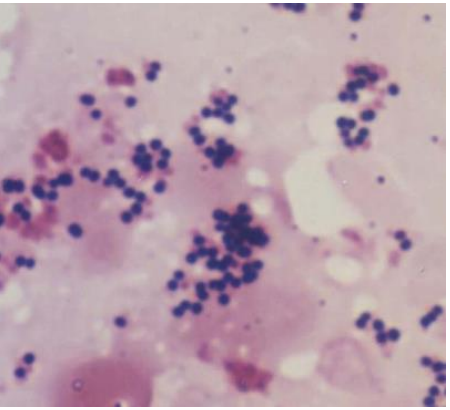


EUCAST
EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

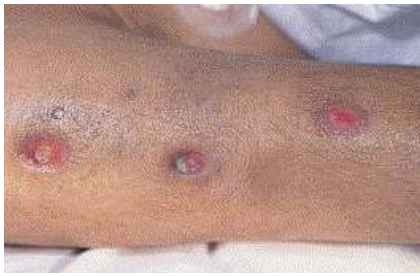
Staphylococcus aureus

Olivier Denis

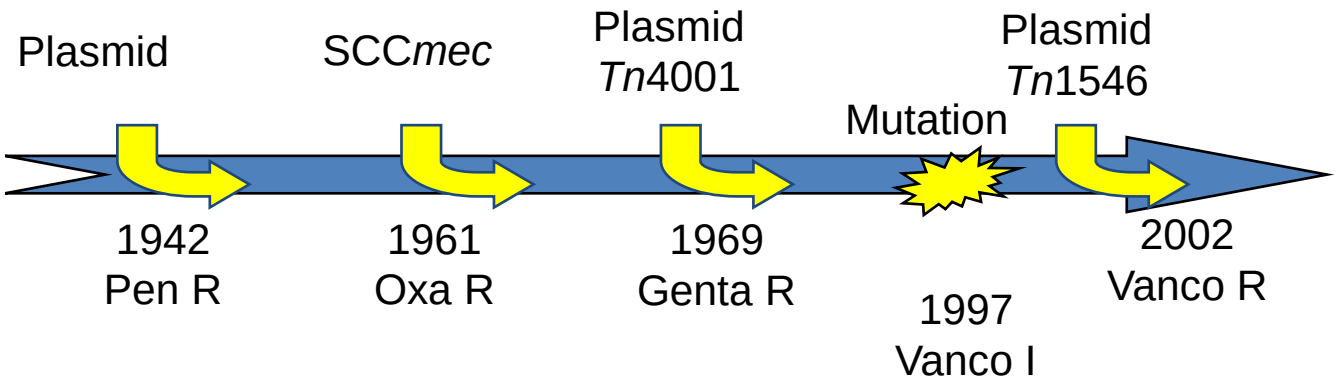
Université Libre De Bruxelles

Hôpital Erasme | ULB

Staphylococcus aureus

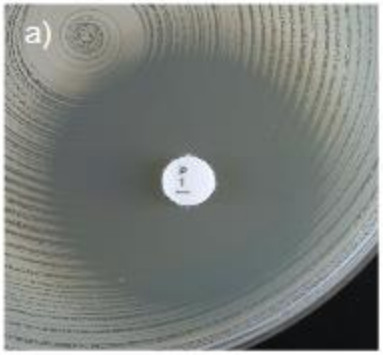


- Major opportunistic pathogen responsible for infections both in hospitals and in the community
- Clinical manifestations
  - Pyogenic infections : Skin and soft tissue infections to endocarditis
  - Toxin mediated diseases : SSSS, SFP, TSS
- Master of creating/picking up resistance determinants

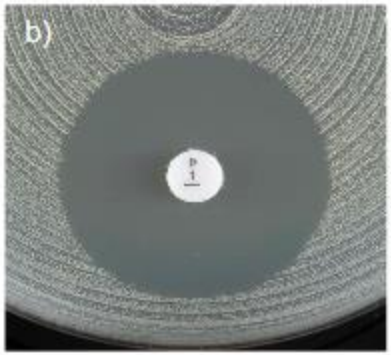


Penicillin resistance

- **Production of penicillinase**
  - Encoding by *blaZ*
  - Inhibited by clavulanic acid
  - Tested by cefinase test or by disk diffusion method
  - Difficult to detect in coagulase negative staphylococci



Susceptible



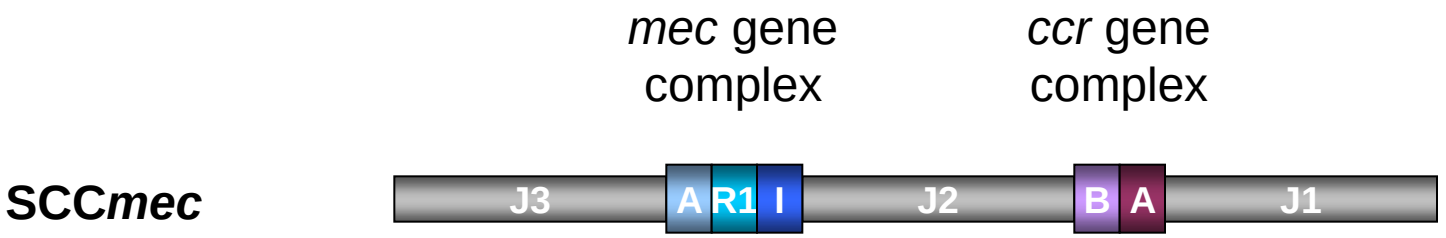
Resistant

Examples of inhibition zones for *Staphylococcus aureus* with benzylpenicillin.
a) Fuzzy zone edge and zone diameter ≥ 26 mm. Report susceptible.
b) Sharp zone edge and zone diameter ≥ 26 mm. Report resistant.

Methicillin-resistant *S. aureus* (MRSA)

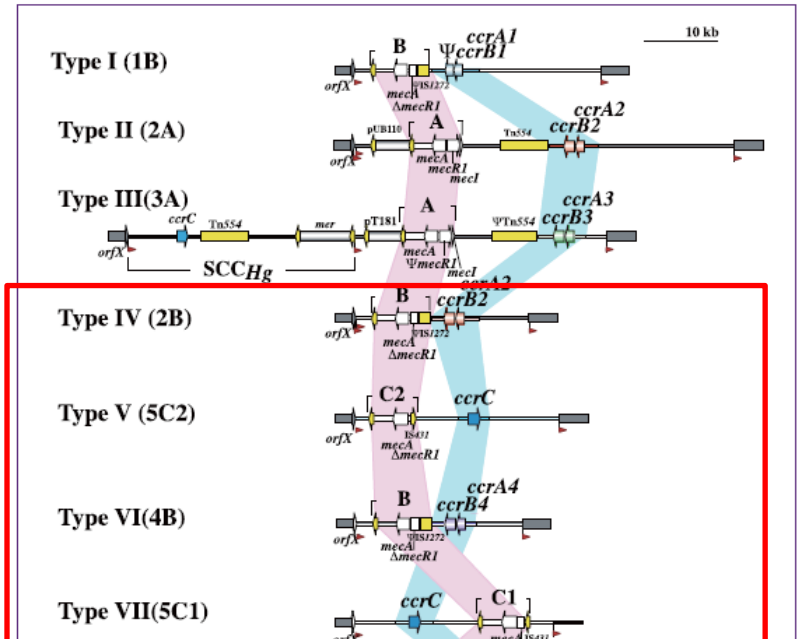
- **Acquisition of *mec* gene encoding PBP2a**
  - PBP2a shows low affinity to β-lactams
  - **Cross-resistance** to all β-lactams, except for the novel anti-MRSA cephalosporins
  - Three different types described: *mecA*, (*mecB)*, *mecC*

- **The *mec* gene is integrated into mobile genetic element**
  - Staphylococcal cassette chromosome *mec* (SCC*mec*)
  - Chromosomal insertion at the $attB_{SCC}$ at the end of orfX
  - Often contain plasmids or transposons carrying resistance genes



Ito t. et al. Antimicrob Agents Chemother 2012;4997

Staphylococcal Cassette Chromosome *mec*



Classification according to

- **Types**: combination of mec and ccr
- **Variants**: difference into j*unkyard regions*.

