Introduction

The incidence and level of penicillin resistance in Streptococcus pneumoniae strains vary greatly from one country to another, and Hungary was found to be one of the 10 main foci of penicillin-resistant (PRSP) in the 1990s. However, little or no levofloxacin resistance was detected in Asia and Europe.1

The aim of this study was to examine the incidence of resistance to penicillin, cefotaxime and levofloxacin in respiratory tract isolates of S. pneumoniae. Levofoxacin activity against S. pneumoniae has been found not to be associated with penicillin susceptibility determined by either the MIC or the bactericidal index and so levofloxacin has been suggested as potential therapy for infections.2 This prompted us to assess the correlation between MICs of penicillin, cefotaxime and levofloxacin for penicillin-non-susceptible respiratory tract isolates of S. pneumoniae.

Materials and methods

Between 1 January and 30 June 2000, a total of 3826 bacterial isolates from 12 062 specimens from the in- and out-patients of Pál Heim Municipal Children’s Hospital and several departments of the University Hospital, Semmelweis University, Budapest, Hungary were examined for the presence of S. pneumoniae.

Samples, except sputa, were taken in Mast transport medium with small swabs (Mast Diagnostica, Reinfeld, Germany) from the eyes and middle ears; the Biotest Transport System with normal sized swabs (Biotest, Dreieich, Germany) was used for other sites. Culture was carried out on 5% sheep blood agar and chocolate agar plates in 5% CO2 at 35–37°C for 24–48 h. S. pneumoniae isolates were identified as described by Ruoff.3 Repeat isolates from individual patients were excluded. The presence of some capsular antigens was tested by Slidex Pneumo latex agglutination reagent (bioMérieux, Lyon, France). S. pneumoniae ATCC 49619 was used as control. Strains were stored in a Mast Microbank System at −80°C.

Susceptibility testing to appropriate antimicrobials was carried out by the disc diffusion method4 with Oxoid discs. Penicillin resistance in S. pneumoniae isolates was screened using 1 µg oxacillin discs (Oxoid, Basingstoke, UK) and strains showing ≥20 mm inhibition zones were assessed as susceptible. Strains exhibiting smaller inhibition zones around the 1 µg oxacillin discs were provisionally identified as penicillin non-susceptible4 and further examined by low concentration penicillin Etest (AB Biodisk, Solna, Sweden). The results were interpreted as described previously.5

Oxacillin disc diffusion tests revealed that 36.7% of 327 Hungarian Streptococcus pneumoniae strains from clinical specimens were not penicillin susceptible. Determination of the MICs of penicillin, cefotaxime and levofloxacin for these strains by Etest confirmed that 30 (9.2%), 19 (5.8%) and four to 11 (1.2–3.4%) were fully penicillin-, cefotaxime- and levofloxacin-resistant, respectively. Most had extremely high MICs. Lower respiratory tract strains were more resistant than those from the upper respiratory tract. Levofloxacin-resistant strains were either penicillin intermediate or resistant, but their MICs did not correlate strongly.
Respiratory strains with penicillin MICs of 0.125 mg/L or higher were further tested for cefotaxime and levofloxacin susceptibility according to the protocol of the EARSS.6

We attempted to apply a linear regression analysis as described by Brueggemann et al.7, but the distribution of MICs of penicillin, cefotaxime and levofloxacin for the S. pneumoniae strains did not correspond to a normal distribution. Instead, therefore, the Spearman rank correlation coefficients were calculated from the MIC data using the software Statistica for Windows, v. 4.5.

Results

Of a total of 3826 clinical isolates, 327 (8.5%) proved to be S. pneumoniae; 75% were isolated from the upper respiratory tract (URT), 15% from the lower respiratory tract (LRT), 4, 2, 1 and 3% from eye, wound, blood stream and urogenital tract infections, respectively. Of these, 221 strains (68%) were isolated from children aged ≤12 years and 106 (32%) from adults. Of the isolates, 72% and 28% were cultured from out- and in-patients, respectively. All strains agglutinated in the Pneumo Slidex test and so were considered as encapsulated pathogenic strains regardless of whether they were associated with clinical infections or colonization only.

Disc diffusion tests showed that 64, 54, 71, 96, 48 and 100% of strains were susceptible to oxacillin, erythromycin, tetracycline, chloramphenicol, sulfamethoxazole/trimethoprim and glycopeptides, respectively. One-hundred and twenty (36.7%) of 327 strains were designated as penicillin non-susceptible with 1 μg oxacillin.

MIC determination for these strains by Etest identified an additional 19 strains as penicillin susceptible (PS) (Figure 1). The NCCLS defines S. pneumoniae strains as PS, penicillin intermediate (PI) and penicillin resistant (PR) if the MICs are ≤0.1, 0.12–1.5 and ≥2.0 mg/L, respectively. Thus, the overall incidence of PS stains was 69.1%. Overall 71 strains (21.7%) showed intermediate and 30 (9.2%) had full resistance to penicillin. Twenty-eight (93.3%) of the 30 PR strains and 68 (95.8%) of the 71 PI strains were isolated from the respiratory tract.

