syndrome and specimen type; consequently, not every isolate was tested against every agent, and susceptibility proportions are reported with agent-specific denominators and 95% confidence intervals. CLSI M100 does not provide applicable *S. pneumoniae* interpretive criteria for ceftazidime, gentamicin, ciprofloxacin, or ofloxacin in the editions used; oxacillin is a 1 µg screening test for penicillin non-susceptibility rather than a therapeutic susceptibility category. These legacy outputs have therefore been removed from the main susceptibility analysis and are retained only as non-interpretive audit labels in [Supplementary Table S3](#). Ampicillin was also excluded from the independently interpretable panel and primary MDR algorithm because its categorical result was a surrogate interpretation derived from penicillin rather than an independent pneumococcal breakpoint; its historical surrogate labels are reported only for audit purposes in [Supplementary Table S3](#).

Definitions and variables

MDR was defined as non-susceptibility (intermediate or resistant) to at least one agent in three or more antimicrobial classes with recognized antipneumococcal activity and an independent applicable CLSI *S. pneumoniae* interpretive

criterion. The ≥ 3 -class threshold follows the resistant-only convention used in the multinational ANSORP pneumococcal surveillance study, in which MDR was defined as resistance to at least three antibiotic classes¹², and is consistent with a subsequent pneumococcus-specific synthesis defining MDR as resistance to three or more antimicrobial classes.¹³ Our primary definition deliberately broadens that resistant-only convention by counting intermediate results as non-susceptible; the resistant-only definition is therefore reported separately as a sensitivity analysis. The consensus definition of Magiorakos et al. cited in the original submission was not applied because it does not provide an organism-specific classification scheme for pneumococci. The seven evaluable classes were penicillins (penicillin only), extended-spectrum cephalosporins (cefuroxime, cefotaxime, ceftriaxone, cefepime), macrolides (erythromycin), lincosamides (clindamycin), phenicols (chloramphenicol), tetracyclines (tetracycline), and glycopeptides (vancomycin). Ampicillin, the oxacillin screen, ceftazidime, gentamicin, ciprofloxacin, and ofloxacin were excluded from the primary MDR algorithm.

A class not tested for a given isolate was treated as missing and was never imputed as susceptible. Isolates with fewer than three evaluable classes would have been excluded from the MDR analysis; no isolate met this criterion. Testing intensity and the complete isolate-level classification for all 58 isolates are provided in [Supplementary Table S1](#). Classification as MDR is robust once ≥ 3 non-susceptible evaluable classes are observed, but classification as non-MDR is conditional on the observed panel because additional untested classes could have been non-susceptible. This verification bias can therefore miss MDR but cannot create a false MDR classification; the primary prevalence is presented as a conservative floor conditional on the clinically directed testing panel, not as an unbiased population prevalence. Four prespecified sensitivity analyses were examined: (1) intermediate results counted as susceptible; (2) the broader nine-category protocol definition; (3) isolates tested against < 5 of the seven evaluable classes reclassified as indeterminate; and (4) non-MDR status accepted only when the observed non-susceptible classes plus all untested classes could not reach the three-class threshold.

Explanatory variables were extracted from medical records using a standardized form: age group, sex, admitting ward (respiratory medicine versus intensive/critical care), presence of any underlying condition, documented antibiotic use in the 30 days before admission, and prior pneumococcal vaccination. Vaccination status

was ascertained from the immunization record when available and otherwise from caregiver report, and was analyzed dichotomously (any documented or reported PCV dose versus none); vaccine product, dose number, dates and schedule completeness were not systematically recorded. Documented antimicrobial exposure in the 30 days before admission was abstracted from the medical record, including treatment documented before referral or transfer where available. Length of hospital stay was defined as the interval in days from admission to discharge, and duration of antimicrobial therapy was recorded separately as the number of days of in-hospital antimicrobial treatment.

Statistical analysis

Categorical variables are summarized as counts and percentages, and continuous variables as medians with interquartile ranges (IQR). Proportions are presented with 95% Wilson score confidence intervals. Associations between candidate variables and MDR status were assessed using Fisher's exact test, with unadjusted odds ratios (OR) and 95% confidence intervals (CI) estimated by the Woolf method with the Haldane–Anscombe correction where a cell contained zero. Length of hospital stay and duration of antimicrobial therapy were compared using the Mann–Whitney U test because subgroup sizes were small and distributions were skewed. Because six comparisons were performed, P values were additionally adjusted for multiplicity using the step-down Holm procedure computed from full-precision (unrounded) P values, and interpretation is based on the adjusted values. With only 20 non-MDR isolates, multivariable modeling was not attempted; all association analyses are exploratory, unadjusted for confounding, and hypothesis-generating rather than confirmatory. Analyses were performed in IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Study flow

During the study period the microbiology register identified 305 children who underwent respiratory or blood culture, of whom 73 had a culture-confirmed *S. pneumoniae* isolate. Fifteen children were excluded: repeat isolates from the same treatment episode ($n = 8$), documented immunodeficiency or immunosuppressive therapy ($n = 5$), and incomplete susceptibility results ($n = 2$); no child was excluded because the medical record was unavailable. Fifty-eight children, each contributing a single isolate, formed the analytic sample ([Fig 1](#)).