SCC*mec*	Type *ccr*		Type *mec*
I (1B)	1	A1B1	B
II (2A)	2	A2B2	A
III (3A)	3	A3B3	A

AAC Journals.ASM.org

Novel Type XII Staphylococcal Cassette Chromosome *mec* Harboring a New Cassette Chromosome Recombinase, CcrC2

Zhaowei Wu,[a] Fan Li,[a] Dongliang Liu,[a] Huping Xue,[a] Xin Zhao[a,b]

College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, People's Republic of China[a]; Department of Animal Science, McGill University, Ste. Anne de Bellevue, Quebec, Canada[b]

Hiramatsu et al. Infect Chemother 2013;45(2):117-136 .

Which penicillins to test for detection of methicillin resistance?

S. aureus with oxacillin MIC > 2 mg/l are mostly MRSA due to the presence of *mecA* gene

***Staphylococcus* spp.**

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

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar
Inoculum: McFarland 0.5
Incubation: Air, 35±1ºC, 18±2h
Reading: Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light (except for benzylpenicillin and linezolid, see below).
Quality control: *Staphylococcus aureus* ATCC 29213

Penicillins[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Benzylpenicillin, *S. aureus*	0.125[1]	0.125[1]	1 unit	26[A,B]	26[A,B]
Benzylpenicillin, *S. lugdunensis*	0.125[1]	0.125[1]	1 unit	26[A]	26[A]
Benzylpenicillin, Coagulase-negative staphylococci	-[2]	-[2]		Note[C]	Note[C]
Ampicillin, *S. saprophyticus*	Note[1,3]	Note[1,3]	2	18[A,D]	18[A,D]
Ampicillin-sulbactam	Note[1,3]	Note[1,3]		Note[A,D]	Note[A,D]
Amoxicillin	Note[1,3]	Note[1,3]		Note[A,D]	Note[A,D]
Amoxicillin-clavulanic acid	Note[1,3]	Note[1,3]		Note[A,D]	Note[A,D]
Piperacillin	Note[1,3]	Note[1,3]		Note[A,D]	Note[A,D]
Piperacillin-tazobactam	Note[1,3]	Note[1,3]		Note[A,D]	Note[A,D]
Ticarcillin	Note[1]	Note[1]		Note[A]	Note[A]
Ticarcillin-clavulanic acid	Note[1]	Note[1]		Note[A]	Note[A]
Phenoxymethylpenicillin	Note[1]	Note[1]		Note[A]	Note[A]
Oxacillin[4]	Note[1,4]	Note[1,4]		Note[A]	Note[A]
Cloxacillin	Note[1]	Note[1]		Note[A]	Note[A]
Dicloxacillin	Note[1]	Note[1]		Note[A]	Note[A]
Flucloxacillin	Note[1]	Note[1]		Note[A]	Note[A]
Mecillinam (uncomplicated UTI only)	-	-		-	-

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

1/A. Most staphylococci are penicillinase producers, which are resistant to benzylpenicillin, phenoxymethylpenicillin, ampicillin, amoxicillin, piperacillin and ticarcillin. Isolates negative for penicillinase and susceptible to methicillin can be reported susceptible to these agents. Isolates positive for penicillinase and methicillin susceptible are susceptible to beta-lactamase inhibitor combinations and isoxazolylpenicillins (oxacillin, cloxacillin, dicloxacillin and flucloxacillin).
Methicillin resistant isolates are, with few exceptions, resistant to all beta-lactam agents.
2/C. No currently available method can reliably detect penicillinase production in coagulase-negative staphylococci.
3/D. Ampicillin susceptible *S. saprophyticus* are *mecA*-negative and susceptible to ampicillin, amoxicillin and piperacillin (without or with a beta-lactamase inhibitor).
4. *S. aureus*, *S. lugdunensis* and *S. saprophyticus* with oxacillin MIC values >2 mg/L are mostly methicillin resistant due to the presence of the mecA or mecC gene. The corresponding oxacillin MIC for coagulase-negative staphylococci other than *S. saprophyticus* and *S. lugdunensis* is >0.25 mg/L.

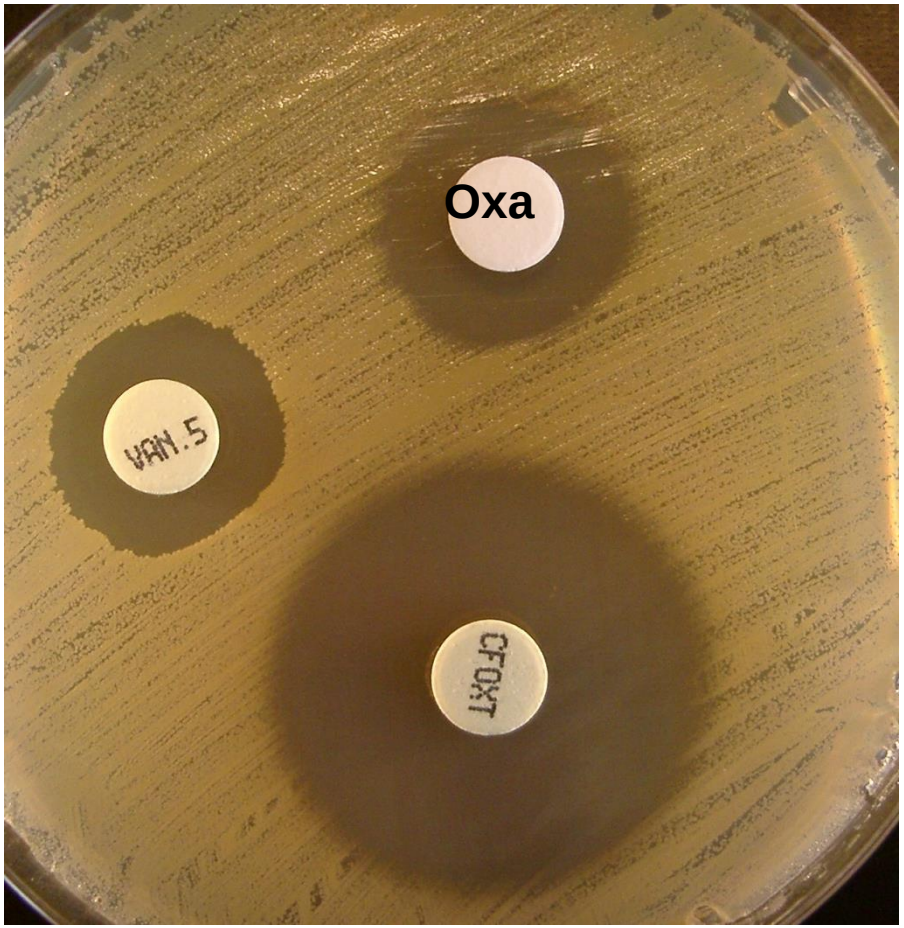
B. For *S. aureus*, disk diffusion is more reliable than MIC determination for detection of penicillinase producers, provided the zone diameter is measured AND the zone edge closely inspected (see pictures below). If the zone diameter is <26 mm, then report resistant. If the zone diameter is ≥26 mm AND the zone edge is sharp, then report resistant. If not sharp, then report susceptible and if uncertain, then report resistant. Chromogenic cephalosporin-based beta-lactamase tests do not reliably detect staphylococcal penicillinase.

Which cephalosporins to test ?

