International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Screening and Molecular Characterization of VISA and VRSA among the MRSA Isolates at a Tertiary Care Centre, Kanpur

Nashra A[1], Sujatha R[2], Deepak Sameer[3], Anil Kumar[4], Ajay Bagoliwal[5]

[1]Ph.D. Scholar, [2]Professor and Head, [3]Tutor, Department of Microbiology, [4]Assistant Professor, Central Research Laboratory, [5]Professor, Department of Community medicine, Rama Medical College Hospital and Research Centre, Mandhana, Kanpur (UP) India.

Corresponding Author: Sujatha R

ABSTRACT

Background: Glycopeptides such as vancomycin are frequently the antibiotics of choice for the treatment of infections caused by methicillin resistant *Staphylococcus aureus* (MRSA). For the last few years incidence of vancomycin intermediate *S. aureus* and vancomycin resistant *S. aureus* (VISA and VRSA respectively) has been increasing in various parts of the world. The present study was carried out to find out the presence of VISA and VRSA among the MRSA isolates and its Molecular characterization at a tertiary care centre, Kanpur.
Objective: Screening and molecular characterization of VISA and VRSA among the MRSA isolates and its prevalence in Kanpur region.
Materials and Methods: Clinical samples were processed from hospitalized patients in Rama Medical College Hospital & Research Centre Mandhana, Kanpur. Out of 140 S. aureus isolates 40 were found MRSA by disk diffusion method, which were then confirmed as VISA and were VRSA by E-test, Vitek and MIC. Bacterial DNA was extracted and PCR for the detection of Mec A and Van A gene was performed.
Results: Out of 140 *S. aureus* isolates, the Prevalence of MRSA was 40 (28.57%) by disk diffusion method, in which 8 (5.7%) were confirmed as VISA and 2 (1.42%) were VRSA by E-test, Vitek and MIC. The molecular characterization of the test isolates detects the presence of Mec A gene, which was confirmed by sequencing but there was no Van A gene detected among these isolates.
Conclusion: The present study reveals the prevalence of MRSA/VISA and VRSA from the Kanpur region and indicates the magnitude of antibiotic resistance in and around the study area. Absence of Van A gene does not rule out that these strains are not VRSA or VISA.
Key words: Molecular characterization, VISA, VRSA, MRSA isolates

INTRODUCTION

Staphylococcus aureus is a leading cause of hospital acquired infection (HAI) gives rise to enormous burden to the health care system significantly affecting the patient's morbidity and mortality.

S. aureus is also leading cause of nosocomial infections, including bacteremia, surgical wound infections, as well as pneumonia. [1-3] About one quarter of healthy people carry one or more strains asymptomatically at any given time, and infections are commonly endogenous being caused by the patient's colonizing strain. [4] Methicillin resistance was first detected in *S. aureus* in 1961, [5] shortly after the agent was introduced clinically; and over the last four decades, there has been a global epidemic of methicillin-resistant *S. aureus* (MRSA). [6,7] MRSA is usually acquired during exposure to hospitals and other healthcare facilities and causes a variety of

serious healthcare-associated infections. The problem is exacerbated by the propensity of the organism to cause cross-infection and its ability to colonize individuals for months or years. Considerable selection pressure for this organism is applied in the hospital setting due to the now intensive use of the many antibiotics, particularly cephalosporins, to which the organism is resistant.

Vancomycin has been regarded as the first-line drug for treatment of MRSA. Unfortunately there has been an increase in the use of this antibiotic for other infections, such as pseudomembranous colitis due to *Clostridium difficile* and coagulase-negative staphylococcal infections in hospitalized patients. [8,9] When this drug was introduced in 1858, it was perceived that there would be no resistance to this antibiotic as resistance was very difficult to induce. [10] However, in 1997 the first strain of *S. aureus* with reduced susceptibility to vancomycin was reported from Japan [11] Since then, there has been an increase in the number of cases with both VISA and VRSA (vancomycin-intermediate and vancomycin-resistant *S. aureus*). This has triggered off alarms in the medical community as *S. aureus* causes life-threatening infections in hospitalized and non-hospitalized patients [12] as Vancomycin is the main antimicrobial agent available to treat serious infections with MRSA but unfortunately, decrease in vancomycin susceptibility of *S. aureus* and isolation of vancomycin-intermediate and resistant *S. aureus* have recently been reported from many countries. [13] Many reports from north India also recorded the emergence of low level and intermediate vancomycin resistance. [32,14-18]

MATERIALS AND METHODS

Study Site and Population

It was a cross sectional study which was carried out among the patients (inpatients and outpatients) conducted in the Department of Microbiology RMCHRC, Mandhana kanpur during the study period for 2 years i.e., from January 2017 to December 2018.

