 DOI: 10.18231/2455-6807.2017.0028

A study on the prevalence of vancomycin resistant and intermediate *staphylococcus aureus* isolated from various clinical Specimen in a tertiary care hospital and detection of their MIC values by E-test

Anjali Kulshrestha[1,*], V. Anamika[2], K. Mrithunjay[3], A.S. Dalal[4], K. Manish[5]

[1]Assistant Professor, NIMS Medical College & Hospital, Jaipur, Rajasthan, [2]Professor, [3]Assistant Professor, [4]Professor & HOD, Geetanjali Medical College & Hospital, [5]Senior Registrar, Dept. of Microbiology, Apollo, New Delhi

***Corresponding Author:**
Email: anjalikulshrestha2185@gmail.com

Abstract

The main antimicrobial used to treat MRSA infections which are life threatening is a glycopeptide antibiotic, Vancomycin. Inturn extensive use of vancomycin in various parts of the world lead to the a rise in *Staphylococcus aureus* strains which are resistant to vancomycin.(1,2) Hence this study was carried out to detect vancomycin resistance in S.aureus isolated from various clinical samples. All 161 *Staphylococcus aureus* strains were screened for Vancomycin resistance by BHI - Vancomycin screen agar [6µg/ml] and Vancomycin disc diffusion method [30µg/disc]. These were further confirmed by E- test. 82 (51%) strains were MRSA determined by Cefoxitin disc diffusion method and MRSA were maximally isolated from pus (61%). 45% strains were MDR-MRSA. Only 5 *S.aureus* strains showed resistance to Vancomycin by disc diffusion method, but all these strains were sensitive to Vancomycin by both Vancomycin screen agar [6µg/ml] and E- test, thus this study also showed 100% sensitivity and specificity for BHI –VSA (6µgm/ml). As current study only indicates the tip of iceberg. Hence we suggest, more and more studies should be undertaken in future to monitor the emergence of resistance to these antibiotic.

Keywords: VSSA, VRSA, MRSA, Multidrug resistance.

Introduction

Staphylococcus aureus is one among the top three pathogens which are responsible for Hospital and Community acquired infections.(3) The *Staphylococcus aureus* infection treatment have become problematic because of continuous rise in resistance to Vancomycin, Methicillin, Penicillin and many other antibiotics, by acquiring several resistance mechanisms. Increased resistance to these antibiotics, therefore is a cause of concern.

In the past few decades MRSA has emerged as an important nosocomial pathogen worldwide. In India, prevalence rate varies from 30-85% in different parts and has now become endemic.(4,5,6) A multi-centric study was done in India involving 17 tertiary care Hospitals reported MRSA prevalence of 41% in 2008-2009.(7)

Increase in MRSA prevalence, inturn lead to the exaggerated use of Vancomycin. Hence the susceptibility to Vancomycin decreased all over the World including India, this was soon followed by *Staphylococcus aureus* strains that were totally resistant to vancomycin.(1,2) Such resistance resulted in serious clinical and public health consequences because currently few licensed alternatives are available to treat VISA and VRSA infections.(8)

To tackle this grave situation constant monitoring of these isolates is important, however there are many hurdles, since most laboratories perform disc diffusion test for antibiotic susceptibility which is not reliable for vancomycin testing.(9) There are ample number of reports showing discrepancies between clinical outcomes of MRSA infections treated with vancomycin and there clinical laboratory susceptibility testing.(10,11)

Keeping the above points in view and also a few reports of VRSA and VISA were documented in India including Rajasthan, the present study was carried out to detect the vancomycin resistance in *S.aureus* isolated from various clinical specimens, to compare the Vancomycin disc diffusion test with BHI- VSA [6µgm/ml] for detecting Vanomycin resistance considering E-Test [MIC] as gold standard, also to determine their antibiotic sensitivity pattern so as to help the clinicians to select correct antimicrobial agents.

Materials and Method

Source of material: The present study was carried out in the department of Microbiology, GMCH, Udaipur during the year 2013-2014. 161 non- duplicate *Staphylococcus aureus* strains isolated from various clinical samples [pus, wound or vaginal swabs, blood, body fluids (csf, pleural fluid, ascitic fluid) urine, sputum, ET secretion etc] were included in the study. Isolates from both in-patients and out-patients were considered.

