

Practical cases

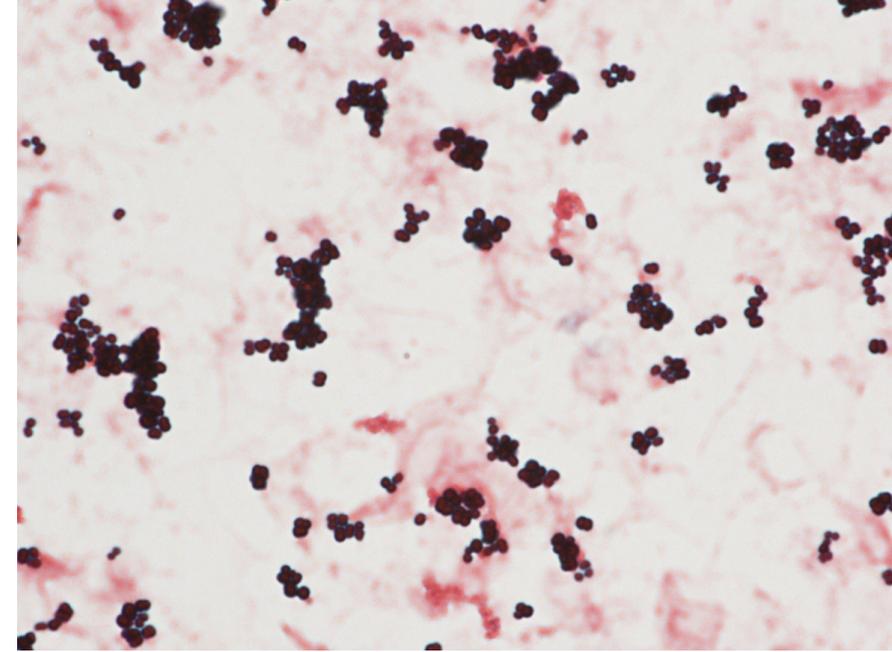
Karianne Wiger Gammelsrud

Nathalie Friberg

Case 1:

Is it an MRSA?

Case 1



- Positive bloodculture: Gram-positive cocci
- Growth next day: *Staphylococcus aureus* (identified by MALDI-TOF)
- AST:

cefoxitin 11 mm (S \geq 22 mm)

R

all other tested antibiotics are

S

- Commercial MRSA-PCR test from 1 isolated CFU from chocolate plate: **MRSA not detected**

Mentimeter 1

What do you do first?

Check for purity

Check QC (quality control) results

Repeat the disk diffusion

Repeat the PCR-test

Mentimeter 2

When you have confirm it is a pure culture of *S. aureus*, how would you proceed?

Repeat the disk diffusion

Repeat the same PCR-test

Do another PCR-test (if possible)

Send the strain to the reference lab

Warn clinicians of the possibility of MRSA

Case 1 – what was actually done?

- Purity and QC were checked; AST and PCR-tests were redone \Rightarrow same results
- Clinicians were warned of MRSA possibility
- Strain was sent to the reference lab and to our lab
- We ran different PCR-tests (commercial and in house)
 - MecA: positive
 - Nuc: positive
 - SCC: negative for commercial tests
- Commercial tests based on detection of MecA and SCC were negative
- Commercial tests based on detection of MecA and Nuc were positive
- Reference lab:
Confirms presence of MecA gene and mutations in the SCC area, Spa type t122

Staphylococcus aureus

No gene detected

= MSSA (wild type)



= **MRSA** (orf + SCC + MEC)



= MSSA (orf + SCC)



= MSSA (orf + SCC + unfunctional MEC)

Our strain:



= **MRSA** (orf + mutation in the SCC + MEC)

Case 1 – and the same has happened in Norway...