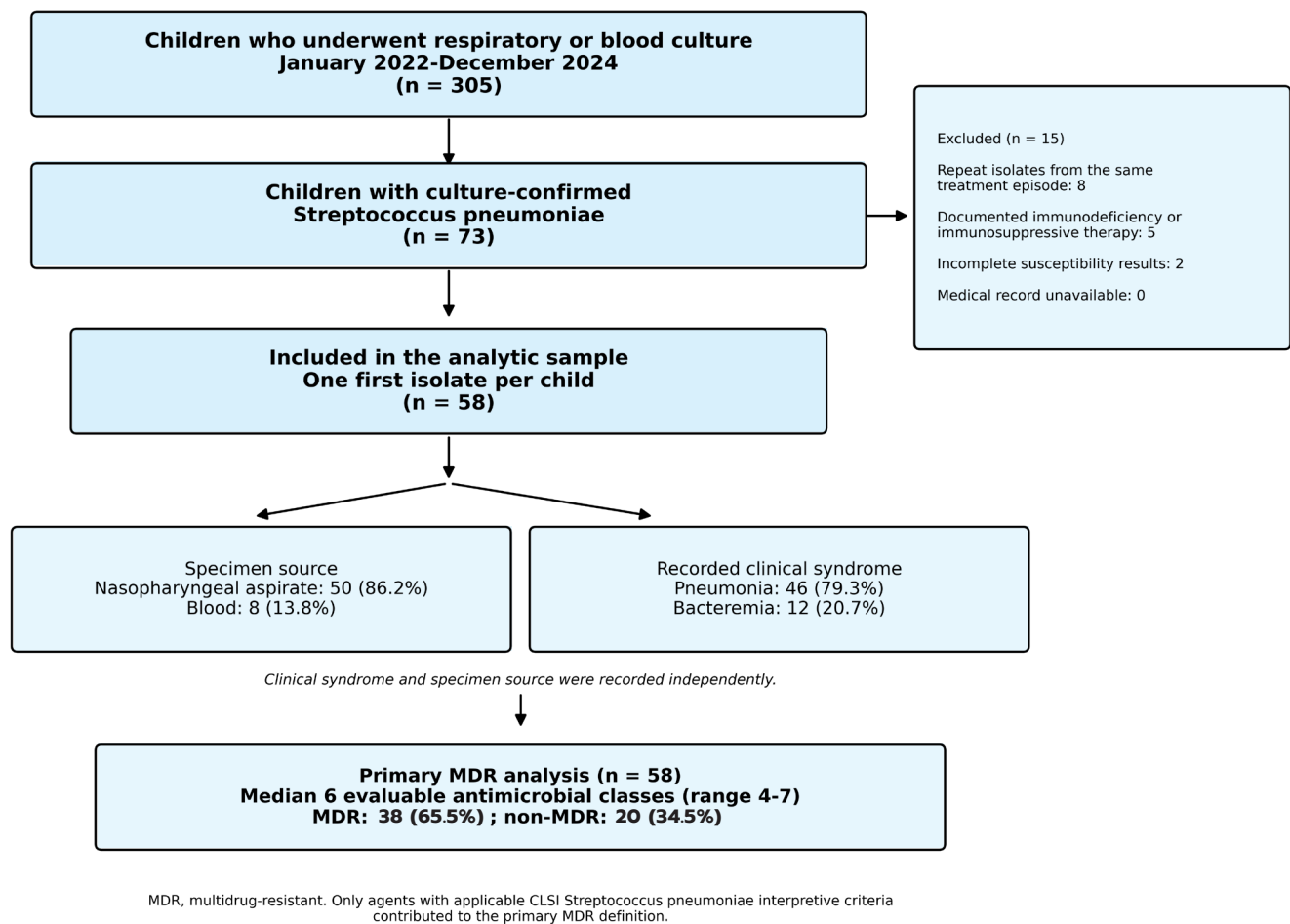


Fig 1. Study flow diagram showing participant selection and primary multidrug-resistance classification of the analytic sample (n = 58)