A total of 140 non repeated pus, wound swab, blood, nasal swab, throat swab, urine etc clinical samples from different anatomic locations received from the patients for bacteriological culture were included in the study.

Isolation and Identification of *Staphylococcus aureus*

The specimens were inoculated on blood agar and mannitol salt agar (HiMedia laboratories private limited, India) and incubated aerobically at 37°C for 48 hours. The strains of *Staphylococcus aureus* were identified on the basis of colony morphology, Gram's stain, and different biochemical tests. [19]

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing was performed by modified Kirby-Bauer disc diffusion technique using Mueller-Hinton agar (HiMedia laboratories private limited, India) following Clinical and Laboratory Standards Institute (CLSI) guidelines. [20] Antibiotic discs used were ciprofloxacin (5μg), clindamycin (2μg), chloramphenicol (30μg), erythromycin (15μg), gentamicin (10μg), tetracycline (30μg), cotrimoxazole (25μg), rifampin (5μg), mupirocin (200μg), and penicillin G (10 units).

Detection of Strains of MRSA by Cefoxitin Disc Diffusion Method

Susceptibility of *Staphylococcus aureus* isolates to cefoxitin (30μg) was determined by modified Kirby-Bauer disc diffusion method following CLSI guidelines. [20] The strains of *Staphylococcus aureus* which were found to be resistant to cefoxitin were screened as MRSA.

Determination of Minimum Inhibitory Concentrations (MICs) of Oxacillin and Vancomycin

MICs of oxacillin (Table 1) and vancomycin for all isolates of *Staphylococcus aureus* were determined by broth microdilution method as described by Andrews [21] and CLSI M07-A9 guidelines. [22] The results were interpreted according to CLSI

guidelines. [20] The concentrations of oxacillin used were 0.0125μg/mL to 128μg/mL and the concentrations of vancomycin used were 2 μg/mL to 128 μg/mL.

Detection of Mec A Gene by Polymerase Chain Reaction (PCR)

Bacterial DNA was extracted by QIAamp DNA KIT by following manufactures guidelines. *S. aureus* previously extracted DNA was used for the amplification of Mec A gene. A volume of 20 µl PCR reaction mixture consisting of 10 µl master mix, 1µl of each forward and reverse primers gene specific for the target gene, 3 µl of DNA template, and the volume was made up by adding nuclease free water. A 336-bp fragment of the Mec A gene was obtained. The forward and revered primers GTTGTAGTTGTCGGGTTTGG and CTTCCACATACCATCTTCTTTAAC were used respectively. The mixture was briefly centrifuged and the tubes were transferred into PCR machine which has been programmed with the following conditions. The initial denaturation step for 7 minutes at 94°C followed by 94°C for 30 sec, 47°C for 40 sec, 72°C for 45 sec, 72°C for 7 min. The total cycles were 37. The PCR programming was very similar as followed by Jonas *et al*., 2002. [23] The PCR products were electrophoresed, stained with 10 µM ethidium bromide, bromophenol blue visualized by using UVtrans illuminator. [23]

The positive cases have shown PCR product of 336 bp.

The strains of *Staphylococcus aureus* which were found to be resistant to cefoxitin were screened as MRSA._(Table No. 1).

Comparison of the phenotypic and genotypic methods for detection of MRSA.

Table 1: Different methods for detection of MRSA

Cefoxitin disc diffusion	Oxacillinbroth microdilution	Polymerase chain reaction
Strains of *S. aureus* having zone of inhibition of ≤21 mm to cefoxitin disc (30μg	Strains of *S. aureus* having oxacillin MIC of ≥4 μg/mL	Strains of *S. aureus* harboring mecA gene

Quality Control

S. aureus ATCC 29213, positive reference strain was used. Mec A negative control strain of *S. aureus* ATCC 43300 as was employed.

