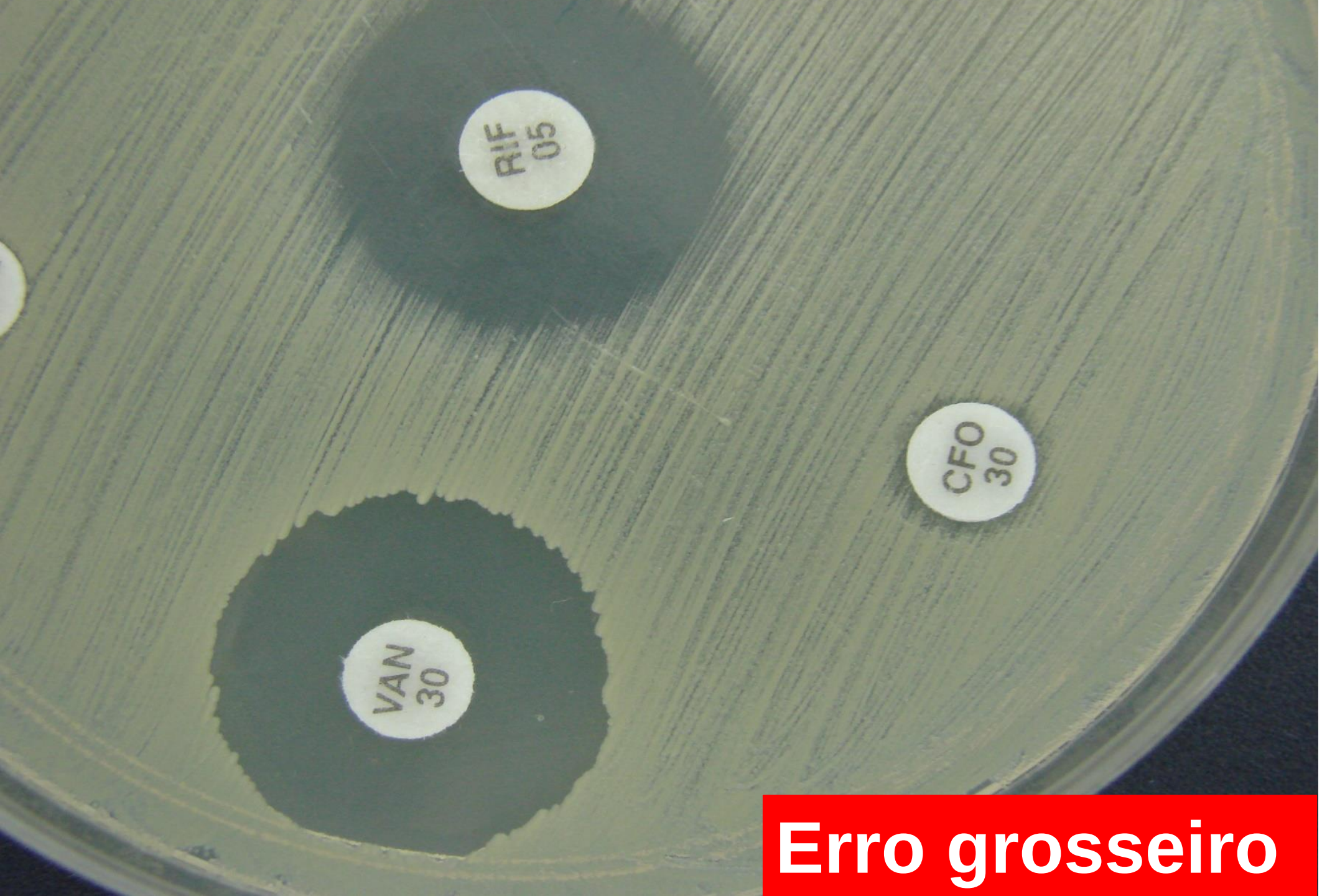
Avaliação da Susceptibilidade aos Glicopeptídeos em *Staphylococcus* no Laboratório Clínico: Erros Sutis e Grosseiros

ALESSANDRO C. O. SILVEIRA



Erro grosseiro

Fenótipos

Isolate	sVISA	Colony	MIC			
			24 h	48 h	72 h	96 h
SI4	no	L	2	3	3	3
SI11	yes	P	2	4	8	12
SI13	no	L	2	4	4	4
10	yes	P	1	1	4	16
36	no	L	2	2	4	4
43	yes	S	1	2	4	8
69	no	L	2	4	4	4
80	no	L	2	3	3	4

Por que o BRCAST é a melhor opção?

Sem fator de confusão

Com critérios interpretativos para teicoplanina

Sem zona cinza

Com guia epidemiológico de detecção

Considera os testes de triagem para hVISA

Importância da detecção do mecanismo	
Necessário para categorização clínica da sensibilidade	Sim
Para propósito de controle de infecção	Sim
Para propósito de saúde pública	Sim

Glicopeptídeos[1]	Ponto de corte p/ CIM (mg/L)				Conteúdo do disco (µg)	Ponto de corte p/ diâmetro do halo (mm)			
	S ≤	I	R >	AIT		S ≥	I	R <	AIT
Teicoplanina[2], *S. aureus*	2	-	>2			Nota[A]	Nota[A]	Nota[A]	
Teicoplanina, estafilococos coagulase negativo	4	-	>4			Nota[A]	Nota[A]	Nota[A]	
Vancomicina[2], *S. aureus*	2	-	>2			Nota[A]	Nota[A]	Nota[A]	
Vancomicina[2], estafilococos coagulase negativo	4	-	>4			Nota[A]	Nota[A]	Nota[A]	

1. A CIM de glicopeptídeos é dependente do método e deve ser determinada por microdiluição em caldo (referência ISO 20776). *S. aureus* com CIM de 2 mg/L para vancomicina estão no limite da distribuição da CIM do tipo selvagem e pode haver diminuição da resposta clínica. O ponto de corte (resistente) foi diminuído para 2 mg/L para evitar que isolados intermediários "GISA" sejam reportados, já que infecções graves por "GISA" não são tratáveis com doses altas de vancomicina ou teicoplanina.

2. Isolados não sensíveis são raros. A identificação e o teste de sensibilidade em isolados não sensíveis devem ser confirmados em centro de referência.

