Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/f903sd11

A STUDY ON EVALUATION OF LABORATORY DETECTION METHODS AND PREVALENCE RATE OF VANCOMYCIN HETERORESISTANCE IN CLINICAL ISOLATES OF STAPHYLOCOCCUS AUREUS IN A TERTIARY CARE HOSPITAL

Dr. Sharmila R[1], Dr. Vanathi S[2], Dr. Malini P[3], Dr. Hemasuganya P[4*]

[1]Tutor microbiology, Government Pudukkottai Medical College, Pudukkottai, Tamilnadu.
[2]Associate professor, Government Pudukkottai Medical College, Pudukkottai, Tamilnadu.
[3]Associate Professor, Government Medical College, Nilgiris, Tamilnadu.
[4*]Assistant professor, Government Pudukkottai medical College, Pudukkottai, Tamilnadu.

[*]**Corresponding author:** Dr. Hemasuganya P
[*]Assistant professor, Government Pudukkottai medical College, Pudukkottai, Tamilnadu.
Email: hemamanoj88@gmail.com

Abstract

Introduction: Heterogenous vancomycin intermediate *Staphylococcus aureus* hVISA is characterized by the presence of a resistant subpopulation, usually at a frequency of 1 in10 ^{6}, in an otherwise fully susceptible population. Increased cell wall thickness is a consistent feature of these isolates though screening of heteroresistance remains difficult, now It has become necessary for all clinical laboratories to establish and validate methods to detect h-VRSA. To detect vancomycin heteroresistant subpopulation of cells present in methicillin resistant *Staphylococcus aureus* isolates. This study aims to evaluate the screening methods in detection of vancomycin resistance among 100MRSA isolates and to find the prevalence rate of vancomycin heteroresistance among MRSA isolates.

Materials and Methods: A cross sectional study including 100 clinically significant methicillin resistant staphylococcus aureus{MRSA} isolates, selected by detection of Methicillin resistance by cefoxitin disc diffusion method. The following menthods are compared for detection of hVISA. Vancomycin screen agar (BHIA6V), E test GRD, vancomycin broth microdilution, modified population analysis profile.

Results and Discussion: Among 100 isolates screened by vancomycin screen agar method heteroresistance was detected in 5 isolates out of which 3 were confirmed as hVISA by modified PAP-AUC method. By BMD 3 out of 100 isolates showed MIC4µg/ml that was identified as VISA .All these 3isolates were identified as hVISA by PAP-AUC method. E STRIP GRD shows 2/100 isolates are identified as VISA that is found to be heteroresistant strains. By Modified population analysis profile the PAP-AUC curve cut-off result was ≥ 0.9 for 3 isolates that were confirmed to be hVISA.

Conclusion: This study infers that the combined use of screen agar – BHIA with 6µg/ml and BMD values is effective combination approach to detect heteroresistant strains but modified PAP-AUC is the gold standard method till date to detect hVISA as it is the only reliable method to confirm the

heterogeneity of vancomycin susceptibility. From this study the prevalence of hVISA was found to be 3% among MRSA isolates.

Keywords: Vancomycin heteroresistance, hVISA detection, Methicillin-resistant Staphylococcus aureus (MRSA), Screening methods, Modified Population Analysis Profile (PAP-AUC)

