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Prevalence of *Streptococcus pneumoniae* serotype 6C among invasive and carriage isolates in metropolitan Salvador, Brazil, from 1996 to 2007

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Abstract

The newly described *Streptococcus pneumoniae* serotype 6C accounted for 2.3% (16/709) of meningitis cases and 3.2% (3/95) of nasopharyngeal isolates from healthy individuals in Brazil. The strains were multidrug resistant (18.8%) and genetically diverse. Despite low serotype 6C prevalence, continuous surveillance is necessary to guide vaccine strategies.

Keywords: *Streptococcus pneumoniae*; Serotype 6C; Epidemiology; Meningitis; Carriage

1. Introduction

Streptococcus pneumoniae is a significant cause of morbidity and mortality especially among children <2 years old and the elderly (World Health Organization [WHO], 2007). The antiphagocytic polysaccharide capsule is the major virulence determinant of *S. pneumoniae* (Kadioglu et al., 2008). Of the 91 known capsular serotypes (Park et al., 2007b), approximately 20 are associated with >80% of invasive pneumococcal disease (IPD) (Hausdorff et al., 2000).

The serotypes 6C and 6A biosynthetic loci are identical except for the presence of different *wci*N genes that encode distinct glycosyl transferases (Park et al., 2007a). The 2 serotypes are not resolved by classic quelling serotyping (Park et al., 2007a). Classically serotyped 6A pneumococci (CS6As) are associated with nasopharyngeal (NP) carriage and IPD in all ages (du Plessis et al., 2008; Granat et al., 2007; Reis et al., 2008).

The currently available 7-valent pneumococcal conjugate vaccine (PCV7) has been highly effective against the 7 serotypes that were predominant in children before its implementation in the United States (CDC, 2008; WHO, 2007). PCV7 contains serotype 6B and cross-protects against 6A; however, recent surveillance data indicates that PCV7 is ineffective against serotype 6C (Carvalho et al., 2009; Park et al., 2008).

We show here the prevalence of serotype 6C within a well-defined collection of CS6As collected in Brazil from meningitis cases and NP carriage.

Antimicrobial susceptibility testing used broth microdilution. MICs of 10 key antibiotics were determined using year 2007 Clinical and Laboratory Standards Institute guidelines (CLSI, 2007). Intermediate penicillin resistance (MICs of 0.12–1.0 μg/mL) or full penicillin resistance (MICs ≥2.0 μg/mL) was considered penicillin nonsusceptible.

CS6As were subtyped using a triplex polymerase chain reaction (PCR) reaction that detects *cps*A (conserved capsular biosynthetic locus; 160 bp), serogroup 6 (250 bp), and the 6C-specific gene *wciN*$_{6C}$ (727 bp) (Carvalho et al., 2009). DNA extraction and PCR were performed as described (http://www.cdc.gov/ncidod/biotech/strep/pcr.

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Table 1
Characteristics of meningitis cases caused by *S. pneumoniae* serotypes 6A and 6C in Salvador, Brazil, 1996 to 2007

Characteristic	No. of isolates (%)		*P*
	6A (*n* = 31)	6C (*n* = 16)[a]	
Age			
<5 years	15/31 (48.4)	4/15 (26.7)	
≥5 years	16/31 (51.6)	11/15 (73.3)	
Male gender	17/31 (54.8)	11/15 (73.3)	
Case fatality rate (no. of deaths/no. of cases)	8/29[b] (27.6)	0/15 (0.0)	0.02
Penicillin nonsusceptible	3/31 (9.7)	1/16 (6.3)	
Sxt nonsusceptible	17/31 (54.8)	12/16 (75.0)	

[a] For 1 patient, the epidemiologic and clinical data were not available (i.e., age, sex, and outcome).
[b] Two patients were transferred to another hospital.

htm). Pulsed-field gel electrophoresis (PFGE) of chromosomal *Sma*I (Sigma, St. Louis, MO) digests was performed as described (McEllistrem et al., 2000; Tenover et al., 1995). Multilocus sequence typing was performed (Enright and Spratt, 1998) on 8 isolates representing the 2 PFGE clusters and 3 PFGE outliers.

CS6As consisting of 47 (6.6%) cerebrospinal fluid (CSF) isolates from a collection of 709 pneumococci identified in metropolitan Salvador, Brazil, during 12 years (1996–2007) of bacterial meningitis surveillance and 9 NP isolates (Reis et al., 2008) were tested by triplex PCR to resolve serotypes 6C and serotype 6A. Of 56 CS6As tested, 16/47 (34%) from meningitis patients were PCR positive for $wciN_{6C}$, indicating that 4.4% (31/709) and 2.3% (16/709) of meningitis cases were caused by serotypes 6A and 6C, respectively. In addition, 6A and 6C represented 6.4% and 3.2% (3/95) of carriage isolates, respectively. Low prevalences of serotype 6C disease and colonization have also been observed in South Africa (du Plessis et al., 2008) and Portugal (Nunes et al., 2009).

