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Screening for Vancomycin-resistant Enterococci (VRE) carriage

This document describes laboratory methods of screening for VRE carriage. It does not include recommendations on indications for screening, sampling methods or equipment used. Infection control measures for established VRE carriage should be determined by national public health and infection control authorities.

Introduction

Enterococci are part of the normal human intestinal microbiota, but can also cause community-acquired and nosocomial infections such as urinary tract infections, intraabdominal infections and endocarditis. *Enterococcus faecium* and *Enterococcus faecalis* are the clinically most relevant species and acquired resistance for vancomycin and other glycopeptides is increasing in these two species (1). Glycopeptide resistance in enterococci is mediated by *van* gene complexes, which cause changes in cell wall precursors. *VanA* and *vanB* are by far the most common acquired resistance genes and are both found on mobile gene elements, enabling horizontal gene transfer (2). Other acquired vancomycin resistance genes are less common and include e.g. *vanD* (3). *Enterococcus gallinarum* and *Enterococcus casseliflavus*, on the other hand, are naturally vancomycin resistant, and carry chromosomal *vanC* genes, but rarely present an infection control problem.
VanA VRE are typically vancomycin resistant with high minimal inhibitory concentrations (MIC; > 64 mg/L) and teicoplanin resistant (MIC> 16 mg/L) while VanB VRE are typically vancomycin resistant (variable MIC, 4 - 32 mg/L), but teicoplanin susceptible (MIC <2 mg/L) (2). While the *vanA* gene complex has mainly been described in *Enterococcus* spp., *vanB* can also be found in anaerobic bacteria (4;5). Therefore, with regard to the detection of VRE, the positive predictive value of detecting *vanB* in a stool sample can be much lower than for *vanA*.
Recently, enterococci harbouring silenced *vanA* gene clusters, thus being phenotypically susceptible, have been described and designated vancomycin variable enterococci (VVE). The detection of VVE is clinically relevant, because reversion to a vancomycin resistant phenotype has been described both after *in vitro* and *in vivo* exposure to vancomycin (6-8). Moreover, VanB type VRE with vancomycin MICs as low as 1 mg/L have been described and designated low-MIC VRE (9). Their low MIC is due to weak induction of *vanB* expression by vancomycin. Still these isolates can mutate to become resistant during glycopeptide exposure both *in vitro* and *in vivo* and thus may cause treatment failure (10;11).
In hospital outbreaks, it has been shown that VRE detected in clinical samples only represent a small part of the VRE burden and that at least 10 times more VRE positive patients may be identified by screening patients for faecal carriage (12). VRE carrier status is often prolonged (several months) (13) and there is no treatment for VRE carrier state.
For VRE screening purposes, rectal swabs or faeces can be used. If the patient has a stoma, a swab from the stoma is used instead of a rectal swab.

Laboratory screening strategies

Screening can be based on selective culturing methods or molecular methods. An overnight enrichment step can be used to increase sensitivity. Often these methods are combined and the precise protocol will depend on the epidemiological situation (outbreak versus non-outbreak situation), the need for rapid results, availability of methods and resources.
In outbreak situations, screening protocols can be tailored to phenotypic characteristics of the outbreak strain, e.g. by enrichment with additional antibiotics.
Based on the current epidemiology of VRE in the Nordic countries, VRE screening should cover the species *E. faecalis* and *E. faecium* and the resistance genes *vanA* and *vanB*.
For surveillance purposes and typing, detection of VRE by molecular methods is usually confirmed by culture.

Figure 1 gives an overview of screening strategies.

