CMPT Clinical Bacteriology Program

Innovation, Education, Quality Assessment, Continual Improvement

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Challenge M141-2

May 2014

Rectal swab – VRE screening – VRE (*Enterococcus faecalis*)

HISTORY

A simulated rectal swab from a 30 female renal unit patient from the US to be admitted to the ICU was sent to category A and B laboratories with request to screen for vancomycin resistant *Enterococcus* (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 QC

The sample was culture positive for 4+ *Enterococcus faecalis* (VRE) and 2+ *Escherichia coli* both organisms viable for 16 days.

SURVEY RESULTS

Reference Laboratories

14/15 (93%) laboratories reported VRE (11 reported *E. faecalis*, three reported *Enterococcus* species) - two of which reported van B, one laboratory indicated that no VRE was isolated.

14/15 (93%) laboratories reported they would notify Infection Control, one reported VRE negative and, therefore, did not notify Infection Control.

Consensus was reached for both components therefore they were suitable for grading.

MAIN EDUCATIONAL POINTS from M141-2

1. It is important to further investigate elevated vancomycin MICs that appear to be in the intermediate range – to rule out *Enterococcus gallinarum* and *Enterococcus casseliflavus*, and to test for vanA and vanB genes should the organism be identified as an *Enterococcus faecium* or *Enterococcus faecalis*.
2. *Enterococcus faecalis* that are vancomycin intermediate should still be notified to Infection Prevention & Control while awaiting for vanA & vanB results so that, where required, appropriate precautions can be taken until final results are available.
3. Reduced susceptibility to vancomycin is commonly associated with 3 genotypes/phenotypes: vanA (high-level resistance), vanB (moderate to high-level resistance), and vanC (low-level, intrinsic resistance).
4. Accurate identification of VRE and timely reporting to the ward and Infection Prevention & Control are critical to the control of VRE in the hospital setting.

Participants

Identification (Table 1) 61/69 (88%) participants correctly reported the presence of VRE in the sample and therefore, were graded 4.

Three laboratories reported *Enterococcus faecalis* with intermediate resistance to vancomycin. These laboratories were graded 3.

Three participants did not report VRE and two did not report results. These laboratories were graded 0.

Grading

Maximum grade: 8

Reporting Vancomycin Resistant *Enterococcus* was graded 4.

Reporting vancomycin intermediate *Enterococcus* was graded 3.

Not reporting VRE was graded 0.

Reporting a VRE or vancomycin intermediate Enterococcus to IC was graded 4.

Not reporting a vancomycin intermediate to IC was graded 0.

Not reporting results is always graded 0.

Table 1. Identification results

Reported results	Total	Grade
VRE (*Enterococcus faecalis*), ± presumptive, ± refer ± group D ± van B	45	4
VRE (*Enterococcus* species), ± refer, ± presumptive ± van B	16	4
Enterococcuc faecalis, vancomycin-intermediate, refer	3	3
screen negative for VRE	3	0
no report	2	0
Sample not normally processed ± refer	13	ungraded
Total	82	

Table 2. Notification to Infection Control

Reported results	Total	Grade
yes	60	4
Yes – *Enterococcus faecalis*, vancomycin-intermediate (refer for additional testing)	2	4
No – *Enterococcus faecalis*, vancomycin-intermediate (refer for additional testing)	1	0
no report	2	0
n/a (reported VRE negative)	3	ungraded
Sample not normally processed, refer	14	ungraded
Total	82	

Infection control notification (Table 2) 59/61 (97%) laboratories that reported VRE indicated they would notify infection control and thus, were graded 4; two laboratories indicated they would refer. Two of the participants that reported the isolate as vancomycin intermediate reported to infection control and were graded 4; the laboratory that reported the isolate as vancomycin intermediate and did not report to infection control was graded 0.

Two laboratories did not send a report and were graded 0 for each component.

COMMENTS ON RESULTS

Overall, participant laboratories continue to do well when screening for VRE. As expected, laboratories that did not isolate VRE did not notify IC/PH.

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. Nevertheless, 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 vancomycin1 where potentially vancomycin resistant enterococci produce colonies which appear to be surrounded by a black halo after 24 h of incubation.

The use of a screening plates 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[1] as it may have excessive breakthrough growth requiring additional follow-up work.

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 identification [3 - 6] making them very cost-effective.

Detection of vancomycin resistance genes found in enterococci directly in patient samples is commercially available but conflicts with the prevalence of vanB gene among non-enterococcal, intestinal colonizers [2,7].

Despite the advantage of rapid results, the molecular tests remain quite expensive and the technology required is not available in most routine clinical laboratories.

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

Tests should be performed to rule out the species that are intrinsically resistant to vancomycin, e.g. *E. gallinarum* and *E. casseliflavus*.

