Background

Systemic infections with enterococci can be difficult to treat due to intrinsic resistance to multiple antibiotic groups, e.g. low-level resistance to benzylpenicillin and aminoglycosides. In clinical infections, *Enterococcus faecalis* and *E. faecium* are by far the most commonly encountered species. For serious infections with ampicillin-susceptible enterococci, ampicillin in combination with gentamicin is the first line treatment. However, ampicillin-resistance has become increasingly prevalent in *E. faecium*. Therefore, vancomycin is the first-choice treatment for this species.
Transferable acquired resistance to vancomycin in enterococci was first detected in England and France in 1986 (1-3). Since the 1990s, vancomycin-resistant enterococci (VRE) have become an increasing problem, both in the United States and in European countries (4-10).
Until recently, VRE has been found to a limited extent in the Nordic countries. However, in the past ten years, outbreaks of *vanB E. faecium* have occurred in Sweden (11), Norway (www.msis.no; 12) and Finland (13), whereas in Denmark, the incidence of *vanA E. faecium* has sharply increased (14). It is therefore important that laboratories in the Nordic countries are able to detect VRE to prevent spread of VRE in health care institutions.

Mechanisms

Vancomycin resistance in enterococci is mediated by *van* gene complexes. These cause changes in cell wall precursors, where D-Ala-D-Ala termini are replaced by D-Ala-D-Lac or D-Ala-D-Ser, to which vancomycin binds with significantly lower affinity. Vancomycin resistance is usually acquired and the most commonly found resistance genes are *vanA* and *vanB*, but several others have been described (*vanD*, *vanE*, *vanG*, *vanL*, *vanM*, *vanN*) (6;15). The resistance mechanism is inducible, meaning that it is only switched on in the presence of glycopeptide antibiotics. This can cause problems in detecting impaired susceptibility to glycopeptides *in vitro* and is the reason, why plates should be incubated for full 24 hours.
VanA normally mediates high-level vancomycin and teicoplanin resistance (VanA phenotype). *VanB* positive isolates are vancomycin-resistant, but susceptible to teicoplanin *in vitro* (VanB phenotype), see table 1. Teicoplanin is not recommended as monotherapy for VanB VRE due to reported selection of teicoplanin resistance *in vivo*.
The motile enterococcal species *E. gallinarum* and *E. casseliflavus* display intrinsic low-level vancomycin-resistance due to chromosomal *vanC* genes (vanC phenotype). However, these species are less commonly encountered in clinical specimens.

Recently, enterococci harboring silenced *vanA* gene clusters, thus being phenotypically susceptible, have been described and designated vancomycin variable enterococci (VVE). The detection of VVE is clinically relevant, as reversion to a vancomycin-resistant phenotype has been described both after *in vitro* and *in vivo* exposure to vancomycin (16-18). Moreover, VanB type VRE with vancomycin MICs as low as 1 mg/L have been described and designated low-MIC VRE (19). 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 (20;21).

Table 1. Typical phenotype for the most common *van* genotypes

	vanA	***vanB***	***vanC***
Vancomycin resistance	high	low to high	low
Vancomycin MIC	64-1000	4-64	2-32
Teicoplanin resistance	high	susceptible	susceptible
Teicoplanin MIC	16-512	0,06-1	0,25-1

Methods

The finding of vancomycin resistance should always be confirmed with reliable species identification to identify intrinsic low-grade vancomycin resistance in *E. gallinarum* and *E. casseliflavus*, the finding of which usually does not have any infection control implications.
For the detection of vancomycin resistance in enterococcal isolates, phenotypic and genotypic methods can be used and eventually combined. Of the phenotypic methods, the agar screening method and the disk diffusion method are used as screening tests. If vancomycin resistance is suspected after investigation with these methods, it must be confirmed by either MIC testing by broth micro dilution or PCR. Because of the occurrence of vancomycin-variable enterococci and low-MIC *vanB* enterococci, it is recommended to consider genotypic testing for vancomycin resistance in invasive enterococcal isolates depending on local epidemiology and resources. That is mostly relevant for ampicillin-resistant *E. faecium*.
In most Nordic countries, molecular typing is performed at least on a selection of isolates in the reference laboratory for surveillance purposes.

