



Screening for Vancomycin-Resistant Enterococci

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Vancomycin-Resistant Enterococci (VRE)

Enterococci

gastrointestinal colonization → (opportunistic) clinical infection

Human pathogens: mainly *E. faecalis* and *E. faecium*

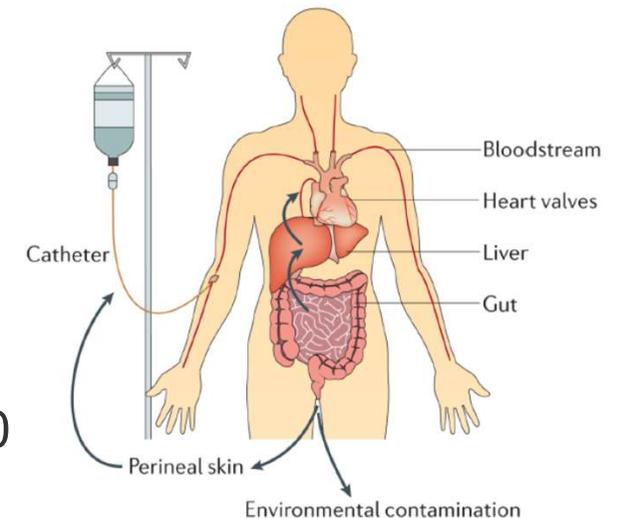
E. faecium mainly nosocomial pathogen

VRE

first detected in 1986

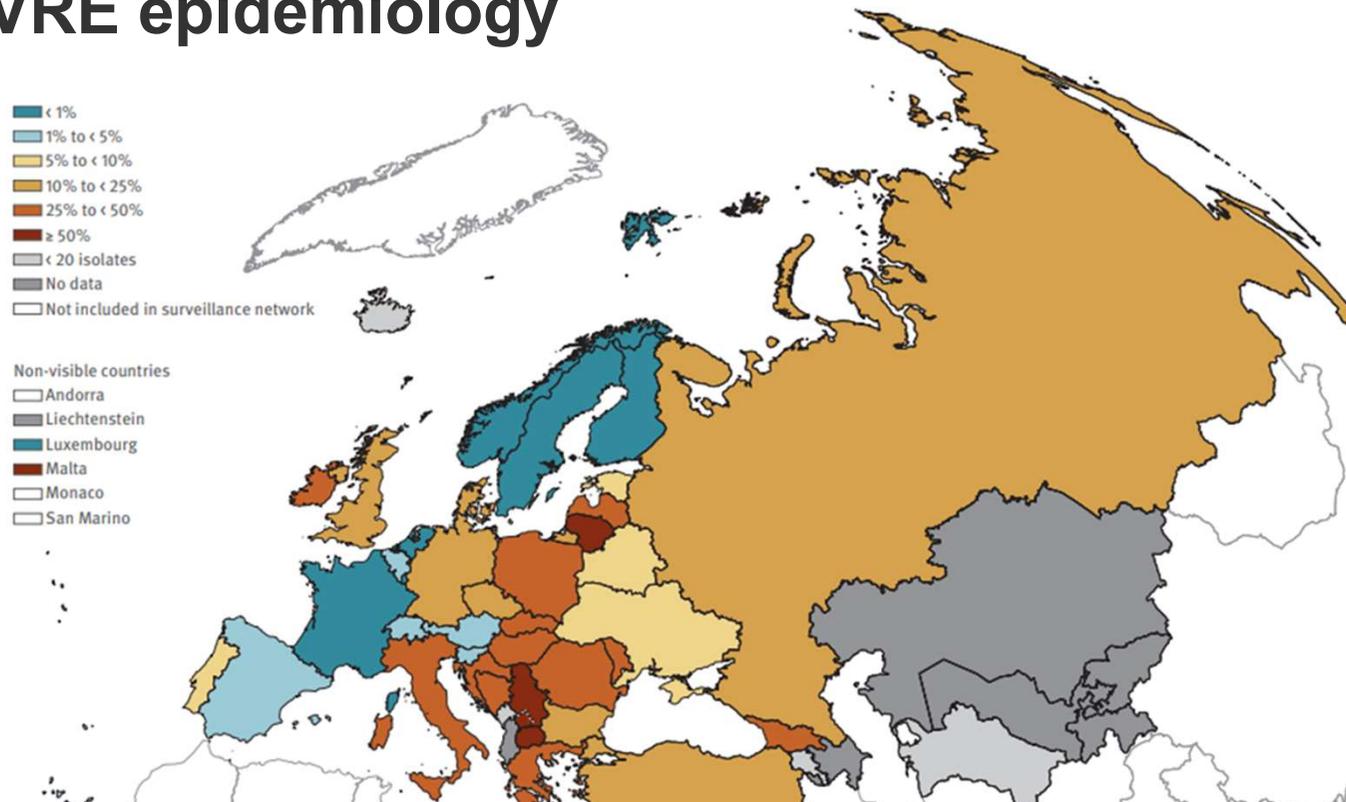
main player Ampicillin R *E. faecium*

ratio VRE in clinical / screening samples: 1 / 5-10





VRE epidemiology



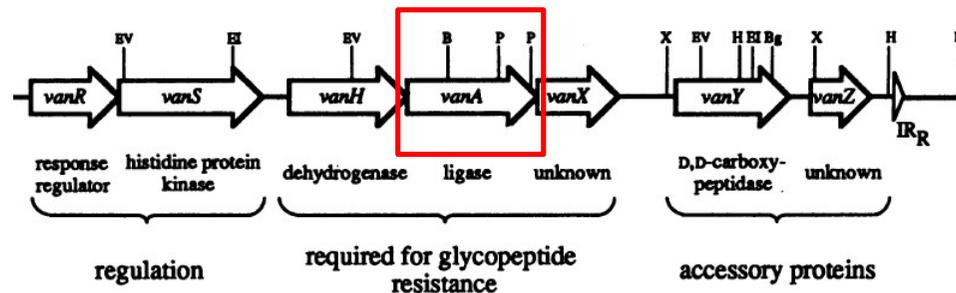
NO: 0,5 %
SE: 0,3 %
FI: 0,4 %
DK: 10,6 %

Fig. 10 *Enterococcus faecium*. Percentage of invasive isolates resistant to vancomycin, by country, WHO European Region, 2021

<https://www.ecdc.europa.eu/sites/default/files/documents/Antimicrobial%20resistance%20surveillance%20in%20Europe%202023%20-%202021%20data.pdf>

Vancomycin resistance / mechanism

Gene complex:



→ Vancomycin cannot bind to cell wall precursors



Different *van* genes: principles and pitfalls

vanA

high level resistance



EASY

(nearly) only described in enterococci
PCR on screening sample: high specificity

Gene can be silent, but reactivated:
vancomycin-variable enterococci (VVE)
diagnosis dependend on PCR

DIFFICULT

vanB

low (-high) level resistance



DIFFICULT

present in many non-enterococci
PCR on screening sample:
low specificity
low positive predictive value

DIFFICULT



- Methods
- EUCASTs disk diffusion method
- Explanatory methods document
