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RESEARCH ARTICLE

A STUDY ON METHICILLIN AND VANCOMYCIN RESISTANT *STAPHYLOCOCCUS AUREUS* FROM TERTIARY CARE HOSPITALS, IN VIDHARBHA REGION, INDIA

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ABSTRACT

Multidrug resistant methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial and community acquired infections and is on the rise. The glycopeptides vancomycin has been proposed as the drug of choice for treating such infections. The present study aimed at identifying the methicillin and vancomycin resistance *staphylococcus aureus* from tertiary care hospitals in Vidarbha region, (M/S) India. This study presents the report of methicillin and vancomycin heteroresistance in *Staphylococcus aureus* isolate from clinical samples. The original isolate was resistant in vitro to methicillin and fewer to vancomycin. Resistance confirmed for all isolates with E-tests using strips of methicillin MIC of >265 mcg/ml and vancomycin MIC of >256 mcg/ml. MRSA were isolated and identified from different clinical samples using conventional methods. Antibiogram of the isolates and MIC were determined following CLSI guidelines. All Multi Drug resistant *Staphylococcus aureus* isolates were MRSA. Only four Vancomycin resistant *Staphylococcus aureus* were found, out of the all MRSA isolates. All MRSA had a Methicillin MIC > 265 mcg/ ml except one showing 5.0 mcg/ml that of three VRSA had a Vancomycin MIC > 265 mcg/ ml and one shows MIC 24 mcg/ml.

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INTRODUCTION

Staphylococcus aureus is one of the most common causes of nosocomial infections, especially pneumonia, surgical site infections and blood stream infections andcontinues to be a major cause of community and hospital acquired infections (Bhateja et al, 2005; Loon, 2000; Proctor RA And Peters G, 1998). Methicillin-resistant *Staphylococcus aureus* (MRSA) was first detected approximately 40 years ago and is still among the top three clinically important pathogens (Stewart et al, 1961). The emergence of high levels of penicillin resistance followed by the development and spread of strains resistant to the semisynthetic penicillins (methicillin, oxacillin, and), macrolides, tetracycline, and aminoglycosides has made the therapy of staphylococcal disease a global challenge (Wootton et al, 2001). The glycopeptides vancomycin was considered to be the best alternative for the treatment of multidrug resistant *Staphylococcus aureus*. However, there are increasing numbers of reports indicating the emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA) strains exhibiting two different resistance mechanisms (Mathews AA et al, 2010;Saderi H et al, 2005). Initially vancomycin Intermediate *Staphylococcus aureus* (VISA) noted in Japan in 1996 and subsequently in United States in 1997 was believed to be due to the thickened cell wall, where many vancomycin molecules were trapped within the cell wall. The trapped molecules clog the peptidoglycan meshwork and finally form aphysical barrier towards further incoming vancomycin molecules (Forbes BA et al, 2007). The second, noted in United States in 2002 among *Staphylococcus aureus*, was identical to the mechanism seen in vancomycin-resistant *Enterococcus*. Vancomycin resistant *Enterococcus faecium* harbours the vanA operon, which contains five genes, *Van*S, -R, -H, -A and –X8. But Tiwari and Sen have reported a VRSA which is *van* gene-negative. Subsequent isolation of VISA and VRSA isolates from other countries including Brazil, France, United Kingdom, Germany, India, and Belgium has confirmed that the emergence of these strains is a global issue (Arthur et al, 1993). The aim of the present study was to identify the emergence of vancomycin resistant *Staphylococcus aureus* (VRSA) and methicillin resistant *Staphylococcus aureus* (MRSA) among *Staphylococcus aureus* isolates from tertiary care hospitals in Vidarbha region, Maharashtra, India, and to determine the sensitivity of these isolates to different antimicrobial agents. Further search is also conducted for the mecA and vanA gene in MRSA and VRSA strains (Pierard D et al, 2004).