Characteristics of the study population

Fifty-eight children met the eligibility criteria (Table 1). The median age was 31 months (IQR 14–60); 26 children (44.8%) were younger than 2 years, and 33 (56.9%) were male. Pneumonia accounted for 46 cases (79.3%) and suspected bacteremia for 12 (20.7%); fifty isolates (86.2%) were recovered from nasopharyngeal aspirates and 8 (13.8%) from blood. Clinical syndrome and specimen source were recorded independently and were not concordant: four children with pneumonia had a positive blood culture, representing bacteremic pneumococcal pneumonia, whereas only 4 of the 12 children with suspected bacteremia (33.3%) had a positive blood culture; the remaining 8 had *S. pneumoniae* recovered only from a respiratory specimen and did not have culture-confirmed bacteremia. Five children (8.6%) had an underlying condition (congenital heart disease, n = 3; malnutrition, n = 2), 16 (27.6%) had received an antibiotic in the 30 days before admission, 17 (29.3%) had a history of pneumococcal vaccination, and 14 (24.1%) were admitted to intensive or critical care. Median length

of hospital stay was 10 days (IQR 7–14) and median duration of antimicrobial therapy 9.5 days (IQR 7–12).

Antimicrobial susceptibility

Resistance was frequent across most classes with applicable interpretive criteria (Table 2). Because the testing panel was selected by the treating team according to clinical syndrome and specimen type rather than applied uniformly to every isolate, agent-specific denominators varied widely (range 21–58 isolates tested), and estimates from different agents are not directly comparable. All 32 isolates tested against tetracycline were resistant (100%). Resistance was also frequent for erythromycin (36/55, 65.5%), penicillin (31/49, 63.3%), and cefepime (12/23, 52.2%). Surrogate ampicillin labels, the oxacillin screen, and historical outputs for agents without applicable pneumococcal interpretive criteria are reported only in Supplementary Table S3 and were excluded from the primary MDR classification.

Among cephalosporins with established CLSI interpretive criteria, ceftriaxone was susceptible in 25

TABLE 1. Baseline characteristics of the 58 children with a culture-confirmed *Streptococcus pneumoniae* isolate.

Characteristic	Total group (n = 58)
Age, months, median (IQR)	31 (14–60)
<2 years	26 (44.8)
2–5 years	18 (31.0)
>5 years	14 (24.1)
Male sex	33 (56.9)
Clinical syndrome: pneumonia	46 (79.3)
Clinical syndrome: suspected bacteremia	12 (20.7)
Specimen: nasopharyngeal aspirate	50 (86.2)
Specimen: blood	8 (13.8)
Pneumonia with positive blood culture	4 (6.9)
Suspected bacteremia with positive blood culture (confirmed)	4 (6.9)
Suspected bacteremia without positive blood culture (clinical only)	8 (13.8)
Underlying condition present	5 (8.6)
Congenital heart disease	3 (5.2)
Malnutrition	2 (3.4)
Prior pneumococcal vaccination	17 (29.3)
Antibiotic use within 30 days before admission	16 (27.6)
Admission to intensive/critical care unit	14 (24.1)
Length of hospital stay, days, median (IQR)	10 (7–14)
Duration of antimicrobial therapy, days, median (IQR)	9.5 (7–12)

Percentages are based on $n = 58$ unless otherwise indicated. Clinical syndrome (pneumonia vs suspected bacteremia) and specimen source (nasopharyngeal aspirate vs blood) were recorded independently and are not interchangeable; suspected bacteremia was microbiologically confirmed by a positive blood culture in only 4 of 12 cases (33.3%).

Abbreviation: IQR, interquartile range

of 36 isolates (69.4%, 95% CI 53.1–82.0), cefuroxime in 30 of 47 (63.8%, 95% CI 49.5–76.0), cefotaxime in 7 of 21 (33.3%), and cefepime in 10 of 23 (43.5%). Because these agents were tested in different, clinically selected subsets rather than a common panel, these are agent-specific descriptive estimates and do not support a cross-agent ranking of activity. Chloramphenicol resistance was 32.0% (8/25), clindamycin resistance was 39.7% (23/58), and all 58 isolates tested against vancomycin were susceptible.

Multidrug resistance and associated factors

Isolates were tested against a median of 11 antimicrobial agents (range 6–15), corresponding to a median of 6

evaluable classes (range 4–7) under the primary definition. Thirty-eight of 58 isolates (65.5%, 95% CI 52.7–76.4) met the primary MDR definition; this estimate is conditional on the observed testing panel and should be regarded as a conservative floor. MDR prevalence was 62.1% (36/58; 95% CI 49.2–73.4) when intermediate results were counted as susceptible and 89.7% (52/58; 95% CI 79.2–95.2) under the broader nine-category protocol definition (Table 4). When the 4 isolates tested against fewer than five of the seven evaluable classes were reclassified as indeterminate, prevalence among the remaining 54 isolates was 70.4% (38/54; 95% CI 57.2–80.9). Under the stricter logical criterion, 9 isolates were definitively non-MDR and 11 were indeterminate; restricting the denominator to the

TABLE 2. Antimicrobial susceptibility of *Streptococcus pneumoniae* isolates, 2022–2024.