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

Vancomycin Resistance

Reduced susceptibility to Vancomycin is commonly associated with three genotypes/phenotypes:

VanA: High-level resistance, Vancomycin MICs ≥ 64 mg/ L, usually ≥ 256 mg/L, with cross resistance to teicoplanin;

VanB: Moderate to high-level resistance, with Vancomycin MICs 16-512 mg/L, most commonly with preservation of susceptibility to teicoplanin;

VanC: Low-level resistance found in *E. gallinarum* and *E. casseliflavus*, with MICs ranging from 2-32 mg/l.

The genes responsible for the vanA and vanB resistance and regulation are located on transposons which explains why Vancomycin resistance has spread so rapidly among different strains of enterococci.[12]

When testing Vancomycin against enterococci, plates should be held a full 24 hours for accurate detection of resistance. Organisms with intermediate zones should be tested by an MIC method.

reliably detect strains exhibiting high level vancomycin resistance (MIC > 128 µg/mL) [9]. VRE with moderate to low vancomycin MICs challenge current phenotypic detection methods. Disk and automated systems have varied in their abilities to detect low to moderate levels of resistance (8-64 µg/mL) [8,10, 11].

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 [2].

REFERENCES

1. Murray, P.R., Baron, E. J., Jorgensen, J.J.,Landry ML,. and Pfaller, M.A., Manual of Clinical Microbiology, 9th ed. ASM Press: Washington, DC, 2007.

2. Klare I, Fleige C, Geringer U, Witte W, Werner G. Performance of three chromogenic VRE screening agars, two Etest((R)) vancomycin protocols, and different microdilution methods in detecting vanB genotype *Enterococcus faecium* with varying vancomycin MICs. Diagn Microbiol Infect Dis. 2012;74:171-176.

3. Ledeboer NA, Kingshuk D, Eveland M, Roger -Dalbert C, Mailler S, et al. Evaluation of a Novel Chromogenic Agar Medium for Isolation and Differentiation of Vancomycin-Resistant *Enterococcus faecium* and *Enterococcus faecalis* Isolates. J Clin Microbiol 2007;45:1556-1560.

4. Grabsch EA, Ghaly-Derias S, Gao W, Howden BP. Comparative Study of Selective Chromogenic (chromID VRE) and Bile Esculin Agars for Isolation and Identification of vanB-containing Vancomycin-Resistant Enterococci from Feces and Rectal Swabs. J Clin Microbiol 2008;46:4034-4036.

5. Pillai DR, Parisian F, Doyle M, Wong A, Richardson SE, Yau YCW. Evaluation of Chromogenic Agar for Screening Vancomycin-resistant *Enterococcus* (VRE). Abstract, 26th ICC, Toronto, Canada, June 2009.

6. AlMohri H, Smith S, VanZuilen L, Colosimo F, Yamamura DLR. Evaluation of a Colorex chromogenic media and Bile-esculin agar with 6 ug vancomycin for the detection of Vancomycin Resistant *Enterococcus faecalis* and *Enterococcus faecium* (VRE). Abstract, 26th ICC, Toronto, Canada, June 2009.

7. Stamper PD, Cai M, Lema C, Eskey K, Carroll KC. Comparison of BD GeneOhm VanR Assay to Culture for Identification of Vancomycin-Resistant Enterococci in Rectal adn Stool Specimens. J Clin Microbiol 2007;45:3360-3365.

8. Hegstad K, Giske CG, Haldorsen B, et al. Performance of the EUCAST Disk Diffusion Method, the CLSI Agar Screen Method, and the Vitek 2 Automated Antimicrobial Susceptibility Testing System for Detection of Clinical Isolates of Enterococci with Low- and Medium-Level VanB-Type Vancomycin Resistance: a Multicenter Study. J Clin Microbiol. 2014;52:1582-1589

9. CNISP Surveillance for Vancomycin Resistant Enterococci (VRE) in Patients Hospitalized in Canadian Acute-Care Hospitals Participating in CNISP 2006 Results Report.

10.Muto CA, Jernigan JA, Ostrowsky BE, Richet HM, Jarvis WR, Boyce JM, Farr BM. SHEA Guideline for Preventing Nosocomial Transmission of Multidrug-Resistant Strains of *Staphylococcus aureus* and *Enterococcus* 2003 Infection Control and Hospital Epidemiology pp362-68.

11.Boyce JM, Opal SM, Chow JW, Zervos MJ, Potter-Bynoe G, et al. Outbreak of multidrug-resistant *Enterococcus faecium* with Transferable vanB Class Vancomycin Resistance. JClin Microbiol 1994;32:1148-1153.