Standard disc testing was used to determine antibiotic susceptibility and erythromycin resistance phenotypes. DNA from test samples was prepared by both Qiagen extraction and rapid boiling. As the efficiencies of the two methods were comparable, the rapid boiling method was used throughout. Primers were as published for erm(A), erm(B), and mef(A/E)5 and erm(TR).4 Donor and acceptor probe-pairs were designed for erm(A), erm(TR), erm(B), and mef(A/E) respectively; ERMADP1, CTGCAACG-GCTTTGGGGTTTCTA and ERMAAP1, AATGGTGAGGATG-GATATAAATATGC; DF-ERMAL, GTCAAGCGAATATAG-CTACCTT and DR-ERMAL1, TGTAGAGGGAATTTGGCTA; ERMA/M1, CGTGTGACCTTTAATTCACCAAGAT and ERMA/M1, TCTAGGTCTTTACTTTAATCAAAAA; ERMD/C1, GTATGGTCCAAGAATAT and ERMD/C1, TACTCTA-ACCTAAAGTGAA; MEFA/ED1, TATCCGTGACATTGG-GAACAGCT and MEFA/EA1, TTCTATCCCCAGCACTCAAGT-CGGT. All donor probes were 3’ end-labelled with fluorescein. Acceptor probes specific for erm(A), erm(B), and erm(C) and erm(TR) were 5’ end-labelled with LC Red 640, and LC Red 705. Each acceptor probe had a 3’-phosphate blocking group.

Each 20 μL PCR mixture consisted of: 2 μL of FastStart DNA Master Hybridisation Probes (Roche Applied Sciences), 1.6 μL of 25 mM MgCl2, 50 pmol of primers, 10 pmol of each probe pair, Master Hybridisation Probes (Roche Applied Sciences), 1.6 strains with the MLSB phenotype. All strains with the M-phenotype controls; similar peaks were produced by the clinical isolates. reported in staphylococci.

The most common resistance gene may be present.4 Many of the existing PCR assays are multiplex,3,4 reducing the number of reactions required. The characteristic melting peaks detected in the different channels are being exploited in developing a multiplex assay, which will reduce further the turn-around time and cost.

References

Loss of meca gene in Staphylococcus epidermidis after prolonged therapy with vancomycin

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Keywords: methicillin resistance, S. epidermidis, gene loss, glycopeptides

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Sir,

The most commonly cultured microorganisms in prosthetic-joint infections are coagulase-negative staphylococci.1 Resistance to β-lactam antibiotics is encoded by the meca gene; this gene is carried on a mobile genetic element, the staphylococcal chromosome cassette mec (SCCmec). Loss or deletion of the meca gene rarely occurs, mainly due to factors affecting the stability of SCCmec. Vancomycin may induce deletion of the meca gene in Staphylococcus aureus, as reported in this journal last year.2 We report the case of an implant-associated infection due to a methicillin-resistant Staphylococcus epidermidis which lost the meca gene after prolonged treatment with glycopeptides.

An 82-year-old man was admitted to our hospital because of a prosthetic-joint associated infection with a sinus tract, 4 months...
after implantation of a total hip prosthesis. *S. epidermidis* resistant to oxacillin, ciprofloxacin, trimethoprim/sulfamethoxazole, gentamicin, clindamycin, erythromycin, fusidic acid and rifampin, but susceptible to tetracycline, teicoplanin and vancomycin grew in biopsies from periprosthetic tissue. Species identification was made using API ID 32 Staph system (bioMérieux, La Balme les Grottes, Montalieu Vercieu, France); the MIC of vancomycin was 2 mg/L determined by Etest (AB Biodisk, Solna, Sweden) in Mueller–Hinton (MH) agar. A spacer-free, two-stage exchange of the prosthesis with an 8 week interval was performed and antimicrobial chemotherapy was administered during the first 6 weeks.\(^1\) The patient was treated with a 5.5 week course of antimicrobial chemotherapy was administered during the implant-free-interval, and a complete recovery was made.

Unfortunately, relapse occurred 4 months after reimplantation. Again, *S. epidermidis* was isolated, but showed two different phenotypes in susceptibility tests. While one strain was indistinguishable from the pathogen causing the primary infection, the second type was susceptible to oxacillin, but otherwise there was no difference in the resistance pattern. Both strains showed no increase in MIC of vancomycin, as revealed by Etest in MH agar. PCR for the mecA gene was negative in the second type, but positive in the original isolate. Interestingly, pulsed-field gel electrophoresis (PFGE) after *Sma*I digestion revealed only a slight difference between the two strains. The distance between two bands at ~140 kb and at ~160 kb was shorter in the mecA-positive strain (arrow).

To our knowledge, in vivo loss of mecA gene in *S. epidermidis* after treatment has only been published in abstract form, without reporting any further details about antimicrobial agents and duration of therapy.\(^3\) Acquiring methicillin resistance is attributed to intraspecies transfer of SCCmec,\(^4\) but little is known about the loss or deletion of the mecA gene. Long-term storage, high temperatures and UV radiation have been described to be factors influencing the stability of SCCmec in vitro.\(^2\) Moreover, experiments have shown that coagulase-negative staphylococci exposed to glycopeptides may lose high-level resistance to oxacillin, without loss of the mecA gene.\(^5\) Recently published findings indicate that the acquisition and/or loss of SCCmec in *S. epidermidis*, may occur in the region of the orfX gene.\(^6\) Our case illustrates that the mecA complex may lose its stability after prolonged antimicrobial treatment.

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The PROTEKT global study (year 4) demonstrates a continued lack of resistance development to telithromycin in *Streptococcus pneumoniae*

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