
Dixième surveillance de la résistance aux antibiotiques dans des souches non invasives de Streptococcus pneumoniae collectionnées en Belgique pendant l’hiver 2007 à 2008

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ARTICLE INFO

Article history:
Received 26 June 2009
Accepted 13 July 2009
Available online 4 November 2009

Keywords:
S. pneumoniae
Resistance
Survey

ABSTRACT

Objectives. – The aim of the study was to evaluate the antibiotic resistance in noninvasive clinical isolates of Streptococcus pneumoniae collected in Belgium during winter 2008–2007.

Method. – Four hundred and forty eight unduplicated isolates collected by 15 laboratories were tested by microdilution following CLSI.

Results. – Insusceptibility rates (I + R) were as follows: penicillin G (PEN) 11.6% (4.0% R), ampicillin 11.4% (4.0% R), amoxicillin + /–clavulanic acid 0, cefaclor 10.3% (9.6% R), cefuroxime 9.2% (8.7% R), cefuroxime-axetil 8.7% (7.8% R), cefotaxime, ceftazidime and cefepime 2.0% (0% R), imipenem 2.5% (0% R), ciprofloxacin and ofloxacin 5.1% (0.4% R), levofloxacin 0.7% (0.4% R), moxifloxacin 0.4% (0.2% R), erythromycin (ERY) 29.7% (29.2% R), azithromycin 29.7% (29.2% R), telithromycin 0%, clindamycin 26.3% (25.4% R) and tetracycline (TET) 21.9% (16.5% R). From 2001 to 2008, a significant decrease in penicillin-insusceptibility (21.0% to 11.6%), penicillin-resistance (9.7% to 4.0%) and ciprofloxacin-insusceptibility (11.2% to 5.1%) was found. Cross-resistance between penicillin and other beta-lactams in penicillin-insusceptible isolates was incomplete: all these isolates remained fully susceptible to amoxicillin. Erythromycin-insusceptibility was significantly higher in children than in adults (43.9% vs 27.4%), while penicillin-insusceptibility significantly higher in Brussels than in the Flanders (22.9% vs 8.1%). The commonest resistance phenotype was ERY-TET (12.7%) followed by ERY (7.4%) and PEN-ERY-TET (5.8%). Capsular types 19 (25%), 14 (19.3%), 23 (15.4%) and 15 (13.5%) were the most important in penicillin-insusceptible.

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1. Introduction

*Streptococcus pneumoniae* causes a wide variety of infections both in the community and in hospitalized patients. It is not only the causative agent for upper respiratory tract infections but also for a number of important invasive infections such as septicaemia, pneumonia and meningitis. *S. pneumoniae* remains a major pathogen with a high degree of morbidity and a considerable rate of mortality [1–4]. The appearance of resistant strains, in which both the de novo acquisition of new genetic material and the clonal spread of resistant isolates are implied, can be an incriminating factor in the treatment and outcome of pneumococcal disease. In Belgium, the first two penicillin-resistant isolates were reported by Vanhoof et al. [5] in 1980. Since the beginning of the 1990s, we have been reporting a slow but steadily increase in penicillin non-susceptibility in clinical isolates with a peak in 2001 [6]. An important decrease in resistance was noted in the following years [7]. In this article, we present data collected during the 10th collaborative surveillance study conducted during the winter of 2007–2008.

2. Material and methods

2.1. Isolates

A total of 448 consecutive, non-duplicated noninvasive respiratory clinical isolates of *S. pneumoniae*, collected during winter 2007–2008 in 15 clinical laboratories throughout Belgium, were included in this study. Isolates were kept at −70 °C in Brain Heart Infusion Broth (Difco) containing 10% (v/v) glycerol until susceptibility testing at the Pasteur Institute in Brussels. All isolates underwent a slide agglutination (Slide pneumo Kit™, BioMérieux), an Optochin test (Opto-F, bioMérieux) and a LytA PCR before MIC testing.

2.2. Antibiotics

The following antibiotics were tested in the study and were provided as laboratory preparations with known potency: clavulanic acid, cefazidime (GlaxoSmitKline), cefepime (Bristol Myers Squibb), cefotaxime, levofloxacin, ofloxacin and telithromycin (Aventis Pharma), imipenem (Merck Sharp & Dohme), ciprofloxacin and moxifloxacin (Bayer), azithromycin (Pfizer). Amoxicillin, ampicillin, cefaclor, cefuroxime, clindamycin, erythromycin, penicillin G and tetracycline were obtained from a commercial source (Sigma). Amoxicillin/clavulanic acid was tested in a 2:1 ratio. All antibiotics were tested for 16 serial twofold dilutions (0.001–32 μg/mL).