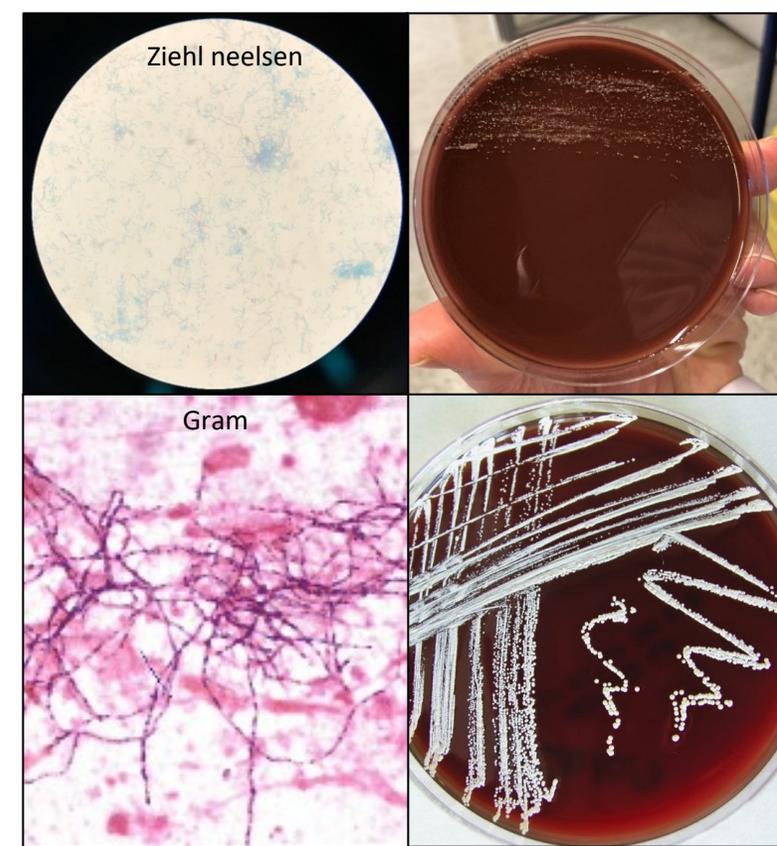
- The reference lab has identified at least two different strains
 - Negative SCCmec på GeneXpert and positive mecA: is then reported negative
 - WGS showed SCCmec 5 for both isolates – otherwise nothing in common:
 - ⇒ Isolate 1 (2020) and isolate (2022) were different spa types and strain types
- OUH-Ullevål (during the week-end) past year:
 - Eye infection in new born.
 - Cefoxitin 16 mm, R genta and cipro. S for the rest of the tested antibiotics
 - GeneXpert: reported as negative (Negative SCCmec and positive mecA)
 - PCR: nuc-positive and MecA-positive
 - Good growth on MRSA screening agar

Case 2 and 3:

Help! It's a *Nocardia* what should we do?

Clinical significance of *Nocardia*

- Gram-positive rod, strict aerobic, typically branched, partially acid-fast, slow-growing
- Environmental microbe (smells like "potato cellar")
- Opportunistic infections, ↑ immunocompromised pts.
 - 1/3 = immunocompetent patients
- Transmission via inhalation or skin inoculation
 - Lung infections: obs immunocompromised, COPD, bronchiectasis and other chronic lung diseases (eg. CF)
 - Can spread locally (eg. empyema, mediastinitis, pericarditis) or hematogenously (brain abscess!)
 - Skin infections: localized, nodular lymphngitis or mycetoma (large tumors) – obs penetrating traumas
 - CNS infections: Brain abscess (most common), meningitis



Case 2

- Brain abscess case
- Culture result: *Nocardia farcinica*

...and during the same week Case 3

- Immunosuppressed patient
- Bloodculture positive with *Nocardia veterana*

Mentimeter

What would you do?

Refuse to perform AST

Try to do something at your own lab

Send the strain to a reference lab

- No EUCAST reference method nor breakpoints for *Nocardia spp*
- No EUCAST MIC distribution in the database for *N. farcinaca* nor *N. veterana*



EUCAST guidance on
When there are no breakpoints in breakpoint tables?
 2024-02-29

The MIC is the basis for any assessment. In the absence of a breakpoint, an assessment can only be made when an accurate and reproducible MIC value can be obtained. Determine the MIC using reference methods or validated surrogate methods.

