



# CMPT Clinical Microbiology Program

Innovation, Education, Quality Assessment, Continual Improvement

## Challenge M091-2

May 2009

Rectal swab for VRE (Vancomycin resistant enterococcus) screen: *Enterococcus faecalis* (VRE), van B positive

### HISTORY

A simulated rectal swab from a 38 year old dialysis patient was sent to category A and B laboratories with request to screen for VRE as per their laboratory protocol.

It was anticipated that laboratories would report *Enterococcus faecalis*, VRE, and would notify Infection Control and/or Public Health (IC/PH)

### CMPT QA

The sample was culture positive for 4+ *Enterococcus faecalis* (VRE) and 4+ *E.coli*, viable for 10 days.

### SURVEY RESULTS

#### Identification Results

Performance on this component of the challenge was good. Of the 92 laboratories submitting results, 90% (93% of category A and 74% of category B) correctly identified or suspected the presence of VRE.

Table 1 details the reports submitted.

The isolate was specifically identified as *E. faecalis* by 80% of the reporting laboratories with the remainder reporting only VRE without species designation. No other species identification was submitted.

One laboratory reported "rectal" as the microorganism isolated and received a grade of zero.

The results of vancomycin susceptibility are considered integral to the reporting of enterococci, which is discussed further in the subsequent section on [ISOLATION AND IDENTIFICATION](#).

#### Report to Infection Control and/or Public Health

Laboratories that recovered or suspected VRE were expected to report their findings to IC/PH.

Performance on this aspect of the challenge was very good as 95% of laboratories reported the appropriate notification. The details of the reported results and the grades received are presented in Table 2.

Four laboratories that submitted positive results for VRE did not notify either agency (this includes a laboratory that reported n/a for the IC/PH communication).

### Grading

#### Maximum grade = 8

Grading was based on identification of the organism, including the presence of vancomycin resistance (maximum 4) and notification of IC/PH (maximum 4).

The sample was considered acceptable for grading on both counts as the Reference Laboratories reached the required consensus of ≥80%.

Fourteen reference laboratories reported the presence of definite (12) or probable VRE (2); one laboratory reported "vancomycin-intermediate *E.faecalis*, refer", and one laboratory reported "*E.faecalis*, refer for VRE confirmation", and 12 of 14 laboratories identified the organism as *E. faecalis*.

One reference laboratory reported "No VRE isolated".

All of the reference laboratories that detected VRE/possible VRE stated they would report to IC/PH.

This sample was processed by 84% of the participants, but 6% of A and 39% of B laboratories do not normally process this type of specimen.

**Table -1** Identification Results for M091-2 *Enterococcus faecalis* (VRE):

Reports submitted	A labs	B labs	Grade
VRE - <i>Enterococcus faecalis</i> , presumptive, refer	44	7	4
VRE - <i>Enterococcus faecalis</i> , van B +/- refer	4		4
VRE- refer +/- presumptive	10	6	4
<i>Enterococcus faecalis</i> - VRE cannot be ruled out, refer	1		4
<i>Enterococcus faecalis</i> , refer for VRE confirmation	2		4
vancomycin-intermediate <i>Enterococcus faecalis</i> , refer	6		4
<i>Enterococcus faecalis</i> , refer +/- snnp	1	1	1
<i>Enterococcus faecalis</i> , not VRE	1		0
group D enterococcus, not VRE	1		0
no VRE isolated ( <i>Enterococcus</i> sp, not VRE)	2	3	0
<i>Enterococcus</i> sp., VRE screen negative, vancomycin sensitive, snnp	0	1	0
only reported "rectal", refer (A); no report (B)	1	1	0
snp, refer	5	12	ungraded
<b>Totals</b>	<b>78</b>	<b>31</b>	

snp: sample not normally processed

As expected, laboratories that did not isolate VRE did not notify IC/PH.

Accurate identification of VRE and informing the ward and infection prevention and control in a timely manner are critical to the control of VRE in the hospital setting.

**ISOLATION AND IDENTIFICATION**

Stool, rectal, or perirectal specimens submitted for VRE surveillance should be inoculated onto selective and/or differential media containing vancomycin. These last media suppresses background normal flora and contain indicators that allow the detection of potential vancomycin resistant enterococci.

Breakthrough growth of other organisms such as *Pediococcus*, *Lactococcus* and *Lactobacillus* is not uncommon on screening. Organisms growing on selective screening media must be further tested to confirm the presence of *Enterococcus* species and the vancomycin resistance.

Several approaches can be used for VRE screening directly from clinical specimens. One medium uses bile esculin azide agar plates containing 6 µg/ml of vancomycin<sup>1</sup>. Potentially vancomycin resistant enterococci produce colonies which appear to be surrounded by a black halo after 24 h of incubation.

