Sample ES-01 (2015) was a simulated blood culture isolate from a patient with associated clinical symptoms (pure culture). Participants were requested to identify any potential pathogen and to perform antimicrobial susceptibility tests via the methods routinely used by the laboratory. This culture contained Enterococcus faecium, a species becoming more commonly associated with multidrug resistances (MDR), particularly in United States (USA) medical centers. Previous enterococcal challenge samples from the API program have featured MDR E. faecalis and E. gallinarum having resistance mechanisms against linezolid, vancomycin and teicoplanin (samples ES-01, 2010 and ES-02, 2011). This current sample was forwarded to participants for susceptibility testing as an educational challenge; grading was not performed on the antimicrobial category results.

Responses of E. faecium, vancomycin-resistant (762; 86.3%), Enterococcus sp. (35; 3.7%), and Gram-positive organism (13; 1.5%) were considered acceptable performance with total acceptability at 91.5%. The most common error was identifying the organism as E. faecalis (46; 5.2%) or another erroneous identification (1-5 occurrences each; 3.3%). This organism was a very typical example of E. faecium and was readily identified by MALDI-TOF (100.0%, but a small sample), BD Phoenix (90.9%; 10/11), and Vitek 2 (94.6%; 366/387), with MicroScan (87.4%; 367/420) and manual biochemical tests (12.5%; 5/40) having lower rates of accuracy at the species level. The acceptable response rates overall by method were: Vitek 2 (96.2%) > MicroScan (94.3%) > BD Phoenix (90.9%) > manual biochemical methods (72.5%). This clinical isolate was originally derived from a bacteremia occurring in a hospitalized patient in Salt Lake City, Utah (2010). This organism had a so-called “Van B resistance profile” that includes resistance to vancomycin, but susceptibility to teicoplanin.

Organism Identification

E. faecium is the second most frequent species of the enterococcus genus isolated from human clinical specimens. They are Gram-positive coci that can occur singly, in pairs, or in short chains, and are facultatively anaerobic with an optimum growth temperature of 35°C. E. faecium strains grow on standard laboratory isolation media and produce small gray/white smooth alpha-hemolytic or non-hemolytic colonies on 5% sheep blood agar plates. All strains are non-motile, catalase negative, can grow in broth with 6.5% NaCl, and will hydrolyze esculin in the presence of 40% bile salts. Most E. faecium strains hydrolyze leucine-β-naphthylamide (LAP) by producing leucine aminopeptidase (LAPase), and L- pyrrolidonyl-β-naphthylamide (PYR) by producing pyrrolidonyl arylamidase (PYRase). Automated and commercially available identification systems such as Vitek, Vitek 2, MicroScan, API and BBL Crystal can be used to accurately identify E. faecium. Recently, Matrix-Assisted Laser Desorption/Ionization Time Of Flight (MALDI-TOF) testing has emerged as a rapid, accurate, and cost effective method for identifying bacterial isolates including E. faecium.

Enterococci grow and survive in many environments and can persist almost anywhere including in soil, food, water, plants, animals, birds, and insects. In humans they are part of the normal flora and are found
ANTIMICROBIAL SUSCEPTIBILITY – CONTEMPORARY SUSCEPTIBILITY TESTS AND TREATMENTS FOR VRE INFECTIONS (cont.)

most often in the gastrointestinal tract and to a lesser extent in the genitourinary tract and oral cavity.\textsuperscript{9,10} Enterococci are known to be important opportunistic pathogens often associated with serious infections including endocarditis.\textsuperscript{5} Among the enterococci, \textit{E. faecalis} and \textit{E. faecium} are the most frequently observed species causing human infections, and account for nearly 90% of all cultures. Enterococci are known to be the pathogen for up to 10% of outpatient urinary tract infections (UTI) and 16% of nosocomial UTIs.\textsuperscript{11}

The SENTRY Antimicrobial Surveillance Program listed enterococci as the fourth leading pathogen from bloodstream infections in North America (10.2%), and fifth in Europe (7.2%).\textsuperscript{6} In UTIs, enterococci were the second ranked pathogen overall, ranking after \textit{E. coli} (47.3%) among UTI isolates.\textsuperscript{11} A higher UTI rate for the enterococci was observed in North America when compared to Europe and Latin America.

