

Susceptibility testing of MRSA including Screening methods

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- **Definition**

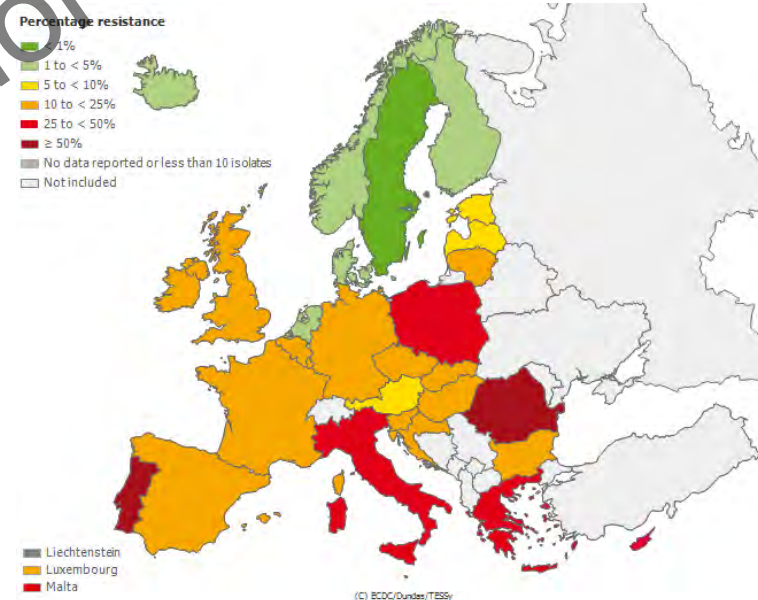
- *S. aureus* with modified PBP, low affinity for β -Lactam-Antibiotics (not Ceftarolin/Ceftobiprol)

- **Mechanism**

- PBP2a coded by *mecA/mecC*

- **Clinical/epidemiological importance**

- infection control
- systemic infections ↓ outcome
- 2013: Austria 9,1 % (EARS-Net)



EARS-Net 2012



- **Phenotype**

- Disk diffusion: Cefoxitin 30µg <22 mm ✓
Oxacillin ✗ (Heteroresistance)
- MIC: Cefoxitin MHK >4 mg/L
Oxacillin MHK >2 mg/L
- Agglutination tests: detect *mecA* coded PBP2a only

- **Molecular biology**

- PCR methods should cover *mecA* and *mecC*



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Detection of *mecC*

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STARTSEITE
AKTUELLES
FACHBEREICHE
KUNDENSERVICE
UNTERNEHMEN
KONTAKT

NATIONALES REFERENZZENTRUM

Aktuelles
IHMT
Hintergrund
AURES
PROHYG 2.0
• NAC-AT

Mitglieder
EUCAST reloaded 1.0
EUCAST reloaded 2.0
• EUCAST reloaded 3.0

Carbapenemase
Libysche Patienten


21. März 2013 - EUCAST reloaded 3.0

Etwa 100 mikrobiologisch interessierte Kolleginnen und Kollegen besuchten am 21. März 2013 die vom NRZ organisierte EUCAST-Veranstaltung am Bundesministerium für Gesundheit in Wien. Aktuelle Informationen zu MecC MRSA, zu Epidemiologie und Diagnostik von Carbapenemase, News aus dem NRZ sowie die Umstellung von CLSI auf EUCAST in der medizinischen Mykologie waren die zentralen Themen der diesjährigen Veranstaltung.

An dieser Stelle möchten wir uns sehr herzlich bei den Vortragenden, aber auch dem Publikum, für die aktive Teilnahme und anregende Diskussion bedanken.

Die von den Referentinnen und Referenten zur Verfügung gestellten Vorträge des Workshops stehen nachfolgend zum Download bereit.

[Vortrag Holmes »](#)
[Vortrag Hartl »](#)
[Vortrag Apfalter »](#)
[Vortrag Willinger »](#)
[Vortrag Metz-Gercek »](#)



Suchbegriff eingeben >> Suche

Kontaktinfos

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Detection

□ Isolates

- 111 isolates containing the mecA variant

□ Methods (not all 111 isolates have been tested in all methods)

➤ Phenotypic

- MIC oxacillin and ceftazidime
 - (BMD, agardilution, Etest)
- Disk diffusion (oxacillin and ceftazidime)
- Chromogenic agars MRSA
 - Brilliance (Oxoid), Chrome ID (bioMerriex), Chrome agar BD, MRSA select
- Vitek (bioMerriex), Phoenix (BD), Microscan (Siemens)
- PBP2a agglutination, Clearview

