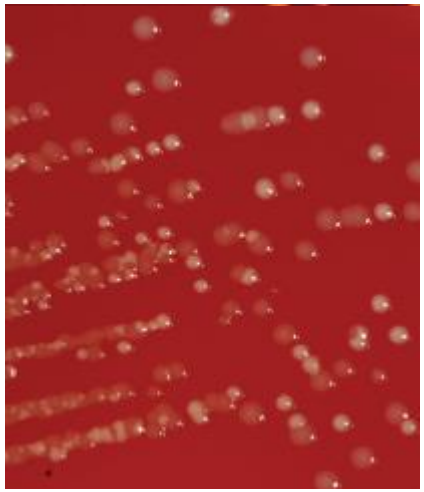
Impact of Mutations in hVISA Isolates on Decreased Susceptibility to Vancomycin, Through Population Analyses Profile - Area Under Curve (PAP-AUC)

Alessandro C. O. Silveira

Introduction

- ❑ Heterogeneous resistance
- ❑ Expression in subpopulations (1 in 10^6)
- ❑ Independent of the MIC and other mecanisms
- ❑ Small Colony Variants (SCV)
- ❑ Nutritionally demanding
- ❑ Slower growth
- ❑ Selective pressure required
- ❑ Intermediate stage for VISA



Howden *et al.*, 2010; Hiramatsu, 2001

Challenges

- There are no breakpoints in CLSI and EUCAST for testing vancomycyn for disk diffusion
- hVISA is not detected by traditional phenotypic methods and there are also no reliable molecular markers
- The presence of mutations in target genes is not always associated with phenotypic expression → ”Molecular Koch Postulates”

Methods

Table 2: Parameters of the main screening tests for the detection of hVISA.

Methodology	Sensitivity	Specificity	PPV[a]	NPV[b]	Accuracy
Etest GRD[c]	66.7%	97.3%	72.3%	96.5%	94.3%
Etestmacromethod	75%	94.6%	60%	97.2%	92.7%
Agar screening[d]	90.9%	93.8%	58.8%	99.1%	93.5%

[a]– Positive predictive value
[b]– Negative predictive value
[c]– Etest glycopeptides resistance detection®
[d]– Agar screening in brain-heart infusion (BHI) with 4 µg/mL vancomycin and 16 g/L pancreatic digest of casein

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Prediffusion

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Bacteriology

Is prediffusion test an alternative to improve accuracy in screening hVISA strains and to detect susceptibility to glycopeptides/lipopeptides?