INTRODUCTION

Staphylococcus aureus plays an important role in cause of many infections severity ranging from minor superficial infections to deep seated infections in which penicillins and cephalosporins are used as empirical therapy. Due to penicillinase production, β-lactam β-lactamase inhibitor combinations and penicillinase stable penicillins are now used in Methicillin Sensitive *Staphylococcus aureus* infections [1-3]. Methicillin resistant *Staphylococcus aureus* conferred by presence of mecA gene that encodes PBP2a, an enzyme that has low affinity for β-lactamase leads resistance to β-lactamase inhibitor and penicillinase resistant penicillins. Vancomycin has been the treatment of choice for serious infections caused by MRSA. But treatment failure of vancomycin in MRSA bacteremia prompted CLSI to lower cutoff value of vancomycin in the interpretive susceptibility criteria of Staphylococcus aureus to vancomycin [2, 4]. Based on minimum inhibitory concentration values by dilution susceptibility tests to vancomycin, *Staphylococcus aureus* strains are classified as [4] vancomycin susceptible *Staphylococcus aureus* (VSSA 2 µg/mL), vancomycin intermediate *Staphylococcus aureus* (VISA 4-8 µg/mL), vancomycin resistant *Staphylococcus aureus* (VRSA ≥16 µg/mL). Heteroresistance refers to presence within a large population of fully antimicrobial susceptible microorganisms of subpopulation with lesser susceptibility [4]. The phenomenon of heteroresistance is observed in *Staphylococcus aureus*, coagulase negative *Staphylococcus*, *Acinetobacter baumanii*, *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, *Enterococcus faecium*, and *Cryptocooccus neoformans.* Heteroresistant vancomycin intermediate *Staphylococcus aureus* is defined as *Staphylococcus aureus* with a vancomycin MIC within susceptible range when tested by routine methods (vancomycin broth dilution MIC ≤ 2 µg/mL) but where a proportion of cells are in vancomycin intermediate range. It is imperative that the laboratory adopts methods to detect hVISA to maximize the vancomycin therapeutic success. The vancomycin MIC breakpoints were lowered for *Staphylococcus aureus* in order to better detect hVISA strain that are potentially associated with vancomycin therapeutic failure. Due to its clinical importance this study aims in detection of vancomycin heteroresistance among *Staphylococcus aureus* isolates using BHIA - vancomycin screen agar and E strip method.

AIMS/ OBJECTIVES

To detect vancomycin heteroresistant subpopulation of cells present in methicillin resistant *Staphylococcus aureus* isolates.To compare laboratory detection method BHIA Vancomycin screen agar with E strip for detection of vancomycin heteroresistance in *Staphylococcus aureus*.

METHODOLOGY

This cross sectional study was conducted at the Institute of Microbiology, Madras Medical College, Rajiv Gandhi Government General Hospital, chennai. All non duplicate MRSA isolates from various samples from inpatients aged ≥18 years with invasive and noninvasive infections. Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 16. Categorical variables were analyzed using chi square test and Fischer's extract test. Continuous variables were analyzed using unpaired T test. P value < 0.05 was considered to be significant.

SAMPLE COLLECTION AND PROCESSING

All the MRSA isolates included in this study were from samples like pus, blood, intravenous catheter tip, sputum, and urine. Cefoxitin (surrogate marker) disc diffusion method for detection of methicillin resistance in *Staphylococcus aureus*.

BHIA 6V [BRAIN HEART INUSION AGAR WITH 6 µg/mL OFVANCOMYCIN]
Screening method for h-VISA described by Hirmatsu *et al*. All plates were spot inoculated with 10 µL of an inoculum suspension prepared with growth from an overnight blood agar plate, with a turbidity equivalent to 0.5 McFarland standard. Incubate at 37ºc for 24-48hrs.
Growth at 24hrs - VRSA.
Growth at 48hrs - h-RSA.

E STRIP TEST:
E test is a quantitative technique for determining the antimicrobial susceptibility (AST) and MIC (in µg/mL). It is a ready to use reagent strip with predefined gradient of antibiotic for determination of precise MIC values of wide range of antimicrobial agents against different organism groups. When E strip is applied to agar surface there is an instant release of antimicrobial agent from the strips (plastic/porous material) to the agar to form a stable and continuous gradient beneath and in the immediate vicinity of the strip.

PROCEDURE:
1. BHIA plates were prepared and dip a sterile nontoxic cotton swab on a wooden applicator in to the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid.
2. After Streaking the entire agar surface of the plate with the swab three times turning the plate at 60º angle between each streaking. Strip was placed using applicator [it was kept at room temperature for 15-30 min before opening].
3. The applicator along with E strip was placed at desired position on the agar plate with test culture. Gently turn the applicator clockwise with fingers so it will detach from strip**.**
4. After incubation, MIC is always read at the point of complete inhibition of all growth, including hazes, micro colonies and isolated colonies which is indicative of heteronature of culture having resistant subpopulation in it .If the ellipse intersects the strip in between 2 dilutions, read the MIC as the value which is nearest to the intersection.

DILUTIONANTIMICROBIALSUSCEPTIBILITYTESTS [11, 27]
Dilution methods can be carried out in 2 ways, broth dilution and agar dilution method. Both broth and agar dilution methods are used to measure quantitatively the invitro activity of an antimicrobial agent against a given bacterial isolate.

