Detection of Vancomycin-resistant Enterococci (VRE)

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28 March 2025

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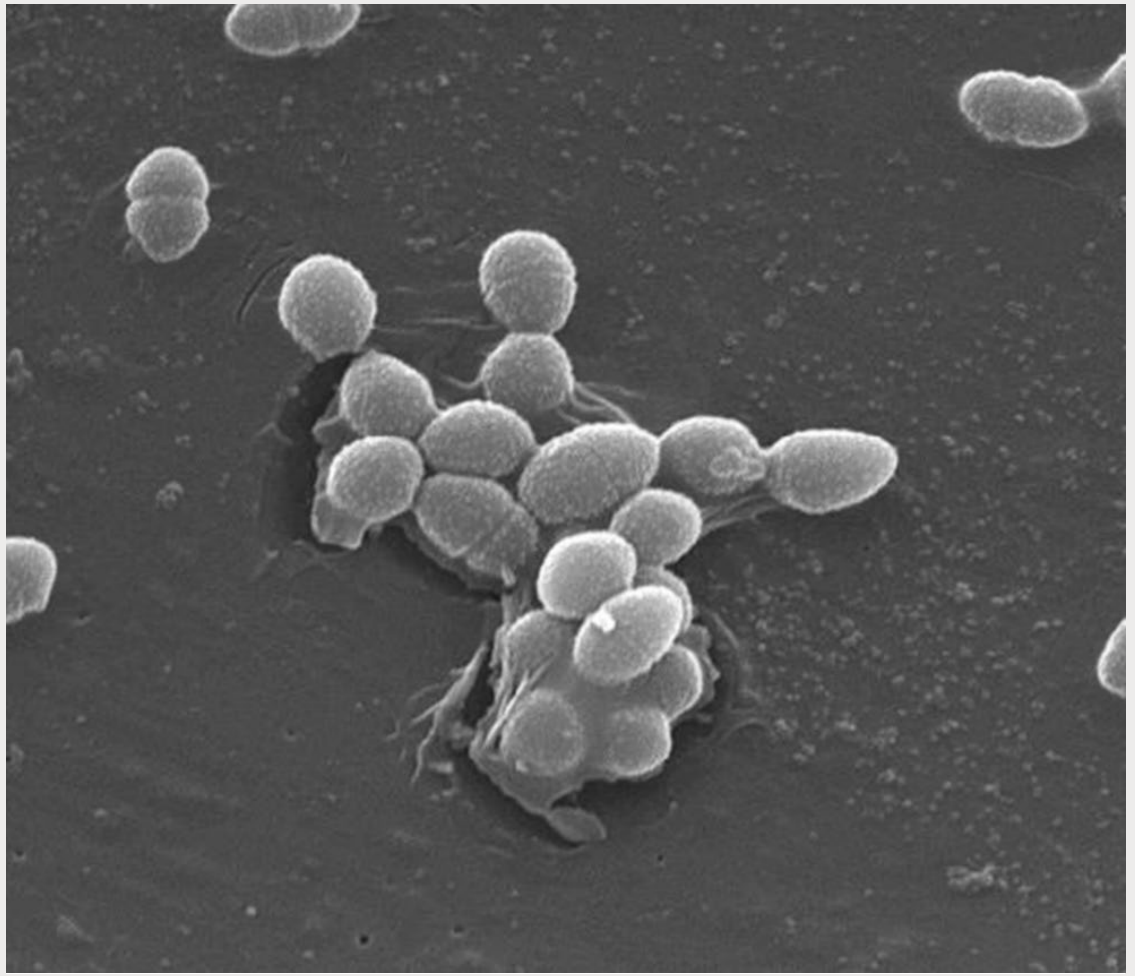
Outline

- Background
- Phenotypic methods of detection
- Genotypic methods of detection
- Treatment

What is VRE?

- Definition: Enterococci resistant to vancomycin (last resort antibiotic)
- Gram-positive cocci, catalase-negative
- Habitat: Normal gut flora; survives harsh conditions (e.g., bile, high salt).
- Common species:
  - *E. faecium* (most common VRE, high resistance).
  - *E. faecalis* (less resistant but more virulent).
- Rare Species: *E. gallinarum, E. casseliflavus* (intrinsic low-level resistance; vanC gene).



