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Serotypes and genotypes of *Streptococcus pneumoniae* isolates from Trinidad and Tobago

Michele Nurse-Lucas[a], **Lesley McGee**[b], **Paulina A. Hawkins**[c], **William H. Swanston**[a], and **Patrick Eberechi Akpaka**[a,*]

[a]Department of Paraclinical Sciences, Faculty of Medical Sciences, The University of the West Indies, St Augustine, Trinidad and Tobago

[b]Respiratory Disease Branch, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA

[c]Rollins School of Public Health, Emory University, Atlanta, Georgia, USA

SUMMARY

Objectives—There are currently 94 known pneumococcal capsular polysaccharide serotypes and their prevalence differs by geographic region and the period studied. *Streptococcus pneumoniae* infections have been diagnosed clinically in Trinidad and Tobago and other Caribbean countries, however data on the serotype and sequence type distributions in this country are limited. The objective of this study was to determine serotypes and multilocus sequence types (MLSTs) of invasive and non-invasive pneumococcal isolates from Trinidad and Tobago.

Methods—Ninety-eight pneumococcal isolates from several regional hospitals in the country were analyzed using both standard microbiological methods and molecular analysis. These isolates included invasive (n = 83) and selected non-invasive (n = 15) strains recovered before (n = 25) and after (n = 73) the introduction of the pneumococcal conjugate vaccine.

Results—More than half of the isolates (54.1%) were recovered from children under 15 years of age, with the largest proportion being from children under 2 years of age (24.5%). The most prevalent serotypes were 19F (18.4%), 6B (15.3%), 23F (14.3%), 3 (11.2%), 19A (6.1%), 6A (5.1%), 14 (5.1%), and 9V (4.1%). The most common serotype/MLST combinations were 6B/ST138 (n = 10, 10.2%), 3/ST180 (n = 5, 5.1%), 23F/ST629 (n = 5, 5.1%), 19F/ST8398 (n = 4, 4.1%), and three each of 6B/ST145, 14/9V/ST156, 9V/ST162, 19A/320, and 3/ST10440.

Conclusions—This report provides the first glimpse of the prevailing pneumococcal sequence types in the country. Most of the isolates represented serotypes in the 10-valent (61.2% of isolates) and 13-valent (83.7%) pneumococcal conjugate vaccines. A detailed population study is warranted to fully determine the circulating pneumococcal sequence types. Furthermore, the implementation of an effective and continuous surveillance system in Trinidad and Tobago is paramount to monitor vaccine impact.

*Corresponding author. Tel.: +1 868 645 3232 ext. 2332; fax: +1 868 663 3797. peakpaka@yahoo.co.uk (P.E. Akpaka).

Keywords

Pneumococcal conjugate vaccine; Invasive pneumococcal disease; Sequence type; Multilocus sequence typing; Trinidad and Tobago

1. Introduction

Streptococcus pneumoniae continues to be a major cause of morbidity and mortality in humans.[1–3] The spectrum of pneumococcal disease ranges from a mild respiratory illness to more severe diseases such as pneumonia and invasive pneumococcal diseases (IPD) such as bacteremia, sepsis, and meningitis. Pneumococcal disease is particularly common in young children under 2 years of age and adults over 65 years of age.[1,4]

There are currently 94 recognized capsular polysaccharide serotypes, including the recently reported serotypes 6C, 6D, 11E, and 20A/20B.[2] Each serotype is distinguished based on the chemical composition of and antigenic differences in the capsular polysaccharide.[2,3] Global surveillance has shown that only a limited number of capsular serotypes cause more than 70–80% of IPD.[4–6] The serotype distribution of IPD isolates differs somewhat by geographic region and the time period studied.[4–6]

Currently, there are two types of pneumococcal vaccine available on the global market: the pneumococcal polysaccharide vaccine (23-valent pneumococcal polysaccharide vaccine (PPV23), Pneumovax, Merck) and the pneumococcal conjugate vaccine (10-valent PCV10 and 13-valent PCV13).[3,5] PPV23 is recommended for use in the elderly and immune-compromised individuals over the age of 2 years, while the PCVs are recommended for children under 2 years and also for older children. In late 2011, PCV13 was also recommended for adults ≥50 years who are clinically at risk, including adults over 65 years of age.[2–4,7,8] PCV7 was formulated to include the most frequent serotypes causing pediatric IPD at that time in developed countries, in particular the USA (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F).[3,4,6,9] Two PCVs are currently available for use in children, PCV10 (PCV7 serotypes plus 1, 5, and 7F; PHiD-CV, Synflorix, GlaxoSmithKline) and PCV13 (PCV7 serotypes plus 1, 3, 5, 6A, 7F, and 19A; Prevnar, Pfizer); these replaced PCV7.[3,4,7,8] The current vaccines provide wider serotype coverage than the previous PCV7 formulation.[2,3,10]

In the Caribbean, as well as other regions, vaccine uptake has been variable despite its approval for use. Although PCV7 was introduced in the USA in 2000, it was not until March 2010 that it was introduced into the national immunization program (NIP) in Trinidad and Tobago for infants at risk of pneumococcal disease, mainly those with immune deficiencies and other chronic diseases. Prior to 2010, PCV7 was only available in the private sector for certain children with medical conditions that put them at higher risk of disease.[11] In 2011, the PCV10 vaccine was introduced, followed by its widespread use in the NIP for all children aged <2 years.[11–13] Subsequently, PCV13 was introduced into the NIP in August 2015, replacing PCV10. Vaccine coverage with PCV10 was reported to be 95% as of December 2014.[14]

PPV23 was licensed in Trinidad and Tobago in 2004, but was only incorporated into the NIP in 2012 for children over 2 years of age with chronic diseases, adults 65 years and above, and persons at risk of pneumococcal disease, including those with HIV/ AIDS.[13,14] Trinidad and Tobago does not qualify for donor support under the Global Alliance for Vaccines and Immunizations (GAVI) program.[15] However, vaccines are provided by the Trinidad and Tobago Government under their immunization program and are purchased through a Pan American Health Organization (PAHO) revolving fund.[13]