Antimicrobial agent	No. tested	Susceptible, n (%)	Intermediate, n (%)	Resistant, n (%)
A. Reportable susceptibility results — agents with applicable CLSI <i>S. pneumoniae</i> interpretive criteria				
Penicillin	49	18 (36.7)	0 (0.0)	31 (63.3)
Cefuroxime	47	30 (63.8)	0 (0.0)	17 (36.2)
Cefotaxime	21	7 (33.3)	6 (28.6)	8 (38.1)
Ceftriaxone	36	25 (69.4)	0 (0.0)	11 (30.6)
Cefepime	23	10 (43.5)	1 (4.3)	12 (52.2)
Erythromycin	55	14 (25.5)	5 (9.1)	36 (65.5)
Clindamycin	58	31 (53.4)	4 (6.9)	23 (39.7)
Chloramphenicol	25	17 (68.0)	0 (0.0)	8 (32.0)
Tetracycline	32	0 (0.0)	0 (0.0)	32 (100.0)
Vancomycin	58	58 (100.0)	0 (0.0)	0 (0.0)
B. Screening test — not a therapeutic susceptibility category				
Oxacillin 1 µg screen	45	Screen-negative: 7 (15.6)		Screen-positive: 38 (84.4)

Denominators vary because testing was clinically directed; percentages are based on the number tested, and agent-specific estimates are descriptive and not directly comparable. Table 2 includes only agents with an independent applicable CLSI *S. pneumoniae* interpretive criterion plus the oxacillin screening test. Surrogate ampicillin labels and historical outputs for ceftazidime, gentamicin, ciprofloxacin, and ofloxacin are reported only in Supplementary Table S3 and were excluded from primary MDR classification. For vancomycin, CLSI provides only a susceptible MIC breakpoint (≤ 1 µg/mL); intermediate and resistant categories are not applicable.

TABLE 3. Susceptibility and multidrug resistance by specimen source.

Outcome	Blood isolates (n = 8)	Nasopharyngeal aspirate isolates (n = 50)
MDR, primary definition	5 (62.5)	33 (66.0)
Penicillin susceptible	3/6 (50.0)	15/43 (34.9)
Ceftriaxone susceptible	4/6 (66.7)	21/30 (70.0)
Erythromycin susceptible	3/8 (37.5)	11/47 (23.4)
Vancomycin susceptible	8/8 (100.0)	50/50 (100.0)

Denominators vary by agent because testing was clinically directed. Blood isolates (n = 8) are presented descriptively only; no comparative inference or claim of equivalence is made from this subgroup.

Abbreviation: MDR, multidrug-resistant

TABLE 4. Prevalence of multidrug resistance under the primary definition and prespecified sensitivity analyses (n = 58).

MDR definition	MDR / total	Prevalence, %	95% CI
Primary: non-susceptibility to ≥ 3 classes with an independent applicable pneumococcal criterion	38/58	65.5	52.7–76.4
Sensitivity 1: intermediate counted as susceptible	36/58	62.1	49.2–73.4
Sensitivity 2: broader nine-category scheme used in the original protocol	52/58	89.7	79.2–95.2
Sensitivity 3: isolates tested against < 5 of 7 evaluable classes reclassified as indeterminate	38/54	70.4	57.2–80.9
Sensitivity 4: definitive non-MDR only where untested classes could not alter the call	38/47	80.9	67.5–89.6

Wilson 95% CIs are shown. Penicillins contribute to the primary definition through penicillin only; surrogate ampicillin results, the oxacillin screen, ceftazidime, aminoglycosides, and fluoroquinolones are counted only in the broader protocol sensitivity analysis. In Sensitivity 3, four isolates tested against fewer than five of seven evaluable classes were reclassified as indeterminate. In Sensitivity 4, non-MDR status required that observed non-susceptible classes plus all untested classes could not reach three; 9 isolates were non-MDR and 11 were indeterminate. Isolate-level classifications are provided in Supplementary Table S1.

Abbreviations: CI, confidence interval; MDR, multidrug-resistant

47 determinate isolates yielded 80.9% MDR (38/47; 95% CI 67.5–89.6).

MDR occurred in 5 of 8 blood isolates (62.5%) and 33 of 50 nasopharyngeal aspirate isolates (66.0%) (Table 3). These results are presented descriptively only; with only eight blood isolates, no comparative inference or claim of equivalence is made. Median length of hospital stay was 9.5 days (IQR 7–14) in children with MDR isolates and 10 days (IQR 7.5–13) in those with non-MDR isolates ($P = 0.95$); median duration of antimicrobial therapy was 8.5 days (IQR 7–12) and 10 days (IQR 7–11), respectively ($P = 0.91$).