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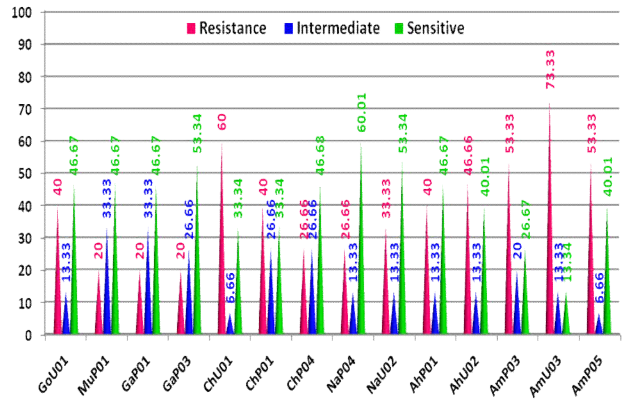


Fig. 1. Antibiogram of Staphylococcus aureus isolates showing different types of antibiotic resistance, intermediate and sensitive pattern in percentage

Prevelence of methicillin and vancomycin resistance in Staphylococcus aureus

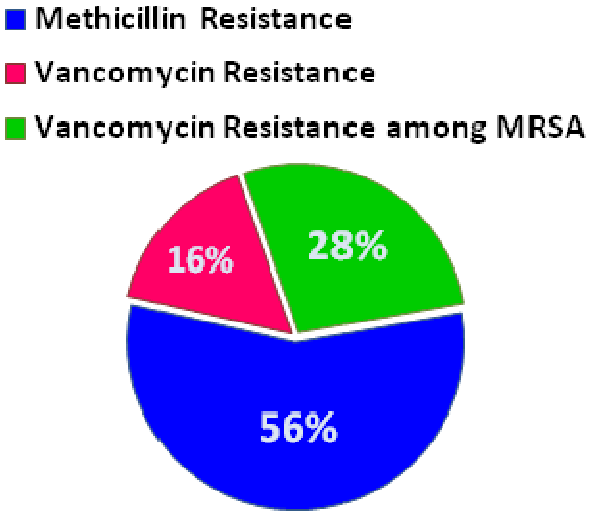


Fig. 2 Represent the Methicillin and vancomycin resistance pattern in percentage with MIC strip test (E-test)

MATERIALS AND METHODS

Bacterial isolates: A total of 21 numbers of *Staphylococcus aureus* isolates were obtained randomly from clinical samples (blood, urine and burn lesion swabs, wound swabs) of admitted patients in tertiary care hospitals, in Vidarbha region between February and September 2016. The study was doneat Centre for Higher Learning and Research in Microbiology, Sardar Patel Mahavidyalaya, Chandrapur (M.S.), India.

Staphylococcus aureus was identified by colony morphology, Gram stain, DNase, catalase and coagulase tests and fermentation of mannitol by conventional methods.

Antibiotic susceptibility testing

The antibiotic resistance profile was determined by the Disc Agar Diffusion (DAD) technique using different antimicrobial agents; Amikacin (30 µg), Ceprofaxacin (5µg), Chloramphenical (30 µg), Erythromycin (15 µg), Gentamicin (10 µg), Lincomycin (2µg), Methicillin (30 µg), Netillin (30 µg), Norfloxacin (10 µg), Oxacillin (1µg), Penicillin G (10 U), Trimethoprime (5µg) , Tetracycline (30 µg), Tobramicin (10 µg), Vancomycin (30 µg) (Hi-media, Mumbai India) according to the guidelines recommended by Clinical and Laboratory Standards Institute (CLSI) (Wayne Pa, 2007, Wayne Pa, 2006). The standard *Staphylococcus aureus* strains NCIM 5522 and NCIM 5521 were used as reference strains (Centre for Higher Learning and Research in microbiology, Sardar Patel Mahavidyalaya, Chandrapur) for MRSA.

Determination of MIC

Minimal inhibitory concentration (MIC) of methicillin and vancomycin was determined by E-test of disc diffusion method using CLSI guidelines (Class II Special Control Guidance Document, 2009). Briefly, Plates of Hi-sensitivity agar (Hi-media) was prepared with forming lawn of inoculums prepared using 18-24 h old culture was spotted with placing gradient strip 0.5 mcg to > 265 mcg / ml of oxacillin and vanomycinon respectively. Plates were incubated overnight at 35^{o}C for 24h before assessing the visible growth (CLSI Performance standards for antimicrobial susceptibility testing M100-S (latest edition); CLSI Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Approved Standard M7-A; CLSI Methods for Dilution Antimicrobial Susceptibility Tests of Anaerobic Bacteria Approved Standard M11-A (Latest edition).