Inclusion criteria: All *Staphylococcus aureus* strains isolated from various clinical specimens, were included in the study.

Exclusion criteria: Except *Staphylococcus aureus*, all other gram positive and gram negative bacteria were excluded.

1. **Isolation & identification of *Staphylococcus aureus* -** All the *S.aureus* isolates were identified by standard procedures which includes gram

staining of the various samples, sample were inoculated on Blood agar, Nutrient agar, MacConkey's agar and culture plates were incubated at 37^0 C aerobically for 24-48 hours. The colonies were identified by performing gram staining, Catalase test (3% H_2O_2) and slide coagulase test from nutrient agar plate. Biochemical tests used for identification were Hugh-Leifson OF media and mannitol fermentation.[12] Tube coagulase was the main test used for identification and was carried out by diluting plasma in normal saline in a ratio 1:6.1 ml of diluted plasma is taken in a test tube, in which 3 to 4 characteristic *S.aureus* colonies were emulsified properly, the tubes were incubated at 37ºC for 4hrs. The test tubes were read after 1, 2, 3 and 4hrs of incubation, if no clumping in the test tube was observed after 4 hrs, then the test tubes were left at room temperature for further incubation.[12]

Determination of antibiotic susceptibility: Antimicrobial susceptibility testing of 161 *Staphylococcus aureus* strains were carried out by modified Kirby–Bauer disc diffusion. Antibiotics tested were Penicillin [10 units], cefoxitin (30 µg), Vancomycin [30µg], Linezolid [30µg], Pristinomycin (Quinupristin/Dalfopristin) [15µg], Gentamicin [10µg], Tetracycline [30µg], Chloramphenicol [30 µg], Ciprofloxacin [5µg], Levofloxacin [5µg], Erythromycin [15µg], Clindamycin [2µg], Rifampicin [5µg] and Cotrimoxazole [1.5/23.75µg]. Cefoxitin disc (30 µgm) was used to detect MRSA. *Staphylococcus aureus* ATCC 25923 was used as control strain. Zone of inhibition of all the antibiotics were measured with scale in reflected light against a black background, to the nearest mm. Interpretation was done according to the Clinical Laboratory Standards Institute guidelines.[9]

Detection of Vancomycin resistance: Following 3 methods were used and comparison between modified Kirby – Bauer disc diffusion method using 30µgm Vancomycin disc and 6 µg/ml BHI vancomycin screen agar was done, considering E-test as gold standard for VISA and VRSA detection. *Enterococcus faecalis* ATCC 51299 and *Staphylococcus aureus* ATCC 25923 were used as control strains for detection of vancomycin susceptibility.

a. **Disc diffusion by Modified Kirby Bauer's Method** using Vancomycin 30µg disc. The zone of inhibition was interpreted according to CLSI guidelines 2007.[13]
b. **BHI vancomycin screen agar plates** containing 6µg/ml vancomycin were prepared. The colonies were emulsified in sterile saline so as to produce a suspension which matches 0.5 McFarland turbidity. Using a micropipette, spot of 10-µL drop onto agar surface was done and were incubated aerobically at 35±2°Cfor 24 hrs. Any growth is examined carefully with transmitted light. More than 1 colony or light film of growth was to be considered for decreased susceptibility to vancomycin.[9]
c. **Determination of MIC values**- by E-test [Epsilometer-test]. According to CLSI guidelines, the laboratory breakpoints of *S.aureus* to determine decreased susceptibility to vancomycin are as follows[9]
   1. Vancomycin sensitive *Staphylococcus aureus* [VSSA]: ≤2 microgram/ml.
   2. Vancomycin intermediate *Staphylococcus aureus* [VISA]: 4-8 microgram/ml.
   3. Vancomycin resistant *Staphylococcus aureus* [VRSA]: ≥16 microgram/ml.