Agents and notes for aerobic bacteria	MIC-values above which therapy with the agent should be discouraged		Notes
	Gram-positive organisms	Gram-negative organisms	
Benzylpenicillin	0.25	0.5	If a beta-lactamase is detected, report resistant without further testing.
Ampicillin, Amoxicillin, Ampicillin-sulbactam, Amoxicillin-clavulanic acid (IV only)	0.5	8	The breakpoint of 8 mg/L pertains to intravenous high dose administration. If a beta-lactamase is detected, the value is only valid for amoxicillin-clavulanic acid and ampicillin-sulbactam.
Piperacillin-tazobactam	1	8	Species specific breakpoints for gram-positive organisms are 0.25 – 1 mg/L, and for gram-negative organisms 8 – 16 mg/L
Cefotaxime	0.5	0.5	Cefotaxime and ceftriaxone – resistance to either excludes the use of both.
Ceftriaxone	0.5	0.5	Cefotaxime and ceftriaxone – resistance to either excludes the use of both.
Ceftazidime	-	4	This is the Enterobacterales R-breakpoint.
Imipenem	2	2	Species specific breakpoints are often 2 mg/L.
Meropenem	2	2	Species specific breakpoints are 0.25 – 2 mg/L
Ciprofloxacin	0.25	0.25	Species specific breakpoints are 0.25 – 1 mg/L.
Levofloxacin	0.5	0.5	Species specific breakpoints are 0.25 – 1 mg/L.
Moxifloxacin	0.25	0.25	Species specific breakpoints are 0.125 – 0.5 mg/L
Clindamycin	0.5	NA	Species specific breakpoints are 0.25 – 0.5 mg/L.
Tetracycline (test tetracycline, report doxycycline, minocycline)	2	2 For Gram-negative organisms other than Enterobacterales	Tetracycline (as a representative for tetracycline, doxycycline, and minocycline) species specific breakpoints are 0.5 – 2 mg/L.
Trimethoprim-sulfamethoxazole	1	1	Species specific breakpoints are 0.5 – 2 mg/L.
Tigecycline	0.5	NA	Species specific breakpoints are 0.125 – 0.5 mg/L.
Rifampicin	0.125	NA	Species specific breakpoints are 0.06 – 0.125 mg/L.
Linezolid	2	NA	Species specific breakpoints are 2 - 4 mg/L
Vancomycin	2	NA	Species specific breakpoints are 2 mg/L.
Dalbavancin	0.125	NA	Species specific breakpoints are 0.125 mg/L.
Daptomycin	1	NA	Species specific breakpoints are 1 mg/L.

- No EUCAST breakpoints for *Nocardia spp*
- No EUCAST MIC distribution in the database for *N. farcinaca* nor *N. veterana*



EUCAST guidance on
When there are no breakpoints in breakpoint tables?
 2024-02-29

- CSLI has *Nocardia* specific breakpoints

	MIC-values above which therapy with the agent should be discouraged		Notes
Agents and notes for aerobic bacteria	Gram-positive organisms	CLSI Nocardia BP	
Benzylpenicillin	0.25		If a beta-lactamase is detected, report resistant without further testing.
Ampicillin, Amoxicillin, Ampicillin-sulbactam, Amoxicillin-clavulanic acid (IV only)	0.5		The breakpoint of 8 mg/L pertains to intravenous high dose administration. If a beta-lactamase is detected, the value is only valid for amoxicillin-clavulanic acid and ampicillin-sulbactam.
Piperacillin-tazobactam	1		Species specific breakpoints for gram-positive organisms are 0.25 – 1 mg/L, and for gram-negative organisms 8 – 16 mg/L
Cefotaxime	0.5		Cefotaxime and ceftriaxone – resistance to either excludes the use of both.
Ceftriaxone	0.5		Cefotaxime and ceftriaxone – resistance to either excludes the use of both.
Ceftazidime	-		This is the Enterobacteriales R-breakpoint.
Imipenem	2	→ 4	Species specific breakpoints are often 2 mg/L.
Meropenem	2		Species specific breakpoints are 0.25 – 2 mg/L
Ciprofloxacin	0.25		Species specific breakpoints are 0.25 – 1 mg/L.
Levofloxacin	0.5		Species specific breakpoints are 0.25 – 1 mg/L.
Moxifloxacin	0.25	→ 1	Species specific breakpoints are 0.125 – 0.5 mg/L
Clindamycin	0.5		Species specific breakpoints are 0.25 – 0.5 mg/L.
Tetracycline (test tetracycline, report doxycycline, minocycline)	2		Tetracycline (as a representative for tetracycline, doxycycline, and minocycline) species specific breakpoints are 0.5 – 2 mg/L.
Trimethoprim-sulfamethoxazole	1	→ 2	Species specific breakpoints are 0.5 – 2 mg/L.
Tigecycline	0.5		Species specific breakpoints are 0.125 – 0.5 mg/L.
Rifampicin	0.125		Species specific breakpoints are 0.06 – 0.125 mg/L.
Linezolid	2	→ 8	Species specific breakpoints are 2 - 4 mg/L
Vancomycin	2		Species specific breakpoints are 2 mg/L.
Dalbavancin	0.125		Species specific breakpoints are 0.125 mg/L.
Daptomycin	1		Species specific breakpoints are 1 mg/L.

