Glycopeptide resistance in *S. aureus* - methods and prevalence of resistance

R. Skov, MD
Bacteriological Surveillance and Infection Control
Statens Serum Institut
Copenhagen, Denmark
Disclosures

- Have received grants, being a speaker, consulting, or being a member of an advisory board for the following companies Leo-Pharma, Novartis, Pfizer, RibX and Targenta
Glycopeptide antibiotics

- Cell wall acting glycosylated peptides
  - binds to the terminal D-alanyl-D-alanine moieties of the stem pentapeptides that attach to \( N \)-acetylglucosamine / \( N \)-acetylmuramic acids
    - prevents crosslinking of the peptidoglycan

- Vancomycin and Teicoplanin
  - Oritavancin

- Large hydrophilic molecules
  - target is just on outside the of the cytoplasmic membrane
    - i.e. molecules have to diffuse though the cell wall
Resistance / reduced susceptibility to vancomycin

Break points

<table>
<thead>
<tr>
<th></th>
<th>S</th>
<th>I</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUCAST</td>
<td>≤2</td>
<td>-</td>
<td>&gt;2</td>
</tr>
<tr>
<td>CLSI</td>
<td>≤2</td>
<td>4-8</td>
<td>≥16</td>
</tr>
</tbody>
</table>

EUCAST warning

- Glycopeptide MICs are method dependent and should be determined by broth microdilution (reference ISO 20776).
- *S. aureus* with vancomycin MIC values of 2 mg/L are on the border of the wild type MIC distribution and there may be an impaired clinical response.
Resistance / reduced susceptibility to vancomycin

- S. aureus with reduced vancomycin susceptibility: SA-RVS
  - Vancomycin intermediate resistant S. aureus – VISA
  - Heterogeneous vancomycin intermediate resistant S. aureus – hVISA
    - i.e. “normal” MIC but resistant subpopulations

- Vancomycin resistant S. aureus – VRSA
Mechanisms of resistance

**VRSA:**
- *vanA* gene positive
  - Change of D-alanyl-D-alanin to D-alanyl-D-lactate
    - 1000 fold decrease in affinity to vancomycin
- Typical MIC > 16 mg/L to vancomycin

**VISA isolates:**
- not a single mutation or acquisition of a single gene
  - Complex! Involves a series of changes!
    - Increased cell wall thickening, increased number of free D-alanyl-D-alanin residues, reduced autolytic activity, mutations in regulators of cell wall synthesis (i.e. *graRS*, *vraSR*), change in transcription profile
- Typical MIC 4-8 mg/L
Mechanisms of resistance

- hVISA - Isolates susceptible by standard MIC testing but have subpopulations expressing reduced susceptibility
  - Same types of resistance mechanisms as VISA isolates
- Typical MIC 1-2 mg/L

Population profile of initial isolate (6000) and after persistent bacteremia / vancomycin therapy (6001)

Howden, AAC, 2006
Susceptibility testing

- Disk diffusion cannot discriminate wildtype/susceptible isolates from isolates with reduced susceptibility
  - No zone brp in EUCAST / CLSI guidelines

- MIC results are method dependent!

