

Original Research Article

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A Prospective Study on the Prevalence and Antibiotic Sensitivity Pattern of Methicillin Resistant *Staphylococcus aureus* isolated from Various Clinical Specimen at a Tertiary Care Post Graduate Teaching Institute

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ABSTRACT

In recent times, the treatment of *Staphylococcus aureus* infection has become problematic because of emergence of resistance to antibiotics which is a cause of concern for all the clinicians and microbiologists worldwide. Infections caused by MRSA have been associated with high morbidity and mortality rates. Hence this study was conducted to know the prevalence of Methicillin resistant *Staphylococcus aureus* [MRSA] isolates in various clinical specimens and to determine the sensitivity of these isolates to different antimicrobial agents. Among the 161 clinical isolates of *S. aureus*, highest isolation was from pus samples (64%), followed by blood (13%) and respiratory secretions (13%). 82 (51%) were identified as MRSA by Cefoxitin disc diffusion method and maximum MRSA were again isolated from pus (61%). Comparatively MRSA prevalence is more in males (73%) and most common affected age group was 21-30 years. All MRSA strains were resistant to penicillin (100%), followed by ciprofloxacin (93%) and erythromycin (61%). We found statistically significant differences in the drug susceptibility pattern of MRSA & MSSA for Penicillin, Clindamycin, Erythromycin, Ciprofloxacin, Co-trimoxazole and Levofloxacin. MDR-MRSA strains in our study is 45%. Hence we suggest, more and more studies in future are needed to fight against rising menace of antibiotic resistance among *Staphylococcus aureus*

Keywords

MSSA, MRSA,
Multidrug
resistance.

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Introduction

Staphylococcus aureus, the most clinically significant species of Staphylococci has been recognized as an important cause of human disease for more than 100 years (Ankur *et al.*, 2013). It is one of the pathogens of greatest concern because of its intrinsic virulence factors, its ability to cause diverse array of life threatening infections, its competency to

adapt to different environmental conditions and its nasal carriage, which accounts for possible spread and re infection (Anupurba *et al.*, 2003). Infections by *Staphylococcus aureus* are continuously challenging the clinicians despite the availability of antibiotics from nearly 70 yrs. This was due to the emergence of various types of antibiotic

resistance mechanisms especially to methicillin and vancomycin, which was the theme of several epidemiological studies (Arora *et al.*, 2010; Assadullah *et al.*, 2003).

With the introduction of Penicillin in 1940, it was thought that we can escape from the deadly threats of Staphylococcal infections, but no sooner in 1944 first strain of *Staphylococcus aureus* resistant to penicillin G appeared in London, mediated by the presence of penicillinase enzyme which hydrolyses the β -lactam ring of penicillin (Astagneau *et al.*, 1996). By 1950s, penicillinase producing strains of *Staphylococcus aureus* were so common that penicillin became useless against Staphylococcal infections. To resolve this issue, in 1960 semi-synthetic penicillin (penicillinase stable penicillin) - like Methicillin came in picture, but availability of these agents did not stem the tide of resistance as within a year the first MRSA was reported from U.K. This was mediated by the presence of PBP-2a which is expressed by an exogenous gene, *mecA* (Bandaru, 2010).

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 (Barber, 1961; Boucher, 2010). A multicentric study done in India involving 17 tertiary care Hospitals reported MRSA prevalence of 41% in 2008-2009 (Clinical and Laboratory Standards Institute, 2012), MRSA is of serious therapeutic concern not only due to its resistance to Methicillin, but also because of resistance to many other antimicrobials that are used on regular basis in Hospitals. Therefore, the most reliable and sustained therapeutic agent against methicillin-resistant *Staphylococcus aureus* (MRSA) strains is Vancomycin. Increasing prevalence of MRSA has lead to the extensive use of vancomycin. This inturn

has lead to the decreased susceptibility to vancomycin. Such resistance is a serious clinical and public health consequences because currently few licenced alternatives are available to treat vancomycin resistant *Staphylococcus aureus* infections.

