Antibiotic susceptibility and serotypes of *Streptococcus pneumoniae* isolates from Hungary

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**Objective:** Hungary has reported one of the highest incidences of penicillin resistance in *Streptococcus pneumoniae* in Europe since the 1970s and is still cited accordingly. However, since the end of the 1990s the resistance of pneumococci in Hungary has not been investigated. In this study we assessed the current situation, particularly to establish whether the incidence of resistance is increasing and if this could be related to the spread of specific strain types.

**Methods:** Isolates of *S. pneumoniae* (n = 304) were collected by five diagnostic laboratories in Hungary in 2000–2002. Their identity was confirmed and their susceptibilities to 16 antibiotics were determined by the agar dilution method according to NCCLS guidelines. Representative strains were serotyped (n = 112).

**Results and conclusions:** We found significantly lower resistance rates for penicillin compared with the data previously reported from Hungary, but the intermediate resistance was high, at 37%. Macrolide resistance was a bigger problem (~40% for erythromycin), although there was full susceptibility to telithromycin. The strains with the highest MICs were isolated from carriers and young children. The fluoroquinolones were very effective, especially moxifloxacin and gatifloxacin. There was full susceptibility to vancomycin and linezolid. We found inconsistencies with previous reports in the survey of the resistance and identification of *S. pneumoniae* in the country. The serotype distribution of the isolates showed a much greater diversity than had previously been reported; however, there was correlation between serotype and resistance.

**Keywords:** *Streptococcus pneumoniae*, Hungary, macrolide resistance, penicillin resistance, serotyping

**Introduction**

Hungary and Spain have consistently reported the highest incidences of penicillin resistance in Europe since the 1970s, but the emergence of multidrug-resistant strains is also a well-documented problem.¹-⁶ The resistance data in Hungary have been available since 1975 from the Annual Reports of the National Public Health Institute.⁷ Marton and colleagues had investigated the pneumococcal problem in Hungary for many years and published their findings on several occasions.¹-³,⁷,⁸,⁹ However, since the end of the 1990s the overall resistance situation in Hungary has not been investigated. As Hungary is still regularly cited as a country with a high incidence of resistance, we wanted to assess the current situation, particularly to establish whether the incidence of resistance is increasing.

**Materials and methods**

**Bacterial strains**

Three hundred and four *Streptococcus pneumoniae* isolates were obtained from five centres in three major cities of...
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Hungary (Budapest, Szeged and Győr) located in different parts of the country during three winter seasons (1999/2000, 2000/2001 and 2001/2002; 98, 123 and 83 isolates, respectively). Seventy-seven per cent of the isolates (n = 234) were from the upper respiratory tract (nasopharynx, sinus), 11% (n = 32) from the lower respiratory tract (sputum, tracheal or bronchial specimen, pleura, ventilation tube), 6% (n = 18) from the eye or ear (conjunctiva, cornea, ear exudates) and 4.4% (n = 13) were from miscellaneous sources (blood culture, pus, abscess, urine and vulva). The origin of seven of the isolates was not known. Patients providing 261 of the isolates had identifiable symptoms commensurate with pneumococcal infection. However, 43 isolates came from children who provided nasopharynx specimens but did not demonstrate infection. However, 43 isolates came from children who provided nasopharynx specimens but did not demonstrate clear clinical symptoms of pneumococcal infection, and were classified as ‘carriers’. The isolates were transported to the UK on active charcoal transport medium and their identity confirmed by optochin susceptibility, colony morphology and by PCR for the presence of the autolysin gene (lytA).10

Very few strains appeared to be optochin resistant.

The control strains for the susceptibility determinations comprised S. pneumoniae NCTC 7465, NCTC 13593 and Escherichia coli NCTC 10418.

Antimicrobial agents

Moxifloxacin and faropenem were kindly provided by Bayer AG (Leverkusen, Germany), and telithromycin by Aventis Pharma Ltd (Bridgewater, NJ, USA). The other antibiotics were purchased from Sigma UK Ltd or their appropriate manufacturer.

