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Pneumococcal serotype distribution and antibiotic susceptibility in Malaysia: A four-year study (2014–2017) on invasive paediatric isolates

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ABSTRACT

Objective: This study was performed to analyze the serotype distribution of *Streptococcus pneumoniae* causing invasive pneumococcal disease (IPD) in children aged 5 years and under in Malaysia and to assess the antimicrobial resistance.

Methods: From 2014 to 2017, a total of 245 invasive *S. pneumoniae* isolates from children ≤5 years of age were received from hospitals all around Malaysia. All isolates were identified and subjected to serotyping and antimicrobial susceptibility testing.

Results: Of the 245 isolates, 117 (48.0%) were from children aged <1 year, 46 (19.05%) were from children aged 1–2 years, and 82 (33.0%) were from children aged 2–5 years. The most common serotypes were 14 (26.9%), 6B (19.6%), 19A (11.8%), 6A (10.6%), and 19F (6.9%) and vaccine coverage was 88.2% for PCV13, 64.1% for PCV10, and 63.3% for PCV7. Resistance to penicillin was 0.2% for non-meningitis cases and 22.2% for meningitis cases; erythromycin resistance was reported in 42.9%, co-trimoxazole in 35.9%, and tetracycline in 42.9%.

Conclusions: Serotypes 14, 6B, 19A, 6A, and 19F were the most common serotypes isolated from children with IPD in Malaysia during this pre-vaccination era. The lack of reports on the serotype distribution has limited action for the implementation of PCV in the national immunization programme (NIP). The information from this study may benefit future policies for the introduction of PCV in the Malaysian NIP and ultimately may reduce the morbidity and mortality among children in Malaysia.

Introduction

Pneumococcus continues to be responsible for various infections worldwide, contributing to the foremost reasons for morbidity and mortality among children under 5 years of age (World Health Organization, 2012). In 2012, the World Health Organization reported that 5% of all-cause child mortality in those under five was due to pneumococcal infections, with an estimated 476 000 deaths among children who were HIV-negative (Wahl et al., 2018). However, in recent years it has been estimated that the worldwide mortality due to pneumococcal infections has declined by 51%, and approximately 50% of all pneumococcal deaths in 2015 occurred in Africa and Asia (Wahl et al., 2018). The incidence of invasive pneumococcal disease (IPD) is estimated at 3.8 per 100 000 cases each year in Malaysia (Maimaiti et al., 2015).

Streptococcus pneumoniae is an encapsulated diplococcus with 98 serotypes. Not all serotypes cause severe pneumococcal disease, but they differ in invasiveness, incidence according to age, geographical distribution, carriage, antimicrobial resistance, and time period of surveillance (Jauneikaite et al., 2012; Ziane et al., 2016; Song et al., 2012; Dagan and Klugman, 2008). In order to reduce the burden of pneumococcal disease, three pneumococcal conjugate vaccines (PCV) have been developed: PCV7, PCV10, and PCV13. In Malaysia, PCV7 was launched in 2006, and was later superseded by PCV13 in 2010. In 2011, the majority of paediatricians in the public and private sectors agreed to the introduction of PCV into the national childhood immunization programme due to its high efficacy; however, these vaccines are only available in the private sector and are not mandatory. The vaccines are expensive and not many in Malaysia can afford to

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vaccinate their children via private practitioners (McNeil and Clarke, 2016).

Pneumococcal antimicrobial resistance has increased rapidly over the past two decades, posing a threat to the management of the disease, especially due to increasing trends in penicillin resistance (Ghaffar et al., 2004; Gladstone et al., 2015; Feikin et al., 2013). However, penicillin resistance in Malaysia seems to be relatively low compared to other drugs (Rohani et al., 2011), whereas macrolide resistance seems to be a more alarming problem in Asian countries (Kim et al., 2016) and also in Malaysia (Arushothy et al., 2016).

