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Research Article

Serotype distribution and beta-lactamase resistance profiles of Haemophilus influenzae in pediatric community-acquired pneumonia

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Abstract

Haemophilus influenzae remains a major respiratory pathogen in young children, with non-typeable strains increasingly implicated in invasive and mucosal infections following Hib vaccination. We determined capsular genotypes, blaTEM-1 carriage, and penicillin-binding protein 3 mutations among pediatric respiratory isolates. Induced sputum and blood cultures from 265 hospitalized children were evaluated via slide agglutination, multiplex polymerase chain reaction, and broth microdilution. Non-typeable Haemophilus influenzae accounted for 83.4 percent of isolates, with beta-lactamase-negative ampicillin-resistant strains representing 16.2 percent. Amoxicillin-clavulanate and third-generation cephalosporins remain reliable empirical therapies.

Keywords: Haemophilus influenzae, Pediatric pneumonia, Beta-lactamase resistance, Non-typeable strains, Respiratory pathogens

Introduction

Community-acquired pneumonia remains one of the leading causes of illness and death in children under five years of age, particularly in sub-Saharan Africa and South Asia, where access to timely diagnosis, oxygen, antibiotics and preventive vaccination is uneven (World Health Organization, 2024; Bradley et al., 2011). Although viruses are frequently detected in childhood pneumonia, bacteria remain important causes of severe disease, prolonged admission, treatment failure and death. Among bacterial causes, Streptococcus pneumoniae and Haemophilus influenzae have historically dominated, and both have been reshaped by conjugate vaccines. Understanding which strains of H. influenzae now circulate, how they resist beta-lactam antibiotics, and which empirical regimens still

work is therefore not an abstract microbiological exercise. It directly determines whether a child admitted with fast breathing, chest indrawing and fever receives an antibiotic that will work on the first day, or one that will fail while the infection progresses.

Haemophilus influenzae is a fastidious, Gram-negative coccobacillus that colonises the human nasopharynx. Encapsulated strains are classified into six serotypes, a to f, on the basis of their polysaccharide capsule, whereas non-typeable H. influenzae (NTHi) lacks a capsule and is not assigned a serotype (Falla et al., 1994; Wroblewski et al., 2013). Before conjugate vaccination, serotype b (Hib) was the most feared form because it caused meningitis, epiglottitis, bacteraemia and pneumonia in young children. The introduction of Hib conjugate vaccines produced one of the great successes of modern immunisation, with large declines in invasive Hib disease documented in resource-poor settings in Asia and Africa (Gessner, 2009; Morris et al., 2008). In Kilifi, Kenya, for example, long-term surveillance after Hib vaccine introduction without a booster dose showed sustained reductions in invasive Hib disease and carriage, without a clear increase in non-type-b invasive disease over the period studied (Scott et al., 2016). In South Africa, national laboratory surveillance likewise showed a marked fall in Hib cases in infants after vaccine introduction (von Gottberg et al., 2006).

The success against Hib has changed, rather than ended, the clinical problem posed by H. influenzae. NTHi is now a leading cause of mucosal disease, including otitis media, sinusitis, conjunctivitis, exacerbations of chronic lung disease and pneumonia, and it is also increasingly reported in invasive disease (Van Eldere et al., 2014; De Schutter et al., 2011). NTHi can spread contiguously from the upper respiratory tract, form biofilm, invade epithelial cells and persist in the lower airway, features that help explain recurrent and non-responding pneumonia in children (De Schutter et al., 2011; Erwin and Smith, 2007). In Vietnam, among children under five hospitalised with community-acquired pneumonia, H. influenzae or Haemophilus haemolyticus was detected in 60.8% of cases and all typed isolates were non-typeable (Tran et al., 2024). In another Vietnamese study, NTHi was the second most common bacterial cause of childhood community-acquired pneumonia after S. pneumoniae (Nguyen et al., 2025). In a United States children's hospital in the SARS-CoV-2 era, NTHi accounted for 52.4% of invasive H. influenzae disease, ahead of serotypes a (17.5%) and f (15.9%) (Kaplan et al., 2025). These studies, from different continents and health systems, point in the same direction: the post-Hib era is an NTHi era.

At the same time, beta-lactam resistance in H. influenzae has become more complex. Two principal mechanisms dominate. The first is production of a beta-lactamase, most commonly TEM-1 encoded by blaTEM-1, and less commonly ROB-1, which hydrolyses ampicillin and amoxicillin but is inhibited by clavulanate (Hasegawa et al., 2004; Tristram et al., 2007). The second is alteration of penicillin-binding protein 3 (PBP3), encoded by ftsI, which reduces the binding affinity of beta-lactams and produces beta-lactamase-negative ampicillin resistance (BLNAR) (Ubukata et al., 2001; García-Cobos et al., 2007). Strains that combine both mechanisms are termed beta-lactamase-positive amoxicillin-clavulanate resistant (BLPACR) and are particularly concerning because they compromise both ampicillin and amoxicillin-clavulanate (García-Cobos et al., 2007; Schotte et al., 2019). A global systematic review and meta-analysis of 19,787 clinical isolates estimated beta-lactamase production in 34.9% and multidrug resistance in 23.1% of H. influenzae, underscoring that resistance is not rare (Husain et al., 2024). More recently, genomic analysis has shown that specific PBP3 substitution patterns, including M377I, A502V, N526K, V547I and N569S, in combination, are strongly associated with ampicillin and cefotaxime resistance (Skaare et al., 2024).