Result

In the present study, a total of 161 non duplicate *Staphylococcus aureus* strains were isolated from various clinical samples. *Staphylococcus aureus* infection was found comparatively more in Male patients i.e. 115 [71%] than in female patients 46 [29%]. The male to female ratio was 2.5:1. Age group of 21-30 yrs and 51-60 yrs were predominantly affected [Fig. 1, 2]. Among all *Staphylococcus aureus* isolates, majority contribution was from Pus samples 103 (64%), followed with blood 23(15%), respiratory secretion 18 (11%) and body fluids 7(4%). Swabs and Urine samples grew only 7(4%) and 3(2%) respectively [Table 1].

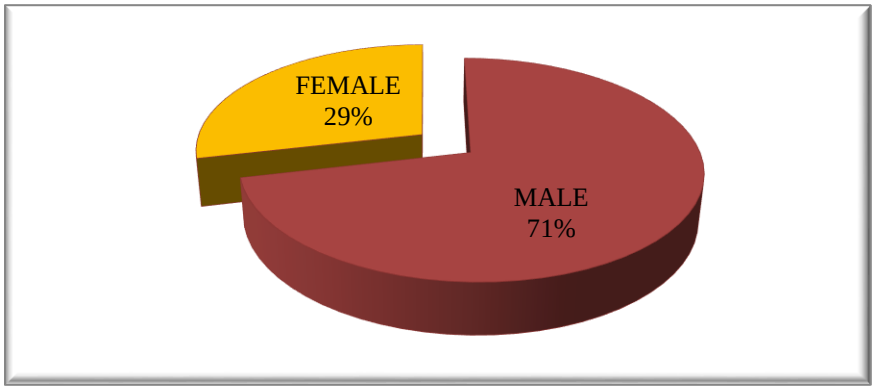

Fig. 1: Distribution of patients with *S. aureus* infection according to gender

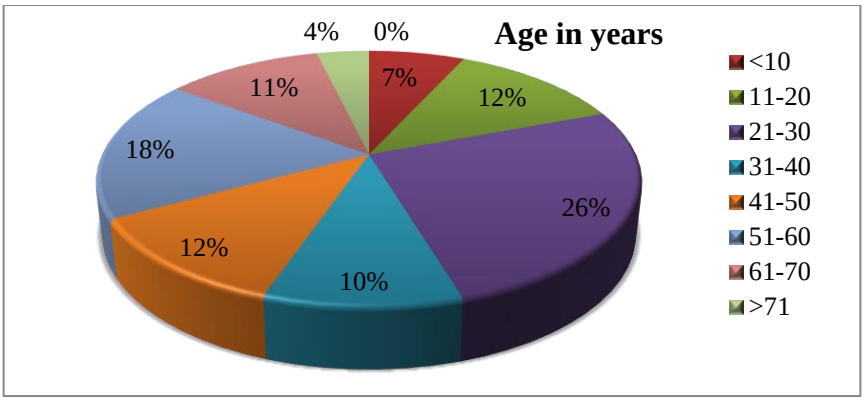

Fig. 2: Distribution of patients with *S. aureus* infection according to the age

Out of total 161 *Staphylococcus aureus* strains, 82 (51%) were found to be MRSA and 79 (49%) were MSSA.[Fig. 3]