Detection

□ Methods – genotypic

➤ In house as well as commercial PCR

- GeneOhm™ StaphSR (BD), GeneXpert™ MRSA (Cepheid) og NucliSENS EasyQ MRSA (bioMérieux)

Results

□ BMD/AD

- Cefox 8-64 mg/L
- Oxacillin 1-64 mg/L / 2-32 mg/L (4 / 6 isolates \leq 2 mg/L)

□ Etest

- Cefox 1-48 mg/L (1 isolate $>$ 6 mg/L)
- Oxacillin 0.75-24 mg/L (5 isolates \leq 2 mg/L)

□ Disk diffusion

- Cefox 10-20 mm
- Oxacillin 6-18 mm (5 isolates $>$ 12 mm)

Results

- ❑ Chromogenic agars MRSA – growth of (24h)
 - Brillance (Oxoid)
 - 108 of 111
 - Chrome ID (bioMerieux),
 - 110 of 111
 - Chrome agar (BD)
 - 88 of 111
 - MRSA select (Biorad)
 - 70 of 111

Results

□ Automated systems

➤ Vitek

- cefoxitin screen 108 of 111
- Oxacillin (≥ 4 mg/L) 21 of 111

➤ Phoenix

- cefoxitin (> 4 mg/L) 83 of 111
- oxacillin (> 2 mg/L) 17 of 111
- Latamoxcef (> 16 mg/L) 99 of 111

➤ Microscan

- cefoxitin (> 4 mg/L) 106 of 110
- oxacillin (> 2 mg/L) 0 of 110
- Cefotaxime (> 4 mg/L) 108 of 110

Detection

❑ Agglutinations assay

- PBP2a detection – negative
 - Some are aberrant positive

❑ Clearview

- Without induction 10 of 111
- With induction 111 of 111

Detection

□ Genotypic methods - All negative!

➤ Commercial methods:

- GeneOhm™ StaphSR (BD), GeneXpert™ MRSA (Cepheid) og NucliSENS EasyQ MRSA (bioMerieux)

➤ Novel PCRs

- *Spa, mecA, mecA_{LGA251}, pvl* – *Direct spa seq.* (Stegger, CMI, online)
- Real Time PCR (Pichon B. et al, JAC, accepted)
- GENSPEED® MRSA PCR test (Greiner Bio-One, Kremsmünster, Austria)



- **Cefoxitin disk diffusion: reliable**
- **Vitek 2: OK, typical profile**
 - Cefoxitin-screen: positive
 - Oxacillin: MHK ≤ 2 , system changes to *R
 - specify with PCR
- **MRSA selektive media: different conclusions**
- **non- β -Laktam antibiotics: susceptible**
- **molecular biology based screening should also cover *mecC***





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Vancomycin

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EUCAST and CLSI Breakpoints for vancomycin

| | EUCAST (S / R) | CLSI (S / R) |
|-----------------------|--|--|
| MIC | $\leq 2 / >2$ mg/L* | $<2 / \geq 16$ mg/L |
| Disk Diffusion | Not reliable | |

