VRE re-visited

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BeLux Meeting Vitek, BacT/Alert users, Groot-Bijgaarden
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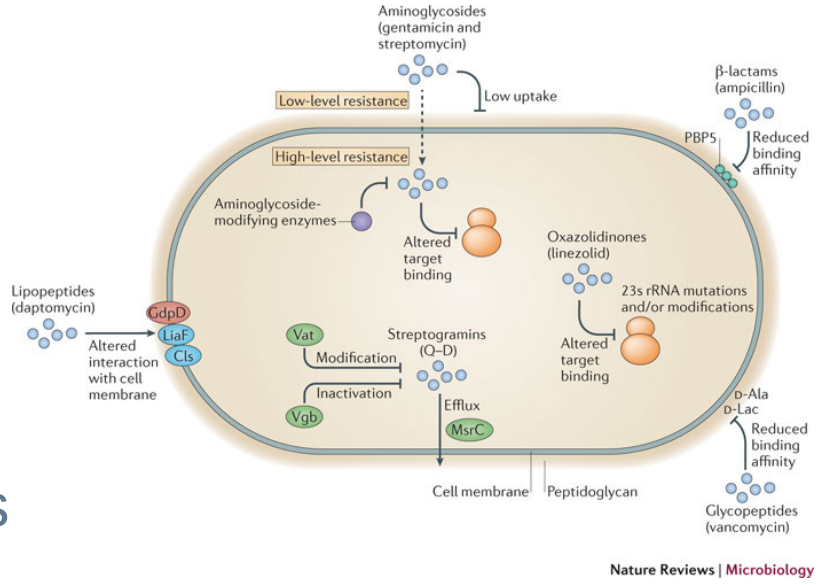
A little history of vancomycin van VRE

- 1958 authorized for use
- End 50's, toxicity, replaced by methicillin
- 1970, re-used due to increasing MRSA and *C. difficile* diarrhea
- 1986, outbreak of VRE in UK
- 1987, first outbreak in the US

How vancomycin works

- Glycopeptide antibiotic (others: teicoplanin, new semi-synthetic: dalbavancin (FDA 2014) telavancin (2009), oritavancin (2014)
- Blocks cell wall synthesis
- By binding to terminal peptides D-alanine-D-alanine
- Bactericidal, extracellular



Enterococci

- Normal flora lower GI tract, skin, vagina, urethra, hepatobiliary tree
- Ratio colonization: infection = 20-40:1 (Tersmette, NTMM, 2015)
- Survival on environmental surfaces
- Risk factor for transmission
  - Diarrhea
  - Discharging wounds
  - Catheterized patients with VRE colonization of the urinary tract

VRE pheno- and genotypes

	Phenotypes				
	vanA	***vanB***	***vanC***	***vanD***	***vanE/G***
Vancomycin MIC (µg/mL)	64 – 1000	4 – 1000	2 - 32	16 – 64	16
Teicoplanin MIC (µg/mL)	15 – 512	0.5 > 32	0.5 - 1	2 -4	0,5
Species	*E. faecium, E. faecalis, (E. raffinosus)*	*E. faecium, E. faecalis*	*E. gallinarum, E. caseliflavus, E. flavescens*	*E. faecium,*	*E. faecalis*
Transferable	Yes	Yes	No	No	No

Laboratory detection of VRE

Laboratory detection

- Antimicrobial susceptibility testing
  - Broth microdilution
  - Disk diffusion
  - Agar screen
- Surveillance screen
- Molecular
  - In house
  - Commercial

Broth microdilution

- Reference standard EUCAST and CLSI

- Commercial version
  - Sensititre (Thermo-Fisher Scientific)
  - Microscan (Beckman Coulter)
  - Phoenix (BD)

VITEK2 (bioMérieux)

- Kobayashi, *et al*. J Med Mic. 2004
  - Panel of 35 Enterococci
  - Very major error 0%, major error 0%
  - But CLSI, R ≥ 32 mg/L

- Hegstad, *et al.* J Clin Mic. 2014
  - Panel of 30 Enterococci vanB low (4 – 8 mg/L) and medium MIC (16 – 32 mg/L)
  - 5 Scandinavian labs, EUCAST
  - Very major error 13%, major error 0%, sensitivity of 87%
  - Lab difference (very major error range 1 – 6)