Although pneumococcal infections are clinically diagnosed and *S. pneumoniae* are routinely isolated from specimens, data on the serotype distribution in the Caribbean, and in Trinidad and Tobago in particular, are limited.[11–13,16–23] The use of molecular methods to determine genotypes of strains in the Caribbean, as in many Latin American countries, has been limited due to financial constraints and other logistical difficulties.[24] To the best of the authors' knowledge, there is no published study that has used multilocus sequence typing (MLST) to characterize pneumococcal isolates from Trinidad and Tobago. The objective of this study was to characterize pneumococcal isolates from both invasive and selected non-invasive sites using serotyping and MLST, and to compare these genotypes to well-described international clonal complexes (http://www.pneumogennet/pmen/).[25]

2. Materials and methods

2.1. Bacterial isolates and country demographics

All pneumococcal isolates (n = 73) obtained from routine clinical specimens submitted to the microbiology diagnostic laboratories of the five major public hospitals in Trinidad and Tobago, during the period 2011 to 2013, were included in this study. The isolates were included in the analysis if they met the following selection criteria: (1) patients with evidence of ongoing infection or confirmed diagnosis (clinical features including fever, elevated C-reactive protein, and elevated white cell count); (2) *S. pneumoniae* as the only pathogen isolated from both invasive and non-invasive sites. Duplicate isolates from the same episode of infection in any one patient were considered to represent the same episode, and therefore were counted once. Selected isolates from non-invasive sites were also included to ascertain a comprehensive picture of the molecular characteristics of pneumococcal infections.

A number of historical pneumococcal isolates (n = 25) from clinical specimens that were collected between 1997 and 2010 were also included in the analysis. Most of the strains were collected from three regional hospitals, prior to the commencement of the SIREVA (Sistema Regional de Vacinas), the Regional System for Vaccines in the Americas project in Trinidad and Tobago.[11,26,27] These historical isolates were included to give some idea of the serotypes and sequence types present in the country prior to the introduction of the PCV7 in the vaccination program in Trinidad and Tobago. The isolates from the earlier period (1997–1998) were not part of a specific study. The pre- and post-vaccination periods were defined as the years 1997 to February 2010 and March 2010 to 2013, respectively.

Demographic and clinical parameters were obtained using a standardized questionnaire. Conventional phenotypic identification was performed at the microbiology laboratories of

the regional hospitals in the country. The twin islands of Trinidad and Tobago are the most southern islands in the Caribbean, located just seven miles off the north eastern coast of Venezuela, and have an estimated population of 1.3 million. It is estimated that 19.4% of the population are less than 14 years old and 9.5% are 65 years old and above. About 13.7% of the population live in urban areas, with 66 000 people in the major capital city of Port of Spain. The majority (68.6%) of the population comprises people of African and Indian descent (34.2% and 34.4%, respectively), with people of mixed race, European, Chinese, and Middle Eastern ancestry adding diversity to the ethnic mix.[28]

All confirmed pneumococcal strains were collected and stored in brain–heart infusion broth with glycerol at −70 °C until further conventional and molecular analysis.

Ethical approval was obtained from the Ethics Committee of the University of the West Indies at St. Augustine. Permission was also granted by the ethics committees of all the regional hospitals where the studies were carried out, namely the North West Regional Health Authority, North Central Regional Health Authority, Eastern Regional Health Authority, South West Regional Health Authority, and Scarborough General Hospital Tobago. Patient records and information were anonymized and de-identified prior to analysis.

2.2. Capsular serotyping

All isolates were serotyped by conventional Quellung reaction method at the US Centers for Disease Control and Prevention (CDC) Streptococcus Laboratory (CDC, Atlanta, GA, USA). Further serotyping was performed using a sequential multiplex PCR method, as described by Pai et al., and updated on the CDC Streptococcus Laboratory website.[29,30] Genomic DNA was extracted from the bacterial isolates using Chelex 100 (Bio-Rad Laboratories, Hercules, CA, USA).

2.3. Multilocus sequence typing analysis

MLST was performed as described by Enright and Spratt, with modified primers (http://www.cdc.gov/streplab/alt-mlst-primers.html).[31] Briefly, the internal fragments of seven housekeeping genes (*aroE*, *gdh*, *gki*, *recP*, *spi*, *xpt*, and *ddl*) of the pneumococcal genome were amplified and sequenced, then the sequence types (STs) were determined by comparing the sequences with alleles from the pneumococcal MLST database (http://pubmlst.org/spneumoniae/). New alleles and STs were submitted to the database curator for assignment. Clonal complexes were defined as groups of STs sharing ≥5 alleles with one or more members of the set and were assigned using eBURST version 3 program (http://eburst.mlst.net) and arbitrarily assigned CC1–CC13.

3. Results

3.1. Bacterial isolates

Ninety-eight *S. pneumoniae* isolates were obtained; 83 were invasive isolates (from blood, pleural fluid, cerebrospinal fluid, and swab from brain tissue) and 15 were non-invasive isolates (ear, eye swabs, and sputum). Most of the invasive isolates were from blood (86.7%,

72/83), while the majority of the non-invasive isolates were from ear swabs (73.3%, 11/15). More than half of the isolates (54.1%, 53/98) were recovered from children under 15 years of age (Table 1), with the largest proportion being from children under 2 years of age (24.5%, 24/98). Twenty-five (25.5%) pneumococcal isolates were recovered before the introduction of the conjugate vaccines (1997 to February 2010) and 73 (74.4%) after (March 2010 to 2013).

3.2. Serotype distribution

Twenty-two different serotypes were detected among the 98 isolates. Only eight serogroups/serotypes were represented by at least four or more isolates (Table 1) and these accounted for 74.4% of the serotypes. These major serotypes included the following: 19F (18.4%), 6B (15.3%), 23F (14.2%), 3 (11.2%), 19A (6.1%), 6A (5.1%), 14 (5.1%), and 9V (4.1%). For the historical isolates (pre-PCV7 period, 1997–2010), the most prevalent serotypes in descending order were 19F ($n = 7$), 3 ($n = 5$), 6B ($n = 4$), 9V ($n = 4$), and 23F ($n = 2$); 7.1% were non-vaccine types (NVT). In the post-PCV7 period (March 2010 to 2013), serotypes 19F, 23F, 6B, 3, 19A, and 6A were the most common, and 32.6% were NVT. Six serotypes (3, 6B, 11A, 14, 19F, and 23F) were identified in both periods. The total coverage rates of PCV7, PCV10, and PCV13 were 60.2%, 61.2%, and 83.7%, respectively; there was a significant 22.5% difference between PCV10 and PCV13 in coverage.