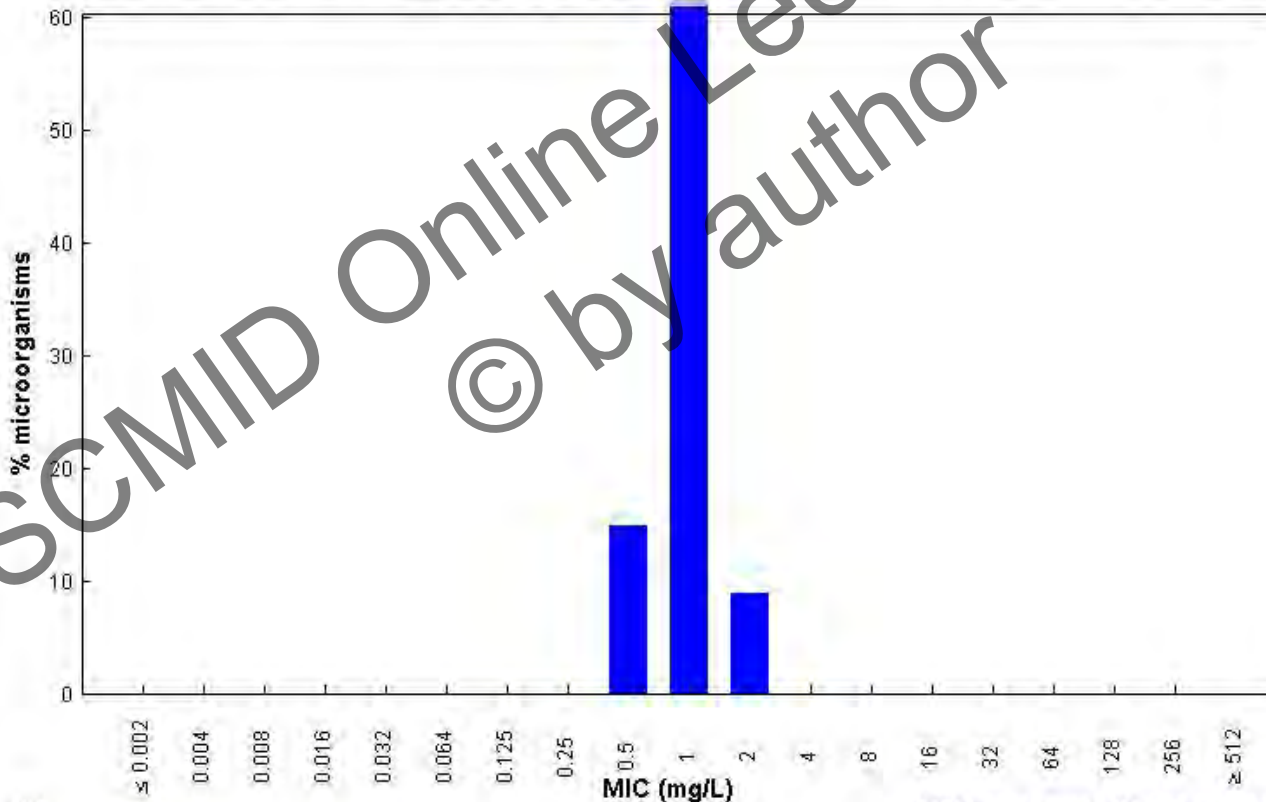
*Glycopeptide MICs are method dependent and should be determined by broth microdilution.

***Isolates with vancomycin MIC of 2 mg/L may have an impaired clinical response.**

Vancomycin Distribution - EUCAST

Vancomycin / *Staphylococcus aureus*
EUCAST MIC Distribution - Reference Database

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC
Epidemiological cut-off: WT ≤ 2 mg/L

87764 observations (33 data sources)
Clinical breakpoints: S ≤ 2 mg/L, R ≥ 2 mg/L

Vancomycin breakpoints

- ❑ Breakpoints have changed during the last years
 - Original breakpoints were developed at a time when resistant isolates were absent
 - Emergence of reports of VISA and hVISA isolates with association to treatment failure
 - Recognition that isolates with MICs of 8 and probably also 4 mg/L are R and not I
 - Although the terms hVISA and VISA is still used to distinguish from VRSA (*vanA* mediated resistance)
 - ie. higher doses will not overcome the reduced susceptibility

Susceptibility / Nomenclature

- ❑ Susceptible (VSSA)
 - MIC \leq 2 mg/L AND no resistant subpopulations
- ❑ Heterogeneous “intermediate” susceptible (hVISA)
 - MIC \leq 2 mg/L WITH resistant subpopulations
 - Clinical significance not fully elucidated
- ❑ “Intermediate” susceptible (VISA)
 - MIC of 4/8 mg/L
 - Should be considered clinical resistant
- ❑ Resistant VRSA
 - MIC $>$ 8 mg/L
 - *vanA* mediated

Vancomycin – resistance rates

- ❑ VRSA - Acquisition of vancomycin resistance genes from *E. faecium* - Vancomycin MIC >8µg/ml
 - 14 Case reports in *S. aureus*, 12 in US
 - http://www.cdc.gov/HAI/settings/lab/vrsa_lab_search_containment.html
 - Iran – 1 case, THE-2, 2005
 - India - 6 cases, intensive care units in 2 tertiary hospitals in hydrabad - 2008

Vancomycin – resistance rates

❑ GISA/VISA

- Thickened cell wall (“Sponge”) - Vancomycin MIC 4-8 mg/L
 - Even with new CSLI definition, <0.1% of strains worldwide

❑ hVISA

- Thickened cell wall (“Sponge”) - Vancomycin MIC ≤ 2 mg/L
 - Prevalence in general very low, however, locally up to 50% - clonal outbreaks