Susceptibility testing

Susceptibility testing was performed by the double dilution method on Mueller–Hinton agar plates (Oxoid, Basingstoke, UK) supplemented with 5% defibrinated horse blood according to NCCLS guidelines.11 Inoculated plates were incubated at 37°C in 5% CO₂. The susceptibility and resistance percentages of the isolates were determined using the breakpoints recommended in the 2002 NCCLS guidelines.11 All of our isolates derived from non-meningitis cases, so we used the appropriate breakpoints for cefotaxime. For telithromycin we followed British Society for Antimicrobial Chemotherapy (BSAC) guidelines, which gave the same breakpoints that were used by several independent authors as well.14-16 For faropenem we used those listed by Schmitz et al.17

Serotyping

Serotyping of 112 mainly penicillin-resistant or -intermediate isolates was performed with the new S. pneumoniae typing antisera purchased from Mast Group Ltd (Bootle, UK).

lytA PCR

The primers used to amplify the lytA gene were the same as described by Nagai et al.,10 but the PCR was performed slightly differently. Each reaction mixture contained 5 µL of template DNA obtained by boiling a loopful of bacteria from the plate in sterile distilled water for 15 min, 5 µL of 10x Taq PCR buffer, 0.2 mM PCR nucleotide mix, 2 mM MgCl₂, 50 pmol primers, 1.25 U Taq DNA polymerase and sterile distilled water to a final volume of 50 µL. The PCR cycling conditions consisted of an initial denaturation of 94°C for 3 min followed by 30 cycles of 94°C for 60 s, 54°C for 60 s and 72°C for 30 s, and then a final extension of 72°C for 10 min. The PCR product was 318 bp. We used several Gram-positive clinical strains (enterococci including vancomycin-resistant enterococci, Staphylococcus aureus including methicillin-resistant S. aureus, coagulase-negative staphylococci, viridans streptococci and lactobacilli) to test the sensitivity and specificity of the method and, in all cases, we obtained a negative PCR result.

Results

The prevalence of high-level resistance to penicillin was surprisingly low (Table 1), but the intermediate resistance was 37%. There was no difference in the amoxicillin or co-amoxiclav results, and together with cefotaxime they showed very good efficacy (97–99% susceptibility). Low MICs were found with faropenem, to which there was almost full susceptibility (99.3%). The resistance to macrolides was very high, ~40% for the macrolides erythromycin and clarithromycin (almost no intermediate resistance was observed), but there was full susceptibility to telithromycin, a new ketolide antibiotic. Importantly, clarithromycin resistance was significantly higher among the penicillin non-susceptible strains (53%) than for the susceptible ones (29%). The fluoroquinolones were very effective, especially the new members of the group, moxifloxacin and gatifloxacin. As expected, strains showed high resistance to trimethoprim–sulfamethoxazole, which confirms that this combination is inadequate therapy in pneumococcal infections. There was full susceptibility to vancomycin as well as to linezolid, the latter representing the new oxazolidinone group with a unique mechanism of protein synthesis inhibition.

MIC data related to the age of the patients

The majority of the strains were isolated from children: 45.2% of all strains were isolated from children under 3 years and 78.3% were isolated from children under 18 years. The prevalence of penicillin non-susceptible isolates was 50% for patients under the age of 3 years, whereas above this age it dropped to 28–30% (Figure 1). Surprisingly, none of the strains isolated from elderly patients (>60 years) showed full
Antibiotic susceptibility of *S. pneumoniae* from Hungary