Disk diffusion (1)

- Removed from CLSI M100 guideline in 2014

- Still in EUCAST
  - Hegstad, *et al.* JCM, 2014
  - 7% very major error, 2.4% major error, sensitivity 93%
  - Agar dependent: BBL 14%, Oxoid 3%

Disk diffusion (2)

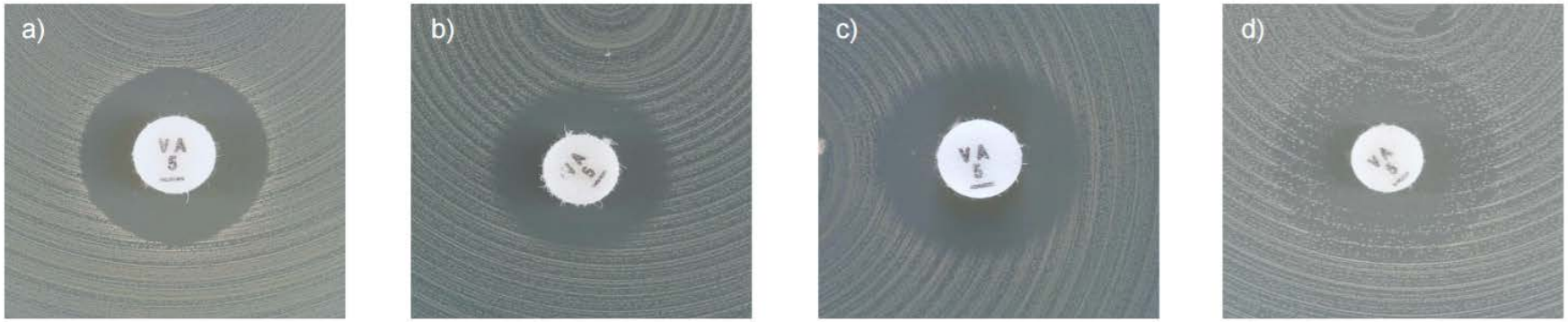
***Enterococcus* spp.**

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

Glycopeptides and lipoglycopeptides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Dalbavancin	IE	IE		IE	IE	A. Vancomycin susceptible enterococci exhibit sharp zone edges and do not exhibit colonies in the inhibition zone. Examine zone edges with transmitted light (plate held up to light). 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 (**see pictures below**) even if the zone diameter is ≥ 12 mm. Isolates must not be reported susceptible before 24 h incubation.
Oritavancin	IE	IE		IE	IE	
Teicoplanin	2	2	30	16	16	
Telavancin	IE	IE		IE	IE	
Vancomycin	4	4	5	12[A]	12[A]	

Disk diffusion (3)

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



Examples of inhibition zones for *Enterococcus* spp. with vancomycin.
a) Sharp zone edge **and** zone diameter ≥ 12 mm. Report susceptible.
b-d) Fuzzy zone edge or colonies within zone. Perform confirmatory testing with PCR or report resistant even if the zone diameter ≥ 12 mm.

CLSI agar screen (1)

- BHI agar with 6 mg/L vancomycin

- Positive control CLSI: *E. faecalis* ATCC 51299
  - MIC 16-64 mg/L
  - Missing 6 -16 mg/L
  - Alternative *E. gallinarum,* stable MIC 8 mg/L

CLSI agar screen (2)

- Very major error 6.6% sensitivity, 0.93

- Variation (Hegstad, *et al.* J Clin Mic. 2014):
  - n=18
  - Between lab in low level vancomycin sensitivity (VME 0 – 5)
  - Between agar (VME: Oxoid 11% vs. Difco 4.2%)

Primary Surveillance Screening

- Ratio colonized: infected: 20:1
- Difco and Bile Esculin Azide agar with vancomycin, limitation:
  - Lactobacilli and Pediococci are not inhibited
  - Sensitivity and specificity
- Chromogenic

