Original Research Article

Methicillin Resistant *Staphylococcus aureus* (MRSA) and Vancomycin Resistant *Staphylococcus aureus* (VRSA) from a rural based tertiary care and teaching hospital in Vadodara district, Gujarat

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Abstract

Background: Staphylococci are ubiquitous being the normal inhabitants of the skin and mucous membranes and the most common cause of human infections all throughout the world, both the community acquired as well as nosocomial infections.

Objectives: Objectives of this study were to determine the prevalence of methicillin resistant *Staphylococcus aures* (MRSA) and detection of emergence of resistance to vancomycin among the *Staphylococcus aureus (S. aureus)* isolates.

Materials and methods: Thus hundred *S. aureus* isolated from various clinical samples were tested for methicillin resistance by cefoxitin disc (30µg) and vancomycin resistance using Ezy MIC – Vancomycin E-test.

Results: The MRSA prevalence was found to be 52%. Of the total MRSA (n=52) 32 were obtained from male and 20 from female; and 36.54% from blood, 28.55%, 15.38%, 11.54% and 3.85% from pus, urine, sputum and body fluids respectively. The MRSA (n=52) were found to be resistant to antibiotics tested routinely but susceptible to levofloxacin (86.54%), doxycycline (92.31%), linezolid (96.15%) and vancomycin (100%). Inducible clindamycin resistance amongst MRSA was found to be 25%. All strains i.e.100% were sensitive to vancomycin indicating zero resistance to vancomycin.
Conclusion: Though we did not find any resistance to vancomcyin in our setup, the prevalence of MRSA is high in our set up and calls for strict implementation of hospital infection control measures to prevent the spread of this organism and infections due to it. In this study E-test proved to be useful for detection of vancomycin resistance.

Key words

Staphylococcus aureus, MRSA, VRSA, E-test.

Introduction

Staphylococci are ubiquitous and the most common cause of human infections all throughout the world, both the community acquired as well as nosocomial infections [1]. They are important pathogens posing challenges to the clinicians in treating infections caused by them due to development of resistance to Penicillin first, then to oxacillin (semi-synthetic compounds) i.e. MRSA (Methicillin Resistant *Staphylococcus aureus*) as well as to other groups of antibiotics like macrolides, aminoglycosides and tetracyclines [2].

Vancomycin was considered to be the treatment of choice for MRSA infections. But now there are increased reports of emergence of vancomycin resistance among the *Staphylococcus aureus* isolates from various parts of the world. Reduced susceptibility to Vancomycin was first reported from Japan in 1996 and subsequently from USA in 1997. Later the isolation of VRSA from various other countries confirmed the emergence of these strains all over the world [2-9]. Thus detection of vancomycin resistance should be carried out for its timely detection and measures to control its spread.

The vancomycin, been a high molecular weight antibiotic, does not diffuse in concentration gradient manner while diffusing through the agar medium when the disc susceptibility test is employed. Moreover it does not differentiate between vancomycin-susceptible isolates from vancomycin-intermediate isolates of *S. aureus* and hence disc diffusion testing is not used for detection of VRSA [10]. But E-test, as recommended by CDC, can be useful for detecting vancomycin resistance in laboratories lacking facilities of genetic studies [11].

A routine testing for MRSA and VRSA detection should be carried out which would be useful for taking control measures to prevent the spread of MRSA and VRSA strains and infections due to them in hospitals.

The objectives of the present study were to determine the prevalence of MRSA and emergence of resistance to vancomycin by MIC-Etest among *Staphylococcus aureus* isolated from the clinical samples from a rural based tertiary hospital in Vadodara district of Gujarat.

Materials and methods

This study was carried out in Clinical Microbiology Laboratory under Dept. of Microbiology at Dhiraj General Hospital after ethical approval from institutional ethical committee. A total of 100 *Staphylococcus aureus (S. aureus),* isolated from various clinical samples received from various wards of the

hospital, were identified by standard methods [12].