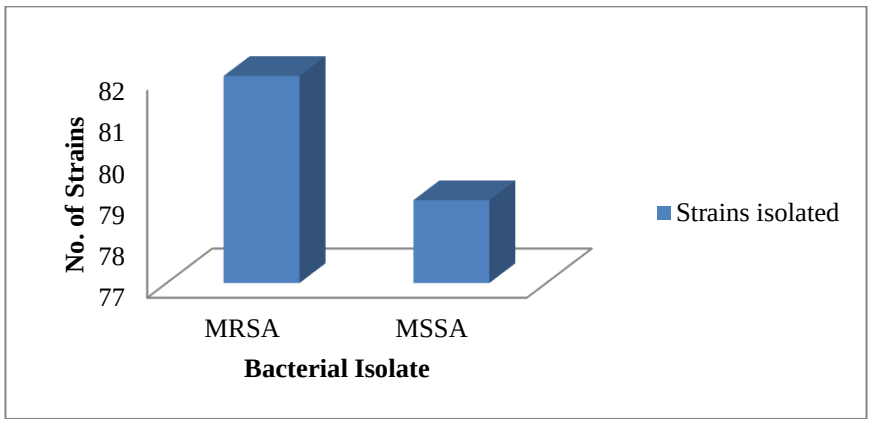

Fig. 3: Total number of MRSA and MSSA in *Staphylococcus aureus* isolates

In a total of 161 *S.aureus* stains, 5 (3%) isolates had shown resistance towards Vancomycin in Disc diffusion. None of the isolates grew on BHI-VSA. So no resistance is reported by this method. E-test was considered as Gold Standard. MIC values of all the 161 isolates fall in between range of ≤2µgm/ml, which is category of sensitive in which 96 [59%] isolates had Vancomycin MIC of 0.5µgm/ml, 62 [39%] isolates with >0.5-1µgm/ml and 3 [2%] isolates had MIC of >1-1.5µgm/ml and no isolates had MIC of >1.5-2 µgm/ml.[Fig. 4]. All of the 5 isolates, which were resistant by Vancomycin disc diffusion, are in range of sensitive MIC. [Fig. 4]

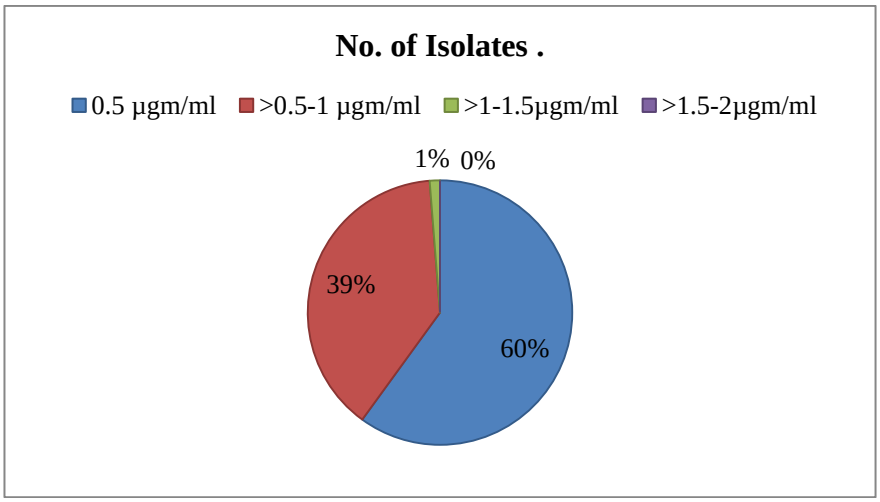

Fig. 4: Distribution of Vancomycin MIC values

In the present study, Vancomycin Screen Agar methods had sensitivity and specificity which was comparable to E-Test in detecting Vancomycin resistance. But the Vancomycin Disc Diffusion method had sensitivity of 96.89% though its specificity was retained to 100%. [Table 3].

All 161 *Staphylococcus aureus* strains were sensitive to Linezolid, Pristomycin, Rifampicin and Chloramphenicol. *Staphylococcus aureus* showed 88% resistance to Pencillin G, followed by 51% resistance to Cefoxitin, 43% each to Co-trimaxazole and Erythromycin, 66% to Ciprofloxacin, 31% to Clindamycin, 16% to Gentamycin and 28% to Levofloxacin and 3% each for both Tetracycline and Vancomycin*[Table 5].

Table 1: Distribution pattern of *S.aureus* isolates in various clinical specimens

Clinical specimen	No of isolates	Percentage
Pus	103	64
Blood	23	15
Sputum/ ETsecretion / Bronchial aspirate	18	11
Body fluids (csf, pleural fluid, ascitic fluid)	7	4
Swabs (Vaginal/wound)	7	4
Urine	3	2
Total	161	100

Table 2: Detection of Vancomycin resistance by different phenotypic methods

Methods	VISA	VRSA	VSSA	Total
Vancomycin disc diffusion (30µg/disc)[66]	NA	5	156	161
Vancomycin screen agar [6µgm/ml]	00	00	161	161
Vancomycin E-test	00	00	161	161

Table 3: Sensitivity and specificity of all 3 methods in detecting resistance to vancomycin

Methods	N	True Positive	False Positive	True Negative	False Negative	Sensitivity (%)	Specificity (%)
VDD*	161	156	5	0	0	96.89	100
VSA	161	161	0	0	0	100	100
E-test#	161	161	0	0	0	100	100

V.D.D*- Vancomycin disc diffusion, V.S.A- Vancomycin Screen Agar,
#E-test as gold standard method for screening VRSA and VISA.

