Intra-hospital dissemination of quinupristin/dalfopristin- and vancomycin-resistant *Enterococcus faecium* in a paediatric ward of a German hospital

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**Objectives:** To demonstrate nosocomial transmission of *Enterococcus faecium* resistant to quinupristin/dalfopristin and vancomycin/teicoplanin among paediatric patients in a German hospital ward.

**Materials and methods:** Multiply-resistant *E. faecium* were isolated from three female patients aged 9 months, 2 and 15 years during a 10 day time span. Antibiotic susceptibilities were determined by microbroth dilution. Clonal relatedness among the isolates was investigated via SmaI-macrorestriction analysis by PFGE, multilocus sequence typing (MLST), and plasmid profiling. Presence of virulence and resistance determinants was tested by polymerase chain reaction (PCR). Selected resistance genes were localized by Southern hybridizations.

**Results:** A single *E. faecium* isolate per patient was investigated. All exhibited resistances to quinupristin/dalfopristin, vancomycin/teicoplanin, streptomycin (high-level), penicillin/ampicillin, erythromycin, oxytetracycline, chloramphenicol, rifampicin and fusidic acid. The isolates were susceptible to linezolid only and intermediately resistant to fluoroquinolones including moxifloxacin. PFGE revealed identical patterns for all three isolates. PCRs for virulence determinants hyaluronidase and enterococcal surface protein, esp, were negative, whereas PCR for the enterocin A gene was positive. MLST identified clonal type [8-5-1-1-1-1-1] belonging to a clonal subgroup C1 of hospital- and outbreak-related *E. faecium*. Southern hybridizations located several resistance genes (*erm(B)*, *vat(D)*, *vanA*) on a large plasmid, which was transferable in mating experiments with an *E. faecium* recipient.

**Conclusions:** These data show routes of dissemination of resistance to multiple antibiotics including streptogramins and glycopeptides in *E. faecium* via vertical and/or horizontal gene transfer. The isolates spread in the absence of a direct selective pressure, as none of the patients had received earlier streptogramin or glycopeptide therapy.

Keywords: quinupristin/dalfopristin, vancomycin, *vanA*, *vat(D)*, VRE

**Introduction**

Enterococci are the third most important nosocomial pathogen worldwide.\(^1\) Strains of vancomycin- and multiply-resistant enterococci raise major concerns in human medicine due to limited treatment options.\(^2\) With the progression of vancomycin resistance, especially among clinical isolates of *Enterococcus faecalis* in the USA, only a few treatment options remain. Two of these antibiotics of last resort are quinupristin/dalfopristin and linezolid. Resistance to these antimicrobials in *E. faecium* is uncommon, and especially for streptogramin resistance, the major reservoir appears to be in agriculture.\(^2\) This is the first description of an intra-hospital spread of quinupristin/dalfopristin- and vancomycin-resistant *E. faecium* among three paediatric patients in a single ward of a German hospital.

**Materials and methods**

**Description of the three cases**

The index case was a 9-month-old patient who was under medical support since birth. As a result of multiple disorders, intensive care treatment including mechanical ventilation was necessary several times. The patient received ceftazidime/cefuroxime for treatment of a urinary tract infection (UTI) of unknown bacterial origin. Bacteriological investiga-
tion revealed *E. faecium* counts of 10 000 cfu/mL urine 3 days after the onset of therapy. Case two was a 15-year-old patient who was hospitalized for 1 month due to a progressing Guillain-Barré syndrome. She received mechanical ventilation via a tracheostomy. The patient was treated with cefuroxime due to a supposed UTI. *Enterococcus* spp. of 10 000 cfu/mL was isolated from the catheter urine (later identified as a mixture of *E. faecalis* and *E. faecium*). Case three was a 2-year-old patient with a progressing peritonitis. The patient received several courses of antibiotic treatment including meropenem, ampicillin/sulbactam, and teicoplanin. *E. faecium* was obtained from wound swabs in low numbers. These three isolates were collected during 10 days, and all patients involved were female. In none of the cases were the enterococci considered to be the cause of infection and no appropriate anti-enterococcal therapy was initiated. The *E. faecium* isolates from the three cases were already identified as multiple resistant in the hospital microbiology laboratory and subjected to further tests for clonal relatedness and other molecular characterizations.

Antibiotic susceptibilities were identified using microbroth dilution according to German standard DIN58940 as already described. According to the resistance phenotype, PCRs for resistance determinants *erm*(B), *vanA/E*, *vanB*, *tetM/L* and *aadE-aphA-3* were carried out. PCRs for virulence markers hyaluronidase (primers *hyl*-1: 5′-GAGTAGAG-GAATATCTTAGC, *hyl*-2: 5′-AGGGTCCAATTCTGTA, enterocin A (entA-F: 5′-TATGGGGGTTACCCTATAG, entA-R: 5′-ACCTA-AAACCCACCTAT), and enterococcal surface protein, *esp* (esp5: 5′-ACGTGGATGTAGAGTTTG, esp6: 5′-GAAATATGTCACCA-CCGTAC) were carried out as described elsewhere. Plasmid analysis and Southern hybridizations were done using digoxigenin-labelled probes and the fluorescence detection system Attophos (Roche Biochemicals, Mannheim, Germany). Resistance determinants were transferred into a rifampicin and fusidic acid high-level resistant recipient (MICs for both >128 mg/L) by filter-mating and transconjugants were selected on agar plates supplemented with oxytetracycline/rifampicin (5/30 mg/L). Fifty transconjugants were randomly chosen and tested on agar plates supplemented with either 30 mg rifampicin or 20 mg fusidic acid excluding high-level rifampicin resistant donor mutants. Phylogenetic analyses including PFGE as well as the corresponding data evaluation were carried out according to recommended schemes. Multilocus sequence typing (MLST) was done for isolate three as described elsewhere.