The MICs of cefotaxime and levofloxacin were determined for the 96 penicillin-non-susceptible respiratory tract isolates (Figure 2). The NCCLS classifies S. pneumoniae strains as cefotaxime susceptible, intermediate and resistant if the MICs are ≤0.5, 1 and ≥2 mg/L, respectively. The strains are defined as levofloxacin susceptible, intermediate and resistant if the MICs are ≤2, 4 and ≥8 mg/L, respectively.

Of all 96 penicillin-non-susceptible respiratory tract strains, 51%, 29% and 20% were cefotaxime susceptible, intermediate and resistant, respectively (Figure 2a).

Seven (7.3%) of the 96 penicillin-non-susceptible strains had intermediate and four (4.2%) had high-level resistance to levofloxacin according to NCCLS criteria (Figure 2b). However, levofloxacin plasma concentrations are close to its MIC for pneumococci. According to BSAC guidelines strains with MICs ≥ 4 mg/L levofloxacin are considered ‘resistant’. Strains with this MIC were over-represented in the groups with penicillin MICs > 32 mg/L, so an assumption that highly penicillin/cefotaxime-resistant strains are not levofloxacin resistant depends on the breakpoints chosen. According to BSAC guidelines, four of the

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Figure 1. MICs of penicillin for 120 S. pneumoniae strains proved to be penicillin nonsusceptible with 1 μg oxacillin disc. Key: susceptible, □; intermediate, □; resistant, ■.

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PI and four of the PR strains (overall 8.3%) should be considered levofloxacin ‘resistant’. Strains isolated from the LRT had considerably higher MICs of both antimicrobials.

The four high-level levofloxacin-resistant stains (MIC ≥ 32 mg/L) were isolated from sputum of in-patients of the Department of Pulmonology, Semmelweis University, Budapest, where levofloxacin has been administered since the autumn of 1999. These strains were penicillin intermediate and remained susceptible to cefotaxime, carbapenems and glycopeptides.

There were 12 strains with extremely high levels of penicillin and/or cefotaxime resistance isolated from the respiratory tract of children under 6 years of age. Most of these had high-level resistance to carbapenems too, and needed increased concentrations of vancomycin within the susceptible range to inhibit their growth. Eight of these strains were susceptible and four were intermediately susceptible (NCCLS guidelines) or resistant (BSAC guidelines) to levofloxacin.

Based on the NCCLS interpretations, increased penicillin resistance in *S. pneumoniae* strains strongly correlated with increased cefotaxime resistance as the Spearman *r* proved to be 0.89, *P* < 0.001. A weak correlation was found between MICs of β-lactams and levofloxacin as the Spearman *r* proved to be 0.37, *P* < 0.001 and 0.45, *P* < 0.001 between penicillin and levofloxacin, and cefotaxime and levofloxacin, respectively.
Discussion

Some 10 years ago Marton et al.,8 published penicillin resistance rates as high as 44–58% in strains of pneumococci isolated from adults and 70% in strains from children in Hungary. Later the rate of PR decreased to 40%.”9 However, no resistance to third-generation cephalosporins was found at that time. Our present study indicates a considerable change in the resistance of Hungarian S. pneumoniae strains to currently used antimicrobials.

The 1 µg oxacillin disc diffusion test has overestimated the prevalence of penicillin resistance in S. pneumoniae, and so, we advise that penicillin MIC determinations are carried out on all clinically significant S. pneumoniae isolates as the first susceptibility test.

Cefotaxime-resistant S. pneumoniae strains have emerged in Hungary; this may be attributable to the introduction, and frequent administration of oral cephalosporins in general practice as well as ceftriaxone use. Smith & Klugman10 analysed the penicillin-binding proteins (PBPs) of some other high-level resistant Hungarian isolates and found them to be clonal. In the hospitals where our patients were treated, penicillin and piperacillin have been used frequently and may be selectors for altered PBP 2B, which leads to high-level cefotaxime resistance.10 The fact that all but two of the 28 PR strains showed intermediate or full resistance to cefotaxime, and that most of the penicillin and cefotaxime highly resistant isolates were also resistant to carbapenems indicates potential for the selection of penicillin-cephalosporin-carbapenem cross-resistant strains of S. pneumoniae.

Under these conditions the therapeutic role of levofloxacin has been appreciated and the question of associated resistance to the β-lactams is of great importance.2 Although the correlation analysis on MICs of penicillin, cefotaxime and levofloxacin for the strains studied indicates a weak association between the two types of resistance, no levofloxacin-intermediate or resistant strain proved to be PS. Six were PR and five were PR. Fluoroquinolones have been widely used in Hungary in hospitals and general practice; therefore, it is not surprising that in as little as 6 months after introducing levofloxacin in the Department of Pulmonology resistant strains have appeared.

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References


