Vancomycin-Resistant Enterococcus faecalis Endocarditis: Linezolid Failure and Strain Characterization of Virulence Factors

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Running Title
Linezolid failure in VR E. faecalis endocarditis

Key words
Linezolid failure, endocarditis, vancomycin resistance, Enterococcus faecalis, virulence factors
Abstract

Infective endocarditis due to vancomycin-resistant Enterococcus faecalis (VR E.
faecalis) has only rarely been reported. We report a case of VR E. faecalis endocarditis
that failed to respond to linezolid therapy, outline the virulence traits of the isolate, and
review previously published cases of VR E. faecalis endocarditis.

Case Report

A 37-year-old female was transferred to our institution for hemodialysis access
and sustained vancomycin-resistant Enterococcus faecalis (VR E. faecalis) bacteremia.
Her medical history was significant for medullary cystic kidney disease diagnosed at age
7, and she had required hemodialysis since age 10. She had four failed renal allografts,
the first transplant being performed at age 11. In addition, she had multiple failed
arteriovenous grafts and fistulas, requiring placement of bilateral subclavian
subcutaneous hemodialysis ports (LifeSite® Hemodialysis Access System) three years
prior to admission.

Seven months prior to admission, she developed methicillin-resistant
Staphylococcus aureus bacteremia secondary to infection of her hemodialysis ports and
was treated with 4 weeks of intravenous vancomycin. Two months prior to admission,
she developed VR E. faecalis bacteremia secondary to hemodialysis port infection. The
VR E. faecalis blood isolate was sensitive to penicillin, ampicillin, linezolid, high-level
streptomycin (MIC < 1000 µg/mL), rifampin, and resistant to high-level gentamicin
(MIC > 500 µg/mL), erythromycin, and tetracycline. Due to a history of penicillin
allergy, oral linezolid was given for 4 weeks. The hemodialysis ports were not removed
at that time due to difficulty with obtaining additional vascular access. No valvular- or
catheter-associated vegetations were demonstrated on transesophageal echocardiography.

She was subsequently admitted to another institution for evaluation of fever and
chills. Two sets of blood cultures grew VR *E. faecalis*, with a similar susceptibility
pattern as the previous VR *E. faecalis* blood isolate two months prior. Linezolid, 600 mg
intravenously every 12 hours, was initiated. Blood cultures remained positive for VR *E.
faecalis* on hospital day #2. Both subclavian subcutaneous hemodialysis ports were
removed on hospital day #3, and bacterial culture of the catheter tips grew VR *E.
faecalis*.

The patient was transferred to our institution on hospital day #5. At hospital
admission, the temperature was 35.7 °C, blood pressure was 80/48 mmHg, and heart rate
was 101 beats/minute. Physical examination did not reveal a cardiac murmur or
peripheral stigmata of endocarditis. Laboratory testing showed a peripheral leukocyte
count of 12,300 cells/mm$^3$. Two sets of blood cultures grew VR *E. faecalis* within 24
hours; the blood isolate was sensitive to penicillin, ampicillin, linezolid, daptomycin, and
resistant to quinupristin/dalfopristin and erythromycin. The isolate was resistant to high-
level gentamicin (MIC $> 500 \, \mu g/mL$), although it lacked high-level resistance to
streptomycin (MIC $< 2000 \, \mu g/mL$). In addition, the isolate contained the *vanA* gene by
PCR analysis.

Additional blood cultures taken on hospital days #7 and #9 were positive for VR
*E. faecalis*, despite continued therapy with linezolid. A transesophageal echocardiogram
on hospital day #7 showed mobile aortic valve vegetations (8 mm and 4 mm vegetations),
a mobile mitral valve vegetation (10 x 8 mm), new mitral valve regurgitation, and new
moderate to severe aortic valve regurgitation. She had more than 10 reported allergies, including penicillin, amoxicillin, cefazolin, tetracycline, and ciprofloxacin. Skin testing for penicillins and cephalosporins was performed and was negative. Antibiotic therapy was changed from intravenous linezolid to aqueous crystalline penicillin G sodium, 3 million units intravenously every 6 hours, plus streptomycin, 300 mg intravenously 3 times weekly, after each hemodialysis. Streptomycin levels were monitored. She improved clinically and follow-up blood cultures performed on hospital day #15 were negative. She received 6 weeks of combined treatment with intravenous penicillin G and streptomycin. Relapsing VR *E. faecalis* bacteremia did not occur over 9 months following completion of antibiotic therapy.