	<i>N. farcinica</i>	<i>N. nova</i>	<i>N. brasiliensis</i>	<i>N. cyriacigeorgica</i>	<i>N. abscessus</i>	<i>N. otitidiscaviarum</i>
Trimethoprim-sulfamethoxazole	S	S	S	S	S	S
Imipenem	S	S	R	S	S	R
Amikacin	S	S	S	S	S	S
Linezolid	S	S	S	S	S	S
Tobramycin	R	R	S	S	S	S
Amoxicillin-clavulanate	S	R	S	R	S	R
Ceftriaxone	R	S	R	S	S	R
Ciprofloxacin	R	R	R	R	R	R
Clarithromycin	R	S	R	R	R	R
Doxycycline	R	R	R	R	S	R
Minocycline	R	R	R	R	S	R
Erythromycin	R	S	R	R	R	R

Common antimicrobial susceptibility patterns for the most common *Nocardia* species are outlined in the table; species are labeled as susceptible if >80% of isolates tested were susceptible and as resistant if >80% of isolates tested were resistant. Such data may be of use when therapy is commenced before antimicrobial susceptibility results are available. The table summarizes data extracted from four studies.^[1-3] The four studies included are not directly comparable because of differences in the number of isolates and species included, antibiotics tested, and the methods of susceptibility testing used. Moxifloxacin was included in only one study; in this report, 81% of 36 *N. farcinica* isolates were susceptible to moxifloxacin.^[2]

Mentimeter

Which option would you choose?

Use BMD method and interpret according to the EUCAST guidance document

Use gradient test according to instructions and interpret according to the EUCAST guidance document

Use BMD method and interpret according to CLSI *Nocardia* breakpoints

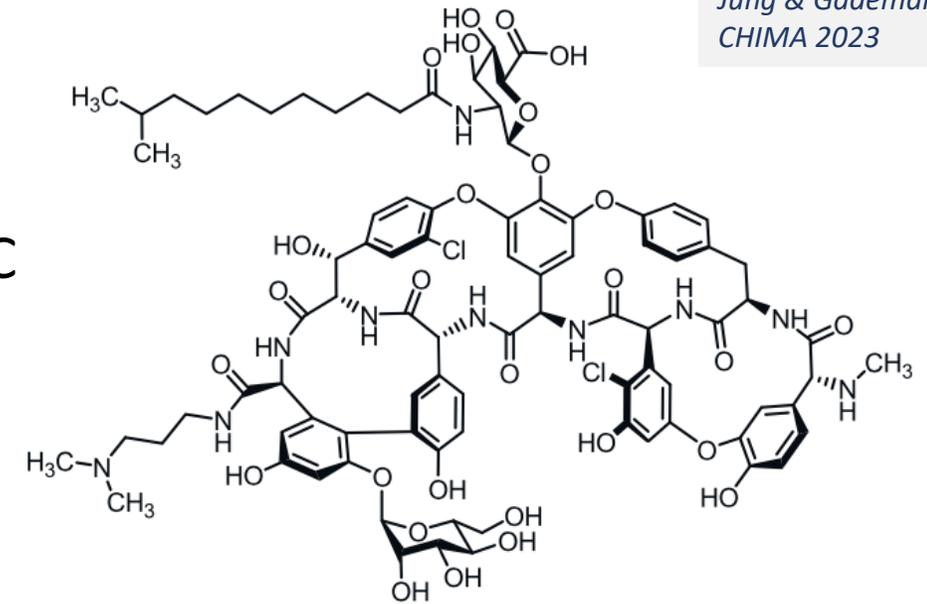
Only refer to the literature (empiric treatment)

Case 4:

Dalbavancin? Are you sure?

