Cfr-mediated linezolid resistance in methicillin-resistant Staphylococcus aureus and Staphylococcus haemolyticus associated with clinical infections in humans: two case reports

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Sir,

Linezolid is a last-resort antimicrobial agent in human medicine approved for the control of serious infections caused by Gram-positive pathogens. Transferable linezolid resistance is based on the multiresistance gene cfr. Although originally found in staphylococci, this gene has meanwhile been reported in various Gram-positive and even Gram-negative bacteria. The emergence of the multiresistance gene cfr in staphylococci is of global concern. Here we report the detection of the cfr gene in a methicillin-resistant Staphylococcus aureus and a methicillin-resistant Staphylococcus haemolyticus strain from two clinical cases of septic shock identified in the same hospital in Spain.

Case 1

A middle-aged patient with no known underlying medical conditions was admitted to the hospital with a fever of 39°C, tachycardia, hypotension and oliguria. The abdominopelvic CT scan showed a peri-rectal and peri-anal liquid collection, which spread to the pre-sacrum and gluteal region, with air content and air–fluid levels in the peri-anal region.

The patient was admitted to the intensive care unit (ICU) with a diagnosis of peri-anal abscess and septic shock. The surgical inspection identified peri-anal fistulae. Several samples were obtained for microbiological analysis. On admission to the ICU, empirical treatment with linezolid and meropenem was started. The surgical inspection revealed peri-anal gangrene, which required a substantial excision of necrotic tissues. The patient’s condition deteriorated, with the development of respiratory distress and refractory septic shock. Refractory septic shock required the addition of vasoactive amines to the treatment. Two days after the patient’s admission, a methicillin-resistant S. aureus (MRSA17) was identified, which was resistant to linezolid, clindamycin, gentamicin, fluoroquinolones and co-trimoxazole, but susceptible to glycopeptides. The unfavourable clinical course continued and the patient died 2 days later.

Case 2

An elderly patient was admitted to the emergency department of the university hospital having suffered a syncope in the street that resulted in a fall to the ground accompanied by frontotemporal trauma. On admission, the patient was unconscious, with miotic and unreactive pupils and apneustic breathing. The patient underwent endotracheal intubation and mechanical ventilation. A CT scan showed a right temporal bone fracture, subarachnoidal and subdural bleeding in the right hemisphere, and posterior interhemispheric and tentorial haematomas.

The patient was admitted to the ICU for haemodynamic stabilization and neurosurgical evaluation. The patient then developed aspiration pneumonia, and viridans group Streptococcus and Escherichia coli were isolated from bronchoalveolar lavage fluid. A control cranial CT scan showed the previously referred findings and a left parieto-occipital infarction. Despite the empirical treatment for aspiration pneumonia with linezolid, clindamycin and tobramycin, the patient developed septic shock. The blood cultures were positive for a Pseudomonas aeruginosa resistant to β-lactams, including carbapenems, but susceptible to aminoglycosides, fluoroquinolones and colistin, and a methicillin-resistant S. haemolyticus (MRSH18) that was resistant to lincosamides, aminoglycosides, fluoroquinolones and linezolid, but susceptible to co-trimoxazole and glycopeptides. The septic shock progressed to refractory shock and the patient died soon after.

Characterization of the methicillin-resistant staphylococci

The two methicillin-resistant Staphylococcus strains—MRSA17 and MRSH18—were characterized and investigated for the genetic basis of linezolid resistance and the location of the corresponding resistance gene. Strain MRSA17 was subjected to multilocus sequence typing (http://saureus.mlst.net), spa typing (http://spaserver.ridom.de) and dru typing (http://dru-typing.org). The dru typing was also
applied to strain MRSH18. Antimicrobial resistance genes were detected by microarray analysis and specific PCR assays.\(^6\)\(^5\) Antimicrobial susceptibility testing was performed by broth microdilution.\(^6\) The linezolid susceptibility testing was performed by VITEK (bioMérieux, Marcy l’Etoile, France). Plasmid transformation experiments were conducted and the plasmids were further characterized by restriction analysis. The genetic environment of the cfr gene was determined by PCR assays and sequence analysis.\(^7\)

Strain MRSA17 belonged to sequence type ST125 within clonal complex 5 and had spa type t067. No dru typing results were obtained in repeated attempts, and sequence analysis of the dru region showed that the variable part, from which the dru type is deduced, had been deleted by the integration of an IS431 element. Strain MRSH18 had the dru type dt11z. Both strains harboured the mecA gene for methicillin resistance. In addition, the MRSA17 strain carried the resistance gene aadD (kanamycin resistance) and the MRSH18 strain the resistance genes aacA–aphD (gentamicin, tobramycin and kanamycin resistance) and aphA3 (kanamycin resistance), respectively. Besides the cfr gene, the penicil exporter gene fexA was also detected in both strains.\(^7\) PCR analysis identified the cfr gene as being inserted in the transposase B gene tnpB of the fexA-carrying transposon Tn558.\(^7\) Sequence analysis revealed exactly the same integration site as previously determined in a human USA300 MRSA strain (ST8, t008) from Ireland (Figure 1a).\(^5\) Transformation experiments confirmed that this truncated Tn558 element was located on ~40 kb plasmids in both strains. Susceptibility testing of the transformants revealed that these plasmids did not carry additional resistance genes and exhibited indistinguishable EcoRI and very similar BglII fragment patterns (Figure 1b).