3.3. Clonal structure

The 98 pneumococcal isolates included in this study represented 55 different STs (Table 2). Nine STs accounted for more than 39.7% of the isolates, including ST138 ($n = 10$), ST180 ($n = 5$), ST629 ($n = 5$), ST8398 ($n = 4$), and ST145, ST156, ST162, ST320, and ST10440 (each accounting for three isolates). Fifteen (15.3%) new STs, including six new MLST alleles, were identified in this study and were assigned to ST 9743–9751, 10439, 10440, 10446, 10449, 10450, and 10455 (Table 2). When compared with STs that have previously been deposited in the MLST database, eight of the 15 were related to previously reported STs. Of these 15 new STs, eight were identified during the period 1997–2009, including ST10440, which was also found during the post-vaccination period (2011–2013).

STs that represented more than one serotype included ST138 (6A, 6B, 19F), ST156 (9V, 14), and ST199 (15B, 19A). Twelve serotypes (4, 7F, 9N, 10A, 15A, 15B, 15C, 16F, 17F, 18C, 22F, 35F) were associated with a single ST, whereas the remaining serotypes consisted of multiple STs. Serotypes 19F and 23F showed the most genetic diversity with 14 and eight STs, respectively. Four STs were isolated during both the pre- and post-PCV periods: STs 62, 156, 180, and 10440. The eBURST analysis revealed 14 clonal complexes (CC) and 17 singletons containing 63 and 35 isolates, respectively (Table 2). Of the new STs, five were singletons and 10 grouped into CCs. Fourteen isolates (14.3%) were identified as being related to seven of the internationally described Pneumococcal Molecular Epidemiology Network (PMEN) clones (PMEN3, PMEN4, PMEN14, PMEN31, PMEN37, PMEN38, and PMEN39; http://www.pneumogen.net/pmen/) (Figure 1).[25]

4. Discussion

This study aimed to describe the observed prevailing serotypes and genotypes of pneumococcal isolates from both invasive and non-invasive pneumococcal diseases in Trinidad and Tobago. IPD is not a notifiable disease in Trinidad and Tobago, and the current laboratory-based surveillance system is based on voluntary rather than mandatory submission of isolates. The annual burden of pneumococcal disease in Latin America and the Caribbean has been estimated to be 1.6 million in children less than 5 years of age.[32] In Trinidad and Tobago, the incidence rates of fever and acute respiratory infections (ARI) during the years 2009–2010 in ages less than 5 was 22 407–25, 202 cases per 100,000 and for ages over 5 years, it decreased from 2447–1163 cases per 100,000 for the same corresponding years.[13]

A number of pneumococcal infections in both children and adult patients were identified during the two time periods, before (1997 to February 2010) and after (March 2010 to 2013) PCV vaccination. A total of 98 isolates were available for analysis, with the majority recovered during the years 2011–2013. There were only 25 isolates available for the period 1997–2010 (21 from 1997–1999 and four from 2007–2010), representing less than 2% of the pneumococcal isolates reported between 2007 and 2010.[11–13,20,21] The majority of the isolates collected during these periods were not available for the current study analysis due to non-viability after storage. This is unfortunate, because it was a missed opportunity to further characterize samples prior to 2010.

The coverage rates of PCV7, PCV10, and PCV13 for pneumococcal isolates encountered in this study were 60.2%, 61.2%, and 83.7%, respectively. The observed 22.5% difference in PCV10 and PCV13 coverage rates is noteworthy. PCV10 provided little added benefit to PCV7, as two of the three serotypes (1 and 5) were rare in Trinidad and Tobago,[11–13,16–22] whereas the additional serotypes in PCV13 (3, 6A, and 19A) contributed to the increase in vaccine coverage that was observed. Data published by the SIREVA network and the Caribbean Public Health Agency (CARPHA), formerly the Caribbean Epidemiology Centre (CAREC), have shown that the most prevalent serogroups/serotypes causing IPD in the Caribbean prior to the NIP in Trinidad and Tobago were 14, 6B, 23F, 18, 19, 19F, 9, 6A, 1, and 23.[16–20,22] Subsequently, serogroups 19 and 9 were identified as 19F and 9V, respectively, by the Instituto Nacional de Salud Bogotá, Bogotá, Colombia.[22] During the same period in Trinidad and Tobago, 12 different serogroups and 22 serotypes were identified, the most prevalent being 14, 6B, 4, 6A, and 15C. Serotypes 14, 6B, 23F, and 4 were recovered from children under 5 years of age.[16–20,23] When compared globally to those observed in Latin America, North America, and Europe, the 10 prevalent serotypes prior to 2010 in the present study were comparably well represented in samples from elsewhere in the pre-PCV7 and early post-PCV7 eras.[2,5,6,9,22,26,33–37]

Serotypes 1 and 5 were absent during the period 1997–2013 in the present analysis. This is similar to North America, where serotypes 1 and 5 are uncommon, but is different from the situation in some European, Asian, and Latin American countries, where they are frequently isolated.[5,6,9,26,34,36–39] Serotype 6C, which has been identified in some countries,[40–43] was absent in the isolates tested in the present study. It is unclear whether 6C may have been

among the serogroup 6A/6B isolates reported by CAREC from 1997 to 1999 and 2007 to 2010, especially among those isolated after the introduction of PCV7.[11–13,16–21] Studies in the USA have shown that previously typed 6A isolates recovered after PCV7 introduction were primarily 6C.[40] Serotype 6C was first described in 2006, however retrospective analyses have indicated that it was in existence as early as 1962. Prior to PCV7, 6C was extremely rare in children <5 years old and was uncommon in adults.[40–42,44] However, 6C increased overall with time throughout the 2000s to become the prevalent serogroup 6 serotype within the US IPD surveillance.[45] Serotype 6C emerged more significantly in adults than in children in the USA during this period,[45] although carriage in children was seen to increase.[46] Studies in Spain and other countries have shown similar increases in both children and adults, but at relatively low levels.[42,47]

