ORIGINAL ARTICLE

Prevalence and Antimicrobial Susceptibility Pattern of Methicillin Resistant *Staphylococcus aureus* From Healthcare And Community Associated Sources

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ABSTRACT

Introduction: Staphylococci are Gram-positive bacteria with diameter of 1 µm. *Staphylococcus aureus* is a versatile human pathogen responsible for nosocomial and community-associated infections which is associated with high morbidity and mortality. MRSA (also known as Mercer) is a bacterium responsible for several infections in humans that are difficult to treat. The study aimed at screening of microbial flora on mobile phones to find out the prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus*. **Materials and Methods:** The present study was carried out in the Department of Medical Microbiology, NIMS Medical College and Hospital, NIMS University, Jaipur during the period of January 2014-June 2014. **Results:** The present study showed males prevalence of MRSA than Females. MRSA was seen among the age group 21-40 years. Maximum MRSA were obtained from pus/wound samples 12 (66.6%). The present study showed higher prevalence rate of MRSA from Doctors mobile phones. Higher prevalence rate of MRSA 58.8% in Hospital associated as compared to community associated sources 40%. HA(MRSA) showed highest resistance to penicillin 100%. The present study showed 35% MDR MRSA in HA. CA(MRSA) showed higher resistance to penicillin and ciprofloxacin 100%. HA and CAMRSA showed vancomycin and Linezolid 0% resistance. **Conclusion:** The study showed a higher prevalence of MRSA in HA as compared to CA sources and indicated high risk of infection in individual exposed to hospital environment. Thus, there is a need to screen individuals in hospitals for risk exposure and infection, to avoid outbreak and cross infection. There is a need of taking good Infection control measures and suggested that microbiological surveillance and monitoring of susceptibility patterns of MRSA may also help in arresting the spread of infections.

Keywords: Infection, Hospital acquired (HA), Community acquired (CA), MRSA, Resistance.

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INTRODUCTION

Staphylococci are Gram-positive bacteria non-motile, non-spore forming, facultative anaerobes that grow aerobically with diameter of 1 µm, which divides in more than one plane to form grape-like clusters. It forms colonies 1-3 mm in diameter, round, smooth, raised, opaque, shiny, easily emulsifible and they show β-haemolysis on blood agar.[1] *Staphylococcus aureus* is a versatile human pathogen responsible for nosocomial

and community-associated infections which is associated with high morbidity and mortality.[2] *S. aureus* causes superficial skin lesions such as boils, and furuncles; more serious infections such as pneumonia, mastitis, meningitis, and urinary tract infections; and deep-seated infections, such as osteomyelitis and endocarditis. *S. aureus* causes food poisoning by releasing enterotoxin into food, and toxic shock syndrome by release of super-antigens into the blood stream.[3] Patients develop sepsis in operation wounds, which take place in the theatre during operation, and others post-operations in the ward.[4]

Methicillin-resistant *Staphylococcus aureus* (MRSA) are a type of staphylococcus or "staph" bacteria that are resistant to many antibiotics.[4] MRSA is a bacterium responsible for several infections in humans that are difficult to treat. MRSA is developed, through the process of natural selection, resistance to beta lactams antibiotics, which include the penicillins (methicillin, dicloxacillin, nafcillin, oxacillin etc.) and the cephalosporins. The incidence of MRSA varies from 25% in western part of India to 50% in South India. Community acquired MRSA (CA-MRSA) has been increasingly reported from India.[5] MRSA prevalence varies greatly with geographical location, type of hospital and studied population. High prevalence has been recorded in tertiary hospitals in US, southern European countries, Asia and South America.[2]

MRSA is a skin infection, with early symptoms on the skin that may look like a bump, pimple or a minor rash. MRSA are major healthcare-associated (HA-MRSA) as well as a community-associated (CA-MRSA) infections causing a wide range of diseases. Hospital associated methicillin resistant *Staphylococcus aureus* (HA-MRSA) is emergence of MRSA from the patients having current or recent hospitalization.

