Sir,

Despite advances in medical care, Streptococcus pneumoniae remains a major cause of community acquired infections. [1,2]. Over recent years, the increasing emergence of penicillin resistant S. pneumoniae (PRSP) is causing serious clinical problems worldwide [3]. In Greece, in two recent reports, the prevalence of PRSP strains recovered from infected children in the periods 1995–1996 and 1997–1998 were 12 and 7.6%, respectively [4,5]. Although this prevalence may not seem to be extremely high, the problems created by these organisms and in particular, the multi-resistance associated with penicillin resistance, gives us great cause for concern. We report the experience in the Penteli Children’s Hospital of Athens, with PRSP in terms of their resistance patterns to other antibiotics, serotypes and characterization of penicillin-binding protein 2B genes (pbp 2b) mutation, using PCR, throughout 1 year (September 1999–October 2000).

All our patients were children aged up to 14 years. Of 207 S. pneumoniae strains isolated, 27 (13%) were resistant to penicillin (PRSP). PRSP were cultured from the nasopharynx (22.8% of all isolates from this source), mastoid pus (2.7%), middle ear (3.7%), sputum (2.7%), conjunctivae (3.7%) and blood (3.7%). Identification of the strains was performed by standard methods (optochin disk, bile solubility) as well as by molecular detection of the specific autolysin gene (lytA) using PCR. Resistance to penicillin was initially determined by the agar diffusion test with a 1 μg oxacillin disk (zone diameter < 20 mm, NCCLS). The MIC of penicillin, cefuroxime, ceftriaxone, cefepime, erythromycin, clari-thromycin, trimethoprim-sulphamethoxazole, clindamycin, vancomycin, rifampicin, sparfloxacin and ciprofloxacin for PRSP strains were performed using a broth dilution method and a commercially available system (Sensititre, West Sussex, UK). Cultures were grown in cation adjusted Müller–Hinton Broth with 2.5% lysed horse blood. Interpretation was based on NCCLS criteria as adjusted for S. pneumoniae. PRSP strains were serotyped by the capsular reaction test with commercially available pneumococcal antisera (Statens Serum Institute, Copenhagen, Denmark). Pbp 2b mutation was characterized by PCR, with a combinational detection of autolysin gene (lytA) and the pbp 2b gene class A or B mutation. Among other related mutations these two play a major role in penicillin resistance, while the simultaneous detection of the pneumococcal autolysin gene is a highly specific identification of S. pneumoniae [6]. PCR was performed as described elsewhere [7] using one set of primers for lytA [8] and three sets of primers derived from the pbp 2b gene (susceptible gene, class A mutation, class B mutation) on the basis of the sequence reported by Dowson et al. [9,10].

The antimicrobial resistance of the PRSP strains is shown in Table 1. The MICs of penicillin for all PRSP was ≥ 0.25 mg/l. High resistance rates were found for cephalosporins. All strains were susceptible to vancomycin and rifampicin. All strains were sensitive to sparfloxacin but only half to ciprofloxacin. About 25 of 27 PRSP strains (92.6%) were resistant to one or more of the other classes of antimicrobials as shown in Table 2. From the 180 penicillin-susceptible S. pneumoniae strains collected in the same period, none was multi-resistant and single-resistance rates were as follows:

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trimethoprim-sulphamethoxazole 17.5%, erythromycin 5%, tetracycline 4% and clindamycin 2.5%. About 27 PRSP strains belonged to six different serotypes: O:23 (9), O:19 (9), O:9 (4), O:15 (2), O:14 (2) and O:6 (1). There was no link between serotypes and resistance phenotypes. All strains were \( \text{pbp } 2b \) class B mutants.

The penicillin resistance rates of our \textit{S. pneumoniae} strains are similar to those reported as increasing in most European countries including Greece [11,12]. The unique pattern of class B mutation in the \( \text{pbp } 2b \) gene correlates well with the MICs of penicillin for all our PRSP (\( \geq 0.25 \) mg/l). This correlation has been reported previously elsewhere [7] suggesting the inclusion of the \( \text{pbp } 2b \) gene in a rapid combinational detection of highly resistant PRSP by PCR in clinical material.

The concurrent high multidrug-resistance (92.6%) of PRSP found in our study is also reported elsewhere [11,13]. This major problem may cause serious difficulties in the clinical treatment of pneumococcal infections, especially in children and immunocompromised individuals. In our experience only vancomycin, rifampicin and the newer quinolone, sparfloxacin proved to be active against PRSP. All serotypes found among our PRSP belonged to those most frequently associated with multidrug-resistance [11,13] and are all included in the commercial glycoprotein conjugate vaccines [14]. Our results support the general concept that special preventive and therapeutic strategies should be implemented for the management of pneumococcal infections. These should include vaccination programmes, as well as therapeutic options offered by new strategies concerning the use of newer quinolone derivatives, ketolides, oxazolidinones and streptogramins.

### References