### Results

All three isolates exhibited an identical antibiotic resistance profile including resistance to quinupristin/dalfopristin (*MIC > 8 mg/L*), vancomycin (512 mg/L), teicoplanin (32–128 mg/L), streptomycin (>2048 mg/L, high-level), gentamicin (16–32 mg/L, low-level), penicillin (16 mg/L), ampicillin (64 mg/L), erythromycin, clindamycin, oxytetracycline (all >8 mg/L), chloramphenicol (16 mg/L), rifampicin and fusidic acid (both 2–4 mg/L). The isolates were only susceptible to linezolid (1 mg/L) and intermediately resistant to fluoroquinolones including moxifloxacin (*MIC 1 mg/L*). PCRs for the appropriate resistance determinants were positive for *vanA/B*, *erm*(B), *tetM*, *tetL*, and *aadE-aphA-3* for all three isolates. Small-macromolecular restriction analysis via PFGE showed an identical pattern for all three isolates indicating clonal distribution of a single strain (Figure 1). Moreover, all three isolates showed an identical plasmid pattern (Figure 2a). Southern hybridizations located determinants for glycopeptide (*vanA*), macrolide-lincosamide-streptogramin B (*erm*(B)), and streptogramin A (*van*(D)) resistances on a single, large plasmid (Figure 2b). All three labelled probes hybridized to a 23 kb large BamHI plasmid fragment suggesting a possible arrangement of these determinants in a composite cluster (not shown).

An *in vitro* filter-mating with isolate three and a rifampicin- and fusidic acid high-level resistant recipient revealed 1.4 × 10⁴ transconjugants per recipient cell. Fifty randomly chosen transconjugants were tested for a co-transfer of other resistance determinants by replica-plating. Thirty-three (66%) were only oxytetracycline resistant whereas the other 17 (34%) were oxytetracycline, vancomycin, erythromycin, and quinupristin/dalfopristin resistant. Five randomly chosen transconjugants were susceptible to penicillin/ampicillin and fluoroquinolones by microbroth dilution indicating clonal relatedness with the recipient (in *E. faecium* ampicillin and fluoroquinolone resistance determinants are not transferred in most matings).

Analysis of phylogenetic relationships of isolate three within the species *E. faecium* by MLST revealed the profile [8-5-1-1-1-1-1]. It included a new allele type for the *adk* gene which was deposited in GenBank (AY205312) and designated allele type adk-8 (in reference to R. J. L. Willems and D. Tribe; www.mlst.net). The corresponding MLST profile suggested that this strain belongs to clonal group C1 (definition of clonal group: 5/7 allele types identical). Subgroup C1 defines a cluster of epidemic *E. faecium* strains disseminated worldwide and isolated mainly from outbreaks or single infections. Most of these isolates also possessed the *esp* gene serving as an epidemic marker. However, when tested by PCR and dot blot hybridizations of genomic DNA, none of the three isolates was *esp* positive. PCR tests for two other virulence determinants frequently found in *E. faecium* hospital isolates, hyaluronidase and enterocin, were negative and positive, respectively.

### Discussion

To our knowledge, this is the first report on quinupristin/dalfopristin-resistant *E. faecium* spreading among patients in a hospital ward.
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Therapy with third-generation cephalosporins is known to select enterococci in the patient’s intestinal flora due to the intrinsic resistance of enterococci to all cephalosporins. This is also likely for the three cases described here. The isolates, however, were not thought to be causing clinical infection (except in case three where the strain might have been involved in a polymicrobial peritonitis). Accordingly, anti-enterococcal therapy was not commenced in any of the affected patients. Quinupristin/dalfopristin had not been used in this hospital during the last 18 months. Therefore resistance to this agent in the E. faecium clone was not selected by antibiotic therapy in the index patient but had been introduced to the hospital from outside. MLST analysis revealed that the investigated E. faecium strain belonged to a group of hospital-adapted and outbreak-related strains. In vitro mating experiments showed conjugative transfer of a multi-resistance plasmid between enterococcal strains suggesting a possible acquisition of this plasmid from an unknown (possibly enterococcal) source by the hospital-adapted E. faecium clone. This has already been described for hospital-adapted E. faecium strains from Finland and Poland acquiring vanA/B plasmids. Reservoirs for resistant enterococci harbouring transferable resistance genes are known to exist outside humans, especially in food animals.

References


Figure 2. Non-digested plasmid DNA from isolates 1–3. (a) Samples resolved in a 1% agarose gel; (b) corresponding Southern blot hybridized with a digoxigenin-labelled probe for vanA; (c) corresponding Southern blot hybridized with a digoxigenin-labelled probe for vat(D); (d) corresponding Southern blot hybridized with a digoxigenin-labelled probe for erm(B). Lanes: M, size marker for orientation; 1–3, isolates 1–3.

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