Antimicrobial Susceptibility Testing: Anti-microbial susceptibility test was performed for all isolates, by modified Kirby-Bauer method according to CLSI guidelines, against Penicillin (10 units), Erythromycin (15µg), Clindamycin (2µg), Gentamicin (10µg), Doxycycline (30 µg), Levofloxacin (5 µg), Linezolid (15 µg) obtained from HiMedia and observed after 18-24 hours after incubation at 37^0C [10].

Detection of MRSA: All isolates were tested for methicillin resistance using Cefoxitin disk (30µg) and incubating plates for 24 hours at 33^0 - 35^0C as per CLSI. The plates were observed for zone of inhibition in a reflected light on a non-reflecting background. Isolates showing zone of inhibition of <21 mm, were interpreted as MRSA according to CLSI **(Figure - 1)**. Control strains used were *S. aureus* – ATCC 25923 (mec-A negative and zone size 23-29 mm) and ATCC 43300 – mec-A positive and zone size ≤21mm) [10].

Figure - 1: MRSA detection by Cefoxitin (30µg) disc).

Detection of Inducible Clindamycin Resistance: Also all the isolates were observed for clindamycin resistance induced by erythromycin by placing Erythromycin (15µg) disk 24 mm adjacent to Clindamycin (2µg) disk on the same AST plate. Plates were observed for flattening of zone of inhibition (formation of D zone) adjacent to Erythromycin indicating an inducible resistance to Clindamycin [10].

Detection of VRSA: CLSI recommends agar dilution method for detection of VRSA. However, CDC recommends [11] Etest as an acceptable method for detection of vancomycin resistance using 0.5 McFarland and 24 hours incubation. Etest combines the concepts of dilution and diffusion principles for susceptibility testing. A predefined concentration gradient antibiotic across 15 two-fold dilutions of a conventional MIC method is immobilized on the surface of the carrier plastic strip [13]. Hence to detect vancomycin resistance among *S. aureus* in our hospital, Ezy-MIC-Etest, obtained from HiMedia Laboratories Pvt. Ltd. Mumbai, was used. According to manufacturer, Ezy-MIC-Etest helps overcomes the problem of differentiating Vancomycin Intermediate *Staphylococcus aureus* (VISA) from Vancomycin Suscptible isolates. VISA can be detected when isolated colonies appear within the zone of inhibition of Vancomycin particularly when 1.0 MacFarland inoculum is used and MIC is read after complete 48 hours of incubation. According to the manufacturer's instructions lawn cultures of all *S. aureus* were made on MHA using 0.5 McFarland standard. An E-test strip containing a concentration gradient of vancomycin ranging from 0.016 - 256 µg/ml was placed on the surface of the agar using an applicator stick provided with the kit. The plates were incubated for 24 hours at 37^0C and observed the next day. The MIC values were read where the edge of inhibition ellipse mean intersected the strip **(Figure - 2)**. The control strains used: *Enterococcus faecalis* ATCC 29212 – Susceptible; *E. faecalis* ATCC 51299 – Resistant; also *S. aureus* ATCC 25923.

The data that was obtained was recorded in Microsoft Excel (2007 version) and analyzed.

The results are expressed in frequency (number) and in percentage.

Figure - 2: Vancomycin MIC by Ezy-MIC (Etest - HiMedia).

