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RESEARCH NOTE

The relationship between serotypes and PFGE genotypes in isolates of *Streptococcus pneumoniae* from Hungary

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ABSTRACT

The relatedness of 112 penicillin-non-susceptible isolates of *Streptococcus pneumoniae* from Hungary was determined by pulsed-field gel electrophor-

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esis (PFGE), serotyping and antibiotic susceptibility tests. The differences in PFGE patterns closely mirrored the changes in resistance. Some genotypes comprised multiple serotypes, and the genetic diversity among certain serotypes was considerable. Generally, serotyping alone was insufficient for epidemiological mapping of pneumococcal isolates. There was considerable serotype diversity, but the five most frequent international serotypes (6, 9, 14, 23, 19) were the most prevalent. In addition, the presence of some well-defined resistant international pneumococcal clones in the Hungarian population was identified.

Keywords Genotypes, Hungary, penicillin-non-susceptible, pneumococci, serotypes, *Streptococcus pneumoniae*

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Streptococcus pneumoniae causes a wide range of serious diseases, and it is essential to determine the epidemiological and genetic relatedness of isolates at the local, national and international levels to control their spread and to develop new vaccines [1]. Phenotypic and genotypic typing methods can be used for the characterisation and epidemiological tracking of these bacteria. The present study used pulsed-field gel electrophoresis (PFGE) for molecular characterisation of penicillin-non-susceptible Hungarian isolates of pneumococci, and then compared the genotypic PFGE results with phenotypic results based on serotypes and antibiotic resistance patterns.

Penicillin-non-susceptible, non-invasive *S. pneumoniae* isolates ($n = 112$), obtained from five clinical laboratories in Hungary during 2000–2002 [2], were included in the study. The identities of all isolates were also confirmed by *lytA* PCR [2,3]. Antibiotic susceptibility testing was performed by the agar dilution method on Mueller–Hinton (Oxoid, Basingstoke, UK) blood agar plates. The sensitivity and resistance breakpoints used were, where available, those recommended by the 2002 NCCLS guidelines [4]. For telithromycin, the following breakpoints were used: susceptible (S), ≤0.5 mg/L; resistant (R), ≥2 mg/L [5,6]. All antibiotics were purchased from Sigma (Poole, UK), except moxifloxacin (Bayer, Leverkusen, Germany) and telithromycin (Aventis Pharma, Bridgewater, NJ, USA).

Serotyping was performed with *S. pneumoniae* typing antisera (Mast Group, Bootle, UK). Chromosomal DNA for PFGE was prepared as described by Hall *et al.* [7], with slight modifications. After digestion with *Apa*I (Promega, Southampton, UK), the fragments were separated in a CHEF-DR II apparatus (Bio-Rad, Hercules, CA, USA), with pulse times of 2–30 s. The NO34OS λ ladder (New England Biolabs, Hitchin, UK) was used as a molecular size marker. The PFGE profiles were analysed with the BioNumerics program v. 2.5 (Applied Maths, Sint-Martens-Latem, Belgium). A PFGE genotype was defined as isolates that showed ≥90% identity in the dendrogram created by the UPGMA/Dice coefficients with a band position tolerance of 2%, in accordance with published interpretive criteria [1]. The presence of the *erm* and *mef* macrolide resistance determinants, when relevant for the international comparison, was tested by PCR with the primers described by Sutcliffe *et al.* [8]. The *mef(E)* and *mef(A)* determinants were distinguished with the *Bam*HI restriction method of Oster *et al.* [9].

The penicillin MICs for the isolates ranged from 0.125 to 16 mg/L, but there were only eight penicillin-resistant isolates (MIC ≥ 2 mg/L). The frequency of macrolide resistance (MIC ≥ 1 mg/L) was 50–54%. All isolates were sensitive to telithromycin, moxifloxacin, vancomycin and linezolid.

The correlation between the serotypes and antibiotic susceptibilities of these isolates has been reported previously [2], but it was important to establish the genetic stability of individual serotypes. Although there were 25 different PFGE genotypes, 70.5% of the isolates belonged to only five genotypes (Table 1). Twelve genotypes contained only one isolate each. While the other major genotypes comprised only one serogroup, the two largest genotypes, A and B, comprised two serogroups each (9/14 and 6A/23F, respectively), and the isolates were clearly sub-clustered according to the serotypes. Additionally, there was a close correlation between the genotypes and the penicillin and macrolide susceptibility levels.

Pneumococcal isolates from Hungary have rarely been serotyped routinely. Instead of the previously reported predominance of serotype