Linezolid resistance in clinical isolates of Staphylococcus aureus

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Sir,

Linezolid is the first oxazolidinone antibiotic to be licensed for treatment of Gram-positive infections. It is active against methicillin-resistant Staphylococcus aureus (MRSA), glycopeptide-intermediate S. aureus (GISA) and vancomycin-resistant enterococci (VRE). It inhibits the formation of the 30S ribosomal subunit. Mutations to the central loop of domain V of 23S rRNA have been associated with resistance to linezolid in several species in vitro. There has been one report of resistance of MRSA in a clinical infection, a patient with dialysis-related peritonitis. We report a second clinical infection due to a resistant MRSA, but unlike the earlier case this was shown to have developed from a susceptible isolate during treatment.

A 52-year-old man underwent thoracotomy and drainage of right-sided empyema. On day 20 post-operation, MRSA susceptible to linezolid was isolated from sputum culture, empyema drain site and the empyema fluid. The thoracic drain was removed and the patient was treated with teicoplanin and rifampicin with good clinical response.

Eleven isolates of MRSA from this patient were collected during his hospital stay and tested in parallel by BSAC disc testing, Etest methodology (AB Biodisk, Solna, Sweden) and NCCLS broth microdilution testing. BSAC disc testing was performed on Iso-Sensitest agar (ISA, Oxoid, Basingstoke, UK). In accordance with the trial protocol, linezolid susceptibility was determined using 30 µg linezolid discs and a zone diameter of ≥18 mm. Etest was performed on Mueller–Hinton agar with 2% sodium chloride using a 0.5 McFarland standard inoculum. Isolates with an MIC ≤4.0 mg/L by this method are considered susceptible to linezolid, and isolates with an MIC ≥8.0 mg/L are resistant. NCCLS broth microdilution methodology was used to determine susceptibility to linezolid and three other antimicrobials, erythromycin, teicoplanin and vancomycin. MICs were determined using the NCCLS broth microdilution method in cation-adjusted Mueller–Hinton broth (Trek Diagnostic Systems Ltd, East Grinstead, UK) with an inoculum of 3–7 × 10^5 cfu contained in 100 µL of medium. After incubation for 16–24 h (24 h for vancomycin) MIC endpoints were read.

The clonal relationship between the isolates was examined using phage typing (Dr B. Cookson, Staphyloccocal Reference Laboratory, Central Public Health Laboratory, Colindale, London, UK) and by pulsed-field gel electrophoresis (PFGE) of Smal-macrodigested genomic DNA. Alleles 1, 2 and 4–6 of the 23S rRNA gene were amplified using a reverse primer common to each allele (SA23SR, 5′-GATCTTATAACCGAAGTTGGG-3′) and a primer unique to the sequence –3 kb upstream of each allele (SA23SS5K1, 5′-CGGCTATTGGATGCAATTGG-3′; SA23SS5K2, 5′-AGCATCTGCGCTTACAAGCAG-3′; SA23SS5K4, 5′-TGATGTTATAGTTTCATACG-3′; SA23SS5K5, 5′-GGTATGCGATAATTTGTCAGC-3′; SA23SS5K6, 5′-AGAACATCTTACACTGGAGAAG-3′). Allele 3 was amplified using a reverse primer –3 kb downstream of the allele (SA23SS5K3, 5′-TGCTACCGCCGCTGCTTC-3′) and a primer (SA23SS5K3, 5′-CACTCACAAAGATTATAAACGC-3′) immediately upstream of the allele. Amplified products were sequenced using five primers covering the complete 23S rRNA gene sequence (SA23SeqR1, 5′-TTGTTAACAGCACAGGTTACG-3′; SA23SeqF1, 5′-GC-CCAAACCACGACGGCAGG-3′; SA23SeqF2, 5′-CAGC-AAAACCTTGAAATGCC-3′; SA23SeqF3, 5′-GAAGACAATTTGATTCCTTGAG-3′; SA23SeqF4, 5′-ACTACCCCTAGCTGTGTTGCG-3′). Nucleotide sequences were aligned using the Clustal method with EditSeq and MegAlign software (DNASTAR, Madison, WI, USA).

The MIC of linezolid for the MRSA was 2 mg/L during treatment. The linezolid-resistant isolate was first detected in a sample 20 days after linezolid treatment finished (Table 1). There were separate populations of MRSA with some
colonies that were linezolid susceptible (MIC 1.0–2.0 mg/L by Etest) and others linezolid resistant (MIC 8.0–32.0 mg/L).

Empyema fluid on day 71 post-operation contained both linezolid-susceptible and -resistant strains, which were resistant and susceptible to erythromycin, respectively. All isolates were susceptible to gentamicin, rifampicin, tetracycline, fusidic acid and trimethoprim, and resistant to ciprofloxacin.

Phage typing of the susceptible and resistant isolates showed them to be indistinguishable E-MRSA 15 (Dr B. Cookson). All isolates had identical banding patterns on PFGE. Sequencing of the resistant isolates demonstrated a G2576T (Escherichia coli numbering) mutation in domain V of 23S rRNA genes compared with susceptible isolates and the S. aureus reference sequence (GenBank accession number X68425). An isolate with an MIC of linezolid of 8 mg/L demonstrated the G2576T mutation in two of six alleles, but the mutation was in five of six alleles in an isolate with an MIC of 32 mg/L. In contrast, the linezolid-susceptible and ATCC strains (25922, 29213 and 43300) had wild-type G2576 in all six alleles. This result differs from the previous clinical report in which the sequence traces suggested that all six alleles were mutated.3

In contrast to the earlier report,4 the linezolid-resistant strain developed from a susceptible strain during treatment. The mutation is the same as that observed in vitro and in the earlier clinical isolate,2,3 and both the mutations and increasing linezolid resistance developed in a stepwise manner during therapy. The previously described relationship between susceptibility to linezolid and erythromycin was also present.5 The development of resistance to linezolid following a 3 week course of treatment indicates that use of this antibiotic should be carefully monitored in deep-seated infections.

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References