Table 1. MIC results of 304 pneumococcal isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt; (mg/L)</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>range</th>
<th>% R</th>
<th>% I</th>
<th>% S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>0.03</td>
<td>0.5</td>
<td>≤0.015–8</td>
<td>2.0</td>
<td>37.0</td>
<td>61.0</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0.03</td>
<td>0.5</td>
<td>≤0.008–8</td>
<td>0.3</td>
<td>2.0</td>
<td>97.7</td>
</tr>
<tr>
<td>Co-amoxiclav 2:1</td>
<td>0.03/0.015</td>
<td>1/0.5</td>
<td>≤0.008/0.004–8/4</td>
<td>0.3</td>
<td>0.7</td>
<td>99.0</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.06</td>
<td>0.5</td>
<td>0.008–2</td>
<td>9.6</td>
<td>18.1</td>
<td>72.3</td>
</tr>
<tr>
<td>Faropenem</td>
<td>0.03</td>
<td>0.25</td>
<td>≤0.004–1</td>
<td>0</td>
<td>0.7</td>
<td>99.3</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0.06</td>
<td>0.5</td>
<td>0.004–2</td>
<td>0</td>
<td>1.3</td>
<td>98.7</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.5</td>
<td>0.5</td>
<td>0.125–1</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0.5</td>
<td>1</td>
<td>0.25–2</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.125</td>
<td>256</td>
<td>0.06–2512</td>
<td>41.7</td>
<td>1.3</td>
<td>57.0</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>0.125</td>
<td>256</td>
<td>≤0.06–2512</td>
<td>38.1</td>
<td>1.0</td>
<td>60.9</td>
</tr>
<tr>
<td>Telithromycin</td>
<td>0.015</td>
<td>0.06</td>
<td>≤0.004–0.5</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>1</td>
<td>1</td>
<td>≤0.5–2</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Ciprofoxacin</td>
<td>1</td>
<td>2</td>
<td>≤0.5–4</td>
<td>3.6</td>
<td>0</td>
<td>96.4</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0.125</td>
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<td>0.06–0.5</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>0.25</td>
<td>0.5</td>
<td>0.125–1</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Co-trimoxazole 1:19</td>
<td>2/38</td>
<td>8/152</td>
<td>0.06/1.88–16/308</td>
<td>46.7</td>
<td>19.2</td>
<td>34.1</td>
</tr>
</tbody>
</table>

R, resistant; I, intermediate; S, susceptible.

Resistance to penicillin (MIC ≥ 2 mg/L) and the intermediate level was only ~7%.

The same large decrease in resistance to clarithromycin was also found in the elderly patients as had been shown for penicillin (Figure 1), but otherwise the resistance rates did not show any significant trend. Interestingly, there was a major decrease in resistance in the age group of 4–18 years.

**MIC data related to other factors**

Penicillin susceptibility was highest among the lower respiratory tract isolates (72%), and only ~60% in the other cases, but the explanation for this is that almost all the lower respiratory tract isolates were derived from adults or elderly people, and as we have already demonstrated penicillin resistance is lower in strains isolated from adults.

For the macrolides the susceptibility data were almost exactly the same in both the upper and lower respiratory tract strains (~59%).

The prevalence of reduced susceptibility to penicillin was higher among the ‘carrier’ group (55.8%); however, this was not significant ($\chi^2 = 2.33, P = 0.13$). Similarly, the clarithromycin insusceptibility was also higher (58.1%), but this again was not significant ($\chi^2 = 2.99, P = 0.08$).

In addition, there were significant shifts (10–20%) in both penicillin and macrolide susceptibility from one season to another. There was no correlation between the sex of patients and the MIC data.

**Serotyping results**

Serotyping was performed on 112 strains, mainly belonging to the penicillin-resistant or -intermediate groups. In the serotyped cohort we included a further two highly penicillin-resistant strains from Hungary, which were of unknown background but were used for comparative purposes. Serotypes 6 and 9 were found most frequently (Table 2), followed by serotypes 14 and 23. There were nine, eight and seven isolates belonging to serotypes 19, 10 and polyvalent antiserum group 3, respectively.