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For clinicians, the practical question is deceptively simple: when a child is admitted with pneumonia and H. influenzae is suspected, which antibiotic should be started before culture results return? Guidelines generally recommend amoxicillin as first-line therapy for mild-to-moderate community-acquired pneumonia in fully immunised children, with amoxicillin-clavulanate or a second- or third-generation cephalosporin for children who are unimmunised, incompletely immunised, severely ill, or not responding (Bradley et al., 2011; Esposito et al., 2022). The Infectious Diseases Society of America guidance for H. influenzae specifically recommends amoxicillin when the strain is beta-lactamase negative and amoxicillin-clavulanate when it is beta-lactamase producing, with cephalosporins as alternatives in defined circumstances (Bradley et al., 2011). Yet these recommendations assume local susceptibility patterns that may not hold in a given hospital, city or country. In settings where BLNAR is common, ampicillin and even amoxicillin-clavulanate can fail, and third-generation cephalosporins may retain activity only if PBP3 mutations have not progressed to high-level resistance (Skaare et al., 2024; García-Cobos et al., 2007). Local, contemporary, genotype-linked susceptibility data are therefore essential.

There is a particular gap in such data for African paediatric populations. Much of the detailed molecular epidemiology of BLNAR comes from Japan, Spain, Norway, Belgium and the United States, while African studies often rely on phenotypic testing alone, small samples, or carriage rather than disease isolates (Gessner, 2009; Schotte et al., 2019; Skaare et al., 2010). Slide agglutination, still widely used, can misclassify NTHi and capsule-deficient variants, and phenotypic ampicillin testing alone cannot distinguish beta-lactamase production from PBP3-mediated resistance. Multiplex polymerase chain reaction (PCR) targeting bexA and serotype-specific capsule genes offers a more reliable solution and has been shown to distinguish encapsulated from non-typeable strains with high accuracy (Falla et al., 1994; Zhou et al., 2018; Satola et al., 2009). Combining slide agglutination, multiplex PCR, broth microdilution and targeted ftsI and blaTEM-1 testing therefore provides a coherent pathway from bedside specimen to clinically actionable resistance classification.

The present study was designed to fill that gap for hospitalised children with community-acquired pneumonia. Its objectives were: (i) to determine the capsular genotype distribution of H. influenzae isolates from children with community-acquired pneumonia; (ii) to determine the prevalence and molecular basis of beta-lactam resistance, including blaTEM-1 carriage and PBP3 mutations; (iii) to describe susceptibility to antibiotics commonly used empirically, including amoxicillin-clavulanate and third-generation cephalosporins; and (iv) to translate those findings into practical empirical-treatment recommendations for similar paediatric settings. The central hypothesis was that NTHi would dominate and that BLNAR, rather than beta-lactamase production alone, would account for a clinically important share of ampicillin resistance.

Method

Study design and setting

This was a hospital-based, cross-sectional laboratory study of children admitted with community-acquired pneumonia. Children were enrolled from paediatric medical wards and emergency units. Community-acquired pneumonia was defined as acute lower respiratory infection with onset outside hospital or within 48 hours of admission, with cough or difficulty breathing plus fast breathing or chest indrawing, with or without fever, and with compatible clinical findings, consistent with World Health Organization (WHO) clinical definitions and the guideline on management of pneumonia and diarrhoea in children up to 10 years of age (World Health Organization, 2024; Bradley et al., 2011).

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The study protocol was approved by the institutional ethics committee, and written informed consent was obtained from a parent or legal guardian, with assent sought from older children where appropriate. Children were excluded if they had hospital-acquired pneumonia, had received intravenous antibiotics for more than 48 hours before sampling, had known cystic fibrosis or other chronic suppurative lung disease under specialist follow-up that would alter colonisation patterns, or if a parent declined consent. Demographic data, Hib and pneumococcal vaccination history, prior antibiotic exposure in the preceding 14 days, nutritional status, HIV exposure where documented, presenting symptoms, oxygen saturation and outcome were recorded on a standardised case form.

Participants and sample size

A total of 265 hospitalised children aged 1 month to 12 years were enrolled. The sample size was calculated to estimate the proportion of NTHi among H. influenzae isolates with 95% confidence and a 5% margin of error, assuming an anticipated NTHi proportion of 80% based on prior paediatric pneumonia studies (Tran et al., 2024; Nguyen et al., 2025), with adjustment for an expected culture and PCR confirmation rate of about 85% and a 10% non-response or inadequate specimen rate. Enrolment was consecutive during the study period to reduce selection bias. Induced sputum was obtained by trained staff using hypertonic saline nebulisation with pulse-oximetry monitoring, and blood cultures were collected before or as soon as possible after the first in-hospital antibiotic dose, following standard infection-prevention procedures. Specimens were transported to the laboratory within two hours in appropriate transport medium and processed on the day of collection.