Detection of Strains of VRSA by Vancomycin Disc Diffusion Method

MHA plates was inoculated with the bacterial suspension which was previously adjusted to 0.5 McFarland standards. Afterward, a 30µg vancomycin disc and a blank disc as a control have been aseptically placed over the surface of the MHA plates at a distance of 5mm and observed the range of the zone diameter for the detection of strains of VISA and VRSA. [24,25]

Identification of VRSA\ VISA through disc E-test strip

E-test (Himedia) for vancomycin MIC was done using Mueller-Hinton agar without NaCl supplementation and Etest strip was been placed. Plates were incubated in ambient temperature. Vancomycin MICs were read after 18 to 19 h of incubation. Isolates obtained of Vancomycin MICs. If it is <=2 µg/mL then it is Sensitive, if it is >= 4-8µg/mL then it is Intermediate and if it is >=16 µg/mL then it is Resistance, according to the CLSI guidelines 2016. [26]

Determination of vancomycin resistance by minimum inhibitory concentration test

The minimum inhibitory concentration (MIC) of vancomycin has been be determined by the tube dilution method. [27-30] Muller-Hinton Broth has been prepared with 2-128 µg/mL of vancomycin. By using a direct colony suspension method, 0.5 McFarland equivalent bacterial inoculums has been prepared in normal saline after culturing for 24 hours on an agar plate. The suspension was further diluted to achieve the desired inoculum concentration. If it is 2 µg/mL then it is Sensitive, if it is >= 4-8µg/mL then it is Intermediate and if it is >=16 µg/mL then it is Resistance, according to the CLSI guidelines 2016. [26] All strains were spotted onto Muller-Hinton plates containing different concentrations of

vancomycin. The plates were incubated for 24 hours at 37^0C and checked for any visible growth. [31]

Genotypic Detection of Van*A* gene by PCR

Detection of *VanA* gene by Polymerase ChainReaction:

Bacterial DNA was extracted by as mentioned above. A volume of 20 µl PCR reaction mixture consisting of 10 µl master mix, 1 µl of each forward and reverse primers gene specific primers were used. 3 µl of DNA template was used and the volume was made up to adding nuclease free water. The primers; CTGGGAAAACGACAATTGCT and TGTACAATGCGGCCGTTACG. The mixture was briefly centrifuged and the tubes was transferred into PCR apparatus which has been programmed with the following conditions: an initial denaturationstep was 7 minutes at 94°C with cycles 35 cycles of amplification were performed as follows: denaturation at 94°C for 45 seconds, annealing at 58°C for 45seconds and extension at 72°C for 1 minute, followed by a final extension step at 72°C for 7 minutes similar to Jonas *et al.*, 2002. [23] The PCR products were electrophoresed, stained with 10 µM ethidium bromide, bromo phenol blue and visualized by using UV trans illumination. [23]

The VanA gene was not found in the PCR product.

Quality Control

Enterococcus faecalis A256 control was used as a positive control whereas *Enterococcus faecalis* ATCC 29212 was used as van A negative control.

RESULTS

Clinical samples were isolated from hospitalized patients in Rama Medical College Hospital & Research Centre Mandhana, Kanpur. Out of 140 *S.aureus* isolates 40 were found MRSA by disk diffusion method, in which 8 were confirmed as VISA and 2 were VRSA by E-test, Vitek and MIC.

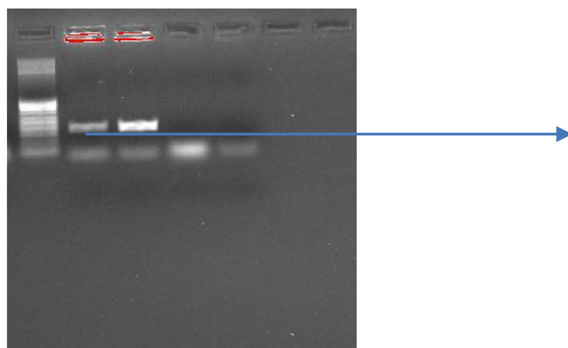
The molecular characterization of the test isolates detects the presence of Mec A gene, which was confirmed by sequencing, The PCR product of the isolates that carried the Mec A gene have been sent to Chromous Biotech Pvt. Ltd., Bengaluru for gene sequencing. No 39, 3rd Floor, Metropolis Business Park, Industrial Area,1st Main, Yelahanka New Town, Bengaluru - 560064. The gene sequences were confirmed by homology of NCBI blast.