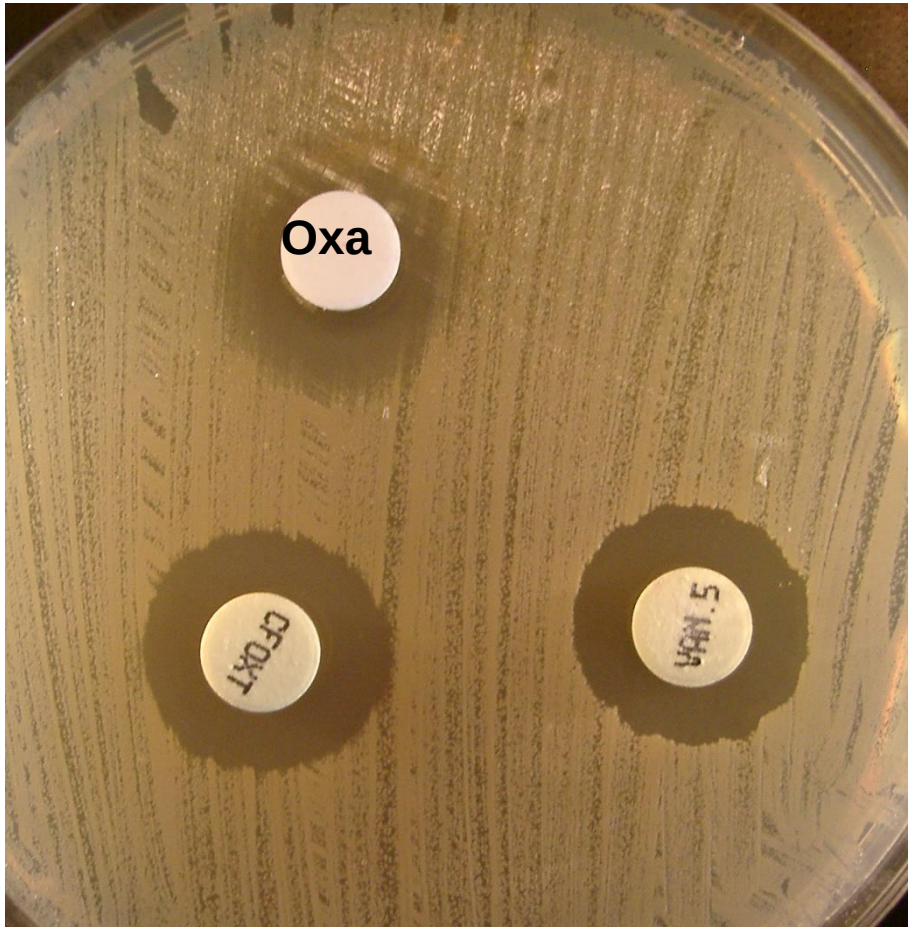
- **Cefoxitin**
  - Interpretation for all penicillins, cephalosporins and carbapenems with the exception of anti-MRSA cephalosporins (ceftaroline, ceftobiprole)
  - Disk diffusion
  - Interpretative criteria according to species

			Disk content	S≥	R<
Cefoxitin (screen), *S. aureus, S. lugdunensis* and *S. saprophyticus*	Note[3]	Note[3]	**30**	22[A]	22[A]
Cefoxitin (screen), Coagulase-negative staphylococci other than *S. lugdunensis* and *S. saprophyticus*	Note[4]	Note[4]	**30**	25[A]	25[A]
Cefoxitin (screen), *S. pseudintermedius*	Note[4]	Note[4]	**30**	35[A]	35[A]

  - MIC values > 4 mg/l can be considered as methicilin-resistant
  - Confirmation
    - PCR for *mecA* and *mecC* genes
    - PBP2a detection by immunochromatographic or latex assay



MSSA



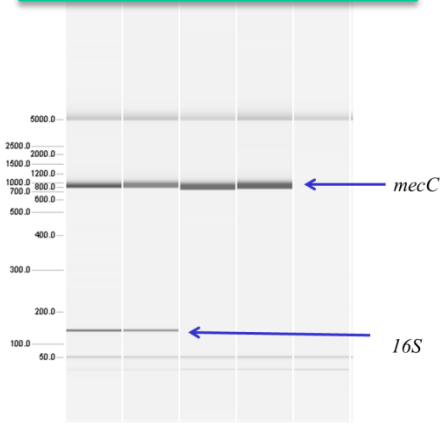
Hetero -MRSA

Methicillin-resistant *S. aureus* ?

CMI oxacillin : 6 mg/l
CMI cefoxitin : 24 mg/l

Negative PCR *mecA*

Positive PCR *mecC*



Less than 1% of MRSA sent to reference lab

Epidemiology and host range

mecC MRSA found in multiple host species across Europe

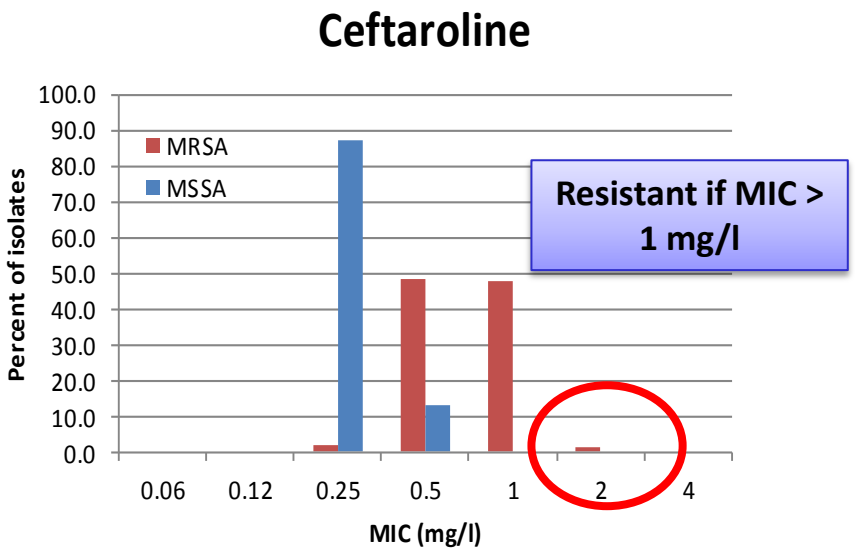
Anti-MRSA cephalosporins

Ceftaroline

- New anti-MRSA cephalosporin
- Increased affinity to PBP2a
- Low emergence of ceftaroline resistant *S. aureus*
  - From mutations in native pbp genes (PBP2 and PBP3) or *mecA*
  - Overexpression of pbp4 gene

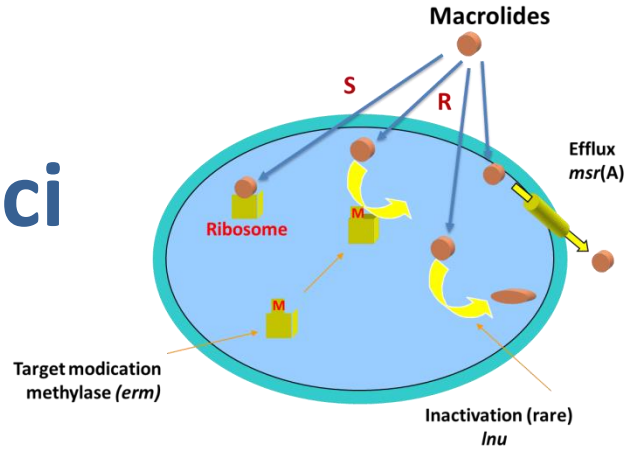
Identification of non-PBP2a resistance mechanisms in *Staphylococcus aureus* after serial passage with ceftaroline: involvement of other PBPs

