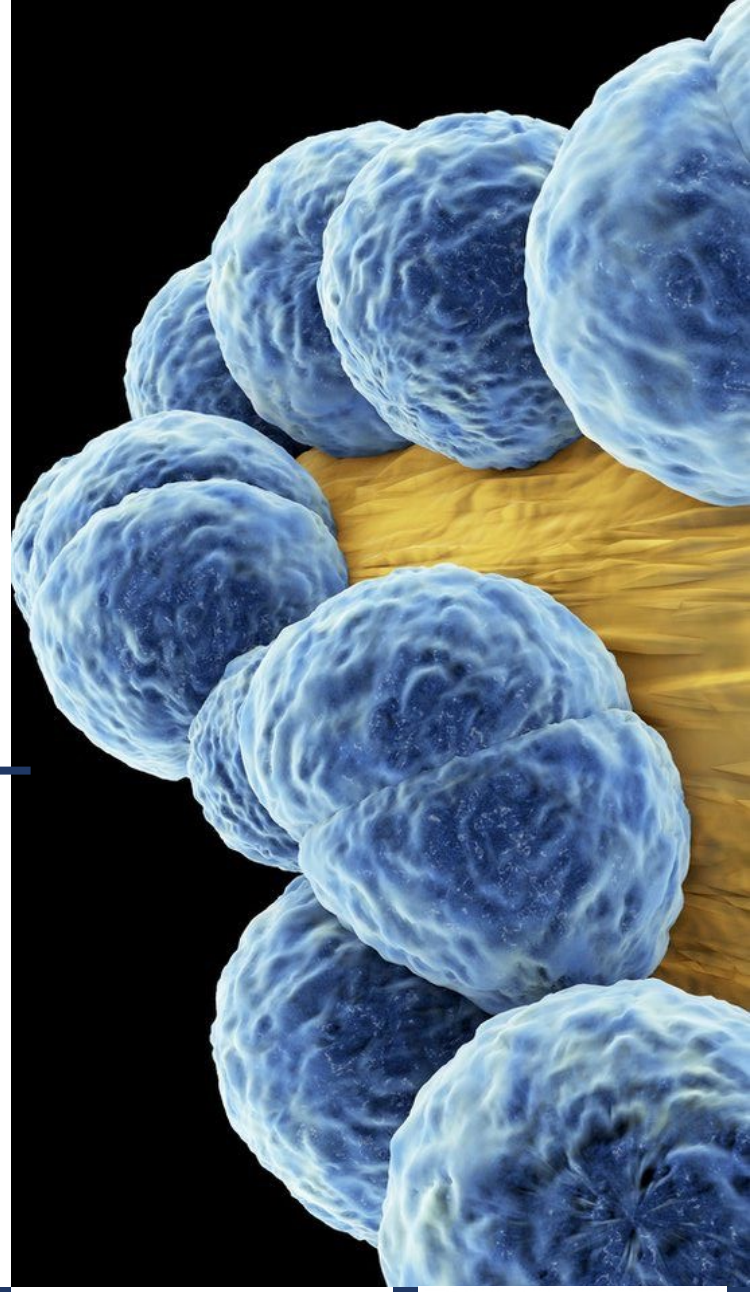
Multi-Drug Resistance Organisms (MDRO): Gram positive bacteria



Lorena Diaz, PhD

Profesor Asociado, Laboratorio de Genómica y Resistencia Microbiana (GeRM), ICIM, Facultad de Medicina, Clínica Alemana - Universidad del Desarrollo

- El presente material médico-científico tiene fines educativos, está dirigido exclusivamente a profesionales de la salud.

- Los conceptos emitidos aquí son responsabilidad del autor y no necesariamente representan las opiniones y recomendaciones de Pfizer.

- Presentación patrocinada por auspiciadores del evento.

Outline

Staphylococcus aureus

- B-lactams Resistance
  - Methicillin/Oxacillin resistance
  - Cefazoline High inoculum effect
- Vancomycin resistance
  - VRSA
  - VISA and hVISA

***Enterococcus* spp.**

- B-lactams Resistance
  - Penicillin resistant Ampicillin susceptible *E. faecalis* (PRASEF)
  - Ampicillin resistance
- Vancomycin Resistance (VRE)

Opinión de experto

AMR mechanisms in Gram positive bacteria

Cell envelope synthesis (membrane and cell wall)

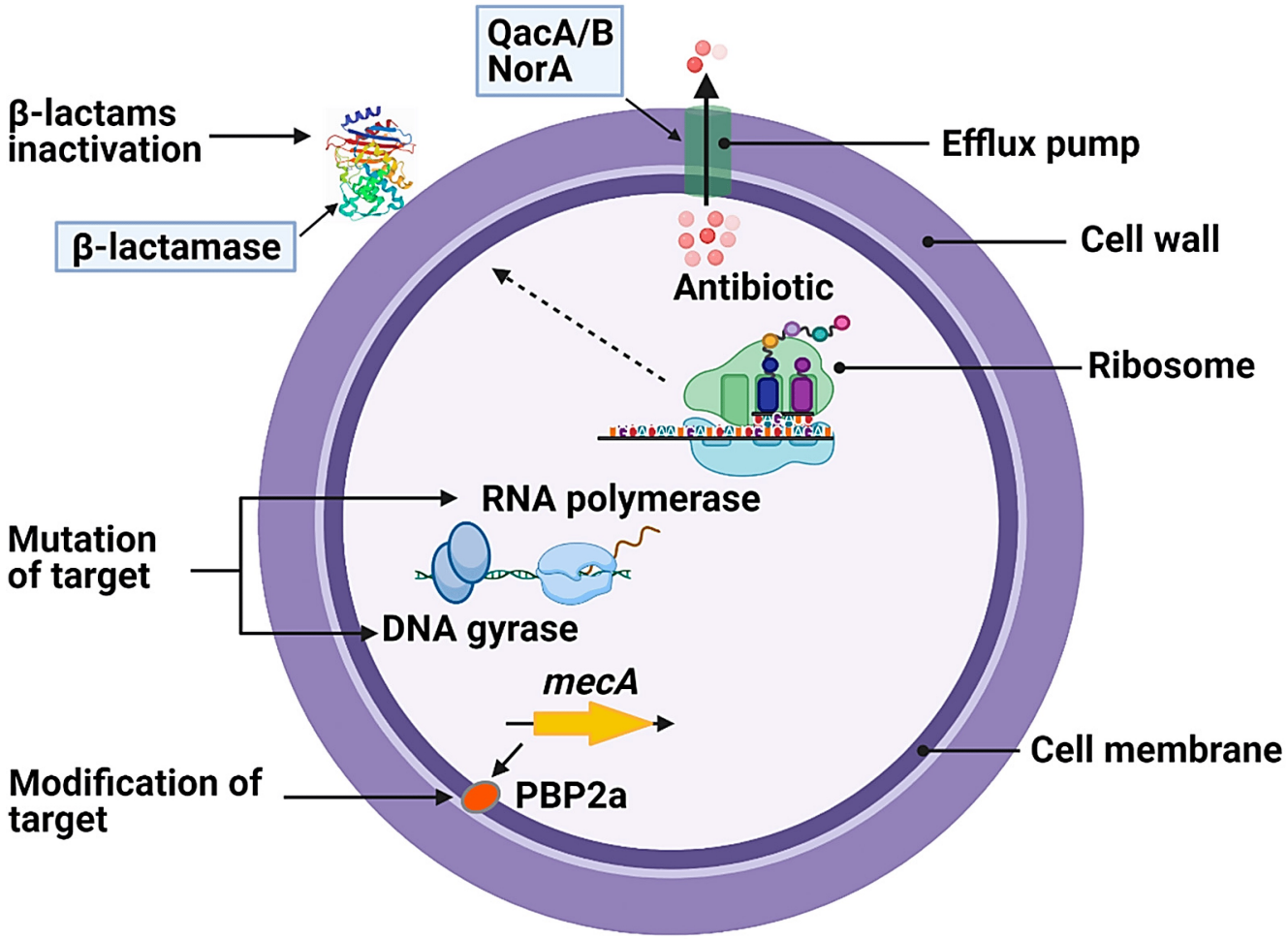
- B-lactms, glycopeptides, lipopeptides

Protein synthesis

- Ribosome large subunit: macrolides, lincosamides, streptogramins, oxazolidinones, phenicols
- Ribosome small subunit: tetracyclines, aminoglycosides

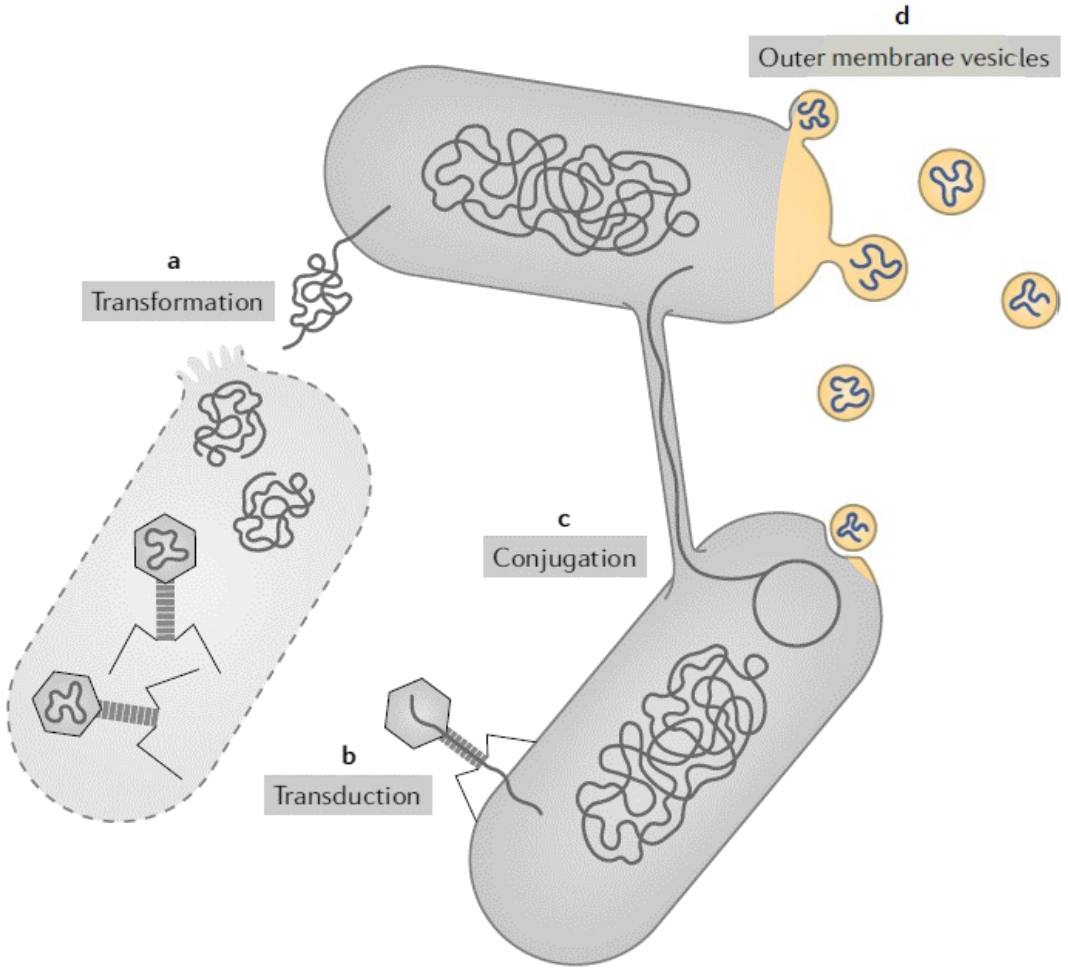
Nucleic acid synthesis:

- DNA: quinolones
- RNA: rifamicins



Lade H, et al. Bacterial Targets of Antibiotics in Methicillin-Resistant *Staphylococcus aureus*. Antibiotics (Basel). 2021 Apr 7;10(4):398.