Testing issues with vancomycin

❑ Disk diffusion

- Can detect VRSA
- **CAN NOT** distinguish susceptible strains (VSSA) from from hVISA or VISA
 - Not recommended by either EUCAST or CLSI

❑ MIC

- Etest results in 1/2-1 dilution higher MICs than Micro broth dilution
- Detection of isolates with reduced susceptibility
 - hVISA
 - RVS-SA

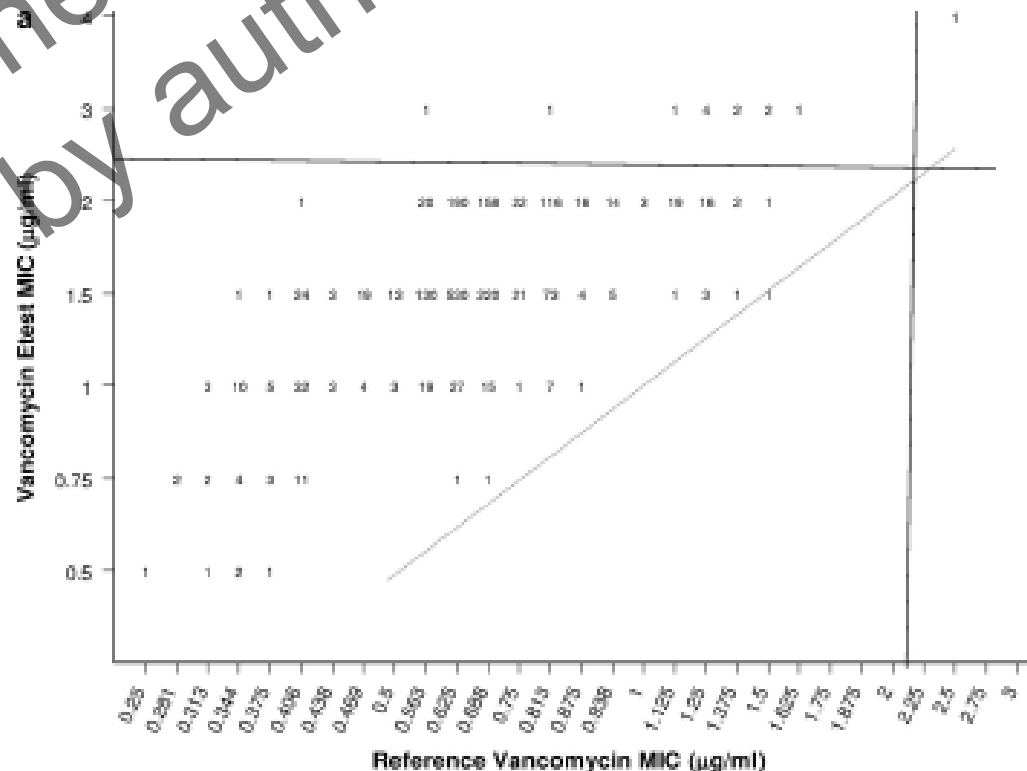
Etest vs BMD

□ Several studies have shown that Etest results in MICs 0.5 to 1.5 double dilutions higher than BMD

➤ In this study 1800 isolates were tested in parallel

Sader AAC, 2009, 3162

- Do not change No. of resistant isolates
- **Far more isolates with MIC of 2 mg/L**
I.e. EUCAST warning



Detection of hVISA

- ❑ No clearcut definition
 - No single gene responsible for the phenotype
- ❑ Present gold standard is population analysis profile PAP-AUC
 - Cumbersome!!
 - Not suited for routine laboratories
- ❑ Several screening assays developed
 - i.e. none is perfect – macro Etest is probably the best presently

Screening assays for hVISA

| Method | Sensitivity | Specificity | Reference(s) |
|--|----------------|---------------|-------------------------|
| Vancomycin broth MIC ^b | 11% | 100% | 372 |
| BHIA + vancomycin at 6 µg per ml, 10 µl of a 0.5-McFarland standard suspension (BHIA6V) ^c | 48 h, 4.5–12% | 48 h, 68–100% | 370, 389, 393 |
| MHA + teicoplanin at 5 µg per ml, 10 µl of a 2-McFarland standard suspension (MHA5T) ^d | 48 h, 65–79% | 48 h, 35–95% | 82, 252, 370, 389, 393 |
| MHA + teicoplanin at 5 µg per ml, 10 µl of a 2-McFarland standard suspension ^e | 48 h, 98% | 48 h, 53% | 82 |
| MHA + vancomycin at 5 µg per ml, 10 µl of a 0.5-McFarland standard suspension | 48 h, 1–20% | 48 h, 59–99% | 370, 372 |
| Simplified PAP ^f | 48 h, 71% | 48 h, 88% | 372 |
| Macromethod Etest (MET) | 48 h, 69–98.5% | 48 h, 89–94% | 174, 289, 370, 372, 389 |
| Etest GRD | 24 h, 70–77% | 24 h, 98–100% | 174, 393 |
| | 48 h, 93–94% | 48 h, 82–95% | |



