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ORIGINAL ARTICLE

Epidemiological & demographic trends of *Haemophilus influenzae* in Malaysia's southern region: data insights from tertiary level hospital and nearby facilities

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Abstract

Introduction: Haemophilus influenzae (HI) is a significant pathogen responsible for respiratory and invasive infections globally. Following the introduction of the *Haemophilus influenzae* serotype B (Hib) vaccine in Malaysia in 2002, cases of Hib-related diseases declined sharply. Still, the prevalence of nontypeable *Haemophilus influenzae* (NTHi) emerged as a public health concern. This study investigates epidemiological and demographic patterns of HI infections in Malaysia's southern region. *Materials and Methods:* Clinical isolates of HI were recovered during routine diagnostic testing and analysed from June 2023 to December 2024. All isolates were identified using conventional laboratory methods, biochemical assays, and Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS). Confirmatory serotyping was outsourced to the National Public Health Laboratory in Sungai Buloh, Selangor. Epidemiological trends were assessed based on demographics, sample types, and seasonal variations. Results: A total of 281 samples were analysed. NTHi accounted for 96.5% of isolates, with sputum as the dominant sample type (56.4%). Infants and elderly individuals constitute the most vulnerable groups. Peaks in sample submissions correlated with monsoon seasons. Foreign nationals had disproportionately higher mortality rates, reflecting challenges in vaccination access. Conclusion: The study underscores the dominance of NTHi infections in post-Hib vaccination settings in Malaysia. Seasonal trends and demographic disparities emphasise the need for tailored public health interventions and infrastructure strengthening to reduce the burden of HI infections.

Keywords: Haemophilus influenzae, nontypeable *Haemophilus influenzae,* epidemiology, serotype, vaccination, respiratory infections, Malaysia

INTRODUCTION

Haemophilus influenzae (HI), a gram-negative coccobacillus, is a major pathogen associated with respiratory and invasive diseases globally. Its highest prevalence is observed in children under five and adults over sixty.[1–3] The organism is classified into six encapsulated serotypes (a–f), with *Haemophilus influenzae* serotype B (Hib) historically associated with severe invasive diseases such as meningitis and septicaemia.[2] Other encapsulated serotypes (a, c–f) are capable of causing invasive infections but are generally less pathogenic and are reported less frequently.[6,19] In contrast, nontypeable *Haemophilus influenzae* (NTHi) lacks a polysaccharide capsule and exhibits substantial genetic and antigenic diversity.[2,9,11] Despite being non-encapsulated, NTHi remains clinically important due to its ability to form biofilms, evade host immunity, and cause a wide spectrum of mucosal and respiratory diseases, including otitis media, sinusitis, acute exacerbations of chronic obstructive pulmonary disease, and invasive infections in immunocompromised individuals.[2,11,15] The introduction of Hib vaccination programmes globally, including Malaysia's incorporation

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of the Hib vaccine into the national children immunisation programme in 2002, marked a pivotal public health achievement. This has led to a substantial decline in Hib-associated illnesses.[3] However, the shift towards NTHi now dominates post-vaccination epidemiology, highlighting new challenges, especially in under-vaccinated or vulnerable populations (infants and the elderly).[1] This study aims to characterise the epidemiological and demographic patterns of HI in the southern region of Malaysia by examining serotype distribution, temporal trends, and age-related disease burden among isolates submitted between 2023 and 2024. The study also compares regional findings with global data to provide a better understanding of the evolving landscape of HI infections.

MATERIALS AND METHODS

Study Site & Design
This study was conducted in the bacteriology laboratory at a tertiary-level hospital in Johor Bahru in a span of 18 months, from the beginning of June 2023 to the end of November 2024. The study employed a convenient sampling method, selecting all non-repetitive isolates from various clinical specimens that were confirmed to be HI. A total of 281 isolates were included in this retrospective laboratory-based descriptive study.

Study Procedure:
All clinical isolates recovered from diagnostic specimens and presumptively identified as HI during routine laboratory processing were included in this study. Specimens were cultured on chocolate agar supplemented with bacitracin (10 units) to inhibit commensal flora and enhance the selective recovery of HI. Cultures were incubated at 35–37°C in 5% CO_2 for 18–24 hours. Colonies demonstrating morphology typical of HI (small, grey, translucent colonies with a smooth surface) were selected for further assessment. Presumptive isolates were evaluated using conventional laboratory methods, which included Gram staining, where HI typically appears as small Gram-negative coccobacilli arranged singly or in short chains. Oxidase testing was performed, with oxidase positivity supporting the presumptive identification. Following conventional phenotypic screening, all presumptive HI isolates were confirmed using Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS) according to the manufacturer's instructions. Only isolates achieving species-level confidence within validated laboratory thresholds were included in the final dataset. Pure and viable isolates were subsequently forwarded to the National Public Health Laboratory (MKAK), Sungai Buloh, Selangor for confirmatory capsular serotyping.