Table 4: Statistical analysis of MIC values in MRSA and MSSA isolates against vancomycin

MIC Values (µgm/ml)	MRSA	MSSA	p Value
0-0.5	26	70	< 0.001
>0.5-1	53	9	< 0.001
>1-1.5	3	0	= 0.086
>1.5-2	0	0	0

Table 5: Antibiogram of *Staphylococcus aureus* strains

Drugs	*Staphylococcus aureus* strains			
	Sensitive	%	Resistant	%
Penicillin G	19	12	142	88
Cefoxitin	79	49	82	51
Ciprofloxacin	54	34	107	66
Levofloxacin	116	72	45	28
Gentamycin	136	84	25	16
Erythromycin	91	57	70	43
Clindamycin	111	69	50	31
Co-trimaxazole	91	57	70	43
Tetracycline	156	97	5	3
Rifampicin	161	100	0	0

Chloramphenicol	161	100	0	0
Vancomycin*	156	97	5	3
Linezolid	161	100	0	0
Quinipristin/ dalphopristin	161	100	0	0

Vancomycin* - According to CLSI guidelines 2007[14]

Discussion

Among161 *Staphylococcus aureus* strain, highest isolation was from pus 103 (64%). Harcharan singh *et al* in Udaipur (65%),[17] Manu Chaudhary *et al* in H.P (63%)[18] and Ankur Goyal *et al* in Agra (66.03%),[19] also reported the highest isolation of *Staphylococcus aureus* from pus. In our study 82 (51%) isolates turned out to be MRSA and 79(49%) as MSSA, from a total of 161 *Staphylococcus aureus* strains. The prevalence of MRSA in our study was 51%, this is comparable with the studies conducted by S.Vidhani and P.L Mehndiratta et al in 2001[20] showing a prevalence rate of 51.6%.

Treatment failure in case of vancomycin, is because of its low bactericidal activity, slow penetration in various tissues and also due to it's continuous rise in MIC values. A continuous rise in MIC values of vancomycin, is inturn leading to increase inhetero-resistant VISA strains. In our study all 161 *Staphylococcus aureus* isolates had MIC values < 2µgm/ml, hence all were sensitive to Vancomycin and were labeled as VSSA according to the CLSI guidelines 2014.[10]

Among 116 *S.aureus* strains, 96(59%) isolates had MIC of ≤ 0.5µgm/ml, 62[39%] had MIC of >0.5-1µgm/ml. Only 3[2%] had MIC of >1-1.5µgm/ml and none of the isolates had MIC values of >1.5µgm/ml. In contrast to this, a study conducted by Asha Pai et al[25] reported 44.6% with MIC of 1 µg/ml and 21.4% with MIC of 2 µg/ml and by Mewar A et al[26] in which one of the strain had MIC of 4µg/ml, 20.4% strains each with MIC <1 µg/ml and 1 µg/ml respectively, 32.6% of the strains had MIC of 2 µg/ml.

According to the guidelines of Infectious Disease Society of America (IDSA) guidelines 2011, state that the isolates having vancomycin MIC >2 µmg/ml, a antibiotic other than Vancomycin should be used. While for isolates with MIC <2 µmg/ml the patient's clinical response should determine the continued use of vancomycin, independent of the MIC. A study conducted by Sakoulas *et al*[27] also verified that S.aureus strains which had an MIC of 1µmg/ml when treated with vancomycin had shown decreased in-vivo response. In our study, 3 (2%) of *Staphylococcus aureus* isolates had MIC in between >1-1.5µgm/ml, even though these are within susceptible range, there might be significant risk of treatment failure if treated with vancomycin; but a clinical follow up was not done in the study for these 3 isolates.