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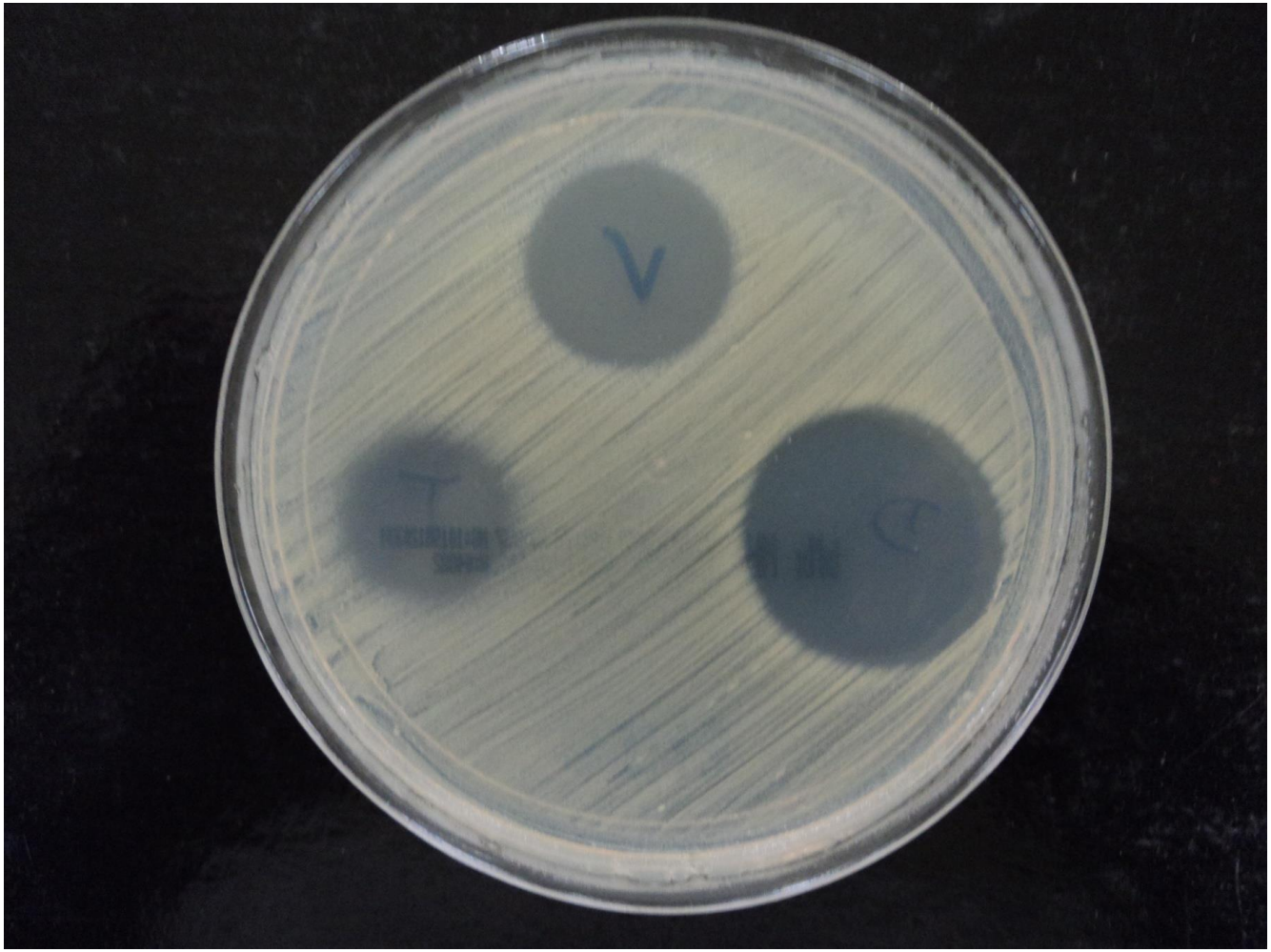
Prediffusion



Sensibility – 91.7 %

Specificity – 83.1 %

Prediffusion

The high negative predictive value (97.1%) allows for testing negative, excluding the possibility of that the phenotype. Taking into account that all positive screening tests should be confirmed with PAP-AUC, the prediffusion method, as a simple and low-cost test, should be considered not only as a useful test to assess the susceptibility of *S. aureus* to glycopeptides, but also as a screening test for hVISA.

Molecular epidemiology

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Original article

Molecular epidemiology of heteroresistant vancomycin-intermediate *Staphylococcus aureus* in Brazil