In the present study, 27% of the 19F isolates were identified as 19F-var on PCR serotyping due to the *wzy* variant gene, which was first observed in Canada and Brazil.[48–50] Identifying this variation in the serotype is important for accurate serotype determination in order to measure the impact of the vaccine. In this study, there was a marginal shift in the serotype distribution, including the emergence of NVTs in the post-PCV7/10 period, in particular serotype 19A. The emergence of 19A was observed globally in the post-PCV7 era, particularly in North America and many Asian countries.[2,3,5,45] However, this serotype was also observed in some countries in the pre-PCV era.[2,36,39] Not all vaccine serotypes provide cross-protection to other serotypes in the same group, as in the case of the 19F antigen in PCV7, which provides little or no protection against 19A.[51] The increased use of both PCV10 and PCV13 in some countries has now resulted in decreases in these serotypes, including 19A and 7F, but continued increases in NVTs like 22F and 33F.[51,52] In the present study, PCV7 NVT and PCV10 NVT increases in serotypes 3, 6A, serogroup 15, and serotype 11A were also seen, as has been reported from the USA and other developed countries.[5,53] Now with the implementation of PCV13 in Trinidad and Tobago, it will be interesting to monitor the NVTs in the post-PCV13 period to assess changes in serotype due to increased valency.

The genotyping data using MLST showed great diversity among isolates from Trinidad and Tobago. While 55 different STs were identified, these were assigned to only 14 CCs and there were large numbers of singletons ($n = 17$). Also, 15.3% of isolates had new STs, which will provide the first MLST-based data for the Caribbean region. All new STs were from invasive sites, with the exception of one recovered from a sputum sample. Serotypes 19F and 23F were more genetically diverse compared to the other serotypes and were associated with distinct STs, including nine of the new STs. Carriage studies have demonstrated that 19F, 23F, and other ‘pediatric’ serotypes colonize the nasopharynx for longer periods as compared to serotypes like 1 and 7F, which colonize for a shorter duration.[2,54,55] Consequently, as they remain longer in the nasopharynx, there is sufficient opportunity for horizontal gene transfer between pneumococcal isolates and other nasopharyngeal organisms, which potentially contributes to this diversity.[56]

The occurrence of STs consisting of more than two serotypes due to horizontal transfer of capsule genes has been well documented,[9,31,33] and there were several examples of capsular switching events in this study: ST138 (6A, 6B, 19F), ST156 (9V, 14), and ST199 (15B,

19A). Eight STs (138, 180, 629, 8398, 145, 156, 162, and 10440) accounted for more than 36% of the isolates, and a number of isolates (14.3%) belonged to well-described international PMEN clones (http://www.pneumogen.net/pmen/). Of the 39 STs reported, 10 from Latin American countries and 29 from North America have been deposited previously in the MLST database (http://pubmlst.org/spneumoniae/). Some of these STs have significant geographic spread, for example ST138 and ST180 have been found to be present in most regions, including the USA and some European and Latin American countries.[2,24,33,57] ST629 has been observed in the USA[30,58] and Germany (described in the MLST database). Available data on genotypes from Venezuela are limited, and the MLST database has 29 isolates deposited since 2007. Among these isolates, only three STs (ST320, ST156, and ST199) were similar to those from the Trinidad and Tobago study. Four STs were observed in both the pre- and post-vaccination periods (1997 to February 2010 and March 2010 to 2013): ST62, 156, 180, and 10440. ST62 (11A) was isolated both in 1998 and 2012, and this ST has been described in invasive isolates from Spain and Italy (described in the MLST database).[33]

When the clonal composition was compared with the USA and Scotland in the pre- and the early post-vaccination periods, variations were observed. For instance, serotype 14 was represented by four STs in the USA and 12 STs in Scotland during the pre-PCV era, and quite striking was the absence of ST156 in Scotland.[33,37,39] ST156 has frequently occurred worldwide and was first described as serotype 9V; this has subsequently also been described as serotype 14 and as other serotypes.[25,57] Furthermore, ST156 is often associated with antibiotic resistance and is one of the well described PMEN clones (Spain^{9V}-ST156, PMEN3) that have spread globally, including within the Latin America region.[25] Isolates belonging to clonal complex 156 (CC156) were also found to modestly emerge within 19A in the post-PCV period in the USA.[33] It is also interesting to see this very enduring clonal complex associated with several serotypes in the post-PCV13 era.[59] In the present study, ST156 was associated with both serotype 9V and 14 (commonly found in South America), and CC156 included closely related ST8398 (19F), ST162 (9V), and ST10449 (14). PMEN4 (Tennessee^{23F}-ST37) was represented by nine STs, all of which were restricted to serotype 23F.[33]

Six isolates were related to the highly successful Taiwan^{19F}-ST236 (PMEN14) lineage, including ST320 (19A). In the USA, the ST320 complex was responsible for a dramatic increase in 19A IPD. 19A/ST320 and related 19A strains became the most commonly recovered strains from IPD during the 2006–2009 post-PCV7 era, and also constituted the majority of multi-resistant strains during this period.[60] Putative vaccine escape ST695 19A variants (n = 2) of the non-resistant major PCV7 type 4 strain were also identified among the study isolates in the post-PCV7 period.[60] Isolates belonging to other PMEN included serotype 15B and 19A capsular variant of Netherlands^{15B}-ST199 (PMEN37), serotype 7F PMEN39 (Netherlands^{7F}-ST191), serotype 4 PMEN38 (Sweden^{4}-ST205), and five ST180 serotype 3 isolates belonging to Netherlands^{3}-ST180 (PMEN31). ST180 was isolated mainly from adults with IPD and was present during the pre-vaccination era and continued to circulate in 2013. One isolate from the pre-PCV7 period was recovered from a postmortem case in 1998. This ST180 clone is the major genotype of serotype 3 isolates globally, but independent lineages among type 3s in this study were also observed. In Europe and North

America, there have been reports of the ST180 clone associated with invasive diseases and increased mortality.[33,37,61,62] ST1262 (15C), which was first observed in the USA study,[33] was also seen in the early post-PCV phase in the present study. The STs associated with 6B (138, 145, 146, 497, and 4241) were also different from those detected in Latin American countries.[57]

Some level of marginal shift in the serotype distribution appeared to be present in this study, including the emergence of the NVTs that have been observed globally. While data suggest the presence of a diverse array of genotypes, some well described global clones and their capsular variants described in other regions of the world, in particular North America, and mainly the USA, are also circulating in Trinidad and Tobago. The STs were slightly different from those in the neighboring Latin American country Venezuela, probably because the Venezuelan isolates have been less studied. This information will continue to provide baseline data for additional studies needed on the molecular characterization of pneumococcal isolates, not only in Trinidad and Tobago, but also throughout the Caribbean region.