MRSA, like all staph bacteria, can be spread from one person to another through casual contact or through contaminated objects. It is commonly spread from the hands of someone who has MRSA.

MRSA that is acquired in a hospital or health care setting is called healthcare-associated methicillin resistant *Staphylococcus aureus* (HA-MRSA). These infections can occur among people who are likely to have cuts or wounds and who have close contact with one another, such as members of sports teams. This type of MRSA is called community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA).

Methicillin-resistant *Staphylococcus aureus* (MRSA) are isolates of *Staphylococcus aureus* which have acquired genes encoding antibiotic resistance to all penicillins including methicillin. Methicillin resistance in *S. aureus* is primarily mediated by the mecA gene, which codes for the modified penicillin-binding protein 2a (PBP 2a or PBP 2'). PBP2a is located in the bacterial cell wall and has a low binding affinity for β-lactams.[6] Although all cells in a population of *S. aureus* may carry the mecA gene, often only a few of the cells will express the gene. *S. aureus* strains expressing the mec a determinant are termed Methicillin Resistance (MRSA).[7]

Now-days, medical professionals extensively use mobile phones which may get contaminated through their hands or when used carelessly in ICU or surgical wards which may act as a source of MRSA to patients and may also pose a danger in spread of infections in the community. The postoperative nosocomial pyogenic MRSA infections are common in most of the surgical wards of the hospital.[8] The result of one study showed that pathogenic bacteria are present on approximately 40% of mobile phones belonging to patients in a hospital, and on approximately 20% of mobile phones belonging to hospital staff.[9]

Mobile phones are continuously used all day long but never cleaned. Furthermore, guidelines for proper disinfection and decontamination of mobile phones are lacking. This can be done by using ethanol, as it is an important industrial ingredient and has widespread use as a base chemical for other organic compounds. These include ethyl halides, ethyl esters, diethyl ether, acetic acid, ethyl amines, and to a lesser extent butadiene. Ethanol is miscible with water and is a good general purpose solvent. Ethanol is used in medical wipes and in most common antibacterial hand sanitizer gels at a concentration of about 62% v/v as an antiseptic. Ethanol kills organisms by denaturing their proteins and dissolving their lipids and is ineffective against bacterial spores.[9]

Studies have been carried out in other part of world to find out the prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* but Paucity of such studies in our area made us to carry out this study. It aimed at screening of microbial flora on mobile phones to find out the prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* of doctors employed in NIMS Medical College and Teaching Hospital located in Jaipur.

AIMS AND OBJECTIVES

To determine the prevalence of Methicillin resistant *Staphylococcus aureus* from healthcare and community associated sources.

To access *in-vitro* antimicrobial susceptibility pattern of Methicillin resistant *Staphylococcus aureus* isolated from healthcare and community associated sources.

MATERIALS AND METHODS

The present study entitled "Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* from healthcare and community associated sources" was carried out in the Department of Medical Microbiology, NIMS Medical College and Hospital, NIMS University, Jaipur during the period of January 2014-June 2014.

Study Sample

A total of 200 samples were collected from health care sources and community associated sources; 50 from Doctor's mobile phones, 50 from pus/wounds and 100 from College going students hand at NIMS medical College and Hospital, Jaipur.

Inclusion and Exclusion Criteria

Patients willing to give written signed consent form were included while Patients not willing to give written signed consent form were excluded from our study.

Sample collection

A total of 50 mobile phone swab samples were collected from different specialty doctors from NIMS Medical College. A total of 50 wound/pus samples were collected from wound, burns and postoperative infections, abscesses. A total of 100 hand swabs were collected from college going students. Samples were immediately transported to the microbiology laboratory for processing within 2 hr of collection. Peptone water was added to the tubes and incubated overnight. Colonies showing characteristics resembling staphylococci were stained by Gram's Method of staining.

Microscopic examination

The Gram staining of isolated bacteria was done and observed microscopically under 100X objective lens. All the colonies showing gram positive cocci arranged in clusters were assumed as staphylococci. These were then subculture onto nutrient agar plates for performing various tests and observing Pigment production. Antibiotic susceptibility testing of the isolates was performed by Kirby-Bauer disc Diffusion method.