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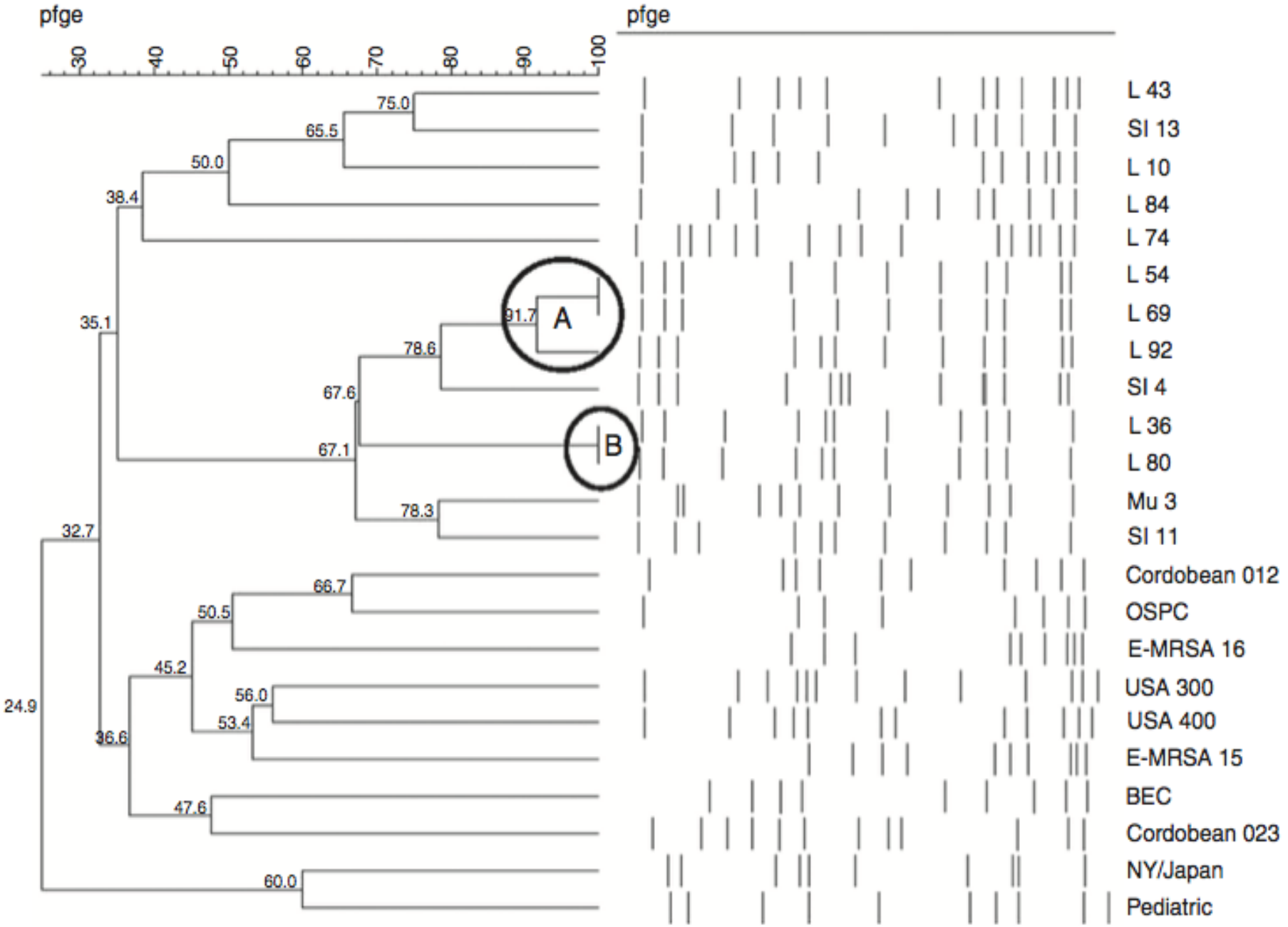
Molecular epidemiology

Table 1 – Epidemiological and clinical characteristics of the 12 hVISA isolates.

Isolate number	City	Institution	Clinical sample	SCC*mec*	Clone	Date
10	Florianópolis	Hospital A	Osteomyelitis	III	Non clonal	23/11/2009
36	Florianópolis	Hospital A	Tracheal aspirate	II	B	03/09/2010
43	Florianópolis	Hospital A	Surgical wound	III	Non clonal	14/10/2010
54	Florianópolis	Hospital A	Osteomyelitis	II	A	04/05/2011
69	Florianópolis	Hospital A	Osteomyelitis	II	A	01/07/2011
74	Florianópolis	Hospital B	Skin lesion	IVc	Non clonal	22/12/2011
80	Florianópolis	Clinic A	Tracheal aspirate	II	B	09/01/2012
84	Florianópolis	Hospital A	Tracheal aspirate	I	Non clonal	25/02/2012
92	Florianópolis	Hospital C	Tracheal aspirate	II	A	18/08/2012
SI4	Blumenau	Hospital D	Blood	I,II	A	28/09/2010
SI11	Blumenau	Hospital D	Tracheal aspirate	II	Non clonal	20/02/2011
SI13	Blumenau	Hospital D	Osteomyelitis	III	Non clonal	12/04/2011