The association of Multidrug resistance with MRSA adds to the problem and the presence of MDR strains in the hospital cannot be neglected. Several studies are prevailing for Methicillin resistance from India but few have worked on Multi-drug resistance. MRSA strain showing resistance towards ≥ 3 antibiotics is defined as MDR strain and its prevalence ranges from 23% - 60% in India (Diep *et al.*,). The emergence of MDR MRSA infections is worrisome in the present therapeutic scenario. Keeping the above points in view, the present study was planned to find out the prevalence of MRSA among isolates of *Staphylococcus aureus* in various clinical specimens along with their antibiotic sensitivity pattern so as to guide the clinicians of our hospital to select appropriate antimicrobial agents and also to make them aware, that if inappropriate use these antibiotics is continued it may lead to impending public health disaster.

Materials and Methods

Source of material

The present study was conducted in the Department of Microbiology, GMCH, Udaipur, during the year 2013-2014. A total of 161 non- duplicate *Staphylococcus aureus* isolates from various clinical specimens [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. Institutional Ethical clearance was obtained. Data regarding age, sex, patient location, history of any clinical

illness etc. was obtained from the requisition form submitted to Microbiology Department, GMCH.

Inclusion criteria

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

Exclusion criteria

Clinical specimen's yielding growth of Gram positive cocci other than *Staphylococcus aureus* and all gram negative bacteria were excluded.

Isolation and identification of *Staphylococcus aureus*

All the isolates were identified by standard procedures (gram staining, catalase test, mannitol fermentation, Hugh-Leifson OF media, slide coagulase and tube coagulase test). Tube coagulase was taken as the main criteria of identification and was performed by diluting plasma in freshly prepared normal saline (1:6). Three to four colonies were emulsified in 1 ml of diluted plasma and the tubes were incubated at 37°C. Readings were taken at 1, 2, 3 and 4 h and further incubated at room temperature if no clot formation was observed (Yogesh *et al.*, 2013).

Determination of antibiotic susceptibility

All *Staphylococcus aureus* isolates were then subjected to antimicrobial susceptibility testing by modified Kirby–Bauer disc diffusion method. 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]. *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 guidelines of Clinical Laboratory Standards Institute.

Detection of methicillin resistance

Cefoxitin disc (30 µgm) was used to detect methicillin resistant isolates as Cefoxitin, which is a potent inducer of the *mecA* regulatory system, is being widely used as a surrogate marker for detection of *mecA* gene-mediated methicillin resistance. MRSA strains exhibiting inducible resistance to methicillin grow much more readily in the presence of cefoxitin than oxacillin, due to an enhanced induction of PBP 2a by cefoxitin. Isolates with zone diameter of ≤ 21 mm were considered resistant to methicillin and zone of ≥ 22 mm were sensitive (Harcharan *et al.*, 2014).

Results and Discussion

In the present study, a total of 161 non duplicate *Staphylococcus aureus* isolates were obtained from various clinical specimens. *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. [Figure 1 and figure 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.[Table1] Out of total 161 *Staphylococcus aureus* strains, 82 (51%) were found to be MRSA

and 79 (49%) were MSSA. Pus shown the highest prevalence of resistance towards methicillin i.e. (61%) followed by Blood (15%), Respiratory secretions (10%), body fluids (5%), Swabs (5%), and Urine (4%). [Figure 3, Figure 4] Comparatively MRSA prevalence is more in males (73%) and most common affected age group was 21-30 years.

Out of 161 *Staphylococcus aureus*, only 19 (12%) strains were sensitive to all antibiotics. [Table 2] Maximum resistance was

shown by MRSA isolates. All the MRSA isolates were resistant to penicillin (100%) and all were sensitive to Linezolid, Pristinomycin, Chloramphenicol and Rifampicin. Among the MRSA strains, least sensitivity was showed by Ciprofloxacin (93%) followed by erythromycin (66%), Co-trimoxazole (54%), Levofloxacin (46%), Clindamycin (46%) and Gentamicin (30%). Tetracycline (6%), Vancomycin (3%) showed good efficacy.