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Linezolid

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EUCAST and CLSI Breakpoints for Linezolid

| | EUCAST (S / R) | CLSI (S / R) |
|----------------|--------------------------|-------------------------------|
| MIC | ≤ 4 / >4 mg/L | ≤ 4 / ≥ 8 mg/L |
| Disk Diffusion | ≥ 17 mm / <17 mm* | ≥ 21 mm / ≤ 20 mm** |

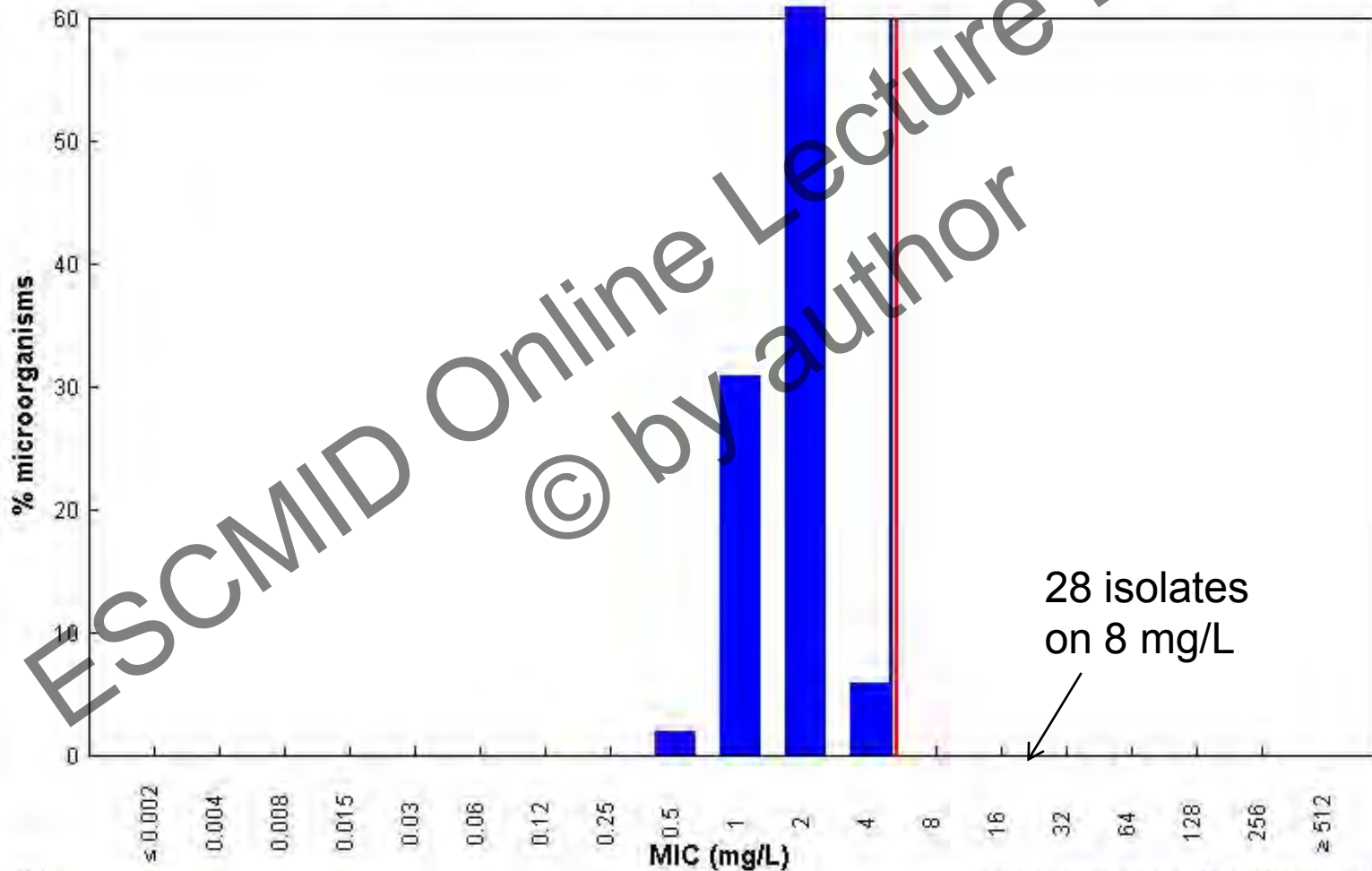
*10 μ g reflected light, tentative breakpoint