Antimicrobial Susceptibility Testing

Participants were asked to perform antimicrobial susceptibility testing on this \textit{E. faecium}. This strain was selected to challenge proper identification and to determine antimicrobial coverage across numerous classes of Gram-positive-active antimicrobial agents. The initial reference laboratory antimicrobial susceptibility testing was conducted using standardized reference broth microdilution methods\textsuperscript{2} and susceptibility was determined by applying CLSI document M100-S25 breakpoints,\textsuperscript{3} where available. The reference laboratory testing reported a total of 30 agents (Table 1) that demonstrated varied antimicrobial activity against this strain. However, the usually potent vancomycin was not active, having a MIC value at > 8 \(\mu\text{g/ml}\).

Table 1. Listing of expected susceptibility testing categorical results for \textit{E. faecium} (blood culture) strain sent as sample ES-01 (2015).

<table>
<thead>
<tr>
<th>Antimicrobials listed by CLSI susceptibility category (Reference MIC in (\mu\text{g/ml}))\textsuperscript{a}</th>
<th>Susceptible</th>
<th>Resistant</th>
<th>No criteria\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dalbavancin (0.06)\textsuperscript{b}</td>
<td>Amoxicillin/Clavulanate (&gt;8)</td>
<td>Cefepime (&gt;16)</td>
<td></td>
</tr>
<tr>
<td>Daptomycin (1)</td>
<td>Ampicillin (&gt;8)</td>
<td>Cefoperazone (&gt;64)</td>
<td></td>
</tr>
<tr>
<td>Doxycycline (2)</td>
<td>Ciprofloxacin (&gt;4)</td>
<td>Ceftaroline (&gt;32)</td>
<td></td>
</tr>
<tr>
<td>Gentamicin (4)\textsuperscript{c}</td>
<td>Erythromycin (&gt;16)</td>
<td>Ceftazidime (&gt;32)</td>
<td></td>
</tr>
<tr>
<td>Linezolid (0.5)</td>
<td>Imipenem (&gt;8)</td>
<td>Ceftixime (&gt;8)</td>
<td></td>
</tr>
<tr>
<td>Oritavancin (0.002)\textsuperscript{b}</td>
<td>Levofloxacin (&gt;4)</td>
<td>Clindamycin (&gt;2)</td>
<td></td>
</tr>
<tr>
<td>Tedizolid (0.12)\textsuperscript{b}</td>
<td>Penicillin (&gt;8)</td>
<td>Meropenem (&gt;32)</td>
<td></td>
</tr>
<tr>
<td>Teicoplanin (≤2)</td>
<td>Piperacillin/Tazobactam (&gt;64)</td>
<td>Moxifloxacin (&gt;4)</td>
<td></td>
</tr>
<tr>
<td>Telavancin (≤0.015)\textsuperscript{b}</td>
<td>Tetracycline (&gt;8)</td>
<td>Oxacillin (&gt;2)</td>
<td></td>
</tr>
<tr>
<td>Tigecycline (0.03)\textsuperscript{b}</td>
<td>Vancomycin (&gt;16)</td>
<td>Trimethoprim/Sulfamethoxazole (2)</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Susceptibility categories determined by CLSI M100-S25 (2015) or USA-FDA product package insert criteria, where appropriate (tigecycline, dalbavancin, oritavancin, telavancin, tedizolid). Original culture source of this strain was from a bacteremia patient in Salt Lake City, Utah in 2010.
\textsuperscript{b} USA-FDA approved agents without susceptibility breakpoint criteria published by CLSI (2015), see product package inserts.
\textsuperscript{c} No high-level resistance, MIC ≤500 \(\mu\text{g/ml}\).
Consensus categorical accuracy (Table 2) ranged from 86.3% (rifampin) to 100.0% (five drugs) with all of the rifampin false-susceptible errors produced by the MicroScan product. The disk diffusion (DD) results, though much smaller in number, had an overall accuracy ranging from 83.3% (penicillin) to 100.0% (seven drugs). False-susceptible vancomycin errors were rare (0.5%), contributed by MicroScan (3) and Vitek 2 (1). False-resistant values (3.3%) for linezolid were determined using the MicroScan system.