Epidemiological surveillance of *S. pneumoniae* is important to detect any changes in serotype distribution or antibiotic resistance, which indirectly affects decisions regarding the choice of vaccine. Current local data on the pneumococcal serotype distribution in Malaysia, especially in children, are limited with only a few reports being published in the last decade (Yatim et al., 2013; Le et al., 2011; Rohani et al., 1999).

This study was performed to analyze the serotype distribution of *S. pneumoniae* causing IPD in children aged ≤5 years in Malaysia and to assess the antimicrobial susceptibility pattern. The PCV coverage rates during the pre-vaccination era in Malaysia were also determined.

Materials and methods

Pneumococcal surveillance in Malaysia

This study was conducted in the Bacteriology Unit of the Institute for Medical Research (IMR), Malaysia under the Surveillance for *S. pneumoniae* in Malaysia programme, which was initiated in the year 2014. This surveillance programme is an effort by the Ministry of Health of Malaysia to survey the distribution of pneumococcal serotypes and antimicrobial susceptibility, in order to provide better patient management and policy development. Invasive pneumococcal isolates from children aged 5 years and under, isolated in the hospital laboratories in Malaysia, were sent to the Bacteriology Unit, IMR for serotyping and antimicrobial susceptibility testing.

Bacterial isolates

A total of 1847 isolates of *S. pneumoniae* were collected between May 2014 and December 2017 from all hospitals around Malaysia. The isolates were obtained from both invasive and non-invasive sites and came from both paediatric and adult patients. This study investigated 245 invasive isolates received from children ≤5 years of age. Invasive *S. pneumoniae* were recovered from normally sterile body sites such as blood and cerebrospinal fluid (CSF). The study group was stratified into three age groups: <1year, 1–2 years, and 2–5 years. Identification of the isolates was confirmed using standard procedures, including Gram staining, optochin sensitivity, and bile solubility tests.

Antibiotic susceptibility

The susceptibility of all isolates to penicillin, ceftriaxone, and cefotaxime was determined by minimum inhibitory concentration (MIC) via the E-test method (bioMérieux, France). Antimicrobial susceptibility to erythromycin, tetracycline, trimethoprim–sulfamethoxazole (co-trimoxazole), and vancomycin was determined by disc diffusion method (Oxoid, USA). All tests were performed following the guidelines and non-meningitis cut-offs for blood specimens and meningitis cut-offs for CSF isolates recommended by the Clinical and Laboratory Standards Institute (CLSI 2017) (Wikler et al., 2007).

S. pneumoniae ATCC 49619 was used as the quality control strain for antimicrobial susceptibility testing. Isolates that were resistant to three or more antimicrobial agents were defined as multidrug-resistant (MDR) *S. pneumoniae*.

Serotyping

Isolates were serotyped by Neufeld's Quellung reaction method using type-specific antisera (Statens Serum Institute, Copenhagen, Denmark), according to the manufacturer's protocol.

Statistical analysis

Statistical comparisons were made using the Chi-square test or Fisher's exact test. SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. Values of $p < 0.05$ were considered to be statistically significant.

Results

Pre-vaccination surveillance programme

In this surveillance study, a total of 52 hospitals in Malaysia sent isolates of pneumococcus isolated in their respective laboratories to IMR. A total of 1847 non-duplicate pneumococcal isolates were collected: 888 (48.1%) were invasive strains and 959 (51.9%) were non-invasive strains.

Clinical and epidemiological characteristics

Among these isolates, 530 (28.7%), were from children aged 5 years and below, of whom 312 (58.7%) were children aged <1 year, 76 (14.3%) were children aged 1–2 years, and 142 (26.8%) were children aged 2–5 years. Of the 530 isolates, 245 (46.2%) were invasive isolates, with 117 (48.0%) from children <1 year old, 46 (19.1%) from children 1–2 years old, and 82 (33.0%) from children 2–5 years old. The median age of the patients in this study was 18.1 months (range 13 days to 4 years and 364 days) and the male to female sex ratio was 1.7:1.