A. O método de disco-difusão não é confiável e não distingue isolados selvagens daqueles com resistência não mediada pelo gene *vanA*.

hVISA

Resistência heterogênea

Manifesta-se em sub-populações (1 em 10^6)

Independe da CIM e outros mecanismos

***Small Colony Variants* (SCV)**

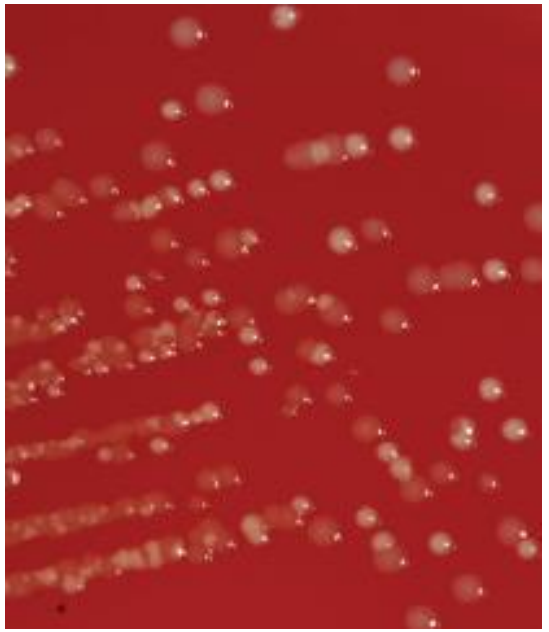
Fator de confusão para o microbiologista

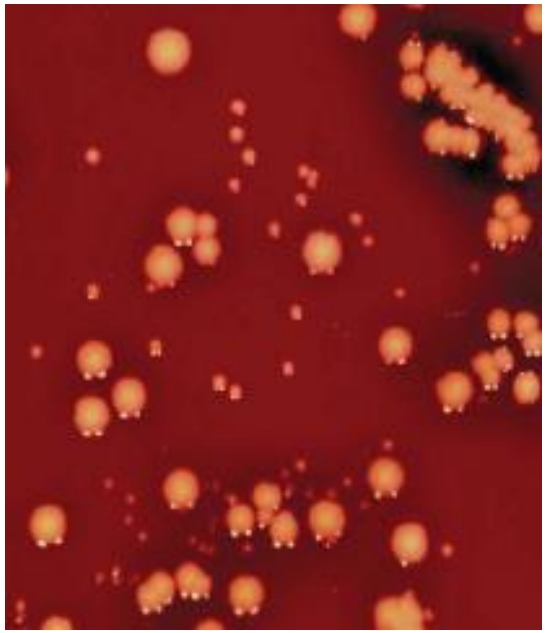
Nutricionalmente exigentes

Crescimento mais lento

Necessidade de pressão seletiva

Estágio intermediário para VISA





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

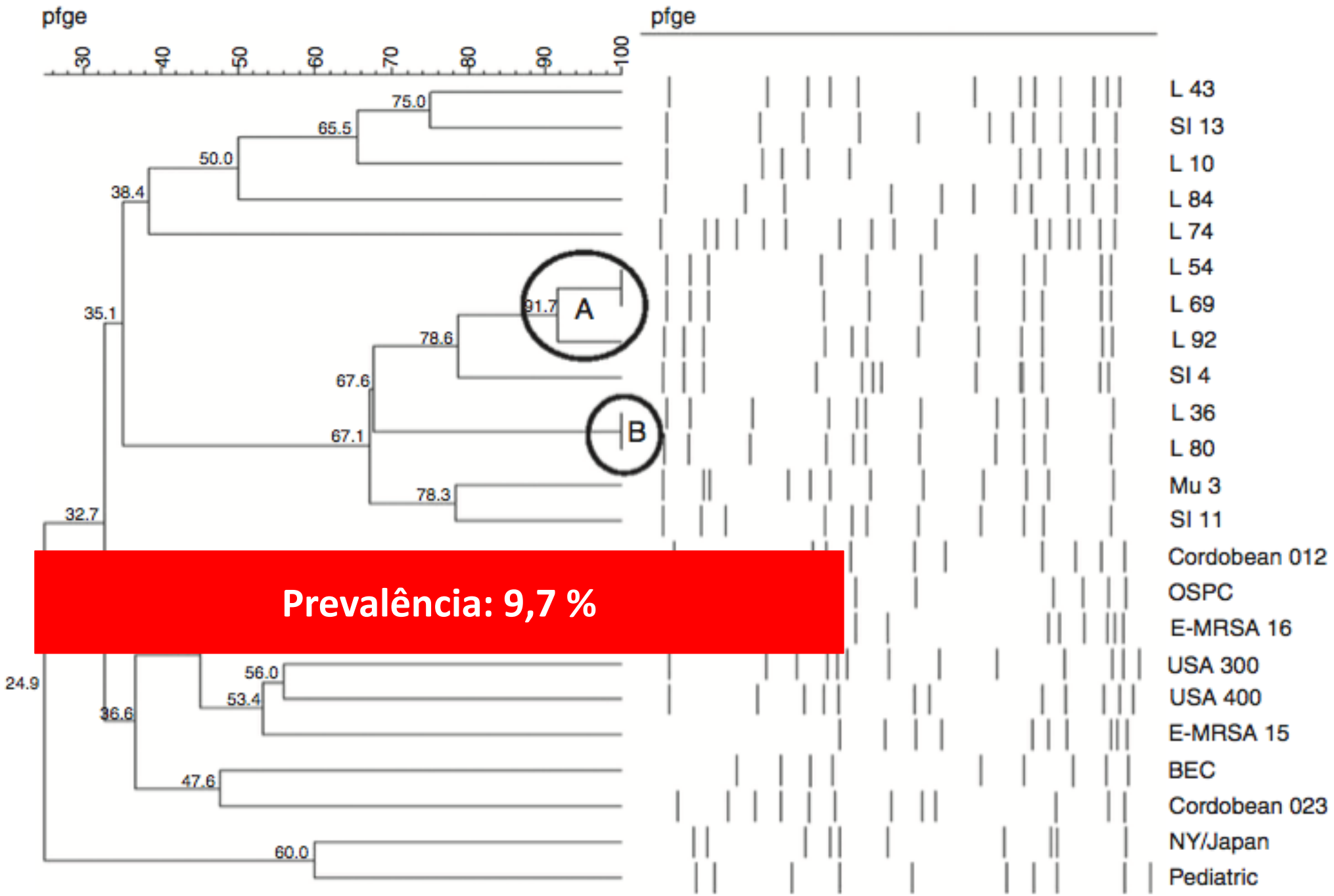
Epidemiologia

RESEARCH ARTICLE

Systematic Review and Meta-Analysis of the Epidemiology of Vancomycin-Intermediate and Heterogeneous Vancomycin-Intermediate *Staphylococcus aureus* Isolates