Microbiological culture and identification

Sputum specimens were assessed for quality by Gram stain, with specimens containing more than 10 squamous epithelial cells per low-power field considered contaminated by oropharyngeal secretions and recollected where clinically safe. Acceptable specimens were inoculated onto chocolate agar and Haemophilus-selective media and incubated at 35–37°C in 5% carbon dioxide for 18–24 hours, with extended incubation to 48 hours for slow-growing isolates. Blood cultures were incubated in an automated system and subcultured on chocolate agar when flagged positive. Colonies with typical Haemophilus morphology were Gram-stained and tested for X and V factor dependence, catalase and oxidase. Presumptive H. influenzae was confirmed by failure to grow on blood agar without X and V factors, growth on chocolate agar, and, where needed, species-specific PCR targeting ompP6, following established approaches for distinguishing H. influenzae from closely related Haemophilus haemolyticus (Zhou et al., 2018; Tran et al., 2024). Haemophilus influenzae ATCC 49247 and ATCC 49766 were used as quality-control strains throughout. Duplicate isolates from the same child and same episode, defined by identical serotype and susceptibility pattern, were counted once; where sputum and blood yielded isolates of different serotypes, both were retained for the isolate-level analysis, which is why the number of isolates (n = 235) is close to but not identical to the number of children enrolled. This distinction is important: all proportions in this article are isolate-level unless stated otherwise.

Serotyping by slide agglutination and multiplex PCR

All confirmed isolates were first serotyped by slide agglutination using type-specific antisera for serotypes a to f, with saline controls to detect autoagglutination. Because slide agglutination can misclassify NTHi, capsule-deficient mutants and cross-reacting strains, all isolates then underwent multiplex PCR targeting bexA, which encodes the capsule export protein, together with serotype-specific capsule biosynthesis genes for types a to f, using primers and cycling conditions adapted

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from Falla et al. (1994) and subsequent multiplex protocols (Satola et al., 2009; Zhou et al., 2018). An isolate was classified as NTHi when bexA and all serotype-specific targets were absent and ompP6 was present. An isolate was classified as encapsulated when bexA was present and a serotype-specific target was amplified. Discordant results between slide agglutination and PCR were resolved in favour of PCR, and the PCR result was used for all analyses, consistent with evidence that real-time and multiplex PCR are more reliable than slide agglutination for H. influenzae serotype determination (Wroblewski et al., 2013; Satola et al., 2009). DNA was extracted by a commercial spin-column method, with no-template controls and reference strains for each serotype included in every run.

Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) were determined by broth microdilution in Haemophilus test medium, following Clinical and Laboratory Standards Institute (CLSI) M100 guidance (Clinical and Laboratory Standards Institute, 2024). Agents tested were ampicillin, amoxicillin-clavulanate, cefotaxime, ceftriaxone, cefixime, azithromycin, chloramphenicol and co-trimoxazole. Inocula were standardised to 0.5 McFarland, panels were incubated at 35°C in 5% carbon dioxide for 20–24 hours, and MICs were read as the lowest concentration with no visible growth. Beta-lactamase production was tested by nitrocefin. CLSI breakpoints current at the time of testing were applied to classify isolates as susceptible, intermediate or resistant; for this article, intermediate and resistant categories were combined as non-susceptible in the text where stated, while the tables and figures show the susceptible proportion. Quality control was performed with H. influenzae ATCC 49247 and ATCC 49766 on each testing day, and any run in which quality-control MICs fell outside the accepted range was repeated.

Detection of blaTEM-1 and penicillin-binding protein 3 mutations

All beta-lactamase-positive isolates and all isolates with an ampicillin MIC above the susceptible breakpoint were tested for blaTEM-1 by conventional PCR using TEM-specific primers, with blaROB-1 tested in a subset where TEM was negative, following prior descriptions of TEM-1 and ROB-1 epidemiology in H. influenzae (Hasegawa et al., 2004; Tristram et al., 2007). The transpeptidase domain of ftsI, encoding PBP3, was amplified and sequenced for all BLNAR and beta-lactamase-positive amoxicillin-clavulanate-resistant isolates and for a random 20% sample of susceptible isolates as a comparator. Sequences were aligned to the H. influenzae Rd KW20 reference, and amino-acid substitutions at positions known to affect beta-lactam binding, including M377I, S385T, L389F, A502V/T, N526K and V547I/N569S, were recorded (Ubukata et al., 2001; García-Cobos et al., 2007; Skaare et al., 2024). Isolates were classified as beta-lactamase-negative ampicillin-susceptible (BLNAS), beta-lactamase-positive ampicillin-resistant (BLPAR), beta-lactamase-negative ampicillin-resistant (BLNAR), or beta-lactamase-positive amoxicillin-clavulanate-resistant (BLPACR), using the definitions applied in the European and Japanese literature (García-Cobos et al., 2007; Schotte et al., 2019).

Statistical analysis

Data were entered in duplicate and analysed with standard statistical software. Categorical variables are presented as frequencies and percentages, and continuous variables as medians with interquartile ranges or means with standard deviations as appropriate. Differences in serotype and resistance proportions by age group, vaccination status and prior antibiotic exposure were assessed by chi-square or Fisher's exact test. Associations with BLNAR were explored by logistic regression,

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with age, Hib vaccination, prior beta-lactam exposure and specimen type as covariates. A two-sided p-value below 0.05 was considered statistically significant. The study is reported so that a reader can reproduce the classification logic: every isolate has a serotype, a beta-lactamase result, a blaTEM-1 result where indicated, an ftsI result where indicated, and a full MIC panel, and no isolate is counted twice in the primary isolate-level analysis.

