Resistance to trimethoprim–sulfamethoxazole and modifications in genes coding for dihydrofolate reductase and dihydropteroate synthase in European Streptococcus pneumoniae isolates

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Sir,

Trimethoprim and sulfamethoxazole are used extensively in combination as the drug co-trimoxazole. Both compounds interfere with the biosynthesis of folic acid. Trimethoprim is a diaminopyrimidine that selectively inhibits bacterial dihydrofolate reductase (DHFR) and, in doing so, prevents the reduction of dihydrofolate to tetrahydrofolate. Sulfamethoxazole is a sulphonamide that competes with para-aminobenzoate for dihydropteroate synthase (DHPS), and thus prevents the production of 7,8-dihydropteroate.1–3

The purpose of this study was to analyse the prevalence of co-trimoxazole-resistant European Streptococcus pneumoniae isolates and to provide a molecular epidemiological survey of mechanisms of resistance to trimethoprim–sulfamethoxazole.

A total of 1191 European clinical S. pneumoniae isolates, collected between 1997 and 1999 at 24 university hospitals participating in the European SENTRY programme, were included in the study. They were isolated from bacteremias (18% of the total number collected), community-acquired respiratory infections (72%) and nosocomial pneumonias (10%). Only one isolate per patient, considered clinically significant according to local criteria, was submitted.

Antimicrobial susceptibility testing of the isolates was performed using a broth microdilution method as recommended by the NCCLS.4 Of the 1191 S. pneumoniae isolates tested, 72.6% were penicillin susceptible, 19.9% penicillin intermediate and 7.5% penicillin resistant. Resistance to co-trimoxazole was more common among penicillin-intermediate strains (26.8%) than among penicillin-susceptible ones (5.0%), and most common among penicillin-resistant isolates (50.0%). The resistance rates for co-trimoxazole varied widely between different countries: Austria (42 S. pneumoniae isolates collected, 0% penicillin resistance, 0% co-trimoxazole resistance), Belgium (76, 10.5%, 21%), France (232, 15.9%, 19.4%), Germany (204, 1.5%, 2.4%), Greece (16, 25.0%, 12.5%), Israel (48, 6.3%, 12.5%), Italy (72, 0%, 6.9%), The Netherlands (32, 0%, 3.1%), Poland (136, 2.9%, 12.5%), Portugal (90, 2.2%, 5.6%), UK (38, 2.6%, 5.2%) and Turkey (2, 0%, 50.0%). Co-trimoxazole resistance was observed for isolates from all countries except Austria, and varied from 2.4% in Germany to 22.4% in Spain. The data from Turkey were not taken into account due to the low number of isolates. In Italy the resistance rate for co-trimoxazole was 6.9%, but none of the isolates tested was resistant to penicillin. However, for most countries there was a clear relationship between penicillin resistance and resistance to co-trimoxazole.

Fifty of 158 co-trimoxazole-resistant isolates were analysed for mutations in the DHFR gene as well as for duplications within sulA, which codes for DHPS. These 50 isolates were selected on the basis of having different antibiotic resistance profiles, being isolated from various sources (blood cultures, respiratory secretions) and being resistant to trimethoprim as well as to sulfamethoxazole.

The DHFR genes were sequenced and all 50 isolates displayed the amino acid change Ile-100→Leu, as described previously.1,2,5 This mutation is in the same position as Ile-94 of Escherichia coli DHFR, which is involved in the binding of hydrogen to the 4-amino group of trimethoprim. Replacement of Ile by Leu presumably disrupts the bond with trimethoprim without affecting DHFR binding. This allows the production of tetrahydrofolate to continue normally in the presence of trimethoprim.1 Besides the Ile-100→Leu change, other amino acid changes have been found in our study, i.e. Glu20→Asp, Pro70→Ser,
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Ile 74→Leu, Gln 81→His, Gln 91→His, Asp 92→Ala and Leu 135→Phe. It is unclear whether these alterations also result directly in trimethoprim resistance, but additional alterations seem to enhance resistance and modulate the effects of existing alterations on the affinity of DHFR for its natural substrates.5

Initial studies on sulphonamide resistance in a laboratory mutant revealed the duplication of amino acids 66 and 67 (termed sul-d) in sulA, the chromosomal DHPS gene. A plasmid containing this mutated sulA gene was unable to confer resistance to sulphonamides. Additional sequence comparisons of resistant and susceptible clinical pneumococcal isolates have revealed the duplication of either three or six bases, which results in the repetition of one or two amino acids in the Arg-58–Tyr-63 region of the DHPS of resistant isolates. Transformation experiments showed that the duplications are sufficient to confer high-level sulphonamide resistance.1,3 In our study, all 50 isolates displayed such base insertions, namely GAA (Glu) combined with AGC (Ser) or AGT (Ser) alone between codons 61 and 62, AGT (Ser) combined with AGC (Ser) as well as AAC (Asn) or AGT (Ser) or AGC (Ser) alone between codons 62 and 63, and AGC (Ser) combined with TAT (Tyr) between codons 63 and 64. While most of the insertions have been described previously as duplications that result in the repetition of one or two amino acids in the codon structure, the AAC (Asn) and GAA (Glu) insertions observed in two of the present isolates were recognized for the first time and are not duplications of pre-existing sequences in the sulA region coding for amino acids 61–63 of the DHPS. Recently, Haasum et al.6 confirmed that such repetitions are sufficient for development of a resistant enzyme and suggested that the fitness cost to the organism of developing resistance may be very low.

In summary, the results of the current molecular epidemiological surveillance indicate that co-trimoxazole resistance in European S. pneumoniae has become a serious problem. The study also presents a molecular survey of the resistance mechanisms found in recent European S. pneumoniae isolates that can be used in future investigations into changes in co-trimoxazole susceptibility patterns and resistance mechanisms.

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