Study Population:
Samples were collected from patients admitted to Hospital Sultanah Aminah, nearby district hospitals, and nearby government clinics. Both inpatients and outpatients were included. Data on demographics (age, nationality, sex, location), sample types, and ward categories were analysed.

Study Analysis:
Descriptive statistics were used which summarise sample distribution, serotype patterns, and demographic characteristics. Seasonal and ward-specific trends were analysed using frequency tables.

RESULTS

Sample Type Distribution
A total of 291 samples were confirmed as HI but only 281 samples were analysed. 10 samples were excluded from the study as these isolates cannot be confirmed by MKAK due to the isolates were non-viable upon receival. Sputum accounted for the majority, which is more than half of our total samples (56.9%, n=160), followed by nasopharyngeal aspirate (NPA) (18.9%, n=53) and lower respiratory tract samples (15.3%, n=43). Other sample types were less frequently submitted as shown in Table 1.

Monthly and Seasonal Trends
Analysis of samples revealed notable seasonal peaks, with the highest numbers recorded in December 2023 (30 samples) and November 2024 (30 samples). Additional trends were observed in October 2023 and July 2024, indicating periodic surges in sample submissions. (Figure 1). The HI samples peaked in December 2023 and November 2024, coinciding with the Northeast Monsoon season.[4] These seasonal fluctuations may be attributed to an increase in respiratory infections during monsoon seasons or colder months, which are known to create conditions conducive to viral infections. Such viral infections can weaken the immune system and predispose individuals to secondary bacterial infections, including those caused by HI.[1,5] This

Table 1: Distribution of Sample Types, Frequency (n), & Percentage (%) of confirmed *Haemophilus influenzae* (HI) infections

Sample Types (n=281)	Frequency (n)	Percentage (%)
Sputum	160	56.9
Nasopharyngeal Aspirate (NPA)	53	18.9
Tracheal Aspirate (TA) / Bronchoalveolar Lavage (BAL) / Blind Bronchoscopy Aspirate (BBA)	43	15.3
Pus	10	3.6
Eye Swab	8	2.8
Blood	4	1.4
CSF	2	0.7
Stool	1	0.4

pattern underscores the role of environmental and seasonal factors in influencing the epidemiology of respiratory pathogens.

Serotype Distribution

Of the 281 samples analysed, 100 % (n=281) were identified as NTHi, reflecting the global pattern observed after the introduction of the Hib vaccine. Notably, no typeable serotypes were detected in the dataset. The dominance of NTHi aligns with worldwide trends in the post-Hib vaccination era, where these strains have become the primary contributors to invasive disease. [1,2,6–9]

