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

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Received 20 April 2009; accepted 19 June 2009

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.

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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 

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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 \textit{wcIN} genes that encode distinct glycosyl transferases (Park et al., 2007b). 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 serotypes 6C and 6A biosynthetic loci are identical except for the presence of different \textit{wcIN} 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 \( \mu \)g/mL) or full penicillin resistance (MICs \( \geq 2.0 \mu \)g/mL) was considered penicillin nonsusceptible.

CS6As were subtyped using a triplex polymerase chain reaction (PCR) reaction that detects \textit{cpsA} (conserved capsular biosynthetic locus; 160 bp), serogroup 6 (250 bp), and the 6C-specific gene \textit{wcIN}_{6C} (727 bp) (Carvalho et al., 2009). DNA extraction and PCR were performed as described (http://www.cdc.gov/ncidod/biotech/strep/pcr).
respectively, of the isolates is inhibited.

\[ S = \text{susceptible}; I = \text{intermediate}; R = \text{resistant}; NA = \text{not applicable}; - = \text{no isolates were identified.} \]

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>MIC (µg/mL)b</th>
<th>No. (%) of isolatesc</th>
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<tr>
<td></td>
<td>S</td>
<td>I</td>
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<tr>
<td>Cefotaxime</td>
<td>0.016–64</td>
<td>0.016</td>
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<tr>
<td>Chloramphenicol</td>
<td>0.016–64</td>
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<tr>
<td>Clindamycin</td>
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<tr>
<td>Erythromycin</td>
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<td>Ofloxacin</td>
<td>0.016–64</td>
<td>1.0</td>
</tr>
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<td>0.016–64</td>
<td>0.031</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.016–64</td>
<td>0.031</td>
</tr>
<tr>
<td>Tetracycline</td>
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<tr>
<td>Sxt</td>
<td>0.0625/1.1875–32/608</td>
<td>1.0</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.016–64</td>
<td>0.5</td>
</tr>
</tbody>
</table>

S = susceptible; I = intermediate; R = resistant; NA = not applicable; - = no isolates were identified.

\[ a \text{ A total of 19 isolates were tested (16 isolates from meningitis patients and 3 isolates from NP carriages).} \]

\[ b \text{ MICs were determined by the broth microdilution method (CLSI, 2007). MIC}_{50} \text{ and MIC}_{90} \text{ concentrations at which the growth of 50% and 90%, respectively, of the isolates is inhibited.} \]

\[ c \text{ The breakpoints used to define susceptibility categories were those recommended by the Clinical Laboratory Standards Institute (CLSI, 2007).} \]
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., 2007), 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., 2007). 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.

Acknowledgments

The authors thank Dr Marise D. Asensi (Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil) for her help in the PFGE performance. They are grateful for the global pneumococcal multilocus sequence typing database (Imperial College London, funded by the Wellcome Trust). This study was supported by grants from the Brazilian National Research Council (CNPq—491345/2005-4 and...
References