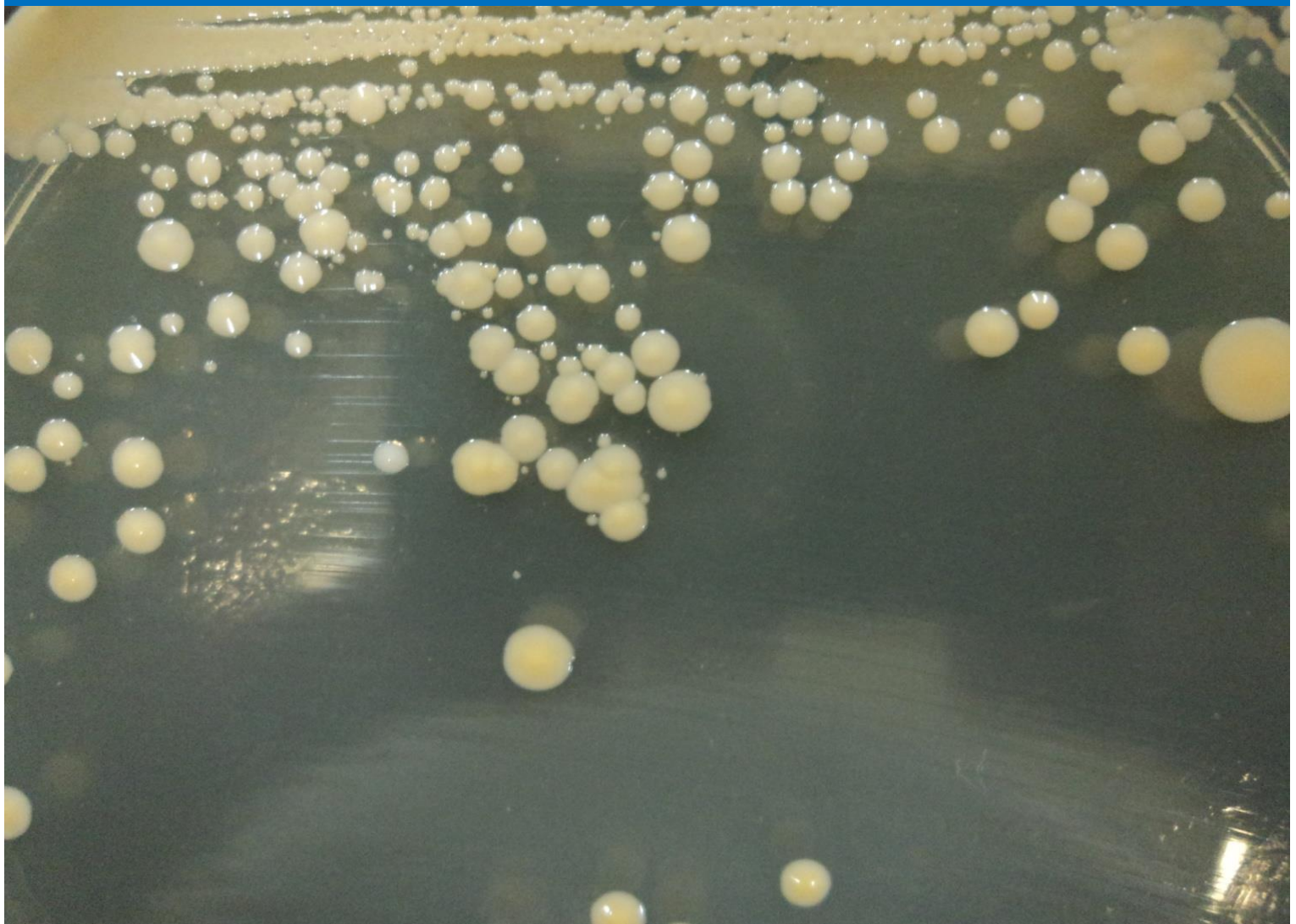
Table 2. Prevalence of hVISA and VISA based on study period, origin of study, and isolate selection.[a]

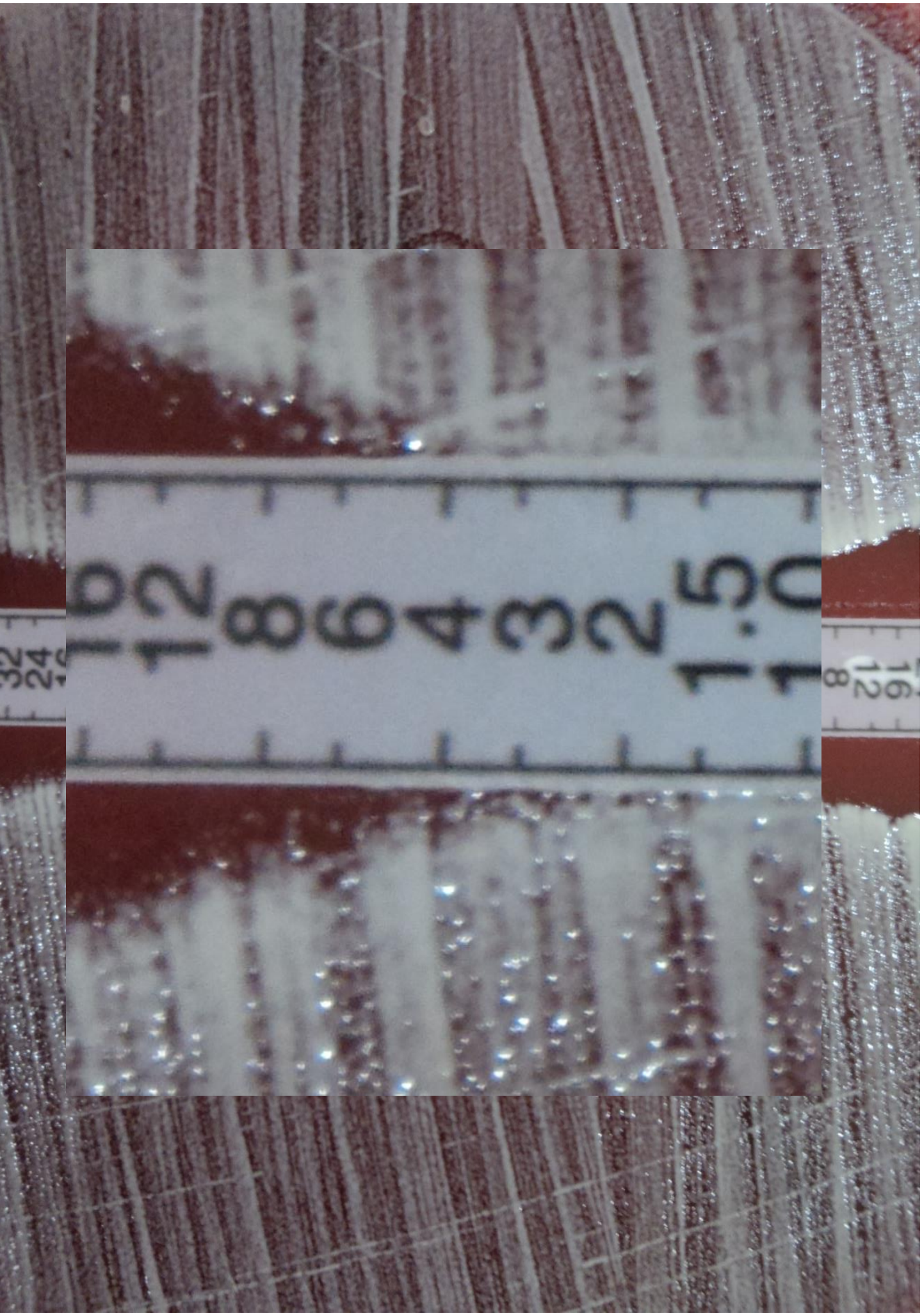
	Category	Subcategory	No. Studies	No. Strains	Prevalence (%) (95% CI)
hVISA	Overall		76	99042	6.05 (4.78–7.48)
	Study period	Before 2006	42	40119	4.68 (3.19–6.41)
		2006–2009	10	6485	5.38 (2.40–9.48)
		2010–2014	5	680	7.01 (2.12–14.42)
	Continent	Asia	35	64692	6.81 (4.76–9.16)
		Europe-America	41	34350	5.60 (3.85–7.64)
	Clinical sample	Blood culture sample	21	5944	9.81 (6.71–13.42)
		All clinical sample	55	93098	4.68 (3.51–6.00)
VISA	Overall		38	68792	3.01 (1.62–4.83)
	Study period	Before 2006	20	13394	2.05 (0.95–3.55)
		2006–2009	4	5630	2.63 (0.29–7.22)
		2010–2014	2	2090	7.93 (0.06–26.67)
	Continent	Asia	18	55362	3.42 (1.10–6.99)
		Europe-America	20	13430	2.75 (1.19–4.91)
	Clinical sample	Blood culture samples	7	2542	2.00 (0.03–6.88)
		All clinical samples	31	66250	3.24 (1.67–5.29)