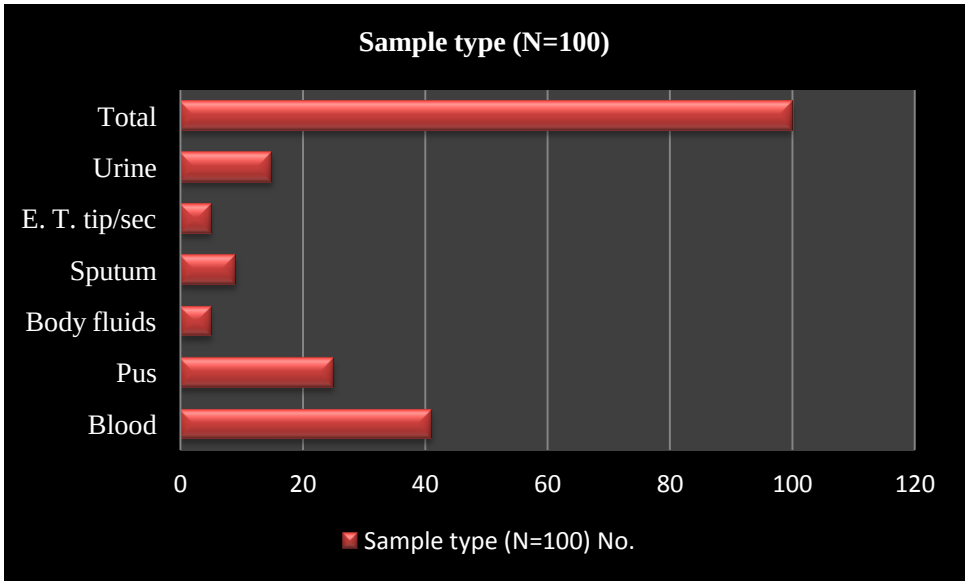
Results

A total of 100 *S. aureus* isolates were tested for oxacillin (methicillin) resistance as well as vancomycin resistance. The details of clinical specimens and wards from which they were recovered are shown in **Chart - 1** and **Chart - 2** respectively. Of the 100 tested, 63 were from male and 37 from female patients.

Of the total 100 *S. aureus* isolates 52 were detected as MRSA and the other 48 as MSSA. Of the 52 MRSA isolates 32 (61.54%) were from male and 20 (38.46%) from female patients. The different sources i.e. samples and wards from which MRSA were recovered are shown in **Chart - 3** and **Chart - 4** respectively. Thus maximum i.e. 36.54% MRSA were isolated from blood samples i.e. patients with suspected/diagnosed sepsis; followed by 28.85% from pus i.e. skin and soft tissue infections, 15.38% from urine – urinary tract infections and 11.54% from sputum samples representing respiratory tract infections and least from body fluids and E.T tips (endotracheal tips) each 3.85%. Maximum numbers of MRSA isolates were obtained from samples of ICU i.e. 38.46%, followed by 15.38% from Medicine, 11.54% Surgery, Orthopedics 9.62% and minimum from Plastic Surgery Ward (1.92%).

Erythromycin induced resistance against clindamycin was seen in only 13 out of MRSA (n=52) strains and 4 out of MSSA strains (n=48).

Chart - 1: Distribution of *S. aureus* isolates from various types of clinical samples.



Susceptibility pattern of the MRSA and MSSA isolates is shown in **Chart - 5**. The MRSA strains were found to show decreased susceptibility against multiple antibiotics as

compared to the MSSA strains. Though MRSA strains were resistant to a number of antibiotics they showed a high susceptibility towards levofloxacin (86.54%), doxycyline (92.31%), linezolid (96.5%) and vancomycin (100%).

All the *S. aureus* isolates (N=100) including MRSA (n=52) and MSSA (n=48), tested for vancomycin resistance, exhibited an MIC of ≤2µg/ml (as shown in Figure 1) and interpreted as susceptible to vancomycin. The MIC values of all the MRSA (n=52) isolates are shown in **Chart - 6**.

Vancomycin resistance was not detected among the *S. aureus* isolates from clinical samples irrespective of resistance to oxacillin. Thus there is no vancomycin resistance in our set up.

Chart - 2: Distribution of *S. aureus* obtained from various wards.