RESULTS AND DISCUSSION

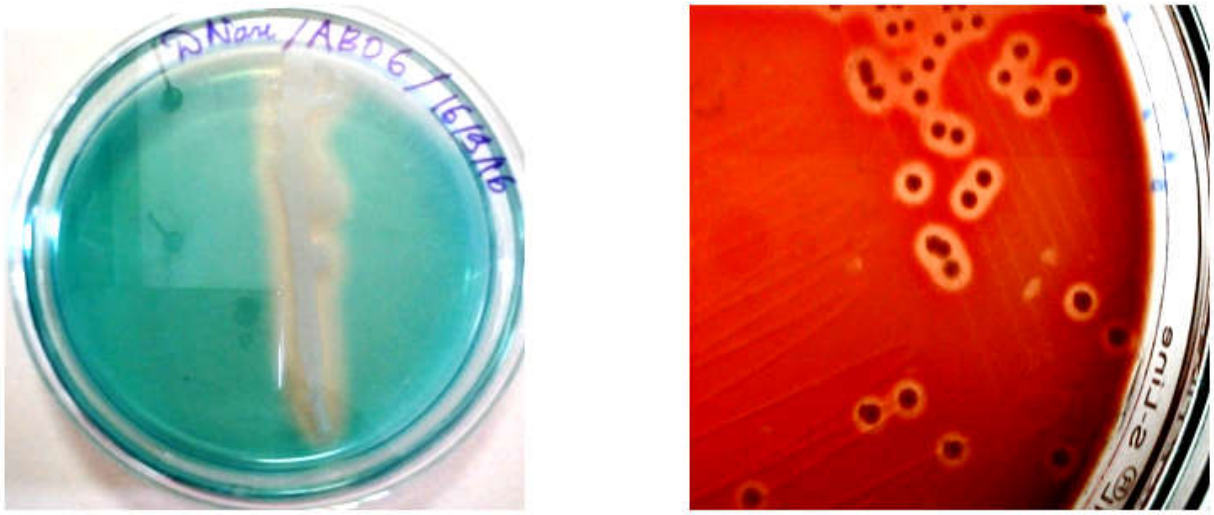
Various clinical samples were analyzed for the infection with *Staphylococcus aureus* and checked for their antibiotic resistance. Throughout the all samples 24 numbers of *Staphylococcus aureus* isolates confirmed including coagulase positive and coagulase negative strains. One of the limitations of the present studywas that, the detection of mecA or PBP 2a which is considered asthe gold standard for detecting the MRSA strains was not done because of technical and economic constrains (Rahbar et al, 2006).

Table No. 1: *Staphylococcus aureus* isolates from different location and different clinical samples

Name of Tertiary Care Hospital	Clinical Sample	S.aureus Isolates	No. of MRSA	No. of VRSA
Civil HospitalGondpimpri	Urine	02	01	NII
Civil Hospital Mul	Blood	01	NIL	NII
	Urine	03	NIL	NII
	Pus	03	01	NII
Civil Hospital Gadchiroli	Pus	02	02	NII
Civil Hospital Chandrapur	Urine	01	01	NII
	Pus	02	02	NII
Civil Hospital Nagpur	Pus	02	01	01
	Urine	01	01	NII
Civil Hospital Aheri &Bharmragad	Pus	02	01	01
	Urine	02	01	NII
Civil Hospital Amravati	Urine	01	01	NII
	Pus	02	02	02
Total		24	14 (58.33%)	04 (16.66%)