Tremendous care should be taken by the clinical microbiology laboratory for determining vancomycin susceptibility, keeping in mind the sensitivity and the specificity of the methods which are used. As routinely used methods like disc diffusion method can not accurately detect the vancomycin resistance. Discrepancies in detection of vancomycin resistance by various phenotypic methods had lead to an adverse effects on patient's management, thereby highlighting the importance of accuracy in detection. For the above mentioned reason, for detection of vancomycin resistance we compared two phenotypic methods i.e. Vancomycin disc diffusion method [30µgm/ml] and 6µgm/ml BHI- vancomycin screen agar.

In the present study Vancomycin disc diffusion test identified 156 vancomycin susceptible *Staphylococcus aureus* isolates giving 100% specificity but misclassified 5 *Staphylococcus aureus* strains as resistant giving sensitivity of 96.89% according to the CLSI guidelines 2007. Horieh Saderi et al[28] in Trehan and Venubabu Thati et al[29] in Hyderabad conducted a study in which disc diffusion test and agar dilution method were used for determining vancomycin susceptibility. In these studies, disc diffusion method was unable to distinguish VISA from VSSA because a zone of inhibition of 20mm was produced by all these strains around the vancomycin disc.

In the present study, 3% strains were resistant to vancomycin by disc diffusion method, this is in contrast to study carried out by Ankur Goyal et al[19] and Yogesh Kumar Gupta et al[31] in which no resistance to vancomycin was documented, but a study carried out by Harcharan Singh et al,[23] showed resistance in 13% strains by Vancomycin disc diffusion method.

Similarly Dr Susmita B et al,[30] carried out a study to detect vancomycin resistance by Vancomycin disc diffusion method and E- Test, in which vancomycin resistance was found in 21 strains by disc diffusion but among them only 4 isolates were detected to be VISA by E-Test. Hence CLSI removed the Vancomycin disc diffusion breakpoints for *Staphylococcus aureus*.[32] Therefore they are not useful for screening of Vancomycin resistance.

When the vancomycin resistance was analysed, in our study none of the *Staphylococcus aureus* isolates grew on Vancomycin screen agar even those resistant on disc diffusion. It identified all 161 *Staphylococcus aureus* strains as VSSA, which were further confirmed by E- test. This shows cent percent specificity and sensitivity of vancomycin screen agar for detecting vancomycin resistance. This is similar to the study conducted by T.A Dhanalakshmi et al,[33] in which no VISA and VRSA were detected out of 250

Staphylococcus aureus isolates by both Vancomycin screen agar [6µg/ml] and E- test thus this study also showed 100% sensitivity and specificity for BHI –VSA (6µgm/ml).

This suggests that vancomycin screen agar is a effective screening test for detecting vancomycin resistance in Staphylococcal isolates in rural hospitals were facilities are not available to perform Vancomycin E- Test or agar dilution method.

In our study no VISA and VRSA was found. This may be due to the fact that the community acquired MRSA (CA-MRSA) unlike the hospital acquired MRSA (HA-MRSA) are known to be sensitive to drugs other than vancomycin. Because of its high cost, vancomycin may not be in use in the peripheral rural setups, thus decreasing the selection pressure for vancomycin resistance.[36]

To conclude, careful and proper use of newer drugs should be done so as is to preserve them for effectiveness treatment of VRSA and MRSA infections. We should undertake more and more such studies in future to fight against rising menace of antibiotic resistance. Also more research should be done to find better treatment policies, effective and cheaper alternative antibiotics in developing countries like ours. The findings of the studies should be shared with hospital infection control committee to help in the formulation of infection control polices and also antibiotic policies. So that the primary care givers can use antibiotics rationally. Also all the laboratories should routinely carry out tests for the detection of MIC values of Vancomycinin *S.aureus* infections so as to guide the clinicians to give appropriate antibiotics to treat patients and also for proper formulation of infection control policies.

