Serotype distribution and antimicrobial resistance patterns of Streptococcus pneumoniae isolated in Tunisia

Streptococcus pneumoniae is the leading cause of bacterial pneumonia, acute otitis media and sinusitis and the most frequent aetiologic agent of bacterial meningitis and bacteremia. It is a major cause of morbidity and mortality among people all over the world (Maraki et al., 2001). Virulence of S. pneumoniae is mainly associated with the presence of capsular polysaccharides, which usually exhibit differences in size, composition, antiphagocytic properties and serotype-specific immunogenicity. There are more than 90 pneumococcal serotypes, but less than a dozen are responsible for most of the infections (Ochoa et al., 2005).

Although penicillin has long been the mainstay of treatment of pneumococcal infections, the prevalence of penicillin resistance has been increasing worldwide. Penicillin resistance is usually associated with resistance to other antibiotics and poses a well-documented risk of therapeutic failure (Ochoa et al., 2005).

The current study was conducted to determine the serotype distribution and antimicrobial susceptibility patterns of S. pneumoniae isolated from patients in Tunisia, in order to plan future treatment and preventive strategies.

Two hundred clinical isolates of S. pneumoniae collected from three university hospitals between 2000 and 2008 were analysed. Consecutive isolates (one per patient) were collected: 125 were invasive clinical isolates [pleural fluid (n=77), cerebrospinal fluid (n=29) and blood (n=19)] and 75 were non-invasive isolates [ear (n=34), sputum (n=20), nose (n=11) and eye (n=10)]. Identification of S. pneumoniae species was based on conventional methods. Antimicrobial susceptibility testing was done by the disc diffusion method according to the Comité de l’Antibiogramme de la Société Française de Microbiologie (CA-SFM). MICs of penicillin, amoxicillin and cefotaxime were assessed by Etest according to the manufacturer’s instructions (AB Biodisk) (CA-SFM). S. pneumoniae ATCC 49619 was used as a control strain. A multiplex PCR scheme was conducted to identify the most predominant serotypes (1, 3, 4, 6A/B, 7F, 7C, 8, 9A/V, 10A, 11A, 12F, 14, 15A, 15B/C, 16F, 17F, 18, 19A, 19F, 20, 22F, 23F, 31, 33, 34, 35B, 35F and 38) (Pai et al., 2006). The $\chi^2$ and Fisher’s exact tests were used for analysing the quantitative variables. All data were analysed with Epi Info software (version 6.0). A P-value of <0.05 was considered statistically significant.

In the present study, as previously reported (Mahjoubi-Rhimi et al., 2003) a high rate of resistance to penicillin and other β-lactams was found. Among the 200 isolates, 48.5% were susceptible to penicillin (PSP) (MIC range 0.008–0.094 μg ml$^{-1}$), 37% were intermediate (PIP) (MIC range 0.125–1 μg ml$^{-1}$) and 14.5% had high-level resistance (PRP) (MIC range 2–32 μg ml$^{-1}$). PIP and PRP strains were more frequent resistant to other antibiotics, with 17.2% resistant to chloramphenicol, 41.4% to tetracyclines, 55.2% to trimethoprim–sulfamethoxazole and 75.8% to erythromycin, versus 14.4%, 6.2%, 4.2% and 47.4%, respectively, in PSP isolates. Reports from other countries showed that PRP or PSP isolates also carry genetic determinants encoding resistance to multiple antibiotics (Nowosiad & Giedrys-Kalema, 2008). In agreement with recent reviews, amino glycosides, pristinamycin, rifampicin and vancomycin had excellent in vitro activity against all our strains (Maraki et al., 2001; Smaoui et al., 2009).

These drugs may be important alternatives for use in the treatment of infections caused by multidrug-resistant S. pneumoniae (Maraki et al., 2001). The most prevalent serotypes were in decreasing order 19F (17%), 19A (11%), 14 (11%), 23F (9.5%), 6B (9%) and 6A (5.5%). These serotypes accounted for 63% of all isolates. Our results were similar to those recorded in several studies performed in different countries, Germany (Reinert et al., 2001), Egypt (Wasfy et al., 2005) and Brazil (Franco et al., 2010), but different to those reported in Spain (Fenoll et al., 2009) and France (Doit et al., 2002). The prevalent coverage of the 7-valent and 23-valent polysaccharide pneumococcal vaccines were 54.5% and 73.5%, respectively.

As reported in an international study (Alance et al., 2007), serotype 6B was significantly associated with invasive disease ($P=0.01$) (Table 1). Only one case, reported in Germany, associated serotype 6B with non-invasive infections (Reinert et al., 2001).