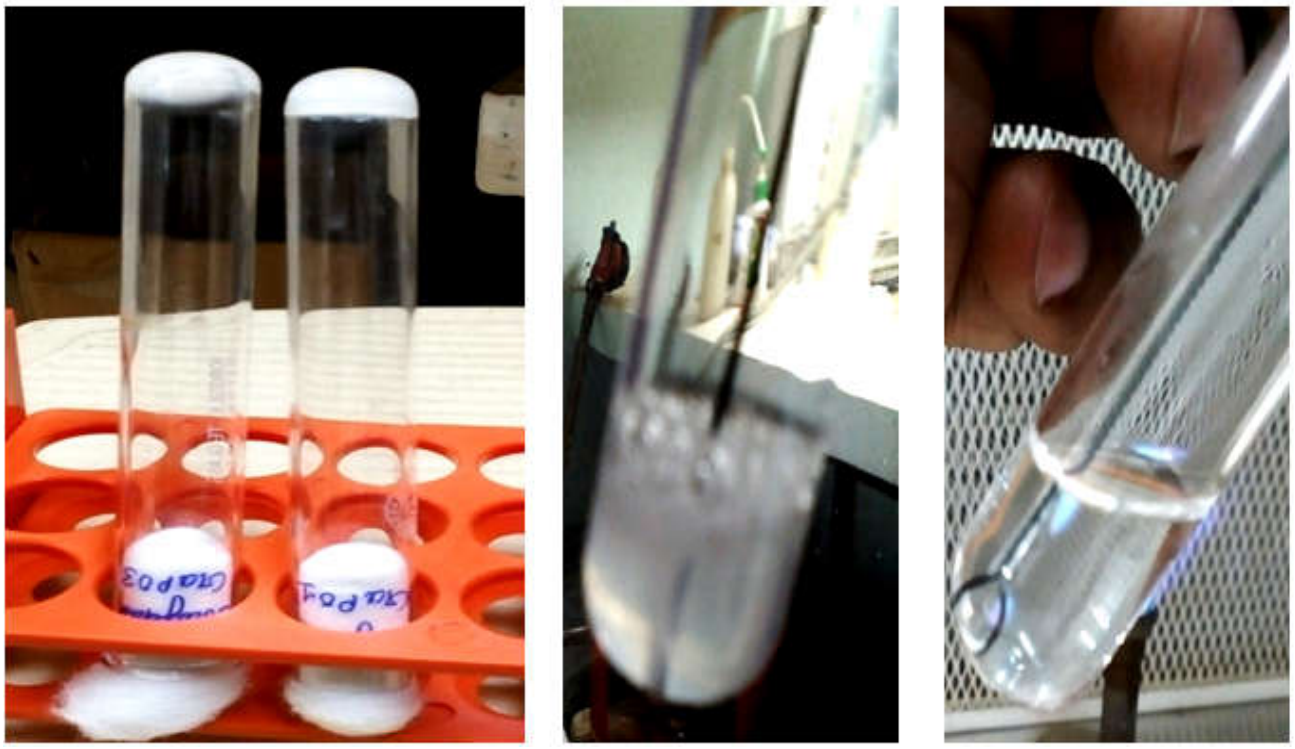
Table No. 2. Biochemical characteristics of MRSA and VRSA isolates

Codes of Isolates	DNase Test	Coagulase Test	Catalase Test
GoU01	Positive√	Negative	Negative
MuP01	Positive√	Negative	Negative
GaP01	Positive√	Positive√	Negative
GaP03	Positive√	Positive√	Negative
ChU01	Positive√	Negative	Negative
ChP01	Positive√	Positive√	Negative
ChP04	Positive√	Positive√	Negative
NaP04	Positive√	Positive√	Positive√
NaU02	Positive√	Positive√	Negative
AhP01	Positive√	Negative	Positive√
AhU02	Positive√	Positive√	Negative
AmP03	Positive√	Positive√	Positive√
AmU03	Positive√	Negative	Positive√
AmP05	Positive√	Positive√	Positive√



A. **B.**

Fig.1. (A) S. *aureus* on DNase Agar Medium (Zone of Clearance around the colonies)
(B) *S. aureus* on Blood Agar(Beta Haemolysis)



A. **B.** **C.**

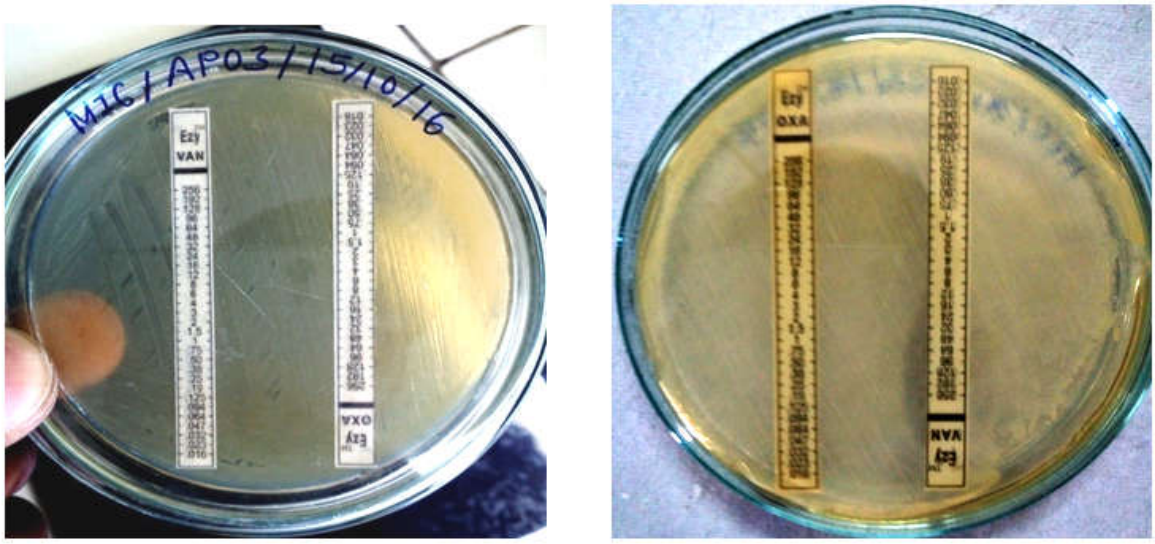
Fig.2. (A) Coagulase Test (with rabbit Plasma)
(B) Catalase tube test (with 3% H_2O_2) Positive, and (C) Negative