Sushmita D. Lahiri† and Richard A. Alm*†



Argudin M et al. JAC 2016
Lahiri SD JAC 2016

MLS resistance in staphylococci

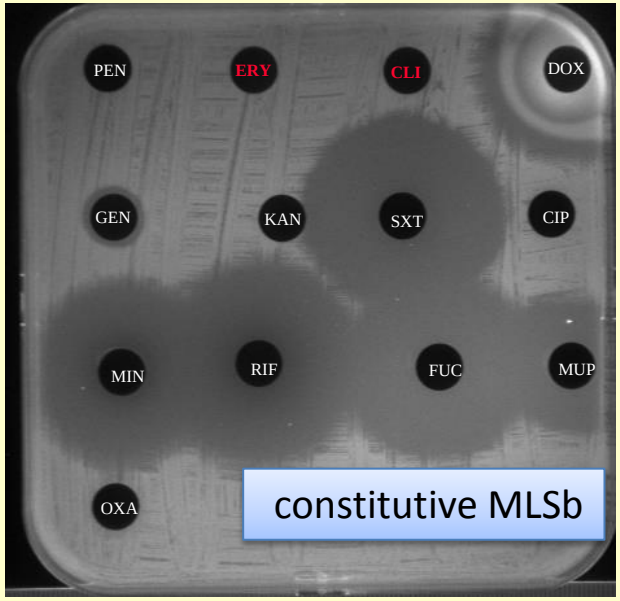


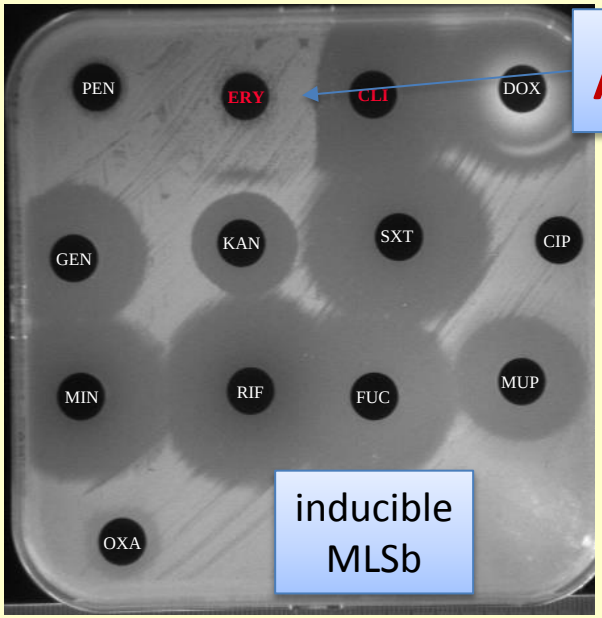
Genes	Mode of action	Resistance phenotype		
			Ery	Clinda
ermA, ermC (other *ermB, ermT)*	Target modification	$MLSb_{i\ ou\ c}$	R	R/s *
msrA/B	Efflux	M	R	S
lnu (rare)	Acetylation	L	S	R

* According to the phenotype inducible or constitutive

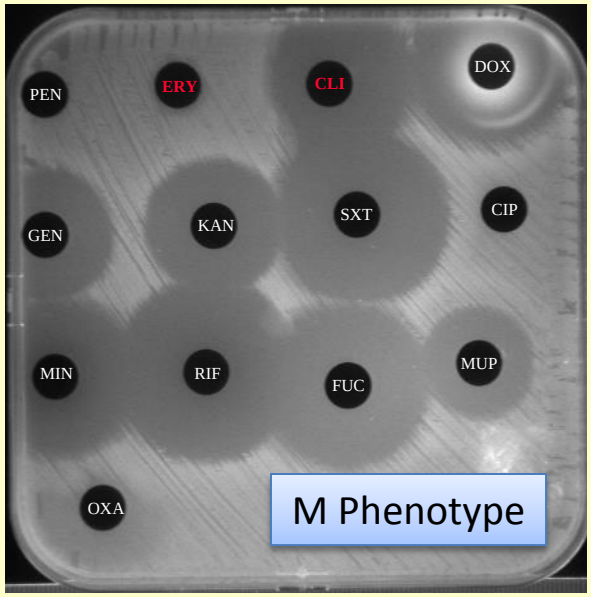
Resistance phenotype to ML

If presence of antagonism between clindamycin and erythromycin, clindamycin should be reported as resistant





Antagonism

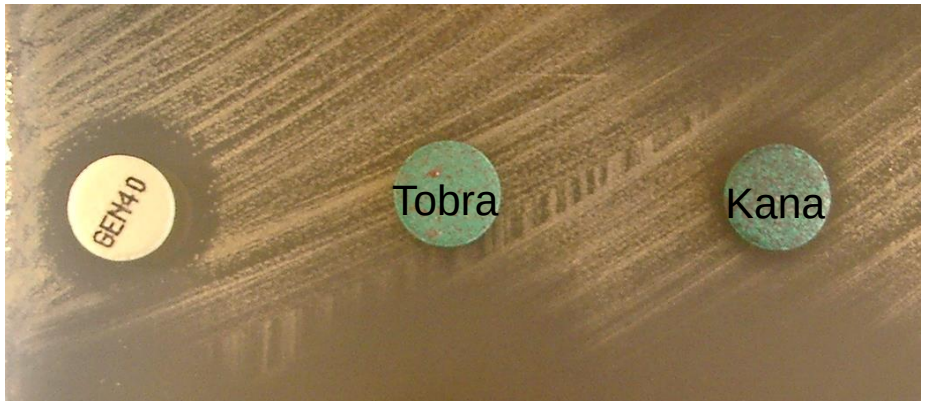


Aminoglycoside modifying enzymes

Genes	Resistance phenotype
aph(3’)	Kana, amika*, isepa*
ant(4’)	Kana, Tobra, amika*, isepa*
aac(6’)-aph(2’’)	Kana, Tobra, Genta, amika*, isepa*, netil*

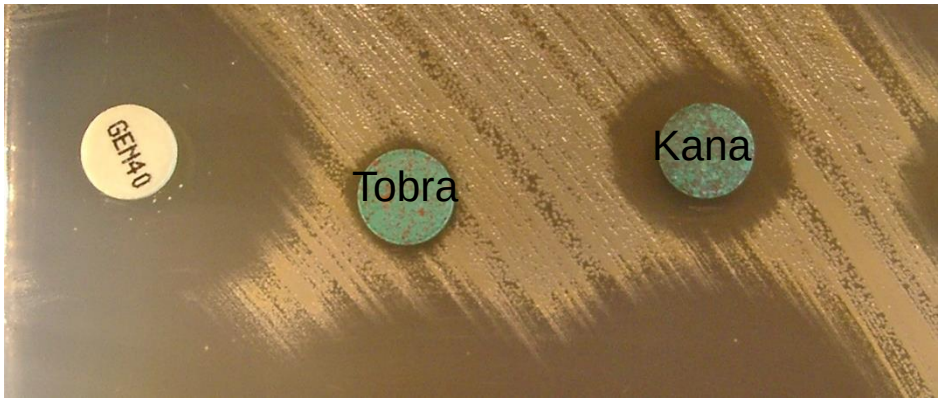
Resistance to amikacin is determined by using **kanamycin** (MIC> 8 mg/l)
Breakpoints are different between S. aureus and coagulase-negative staphylococci

Aminoglycosides resistance



Phenotype KTG

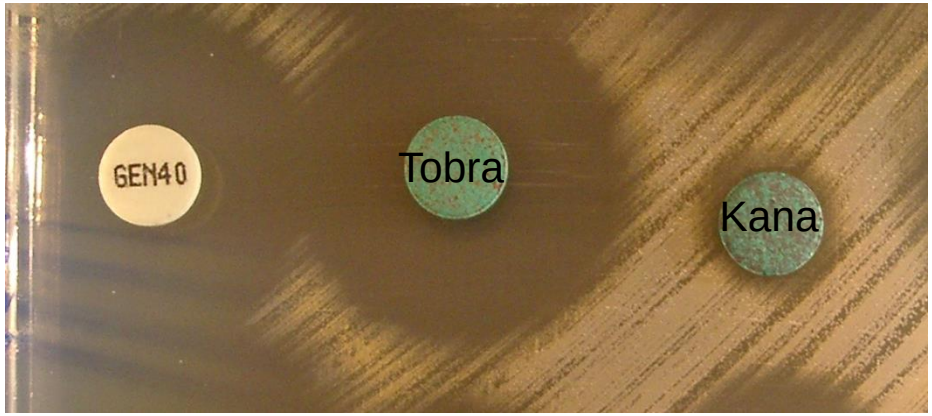
aac6'-aph2 ''



Phenotype KT

ant4'

Resistance to amikacin can be deducted by kanamycin resistance (MIC > 8 mg/L)



Phenotype K

aph3'

Vancomycin resistance in *Staphylococcus aureus*

Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility

J Antimicrob Chemother 1997; **40:** 135–136

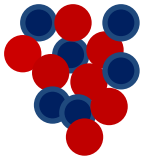
K. Hiramatsu[a*], H. Hanaki[a], T. Ino[b], K. Yabuta
T. Oguri[c] and F. C. Tenover[d]

