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Detection of vancomycin intermediate and vancomycin resistance in clinical isolates of *Staphylococcus aureus* in Tertiary Care Hospital

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Abstract
Introduction: *Staphylococcus aureus* is a major cause of variety of infections in health-care setting and community. In 1997 first case of infection by *S. aureus* with reduced susceptibility to vancomycin was documented in Japan. Soon several countries reported similar cases of infection due to this mutated pathogens.
Objective: To isolate and identify *Staphylococcus aureus* and to detect VISA and VRSA from *staphylococcus aureus* isolates from clinical specimens.
Material and Methods: A total of 400 Staphylococcal species isolated from the various clinical specimens were included in the study. Specimens were inoculated on Blood agar, Macconkey agar and Thioglycollate broth. The inoculated media were incubated aerobically at 37º C for 18-24 hours. Isolates were identified and confirmed as per laboratory steps of procedure (SOP) by the conventional methods. Vancomycin screen agar test, Epsilometer test (E-test) and Microbroth dilution were performed to detect VISA and VRSA strains. All the isolates were subjected to Antimicrobial susceptibility testing on Mueller-Hinton agar (MHA) by Kirby Bauer disc diffusion method.
Results: Out of 400 cases most of the cases belong to 21-30 years of age (28%). In E test most (64.75%) were found to be sensitive to Vancomycin with MICs of 1 µg/ml and 141 isolates (35.25%) were 2µg/ml while in microbroth dilution mostly (70%) was found to be sensitive to Vancomycin with MICs of 1 µg/ml, followed by 2 µg/ml (30%). Overall *Staphylococcus aureus* were 100% sensitive to Vancomycin and Linezolid both in Kirby-Baur disc diffusion while Erythromycin were least sensitive (47.5%).
Conclusion: Our results suggested that prevention of emergence and transmission of VISA/VRSA in each community is needed.

Keywords: epsilometer test, microbroth dilution, *Staphylococcus aureus*, VISA, VRSA

1. Introduction

Staphylococcus aureus is a major cause of variety of infections in health-care setting and community. It causes a variety of clinical conditions ranging from asymptomatic colonization to different kinds of infections ranging from superficial skin infection to severe infection such as sepsis. Over the last decade, methicillin resistant *S. aureus* (MRSA) strains have become endemic in hospitals worldwide. In addition, it is now incipient community pathogen in many geographical regions [1].

Methicillin resistance in *S. aureus* is mediated by expression of *mecA* gene which results production of modified Penicillin–binding proteins (PBP2a). In addition recently other genes such as *femA* and auxiliary genes are known which can contribute to MRSA resistance [2].

In 1980s, because of widespread occurrence of MRSA, empiric therapy for staphylococcal infections (particularly nosocomial sepsis) was changed to vancomycin in many health care institutions.

In 1997 first case of infection by *S. aureus* with reduced susceptibility to vancomycin was documented in Japan. Soon several countries reported similar cases of infection due to this mutated pathogens [3-5]. The first case of vancomycin-resistant *S. aureus* (VRSA) was reported from the USA in 2002 [6]. Several other reports of isolated cases of VRSA infection have also been documented over the years. Some workers have reported vancomycin resistant staphylococcal stains from Brazil [6] and Jordan [7].

In 2006, due to increase in vancomycin treatment failure CLSI revised the vancomycin breakpoint. According to recent CLSI guidelines MIC of vancomycin was changed from ≤4 µg/ml to ≤2 µg/ml and the isolate having this MIC is considered as VSSA while for VISA the MIC of vancomycin which was initially 8-16 µg/ml was revised to 4-8 µg/ml [8]. The vancomycin MIC for defining VRSA according to which instead of a MIC value of ≥32 µg/ml, isolates with a MIC of ≥16 µg/ml are considered as VRSA [8].

This study was undertaken to find magnitude of this problem and to reduce therapeutic failure in such detection of VISA and VRSA will help in modification of treatment in patients and make the clinicians aware of this impending public health disaster, if inappropriate use of this glycopeptide is continued.

2. Material and Methods

The present study was conducted in Bacteriology Laboratory of the Department of Microbiology SMS Medical College & Attached Hospital, Jaipur, Rajasthan from April 2017 to March 2018. A total of 400 Staphylococcal species isolated from the various clinical specimens were included in the study. Specimens were inoculated on Blood agar, Mac Conkey agar and Thioglycollate broth as per the standard laboratory protocol. The inoculated media were incubated aerobically at 37º C for 18-24 hours. Isolates were identified and confirmed as

per laboratory steps of procedure (SOP) by the conventional morphological and biochemical tests which included: Colony morphology, Gram staining, Catalase test, Coagulase test (both slide and tube coagulase tests), Mannitol fermentation, Oxidative fermentation test. Vancomycin screen agar test, Epsilometer test (E-test) and Microbroth dilution were performed to detect VISA and VRSA strains. All the isolates were subjected to Antimicrobial susceptibility testing on Mueller-Hinton agar (MHA) by Kirby Bauer disc diffusion method as recommended by CLSI guidelines (2016).