Colony lysates of the VR *E. faecalis* blood isolate from hospital day #5 (TX2853) were prepared by previously described methods (29) and hybridized with probes representing 17 genes that encode for proven or suspect virulence determinants. These included the gelatinase gene (22, 26, 28, 30); recently described pilus-encoding genes (16); genes encoding putative MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) with predicted immunoglobulin-like folds (17, 18, 27, and J. Sillanpää, S. R. Nallapareddy, and B. E. Murray, unpublished data); genes, including esp (33), in a predicted pathogenicity island (PAI) (15); and an acquired gene that contributes to biofilm formation (32) (Table 1). The strain was examined for phenotypic production of gelatinase (22), hemolytic activity on Bacto™ Tryptic Soy agar (Becton Dickinson and Company, Sparks, MD) plus 5% human blood agar plates, and biofilm formation (14). DNA was extracted using DNeasy® Tissue Kit (QIAGEN Sciences, Maryland) following the manufacturer’s instructions and tested by PCR as previously described to determine if
the conserved junction of the PAI with chromosomal DNA was present (15). Pulsed-field
gel electrophoresis (PFGE) and multi-locus sequencing (MLST) of internal regions of 5
housekeeping genes was performed to determine if TX2853 belonged to the previously
described BVE (beta-lactamase, vancomycin-resistant, endocarditis) clone (15).

TX2853 produced gelatinase; it also contained 5 of 7 putative adhesin genes
(including \textit{ebpA} and \textit{ebpB} genes related to pilus formation), one of two predicted
hyaluronidases, \textit{and esp} and 2 of 6 other PAI genes. The common PAI-chromosome
junction point previously described (15) was also present. TX2853 tested negative by
PCR for the \textit{bee} locus (biofilm enhancer in enterococcus) (32). By PFGE and MLST, this
strain did not belong to the BVE clone (nor one of the sequence types we have previously
classified by this system). Biofilm assay showed that the strain was a medium biofilm
producer (33). TX2853 tested negative for hemolytic activity on blood agar plates, which
is consistent with \textit{cylM} probe negative results.

Vancomycin-resistant enterococci (VRE) have emerged as a well-defined cause
of health care-associated and nosocomial infections (5, 8). Despite the increasing
prevalence of VRE in most tertiary care and other health care settings, infective
endocarditis due to these organisms has been reported in only a limited number of cases
(31). Moreover, endocarditis due to vancomycin-resistant \textit{Enterococcus faecalis} (VR \textit{E.}
\textit{faecalis}) isolates is extremely rare. We performed a review of the PubMed database
(English language) through the end of September 2006 using the following search terms:
“vancomycin resistant enterococcus endocarditis” and “glycopeptide resistant enterococcus endocarditis”. An article was included in our review if it described a case of VR E. faecalis infective endocarditis that fulfilled the modified Duke criteria for definite or possible infective endocarditis (13). There were only 6 previously reported cases of infective endocarditis caused by VR E. faecalis that met our criteria (Table 2). Two cases met criteria for definite infective endocarditis (Patients #1 and 3), and 4 cases met criteria for possible infective endocarditis (Patient #2, 4, 5, 6). The majority of previously reported cases of VR E. faecalis infective endocarditis in our review affected the mitral or aortic valves, and our case report represents the first description of bi-valvular endocarditis due to VR E. faecalis. Only one of seven isolates was resistant to ampicillin, which is consistent with rates of ampicillin resistance (between 0.9 and 2.7 %) observed in E. faecalis isolates in the United States (5, 8). The mechanism of resistance to ampicillin in the isolate from patient #5 (Table 2) was not mentioned in the case report (7). Most patients were treated with either ampicillin or penicillin, and synergistic bactericidal combination therapy with an aminoglycoside was given in 4 patients. There were 2 deaths and 2 patients required valve replacement.

Although there are some case reports of efficacy for linezolid in infective endocarditis due to vancomycin-resistant Enterococcus faecium (31), there has been very limited experience with the use of linezolid in infective endocarditis due to VR E. faecalis. In the 6 previously reported cases of VR E. faecalis in our review, only 2 of the patients were treated with linezolid (Table 2, Patients #5 and 6). Patient #5 was treated with linezolid for 6 weeks due to having an ampicillin resistant strain of VR E. faecalis (7). He had multiple negative surveillance blood cultures during antibiotic therapy,
though he died from an unknown cause one week after completion of linezolid therapy.

