Susceptibility testing and screening of vancomycin-resistant enterococci

Christian G. Giske
Consultant physician/Associate professor
Clinical microbiology
Karolinska University Hospital
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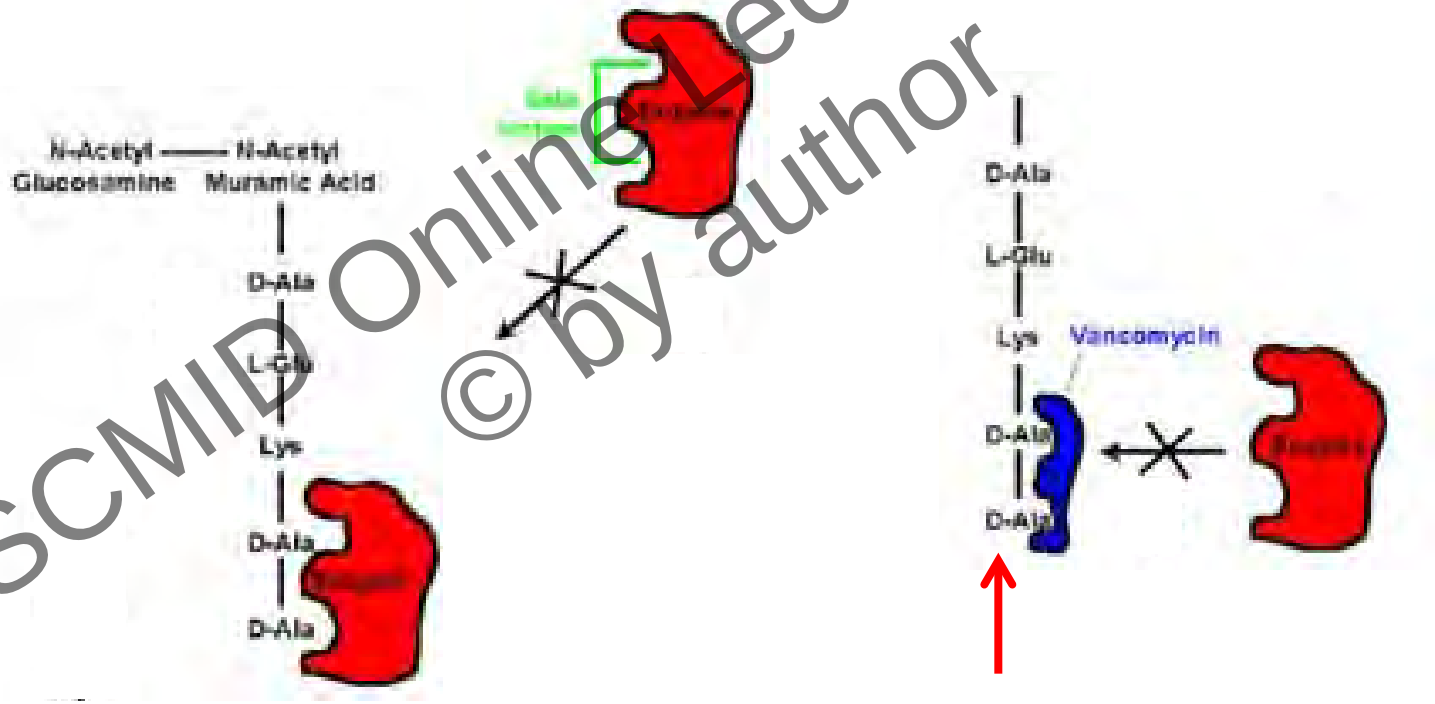
Mechanisms of vancomycin-resistance in enterococci



Van-enzymes:
conversion of D-Ala

EUCAST breakpoints for enterococci

Glycopeptides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbers for comments on MIC breakpoints Letters for comments on disk diffusion
	S ≤	R >		S ≥	R <	
Teicoplanin	2[1]	2	30	16[A]	16[A]	**1.** The susceptible breakpoint for vancomycin has been raised to 4 mg/L to avoid dividing the wild type MIC distributions of some species. The resistant breakpoint for teicoplanin has been reduced to 2 mg/L to avoid erroneous reporting of isolates with *vanA*-mediated resistance. **A**. Glycopeptide susceptible enterococci exhibit sharp zone edges. Suspect resistance when the zone edge is fuzzy or colonies grow within the inhibition zone. Some *vanB* isolates (vancomycin resistant, teicoplanin susceptible) are particularly difficult to detect with disk diffusion.
Vancomycin	4[1]	4	5	12[A]	12[A]	

MIC values of typical *van*-genes

	vanA	***van*B**	***vanC***
Vancomycin resistance	High	Variable	Low
Vancomycin MIC	64-1024	4-1024	2-32
Teicoplanin resistance	High	No	No
Teikoplanin MIC	16-512	0.06-1	0.25-1

Acquired vancomycin resistance in enterococci

- *vanA*, *B*, *D*, *E*, *G, L, M* and *vanN*
- *vanB*
  - → resistant to vancomycin (MIC 4-1024 mg/L)
  - → susceptible to teicoplanin (MIC ≤ 2 mg/L)

Importance of species determination

Species	Van-genes	Arabinose	Motility	Pigment
E. faecalis	non *vanC*	-	-	-
E. faecium	Non *vanC*	+	-	-
E. gallinarum	*vanC1*	+	+	-
E. casseliflavus	*vanC2*	+	+	Yellow

Which methods are available for susceptibility testing?