There were no significant differences between serotypes 6A and 6C with respect to ages and genders of meningitis patients. Serotype 6C meningitis cases were associated with a significantly lower case fatality rate than serotype 6A cases (0% [0 of 15 cases] versus 28% [8 of 29 cases], respectively, $P = 0.02$) (Table 1). These findings are in contrast to observations in South Africa (du Plessis et al., 2008) where significantly different case fatality rates between serotypes 6A and 6C were not observed.

Table 2 lists the antibiotic susceptibility of all serotype 6C isolates identified. Among meningitis case CS6As, 71% (22 of 31) of 6A and 81.3% (13 of 16) of 6C were nonsusceptible to at least one antibiotic. Similar proportions of serotype 6A and 6C meningitis isolates were nonsusceptible to penicillin or trimethoprim/sulfamethoxazole (Sxt). Among 6C isolates, 6.3% (1 of 16) and 75% (12 of 16) were nonsusceptible to penicillin and Sxt, respectively, whereas 10% of 6A isolates were penicillin nonsusceptible and 55% were Sxt nonsusceptible. The high frequency of Sxt resistance among meningitis CS6As reflects widespread use of this drug in Brazil (Reis et al., 2008). Multidrug resistance was found among 19.4% (6 of 31) and 18.8% (3 of 16) of serotype 6A and 6C meningitis isolates, respectively. Of CS6As obtained during a NP carriage study of a healthy population in Salvador (Reis et al., 2008), 2/3 (67%) 6C isolates and 1/6 6A (16.7%) isolates were multidrug resistant.

PFGE of serotype 6C chromosomal digests revealed 2 clusters and 6 outlier patterns (Fig. 1). Cluster I (black stars) represented 8 meningitis isolates, whereas cluster II (white stars) represented 2 meningitis isolates and 3 NP carriage isolates. PFGE cluster I isolates were associated with 2 newly identified sequence types (STs), ST3929 and ST3930, which differed by only 2 loci. ST3930 differed by only 1 or 2 loci from 5 CS6As (NP and lower respiratory tract isolates) recovered in Finland, the United States, and Poland (http://www.mlst.net). PFGE cluster II isolates were associated with ST812, which is a single locus variant (SLV) of ST753 from a CS6A meningitis isolate recovered in Brazil and a double locus variant of ST2789 from a type 6C carriage isolate

Table 2
Antimicrobial susceptibilities of *S. pneumoniae* serotype 6C isolates in metropolitan Salvador, Brazil, from 1996 to 2007[a]

Antimicrobial agents	MIC (μg/mL)[b]			No. (%) of isolates[c]		
	Range	MIC_{50}	MIC_{90}	S	I	R
Cefotaxime	0.016–64	0.016	0.031	19 (100)	-	-
Chloramphenicol	0.016–64	2.0	4.0	17 (89.5)	NA	2 (10.5)
Clindamycin	0.016–64	0.031	0.062	19 (100)	-	-
Erythromycin	0.016–64	0.062	0.25	18 (94.7)	-	1 (5.3)
Ofloxacin	0.016–64	1.0	2.0	19 (100)	-	-
Penicillin	0.016–64	0.031	0.062	18 (94.7)	1 (5.3)	-
Rifampicin	0.016–64	0.031	0.062	19 (100)	-	-
Tetracycline	0.016–64	0.5	8.0	15 (78.9)	-	4 (21.1)
Sxt	0.0625/1.1875–32/608	1.0	2.0	6 (31.6)	11 (57.9)	2 (10.5)
Vancomycin	0.016–64	0.5	0.5	19 (100)	NA	-

S = susceptible; I = intermediate; R = resistant; NA = not applicable; - = no isolates were identified.
[a] A total of 19 isolates were tested (16 isolates from meningitis patients and 3 isolates from NP carriages).
[b] MICs were determined by the broth microdilution method (CLSI, 2007). MIC_{50} and MIC_{90} concentrations at which the growth of 50% and 90%, respectively, of the isolates is inhibited.
[c] The breakpoints used to define susceptibility categories were those recommended by the Clinical Laboratory Standards Institute (CLSI, 2007).

