highly rifampicin-resistant Escherichia coli laboratory strain as recipient, followed by plasmid DNA analysis, showed that all isolates harboured the same two plasmids, with approximate sizes of 110 and 50 kb. The plasmids were readily segregated, were both self-transmissible, and conferred distinct multiresistant phenotypes. The 110 kb plasmid was similar to the SHV-5-encoding plasmids that belong to incompatibility group L/M (IncL/M) and are widely disseminated among clinical enterobacteria in Europe, and especially in the Mediterranean region. PCR assays based on published sequences of the above mentioned IncL/M plasmids (AJ245670 and AF550679 in GenBank) and sequencing of the amplicons, showed that the 110 kb plasmid carried a blaSHV-5 gene adjacent to an integrase similar to In-T3, that contained aacC1, dhfr1, aada and sul2. This plasmid also mediated resistance to chloramphenicol, but the respective determinant was not examined. The antibiotic resistance region of the 50 kb plasmid included an intact class 1 integron containing blaSHV-5, aacA4, dhfr1, aada and sul2, as described previously (AY339625 in GenBank). This plasmid was similar to a group of IncN, VIM-1-encoding plasmids, found recently in clinical enterobacteria in Greece. In addition, a mutation in the gyrA gene associated with resistance to quinolones (Ser-83→Phe) was detected in all isolates. gyrB and parC were not examined.

Panresistant K. pneumoniae have been noticed in the intensive care units of various Athens hospitals since 2001, coinciding with the emergence of MBL-positive enterobacteria. We have shown that this phenotype can be caused by the acquisition of two multiresistant plasmids, able to co-exist within a single cell, since they belong to different incompatibility groups. Until now, VIM-1-producing K. pneumoniae were susceptible to aztreonam—a poor substrate for the VIM-1 MBL—and gentamicin, which remains unaffected by the AMG-modifying enzymes encoded by the VIM-1 plasmids. However, in the isolates presented here, these two drugs are also rendered inactive, by expression of blaSHV-5 and aacC1, carried by the 110 kb plasmid. Another notable characteristic of these isolates was their high-level resistance to carbenapenem, contrary to the majority of VIM-producing enterobacteria, where carbenapenem MICs remain within the susceptible range. Carbenapenemase activity of cell extracts, assessed by spectrophotometry, did not reveal significant differences in the VIM-1 amounts produced by these highly resistant isolates, compared with the less resistant ones. Therefore, it is possible that mutations affecting intracellular carbenapenem accumulation contribute to the elevated MICs, but this was not examined further.

PFGE typing suggested that the isolates may be genetically related. This clonal hypothesis is not contradicted by the identical gyrA mutations. Despite the propitious and ubiquitous antibiotic selection pressure in the nosocomial environment, these panresistant strains still only account for a small fraction of all VIM-positive K. pneumoniae isolated. Nevertheless, emergence of other enterobacterial clones with a similar phenotype can be expected, given that both plasmids are endemic in this country.

The limited number of cases and the serious nature of patients’ conditions that led to frequent changes in antibiotic treatment, do not allow us at present to make any firm suggestions for an optimal therapeutic scheme. So far, colistin, various antibiotic combinations, and non-conventional dosing schemes have been proposed for the treatment of life-threatening infec-
tions caused by panresistant non-fermenting bacilli. Our isolates appeared susceptible to colistin by disc diffusion. However, we also determined the activity of aztreonam in the presence of clavulanic acid (2 mg/L) and tazobactam (4 mg/L), and found that both inhibitors partly restored activity of the antibiotic, lowering the MICs to 1–2 mg/L. This approach could therefore also be of clinical relevance.

References


The Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dki072
Advance Access publication 10 March 2005

Emergence of Staphylococcus hominis strains expressing low-level resistance to quinupristin/dalfopristin in Greece

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Keywords: streptogramins, staphylococci, resistance, Greece

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Sir,

Quinupristin/dalfopristin, a semi-synthetic derivative of pristinamycin IA (streptogramin B) and pristinamycin IIA (streptogramin A), respectively, was introduced in Greek hospitals in 2002, for the treatment of infections caused by multiresistant Gram-positive bacteria such as vancomycin-resistant enterococci and teicoplanin-resistant staphylococci. In Greece, where natural mixtures (pristinamycin, synergistin, etc.) have not been used orally and topically, and virginiamycin has been never used as a growth promoter in animal feed, staphylococci resistant to streptogramins were not isolated until 2002. This is the first report of the emergence of staphylococci resistant to quinupristin/dalfopristin in Greece.

A total of 850 staphylococci [350 Staphylococcus aureus and 500 coagulase-negative staphylococci (CoNS)] were tested for their susceptibility to quinupristin/dalfopristin. The isolates were recovered during 2002–2004 from clinical specimens (blood, pus, etc.) from individual patients in two tertiary care hospitals, University Hospital of Patras and University Hospital of Larissa, located in south–western and in central Greece, respectively. These institutions are hospitals with 750 and 600 beds, respectively, about 229,667 ambulatory visits and 107,000 admissions per year and they cover an area of 2,500,000 inhabitants, roughly 25% of the total population of Greece. The identification of isolates was carried out by Gram-stain, catalase and coagulase production, and the API Staph System (bioMérieux SA, Lyon, France). The susceptibility of isolates to antimicrobial agents (ampicillin, oxacillin, trimethoprim/sulfamethoxazole, ofloxacin, clindamycin, erythromycin, gentamicin, tobramycin, rifampicin, tetracycline, fusidic acid, vancomycin, linezolid and quinupristin/dalfopristin) was determined by the disc diffusion method.2 MIC determination of quinupristin/dalfopristin was assessed by Etest, according to the procedures of the manufacturer, and by the reference agar dilution method.3 The classification of isolates as susceptible or resistant was carried out according to the NCCLS criteria (susceptible = 1 mg/L, resistant = 4 mg/L). Isolates with MICs = 1 mg/L were tested for the presence of genes encoding resistance to streptogramin A [vat(A), vat(B), vat(C), vga(A), vga(B), vga(AV)] and streptogramin B [erm(A), erm(C), msr, vgb(A), vgb(B)] by PCR.4,5 The presence of the mecA gene was also detected by PCR.5 The clonality of the isolates was determined by pulsed-field gel electrophoresis (PFGE) of SmaI DNA digests.6

Among S. aureus isolates, none was found to express resistance to quinupristin/dalfopristin. Their MICs ranged from 0.19 to 0.75 mg/L (mean value 0.35 mg/L). The majority of CoNS, resistant against erythromycin, clindamycin, tetracycline, fusidic acid, vancomycin, linezolid and quinupristin/dalfopristin, was determined by disc diffusion method. This approach could therefore also be of clinical relevance. The MICs to 1–2 mg/L. This approach could therefore also be of

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