Table No. 3. Antibiogram of all *Staphylococcus aureus* Isolates

Antibiotics / S. aureus isolates	GoU01	MuP01	GaP01	GaP03	ChU01	ChP01	ChP04	NaP04	NaU02	AhP01	AhU02	AmP03	AmU03	AmP05
Oxacillin	Resistant	Inter-	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Methicillin	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Ciproflaxacin	Resistant	Inter-mediate	Inter-mediate	Inter-mediate	Resistant	Inter-mediate	Inter-mediate	Inter-mediate	Inter-mediate	Inter-mediate	Resistant	Inter-mediate	Inter-mediate	Resistant
Erythromycin	Resistant	Inter-mediate	Sensitive	Sensitive	Resistant	Resistant	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Penicillin	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Tetracyclin	Resistant	Resistant	Sensitive	Sensitive	Sensitive	Inter-mediate	Sensitive	Sensitive	Resistant	Resistant	Inter-mediate	Inter-mediate	Resistant	Sensitive
Amikacin	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Chloramphenicol	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Inter-mediate	Resistant
Gentamycin	Sensitive	Sensitive	Inter-mediate	Sensitive	Resistant	Inter-mediate	Inter-mediate	Sensitive	Sensitive	Sensitive	Resistant	Sensitive	Resistant	Sensitive
Lincomycin	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant
Netillin	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Trimethoprim	Sensitive	Sensitive	Inter-mediate	Inter-mediate	Resistant	Resistant	Resistant	Inter-mediate	Inter-mediate	Inter-mediate	Inter-mediate	Inter-mediate	Resistant	Resistant
Tobramycin	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Sensitive
Vancomycin	Inter-mediate	Inter-mediate	Inter-mediate	Inter-mediate	Inter-mediate	Inter-mediate	Inter-mediate	Resistant	Sensitive	Resistant	Sensitive	Resistant	Resistant	Sensitive
Norfloxacin	Inter-mediate	Inter-mediate	Inter-mediate	Inter-mediate	Resistant	Resistant	Inter-mediate	Sensitive	Sensitive	Sensitive	Inter	Resistant	Resistant	Inter-mediate

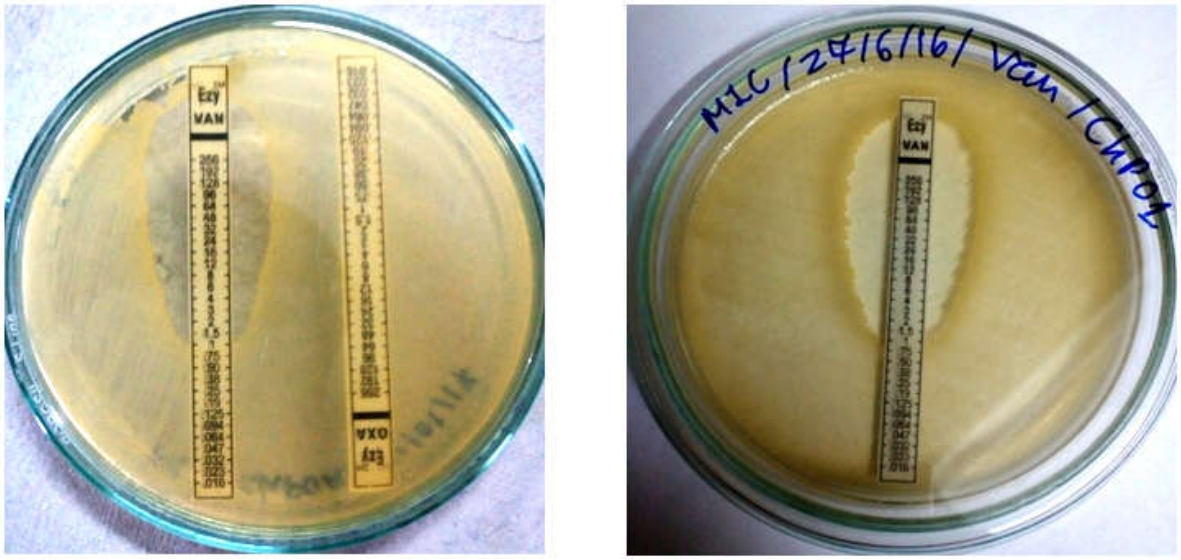
Table No. 4. Results obtained with E-Test of *Staphylococcus aureus* isolates

