Antibiotic sensitivity of the *Enterococcus* spp.

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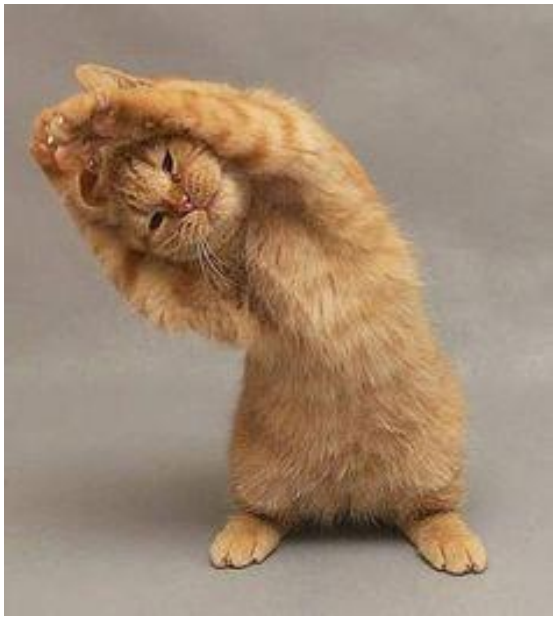
A bit of warming up

- Which of the following antibiotics doesn't belong to the same group as vancomycin:
  - Teicoplanin
  - Dalbavancin
  - Telavancin
  - Oritavancin
  - Fidaxomicin



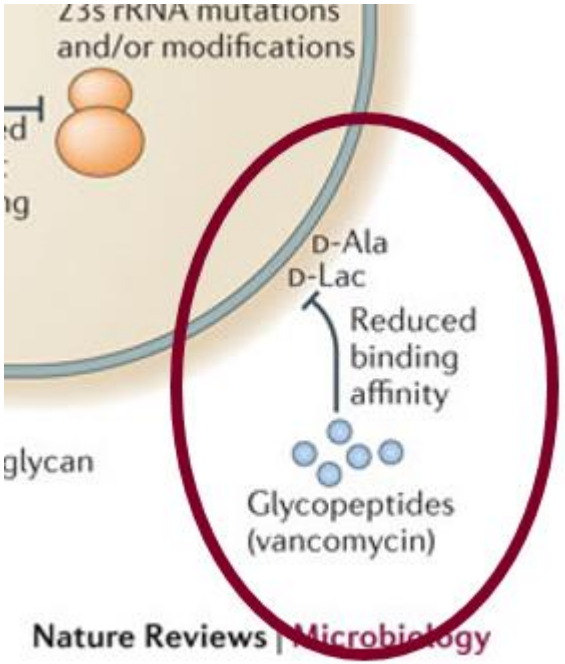
Enterococci and new glycopeptides

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

Vancomycin

- Glycopeptide antibiotic

- A little history
  - 1958 authorized for use
  - End 50's, toxicity, replaced by methicillin
  - 1970 re-used due to increasing MRSA and *C. difficile* diarrhea



Enterococci and VRE (1)

- Normal flora lower GI tract, skin, vagina, urethra, hepatobiliary tree
- 3rd most comon nosocomial pathogen
- Survival on environmental surfaces
- Ratio colonization: infection = 20-40:1 (Tersmette, NTMM, 2015)

Enterococci and VRE (2)

- First outbreaks:
  - 1986, UK
  - 1987, US

- Risk factor for transmission
  - Diarrhea
  - Discharging wounds
  - Catheterized patients with VRE colonization of the urinary tract

VRE genotypes and phenotypes

	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. faecium, E. faecalis*	*E. gallinarum, E. caseliflavus, E. flavescens*	*E. faecium,*	*E. faecalis*
Transferable	Yes	Yes	No	No	No

Laboratory detection

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

EUCAST or CLSI

- Which vancomycin MIC for Enterococcus spp. is higher, EUCAST or CLSI?
  - CLSI, R ≥ 32 mg/L (vs. EUCAST R >4 mg/L)

EUCAST vs. CLSI

- Performance of commercial broth microdilution depends on EUCAST or CLSI breakpoints and also on lab