BROTH DILUTION PROCEDURE
Macro dilution: Uses broth volume of 1mL in standard test tubes. Microdilution: Uses about 0.05 to 0.1 mL total broth volume and can be performed in a microtiter plate or tray. In this study the following broth is used as basal medium for control and test strains in micro broth dilution method. Muller Hinton broth II (cation adjusted) with 2% sodium chloride: Muller Hinton broth No 2 control cation is used in the susceptibility testing of rapidly growing aerobic and facultative anaerobic bacteria. This medium has low thymine and thymidine content and also the calcium and magnesium ion concentration is adjusted. Beef extract and casein acid hydrolysate provide nitrogenous compounds, carbon, sulphur and other essential nutrients (Thymine and Thymidine inhibits sulfonamides and trimethoprim, ca and mg interferes with aminoglycosides activity).

AGARDILUTIONPROCEDURE [27]
In this method, antimicrobial agent is incorporated into the agar medium with each plate containing different concentrations of the agent. Prepare intermediate (10x) antimicrobial agent solutions by making successive1:2, 1:4…dilutions by making serial two fold dilutions. Add 1 part of 10x antimicrobial solution to nine parts of molten agar. Add appropriate dilutions of antimicrobial solution to molten test agars that have been at water bath at 45-50ºC. Mix agar and antimicrobial

solution well and pour into petridishes for agar depth of 3-4 mm. Pour plates quickly to prevent partial solidification in mixing container. Avoid generating bubbles when mixing. Allow the agar to solidify at room temperature, store them in sealed plastic bags at 2-8ºC and it can be used up to 5 days. Allow plates to equilibrate to room temperature before use. In this study vancomycin agar dilution is performed in BHIA at following concentrations 0, 0.5, 1, 2, and 4µg/mL.

MODIFIED POPULATION ANALYSIS PROFILE [4, 6, 8]
It was performed as described by Wotton *et al*.

INOCULUM:
Culture was grown overnight in BAP. Inoculum was prepared from direct colony suspension of 0.5 McFarland standards.

PROCEDURE:
The culture plates BHIA with vancomycin at 0.25, 0.5, 1, 2, 4, and 8µg/mL were prepared for agar dilution method. The inoculum was manually spiral plated to BHIA plates with increasing vancomycin concentrations [4, 6]. The control organism Mu 3 (ATCC 700698) was run in parallel with test organism.

INTERPRETATION:
Colonies were counted at end of 48 hrs incubation at 37ºC and a viable count was plotted against the vancomycin concentrations on a logarithmic paper. Using plotted graph of CFU/ml vs. vancomycin concentrations for each isolate AUC is calculated.

RESULT:
A ratio of AUC of test organism to AUC of Mu3 was determined and classified as follows [4]:

CUT OFF VALUES–AUC RATIO	STRAIN
<0.9	VSSA
≥0.9to<1.3	hVISA
≥1.3	VRSA

This cross sectional study was conducted in the Institute of Microbiology Madras Medical College at the Rajiv Gandhi Government General Hospital. A total of 100 clinically significant, consecutive, non-duplicate isolates of MRSA isolates from various clinical specimens were included in this study. All the isolates were identified by standard microbiological procedures.

Table: 1 Age wise distribution of patients with MRSA isolates (n=100)

Age	Number	Percentage (%)
≤20 years	7	7
21-40 years	34	34
41-60 years	38	38
61-80 years	20	20
>80 years	1	1
Total	100	100

The maximum number of isolates were from the patients in the age group of 41-60 years (38%) followed by 21-40 years (34%). Specimen (62%) followed by blood samples.

Table: 2 Distribution of MRSA isolates among various clinical syndromes (N=100)

Clinical Infections	No. of isolates (N=100)	Percentage
Blood stream infections	35	35 %
Skin and soft tissue infections	58	58 %
Lower respiratory tract infections	3	3 %
Bone and joint infections	2	2 %
Ear infections	2	2 %
Total	100	100

Table: 3 Antibiotic sensitivity pattern of MRSA isolates (n=100)

