Spectrum of vancomycin and susceptibility testing

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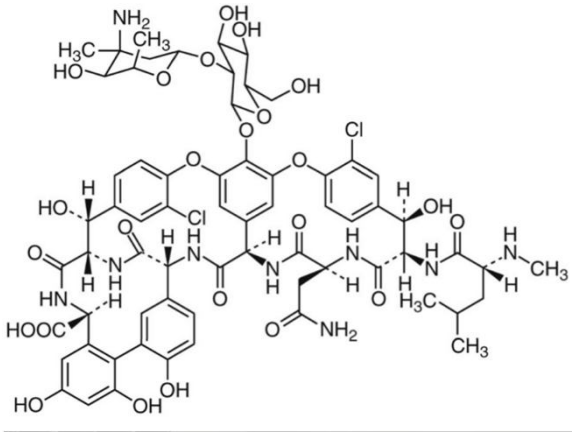
Glycopeptides



- **Vancomycin**
  - 1958 < *Amycolatopsis orientalis*
  - High toxicity ⇒ limited use until the end of 1970s
  - ↑ infections with Gram positive
  - Emergence of multi-R Gram positive but still vancoS
  - Oral vancomycin use and better formulation

- **Teicoplanin**
  - End 1970s < *Actinoplanes teichomycetus*
  - Only in Europe

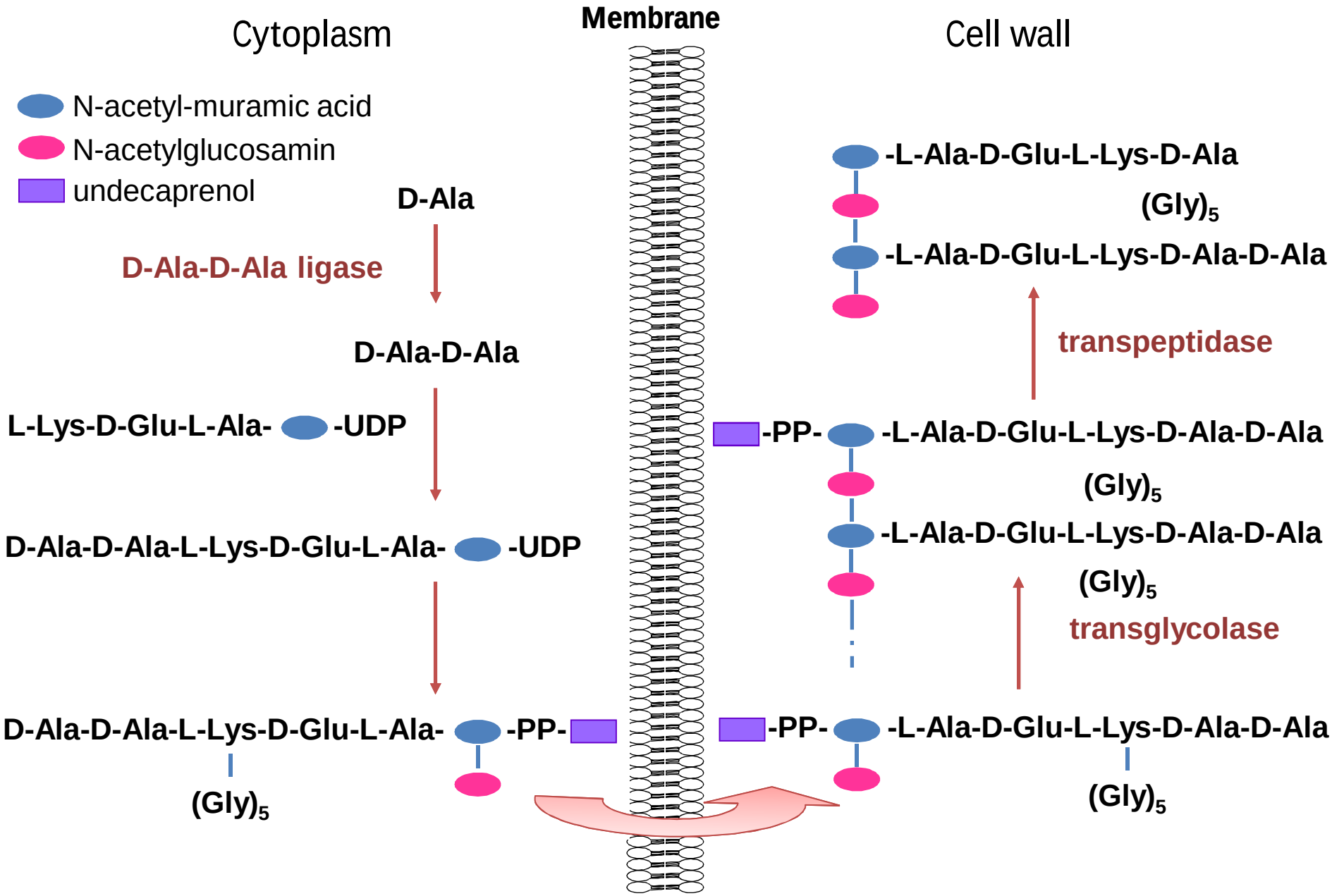
- **Avoparcin**
  - 1970: Growth promoter for livestock animals
  - Forbidden in 1997 in Europe following the emergence of glycopeptide resistant enterococci

Mode of action of glycopeptides

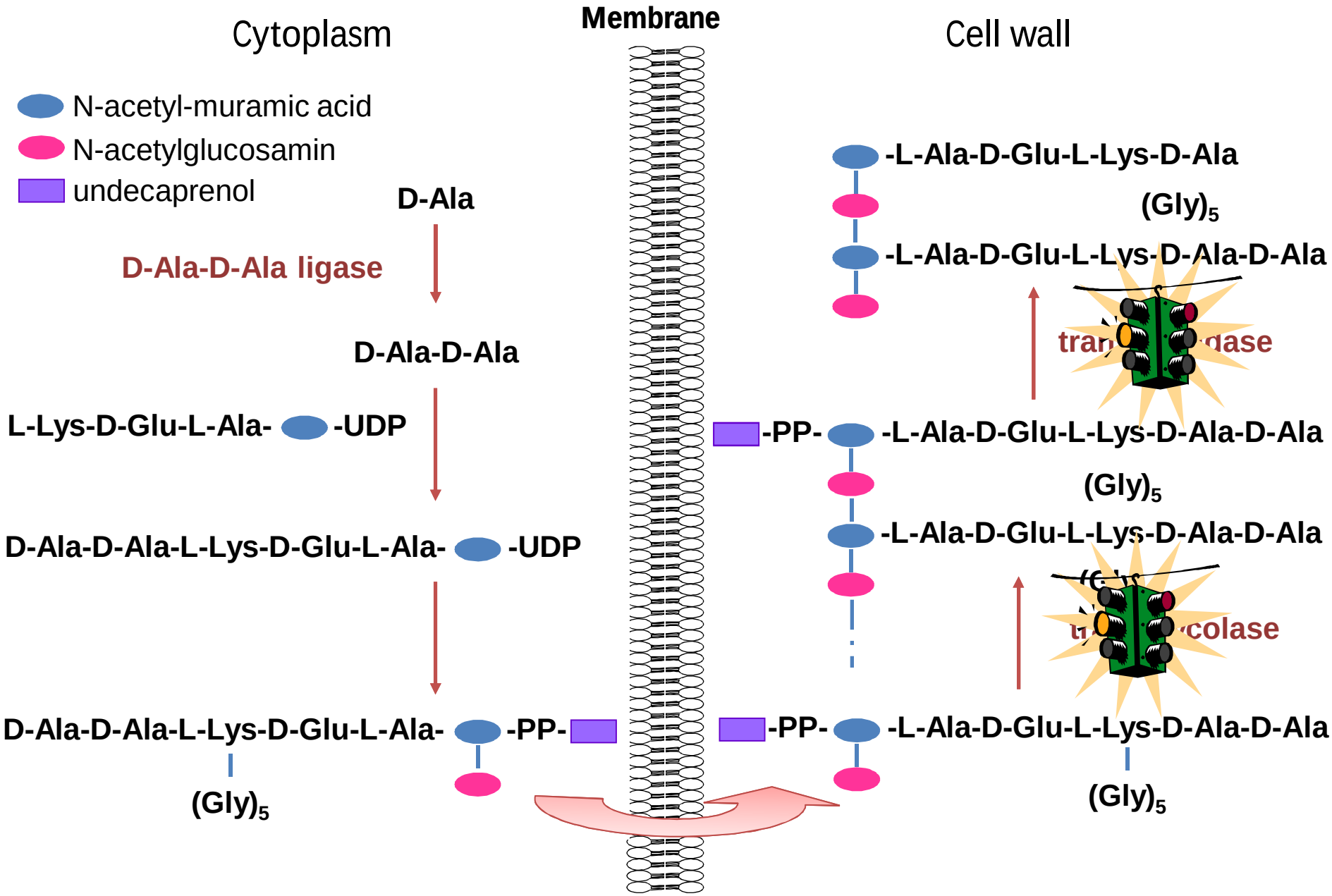
- **Interfere with cell wall synthesis**
  - Binding to terminal D-alanine-D-alanine at the end of pentapeptide
  - Inhibition of transglycosylation by preventing incorporation of new subunits into the growing cell wall

- **Spectrum of antimicrobial activity**
  - Restricted to Gram-positive organisms including *Staphylococci*, *Streptococci*, *Enterococci*, *Clostridium*
  - Inherently resistant: *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Erysipelothrix spp, …* and few species of *Enterococci*
  - Inactive against Gram-negative bacteria

Peptidoglycan biosynthesis



Peptidoglycan biosynthesis



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Glycopeptide breakpoints for *Staphylococci*

	MIC (mg/L) for					
	Vancomycin			Teicoplanin		
	S	I	R	S	I	R
EUCAST	≤2		>2	≤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

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Definitions for glycopeptide non-susceptible *S. aureus*

- **According to the mechanism of resistance**
  - VanA-mediated high-level glycopeptide resistance
  - Non-VanA-mediated low level resistance to glycopeptide