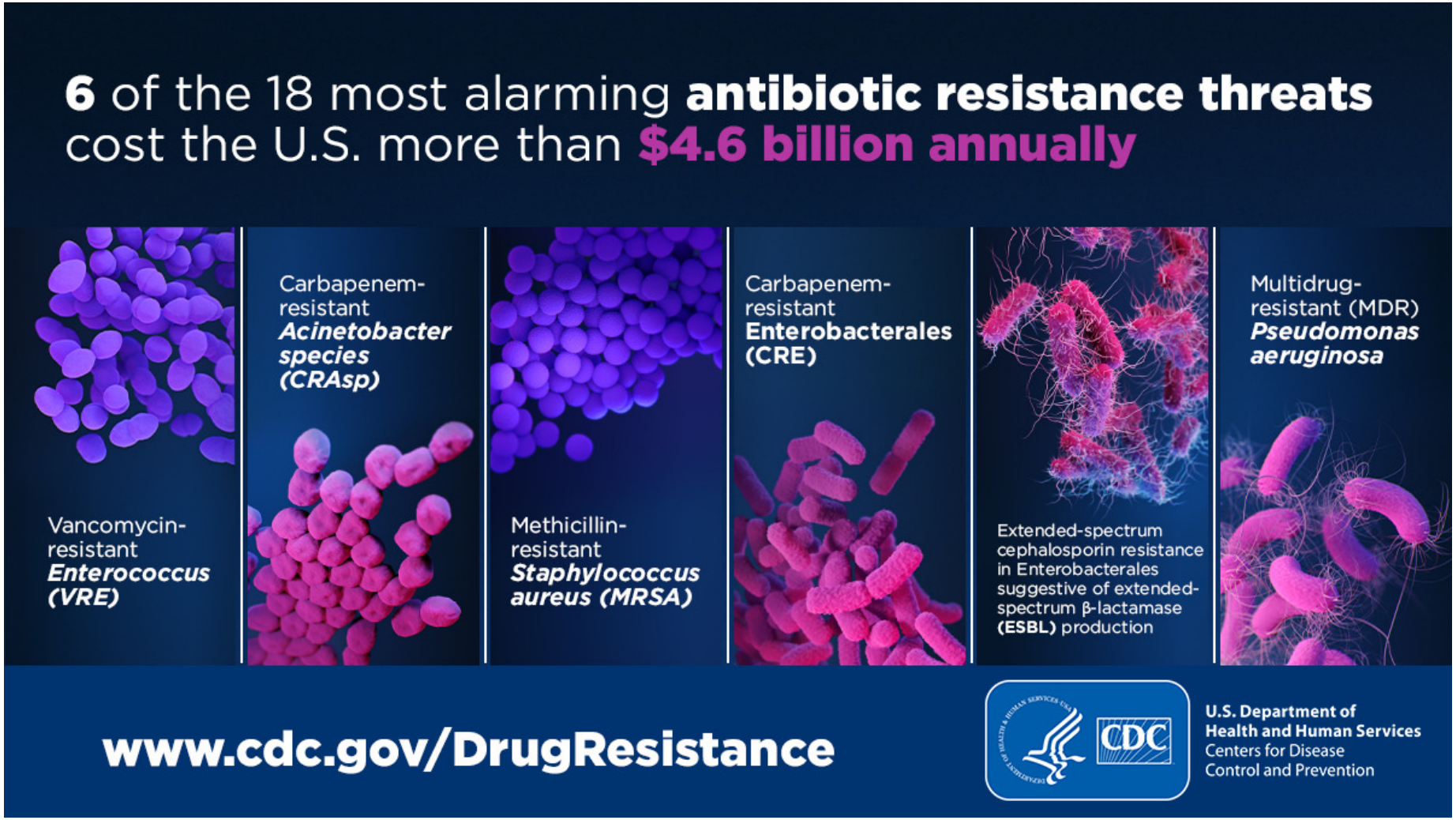
AMR transferability



Courtesy Ana M. Gonzalez PhDc

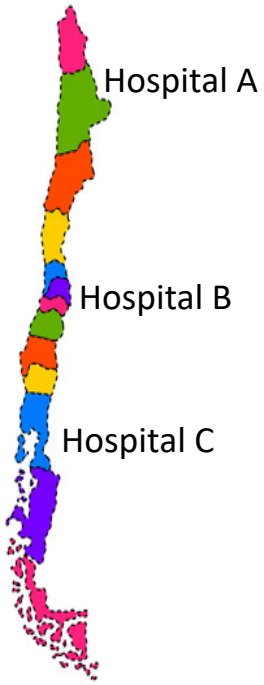
1. Tran F, et al. Genetic cargo and bacterial species set the rate of vesicle-mediated horizontal gene transfer. Sci Rep. 2017 Aug 18;7(1):8813; **2.** Brito IL. Examining horizontal gene transfer in microbial communities. Nat Rev Microbiol. 2021 Jul;19(7):442-453.

Most alarming MDRO

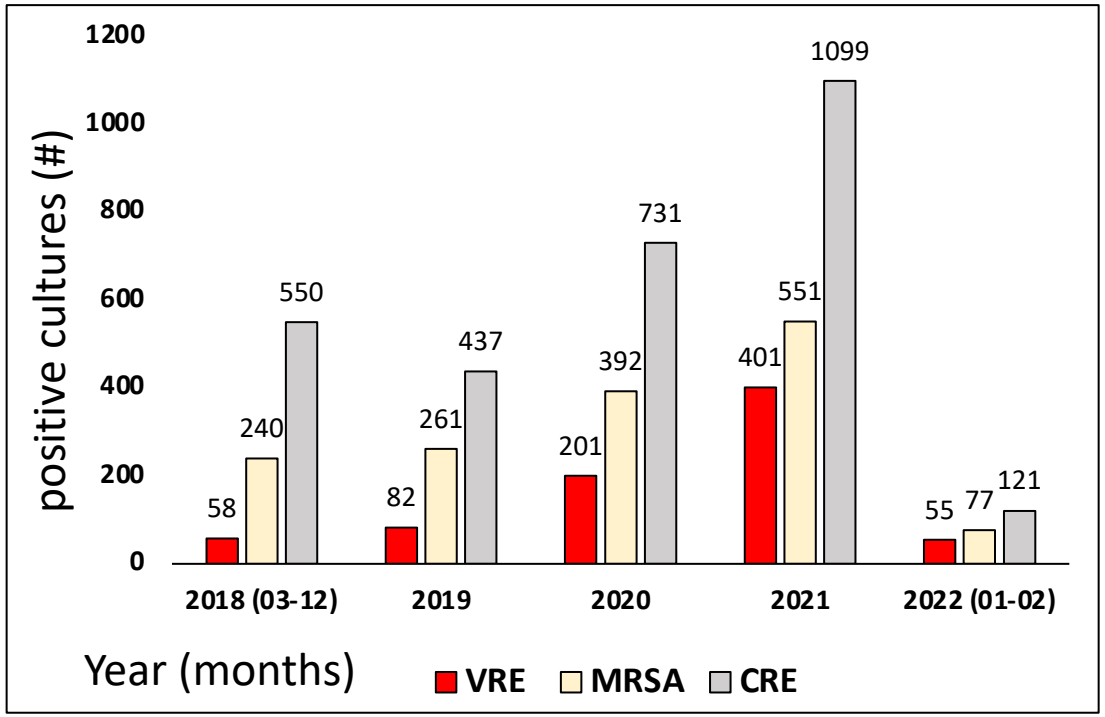


Centers for Disease Control and Prevention (CDC). Drug Resistance. CDC website. Accessed October 19, 2023. Disponible en: www.cdc.gov/drugresistance

