

Vancomycin-resistant *Enterococcus* sp.

Infection with vancomycin-resistant *Enterococcus* is associated with increased morbidity and mortality. The majority of VRE are associated with the species *Enterococcus faecium* (77%) and *E. faecalis* (9%), with the remaining 14% of isolates representing species less frequently implicated in serious infections, including *E. gallinarum*, *E. casseliflavus*, *E. avium*, and *E. raffinosus*.

Resistance to glycopeptides in *Enterococcus* spp. is mediated by the vancomycin resistance (Van) operon. This operon may be carried on the chromosome or on a plasmid. Nine variants have been identified in the ligase gene: *vanA*, *vanB*, *vanC*, *vanD*, *vanE*, *vanG*, *vanL*, *vanM*, and *vanN*. The most frequent are *vanA* and *vanB*.

E. faecium and *E. faecalis* with acquired vancomycin resistance (VRE) are multidrug resistant bacteria which can be responsible for health care-associated infections. Detection of this resistance trait is particularly important for the prevention and epidemiological surveillance of these infections.

The *vanA* gene procures mostly a high-level resistance to vancomycin and teicoplanin, while isolates with the *vanB* gene are mostly still susceptible to teicoplanin *in vitro*. Pathogenicity of VRE is low except for immunocompromised patients, but they are highly transmissible within hospital settings (especially *E. faecium*) which is the reason to implement infection control measures in order to limit their spread.

Clinical sample

From isolated colonies:

- Identification tests:
 - MALDI-TOF
 - or
 - at the genus *Enterococcus* level: Gram + cocci, catalase -, PYR test +
 - at the species level: Vitek (GP ID card) or Phoenix (PMIC/ID Panels) for example
- Susceptibility/detection tests:

- Disk diffusion test of vancomycin and teicoplanin (EUCAST v. 9.0, 2019)

	S ≥	R <
Vancomycin (5 µg)	12 mm	12 mm
Teicoplanin (30 µg)	16 mm	16 mm

According to EUCAST guidelines: If the zone edge is fuzzy, colonies grow within the zone or if you are uncertain, then perform confirmatory testing with PCR or report resistant, even if the zone diameter is ≥ 12 mm. Isolates must not be reported susceptible before 24 h incubation.

- MIC with antibiotic gradient tests (e.g. Etest®) of vancomycin and teicoplanin (EUCAST v. 9.0):

	S	R
Vancomycin	≤4	>4
Teicoplanin	≤2	>2

Some strains of VRE confer a low MIC values. If suspected, the bacterial suspension can be increased to McFarland 2.0 and the incubation time extended to 48 h. The detection of the *van* genes or the broth microdilution method should be done to confirm the resistance.

- EUCAST posted a warning against the use of vancomycin Etest™ (bioMérieux) and MTS™ (Liofilchem) for vancomycin MIC determination in *E. faecalis* and *E. faecium* with low-level vancomycin resistance. The warning was extended to the M.I.C.E from Oxoid/Thermofisher. Until further notice, the determination of MIC should be done using a broth microdilution method. No further notice has been reported at that time (15.02.2024).
- In case of ambiguous results, perform confirmatory PCR testing for *vanA* and *vanB* genes if available in-house or send the strain to a laboratory with available testing methods (Xpert *vanA/vanB* or *vanR* PCR BD max kits).
- Daptomycin susceptibility testing.
The CLSI guidelines define daptomycin non-susceptible *Enterococcus faecium* when the MIC is >4 mg/L whereas the EUCAST guidelines does not provide information on that antibiotic. Several studies consider resistance when the MIC is ≥8 mg/L. It is recommended to perform/confirm daptomycin susceptibility using broth microdilution technique. This is partly due to the fact that a determined concentration of Ca⁺⁺ is needed for DAP activity.

Screening sample (colonization)

Chromogenic agar should be used for the analysis of screening samples for VRE. All chromogenic agar from different manufactures have similar performances. The incubation time and supplementary tests might be different from one agar to another (Suwantararat et al., 2014, J Clin Microbiol.). Identification and susceptibility testing should be done on characteristic colonies similarly to clinical sample or according to manufacture recommendation.

An enrichment broth increases the sensitivity of detection at least of 10 to 15%.(for example by adding vancomycin 1 mg/L) It is particularly helpful in patients expected to have very low concentrations of VRE in the digestive tract (e.g. contact patient and no antibiotic treatment).

The combination of the two methods, direct inoculation on agar plates and enrichment broth, allows a fast detection of those heavily colonized or infected VRE carriers and in the same time ensures the capability of detecting VRE carriers with low concentrations of VRE in the digestive tract.

During an outbreak, or to screen for contact patients of an index case, detection of VRE using a rapid PCR test directly on the rectal swab should be considered, as the turnaround time is reduce to few hours instead of two days. The negative predictive value is usually very high. However, the positive predictive value for a *vanB* result is low because of the presence of this gene in anaerobic commensal bacteria present in human faeces (e.g. *Clostridium* sp.; Ballard et al. 2005, Antimicrob. Agents and Chemother.).

15/02/2024