Swensson, JCM 2009, 2013
Comparison of BMD and commercial systems

- 129 S. aureus isolates with MICs between 0.5 and 8 mg/L

**TABLE 1. Vancomycin MICs and MIC categories determined by three reference methods and six commercial systems**

<table>
<thead>
<tr>
<th>Method or system</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>S</th>
<th>I</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMIC-Difco</td>
<td>7</td>
<td>53</td>
<td>24</td>
<td>36</td>
<td>9</td>
<td></td>
<td>65.1</td>
<td>34.9</td>
<td>0</td>
</tr>
<tr>
<td>BMIC-BBL</td>
<td>9</td>
<td>53</td>
<td>26</td>
<td>38</td>
<td>6</td>
<td></td>
<td>68.2</td>
<td>31.8</td>
<td>0</td>
</tr>
<tr>
<td>Agar dilution</td>
<td>9</td>
<td>54</td>
<td>33</td>
<td>29</td>
<td>4</td>
<td></td>
<td>74.4</td>
<td>25.6</td>
<td>0</td>
</tr>
<tr>
<td>Ettest</td>
<td>4</td>
<td>22</td>
<td>48</td>
<td>47</td>
<td>8</td>
<td></td>
<td>57.4</td>
<td>42.6</td>
<td>0</td>
</tr>
<tr>
<td>MicroScan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>57.4</td>
<td>42.6</td>
<td>0</td>
</tr>
<tr>
<td>Phoenix</td>
<td>9</td>
<td>55</td>
<td>42</td>
<td>23</td>
<td></td>
<td></td>
<td>49.6</td>
<td>50.4</td>
<td>0</td>
</tr>
<tr>
<td>Sensititre</td>
<td>64</td>
<td>33</td>
<td>28</td>
<td>1</td>
<td>1</td>
<td></td>
<td>76.4</td>
<td>22.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Vitek Legacy</td>
<td>46</td>
<td>71</td>
<td></td>
<td></td>
<td>1</td>
<td>10</td>
<td>92.2</td>
<td>26.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Vitek 2</td>
<td>68</td>
<td>24</td>
<td>29</td>
<td>8</td>
<td></td>
<td></td>
<td>71.3</td>
<td>28.7</td>
<td>0</td>
</tr>
</tbody>
</table>

*Boldface indicates that the MICs for the isolates were less than or equal to the MIC listed.*

Swensson, JCM 2009, 2013
Comparison of BMD and commercial systems

- 129 *S. aureus* isolates with MICs between 0.5 and 8 mg/L

<table>
<thead>
<tr>
<th>Method or system</th>
<th>No. of results with vancomycin MIC (µg/mL)</th>
<th>% of results with vancomycin category</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>BMIC-Difco</td>
<td>7</td>
<td>53</td>
</tr>
<tr>
<td>BMIC-BBL</td>
<td>9</td>
<td>53</td>
</tr>
<tr>
<td>Agar dilution</td>
<td>9</td>
<td>54</td>
</tr>
<tr>
<td>Etest</td>
<td>4</td>
<td>22</td>
</tr>
<tr>
<td>MicroScan</td>
<td>9</td>
<td>55</td>
</tr>
<tr>
<td>Phoenix</td>
<td>64</td>
<td>33</td>
</tr>
<tr>
<td>Sensititre</td>
<td>46</td>
<td>71</td>
</tr>
<tr>
<td>Vitek Legacy</td>
<td>68</td>
<td>24</td>
</tr>
<tr>
<td>Vitek 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Boldface indicates that the MICs for the isolates were less than or equal to the MIC listed.*

Swenson, JCM 2009, 2013
Gradient tests vs BMD

Several studies have confirmed that Etest (biomerrieux) results in MICs 0.5 to 1.5 double dilutions higher than BMD

- No studies with M.I.C.E (Thermofisher) or the MIC Test strip (Liofilchem)
- 1800 isolates were tested in parallel
- Far more isolates with MIC of 2 mg/L i.e. EUCAST warning!
  - Do not change No. of resistant isolates

Sader AAC, 2009, 3162
Comparison of BMD and commercial systems

- 129 *S. aureus* isolates with MICs between 0.5 and 8 mg/L
Comparison of BMD and commercial systems

- 129 *S. aureus* isolates with MICs between 0.5 and 8 mg/L

<table>
<thead>
<tr>
<th>Method or system</th>
<th>No. of results with vancomycin MIC (µg/mL) of a:</th>
<th>% of results with vancomycin category of b:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>BMIC-Difco</td>
<td>7</td>
<td>53</td>
</tr>
<tr>
<td>BMIC-BBL</td>
<td>9</td>
<td>53</td>
</tr>
<tr>
<td>Agar dilution</td>
<td>9</td>
<td>54</td>
</tr>
<tr>
<td>Etest</td>
<td>4</td>
<td>22</td>
</tr>
<tr>
<td>MicroScan</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Phoenix</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensititre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitek Legacy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitek 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Boldface indicates that the MICs for the isolates were less than or equal to the MIC listed.
Comparison of BMD and commercial systems

- 129 *S. aureus* isolates with MICs between 0.5 and 8 mg/L
Which MIC?

- For the vast majority of clinical trials outcome and thereby approval of antibiotics are correlated to broth micro dilution (BMD) MIC (ISO 20776)
  - Gradient tests and automated systems are manufactured to give similar results as BMD also to comply with ISO 20776-2.