Antibiotic sensitivity pattern	Sensitive	Resistant	Sensitive %	Resistant %	P value Fishers Exact Test
Penicillin [10 U]	0	100	0.00	100.00	<0.0001
Amikacin [30µg]	44	56	44.00	56.00	0.3953
Ciprofloxacin [5µg]	39	61	39.00	61.00	0.1176
Cefoxitin [30µg]	0	100	0.00	100.00	<0.0001
Co-Trimoxazole [1.25/23.75]	2	98	2.00	98.00	<0.0001
Amoxyclav [20/10µg]	0	100	0.00	100.00	<0.0001
Tetracycline [30µg]	37	63	37.00	63.00	0.0637
Chloramphenicol [30µg]	99	1	99.00	1.00	>0.9999
Linezolid [30µg]	100	0	100.00	0.00	>0.9999
Rifampicin [5µg]	100	0	100.00	0.00	>0.9999
Tigecycline [15µg]	100	0	100.00	0.00	>0.9999

Table: 4 Screening Method-Brain Heart Infusion Agar with 6µg/mL of vancomycin (n=100)

Screening Method :Brain Heart Infusion Agar - 6µg/mL of vancomycin	No. of isolates	Percentage (%)
Growth at 48hrs	5	5
No Growth	95	95
Total	100	100

Among100 isolates heteroresistance was detected in 5 isolates as demonstrated by presence of growth at 48hrs.

Table: 5 Minimum inhibitory concentration values by broth micro dilution method and E-strip GRD (n=100)

MIC values (µg/mL)	No. of isolates	Percentage
0.25	5	5
0.5	22	22
1	48	48
2	22	22
4	3	3
Total	100	100

Minimum Inhibitory Concentration Values (µg/mL)	Vancomycin	Teicoplanin
0.5	36	39
0.75	19	20
1	19	12
1.5	19	18
2	4	3
3	1	6
4	2	2
Total	100	100

Table: 6

Area Under the Curve	Number	Percentage
AUC < 9.0	97	97.00
AUC ≥ 9.0	3	3.00
Total	100	100

Out of 100 MRSA isolates 3 were identified as hVISA by PAP-AUC method.

Table: 7

BHIA vs. AUC	AUC Sensitive	AUC Resistant	Total
No growth (Negative)	95	0	95
Growth (Positive)	2	3	5
Total	97	3	100
Sensitivity	97.93%		
Specificity	100%		
PPV	60%		
NPV	100%		

Table: 8

BMD vs. AUC	AUC Sensitive	AUC Resistant	Total
Negative (<2 µg/ml)	75	0	75
Positive (≥ 2µg/ml)	22	3	25
Total	97	3	100
Sensitivity	77.08%		
Specificity	100%		
PPV	12%		
NPV	100%		

Table: 9

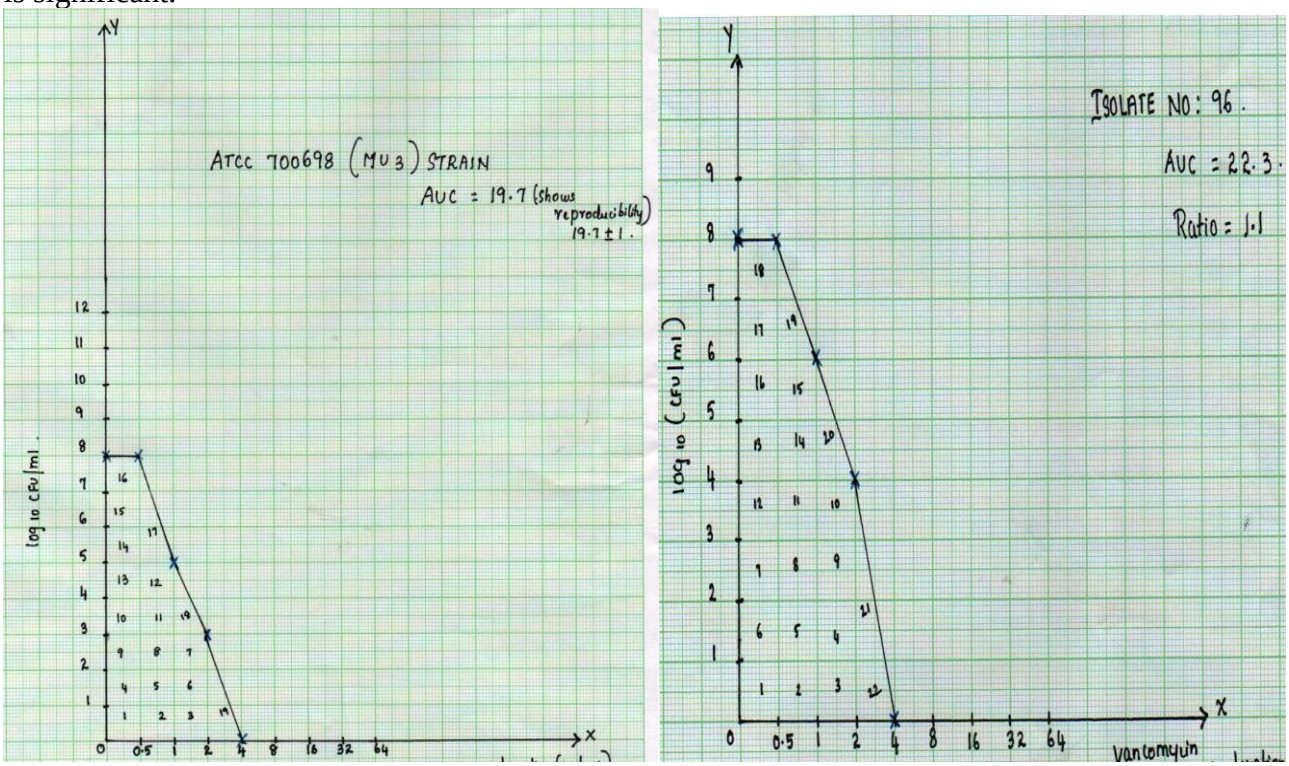
E-strip GRD vs. AUC	AUC Sensitive	AUC Resistant	Total
E-strip GRD Negative (< 2 µg/mL)	86	0	86
E-strip GRD Positive (≥ 2 µg/mL)	11	3	14
Total	97	3	100
Sensitivity	88.54%		
Specificity	100%		
PPV	21.42%		
NPV	100%		