Table.1 Distribution pattern of *Staphylococcus 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 Antibiogram of *Staphylococcus aureus* strains

Drugs	<i>Staphylococcus aureus</i> 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-trimoxazole	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^[66]

Table.3 Antibiogram of MRSA and MSSA

Drugs	MRSA [n=82]		MSSA [n=79]	
	Sensitive (%)	Resistance (%)	Sensitive (%)	Resistance (%)
Penicillin G	0 (0)	82 (100)	19 (24)	60 (76)
Ciprofloxacin	6 (7)	76 (93)	48 (61)	31 (39)
Levofloxacin	44 (54)	38 (46)	72 (91)	7 (9)
Gentamycin	57 (70)	25 (30)	79(100)	0 (0)
Erythromycin	28 (34)	54 (66)	63 (80)	16 (20)
Clindamycin	44 (54)	38 (46)	67 (85)	12 (15)
Co-trimaxazole	38(46)	44(54)	53(67)	26(33)
Tetracycline	77 (94)	5 (6)	79 (100)	0 (0)
Rifampicin	82 (100)	0 (0)	79 (100)	0 (0)
Chloramphenicol	82 (100)	0 (0)	79 (100)	0 (0)
Vancomycin*	82 (100)	5(3)	79 (100)	0 (0)
Linezolid	82 (100)	0 (0)	79 (100)	0 (0)
Quinipristin/dalphopristin	82 (100)	0 (0)	79(100)	0 (0)

Vancomycin* - According to CLSI guidelines 2007^[66]

Table.4 Statistical analysis of antibiotic resistance pattern of MRSA and MSSA by using software SPSS

Drugs	MRSA Resistance (%)	MSSA Resistance (%)	p value	Statistical Significance
Penicillin G	82 (100)	60 (76)	<0.001	Significant
Ciprofloxacin	76 (93)	31 (39)	<0.001	Significant
Levofloxacin	42 (51)	15 (19)	<0.001	Significant
Erythromycin	50 (61)	20 (25)	<0.001	Significant
Clindamycin	38 (46)	10 (13)	<0.001	Significant
Co-trimoxazole	44(54)	26(33)	<0.001	Significant

Figure.1 Sex wise distribution of patients with *Staphylococcus aureus* infection

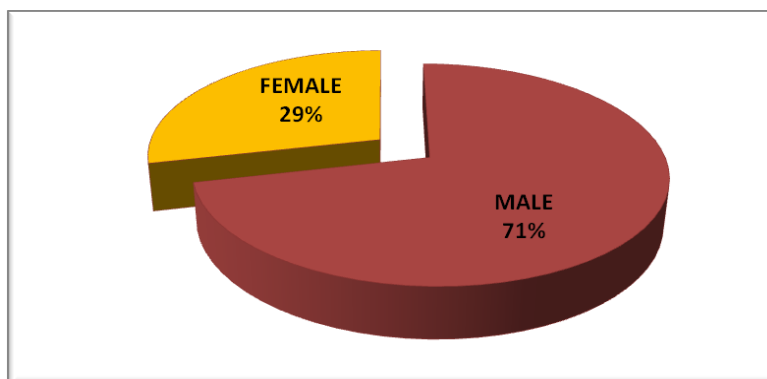


Figure.2 Age wise distribution of patients with *Staphylococcus aureus* infection