Of the six isolates with a penicillin MIC of ≥2 mg/L found in our survey, two isolates (MIC 2 mg/L) were serotypes 6 and 14. The four remaining isolates (MIC 4–16 mg/L) were all

![Figure 1. *S. pneumoniae* penicillin and clarithromycin resistance related to age. Grey columns, penicillin; black columns, clarithromycin. R + I, resistant + intermediate.](image-url)
serotype 19 (Table 2). The two strains of unknown origin (MIC 16 mg/L) were also serotype 19, suggesting that the few high-level resistant strains in Hungary derive from serotype 19. In strains with intermediate penicillin MICs, serotypes 6, 9, 14 and 23 were predominant, the former two being more prevalent in the lower MIC categories (0.25 mg/L), whereas the latter two were more prevalent in the strains with MIC 0.5–1 mg/L. Interestingly we found that three isolates with serotype 19 had a penicillin MIC as low as 0.25 mg/L.

We found a significant correlation of macrolide susceptibility with serotype (Table 2). Serotypes 6 and 19 were found exclusively among the macrolide-resistant isolates, and serotype 19 was always linked with very high resistance (six strains with MICs ≥ 512 mg/L of both erythromycin and clarithromycin, and one isolate with an MIC of 16 mg/L of clarithromycin and 128 mg/L of erythromycin). On the other hand, serotypes 9 and 23 were found almost entirely among the macrolide-susceptible isolates. The prevalence of serotype 14 did not show any significant correlation with resistance.

As the majority of tested isolates were isolated from children, we could not find any significant correlation between age and serotype of the isolated strains. There was also no correlation between body site of isolation and serotype of the isolate.

Discussion

Currently, the antibiotic resistance patterns of *S. pneumoniae* isolates vary widely from one country to another within Europe. Rates of resistance to penicillin have been reported to be increasing in countries such as France, Spain and Portugal, as well as in the central eastern European countries such as Hungary, Slovak Republic, Poland, Romania, Bulgaria, Slovenia and Croatia, but resistance has remained at very low levels in other central European countries (Austria, Germany, Czech Republic and Poland). This remarkable difference may have occurred because of the unlimited use of antibiotics, the lack of antibiotic policy and control in these countries, or as a result of the different antibiotic distribution policies.

We would suggest that they may also reflect the different methods used to confirm *S. pneumoniae* identity. We used a PCR-based method in order to show the presence of the pneumococcal-specific autolysin gene to confirm the identification of all our strains before including them in this study. Many other Gram-positive α-haemolytic small colonial bacteria may exhibit optochin susceptibility and these can be read phenotypically as *S. pneumoniae*. We believe that some genotypic identification is essential to obtain an accurate identification of *S. pneumoniae*.

We found that most of the isolates derived from children. This tendency had been shown earlier in Hungary and, more recently, Nagai et al. observed a similarly high incidence of pneumococcal infections among children in Hungarian samples in an eastern European study. Paediatric strains have also been shown worldwide to be more resistant than those from adults, and our findings in Hungary support this.

The higher resistance proportion among the healthy carriers has already been observed in South Africa and Spain. This could be associated with the fact that children carry pneumococci more frequently than adults, and all the 43 carriers within our cohort were children, 67.4% under the age of 3 years. This suggests that carriers can be dangerous for other vulnerable members of the community (e.g. elderly, infants) and they can have an important role in the transmission of nosocomial infections.

The prevalence of high-level resistance to penicillin obtained in this study was surprisingly low compared with the data reported previously from Hungary. This can be explained by the fact that these data were based on the Annual Reports of the National Public Health Institute. When we re-examined the archived data, we found that the so-called ‘resistant’ strains were actually a combination of resistant and intermediate strains. The error was compounded by the fact that the definitions used by the regional public health laboratories were different from those used by the university and individual hospital laboratories (Figure 2). Our data were based purely on MICs, and thus show a much lower incidence of resistance (Figure 2).