Table: 10 Pattern of Area Under the Curve ratio of MRSA isolates (n=100)

Area Under the Curve Ratio	Sensitive	Resistant	Combined	Sensitive%	Resistant%	Combined%
AUC < 0.3	2	0	2	2.06	0.00	2.00
AUC 0.3–0.6	78	0	78	80.41	0.00	78.00
AUC 0.6–0.9	17	0	17	17.53	0.00	17.00
AUC ≥ 9.0	0	3	3	0.00	100.00	3.00

Area Under the Curve Ratio Distribution	Sensitive	Resistant	Combined
Mean	0.48	0.98	0.49051269
SD	0.10	0.17	0.14
P value Unpaired t-Test			<0.0001

The gold standard modified PAP-AUC showed P value using Unpaired t-Test was < 0.0001 which is significant.



The following graph shows AUC >0.9 that is hVISA strain where X axis- vancomycin concentrations (µg/mL) and Y axis - log10 CFU/mL.

DISCUSSION

This study titled "A Study on Evaluation of Laboratory detection Methods and Prevalence Rate of Vancomycin heteroresistance in clinical isolates of *Staphylococcus aureus* in a Tertiary Care Hospital*"* was conducted at the Institute of Microbiology, Madras Medical College, chennai. This study aims to evaluate the screening methods in detection of vancomycin heteroresistance and to find its prevalence rate of among 100 clinically significant MRSA isolates. The isolates were identified by standard microbiological procedures. The maximum numbers of isolates were from the patients in the age group of 41-60 years followed by 21-40years. (Table 1) 70 % of MRSA isolates were obtained from male patients. Most of the MRSA isolates are isolated from pus specimen in patients with skin and soft tissue infections (62 %) followed by blood samples (30 %) from blood stream infections. (Table2) Methicillin resistance among *Staphylococcus aureus* isolates is

confirmed by cefoxitin disc diffusion method. The susceptibility pattern of MRSA isolates had been determined by Kirby Bauer disc diffusion method. The following is the susceptibility pattern of MRSA isolates to various antibiotics used in the panel. MRSA isolates were sensitive to Chloramphenicol (99 %), Linezolid (100 %), Rifampin (100 %), and Tigecycline (100 %). Majority of the isolates are resistant to Co-trimoxazole 98 % followed by Tetracycline 63 % and Amikacin 56 %, Ciprofloxacin 39 % respectively.

