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# Letters to the Editor

## Gentamicin-Resistant *Enterococcus faecalis* Sequence Type 6 with Reduced Penicillin Susceptibility: Diagnostic and Therapeutic Implications▽

In 2007, 20 *Enterococcus faecalis* isolates from bloodstream infections diagnosed at seven hospitals in the North Denmark region were recorded by the regional clinical microbiology laboratory as being penicillin resistant (MICs ≥ 16 μg/ml) and ampicillin susceptible (MICs ≤ 4 μg/ml) using Etest (AB bioMérieux, Solna, Sweden). Seventeen and two isolates were additionally resistant to gentamicin (MICs ≥ 1,024 μg/ml) and imipenem (MIC = 32 μg/ml), respectively (Table 1).

Due to the unusual susceptibility pattern obtained by Etest, the penicillin MICs were measured by broth microdilution (BMD) according to the Clinical and Laboratory Standards Institute (CLSI) (1). Twofold dilutions of penicillin G sodium salt (Sigma-Aldrich Denmark A/S, Copenhagen, Denmark) in the range between 0.5 and 32 μg/ml were prepared using Mueller-Hinton broth (MHB, Oxoid A/S, Greve, Denmark). Thirteen *E. faecalis* blood culture

TABLE 1. Origin, MICs determined by Etest, and genotypes based on partial (*gdh* gene) or full multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) of 33 *E. faecalis* isolates from bloodstream infections in the North Denmark region in 2007

| Strain ID | Site of infection[a] | Origin[b] | Hospital[c] | Etest MIC (μg/ml) for[d]: | | | | | MLST | | PFGE profile |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | AMP | PEN | IPM | GEN | VAN | *gdh* | ST | |
| Isolates with reduced susceptibility to penicillin | | | | | | | | | | | |
| B69486 | AVP | C | 4 | 1.5 | >32 | 6 | >1024 | 3 | 12 | 6 | A1 |
| B135456 | Urinary tract | N | 6 | 4 | >32 | 8 | 96 | 2 | 12 | 6 | A1 |
| B33621 | Urinary tract | N | 7 | 1 | 16 | 8 | 1024 | 3 | 12 | 6 | A1 |
| B37814 | Undetermined | N | 8 | 1.5 | >32 | 8 | >1024 | 3 | 12 | 6 | A1 |
| B63792 | Urinary tract | NH | 8 | 1.5 | >32 | 6 | >1024 | 4 | 12 | 6 | A1 |
| B71580 | HBP tract | HC | 8 | 1.5 | >32 | 8 | >1024 | 4 | 12 | 6 | A1 |
| B98380 | Undetermined | C | 1 | 3 | 16 | 4 | >1024 | 2 | 12 | 6 | A2 |
| B15725 | Undetermined | N | 8 | 1.5 | 32 | 32 | >1024 | 3 | 12 | 6 | A2 |
| B72715 | Urinary tract | N | 4 | 2 | >32 | 6 | 1024 | 3 | 12 | 6 | A3 |
| B37093 | Undetermined | N | 8 | 1 | >32 | 4 | >1024 | 3 | 12 | 6 | A3 |
| B33672 | Perirenal abscess | N | 3 | 1 | 32 | 4 | 1024 | 3 | 12 | 6 | A4 |
| B54027 | IV catheter | N | 8 | 3 | >32 | 6 | >1024 | 3 | 12 | 6 | A4 |
| B147794 | Diabetic gangrene | N | 8 | 0.75 | >32 | 4 | >1024 | 2 | 12 | 6 | A4 |
| B110274 | Undetermined | N | 8 | 3 | >32 | 4 | >1024 | 3 | 12 | 6 | A5 |
| B85040 | Urinary tract | HC | 8 | 1.5 | 16 | 4 | >1024 | 3 | 12 | 6 | A6 |
| B67010 | Endocarditis | N | 8 | 1.5 | >32 | 8 | >1024 | 3 | 12 | 6 | A7 |
| B37957 | Surgical wound | N | 8 | 1.5 | >32 | 6 | >1024 | 3 | 12 | 6 | A8 |
| B84847 | HBP tract | N | 5 | 1.5 | >32 | 2 | 16 | 3 | 2 | 23 | ND[e] |
| B56765 | Undetermined | N | 8 | 4 | >32 | 32 | >1024 | 4 | 4 | 28 | ND |
| B16457 | Undetermined | N | 8 | 4 | 32 | 8 | 96 | 4 | New | New | ND |
| Isolates with full susceptibility to penicillin | | | | | | | | | | | |
| B84828 | HBP tract | N | 2 | 2 | 4 | 2 | 16 | 3 | 12 | New | ND |
| B115329 | Abdomen | N | 4 | 2 | 3 | 2 | 24 | 2 | 12 | New | ND |
| B104838 | Urinary tract | N | 8 | 2 | 2 | 2 | 12 | 3 | 1 | ND | ND |
| B56478 | Urinary tract | C | 3 | 1.5 | 4 | 1.5 | 64 | 3 | 3 | ND | ND |
| B96280 | Urinary tract | N | 8 | 1 | 1 | 2 | 12 | 3 | 3 | ND | ND |
| B30537 | Undetermined | HC | 8 | 1.5 | 4 | 2 | >1024 | 3 | 5 | ND | ND |
| B150692 | Endocarditis | N | 8 | 0.5 | 1.5 | 3 | 16 | 3 | 5 | ND | ND |
| B126859 | Urinary tract | N | 4 | 3 | 6 | 2 | 16 | 3 | 7 | ND | ND |
| B70401 | Biliary tract | N | 8 | 2 | 12 | 2 | 48 | 4 | 7 | ND | ND |
| B123830 | IV catheter | N | 8 | 3 | 3 | 2 | 24 | 4 | 7 | ND | ND |
| B75968 | Undetermined | HC | 7 | 1.5 | 2 | 2 | 48 | 3 | 16 | ND | ND |
| B28054 | Pancreas | N | 8 | 1 | 6 | 2 | 48 | 3 | 20 | ND | ND |
| B128229 | Surgical wound | N | 8 | 3 | 12 | 1.5 | 24 | 4 | 27 | ND | ND |