Table 2. Participant performance for selected agents (≥40 responses by one or both tests) listed by disk agar diffusion (DD) and quantitative MIC methods for ES-01 (2015), an Enterococcus faecium bloodstream infection isolate.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Acceptable category</th>
<th>DD No.</th>
<th>% correct</th>
<th>MIC No.</th>
<th>% correct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>Resistant</td>
<td>13</td>
<td>92.3</td>
<td>755</td>
<td>99.3</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Susceptible</td>
<td>0</td>
<td>-</td>
<td>43</td>
<td>100.0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Resistant</td>
<td>7</td>
<td>100.0</td>
<td>251</td>
<td>100.0</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>Susceptible</td>
<td>0</td>
<td>-</td>
<td>183</td>
<td>100.0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Resistant</td>
<td>5</td>
<td>100.0</td>
<td>306</td>
<td>99.7</td>
</tr>
<tr>
<td>Gentamicin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Susceptible</td>
<td>9</td>
<td>88.9</td>
<td>208</td>
<td>97.6</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>Resistant</td>
<td>8</td>
<td>100.0</td>
<td>278</td>
<td>99.3</td>
</tr>
<tr>
<td>Linezolid</td>
<td>Susceptible</td>
<td>2</td>
<td>50.0</td>
<td>675</td>
<td>98.2</td>
</tr>
<tr>
<td>Penicillin</td>
<td>Resistant</td>
<td>6</td>
<td>83.3</td>
<td>571</td>
<td>100.0</td>
</tr>
<tr>
<td>Quinupristin/Dalfopristin</td>
<td>Susceptible</td>
<td>0</td>
<td>-</td>
<td>165</td>
<td>100.0</td>
</tr>
<tr>
<td>Rifampin</td>
<td>Resistant</td>
<td>2</td>
<td>100.0</td>
<td>131</td>
<td>86.3</td>
</tr>
<tr>
<td>Streptomycin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Susceptible</td>
<td>1</td>
<td>100.0</td>
<td>162</td>
<td>99.4</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Resistant</td>
<td>8</td>
<td>100.0</td>
<td>276</td>
<td>98.2</td>
</tr>
<tr>
<td>Tigecycline&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Susceptible</td>
<td>0</td>
<td>-</td>
<td>102</td>
<td>99.0</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Resistant</td>
<td>16</td>
<td>93.8</td>
<td>801</td>
<td>99.5</td>
</tr>
</tbody>
</table>

a. Correct categorical interpretation was determined by the reference MIC result, using the M07-A10, M100-S25 and USA-FDA breakpoint criteria (tigecycline), or participant consensus (chloramphenicol, quinupristin/dalfopristin, rifampin, streptomycin).

b. Susceptibility indicates potential synergistic activity in combination with cell wall active agents.

c. Breakpoint as published in the USA-FDA approved product package insert.

Some participants continue to report antimicrobials for systemic infections (blood culture for this challenge) that are only active/indicated for urinary tract infections, such as nitrofurantoin (40), norfloxacin, and trimethoprim/sulfamethoxazole (mostly for Vitek 2 users). This practice could result in compromised patient therapy. Also, some laboratories reported susceptibility testing results for the cephalosporins and other agents (clindamycin, oxacillin, etc.) having no published breakpoint criteria; see Table 1.

Only one laboratory reported a teicoplanin result (susceptible); no results were offered for recently approved potent agents dalbavancin, oritavancin, tedizolid, and telavancin. All of these lipoglycopeptides or oxazolidinones were active against this Van B VRE strain (Table 1).
Participants are reminded that susceptibility testing profiles can enhance accurate species identification, as an enterococcal isolate that is a VRE, ampicillin-resistant and quinupristin/dalfopristin-susceptible has a very high likelihood of being an *E. faecium*. All participants having submitted erroneous *E. faecalis* identifications, as well as producing susceptible results for vancomycin, should re-examine their methods for technical errors or procedural flaws. Generally, susceptibility testing performance was very acceptable.