(Table3) Different methods have been proposed to detect VRSA and hVISA with varying degrees of sensitivity and specificity. This study evaluates the performances of three screening methods for detecting clinical isolates of *Staphylococcus aureus* strains with reduced susceptibility to Glycopeptides. In this study, VRSA and hVISA were screened by vancomycin screen agar, BMD and E-strip GRD and confirmed by modified PAP-AUC method. The screening method as described by Hiramatsu *et al.* is simple to perform and is ideal for screening large number of isolates and hence included in CLSI 2024 as screening method to detect vancomycin resistance among *Staphylococcus aureus* isolates [11]. According to this method, vancomycin resistance screening was performed by using BHI agar plates with 6 mg/mL of vancomycin. Heteroresistance was detected in 5 isolates as demonstrated by the presence of growth at 48 hrs (Table 4). Among these 5 isolates two isolates exhibited false positive results on confirmation with PAP which is the gold standard test for detection of heteroresistance. Using the modified PAP analysis as reference test, the screening agar showed sensitivity of 97.93 % and specificity100 % with PPV of 60 % and NPV 100 %. (Table 10) The results of this study are similar to the Indian study done by R Niyer and V Hittinahalli [4], in which the screen agar sensitivity and specificity of the Hiramatsu screening method were found to be 100 % and 93.8 % respectively [3]. In this study, Vancomycin MICs were determined for all isolates by broth microdilution and E- trip GRD [9] it was found that MIC values correlated well with the heteroresistance detected by PAP method. Higher the MIC,more the likelihood of detecting heteroresistance [7, 8].

In a study done by Sarah W. Satola *et al*, reducing the MIC cut-off of vancomycin in broth micro dilution including 2 µg/mL for screening heteroresistance resulted in the highest sensitivity (97 %) giving an hVISA prevalence of 12 %. Though decreasing the MIC cut-off increased the testing sensitivity, there was a corresponding decline in specificity and test accuracy. Various studies have reported that of all the testing methods used for detection of hVIS , vancomycin BMD using a MIC cut-off of 2 µg/ml was the most effective single test with 82% of hVISA detection rate. The addition of PAP-AUC analysis for the isolates having cut-off of 2 µg/mL in vancomycin BMD was the most effective combination for hVISA detection, increasing the efficacy to 100 %. For laboratories that do not have facilities to perform BMD or PAP-AUC, tests can be done by the GRD or standard E-test (with a MIC cut-off of 2 µg/mL) as screening test with high predictive values [7, 9, 10] but to be confirmed by PAP-AUC analysis at referral centres.

In the study by K. Riederer *et al*, the sensitivity, specificity, PPV and NPV of E-strip GRD in detection of hVISA is 70.08 %, 85.8 %, 30 %, and 97 % respectively [29]. From this study we found with reference to the CLSI 2024 break points, 2 of 100 MRSA isolates had been identified as VISA by the E test GRD (MIC4 µg/mL) and 3of 100 isolates were identified as VISA by broth micro dilution method and all these isolates were also found to be heteroresistant strains by PAP-AUC method. One MRSA isolate which was tested to have vancomycin MIC of 3µg/mL by E-strip GRD was also confirmed to be hVISA by PAP-AUC. The sensitivity, specificity, PPV and NPV of vancomycin BMD in detection of hVISA is 77.08 %, 100 %, 12 %, and 100 % respectively and E-strip GRD with 88.54 %, 100 %, 21.42 %, 100 % respectively.(Table 6) The results of all the screening methods were confirmed with a gold standard method-modified PAP method. The gold standard PAP-AUC ratio maximum isolates falls between ratios 0.3 to 0.6 (78%) which were confirmed to be VSSA strains. It correlated with other screening assays like vancomycin BMD and E-strip GRD.3% of isolates showed AUC ratio ≥ 0.9 that were confirmed to be hVISA strains. P value using Unpaired t-Test for Modified PAP-AUC was < 0.0001 which is significant.

All the studies that aimed at detection of h-VISA have employed modified PAP method as a confirmatory test, though it is labour intensive and requires technical expertise and manual dexterity. In this study the hVISA was isolated from 3 patients including one patient who was diagnosed with BSI and was receiving vancomycin and imipenem for three days but expired. Other two hVISA were isolated from pus collected from a patient with SSI and sinus tract discharge from a patient with chronic osteomyelitis. Therapeutic failure was observed with vancomycin in the patient with chronic osteomyelitis but showed clinical improvement after escalation to linezolid therapy. A study done by Alessandra *et al*, showed vancomycin screen agar along with MIC cut-off values ≥ 2μg/mL is an effective combined approach for identifying heteroresistance among *Staphylococcus aureus* [9]. From this study, it is concluded that using vancomycin screen agar combined with BMD is effective approach for screening vancomycin heteroresistance among *Staphylococcus aureus* isolates and that Modified PAP-AUC is the only gold standard method for confirmation of hVISA isolates.