References

1. Centers for Disease Control and Prevention *Staphylococcus aureus* resistant to vancomycin – United States, 2002. Morb Mortal Wkly Rep MMWR. 2002; 51: 565–567.
2. Tiwari HK, Sen MR et al. Emergence of vancomycin resistant Staphylococcus aureus (VRSA) from a tertiary care hospital from northern part of India. Infect Dis. 2006;6:156**.**
3. Koneman Elmer, Winn Washington, Allen Staphen, Procop Gary editors. Color Atlas & Textbook of Diagnostic Microbiology, 6th edition.2006: 643 – 648.
4. Hafiz S, Hafiz AN, Ali L, Chughtai AS, Memon B: Methicillin resistant Staphylococcus aureus: a multicentre study. JPMA 2002; 52:312.
5. Nitish K S, Raina G, Shrikala B, and Gopalkrishna B K: Nosocomial Infections and Drug Susceptibility Patterns in Methicillin Sensitive and Methicillin Resistant Staphylococcus aureus. Clin Diagn Res. 2013; 7: 2178–2180.
6. Priyanka Chauhan, Prabhakar S. Bais and Nidhi Gupta et al.Prevalance of Methicillin resistant Staphylococcus aureus (mac A gene) among the patients admitted in Intensive care Unit. Int. J. Bioassays, 2013, 02 (09), 1256-1259.
7. Sangeeta Joshi, Pallab Ray, Vikas Manchanda, Jyoti Bajaj, D.S. Chitnis, Vikas Gautam. Indian network for surveillance of Antimicrobial Resistance (INSAR) Group, India. Methicillin resistant Staphylococcus aureus (MRSA) in India: Prevalence & susceptibility pattern. Indian J Med Res 2013;137(2):363– 369.
8. Schweiger ES, Scheinfeld NS, Tischler HR, Weinberg JM et al. Linezolid and quinupristin/dalfopristin: novel antibiotics for Gram-positive infections of the skin. J Drugs Dermatol. 2003;2: 378-83.
9. Clinical and Laboratory Standards Institute [CLSI]. Performance Standards for Antimicrobial Susceptibility Testing. Twenty- second Informational Supplement. M100-S22. 2014; 32(1).
10. Hsu DI, Hidayat LK, Quist R, Hindler J, Karlsson A, Yusof A, et al. Comparison of method-specific vancomycin minimum inhibitory concentration values and their predictability for treatment outcome of methicillin-resistant Staphylococcus aureus (MRSA) infections. Int J Antimicrob Agents 2008;32:378-85.
11. Neoh HM, Hori S, Komatsu M, Oguri T, Takeuchi F, Cui L, et al. Impact of reduced vancomycin susceptibility on the therapeutic outcome of MRSA bloodstream infections. Ann Clin Microbiol Antimicrob 2007;6:13.
12. Baird. Staphylococcus: Cluster-forming gram-positive cocci. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie and McCartney Practical Medical microbiology. 14th edn. Edinburg: Churchill Livingstone. 1996:245-261.
13. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 17th informational supplement M100-S17, CLSI, Wayne, PA, 2007.
14. Lowy, F. D. Staphylococcus aureus infection. N. Engl. J. Med. 1998. 339:520-53**2.**
15. Ratnaraja NV, Hawkey PM. Current challenges in treating MRSA: What are the options? Expert Rey Anti-Infect Therapy. 2008;6: 601-18.
16. Boucher HW. Challenges in anti-infective development in the era of bad bugs, no drugs: A regulatory perspective using the example of BSI as an indication, Clinical Infectious Diseases. 2010;50:S4-9.
17. Harcharan Singh, Meena Atray, and Pankaj Kumar Modi et al.Antibiotic susceptibility pattern of Methicillin resistance Staphylococcus aureus in tertiary care center at Southern Rajasthan. IJPSR, 2014; 5(2): 607-611.
18. Manu Chaudhary and Anurag Payasi. Prevalance of Heterogeneous Glycopeptide intermediate resistance in Methicillin resistant Staphylococcus aureus. American Journal of Infectious diseases.2013; 9(3):63-70.
19. Ankur Goyal, Manish Kumar Diwakar, Suneel Bhooshan, Sapna Goyal, Arti Agrawal, et al. Prevalence and Antimicrobial Susceptibility Pattern of Methicillin-resistant Staphylococcus aureus [MRSA] isolates at a Tertiary Care Hospital in Agra, North India – A systemic annual review. Journal of Dental and Medical Sciences (IOSR-JDMS). 2013;11(6): 80-84.
20. Vidhani S, Mathur MD, Mehndiratta PL, Rizvi M. Methicillin resistant Staphylococcus aureus: the associated risk factors. Indian J Pathol Microbiol 2003;46(4):676-679.
21. Majumder D, Sarma Bordoloi JN, Phukan AC, et al. Antimicrobial susceptibility pattern among methicillin resistant Staphylococcus isolates in Assam. Ind. J. Med. Microbiol. 2001; 19(3):21-27.
22. Assadullah S, Kakru DK, Thoker MA, Bhat FA, Hussain N, Shah A et al. Emergence of low level vancomycin