3. Results

Out of 400 cases most of the cases belong to 21-30 years of age (28%) followed by ≤10 years of age 17.5%, 11-20 years of age 14%. Out of 400 cases 269 (67.25%) were male and 131 (32.75%) were female. Male to female ratio was 2.05:1. Out of 400 isolates most 149(37.2%) were from urine followed by blood 97(24.2%) while the least was throat swab 06(1.5%)

MIC value of E -Test

In E test out of 400 isolates 259(64.75%) were found to be sensitive to Vancomycin with MICs of 1 µg/ml and 141 isolates (35.25%) were 2µg/ml while none of the isolates were with MICs of 3 µg/ml and 4µg/ml.

Micro broth dilution

In microbroth dilution out of 400 isolates 280 (70%) were found to be sensitive to Vancomycin with MICs of 1 µg/ml, followed by 2 µg/ml (30%). None of the sample was found to be MIC value 4µg/ml or ≥ 4µg/ml.

Antibiogram of *Staphylococcus aureus*

Out of 400 isolates all *Staphylococcus aureus* were 100% sensitive to Vancomycin and Linezolid both. We also observed that out of 400 most (86.3%) were sensitive to gentamycin followed by Piperacillin/Tazobactum (72.5%), Ciprofloxacin (69.5%) while Erythromycin were least sensitive (47.5%)

Magnitude of MRSA

Out of 400 *staphylococcus aureus* isolates, 192(48%) are MSSA and 208(52%) are MRSA

Table 1: showing MIC value of E –Test

MIC Value	Number	Percentage %	Inference
1	259	64.75	VSSA
2	141	35.25	VSSA
3	0	0	VSSA
4	0	0	VSSA
Total	400	100	VSSA

Table 2: showing MIC according to Micro broth dilution

MIC value(µg/ml)	Number	Percentage %	Interference
1	280	70	VSSA
2	120	30	VSSA
4	00	00	VSSA
8	00	00	VSSA
16	00	00	VSSA
Total	400	100	VSSA

Table 3: Susceptibility pattern of *S. aureus* according to Micro broth dilution

Types	Number	Percentage %
VSSA	400	100
VISA	00	0.0
VRSA	00	0.0
Total	400	100

Table 4: Antibiogram of *Staphylococcus aureus*

	Antibiotic Susceptibility Pattern			
Antibiotics (Potency)	Resistant	Resistant %	Sensitive	Sensitive %
Amikacin 30mcg	156	39	244	61
Augmentin 20/10mcg	163	40.7	237	59.3
Cefepime 30mcg	176	44	224	56
Cefoxitin 30mcg	208	52	192	48
Ciprofloxacin 5mcg	122	30.5	278	69.5
Clindamycin 2mcg	180	45	220	55
Cotrimoxazole 1.25	170	42.5	230	57.5
Doxycycline30mcg	169	42.2	231	57.8
Erythromycin 15mcg	210	52.5	190	47.5
Gentamycin 10mcg	51	12.7	349	86.3
Linezolid 30mcg	0	0.00	400	100
Piperacllin/Tazobactam 100/10mcg	110	27.5	290	72.5
Teicoplanin 30mcg	129	32.2	271	67.8
Vancomycin 30mcg	0	0.00	400	100

4. Discussion

The rate of resistant strains occurrence is associated to lack of strict measures in the use of antibiotics in hospitals by both health practitioners and the community. It is a well-known fact that development of antibiotics resistance in most organisms is associated with unjustified, irrational and irregular use of antibiotics by the human population, over the counter accessibility without recommendations and unrestricted use of antimicrobials in poultry, farm animals, and fisheries [9].

In this study we found that most of the cases 28% belong to 21-30 years of age followed by ≤10 years of age 17.5% and the least was 71-80 years of age 1%. Our results are in accordance with Anjali Kulshrestha *et al.* [10] who also reported 21-30 yrs of age group were predominantly affected followed by 51-60 years of age. A study conducted by Anowai C.O *et al.* [11] who reported high isolation rate was observed in age group 20-29 years (32.9%) followed by 30-39 years (28.8%).

In this study out of 400 cases 269 (67.25%) were male and 131 (32.75%) were female. Male to female ratio was 2.05:1. Our results are in accordance with Anjali Kulshrestha [10]

who reported male to female ratio 2.5:1.There is various studies who reported male outnumbered female[12]. However Anowai C.O *et al.* [11] reported female outnumbered male.