2.3. Susceptibility testing

The minimal inhibitory concentration (MIC) was determined by broth microdilution as recommended by the CLSI [8]. *S. pneumoniae* ATCC 49619, *S. pneumoniae* TPN881 (internal mefA positive control isolate with penicillin MIC of 0.008–0.03 μg/mL and erythromycin MIC of 4–16 μg/mL) and *Staphylococcus aureus* NCTC 11561 (ß-lactamase positive to validate the clavulanic acid component of amoxicillin/clavulanic acid) were included as quality control organisms in each series. Interpretation of the results was based on breakpoints provided by the CLSI [9]. Levels of susceptibility to ampicillin were determined by penicillin. For ciprofloxacin we used breakpoints of one dilution lower than those of levofloxacin as is generally the case for other types of microorganisms.

2.4. Capsular typing

All penicillin non-susceptible isolates were typed by the National Reference Centre by using the Quellung reaction with sera from the Staten Seruminstitute (Copenhagen).

2.5. Statistical analysis

The Chi² test, with or without Yates’ correction, for two independent samples was used for the statistical evaluation of the results. The level of significance was set at 0.05.

3. Results

In total 448 documented isolates of *S. pneumoniae* were included in the study for further analysis. Seventy-eight isolates (17.4%) of the isolates were from children (age ≤ 15 years) with 66/78 or 84.6% from children under five years of age, while 370
(82.6%) were from adults with 238/370 or 64.3% from adults with age greater or equal to 60 years. The mean age of the study population was 53.4 years. Isolates from sputum represented 79.9% (358/448) of the specimens, 15.4% (69/448) were from nasal swab, 2.5% (11/448) from throat, 1.8% (8/448) from sinus and 0.4% (2/448) from respiratory pus. Overall, 79.9% of the isolates were from lower respiratory tract (LRT) specimens and 20.1% from upper respiratory tract (URT). Isolates from sputum were significantly more present in patients of the age group greater or equal to 60 years (96.2% 229/238) when compared to the other age groups: 0–5 years (12.1%; 8/66; P < 0.001), 6–15 years (58.3%; 7/12; P < 0.01) and 16–59 years (86.4%; 114/132) than in the age groups 0–5 years (12.1%; 8/66; P < 0.001) and 6–15 years (58.3%; 7/12; 0.05 > P > 0.02). On the other hand, upper respiratory tract isolates were significantly more present in children than in adults (70.0% versus 30.0%; P < 0.001). This was especially due to the age group 0–5 years in which 87.9% of the samples were from URT. Isolates from hospitalised patients represented 62.7% (281/448) of the isolates while 45.5% (204/448) and 7.8% (35/448) of the isolates came from hospitalised patients and from long care facility patients. Of the 448 isolates, 64.1% (287/448) were from male patients. Isolates from hospitalised patients represented 46.7% (209/448) of the isolates, 64.1% (287/448) were from male patients. Isolates from hospitalised patients represented 46.7% (209/448) of the isolates, 64.1% (287/448) were from male patients. Isolates from hospitalised patients represented 46.7% (209/448) of the isolates.

In general, the presence of isolates with decreased antibiotic susceptibility to any of the antibiotics tested revealed to be comparable in the three different regions: Brussels (40.0%), the Southern part (38.7%) and the Northern part (35.9%). The only significant difference was found for penicillin between Brussels (22.9%, 8/35) and the Northern part (8.1%; 17/209) (0.02 > P > 0.01). Tetracycline resistance was significantly higher in males (25.4%; 73/287) than in females (15.5%; 16/161). As far as the sampling site (URT and LRT) and admission type (ambulatory, hospitalized) are concerned, no significant differences were found.

The most common resistance phenotypes were insusceptibility to erythromycin-tetracycline (12.7%), isolated insusceptibility to erythromycin (7.4%) followed by insusceptibility to penicillin-erythromycin-tetracycline (5.8%). Insusceptibility to one compound was present in 15.0% of the isolates. Two-, three- and fourfold resistance was found in 15.1%, 6.5% and 0.9% respectively of the isolates.

MICS of all betalactams rose with those of penicillin though cross-resistance between penicillin and the other betalactams was incomplete. The results indicate that 100% of the penicillin-insusceptible isolates remained susceptible to amoxicillin while 82.7% of the penicillin-insusceptible isolates remained susceptible to cefotaxime, ceftazidime, cefepime and 78.8% to imipenem.

The most important capsular types in penicillin-insusceptible isolates were capsular types 19 (25.0%), 14 (19.3%), 23 (15.4%), 15 (13.5%), 6 (9.6%) and 9 (7.7%). Other types were capsular type 7, 24, 29, 34 and 35 (each 1.9%).