    ⇒ GISA and hGISA for isolates with non-VanA-mediated low-level resistance

- **Glycopeptide resistant *S. aureus* (GRSA)**
  - Isolates with high-level resistance to vancomycin (MIC >8 mg/L).
- **Glycopeptide intermediate *S. aureus* (GISA)**
  - Isolates with low-level resistance to vancomycin (MIC 4 - 8 mg/L).
- **Heterogeneous glycopeptide intermediate *S. aureus* (hGISA)**
  - Isolates susceptible to vancomycin (MICs ≤2mg/L) but with subpopulations (1 in 10^6 cells) with vancomycin MIC >2 mg/L by population analysis

http://www.eucast.org/expert_rules/

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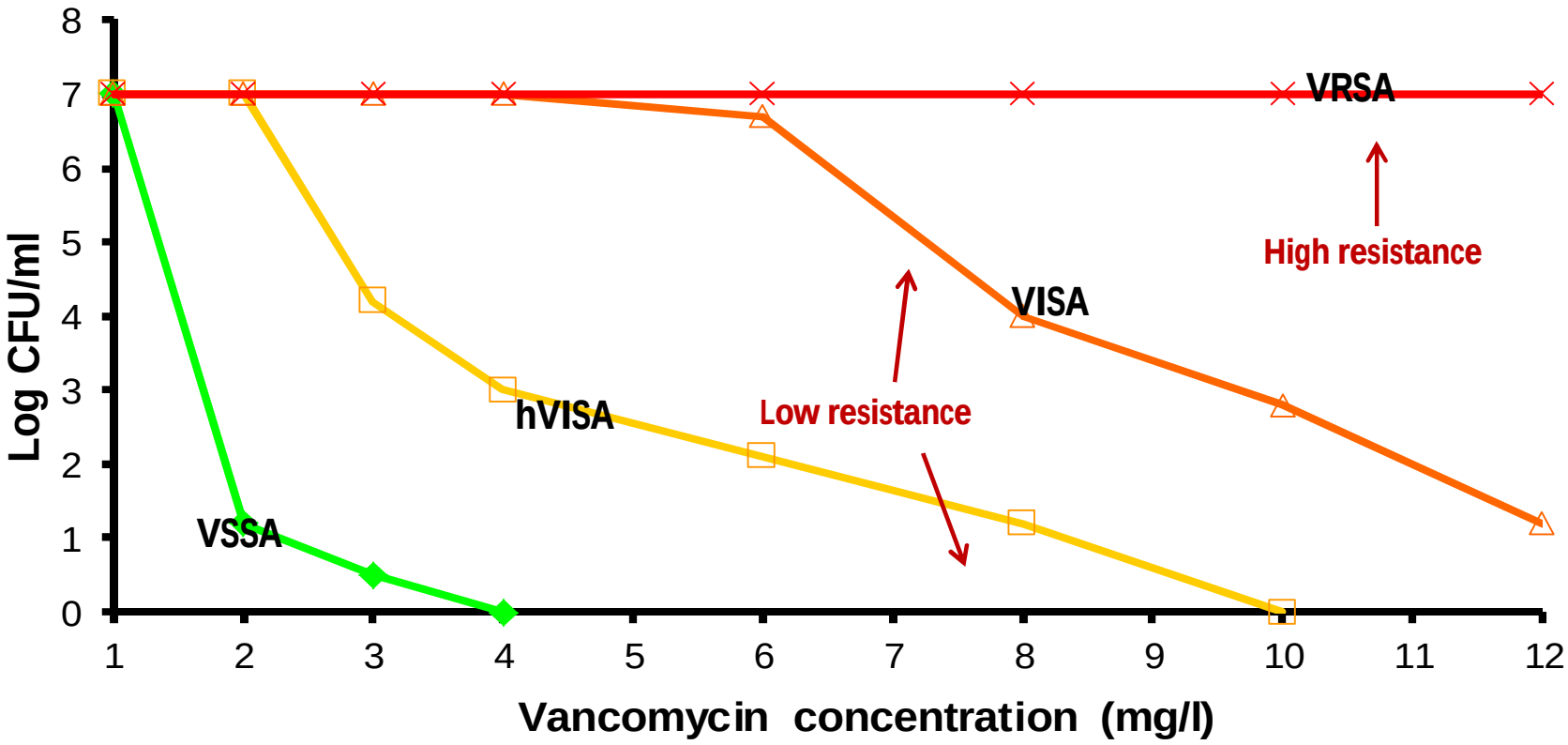
Population analysis
VSSA, hVISA, VISA et VRSA



from Liu c. et al. 2003. Antimicrob Agents Chemother. 47:3040

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[b], T. Oguri[c] and F. C. Tenover[d]

[a]*Department of Bacteriology;* [b]*Department of Pediatrics, Juntendo University, Tokyo;* [c]*Clinical Laboratory, Juntendo Hospital, Tokyo, Japan;* [d]*Nosocomial Pathogens Laboratory, Centers for Disease Control and Prevention, Atlanta, GA, USA*

The MRSA strain
purulent discharge at
debridement sample,
the broth microdilu
most reliable antimi
emergence of resista
been predicted[3,4] bas
vancomycin in enter
vanA-containing plas
has been demonstra
carry *vanA* or *vanB* g
of DNA. The exact r
susceptibility to van
but it may be due to
cell wall synthesis.[5]

Mechanism of low level resistance

- **Genetic environment**
  - Multiple point mutations leading to modified peptidoglycans synthesis including *walkR, vraSR*, *yvqF* and *rpoB*
  - Directly or indirectly involved with biosynthesis/metabolism of the cell wall including systems controlling the transcription of genes in its synthesis
  - No *van* genes and not linked to methicillin resistance
- **↑ Thickness of cell wall**
  - ↑ synthesis of peptidoglycan, ↓ autolytic activity, ↑ residues D-Alanyl-D-Alanine

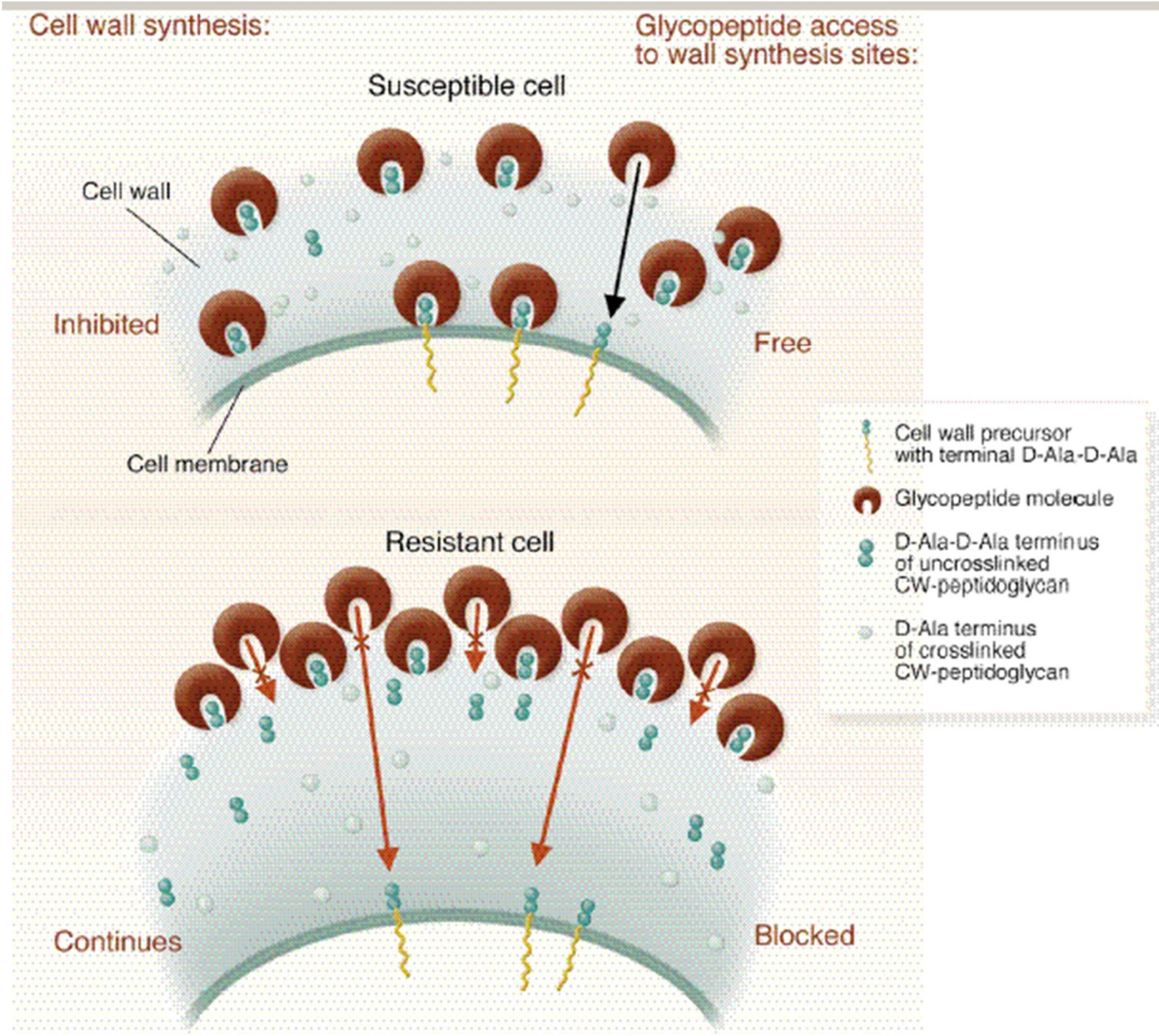
Absorption of GLYCOPEPTIDES into bacterial cell wall before extern membrane surface

⇒ **Low level resistance and reversible**

Teicoplanin > vancomycin

MIC to vancomycin 4 – 8 mg/L

Gardette J et al. J Clin Inverst. 2014
Hiramatsu K. 2001. The Lancet Infect. Dis. 1:147


Cell wall synthesis:
Glycopeptide access to wall synthesis sites:
Susceptible cell
Cell wall
Inhibited
Free
Cell membrane
Resistant cell
Continues
Blocked
Cell wall precursor with terminal D-Ala-D-Ala
Glycopeptide molecule
D-Ala-D-Ala terminus of uncrosslinked CW-peptidoglycan
D-Ala terminus of crosslinked CW-peptidoglycan

