Serotype prevalence and antibiotic resistance in *Streptococcus pneumoniae* clinical isolates among global populations

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ABSTRACT

Streptococcus pneumoniae remains a leading cause of disease in children and adults. Serotypes differ in invasiveness, virulence, and antibiotic resistance; therefore, serotype surveillance is necessary to monitor the burden of pneumococcal disease, especially in the setting of pneumococcal vaccination programs. The Tigecycline Evaluation Surveillance Trial, (TEST), is an on-going global antibiotic susceptibility surveillance program. Serotypes and antibiotic susceptibilities of 2173 invasive *S. pneumoniae* in this existing database during 2004–2008 were evaluated. Worldwide, serotypes 19A (28%), 19F (10%) and 14 (9%) were the most common in children under 5 years. In adults over 16 years, 19A (13%), 3, 6A and 7F (all 7%) were most common. Serotypes 19A, 6A, 19F, 6B, 15A, 9V, and 14 exhibited significantly higher levels of erythromycin resistance ($P < 0.05$), while 19A, 19F, 35B, 6A, 6B, 23A, 9V, 15A, and 14 demonstrated higher rates of penicillin resistance ($P < 0.05$). This analysis of an existing pathogen database provides a snapshot of global serotype data and describes the consequential issue of antibiotic resistance in specific serotypes, many of which are increasingly common causes of invasive pneumococcal disease.

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Streptococcus pneumoniae represents a leading cause of morbidity and mortality worldwide in both children and adults, causing pneumonia, bacteremia, sepsis syndromes, otitis media (primarily in children) and meningitis [1–11]. The burden of disease is born disproportionately by the young and the old. The risk of pneumococcal disease increases as individuals age but the risk increases most rapidly after age 50 [12–14]. In addition, *S. pneumoniae* has at least 92 known serotypes, with varying geographic and age group distributions among populations [8,15–17]. Individual serotypes vary in their ability to cause invasive disease and their association with antibiotic resistance [14,18–24]. A number of multivalent vaccines have been developed to target the burden of disease caused by specific pneumococcal serotypes. The pneumococcal conjugate vaccines (PCV7, PCV10, PCV13) elicit a T cell-dependent immune response; the plain polysaccharide vaccine (PPSV23) elicits an immune response via a T cell-independent mechanism. Use of these different vaccines has resulted in variable vaccine-type, serotype-specific reductions in invasive pneumococcal disease (IPD) [19,25,26]. Some studies have reported changes in the prevalence of non-vaccine-related serotypes felt likely due to antibiotic and vaccine selection combined with natural serotype variation [27–29]. Up-to-date data on serotype distribution of IPD is necessary for recommending bodies, physicians, and patients who must weigh the potential advantages of a conjugate mechanism of action against the larger valency of the polysaccharide vaccine.

The intent of this study was to provide seroepidemiology and antibiotic resistance data for *S. pneumoniae* clinical isolates obtained from patients with invasive infections worldwide including regions where a paucity of information is available i.e. Africa, Asia/Pacific and Middle Eastern regions. The Tigecycline Evaluation Surveillance Trial (TEST) is an on-going global antimicrobial surveillance program begun in 2004 that monitors antibiotic susceptibility patterns of Gram negative and Gram positive bacteria [30]. In this analysis, we report on the serotypes of *S. pneumoniae* isolates from IPD collected in 47 countries throughout the world through the TEST program from 2004 to 2009.