At least three to five well isolated colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a loop, and the growth was transferred into a tube containing 2 mL of Peptone water. The plates were inoculated and the bacteria from the swab were inoculated onto the dried surface of a Muller-Hinton agar plate by streaking the swab three times over entire surface, rotating the plate three times approximately 60° each time to ensure an even distribution of inoculum.[7] Test for Methicillin resistance was performed by Kirby Bauer disc diffusion method using Cefoxitin disc (30µg) incubated at 37°C for 18 hrs. An Inhibition zone of 21 mm or less around indicates methicillin resistant *Staphylococcus aureus*.[24] Antimicrobial susceptibility test MRSA strains detected by Kirby-Bauer disk diffusion method as per CLSI guidelines. The following antibiotics like Amikacin, Amoxyclav, Clindamycin, Vancomycin, Ciprofloxacin etc were used in study. After overnight incubation, the zone diameter (including the 6-mm. disk) was measured with a ruler (millimeter scale) on the undersurface of the Petri dish. Each Zone size was interpreted as Sensitive, Intermediate, and Resistant according to given standard zones compared to that of the manufacturer interpretation charts and according to the guidelines of the Clinical Laboratory and Standards Institute (CLSI).

OBSERVATION AND RESULTS

In present study *S. aureus* and MRSA was isolated from the different samples and antibiotic sensitivity was performed on the isolates. Obtained results are presented in different tables along with the charts as below.

Above Figure 1 shows that a total of 200 samples were collected out of which, 100 samples were taken from hospital associated sources i.e., 50 (25%) from pus/wound and 50 (25%) from doctors mobile phones and 100 (50%) from community acquired i.e., students hands.

The above Figure 2 shows the 50 samples were taken from pus, out of which 35 (70%) were male and 15 (30%) were female. 50 samples of doctors mobile phone were taken, 30 (60%) samples were of male and

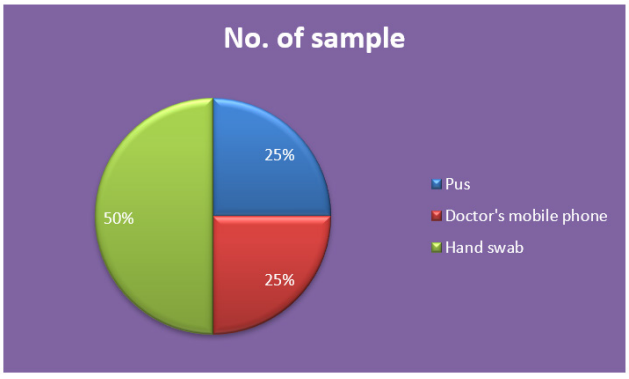

Figure 1: Distribution of samples.

20 (40%) were of female. And in the community a total of 100 samples were taken, 50 (50%) samples were of male and 50 (50%) samples were of female.

The above Figure 3 shows the highest incidence of *S. aureus* in males i.e. 28 (24.34%) as compared to females 11 (12.94%).

The above Figure 4 shows the highest number of *S. aureus* isolated from males as compared to females and the highest MRSA prevalence rate in males (69.2%) as compared to females (60%) in pus samples.

The above Figure 5 shows the highest Isolation rate of *S. aureus* in males as compared to females and highest prevalence of MRSA in males (58.3%) as compared to females (25%) on Doctor's mobile phones.

The above Figure 6 shows the highest prevalence of *S. aureus* and MRSA in males (66.6%) as compared to females (0%) From students Hand swabs.

The above Table 1 revels the percentage of MRSA in relation to age, the highest MRSA Prevalence rate observed in the age group of 21-40 yr followed by 0-20 yr and 41-60 yr and the least in the age group of 61-70 yr from pus sample, For Doctor's mobile phone highest MRSA prevalence rate was observed in the age group of 21-40 yr followed by 61-70 yr and 41-60 yr. From Students Hand it was observed in the age group of 21-40 yr.