**30 μ g, transmitted light



Linezolid / *Staphylococcus aureus* EUCAST MIC Distribution - Reference Database 2012-09-24

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC
Epidemiological cut-off: WT ≤ 4 mg/L

62420 observations (22 data sources)
Clinical breakpoints: S ≤ 4 mg/L, R ≥ 4 mg/L

Linezolid

Resistance mechanisms

□ Resistance mechanisms

- Alterations in the binding to the peptidyl transferase center (PTC) of the Ribosome
 - Mutations in the 23S rRNA
 - Alterations in the ribosomal proteins L3 and L4
- Plasmid mediated *cfr* methyltransferase gene

Linezolid - prevalence of resistance

□ Resistance level is still very low

➤ ZAPPS study:

- 5769 isolates from 57 sites / 34 countries outside US
 - 2,831 *S. aureus*
 - 656 CoNS
- No resistance in *S. aureus*
- 7 CoNS resistant
 - 5 with 23S mutation
 - 1 with *cfr*
 - 1 with *cfr* and 23S mutation
 - » In addition 4 CoNS isolates carried the *cfr* gene but had an MIC of 4 mg/L
- 2 *E. faecium* isolates
 - 23S mutation

Linezolid - resistance

- ❑ Dissemination of the plasmid mediated *cfr* gene in staphylococci, and enterococci is worrisome
 - Nosocomial outbreaks in *S. aureus* from Spain
 - LA-MRSA of ST 398 and ST9
 - CoNS in Southern Europe and USA
 - *E. faecium* in Spain

Linezolid testing Issues

- ❑ Resistance is still rare!
 - Resistance by disk diffusion should be confirmed by MIC
- ❑ Broth Microdilution
 - Tendency to produce a one well trailing effect
- ❑ Etest, disk diffusion and automated systems
 - Tendency to undercall resistance

Accuracy of Linezolid Testing by Six Methods

- ❑ 100 isolates (**25 *S. aureus***, 25 CoNS, 25 *E. faecalis*, 25 *E. faecium*)
 - 32 of 100 organisms were non-susceptible or resistant (**7/25 *S. aureus* non-susceptible**)
- ❑ Disk diffusion
 - Reflected light
 - Transmitted light
- ❑ Etest
 - Using 90% inhibition
- ❑ Automated systems

Accuracy of Linezolid Testing *S. aureus*

❑ Disk diffusion

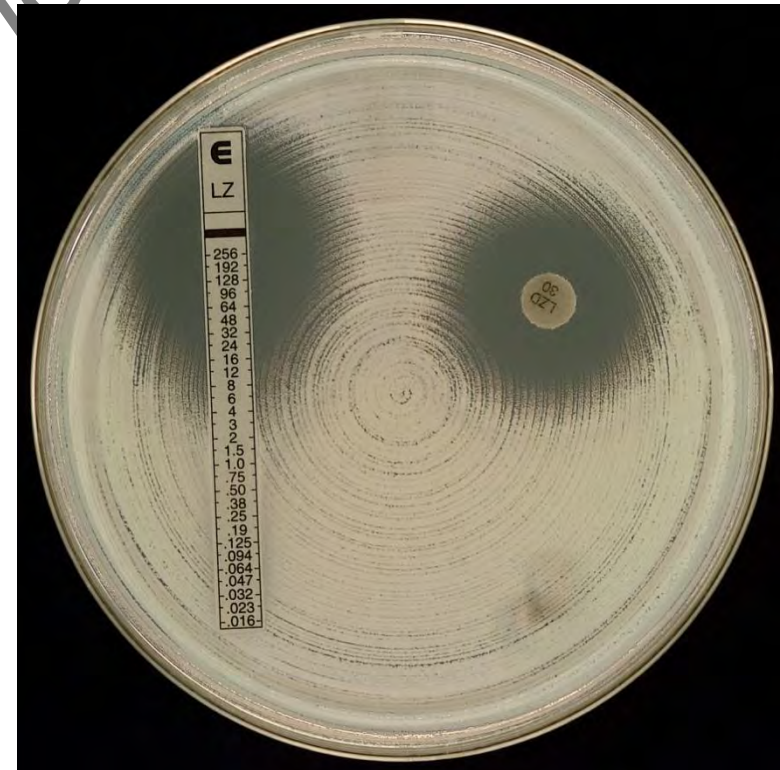
- Reflected light: 4/18 Very Major errors (VME)
- Transmitted light: 1/18 VME