Serotype distribution and coverage of PCVs

The 245 invasive pneumococcal isolates in this study were distributed among 24 different serotypes. Serotypes 14 (66/245; 26.9%), 6B (48/245; 19.6%), 19A (29/245; 11.8%), 6A (26/245; 10.6%), and 19F (17/245; 6.9%) were the predominant types, covering approximately 75% (186/245) of the total invasive isolates. About 7.8% of the isolates were non-vaccine serotypes (11A, 11C, 15A, 15B, 15C, 19B, 23A, 8, 6C). Non-typeable serotypes accounted for 4.9% (12/245) of the isolates. Two hundred thirty-six *S. pneumoniae* were isolated from blood and nine from CSF. Serotypes 14 (27.5%), 6B (19.1%), 19F (11.9%), and 6A (10.6%) were predominantly isolated from blood specimens ($n = 164$). Serotype 6B ($n = 4$) was predominant among CSF specimens. PCV10 covered 62.9% of the serotypes and PCV13 covered 86.1% of the serotypes (Figure 1).

The majority of invasive isolates were isolated from patients in the age group <1 year, with serotype 14 being the most common (25.0%), followed by serotype 6B (22.4%) and 6A (11.8%), respectively. Serotypes 14 and 6B remained the predominant serotypes for both the 1–2 years age group and the 2–5 years age group.

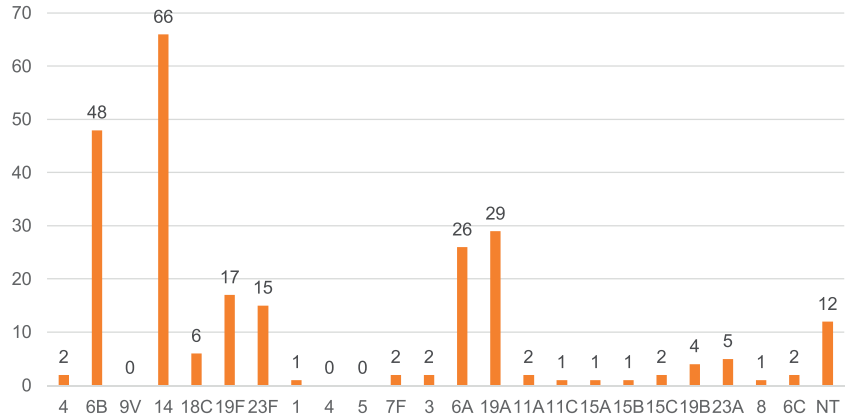

Figure 1. Serotype distribution of invasive *S. pneumoniae* isolates. Figure represents the distribution of *S. pneumoniae* serotypes in children <5 years old according to PCV representation. PCV7, 7-valent pneumococcal conjugate vaccine; PCV10, 10-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine.

Antibiotic susceptibility

Penicillin resistance was seen in two of nine (22.2%) isolates from CSF (meningitis breakpoint). Only 0.2% of the blood isolates (236/245) were resistant, as analysed using the non-meningitis breakpoints (CLSI, 2017).

However, 105/245 (42.9%) invasive *S. pneumoniae* isolated from children ≤5 years of age were resistant to erythromycin, with serotype 14 (41.9%) and serotype 6B (21.8%) being the predominant erythromycin-resistant serotypes. Resistance to co-trimoxazole and tetracycline was 35.9% and 42.5%, respectively (Table 1). Serotype 6B isolates were most resistant to co-trimoxazole (22.8%) and tetracycline (31.1%).

Although, serotype 14 was the predominant serotype among invasive isolates, serotype 6B was predominant among CSF isolates. Serotype 6B isolates, which represented about 19.6% of all invasive isolates, showed the highest resistance pattern as well, with resistance to erythromycin at 50.0%, tetracycline at 52.1%, and co-trimoxazole at 54.2%.