GENOTYPIC METHOD

Mec A Gene

Molecular characterization and sequencing of MRSA isolates confirmed the presence of MecA gene.

DNA isolated



Lane 1 DNA Ladder,
Lane 2 Sample Positive for Mec A gene gene Lane 3 is Positive control
Lane 4 is Negative control

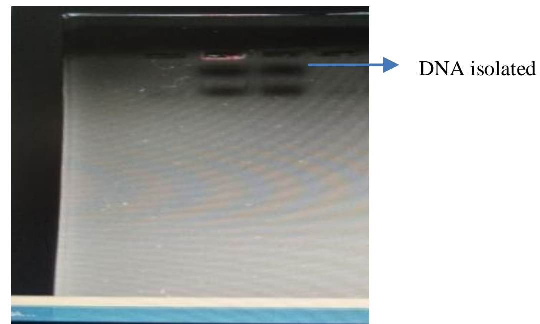
Detection of Mec A gene; 1st lane is DNA ladder; lane 2 is sample positive for Mec A gene, lane 3 is positive control and lane 4 is negative control

GENOTYPIC METHOD

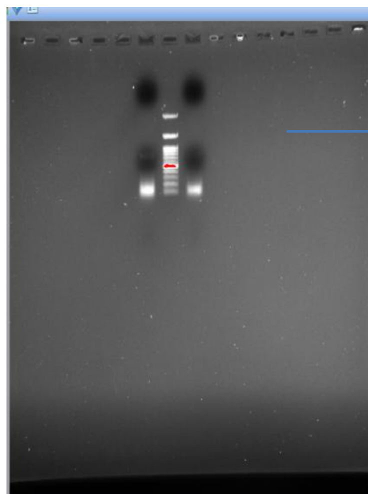
Van A Gene

Bacterial DNA was extracted by QIAamp DNA KIT by following manufactures guidelines. *S. aureus* previously extracted DNA was used for the amplification of Van A gene.

The VanA gene was not found in the PCR product.



DNA Isolates



Lane 1 is positive control,

Lane 2 is the DNA ladder

Lane 3 is sample negative for VanA gene

Lane 4 is negative control

Absent of Van A gene

Van A gene was not detected in VRSA and VISA among MRSA isolates.

DISCUSSION

The increase in isolates of *S.aureus* with resistance to methicillin and decreased susceptibility to vancomycin has created concern for development of new anti-staphylococcal agents that kills resistant mutants. Emergence of VRSA/VISA may be due to buildings of selective pressure of vancomycin. The true mechanism of vancomycin resistance in *S. aureus* is not known. It was initially feared that *S. aureus* would acquire the *van* gene that code for vancomycin resistance in *Enterococcus* spp; this phenomenon was successfully accomplished in the laboratory. [32] Further, Showsh et al, 2001 [33] have demonstrated the presence of sex pheromone in *S. aureus* that promotes plasmid transfer in *Enterococcus* spp. Release of these pheromones by *S. aureus* with proximity to vancomycin-resistant enterococci causes the transfer of plasmids encoding *van* gene to the *S. aureus*.

As a whole comparison between phenotypic and PCR-based methods for detection of MRSA, is shown in the present study results, it is still leaning towards the sensitivity of molecular techniques. However, an important opinion indicates that methicillin-resistant can be due to not only to the presence of the Mec A gene alone; but by a cluster of this gene and *ica*gene (Memmi *et al*., 2008). [34] This was also similar to our study in case of PCR-based methods for detection of VRSA and VISA among the MRSA isolates, where no Van A gene was detected.