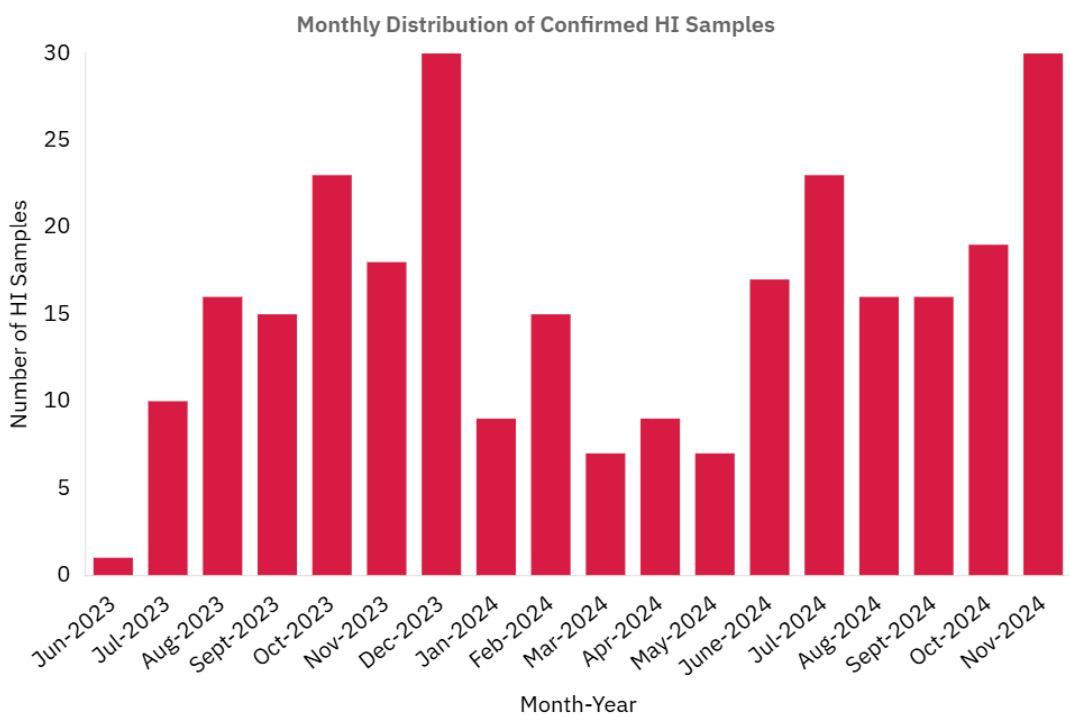

Figure 1. Monthly Distribution of confirmed *Haemophilus influenza* (HI) Sample Submissions for June 2023 to November 2024

Age Group, Sex and Nationality Disparities Analysis

When stratified by nationality, the majority of HI cases occurred among Malaysian patients across all age groups (Figure 2). Foreign nationals constituted only a very small proportion of cases, with isolated occurrences recorded in the <1 year, 1–5 years, 6–18 years, 19–40 years and aged ≥65 years groups. Overall, the distribution demonstrates that HI infections in this region predominantly affect Malaysian residents, with foreigner-associated cases representing only a minor fraction of the total caseload. The highest burden was observed in children aged 1–5 years (n=75) and adults aged 41–64 years (n=72), reflecting vulnerabilities linked to immunological immaturity or senescence.[10] Extreme age groups (infants and elderly) are at higher risk for HI infections also likely due to immature or weakened immune systems.[8,10,11] Males comprised most cases, accounting for approximately 56.7% (166 individuals), while females represented 42.9% (125 individuals), as illustrated in Figure 3.

Among the 281 cases analysed, Malaysians constituted the vast majority, with 276 cases (98.2%), while foreigners accounted for only 5 cases (1.8%). Despite their smaller representation, foreign nationals experienced disproportionately higher mortality rates, highlighting significant disparities in healthcare outcomes. These differences likely stem from limited access to vaccination programmes and healthcare services among foreign populations, which exacerbate their vulnerability to severe disease outcomes.[12] Addressing these gaps is crucial to improving health equity and reducing the burden of infections in marginalised groups.[13,14]

Critical vs. Non-Critical Ward Analysis

The majority of samples were collected from general wards, which accounted for 48.0% (135 cases) of submissions, followed by clinics with 37.4% (105 cases). In contrast, specialised wards for critical care, including ICU (Intensive Care Unit), NICU (Neonatal Intensive Care Unit, and PICU (paediatric Intensive Care Unit) contributed only 7.5% (21 cases), with even fewer samples originating from wards such as special care nursery and forensic units. The lower proportion of critical care cases may reflect the effectiveness of timely interventions for non-critical patients, preventing the progression to severe disease. This distribution highlights the reliance on general wards and clinics for sample collection while underscoring the importance of early diagnosis and treatment.