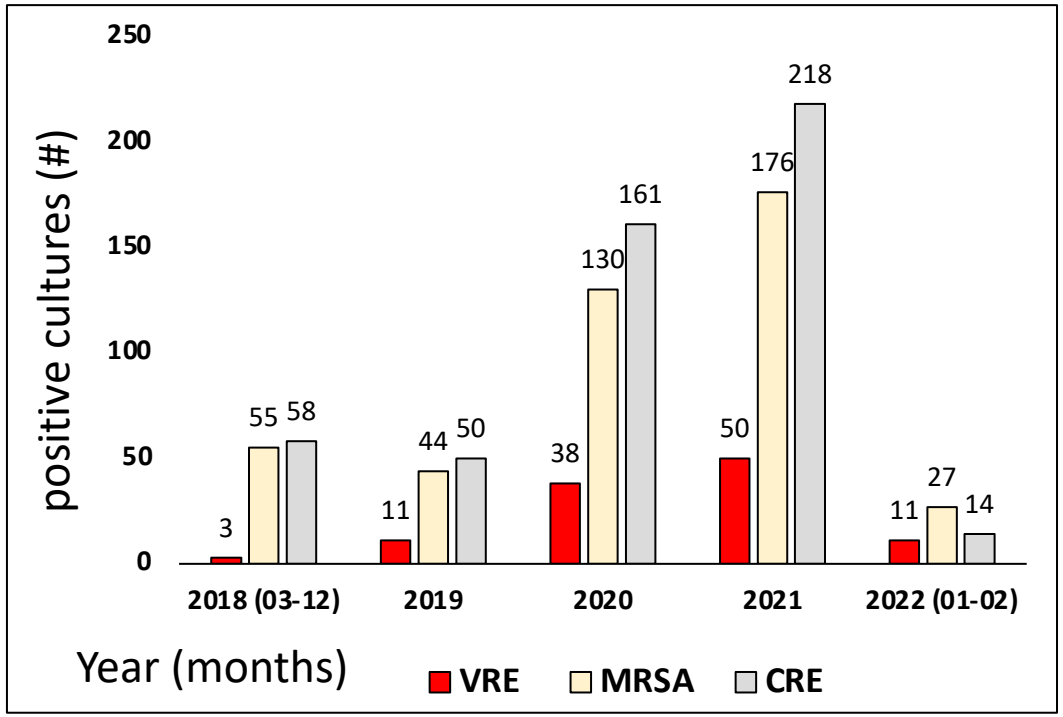
Most alarming MDRO in Chile



All cultures



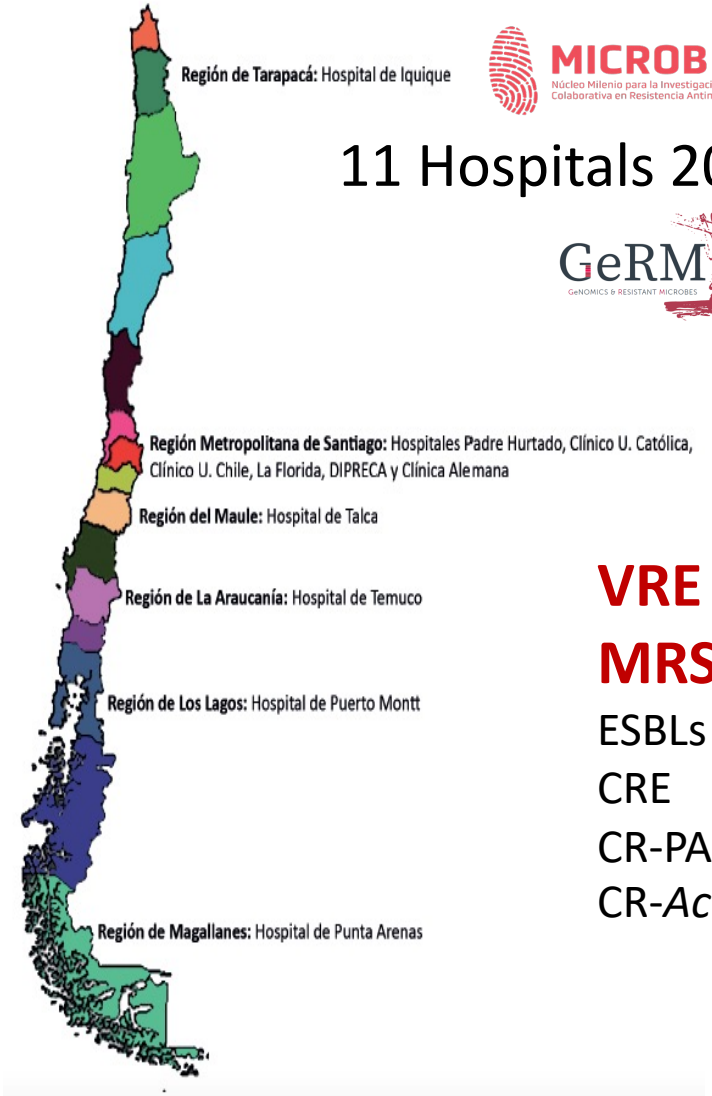
Blood cultures



This report is based on databases including the extraction of all positive cultures processed in three tertiary hospitals in Chile from March 2018 until February 2022. Data on file

Antibiotic resistance data in Chile

- 2012 – 2020
- One isolate per patient
- Resistance: Clinical Laboratory Standards Institute (CLSI), EUCAST (European Committee on Antimicrobial Susceptibility Testing).



MICROB-R
Núcleo Milenio para la Investigación Colaborativa en Resistencia Antimicrobiana

11 Hospitals 2018 - 2023

GeRM
GeNOMICS & RESISTANT MICROBES

VRE

MRSA

ESBLs

CRE

CR-PAE

CR-*Acinetobacter*

Staphylococcus aureus resistance

- B-lactams Resistance
  - Methicillin/Oxacillin resistance
  - Cefazoline High inoculum effect
- Vancomycin resistance
  - VRSA
  - VISA and hVISA

Methicillin Resistant *S. aureus* (MRSA)

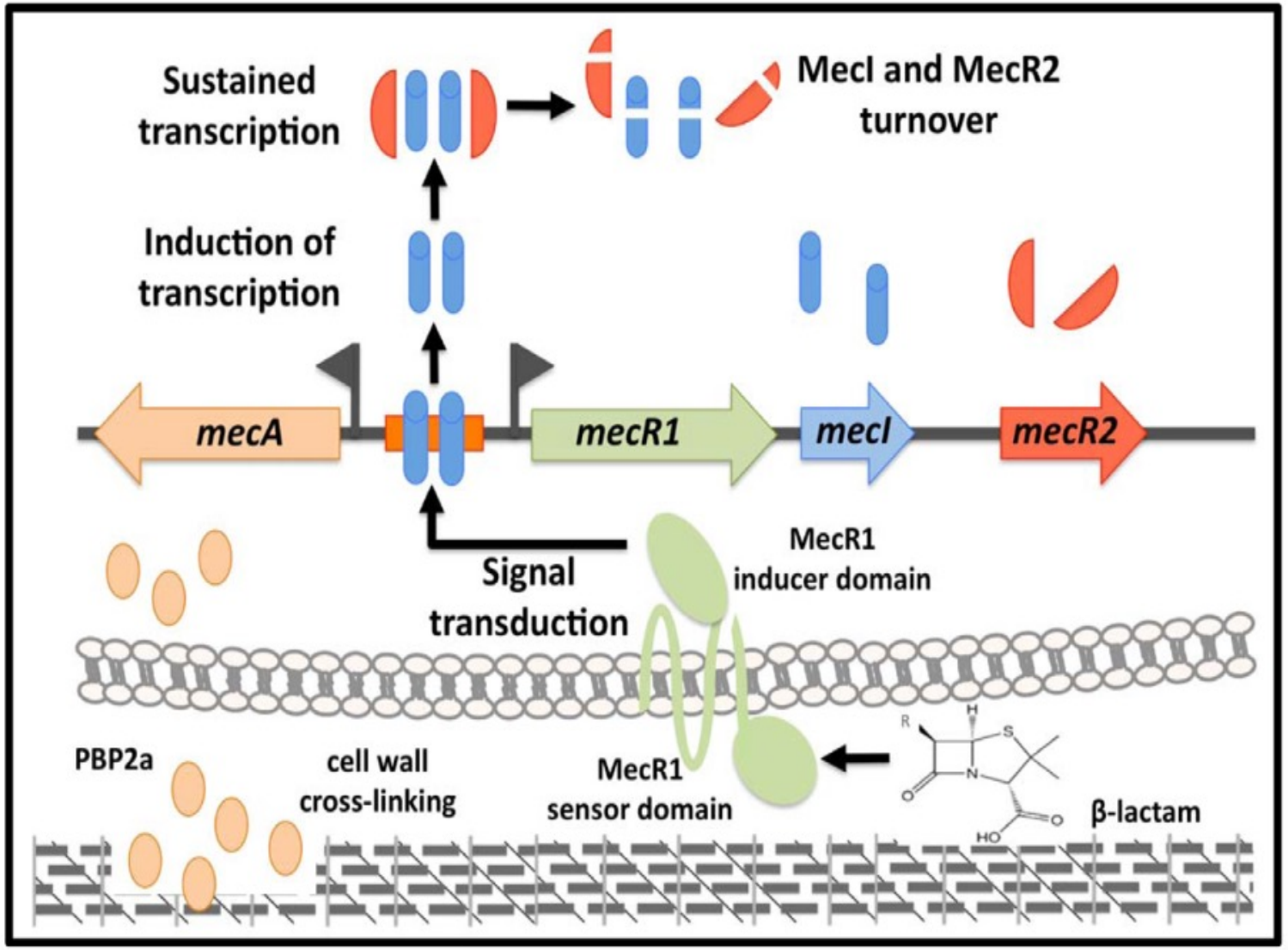
- MRSA is resistant to all B-lactams but susceptible to Ceftaroline and Ceftobiprole
- Oxacillin resistance in Chile 2012 – 2020: **36 – 55 %**

Mechanism

Overexpression of BlaZ

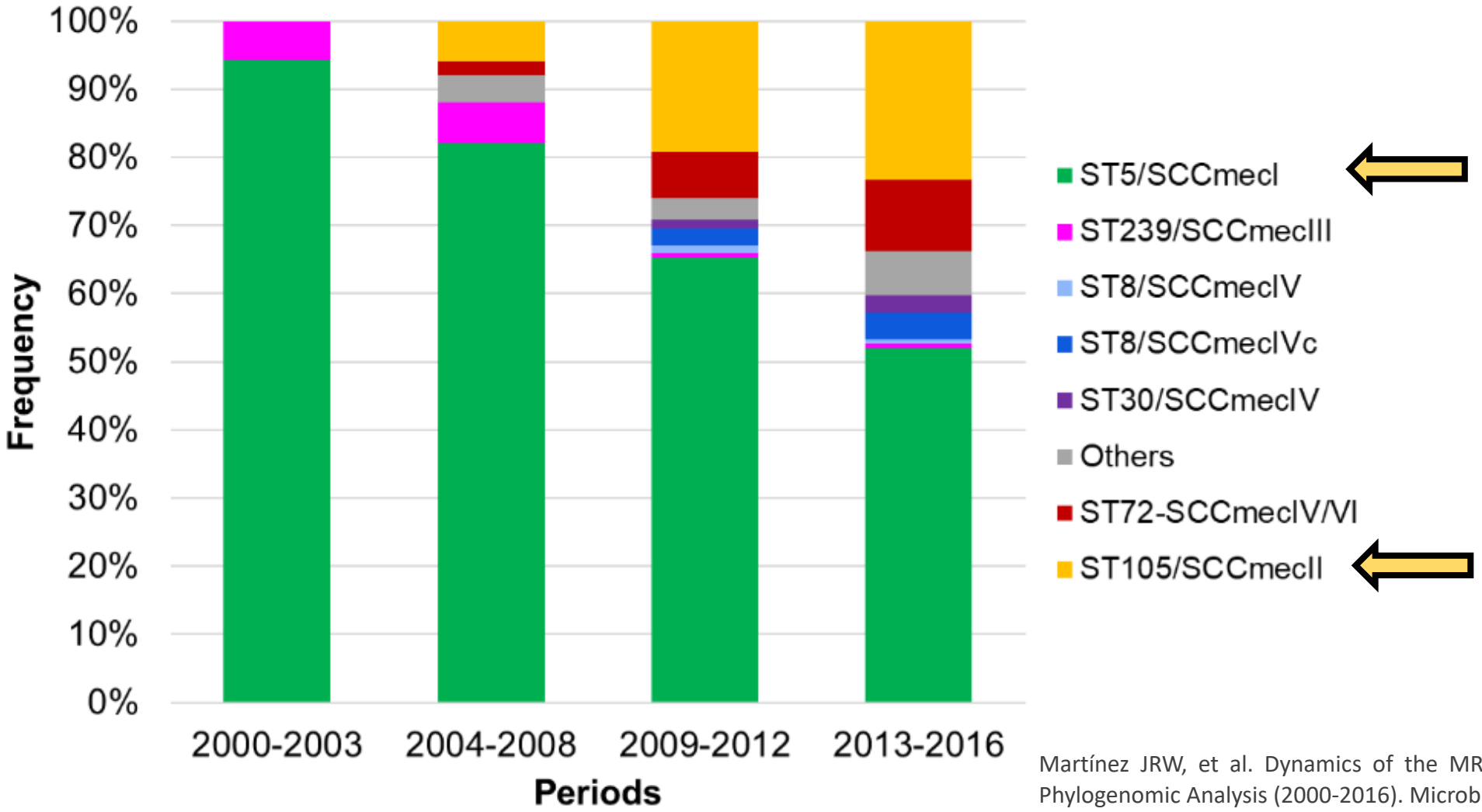
Acquisition of *mecA* → PBP2a

mecA and regulatory genes are found in SCC*mec*



1. Arêde P, el al. The anti-repressor MecR2 promotes the proteolysis of the mecA repressor and enables optimal expression of β-lactam resistance in MRSA. PLoS Pathog. 2012;8(7):e1002816;
2. Saber H, et al. A Review of Staphylococcal Cassette Chromosome *mec* (SCC*mec*) Types in Coagulase-Negative Staphylococci (CoNS) Species. Malays J Med Sci. 2017 Oct;24(5):7-18.