**Epidemiology and Treatment of *E. faecium* Infections Including VRE**

Enterococci have been reported as the third most prevalent cause of hospital-associated infection (HAI) and the second most prevalent cause of central line-associated bloodstream infection (CLA-BSI) in hospitalized patients in the USA.\(^{12}\) *E. faecium* is the causative species in a greater proportion of infections (5.6% of HAI, 8.2% of CLA-BSI) than *E. faecalis* (3.5% of HAI, 5.5% of CLA-BSI). Vancomycin resistance is much greater among *E. faecium* (78.9%) compared to *E. faecalis* (7.5%), and Van A has been reported as the predominant resistance phenotype (\('80%\) over the Van B phenotype (\('20%\)) in *E. faecium* isolated in North America.\(^{10,12}\) VRE are an increasing problem in the USA. VRE-associated hospitalizations are estimated to have doubled from 2000 to 2006, and VRE now represent >30% of ICU enterococcal isolates.\(^{13}\) Importantly, bacteremia due to VRE has been shown to be a significant predictor of mortality.\(^{14}\)

Treatment options for VRE are very limited and are even more challenging for patients with BSI. Unlike with *E. faecalis* isolates, *E. faecium* isolates are almost always resistant to ampicillin\(^{10}\) and hence treatment options for vancomycin-resistant *E. faecium* BSI’s are even further diminished. Until recently, the available drugs with activity against VRE were daptomycin, linezolid, quinupristin-dalfopristin (*E. faecium* only), and tigecycline; however, limited clinical data exist to support the most efficacious choice among these treatment options. Of these potential regimens, linezolid and daptomycin are the two agents most frequently used to treat VRE-BSI, although daptomycin is not approved for this purpose.\(^{13}\) With the caveat of limited data, a recent meta-analysis of studies examining the comparative efficacy of linezolid and daptomycin for the treatment of VRE-BSI showed that linezolid therapy was associated with a lower mortality than treatment with daptomycin.\(^{15}\) Telavancin, dalbavancin, and oritavancin are three newly released compounds. Although not approved for VRE or BSI, they have demonstrated in vitro activity against Van B-phenotype isolates and are less active against Van A-phenotype VRE.\(^{16,17}\) Oritavancin has exhibited a greater breadth of activity against Van B and many Van A isolates.\(^{18}\)

**Glycopeptide Resistance Mechanisms**

As previously noted in ES-02 (2011):

> Van enzymes can be distinguished on the basis of the level and inducibility of glycopeptide resistance. The VanA type is characterized by acquired resistance to high levels of vancomycin and teicoplanin, and it is induced by both drugs. The VanB type is defined by
acquired resistance to various concentrations of vancomycin, but not to teicoplanin and is
induced only by vancomycin.\textsuperscript{[19]} However, isolates demonstrating a VanB phenotype with a
\textit{vanA} genotype have been observed in the East Asia region (Japan, China, Korea and
Taiwan)\textsuperscript{[20]} and in the USA. These acquired \textit{van} genes are carried by large transposon
elements, which are usually associated with transmissible plasmids\textsuperscript{[19,21]} These mobile
elements contribute to genomic plasticity of bacterial organisms and explain the
dissemination of \textit{van} genes in \textit{E. faecalis}, \textit{E. faecium} and other enterococcal species,
including \textit{E. avium}, \textit{E. casseliflavus}, \textit{E. durans}, \textit{E. gallinarum} and \textit{E. raffinosus}. In addition,
transfer of \textit{VanA} resistance to non-enterococcal species such as \textit{Staphylococcus aureus}, has
been documented, thus producing VRSA.\textsuperscript{[19,21,22]}

The recently approved lipoglycopeptides (dalbavancin, oritavancin, telavancin) retain potent activity
against the Van B strains, regardless of species affected.\textsuperscript{[16-18]}

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