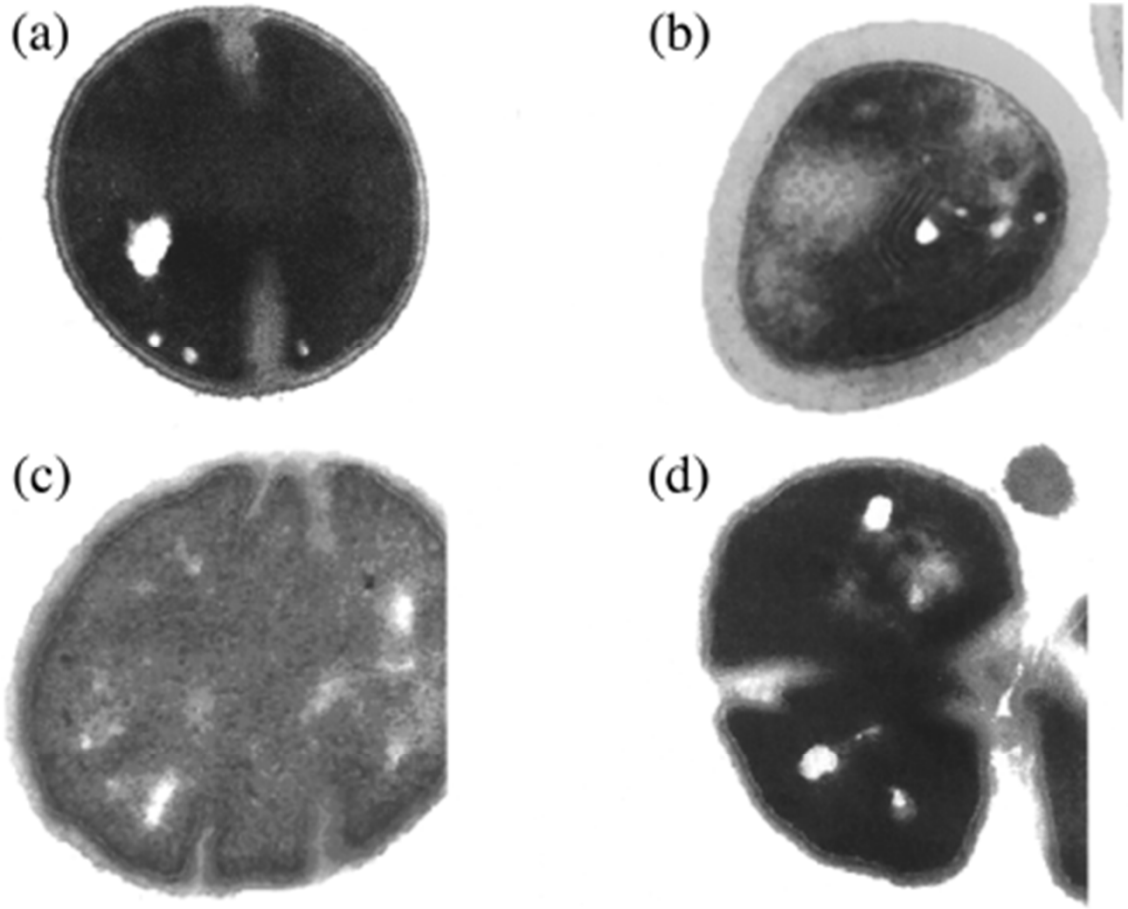


Figure 3. Transmission electron micrographs of (a) *S. aureus* ATCC 29213, (b) VISA strain P1V44, (c) hetero-VISA strains P1V69 and (d) MRSA P39575. Magnification ×60 000.

Epidemiological and clinical characteristics of VISA infections

- **Epidemiology**
  - Emergence of teicoplanin resistant strains in 1990s
  - Worldwide dissemination but at **low frequency (<1%)**
- **Clinical aspects**
  - Asymptomatic carriage
  - From skin and soft tissue infections to endocarditis
  - Persistence of infection ++++
  - High mortality but no necessary linked to VISA
- **Host factors**
  - Comorbidities ++++
  - Foreign bodies, undrained abscess, immunosuppression (diabetes, renal failure, neoplasia,...)
- **Factors linked to glycopeptides**
  - Previous treatment (1 week to 1 year) for long period, often intermittently

Kaatz G.W. et al. 1990. J Infect. Dis.162:103
Fridkin SK. et al. 2003. Clin. Infect. Dis. 36:429
Geisel R. et al. 2001. European J Clin. Microbiol. 2001:685

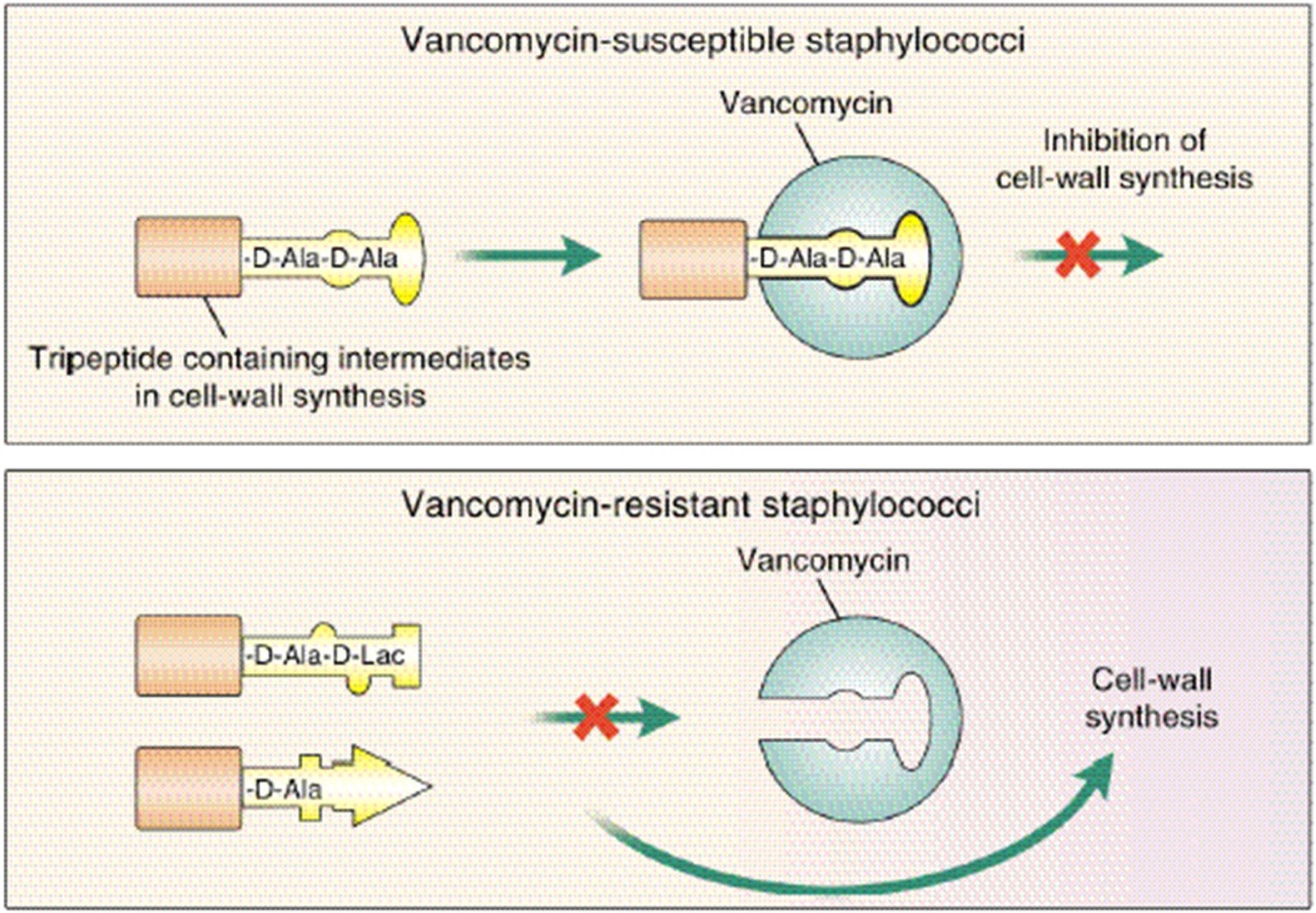
Mechanism of high level resistance

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

VRSA—doomsday superbug or damp squib?

The most extreme form of vancomycin resistance is vancomycin-resistant *Staphylococcus aureus* (VRSA). VRSA has become synonymous with the *van*A gene, which is transferred from *Enterococcus faecalis*. This gene encodes for high-level glycopeptide resistance. There are many reports of VRSA worldwide, most recently from a Pennsylvania hospital,[1] but there are arguably only 11 well characterised examples from published work about the transfer of *van*A to meticillin-resistant *S aureus* (MRSA)—nine from the USA[2,3] and seven from the state of Michigan. The first sample was isolated in Michigan in 2002, the second in Pennsylvania that same year,

Gould IM Lancet Infect. Dis 2012


Vancomycin-susceptible staphylococci
Vancomycin
Inhibition of
cell-wall synthesis
-D-Ala-D-Ala
-D-Ala-D-Ala
Tripeptide containing intermediates
in cell-wall synthesis
Vancomycin-resistant staphylococci
Vancomycin
-D-Ala-D-Lac
-D-Ala
Cell-wall
synthesis

[redacted]

Although vancomycin is often prescribed for the treatment of meticillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *S aureus* (VRSA) infections remain rare, with only few cases confirmed worldwide—mostly in the USA.[1] Here, we report the isolation and preliminary characterisation of the first VRSA strain in Europe isolated from a patient in Portugal.

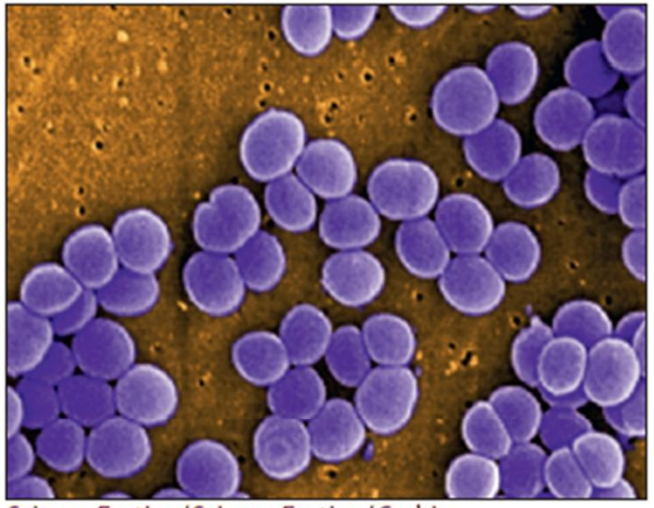
A 74-year-old woman with diabetes [redacted] haemodialysis, and peripheral vascular disease conditioning critical limb ischaemia underwent endovascular revascularisation and amputation of two gangrenous toes. Previous cultures of the wound amputation site

acquisition of the vancomycin-resistance determinant. The VRSA was resistant to erythromycin, clindamycin, gentamicin, and ciprofloxacin, and susceptible to co-trimoxazole, tetracycline, tygecycline, linezolid, daptomycin, quinupristin/dalfopristin, fusidic acid, cloramphenicol, rifampicin, and mupirocin.

Precautions were reinforced. The patient is clinically well, and is being treated with daptomycin, rifampicin, and amikacin, and aggressive wound care. An epidemiological investigation is ongoing, but so far transmission of VRSA from this patient to contacts at home, other patients or health-care workers from the dialysis unit was not detected.

The identification of VRSA is particularly worrying since Portugal is a country with one of the highest prevalences of MRSA and VRE in Europe.[4]

In all cases of VRSA detected so far there was no spread of the strain



Science Faction/Science Faction/Corbis

Control. Antimicrobial resistance surveillance in Europe 2011. http://ecdc.europa.eu/en/publications/Publications/Forms/ECDC_DispForm.aspx?ID=998 (accessed May 21, 2013).

5 Espadinha D, Faria NA, Miragaia M, Lito LM, Melo-Cristino J, de Lencastre H. Extensive dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA) between the hospital and the community in a country with a high prevalence of nosocomial MRSA. *PLoS ONE* 2013; **8**: e59960.