Methicillin Resistant *S. aureus* (MRSA) in Chile



Martínez JRW, et al. Dynamics of the MRSA Population in a Chilean Hospital: a Phylogenomic Analysis (2000-2016). Microbiol Spectr. 2023 Aug 17;11(4):e0535122.

Staphylococcus aureus resistance

- B-lactams Resistance
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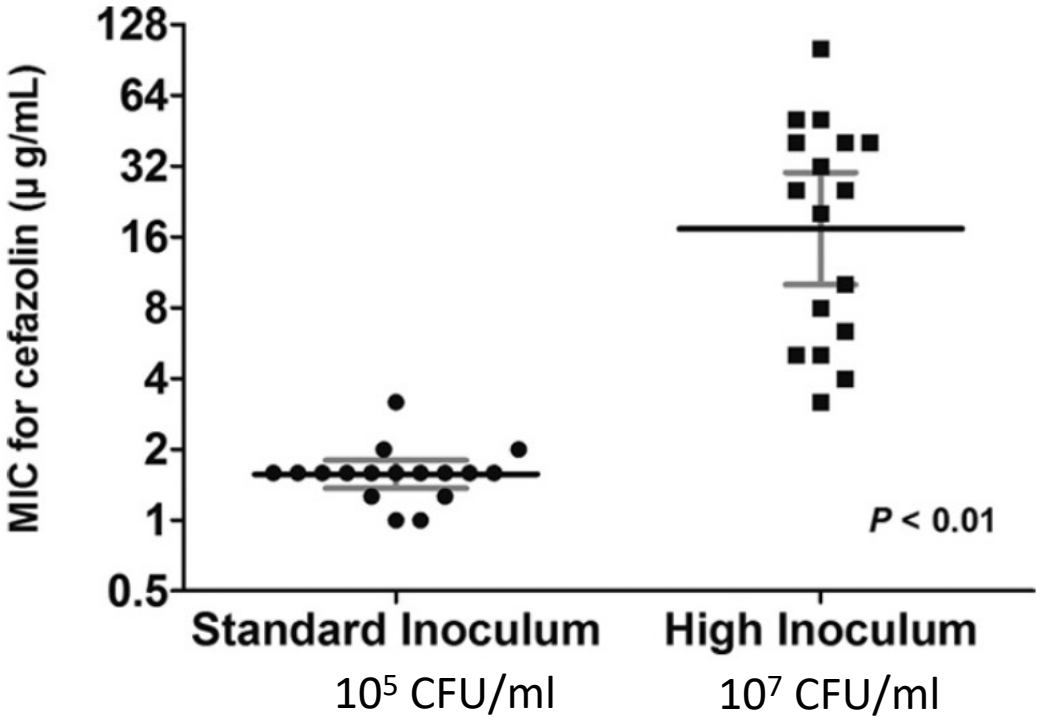
Cefazolin High Inoculum (CzIE) Effect in MSSA

- Infections caused by MSSA are more frequently reported than those caused by MRSA
- The IDSA recommends B-lactams as the cornerstone of therapy for infections caused by MSSA: **Cefazolin**.
- CzIEimits the therapeutic efficacy of cefazolin in deep-seated MSSA infections.
- Frequency of CzIE:
  - 3 to 20% of MSSA isolates from Asia and the United States.
  - Up to **30 to 54% in MSSA isolates from Latin America**.

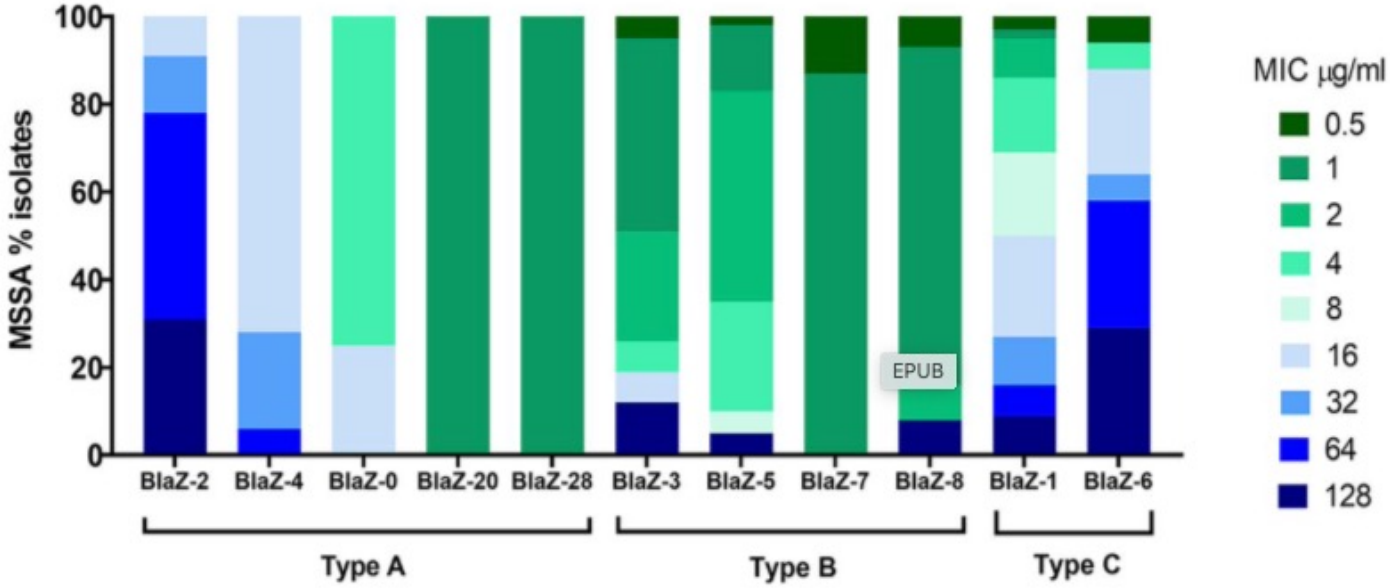


CzIE: A marked increase in the MIC to cefazolin when using a high bacterial inoculum, instead of the standard.

Loubet P, *et al*. Clin Microbiol Infect24:125–132.
Li J, *et al*. Pharmacotherapy 37:346–360.
Miller WR *et al.* Open Forum Infect Dis. 2018 May 23;5(6):ofy123.
Rincon S, *et al*. J Antimicrob Chemother 2013. 68:2773–2778.
Lee SH *et al*. Antimicrob Agents Chemother. 2016 Oct 21;60(11):6928-6932

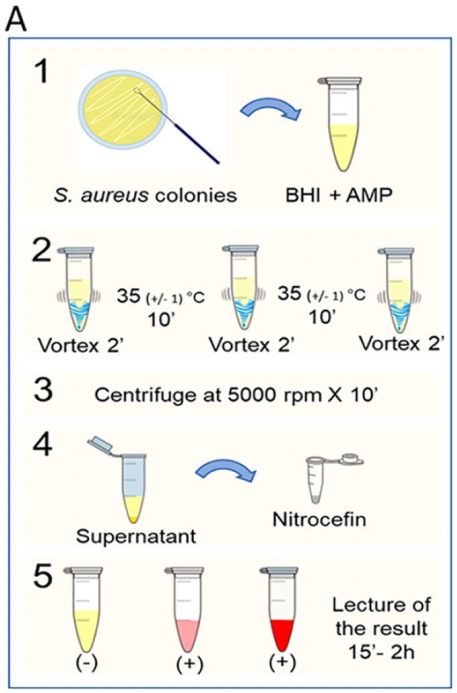
Cefazolin High Inoculum Effect in MSSA

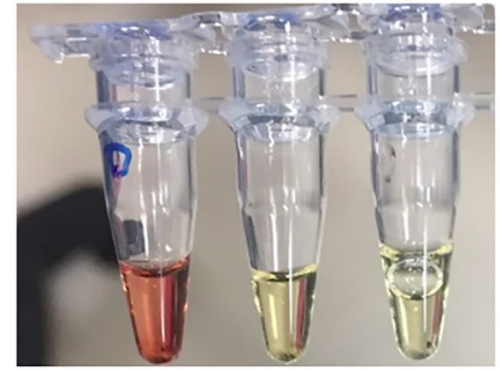
CzIE is mediated by BlaZ



Cefazolin MIC values at high inoculum in relevant allotypes of BlaZ. Distribution of cefazolin MIC values at high inoculum in relevant allotypes of BlaZ Type A, Type B, and Type C.

Nitrofecin-based rapid test for the detection of CzIE





1. Carvajal LP, et al. Novel Insights into the Classification of Staphylococcal β-Lactamases in Relation to the Cefazolin Inoculum Effect. Antimicrob Agents Chemother. 2020 Apr 21;64(5):e02511-19;
2. Rincón S, et al. A Test for the Rapid Detection of the Cefazolin Inoculum Effect in Methicillin-Susceptible Staphylococcus aureus. J Clin Microbiol. 2021 Mar 19;59(4):e01938-20.