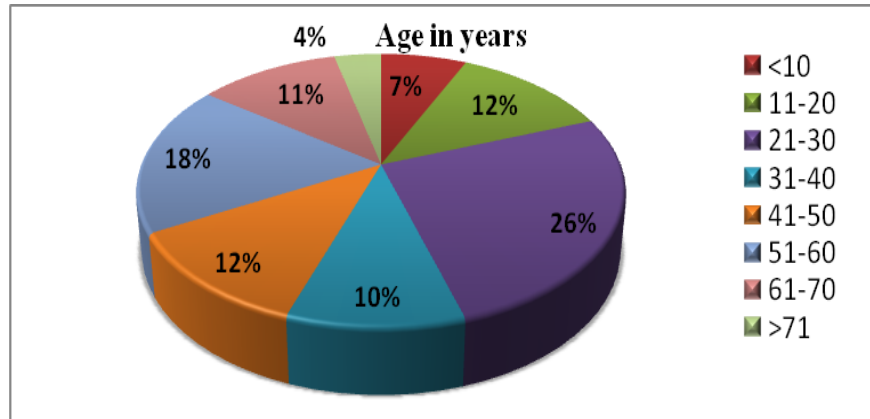


Figure.3 Total number of MRSA and MSSA in *Staphylococcus aureus* isolates

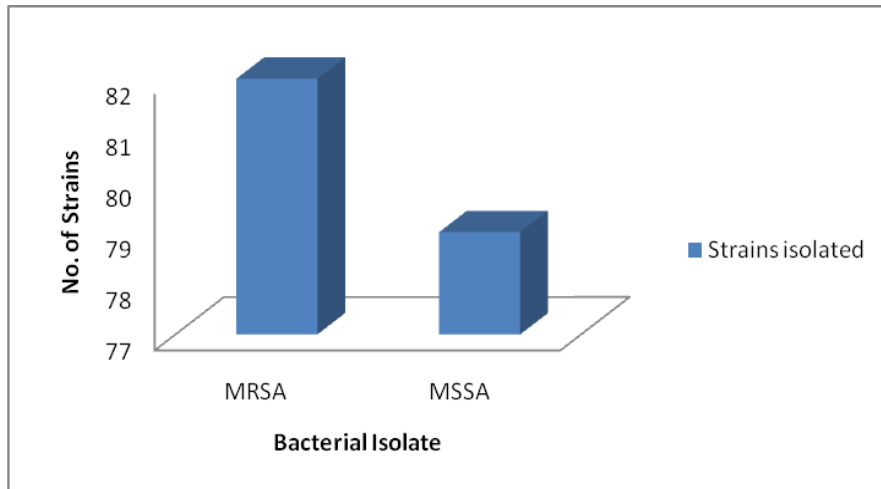
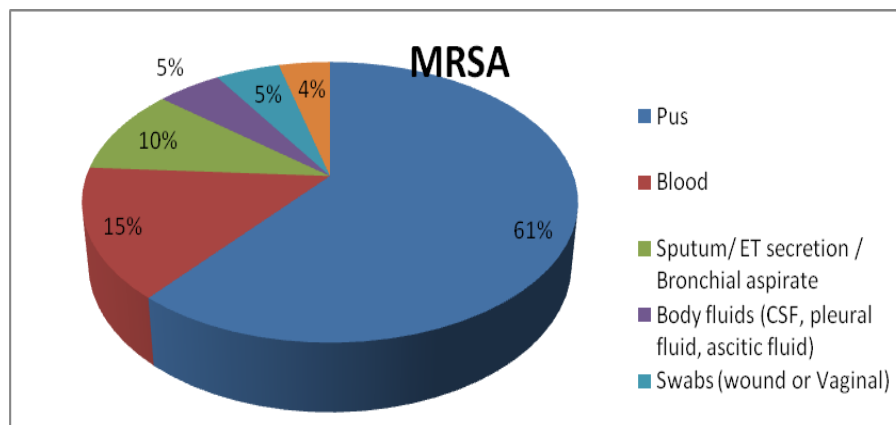


Figure.4 Specimen wise distribution of MRSA



The sensitivity pattern of MSSA was quite different from the MRSA strains. All the MSSA strains showed cent percent sensitivity to Gentamicin, Tetracycline, Vancomycin, Linezolid, Pristinomycin, Chloramphenicol and Rifampicin. Levofloxacin showed the highest sensitivity (91%) among MSSA strains followed by Clindamycin (85%), Erythromycin (80%), Co-trimoxazole (67%), Ciprofloxacin (61%) and showed least sensitivity to penicillin (24%).(Table 3) Among all above mentioned drugs, antibiotic resistance for MRSA and MSSA was statistically significant only for Pencillin, Clindamycin, Erythromycin, Ciprofloxacin, Co-trimozole and Levofloxacin.(Table 4).