Health professionals in Syria

The US Centers for Disease Control and Prevention, UNICEF, and WHO, with support from the Office of Foreign Disaster Control, trained 50 staff from Syrian and Jordanian ministries

Challenge for detection of glycopeptide resistance in *Staphylococci*

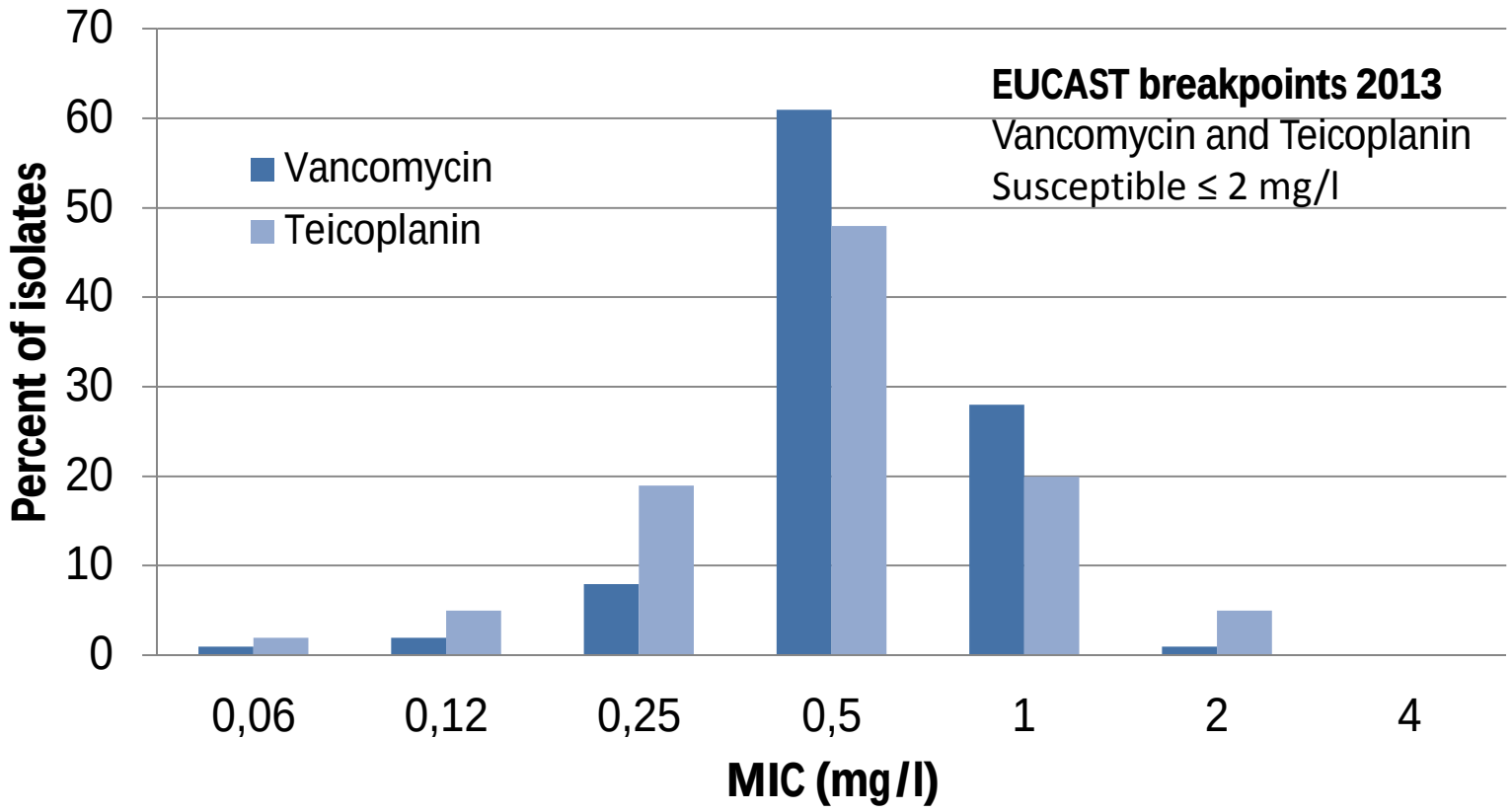
- **Disk diffusion**
  - cannot be used for GISA and hGISA detection
- **Detection of hGISA**
  - As proven difficult
  - Divided into screening and confirmation
  - Screening: macromethods, GRD, agar screen
  - Reversible

MIC determination for GISA and GRSA

- **Broth microdilution = gold standard**

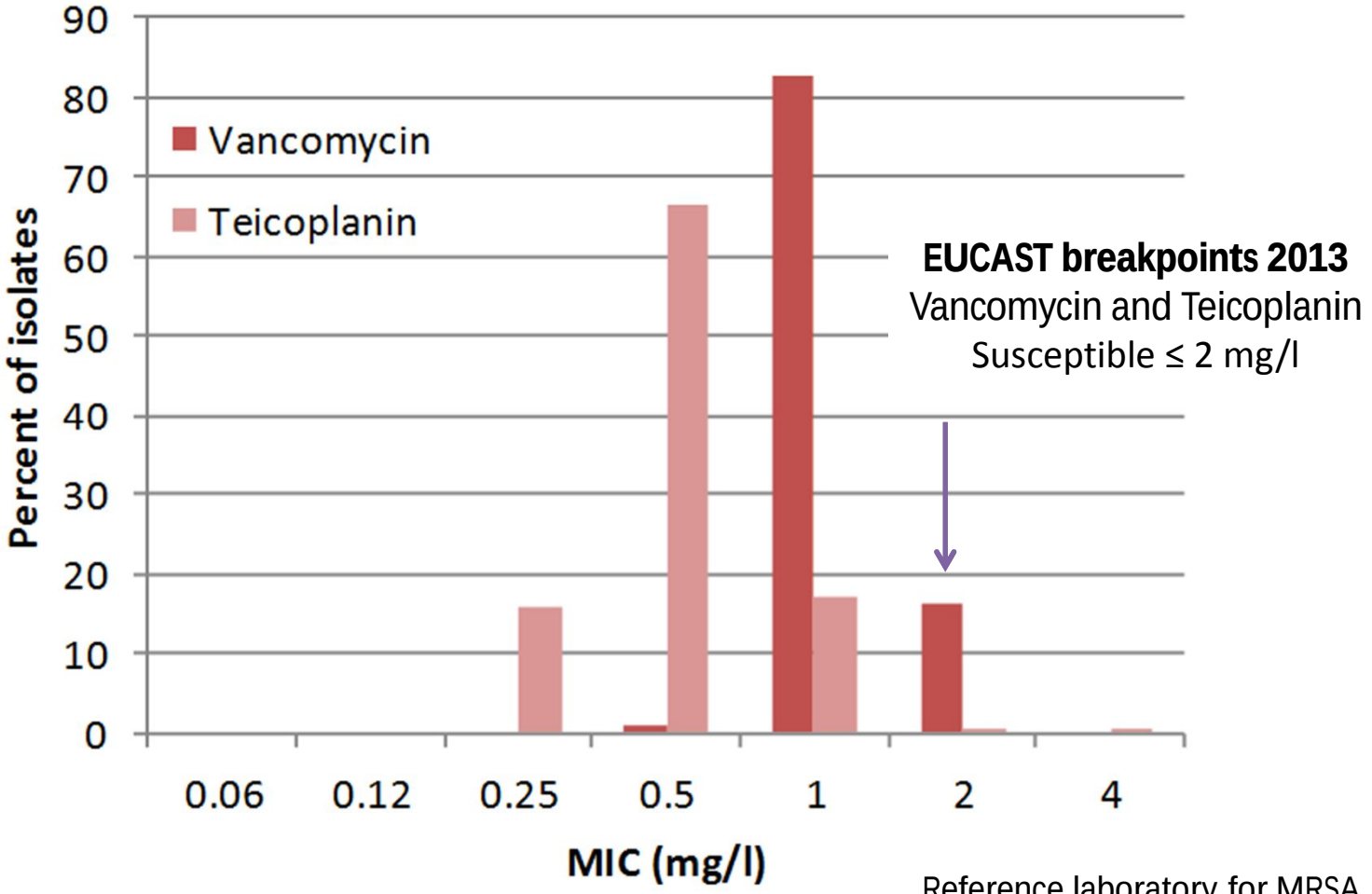
- **May also be determined by**
  - Gradient strip methods (E-test), agar dilution and automated systems
  - E-tests show MICs with 0.5-1 two-fold dilution steps higher than broth microdilution
  - Isolates with MICs >2 mg/L should be referred to the NRC
  - hGISA not detected by MIC determination

MIC distribution to glycopeptides for 313 MRSA isolates, Belgian hospitals, 2011



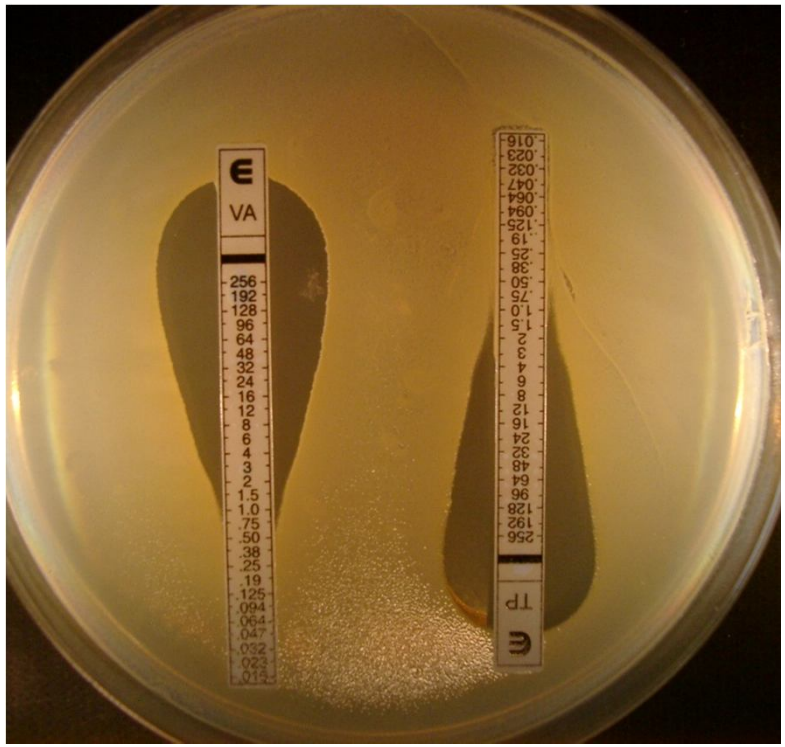
Reference laboratory for MRSA , 2011

MIC distribution to glycopeptides for 288 MRSA isolates, Belgian hospitals, 2013-14