In our study, a strong correlation between serotypes and antimicrobial resistance patterns was observed. The six most common serotypes, except serotype 6A, were associated with high rates of resistance to penicillin (ranging from 44.4% in serotype 6B to 73.5% in serotype 19F), whereas the less commonly isolated serotypes (35F, 4, 1, 34, 10A, 15A, 35B, 8, 11A, 16F, 31, 33F, 20, 17F and 3F) were PSP (Table 1). Similar results were also reported in Taiwan (Lauderdale et al., 2006) and China (Liu et al., 2008). All strains with serotypes 24F and 9A were PIP or PRP. More than 50% of serotypes 19F and 14 were non-susceptible to amoxicillin and 59.6% of cefotaxime-resistant strains belonged to serotypes 19F, 19A, 14, 23F and 6B (Table 1). The six predominant serotypes/groups exhibited high rates of resistance to erythromycin and clindamycin (59–77.2%), tetracycline (38.8–70.5%) and trimethoprim–sulfamethoxazole (40.9–68.1%). Serotypes 19F (the most frequent), 19A and 23F were significantly more likely to be multidrug-resistant compared with all other serotypes combined ($P=0.0002$, $P=0.02$ and $P=0.05$, respectively) (Table 1).
The present study documents the increasing problem of antimicrobial resistance in S. pneumoniae and points out the need for continuous surveillance for changes in the susceptibilities to antimicrobial agents that are used to manage pneumococcal infections. We also found that most serotypes of the strains recovered from Tunisian patients are included in the 23-valent pneumococcal vaccine, indicating the potential usefulness of this vaccine in the Tunisian population.

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**Table 1. Distribution of serotypes and antimicrobial resistance of S. pneumoniae isolated in Tunisia**

Values represent number of isolates.

<table>
<thead>
<tr>
<th>Serotype/serogroup</th>
<th>Non-invasive</th>
<th>Invasive</th>
<th>PIP</th>
<th>PRP</th>
<th>CNS*</th>
<th>Multidrug-resistant (%)†</th>
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</thead>
<tbody>
<tr>
<td>19F (n=34)</td>
<td>15</td>
<td>19</td>
<td>25</td>
<td>3</td>
<td>9</td>
<td>25 (73.5)</td>
</tr>
<tr>
<td>19A (n=22)</td>
<td>11</td>
<td>11</td>
<td>9</td>
<td>5</td>
<td>7</td>
<td>15 (68.1)</td>
</tr>
<tr>
<td>14 (n=22)</td>
<td>13</td>
<td>9</td>
<td>8</td>
<td>7</td>
<td>10</td>
<td>14 (63.6)</td>
</tr>
<tr>
<td>23F (n=19)</td>
<td>4</td>
<td>15</td>
<td>10</td>
<td>2</td>
<td>5</td>
<td>13 (68.4)</td>
</tr>
<tr>
<td>6B (n=18)</td>
<td>2</td>
<td>16</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>8 (44.4)</td>
</tr>
<tr>
<td>6A (n=11)</td>
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<td>8</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1 (9)</td>
</tr>
<tr>
<td>9V (n=9)</td>
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<td>8</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>4 (44.4)</td>
</tr>
<tr>
<td>35F (n=8)</td>
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<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24F (n=8)</td>
<td>2</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>4 (50)</td>
</tr>
<tr>
<td>4 (n=7)</td>
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</tr>
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<td>6</td>
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</tr>
<tr>
<td>18C (n=7)</td>
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<td>5</td>
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<td>0</td>
<td>4 (57.1)</td>
</tr>
<tr>
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<td>2</td>
<td>1</td>
<td>2 (50)</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>1 (33.3)</td>
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<td>35B (n=3)</td>
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</tr>
<tr>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
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<td>0</td>
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</tr>
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<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>3F (n=1)</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>23B (n=1)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total (n=200)</td>
<td>75</td>
<td>125</td>
<td>74</td>
<td>29</td>
<td>39</td>
<td>90 (45)</td>
</tr>
</tbody>
</table>

*CNS, Cefotaxime non-susceptible.
†Multidrug resistance was defined as resistance to penicillin and to two or more classes of antimicrobial agents.

The present study documents the increasing problem of antimicrobial resistance in S. pneumoniae and points out the need for continuous surveillance for changes in the susceptibilities to antimicrobial agents that are used to manage pneumococcal infections. We also found that most serotypes of the strains recovered from Tunisian patients are included in the 23-valent pneumococcal vaccine, indicating the potential usefulness of this vaccine in the Tunisian population.

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