Tigecycline-resistant *Enterococcus faecalis* strain isolated from a German intensive care unit patient

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Sir,

Tigecycline is a member of the new group of glycyclines and a promising new antibiotic of last resort, active against many bacteria including *Enterococcus* spp.1,2 Tigecycline acts in a similar way to tetracyclines by binding to the 30S subunit of the bacterial ribosome and thus inhibiting protein biosynthesis;1 however, different binding capacities and kinetics also allow activity against tetracycline-resistant bacteria.1 Enterococcal isolates displaying MICs of tigecycline of ≤0.25 mg/L are considered susceptible.3 The epidemiological cut-off value (breakpoint) for tigecycline is >0.5 mg/L for enterococci.3 Here, we report the first case of a tigecycline-resistant *Enterococcus* from a German hospital patient isolated in 2007.

The 65-year-old patient underwent an intra-abdominal surgery in October 2006 following a femoral neck fracture. Soon after, she suffered from several post-operative complications including colon perforation, peritonitis, nosocomial pneumonia after long-term ventilation and renal failure. The patient showed septic signs for weeks and months. She died of multorgan failure in June 2007. She was treated with several courses of antibiotics; including, among others, tigecycline for more than 2 weeks to treat multiresistant *Stenotrophomonas maltophilia* isolated from tracheal secretion. Soon after, enterococci were isolated from catheter urine samples with colony counts of $1 \times 10^5$ cfu/mL in January 2007. Species identification revealed *Enterococcus faecalis*. Initial Etest for tigecycline resulted in an MIC of 2 mg/L. The strain (UW6940) was sent to the Robert Koch Institute for confirmation and further characterizations.

Tigecycline susceptibility was determined by broth microdilution and Etest. Non-susceptibility to tigecycline is mediated via efflux porters in *Acinetobacter baumannii* or via mutations in Tet(A)-mediating tetracycline efflux, e.g. in *Escherichia coli*. We tested MICs of tigecycline in the presence and absence of several efflux pump inhibitors.4 Concentrations were chosen as given in the literature and were as follows: reserpine, 20 mg/L; verapamil and omeprazole, 60 mg/L; and prochlorperazine, 2 mg/L (16 mg/L prochlorperazine alone already inhibited growth of the test strain). A single tetracycline resistance gene, *tetX*, encodes an oxygen-dependent mono-oxygenase conferring tigecycline resistance.5 So, MICs of tigecycline were determined under anaerobic and aerobic growth conditions. In addition, we tested amplification of a PCR product specific for *tetX* (primers: tetX-F: CAATAATTGGTGGTGGACCC; tetX-R: TTCTTACC TTGGACATCCC; 468 bp) with DNA from strain UW6940 and a reference *E. coli* strain Em24 pBSJ possessing *tetX* cloned into a pBR328 plasmid vector (kindly provided by Professor M. Roberts, WA, USA). Tigecycline interacts with different target nucleotides of the ribosomal 16S rRNA.1 Mutations at those positions could render UW6940 non-susceptible to tigecycline. We sequenced the 16S rDNA of UW6940 and compared it with the sequence of the tigecycline-susceptible, fully sequenced *E. faecalis* V583. Strain UW6940 was grown for 2 weeks (~400–500 generations) on brain heart infusion (BHI; Difco labs, Sparks, MI, USA) agar plates and in BHI liquid broth in the absence of any selective pressure to test the stability of tigecycline resistance. Transferability of the resistance trait was identified by in vitro filter- and broth-mating using tigecycline-susceptible *E. faecalis* recipient strains JH2-2 and OG1X. The MLST sequence type (ST) was indentified and allocated using a web-based internet service (http://efaecalis.mlst.net/).

Resistance to tigecycline in strain UW6940 was confirmed by Etest, revealing an MIC of 2 mg/L (Table 1). MIC determination in broth medium revealed an MIC of 1 mg/L. Tigecycline MICs for susceptible reference strains *E. faecium* ATCC 19434 and *E. faecalis* ATCC 29212 were between 0.047 and 0.125 mg/L (Table 1). Addition of several efflux pump inhibitor substances did not show any effect (always 1 mg/L), except with omeprazole where addition of it resulted in a several-fold increased MIC (64 mg/L with omeprazole versus 1 mg/L without omeprazole). This intriguing antagonistic effect of omeprazole was obviously concentration-dependent and strain-specific since it did not appear at lower concentrations (20 mg/L) and with enterococcal and staphylococcal reference strains (data not shown). The MIC of tigecycline was not influenced by aerobic or anaerobic growth conditions. In addition, we failed to amplify by PCR an internal fragment specific to the *tetX* resistance gene with DNA from UW6940, whereas we were able to demonstrate an

