Genotypic characterization of *Streptococcus pneumoniae* serotype 19F in Malaysia

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**A B S T R A C T**

*Streptococcus pneumoniae* is an epidemiologically important bacterial pathogen. Recently, we reported the antibiotic susceptibility patterns of a limited collection of pneumococcal isolates in Malaysia with a high prevalence of erythromycin resistant strains. In the present study, 55 of the pneumococcal isolates of serotype 19F were further analysed by pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). The generated genotypic patterns were then correlated with the antibiograms previously reported. Forty-seven different PFGE profiles (PTs) were obtained, showing that the isolates were genetically diverse. MLST identified 16 sequence types (STs) with ST-236 being predominant (58.2%), followed by ST-81 (10.3%). Among the ST-236 isolates, 22 were erythromycin resistant *S. pneumoniae* (ERSP) and 15 were trimethoprim/sulfamethoxazole (TMP/SMX) resistant. The high prevalence of erythromycin resistant serotype 19F isolates of ST-236 in this study has also been reported in other North and South East Asian countries.

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1. Introduction

*Streptococcus pneumoniae* remains a major cause of otitis media, meningitis and sepsis worldwide (Center for Disease Control and Prevention, 2012). Pneumococcal infections are most common among the very young and elderly as well as those with chronic medical conditions. Although many serotypes are associated in pneumococcal infections, studies have shown that serotype 19F is predominant in Malaysia (Rohani et al., 1999, 2011; Desa et al., 2003; Song et al., 2004a,b; Nathan et al., 2013). Although antibiotic treatment for pneumococcal diseases is available, this therapeutic option needs to be reviewed as the morbidity and mortality rates in pneumococcal infections continue to increase (Le et al., 2011, 2012). Following the recommendation by the Strategic Advisory Group of Experts (SAGE) at World Health Organization (WHO) in 2007 on immunization, the pneumococcal conjugate vaccine (PCV) should be a priority for inclusion in national childhood immunization programmes. The current PCVs (Prevenar™ and Synflorix™) include serotype 19F and therefore their usage will most likely benefit populations at risk of pneumococcal disease where the serotype is prevalent. In the United States, where PCVs have been included as part of the routine vaccination schedule for some time, it was demonstrated that vaccination of specific populations not only provides immunized individuals with protection against pneumococcal disease, but also reduces the risk of developing pneumococcal illness in unimmunized individuals via herd immunity (Center for Disease Control and Prevention, 2005).

Meanwhile, rapid and discriminative subtyping methods are essential for determining the epidemiology of pathogenic strains and are useful in the design of rational pathogen control methods. Genetic analysis methods such as pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) are more discriminative than serotyping alone (Yano et al., 2000). Nevertheless, the combined use of both phenotypic and genotypic methods will provide a clearer picture on the dissemination and characteristic

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pattern of pneumococcal strains locally and globally (Hermans et al., 1995). Our previous investigation on the epidemiology of S. pneumoniae isolates (serotypes 1 and 19F) collected between 2008 and 2010 in Malaysia showed that there was a high prevalence of macrolide (erythromycin) resistant strains (Nathan et al., 2013). In the last decades, penicillin resistant S. pneumoniae was predominant (Rohani et al., 1999, 2011; Desa et al., 2003; Song et al., 2004a,b; Jauneikaitė et al., 2012; Nathan et al., 2013). This changing pattern and with the increasing trend in pneumococcal morbidity and mortality rates is a public health concern. Therefore our previous investigation was extended to include molecular typing, with a special focus on serotype 19F and its antibiogram profile.

2. Materials and methods

2.1. Bacterial strains

A collection of consecutive single clinical isolates of S. pneumoniae serotype 19F (n = 55), collected from various Malaysian hospitals were used in this study (Nathan et al., 2013). The minimum inhibitory concentration (MICs) of six antibiotics (penicillin, amoxicillin/clavulanic acid, cefotaxime, ceftriaxone, trimethoprim/sulfamethoxazole (TMP/SMX) and erythromycin) was previously determined by Etest and isolates were analysed by PFGE and MLST. The source of isolates included sputum, nasal swab, throat swab, tracheal aspirate (TA), nasopharyngeal aspirate and MLST. The source of isolates included sputum, nasal swab, throat swab, tracheal aspirate, ear swab, pus, pleural fluid, blood and cerebrospinal fluid (CSF). Clinical data of patients were no longer traceable and thus correlations with invasive cases were not possible.

2.2. Pulsed field gel electrophoresis (PFGE)

PFGE was performed according to a previously published protocol (Desa et al., 2003). Briefly, the chromosomal DNA of S. pneumoniae was digested with 10 units of SmaI. The digested chromosomal DNA was separated on a CHEF-DR II apparatus (Bio-Rad Laboratories) for 23 h with pulse times ramped from 1 to 35 s at 200 V. XbaI-digested Salmonella Braenderup strain H9812 was used as the molecular size standard. Cluster analysis of PFGE profiles were analyzed with BioNumerics Version 6.0 (Applied Maths, Kortrijk, Belgium) based on the unweighted pair group method with arithmetic averages (UPGMA) with tolerance parameter of 1% in band positions and a pattern optimization parameter of 1%. All PFGE profiles were assigned with an arbitrary designation and the differences were defined by the Dice coefficient of similarity (F: 0–1). Gels were photographed under UV-light after staining with ethidium bromide (0.5 µg/mL). Isolates were grouped into clusters at a similarity of ≥ 75%.