pfge
30
40
50
60
70
80
90
100
75.0
65.5
50.0
38.4
35.1
91.7
A
78.6
67.6
67.1
B
78.3
32.7
66.7
50.5
45.2
56.0
53.4
24.9
36.6
47.6
60.0
pfge
L 43
SI 13
L 10
L 84
L 74
L 54
L 69
L 92
SI 4
L 36
L 80
Mu 3
SI 11
Cordobean 012
OSPC
E-MRSA 16
USA 300
USA 400
E-MRSA 15
BEC
Cordobean 023
NY/Japan
Pediatric

Complete Genome

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Complete Genome Sequence of *Staphylococcus aureus* FCFHV36, a Methicillin-Resistant Strain Heterogeneously Resistant to Vancomycin

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We report here the sequence of the entire chromosome of *Staphylococcus aureus* strain FCFHV36, a methicillin-resistant strain heterogeneously intermediate to vancomycin, bearing a type II staphylococcal chromosome cassette *mec* element (SCC*mec*), belonging to multilocus sequence type (MLST) 105, and isolated from a vertebra of a patient with osteomyelitis.

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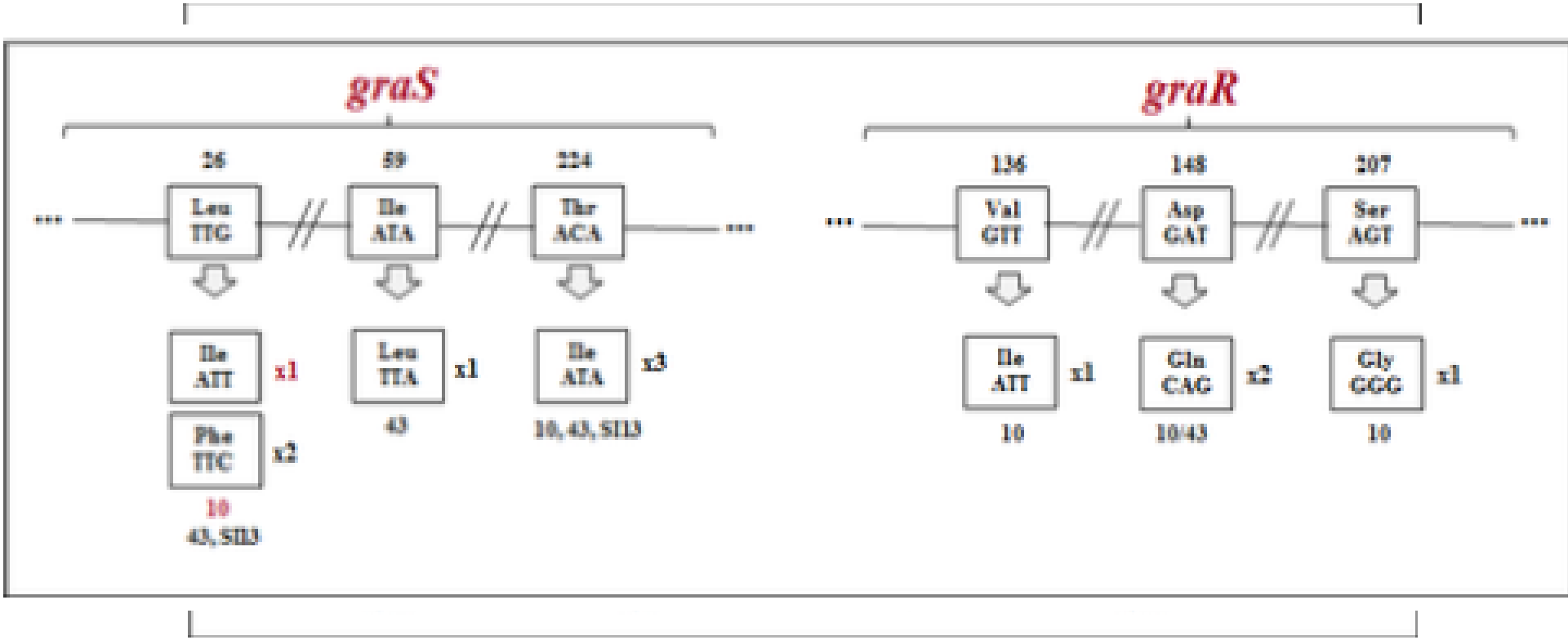
Present Study - Methods