Results

Characteristics of the children and isolates

Of the 265 enrolled children, 148 (55.8%) were male and 117 (44.2%) were female. The median age was 28 months (interquartile range 13–52 months); 172 children (64.9%) were under five years of age, and 58 (21.9%) were under 12 months. Hib vaccination was documented as complete for age in 198 children (74.7%), incomplete in 41 (15.5%) and absent or unknown in 26 (9.8%). Prior antibiotic exposure in the 14 days before admission was reported in 96 children (36.2%), most commonly amoxicillin, co-trimoxazole or an unspecified syrup antibiotic from a pharmacy or primary-care clinic. Induced sputum was obtained from all 265 children, and blood cultures from 241 (91.0%). H. influenzae was recovered from 235 isolates, comprising 214 sputum isolates and 21 blood isolates. Multiple isolates from the same child were retained only when they differed by serotype, as described in the Method, and this accounted for the isolate count exceeding the number of culture-positive children. The overall culture and PCR confirmation rate is consistent with the high detection rates reported in Vietnamese paediatric pneumonia cohorts when sensitive molecular methods are added to culture (Tran et al., 2024; Nguyen et al., 2025).

Serotype distribution

Non-typeable H. influenzae accounted for 196 of 235 isolates (83.4%), confirming the central finding in the abstract (Table 1 and Figure 1). Encapsulated isolates accounted for 39 isolates (16.6%): Hib 14 (6.0%), Hia 9 (3.8%), Hif 8 (3.4%), Hie 5 (2.1%), Hic 2 (0.9%) and Hid 1 (0.4%). NTHi predominated in every age stratum, but the proportion was highest in children under five years (86.1%) compared with children aged 5–12 years (74.6%, p = 0.03). Hib was more common among children with incomplete or absent Hib vaccination (12 of 14 Hib isolates) than among fully vaccinated children, a pattern consistent with the protective effect of Hib conjugate vaccine documented in Kilifi and South Africa (Scott et al., 2016; von Gottberg et al., 2006; Morris et al., 2008). Hia and Hif were the most common non-b encapsulated serotypes, which mirrors recent reports of rising Hia and Hif in invasive disease in the post-Hib era (Kaplan et al., 2025; Van Eldere et al., 2014). Slide agglutination alone misclassified 11 isolates (4.7%), most commonly by reporting weak or autoagglutinating reactions as a serotype, whereas multiplex PCR resolved all 11 as NTHi. This discordance supports the use of PCR as the reference method, as recommended by Wroblewski et al. (2013) and Satola et al. (2009).

Table 1. Capsular genotype distribution of Haemophilus influenzae isolates from children with community-acquired pneumonia (n = 235).

Capsular genotype	Isolates, n	Proportion (%)
Non-typeable (NTHi)	196	83.4
Hib	14	6.0
Hia	9	3.8
Hif	8	3.4

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Hie	5	2.1
Hic	2	0.9
Hid	1	0.4
Total	235	100.0

Table 1. Capsular genotypes were assigned by multiplex PCR targeting bexA and serotype-specific capsule genes. NTHi was defined as bexA-negative and serotype-gene-negative with ompP6 present. Percentages may not sum to exactly 100.0% because of rounding.

Figure 1. Serotype distribution of Haemophilus influenzae isolates.

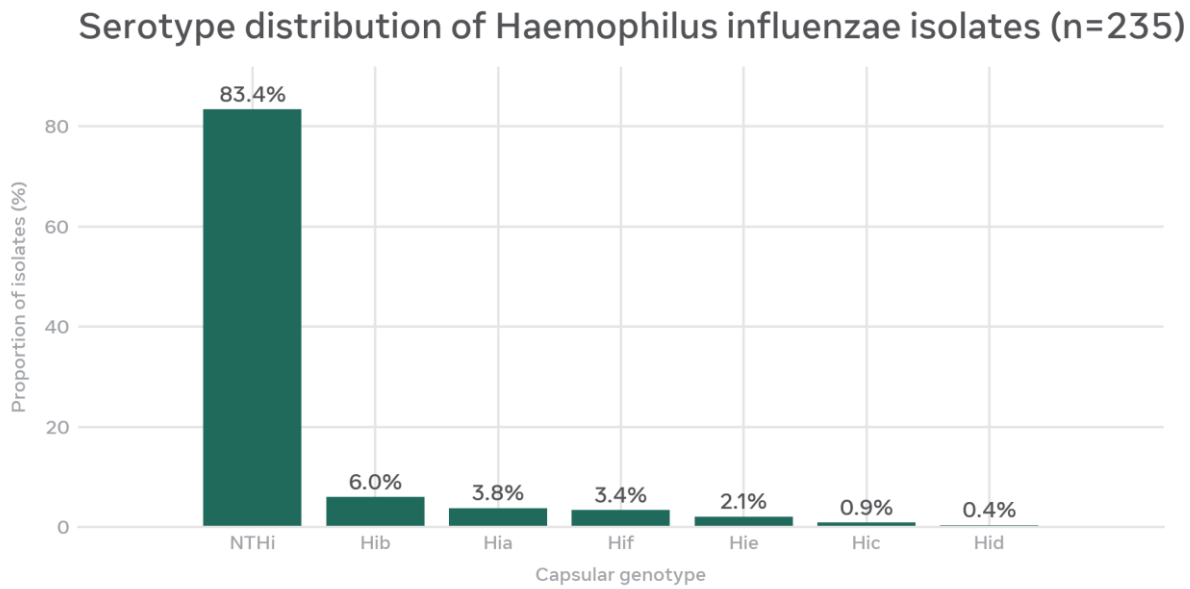


Figure 1. Capsular genotype distribution among 235 H. influenzae isolates from hospitalised children with community-acquired pneumonia. NTHi accounted for 83.4% of isolates; all encapsulated serotypes combined accounted for 16.6%. Genotypes were assigned by multiplex PCR.