2.3. Multilocus sequence typing (MLST)

MLST was performed for all 55 isolates utilizing seven housekeeping as previously described (Enright and Spratt, 1998). PCR products were sequenced at 1st BASE Laboratory (Malaysia). Sequences were submitted to the MLST database website (http://spneumoniae.mlst.net) for assignment of allelic profiles and sequence types (STs).

3. Results

3.1. PFGE typing

Generally, SmaI-digested genomic DNA of the 55 S. pneumoniae isolates showed diverse fingerprint patterns; 47 distinct PFGE profiles (PTs) comprising 11–24 discernible restriction fragments. Based on the interpretation proposed by Tenover et al. (1995), isolates with a similarity of ≥ 75% were assumed as closely related and grouped in a single cluster. This resulted in 10 clusters with at least 2 isolates each: Clusters A–J in sequence consists of 5, 12, 5, 3, 4, 2, 3, 2 and 2 isolates respectively (Fig. 1).

3.2. MLST

MLST of the 55 pneumococcal isolates yielded 16 distinct STs, the most predominant was ST-236 (58.2%, n = 32) followed by ST-81 (10.3%, n = 6), ST-271 (5.5%, n = 3), ST-5821 (3.6%, n = 2). The remaining STs were singletons.

3.3. Combined analysis

A dendrogram based on combined PTs, STs and resistotypes is shown in Fig. 1. Five isolates of Cluster A were from different sources (sputum, tracheal aspirate, eye and ear) and different years. These isolates were susceptible to all the six antibiotics and of ST-236. Cluster B comprised 12 isolates from different sources (sputum, tracheal aspirate, nasal swab, nasopharyngeal aspirate, pus and ear) and years. These isolates were resistant to erythromycin or TMP/SMX or both the antibiotics and of ST-236. Cluster C consisted of 5 very closely related isolates (blood and pleural fluid) (F = 0.84) and were variably resistant to cefotaxime, erythromycin or TMP/SMX or to all three antibiotics. They were all of ST-236. Cluster D comprised three isolates (blood) (F = 0.81) and all were susceptible to the antibiotics tested, and again of ST-236. Cluster H consisted of 3 indistinguishable isolates (blood) (F = 1) of ST-81, and have the same resistant pattern towards erythromycin and TMP/SMX.

4. Discussion

Infections caused by S. pneumoniae continue to be a problem in Malaysia. Although several studies have documented the antibiotic susceptibility of S. pneumoniae, reports comparing resistance trends and their genetic characteristics between periods of time in Malaysia remain scarce (Rohani et al., 1999, 2011; Desa et al., 2003; Song et al., 2004a,b). In Malaysia, serotype 19F is one of the commonly prevalent serotypes associated with pneumococcal infections in both children and adults (Desa et al., 2003; Song et al., 2004a,b). Early findings in some regions in North and Southeast Asia (Vietnam, Taiwan, Korea, Hong Kong, China and Malaysia) have similarly demonstrated increase in macrolide resistance and serotype 19F S. pneumoniae is among the associated serotypes (Desa et al., 2003; Hsueh et al., 2003; Waites et al., 2003; Ko et al., 2004; Song et al., 2004a; Jenkins and Farrell, 2009; Lynch and Zhanel, 2010; Rohani et al., 2011; Jauneikaitė et al., 2012; Kim et al., 2012; Nathan et al., 2013). The isolates in this study belong to some different STs but the majority were of ST-236. On the other hand, the PFGE clustering pattern shows that the majority were genetically diverse but a few clonally related groups were obvious, even though some of the isolates in the respective clusters were cultured at different times (Fig. 1).

As far as global dissemination is concerned, MLST can be utilized to infer the spread of particular STs to a certain extent due to the portability of the sequence data. ST-236 is observed as having a preponderance over other STs in Southeast Asia (Ko and Song, 2004; Song et al., 2004a; Yang et al., 2008). The divergence of ST-236 as indicated by the PFGE analysis in this study exemplifies the evolutionary changes in Malaysia. Globally, ST-236 may be linked back to the major clone ST-236 reported in last few years.
(Mal P9, Taiwan19F-14 clone) (Ko and Song, 2004; Song et al., 2004a; Yang et al., 2008). The high proportions of ST-236 isolates in this study (59.3%) were also observed in Taiwan with similar serotypes. Nevertheless, this phenomenon is distinct for ST-81 found in this study whereby ST-81 has been previously reported to be associated with serotype 23F (Spain23F-1 clone) (Ko and Song, 2004). In the present study, MLST analysis showed that two STs (ST-81 and ST-236) comprised the majority of erythromycin-resistant *S. pneumoniae*.

Based on similar serotypes and ST-236, along with erythromycin resistance, the dissemination of some serotype 19F strains in this country may be associated with the Taiwan clones. Further studies are required to establish such a connection. The major limitation of this study is the low number of isolates and thus the current information may not present the actual epidemiological situation of *S. pneumoniae* epidemiology in Malaysia. In addition the isolates were based on our available culture collection which was not properly sampled for epidemiological purposes, and thus correlation with the locations (states) was also not discussed. Nevertheless, considering that the origins of isolates were varied throughout Malaysia, this data may suggest the potential prevailing STs among the common serotype with potential inheritance...
from other circulating clones in nearby regions. This may highlight the importance of PCVs which have yet to be fully implemented in Malaysia.

Conflict of interest

SCC currently receives unrestricted research funding from Pfizer Vaccines (previously Wyeth Vaccines) and has received consulting fees from GlaxoSmithKline. SCC has received financial assistance from vaccine manufacturers to attend conferences. All grants and honoraria are paid into accounts within the respective NHS Trusts or Universities, or to independent charities.

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References