In addition, the Alexander Project found significantly lower penicillin resistance percentages when they tested 127 Hungarian strains isolated in 1996 (11.8% resistant, 24.4% intermediate), and made a particular note about the difference between their results and those reported from Hungary. Similarly, lower resistance percentages were reported by the European Antimicrobial Resistance Surveillance System (EARSS) in 2001 with even lower values (8% resist-

<table>
<thead>
<tr>
<th>Serotype</th>
<th>All strains</th>
<th>Penicillin</th>
<th>Clarithromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>6</td>
<td>21</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>9</td>
<td>18</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>14</td>
<td>15</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>23</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>19</td>
<td>9</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Poly 3*</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

Data in the table are the number of isolates.

*Serotype 11, 15 or 16.
S, susceptible; I, intermediate; R, resistant.

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![Figure 2. Comparison of penicillin resistance data. Black squares, regional public health laboratories data, taken directly from the Annual Reports; black triangles, university and individual hospital laboratories data, taken directly from the Annual Reports; black circles, data obtained in this study.](image)

...ant, 14% intermediate), but only 36 strains were examined (www.eارss.rivm.nl).

Although there is an excessive consumption of β-lactams by the Hungarian community, the very high rate of penicillin resistance reported in *S. pneumoniae* isolates can also be attributed to a number of methodology and identification problems. Colony morphology, microscopic examination and bile solubility were routinely used for identification in previous studies; however, optochin susceptibility testing was only rarely employed. Up until the early 1980s, ‘resistance’ to penicillin had been defined with 3 IU penicillin discs. Then, in the late 1980s, both 3 IU and 10 μg penicillin and 5 or 10 μg oxacillin discs were used. The laboratories started to use a 1 μg oxacillin disc in the early 1990s. The Etest has recently been introduced, but mostly in the university and individual hospital laboratories. All these factors suggest that the previous figures from Hungary may have been misleading.

Despite our criticism about the previous reports from Hungary that the prevalence of high-level penicillin resistance in the country might not be as high as has been reported, the country does face a problem with penicillin intermediate resistance, and it is only a question of time before these strains become fully resistant. However, it is certainly important to emphasize that there is already a severe problem with macrolide resistance in Hungarian isolates, and it should be seriously considered whether the older members of this antibiotic group should be not be used as first choice in treatment of pneumococcal infections prescribed by the family doctors without the result of susceptibility tests. Surprisingly, β-lactam antibiotics could have a much wider role in empirical treatment despite the general perception of resistance in Hungary.

The majority of penicillin-resistant strains found worldwide belong to the serogroups 6, 9, 14, 19 and 23. These five serotypes have also been associated with carriage (mainly by young children). In Hungary, the dominant serotype among penicillin-resistant strains has been reported previously to be 19A. In this study we found that although serotype 19 was predominant among the few highly resistant isolates (MIC ≥ 4 mg/L), as the MIC decreased, particularly to the intermediate level, different serotypes appeared, particularly the international serotypes 6, 9, 14 and 23. We also found three serotype 19 isolates with low penicillin MICs (0.25 mg/L), and the proximity of their isolation dates suggested that they were epidemiologically related to one another. Only one serotype 19 strain with a low MIC has previously been reported from Hungary.

Generally, the Hungarian pneumococci serotyped by us show a greater diversity than has been reported previously. This might seem obvious when we consider that the previously typed strains had been isolated predominantly from a closed Budapest community of children attending a single children’s hospital, whereas our strains were isolated from many unrelated communities. The serotypes do not always show a close correlation with the resistance pattern; therefore, it is not certain that this method alone is sufficient for epidemiological studies.

In Hungary the vaccine currently available is the 23-valent purified polysaccharide pneumococcal vaccine, which is used in the USA and Europe. It can be given on request and is recommended to the elderly. Ideally, this should cover all the important serotypes, but it has two major disadvantages; first, it is ineffective in small children (<2 years of age), and secondly, the response to the non-conjugated vaccine is rather poor. The new 7-, 9- and 11-valent protein conjugate vaccines, which are immunogenic in children <2 years of age, contain all the five most prevalent serotypes (6, 9, 14, 19 and 23), so their use in Hungary could be considered, but this requires a major study.

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