In exploratory unadjusted analyses (Table 5), absence of a documented underlying condition was the only characteristic associated with MDR status after Holm correction for the six comparisons. None of the five children with an underlying condition had an MDR isolate, compared with 38 of 53 children without one (OR 0.04, 95% CI 0.002–0.70; unadjusted $P = 0.0034$; Holm-adjusted $P = 0.020$). This estimate is fragile because it is anchored by a zero cell in a group of only five children. Pneumococcal vaccination was not associated with MDR after correction (8/17 vs 30/41; OR 0.33, 95% CI 0.10–1.06; unadjusted $P = 0.073$; Holm-adjusted $P = 0.367$). Age

group, sex, admitting ward, and antibiotic use in the preceding 30 days were not associated with MDR status.

DISCUSSION

In this three-year, single-center series of *S. pneumoniae* isolates from hospitalized children in the Mekong Delta, approximately two in three isolates were multidrug-resistant under an organism-appropriate definition restricted to agents with an independent pneumococcal interpretive criterion, resistance to tetracycline was universal, and resistance to penicillin and erythromycin each exceeded 60%. Vancomycin retained complete in vitro activity. A central methodological finding is that the MDR estimate moved from 89.7% under the broader protocol definition to 65.5% under the primary definition when surrogate and non-interpretable results were excluded, underscoring the importance of a transparent organism-specific MDR algorithm.

The resistance profile for penicillin, oxacillin, and erythromycin is broadly consistent with earlier Vietnamese data and with reports from elsewhere in East and Southeast Asia, a region that has long carried among the highest pneumococcal resistance burdens globally.^{6,14–18} The MDR prevalence of 65.5% falls within the range of published

TABLE 5. Exploratory unadjusted analysis of characteristics associated with multidrug-resistant status (n = 58).

Characteristic	MDR, n (%)	Non-MDR, n (%)	Unadjusted OR (95% CI)	P value	Holm-adjusted P
Age ≤5 years (vs >5 years)	29 (65.9)	15 (34.1)	1.07 (0.31–3.78)	1.000	1.000
Male sex (vs female)	24 (72.7)	9 (27.3)	2.10 (0.70–6.30)	0.265	1.000
Respiratory ward (vs ICU)	29 (65.9)	15 (34.1)	1.07 (0.31–3.78)	1.000	1.000
Underlying condition (vs none)	0 (0.0)	5 (100.0)	0.04 (0.00–0.70)	0.003	0.020
Antibiotic use ≤30 days (vs none)	11 (68.8)	5 (31.2)	1.22 (0.36–4.19)	1.000	1.000
Pneumococcal vaccination (vs none)	8 (47.1)	9 (52.9)	0.33 (0.10–1.06)	0.073	0.367

Percentages are row percentages. ORs compare each category with the reference shown in parentheses; Haldane–Anscombe correction was used for zero cells. P values are from two-sided Fisher exact tests; Holm-adjusted values use the step-down Holm procedure for six comparisons computed from full-precision (unrounded) P values. The association involving underlying condition is anchored by a zero cell in only five children and should be interpreted cautiously; no other association remained significant after multiplicity adjustment.

Abbreviations: CI, confidence interval; ICU, intensive/critical care unit; MDR, multidrug-resistant; OR, odds ratio

estimates from the region, although direct comparison is limited by differences in isolate selection and susceptibility panels. Three explanations are plausible and mutually compatible. First, as the sole tertiary referral center for the region, our hospital receives children who have often failed community treatment, enriching the sample for resistant organisms. Second, antimicrobials remain widely available without prescription in Vietnam, and more than a quarter of our patients had documented antibiotic exposure in the preceding month. The same pattern has been described in neighbouring Thailand, where antimicrobial dispensing by drug stores outside formal prescription is common and frequently directed at self-limiting illnesses, and where short courses of antimicrobials in primary care have been shown to select for resistance in the treated individual for up to a year.¹⁹ Third, the highly resistant clones and serotypes described across the region, notably 19A and 19F, are known to circulate in Vietnam.^{5,17,20} None of these mechanisms was measured directly here, and our estimate should be read as descriptive rather than explanatory. Comparison with previously published Vietnamese estimates approaching 100% must also be made with caution, because those series applied broader and largely undocumented agent panels; the incomparability is itself a finding that argues for a standardized surveillance panel. The same problem is documented at global scale: among countries reporting to the WHO Global Antimicrobial Resistance and Use

Surveillance System, both the isolates selected for testing and the agents included vary widely, which limits the comparability of resistance estimates between settings.³

Two aspects of the susceptibility profile carry stewardship implications, framed here as surveillance and laboratory-practice observations rather than treatment guidance. No vancomycin non-susceptibility was detected; this finding does not establish an empirical-treatment recommendation. Ceftazidime has no applicable *S. pneumoniae* interpretive criterion in the CLSI M100 editions used.¹¹ Accordingly, as a laboratory-stewardship measure, routine ceftazidime testing or interpretation for *S. pneumoniae* should be avoided, and its continued presence in local pneumococcal panels should be reconsidered by the laboratory.