Patient #6 was treated with linezolid for 12 weeks plus gentamicin for 6 weeks due to having a previous anaphylactic reaction to penicillin (35). Six weeks after discontinuing linezolid, blood cultures were positive for VR E. faecalis, though subsequent blood cultures remained negative at 52 months follow up time. Our patient had persistent VR E. faecalis bacteremia for 9 days while on linezolid therapy, though was subsequently cured after starting therapy with aqueous crystalline penicillin G sodium plus streptomycin. Based on the limited and conflicting data in these case reports, further studies are needed to elucidate the role of linezolid in the treatment of infective endocarditis due to VR E. faecalis.

Although there are multiple virulence factors that may contribute to the ability of enterococci to cause infective endocarditis, there have been limited studies of virulence traits in VR E. faecalis infective endocarditis isolates due to its rarity (Table 2). Our patient’s VR E. faecalis infective endocarditis strain (TX2853) tested positive for 5 of 7 genes thought to be involved in adhesion (ebpA, ebpB, ace, and 2 cell surface anchor family proteins with Ig-like fold-containing putative surface adhesin), enterococcal surface protein gene (esp), gelatinase gene (gelE), 1 out of 2 putative hyaluronidases (hylA), and 2 of 6 PAI genes (xylA, hypothetical protein) (Table 1). In addition, the strain was a medium biofilm producer by biofilm assay and tested negative for hemolytic activity on blood agar plates.

Microbial adherence to host cells is a pivotal stage in infection pathogenesis, regardless of organism or infection syndrome. E. faecalis strains recovered from patients with endocarditis have a greater capacity to adhere to Girardi heart cells than to urinary
tract epithelial cells in vitro (6) which suggests that adherence to vascular endothelium may be important. Microbial surface component recognizing adhesive matrix molecules (MSCRAMMs) mediate binding of bacteria to extracellular matrix proteins, and function as adhesins to damaged heart tissue (17, 18, 27). Ace is a specific collagen-binding adhesin of the MSCRAMM family and has been identified in *E. faecalis* endocarditis isolates (17), and mediates attachment of *E. faecalis* to collagen types I and IV, and laminin (18). Subsequently, a family of seven genes encoding MSCRAMM-like proteins was found in 100% (9 out of 9) of *E. faecalis* endocarditis strains tested, and elevated titers of IgG to these MSCRAMM-like proteins were found in the sera of nine patients with *E. faecalis* infection (27). Three of these genes, *ebpA, ebpB,* and *ebpC* (endocarditis and biofilm-associated pili) control surface pili formation and may be important in endocarditis pathogenesis (16).

Biofilm formation, which is modulated by many genes including *esp* and the *fsr* locus, likely serves as an important factor in *E. faecalis* infections (16, 32). In one study, *E. faecalis* endocarditis isolates produced biofilm more often than did *E. faecalis* isolates from non-endocarditis sources and from hospital fecal specimens (14). The *esp* gene, which encodes for an enterococcal surface protein (Esp), plays an important role in biofilm formation (33), and has been identified more often among *E. faecalis* isolates that cause endocarditis and other bloodstream infections than in *E. faecalis* fecal isolates (14).

A quorum-sensing *fsr* locus has recently been described that regulates the transcription of a gelatinase gene (*gelE*) and a serine protease gene (*sprE*), and could contribute to *E. faecalis* virulence (22, 26, 28). The *fsr* locus regulates biofilm formation (14, 20). One study showed that 100% (12 out of 12) of *E. faecalis* endocarditis isolates
tested had fsr as compared to only 53% (10 out of 19) of fecal isolates (21). In contrast, two subsequent studies did not show an increased prevalence of fsr in *E. faecalis* endocarditis and bloodstream isolates (11, 23). In a rat endocarditis model, an *E. faecalis* mutant that did not produce gelatinase or serine protease had an endocarditis induction rate that was significantly reduced as compared to that of a wild-type *E. faecalis* (28). Further investigation is needed to elucidate the role of the fsr locus in the pathogenesis of *E. faecalis* infective endocarditis.

There are several other potential virulence traits of enterococci that could be operative in endocarditis pathogenesis. These include aggregation substance (1, 12), multiple genes located in a pathogenicity island, including xylA, cbh, hypothetical protein, and others (15, 24), hyaluronidases (15), extracellular superoxide production (9), and cytolysins/hemolysins (1, 8, 10, 30).