These results confirmed that the cfr gene was located on structurally closely related plasmids in strains MRSA17 and MRSH18. The plasmid location supports the spread of cfr-mediated linezolid resistance across strain and species borders. Sequence analysis of the cfr region and comparison with a similar plasmid from an MRSA USA300 strain confirmed this observation.

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**Transparency declarations**

S. M. and R. E. are employees of Alere Technologies GmbH and do not hold any stocks or options. All other authors: none to declare.

**References**

Insertion sequence IS18 mediates overexpression of bla_{OXA-257} in a carbapenem-resistant Acinetobacter bereziniae isolate

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Sir,

Acinetobacter bereziniae, previously known as Acinetobacter genomic species 10, has been isolated primarily from clinical specimens and the hospital environment and more rarely from various other sources, including vegetables, soil and animals. Antibiotic resistance is rarely reported in this species. Over the last decade, carbapenem resistance in Acinetobacter spp., mainly Acinetobacter baumannii, has emerged as a threat in hospitals around the world. The most widespread mechanism resulting in carbapenem resistance in Acinetobacter spp. is mediated through carbapenem-hydrolysing class D β-lactamases, also known as oxacillinases. The overexpression of bla_{OXA} genes is often associated with insertion sequences (IS) located upstream and providing strong promoters. Carbapenem resistance in A. bereziniae has previously been associated with the metallo-β-lactamases IMP, SIM and VIM or overexpression of OXA-229, a variant of the intrinsic OXA-228-like, which was mediated by a mutated promoter. To date, OXA-228-like has not been associated with an IS.

In the present study, we investigated a carbapenem-resistant Acinetobacter strain isolated from the bronchial secretions of a patient in Germany in 2012. Isolate KH243 was initially identified as Acinetobacter guillouiae by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. However, rpoB sequencing revealed 100% similarity to the A. bereziniae type strain CIP 70.12 (accession no. DQ207475). By Etest, carbapenem MICs were 12 and >32 mg/L for imipenem and meropenem, respectively. Multiplex PCR for OXA subclasses that are associated with carbapenem resistance in Acinetobacter spp. (OXA-51, OXA-23, OXA-40, OXA-58, OXA-143 and OXA-235) was negative. Based on published A. bereziniae sequences, primers were designed to amplify and sequence the intrinsic bla_{OXA} and its surrounding region from isolate KH243 (Table 1). PCR revealed an unexpected large amplicon of ~2.1 kb. Sequencing of the purified PCR product by primer walking identified IS18 40 bp upstream of a novel bla_{OXA-228} variant, which was numbered bla_{OXA-257} by the Lahey β-lactamase database (http://www.lahey.org/Studies/) and was submitted to GenBank. OXA-257 possessed six amino acid differences compared with OXA-228. The IS18:bla_{OXA-257} nucleotide sequence reported in this paper has been submitted to EMBL/GenBank under accession number KCS67681.

The IS18 insertion element encoded a transposase that harboured eight amino acid changes compared with the IS18 sequence available in the IS database (http://www-is.biotoul.fr/). IS18 was flanked by a 3 bp target site duplication (TTT) and 26 bp imperfect inverted repeats. In Acinetobacter spp., IS are often located upstream of bla_{OXA} genes and, by providing strong promoters, lead to overexpression of the OXA, resulting in carbapenem resistance. For example, the intrinsic bla_{OXA-51}‐like and the acquired bla_{OXA-58}‐like in A. baumannii are often associated with ISAba1 and ISAba3, respectively. IS18 has also been associated with bla_{OXA-58}‐like. Other IS elements include ISAcrA3, which was recently identified and overexpressed bla_{OXA-2} in a carbapenem-resistant Acinetobacter radioresistens isolate.

Two predicted promoters were found upstream of bla_{OXA-257} with both −35 boxes located within the right inverted repeat of IS18. One was a hybrid promoter based on those previously described in A. bereziniae isolates Nec (bla_{OXA-229} and Baz (bla_{OXA-228}). The −35 and −10 boxes were identical to the −35 box in Nec and the −10 box in Baz, respectively, and were conserved in A. baumannii and A. bereziniae

Table 1. Primers used in this study

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