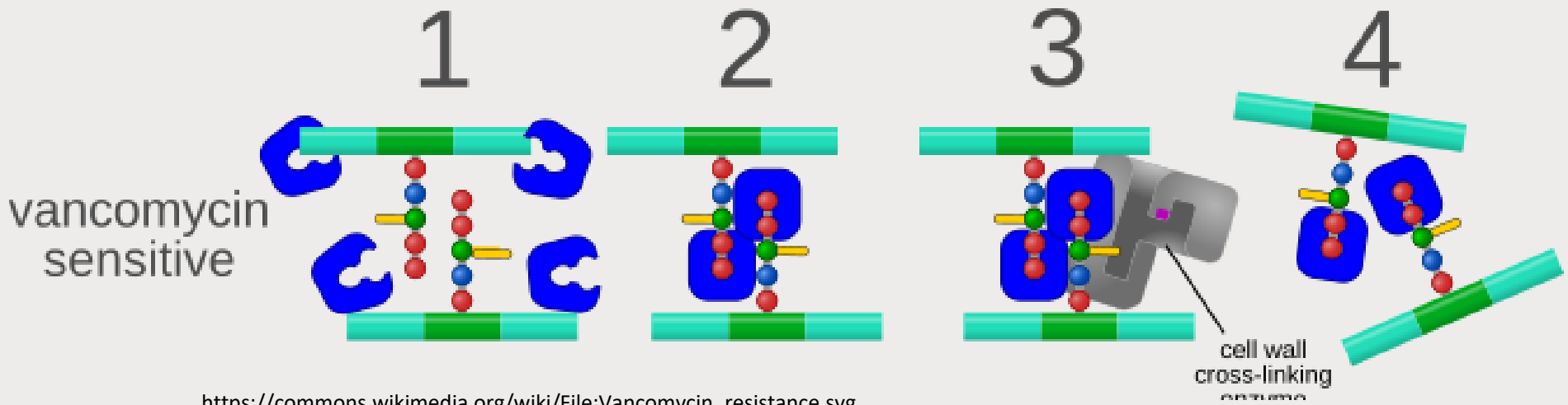
Janice Haney Carr Content Providers(s): CDC/ Pete Wardell

Vancomycin: Mode of action

Vancomycin blocks the peptidoglycan cross linkage: Weakens cell wall

Binding to Peptidoglycan Precursors:

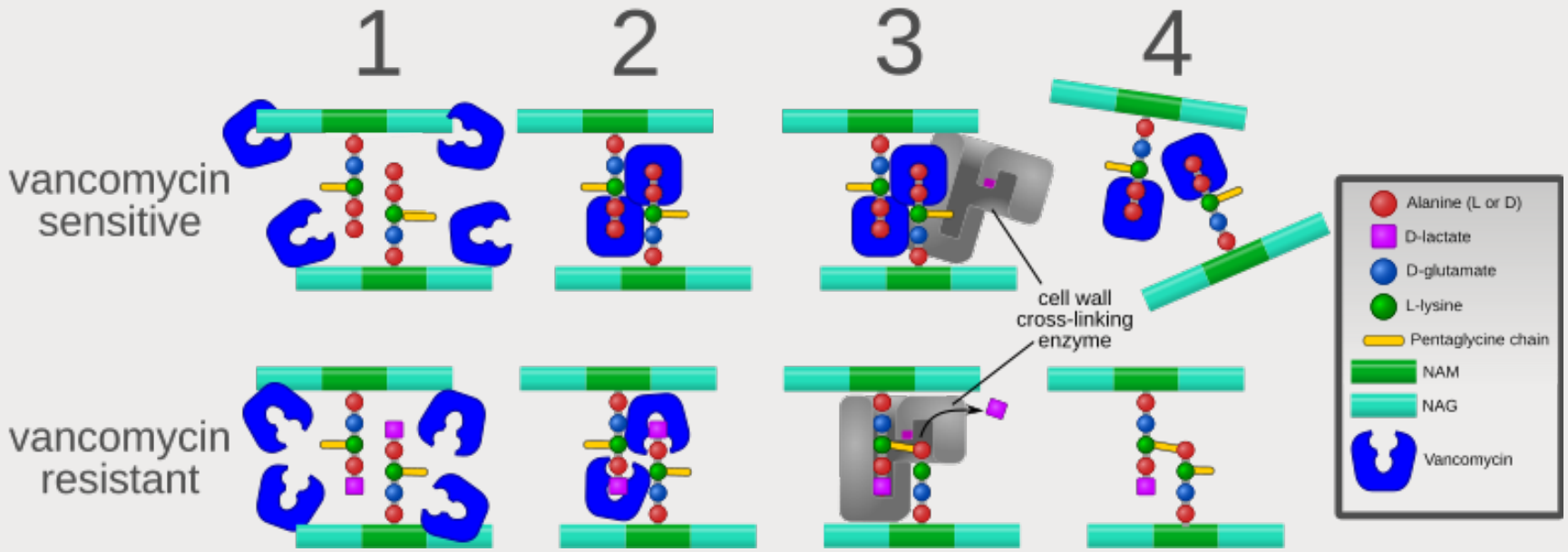
- Vancomycin **binds tightly** to the **D-Ala-D-Ala** residues of lipid II, a key intermediate in bacterial cell wall synthesis.
- This prevents **transpeptidase** (e.g., penicillin-binding proteins, PBPs) and **transglycosylase** enzymes from cross-linking peptidoglycan chains