Dalbavancin

- Lipoglycopeptide with greater potency than VAN/TEIC
- Longer half-life than other glycopeptides
- Only IV administration
- Spectrum: **Gram-positive bacteria**, e.g.
S. aureus (both MSSA and MRSA), CoNS,
Group A, B, C and G betahemolytic streptococci
Streptococcus anginosus group
Enterococcus spp. (?)
Corynebacterium spp. (?)
- Official use: labeled indication for skin and soft tissue infections



Dalbavancin (Dalvance®)
Year of approval: 2014
Indication: ABSSSI
Administration: IV
Spectrum: Gram-positive
MW: 1817

Case 4: Why so popular?

Long half-life: $t_{1/2} = 7-14$ (even longer?) **days** \Rightarrow dosing = 1 injection/week!

Greater potency than vanco/teico (especially *Staph*)

Popular indications (more and more publications):

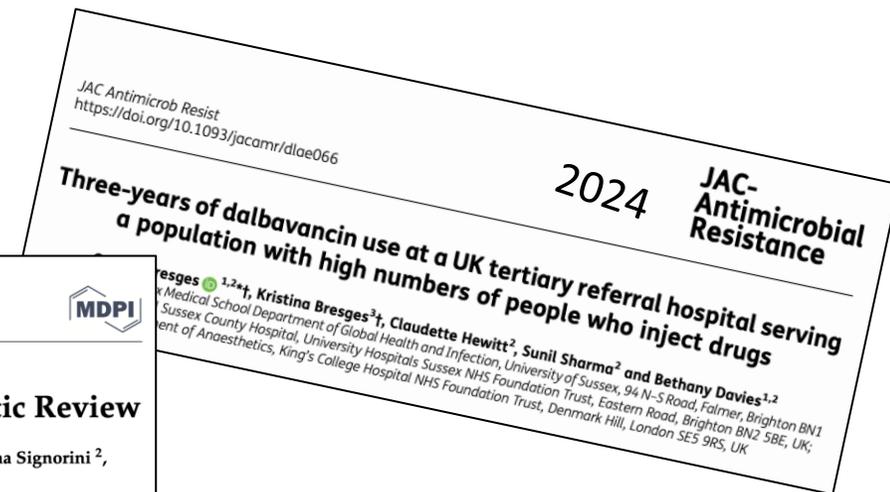
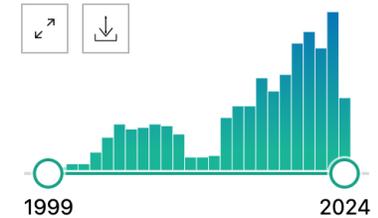
- Bone and joint infections, implant-associated infections, endocarditis, blood stream infections. ***Iv. drug users***

PubMed®

736 publications

MY NCBI FILTERS

RESULTS BY YEAR



Mentimeter: Dalbavancin vs *Staphylococcus*

The clinician calls and wants you to test dalbavancin on a coagulase-negative *Staphylococcus* from a prosthetic infection.

What do you do?

- Nothing (no test available in our lab)
- Use a dalbavancin gradient test
- Infer dalbavancin from vancomycin and teicoplanin gradient test results
- Use a dalbavancin BMD
- Infer dalbavancin from vancomycin and teicoplanin BMD results

Case 4: How to test *Staphylococcus* spp.?

- BMD = gold standard, not enough data on gradient tests (maybe underestimating MICs?)
- Currently EUCAST/NordicAST:
 - CoNS: You can infer DAL from VAN and TEIC (S=S)
 - *S. aureus*: You can infer DAL from VAN (S=S)
- However, EUCAST is reviewing the guidance to infer DAL from VAN and/or VAN + TEIC since there are studies showing DAL R in VAN S isolates for *S. aureus* and CoNS.

Etest® warning from Biomerieux: Teico Etest not for CoNSs!