Susceptible population

Hetero low-level resistance (hVISA)

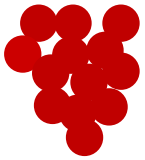
Heterogenous population

10^{-6} to 10^{-9} resistant bacteria



Low-level resistance (VISA)

Homogenous population



High resistance (VRSA)

Homogenous population

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

Glycopeptide breakpoints for *Staphylococci*, 2016

	MIC (mg/L) for					
	Vancomycin			Teicoplanin		
	S	I	R	S	I	R
EUCAST for *S. aureus*	≤2		>2	≤2		>2
EUCAST for CoNS	≤4		>4	≤4		>4
CLSI for *S. aureus*	≤2	4-8	≥16	≤8	16	≥32
CLSI for CoNS	≤4	8-16	≥32	idem		

http://www.eucast.org/clinical_breakpoints/
CLSI 2014 M100-S24

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Low level resistance

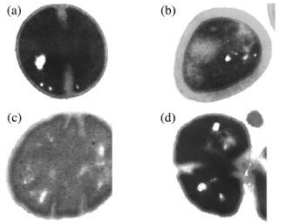


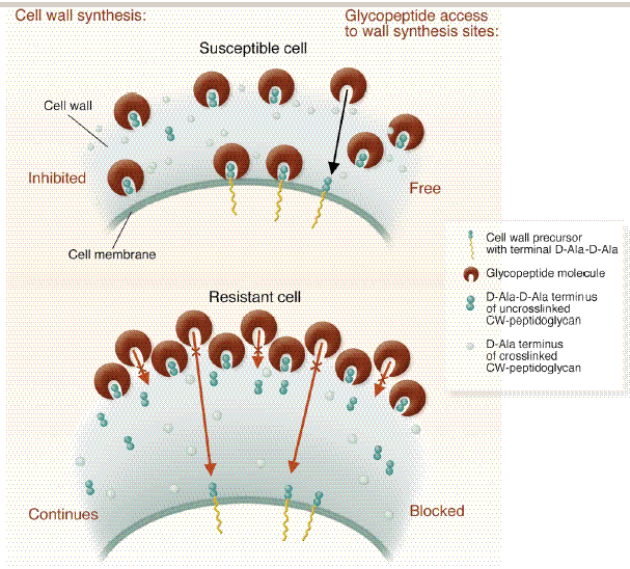
Figure 3. Transmission electron micrographs of (a) *S. aureus* ATCC 29213, (b) VISA strain P1V44, (c) hetero-VISA strains P1V69 and (d) MRSA P39575. Magnification ×60 000.

- **Genetic environment**
  - Multiple point mutations leading to modified peptidoglycans synthesis
  - No *van* genes and not linked to methicillin resistance
- **↑ Thickness of cell wall**
  - ↑ synthesis of peptidoglycan , ↓ autolytic activity, ↑ residues D-Alanyl-D-Alanine
- **Absorption of GLYCOPEPTIDES** into bacterial cell wall before extern membrane surface

⇒ **Low level resistance and reversible**

Teicoplanin > vancomycin

MIC to vancomycin 4 – 8 mg/L

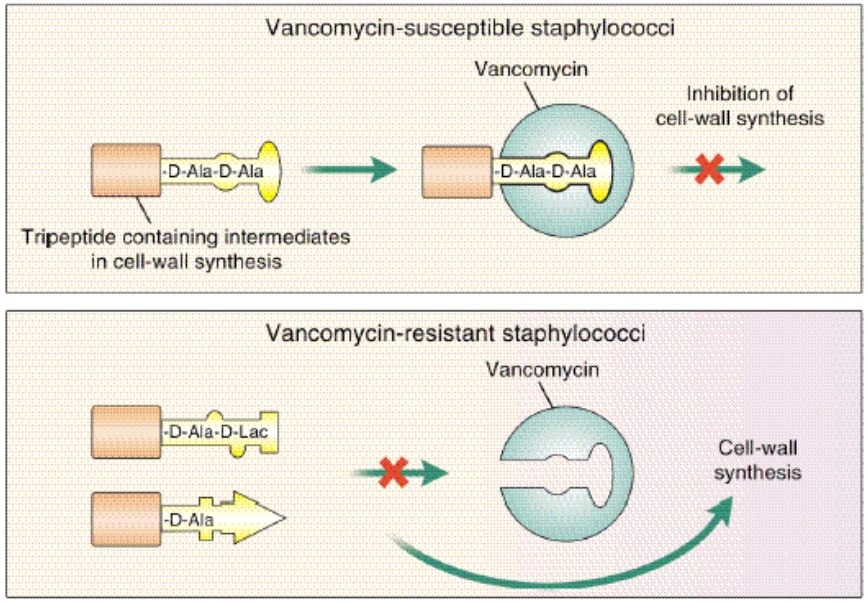


Denis O. et al. JAC 2002;50:755

Hiramatsu K. 2001. The Lancet Infect. Dis. 1:147

Mechanism of high level resistance

- Transfer of Tn*1546* carrying *vanA* ***gene***
  - *E. faecalis* $\Rightarrow$ *S. aureus*
- Modification of peptidoglycan synthesis
  - Substitution of D-Ala-D-Ala by D-Ala-D-Lac
  - $\downarrow\downarrow$ affinity to vancomycin 10^3
- High level resistance
  - Vancomycin >> teicoplanin
  - MIC vancomycin > 16-256 mg/l



- Frequency
  - Only about 30 cases reported from USA, India, Iran, Brazil and Portugal

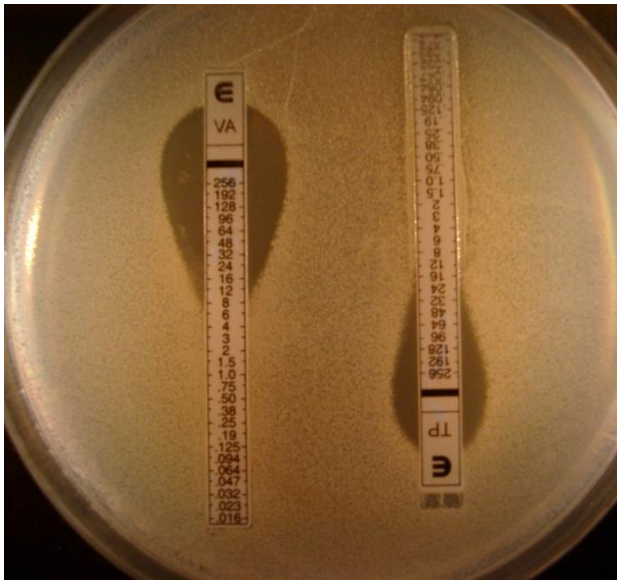
Gould IM Lancet Infect. Dis 2012

Challenge for detection of glycopeptide resistance in *Staphylococci*

- **Disk diffusion**
  - Cannot be used for (h-)GISA
- **Detection of (h-)GISA**
  - As proven difficult
  - Divided into screening and confirmation
  - Screening: macromethods, GRD, agar screen
  - Reversible phenotype

MIC determination for GISA and GRSA

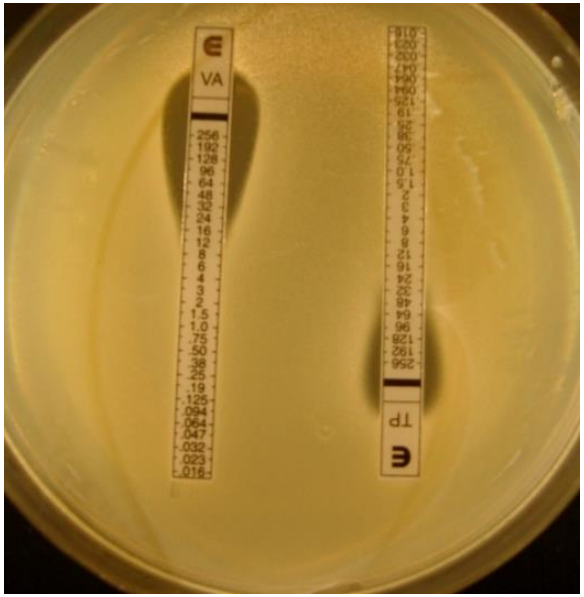
- **Broth microdilution = gold standard**
- **May also be determined by**
  - Gradient strip methods (E-test), agar dilution and automated systems
  - E-tests show MICs with 0.5-1 two-fold dilution steps higher than broth microdilution
  - Isolates with MICs >2 mg/L should be confirmed