S. aureus Isolates	Oxacillin MIC Strip test	Vancomycin MIC Strip test	Conclusion
GoU01	> 265 mcg /ml	1mcg	Methicilin Resistant
MuP01	>265 mcg /ml	1.5 mcg /ml	Methicilin Sensitive
GaP01	> 265 mcg /ml	1.0 mcg /ml	Methicilin Resistant
GaP03	>265 mcg /ml	1.0 mcg /ml	Methicilin Resistant
ChU01	> 265 mcg /ml	1.0 mcg /ml	Methicilin Resistant
ChP01	> 265 mcg /ml	1.0 mcg /ml	Methicilin Resistant
ChP04	>265 mcg /ml	1.0 mcg /ml	Methicilin Resistant
NaP04	>265 mcg /ml	24 mcg /ml	Vancomycin Resistant
NaU02	>265 mcg /ml	1.2 mcg / ml	Methicilin Resistant
AhP01	>265 mcg /ml	>265 mcg / ml	Vancomycin Resistant
AhU02	>265 mcg /ml	1.0 mcg /ml	Methicilin Resistant
AmP03	>265 mcg / ml	>265 mcg / ml	Vancomycin Resistant
AmU03	5.0 mcg / ml	>265 mcg / ml	Vancomycin Resistant
AmP05	>265 mcg / ml	1.0 mcg / ml	Methicilin Resistant



A. B.

Fig. 3. (A) & (B) E-test with Oxacillin MIC Strip And Vancomycin MIC Strip (No Zone of Inhibition)



C. D.

Fig. 4. (C) & (D) E-test with Vancomycin MIC Strip showing zone of inhibition

All the *Staphylococcus aureus* isolates were further analyzed for their pathogenic characterization along with antibiotic sensitivity test (Disc Agar Diffusion method) and resistance confirmed with E-test. Vancomycin resistance has been perceived as a fearsome threat to the already challenging therapy of MRSA and MDR-MRSA (Tiwari HS et al, 2008). Along with all representative data and the results obtained, it seen that a serious need for more study and research including molecular characterization of MRSA as well as VRSA (Prax M et al, 2013). Also there is a need to do more research and studies at the plenty of knowledge to knowing the exact mechanism of acquiring antibiotic resistance in *Staphylococcus aureus* along with proper treatment of patients against such an infection from *Staphylococcus aureus* in tertiary care hospitals.The morphological, biochemical and MIC test observation found for all among the MRSA and VRSA isolates as well as pictorial data as fallows.

Conclusion

From the above results and discussion this is concluded that, *Staphylococcus aureus* were shows higher prevalence in pus or wound swabs and next in urine samples. The resistant pattern shown by these isolats where checked by the standard Disc Agar Diffusion test as methicillin resistant *Staphylococcus aureus* (MRSA) were shown 58.33%and vancomycin resistant *Staphylococcus aureus* (VRSA) 16.66%out of all *Staphylococcus aureus* isolates and only 28.57% shows vancomycin resistant out of all MRSA. All the MRSA and VRSA isolates had shown multi drug resistance where MIC confirmed with E-test (CLSI, Approved Standard, M7-A (Latest edition) shows resistant against methicillin 92.85% and 28.57% against vancomycin. But all the MRSA and VRSA were found susceptible to Amikacin, Netilin and shows 100 % resistant against penicillin-G.

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