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

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

Disk diffusion

- Is disk diffusion a valid method to detect VRE?
  - Yes
  - No

Disk diffusion

- Removed from CLSI M100 guideline in 2014
- Still in EUCAST
  - Hegstad, JCM, 2014
    - 7% very major error, 2.4% major error
    - Agar dependent, very major error: BBL 14%, Oxoid 3%

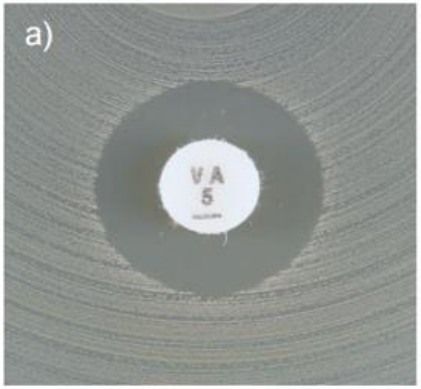
***Enterococcus* spp.**

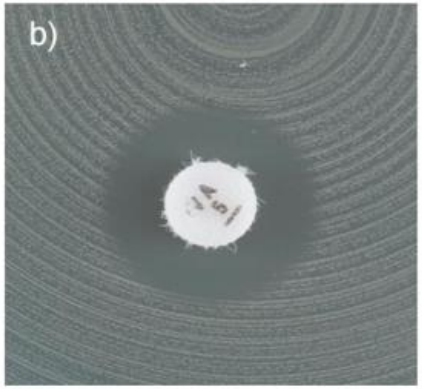
EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

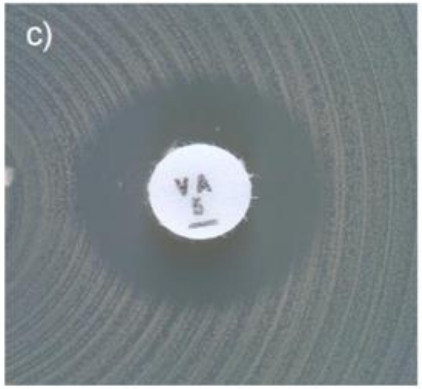
Glycopeptides and lipoglycopeptides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Dalbavancin	IE	IE		IE	IE	A. Vancomycin susceptible 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]	

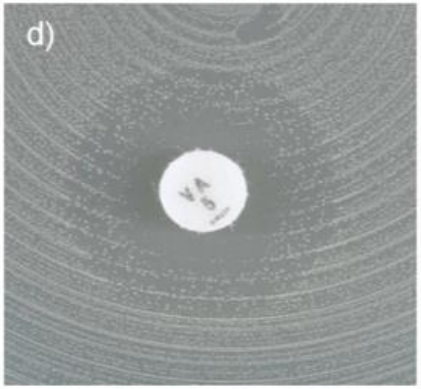
A bit more practical, disk diffusion (1)

- A) S or R?
- B) S or R?
- C) S or R?
- D) S or R?