1. Materials and methods

1.1. Strains

A total of 2173 isolates of *S. pneumoniae* collected from 2004–2009 were used in this study. Global investigators enrolled in the TEST program collected clinically relevant causative agents from a variety of sources. Only one isolate per patient was accepted. Individual sites were requested to collect and ship a total of 15 *S.*

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pneumoniae per year. The majority of isolates included in this analysis were collected from sterile body sites including blood ($n = 1673$, 76.9%), body fluids ($n = 241$, 11.1%), sterile respiratory sites, including lungs, bronchia, and sinuses ($n = 219$, 10.1%), and other sterile body sites including central nervous system, lymph nodes, and ovary ($n = 39$, 1.8%). One isolate, originally misclassified, was from a trans-tracheal aspirate. Of the 2173 isolates, 58.9% ($n = 1279$) were isolated in North America (NA), 21.5% ($n = 468$) in Europe, 6.6% ($n = 144$) in Latin America (LA), 3.9% ($n = 84$) in Middle East, 3.8% ($n = 83$) in South Pacific, 3.4% ($n = 73$) in Asia, and 1.9% ($n = 42$) in Africa. The identification of each isolate was confirmed following standard procedures [31].

1.2. Antimicrobial susceptibility

Minimum inhibitory concentrations (MICs) were determined by the Clinical and Laboratory Standards Institute (CLSI) recommended broth microdilution testing method [32] using MicroScan (Siemens Medical Solutions Diagnostics, West Sacramento, CA) or TREK (ThermoFisher Scientific, Pittsburgh, PA, USA) panels for penicillin and levofloxacin. Erythromycin MICs were determined on broth microdilution panels produced by Laboratories International for Antibiotic Studies (International Health Management Associates, Schaumburg, IL). MIC interpretive criteria followed published CLSI guidelines [33], using parenteral meningitis breakpoints for penicillin (resistant >0.06 μg/mL).

1.3. Serotyping

Based on the seven- reaction sequential multiplex PCR described previously [34], a PCR amplification was developed using five reactions to identify the pneumococcal serotype, followed by confirmation via the conventional Quellung (capsular swelling) reaction using commercial type-specific antisera (Statens Serum Institute (Copenhagen, Denmark) [35]. For DNA extraction, pneumococcal isolates were subcultured on blood agar plates, and incubated overnight at 37 °C with 5% CO_2. A 10 μL loop was used to transfer bacterial cells to 50 μL of nuclease-free water. The suspensions were heated at 95 °C for 10 min, and then stored at −20 °C. 92 isolates were extracted in a single run using 96-well plates. Multiplex-PCR was done in a 20-μL volume that contained 2X QIAGEN Multiplex PCR Master Mix (Qiagen, Valencia, CA), 0.2 μM of each primer and 2 μL crude DNA extract. Thermocycling was done in a Bio-Rad iCycler apparatus (Bio-Rad, Hercules, CA) with the following conditions: 95 °C for 15 min, 30 amplification cycles of 94 °C for 30 s, 55 °C for 90 s, and 72 °C for 90 s; and a final extension step at 72 °C for 10 min. The amplified products were analyzed on 2% Tris-Acetate-EDTA gels at 150 V for 1 h. A 100 bp ladder (Invitrogen, Carlsbad, CA) was run alongside unknown samples in each gel, as well as an internal *cps* control using primers targeted at loci within the *cps* gene, and a positive control mixture per reaction which also served as a second molecular weight marker to better identify serotypes in the unknown samples. All PCR serotype results were confirmed by Quellung reaction. Isolates that were negative by PCR were serotyped by Quellung reactions alone.

1.4. Statistical analysis

Contingency tables were constructed to look at the relationship between serotype and age group, or between resistance and serotype. Overall tests of significance were conducted using Fisher's exact tests, but significance was determined based on a Monte Carlo simulation of the null distribution, to avoid an application of the asymptotic distribution under questionable circumstances. If these overall relationships were significant, specific serotypes were compared to all other serotypes combined using Fisher's exact

test. Statistical analyses were performed with SAS v. 9.3. *P* values of <0.05 were regarded as significant for comparisons. A preliminary analysis of the data was presented at the 5th Vaccines and ISV Annual Global Congress, October 2011, in Seattle, WA, USA using a different method of data analysis.