https://commons.wikimedia.org/wiki/File:Vancomycin_resistance.svg

Vancomycin Resistance

- Resistance to vancomycin is mainly mediated by the modification of target
  - Encode enzymes that replace **D-Ala-D-Ala** (vancomycin binding site) with **D-Ala-D-Lactate** (*vanA/B/D/M*) or **D-Ala-D-Serine** (*vanC/E/G*), reducing vancomycin affinity.



Types of *van* genes in VRE

- **1. High-Level Resistance (Acquired)**

Gene	Resistance Profile	Genetic Location	Regulation	Clinical Impact
vanA	High-level (MIC ≥64 µg/mL) to **vancomycin & teicoplanin**	Plasmid (Tn1546 transposon)	Inducible (vanR/vanS)	Most prevalent; epidemic spread in hospitals
vanB	Variable (MIC 4–1024 µg/mL) to **vancomycin only** (teicoplanin-sensitive)	Plasmid or chromosome (Tn1549-like)	Inducible (vanRB/vanSB)	Hospital outbreaks; strain-dependent
vanD	Moderate (MIC 64–256 µg/mL) to both drugs	Chromosomal (rarely mobile)	Constitutive	Rare; reported in E. faecium
vanM	High-level (similar to vanA)	Plasmid	Inducible	Emerging in Asia (E. faecium)

Types of *van* genes in VRE

- **1. Low-Level Resistance (Intrinsic)**

Gene	Species	Resistance Level	Mobility	Notes
vanC	E. gallinarum (C1), E. casseliflavus (C2/C3)	Low (MIC 4–32 µg/mL)	Chromosomal (non-transferable)	Natural resistance; less clinically significant
vanE	E. faecalis	Low (MIC 16 µg/mL)	Chromosomal	Rare; D-Ala-D-Ser pathway
vanG	E. faecalis	Low (MIC 12–16 µg/mL)	Chromosomal	Inducible; limited reports

Key Difference:

- *vanA/B/D/M* are **acquired** (horizontal transfer via plasmids/transposons), while *vanC/E/G* are **intrinsic** (species-specific).

Vancomycin-resistant enterococci (VRE)

- Most common types
  - *vanA*
  - *vanB*

Glycopeptide	MIC (mg/L)	
	VanA	VanB
Vancomycin	64-1024	4-1024
Teicoplanin	8-512	0.06-1

Enterococci - identification

- Important to distinguish between different *Enterococcus* species
  - *E. faecium* from *E. gallinarium*
  - *E. faecium* from *E. casseliflavus*

- **Why specie identification matters?**
  - **Clinical Relevance**:
    - *E. faecium* (*vanA/vanB*): High-level resistance, major nosocomial pathogen.
    - *E. gallinarum* & *E. casseliflavus* (*vanC*): Intrinsic low-level resistance, less clinically significant.
  - **Misidentification Risks**:
    - False reporting of *vanC* species as *vanA/B* can lead to unnecessary isolation/infection control measures.

Identification workflow

1. **Screen**: AST, Breakpoint agar, Chromogenic agar (e.g., chromID® VRE) for presumptive VRE.

2. **Confirm**:
    1. MALDI-TOF for species ID.
    2. PCR for *vanA/B* if *E. faecium* is identified.

3. **Supplement**: MGD/motility tests if MALDI-TOF is unavailable.

AST: MIC determination

- Agar dilution
- Broth microdilution

- EUCAST - MIC >4mg/L = VRE
- CLSI – MIC >4mg/L – biochemical tests for identification