❑ Etest

- 5/18 VME
 - For CoNS. 1/17 VME

❑ Automated systems

- 1/4/1 VME for Microscan,
Phoenix and Vitek2



Linezolid testing Issues

- ❑ Susceptibility testing using both disk diffusion and Etest remains a problem for many strains due to fuzzy zone edges
- ❑ Should be a focus for quality control schemes like NEQUAS



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Daptomycin

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EUCAST and CLSI Breakpoints for Daptomycin

| | EUCAST (S / R) | CLSI (Susceptible only) |
|-----------------------|---------------------------|------------------------------------|
| MIC | ≤ 1 / >1 mg/L | ≤ 1 mg/L |
| Disk Diffusion | Not reliable | |

Resistance rates

- ❑ Resistance / non-susceptibility is quite rare and resistant values should be confirmed by a reference laboratory

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Resistance Mechanism

- ❑ Whole genome sequence of 33 isolates
 - 21 isogenic pairs/series of clinical isolates
 - 12 laboratory derived mutants
- ❑ 3 main types of mutations were identified
 - All with the net effect of charge of the cell membrane leading to repulsion of daptomycin

| Gene | Function | Result |
|-------------|-------------------------------|---------------------------------|
| <i>mprF</i> | Large membrane protein | More Lysine-PG |
| <i>cls2</i> | Anionic membrane phospholipid | Decreased amount of cardiolipin |
| <i>pgsA</i> | CDP-diacylglycerol to PG | Increased production of PG |

Daptomycin testing issues

- ❑ Can only be tested by MIC and not by disk diffusion
 - Many laboratories do not test for daptomycin primarily
- ❑ Mechanism of action is dependant of Ca^{++} level
 - Reflected when MIC testing is performed
 - Low Ca^{++} level results in elevated MICs
- ❑ Need for standardization of both broth media and agar media (Etest)
 - 50 mg/L for broth micro dilution
 - 25-40 mg/L for agar media used with Etest

Ca⁺⁺ contents in commercial MH agar

Manufacturer mg/L

- Mast (N=1) 19
- EO (N=1) 21
- Becton Dickinson (N=3) 29-31
- Oxoid (N=4) 29-36
- Biorad (N=2) 63-64
- bioMerieux (N=3) 63-64

Dependency of Ca⁺⁺ using Etest on Mueller Hinton agar

| BMD MIC (mg/L) | MHA [Ca ⁺⁺] (mg/L) | Number of Etest MICs with a doubling dilution difference compared with BMD MICs of: | | | | | | | | | | | | |
|----------------|--------------------------------|---|------|----|------|----|------|----|-----|----|-----|---|-----|---|
| | | -3 | -2.5 | -2 | -1.5 | -1 | -0.5 | 0 | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 |
| 0.5* | <20 | | | | | | | | | 1 | 6 | 3 | 1 | 1 |
| | 20 | | | | | | | | 5 | 5 | 2 | | | |
| | 25-30 | | | | | | 1 | 3 | 5 | 3 | | | | |
| | 40 | | | | | 2 | 1 | 4 | 5 | | | | | |
| | 50 | | | | | 7 | 3 | 1 | 1 | | | | | |
| 1 | <20 | | | | | | | | | 3 | 10 | 1 | | |
| | 20 | | | | | | | | 2 | 9 | 3 | | | |
| | 25-30 | | | | | | | 2 | 7 | 10 | | | | |
| | 40 | | | | | | | 4 | 9 | 1 | | | | |
| | 50** | | | | | 1 | 1 | 4 | 7 | 1 | | | | |
| 2 | <20 | | | | | | | | 4 | 9 | 1 | | | |
| | 20 | | | | | | | 3 | 7 | 4 | | | | |
| | 25-30 | | | | | | | 9 | 5 | | | | | |
| | 40 | | | | | | 3 | 8 | 3 | | | | | |
| | 50*** | | | | | 1 | 3 | 8 | 1 | | | | | |
| Total | <20 | | | | | | | | 4 | 13 | 17 | 4 | 1 | 1 |
| | 20 | | | | | | | 3 | 14 | 18 | 5 | | | |
| | 25-30 | | | | | | 1 | 14 | 12 | 13 | | | | |
| | 40 | | | | | 2 | 4 | 16 | 17 | 1 | | | | |
| | 50 | | | | | 8 | 6 | 13 | 9 | 1 | | | | |