One of the limitations of this study was the relatively small number of isolates used in the analysis, particularly for the pre-vaccination period (1997 to February 2010), which may not have reflected the true picture of circulating pneumococcal serotypes in Trinidad and Tobago. The isolates were recovered from patients seen and treated at the regional hospitals and this may have led to a bias towards the more severe cases. Despite these limitations, the information obtained from this study provides the first insight into the prevailing genotypes of pneumococcal isolates in the country. The findings also suggest that PCV13 could provide greater serotype coverage than PCV10 for serotypes currently causing IPD in Trinidad and Tobago. A more detailed population study is required post-PCV13 introduction in order to obtain a comprehensive picture of the circulating pneumococcal genotypes in the country.

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References

1. O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. Lancet. 2009; 374:893–902. [PubMed: 19748398]
2. Song JY, Nahm MH, Moseley MA. Clinical implications of pneumococcal serotypes: invasive disease potential, clinical presentations, and antibiotic resistance. J Korean Med Sci. 2013; 28:4–15. [PubMed: 23341706]
3. Tan T. Pediatric invasive pneumococcal disease in the United States in the era of pneumococcal conjugate vaccines. Clin Microbiol Rev. 2012; 25:409–19. [PubMed: 22763632]

4. World Health Organization. Pneumococcal conjugate vaccine for childhood immunization—WHO position paper. Wkly Epidemiol Rec. 2012; 87:129–44. [PubMed: 24340399]
5. McIntosh ED, Reinert RR. Global prevailing and emerging pediatric pneumococcal serotypes. Expert Rev Vaccines. 2011; 10:109–29. [PubMed: 21162625]
6. Johnson HL, Deloria-Knoll M, Levine OS, Stoszek SK, Hance LF, Reithinger R, et al. Systematic evaluation of serotypes causing invasive pneumococcal disease among children under five: the Pneumococcal Global Serotype Project. PLoS Med. 2010; 7:e1000348. [PubMed: 20957191]
7. Advisory Committee on Immunization Practices licensure of a 13-valent pneumococcal conjugate vaccine (PCV13) and recommendations for use among children. MMWR Morb Mortal Wkly Rep. 2010; 59:258–61. [PubMed: 20224542]
8. Tomczyk S, Bennett NM, Stoecker C, Gierke R, Moore MR, Whitney CG, et al. Use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine among adults aged ≥65 years: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR. 2014; 63:822–5. [PubMed: 25233284]
9. Hausdorff WP, Bryant J, Paradiso PR, Siber GR. Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, Part 1. Clin Infect Dis. 2000; 30:100–21. [PubMed: 10619740]
10. Jauneikaite E, Jefferies JM, Hibberd ML, Clarke SC. Prevalence of *Streptococcus pneumoniae* serotypes causing invasive and non-invasive disease in South East Asia: a review. Vaccine. 2012; 30:3503–14. [PubMed: 22475858]
11. [accessed March 5, 2015] Caribbean Epidemiology Centre annual report. 2008. CAREC/PAHO/WHO. Published 2009. Available at: http://carpha.org/downloads/200820CAREC20Annual20Report.pdf
12. [accessed March 5, 2015] Caribbean Epidemiology Centre annual report. 2009. CAREC/PAHO/WHO. Published 2010. Available at: http://carpha.org/downloads/200920CAREC20Annual20Report.pdf
13. [accessed March 5, 2015] Caribbean Epidemiology Centre annual report. 2010. CAREC/PAHO/WHO. Published 2011. Available at: http://carpha.org/downloads/201020CAREC20Annual20Report.pdf
14. World Health Organization. Vaccine-preventable diseases: monitoring system. 2014 global summary. WHO; Published online 2014 (Updated June 2015)Available at: http://apps.who.int/immunization_monitoring/globalsummary [accessed February 27, 2016]
15. GAVI Alliance. Countries approved for support. Geneva, Switzerland: GAVI Alliance; Mar. 2015 Published June 2015. Available at: http://www.gavialliance.org/results/countries-approved-for-support [accessed August 8, 2014]
16. [accessed March 5, 2015] Caribbean Epidemiology Centre annual report. 2001. CAREC/PAHO/WHO. Published 2002. Available at: http://carpha.org/downloads/200120CAREC20Annual20Report.pdf
17. [accessed March 5, 2015] Caribbean Epidemiology Centre annual report. 2002. CAREC/PAHO/WHO. Published 2003. Available at: http://carpha.org/downloads/200220CAREC20Annual20Report.pdf
18. [accessed March 5, 2015] Caribbean Epidemiology Centre Annual Report. 2005. CAREC/PAHO/WHO; Published 2006. Available at: http://carpha.org/downloads/2005CAREC20Annual20Report.pdf
19. [accessed March 5, 2015] Caribbean Epidemiology Centre Annual Report. 2006. CAREC/PAHO/WHO. Published 2007. Available online at: http://carpha.org/downloads/2006CAREC20Annual20Report.pdf
20. [accessed March 5, 2015] Caribbean Epidemiology Centre Annual Report. 2007. CAREC/PAHO/WHO. Published 2008. Available online at: http://carpha.org/downloads/2007CAREC20Annual20Report.pdf
21. [accessed March 5, 2015] Caribbean Epidemiology Centre Annual Report. 2011. CAREC/PAHO/WHO; Published 2012. Available online at: http://carpha.org/downloads/2011CAREC20Annual20Report.pdf