- Mutations were evaluated in ten clinical isolates of hVISA, from the state of Santa Catarina, Southern Brazil
- Sequences were analyzed on MiSeq equipment (Illumina, Inc) and compared by BioEdit Sequence Alignment Editor version 7.2.5, using as reference the VSSA isolate sequenced in this study and strain VSSA N315 (GenBank BA00018.3)
- Mutations in four target-genes were evaluated, before exposure to 2 µg/mL of vancomycin, and related to phenotypic expression by Population Analyses Profile - Area Under Curve (PAP-AUC)
  - *wal*KR
  - *vra*SR
  - *gra*SR
  - *rpo*B

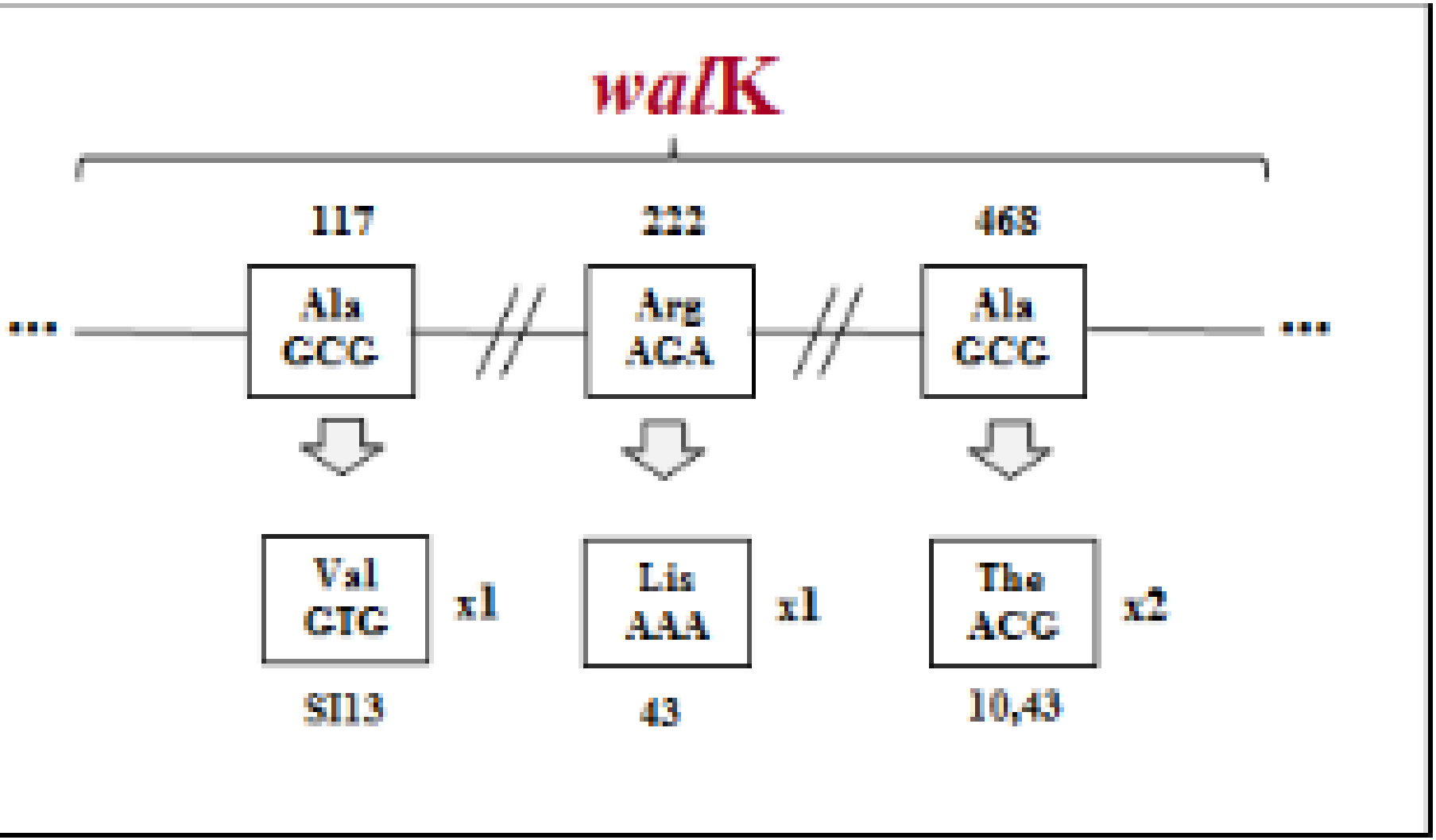
PCR

1 - *hu* (161 bp); 2 - *pta* (193 bp); 3 - *tpi* (173 bp); 4 - *gyr*B (153 bp); 5- *gra*SR (196 bp); 6 - *vra*SR (149 bp); 7 - *wal*KR (188 bp); 8 - *rpo*B (153 bp); 9 – molecular size standard(100 bp).