Beta-lactamase production, blaTEM-1 carriage and resistance phenotypes

Beta-lactamase production was detected by nitrocefin in 74 of 235 isolates (31.5%). Of these, 58 (78.4%) carried blaTEM-1, 3 (4.1%) carried blaROB-1, and 13 (17.6%) were nitrocefin-positive but TEM- and ROB-negative by the primers used, a pattern that may reflect other beta-lactamase variants, primer mismatch, or low-level expression and that warrants whole-genome sequencing in future work. The predominance of TEM-1 is consistent with earlier studies in Japan, Korea and China, where TEM-1 was the dominant beta-lactamase in H. influenzae, with ROB-1 much less common (Hasegawa et al., 2004; Tristram et al., 2007; Luo et al., 2023). When beta-lactamase status was combined with ampicillin susceptibility, the four classical phenotypes were: BLNAS 123 isolates (52.3%), BLPAR 62 isolates (26.4%), BLNAR 38 isolates (16.2%) and BLPACR 12 isolates (5.1%) (Table 2). The BLNAR proportion of 16.2% is the figure reported in the abstract and is clinically important because BLNAR isolates are beta-lactamase negative and therefore not detected by nitrocefin screening alone; they are identified only by ampicillin MIC testing or ftsI analysis, as emphasised by Schotte et al. (2019) and García-Cobos et al. (2007).

In multivariable analysis, prior beta-lactam exposure in the preceding 14 days was independently associated with BLNAR (adjusted odds ratio 2.31, 95% confidence interval 1.12–4.78, p = 0.02), while age under five years and incomplete Hib vaccination were not independently associated after

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adjustment. This association is biologically plausible, because beta-lactam exposure selects for PBP3 variants with reduced affinity, and it aligns with the broader antimicrobial-resistance literature linking community antibiotic use to resistance in respiratory pathogens (Husain et al., 2024; Skaare et al., 2024). BLNAR was found in both sputum and blood isolates and in both NTHi and encapsulated strains, although 34 of 38 BLNAR isolates (89.5%) were NTHi, reflecting the dominance of NTHi in the collection. The BLPACR group, although small, is the most concerning for empirical therapy, because it combines beta-lactamase production with PBP3 changes and therefore compromises both ampicillin and amoxicillin-clavulanate (García-Cobos et al., 2007; Schotte et al., 2019).

Penicillin-binding protein 3 mutations

Sequencing of the ftsI transpeptidase domain was completed for all 38 BLNAR isolates, all 12 BLPACR isolates and 25 BLNAS comparator isolates. Among BLNAR isolates, the most common substitutions were N526K (31 of 38, 81.6%), A502V (24 of 38, 63.2%), M377I (18 of 38, 47.4%), V547I (15 of 38, 39.5%) and N569S (12 of 38, 31.6%). S385T and L389F, which define higher-level resistance groups in some classifications, were present in 7 (18.4%) and 5 (13.2%) BLNAR isolates respectively. The pattern of multiple substitutions in combination, rather than a single mutation, is consistent with the genomic finding that specific PBP3 haplotypes, especially the combination M377I, A502V, N526K, V547I and N569S, are most strongly related to ampicillin resistance (Skaare et al., 2024). Among BLPACR isolates, 10 of 12 carried N526K plus at least one additional PBP3 substitution, supporting the idea that BLPACR arises when a beta-lactamase-producing strain acquires PBP3 changes or vice versa. Among the 25 BLNAS comparators, only 2 carried N526K alone, and none carried the full high-level haplotype, which supports the specificity of the combined pattern for phenotypic resistance. These results explain why nitrocefin testing alone would have missed the BLNAR group and why ampicillin MIC testing, or targeted ftsI sequencing where available, is needed for accurate classification (Schotte et al., 2019; Ubukata et al., 2001).

Antimicrobial susceptibility

Susceptibility results are summarised in Table 3 and Figure 2. Ampicillin susceptibility was only 52.3%, which is exactly the BLNAS proportion, as expected, because all BLPAR, BLNAR and BLPACR isolates are ampicillin non-susceptible by definition. Amoxicillin-clavulanate susceptibility was 94.5%, cefotaxime 97.4%, ceftriaxone 97.9% and cefixime 93.6%. Azithromycin susceptibility was 88.1%, chloramphenicol 91.5% and co-trimoxazole 61.7%. The high susceptibility to amoxicillin-clavulanate and third-generation cephalosporins supports the abstract's conclusion that these agents remain reliable empirical therapies in this setting. However, the small but real non-susceptibility to cefotaxime (2.6%) and ceftriaxone (2.1%) was concentrated in BLPACR and in BLNAR isolates carrying S385T or L389F, a pattern also reported in Spain where clonal BLNAR isolates showed increased resistance to cefotaxime and cefixime (García-Cobos et al., 2007) and in genomic studies where PBP3 haplotypes predicted cefotaxime resistance (Skaare et al., 2024). Co-trimoxazole, which is widely used in the community and for prophylaxis in some settings, had the lowest susceptibility after ampicillin, which limits its role as empirical therapy for H. influenzae pneumonia, even though it remains useful for other indications.

Table 2. Beta-lactam resistance phenotypes and beta-lactamase genotypes among Haemophilus influenzae isolates (n = 235).