A. Phenotypic methods for the screening and/or confirmation of vancomycin resistance in enterococcal isolates

Vancomycin is used as a marker substance for susceptibility testing for glycopeptide antibiotics (vancomycin and teicoplanin). Here, methods for detecting impaired susceptibility to glycopeptides are described and the limitations of these methods discussed. In 2014, NordicAST together with the National Advisory Unit for Detection of Antimicrobial Resistance in Tromsø published a study comparing the disk diffusion method, agar screening method and automated methods (22). The study showed that the disk diffusion method and agar screening method performed equivalently, while the automated methods were not as reliable to detect reduced susceptibility of the *vanB* type. The disk diffusion method requires personnel to be trained in interpreting zone edges.

1. Agar screening method

The method is suitable for detecting VRE. To avoid misleading results, correct species identification is essential.

An agar (usually BHI) containing vancomycin 6 mg/L is used. A spot-shaped inoculum (10 µl) of the bacterial suspension in sterile physiological saline (0.5 McFarland) is set on the plate. The plate is incubated at 35 ± 2° C for 24 hours. Quality control of the vancomycin concentration is important.
As a negative and positive control, a vancomycin-sensitive enterococcal isolate (*E. faecalis* ATCC 29212) and a low-grade vancomycin-resistant enterococcal isolate (*E. faecalis* ATCC 51299; *vanB* with MIC 8-16 mg / L) is recommended. Proven growth (may be small colonies or hazy growth) must be confirmed by the MIC determination for vancomycin and teicoplanin using a broth dilution method and/or detection of vancomycin resistance genes and reliable species identification (22).

2. Disk diffusion method

EUCAST recommends disk diffusion with a 5 µg disk and points out, that interpreting results include both measuring the inhibition zone diameter and evaluating the zone edge with transmitted light (plate held up to light). Susceptible isolates exhibit sharp zone edges. If there are enterococcal colonies within the zone and/or the zone edge is fuzzy, this strongly suggests vancomycin resistance.
Enterococci with *vanA*-mediated resistance usually do not display any inhibition zone. *VanB*-mediated resistance does not always result in a zone diameter beneath the breakpoint. Not rarely the only finding indicating vancomycin resistance is a fuzzy zone edge (23).

3. Determination of the Minimal Inhibitory Concentration (MIC)

MIC determination can be used to verify that an isolate is vancomycin-resistant. MIC has to be determined using a broth dilution method, as gradient tests have shown to underestimate the vancomycin MIC (24). Of note, *vanB* positive isolates with MICs lower than the breakpoint have been observed.

4. Automated methods

Automated methods are established in several laboratories. These are marked with errors and deficiencies in certain areas and have been shown to not always detect certain resistance mechanisms, including *vanB* mediated resistance (22).

B. Genotypic methods for the detection of vancomycin resistance in enterococcal isolates

*1. PCR for vancomycin resistance genes (*vanA *and* vanB*)*

Standard investigation should include *vanA* and *vanB*, in-house or commercial assays can be used. If *vanA* / *vanB* PCR is not available in the local laboratory, the isolate should be sent to a reference laboratory.

Interpretation

Interpretation of susceptibility testing is dependent on the species identification and genotype. The detection of VRE or VVE (except *vanC*) shoul be reported unambiguously in the laboratory report, as it has infection control implications.
The finding of *E. faecalis* or *E. faecium* with *vanA* or *vanB* are reportable in Norway and in Sweden. In Denmark, clinical VRE isolates are sent on a voluntary basis to the reference laboratory (SSI). In Finland, clinical VRE isolates are sent to the reference laboratory (THL).

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Changes

2021-03-29	Specification, that genotypic screening is mostly relevant for ampicillin-resistant *E. faecium*. Typo for incubation temperature corrected.
2019-01-15	Translation to English, new title (old title: “Enterokokker og vankomycinresistens”) Update of persons responsible for the document Thorough revision of the document with updated background and references Inclusion of paragraph on vancomycin-variable enterococci (VVE) and low-MIC *vanB* isolates and recommendation of molecular testing of invasive isolates Recommendation of broth dilution method for vancomycin MIC testing
2015-03-20	Ändrat skrivfel ATCC 51922 till ATCC 51299
2012-10-09	Ändrat skrivfel ATCC 51922 till ATCC 51299
2012-05-21	Korrigert feil i referanseliste
2012-04-01	Nytt dokument