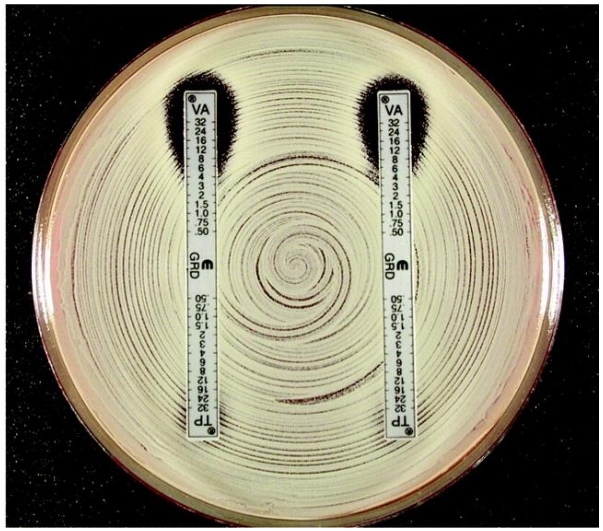
HIP5827

Screening method for GISA

- **Macromethod**
  - Should not be reported as MICs
  - Does not differentiate between hGISA and GISA
  - High inoculum (2,0 McF) on BHI agar for 48h



- **GISA detection by gradient test**
  - Double strip vancomycin and teicoplanin
  - 0.5 McF on MH agar for 24 and 48h



Laboratory performance of methods for detection hGISA

TABLE 5. Laboratory detection of hVISA and accuracy of methods compared to those of modified population analysis/area under the curve[a]

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% 48 h, 93–94%	24 h, 98–100% 48 h, 82–95%	174, 393

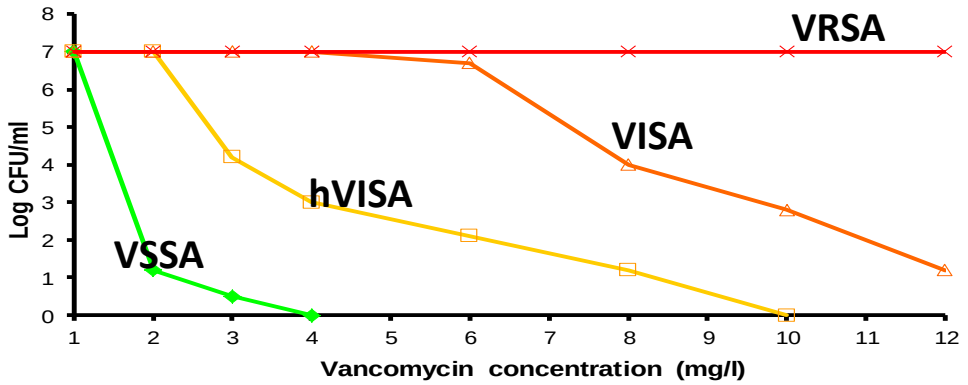
As low prevalence, low positive predictive value

[a] In all studies, vancomycin … or calculating sensitivity and specificity.
[b] Evaluation of vancomycin … cted by determinations of broth MIC.
[c] BHIA6V is the screening plate recommended by the CDC and the Clinical and Laboratory Standards Institute for the detection of VRSA and VISA strains with vancomycin MICs of ≥8 μg per ml (http://www.cdc.gov) (53), which is spot inoculated with 10 μl from a 0.5-McFarland-standard suspension and read at 24 and 48 h. The culture is considered positive if there is growth of 2 or more colonies.
[d] MHA5T is the screening plate recommended by the Comité de l'Antibiogramme de la Société Française de Microbiologie (http://www.sfm.asso.fr),which is spot inoculated with 10 μl from a 2-McFarland-standard suspension and read at 24 and 48 h. The culture is considered positive if there is growth of 1 or more colonies.
[e] This analysis included some isolates with a hetero-teicoplanin-resistant but vancomycin-susceptible phenotype by population analysis.
[f] Simplified PAP consists of inoculating BHIA with 4 μg per ml of vancomycin with 10 μl from a 0.5-McFarland-standard suspension and reading at 24 and 48 h for any growth.

Howden BP et al. Clin Microbiol Reviews 2010

Confirmation test

- **Population analysis profile-area under curve (PAP-AUC)**
  - Isolate screening positive for reduced susceptibility
  - Not identified as GRSA or GISA by MIC determination
- **Method**
  - Population analysis for vancomycin
  - Determine ratio of AUC of test organism vs Mu3 (ATCC 700698)



Linezolid resistance

- Mechanisms of resistance
  - Mutations in domain V of 23S rRNA (G2576T) or other genes encoding ribosomal proteins
  - **Transferable mechanisms on plasmids**
    - **Methylation of nucleotide A2503 encoded by** *cfr* gene located on plasmids resistance to $PhLOPS_A$
    - ABC transporter encoded by *optrA* gene only *in S. sciuri* resistance to PhO
- Described in *S. aureus* and CoNS isolates from animals and humans including Belgium
- Resistance: rare (<1%) but outbreaks occurred

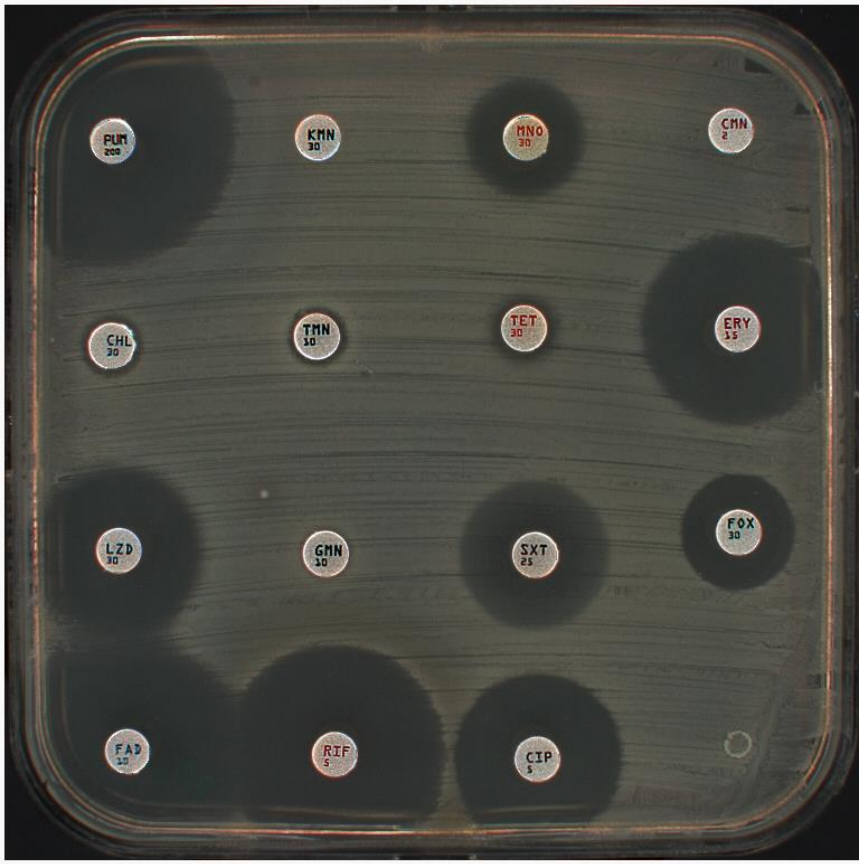
Vanderhaeghen W, et al. JAC 2012
Diaz L et al. AAC 2012
Morales G et al. CID 2012
Sánchez García M et al. JAMA 2010

Clinical Outbreak of Linezolid-Resistant *Staphylococcus aureus* in an Intensive Care Unit

Miguel Sánchez García, MD, PhD

Context Linezolid resistance is extremely uncommon in *Staphylococcus aureus*.