- EUCAST warning
  - Glycopeptide MICs are method dependent and should be determined by broth microdilution (reference ISO 20776-2).
Detection of hVISA

- Present gold standard is population analysis profile PAP-AUC
  - Cumbersome!!
    - Not suited for routine laboratories
    - Require a spiral plater
  - Long assay time
    - >3 days

- Several screening assays developed
  - i.e. none is perfect
Screening assays for hVISA

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin broth MIC$^b$</td>
<td>11%</td>
<td>100%</td>
<td>372, 389, 393</td>
</tr>
<tr>
<td>BHIA + vancomycin at 6 μg per ml, 10 μl of a 0.5-McFarland-standard suspension (BHIA6V)$^c$</td>
<td>48 h, 4.5–12%</td>
<td>48 h, 68–100%</td>
<td>370, 389, 393</td>
</tr>
<tr>
<td>MHA + teicoplanin at 5 μg per ml, 10 μl of a 2-McFarland-standard suspension (MHA2ST)$^d$</td>
<td>48 h, 65–79%</td>
<td>48 h, 35–95%</td>
<td>82, 252, 370, 389, 393</td>
</tr>
<tr>
<td>MHA + teicoplanin at 5 μg per ml, 10 μl of a 2-McFarland-standard suspension$^e$</td>
<td>48 h, 93%</td>
<td>48 h, 53%</td>
<td>82</td>
</tr>
<tr>
<td>MHA + vancomycin at 5 μg per ml, 10 μl of a 0.5-McFarland-standard suspension</td>
<td>48 h, 1–20%</td>
<td>48 h, 59–99%</td>
<td>370, 372</td>
</tr>
<tr>
<td>Simplified PAP$^f$</td>
<td>48 h, 71%</td>
<td>48 h, 88%</td>
<td>372</td>
</tr>
<tr>
<td>Macromethod Etest (MET)</td>
<td>48 h, 69–98.5%</td>
<td>48 h, 89–94%</td>
<td>174, 289, 370, 372, 389</td>
</tr>
<tr>
<td>Etest GRD</td>
<td>24 h, 70–77%</td>
<td>24 h, 98–100%</td>
<td>174, 393</td>
</tr>
<tr>
<td></td>
<td>48 h, 93–94%</td>
<td>48 h, 82–95%</td>
<td></td>
</tr>
</tbody>
</table>
VRSA

- USA - 12 cases
  - First discovered in 2002
    - 8 from Michigan!
    - 2 most recent from Delaware (2010)

- Iran – 1 case
  - THE-2, 2005

- India - 6 cases
  - From intensive care units in 2 tertiary hospitals in Hyderabad - 2008

http://www.cdc.gov/HAI/settings/lab/vrsa_lab_search_containment.html
Highly variable prevalence's are reported in the literature including within countries

Illustrated by data from Australia

- **Melbourne (Austin)** – 117 MRSA
  - hVISA 56 isolates (48%)
  - VISA 2 isolates (2%)
- **Sydney** 401 MRSA BSI
  - hVISA 46 (11.5%) (almost all ST239)
  - VISA 2 (0.5%)
- **Australia – general** – 532 SAB / 202 MRSA
  - hVISA 2 isolates 0.4% / 1%
  - VISA 0 isolates

Horne AAC, 2009, 3447
Val Hal, PloSOne, 2011
Holmes, JID, 2011, 340
Prevalence og VISA /hVISA

- In General countrywide studies finds very rates of hVISA and VISA even from Japan
  - Reviewed by Howden et al

- Italy – 1284 MRSA isolates (2005-7)
  - 139 had MICs between 1 mg/L and 2 mg/L
  - hVISA 36 isolates (PAP-AUC) 26% / 3%

- Korea – 37,586 isolates (2001-6) screened by BHI + 4 mg/L vancomycin
  - hVISA -15 0.04%
  - VISA – 18 0.04%

- USA – Detroit
  - 485 MRSA blood isolates (1996-2006)
    - hVISA 33 isolates 6.8%
    - VISA 7 isolate 1.4%

Howden, CMR, 2010, 99
Campanile F, IJAA, 2010, 415
Riederer, JCM, 2011
Chung, J Micro Bio, 2010, 637
Vancomycin MIC creep

- A number of institutions have reported an increase in MIC over time especially when using Etest.
- Other studies have, however, not been able to confirm this – including a large study using isolates.