- Method development

Method document in quality control

Method documents in quality control are available [here](#).

Explanatory methods documents

Visa 50 rader

Kod	Titel
Anaerobes	Anaerobic bacteria and resistance
Pseudomonas	Detection of carbapenemases in Pseudomonas aeruginosa
Enterococcus spp.	Detection of glycopeptide-resistance in enterococcal isolates
Staphylococcus spp.	Detection of methicillin resistance in staphylococci
Streptococcus pneumoniae	Detection of reduced penicillin susceptibility in Streptococcus pneumoniae
CPE detection	Enterobacterales: Detection of carbapenemases
ESBL/AmpC detection	Enterobacterales: Detection of classical ESBL (ESBLA) and plasmid-mediated AmpC (ESBLM-C)
Haemophilus	Haemophilus influenzae and beta-lactam resistance
Enterococci and viridans streptococci	High level aminoglycoside resistance in enterococci and viridans group streptococci
Staphylococci and streptococci	Inducible clindamycin-resistance in staphylococci and streptococci
CPE screening	Screening for carbapenemase-producing Enterobacteriaceae (CPE)
ESBL screening	Screening for ESBL and pAmpC producing Enterobacterales (EPE) carriage
MRSA	Screening for Methicillin resistant Staphylococcus aureus (MRSA)
VRE	Screening for Vancomycin-resistant Enterococci (VRE) carriage

Visar 1 till 14 av totalt 14 rader 1



Methods

EUCASTs disk diffusion method

Explanatory methods document

Method development

Method document in quality control

Method documents in quality control are available [here](#).