We have found 40 MRSA strains from 140 *S.aureus* isolates, of which 8 were confirmed to be VISA and 2 VRSA by MIC

method . Of total eight VISA strains, MIC ranges>= 4-8µg/mL

One VRSA (vancomycin MIC >=32µg/ml) strain was isolated from Pus swab; the other VRSA (vancomycin MIC >=32 µg/ml) strain was isolated from Blood. Both the isolates were also found to be resistant to several other antimicrobials such as gentamicin, tobramycin, amikacin, norfloxacin, ciprofloxacin, erythromycin, tetracycline, trimethoprim/sulfamethoxazole and cefoperazone/sulbactam. The emergence of the glycopeptide resistance is of great concern. Though first case of VRSA was reported in 2002 in USA. [35]

In the current study all strains of VRSA and VISA among the MRSA isolates were positive for Mec A gene by PCR which was confirmed by sequencing, whereas, negative for van A gene by PCR. Therefore the absence of van A gene in the present isolates does not rule out that these strains are not VRSA or VISA. There is another hypothesis which says that cell wall thickening is responsible for the development of vancomycin resistance. The mechanism of vancomycin resistance has been extensively studied with the first clinical VRSA strain, Mu50. [36-39]

This was similar to the other study where they had reported six VISA and two VRSA strains from clinical samples, but none of these isolates was found to have either vanA or vanB by PCR. [39]

Biochemical and transmission electron microscopy (TEM) examination of the Mu50 cell, suggested that it produces increased amounts of peptidoglycan. More murein monomers and more layers (probably 30-40 layers as judged by cell-wall thickness observed with TEM) of peptidoglycan are considered to be present in the cell wall. As a result, more vancomycin molecules are trapped in the peptidoglycan layers before reaching the cytoplasmic membrane where peptidoglycan synthesis occurs. Moreover, a higher concentration of vancomycin would be required to saturate all the murein monomers that are supplied at an increased rate in Mu50. Besides the vancomycin-trapping mechanism, designated "affinity trapping,". [40-42] There are many other study which says though there is VISA isolates with the vanA genotype, that was probably not the mechanism of intermediate resistance to vancomycin. Instead, as in other VISA isolates, a thick cell wall resulted in resistance. An unstable vancomycin resistance phenotype in such isolates [43] and heterologous expression of the enterococcal *vanA* operon in MRSA [44] have already been reported. The VISA strains with the *vanA* genotype colonizing the anterior nares may be a potent source of VRSA with reduced expression of the vancomycin resistance gene. When exposed to the appropriate selective step-up pressure, these isolates may eventually take a resistant form.

CONCLUSION

Antibiotic misuse is very common now a days, hence immediate response from the concerned authorities to check further emergence and spreading of these VRSA\VISA strains is alarming. A strict regulation on irrational antibiotic usages might be an appropriate and effective approach. Moreover, nationwide surveillance program should be carried out to map the vancomycin susceptibility pattern in this country. For this, all strains with the vancomycin MIC 4µg/ml should be earmarked and sent to the reference laboratory for further characterization. This helps to identify the potential areas which are already under the major threat of VRSA/VISA emergence and therefore draw more focused attention of Government for prompt tackling of this problem.

REFERENCES

1. Pfaller MA, Jones RN, Doern GV, Kugler K. Bacterial pathogens isolated from patients with bloodstream infection: Frequencies of occurrence and antimicrobial susceptibility patterns from the SENTRY antimicrobial surveillance program (United States and Canada, 1997) Antimicrob Agents Chemother. 1998;42:1762–70.