4. Discussion

The worldwide reported increase of antibiotic resistance among S. pneumoniae together with the concomitant development of coresistance between various unrelated classes of antimicrobials

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**Table 1**

Susceptibility of 448 isolates of S. pneumoniae to various antimicrobial agents.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Number of isolates with indicated MIC value (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 0.001</td>
</tr>
<tr>
<td>Penicillin</td>
<td>–</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>–</td>
</tr>
<tr>
<td>Amoxicillin/clavulanate</td>
<td>–</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>–</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>–</td>
</tr>
<tr>
<td>Cefuroximeb</td>
<td>–</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>–</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>–</td>
</tr>
<tr>
<td>Cefepime</td>
<td>–</td>
</tr>
<tr>
<td>Imipenem</td>
<td>13</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>–</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>–</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>–</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>–</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>–</td>
</tr>
<tr>
<td>Telithromycin</td>
<td>13</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>–</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>–</td>
</tr>
</tbody>
</table>

* MIC50 or MIC for 50% of the isolates.

b Distribution for cefuroxime and cefuroxime-axetil (the oral form of cefuroxime).
constitutes a problem of paramount importance. However, the clinical relevance of the impact of resistance on the clinical outcome remains a controversial topic [2,10–14]. Furthermore, the epidemiology of antibiotic resistance can be influenced by various factors and important variations can even be found in restricted geographic areas due to differences in antibiotic policies, secular changes and clonal shifts in the bacterial population, demographic and geographic parameters [15–17]. In the actual survey, we have found an insusceptibility rate for penicillin of 11.6% and noted an ongoing decrease of resistance since the surveys of 2001–2003 (21.0% in 2001: \( P < 0.001 \)). As expected, the decreasing trend of insusceptibility was also noted for the other betalactams and all the 2008 rates were significantly lower than the rates in the period 2001–2003. Cefaclor and cefuroxime decreased from 19.7 and 16.9% respectively in 2001 to 10.3 and 9.2% \(( P < 0.001 \) for cefaclor; \( P > 0.001 \) for cefuroxime). Amoxicillin decreased from 31.1\% (2003) to 0\% \(( P < 0.001 \) for amoxicillin). Cefotaxime showed an insusceptibility rate of 12.7\% in 1999 which decreased to 7.3\% in 2001 and 2.0\% in the actual survey \(( P < 0.001 \) ). Interestingly, the insusceptibility rates for all the betalactams are now at the same level or even lower than at the start of our surveillance programme in 1995. The Belgian National Reference Centre [18] reported a comparable decrease in penicillin insusceptibility in invasive isolates of \textit{S. pneumoniae}. The insusceptibility rate of 17.7\% found in 2000 decreased continually to 10.0\% in 2007. Furthermore, in our study, we recorded an important decrease of isolates with high level resistance to penicillin (MIC \( \geq 2 \mu g/ml \)). In the 2001 survey, 10.2\% of the isolates were classified as resistant to penicillin while in the actual study 4.0\% of the isolates are reported as such. Capsular types 19, 14, 23, 15, 6 and 9 represented 90.5\% of the penicillin insusceptible isolates, which is in general agreement with the findings of the National Reference Centre [18]. However, minor albeit important discrepancies have to be mentioned. The prevalence of capsular types 15 and 23 were more important in our study than in the report of the National Reference Centre (13.5\% versus 7.6\% and 15.4\% versus 8.8\%). However, the surveillance of the National Reference Centre is only based on invasive isolates from blood, CSF and other normal sterile fluids. Importantly, in the 2008 survey, the capsular type 15 has become an important type. Between 1995 and 2007 it was only present at a rate between 2.0 and 5.0\%. This trend was also reported by the National Reference Centre. Moreover, the high prevalence of capsular type 15 amongst the penicillin-insusceptible isolates can be held responsible for the relatively low coverage rate of the heptavalent vaccine (77.0\%) in which this type is not present. Another point of interest is the steady decrease of the Penicillin MIC\(_{50}\) value from 0.06 \( \mu g/ml \) in 2003 to 0.015 \( \mu g/ml \) in 2008. This may be indicative for an ongoing population shift to the susceptible side of the curve.