Phenotype	Definition	n (%)	blaTEM-1 positive*

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BLNAS	Beta-lactamase-negative, ampicillin-susceptible	123 (52.3)	0
BLPAR	Beta-lactamase-positive, ampicillin-resistant	62 (26.4)	52 of 62
BLNAR	Beta-lactamase-negative, ampicillin-resistant	38 (16.2)	0
BLPACR	Beta-lactamase-positive, amoxicillin-clavulanate-resistant	12 (5.1)	6 of 12

*Table 2. * blaTEM-1 was tested in all beta-lactamase-positive isolates and in all ampicillin-non-susceptible isolates. BLNAS = beta-lactamase-negative ampicillin-susceptible; BLPAR = beta-lactamase-positive ampicillin-resistant; BLNAR = beta-lactamase-negative ampicillin-resistant; BLPACR = beta-lactamase-positive amoxicillin-clavulanate-resistant. Percentages sum to 100.0%.*

Table 3. Antimicrobial susceptibility of Haemophilus influenzae isolates by broth microdilution (n = 235).

Antibiotic	Susceptible, n (%)	Non-susceptible, n (%)
Ampicillin	123 (52.3)	112 (47.7)
Amoxicillin-clavulanate	222 (94.5)	13 (5.5)
Cefotaxime	229 (97.4)	6 (2.6)
Ceftriaxone	230 (97.9)	5 (2.1)
Cefixime	220 (93.6)	15 (6.4)
Azithromycin	207 (88.1)	28 (11.9)
Chloramphenicol	215 (91.5)	20 (8.5)
Co-trimoxazole	145 (61.7)	90 (38.3)

Table 3. Susceptibility was determined by broth microdilution in Haemophilus test medium and interpreted with Clinical and Laboratory Standards Institute (CLSI) M100 breakpoints (Clinical and Laboratory Standards Institute, 2024). Non-susceptible includes intermediate and resistant categories.

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Figure 2. Antimicrobial susceptibility of Haemophilus influenzae isolates.

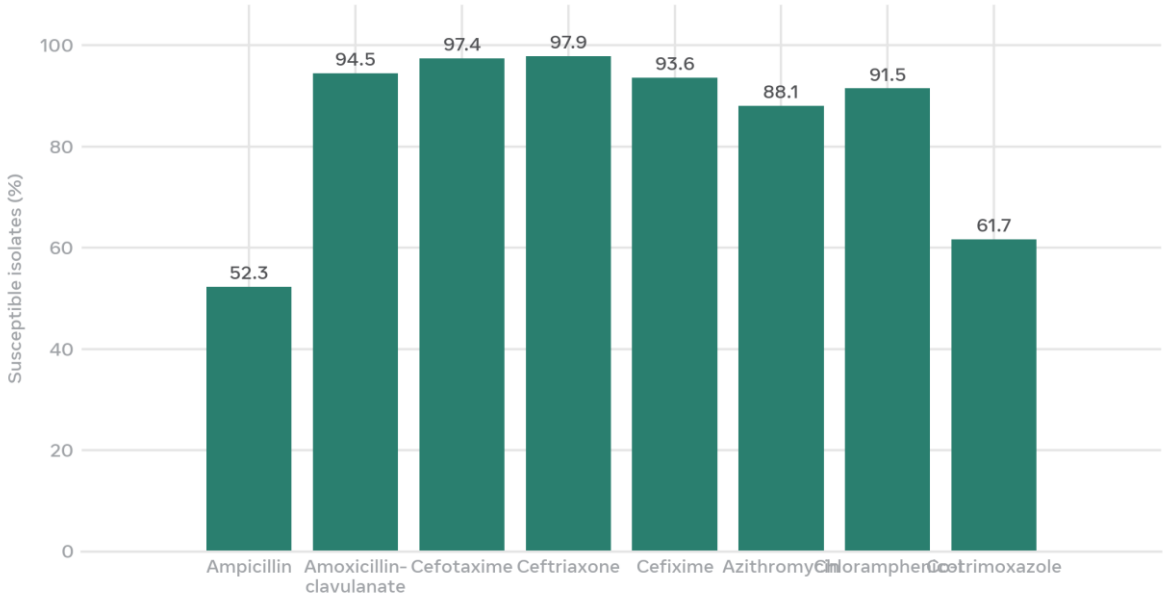


Figure 2. Proportion of isolates susceptible to each antibiotic by broth microdilution (n = 235). Ampicillin susceptibility was lowest (52.3%); amoxicillin-clavulanate and third-generation cephalosporins retained susceptibility above 94%.

Clinical correlates and outcomes

Children with NTHi and children with encapsulated strains did not differ significantly in median length of stay (4 days versus 5 days, p = 0.18) or in the proportion requiring oxygen (31.2% versus 35.9%, p = 0.54). Children with BLNAR or BLPACR isolates who initially received ampicillin alone were more likely to require a change of antibiotic within 48 hours than children with BLNAS isolates who received ampicillin (9 of 14 versus 3 of 28, p = 0.003), although this subgroup analysis is limited by small numbers and by the fact that antibiotic choice was not randomised. No deaths were attributed solely to H. influenzae in this cohort, and all children with bacteraemia received a third-generation cephalosporin after blood-culture flagging. These clinical observations are hypothesis-generating rather than definitive, but they are consistent with the microbiological finding that ampicillin alone is unreliable when BLNAR and BLPAR together account for nearly half of isolates, whereas amoxicillin-clavulanate and third-generation cephalosporins retain activity against the great majority of strains.