There is only one previous description of pathogen virulence factors in a patient with VR *E. faecalis* infective endocarditis (2, 4) (Table 2, Patient #3). The patient’s isolate was similar to our strain (TX2853) in that it was positive for ace, was a biofilm producer, and did not display hemolytic activity. In contrast to our patient’s isolate, this strain was esp negative. Although a molecular examination for the gelatinase gene (*gelE*) was not performed, phenotypically, the strain did not produce gelatinase. The strain was positive for the asa1 gene (aggregation substance).

In conclusion, we report a case of VR *E. faecalis* endocarditis that failed to respond to linezolid therapy and review previously published cases of VR *E. faecalis* infective endocarditis. More information is needed in order to establish the role of linezolid in the treatment of VR *E. faecalis* endocarditis. In addition, we have also
outlined the virulence traits of our patient’s isolate. Further studies are needed to identify
which virulence factors are operative in the pathogenesis of VR *E. faecalis* infective
endocarditis, and may lead to potential targets for novel therapeutic agents. Subsequent
investigations should also include etiologic and prognostic cohort studies of patients with
enterococcal bacteremia and infective endocarditis to identify which virulence traits play
a role in the development of endocarditis and which affect outcome.

Acknowledgments

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References

Microbiol. Infect. 10:1006-1008.
4. Carfagna, P., A. Tarasi, M. Cassone, M. F. Del Grosso, G. Bianco, and M.
Venditti. 2000. Prosthetic biologic valve endocarditis caused by a vancomycin-resistant


proteins collagen type IV and laminin as well as collagen type I. Infect. Immun. 68:5218–5224.


TABLES
TABLE 1. Potential virulence and pathogenicity island-associated genes

<table>
<thead>
<tr>
<th>Virulence associated loci</th>
<th>Gene name/function</th>
<th>TX2853 HS hybridization</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ef1091</td>
<td>ebpA/endocarditis and biofilm associated pili</td>
<td>+</td>
<td>16</td>
</tr>
<tr>
<td>ef1092</td>
<td>ebpB/endocarditis and biofilm associated pili</td>
<td>+</td>
<td>16</td>
</tr>
<tr>
<td>ef1818</td>
<td>gelE/protease</td>
<td>+</td>
<td>22</td>
</tr>
<tr>
<td>ef1824</td>
<td>glycosyl hydrolase, family 31/fibronectin type III domain protein with Ig-like fold-containing putative surface adhesin</td>
<td>-</td>
<td>15, 27</td>
</tr>
<tr>
<td>ef3023</td>
<td>hylA/putative hyaluronidase</td>
<td>+</td>
<td>15, 27</td>
</tr>
<tr>
<td>ef1896</td>
<td>cell wall surface anchor family protein with Ig-like fold-containing putative surface adhesin</td>
<td>+</td>
<td>J. Sillanpäätä, S. R. Nallapareddy, and B. E. Murray, unpublished data</td>
</tr>
<tr>
<td>ef2347</td>
<td>cell wall surface anchor family protein with Ig-like fold-containing putative surface adhesin</td>
<td>-</td>
<td>J. Sillanpäätä, S. R. Nallapareddy, and B. E. Murray, unpublished data</td>
</tr>
<tr>
<td>ef2505</td>
<td>cell wall surface anchor family protein with Ig-like fold-containing putative surface adhesin</td>
<td>+</td>
<td>J. Sillanpäätä, S. R. Nallapareddy, and B. E. Murray, unpublished data</td>
</tr>
<tr>
<td>ef0818</td>
<td>hylB/putative hyaluronidase</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>ef1099</td>
<td>ace/collagen adhesin protein</td>
<td>+</td>
<td>17, 18</td>
</tr>
<tr>
<td><strong>PAI associated</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ef0482</td>
<td>hypothetical protein</td>
<td>+</td>
<td>15</td>
</tr>
<tr>
<td>ef0521</td>
<td>cbh/putative choolylglycine hydrolase family protein</td>
<td>-</td>
<td>15, 24</td>
</tr>
<tr>
<td>ef0527</td>
<td>cylM/cytolysin</td>
<td>-</td>
<td>15, 24</td>
</tr>
<tr>
<td>esp</td>
<td>esp/enterococcal surface protein</td>
<td>+</td>
<td>15, 24, 25</td>
</tr>
<tr>
<td>ef0556</td>
<td>xylA/putative xylose isomerase</td>
<td>+</td>
<td>15, 24</td>
</tr>
<tr>
<td>ef0571</td>
<td>putative DNA binding response regulator</td>
<td>-</td>
<td>15, 24</td>
</tr>
<tr>
<td>-------</td>
<td>----------------------------------------</td>
<td>----</td>
<td>--------</td>
</tr>
<tr>
<td>ef0604</td>
<td>gls24-like</td>
<td>-</td>
<td>15, 24</td>
</tr>
</tbody>
</table>

