



Comparison of vancomycin and teicoplanin disk and agar screen procedures for detection of vancomycin intermediate *S. aureus*

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Revised Abstract

Background: Agar screen is recommended for detection of possible vancomycin intermediate *S. aureus* (VISA). Vancomycin agar is used in the CLSI method and teicoplanin agar is used in some countries and by the EARSS surveillance program for detection of suspected VISA. Vancomycin or teicoplanin disk testing has not been reliable in detecting VISA. This study was undertaken in order to compare vancomycin and teicoplanin disk and agar screen results against vancomycin susceptible and intermediate *S. aureus*. **Method:** 40 *S. aureus* (20 VISA and 20 MRSA), 3 *S. aureus* and 2 *E. faecalis* QC strains were tested by vancomycin screen using 10µL inoculum (0.5 McFarland) on Brain Heart Infusion agar (BHIA) with 6 µg/mL vancomycin and by teicoplanin screen using 10 µL inoculum (2 McFarland) on Mueller Hinton agar (MHA) with 5 µg/mL teicoplanin, at 24 and 48 hours. Each strain was also tested by CLSI and BSAC disk methods using vancomycin 5 µg disks and 2 lots of teicoplanin 30 µg and vancomycin 30 µg disks. Vancomycin MICs for all study strains were confirmed by CLSI broth microdilution and Etest. **Results:** 19 and 16 of 20 VISA were detected at 48 hours by teicoplanin and vancomycin screen, respectively. 4 susceptible strains grew on teicoplanin screen and 0 grew on vancomycin screen plates. Disk performance was very poor with standardized CLSI and BSAC methods. However, detection of 12 of 20 VISA was obtained using CLSI disk method with vancomycin 5 µg disk and susceptible breakpoint of ≥ 12 mm.

Number of strains at each MIC (µg/mL)

Method	8	4	2	1
Broth Microdilution (CLSI)	1	19	8	12
Number resistant by CLSI/BSAC disk:				
Vancomycin 5 µg (Mast)	1/0	11/6	0/0	0/0
Teicoplanin 30 µg (Mast)	1/0	6/0	2/0	0/0
Teicoplanin 30 µg (Oxoid)	0/0	3/0	1/0	0/0
Vancomycin 30 (Mast)	0/0	1/0	0/0	0/0
Vancomycin 30 (Oxoid)	0/0	0/0	0/0	0/0
Number positive growth by agar screen (24/48 hours)				
Teicoplanin (5 µg on MHA)	1/1	17/18	4/4	0/0
Vancomycin (6 µg on BHIA)	1/1	12/15	0/0	0/0

Conclusion: Greater sensitivity was obtained with the teicoplanin compared to the vancomycin screen method for detection of VISA. Current vancomycin and teicoplanin disk methods are not reliable for detection of VISA.

Introduction

Agar screen methodologies are widely used for detection of vancomycin intermediate and resistant *Staphylococcus aureus*. A vancomycin screen method using brain heart infusion agar is recommended by CLSI. Alternative methods for detecting glycopeptide non-susceptible strains, utilizing teicoplanin containing agar, are used in Europe. In this study, the teicoplanin agar screen method recommended by the European Antimicrobial Resistance Surveillance System (EARSS) and the CLSI vancomycin agar screen method were compared against 20 VISA and 20 MRSA. Both vancomycin and teicoplanin disk results (utilizing CLSI and BSAC methodologies) and Etest MICs were also determined for each study isolate.

Materials & Methods

Agars:

Mueller Hinton Agar (MHA), BD Biosciences, Sparks, MD
IsoSensitest Agar (ISA), Oxoid Ltd., Cambridge, U.K.
Vancomycin screen plates – Brain Heart Infusion (BHI) Agar + 6 µg/mL vancomycin, BD Biosciences
Teicoplanin screen plates – Mueller Hinton Agar (MHA) + 5 µg/mL teicoplanin, LSI prepared with MHA, BD Biosciences

Disks:

Vancomycin (5 µg)–Mast Group Ltd., Merseyside, UK
Vancomycin (30 µg)–Oxoid Ltd. and Mast Group Ltd.
Teicoplanin (30 µg)–Oxoid Ltd. and Mast Group Ltd.

Etest Strips:

Vancomycin (VA) 0.016-256 µg/mL (AB Biodisk, Piscataway, NJ)
Teicoplanin (TMO) 0.016-256 µg/mL (AB Biodisk, Piscataway, NJ)

Bacterial Strains

20 VISA (Vancomycin MICs from 4 to 8 µg/mL, confirmed VISA by CDC)
20 MRSA (Vancomycin MICs from 1 to 2 µg/mL)
S. aureus ATCC 29213 and ATCC 25923

Testing Site:

Laboratory Specialists, Inc., Westlake, OH

Etest Procedure:

Each isolate was tested according to manufacturer's instructions.