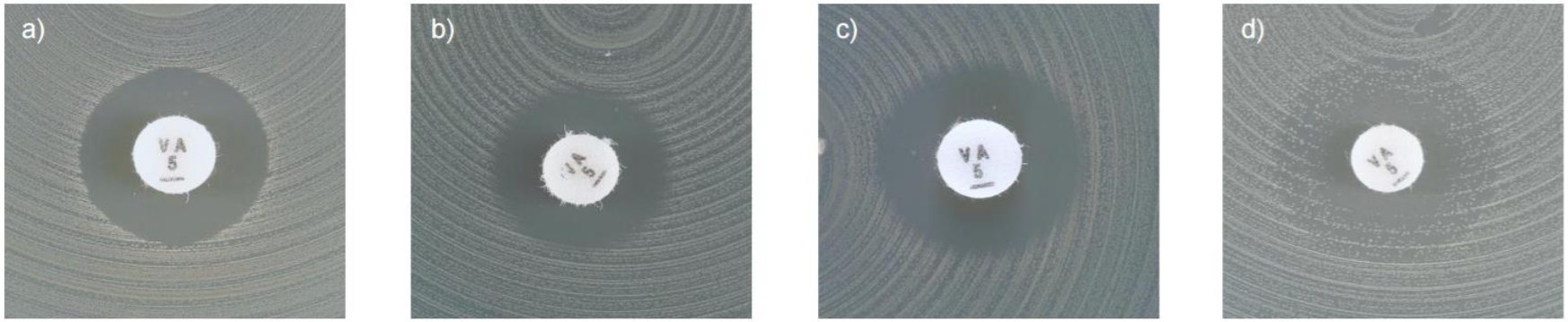








A bit more practical, disk diffusion (2)



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.

A bit more practical, 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

From the study books…..

- Which of the following microorganisms are vancomycine resistant?
  - *Gemella*
  - *Pediococcus*
  - *Lactobacillus*
  - *Erysipelothrix*

Why important to know this?

- The performance of agar with vancomycin from samples can be influenced by these bacteria.

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
Jenkins, JCM, 2011	Stools (142)	BEAV with 6 mg/L vanco	24	86.2	91.7
			48	96.5	84.5

Quite difficult

- Cepheid Xpert *van*A/*van*B Assay
  - Spec 92.6% (*van*A), 14.7% (*van*B) vs. PCR (50 stool samples) (Gazin, *et al.* EJCMID 2012)

- Which bacteria can have transposons with *van*B?
  - *Clostridium* spp.,
  - *Eggerthella lenta*
  - *Ruminococcus* spp.
  - *Oerskovia turbata*

A question, may be for escaping internist (1)

- 69 y.o. man
- Hx/ DM, colon adenocarcinoma
- Previous admission 3 weeks ago/ SBP, *E. faecium vanA* (GeneXpert), but S to vancomycin (MIC = 1 µg/ml)
- HPx/ sepsis and bleeding esophageal varices

A question, may be for escaping internist (2)

- Antibiotic history/
  - D 1-2: pip/tazo
  - D 2 -10: cipro
  - D 12-17: pip/tazo and vancomycin (d12-14) because of suspected recurrent sepsis
- Culture/
  - Swab d22&24: vanco >256, teicoplanin > 256
- FU/
  - d29: died variceal bleeding

Vancomycin variable *Enterococci* (1)

- Vancomycin variable *Enterococci*
  - 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

Which of the folowing is/ are exceptional?

- Which of the following statements is/ are exceptional?
  - *E. faecium* R to linezolid
  - *E. faecium* R to teicoplanin but not vancomycin
  - *E. gallinarum* R to vancomycin
  - *E. casseliflavus* with vanC genes

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)

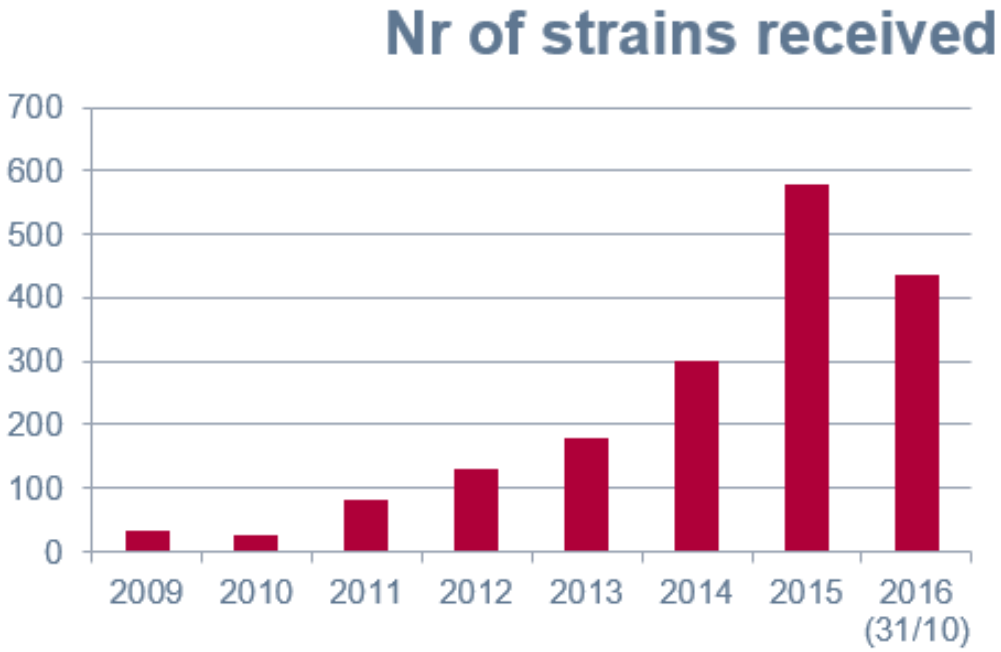
- NRC 2.3% (2013 – 2015)
  - 8 *E. faecalis* (all vancomycin S), MIC 8 mg/L
  - 11 *E. faecium* (9 vancomycin R), MIC 8 mg/L