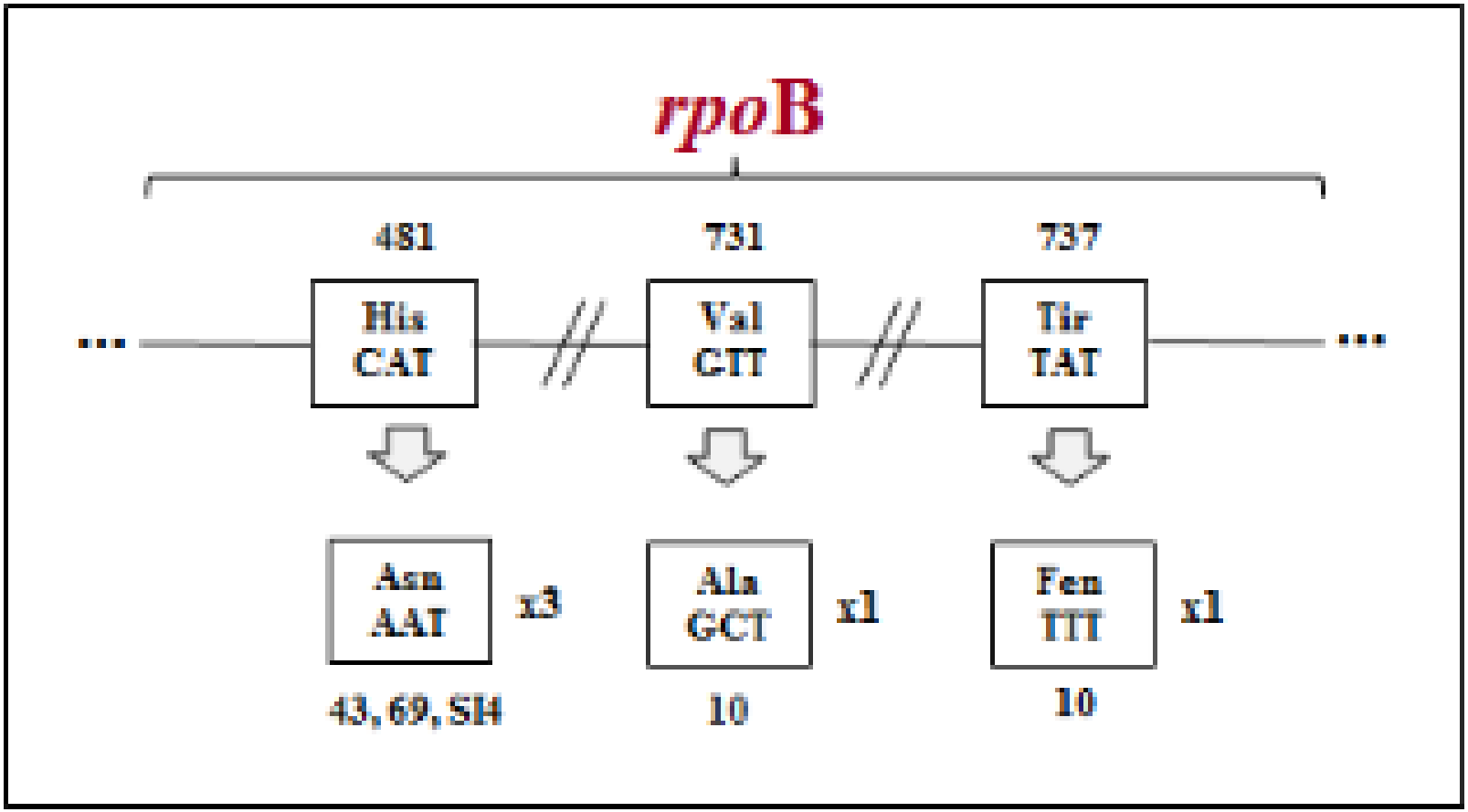
Mutations in *graS* and *graR*



Mutations in *walK*

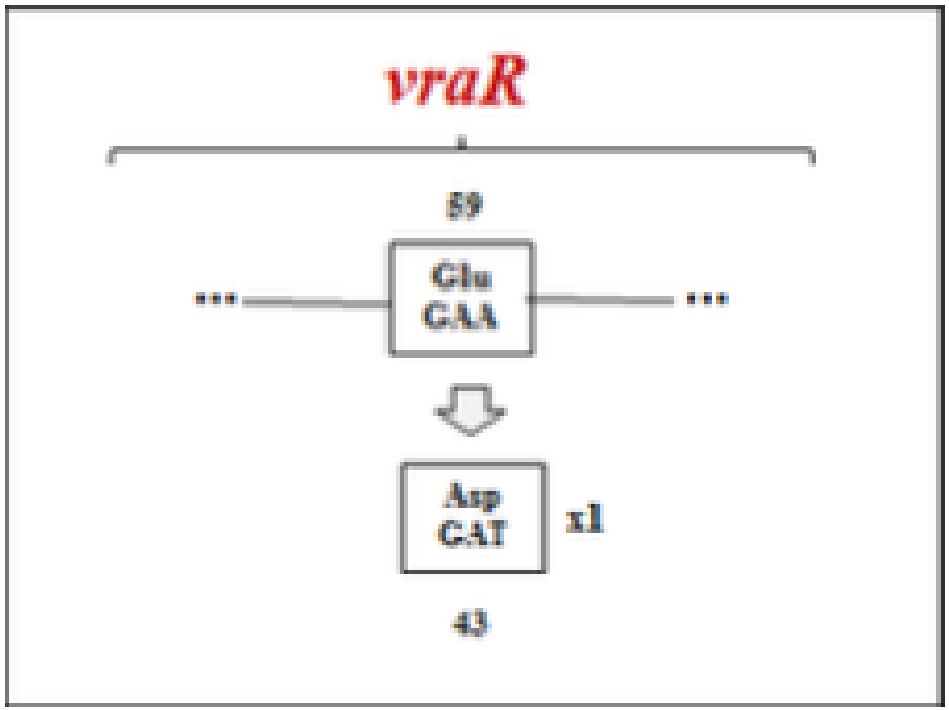


Mutations in *rpo*B



Mutations in *vra*SR

- ❑ No silent mutations were found in *vra*SR
- ❑ No mutations were observed in *vra*S