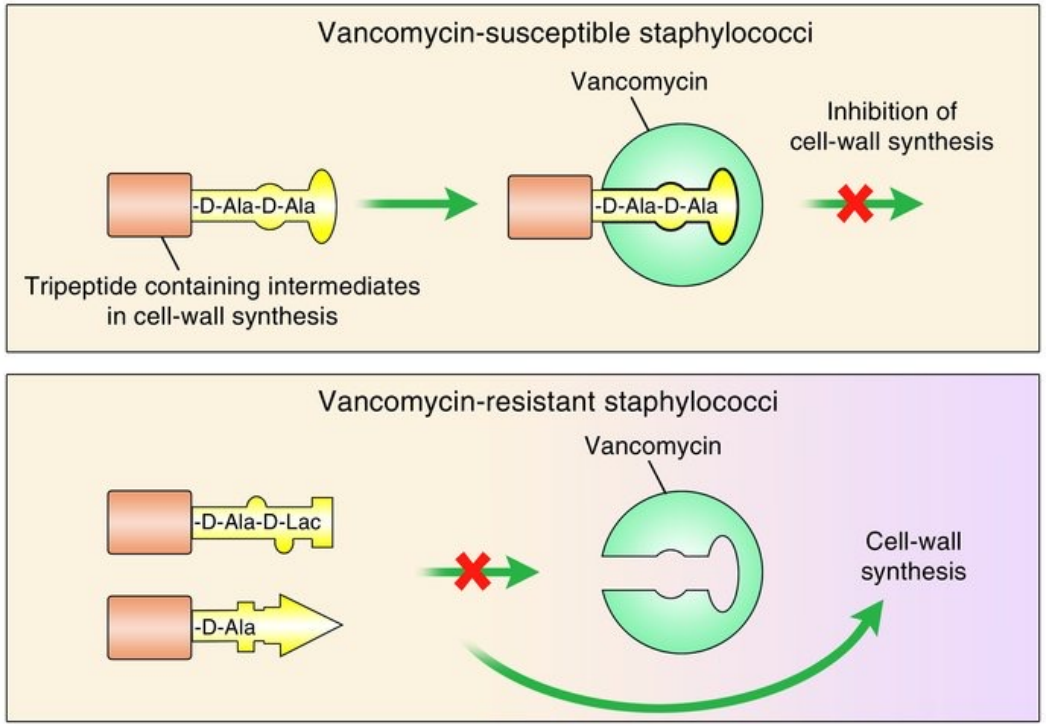
Staphylococcus aureus resistance

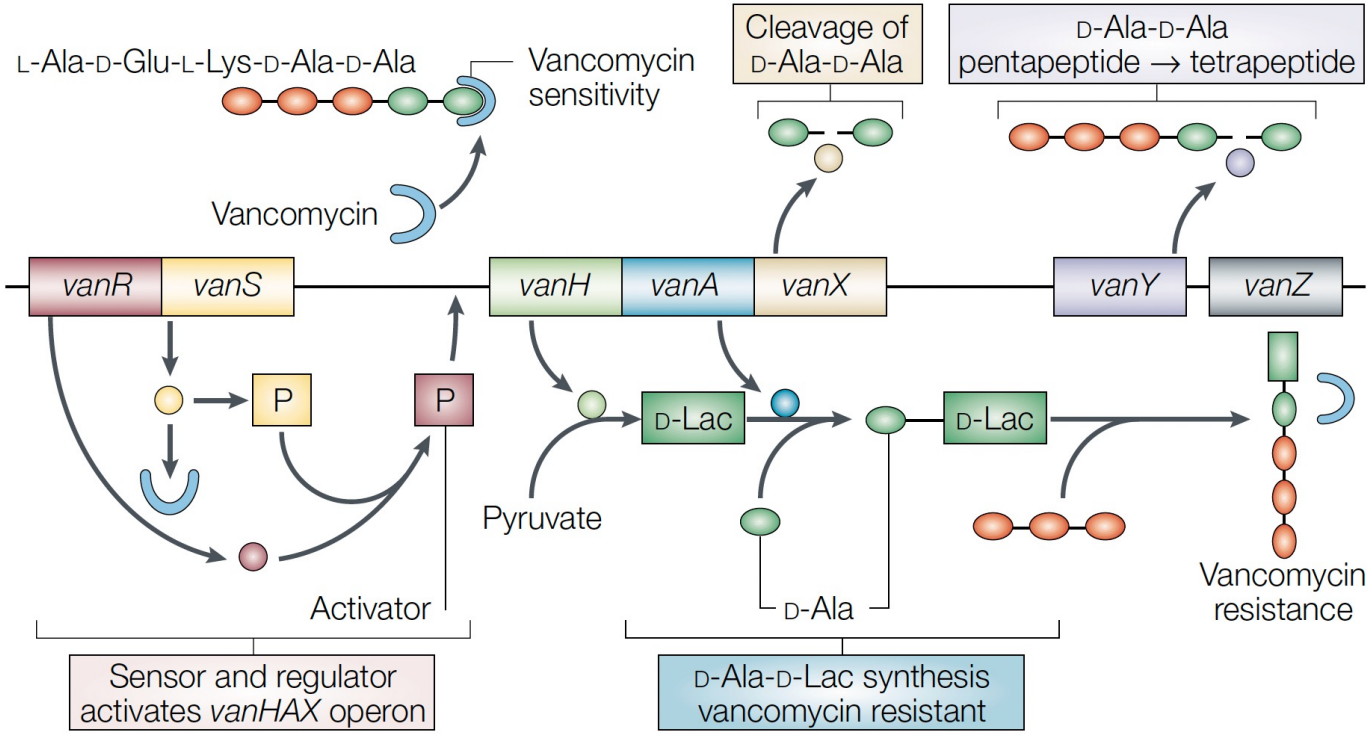
- B-lactams Resistance
  - Methicillin/Oxacillin resistance
  - Cefazoline High inoculum effect
- Vancomycin resistance
  - VRSA
  - VISA and hVISA

Vancomycin Resistant *S. aureus* (VRSA)

Synthesis of a cell wall precursor that ends in D-Ala-D-Lac dipep-tide rather than D-Ala-D-Ala.
The novel cell wall precursor is synthesized, allowing continued peptido- glycan assembly

VanA cluster of genes has been acquired from *E. faecalis*





1. Lowy FD. Antimicrobial resistance: the example of Staphylococcus aureus. J Clin Invest. 2003 May;111(9):1265-73;
2. Hughes D. Exploiting genomics, genetics and chemistry to combat antibiotic resistance. Nat Rev Genet. 2003 Jun;4(6):432-41.

Vancomycin Resistant *S. aureus* (VRSA)

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Transferable Vancomycin Resistance in a Community-Associated MRSA Lineage

Flávia Rossi, M.D., Ph.D., Lorena Diaz, Ph.D., Aye Wollam, B.Sc., Diana Panesso, Ph.D., Yanjiao Zhou, Ph.D., Sandra Rincon, M.Sc., Apurva Narechania, M.A., Galen Xing, Thais S.R. Di Gioia, M.D., André Doi, M.D., Truc T. Tran, Pharm.D., Jinnethe Reyes, M.Sc., Jose M. Munita, M.D., Lina P. Carvajal, B.Sc., Alejandra Hernandez-Roldan, M.Sc., Denise Brandão, M.D., Inneke Marie van der Heijden, Ph.D., Barbara E. Murray, M.D., Paul J. Planet, M.D., Ph.D., George M. Weinstock, Ph.D., and Cesar A. Arias, M.D., Ph.D.

C BR-VRSA Plasmid pBRZ01

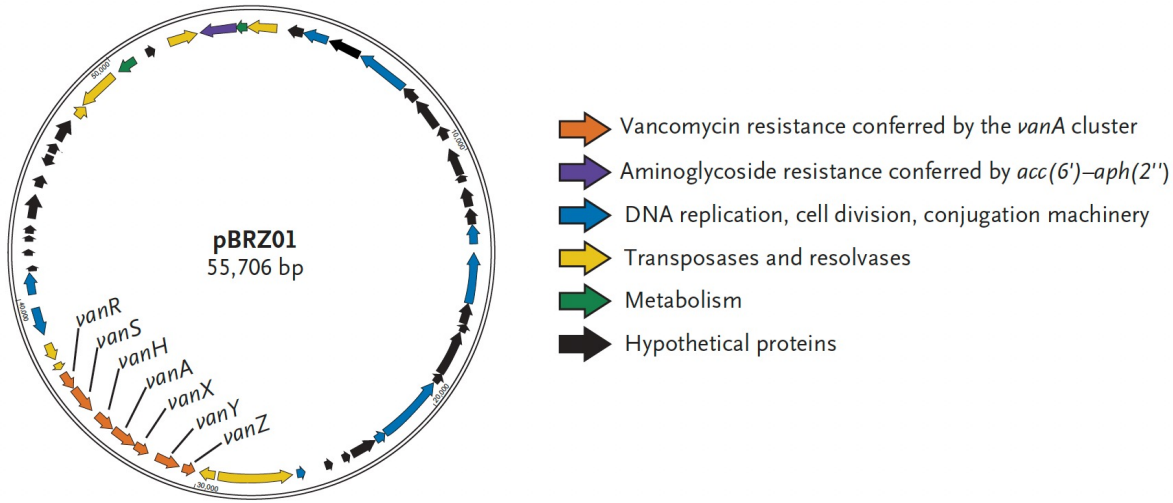


Table 1. *Staphylococcus aureus* strains used in analysis of methicillin and vancomycin resistance, Brazil*

Strain	Strain characteristics	MIC, μg/mL		Reference
		Vancomycin	Gentamicin	
VS-MRSA	Isolated from the bloodstream of a patient in Brazil	0.5	0.5	(*1*)
VR-MRSA	Isolated from the blood of the same patient above and carrying *vanA*-containing pBRZ01	>256	32	(*1*)
VS-MSSA	Isolated from the blood of the same patient 13 d after isolation of VR-MRSA	1	0.75	This study
VR-MSSA	Isolated from the same blood culture as VS-MSSA	256	48	This study

35-year-old man from São Paulo with mycosis fungoides, cocaine addiction, and diabetes mellitus. MRSA Bloodstream infection

1. Panesso D, et al. Methicillin-Susceptible, Vancomycin-Resistant Staphylococcus aureus, Brazil. Emerg Infect Dis. 2015 Oct;21(10):1844-8; **2.** Rossi F. Transferable vancomycin resistance in a community-associated MRSA lineage. N Engl J Med. 2014 Apr 17;370(16):1524-31.

Staphylococcus aureus resistance

- B-lactams Resistance
  - Methicillin/Oxacillin resistance
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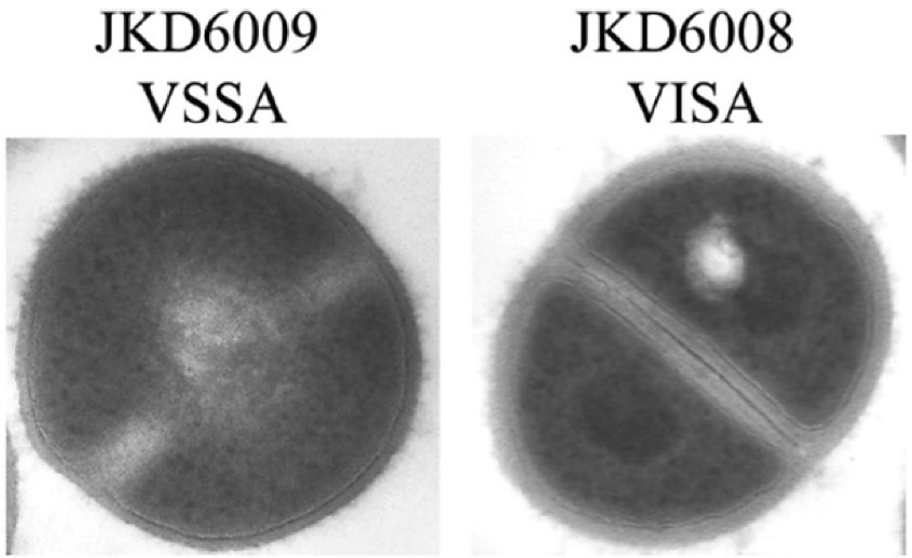
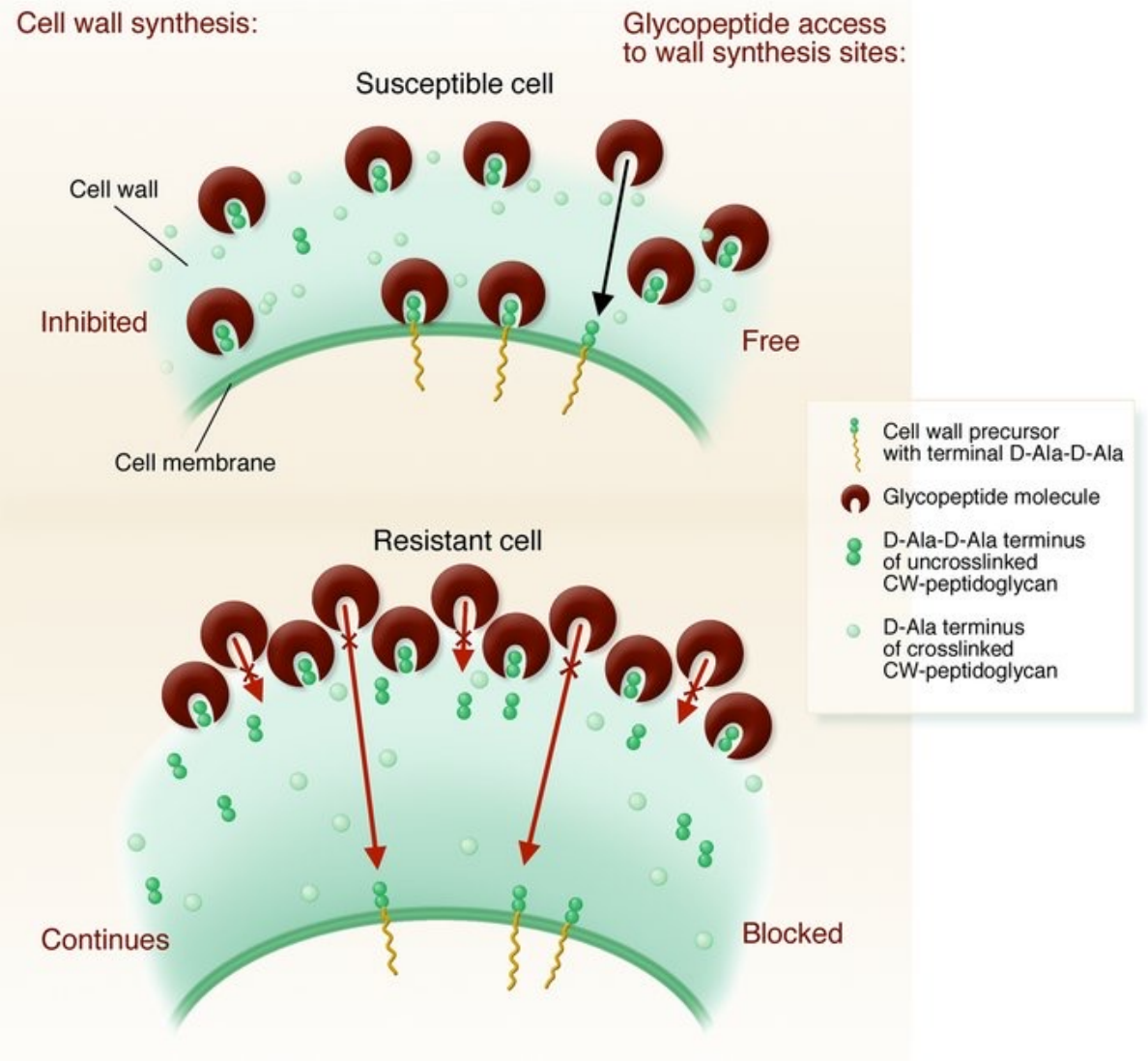
Vancomycin Intermediate *S. aureus* (VISA)