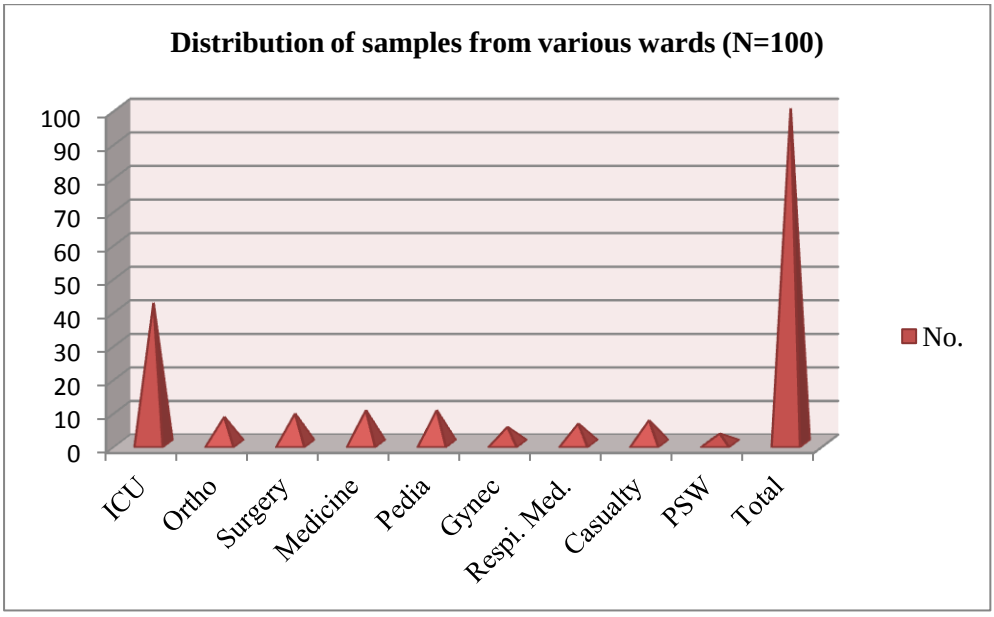


Chart - 3: Distribution of MRSA based on recovery source.