Ceftriaxone susceptibility was 69.4% (25/36; 95% CI 53.1–82.0). This agent-specific estimate is not directly comparable with the other cephalosporin percentages because the agents were tested in different, clinically selected subsets. We deliberately refrain from translating it into an empirical-therapy recommendation. Susceptibility testing was clinically directed rather than protocol-driven, and 22 of 58 isolates were not tested against ceftriaxone; empirical regimen selection also requires consideration of clinical syndrome, disease severity, dosing, the possibility of meningitis, and current national and institutional guidance, none of which this study was designed to evaluate.

Fewer MDR isolates were recovered from previously vaccinated children (8/17, 47.1%) than from unvaccinated children (30/41, 73.2%), but this association did not survive multiplicity adjustment (OR 0.33, 95% CI 0.10–1.06; Holm-adjusted $P = 0.367$) and is not presented as a positive finding. A protective direction is biologically coherent because conjugate vaccines target serotypes disproportionately represented among resistant lineages^{21,22}, and Vietnamese evidence supports direct PCV protection against invasive pneumococcal disease despite low private-sector coverage.⁵ Nevertheless, the estimate is sparse and unadjusted; vaccination in a setting where PCV was privately purchased may also mark household income, health literacy, and access to care. Vaccination status was partly caregiver-reported, without vaccine product or dose number, and no serotyping or molecular resistance testing was performed. Because this case-only hospital series had no disease-free comparison group, it cannot estimate vaccine effectiveness or support inference about vaccination policy.

The direction of this association is not universal. A multicenter study in Bogotá following PCV10 introduction and a study of immunocompromised patients in Tunisia receiving PCV13 both reported higher resistance among vaccinated individuals^{23,24}, consistent with serotype replacement by resistant non-vaccine types where vaccine pressure has been sustained. Our finding should therefore be treated as setting-specific and hypothesis-generating, not as evidence that vaccination reduces resistance.

This study has several limitations. It was conducted at a single center, which constrains generalizability. Most isolates (50/58) were recovered from nasopharyngeal aspirates rather than a sterile site; because *S. pneumoniae* colonizes the nasopharynx, some may represent colonization rather than the causative pathogen, and only the 8 blood isolates represent unequivocally invasive disease.²⁵ Eight children with a clinical diagnosis of suspected bacteremia had no positive blood culture, so their syndrome classification was clinical rather than microbiological. Viral co-pathogens were not systematically ascertained: multiplex testing for respiratory syncytial virus, influenza, adenovirus, and rhinovirus was performed only when clinically requested, SARS-CoV-2 was not part of the routine panel, and viral results were not captured as a structured study variable; therefore, unmeasured viral co-infection may have contributed to clinical outcomes independently of pneumococcal resistance. The susceptibility panel was clinically directed rather than standardized, so selective-testing verification bias remains; incomplete testing can miss MDR among isolates classified as non-MDR, making the primary 65.5% estimate conditional on the observed

panel. Numerical MIC values and inhibition-zone diameters could not be retrieved, so the categorical results are legacy laboratory reports that cannot be independently re-derived or re-interpreted under another CLSI edition. Trimethoprim–sulfamethoxazole was not included in the panel, which may further bias the MDR estimate downward. The sample was small, with only 20 non-MDR isolates, so association analyses were underpowered and unadjusted for confounding; the sole adjusted association (absence of an underlying condition) rests on a zero cell in five children. Finally, no serotyping or molecular characterization was performed.

Set against these constraints, the study has genuine strengths: laboratory confirmation of *S. pneumoniae* recovery in every included child, a single unchanged laboratory workflow across three years, inclusion of all eligible children rather than a convenience subset, and public deposition of the anonymized dataset together with a full isolate-level classification that allows the MDR outcome to be independently reconstructed. The institutional context was stable: no pneumococcal outbreak or change in pneumococcus-specific infection-control or formal hospital-wide antimicrobial-stewardship policy occurred during the study period.