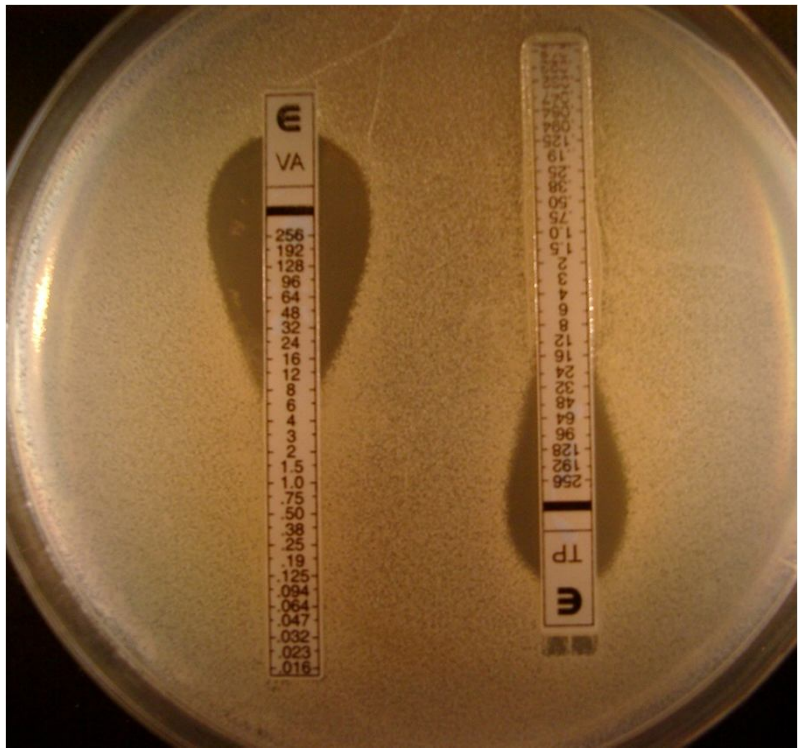


Reference laboratory for MRSA , 2011

MICs to glycopeptides using E-test
O,5 McF + MH + 24h



ATCC29213

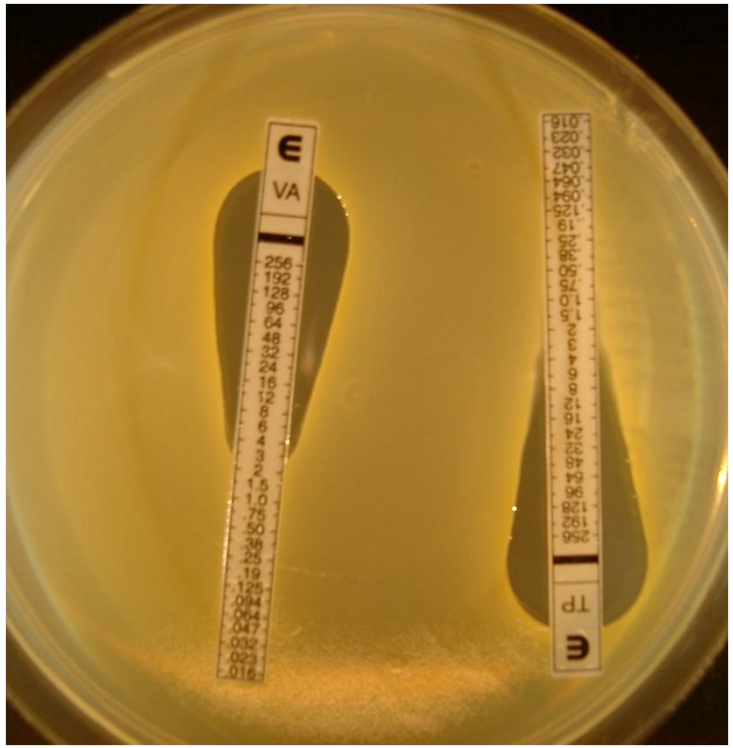


HIP5827

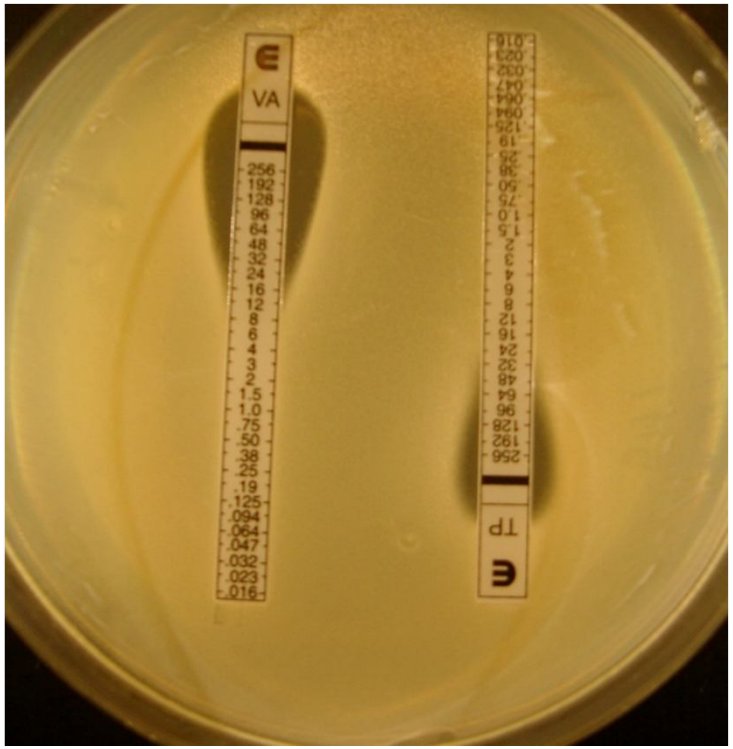
Macro gradient test or Macromethod

- **Screening method for reduced vancomycin susceptibility**
  - Should not be reported as MICs
  - Does not differentiate between hGISA, GISA and GRSA
- **Methods**
  - High inoculum (2,0 McFarland)
  - Incubate on BHI agar for 48h
- **Interpretation**
  - Teicoplanin ≥12 mg/L: GRSA, GISA or hGISA
  - Teicoplanin and vancomycin 8 mg/L: GRSA, GISA or hGISA
  - Teicoplanin <8 mg/L: Not GRSA, GISA or hGISA

MICs to glycopeptides using E-test
2 McF + BHI + 48h

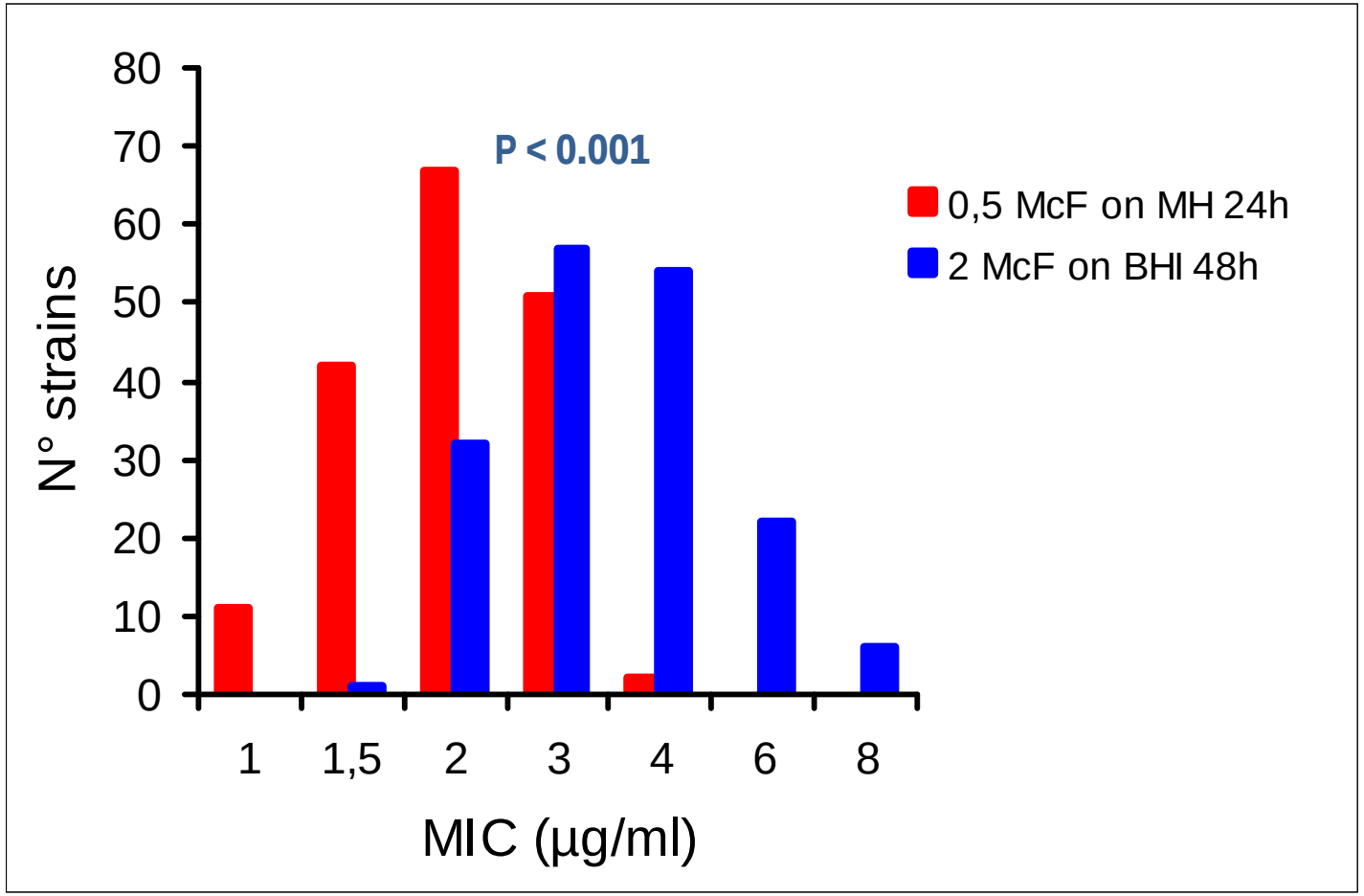


ATCC29213



HIP5827

MIC determination for vancomycin by E-test comparison of two methods, Belgium EARSS 1999



Glycopeptide resistance detection (GRD) gradient test

- Double strip vancomycin and teicoplanin
- 0.5 McF on MH agar for 24 and 48h
- **Interpretation**
  - Positive if the GRD strip with ≥8 mg/L for either vancomycin or teicoplanin
  - Suspected reduced susceptibility to glycopeptides

Teicoplanin screen agar

Teicoplanin screen agar
5 mg/l

- Mueller Hinton plate
- Inoculum of 2.0 McFarland standard
- 10 µl spotted
- Incubation for for 24 to 48 h at 35°C
- Interpretation
  - Positive if > 2 colonies
  - Suspected reduced susceptibility to glycopeptides