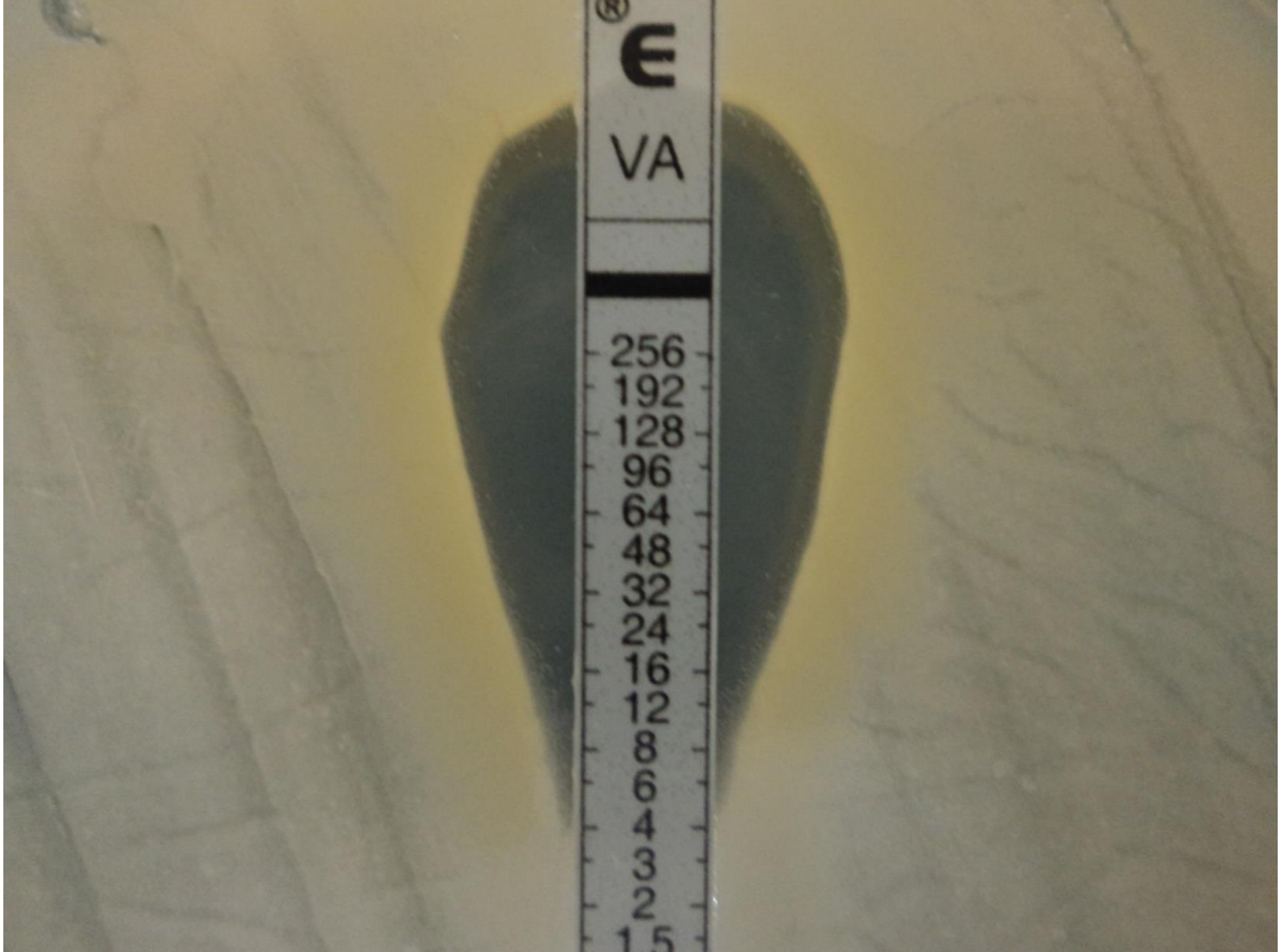


Prevalência: 9,7 %

Triagem em ágar com teicoplanina






®E
VA
256
192
128
96
64
48
32
24
16
12
8
6
4
3
2
1.5

Diagnostic Microbiology and Infectious Disease 79 (2014) 401–404

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

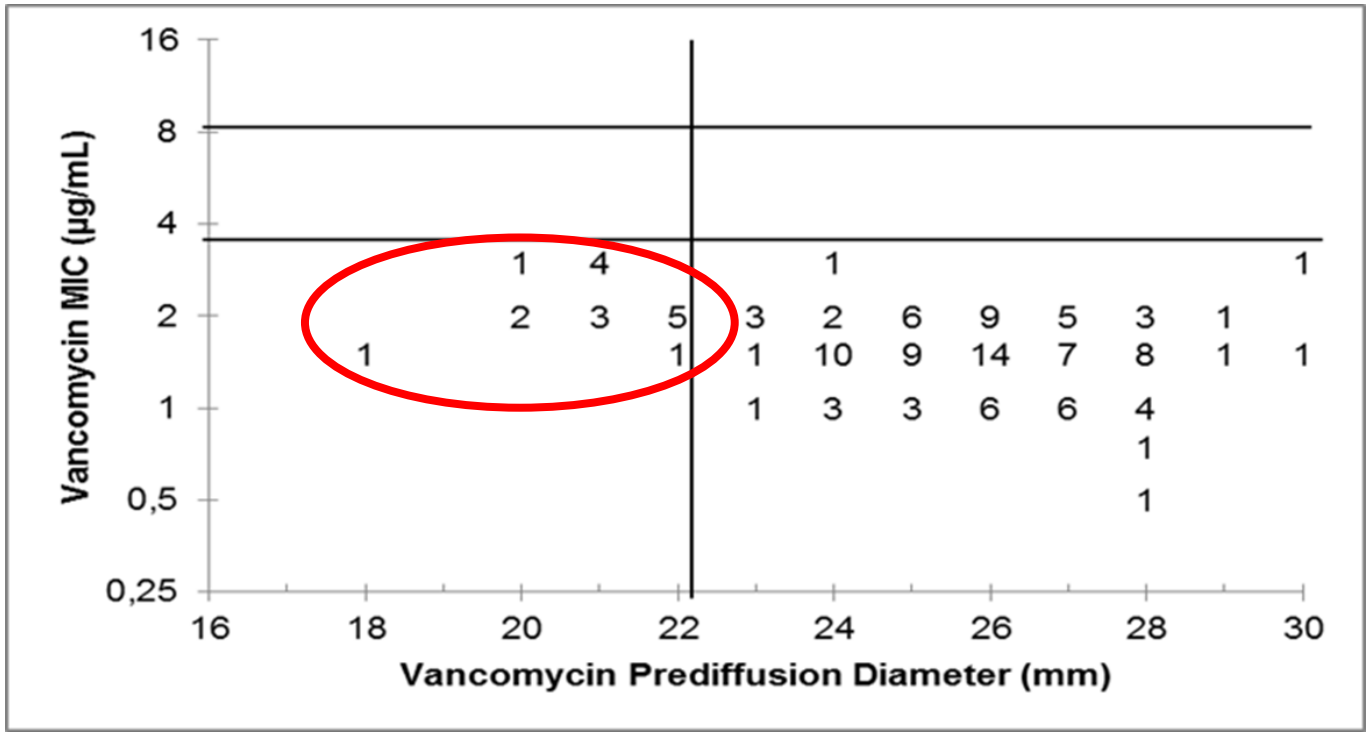
journal homepage: www.elsevier.com/locate/diagmicrobio