Although, heteroresistance has been reported in strains with vancomycin MICs as low as 0.5 μg/mL [5], in the present study heteroresistance was demonstrated in clinical MRSA isolates with vancomycin MICs of ≥ 2μg/mL.

PREVALENCE OF VRSA, VISA AND hVISA

No VRSA strains were detected in this study. With reference to CLSI 2016 vancomycin MIC interpretive guidelines, 3 % of MRSA isolates were detected as VISA by BMD where as only 2 % of MRSA isolates were tested to be VISA by E-strip GRD.

In the study by R. Niyer, it is implied that prevalence of hVISA among MRSA isolates is 0.5 to 20 % depending on the accuracy of detection methods [4]. In the study by Margaret M. Fitzgibbon *et al* the overall proportion of hVISA isolates was 5.6 %. In a study of MRSA isolates in Ireland in1999, the prevalence was 12% [14]. All the three isolates showed AUC ratio ≥ 0.9 by modified PAP method that is confirmed as hVISA. From this study the prevalence of hVISA was found to be 3 % among MRSA isolates. Although the transmission of VISA infections have not yet been documented, clinical failure in treatment with vancomycin heteroresistant MRSA isolates are reported by many studies including the present study which implies that laboratories need to identify the heteroresistant strains. Interim guidelines have also been issued by CDC for strain identification and infection control measures.

SUMMARY

This cross-sectional study was conducted in the Institute of Microbiology, Madras Medical College recruiting patients from various other departments at the Rajiv Gandhi Government General Hospital, Chennai. This study aims to evaluate the screening methods in detection of vancomycin resistance among 100 MRSA isolates and to find the prevalence rate of vancomycin heteroresistance among MRSA isolates. A total of 100 clinically significant, consecutive, non-duplicate isolates of MRSA isolates from various clinical specimens were included in this study. All the isolates were identified by standard microbiological procedures. The maximum number of isolates was from the patients in the age group of 41-60 years followed by 21- 40years.70 % of MRSA isolates were obtained from male patients. Most of the MRSA isolates are isolated from pus specimens in patients with skin and soft tissue infections (62 %) followed by blood samples (30 %) from blood stream infections.

MRSA isolates by Kirby Bauer disc diffusion method showed following susceptibility pattern: chloramphenicol (99 %), linezolid (100 %), Rifampin (100%), and Tigecycline (100 %). Majority of the isolates were resistant to co-trimoxazole (98 %), followed by tetracycline 63 % and Amikacin 56 %, ciprofloxacin 39 %. Among 100 isolates screened by vancomycin screen agar method heteroresistance was detected in 5 isolates as demonstrated by presence of growth at 48 hrs out of which 3 were confirmed as hVISA by modified PAP-AUC method. Out of 100 MRSA isolates 48 isolates showed vancomycin MIC as 1 μg/mL by BMD method. 3 out of 100 isolates showed MIC 4

µg/mL that was identified as VISA. All these 3 isolates were identified as hVISA by PAP-AUC method. Most of the isolates shows MIC ≤ 2µg/mL for vancomycin (97 %) and teicoplanin (92 %) by E-strip GRD. 2/100 isolates are identified as VISA that are found to be heteroresistant strains. One isolate showing 3 µg/mL as identified as hVISA by PAP-AUC method. By Modified population analysis profile the PAP-AUC curve cut-off result was ≥ 0.9 for 3 isolates that were confirmed to be hVISA. Considering PAP-AUC as gold standard, the BMD and E- trip GRD had a better specificity than the vancomycin screen agar. The vancomycin screen agar however had a good sensitivity. From this study, it is concluded that using vancomycin screen agar combined with BMD is an effective approach for screening vancomycin heteroresistance among *Staphylococcus aureus* isolates and Modified PAP-AUC method is necessary to confirm hVISA isolates.