Primary Surveillance Screening, literature 2010 - 2015

Reference	Specimens type (n)	VRE media	Incubation time (h)	Sn	Sp
Suwantarat, JCM, 2014.	Stools (396)	Spectra VRE	24	93.9	99.7
		ChromID (bioMérieux)	48	94.9	99.7
		VRE Select (BioRad)	24	91.9	99.7
		HardyChrom VRE	48	88.9	99.7
		InTray Colorex VRE	24	91.9	98.3
Jenkins, JCM, 2011	Stools (142)	BEAV with 6 mg/L vanco	24	86.2	91.7
			48	96.5	84.5
		Campy agar with 5 antibiotics	24	96.5	91.7
			48	100	89.3

Molecular detection (1)

- Roche LightCycler VRE detection kit
  - Sens 74%, spec 92% (Mehta, *et al*. 2008)
- Cepheid Xpert *van*A/*van*B Assay
  - Sens 74% (vanA 73.9%, vanB 87.5%), spec 92% (vanA 92.6%, van B 14.7%) (Gazin, *et al.* 2012 and Mehta, *et al.* 2008)
- NanoCHIP infection control panel assay (Savyon Diagnostics)
  - Sens and spec 100% (Greenberg, *et al.* 2012)

Molecular detection (2)

- 3Plex VRE and 5Plex VRE detection kit (Ausdiagnostics)
  - Sens 84%, spec 99% (Cekin, *et al.* 2013)

- PCR (Seeplex VRE) vs. chromogeen (Seo, *et al.* JMM. 2011)
  - 13 culture-positive and PCR-negative
  - 30 culture-negative and vanA PCR-positive
  - 3 culture-negative and vanB PCR- positive
  - Sensitivity 98.2 %, specificity 99.6 %

Molecular detection (3)

- False positive problems:
  - *van*B-containing transposons Tn5382 and Tn1549 in *Clostridium* spp., *Eggerthella lenta* and *Ruminococcus* spp.
  - *van*A genes: *Bacillus circulans, Arcanobacterium haemolyticum, Oerskovia turbata*

National Reference Center

Tests performed @ NRC (1)

- In all:
  - Confirmation of ID (*E. faecium, E. faecalis, E. casseliflavus, E. gallinarum, E. raffinossus, E. durans, E. hirae, E. avium, (E.mundtii, E. gilvus)*)
  - Confirmation of susceptibility
    - MIC ampicilin, vancomycin, teicoplanin, linezolid and tigecycline

- On request:
  - High level resistance (gentamycin, streptomycin)

Tests performed @ NRC (2)

- Genotypic detection of resistence genes:
  - All species: if MIC ≥ 4 → PCR *vanA, vanB (*if neg*:* PCR *vanD, vanE, vanG)*
  - All *E. faecium* MIC < 4 → PCR *vanA* to exclude VVE
  - All *E. gallinarum*/ *E. casseliflavus* → PCR *vanA, vanB, vanC*

- In case of outbreak investigation:
  - Was: Pulsed Field Gel Electrophorese (PFGE) and Multi Locus Sequence Type (MLST)
  - Since November 2016, only: WGS MLST

WGS MLST

- Classical MLST
  - Typically 7 loci (housekeeping genes), sequenced using Sanger technology.

- WGS MLST
  - More loci (1500 – 2500) → higher typing resolution
  - At present a bit more expensive than classical

Exceptional resistance

Vancomycin variable *Enterococci* (1)

- VVE
  - Initially S to vancomycin
  - But possesses *van*A gene
  - Can develop *in vitro* and *in vivo* resistance to vancomycin
  - First described in Quebec (Gagnon, *et al.* JAC. 2011)
  - Lacking the *vanR* and *vanS*

- Questions
  - Molecular testing in all *E. faecium*?
  - Need to screen? How?
  - How to treat?