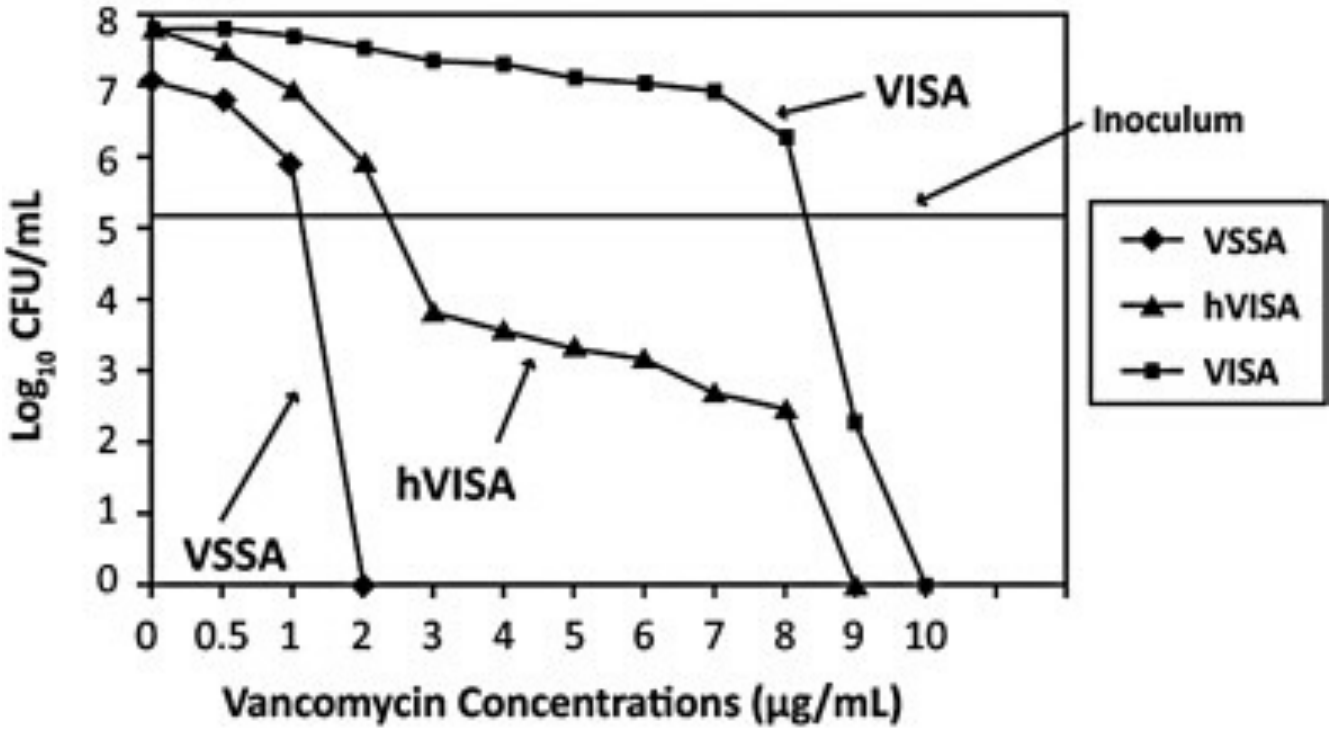




1. Howden BP, et al. Reduced vancomycin susceptibility in Staphylococcus aureus, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. Clin Microbiol Rev. 2010 Jan;23(1):99-139; **2.** Lowy FD. Antimicrobial resistance: the example of Staphylococcus aureus. J Clin Invest. 2003 May;111(9):1265-73.

Hetero- Vancomycin Intermediate *S. aureus* (hVISA)

Population anaysis profile (PAP)



Howden BP, et al. Clin Microbiol Rev2010; 23: 99–139.

- MIC within the **susceptible** range (≤2 mg/L)
- hVISA strains contain subpopulations with decreased susceptibility to vancomycin
- Not detected by routine susceptibility methods
- hVISA strains are associated with vancomycin therapeutic failure and persistent infections.

Kim T, et al. Eur J Clin Microbiol Infect Dis 2017; 36: 1473–81
Yang CC, et al. Sci Rep 2018; 8: 1–7
Howden BP, et al. Antimicrob Agents Chemother 2006; 50: 3039–47

Hetero- Vancomycin Intermediate *S. aureus* (hVISA)

hVISA in Latin America

GRD test

Country	2006–08[a] MRSA (*n*)	2006–08[a] hVISA, *n* (%)	2011–14[a] MRSA (*n*)	2011–14[a] hVISA, *n* (%)
Brazil	—	—	126	1 (0.8)
Peru	178	6 (3.4)	84	16 (19.0)
Chile	—	—	74	9 (12.2)
Guatemala	—	—	74	ND
Argentina	—	—	60	3 (5.0)
Colombia	318	2 (0.6)	41	ND
Venezuela	69	—	33	ND
Ecuador	86	1 (1.2)	29	1 (3.4)
Mexico	—	—	17	ND
Total	651	9 (1.4)	538	30 (5.6)

ND, not determined.
[a]Period of time of multicentre study surveillance.

Castro BE, et al. Detection of heterogeneous vancomycin intermediate resistance in MRSA isolates from Latin America. J Antimicrob Chemother. 2020 Sep 1;75(9):2424-2431.

Enterococcus spp. Resistance

- *Enterococcus faecalis*
  - Penicillin resistant Ampicillin susceptible *E. faecalis* (PRASEF)
  - Ampicillin resistance
- Vancomycin Resistance

Penicillin-R Ampicillin-S *E. faecalis* (PRASEF)

- Very unusual resistance phenotype, have been reported in various countries
- The CLSI and EUCAST guidelines state that the susceptibility to ampicillin may predict susceptibility to amoxicillin, piperacillin, and imipenem for *E. faecalis*
- Studies have demonstrated that this rule may not be applicable to the penicillin resistant isolates.

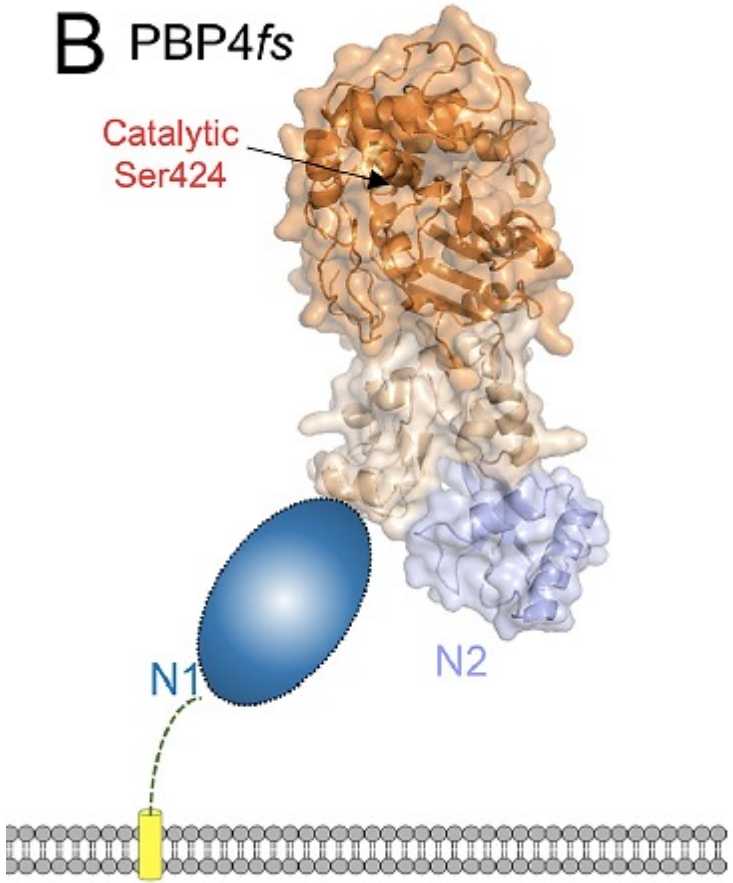
Ampicillin can predict *in vitro* susceptibility to amoxicillin but not to imipenem and piperacillin

1. Infante VHP, et al. Effect of sonic stimulation on Bacillus endospore germination. FEMS Microbiol Lett. 2016;363(7):fnv217; **2.** Conceição N, et al. Beta-lactams susceptibility testing of penicillin-resistant, ampicillin-susceptible Enterococcus faecalis isolates: a comparative assessment of Etest and disk diffusion methods against broth dilution. J Clin Microbiol. 2012 Nov;50(11):3729-31.