INFORMATION TO THE CUSTOMERS
4340 - MAR - ETEST® TEICOPLANIN TP 256 - Follow up of the FSCA 3710
and the GCS info 3808

Dear customer,
You are a user of ETEST® products, and we thank you for choosing bioMérieux solutions.
In 2017, some internal investigations led us to set up some restrictions for the usage of ETEST® TEICOPLANIN TP 256 WW with *Staphylococcus* coagulase negative strains. A letter was issued regarding the lots you were using at this time and a supplementary insert was added in the Technical Library to inform about this new restriction of use (supplementary insert O4957601).
The decision has been taken to remove definitively from the Intended Use of ETEST TEICOPLANIN TP 256 the *Staphylococcus* coagulase negative strains. Laboratories can continue to use ETEST TP 256 for the other microorganisms claimed: *Staphylococcus aureus* and *Enterococcus* spp.

2024

JAC Antimicrob Resist
<https://doi.org/10.1093/jacamr/dlae063>

MIC determined
w/gradient strips

JAC-
Antimicrobial
Resistance

The MIC distribution of dalbavancin differs between different
coagulase-negative staphylococci

Sara Svensson^{1†}, Paulina Hagstedt^{1†} and Bo Söderquist^{1,2*}

Enterococci: What do we know?



- For enterococci, the recommendation that isolates that are **wild type to both vancomycin and teikoplanin are wild type to dalbavancin** works as far as we know
- vanA-positive = R, vanB-positive = ? (need to test dalbavancin)

This warning was issued May 2019.

Vancomycin susceptibility testing in *Enterococcus faecalis* and *E. faecium* using MIC gradient tests – a modified warning 21 May, 2019.

Original warning was issued 10 July, 2018, against the use of gradient tests for the detection of vanB-positive Enterococci. Several studies (Norwegian Reference Laboratory, Tromsø, Norway; The EUCAST Development Laboratory, Växjö, Sweden; Robert Koch Institute, Wernigerode, Germany) show that the use of MIC gradient tests with standard inoculum and incubation fail to detect glycopeptide resistance in low-level resistant enterococci (see posters 1754 and 1764, ECCMID 2019). Confirmation of suspected vancomycin resistance with gradient tests, can be significantly improved by the use of a macro method (BHI-medium, McF 2.0 and 48 hours incubation; see poster 1764, ECCMID 2019). Uncertain results should be confirmed with a molecular test for vanA and vanB.

01 Apr 2024

Dalbavancin MIC distributions and ECOFFs

Dalbavancin MIC distributions and ECOFFs, particularly for *Enterococcus faecalis* and *E. faecium*, made available. This is in response to frequent requests for more information on dalbavancin MIC distributions. [\[more\]](#)

Antimicrobial: Dalbavancin (Method: MIC)

	0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	Distributions	Observations	(T)ECOFF	Confidence interval
<i>Clostridium perfringens</i>	0	0	0	3	7	8	2	0	0	0	0	0	0	0	0	0	0	0	0	1	20	ID	
<i>Clostridium ramosum</i>	0	0	0	0	0	0	0	1	1	13	0	0	0	0	0	0	0	0	0	1	15	ID	
<i>Enterococcus avium</i>	0	0	0	0	0	12	8	0	0	0	0	0	0	0	0	0	0	0	0	1	20	-	
<i>Enterococcus casseliflavus</i>	0	0	0	0	2	9	7	2	0	0	0	0	0	0	0	0	0	0	0	1	20	-	
<i>Enterococcus durans</i>	0	0	0	11	4	5	0	0	0	0	0	0	0	0	0	0	0	0	0	1	20	-	
<i>Enterococcus faecalis</i>	0	0	0	371	2708	1068	97	5	4	5	13	39	10	53	0	0	0	0	0	5	4373	0.125	0.03 - 0.25
<i>Enterococcus faecalis</i> ATCC 29212	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	1	20	ID	
<i>Enterococcus faecium</i>	0	0	0	131	352	607	234	54	29	51	109	402	120	183	0	0	0	0	0	4	2272	(0.25)	0.125 - 0.5
<i>Enterococcus gallinarum</i>	0	0	0	0	1	2	6	9	0	0	2	0	0	0	0	0	0	0	0	1	20	-	

More MIC-distribution info: See mini-oral presentation later today 😊!