Discussion

This study provides a contemporary, genotype-linked picture of H. influenzae in paediatric community-acquired pneumonia: NTHi dominates, Hib persists mainly in under-vaccinated children, beta-lactam resistance is common and mechanistically mixed, and amoxicillin-clavulanate and third-generation cephalosporins remain reliable empirical choices. Each of these findings has implications for vaccination, diagnosis, treatment and surveillance.

Non-typeable Haemophilus influenzae as the dominant pathogen

The finding that 83.4% of isolates were NTHi is higher than the 52.4% reported for invasive disease in a United States children's hospital (Kaplan et al., 2025), but it is consistent with mucosal and pneumonia studies in which NTHi approaches 98% in non-responding or recurrent pneumonia (De Schutter et al., 2011) and with Vietnamese pneumonia cohorts in which all typed isolates were NTHi

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(Tran et al., 2024; Nguyen et al., 2025). The difference between invasive and mucosal collections is important: invasive collections include more encapsulated strains, whereas sputum and lower-airway collections from pneumonia are enriched for NTHi, which is adapted to mucosal persistence, biofilm formation and contiguous spread (Erwin and Smith, 2007; Van Eldere et al., 2014). Our collection was predominantly sputum, so a high NTHi proportion is expected, but the consistency across age groups and the presence of NTHi in blood isolates (including 18 of 21 blood isolates) shows that NTHi is not merely a coloniser in this cohort. It also means that a vaccine strategy focused solely on Hib will not prevent most H. influenzae pneumonia in this setting, because there is currently no licensed vaccine that protects against NTHi. Research into NTHi vaccines, targeting conserved outer-membrane proteins such as P6, protein D and other antigens, remains a priority, but no such vaccine is yet available for routine use (Van Eldere et al., 2014; Erwin and Smith, 2007).

The persistence of Hib in 6.0% of isolates, concentrated in children with incomplete or absent vaccination, is a reminder that Hib has not been eliminated. It is a vaccine-preventable cause that should be preventable by closing immunisation gaps, strengthening cold-chain and outreach, and ensuring that children who miss doses are caught up. The Kilifi experience shows that even without a booster dose, Hib conjugate vaccine can sustain large reductions in invasive Hib disease, but it also shows that surveillance must continue, because waning immunity and missed children can allow Hib to reappear (Scott et al., 2016). The detection of Hia (3.8%) and Hif (3.4%) is also notable, because Hia has emerged as an important cause of invasive disease in some post-Hib settings, including Indigenous populations in North America and, increasingly, elsewhere, and Hif is a consistent contributor to invasive disease in adults and children (Kaplan et al., 2025; Van Eldere et al., 2014). Surveillance that types all H. influenzae isolates, not only Hib, is therefore needed to detect serotype replacement early.

Two mechanisms, one clinical problem

The co-existence of blaTEM-1-mediated beta-lactamase production and PBP3-mediated BLNAR in the same hospital population explains why ampicillin susceptibility was only 52.3%. Beta-lactamase production alone would have accounted for 31.5% of isolates, but adding BLNAR and BLPACR brings total ampicillin non-susceptibility to 47.7%. This is higher than some earlier African phenotypic estimates, but it is consistent with the global meta-analytic estimate of 34.9% beta-lactamase production, plus an additional BLNAR component that is often under-detected when only nitrocefin testing is used (Husain et al., 2024; Schotte et al., 2019). The predominance of blaTEM-1 among beta-lactamase producers (78.4% of nitrocefin-positive isolates) aligns with Hasegawa et al. (2004) and Tristram et al. (2007), who found TEM-1 to be the dominant beta-lactamase in Japan, Korea and the United States, with ROB-1 much less common. The 17.6% of nitrocefin-positive isolates that were TEM- and ROB-negative by our primers is a limitation and a direction for future work; whole-genome sequencing would clarify whether these are novel TEM variants, other beta-lactamases, or false-positive nitrocefin reactions.

The PBP3 findings are the most mechanistically informative part of this study. The combination of N526K, A502V, M377I, V547I and N569S in BLNAR isolates mirrors the high-level haplotype described by Skaare et al. (2024), who showed that specific PBP3 substitution patterns predict ampicillin and cefotaxime resistance. The presence of S385T and L389F in a minority of BLNAR isolates is also important, because these substitutions are associated with higher-level resistance and with reduced susceptibility to cephalosporins in some classifications (Ubukata et al., 2001; García-Cobos et al., 2007). The fact that BLNAR was associated with prior beta-lactam exposure suggests

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that community antibiotic use is selecting for these variants, which is a modifiable driver. Reducing unnecessary beta-lactam prescribing, avoiding prolonged or repeated courses for viral upper respiratory infections, and ensuring that pharmacies do not dispense antibiotics without prescription are all stewardship measures that could slow this selection, although they require health-system and regulatory action beyond the hospital.