- ETEST can be performed using manufacturers guidelines

Disk diffusion testing

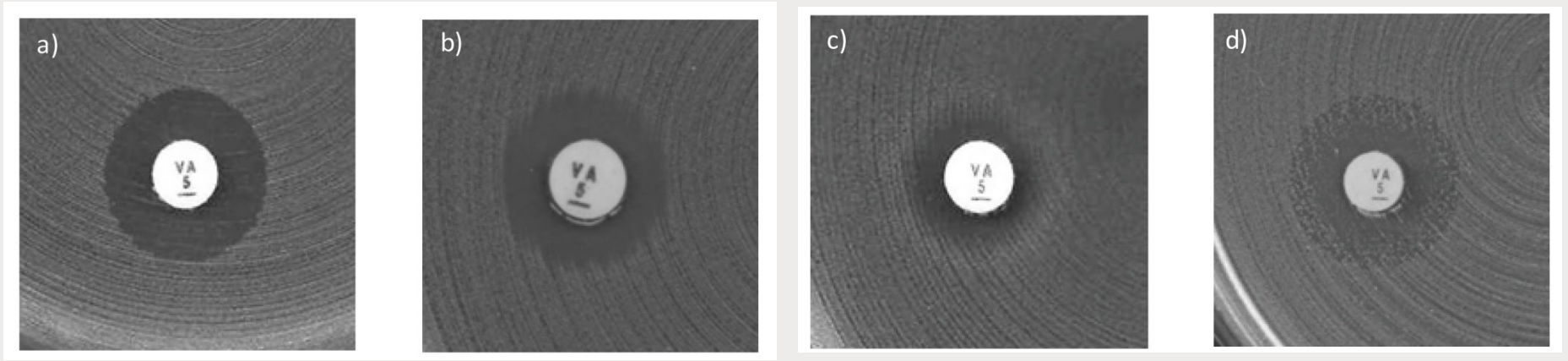
- CLSI - Vancomycin 30µg
- EUCAST – Vancomycin 5µg

- Incubation: 35°C+/-1°C for full 24 hours
- Reading: transmitted light

- Vancomycin <16mm – CLSI
- Vancomycin <12mm - EUCAST

Disk diffusion testing

- **Sharp zones** above breakpoint - **report susceptible**
- **Fuzzy edges** and/or **microcolonies within the zone** regardless the zone size **- report resistant** (only report susceptible after confirmation by MIC determination)



Vancomycin **susceptible**

Vancomycin **resistant**

Vancomycin **resistant**

Vancomycin **resistant**

EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2,0, July 2017

Breakpoint agar

- Brain Heart Infusion agar + **6mg/L vancomycin**
  - 10µl of inoculum standardized using 0,5 McFarland suspension
  - Incubation: 35°C+/-1°C for 24hours
- Growth of one or more colonies = **positive test**
- Commercial alternatives
  - chromID® VRE (bioMérieux)
  - VRE Select (Bio-Rad)
  - Brilliance™ VRE (Oxoid)

EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 1,0, July 2013

Confirmatory tests: Biochemical

- Key biochemical tests:

Test	E. faecium	E. faecalis	E. gallinarum	E. casseliflavus
L-Arabinose Fermentation	– (some +)	–	+	+
Motility	–	–	+	+
PYR (Pyrrolidonyl Arylamidase)	+	+	–	–
MGD Fermentation (Methyl-α-D-glucopyranoside)	–	–	+	–
Pigment Production	–	–	–	**Yellow** (on TSA)

- Arabinose testing is outdated as a standalone method due to poor specificity:
  - Use MALDI-TOF/PCR for definitive ID.
  - If biochemical tests are needed, combine arabinose + motility + MGD + PYR.

Confirmatory tests: Genomic

- Detection of *vanA and vanB* gene by PCR

- In-house or commercial tests

Method	Description	Turnaround Time
PCR (vanA/vanB)	Detects resistance genes directly (e.g., **Xpert® VRE**, **BD Max™**).	**1–4 hours**
Multiplex NAAT	Panels including VRE (e.g., **BioFire® FilmArray**).	**1–2 hours**

Confirmatory tests: WGS

- **Detection of Resistance Mechanisms**
  - Identifies vancomycin resistance genes (vanA, vanB, vanD, vanM).
  - Detects co-resistance (e.g., linezolid (cfr), daptomycin (liaSR mutations)).

- **Outbreak Investigation & Transmission Tracking**
  - SNP (Single Nucleotide Polymorphism) Analysis:
  - Determines if isolates are clonally related (≤20 SNPs = likely outbreak).

- **Plasmid Analysis:**
  - Tracks horizontal gene transfer (e.g., vanA on Tn1546).

Treatment options for VRE

- First line options

Antibiotic	Dose	Key Considerations
Linezolid (PO/IV)	600 mg q12h	Myelosuppression risk
Daptomycin (IV)	6–12 mg/kg q24h	Not for pneumonia (poor lung penetration)
Tigecycline (IV)	100 mg load, then 50 mg q12h	Low serum levels (avoid bacteremia)

- Alternative/Add-On Therapies
  - UTI: Fosfomycin or nitrofurantoin (E. faecalis only).
  - Bacteremia/Endocarditis: High-dose daptomycin + β-lactam (if susceptible).
  - Severe Infections: Combination therapy (e.g., daptomycin + ceftaroline).

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