In this study out of 400 cases most 149(37.2%) were isolated from urine followed by blood 97(24.2%), pus 45(11.2%), CSF 22(5.5%) and the least was throat swab 6(1.5%). Our findings are in accordance with Anowai C.O *et al.* [11] who also reported most of the cases were isolated from urine. In a similar study carried out by Shittu and Lin [13] reported more than 80% of the total numbers of isolates recovered were from infected wounds. Adetayo *et al.* [14] reported that 52.2% of *S. aureus* from clinical specimens were recovered from urine specimens, 30.4% from wounds swab, 13.0% ear swab and 4.3% from nasal swab. These findings showed that *S. aureus* is ubiquitous in its distribution, and although it is a normal flora of the anterior nares, skin, and genital tract of humans, it can cause infections in virtually any organ or system of the body; infections of wounds, skin, soft tissue, blood, and the lower urinary tract are particularly common.

In this study we found that all *Staphylococcus aureus* were 100% sensitive to Vancomycin and Linezolid both. We also observed that out of 400 most (86.3%) were sensitive to gentamycin followed by Piperacillin/Tazobactum (72.5%), Ciprofloxacin (69.5%), Teicoplanin (67.8%) while Erythromycin were least sensitive (47.5%). The findings on vancomycin and linezolid substantiated other reports that Vancomycin remains the reference standard for the treatment of systemic infection caused by MRSA, as a result of its relatively clean safety profile, its durability against the development of resistance, and, for many years, the lack of other approved alternatives.

In this study we found that out of 400 isolates 208(52%) were Cefoxitin resistant which confirms that 52 % *Staphylococcus aureus* were MRSA while 48% were MSSA. We also observed that all the MRSA strains were sensitive to Linezolid, and Vancomycin. Our results are in accordance with Anjali Kulshrestha *et al.* [10] who reported 51% MRSA strain in their study. Vidhani and Mehndiratta *et al.* [15] showing a prevalence rate of 51.6% and almost comparable to the study conducted by Majumdar *et al.* [16], in 2001 and Assadullah *et al.* [17], in 2003 showing 52.9% prevalence rate. However Verma *et al.* [18] reported a higher prevalence rate of 80.89% in Indore and Mehta *et al.* [19] reported a lower prevalence rate of 24% in Chandigarh. This variation in prevalence may be because of several factors like different Geographical and environmental conditions, population group under study, healthcare facilities available in the particular hospital, implementation and monitoring of infection control committee, rationale antibiotic usage which varies from hospital to hospital. We have 52% prevalence rate of MRSA in our hospital
setup. It therefore calls in for better vigilance an implementation of more effective MRSA surveillance programmed complemented with improved infection control practices [10].

In this study we performed vancomycin screen agar test for all the isolates. Out of 400 isolates we did not found any growth on this agar. All the isolates were vancomycin susceptible. Similarly Yogesh Kumar Gupta *et al.* [20], and Ankur Goyal *et al.* [21], had reported no vancomycin resistance by Disc diffusion in Rajasthan and Agra respectively.

The E- test is basically an agar diffusion method. It consists of plastic strip calibrated with a MIC scale in µg/ml and codes to identify the antimicrobial agent. The E-test is a preformed, predefined, stable and it is not dependent on diffusion [22].

In this study out of 400 isolates 228(57%) were found to be sensitive to Vancomycin with MICs of 2 µg/ml, followed by 81(20.25%) were 3 µg/ml while 91 (22.75%) isolates were 4µg/ml. All the isolates which are processed for observing Glycopeptide Resistance (Vancomycin) were found to be susceptible to Vancomycin. No VISA or VRSA strains were observed. Our results are in accordance with Flora Grace M [22] who also reported no VISA or VRSA strains were observed in their study.

In 2012, the CLSI defined the vancomycin MIC ≥ 16 mg/mL for VRSA, based on the broth microdilution method [23].

In this study we found that out of 400 isolates 280 (70%) were found to be sensitive to Vancomycin with MICs of 1 µg/ml, followed by 2 µg/ml (30%). None of the sample was found to be MIC value 4µg/ml or more. Our results are in accordance with Anjali *et al.* [10] who also reported all the isolates were vancomycin susceptible. None of the isolates were either intermediate or resistant to vancomycin.

At present when the infections due to MRSA have become a serious public health concern; the development and rapid spread of resistance of *S. aureus* to the reserve drug (vancomycin) is very fearsome and immediate actions should to be taken by the responsible authorities to halt it [24].

5. Conclusion

We need to encourage and facilitate adherence to recommended prevention and control guidelines, conduct active surveillance to detect the emergence of these organisms, and ensure vigorous antibiotic stewardship by health care providers. Our results suggested that prevention of emergence and transmission of VISA/VRSA in each community is needed. So, use of proper infection-control practices, appropriate antimicrobial agent management, maintaining a clean environment and increased awareness can control the spread of antimicrobial-resistant microorganisms, including VRSA.

6. References

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