*Includes one isolate with an MIC of 0.25 mg/L

**2 results not available

***1 result not available

Etest on ISA

Etest on BHIA

| BMD MIC (mg/L) | BHIA [Ca ²⁺] (mg/L) | Number of Etest MICs with a doubling dilution difference compared with BMD MICs of: | | | | | | | | | | | | |
|----------------|---------------------------------|---|------|----|------|----|------|---|-----|----|-----|----|-----|---|
| | | -3 | -2.5 | -2 | -1.5 | -1 | -0.5 | 0 | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 |
| 0.5* | <25 | | | | | | | | | | | 8 | 7 | 2 |
| | 25-30 | | | | | | | | 2 | 2 | 3 | 2 | 3 | |
| | 40** | | | | | | | | 4 | 1 | 3 | 2 | | |
| | 50 | | | | | | | | 2 | 5 | 3 | 1 | 1 | |
| 1 | <25 | | | | | | | | | | 5 | 8 | 1 | |
| | 25-30 | | | | | | | | 4 | 3 | 6 | 1 | | |
| | 40 | | | | | | | | 1 | 3 | 3 | 1 | 1 | |
| | 50 | | | | | | | | 2 | 7 | 5 | | | |
| 2 | <25 | | | | | | | | | 4 | 8 | 2 | | |
| | 25-30 | | | | | | | | 2 | 5 | 6 | 1 | | |
| | 40 | | | | | | | 1 | 3 | 7 | 2 | 1 | | |
| | 50 | | | | | | | 2 | 3 | 8 | 1 | | | |
| Total | <25 | | | | | | | | | 4 | 13 | 18 | 3 | 2 |
| | 25-30 | | | | | | | | 2 | 11 | 11 | 9 | 4 | 3 |
| | 40 | | | | | | | 1 | 4 | 14 | 11 | 5 | 3 | |
| | 50 | | | | | | | 2 | 7 | 20 | 9 | 1 | 1 | |

*Includes one isolate with an MIC of 0.25 mg/L
 **2 results not available

| BMD MIC (mg/L) | ISA [Ca ²⁺] (mg/L) | Number of Etest MICs with a doubling dilution difference compared with BMD MICs of: | | | | | | | | | | | | | | | | |
|----------------|--------------------------------|---|------|----|------|----|------|---|-----|---|-----|---|-----|----|----|----|---|---|
| | | -3 | -2.5 | -2 | -1.5 | -1 | -0.5 | 0 | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 | | | | |
| 0.5* | <20 | | | | | | | | | | | 1 | 5 | 4 | 1 | 1 | | |
| | 20 | | | | | | | | | | | 8 | 1 | 3 | | | | |
| | 25-30 | | | | | | | | | | | 7 | 2 | 2 | 1 | | | |
| | 40 | | | | | | | | | | | 2 | 7 | 1 | 1 | | | |
| 1 | <20 | | | | | | | | | | | | 3 | 8 | 3 | | | |
| | 20 | | | | | | | | | | | | 12 | 2 | | | | |
| | 25-30 | | | | | | | | | | | | 7 | 7 | | | | |
| | 40 | | | | | | | | | | | | 4 | 7 | 3 | | | |
| 2 | <20 | | | | | | | | | | | | | | | | | |
| | 20 | | | | | | | | | | | | | | | | | |
| | 25-30 | | | | | | | | | | | | | | | | | |
| | 40 | | | | | | | | | | | | | | | | | |
| Total | <20 | | | | | | | | | | | | 2 | 10 | 18 | 8 | 1 | 1 |
| | 20 | | | | | | | | | | | | 5 | 29 | 3 | 3 | | |
| | 25-30 | | | | | | | | | | | | 6 | 21 | 10 | 2 | 1 | |
| | 40 | | | | | | | | | | | | 15 | 19 | 4 | 2 | | |
| Total | 50 | | | | | | | | | | | | 1 | 4 | 20 | 10 | 3 | |

*Includes one isolate with an MIC of 0.25 mg/L
 **2 results not available

Daptomycin and automated systems

- ❑ Evaluations have generally shown good performance for both *S. aureus* and enterocococci
 - MicroScan, Phoenix, Sensititre, Vitek

Thank you for your attention

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