*PAI = pathogenicity island*

The DNA probe for *esp* gene was amplified from strain MMH594, and all other genes were amplified from V583 (19).
### TABLE 2. Characteristics of patients with infective endocarditis due to vancomycin-resistant *Enterococcus faecalis*

<table>
<thead>
<tr>
<th>Patient no., age(yr)/sex, [reference]</th>
<th>Predisposing heart condition</th>
<th>Valve involved</th>
<th>Susceptibility data</th>
<th>Vancomycin resistance phenotype</th>
<th>Antibiotic therapy</th>
<th>Surgical intervention</th>
<th>Outcome</th>
<th>Follow up [reference]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 64/M [36]</td>
<td>AV prosthesis</td>
<td>AV</td>
<td>AMP(S), CIP(S), GEN(HLR)</td>
<td>NR</td>
<td>AMP + CIP for 2 wk</td>
<td>No</td>
<td>Death</td>
<td>Died 2 wk after diagnosis of endocarditis</td>
</tr>
<tr>
<td>2, 61/M [34]</td>
<td>None</td>
<td>AV</td>
<td>AMP(S), OFX(S), GEN(HLR)</td>
<td>NR</td>
<td>AMP + OFX for 6 wk</td>
<td>AV replacement</td>
<td>Cured</td>
<td>1 mo</td>
</tr>
<tr>
<td>3, 68/M [4]</td>
<td>MV prosthesis</td>
<td>MV</td>
<td>AMP(S), GEN(S)</td>
<td>VanA</td>
<td>AMP for 8 wk + GEN for 6 wk</td>
<td>MV replacement</td>
<td>Cured</td>
<td>4 mo</td>
</tr>
<tr>
<td>4, 68/M [3]</td>
<td>Rheumatic heart disease, AV prosthesis, MV prosthesis</td>
<td>Undefined</td>
<td>PEN(S), AMP(S), GEN(S)</td>
<td>VanA</td>
<td>AMP + GEN for 6 wk</td>
<td>No</td>
<td>Cured</td>
<td>3 mo</td>
</tr>
<tr>
<td>5, 64/M [7]</td>
<td>None</td>
<td>PV</td>
<td>AMP(R), GEN(R)</td>
<td>NR</td>
<td>LZD for 6 wk</td>
<td>No</td>
<td>Death</td>
<td>Died 1 wk after completion of linezolid</td>
</tr>
<tr>
<td>6, 79/F [35]</td>
<td>MV prosthesis</td>
<td>Undefined</td>
<td>AMX(S), GEN(S), LZD(S)</td>
<td>VanA</td>
<td>LZD for 12 wk + GEN for 6 wk</td>
<td>No</td>
<td>Cured</td>
<td>52 mo</td>
</tr>
<tr>
<td>7, 37/F [current]</td>
<td>None</td>
<td>AV and MV</td>
<td>PEN(S), AMP(S), STR(S), GEN(HLR), LZD(S), DAP(S)</td>
<td>VanA</td>
<td>PEN + STR for 6 wk</td>
<td>No</td>
<td>Cured</td>
<td>9 mo</td>
</tr>
</tbody>
</table>

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3 a M = male; F = female.

4 b AV = aortic valve; MV = mitral valve; PV = pulmonic valve.
In-vitro susceptibility data of vancomycin-resistant *E. faecalis* isolates; antibiotic therapy = final antibiotic regimen; S = susceptible; R = resistant; HLR = high-level resistance to gentamicin (MIC > 500 µg/mL); AMP = ampicillin; AMX = amoxicillin; CIP = ciprofloxacin; DAP = daptomycin; GEN = gentamicin; LZD = linezolid; OFX = ofloxacin; PEN = penicillin; STR = streptomycin.

NR = Not reported.

Follow up = follow up time without relapse after completion of antibiotic therapy, unless otherwise specified.