MDR pneumococcal strains were 7.4% and 7.2% for PCV7 and PCV10 vaccine isolates, respectively, and accounted for about 13.6% for PCV13 isolates. Emerging serotypes (11C, 15A, 15C, 19B, 23A, 8, and 6C) accounted for 13.8% of MDR.

Discussion

This study analysed the serotype distribution, antimicrobial resistance, and PCV coverage of invasive pneumococcal isolates from children ≤5 years of age during the pre-vaccination era in Malaysia. In accordance with other publications (Mokaddas and Albert, 2012), the most common sources of invasive pneumococcal isolates in this study were blood (13.1%) and CSF (9.2%). A male predominance was noted among the study population.

Five major serotypes – serotypes 14, 6B, 19A, 6A, and 19F – were found to account for about three-quarters of *S. pneumoniae* isolates causing IPD among children; these serotypes are covered by the PCV13 vaccine. In comparison to a previous study reported by Rohani et al. in 2011, there seems to have been a reduction in serotypes 19F, 23F, and 6A in Malaysia, which indicates a natural serotype shift despite the lack of an immunization policy in Malaysia. The prevalence of serotype 19A in Malaysia is high, in contrast to the findings of other studies, which have reported an increase in 19A serotype after the introduction of PCV. A predominance of serotype 19A has been reported in Korea, Malaysia, Taiwan, Thailand, Saudi Arabia, Hong Kong, India, Japan, the Philippines, and Vietnam, with ST320 being the most prevalent from Malaysia (Shin et al., 2011). A longitudinal investigation of serotypes and genotypes may reveal the emergence and dissemination mechanisms for serotype 19A in Malaysia.

Non-typeable serotypes, which may reflect the presence of non-encapsulated pneumococcal isolates, is not common for invasive infections. However, there have been reports of rare cases in which non-encapsulated isolates have caused invasive infections, especially in children and the immunocompromised (McNeil and Clarke, 2016). In Malaysia, the PCV vaccine has to be obtained via private practitioners; therefore, the number of fully vaccinated children with PCV could not be determined and was deemed insignificant to measure the efficacy of the vaccine (Shin et al., 2011). However, despite this, natural fluctuations among circulating serotypes may occur even without the effect from the conjugate vaccine.

Since the first case of pneumococcal penicillin resistance reported in the 1960s, the emergence and spread of penicillin and MDR pneumococci has become a serious concern worldwide. The Asian Network for Surveillance of Resistant Pathogens (ANSORP) study (Kim et al., 2016) reported zero resistance for non-meningitis isolates and about 23.1% for meningitis isolates with

Table 1
Antimicrobial resistance pattern among invasive *Streptococcus pneumoniae* isolated from children less than 5 years old in Malaysia (2014–2017).

Antibiotic	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	S %	I %	R %
Penicillin (meningitis) (S: ≤0.06 μg/ml; R: ≥0.12 μg/ml)	0.003–1.0	0.008	0.38	77.8	0	22.2
Penicillin (non-meningitis) (S: ≤2 μg/ml; I: 4 μg/ml; R: ≥8 μg/ml)	0.002–12.0	0.032	0.38	97.4	2.3	0.2
Ceftriaxone (S: ≤1 μg/ml; I: 2 μg/ml; R: ≥4 μg/ml)	0.003–8.0	0.012	0.125	94	3.7	2.2
Cefotaxime (S: ≤1 μg/ml; I: 2 μg/ml; R: ≥4 μg/ml)	0.002–12.0	0.016	0.125	94.1	3.6	1.9
Co-trimoxazole (S: ≥19 mm; I: 16–18 mm; R: ≤15 mm)	–	–	–	56.4	7.7	35.9
Erythromycin (S: ≥21 mm; I: 16–20 mm; R: ≤15 mm)	–	–	–	55.3	1.8	42.9
Vancomycin (S: ≥17 mm)	–	–	–	100.0	0.0	0.0
Tetracycline (S: ≥28 mm; I: 25–27 mm; R: ≤24 mm)	–	–	–	55.3	1.8	42.9