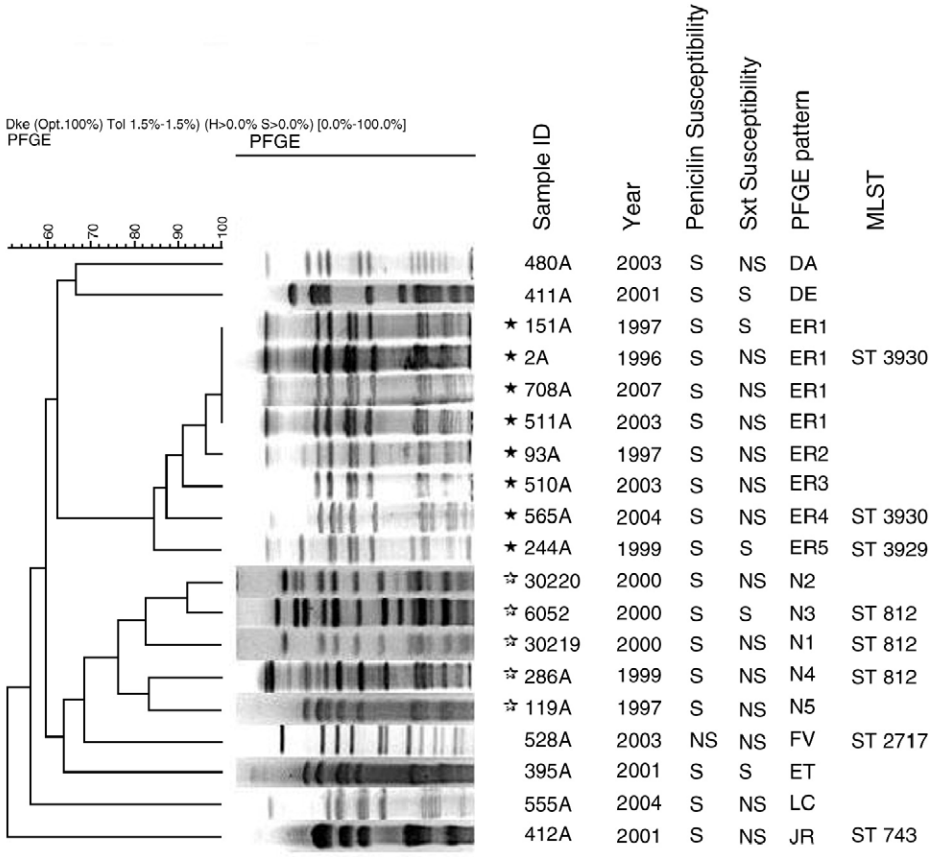

Fig. 1. PFGE analysis showing serotype 6C isolates recovered from meningitis cases and carriage. Cluster I (*black stars*) comprised 8 meningitis isolates (nos. 151A, 2A, 708A, 511A, 93A, 510A, 565A, and 244A) belonging to ST3930; cluster II (*white stars*), comprised 2 meningitis isolates (nos. 286A and 199A) and 3 6C NP carriage isolates (nos. 30220, 6052, and 30219) belonging to ST812. The other 6 isolates are genetic outliers as judged by PFGE relationships. S = susceptible; NS = nonsusceptible.

recovered in Portugal (Nunes et al., 2009). The PFGE outlier correlated with ST2777, which was also from a type 6C CSF isolate recovered in Brazil (http://www.mlst.net). It is interesting that ST2777 is an SLV of ST338 from the clone Colombia^{23F}-26 that is associated with antibiotic-nonsusceptible 23F and 23A isolates (Pai et al., 2005). The final PFGE outlier (ST743) was previously associated with serotype 34 meningitis and NP isolates (http://www.mlst.net). Overall, these data are consistent with other studies indicating both a high degree of genetic diversity within serotype 6C and its long-term existence within the species (Jacobs et al., 2009; Nunes et al., 2009; Park et al., 2007a). In our study, the first 6C isolate identified was isolated in March 1996 (strain no. 2A). Vaccine pressure could potentially select for the emergence of preexisting 6C clones and 6C variants that arise through serotype switching.

In Brazil, PCV7 will probably be implemented within the next several years in young children (Brasil, 2008). Although PCV7 does not protect against 6C disease (Carvalho et al., 2009; Park et al., 2008), we found that the prevalence of serotype 6C among meningitis isolates is low (2.3%). Nonetheless, serotype replacement in disease incidence is a concern, where non-PCV7 serotypes such as type 6C could possibly emerge as important pathogens due to removal of vaccine serotype strain competitors from the NP reservoir (Hicks et al., 2007; Moore et al., 2008). A slight increase of 6C IPD has been documented in the post-PCV7 era among adults in the United States, where 6C has become the prevalent serogroup 6 serotype (Carvalho et al., 2009; Moore et al., 2008; Park et al., 2008). We emphasize that these data showing a predominance of 6C from the United States primarily indicate high efficacy of PCV7 against serotypes 6A and 6B rather than 6C emergence. Continuous pneumococcal serotype surveillance is necessary to evaluate the impact and suitability of current conjugate vaccines in developing countries such as Brazil.

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