CONCLUSION

Among hospitalized children in the Mekong Delta of Vietnam, *S. pneumoniae* showed frequent antimicrobial resistance, with 65.5% of isolates meeting the primary MDR definition restricted to agents with an independent applicable pneumococcal criterion; this is a conservative estimate conditional on the observed testing panel. Because 50 of 58 isolates were recovered from nasopharyngeal aspirates and only 4 of 12 clinically suspected bacteremia cases were confirmed by a positive blood culture, the findings should be interpreted as institution-level surveillance of clinically obtained *S. pneumoniae* rather than a resistance profile restricted to confirmed invasive disease. Agent-specific susceptibility percentages should not be ranked because testing subsets differed. As a laboratory-stewardship measure, ceftazidime should not be tested or interpreted for *S. pneumoniae* under the CLSI framework used. Absence of an underlying condition was the only characteristic associated with MDR after Holm adjustment, but that finding depends on a zero cell in five children and is hypothesis-generating; pneumococcal vaccination and the remaining characteristics were not associated after multiplicity adjustment. Standardized, organism-appropriate susceptibility panels are needed for more reliable MDR surveillance.

Data Availability Statement

The anonymized dataset supporting the findings of this study is publicly available in the Zenodo repository at <https://doi.org/10.5281/zenodo.21600133>, under a Creative Commons Attribution 4.0 International license. The deposited file contains categorical susceptibility interpretations only, together with a codebook defining every variable and value code; numerical MIC values and inhibition-zone diameters were not retrievable and are therefore not included. All prevalence estimates, odds ratios, confidence intervals, and multiplicity-adjusted P values reported in this manuscript are reproducible from the deposited dataset.

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Conflict of Interest

The authors declare no competing financial interests or personal relationships that could have influenced the work reported in this article.

Registration Number of Clinical Trial

Not applicable.

Author Contributions

Conceptualization and methodology, N.Q.B. and H.T.N.; Investigation, K.Q.T., L.T.N.T. and L.D.P.; Data curation, D.K.N., D.T.B., N.T.T., T.N.L. and T.N.; Formal analysis, N.Q.B.; Writing — original draft, N.Q.B.; Writing — review and editing, D.K.N., D.T.B., N.T.T., T.N.L., T.N. and H.T.N.; Supervision and project administration, K.Q.T., L.T.N.T., L.D.P. and H.T.N. All authors have read and agreed to the published version of the manuscript.

Use of Artificial Intelligence

During the preparation of this work the authors used Grammarly (Grammarly Inc.) and Claude (Anthropic) to improve language, readability, and manuscript formatting.

These tools were not used to generate, analyze, or interpret data, or to draw scientific conclusions. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Institutional Review Board Statement

This study was approved by the Biomedical Research Ethics Committee of Can Tho University of Medicine and Pharmacy (approval no. 23.101.SV/PCT-HĐĐĐ, 25 December 2023; study code 23.KY.042) and was authorized by the Director of Can Tho Children's Hospital. The approval covered retrospective review of 2022–2023 records and stored isolate data with a waiver of individual consent; written informed consent from a parent or legal guardian, and age-appropriate assent, were obtained for the prospective 2024 component. All procedures were conducted in accordance with the ethical standards of the responsible institutional committee and with the 1964 Declaration of Helsinki and its later amendments.

REFERENCES

1. GBD 2021 Lower Respiratory Infections and Antimicrobial Resistance Collaborators. Global, regional, and national incidence and mortality burden of non-COVID-19 lower respiratory infections and aetiologies, 1990–2021: a systematic analysis from the Global Burden of Disease Study 2021. *Lancet Infect Dis.* 2024;24(9):974-1002.
2. World Health Organization. WHO bacterial priority pathogens list, 2024: bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. Geneva: World Health Organization; 2024.
3. World Health Organization. Global antimicrobial resistance and use surveillance system (GLASS) report 2021. Geneva: World Health Organization; 2021.
4. Vu TVD, Choisy M, Do TTN, Nguyen VMH, Campbell JI, Le TH, et al. Antimicrobial susceptibility testing results from 13 hospitals in Viet Nam: VINARES 2016–2017. *Antimicrob Resist Infect Control.* 2021;10(1):78.
5. Truong HC, Pham QD, Phan TV, Vo DTT, Nguyen PD, Nguyen HT, et al. Effectiveness of pneumococcal conjugate vaccines against invasive pneumococcal disease in Vietnamese children prior to national introduction: a matched case-control study. *Vaccine.* 2026;77:128349.
6. Tran-Quang K, Nguyen-Thi-Dieu T, Tran-Do H, Pham-Hung V, Nguyen-Vu T, Tran-Xuan B, et al. Antibiotic resistance of *Streptococcus pneumoniae* in Vietnamese children with severe pneumonia: a cross-sectional study. *Front Public Health.* 2023;11:1110903.
7. Bui AS, Duong DC, Le THH, Do NA. Frequency of *erm(B)* and *mef(A)* genes among macrolide-resistant *Streptococcus pneumoniae* strains isolated from children under 5 years with pneumonia in Nghe An province, 2019–2021. *Vietnam J Sci Technol.* 2023;65(2): 9-13. Vietnamese.
8. Wambugu P, Shah MM, Nguyen HA, Le KA, Le HH, Vo HM,