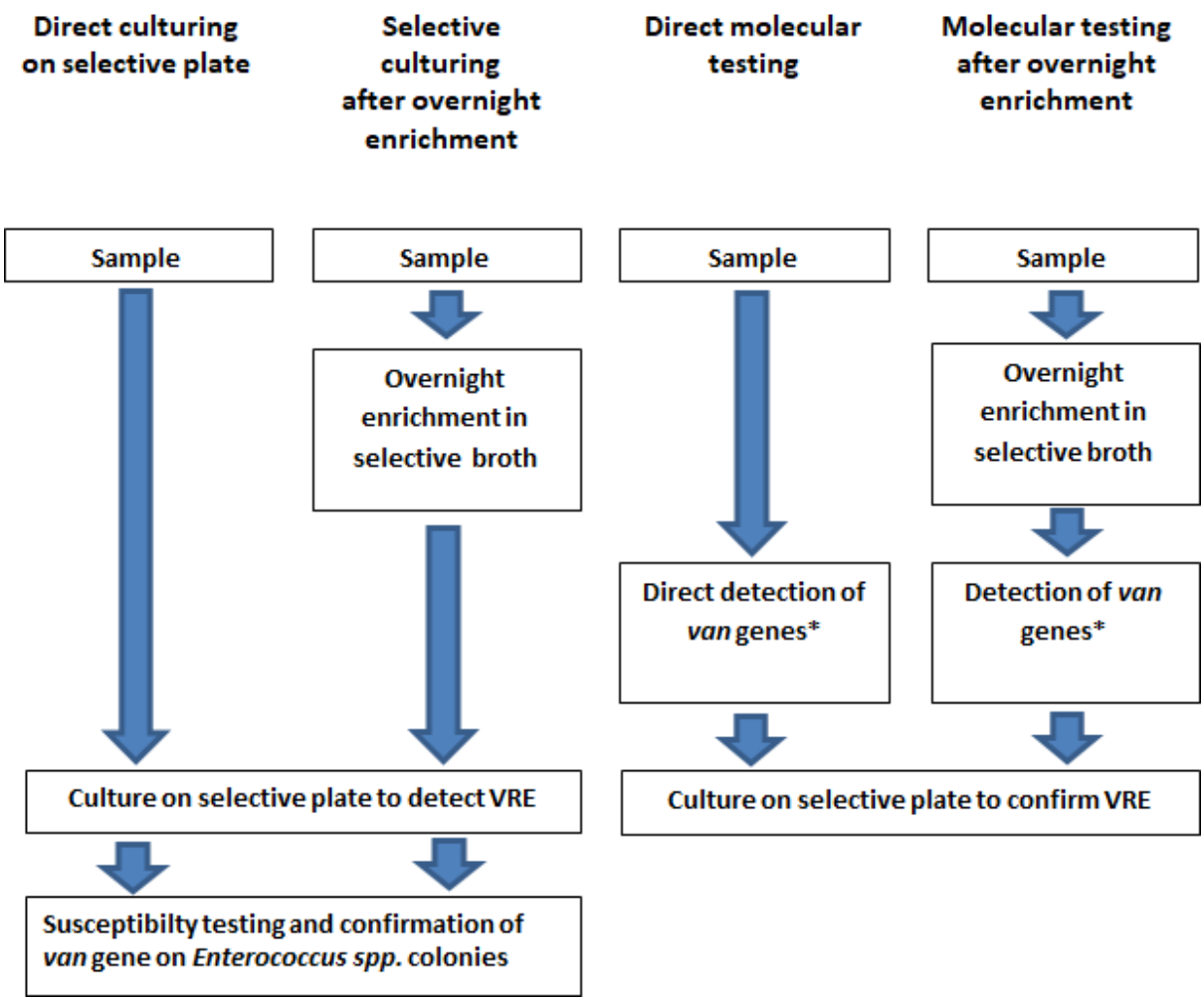

Figure 1: Overview of different screening strategies.
*A positive *vanA* result directly on the sample or after enrichment can be used to predict VRE carriage (high positive predictive value), whereas a positive *vanB* results has low specificity and must be confirmed by culture.

Description of the methods

1.Culture-based detection of VRE

Culture-based VRE screening relies on selective plates, e.g. Bile Esculin Azide agar or chromogenic plates containing vancomycin. Plates are usually incubated for 48 hours, meaning that a negative result can be obtained in 2 days, if the sample is plated directly on the selective plate and in 3 days, if overnight enrichment is used.
Overnight enrichment in growth media (e.g. BHI) supplemented with vancomycin increases sensitivity (14). If enrichment is used, the sample is transferred into the selective enrichment broth. Vancomycin concentration should be low enough (4 mg/L) to ensure the growth of *vanB* positive isolates with low vancomycin MICs. Addition of 10 mg/L oxgall to media not containing bile, also when confirming vancomycin resistance by MIC testing, have shown to increase both the sensitivity and specificity in detection of low-MIC VRE (9). Addition of aztreonam (e.g. 60 mg/L) inhibits the growth of Gram negative organisms. The broth should be incubated overnight at 35-37° C in ambient air and the next day, an aliquot of incubated enrichment broth (recommended 100µl) is plated on a selective VRE agar plate. In an outbreak situation, it is important to ensure that the outbreak strain is not inhibited by the enrichment broth and/or selective plates used.
Several studies of chromogenic plates have shown high specificity and sensitivity (14-16), but *vanB* positive isolates with a low vancomycin MIC can be inhibited (17). As an alternative to a chromogenic plate, other vancomycin-containing agar plates may be used or an agar plate with a vancomycin disk. The plate is incubated at 35-37° C in ambient air and should be read after 1 and 2 days incubation to increase sensitivity (18).
Growth of VRE-like colonies on the chromogenic or other selective plate has to be confirmed by species identification and susceptibility testing for vancomycin and teicoplanin. Confirmation of resistance genes with PCR is recommended.

2.Molecular detection of VRE

Molecular methods are based on detection of the resistance genes *vanA* and *vanB* by PCR, with the potential of providing

results within hours. PCR can either be performed directly on the sample, or on enrichment broth.
There are several commercial assays and in-house methods available. For different assays and in various settings, sensitivities of 43.5% to 100% and specificities from 79.2% to 99.7% have been reported for the detection of *vanA* (18-27). As the *vanB* gene is not unique to enterococci and can be found in other bacterial species in the human gut, the diagnostic accuracy of *vanB* detection is poor due to low specificity (20;23;26) and positive *vanB* results must therefore be confirmed by culture. However, lowering the cycle threshold (CT) cut-off value has been shown to increase the positive predictive value for a positive vanB PCR result, when performed on enrichment broth (28).

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