Detection of Methicillin Resistant *Staphylococcus aureus* with Reduced Susceptibility to Vancomycin

Thesis

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Maha Soliman Abdel Hamid

List of Contents

List of Abbreviations

Abb.	Full term
BHI	*Brain heart infusion*
BHIAVA	*Brain heart infusion agar with vancomycin*
BMD	*Broth Microdilution*
CDC	*Centers for disease control and prevention*
CLSI	*Clinical and laboratory standards institute*
DHSS	*Department of health and social serives*
DT	*Doubling time*
E-test GRD	*Epsilometer test glycopeptide resistant detection*
E-test	*Epsilometer*
EUCAST	*European committe for antimicrobial susceptibility testing*
GISA	*Glycopeptide intermediate staph. aureus*
hGISA	*Heterogenous glycopeptide intermediate staph. aureus*
hVISA	*Heterogenous vancomycin intermediate staphylococcus aureus*
MALDI-TOF	*The matrix*–assisted laser desorption ionization – time of flight mass spectrometry
MET	*Macromethod E-test*
MHA	*Muller Minton Agar*
MHA5T	*Muller hinter agar with 5mg teicoplanin*
MIC	*Minimal inhibitory concentration*
MRSA	*Methicillin resistant staphylococcus aureus*

List of Abbreviations (Cont...)

Abb.	Full term
NCCLS	*National communitte for clinical laboratory standards*
PAP	*Population analysis profile*
PAP-AUC	*Population analysis profile-area under the curve*
PBP	*Penicillin binding protein*
PG	*Peptidoglycan*
QD	*Quinpristin-dalfopristin*
SVISA	*Slow vancomycin intermediate staph. aureus*
TMP-SMX	*Trimethroprism-sulphamethoxozole*
TSB	*Tyrptone soya broth*
VISA	*Vancomycin intermediate staph. aureus*
VRSA	*Vancomycin resistant staph. Aureus*

List of Tables

List of Tables (Cont...)

List of Figures

INTRODUCTION

Staphylococcus aureus (S.aureus) is a major cause of hospital acquired infections, causing high morbidity and mortality throughout the world. The proportion of methicillin resistant *Staphylococcus aureus (*MRSA) has risen worldwide during the last decades. The recommended treatment for multiresistant MRSA are glycopeptides, particularly vancomycin ***(Wootton et al., 2001).***

Since the emergence of vancomycin resistance in enterococci in 1988 and its in vitro demonstration that its resistance genes *(van A and van B)* are transmissible to other bacterial species including *S.aureus,* emergence of vancomycin resistance in clinical *Staphylococci* has become a great concern ***(Tenover et al., 1998).*** *Staphylococcus aureus* isolates with reduced susceptibility to vancomycin, including those with intermediate susceptibility, are usually associated with worse treatment outcomes ***(Lodise ., 2008).***

Initial reports of reduced vancomycin susceptibility in clinical isolates of *S. aureus* from Japan in 1997 generated significant concern in the medical community. Since that time there has been uncertainty regarding optimal laboratory detection and the clinical relevance of reduced vancomycin susceptibility in *S. aureus.* So Clinical and Laboratory Standards Institute (CLSI) changes the minimal inhibitory concentration (MIC) breakpoints for vancomycin against *S.*

aureus, and there has been increased concern regarding the efficacy of vancomycin for the treatment of *S. aureus* infections ***(Howeden et al., 2010).***

In January 2006, the Clinical and Laboratory Standards Institute (CLSI) updated MIC breakpoints for vancomycin susceptibility testing for *S. aureus* such that an MIC less than 2 ug/mL is considered to represent susceptibility to vancomycin, 4-8 ug/mL intermediate susceptibility and greater than 16 ug/L resistant to vancomycin Additionally, in 2009, the CLSI altered the guidelines for *Staphylococci* such that disk diffusion was no longer an acceptable means for testing vancomycin susceptibility in these organisms ***(Burnham et al., 2010).***

According to CLSI, broth microdilution (BM) is considered the gold standard to determine vancomycin MIC. However, because it is time- consuming, a considerable number of clinical laboratories do not use it as routine methodology. Other techniques have been widely used, with variable sensitivity and specificity, such as E-test and automated systems ***(Rossatto et al., 2014).***

The definition and optimal laboratory detection of hetergenous vancomycin intermediate *S. aureus* (hVISA) remain uncertain. Essentially, hVISA isolate is *a S. aureus* isolate with a vancomycin MIC within the susceptible range when tested by routine methods, but where a proportion of the

population of cells are in the vancomycin-intermediate range ***(Raybak et al., 2015).***