Explanatory methods documents

Visa 50 rader

Kod Titel

Q Sök

Metoddokument: VRE
 Titel: Screening for Vancomycin-resistant Enterococci (VRE) carriage

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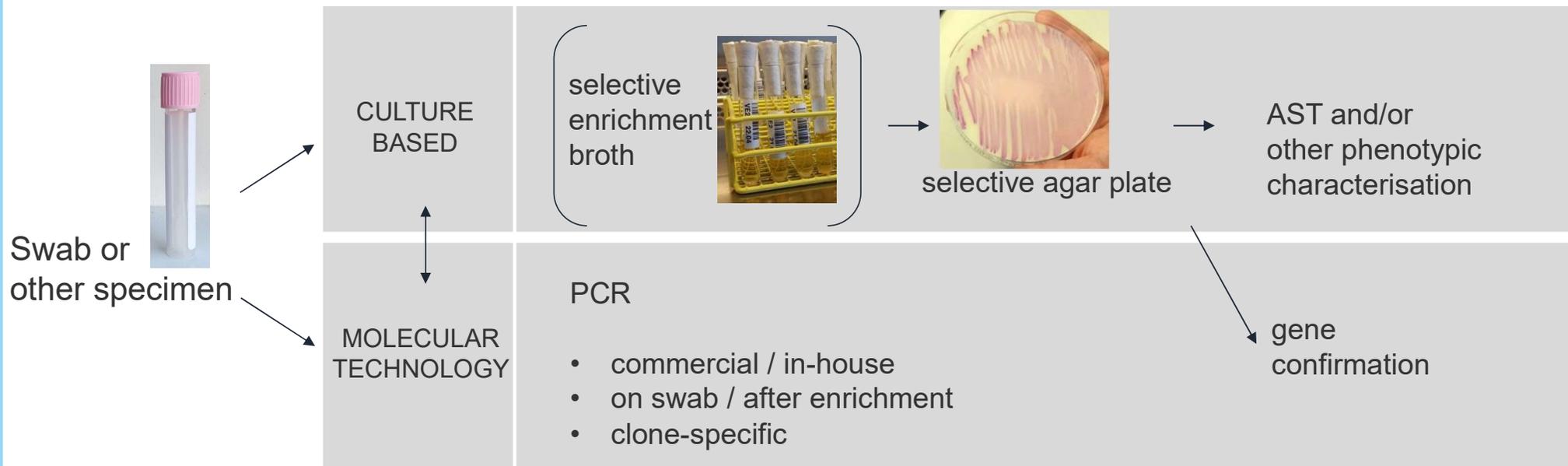
Screening for Vancomycin-resistant Enterococci (VRE) carriage

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.

ESBL screening	Screening for ESBL and pAmpC producing Enterobacteriales (EPE) carriage
MRSA	Screening for Methicillin resistant Staphylococcus aureus (MRSA)
VRE	Screening for Vancomycin-resistant Enterococci (VRE) carriage

Visar 1 till 14 av totalt 14 rader

Första Föregående 1 Nasta Sista





One size does not fit all

Exact protocol

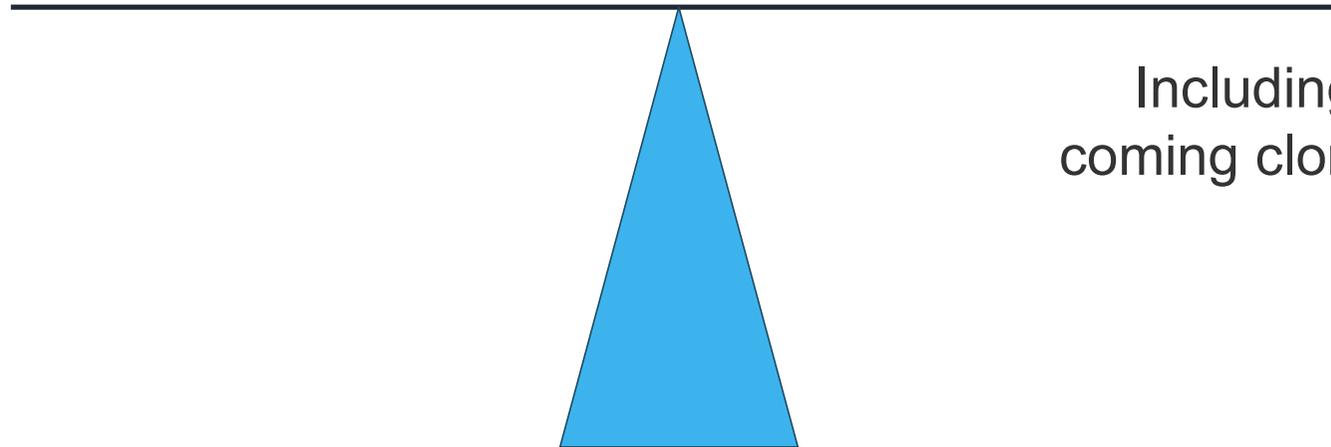
- Depending on local epidemiology
- Depending on local needs for rapid reports
- Depending on resources/availabilities

Balancing screening test

Selective and
targeted

Broad enough
to cover all
relevant types /
clones

Including
coming clones!