- et al. Molecular epidemiology of *Streptococcus pneumoniae* detected in hospitalized pediatric acute respiratory infection cases in Central Vietnam. *Pathogens*. 2023;12(7):943.
9. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP; STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet*. 2007;370(9596):1453-7.
10. Tacconelli E, Cataldo MA, Paul M, Leibovici L, Kluytmans J, Schröder W, et al. STROBE-AMS: recommendations to optimise reporting of epidemiological studies on antimicrobial resistance and informing improvement in antimicrobial stewardship. *BMJ Open*. 2016;6(2):e010134.
11. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 32nd, 33rd and 34th eds. CLSI supplement M100. Wayne (PA): Clinical and Laboratory Standards Institute; 2022, 2023, 2024.
12. Song JH, Jung SI, Ko KS, Kim NY, Son JS, Chang HH, et al. High prevalence of antimicrobial resistance among clinical *Streptococcus pneumoniae* isolates in Asia (an ANSORP study). *Antimicrob Agents Chemother*. 2004;48(6):2101-7.
13. Kim L, McGee L, Tomczyk S, Beall B. Biological and epidemiological features of antibiotic-resistant *Streptococcus pneumoniae* in pre- and post-conjugate vaccine eras: a United States perspective. *Clin Microbiol Rev*. 2016;29(3):525-52.
14. Cai K, Wang Y, Guo Z, Xu X, Li H, Zhang Q. Clinical characteristics and antimicrobial resistance of pneumococcal isolates of pediatric invasive pneumococcal disease in China. *Infect Drug Resist*. 2018;11:2461-9.
15. Nguyen TH, Nguyen VA, Pham TL, Le HLH. Antibiotic resistance status of *Streptococcus pneumoniae* causing respiratory infections in children under 6 years at Duc Giang General Hospital, 2023. *Vietnam Med J*. 2024;541(3):339-43. Vietnamese.
16. Oh H, Heo ST, Kim M, Kim YR, Yoo JR. Antimicrobial susceptibility trends of *Streptococcus pneumoniae* by age groups over recent 10 years in a single hospital in South Korea. *Yonsei Med J*. 2021;62(4):306-14.
17. Pan F, Han L, Huang W, Tang J, Xiao S, Wang C, et al. Serotype distribution, antimicrobial susceptibility, and molecular epidemiology of *Streptococcus pneumoniae* isolated from children in Shanghai, China. *PLoS One*. 2015;10(11):e0142892.
18. Zhao W, Pan F, Wang B, Wang C, Sun Y, Zhang T, et al. Epidemiology characteristics of *Streptococcus pneumoniae* from children with pneumonia in Shanghai: a retrospective study. *Front Cell Infect Microbiol*. 2019;9:258.
19. Thamlikitkul V. STOP antimicrobial resistance: everybody's business. *Siriraj Med J*. 2014;66(6):234-40.
20. Peng S, Ren H, Deng J, Zhao N, Li Y, Li M, et al. Genotypic and phenotypic characteristics of *Streptococcus pneumoniae* from community-acquired pneumonia patients and healthy asymptomatic participants in Sichuan province, China. *BMC Infect Dis*. 2021;21(1):1030.
21. Song JY, Nahm MH, Moseley MA. Clinical implications of pneumococcal serotypes: invasive disease potential, clinical presentations, and antibiotic resistance. *J Korean Med Sci*. 2013;28(1):4-15.
22. Marimon JM, Ardanuy C. Epidemiology of pneumococcal diseases in Spain after the introduction of pneumococcal conjugate vaccines. *Enferm Infecc Microbiol Clin*. 2021;39(3):142-50.
23. Gutiérrez-Tobar IF, Londoño-Ruiz JP, Mariño-Drews C, Beltrán-Higuera S, Camacho-Moreno G, Leal-Castro AL, et al. Epidemiological characteristics and serotype distribution of culture-confirmed pediatric pneumococcal pneumonia before and after PCV10 introduction: a multicenter study in Bogotá, Colombia, 2008-2019. *Vaccine*. 2022;40(20):2875-83.
24. Raddaoui A, Simões AS, Baaboura R, Félix S, Achour W, Ben Othman T, et al. Serotype distribution, antibiotic resistance and clonality of *Streptococcus pneumoniae* isolated from immunocompromised patients in Tunisia. *PLoS One*. 2015;10(10):e0140390.
25. Bogaert D, De Groot R, Hermans PWM. *Streptococcus pneumoniae* colonisation: the key to pneumococcal disease. *Lancet Infect Dis*. 2004;4(3):144-54.