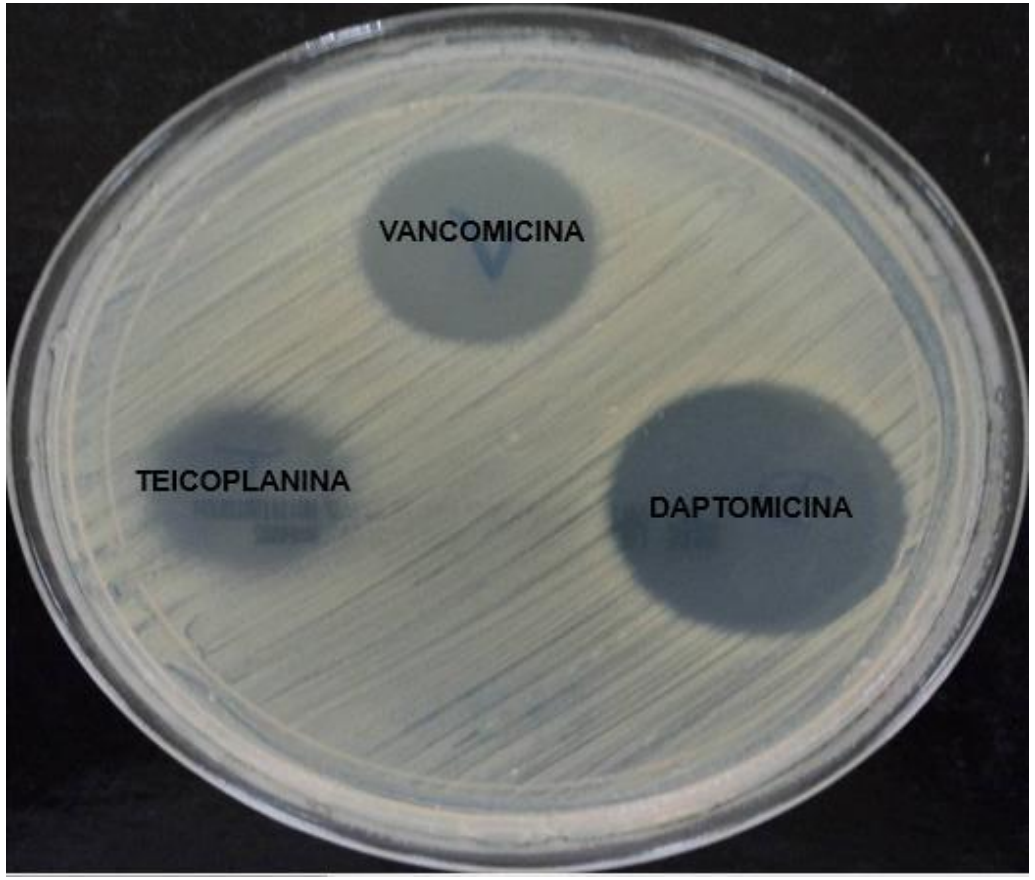
Bacteriology

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

Alessandro Conrado de Oliveira Silveira [a,b,*], Gustavo Enck Sambrano [a], Thiago Galvão da Silva Paim [a], Juliana Caierão [a], Caio Mauricio Mendes de Cordova [b], Pedro Alves d'Azevedo [a]

[a] Federal University of Health Sciences of Porto Alegre, RS, Brazil
[b] University Regional of Blumenau, SC, Brazil





Valor preditivo negativo: 97,1 %

JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2011, p. 1489–1494 Vol. 49, No. 4
0095-1137/11/$12.00 doi:10.1128/JCM.02302-10

Performance of Various Testing Methodologies for Detection of Heteroresistant Vancomycin-Intermediate *Staphylococcus aureus* in Bloodstream Isolates▽

Sebastian J. van Hal,[1,2]†* Michael C. Wehrhahn,[1]† Thelma Barbagiannakos,[1] Joanne Mercer,[1] Dehua Chen,[1] David L. Paterson,[3] and Iain B. Gosbell[1,2]

Department of Microbiology & Infectious Diseases, Sydney South West Pathology Service-Liverpool Hospital, Locked Bag 7090, Liverpool BC NSW 1871,[1] Antibiotic Resistance & Mobile Elements Group, School of Medicine, University of Western Sydney, Sydney,[2] and University of Queensland Centre for Clinical Research (UQCCR), Brisbane,[3] Australia

TABLE 2. Comparison of hVISA detection methods to PAP-AUC at hVISA prevalence of 12%[c]

Method (MIC cutoff [μg/ml])	No. of isolates[a]		Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	% Accuracy[b]
	hVISA (TP = 55)	VSSA (TN = 399)					
Etest (≥1.5)	50	263	91	66	98	27	69
BMD (≥1.5)	49	335	89	84	98	43	85
MET	49	218	89	55	97	21	59
BMD (≥2)	45	388	82	97	97	80	95
Etest (≥2)	39	375	71	94	96	62	91
GRD Etest	39	375	71	94	96	62	91
Vitek2 (≥2)	14	382	25	96	90	45	87

TABLE 3. Sensitivity and specificity values for the different methods in determining hVISA

Method	Correct no. of hVISA isolates identified (n = 21)	No. of false positives	No. of false negatives	% sensitivity	% specificity
Etest macromethod, 24 h	3	0	18	14	100
Etest macromethod, 48 h	12	5	9	57	96
Etest GRD, 24 h	9	1	12	43	99
Etest GRD, 48 h	12	3	9	57	97
BHI screen agar, 0.5 McFarland, 24 h	9	0	12	43	100
BHI screen agar, 0.5 McFarland, 48 h	19	8	2	91	94
BHI screen agar, 2 McFarland, 24 h	16	13	5	76	89
BHI screen agar, 2 McFarland, 48 h	17	39	0	100	67