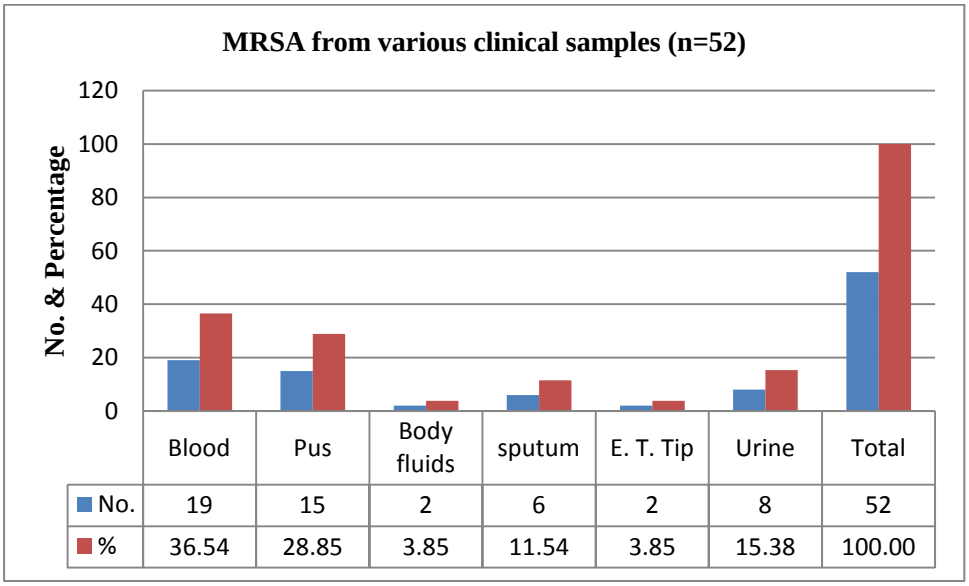
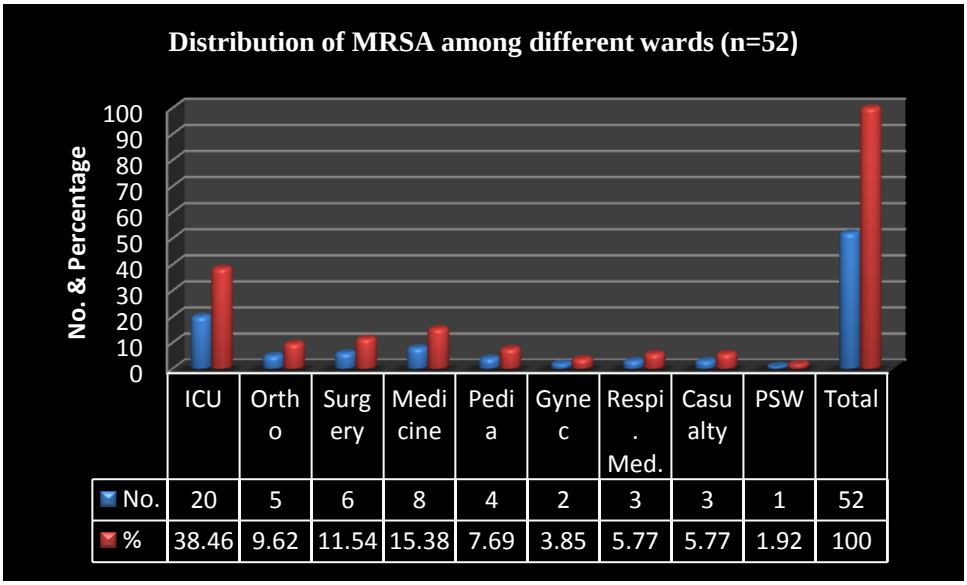
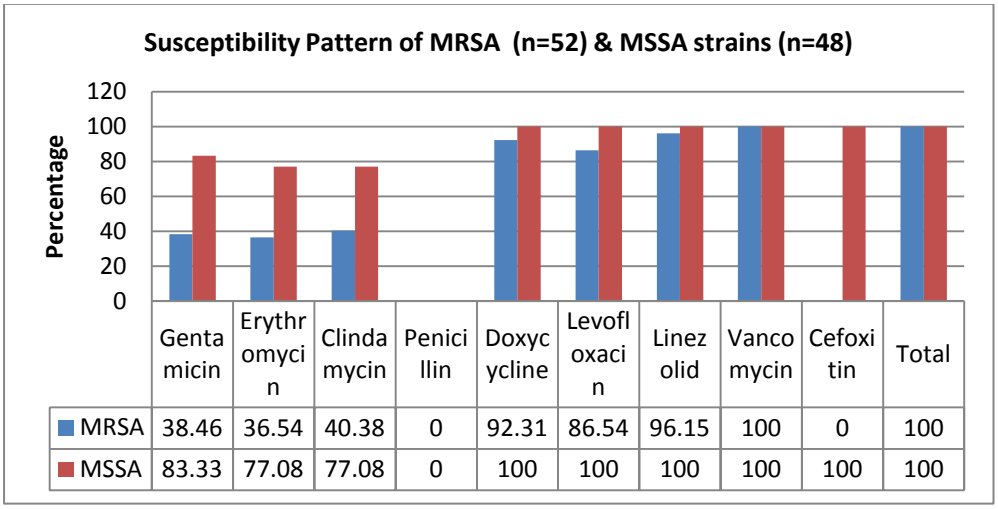


Chart - 4: Distribution of MRSA among different Wards.