[a] AVP, aorthic vascular prosthesis; HBP, hepato-biliary-pancreatic.
[b] N, nosocomial onset; C, community onset; HC, healthcare associated; NH, nursing home associated.
[c] 1, Brovst Hospital; 2, Dronninglund Hospital; 3, Farsø Hospital; 4, Frederikshavn Hospital; 5, Hjørring Hospital; 6, Hobro Hospital; 7, Aalborg Hospital section North; 8, Aalborg Hospital section South.
[d] AMP, ampicillin; PEN, penicillin; IPM, imipenem; GEN, gentamicin; VAN, vancomycin.
[e] ND, not determined.

isolates from hospitalized patients obtained during the same year were randomly selected for comparative purposes based on their susceptibility to penicillin determined by Etest (MICs $\leq$ 12 μg/ml) (Table 1). All 20 isolates were reported as being penicillin resistant according to Etest and the 13 control isolates were found to be penicillin susceptible by BMD based on the CLSI interpretive MIC breakpoint (S $\leq$ 8 μg/ml) and fell into the wild-type *E. faecalis* MIC distribution (1 to 16 μg/ml), provided by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). However, using BMD according to CLSI standards, the penicillin MICs for the isolates that were reported to be penicillin resistant according to Etest were two- to eightfold higher (4 to 8 μg/ml) than those for the control isolates reported to be susceptible by Etest (1 to 2 μg/ml), with the exception of a single isolate displaying a MIC of 2 μg/ml. Gentamicin resistance (MIC $\geq$ 1,024 μg/ml) was confirmed in 17 of the 20 isolates displaying reduced susceptibility to penicillin, whereas the two isolates displaying imipenem resistance were susceptible to this agent (MICs $\leq$ 2 μg/ml).

Multilocus sequence typing (MLST) (2) showed that most (17/20) *E. faecalis* isolates with reduced penicillin susceptibility belonged to sequence type 6 (ST6), whereas none of the control isolates belonged to ST6, as shown by partial (*gdh* gene) MLST typing. Pulsed-field gel electrophoresis (PFGE) (3) demonstrated closely related patterns ($\leq$5-band difference) among the 17 ST6 isolates with reduced penicillin susceptibility (data not shown). Eight PFGE profiles (A1 to A8) were designated on the basis of minor band differences, and the most common profile (A1) was found in six isolates originating from four hospitals (Table 1).

We report the occurrence in several Danish hospitals of a distinctive penicillin susceptibility phenotype in *E. faecalis* consisting of reduced penicillin susceptibility and full ampicillin susceptibility. Most isolates displaying this phenotype were additionally resistant to gentamicin, belonged to type ST6, and had indistinguishable or closely related PFGE patterns, indicating the occurrence of a clonal outbreak. Despite the increased MICs of penicillin and imipenem obtained by Etest, the ST6 isolates were susceptible according to CLSI standards. The results draw attention to the ambiguity of penicillin and imipenem MICs determined by concentration gradient diffusion assays such as Etest. In laboratories where these assays are routinely employed for susceptibility testing of *E. faecalis*, resistant isolates should be confirmed by broth dilution. As long as the mechanism leading to *in vitro*-reduced penicillin susceptibility in *E. faecalis* ST6 remains unknown, the use of penicillin should be considered with caution for treatment of infections caused by strains displaying this atypical phenotype.

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**Luca Guardabassi***
**Jesper Larsen**
*Department of Veterinary Disease Biology*
*Faculty of Life Sciences*
*University of Copenhagen*
*Stigbøjlen 4, DK-1870*
*Frederiksberg, Denmark*

**Robert Skov**
*Department of Microbiological Surveillance and Research*
*Statens Serum Institut*
*Copenhagen, Denmark*

**Henrik C. Schønheyder**
*Department of Clinical Microbiology*
*Aalborg Hospital*
*Aarhus University Hospital*
*Aalborg, Denmark*

*Phone: 45 3533-2745
Fax: 45 3533-2757
E-mail: lg@life.ku.dk

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