Tests performed @ NRC

- Phenotypic and antimicrobial susceptibility confirmation

- 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*

Can you help us?



Need to explain this number

- Surveillance?

- A very 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

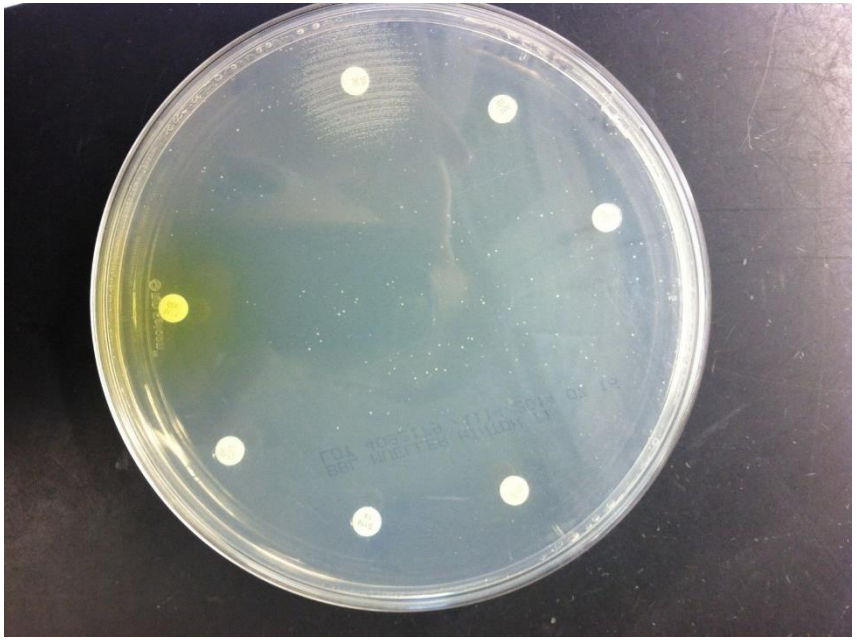
- Thanks to
  - Prof. Dr. H. Goossens
  - K. Loens, Ph.D.
  - Prof. Em. M. Ieven
  - Bea Jans

Vancomycin dependent *Enterococci*

- 34 y.o. woman
- Hx/ IVDU
- HPI/ Endocarditis MRSA treated with vancomycin
- 5 weeks later UTI

Vancomycin dependent *Enterococci*

Antibiogram growth around the vancomycin disk but no growth around the ampicillin, ciprofloxacin, doxycycline



Courtesy of Bella Goyal, WUSM, Medical Microbiology Question

Vancomycin dependent Enterococci

- Fraimow HS, Jungkind DL, Lander DW, Delso DR, Dean JL. Urinary tract infection with an *Enterococcus faecalis* isolate that requires vancomycin for growth. Ann Intern Med. 1994 Jul 1;121(1):22-6.

- Van Bambeke F, Chauvel M, Reynolds PE, Fraimow HS, Courvalin P. Vancomycin-dependent *Enterococcus faecalis* clinical isolates and revertant mutants. Antimicrob Agents Chemother. 1999 Jan;43(1):41-7.