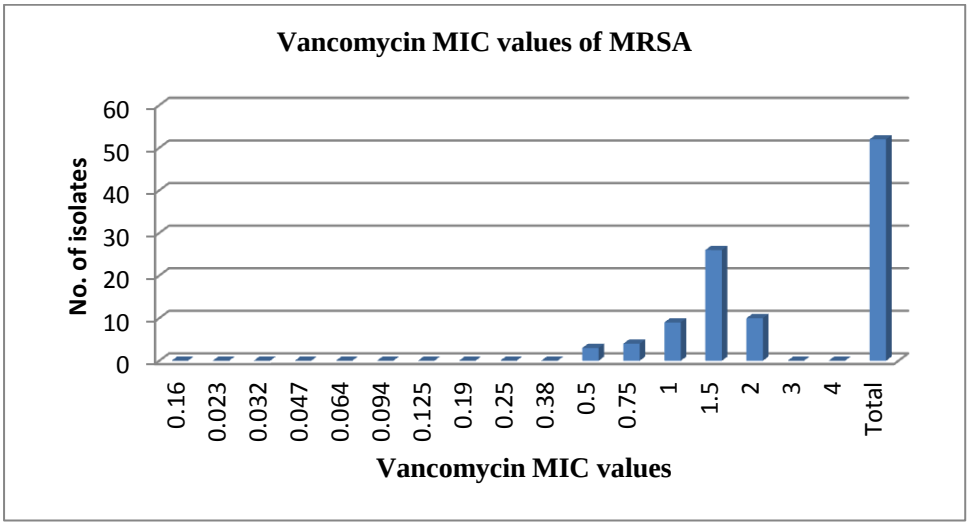
	ICU	Ortho	Surgery	Medicine	Pedia	Gynec	Respi. Med.	Casualty	PSW	Total
No.	20	5	6	8	4	2	3	3	1	52
%	38.46	9.62	11.54	15.38	7.69	3.85	5.77	5.77	1.92	100

Chart - 5: Sensitivity Pattern of MRSA (n=52) and MSSA (n=48) to various antibiotics tested(in percentage).



	Gentamicin	Erythromycin	Clindamycin	Penicillin	Doxycycline	Levofloxacin	Linezolid	Vancomycin	Cefoxitin	Total
MRSA	38.46	36.54	40.38	0	92.31	86.54	96.15	100	0	100
MSSA	83.33	77.08	77.08	0	100	100	100	100	100	100

Chart - 6: MIC values of the MRSA isolates (n=52).



Discussion

In the present study the prevalence of MRSA was found to be 52% which is similar to a study by S Anupurba, et al. in 2003 who reported prevalence of 54.85% from a hospital in eastern Uttar Pradesh [15]. **Table - 1** shows the comparison of MRSA prevalence of the present study with other studies from India.

In the present study maximum number of MRSA were isolated from blood samples i.e. 36.54% which is different from other studies that reported 15%, 13.7%, 9.16% [16, 18, 19], who found maximum number of MRSA from pus samples; followed by 28.85% from pus i.e. skin and soft tissue infections which is similar to a study by Shrikanth, et al. [18] - 27.5% but lower to 71% and 66.03% [16, 19] ; followed by 15.38% from urine i.e. urinary tract infections which is higher as compared to 3.51% [16] and similar to 11.45% and 17.2% [18, 19]. Also 11.54% from sputum samples representing respiratory tract infections versus 4.09% by Trivedi MB, et al. [16], 10.3% by Shrikanth, et al. [18], 8.02% by Goyal A, et al. [19] and least from body fluids and E.T tips (endotracheal tips) i.e. 3.85% versus 5.26% from other samples by Trivedi MB, et al. [16], 0.76% by Goyal A, et al. [19], and 6.8% by Shrikanth, et al. [18]. The highest prevalence of MRSA was observed in ICU i.e. 38.46% followed by 15.38% from Medicine versus 12.44% and 10.88% respectively, reported by Goyal A, et al. in their study [19].

Table - 1: Comparison of MRSA prevalence with Indian studies.

Place	Author	Prevalence
Hyderabad	Thati Venu, et al. [2]	79.6%
Varanasi	Tiwari HK, et al. [3]	40.61%
Ahmedabad	Minal Trivedi, et al. [16]	20.25%
Jamnagar	Nutanbala N. Goswami, et al. [17]	29.17%
Gulbarga	Shrikanth, et al. [18]	32.2%
Agra	Ankur Goyal, et al. [19]	32.6%
Vishakhapatnam	Bandaru Narasinga Rao, et al. [20]	45%
Karnataka	Hanumanthappa, et al. [21]	43%
Vadodara	Present study	52%