2. Results

Among the 2173 worldwide isolates evaluated in this study, serotype 19A was the most common in all age groups (15.6%), ranging from 25.9% in children <2y to 12.9% in adults >65y (Table 1). Serotypes 19F (9.4%, 9.6%) and 14 (9.4%, 7.5%) were the next most common in children <2y and 2–4y, respectively. In children 5–17y, serotype 1 was significantly more common than in other age groups ($P < 0.05$). In adults 18–65y and >65y, serotypes 6A (6.3%, 7.7%), 7F (7.1%, 6.4%) and 3 (6.2%, 8.5%), respectively, were the most common after 19A.

Worldwide, 724 of 2171 isolates tested (33.3%) were resistant to penicillin (MIC > 0.06 μg/mL), 468 of 2048 isolates tested (22.9%) were resistant to erythromycin (MIC ≥ 1 μg/mL), and 331 of 2046 isolates tested against both (16.2%) were resistant to both erythromycin and penicillin, with serotypes 19A, 6A, 19F, 14, 6B, 9V, and 15A more likely to be resistant to both these drugs ($P < 0.05$). Serotypes 19A, 6A, 15A, 19F, 9V, 6B and 14 exhibited significantly higher levels of erythromycin resistance ($P < 0.05$), while 19A, 6A, 19F, 14, 6B, 9V, 35B, 23A, and 15A demonstrated higher rates of penicillin resistance ($P < 0.05$). Ten isolates (0.5%) of varying serotypes were levofloxacin resistant (data not shown).

The most common serotypes found in Latin America ($n = 144$) were 14 (13.2%), 1 (10.4%) and 6A (9.0%) (Table 2). Out of 12 isolates from children <2, two each were serotypes 6B, 19A and 19F (all 16.7%). In the 2–4y and 5–17y age groups, serotype 14 (28.6% and 20.0%, respectively) was most common. In adults 18–65y, serotypes 1 (15.3%) and 14 (12.5%) were more frequent, while 6A (12.9%) was most common in adults >65y. 15.3% of all isolates were erythromycin resistant and 34.7% were resistant to penicillin.

19F and 6B were the most common serotypes in 42 isolates from S. Africa/Mauritius (both 14.3%), followed by 4 and 23F (both 9.5%) (Table 3). 23.8% of the isolates from this region were resistant to erythromycin, while 64.3% were resistant to penicillin.

In the Asia/Pacific region ($n = 156$), serotypes 3 and 19A (10.8% and 10.3%) were the most common overall, followed by 14 (8.3%) and 19F (7.7%) (Table 4). In children <2y, 18.2% of isolates were serotype 14. In adults 18–65y, serotypes 3, 14, and 22F were most common (9.0% each), with 19A (15.4%) more common in adults >65y. 28.8% of all isolates were erythromycin resistant, while 27.6% were penicillin resistant. For all age groups, serotype 14 exhibited significantly higher levels of erythromycin resistance ($P < 0.05$).

Of 84 isolates from the Middle East, 14.3% were 19A and 10.7% were serotype 1 (Table 5). Serotype 14 exhibited significantly higher levels of erythromycin resistance ($P < 0.05$), while serotypes 19A, 19F, and 6B were more likely to be resistant to penicillin ($P < 0.05$).

In North America ($n = 1279$), serotype 19A (21%) was the most common and was significantly more likely to be found in pediatric isolates (age groups <2, 2–4, and 5–17) than adults 18–65 or >65 ($P < 0.05$) (Table 6). Serotypes 19A, 6A (7.3%), 19F (3.8%), 15A (2.7%), 9V (1.8%) and 14 (1.5%) exhibited significantly higher levels of erythromycin resistance ($P < 0.05$), while 19A, 6A, 23A (4.2%), 15A, 6B (2.0%), 19F, 35B (3.0%), 9V (1.8%) and 14 (1.5%) demonstrated higher rates of penicillin resistance ($P < 0.05$). Overall in NA, 23.5% of isolates were erythromycin resistant and 38.5% were penicillin resistant. Seven isolates (0.5%) of varying serotypes were levofloxacin resistant (data not shown).