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

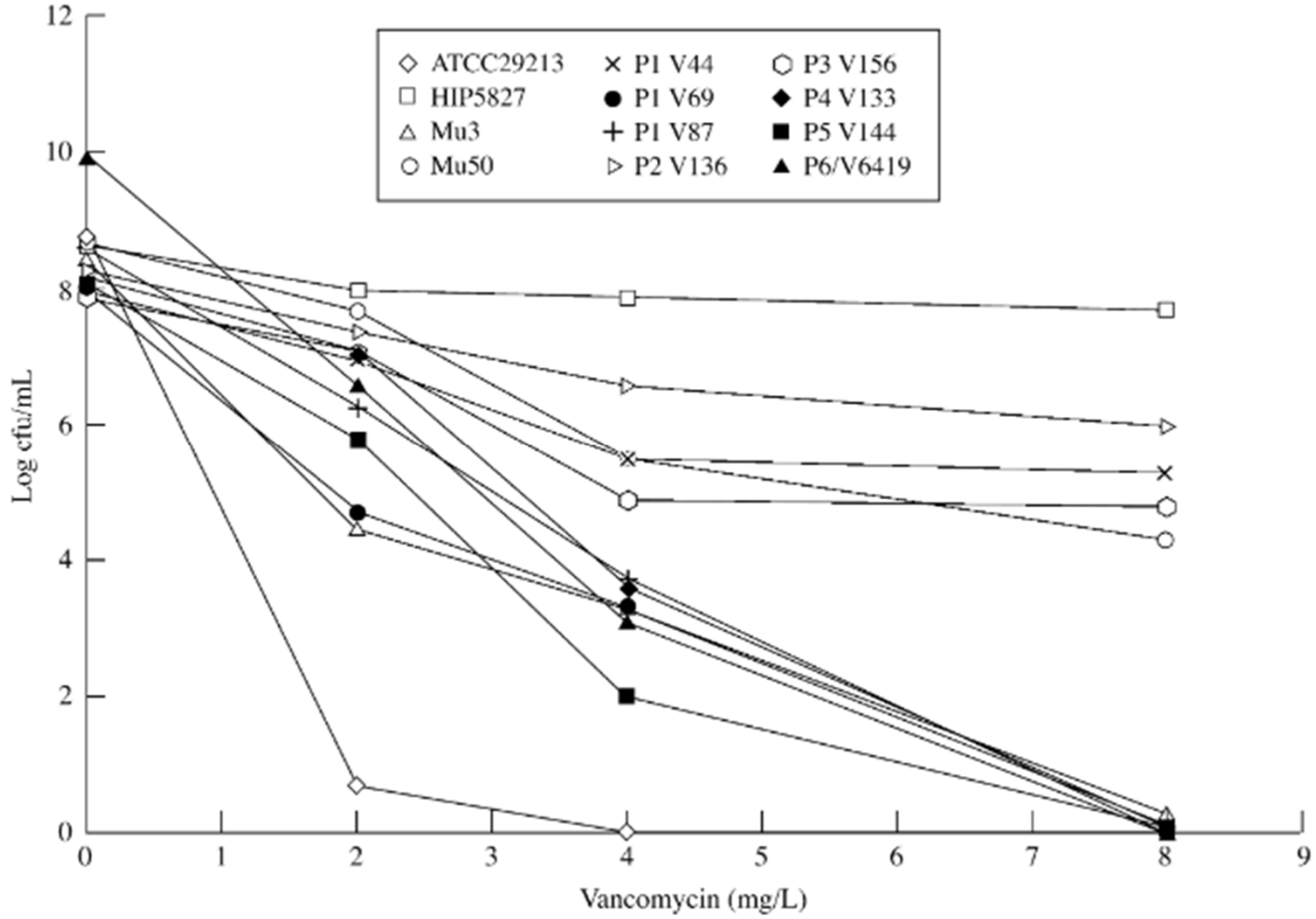
[a] In all studies, vancomycin population analysis/area under the curve (PAP/AUC) was considered the "gold standard" for calculating sensitivity and specificity.
[b] Evaluation of vancomycin broth MICs included detection of VISA and hVISA. By definition, hVISA will not be detected by determinations of 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

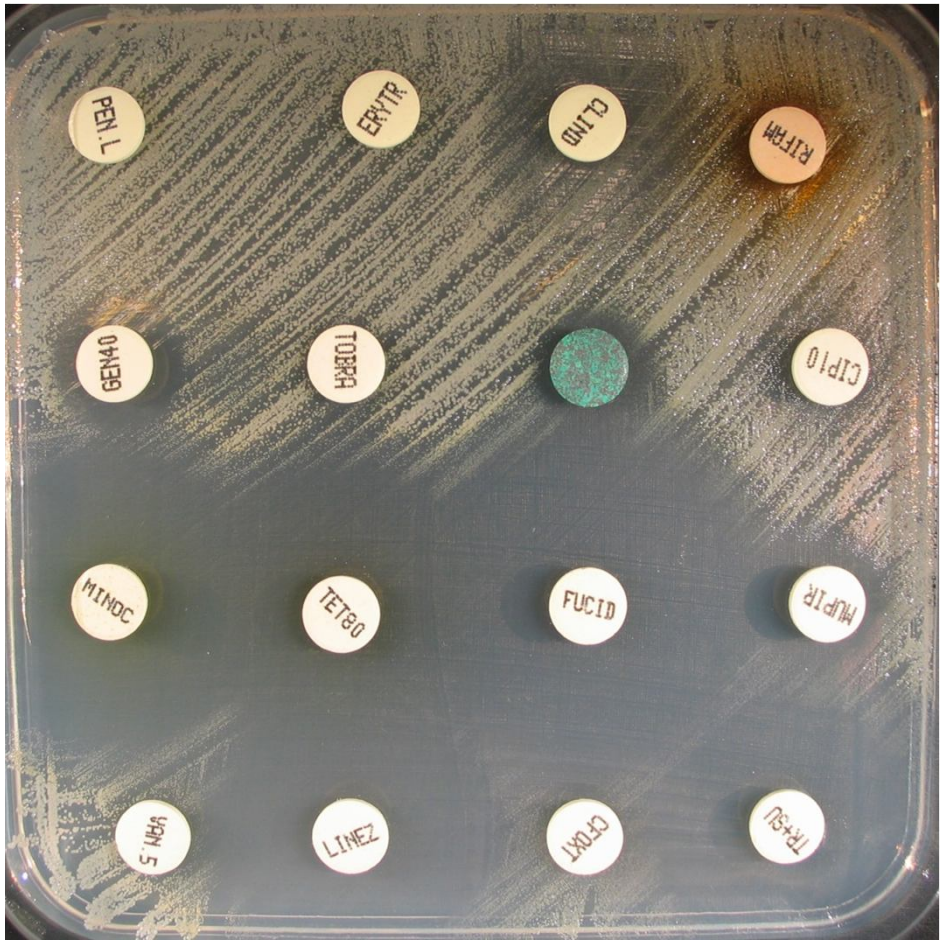
- **Population analysis profile-area under curve (PAP-AUC)**
  - Isolate screening positive for reduced susceptibility
  - Not identified as GRSA or GISA by MIC determination
- **Method**
  - Population analysis for vancomycin
  - Determine ratio of AUC of test organism vs Mu3 (ATCC 700698)
- **Interpretation**
  - hGISA if AUC ratio is 0.9 and vancomycin MIC is 2 mg/L

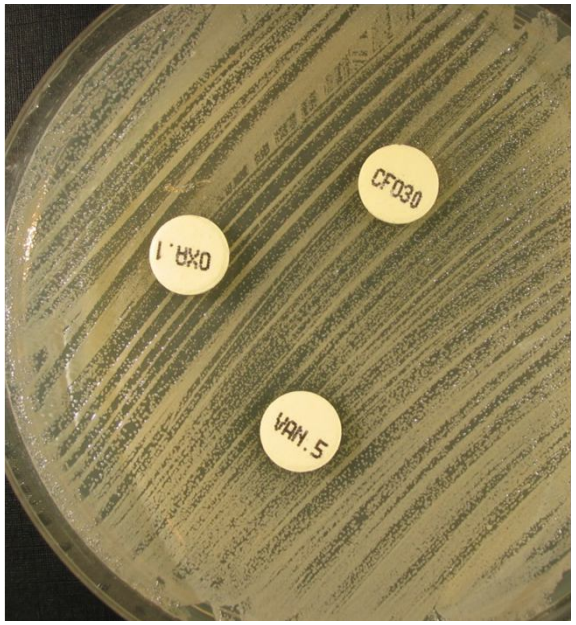
Population analysis of Belgian VISA and hetero-VISA strains and reference strains



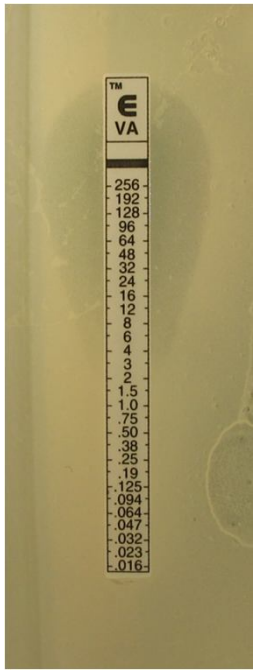
Denis O. et al. J Antimicrob Chemother 2002

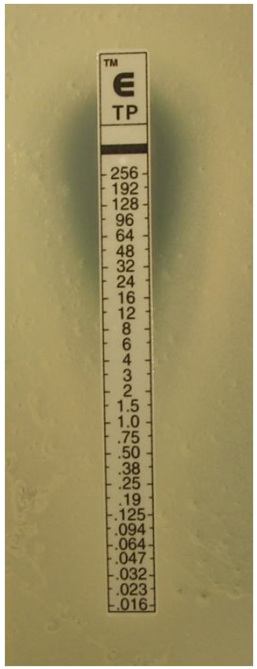
Detection of GRSA





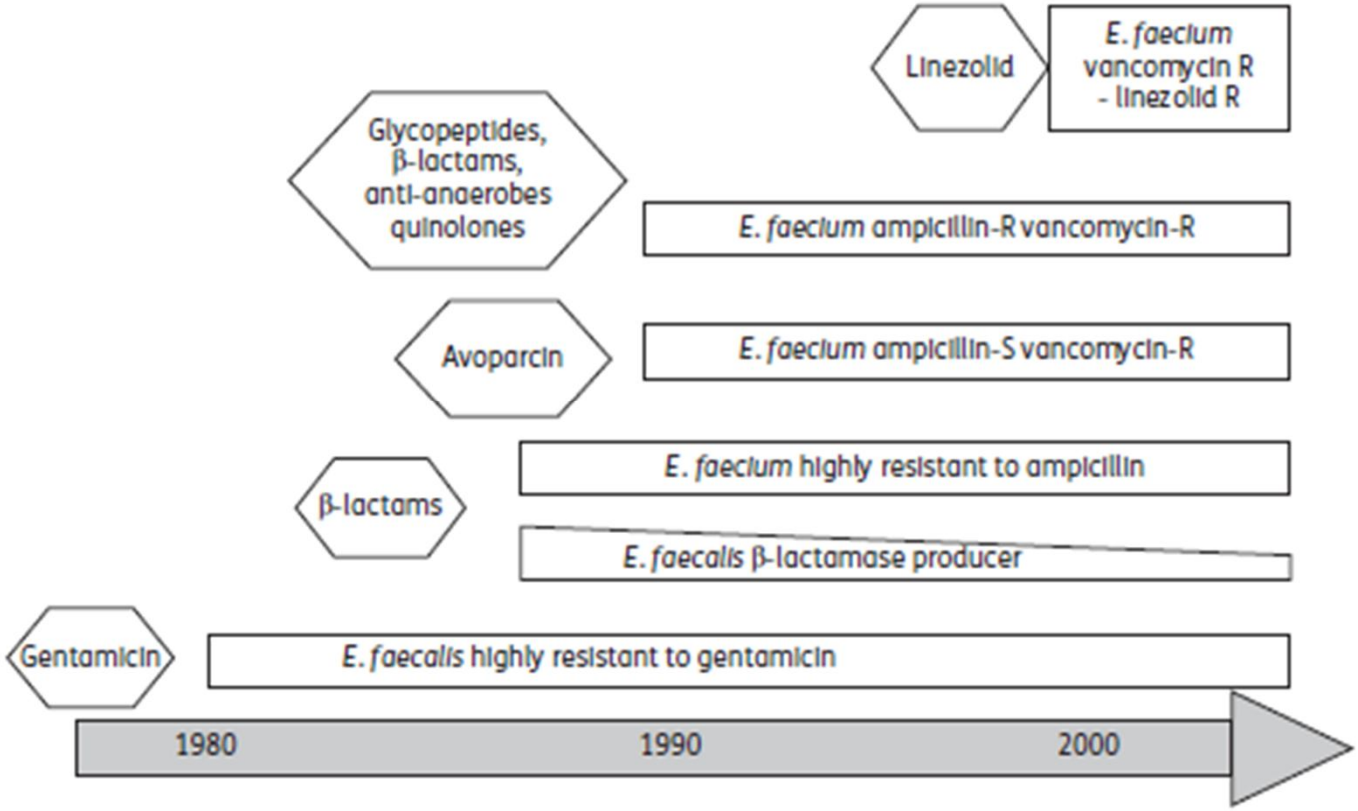
GRSA





Should be confirmed by PCR for the presence of *van* genes

The walk of enterococci towards multiple antibiotic resistance



Cattoir V et al. JAC 2012

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European Society of Clinical Microbiology and Infectious Diseases

