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

- **vanA**
  - R to both dalbavancin and telavancin
  - S to oritavancin

- **vanB**
  - 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 common nosocomial pathogen
- Survival on environmental surfaces
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
<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>vanA</th>
<th>vanB</th>
<th>vanC</th>
<th>vanD</th>
<th>vanE/G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin MIC (µg/mL)</td>
<td>64 – 1000</td>
<td>4 – 1000</td>
<td>2 - 32</td>
<td>16 – 64</td>
<td>16</td>
</tr>
<tr>
<td>Teicoplanin MIC (µg/mL)</td>
<td>15 – 512</td>
<td>0.5 &gt; 32</td>
<td>0.5 - 1</td>
<td>2 -4</td>
<td>0.5</td>
</tr>
<tr>
<td>Species</td>
<td>E. faecium, E. faecalis</td>
<td>E. faecium, E. faecalis</td>
<td>E. gallinarum, E. caseliflavus, E. flavescens</td>
<td>E. faecium, E. faecalis</td>
<td></td>
</tr>
<tr>
<td>Transferable</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
Laboratory detection

- Antimicrobial susceptibility testing
  - Broth microdilution
  - Disk diffusion
  - Agar screen

- Surveillance screen

- Molecular
  - In house
  - Commercial
• 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 vanB* 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%

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### Enterococcus spp.

<table>
<thead>
<tr>
<th>Glycopeptides and lipoglycopeptides</th>
<th>MIC breakpoint (mg/L)</th>
<th>Disk content (µg)</th>
<th>Zone diameter breakpoint (mm)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S ≤</td>
<td>R &gt;</td>
<td>S ≥</td>
<td>R &lt;</td>
</tr>
<tr>
<td>Dalbavancin</td>
<td>IE</td>
<td>IE</td>
<td>IE</td>
<td>IE</td>
</tr>
<tr>
<td>Oritavancin</td>
<td>IE</td>
<td>IE</td>
<td>IE</td>
<td>IE</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>2</td>
<td>2</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>Telavancin</td>
<td>IE</td>
<td>IE</td>
<td>IE</td>
<td>IE</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>12^a</td>
</tr>
</tbody>
</table>

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

Notes
- Numbered notes relate to general comments and/or MIC breakpoints.
- Lettered notes relate to the disk diffusion method.

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.
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
• Which of the following microorganisms are vancomycin resistant?
  • Gemella
  • Pediococcus
  • Lactobacillus
  • Erysipelothrix
Why important to know this?

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

<table>
<thead>
<tr>
<th>Reference</th>
<th>Specimens type (n)</th>
<th>VRE media</th>
<th>Incubation time (h)</th>
<th>Sn</th>
<th>Sp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suwantarat, JCM, 2014</td>
<td>Stools (396)</td>
<td>Spectra VRE</td>
<td>24</td>
<td>93.9</td>
<td>99.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ChromID (bioMérieux)</td>
<td>48</td>
<td>94.9</td>
<td>99.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VRE Select (BioRad)</td>
<td>24</td>
<td>91.9</td>
<td>99.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HardyChrom VRE</td>
<td>48</td>
<td>88.9</td>
<td>99.7</td>
</tr>
<tr>
<td>Jenkins, JCM, 2011</td>
<td>Stools (142)</td>
<td>BEAV with 6 mg/L vanco</td>
<td>24</td>
<td>86.2</td>
<td>91.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>48</td>
<td>96.5</td>
<td>84.5</td>
</tr>
</tbody>
</table>
Quite difficult

- Cepheid Xpert vanA/vanB Assay
  - Spec 92.6% (vanA), 14.7% (vanB) vs. PCR (50 stool samples) (Gazin, et al. EJCMID 2012)

- Which bacteria can have transposons with vanB?
  - Clostridium spp.,
  - Eggerthella lenta
  - Ruminococcus spp
  - Oerskovia turbata
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 *vanA* 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?
• 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 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

- 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 resistance 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?

Nr of strains received

<table>
<thead>
<tr>
<th>Year</th>
<th>Strains Received</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>50</td>
</tr>
<tr>
<td>2010</td>
<td>70</td>
</tr>
<tr>
<td>2011</td>
<td>80</td>
</tr>
<tr>
<td>2012</td>
<td>100</td>
</tr>
<tr>
<td>2013</td>
<td>150</td>
</tr>
<tr>
<td>2014</td>
<td>200</td>
</tr>
<tr>
<td>2015</td>
<td>600</td>
</tr>
<tr>
<td>2016</td>
<td>300</td>
</tr>
</tbody>
</table>

(31/10)
Need to explain this number

- Surveillance?

- A very short survey
  - Do you screen?
  - If yes, in response to an outbreak?
  - If yes, specific department (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