22. PAHO. Informe Regional de SIRVEA11: Datos por país y por grupos de edad sobre las características de los aislamientos de *Streptococcus pneumoniae*, *Haemophilus influenzae* y *Neisseria meningitidis* en procesos invasores 2000–2005. Washington DC: OPS; 2007.
23. Orrett FA. Pneumococcal infections in Trinidad: patterns of antimicrobial susceptibility: 1994–2002. Japan J Infect Dis. 2005; 58:20–4. [PubMed: 15728985]
24. Inverarity D, Diggle M, Ure R, Johnson P, Altstadt P, Mitchell T, et al. Molecular epidemiology and genetic diversity of pneumococcal carriage among children in Beni State, Bolivia. Trans R Soc Trop Med Hyg. 2011; 105:445–51. http://dx.doi.org/10.1016/j.trstmh.2011.04.013. [PubMed: 21714978]
25. McGee L, McDougal L, Zhou J, Spratt BG, Tenover FC, George R, et al. Nomenclature of major antimicrobial-resistant clones of *Streptococcus pneumoniae* defined by the pneumococcal molecular epidemiology network. J Clin Microbiol. 2001; 39:2565–71. [PubMed: 11427569]
26. Di Fabio JC, Castaneda E, Leal L, Castillo O. PAHO SIREVA-VIGIA Study Group. Evolution of *Streptococcus pneumoniae* serotypes and penicillin susceptibility in Latin America, Sireva-Vigia Group, 1993–1999. Pediatr Infect Dis J. 2001; 20:259–67.
27. Garcia S, Levine OS, Cherian T, Gabastou JM, Andrus J. Working Group members. Pneumococcal disease and vaccination in the Americas: an agenda for accelerated vaccine introduction. Rev Panam Salud Publica. 2006; 19:340–8. [PubMed: 16805976]
28. Central Intelligence Agency (CIA). [accessed February 2015] The World Fact Book. Trinidad and Tobago. People and Society. 2015. Available at https://www.cia.gov/library/publications/resources/the-world-factbook/geos/td.html
29. Pai R, Gertz R, Beall B. Sequential multiplex PCR approach for determining capsular serotypes of *Streptococcus pneumoniae* isolates. J Clin Microbiol. 2006; 44:124–31. [PubMed: 16390959]
30. Centers for Disease Control and Prevention. Multiplex PCR for deducing serotypes from pneumococcal isolates/PCR serotype deduction protocols/PCR deduction of pneumococcal serotypes. Atlanta, GA: CDC; 2014. Available at: http://www.cdc.gov/ncidod/biotech/strep/pcr.htm [accessed March 23, 2015]
31. Enright MC, Spratt BG. A multilocus sequence typing scheme for *Streptococcus pneumoniae*: identification of clones associated with serious invasive disease. Microbiology. 1998; 144:3049–60. [PubMed: 9846740]
32. Constenla, D., Gomez, E., de la Hoz, FP., O'Loughlin, R., Sinha, A., Valencia, JE., Valenzuela, MT. The burden of pneumococcal disease and cost-effectiveness of a pneumococcal vaccine in Latin America and the Caribbean: a review of the evidence and a preliminary economic analysis. Washington, DC: The Albert B. Sabin Vaccine Institute, Pan American Health Organization, GAVI's Pneumococcal Accelerated Development Introduction Plan at Johns Hopkins (PneumoADIP), Centers for Disease Control and Prevention; 2007. Available at: http://www.ispch.cl/sites/default/files/document1.pdf or www.sabin.org [accessed February 27, 2016]
33. Beall B, McEllistrem MC, Gertz RE Jr, Wedel S, Boxrud DJ, Gonzalez AL, et al. Pre-and post-vaccination clonal compositions of invasive pneumococcal serotypes for isolates collected in the United States in 1999, 2001, and 2002. J Clin Microbiol. 2006; 44:999–1017. [PubMed: 16517889]
34. Castaneda E, Leal L, Castillo O, Di Fabio JC. Pneumococcal Study Group in Colombia. Distribution of capsular types and antimicrobial susceptibility of invasive isolates of *Streptococcus pneumoniae* in Colombian children. Microbiol Drug Resist. 1997; 3:147–52.
35. Centers for Disease Control and Prevention. Geographic variation in penicillin resistance in *Streptococcus pneumoniae* - selected sites, United States, 1997. MMWR. 1999; 48:656–61. [PubMed: 10488781]
36. Castaneda E, Agudelo CI, De Antonio R, Rosselli D, Calderon C, Ortega Barria E, Colindres RE. *Streptococcus pneumoniae* serotype 19A in Latin America and the Caribbean: a systematic review and meta-analysis, 1990–2010. BMC Infect Dis. 2012; 12:124. [PubMed: 22639955]
37. Clarke SC, Jefferies JM, Smith AJ, McMenamin J, Mitchell TJ, Edwards GF. Pneumococci causing invasive disease in children prior to the introduction of pneumococcal conjugate vaccine in Scotland. J Med Microbiol. 2006; 55:1079–84. [PubMed: 16849728]