Glycopeptide breakpoints for *Enterococci*

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

http://www.eucast.org/clinical_breakpoints/

CLSI 2014 M100-S24

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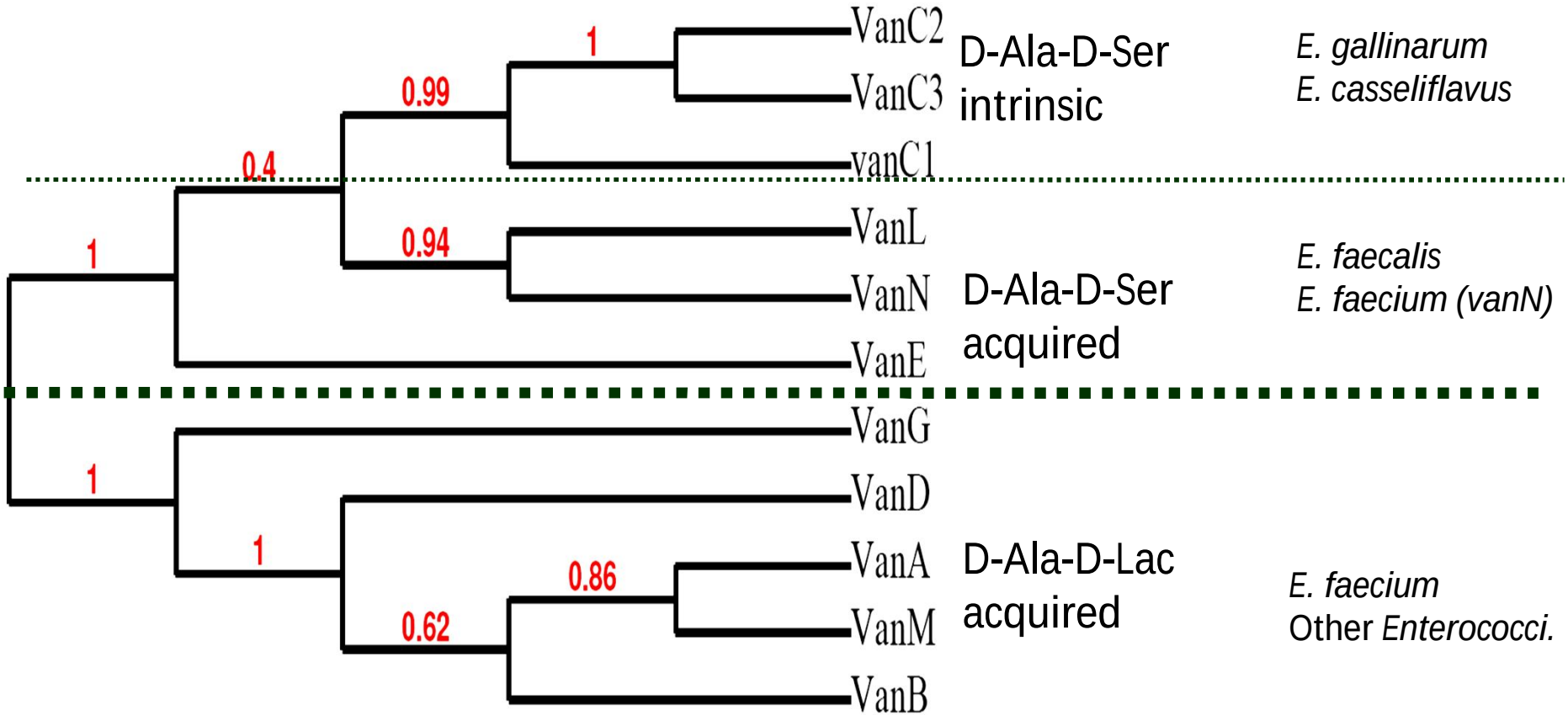
Glycopeptide resistance in *Enterococci*

Glycopeptides

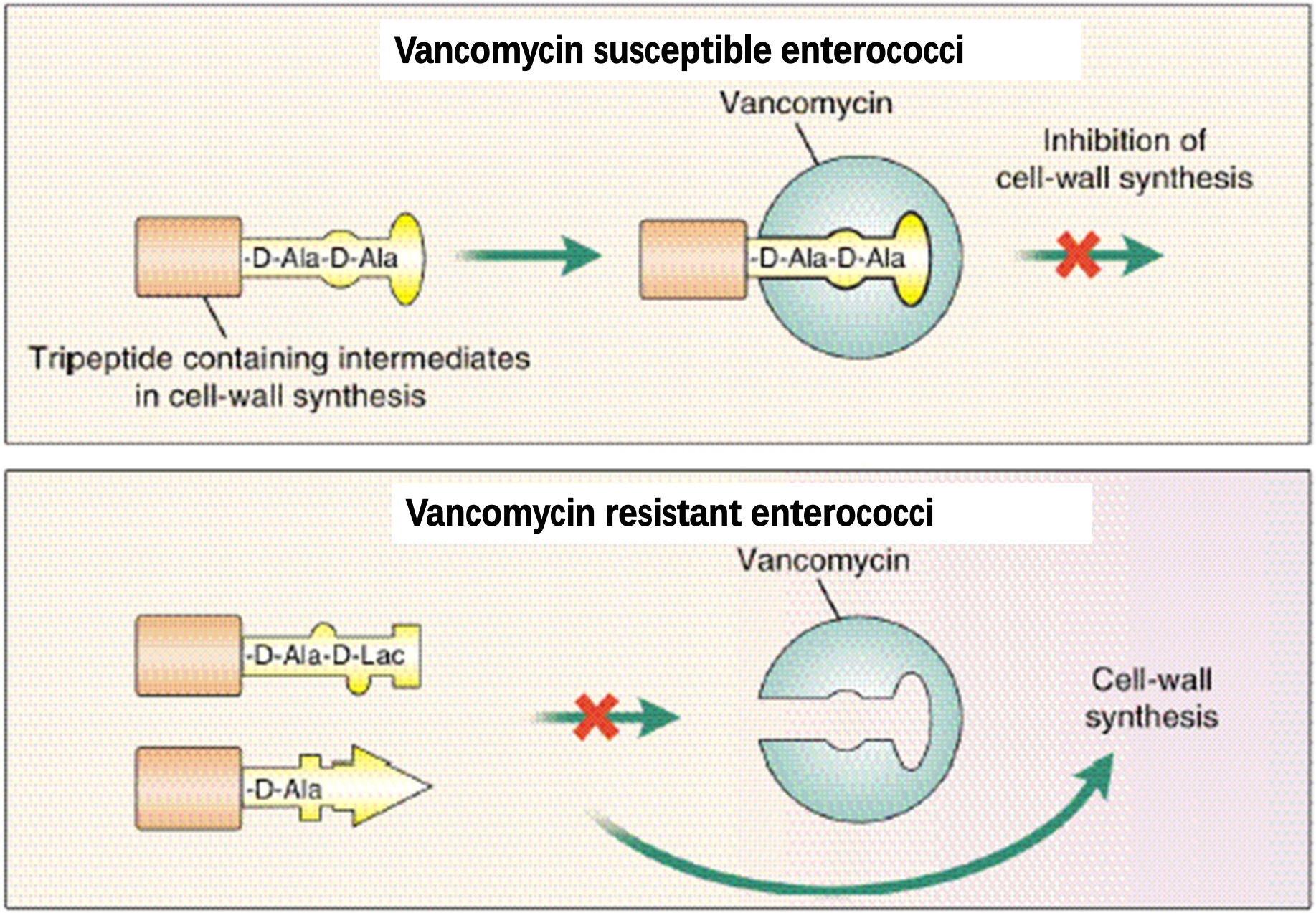
- Natural phenotype
  - Susceptible except few non pathogenic species
  - Intrinsic resistant chromosomally mediated*: E. gallinarum* and *E. casseliflavus*
- Acquired resistance phenotype
  - New precursor of peptidoglycane with reduced affinity to glycopeptides
  - Eight *van* genes with variable MIC to vancomycin and teicoplanin

Glycopeptide	MIC (mg/l)	
	vanA	*vanB*
Vancomycin	64-1024	4-1024
Teicoplanin	8-512	0.06-1