Staphylococcus aureus is a major human pathogen and is one of the commonest causative agent of Community and Hospital acquired infections. The treatment of *Staphylococcus aureus* infection has become problematic because of emergence of resistance to Penicillin, Methicillin, Vancomycin and many other antibiotics, by acquiring several resistance mechanisms. Increased antimicrobial resistance for such an organism is, therefore 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 (Lowy, 1998). A multicentric study done in India involving 17 tertiary care Hospitals reported MRSA prevalence of 41% in 2008-2009 (Mallick *et al.*, 2010). MRSA is of serious therapeutic concern not only due to its resistance to Methicillin, but also because of resistance to many other antimicrobials that are used on regular basis in Hospitals. Therefore, the most reliable and sustained therapeutic agent against methicillin-resistant *Staphylococcus aureus* (MRSA) strains is Vancomycin. Extensive use of this antibiotic inturn lead to

the emergence of Vancomycin intermediate *Staphylococcus aureus* [VISA] and Vancomycin resistant *Staphylococcus aureus* [VRSA] strains in various parts of the world (Manu *et al.*, 2013).

In the present study a total of 161 non-duplicate *Staphylococcus aureus* strains were isolated from various clinical specimens. Among all these samples highest isolation was from pus 103 (64%). Harcharan singh *et al.*, (2014) in Udaipur (65%), Manu Chaudhary *et al.*, (2013) in H.P (63%) and Ankur Goyal *et al.*, in Agra (66.03%), also reported the highest isolation of *Staphylococcus aureus* from pus.

All the *Staphylococcus aureus* strains were screened for Methicillin resistance using Cefoxitin disc diffusion method. Since it is a potent inducer of *mecA* gene, hence considered as a surrogate marker for detection of methicillin resistance by CLSI 2012, 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 rate of MRSA in our institute is 51%, which is similar to the studies conducted by Vidhani and Mehndiratta *et al.*, in 2001 showing a prevalence rate of 51.6% and almost comparable to the study conducted by Majumdar *et al.*, in 2001 and Assadullah *et al.*, in 2003 showing 52.9% prevalence rate. The higher rate in their studies may be attributed to the fact that the studies were conducted at a tertiary care multispecialty center with more and more patients coming from pheriphery and small nursing homes, where injudious use of antibiotics and inadequate infection control policies are prevalent. Verma *et al.*, in 2000 reported a higher prevalence rate of 80.89% in Indore and Mehta *et al.*, in 2007 reported a lower prevalence rate of 24% in Chandhigarh. 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 51% prevalence rate of MRSA in our hospital setup. It therefore calls in for better vigilance and implementation of more effective MRSA surveillance programme complemented with improved infection control practices.

In the present study, maximum MRSA were isolated from pus 50 (61%), followed by blood 13(15%), respiratory secretions 8 (10%), Swabs and body fluids 4(5%) each and least from Urine 3(4%). This pattern correlates with studies conducted by Vidya Pai *et al.*, in 2010 and Nitish Kumar Sharma *et al.*, 2013. This is due to the reason that *Staphylococcus aureus* accounts for most of the skin and soft tissue infections, septicemia and also respiratory tract infection.

Comparatively MRSA prevalence was more in males (73%) than in females (23%) in our study. Similar findings was also reported by Rao *et al.*, (2010) and Abhishek Mewar *et al.*, The increased rate of MRSA infections among males could be due to their more outdoor activities, inturn exposing them to contaminated environment and also compared to females, accidental injuries are more common among men.