Enterococcus spp. Resistance

- *Enterococcus faecalis*
  - Penicillin resistant Ampicillin susceptible *E. faecalis* (PRASEF)
  - Ampicillin resistance
- Vancomycin Resistance

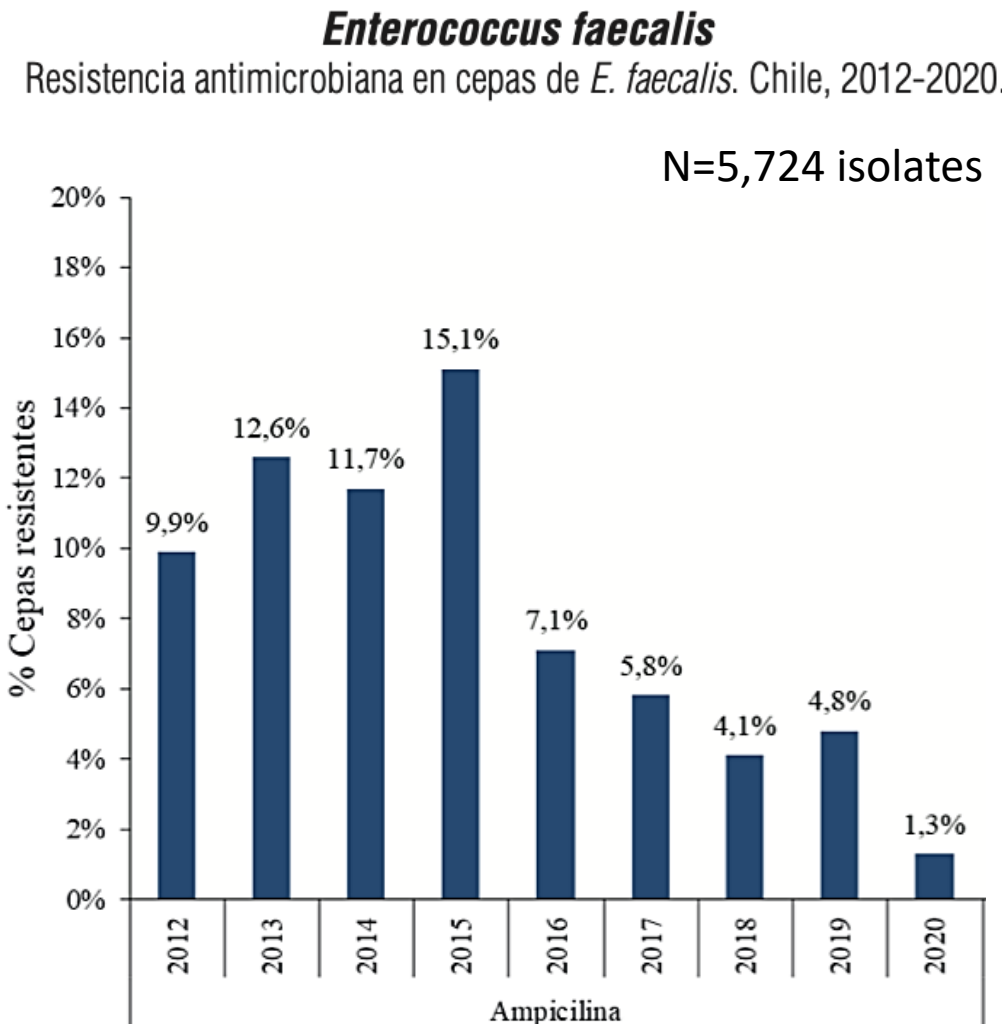
Ampicillin resistance in *E. faecalis*



- B-lactamase production
- Alterations in PBP4
  - Alterations in PBP4 gene promoter causing over expression of PBP4
  - Alterations in PBP4 protein sequence affecting

1. Moon TM, et al. Structural and functional analysis of the Staphylococcus aureus virulence factor Sbi with broad specificity for immunoglobulin domains. J Biol Chem. 2018 Nov 30;293(48):18574-18584;
2. Rice LB, et al. Structural and Regulatory Changes in PBP4 Trigger Decreased β-Lactam Susceptibility in Enterococcus faecalis. mBio. 2018 Apr 3;9(2):e00361-18.

Ampicillin resistance in *E. faecalis*



MICs in VRE *faecalis* from GeRM collection

SCL	AMP	PEN	IMI	PIP	CRO	VAN	TEI	LNZ	DAP
10298	8	16	8	64	>16384	>256	>64	0.75	1,5
11142	8	16	8	256	>16384	>256	64	1	0,75
12003	8	16	8	256	>16384	>256	>64	1.5	1
12774	4	16	8	128	>16384	>256	>64	2	0,064
12776	4	16	8	128	>16384	>256	>64	1.5	0,19
12785	4	16	8	128	>16384	>256	>64	1.5	0,5
12812	4	16	8	256	>16384	>256	>64	1.5	0,25
12838	4	16	8	256	>16384	>256	>64	2	0, 094
12886	8	16	8	128-256	>16384	>256	>64	1.5	0,25
13695	8	16	8	64	>16384	>256	>64	0.5	0,19
15927	8	16	8	256	>16384	>256	>64	0.75	1
15946	8	16	8	256	>16384	>256	>64	1	0,094
15967	8	16	8	256	>16384	>256	>64	1	0,19
15987	8	32	8	256	>16384	>256	>64	1	1
16003	8	16	8	256	>16384	>256	>64	2	
16131	8	32	8	256	>16384	>256	>64	2	0,125
16138	8	32	8	256	>16384	>256	>64	2	1
16196	4	16	4	128	>16384	>256	>64	0.5	1
17502	4	16	8	64	>16384	>256	64	2	1,5
17504	8	16	8	128	>16384	>256	>64	2	0,5
17506	4	16	8	64	>16384	>256	64	2	0,5

Enterococcus spp. Resistance

- *Enterococcus faecalis*
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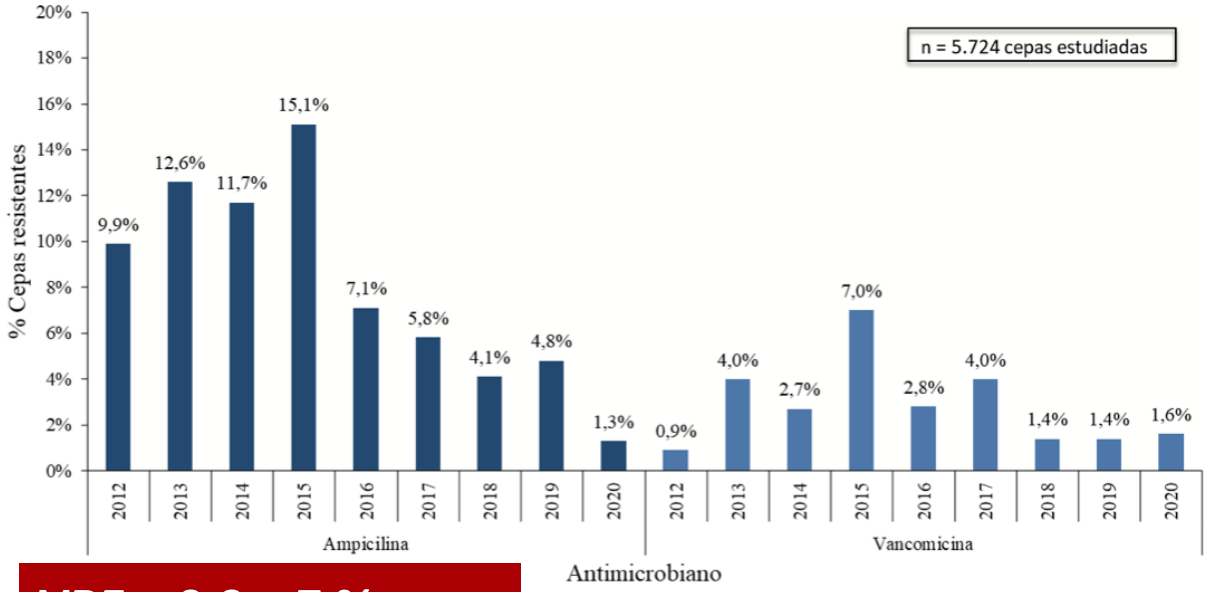
  - Ampicillin resistance
- Vancomycin Resistance

Vancomycin Resistance Enterococci (VRE)

Enterococcus faecalis

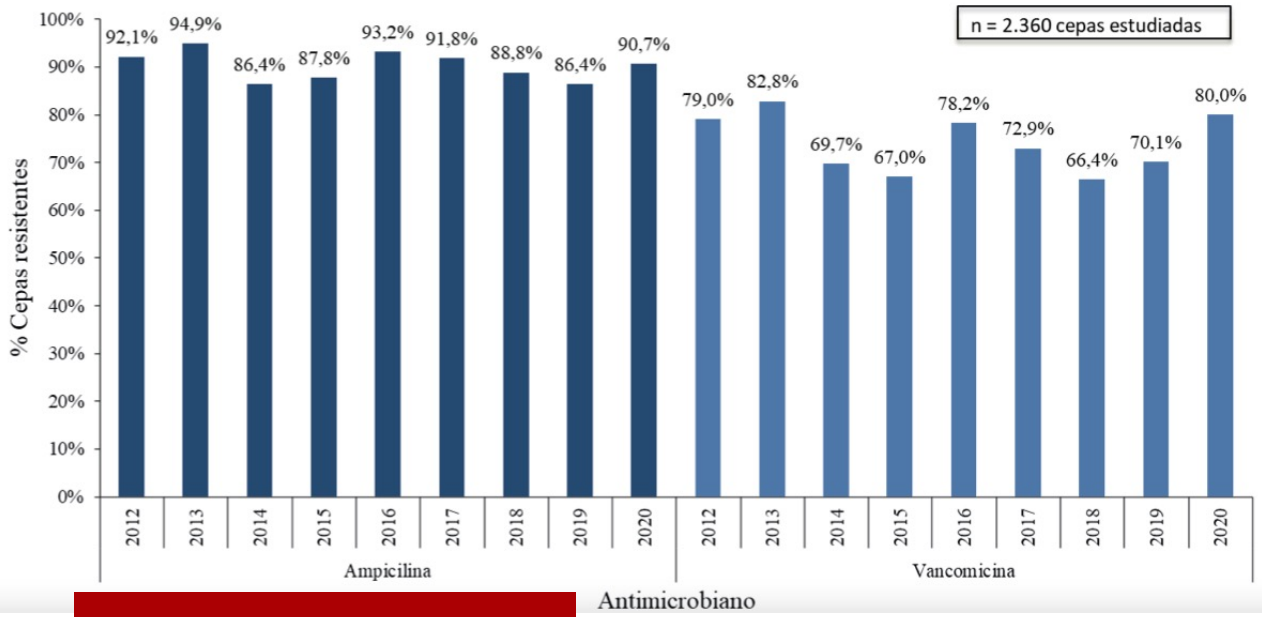
Resistencia antimicrobiana en cepas de *E. faecalis*. Chile, 2012-2020.