38. Lovegren M, Spika JS, Talbot JA. Invasive *Streptococcus pneumoniae* infections: serotype distribution and antimicrobial resistance in Canada, 1992–1995. Can Med Assoc J. 1998; 158:327–31. [PubMed: 9484256]
39. Jefferies JM, Smith AJ, Edwards GF, McMenamin J, Mitchell TJ, Clarke SC. Temporal analysis of invasive pneumococcal clones from Scotland illustrates fluctuations in diversity of serotype and genotype in the absence of pneumococcal conjugate vaccine. J Clin Microbiol. 2010; 48:87–96. [PubMed: 19923488]
40. Carvalho MD, Pimenta FC, Gertz RE, Joshi HH, Trujillo AA, Keys LE, et al. PCR-based quantitation and clonal diversity of the current prevalent invasive serogroup 6 pneumococcal serotype, 6c, in the United States in 1999 and 2006 to 2007. J Clin Microbiol. 2009; 47:554–9. [PubMed: 19116353]
41. McEllistrem CM, Nahm MH, Novel CM. Novel pneumococcal serotypes 6C and 6D: anomaly or harbinger. Clin Infect Dis. 2012; 55:1379–86. [PubMed: 22903767]
42. Green MC, Mason EO, Kaplan SL, Lamberth LB, Stovall SH, Givner LB, et al. Increase in prevalence of Streptococcus pneumoniae serotype 6C at eight children's hospitals in the United States from 1993 to 2009. J Clin Microbiol. 2011; 49:2097–101. [PubMed: 21450963]
43. van der Linden M, Winkel N, Küntzel S, Farkas A, Perniciaro SR, Reinert RR, Imöhl M. Epidemiology of *Streptococcus pneumoniae* serogroup 6 isolates from IPD in children and adults in Germany. PLoS One. 2013; 8:e60848. [PubMed: 23593324]
44. Jacobs MR, Bajaksouzian S, Bonomo RA, Good CE, Windau AR, Hujer AM, et al. Occurrence, distribution, and origins of *Streptococcus pneumoniae* serotype 6C, a recently recognized serotype. J Clin Microbiol. 2009; 47:64–72. [PubMed: 18971356]
45. Moore MR, Link-Gelles R, Schaffner W, Lynfield R, Lexau C, Bennett NM, et al. Effect of use of 13-valent pneumococcal conjugate vaccine in children on invasive pneumococcal disease in children and adults in the USA: analysis of multisite, population-based surveillance. Lancet Infect Dis. 2015; 15:301–9. [PubMed: 25656600]
46. Desai AP, Sharma D, Crispell EK, Baughman W, Thomas S, Tunali A, et al. Decline in pneumococcal nasopharyngeal carriage of vaccine serotypes after the introduction of the 13-valent pneumococcal conjugate vaccine in children in Atlanta, Georgia. Pediatr Infect Dis J. 2015; 34:1168–74. [PubMed: 26226445]
47. Rolo D, Fenoll A, Ardanuy C, Calatayud L, Cubero M, de la Campa AG, Linares J. Trends of invasive serotype 6C pneumococci in Spain: emergence of a new lineage. J Antimicrob Chemother. 2011; 66:1712–8. [PubMed: 21628304]
48. Pimenta FC, Gertz RE Jr, Roundtree A, Yu J, Nahm MH, McDonald RR, et al. Rarely occurring 19A-like cps locus from a serotype 19F pneumococcal isolate indicates continued need of serology based quality control for PCR-based serotype determinations. J Clin Microbiol. 2009; 47:2353. [PubMed: 19439547]
49. Menezes AP, Reis JN, Ternes YM, Andrade AL, Pimenta FC, Carvalho MD, et al. Update of pneumococcal PCR serotyping assay for detection of a commonly occurring type 19F *wzy* variant in Brazil. J Clin Microbiol. 2013; 51:2470–1. [PubMed: 23658255]
50. Siira L, Kaijalainen T, Lambertsen L, Nahm MH, Toropainen M, Virolainen A. From Quellung to multiplex PCR, and back when needed, in pneumococcal serotyping. J Clin Microbiol. 2012; 50:2727–31. [PubMed: 22692742]
51. van der Linden M, Perniciaro S, Imohl M. Increase of serotypes 15A and 23B in IPD in Germany in the PCV13 vaccination era. BMC Infect Dis. 2015; 15:207. [PubMed: 25940580]
52. Feikin DR, Kagucia EW, Loo JD, Link-Gelles R, Puhan MA, Cherian T, et al. Serotype-specific changes in invasive pneumococcal disease after pneumococcal conjugate vaccine introduction: a pooled analysis of multiple surveillance sites. PLoS Med. 2013; 10:e1001517. [PubMed: 24086113]
53. Moore CE, Paul J, Foster D, Mahar SA, Griffiths D, Knox K, et al. Reduction of invasive pneumococcal disease 3 years after the introduction of the 13-valent conjugate vaccine in the Oxfordshire region of England. J Infect Dis. 2014; 210:1001–11. [PubMed: 24719477]
54. Garcia Rodriguez JA, Fresnadillo Martínez MJ. Dynamics of nasopharyngeal colonization. J Antimicrob Chemother. 2002; 50(Suppl S2):59–73.

55. Örtqvist A, Hedlund AJ, Kalin M. *Streptococcus pneumoniae*: epidemiology, risk factors, and clinical features. Semin Respir Crit Care Med. 2005; 26:563–74. [PubMed: 16388428]

56. Hsieh YC, Wang JT, Lee WS, Hsueh PR, Shao PL, Chang LY, et al. Serotype competence and penicillin resistance in *Streptococcus pneumoniae*. Emerg Infect Dis. 2006; 12:1709–14. [PubMed: 17283621]

57. Zemlickova H, Crisostomo I, Brandileone MC, Camou T, Castaneda E, Corso A, et al. Serotype and clonal types of penicillin-susceptible *Streptococcus pneumoniae* causing invasive disease in children in five Latin American countries. Microb Drug Resist. 2005; 11:195–204. [PubMed: 16201921]

58. Gertz RE, McEllistrem MC, Boxrud DJ, Li Z, Thompson TA, Facklam RR. Clonal distribution of invasive pneumococcal isolates from children and selected adults in the United States prior to 7-valent conjugate vaccine introduction. J Clin Microbiol. 2003; 41:4194–216. [PubMed: 12958247]

59. Metcalf BJ, Gertz RE Jr, Gladstone RA, Walker H, Sherwood LK, Jackson D, et al. Strain features and distributions in pneumococci from children with invasive disease before and after 13 valent conjugate vaccine implementation in the United States. Clin Microbiol Infect. 2016; 22:60.e9–60.e29.

60. Beall BW, Gertz RE, Hulkower RL, Whitney CG, Moore MR, Brueggemann AB. Shifting genetic structure of invasive serotype 19A pneumococci in the United States. J Infect Dis. 2011; 203:1360–8. [PubMed: 21398395]

61. Harboe ZB, Thomsen RW, Riis A, Valentiner-Branth P, Christensen JJ, Lambertsen L, et al. Pneumococcal serotypes and mortality following invasive pneumococcal disease: a population-based cohort study. PLoS Med. 2009; 6:e1000081. [PubMed: 19468297]

62. Inverarity D, Lamb K, Diggle M, Robertson C, Greenhalgh D, Mitchell TJ, et al. Death or survival from invasive pneumococcal disease in Scotland: associations with serogroup and multilocus sequence types. Med Microbiol J. 2011; 60:793–802.

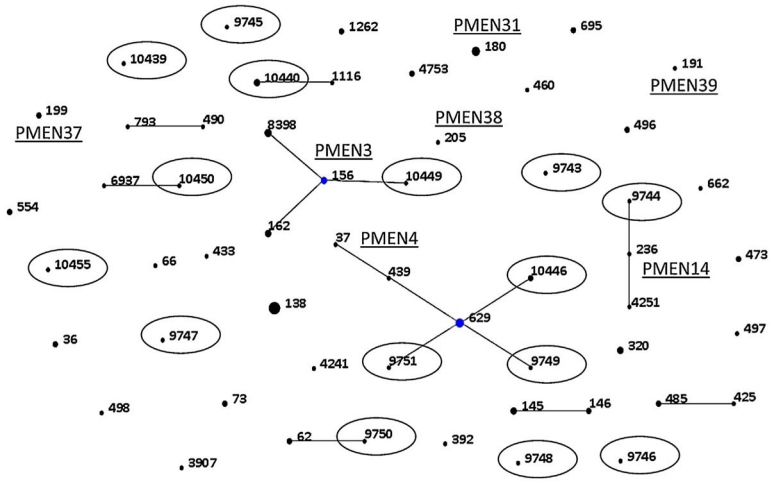

Figure 1.

eBURST diagram showing a population snapshot of 98 isolates from Trinidad and Tobago with complete MLST data. Each ST is represented by a point, with the size of the point representing the number of isolates associated with that ST within the dataset. Isolates (14.3%) were related to seven of the internationally described PMEN clones (http://www.pneumogen.net/pmen/). STs circled represent new STs identified among isolates in this study.