Results

- Eight mutations were found in *gra*SR genes, one in the *vra*SR gene, four in the *wal*KR gene and three in the *rpo*B gene.
- The most prevalent mutations were *gra*SR T224I and *rpo*B H481N, followed by *gra*SR D148Q and *wal*KR A468T mutations.
- Considering the response profile to vancomycin, there was a statistically significant increase ($p < 0.05$), after induction of vancomycin resistance, in three isolates.

Results

- Among the genes analyzed, the *gra*SR complex (the mutations in L26F and T224I were found in the three isolates) was the one that demonstrated the greatest impact in the decrease of the susceptibility to vancomycin.
- No mutation in *wal*KR and *rpo*B was found simultaneously in all three isolates.

Summary of mutations

Isolate	*graS*	*graR*	*vraS*	*vraR*	*walK*	*walR*	*rpoB*
10	L26I T224I	V136I D148Q S207Q	-	-	A468T	-	V731A Y737F
36	-	-	-	-	-	-	-
43	L26F I59L T224I	D148Q	-	E59D	R222K A468T	-	H481N
69	-	-	-	-	-	-	H481N
80	-	-	-	-	-	-	-
SI4	-	-	-	-	-	-	H481N
SI11	-	-	-	-	-	-	-
SI13	L26F T224I	-	-	-	A117V	-	-

PAP-AUC 0.92 to 1.09

PAP-AUC 0.98 to 1.17

PAP-AUC 1.19 to 1.37

Gene	Samples	SCCmec*	PAP ratio	Amino acid Changes	Phenotypes	Previous studies
graS	10	III	0.92	L26I	VSSA	YOO et al., 2013; ROCH et al., 2014
	43 SI13	III III	0.98 1.19	L26F	VISA/ hVISA/ VSSA	YOO et al., 2013; ROCH et al., 2014
	43	III	0.98	I59L	VISA/ hVISA	YOO et al., 2013; ROCH et al., 2014
	10 43 SI13	III III III	0.92 0.98 1.19	T224I	VISA/ hVISA/ VSSA	YOO et al., 2013; ROCH et al., 2014
graR	10	III	0.92	V136I	VSSA	YOO et al., 2013
	10 43	III III	0.92 0.98	D148Q	VISA/ hVISA/ VSSA	NEOH et al., 2008; HOWDEN et al., 2010b; DODDANGOUDAR et al., 2011; DODDANGOUDAR et al., 2012; YOO et al., 2013; ROCH et al., 2014
	10	III	0.92	S207G	-	N.D.
vraR	43	III	0.98	E59D	VISA/ VSSA	YOO et al., 2013; ROCH et al., 2014
walK	SI13	III	1.19	A117V	-	N.D.
	43	III	0.98	R222K	VISA	SHOJI et al.,2011; HIRAMATSU et al., 2014;
	10 43	III III	0.92 0.98	A468T	VISA	SHOJI et al., 2011; HIRAMATSU et al., 2014
rpoB	43 69 SI4	III II I e II	0.98 0.93 1.14	H481N	VISA/ hVISA	CUI et al., 2010; WATANABE et al., 2011; HAFER et al., 2012; HIRAMATSU el al., 2014
	10	III	0.92	V731A	VISA	HIRAMATSU el al., 2014
	10	III	0.92	Y737F	VISA	WATANABE et al., 2011; HIRAMATSU el al., 2014

Conclusions

Our findings demonstrate that no single mutation was found in any isolate, responsible for characterization of the phenotype. Mutations in *wal*KR and *rpoB* genes, alone, did not demonstrate relevance. Although mutations in the *gra*SR gene have shown the greatest impact on decreased susceptibility, the sum of the performance of other genes may also lead to the development of the hVISA phenotype.

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Thanks!