Agar Screen and Disk procedure:

Procedure	Method	Media	Inoculum	Read Time (h)
Vancomycin Screen	CLSI	BHI – 6 µg/mL vancomycin	10 µL of a direct colony suspension, turbidity equivalent to a 0.5 McFarland, to plate	24, 48
Teicoplanin Screen	EARSS	MHA – 5 µg/mL teicoplanin	10 µL of a stationary phase culture to plate	24, 48
Vancomycin, Teicoplanin Disk	CLSI	MHA	Agar plate streaked with a direct colony suspension, turbidity equivalent to a 0.5 McFarland	24
Vancomycin, Teicoplanin Disk	BSAC	ISA	Agar plate streaked with a 1:10 dilution of a direct colony suspension, turbidity equivalent to a 0.5 McFarland	24

Breakpoints used for Analysis:

Vancomycin and Teicoplanin MIC ≤ 2 Susceptible, 4-8 Intermediate
Vancomycin and Teicoplanin 30 µg disks ≥ 15 Susceptible
Vancomycin 5 µg disks ≥ 12 Susceptible

Results

Etest:

- Of the 40 *S. aureus*, 39 (97.5%) of Etest vancomycin MICs were within ± 1 doubling dilution of the broth microdilution reference MICs.
- Of the 20 VISA, 18 (90%) were detected by Etest. Etest MICs for two strains with broth microdilution MICs of 4 mcg/mL were 1 and 2 mcg/mL.

Disk (See Table 1, Figures 1-2):

- Of total vancomycin 30 µg disk results (both methods), only one VISA was detected.
- No VISA were detected with teicoplanin 30 µg disk by BSAC method
- 6 and 3 VISA were detected with teicoplanin 30 µg disk (Mast and Oxoid disks, respectively)
- 12 and 6 VISA were detected with vancomycin 5 µg disk by CLSI and BSAC methods, respectively
- If susceptible breakpoint is ≥ 14 mm for vancomycin 5 µg disk, all but one VISA would be detected using BSAC method.

Table 1: Number of Strains Resistant at each Vancomycin MIC by CLSI/BSAC Disk Methodologies

Disk Method (CLSI/BSAC)	Vancomycin MIC (µg/mL)				False S	False NS
	8	4	2	1		
Vancomycin 5 mcg (Mast)	1/1	14/9	0/0	0/0	25/50%	0/0%
Teicoplanin 30 mcg (Mast)	1/0	8/0	2/0	0/0	55/100%	10/0%
Teicoplanin 30 mcg (Oxoid)	0/0	6/0	1/0	0/0	70/100%	5/0%
Vancomycin 30 (Mast)	0/0	5/0	0/0	0/0	75/100%	0/0%
Vancomycin 30 (Oxoid)	0/0	0/0	0/0	0/0	100/100%	0/0%