ECCMID 2023 / Late Breakers, Outbreaks and public health emergencies

O1147: Analysis of a current vancomycin resistant *Enterococcus faecium* (VREfm) outbreak implies the need for an adapted diagnostic algorithm

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Multiclonal outbreak, vanA and vanB

2 vanB clones grew badly on Brilliance VRE agar, exact mechanism remains unclear

Adaption of diagnostic algorithm:

positive *vanB* PCR on enrichment media (Ct-value <30) handled as VRE-positive
change of chromogenic media (CHROMAgar VRE)



Comparison of 5 selective chromogenic plates

187 *E. faecium* isolates, 119 *van* gene positive (incl. 14 vancomycin S *vanB*)
 Plates streaked with 10 µL bacterial suspension McF 0.5

Incubation [Hours]	ChromID VRE (BioMérieux)		CHROMagar VRE (CHROMagar)		Brilliance VRE (ThermoFisher)		VRESelect (Bio-Rad)		Chromatic VRE (Liofilchem)	
	24	48	24	48	24	48	24	48	24	48
Sensitivity [%]	95.0	95.0	95.0	95.0	95.0	95.0	94.1	95.0	91.6	93.3
(95% CI)	(88.9–97.9)	(88.9–97.9)	(88.9–97.9)	(88.9–97.9)	(88.9–97.9)	(88.9–97.9)	(88.9–97.9)	(88.9–97.9)	(84.7–95.7)	(86.8–96.8)
Specificity [%]	98.5	98.5	98.5	95.6	95.6	94.1	97.1	95.6	100.0	98.5
(95% CI)	(91.0–99.9)	(91.0–99.9)	(91.0–99.9)	(86.8–98.9)	(86.8–98.6)	(84.7–98.1)	(88.8–99.5)	(86.8–98.9)	(93.3–100.0)	(91.0–99.9)



Clonal comparison VREfm Clinical* versus screening isolates

**vanA/vanB* PCR on all clinically significant *E. faecium*
→ no phenotypic bias



clinical VRE isolates
screening VRE isolates



A reminder: the EUCAST warning



This warning was issued May 2019.

Vancomycin susceptibility testing in *Enterococcus faecalis* and *E. faecium* using MIC gradient tests – a modified warning 21 May, 2019.

Original warning was issued 10 July, 2018, against the use of gradient tests for the detection of vanB-positive Enterococci.
Several studies (Norwegian Reference Laboratory, Tromsø, Norway; The EUCAST Development Laboratory, Växjö, Sweden; Robert Koch Institute, Wernigerode, Germany) show that the use of MIC gradient tests with standard inoculum and incubation fail to detect glycopeptide resistance in low-level resistant enterococci (see posters 1754 and 1764, ECCMID 2019). Confirmation of suspected vancomycin resistance with gradient tests, can be significantly improved by the use of a macro method (BHI-medium, McF 2.0 and 48 hours incubation; see poster 1764, ECCMID 2019). Uncertain results should be confirmed with a molecular test for vanA and vanB.
We remind users of the EUCAST standard disk diffusion test for vancomycin in *Enterococcus* spp. to measure the zone (suspect resistance if <12 mm), and to note whether the zone edge is sharp or fuzzy (suspect resistance if fuzzy) and to take into account any colonies inside the inhibition zone (suspect resistance if colonies in zone). Either of these phenomena indicates glycopeptide resistance and a PCR should be performed to confirm or exclude the presence of vanA and vanB.

68 *vanB* isolates, MIC 2-8 overrepresented:

3 different gradient tests

Sensitivity 61-63%

increased to 89-96% using "macromethod" (higher inoculum, richer medium, longer incubation)

Reference broth microdilution

Sensitivity 72% after 24h, increased to 100% after 48h



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Conclusions

- *vanA* = easy: high resistant, only in enterococci
- *vanB* = more difficult, low (-high) resistant, prevalent in other bacteria
- VVE = *vanA* positive, but phenotypical susceptible, very difficult
- VRE screening algorithm to be adapted to local epidemiology and needs
- Be aware of new clones and changing epidemiology!