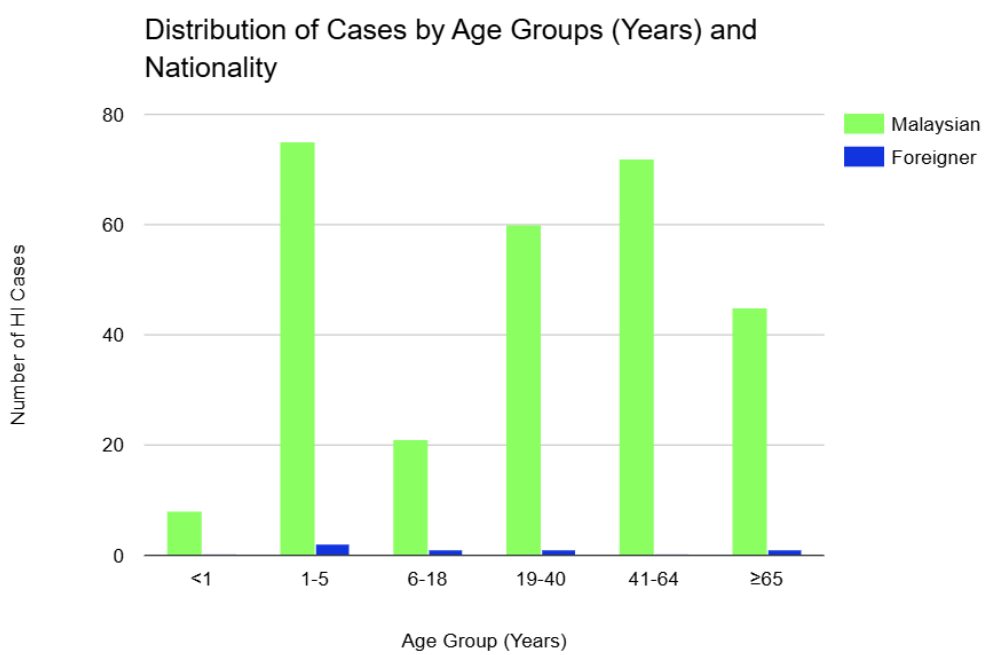

Figure 2. Distribution of *Haemophilus influenza* (HI) cases across age groups stratified by nationality.

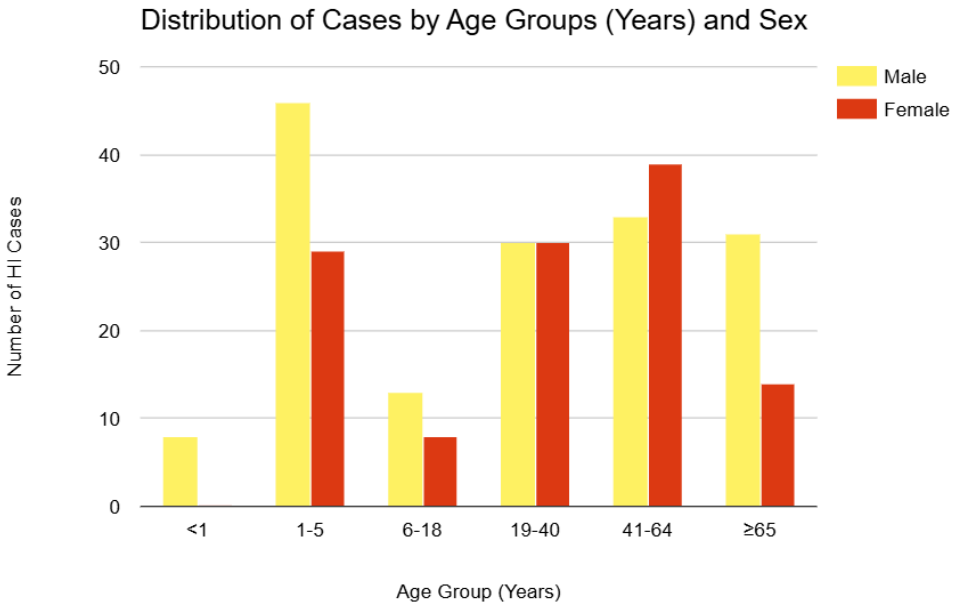

Figure 3. Bar chart displaying the distribution of age groups by sex. Each bar represents the frequency of individuals within a specific age group, categorised by sex (male and female).

DISCUSSION

Malaysia's robust public healthcare system and immunisation programme have significantly reduced invasive Hib infections since the introduction of Hib vaccination as a combination vaccine with diphtheria, tetanus, acellular pertussis, and inactivated polio (DTaP-IPV-Hib) in 2002. This achievement mirrors trends observed globally in post-vaccination settings, where the suppression of Hib has led to a shift toward NTHi as the dominant strain. Our findings show that NTHi accounted for 100% of all HI cases, aligning with global data, underscoring the epidemiological shift and emphasising the need for continued vigilance against this emerging pathogen.[2,6–8]

Following the success of Malaysia's national Hib immunisation programme, the epidemiology of HI has shifted, with NTHi now accounting for most clinical cases. NTHi lacks a polysaccharide capsule and shows marked antigenic variability, forms robust biofilms, and employs multiple immune-evasion strategies.[2,11,19] These features have hindered vaccine development and remain key reasons why no licensed NTHi vaccine is currently available.

The predominance of NTHi presents several clinical and public health challenges. Unlike Hib, no vaccine exists for NTHi, leaving vulnerable populations, including infants and the elderly, unprotected. NTHi is a significant cause of respiratory diseases such as pneumonia, otitis media, and exacerbations of chronic obstructive pulmonary disease (COPD).[15] It also has invasive potential, particularly in immunocompromised individuals or those with limited access to healthcare. This lack of vaccine coverage and the growing antibiotic resistance of NTHi strains highlight an urgent need for preventive and therapeutic interventions.