Most of the MRSA strains were isolated from 21-30 yrs of age group (ie 32%) and in 51-60yrs (ie 26%), indicating MRSA infection is more common in working and old age group. The reason for this may be that younger age group are more involved in outdoor activities inturn exposing them to contaminated environment and in older age group it may be due to waning immunity, hormonal abnormalities and co-morbid conditions. Similar pattern of affected age group was also reported by Ankur Goyal *et al.*, in 2013.

Isolation was more from the IPD patients (88.8%) than from the OPD patients (11.2%). Among IPD patients, highest prevalence was seen in orthopedics, oncology and surgical wards. Similarly Mallick and Basak reported 84.8% MRSA from IPD patients, in which maximum strains were isolated from surgical and orthopedics ward. This might be because the patients in these wards are usually with open wounds and are debilitated. They undergo multiple interventions in the hospital which further increases the risk of MRSA infection due to multiple people involvement as well as prolonged stay in the wards. Along with these factors, the patient is usually on multiple antibiotics.

Resistance of MRSA to a wide range of antibiotics is well documented³⁵. In the present study antibiotic resistance is significantly more in MRSA strains of *Staphylococcus aureus* as compared to MSSA strains. Statistically significant difference was observed for Penicillin, Fluoroquinolones, Erthromycin, Clindamycin and Co-trimoxazole. Such similar statistically significant difference between the sensitivity pattern of MRSA and MSSA was also reported by Vidhani *et al.*, (2013), Shilpa Arora *et al.*, (2013) Majumder *et al.*, (2013).

For Penicillin 100% resistance was observed for MRSA strains in our study and such similar finding was also reported by Gupta *et al.*, (2010), Anupurba *et al.*, The resistance rate of Ciprofloxacin in MRSA strains was high [93%], consistent with the resistance rate of Pulimood *et al.*, [90%] and Udaya Shankar *et al.*, [95.8%]. Our study had also showed high degree of resistance to Erythromycin and Co-trimoxazole. Such high resistance pattern observed for these antibiotics in our study may be due to the differential clonal expansion and indiscriminate empirical use of these drugs. The present study also showed a low level of resistance to Clindamycin, similar to the study conducted by Ankur

Goyal *et al.*, (2013). This may be due to the antibiotic recycling and the dependence of the clinicians more on beta lactam drugs. In the light of the present study, we would recommend use of Clindamycin for the management of soft tissue infections, with MRSA strains that are sensitive to Clindamycin since it has better soft tissue penetration and no renal dosing adjustments and thus by reserving Vancomycin for life threatening infections.

The association of Multidrug resistance with MRSA adds to the problem and the presence of MDR strains in the Hospital can't be neglected. Several studies are prevailing for Methicillin resistance from India but few have worked on Multi-drug resistance. MRSA strain showing resistance towards ≥ 3 antibiotics is defined as MDR strain. MDR MRSA strains in our study is 45%, a study conducted by Majumder *et al.*, (2013) reported 23.2% MDR- MRSA, 32% by Anupurba *et al.*, and as high as 63.6% by Rajaduraipandi *et al.*, (2000). The emergence of MDR MRSA infections is worrisome in the present therapeutic scenario, hence a continuous antibiotic surveillance is required for all the *Staphylococcus aureus* isolates. This inturn will help in formulation of effective antibiotic policies in the health care set-up or else the threat will increase.

In the present study 3% resistance was observed for Vancomycin by disc diffusion test, Harcharan Singh *et al.*, (2013) conducted a study in Udaipur and observed a higher resistance of 13% to Vancomycin by using Disc diffusion method only. Similarly Yogesh Kumar Gupta *et al.*, and Ankur Goyal *et al.*, had reported no vancomycin resistance by Disc diffusion in Rajasthan and Agra respectively.

To conclude, the result of our present study indicated high antibiotic resistance to

commonly used antibiotics by MRSA isolates. The increase in vancomycin resistance among MRSA and MDR- MRSA and excessive use of antimicrobial agents has worsened the sensitivity. Hence prudent and responsible usage of newer antibiotics is advocated to preserve their continued effectiveness in the management of difficult to treat infections caused by MRSA and VRSA. 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.

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