Research Article

Evaluation of the Accuracy of Phenotypic Methods for the Detection of Heteroresistant Vancomycin-Intermediate *Staphylococcus aureus* (hVISA)

Silveira ACO[1,2]*, Da Cunha GR[1], Caierão J[1], De Cordova CMM[2], and d'Azevedo PA[1]

[1]*Department of Health Sciences, Federal University of Health Sciences of Porto Alegre, Brazil*

[2]*Department of Pharmaceutical Sciences, Regional University of Blumenau, Brazil*

JSM Microbiology 4(1): 1031 (2016)

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

Erro sutil

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

Rapid Detection of Vancomycin-Intermediate *Staphylococcus aureus* by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

Cheryl A. Mather,[a,d*] Brian J. Werth,[b] Shobini Sivagnanam,[c] Dhruba J. SenGupta,[a] Susan M. Butler-Wu[a*]

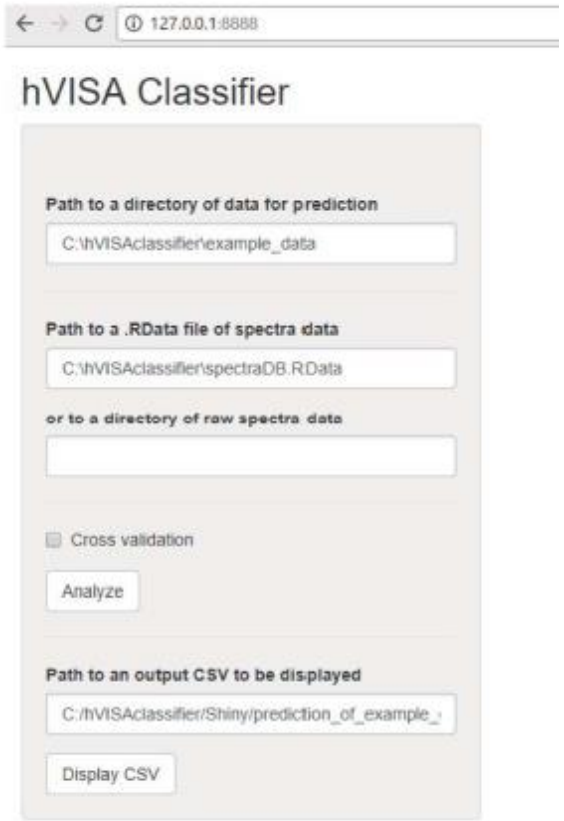
April 2016 Volume 54 Number 4 Journal of Clinical Microbiology jcm.asm.org 883

RESEARCH ARTICLE

Rapid and easy detection of low-level resistance to vancomycin in methicillin-resistant *Staphylococcus aureus* by matrix-assisted laser desorption ionization time-of-flight mass spectrometry

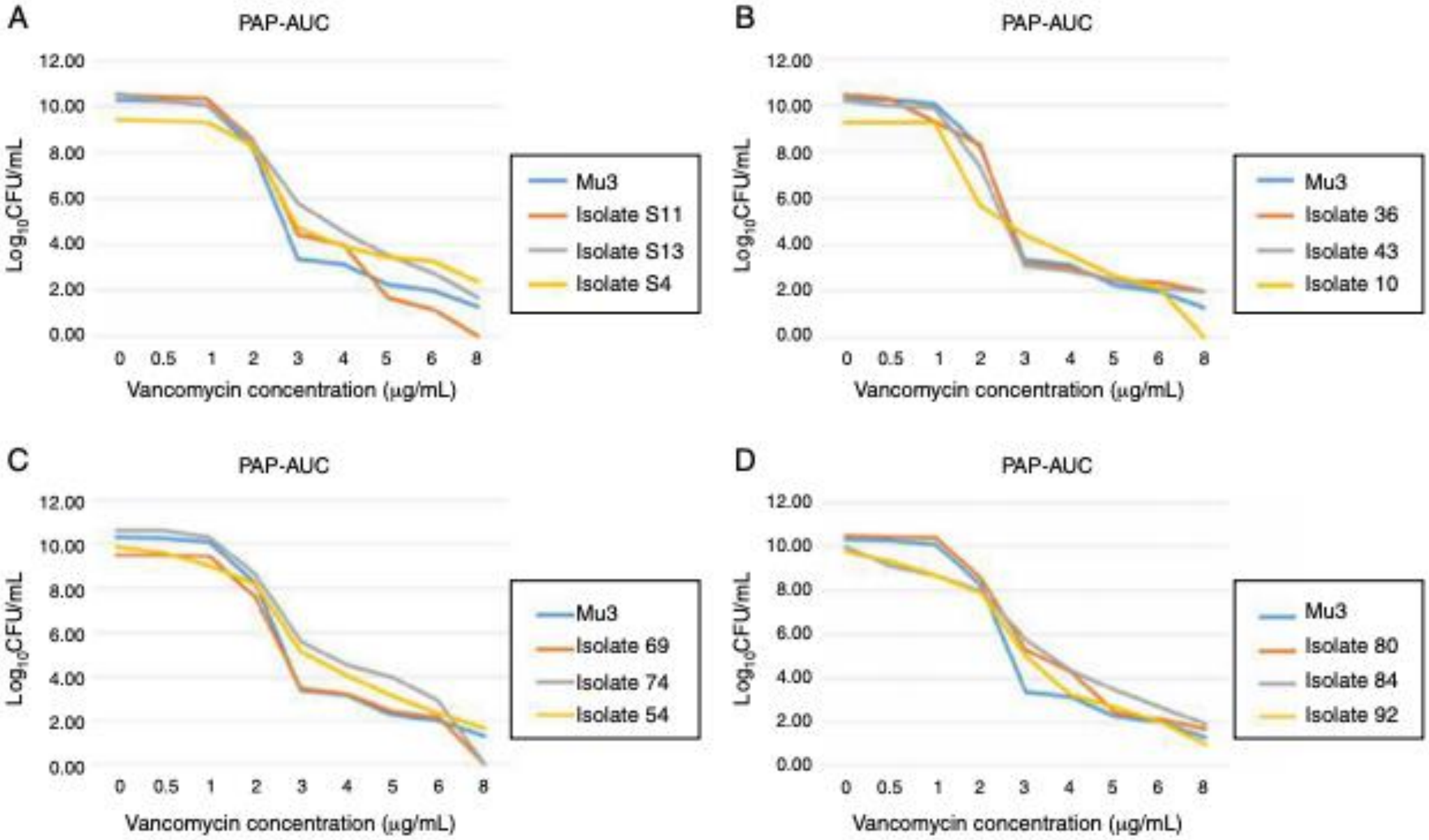
Kota Asakura[1], Takuya Azechi[1], Hiroshi Sasano[1], Hidehito Matsui[2], Hideaki Hanaki[2], Motoyasu Miyazaki[3], Tohru Takata[4], Miwa Sekine[5], Tomoiku Takaku[6], Tomonori Ochiai[6], Norio Komatsu[6], Keigo Shibayama[7,8], Yuki Katayama[5☯]*, Koji Yahara[8☯]*