Standardized reference methods for susceptibility testing, such as CLSI broth microdilution, agar dilution, and standard E-test methods, fail to detect hVISA, in part due to the small inoculum, the relatively poor support of growth on Mueller-Hinton agar plates, or a combination of both. Inoculum size is critical to detection of the minor subpopulation of resistant cells. Additionally, hVISA strains are notoriously slow growing, with thickened cell walls and unique pleomorphic features, such as small-colony variants. Screening for hVISA by the population analysis profile-area under the curve (PAP-AUC) method has been the most reliable and reproducible approach but is labor-intensive, costly, and unsuitable for routine use in clinical laboratories ***(Howeden et al., 2010)***.

A variety of alternative methods for detection of the heteroresistant phenotype have been evaluated with varying success e.g. standard E-test, E-test GRD, E-test macromethod, BHI screen agar plates ***(Satola et al., 2011).***

In addition to knowing the appropriate testing methodologies, all laboratories should develop a step by step problem-solving procedure or algorithm for detecting VRSA specifically for their laboratory **(*CDC, 2015).***

AIM OF THE WORK

- To detect the efficacy of phenotypic and automated methods for detection of MRSA with reduced susceptibility to vancomycin
- To determine the best MIC concentration in vancomycin screening agar for detection of VISA among MRSA isolates.

Chapter 1
STAPYLOCOCCUS AUREUS WITH REDUCED SUSCEPTIBILITY TO VANCOMYCIN

Stapylococcus aureus with reduced susceptibilibty to vancomycin is the term that contain both glycopeptide intermediate *Staphylococcus aureus* (GISA) and heterogeneous glycopeptide intermediate *Staphylococcus aureus* (HGISA) ***(Devi et al., 2015).***

Definition

Centres for Disease Control and prevention (CDC) definitions for classifying isolates of *S. aureus* with reduced susceptibility to vancomycin are based on the laboratory breakpoints published by the Clinical and Laboratory Standards Institute (formerly NCCLS), M100-S16; Jan 2006 ***(CDC, 2015).***

- Vancomycin-susceptible S. aureus (VSSA): Vancomycin MIC:≤ 2 µg/ml.
- Vancomycin-intermediate S. aureus (VISA): Vancomycin MIC: = 4-8 µg/ml.
- Vancomycin-resistant S. aureus (VRSA): Vancomycin MIC: ≥ 16 µg/ml. (Table 1)

Table (1): Broth microdilution method for detection of *Stapylococcoi aureus* with reduced susceplibility to vancomycin

Vancomycin interpretation	Phenotypes	Broth Microdilution method (Reference method recommended by CLSI, EUCAST, etc)		
		CLSI interpretation prior to 2006 (in µg/ml)	CLSI interpretation after 2006 (in µg/ml)	EUCAST interpretation till 2015 (in µg/ml)
Susceptible	VSSA	≤ 4	≤ 2	≤ 2
*Heteroresistant	*hVISA	-	-	-
Intermediate	VISA	8-16	4-8	Excluded from the definition
Resistant	VRSA	≥ 32	≥ 16	>2
*Heteroresistant subpopulations remain within susceptible range of vancomycin MIC (1-2 µg/ml)				

(Devi et al., 2015)

Table (2): CLSI and EUCAST breakpoints for vancomycin

Characteristics	hVISA	VISA	VRSA
MIC	1-2 µg/ml	4-8 µg/ml	≥ 16 µg/ml
Mechanism of resistance	Cell wall thickening and hyperproduction of glycopeptide binding targets	Cell wall thickening and hyperproduction of glycopeptide binding targets.	Substitution of D-Ala-D-Ala with D- Ala-D-Lac
Gene encoding for resistance	Endogenous resistance-Chromosomal mutation	Endogenous resistance-Chromosomal mutation	Van A
Recommended methods for detection in CLSI guidelines	-	Vancomycin MIC: E-test, Microbroth dilution method	Vancomycin MIC: E-test, Microbroth dilution method
Recommended methods for detection in EUCAST guidelines	Screening methods (hVISA, VISA and VRSA): Macro E-test, Glycopeptide resistance detection test and Teicoplanin screening agar. Confirmatory testing for hVISA/VISA: Population analysis profile-Gold standard		

(Devi et al., 2015)