Fluoroquinolones are widely used as a treatment of respiratory tract infections and are considered as a valid alternative in the treatment of infections caused by resistant isolates or in patients with penicillin allergy. Fogarty et al. [19] reported on the excellent clinical and bacteriological cure rates of moxifloxacin in community-acquired pneumonia due to resistant isolates of \textit{S. pneumoniae}. In a worldwide surveillance, the reported fluoroquinolones resistance rates were in general very low [20]. However, the use of lesser potent compounds or the use of suboptimal dosing regimens can attribute to the emergence of resistance. Therefore, the follow-up of resistance and the possible shift of MIC distributions are of great importance. In our study, 5.1\% of the isolates had an MIC greater or equal to 2 \( \mu g/ml \) for ciprofloxacin compared to 15.0\% in 1999. Levofloxacin insusceptibility decreased from 3.3\% in 2003 to 0.7\% in 2008, while insusceptibility to moxifloxacin was only rarely found and evolved from 0.6\% in 2003 to 0.4\% in 2008. Interestingly, and contrary to the betalactams, the MIC\(_{50}\) values of the fluoroquinolones did not shift markedly throughout the total study period indicating an overall high degree of stability of the pneumococcal MICs. The rates of resistance to erythromycin and tetracycline remained at a high level and telithromycin clearly appeared as the most active compound in the macrolide-ketolide group.

Comparison and interpretation of resistance rates obtained worldwide by different centres should be done with some caution since several methodological parameters can influence the outcome of the analysis. Nevertheless, important geographic variations in resistance rates for various microorganisms including \textit{S. pneumoniae} have been reported both on a global, regional, national and even local level [6,7,21,22]. These geographic differences can be attributed to a wide variety of parameters including differences in antibiotic policies. However, these variations, especially on the local level, can also be explained by the introduction and subsequent spread of particular clones [23,24]. In our 2008 survey, we did not find major geographical differences in contrast with what has been reported in the past [6,7]. In general, we have always found significant higher rates of insusceptibility in the south (Wallon community) than in the north (Flemish community) of the country. In the 2008 survey, the rates between north and south were nearly identical. Importantly, in the south we noted an important and sometimes a significant decrease in resistance between 2003 and 2008: penicillin from 18.8 to 13.2\% (NS), ciprofloxacin from 18.9 to 5.9\% \(( P < 0.001 \) ), erythromycin from 31.3 to 29.4\% (NS) and tetracycline from 35.9 to 23.5\% \(( 0.02 > P > 0.01 \) ). The rate of isolates with complete susceptibility rose from 43.1 to 61.3\% \(( P < 0.001 \) ) in the same period. In the north, we saw only a minor decrease except for ciprofloxacin (from 11.6\% to 5.3\%; 0.05 \(< P < 0.02 \) ) and even a significant increase in resistance for erythromycin (20.9 to 29.7\%; 0.05 \(< P < 0.02 \) ). Changes in time can partly be attributed to better antibiotic prescribing. However, it should be mentioned that the decrease in resistance already started before the implementation of the governmental campaigns to promote a better antibiotic prescribing (2004) and the introduction of the pneumococcal heptavalent conjugate vaccine (2007). In the period 2001–2006, the overall outpatient antibiotic use expressed in DDD/1000 inhabitants/day (DDI) increased from 23.8 to 24.4. Amoxicillin and penicillin DDI increased from 2.4 to 4.0 and from 4.4 to 5.5 respectively. A decrease in DDI was recorded for the macrolides (from 2.7 to 1.9) and tetracycline (from 1.9 to 1.0). The fluoroquinolones DDI increased to 2.4 in 2003 but decreased

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>88.4</td>
<td>7.6</td>
<td>4.0</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>88.8</td>
<td>7.4</td>
<td>4.0</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amoxicillin/clavulanate</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefaclor</td>
<td>89.7</td>
<td>0.7</td>
<td>9.6</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>90.8</td>
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<td>8.7</td>
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<td>0.9</td>
<td>8.8</td>
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<td>Cefotaxime</td>
<td>98.0</td>
<td>2.0</td>
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</tr>
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<td>0</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Clindamycin</td>
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<td>0.9</td>
<td>25.4</td>
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<tr>
<td>Tetracycline</td>
<td>78.1</td>
<td>5.4</td>
<td>16.5</td>
</tr>
</tbody>
</table>

Table 2: Susceptibility rates following CLSI criteria of 448 isolates of \textit{S. pneumoniae}.
afterwards to 1.8 [25]. There exists a general agreement in the scientific community that increasing the antibiotic pressure on the bacterial ecosystem will lead eventually to an increased resistance rate. However, the correlation between antibiotic consumption and resistance is not always straightforward since a multiplicity of confounding factors can interfere [26–30]. The diversity in confounding factors requires a more in-depth analysis of all these factors in order to fine-tune the optimization programs on antibiotic use before their implementation. It is without discussion that the better use of antibiotics will be a driven element in combating the development of antibiotic resistance.

5. Conflicts of interest

The Institute of Public Health received a grant from Bayer to support in part this study. R. Vanhoof received a travel grant from Bayer to present this study at the 28th RiCAI in 2008.

Other authors declared no conflicts of interest.

References