Total # of Strains at each MIC

1 19 8 12

Intermediate Susceptible

Figure 1: Number of results at MIC/zone by CLSI method

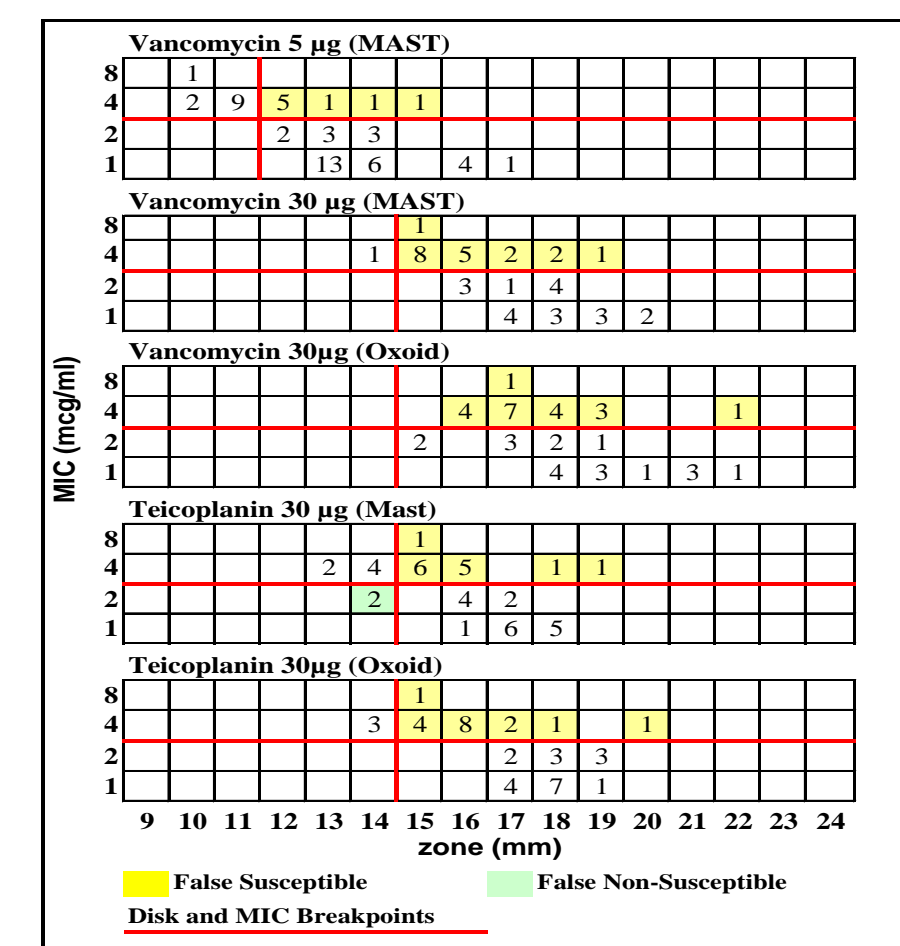
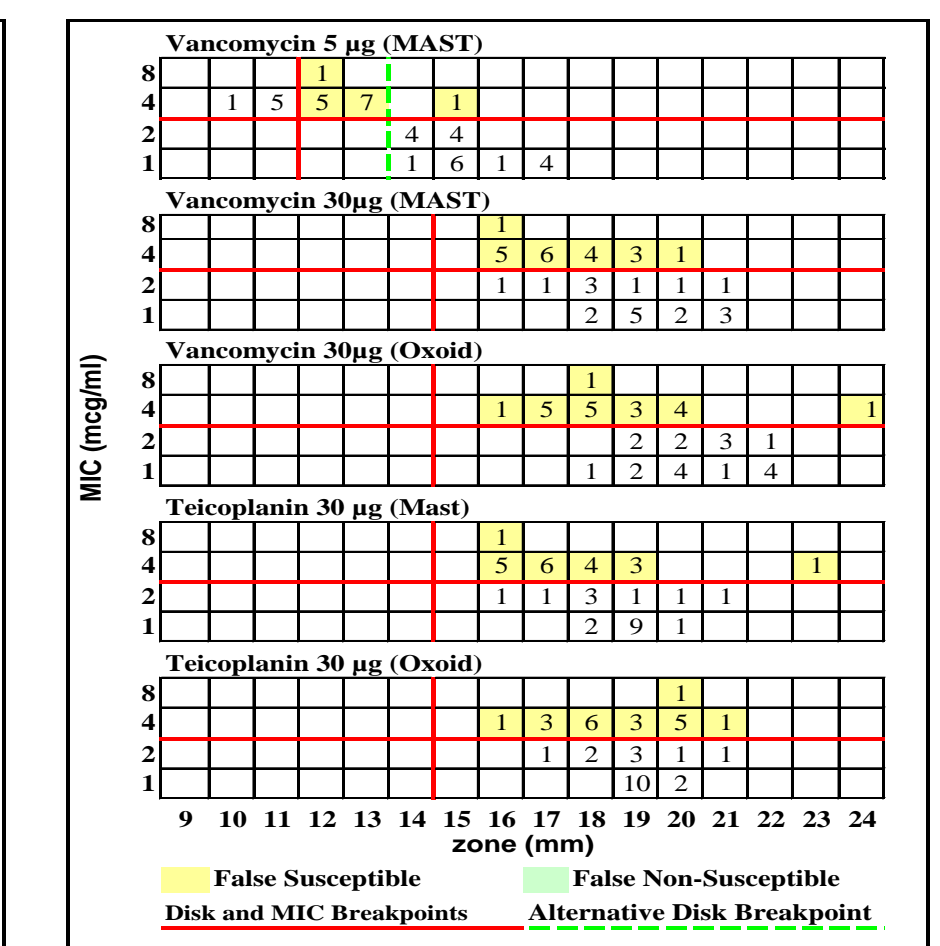


Figure 2: Number of results at MIC/zone by BSAC method



Agar Screen (See Table 2):

- Teicoplanin: 19 and 18 of 20 VISA were detected at 48 and 24 hours, respectively
- Vancomycin: 16 and 13 of 20 VISA were detected at 48 and 24 hours, respectively
- 4 susceptible strains grew on teicoplanin screen and 0 grew on vancomycin screen plates

Table 2: Number of Strains Positive at each Vancomycin MIC by Teicoplanin and Vancomycin Agar Screen Methodologies

Agar dilution method	Vancomycin MIC (µg/mL)				False Neg	False Pos
	8	4	2	1		
Teicoplanin (24h)	1	17	4	0	10.0%	20.0%
Teicoplanin (48h)	1	18	4	0	5.0%	20.0%
Vancomycin (24h)	1	12	0	0	35.0%	0.0%
Vancomycin (48h)	1	15	0	0	20.0%	0.0%

Total # of Strains at each MIC

1 19 8 12

Intermediate Susceptible

Conclusions

- Greater sensitivity was obtained with the teicoplanin screen method compared to the vancomycin screen method for detection of VISA
- Current vancomycin and teicoplanin disk methods are not reliable for detection of VISA and elimination of these methods for *S. aureus* from standardized disk guidelines is suggested.
- In this study, the best disk performance was achieved with vancomycin 5 µg disk, BSAC method and susceptible breakpoint of ≥ 14 mm, and may be an alternative disk method that would require further evaluation.

References

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