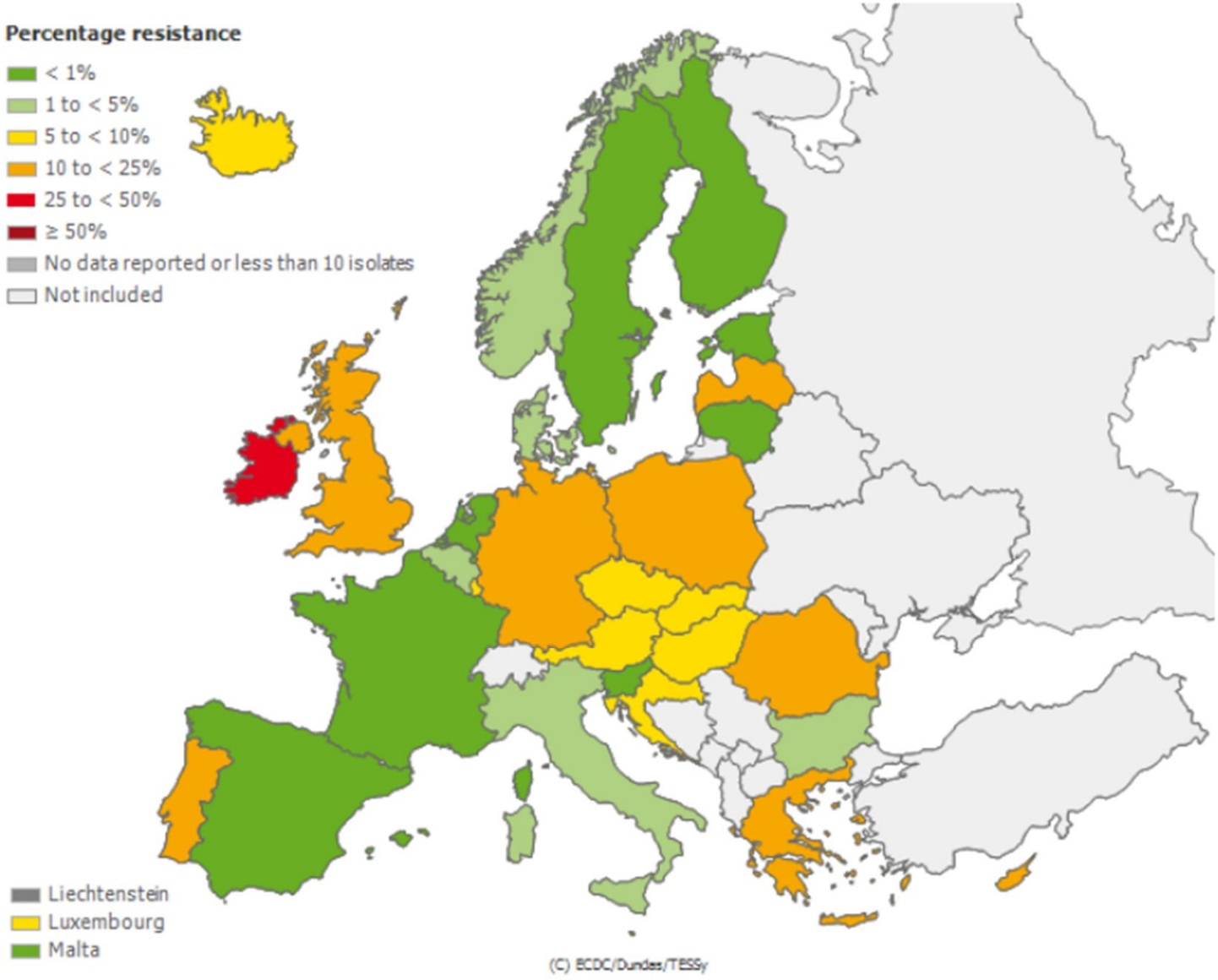
Phylogenetic analysis of 11 *van* genes



Lebreton F et al. Antimicrob. Agents Chemother, 2012; 55: 4606–4612


Vancomycin susceptible enterococci
Vancomycin
Inhibition of
cell-wall synthesis
-D-Ala-D-Ala
-D-Ala-D-Ala
Tripeptide containing intermediates
in cell-wall synthesis
Vancomycin resistant enterococci
Vancomycin
-D-Ala-D-Lac
-D-Ala
Cell-wall
synthesis

Proportion of vancomycin resistance among *E. faecium* isolates from bacteraemia, 2013



Methods for detection of glycopeptide resistance in *Enterococci*

- **Methods**
  - MIC determination, disk diffusion, breakpoint agar method and automated system
- ***vanB*-mediated resistance**
  - Detection more challenging
- **Accurate identification !**
  - *E. gallinarum* or *E. casseliflavus* may be erroneously perceived as *E. faecium*

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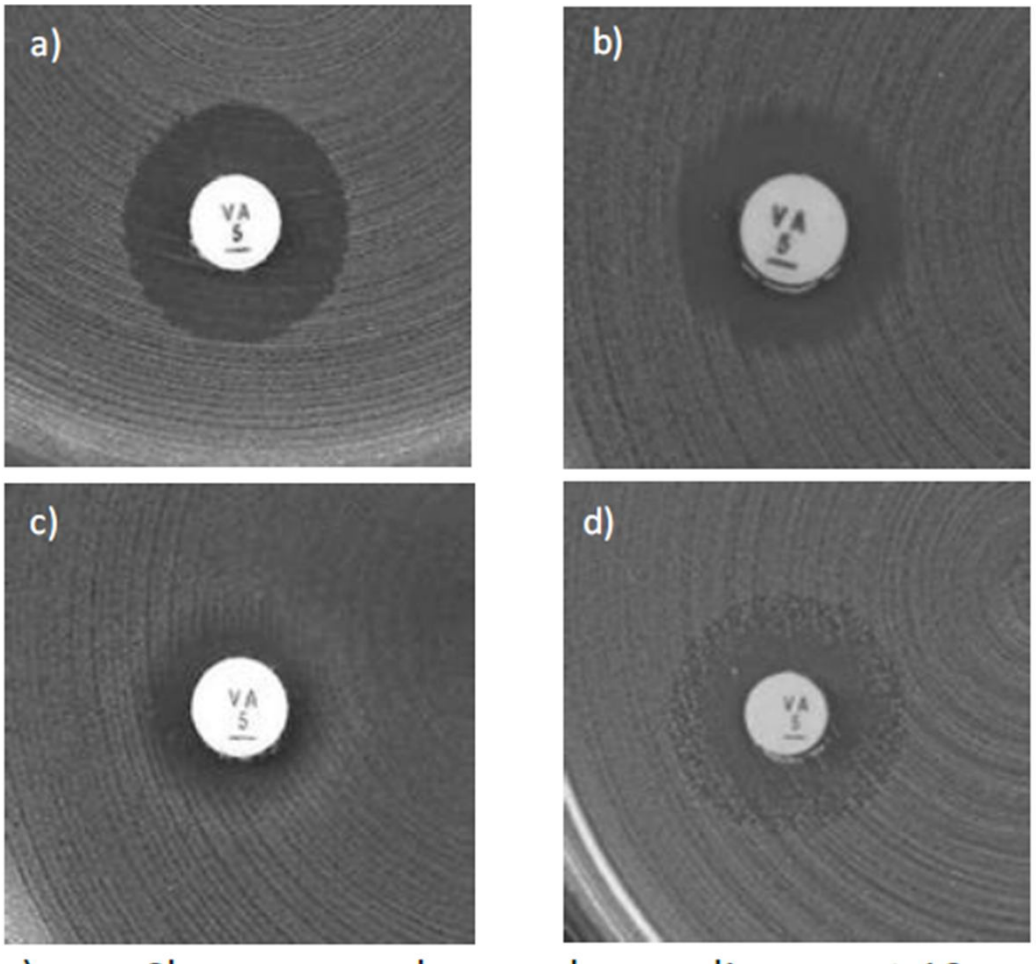
Reading of vancomycin disk diffusion tests for *Enterococci*



a) **Sharp zone edges and zone diameter ≥12 mm**. Report as susceptible.

b-d) **Fuzzy zone edges and/or colonies within the zone.** Report as resistant regardless of zone diameter

5 µg vancomycin
Incubation 24h

http://www.eucast.org/expert_rules/

Criteria are different for CLSI

Breakpoint agar method

BHI supplemented with 6 mg/l vancomycin

10 µl of 0.5 McF suspension

Incubation 24h at 35°C

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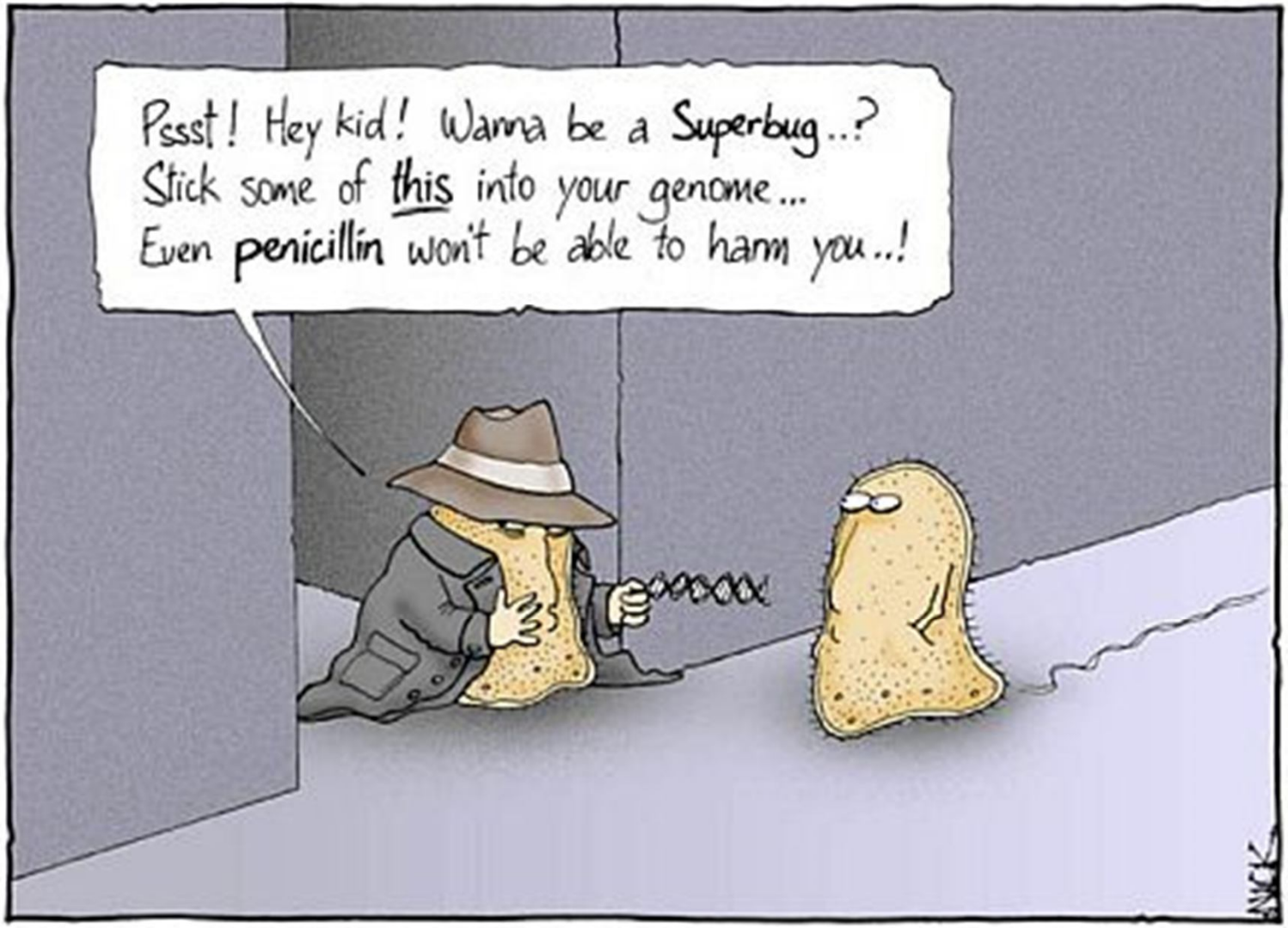
Thank you for your attention


Pssst! Hey kid! Wanna be a Superbug..?
Stick some of this into your genome...
Even penicillin won't be able to harm you..!