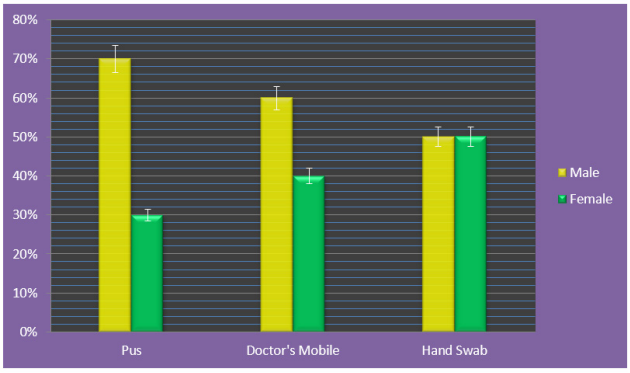

Figure 2:Distribution of samples in relation to gender.

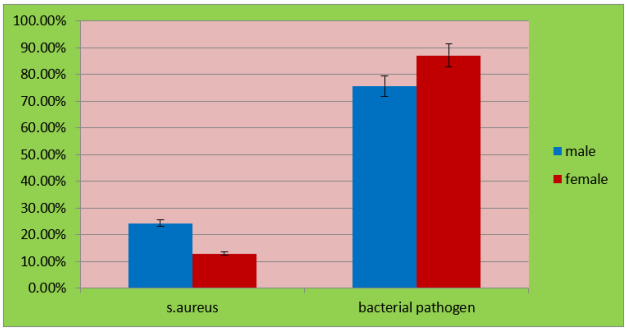

Figure 3: Incidence of Isolates in relation to gender.

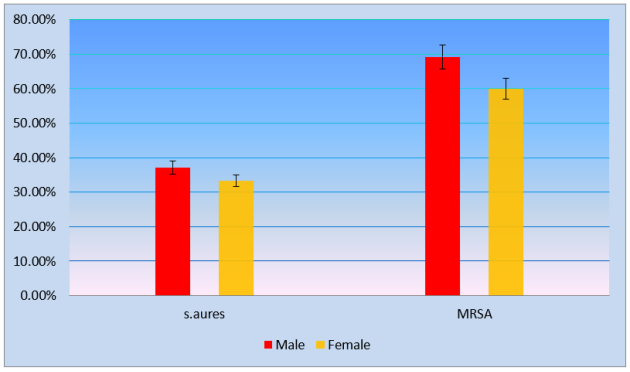

Figure 4: Distribution of Isolates in relation to sex in pus sample.

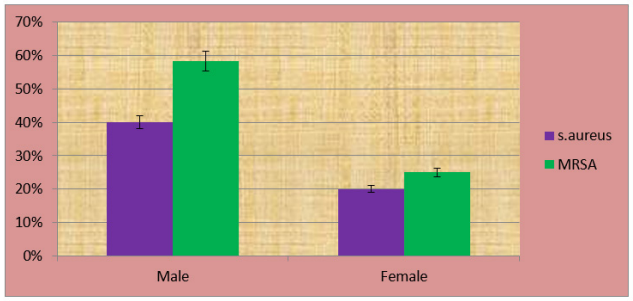

Figure 5: Distribution of isolates in relation to sex from Doctor's mobile phone.

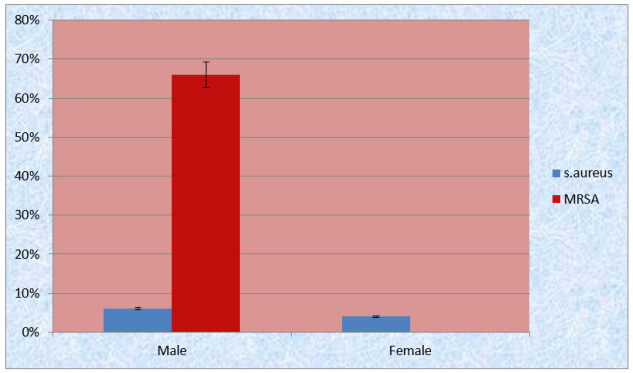

Figure 6: Distribution of isolates in relation to sex in student's hand.