2. Giacometti A, Cirioni O, Schimizzi AM, Del Prete MS, Barchiesi F, D'Errico MM, et al. Epidemiology and microbiology of surgical wound infections. J Clin Microbiol. 2000;38:918–22.
3. Hoban DJ, Biedenbach DJ, Mutnick AH, Jones RN. Pathogen of occurrence and susceptibility patterns associated with pneumonia in hospitalized patients in North America: Results of the SENTRY Antimicrobial Surveillance Study (2000) Diagn Microbiol Infect Dis. 2003;45:279–85.
4. Von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of Staphylococcus aureus bacteremia. Study Group. N Engl J Med. 2001;344:11–26.
5. Dowling HF. The newer penicillins. Clin Pharmacol Ther. 1961;2:572–80.
6. Wenzel RP, Nettleman MD, Jones RN, Pfaller MA. Methicillin-resistant Staphylococcus aureus: Implications for the 1990s and effective control measures. Am J Med. 1991;91:221S–7S.
7. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of methicillin-resistant Staphylococcus aureus as a public-health threat. Lancet. 2006; 368:874–85.
8. Ena J, Dick RW, Jones RN, Wenzel RP. The epidemiology of intravenous vancomycin usage in a university hospital: A 10 year study. JAMA. 1993;269:598–602.
9. Cunha BA. Vancomycin. Med Clin North Am. 1995;79:817–31.
10. Moellering RC. The spectre of glycopeptide resistance: Current trends and future considerations. Am J Med. 1988;104:3S–6S.
11. Hiramatsu K, Hanaki H, Ino T, Tenover FC. Methicillin resistant S aureus clinical strain with reduced vancomycin susceptibility. J Antimicrob Chemother. 1997;40:135–8.
12. Fridkin SK. Vancomycin intermediate and resistant S aureus: What infectious disease specialists need to know. Clin Infect Dis. 2001;32:429–39.
13. Benjamin PH, John KD, Paul DR. J, Timothy PS, Grayson ML.Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin- intermediate strains: Resistance mechanisms, laboratory detection, and clinical implications. Clin Microbiol Rev. 2010;23:99–139.
14. Assadullah S, Kakru DK, Thoker MA, Bhat FA, Hussain N, Shah A. Emergence of low level vancomycin resistance in MRSA. Indian J Med Microbiol. 2003;21:196–8.
15. Menezes GA, Harish BN, Sujatha S, Vinothini K, Parija SC. Emergence of vancomycin-intermediate *Staphylococcus species* in southern India. J Med Microbiol. 2008;57:911–2.
16. Bhateja P, Mathur T, Pandya M, Fatma T, Rattan A Detection of vancomycin resistance *Staphylococcus aureus*: A comparative study of three different phenotyic screening methods. Indian J Med Microbiol. 2005;23:52–5.
17. Bijiyani B, Purva M. Erroneous reporting vancomycine susceptibility for *Staphylococcus* spp.Vitek software version 2.01. Jpn J Infect Dis. 2009;62:298–9.
18. Veer P, Chande C, Chavan S, Wabale V, Chopdekar K, Bade J, et al. Increasing levels of minimum inhibitory concentration vancomycin in methicillin resistant *Staphylococcus aureus* alarming bell for vancomycin abusers. Indian J Med Microbiol. 2010;28:413–4.
19. Forbes B. A., Sahm D. F., Weissfeld A. S. *Bailey and Scott's Diagnostic Microbiology*. 12th. Maryland Heights, Mo, USA: Mosby Inc; 2007.
20. Clinical and Laboratory Standards Institute. *CLSI Document*. M100-S23. Wayne, Pa, USA: CLSI; 2013. Performance standards for antimicrobial susceptibility testing: twenty third informational supplement edition.
21. Andrews J. M. Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy*. 2001;48(1)5–16. doi: 10.1093/jac/48.suppl_1.5.
22. Clinical and Laboratory Standards Institute. *CLSI Document M07-A9. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard-Ninth Edition*. Wayne, Pa, USA: CLSI; 2012.
23. Jonas D, Speck M, Daschner FD, Grundmann H.Rabid PCR-based identification of methicillin resistant *Staphylococcus aureus* from screening swabs. J Clin Microbiol 2002; 40(5):1821-23.