Of the total 52 MRSA strains 38.46% were sensitive to Gentamicin versus 10.53% by Trivedi MB, et al. [16], 34.67% by Goyal A, et al. [19] and 32.7% by Shrikanth, et al. [18]; 36.54% were sensitive to Erythromycin which is higher compared to 32.7% by Shrikanth, et al. [18] but lower to 12% by Goyal A, et al. [19]; 40.38% were sensitive to Clindamycin versus 49.33% by Goyal A, et al. [19] and 29.3% by Shrikanth, et al. [18]. None of the MRSA strains were sensitive to Penicillin which is comparable to Rao BN [20] and much lower to 25% reported by Goswami NN, et al. [17] from Gujarat. The percentage of MRSA strains sensitive to Doxycycline was 92.31% which is higher compared to 79.04% reported by Goyal A, et al. [19]; 86.54% were susceptible to Levofloxacin which is closer to 80.7% by Trivedi MB, et al. [16] but higher compared to 60.42% by Goswami NN, et al. [17]; 96.15% were found sensitive to Linezolid which is comparable to 99.42% by Trivedi MB, et al. [16] and 100% by Goswami NN, et al. [17]. All the MRSA i.e. 100% strains were sensitive to Vancomycin, a significant finding of our study, is comparable to Trivedi MB, et al. [16], Goyal A, et al. [19] and higher compared to 61.4% reported by Goswami NN, et al. [17] and 96.5% reported by Shrikanth, et al. [18] while Tiwari HK, et al. reported 0.25% VRSA and 0.76% VISA from Varanasi [3]. Inducible clindamycin resistance among MRSA (n=52) was 25% and MSSA (n=48) was (8.33%)

which is quite lesser to that reported by Ghosh S i.e.54.54% and 37.14% in MRSA and MSSA respectively [21].

Of the total MSSA (n=48) 83.33% were sensitive to Gentamicin which is comparable to 85.64% reported by Trivedi MB, et al. [16] and 86.05% by Goyal A, et al. [19], lower compared to 91.8% by Shrikanth, et al. [18] and higher than 77.78% by Goswami N, et al. [17]; 77.08% were susceptible to Erythromycin which is similar to 77.78% reported by Goswami N, et al. [17], lower compared to 82.8% by Shrikanth, et al. [18] but higher to 67.35% by Goyal A, et al. [19]; 77.08% were found sensitive to Clindamycin which is comparable to 77.01% by Goyal A, et al. [19], lower compared to 83.6% by Shrikanth, et al. [18] and higher compared to 44.44% reported by Goswami NN, et al. [17]. None of the MSSA strains were found susceptible to Penicillin which is lower compared to 55.56% reported by Goswami NN, et al. [17]. All MSSA strains were100% susceptible to doxycycline which is higher compared to 84.8% by Goyal A, et al. [19]. The MSSA strains were 100% susceptible to levofloxacin which is similar to that reported by Goswami NN, et al. [17] and closer to 99.13% by Trivedi MB, et al. [16]. Sensitivity of MSSA strains towards linezolid was 100% which is similar to that reported by Trivedi MB, et al. [16].

All the 100 *S. aureus* isolates i.e. 100% were sensitive to Cefoxitin and Vancomycin which is comparable to findings of Trivedi MB, et al.; Goswami NN, et al.; Shrikanth, et al.; and Goyal A, et al. [16-19]. Thus no VRSA was detected in our setup which is an important finding when a number of studies have reported resistance to vancomycin from the country as well as abroad. It also proves that vancomycin can be used as the drug of choice for MRSA infections in our set up. Moreover, in the present study Etest was found to be useful, convenient and easy method to perform and interpret vancomycin resistance allowing detection in our setup which was not done otherwise.

Conclusion

Though resistance to Vancomycin among *Staphylococcus aureus* isolates is not seen in our set up, the MRSA prevalence of 52% is quite higher and calls for measures to control its spread in hospital and infections due to it. Moreover regular testing to detect vancomycin resistance should be carried out so that emergence of any resistant strain is detected, especially among the MRSA strains.

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