The use of an agar screening plate with brain heart infusion (BHI) agar, incorporating 6 µg/mL of vancomycin, is useful for screening isolates for vancomycin resistance, but not recommended for use with clinical specimens<sup>1</sup>.

Chromogenic media for the rapid detection of VRE, have recently been reported to speed up the time to detection and to decrease the additional testing necessary to confirm identifica-

tion.<sup>3,4,5,6</sup> Chromogenic media have been shown to be very cost-effective when compared to the cost and time required for additional testing.

Molecular methods, for the detection of the most common vancomycin resistance genes found in enterococci directly in patient samples are also becoming commercially available. Although vanB can be found in other microorganisms found in the gastrointestinal tract, further testing is required when tests are positive for vanB.<sup>7</sup> Despite the advantage of rapid results, the molecular tests remain quite expensive and the technology required is not available in most routine clinical laboratories.

Identification of *Enterococcus* sp is confirmed by catalase (negative) PYR (positive) and LAP (positive)<sup>1</sup> tests.

The two most common species, *E. faecalis* and *E. faecium* can be differentiated using arabinose, tellurite and pyruvate tests (*E. faecalis*: - + +, and *E. faecium*: + - - respectively)<sup>1</sup>.

Tests should be performed to rule out the species that are intrinsically resistant to vancomycin, e.g. *E. gallinarum* and *E. casseliflavus*. These tests include the ability of the isolate to acidify methyl-α-D-glucopyranoside (MGP), the presence of yellow pigment in the colonies, and the testing of the organisms for motility.<sup>1</sup>

Enterococci with intrinsic vancomycin resistance usually have vancomycin MICs of 2 - 32 µg/mL and contain the vanC genes which are not transferable. Enterococci with vanC genes have not been associated with nosocomial outbreaks, and are not considered true VRE.

The identification and confirmation of VRE has been discussed in recent CMPT critiques and participants are referred to [M063-4](#) and [M084-1](#) for more details.

**Vancomycin Resistance**

Reduced susceptibility to vancomycin in enterococci is defined by the Clinical Laboratory Standards Institute (CLSI) as strains with demonstrating either vancomycin-intermediate resistance (vancomycin MICs ≥4 and ≤ 32), or vancomycin resistance (vancomycin MICs >32)<sup>1,2</sup>.

Reduced susceptibility to vancomycin is commonly associated with 3 genotypes/phenotypes:

1. **VanA:** high-level resistance, vancomycin MICs ≥ 64 µg/mL, usually ≥ 256 µg/mL, with cross resistance to teicoplanin,
2. **VanB:** moderate to high-level resistance with vancomycin MICs 16-512 µg/mL, most commonly with preservation of susceptibility to teicoplanin, and
3. **VanC:** low-level resistance found in *E. gallinarum* and *E. casseliflavus*, with MICs ranging from 2-32 µg/mL.

Recently, **VanD**, **VanE** and **VanF** phenotypes have been identified but these are still quite rare.

**Table-2:** Notification of Infection Control or Public Health

Report to IC or PH	No of labs	%	grading
Laboratories reporting VRE or referring for confirmation	<b>82</b>	<b>90</b>	
Reported to IC (34 labs also reported to PH)	76	93	4
Reported only to PH	2	2	4
Not reported	4	5	0
Laboratories reporting no VRE isolated – Not reported	<b>8</b>	<b>9</b>	ungraded
Laboratory reporting "rectal", refer (reported to IC and PH)	<b>1</b>	<b>1</b>	0
No report received	<b>1</b>		0
snp	<b>17</b>	<b>16</b>	ungraded
<b>Total</b>	<b>109</b>		

snp: sample not normally processed

### Confirmation of Vancomycin Resistance

Vancomycin resistance should be confirmed by performing vancomycin MIC.

Traditional methods have used broth or agar dilution and disk diffusion. All of these methods reliably detect strains exhibiting high level vancomycin resistance (MIC  $\geq$  128  $\mu\text{g}/\text{mL}$ )<sup>9</sup>. However, disk and automated systems have varied in their abilities to detect low to moderate levels of resistance (8-64  $\mu\text{g}/\text{mL}$ )<sup>10, 11</sup>.

CLSI recommends that the procedures and interpretive criteria used in disk diffusion testing for vancomycin and teicoplanin should be followed to ensure reliable detection of VRE<sup>2</sup>.

The isolate sent in this challenge was unusual in that it was a vanB bearing *E. faecalis*. Participating laboratories reported MICs ranging from 6 to >256  $\mu\text{g}/\text{mL}$ , with both the mode and median results of 8-16  $\mu\text{g}/\text{mL}$ .