Vancomycin variable *Enterococci* (2)

- VVE received by the NRC
  - Case 1
    - UTI
    - Discrepancy MIC Vitek2 and E-test
    - E-test: vancomycin 8 mg/L, teicoplanin 1 mg/L by Vitek2, MIC vancomycin 2 mg/L and teicoplanin 2 mg/L by another tests
  - Case 2
    - Sepsis oncology patient
    - Treated with vancomycin, but without success

Linezolid R *Enterococci*

- EUCAST exceptional resistance *Enterococcus* spp.
  - R to daptomycin, linezolid and/or tigecycline.
  - R to teicoplanin but not vancomycin

- Two resistance mechanisms
  - Point mutation 23S rDNA (Alonso, *et al.* J Mic Met, 2014)
  - *Cfr* gene (Diaz, *et al*. AAC, 2012) and *optr*A

- NRC 2.3% (2013 – 2015)
  - 8 *E. faecalis* (all vancomycin S), MIC 8 mg/L
  - 11 *E. faecium* (9 vancomycin R), MIC 8 mg/L

vanB with vanA phenotype

- vanB genotype
- Resistant to vancomycine and teicoplanine

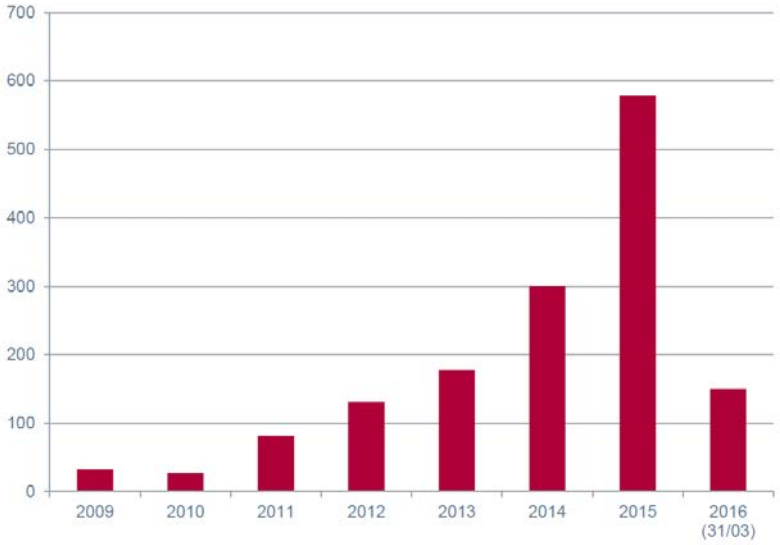
Enterococci and new glycopeptides

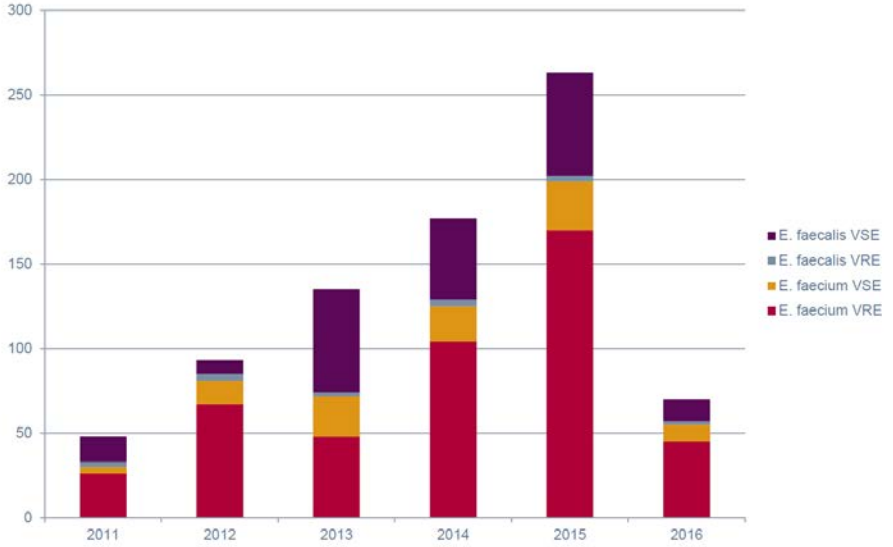
- *van*A
  - R to both dalbavancin and telavancin,
  - S to oritavancin

- *van*B
  - S to both dalbavancin, oritavancin and telavancin,

Survey, help needed

Number strains received and infection





Need to explain this number

- Surveillance?
- Please help us to fill in a very short survey

Survey

- Short survey
  - Do you screen?
  - If yes, in response to an outbreak?
  - If yes, specific departement (intensive care, dialysis?)
  - How do you screen? (chromogenic plates? culture + antibiogram? rapid molecular tests?)
  - Since when?

- Please help us….

Thank you for your attention

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