resistance in MRSA. Indian J Med Microbiol. 2003;21:196–198.
23. Verma S, Joshi S, Chitnis V, et al. Growing problem of Methicillin resistant Staphylococci: Indian Scenario. Ind. J. Med. Sci. 2000; 54(12): 535-40.
24. Mehta A, Rodrigues C, Kumar R, et al. A pilot programme of MRSA surveillance in India (MRSA Surveillance Study group). Postgrad. Med. J. 1996; 42(1): 1-3.
25. Asha Pai K.B. Sweetha N.N., Sanjeev H., Rekha R., Vimal K.K. & Ganesh H.R. et al. Increased Vancomycin minimum inhibitory concentration (MIC) among MRSA isolates in a tertiary care Hospital. Nitte University Journal of Health Science Vol. 3, No.4, December 2013.
26. Mewara A, Gautam V, Kaur H, Ray P. *In vitro* evaluation of antibiotics for methicillin-resistant *Staphylococcus aureus* from north India. Indian J Med Res.2014;139:319-22.
27. Sakoulas G, Moise-Broder PA, Schentag J, Forrest A, Moellering RC Jr, Eliopoulos GM. Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. *J Clin Microbiol* 2004; *42*:2398-402.
28. Horieh Saderi, Parviz Owlia, Zohreh Maleki, Mehri Habibi, Nayere Rahmati. Susceptibility to Vancomycin in Staphylococcus aureus isolated from patients of four University-Affiliated Hospitals in Tehran. Iranian Journal of Pathology (2008);3(3):161 – 166.
29. Venubabu Thati, Channappa T. Shivannavar, and Subhaschandra M. Gaddad et al. Vancomycin resistance among methicillin resistant *Staphylococcus aureus* isolates from intensive care units of tertiary care hospitals in Hyderabad. Indian J Med Res. Nov 2011; 134(5): 704–708.
30. Dr. Susmita Bhattacharya, Dr. Kuhu Pal, Dr. Mitali Chatterjee, Dr. Mandira Banerjee, Dr. Pratip Kumar Kundu, Dr. Swapan Kumar Niyogi, et al. Vancomycin intermediate Staphylococcus aureus isolated from a tertiary care hospital in Kolkata. IOSR Journal of Dental and Medical Sciences. 2013;5(2):19-23.
31. Dr. Yogesh Kumar Gupta, Dr. Garima Gupta, Dr. S.P. Garg, Dr. Prem Singh Nirwan, et al. Prevalence and antimicrobial susceptibility pattern of Methicillin resistant Staphylococcus aureus isolated at a tertiary care institute in North West Region of Rajasthan. International Research Journal of Pharmaceutical and Applied Sciences. 2013;3(6):13-16.
32. CLSI. 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 7th ed. CLSI document M7-A8. CLSI, Wayne, PA.
33. Dhanalakshmi T.A, Umapathy B.L, Mohan D.R, et al. Prevalence of Methicillin, Vancomycin and Multidrug Resistance among Staphylococcus aureus. Journal of Clinical and Diagnostic Research. 2012 August;6(6): 974-977.

How to cite this article: Kulshrestha A., Anamika V., Mrithunjay K., Dalal A.S., Manish K. A study on the prevalence of vancomycin resistant and intermediate staphylococcus aureus isolated from various clinical Specimen in a tertiary care hospital and detection of their MIC values by E-test. International Journal of Medical Microbiology and Tropical Diseases 2017;3(3):119-125.