PLOS ONE | https://doi.org/10.1371/journal.pone.0194212 March 9, 2018



create CSV file -> prediction_of_example_data_out.csv

sample	prediction
C:\hVISAclassifier\example_data\hVISA\0916-MRSA5\0_B10\1\1SLin\fid	hVISA
C:\hVISAclassifier\example_data\hVISA\9_hVISA_MRSA5\0_E6\1\1SLin\fid	hVISA
C:\hVISAclassifier\example_data\hVISA\MRSA239-3\0_F10\1\1SLin\fid	hVISA
C:\hVISAclassifier\example_data\hVISA\MRSA81-3\0_D6\1\1SLin\fid	hVISA
C:\hVISAclassifier\example_data\VISA\MRSA372\0_D11\1\1SLin\fid	VISA
C:\hVISAclassifier\example_data\VISA\T11\0_B3\2\1SLin\fid	VISA
C:\hVISAclassifier\example_data\VSSA\FU_97\0_F11\1\1SLin\fid	VSSA
C:\hVISAclassifier\example_data\VSSA\VSSA104\0_C1\1\1SLin\fid	VSSA


A
PAP-AUC
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Log10CFU/mL
0 0.5 1 2 3 4 5 6 8
Vancomycin concentration (μg/mL)
Mu3
Isolate S11
Isolate S13
Isolate S4
B
PAP-AUC
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Log10CFU/mL
0 0.5 1 2 3 4 5 6 8
Vancomycin concentration (μg/mL)
Mu3
Isolate 36
Isolate 43
Isolate 10
C
PAP-AUC
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Log10CFU/mL
0 0.5 1 2 3 4 5 6 8
Vancomycin concentration (μg/mL)
Mu3
Isolate 69
Isolate 74
Isolate 54
D
PAP-AUC
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Log10CFU/mL
0 0.5 1 2 3 4 5 6 8
Vancomycin concentration (μg/mL)
Mu3
Isolate 80
Isolate 84
Isolate 92

RESEARCH ARTICLE

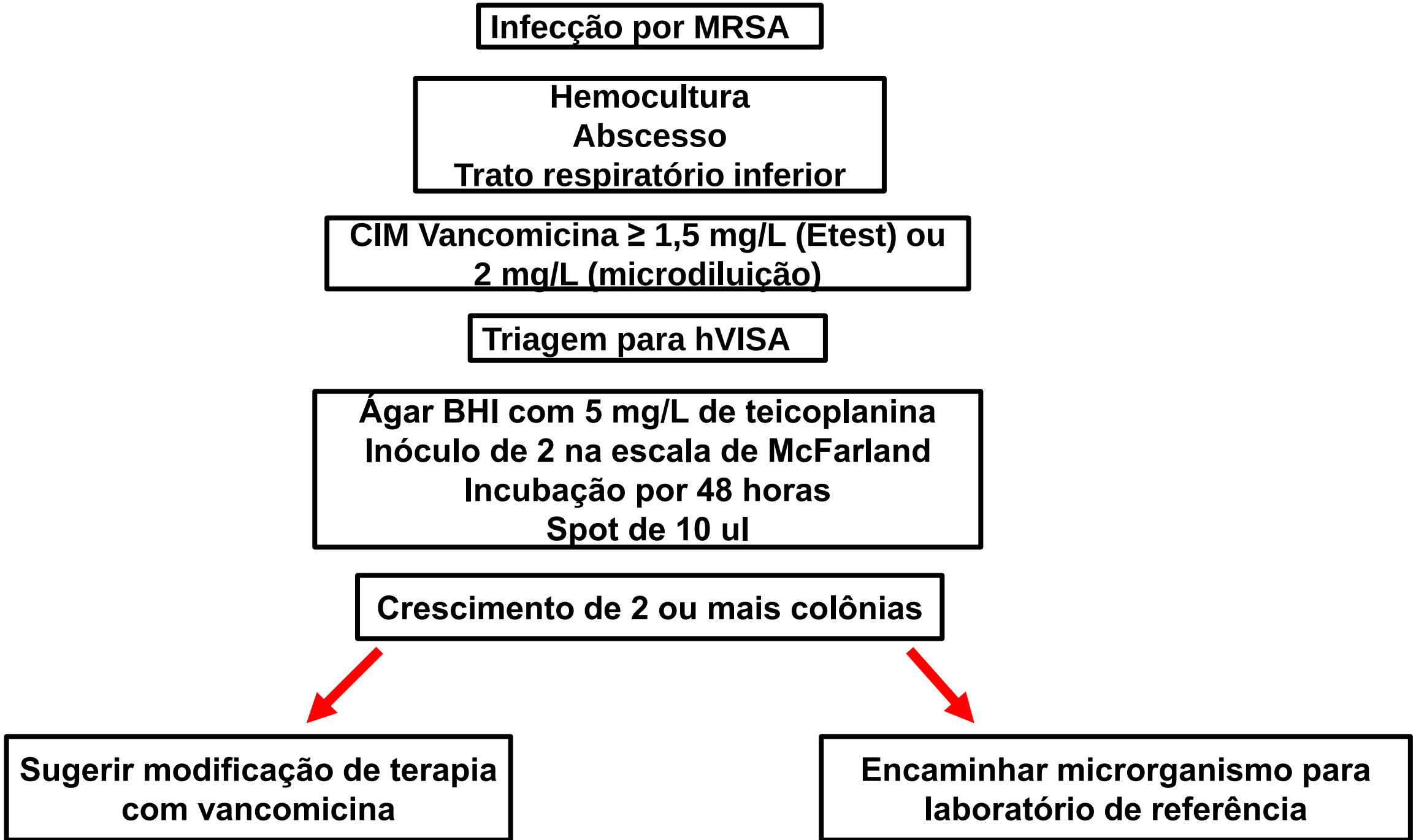
Clinical and Microbiological Characteristics of Heteroresistant and Vancomycin-Intermediate *Staphylococcus aureus* from Bloodstream Infections in a Brazilian Teaching Hospital

PLOS ONE | DOI:10.1371/journal.pone.0160506 August 30, 2016

Thaina Miranda da Costa[1], Priscylla Guimarães Migueres Morgado[1], Fernanda Sampaio Cavalcante[1], Andreia Paredes Damasco[1], Simone Aranha Nouér[2], Kátia Regina Netto dos Santos[1]*

Table 4. Microbiological characteristics of six *Staphylococcus aureus* isolates presenting vancomycin MIC of 2 mg/L with at least one screening test positive to detect heterogeneous vancomycin intermediate resistance (hVISA).