MIC, minimum inhibitory concentration; S, sensitive; I, intermediate; R, resistant.

regard to pneumococcal isolates in Malaysia. A similar trend was observed in the present study, whereby resistance to penicillin was 0.2% for non-meningitis isolates and 22.2% for meningitis isolates. However, when compared to other Asian countries, Thailand, Vietnam, China, and South Korea have reported relatively low penicillin resistance for meningitis IPD. Resistance to other β-lactam drugs was also found to be low, with ceftriaxone 2.2% and cefotaxime 1.9%.

In this study, a high rate of resistance to antibiotics other than penicillin was found. The erythromycin resistance rate at 42.9% is still comparatively lower than the rates reported in studies performed in India (71.4%), Tunisia (64.7%), and Korea (73.3%) (Ktari et al., 2017; Ganaie et al., 2016; Song et al., 2004). Besides erythromycin resistance, tetracycline and co-trimoxazole resistance was found to be 42.9% and 35.9%, respectively. Similar resistance rates have also been reported in a related study among children less than 5 years of age (Ktari et al., 2017; Ganaie et al., 2016). MDR *S. pneumoniae* (MDRSP) is also being reported progressively in Malaysia and other Asian countries (Ganaie et al., 2016; Song et al., 2004). It was noted in the present study that serotypes 6B and 19A demonstrated resistance to three or more of the antimicrobials tested. The most frequent combination of antimicrobial resistance contributing to MDR was erythromycin, co-trimoxazole, and tetracycline resistance, which accounted for 21.2% of the isolates.

The severity of the disease and patient outcomes of the children included in this study remain unknown, since the focus for surveillance was on the pneumococcal isolates, their serotypes and antimicrobial resistance. However, based on a few reports, the clinical features of IPD in children have shifted towards more focal infections requiring hospitalization (Ricketson et al., 2018). It has been concluded that the rate of IPD has declined, but that the disease appears to be more severe (Ricketson et al., 2018).

The findings of this study highlight the serotype distribution and antimicrobial resistance of pneumococcal isolates from children in the risk age group during the pre-PCV vaccination era in Malaysia. Awareness of pneumococcal disease has increased over the years, yet without sufficient evidence, it will not be possible to decide on the appropriate PCV to introduce into the Malaysian national immunization policy. Based on the serotype distribution in this study, vaccine coverage for PCV13 is 88.2%, for PCV10 is 64.1%, and for PCV7 is 63.3% in Malaysia.

A limitation of this study was the difficulty obtaining the full cooperation of all participating hospitals in sending the invasive pneumococcal isolates to be serotyped and screened for antimicrobial resistance, since the study was done via a surveillance programme. Going forward, it is planned to continue pneumococcal surveillance in Malaysia for PCV introduction without making any methodological changes. Stable surveillance before the introduction of PCV will help to overcome the limitations of missing specific denominators.

In conclusion, this study demonstrated that serotypes 14, 6B, 19A, 6A, and 19F are the most common serotypes of *S. pneumoniae* isolated from children with IPD in Malaysia. The serotype distribution showed that the majority of the serotypes circulating in Malaysia are covered by PCV13. Therefore, it is believed that the introduction of PCV in the national immunization programme, with continuous surveillance of the circulating serotypes and resistance clones, is important to reduce the morbidity and mortality caused by *S. pneumoniae* in Malaysia, especially among children.

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Ethical considerations

This study did not involve any types of intervention in diagnosis and treatment, thus written informed consent was not considered necessary for the study.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Author contributions

RA, NA, FA, and RH conceived and designed the experiments. RA, NA, and CRCA performed the experiments. RA and NA analysed the data. RA wrote and reviewed the paper.

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