Table 1. MICs (mg/L) of tigecycline tested by Etest and broth microdilution

<table>
<thead>
<tr>
<th>Strain</th>
<th>Etest</th>
<th>Broth microdilution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BHI agar</td>
<td>MH agar</td>
</tr>
<tr>
<td><em>E. faecalis</em> UW6940</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>S. aureus</em> NCTC 6571</td>
<td>0.064/0.094</td>
<td>≤0.008</td>
</tr>
<tr>
<td><em>E. faecium</em> ATCC 19434</td>
<td>0.047</td>
<td>≤0.008</td>
</tr>
<tr>
<td><em>E. coli</em> ATCC 25922</td>
<td>0.094</td>
<td>0.094</td>
</tr>
<tr>
<td><em>S. aureus</em> ATCC 29213</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td><em>E. faecalis</em> ATCC 29212</td>
<td>0.125</td>
<td>0.125</td>
</tr>
</tbody>
</table>

BHI, brain heart infusion (Difco); MH, Mueller–Hinton (Difco); IST, Iso-Sensitest (Oxoid, Basingstoke, UK).

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expected PCR product with DNA from the tetX-positive reference strain.

The ~1.5 kb 16S rDNA fragment was identical in E. faecalis V583 and UW6940 (data not shown). The sequenced fragment covered all relevant sites of interaction between tigecycline and the 16S rDNA of the 30S ribosomal subunit in the model bacterium Thermus thermophilus.1

The strain was grown for 2 weeks (~400–500 generations) on BHI agar plates and in BHI liquid broth without any selective pressure in order to test the stability of tigecycline resistance. Progenies were sampled after the first and second weeks of passage. MICs of tigecycline for all progenies remained stable at 1 mg/L (data not shown).

The resistance trait was not transferable in vitro irrespective of the test method or recipient used.

Molecular typing using MLST revealed ST6. ST6 belongs to MLST clonal complex 2 combining mainly isolates from hospital outbreaks.6

Our preliminary results show stable tigecycline resistance in an epidemic, hospital-adapted E. faecalis strain type from an intensive care unit patient after prolonged tigecycline therapy. The basis of tigecycline resistance is not due to mutations in the 16S rDNA, tetX-encoded or efflux-mediated. The exact mechan-ism of resistance could not be elucidated.

Acknowledgements

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Transparency declarations

None to declare.

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Nosocomial bloodstream infections due to metallo-β-lactamase-producing Pseudomonas aeruginosa

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Sir,

The worldwide emergence of metallo-β-lactamases (MBLs) has challenged antimicrobial therapy against Pseudomonas aeruginosa, a leading pathogen causative of nosocomial infections.1 In a recent cohort, we showed that the production of this type of enzyme was associated with increased mortality rates in patients with nosocomial infections due to P. aeruginosa.1

Bacterial bloodstream infections (BSIs) are serious infections associated with significant mortality and healthcare costs. Only two reports have analysed mortality of patients with MBL-producing P. aeruginosa (MBL-PA) BSIs.2,3 However, both were limited by a small number of patients and did not compare mortality rates with mortality rates of patients with MBL-PA and non-MBL-PA BSIs. Owing to the importance of BSI, in the current study, we describe the clinical characteristics, treatments and mortality of the subset of patients from our cohort with nosocomial MBL-PA BSIs.

Patients from a contemporary cohort performed at two tertiary-care teaching hospitals in Porto Alegre, southern Brazil,1 who presented positive blood cultures for P. aeruginosa were included in the study. Microbiological and molecular procedures are described in detail elsewhere.1 The Ethics Review Boards of both hospitals have approved this study. Written informed consent was obtained from each participant. Variables were compared using the χ² or Fisher exact test for categorical variables and the Student’s t-test for continuous variables. All tests were two-tailed and a P value ≤0.05 was considered significant.

A total of 44 patients were included in the study. Twenty-one (47.7%) had MBL-PA BSIs (Table 1). The overall hospital mortality of patients with P. aeruginosa BSI was 52.3% (23 of 44):