CONCLUSION

Though screening of heteroresistance remains difficult, now it has become necessary for all clinical laboratories to establish and validate methods to detect hVRSA. Although VRSA and h-VRSA have not become a prevalent problem, as they are capable of transformation into the homogenously resistant strains it is essential to detect this phenotype and institute appropriate infection control measures to prevent the dissemination of such strains in a tertiary care facility. As MIC creep trend keeps changing over in every healthcare setting and as detection of vancomycin resistance remains low, it is associated with vancomycin treatment failure. This can be overcome by imparting screening methods in laboratory to detect this heteroresistant population. The E-test GRD is an expensive test to be performed on all isolates and on other hand population analysis is labour-intensive, time-consuming. Therefore these tests are less suitable for screening and so the screen agar is be suitable for screening large number of isolates for routine use. In conclusion, since E-test screening methods for detecting hVISA and VISA are suboptimal, and since population analysis is not practical, screening for isolates with reduced susceptibility on vancomycin-supplemented BHI agars provides a reliable alternative method.

This study infers that the combined use of screen agar – BHIA with 6 µg/mL and BMD values is effective combination approach to detect heteroresistant strains but modified PAP-AUC is the gold standard method till date to detect hVISA as it is the only reliable method to confirm the heterogeneity of vancomycin susceptibility [11, 12].

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

None.

REFERENCES:

1. Anne Yusof; Evaluation of a New Etest Vancomycin-Teicoplanin Strip for Detection of Glycopeptide-Intermediate Staphylococcus aureus (GISA), in Particular, Heterogeneous GISA; J Clin Micro biol. 2008 Sep; 46(9): 3042–3047.
2. K. Riederer, S. Shemes, P. Chase, A. Musta, A. Mar, and R. Khatib; Detection of Intermediately Vancomycin-Susceptible and Heterogeneous Staphylococcus aureus Isolates: Comparison of Etest and Agar Screening Methods; doi:10.1128/JCM.01435-10
3. Hayati Güneş1, Emel Sesli Çetin2, Selçuk Kaya2 and Buket Cicioğlu Arıdoğan2 ;Investigation of reduced glycopeptid susceptibility among methicillin resistant staphylococci; DOI: 10.5897/AJMR11.1445 .
4. Benjamin P. Howden,1,2,3,4* John K. Davies,3 Paul D. R. Johnson, Timothy P. Stinear,3,4* and M. Lindsay Grayson1,5,6;Reduced Vancomycin Susceptibility in Staphylococcus aureus, Including Vancomycin-Intermediate and Heterogeneous Vancomycin-Intermediate Strains:

Resistance Mechanisms, Laboratory Detection, and Clinical Implications; doi:10.1128/CMR .00042-09.

5. Adina C. Musta, Kathleen Riederer, Stephen Shemes, Patrick Chase, Jinson Jose; Vancomycin MIC plus Heteroresistance and Outcome of Methicillin-Resistant Staphylococcus aureus Bacteremia: Trends over 11 Years;doi:10.1128/JCM.02135-08.
6. RN Iyer, V Hittinahalli; modified pap method to detect heteroresistance to vancomycin among MRSA isolates at a tertiary care hospital; IJMM(2008) ,26(2):176-9.
7. CLSI GUIDELINES: M100-S38 Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement
8. Catherine liu and henry; Staphylococcus aureus with heterogenous resistance to vancomycin, epidemology, clinical significance and critical assesment of diagnostic methods; antimicrob agents chemother.2003 oct;47(10):3040-3045.
9. Sebastian, michael; performance of various testing methodologies for detection of heteroresistant vancomycin intermediate sthaphylocoocus aureus in blood stream isolates; j clin microbiol.2011 apr;49(4):1489-1494.
10. Collee JG, Fraser AG, Marion BP, Simmons A, editors. Mackie and McCartney practical medical microbiology, 14thed.Edinburgh, Churchill Livingstone, 1996.
11. Leonard, S. N., K. L. Rossi, K. L. Newton, and M. J. Rybak. 2009. Evaluation of the Etest GRD for the detection of Staphylococcus aureus with reduced susceptibility to glycopeptides. J. Antimicrob. Chemother. 63:489–492
12. Walsh, T. R., et al. 2001. Evaluation of current methods for detection of staphylococci with reduced susceptibility to glycopeptides. J. Clin. Microbiol.39:2439–2444.