Table 1: Distribution of Isolates in relation to Age

Age	Pus		Doctor's mobile		Students Hand	
	S. aureus	MRSA	*S. aureus*	MRSA	*S. aureus*	MRSA
0-20	6 (50%)	4(67%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)
21-40	10 (37%)	7(70%)	8 (22.8%)	5 (63%)	4 (4.2%)	2(50%)
41-60	2 (28%)	1(50%)	6 (60%)	2(33.3%)	0 (0%)	0 (0%)
61-70	0 (0%)	0(0%)	2 (40%)	1 (50%)	0 (%)	0 (0%)

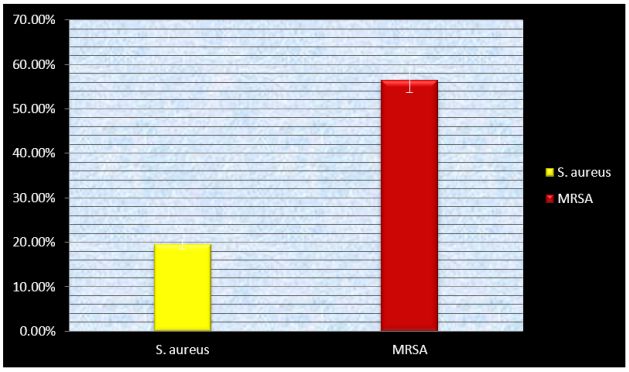

Figure 7: Prevalence of *S. aureus* and MRSA.

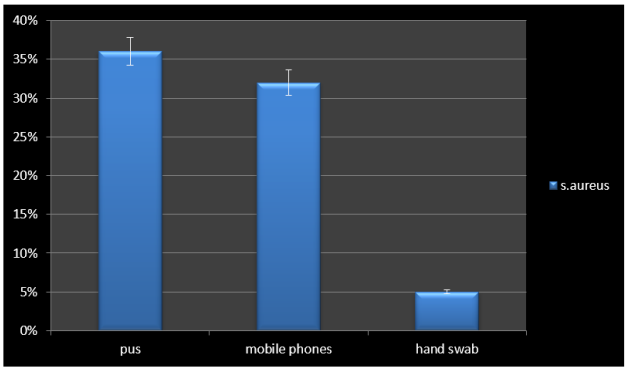

Figure 8: Distribution of *S. aureus* in various samples.

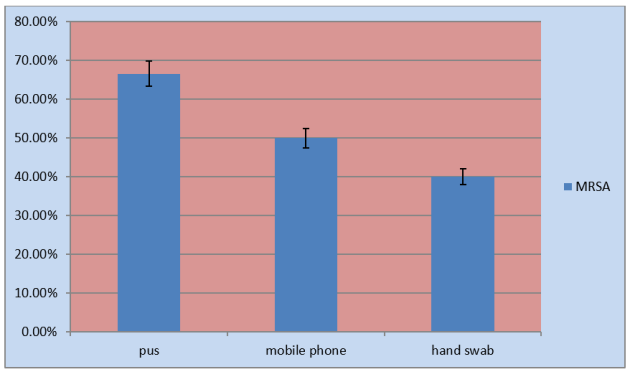

Figure 9: Percentage of MRSA.

The above Figure 7 shows overall prevalence of *S. aureus* 19.5% was determined and MRSA prevalence rate was 56.41% as shown in Table and chart.4.8

The above Figure 8 shows the highest incidence of *S. aureus* in pus/wound samples (36%) followed by Doctors mobile phone (32%) and the least from Hand swabs (5%) taken from students.

The above Figure 9 shows highest MRSA prevalence rate was observed in pus/wound swab (66.6%) followed by Doctors mobile phone (50%) and students Hand swab (40%).