Our data underline geographic disparities in healthcare access across Malaysia. While urban areas like Johor Bahru benefit from advanced facilities, rural districts face significant challenges, including limited diagnostic capabilities and delays in sample transportation. Most samples in our study were submitted from general wards (48.0%) and clinics (37.4%), reflecting the reliance on primary healthcare facilities for diagnosis. These challenges likely contribute to delayed detection and management, which can exacerbate infection severity.[16,17] Strengthening rural healthcare infrastructure through investment in advanced diagnostics, training, and logistics is crucial for reducing disparities and improving outcomes.

Foreign nationals, who represent a small proportion (1.8%) of cases, exhibited higher mortality rates and critical care admissions, likely due to barriers in accessing healthcare services, including vaccination programmes.[18] Malaysia's significant population of migrant workers and refugees, particularly in industrialised states like Johor, requires targeted public health initiatives. Outreach vaccination programmes and subsidised healthcare for these groups could mitigate disparities and reduce the burden of severe infections.

Malaysia's tropical climate, with its monsoon seasons, affects patterns of respiratory infections. HI cases peak during the Northeast Monsoon (November to March) when viral respiratory infections are more common, making people more likely to get secondary bacterial infections.[17] Our data, which indicate infection peaks in December 2023 and November 2024, are consistent with these trends. Seasonal preparedness, including enhanced vaccination campaigns and public awareness during monsoon seasons, could help mitigate these spikes.

Children of one to five years of age (26.7% of cases) and adults of 41 to 64 years of age (25.6% of cases) represent the most vulnerable groups. The high incidence of HI rates in young children aged one to five years old is because their immune systems are still developing, hence lacking mature antibodies to fight the bacteria after maternal immunity fades. On the other hand, the presence of comorbidities in middle-aged adults increases their vulnerability to HI.[19] Addressing these vulnerabilities requires strategies such as booster vaccinations for older adults and enhanced surveillance for paediatric cases to close gaps in protection and care.

The higher prevalence of HI infections among males raises important questions. Behavioural factors, such as higher smoking rates and occupational exposures in males, likely increase their susceptibility to respiratory infections.[20] Differences in healthcare-seeking behaviours may also contribute, with males potentially presenting with more severe symptoms.[21] Biological differences, including hormonal and immune system variations, may further influence this disparity. Culturally tailored interventions, including workplace health campaigns and targeted education, could address these gender-specific risk factors.

Diagnostic limitations remain a significant barrier, particularly in rural settings. Improper collection, handling, and transportation of samples led to the rejection of 3.4% of samples in our study, potentially delaying or missing diagnoses. Investments in healthcare provider training and improved logistics for sample transport are essential to enhancing diagnostic accuracy and patient outcomes.[22]

This study highlights several key areas that warrant further research and focus public health efforts. Firstly, it is regarding the shift of Hib towards NTHi as the prominent strain. Although NTHi is associated with a lower mortality rate compared with Hib, invasive disease may present as bacteraemia, pneumonia, and less commonly, meningitis. The fatality rates for invasive NTHi infections are approximately 10–20%.[23] At present, there is no vaccine for NTHi. Thus, antibiotic treatment is the only effective way to handle illness caused by these strains.[24] Hib isolates were more closely linked and had lower rates of resistance to antibiotics such as ampicillin in comparison to NTHi. NTHi possesses a high diversity of genes and has adopted extensive multidrug resistance mechanisms. Hence, the local antimicrobial susceptibility data are crucial to control diseases caused by HI.[25]

Secondly, targeted interventions are essential to address disparities in healthcare access, particularly for rural populations and foreign nationals, to reduce infection rates and enhance health outcomes. Lastly, enhancing surveillance systems and preparedness measures, including the monitoring of seasonal trends will be vital for guiding effective public health responses and optimising treatment strategies.

CONCLUSION

This study provides valuable insights into the epidemiological patterns of HI infections at Hospital Sultanah Aminah and surrounding district hospitals and clinics. The findings highlight several key trends, including the prevalence of NTHi following the Hib vaccination, seasonal peaks during the monsoon period driven by secondary bacterial infections, and a disproportionate impact on young children less than 5 years old and middle-aged adults. Additionally, geographic disparities in healthcare access, particularly in rural regions, were identified. Mitigation of the burden of HI in Malaysia primarily involves public health measures, good personal hygiene, and infection control, as there is currently no specific vaccine available for NTHi.

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