Isolatenumber	Methicillin resistance/SCC*mec* type	Broth microdilution test MIC (mg/L)[a]			Screening plates (48h of incubation)				Etest macro[b] (μg/mL)		Etest GRD[b] (μg/mL)		PAP-AUC ratio[c]	Interpretation (Clonality)
		TEI	OXA	DAP	BHIa3	BHIa4	BHI4ca	BHIa6	VAN	TEI	VAN	TEI		
1594	SCC*mec*II	0.5	128	2	+	+	+	-	3	16	1	3	1.15	hVISA (USA100/ ST5/CC5)
1636	SCC*mec*IV	0.25	8	1	+	-	-	-	4	4	1	1.5	0.50	False positive
1588	MSSA	0.25	0.5	1	-	-	-	-	4	12	1	3	ND	NA
1595	MSSA	0.5	0.5	2	-	-	-	-	3	16	0.5	3	ND	NA
1622	MSSA	0.25	0.25	1	+	-	-	-	2	2	0.75	1.5	ND	NA
1691	MSSA	0.25	≤0.2	1	+	-	-	-	6	4	0.75	3	ND	NA


Infecção por MRSA
Hemocultura
Abscesso
Trato respiratório inferior
CIM Vancomicina ≥ 1,5 mg/L (Etest) ou
2 mg/L (microdiluição)
Triagem para hVISA
Ágar BHI com 5 mg/L de teicoplanina
Inóculo de 2 na escala de McFarland
Incubação por 48 horas
Spot de 10 ul
Crescimento de 2 ou mais colônias
Sugerir modificação de terapia
com vancomicina
Encaminhar microrganismo para
laboratório de referência

Mortalidade

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2011, p. 405–410 Vol. 55, No. 1
0066-4804/11/$12.00 doi:10.1128/AAC.01133-10

Systematic Review and Meta-Analysis of the Significance of Heterogeneous Vancomycin-Intermediate *Staphylococcus aureus* Isolates[▽]

Sebastiaan J. van Hal[1]* and David L. Paterson[2]

Study or Subgroup	hVISA Events	hVISA Total	VSSA Events	VSSA Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Bae et al (4)	8	19	16	65	8.7%	2.23 [0.76, 6.50]
Bert et al (5)	1	13	6	35	6.2%	0.40 [0.04, 3.71]
Charles et al (8)	1	5	17	48	5.3%	0.46 [0.05, 4.41]
Fong et al (16)	5	10	19	30	9.9%	0.58 [0.14, 2.46]
Horne et al (24)	12	58	11	56	18.4%	1.07 [0.43, 2.67]
Maor et al (37)	14	27	103	223	22.2%	1.25 [0.56, 2.79]
Musta et al (41)	14	43	67	242	28.3%	1.26 [0.63, 2.53]
Neoh et al (42)	2	2	8	16	0.9%	5.00 [0.21, 120.44]
Total (95% CI)		**177**		**715**	**100.0%**	**1.18 [0.80, 1.72]**
Total events	57		247			

Heterogeneity: Chi² = 4.75, df = 7 (P = 0.69); I² = 0%
Test for overall effect: Z = 0.84 (P = 0.40)

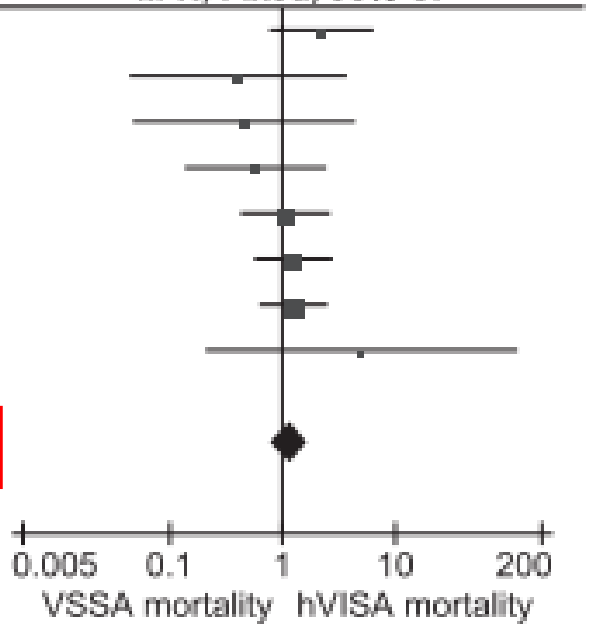


FIG. 2. Forest plot (using Mantel-Haenszel analysis) of 30-day mortality in hVISA- compared to VSSA-infected patients with "events" denoting deaths in each group. Squares indicate point estimates, and the size of the square indicates the weight of each study.

Mortalidade

Table 3. Clinical characteristics of the six patients with bloodstream infections caused by vancomycin intermediate *Staphylococcus aureus* (VISA) isolates.

Isolate number	Cause of admission	Gender	Charlson score	Dialysis	Ward	Previous therapy	Treatment after bacterial isolation	Outcome (number of days from therapy to outcome); Type of VISA infection
1579	Community-acquired Pneumonia	M	6	Acute hemodialysis	ICU	amx + sul + azm// tzp // tei + tzp //tei //van + mem + flu	van + mem + flu	Death (6); VAP infection
1582	Bacteremia (VISA) due to dialysis	F	4	Peritonial dialysis	Nephrology	Amk	van + amk//van	Discharge (13); Bacteremia related to dialysis
1616	Liver transplantation	F	4	Acute hemodialysis	ICU	nor + cro // sul + flu + amx// van + pmb + mem	Van	Death (4); Bacteremia due to central vascular catheter
1698	Infection (VISA) after vascular surgery	M	3	No	Internal Medicine	van // sxt // fep// tei	tei // dap // sxt + dap // gen + dap// dap // dap + fep // cip	Discharge (59); Nosocomial osteomyelitis with endocarditis
1638	Cancer	F	6	No	Oncology	fep // fep + van	fep // fep + van // oxa	Discharge (36); Bacteremia due to peripheral vascular catheter
1645	Bacteremia due to hemodialysis	M	2	Chronic hemodialysis	Nephrology	Cip	cfz // oxa + amk// cfz	Discharge (16); Bacteremia due to central vascular catheter

Perspectivas Futuras

Realização da microdiluição em caldo

Estudos clínicos para avaliar impacto na mortalidade

Programa de detecção e monitoramento da resistência, em parceria com o CGLAB

Epidemiologia molecular de MRSA no Brasil

Validação da pré-difusão

Estudo do MALDI-TOF com amostras brasileiras

Utilização de outras alternativas terapêuticas: tigeciclina, daptomicina, linezolida, clindamicina, sulfametoxazol-trimetoprim

Uso de marcador molecular

Conclusões

A detecção da diminuição da susceptibilidade à vancomicina é um grande desafio

Negligenciada no Brasil

Cara ➔ elitizada

Problema grave de saúde pública

Associada à morbidade e mortalidade

Imprescindível a criação de programas públicos para detecção, controle e monitoramento

Fundamental para diminuição de mortalidade e custos em saúde

Obrigado!

Alessandro C. O. Silveira
alessandro.silveira@dasa.com.br
acosilveira@furb.br
(47) 99974-1213