A total of 34(34%) *S. aureus* isolated from hospital associated and 5(5%) community associated sources and a total of 20(58.8%) MRSA isolated from hospital and 2(40%) community associated sources as shown in Figure 10.

Above Table 2 shows that the higher prevalence rate of MRSA from the department of Anaesthesia 100% Followed by surgery and gynecology 66.6% and pediatrics, Medicine and orthopedics 50%.

In the present study, HA-MRSA shows 100% resistance to Cefoxitin, and Penicillin G followed by Erythromycin, Gentamycin 70%, Ceftazidime, co-trimoxazole 50%, Amoxyclav, Amikacin, teicoplanin and ciprofloxacin 35%-45% and clindamycin 25% and vancomycin, Linezolid showed 0% resistance as shown in the above Table 3.

The data in Table 4 shows 100% resistant to Cefoxitin and Penicillin Followed by Amoxyclav, erythromycin and Gentamycin 50%.

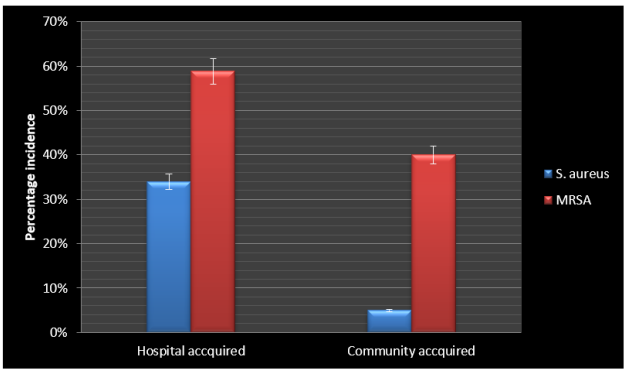

Figure 10: Prevalence of *S. aureus* MRSA in HA and CA sources.

Table 2: Specialty wise doctor's mobile phone analyzed.

Specialty	*S. aureus*	MRSA	Total no. of sample
Anesthesia	1	1 (100%)	1
Surgery	3	2 (66.6%)	8
Gynecology	3	2 (66.6%)	12
Medicine	2	1 (50%)	7
Pediatrics	2	1 (50%)	6
Orthopedics	2	1 (50%)	5
Psychiatry	1	0 (0%)	2
Nephrology	0	0 (0%)	1
Microbiology	2	0 (0%)	2
Pathology	0	0 (0%)	3
Dermatology	0	0 (0%)	2
IVF	0	0 (0%)	1

Table 3: Antibiotic resistance pattern in Hospital associated sources.

Antibiotics	Potency (μ g)	Wound swabs	Doctor's cell phone	Total HA
Cefoxitin	30	12 (100%)	8 (100%)	100%
Penicillin G	10 units	12 (100%)	8 (100%)	100%
Gentamycin	10	9 (75%)	4 (50%)	70%
Erythromycin	15	9 (75%)	5 (62.5%)	70%
Ceftazidime	30	7 (58.3%)	4 (50%)	55%
Co-Trimoxazole	25	6 (50%)	4 (50%)	50%
Amikacin	30	4 (33.33%)	4 (50%)	45%
Amoxyclav	30	5 (41%)	3 (37.5%)	40%
Teicoplanin	30	5 (41%)	3 (37.5%)	40%
ciprofloxacin	5	4 (33.33%)	3 (37.5%)	35%
Clindamycin	2	3(25%)	2(25%)	25%
vancomycin	30	0 (0%)	0 (0%)	0%
Linezolid	30	0 (0%)	0 (0%)	0%

Table 4: Antibiotic resistance pattern in community associated MRSA.