- Disk diffusion
- Breakpoint agar (including commercial agars)
- MIC-determination
- Automated methods

Recently: methods have been compared in a Nordic study (The NordicAST VRE study)

Reading instructions disk diffusion

- Examine with transmitted light (plate held up to light).
  → Fuzzy zone edges and colonies within zone indicate vancomycin resistance. If the zone diameter is ≥ 12 mm and the zone edge is fuzzy, investigate further.

E. faecalis
non-VRE

E. faecium
VRE

NordicAST-study: blinded material containing 27 isolates with a *van* genotype and 3 without

Species	*van* genotype	Vancomycin MIC range mg/L	No. of strains (No. of PFGE types)
E. faecalis	B1	8 - 24	3 (3)
E. faecalis	B2	4	1 (1)
E. faecalis	E	16	1 (1)
E. faecalis	G	16	1 (1)
E. faecalis ATCC 29212	-	3	3 (1)
E. faecalis ATCC 51299	B	16	3 (1)
E. faecium	B1	>256	1 (1)
E. faecium	B2	6 - >256	17 (15)

Courtesy of Kristin Hegstad, University of Tromsø, Norway

Participation and methods

- 34 Scandinavian labs participated
  - → 13 Norwegian
  - → 12 Swedish
  - → 9 Danish
- Methods

	No. of labs
→ Disk diffusion (6mm disks)	32
→ Disk diffusion (Neosensitabs)	2
→ BHI agar screen vanco 6 mg/L	21
→ VRE Chrom Agar	14
→ Vitek	7
→ Phoenix	1

Results automated systems

Method (n=no. of labs)	False susceptible samples	False resistant samples
Vitek (n=7)	25 (13%)	0 (0%)
Phoenix (n=1)	4 (15%)	0 (0%)

- False susceptible = classified as S when containing *van* genotype
- False resistant = classified as R when containing no *van* genotype

Results disk diffusion

Method (n=no. of labs)	False susceptible samples	False resistant samples
6 mm disks (n=32)	60 (6,9%)	4 (4,2%)
Neosensitabs (n=2)	9 (17 %)	0 (0%)

- False susceptible = classified as S when containing *van* genotype
- False resistant = classified as R when containing no *van* genotype

If zone edge was fuzzy or colonies grew within the zone, the sample was considered R even though the zone size suggested S

Results agar screen

Method (n= no. of labs)	False susceptible samples	False resistant samples
BHI vanco 6 (n=21)	47 (8,3%)	4 (6,3%)
VRE ChromAgar (n=14)	8 (2,1%)	6 (14%)

- False susceptible = No growth when containing *van* genotype
- False resistant = Growth when containing no *van* genotype

Summary

Method	False susceptible samples	False resistant samples
Vitek	25 of 189 (13%)	0 of 21 (0%)
Phoenix	4 of 27 (15%)	0 of 3 (0%)
BHI vanco 6	47 of 567 (8,3%)	4 of 63 (6,3%)
VRE ChromAgar	8 of 378 (2,1%)	6 of 42 (14%)
Disk diffusion 6mm	60 of 864 (6,9%)	4 of 96 (4,2%)
Neosensitabs	9 of 54 (17 %)	0 of 6 (0%)