VRE_{fs}: 0.9 – 7 %

Enterococcus faecium

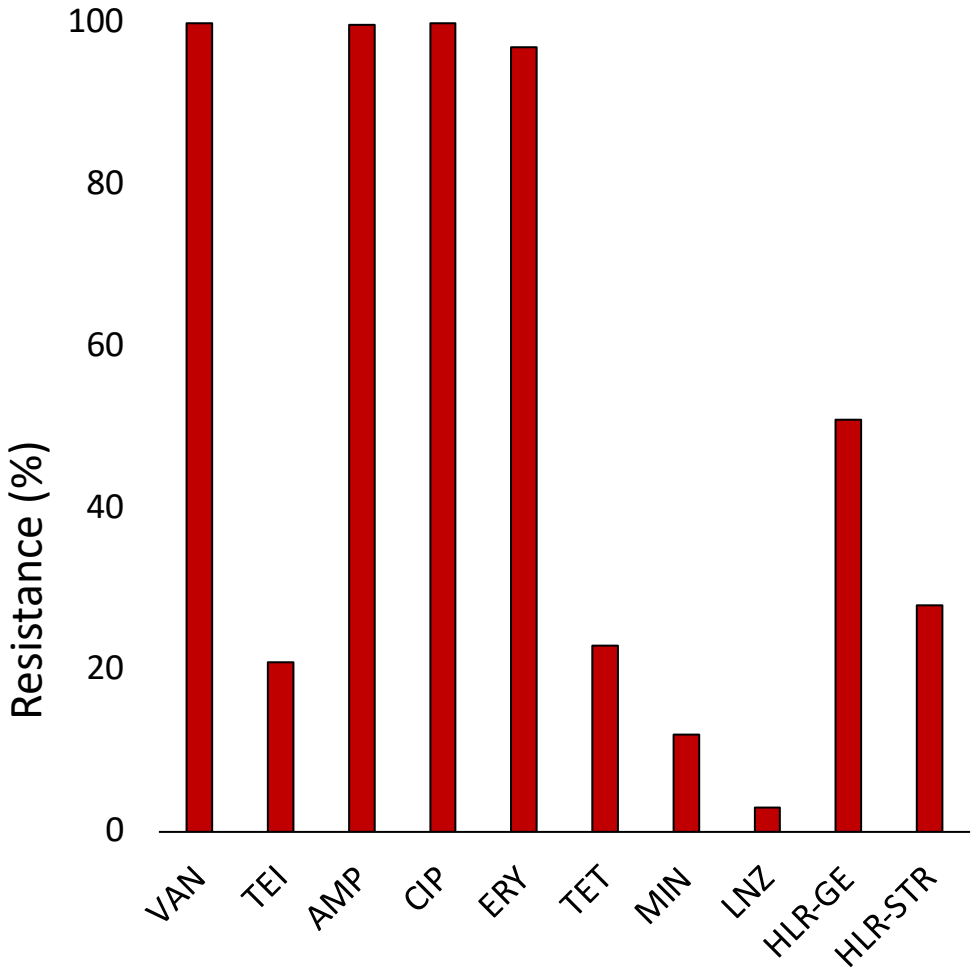
Resistencia antimicrobiana en cepas de *E. faecium*. Chile, 2012-2020.



VRE_{fm}: 66 – 83 %

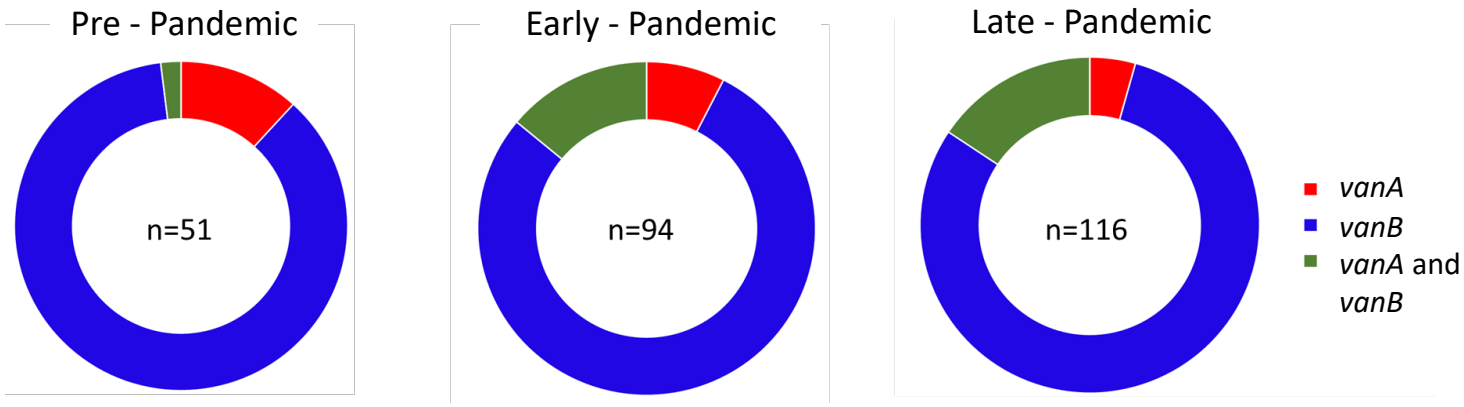
Instituto de Salud Pública de Chile. Boletín de Resistencia Antimicrobiana. Publicado el 1 de septiembre de 2022. Consultado en octubre de 2023. Disponible en: https://www.ispch.cl/wp-content/uploads/2022/09/BoletinRAM_FINAL-1-1.pdf

VRE *faecium* in Chile

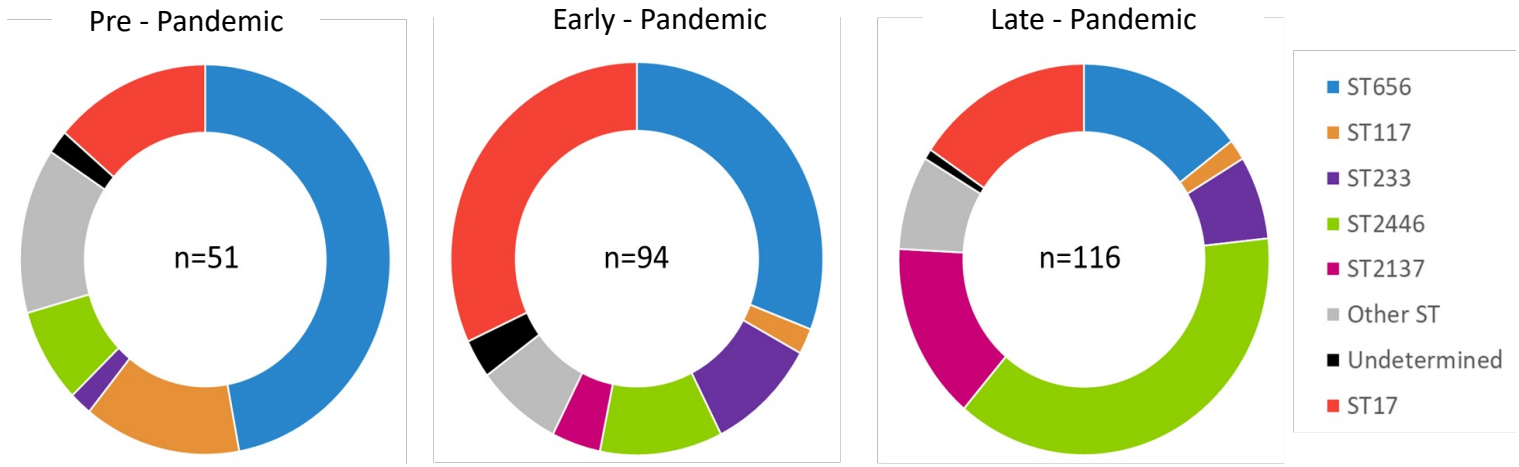


N=762

Confirmation of the presence of *vanA* and/or *vanB* clusters in 261 VREfm genomes



STs from 261 Chilean VREfm genomes



The team

GeRM Lab:

Jose M. Munita, MD
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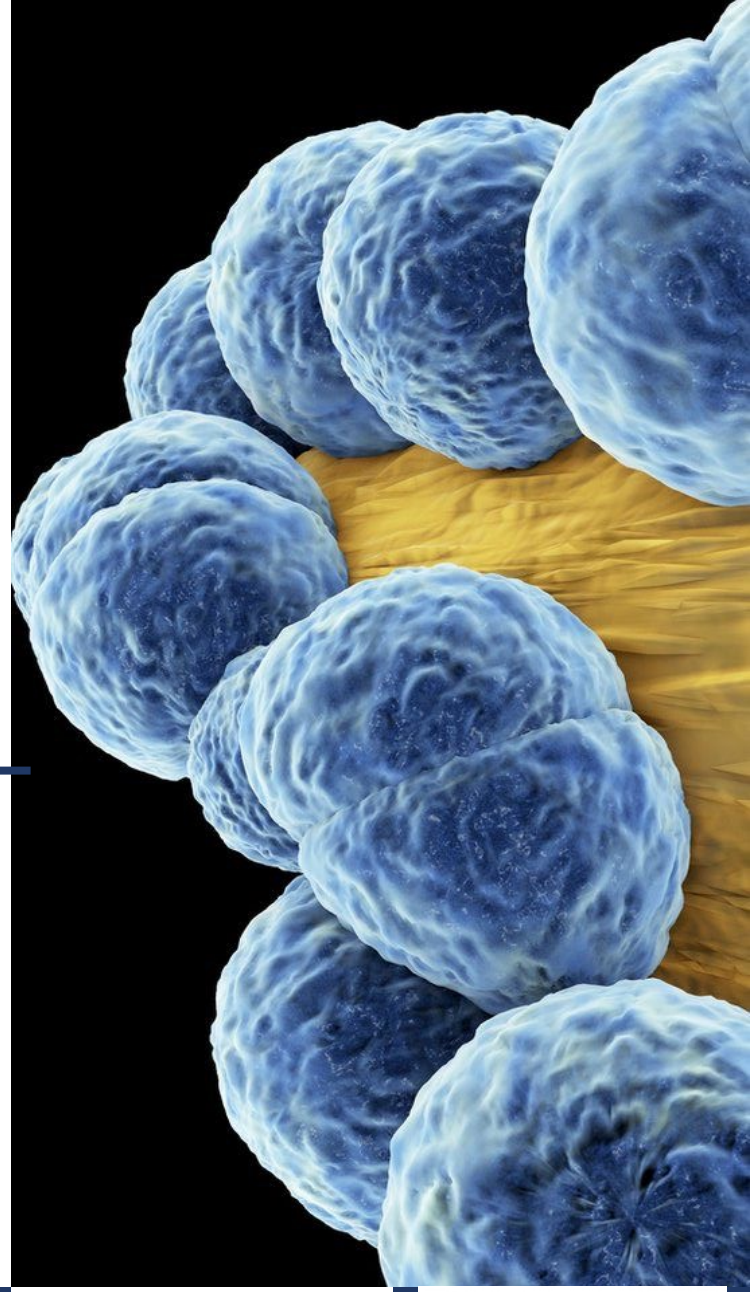
UNIVERSIDAD EL BOSQUE

GeRM GENOMICS & RESISTANT MICROBES
MICROB-R Núcleo Milenio para la Investigación Colaborativa en Resistencia Antimicrobiana
ICIM UDD INSTITUTO DE CIENCIAS E INNOVACIÓN EN MEDICINA Facultad de Medicina Clínica Alemana - Universidad del Desarrollo

Multi-Drug Resistance Organisms (MDRO): Gram positive bacteria



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