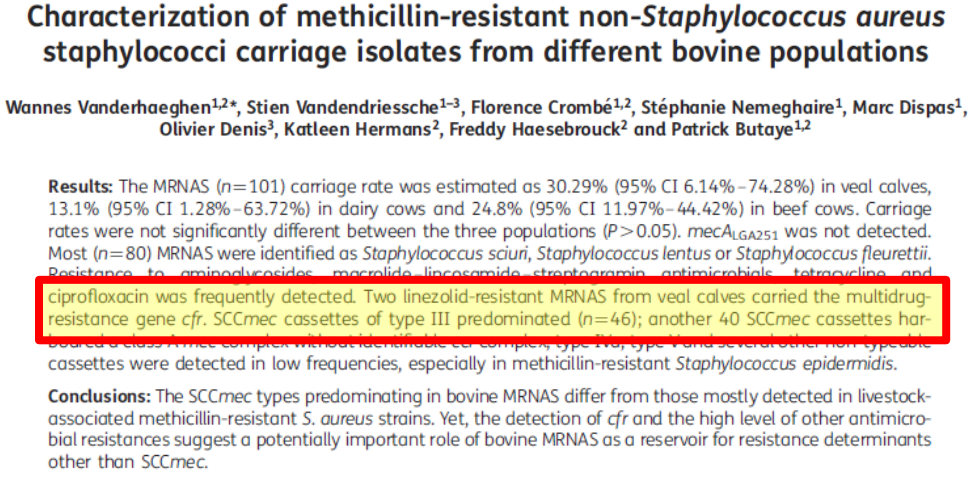
cfr-Positive MRSA ST398



- LA-MRSA ST398
- Resistance to chloramphenicol and clindamyin
- Linezolid susceptible ?
- Not detected by disk diffusion using CLSI guidelines
- MIC to linezolid = 12 mg/L

Emergence of cfr-positive *S. aureus* in Belgium

- Humans
  - 1464 S. aureus isolates from 2013 to 2015 sent by 167 laboratories
  - 30 resistant to chloramphenicol, clindamycin and/or linezolid
  - One cfr-positive MRSA belonging to CC398 collected from patient with SSI
  - Linezolid MIC = 12 mg/l
- Animals
  - Occasionally found in *S. aureus* and non *S. aureus*
  - Pigs, veals



Characterization of methicillin-resistant non-*Staphylococcus aureus* staphylococci carriage isolates from different bovine populations

Wannes Vanderhaeghen[1,2]*, Stien Vandendriessche[1–3], Florence Crombé[1,2], Stéphanie Nemeghaire[1], Marc Dispas[1], Olivier Denis[3], Katleen Hermans[2], Freddy Haesebrouck[2] and Patrick Butaye[1,2]

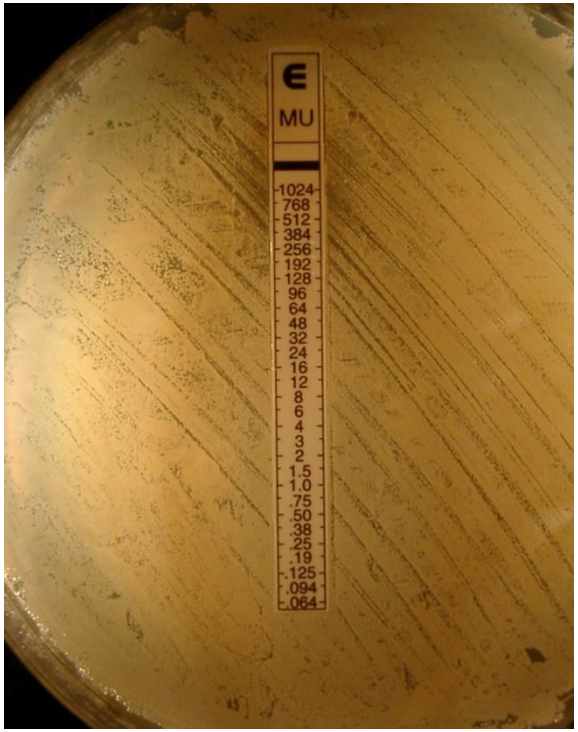
Results: The MRNAS ($n=101$) carriage rate was estimated as 30.29% (95% CI 6.14%–74.28%) in veal calves, 13.1% (95% CI 1.28%–63.72%) in dairy cows and 24.8% (95% CI 11.97%–44.42%) in beef cows. Carriage rates were not significantly different between the three populations ($P>0.05$). $mecA_{LGA251}$ was not detected. Most ($n=80$) MRNAS were identified as *Staphylococcus sciuri*, *Staphylococcus lentus* or *Staphylococcus fleurettii*. Resistance to aminoglycosides, macrolide–lincosamide–streptogramin, antimicrobials, tetracycline and ciprofloxacin was frequently detected. Two linezolid-resistant MRNAS from veal calves carried the multidrug-resistance gene *cfr*. SCC*mec* cassettes of type III predominated ($n=46$); another 40 SCC*mec* cassettes harboured a class A *mec* complex without identifiable *ccr* complex, type IVa, type V and several other non-typeable cassettes were detected in low frequencies, especially in methicillin-resistant *Staphylococcus epidermidis*.

Conclusions: The SCC*mec* types predominating in bovine MRNAS differ from those mostly detected in livestock-associated methicillin-resistant *S. aureus* strains. Yet, the detection of *cfr* and the high level of other antimicrobial resistances suggest a potentially important role of bovine MRNAS as a reservoir for resistance determinants other than SCC*mec*.

Vanderhaegen W et al. JAC 2013
Peeters LEJ et al. Vet Microbiol 2015
Angeles Argudin M et al Res Vet Science 2015