VRE with vancomycin MICs in this range are not common and its identification could be problematic.

The reference laboratory that reported no VRE present used a medium containing 8  $\mu\text{g}/\text{mL}$  vancomycin and reported no growth. Media with vancomycin 6  $\mu\text{g}/\text{mL}$  has been noted to have excessive breakthrough growth requiring additional follow-up work, and media with 8  $\mu\text{g}/\text{mL}$  is preferred by some laboratories.

## CLINICAL RELEVANCE

Enterococci normally inhabit the bowel of humans and a broad range of animals, and are present in lesser numbers in the genital and oral flora. Of the 20 or more enterococcal species, only *E. faecalis* and *E. faecium* commonly colonize and infect humans in detectable numbers. *E. faecalis* is isolated from approximately 80% of human infections, and *E. faecium* from the remainder. Infections from other enterococcal species are rare<sup>8</sup>.

VRE is associated primarily with exposure in healthcare settings. The 2006 Canadian Nosocomial Infection Surveillance Program (CNISP) for VRE reported that 96.2% were healthcare-associated including acute care hospitals, dialysis units and long-term care facilities; 3.8% of VRE cases were reported as community-acquired when the origin could be identified<sup>9</sup>.

The situation is different in Europe, where the

gram positive glycopeptide antibiotic avoparcin was used for years as a growth promoter in the animal industry. For this reason, VRE has been identified in the gastrointestinal tract of healthy individuals and farm animals and on processed meat products in grocery stores across Europe<sup>10</sup>.

The numbers will vary from year to year, but generally, only about 5% of patients colonized with VRE develop infections.<sup>9</sup>

Several studies have identified risk factors for VRE infection and/or colonization. These factors include previous, multiple antibiotic and vancomycin therapy, indwelling foley catheters, central venous catheterization, renal insufficiency, increased duration of hospital stay, multiple hospital admissions, and the elderly population<sup>9</sup>

Strains with high vancomycin MICs are fairly easy to detect using most screening methods.

However, the isolate sent in this challenge was an unusual VRE: *E. faecalis* with a vanB gene and an MIC in the range of 8-16 mg/L (considered vancomycin intermediate). Although the VanB phenotype can demonstrate vancomycin MICs this low, MICs in this range are more commonly associated with the intrinsic vancomycin resistance found in strains of *E. gallinarum* or *E. casseliflavus*.

Strains with vancomycin MICs  $\geq$  4 mg/L should be investigated and speciated. If determined to be *E. gallinarum* or *E. casseliflavus*, these are not considered true VRE and are therefore not an issue for infection control. However, *E. faecalis* or *E. faecium* harbouring the VanB genotype can transmit this gene and be the source of potential outbreaks. In one reported outbreak due to a single clone of *E. faecium* with transferable VanB genotype, MICs of the isolates ranged from 8-256 mg/L.<sup>11</sup>

Surveillance cultures for VRE are reported as positive or negative for the presence of the organism without reporting other antimicrobials.

VRE isolated from rectal or other surveillance cultures usually indicate colonization and not infection and do not require specific treatment. There are no decolonization regimens to eradicate VRE carriage.

Regardless of colonization or infection status, patients with VRE are cared for with the same infection control precautions to prevent spread to others.

## VRE in Canada

Laboratory-based surveillance (*E. faecium* or *E. faecalis* with MIC > 8  $\mu\text{g}/\text{mL}$ ) has seen a steady increase in the isolation of VRE in Canada<sup>9</sup>.

From 1999 to 2006 the number has more than tripled (0.37 cases per 1000 patient admissions in 1999 to 1.14 cases per 1000 patients in 2006). Infection rates however have remained stable along this time at lower than 0.1 cases per 1000 patient admissions.

The VRE found in many parts of the world are primarily *E. faecium* and have high vancomycin MICs, indicating the presence of the VanA gene. In a report of isolates from Canadian ICUs (n=17), VRE accounted for 6.7% of enterococcal isolates; 88.2% had the vanA genotype and 76.5% were *E. faecium*.<sup>13</sup>

These precautions generally include private room or cohort with similarly colonized patients, appropriate barrier precautions, environmental control of equipment and supplies and special housekeeping procedures for room cleaning at the time of discharge.<sup>12</sup>

The Committee recommends that all Proficiency Testing samples should be processed as routine samples even when there is a staff shortage or high workload.

## REFERENCES

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## ADDITIONAL READING

14. CMPT Critique [M063-4](#) November 2006. Perianal swab for screening: VRE positive (vancomycin-resistant *Enterococcus faecium*).
15. CMPT Critique [M084-1](#) February 2009. Midstream urine:  $\geq 100 \times 10^6$  CFU/L *Enterococcus faecalis*.