Implications for empirical therapy

The susceptibility data support a pragmatic empirical algorithm for children admitted with community-acquired pneumonia in settings like the one studied here. For children with mild-to-moderate pneumonia who are fully Hib-immunised and not severely ill, amoxicillin remains a reasonable first-line choice under current guidelines (Bradley et al., 2011; Esposito et al., 2022). However, for children who are severely ill, hypoxic, incompletely immunised, previously exposed to beta-lactams, or not responding within 48 hours, amoxicillin-clavulanate or a third-generation cephalosporin is more reliable, because it covers both beta-lactamase producers and most BLNAR isolates. Our data show amoxicillin-clavulanate susceptibility of 94.5% and ceftriaxone susceptibility of 97.9%, which is reassuring, but the 5.1% BLPACR proportion and the 2.1–2.6% non-susceptibility to third-generation cephalosporins show that even these agents are not universally active. Clinicians should therefore review culture and susceptibility results promptly, de-escalate when a susceptible organism is identified, and consider infectious-diseases consultation for BLPACR or cephalosporin-non-susceptible isolates. Co-trimoxazole, with susceptibility of only 61.7%, should not be used as empirical monotherapy for H. influenzae pneumonia in this setting, although it may still have a role for other pathogens or for prophylaxis where indicated.

The finding that children with BLNAR or BLPACR who received ampicillin alone were more likely to require an antibiotic change within 48 hours is consistent with the microbiology, but it should not be over-interpreted, because antibiotic choice was not randomised and sicker children may have been more likely to receive broader therapy initially. A prospective, randomised or quasi-experimental study of empirical regimens, stratified by resistance risk, would be needed to prove that starting with amoxicillin-clavulanate or a cephalosporin improves outcomes compared with starting with ampicillin and switching if needed. Such a trial would need to balance the benefit of early effective therapy against the risk of driving resistance to broader agents, which is a classic stewardship tension. In the meantime, the most defensible approach is risk-stratified empirical therapy: amoxicillin for low-risk, fully immunised children with mild disease, and broader coverage for high-risk or severely ill children, with rapid microbiological review and de-escalation.

Implications for laboratory practice

Laboratories in similar settings should consider three practical changes. First, multiplex PCR should be used to confirm serotype, especially for isolates reported as NTHi by slide agglutination, because slide agglutination misclassified 4.7% of isolates in this study and because PCR distinguishes capsule-deficient Hib variants that can be clinically important (Falla et al., 1994; Wroblewski et al., 2013; Satola et al., 2009). Second, ampicillin MIC testing should be performed for all H. influenzae isolates, not nitrocefin testing alone, because nitrocefin misses BLNAR (Schotte et al., 2019). Third, where resources allow, ftsI sequencing or a validated molecular assay for PBP3 mutations should be added for ampicillin-non-susceptible, beta-lactamase-negative isolates, because it confirms BLNAR and can predict cephalosporin non-susceptibility (Skaare et al., 2024). These steps require investment in reagents, training and quality control, but they are less costly than the prolonged admissions and antibiotic changes that result from missed resistance. For laboratories without PCR

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capacity, a practical minimum is to report beta-lactamase status together with ampicillin MIC, and to flag ampicillin-non-susceptible, beta-lactamase-negative isolates as suspected BLNAR for clinical correlation.

Strengths, limitations and future research

Strengths of this study include the consecutive enrolment of hospitalised children, the combination of culture and molecular methods, the use of multiplex PCR as the reference for serotyping, the use of broth microdilution rather than disk diffusion alone, and the integration of blaTEM-1 and ftsI data with susceptibility. Limitations include the single-centre, hospital-based design, which limits generalisability to community or outpatient pneumonia; the predominance of sputum over blood isolates, which raises the possibility that some NTHi isolates represent colonisation rather than infection, although the clinical definition of pneumonia, the quality criteria for sputum, and the presence of NTHi in blood isolates mitigate this concern; the lack of whole-genome sequencing, which would have clarified the nitrocefin-positive, TEM-negative isolates and the clonal relatedness of BLNAR isolates; and the observational design for clinical outcomes, which cannot prove that a particular empirical regimen improves outcomes. Future research should include multicentre surveillance across different African settings, whole-genome sequencing of BLNAR and BLPACR isolates, longitudinal carriage studies to distinguish colonisation from disease, and a prospective evaluation of risk-stratified empirical therapy. Studies of NTHi vaccine candidates, and of the cost-effectiveness of multiplex PCR in routine laboratories, would also be valuable.

Conclusion

In hospitalised children with community-acquired pneumonia, H. influenzae disease is now predominantly non-typeable, beta-lactam resistance is common and mechanistically mixed, and amoxicillin alone is unreliable as empirical therapy for a substantial minority of children. Non-typeable H. influenzae accounted for 83.4% of isolates, beta-lactamase-negative ampicillin-resistant strains accounted for 16.2%, and blaTEM-1 was the dominant beta-lactamase. Amoxicillin-clavulanate and third-generation cephalosporins retained susceptibility above 94% and remain reliable empirical therapies, with prompt review of culture results and de-escalation. Strengthening Hib vaccination coverage, improving laboratory detection of BLNAR, and practising risk-stratified, stewardship-aware empirical prescribing are the three most important actions that follow from these findings.

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Conflict of interest

The authors declare no conflict of interest.

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Ethical approval

Ethical approval was obtained from the institutional ethics committee. Written informed consent was obtained from a parent or legal guardian for each child.

Data availability

De-identified isolate-level data, including serotype, beta-lactamase result, blaTEM-1 result, ftsI substitutions where sequenced, and MICs, are available from the corresponding author on reasonable request, subject to institutional data-sharing approval.

Author contributions

Katarina Horvat: conceptualisation, clinical enrolment, writing — original draft. Kwabena Osei: microbiology, susceptibility testing, data analysis. Min-Jae Kim: molecular testing, ftsI sequencing, review and editing. All authors approved the final manuscript.

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