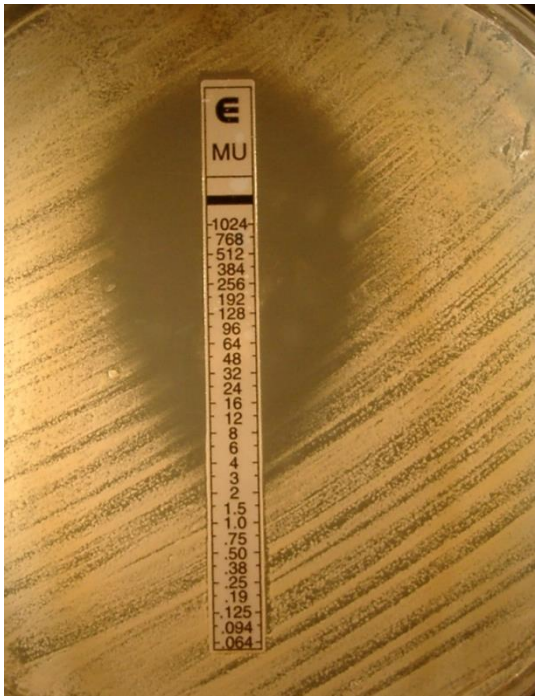
Mupirocin resistance

MRSA + *mupA*



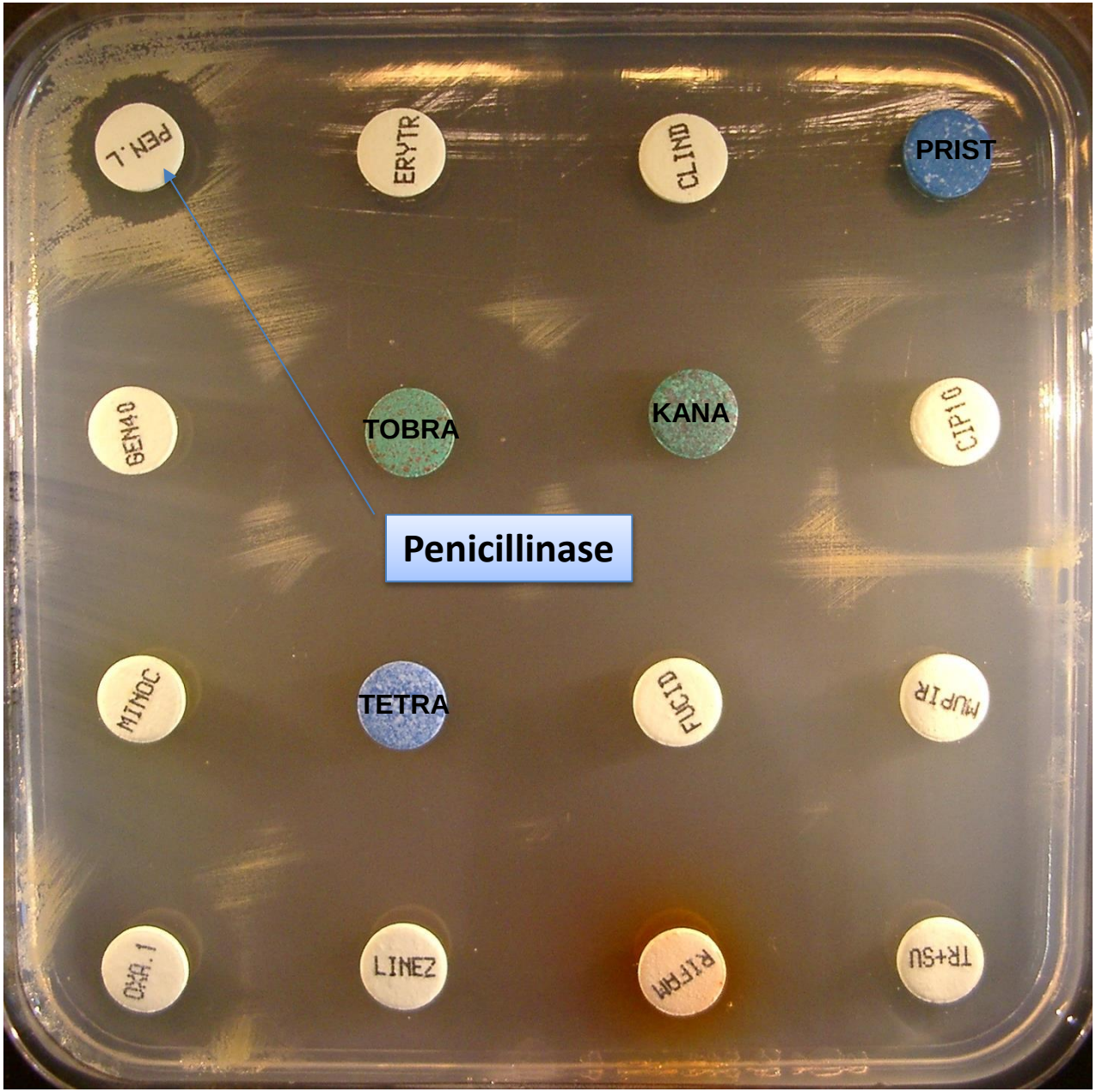
High-level of mupirocin resistance confered by *mupA* > 256 mg/l

MRSA + *mupA* négatif



Low-level resistance to mupirocin (2-256 mg/l)

Few examples


PEN.L
ERYTR
CLIND
PRIST
GEN40
TOBRA
KANA
CIP10
Penicillinase
MINOC
TETRA
FUCID
MUPIR
CHA.1
LINEZ
RIFAM
TR+SU


CMN
29 - S - 0.06
ERY
30 - S - 0.12
FOX
16 - R - 25
32 - S - 0.12
TET
32 - S - 0.12
SXT
32 - S - 0.5
CIP
6 - R - 32
23 - S - 4
TOB
25 - S - 0.06
GMN
24 - S - 0.06
RIF
35 - S - <0.0
PUM
35 - S -
CHL
27 - S - 1
LIN
32 - S - <0.0
FAD
35 - S -

MRSA

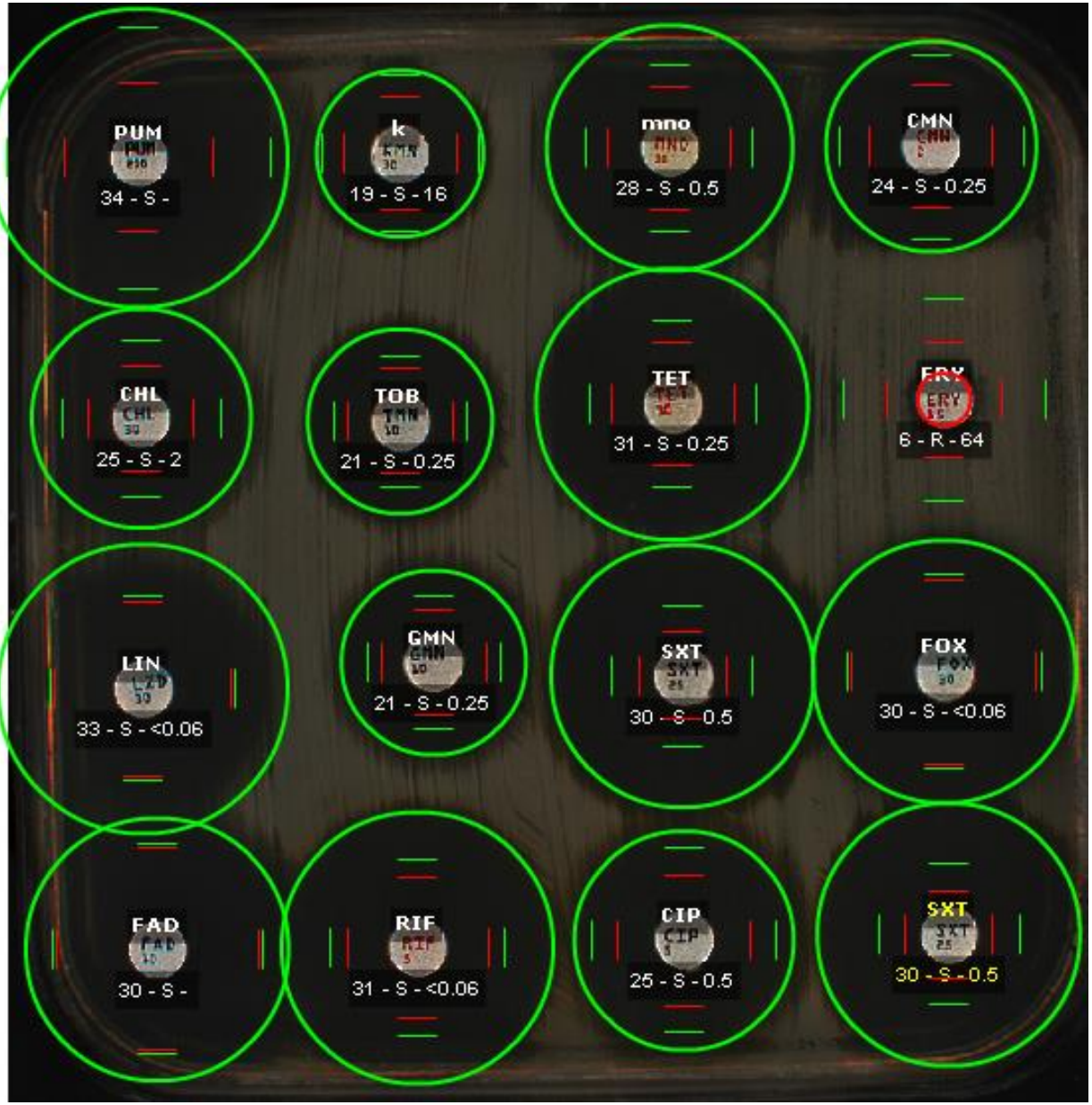
MRSA

Phenotype GTK
aac(6')-aph(2'')

⇒ resistance to amikacin

MRSA

constitutive MLS_b
+ *ermA*



MSSA

inducible MLS_b
ermC

MRSA
M phenotype

Acknowledgements

Hôpital Erasme ULB Bruxelles

- Sandrine Roisin
- Magali Dodemont
- Claire Nonhoff
- Ariane Deplano
- Maria Argudin
- Ricardo De Mendonca

Thank you for your attention