Edwards, JCM, 2012, 318
Vancomycin MIC creep of MIC

- Comparison of vancomycin MICs from 2006-2010 in Scotland
  - Etest and Vitek 2 performed prospectively 2007-10
    - N=102
  - Etest, Vitek 2 and BMD performed on strains after storage 2006-10 (N=208 – including the strains above)

- Results
  - Prospective testing
    - Etest mean MIC 1.08 mg/L
    - Vitek 2 mean MIC 0.56 mg/L
Vancomycin MIC creep of MIC

- Retrospective vs prospective testing
Reduced Clinical efficacy despite Susceptible MIC?

Several papers have shown that the clinical efficacy is related to the MIC even within the susceptible range (i.e. ≤2 mg/L)
Studies supporting lowering of the breakpoints for vancomycin

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Method</th>
<th>Interpretive MIC</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sakoulas JCM 2004</td>
<td>30</td>
<td>CLSI</td>
<td>&gt; 0.5</td>
<td>Failure</td>
</tr>
<tr>
<td>Moise CID 2004</td>
<td>63</td>
<td>CLSI</td>
<td>&gt; 0.5</td>
<td>Failure</td>
</tr>
<tr>
<td>Moise AACH 2007</td>
<td>34</td>
<td>CLSI</td>
<td>&gt; 0.5</td>
<td>Failure</td>
</tr>
<tr>
<td>Hidayat AIM 2006</td>
<td>95</td>
<td>E-test</td>
<td>&gt;1</td>
<td>Failure</td>
</tr>
<tr>
<td>Lodise AACH 2008</td>
<td>92</td>
<td>E-test</td>
<td>&gt;1</td>
<td>Failure</td>
</tr>
<tr>
<td>Soriano CID 2008</td>
<td>414</td>
<td>E-test</td>
<td>&gt;1</td>
<td>Mortality</td>
</tr>
<tr>
<td><em>Musta JCM 2009</em></td>
<td>489</td>
<td>E-test</td>
<td>&gt;1</td>
<td>Mortality</td>
</tr>
<tr>
<td>Kullar R CID 2011</td>
<td>320</td>
<td>E-test</td>
<td>&gt;1</td>
<td>Failure</td>
</tr>
<tr>
<td>Haque Chest 2010</td>
<td>163</td>
<td>E-test</td>
<td>&gt;1</td>
<td>Mortality</td>
</tr>
<tr>
<td>Young Yoon JAC 2010</td>
<td>63</td>
<td>Vitek</td>
<td>≥ 2</td>
<td>PB</td>
</tr>
<tr>
<td>Sheng-Hsiang JAC 2010</td>
<td>277</td>
<td>CLSI</td>
<td>≥ 2</td>
<td>Mortality (PB)</td>
</tr>
</tbody>
</table>
Lowering the breakpoints?

- Lowering the breakpoint to
  - $S < 1 \text{ mg/L}$ for BMD
  - $S < 1.5 \text{ mg/L}$ for Etest

- 83% / 90% of the isolates from Sader et al. would be I/R
  - Does not correspond with clinical perception!

EUCAST warning

- $S. aureus$ with vancomycin MIC values of 2 mg/L are on the border of the wild type MIC distribution and there may be an impaired clinical response.

Tenover, Moellering, CID, 2007, 1208
Summary

- The prevalence of VRSA and VISA isolates are still low in most part of the world.
- The prevalence of hVISA varies in general relatively low but can locally be up 50% of MRSA isolates.
- There are increasing evidence that strains with MIC > 1 mg/L are associated with poorer outcome – but large definitive prospective studies is needed.
  - Especially as MIC determination is highly method dependant.
Summary

- BMD is in my opinion till the reference method for MIC determination
- For hVISA the gold standard continues to be the PAP-AUC method but it is cumbersome
  - Requires a spiral plater!
  - A number of initial screening methods exists but none are perfect!
- The phenotype of hVISA is not stable and may be lost during storage which needs to be taken into account into future studies
Acknowledgements

- Staphylococcus Laboratory
  - Anders Rhod Larsen, Jesper Larsen, Andreas Petersen, Marit Sørum, Julie Hindsberg, Lone Ryste Hansen, Nadia Olsen, Stine Freese Madsen

- Benjamin Howden, Melbourne, Australia
- Brandi Limbargo, CDC, Atlanta, US
- Stefania Stefani, Catania, Italy
- Alex Soriano, Barcelona, Spain