Table 1

Age and serotype distribution of invasive and non-invasive pneumococcal isolates from Trinidad and Tobago

Age (years)	No. of isolates (%)	Serotype (No. of isolates)
<2	24 (24.5%)	6B (6), 9V (2), 14 (1), 19F (8), 23F (2), 3 (1), 10A (2), 16F (1), 22F (1)
2 to <5	18 (18.4%)	6B (4), 19F (3), 23F (5) 6A (2), 19A (3), 15C (1)
5 to <15	11 (11.2%)	14 (1), 19F (2), 23F (3), 3 (1), 6A (1), 15C (1), 18C (1)
15 to <24	1 (1.0%)	11A (1)
24 to <45	15 (15.3%)	9V (2), 6B (1), 14 (2), 19F (3), 23F (1), 3 (1), 19A (1), 11A (1), 15A (1), 17F (1), 35F (1)
45 to <60	11 (11.2%)	6B (2), 14 (1), 23F (2), 7F (1), 3 (2), 6A (1), 19F (1), 20 (1), 11A (1)
>60	18 (18.4%)	4 (1), 6B (2), 18C (1), 19F (1), 23F (1), 3 (6), 6A (1), 19A (2), 9N (1), 15B (1), 15A (1)

Table 2

Distribution of genotype (ST) among the pneumococcal serotypes from Trinidad and Tobago, 1997–2013

Serotype	No. of isolates	Sequence type (No. of isolates)	MLST_*aroe-gdh-gki-recp-spi-xpt-ddl*	Clonal complex
4	1	205	10-5-4-5-13-10-18	Singleton
6B	15	138 (8)	7-5-8-5-10-6-14	Singleton
		145 (3)	7-6-1-1-6-15-14	5
		146 (2)	7-6-1-2-6-15-14	5
		497 (1)	7-25-4-2-48-20-28	12
		4241 (1)	15-16-19-10-6-20-26	Singleton
9V	4	156 (1)	7-11-10-1-6-8-1	2
		162 (3)	7-11-10-1-6-8-14	2
14	5	156 (2)	7-11-10-1-6-8-1	2
		554 (2)	8-8-4-15-39-12-26	Singleton
		10449 (1)	7-11-10-1-**451**-8-1	2
18C	2	496	42-35-29-36-9-39-18	13
19F	18	138[a] (1)	7-5-8-5-10-6-14	Singleton
		236 (1)	15-16-19-15-6-20-26	3
		425 (1)	1-5-1-5-1-1-8	6
		485 (2)	1-5-1-1-1-1-8	6
		4251 (1)	15-16-19-10-6-20-26	3
		793 (1)	7-13-9-1-6-19-14	11
		3907 (1)	1-5-214-12-1-79-8	Singleton
		6937 (1)	2-5-4-5-27-20-5	9
		9743 (1)	1-5-106-12-10-4-14	Singleton
		9744 (1)	15-16-19-15-6-4-26	3
		9746 (1)	18-43-4-1-6-4-8	10
		9748 (1)	18-5-4-1-6-11-8	10
		8398[a] (4)	7-11-10-1-8-8-1	2
		10450 (1)	**327**-5-4-5-27-20-5	9
23F	14	36 (2)	1-8-4-1-1-4-6	Singleton
		37 (1)	1-8-6-2-6-4-6	1
		439 (1)	1-8-9-2-6-4-6	1
		629 (5)	1-8-9-10-6-4-6	1
		9747 (1)	2-13-2-4-9-1-1	4
		9749 (1)	1-8-9-6-6-4-6	1
		9751 (1)	1-2-9-10-6-4-6	1
		10446 (2)	1-8-9-**294**-6-4-6	1
7F	1	191	8-9-2-1-6-1-17	Singleton
3	11	180 (5)	7-15-2-10-6-1-22	Singleton
		1116 (1)	1-26-28-11-13-1-14	14
		662 (1)	5-35-29-12-9-39-18	13
		10440 (3)	1-26-28-11-**452**-1-14	14

Serotype	No. of isolates	Sequence type (No. of isolates)	MLST_*aroe-gdh-gki-recp-spi-xpt-ddl*	Clonal complex
		10455 (1)	2-12-19-1-6-**615**-22	Singleton
6A	5	473 (2)	7-25-4-4-15-20-28	12
		138 (1)	7-5-8-5-10-6-14	Singleton
		460 (1)	5-7-4-10-10-1-27	7
		490 (1)	2-13-9-1-6-19-14	11
19A	6	320 (3)	4-16-19-15-6-20-1	3
		695 (2)	16-13-4-4-6-113-18	Singleton
9N	1	199 (1)	8-13-14-4-17-4-14	Singleton
		66	2-8-2-4-6-1-1	4
10A	2	4753	5-241-4-2-10-1-27	7
11A	3	62 (2)	2-5-29-12-16-3-14	8
		9750 (1)	2-5-29-12-16-1-14	8
15A	2	73	2-13-2-15-6-1-1	4
15B	1	199	8-13-14-4-17-4-14	Singleton
15C	2	1262	7-41-2-6-10-26-1	Singleton
16F	1	10439	1-10-62-10-15-1-31	Singleton
17F	1	392	7-5-1-1-6-31-14	5
20	1	9745	5-31-8-**295**-9-1-**665**	Singleton
22F	1	433	1-1-4-1-18-58-17	Singleton
35F	1	498	2-7-4-16-10-40-27	Singleton

STs underlined refer to new STs identified in this study. New alleles in this study are in bold.

[a]These serotype 19F isolates are *wzy* variants described by Pimenta et al.[48]