Antibiotics	Conc.(μg)	Resistant
Cefoxitin	30	2 (100%)
Penicillin G	10 units	2 (100%)
ciprofloxacin	5	2 (100%)
Erythromycin	15	1 (50%)
Gentamycin	10	1 (50%)
Amoxyclav	30	1 (50%)
Ceftazidime	30	0 (0%)
Clindamycin	2	0 (0%)
Amikacin	30	0 (0%)
Co-trimoxazole	25	0 (0%)
vancomycin	30	0 (0%)
Linezolid	30	0 (0%)
teicoplanin	30	0 (0%)

DISCUSSION

The present study was conducted in the Department of Microbiology, NIMS Medical College and Hospital, NIMS University, Jaipur Rajasthan from January–June 2014. The purpose of this study was to analyze prevalence of Methicillin resistant *Staphylococcus aureus* in Hospitals and community acquired sources with their antimicrobial resistance patterns. In the present study a total of 200 samples were collected, of which 100 samples were from Hospital associated sources. Out of these 50 (25%) were from pus/wound and 50 (25%) from doctors mobile phones. The other 100 (50%) samples were taken from community associated sources i.e., from students hands.

Comparison on basis of gender

A total of 18 (36%) *S. aureus* were isolated from pus. Out of which 13 (37.14%) *S. aureus* was from males and 5 (33.3%) *S. aureus* was from females. Incidence of *S. aureus* from doctor's mobile phone was higher in males 12 (40%) as compared to Females 4 (20%). It is a well known fact that organisms like *Staphylococcus aureus* resist drying and thus can survive and multiply rapidly in the warm environments like mobile phones.[11] Incidence of community associated *S. aureus* from Students hand, was higher in males 3 (6%) as compared to females 2 (4%). Study done by Tambekar DH *et al.*[12] reported high prevalence of *S. aureus* in Males 61% and 39% of *S. aureus* in females, which is comparable to our study.

The present study showed higher incidence of MRSA in males 9 (69.2%) as compared to females 3 (60%) in pus samples, which was in concordance with the study of Rao BN *et al.*[13] who reported higher incidence of MRSA in male patients 60% as compared to female 40% however it was contradictory to our of Goyal A *et al.*[14]

Prevalence of MRSA on Doctors mobile phones in males was higher 7 (58.3%) as compared to females 1 (25%) which is in concordance with the study of Tambekar DH *et al.*[12] The reason might be that female doctors often keep their phones in purses and use them less frequently during their duties while male doctors keep their mobile phone in their pockets and use them frequently anywhere, anytime whenever it is needed and thus contaminate it. Moreover, these contaminated mobile phones and hands of the healthcare professionals may also pose a danger in the spread of infection to the community.[12] Incidence of CA-MRSA in males 2 (66%) was higher as compared to female 0 (0%) however contradictory to findings of Shen H *et al.*[15]

Comparison on basis of age group

In the present study most of the MRSA strains were isolated in age group 21-40 years (70%) followed by 0-20 age group (66.6%), 41-60 age group 50% and no isolates were found from 61-70 age group in pus samples which was comparable to the study done by Goyal A *et al.*[14] In present study a total of 18(36%) *S. aureus* was isolated from pus samples. Our study is comparable to the study done by Parween S *et al.*[18] and contradictory to findings of Surpur RR *et al.*[19] The present study showed 16 (32%) *S. aureus* isolated from doctors mobile phone which is comparable to studies done by Amadi EC *et al.*[20] and contradictory to findings of Badr RI.[21] The present study showed *S. aureus* 5(5%) from students hands which is comparable to findings of Tambekar *et al.*[12] and contradictory to findings of Renushri *et al.*[22]

The present study showed 66.6% MRSA from pus samples which are comparable to the study done by Rao BN *et al.*[13] In the present study, 50% MRSA were reported from Doctor's mobile phone which is comparable to the study of Badr RI *et al.*[21] While contradictory to findings of Padmaja N *et al.*[23] MRSA is weakened immune systems patients and are at a greater risk of infection than the general public. The prevalence rate of CA-MRSA was found to be 40% which is in accordance with the study done by Tambekar DH *et al.*[12] however Renushri *et al.*[22] reported lower prevalence rate of MRSA.

