bacteria to their young through regurgitation feeding and throughout the Antarctic ecosystem via faecal contamination.

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Transparency declarations

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References


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Macrolide resistance among invasive Streptococcus pneumoniae in Slovenia

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Sir,

The worldwide increase in antibiotic resistance of Streptococcus pneumoniae has become a serious problem in recent years, including the increasing resistance to macrolides. In Slovenia, the epidemiological situation of invasive diseases, caused by S. pneumoniae, has been constantly monitored since 1993.

The objectives of this study were to characterize the macrolide-resistant clinical isolates of invasive S. pneumoniae in Slovenia and to investigate the genetic basis of macrolide resistance.

A total of 1448 invasive isolates of S. pneumoniae, recovered from blood cultures and from cerebrospinal fluid, were collected in Slovenia from 1998 to 2007. Of these, 399 isolates (27.6%) were obtained from children (0–14 years of age) and 1049 (72.4%) from adult patients. The isolates were serotyped by Neufeld’s Quellung reaction using antiserum provided by the Statens Serum Institut, Copenhagen, Denmark. Antibiotic susceptibility was determined following the recommendations of the CLSI. The presence of erm(A), erm(B) and mef(A) genes were determined by PCR with primers and reaction conditions according to Sutcliffe et al. Isolates negative for the acquired macrolide genes had their domain V and II from all four 23S rRNA alleles and the genes coding for ribosomal proteins L4 and L22 sequenced.

A rise of macrolide resistance (MIC ≥ 1 mg/L) in invasive S. pneumoniae from 4.7% in 1998 to 16.8% in 2007 was observed in Slovenia. The rate of resistance increased 5.7-fold in the case of isolates from children, from 4.3% in 1998 to 24.6% in 2007 (Table 1). At the same time, however, the consumption of macrolides in Slovenia decreased by 36% from 1999 to 2007 (from 3.81 to 2.43 defined daily doses per 1000 inhabitants per day), which had no influence on the increase in resistance.

Fifty-nine percent of the macrolide-resistant pneumococci showed the constitutive MLSB phenotype and 41% the M phenotype. No erythromycin–clindamycin inducible resistant strains were found.

The most prevalent determinant of macrolide resistance was the erm(B) gene, which was present in 86 isolates (55%), followed by the mef(A) gene, which was present in 61 isolates (39%). The erm(A) gene was not found.

The erythromycin MICs for the isolates carrying the erm(B) gene were ≥256 mg/L and for the isolates with the mef(A) gene, they ranged between 2 and 32 mg/L, except for a single isolate with MIC ≥ 256 mg/L. In this isolate, we also found a mutation in the rrl gene coding for 23S rRNA, A2059G in all four alleles. Three other isolates also harboured mutations in the rrl gene; we demonstrated the A2059G substitution in three of four alleles, A2058G in all four alleles and A2058C in two of four alleles. Isolates negative for the acquired macrolide genes had their domain V and II from all four 23S rRNA alleles and the genes coding for ribosomal proteins L4 and L22 sequenced.

The acquisition of a resistance determinant often provides a selective advantage for a pneumococcal strain and promotes its clonal spread. It is not surprising therefore that, among...
macrolide-resistant isolates, only a few different serotypes were found. In Slovene invasive macrolide-resistant pneumococcal isolates, the most prevalent serotypes were serotype 14 (35%), 6B (18%), 19F (9%), 9V (9%), 19A (8%) and 6A (4%). In the observed period, the \textit{erm}(B) gene predominated in the resistant isolates of serotypes 6B (29 of 30; 96.7%), 19A (11 of 12; 91.7%), 19F (11 of 13; 84.6%) and 6A (5 of 6; 83.3%), whereas the \textit{mef}(A) gene predominated in serotypes 14 (40 of 55; 72.7%) and 9V (13 of 14; 92.9%).

Multiple antimicrobial resistance (co-resistance to three or more different classes of antibiotics) was very common among macrolide-resistant invasive pneumococci in Slovenia. Of the 156 erythromycin-resistant invasive pneumococci, 53.8% were multiresistant. The strains carrying the \textit{erm}(B) gene were multi-resistant in 62/86 of the cases (72.1%). The most frequent co-resistance pattern was the resistance to penicillin, tetracycline and trimethoprim/sulfamethoxazole. This was the case for 39 of 86 isolates with the \textit{erm}(B) gene. The \textit{mef}(A) gene was less often associated with multiresistance (36.1%). The most frequent co-resistance pattern in the isolates with \textit{mef}(A) was the resistance to penicillin and trimethoprim/sulfamethoxazole (21 out of 61).

Resistance surveillance and detection of resistance determinants have become necessary because of increasing resistance of invasive pneumococci, their clonal spread and multiresistance. The results obtained in the present study represent the basis for a further molecular research, survey and control over the spread of resistance of invasive pneumococci in Slovenia.

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