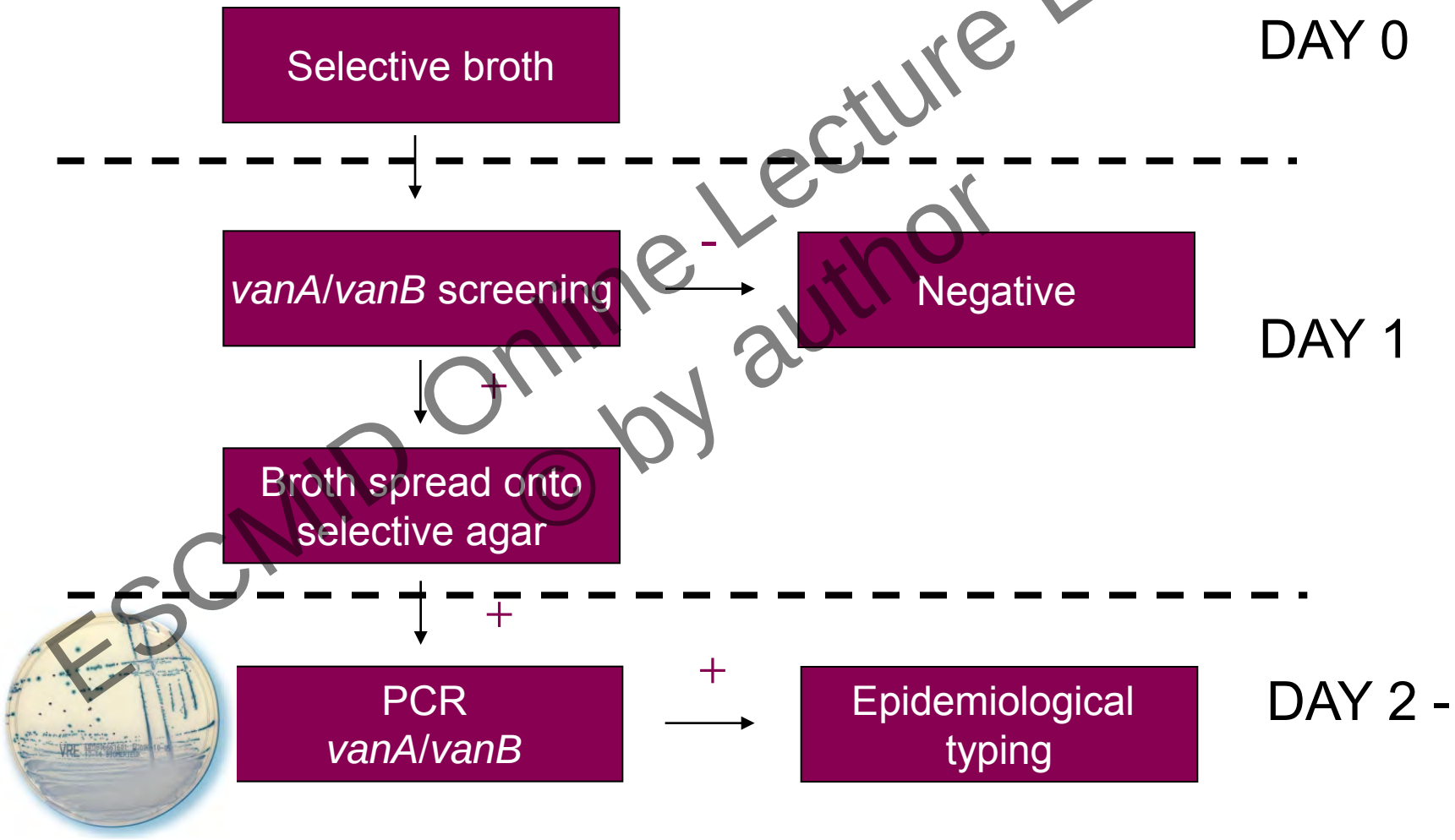
Comparing results by countries

Method	Country (no. of labs)	False susceptible samples in %	False resistant samples in %
Disk diffusion	Sweden (12)	1,5	0
	Norway (13)	10	2,6
	Denmark (9)	12	11
BHI vanco 6	Sweden (1)	3,7	0
	Norway (13)	7,1	0
	Denmark (7)	11	19
ChromID VRE	Sweden (7)	1,6	14
	Norway (6)	3,1	17
	Denmark (1)	0	0

Screening for VRE

- Usually done with either chromogenic agar containing vancomycin (commercial or in-house) or with enrichment in broth prior to streaking the samples on selective agar
- Sometimes combined with molecular biology or with broths changing colours when enterococci are growing
- Final step is usually always cultivating the broth on selective agar to find an isolate

VRE screening at Karolinska



What can generally be reommended regarding screening?

- VRE can be combatted!
  - → UK: drop in number of invasive cases of VRE (28.4% of *E. faecium* 2008 and 10.4% in 2010, according to EARS-Net)
  - → Detection of *vanB* can be challenging, but is possible with commercial agars
  - → Hospitals will benefit from establishing screening routines
- Selective broth increases senstivity, but also increases time consumption
- Pure molecular approaches are not appropriate as *van*-genes can also be seen in other species
- PCR is not strictly necessary if the quality of species determination is good

Conclusions

- The EUCAST disk method has good performance for detection of VRE, including *vanB*
  - → Training in the methodology is required to obtain good results
  - → Results should be confirmed with MIC-testing (but this should not delay reporting)
- Automated susceptibility testing and breakpoint agars have lower performance than disk diffusion
- Screening for VRE is strongly recommended, and the best approach is to use enrichment broth prior to cultivation on selective agar