Comparison of HA (pus/wounds and Doctor's mobile phones) and CA (students hands) sources

In the present study prevalence rate of MRSA was reported to be 58.8% in Hospital Associated sources while it was 40% in community associated sources. The prevalence rate of MRSA in Hospital Associated sources in our study was 58.8%. Few studies from India observed no MRSA in community. Our study showed t prevalence rate of CAMRSA to be 40% which was similar to the findings of Tambekar DH *et al.*[12] In the present study out of 39 (19.5%) *S. aureus* 22 (56.41%) were methicillin resistant. our study is comparable to the study done by Majumdar D *et al.*[24] and contradictory to findings of Mehta AP *et al.*[25]

The present study HA-MRSA showed Penicillin G 100% resistance followed by Erythromycin, gentamycin 70% and Ceftazidime, co-trimoxazole 50-55%. Our study showed highest resistance to penicillin G which is similar to the Study done by chandrashekar DK *et al.*[27] Our study showed 70% resistance to gentamycin, which was quite similar to the study done by Rajaduraipandi K *et al.*[28] The Ceftazidime resistance pattern in this study was 55% which is camparable with the study done dy Tambekar DH *et al.*[12] In the present study 50% of hospital acquired MRSA strains were resistant to co-trimoxazole which is comparable to study done by parviz V *et al.*[30]

Our study showed 35% resistance to Ciprofloxacin which is comparable to study done by Rajaduraipandi K *et al.*[28] The present study showed 40% resistance to Amoxyclav and amikacin Which is quite similar to study done by Gupta YK *et al.*[31] Our study showed 0% resistance to Linezolid and Vancomycin Indicating that they were the most effective antibiotics to HAMRSA and CAMRSA. Our study coincides with Gupta YK *et al.*[31] The present study showed 35% resistance to Teicoplanin which was comparable to findings of Goyal A *et al.*[14]

The present study showed CA MRSA 100% resistance to penicillin G, ciprofloxacin and cefoxitin followed by gentamycin and erythromycin 50% which is comparable to the findings of Okwu MU *et al.*[32] while contradictory to the findings of Surpur RR *et al.*[19] In the present study 58.8% were HA MRSA strains isolated, out of which 35% were Multi drug resistant. The present study coincides with study done by Rao BN *et al.*[13] while contradictory to the findings of Rajaduraipandi K *et al.*[28] From the above study it was clear that hospital acquired strains were more resistant to antimicrobials than community accquired strains. In the present study linezolid and Vancomycin were found to be useful drugs in treating MRSA infections and similar findings were observed by Rajaduraipandi K *et al.*[28] with 100% sensitivity to both the drugs.

CONCLUSION

In the present study males showed more prevalence of MRSA than Females. The maximum percentage of MRSA was seen among the age group 21-40 years. HA and CAMRSA showed vancomycin and Linezolid 0% resistance. Vancomycin and Linezolid may be used as the drug of choice to treat MDR MRSA infections. The study showed a higher prevalence of MRSA in HA as compared to CA sources and indicated high risk of infection in individual exposed to hospital environment. Thus, there is a need to screen individuals in hospitals for risk exposure and infection, to avoid outbreak and cross infection. There was higher degree antibiotic resistance observed in MRSA from HA sources.

This may be due to indiscriminate use of multiple antibiotics, prolonged hospital stay, intravenous drug abuse, over the counter availability of antibiotics, self-medication and inappropriate use of antibiotics are few important risk factors for MRSA acquisition. Moreover improper handling of mobile phone by doctors may spread MRSA through handling or treating the patients. Thus the control of MRSA is essential this can be achieved by avoiding use of mobile phone by doctor's while handling or treating patients and care must be taken while wound dressing. Healthcare workers in hospitals should adhere to infection control practices such as hand washing after every activity. Proper hand hygiene also prevents the spread of MRSA in community setting.

To conclude, there is a need of taking good Infection control measures such as proper hand hygiene, avoiding mobile phone usage while wound dressing and treating patients. It is suggested that microbiological surveillance and monitoring of susceptibility patterns of MRSA may also help in arresting the spread of infections.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

Ethical Approval

The study was approved by Institutional Ethics Committee.

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