24. Kaiser ML, Thompson DJ, Malinoski D, Lane C, Cinat ME. Epidemiology andrisk factors for hospital-acquired methicillin-resistant Staphylococcus aureus among burn patients. J Burn Care Res 2011; 32:429-34.
25. Schweizer M, Ward M, Cobb S, McDanel J, Leder L, Wibbenmeyer L, et al. The epidemiology of methicillin-resistant Staphylococcus aureus on a burn trauma unit. Infect Control Hosp Epidemiol 2012; 33:1118-25.
26. Clinical and Laboratory Standards Institute. CLSI *Document M100*. Performance standards for antimicrobial susceptibility testing. Wayne, Pa, USA: CLSI; 2016..
27. Alam SMS, Kalam MA, Munna MS, Munshi SK, Noor R. Isolation of pathogenic microorganisms from burn patients admitted in Dhaka Medical Collage and Hospital and demonastration of their drug-resistant traits. Asian Pac J Trop Dis 2014; 4:402-7.
28. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved Standard-7th Edition. Wayne (PA): CLSI; 2006.
29. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; 19th informational supplement. CLSI M100-S19 Clinical and Laboratory Standards Institute. Wayne (PA): CLSI; 2009.
30. National Committee for Clinical Laboratory Standards (NCCLS). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A5. 5th ed. Wayne (PA): NCCLS; 2000.
31. Sharmin M, Nur IT, Acharjee M, Munshi SK, Noor R. Microbiological profiling and the demonstration of in vitro antibacterial traits of the major oral herbal medicines used in Dhaka Metropolis. SpringerPlus 2014; 3:739.
32. Noble WC, Virani Z, Cree RC. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. FEMS Microbiol Lett. 1992; 72:195–198. doi: 10.1111/j.1574-6968.1992.tb05089.x
33. Showsh SA, De Boever RH, Clewell DB. Vancomycin resistance plasmid in *Enterococcus faecalis* that encodes sensitivity to a sex pheromone also produced by *Staphylococcus aureus*. Antimicrob Agents Chemother. 2001; 45:2177–2178. doi: 10.1128/AAC.45.7.2177-2178.2001.
34. Memmi, G Filipe SR, Pinho, MG., Fu Z. Cheung, A. *Staphylococcus aureus* PBP4 is essential for betalactam resistance in community-acquired methicillin-resistant strains. Antimicrob Agents Chemother 2008; 52: 3955-66.
35. Centers for Disease Control and Prevention *Staphylococcus aureus* resistant to vancomycin – United States, 2002. Morb Mortal Wkly Rep MMWR. 2002; 51:565–567.
36. Hanaki H, Kuwahara-Arai K, Boyle-Vavra S, Daum RS, H Labischinski H, Hiramatsu K. Activated cell-wall synthesis is associated with vancomycin resistance in methicillin-resistant *Staphylococcus aureus* clinical strains Mu3 and Mu50. J Antimicrob Chemother. 1998; 42:199–209. doi: 10.1093/jac/42.2.199.
37. Hanaki H, Labischinski H, Inaba Y, Kondo N, Murakami H, Hiramatsu K. Increase in glutamine-non-amidated muropeptides in the peptidoglycan of vancomycin-resistant *Staphylococcus aureus* strain Mu50. J Antimicrob Chemother. 1998; 42:315–320. doi: 10.1093/jac/42.3.315.
38. Cui L, Murakami H, Kuwahara-Arai K, Hanaki H, Hiramatsu K. Contribution of a thickened cell wall and its gultamine nonamidated component to the vancomycin resistance expressed by *Staphylococcus aureus* Mu50. J Antimicrob Chemother. 2000; 44:2276–2285. doi: 10.1128/AAC.44.9.2276-2285.2000.
39. Tiwari HK et al. Emergence of vancomycin resistant staphylococcus aureus (VRSA) from a tertiary care hospital from northern part of India BMC Infectious Diseases 2006; 6:156.
40. Marchese A, Balistreri G, Tonoli E, Debbia EA, Schito GC. Heterogeneous vancomycin resistance in methicillin-resistant *Staphylococcus aureus* strains isolated in a large Italian hospital. J Clin Microbiol. 2000; 38:866–869.
41. Rotun SS, McMath V, Schoonmaker DJ, Maupin PS, Tenover FC, Hill BC, Ackman DM. *Staphylococcus aureus* with reduced susceptibility to vancomycin isolated from a patient with fatal bacteremia. Emerg Infect Dis. 1999; 5:147–149.

42. Hiramatsu K. Vancomycin resistance in staphylococci. Drug Resistance Updates. 1998; 1:135–150. doi: 10.1016/S1368-7646(98)80029-0.
43. Liu C, Chambers HF. Staphylococcus aureus with heterogeneous resistance to vancomycin: epidemiology, clinical significance, and critical assessment of diagnostic methods. Antimicrob. Agents Chemother. 2003; 47:3040–3045.
44. Perichon B, Courvalin P. 2004. Heterologous expression of the enterococcal *van A* operon in methicillin resistant Staphylococcus aureus. Antimicrob. Agents Chemother. 2004;48:4281–4285.

How to cite this article: Nashra A, Sujatha R, Sameer D et.al. Screening and molecular characterization of VISA and VRSA among the MRSA isolates at a tertiary care centre, Kanpur. Int J Health Sci Res. 2019; 9(5):46-54.

******