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Prevalence and antimicrobial susceptibility of methicillin resistant *Staphylococcus aureus* in tertiary care hospital

Sunita Chandrakar[1], Sk. Khairul Enam[2], Animesh Panda[3], Smita Bawankar[3], Dhruba Hari Chandi[4]

[1]Professor, Department of Pharmacology, CCM Medical College and Hospital, Durg, [2]Assistant Professor, Department of General Surgery, CCM Medical College and Hospital, Durg, [3]Assistant Professor, Department of Microbiology, CCM Medical College and Hospital, Durg, [4]Demonstrator, Department of Microbiology, CCM Medical College and Hospital, Durg

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ABSTRACT

Background: Methicillin resistant *Staphylococcus aureus* (MRSA) infections are important causes of morbidity, mortality in hospitals and the community worldwide. MRSA has been known to acquire resistance to most antibiotics like β- lactams and aminoglycosides so these strains are more virulent. **Aims and Objectives:** Therefore the knowledge and determination of prevalence of MRSA and their current antimicrobial profile becomes necessary to the clinicians to avoid clinical complications from community-acquired and hospital acquired MRSA infection. **Materials and Methods:** A total of two hundred five different samples from different clinical specialties were collected and processed by appropriate microbiological technique like staining, culture, biochemical test and antimicrobial susceptibility test by the use of different antibiotic discs. **Results:** The present study shows that 47 MRSA strains out of 105 men comprising 44.76%, whereas in case of females it was 29% i.e, 29 MRSA strains out of 100 cases. MRSA percentage of males was higher than the MRSA percentage of female. It proves that men were more prone to acquire the infection by MRSA strain. Out of 205 clinical samples, 76 were identified as MRSA so the prevalence rate was 37.07% but all the multidrug resistance MRSA were mostly sensitive towards antibiotics like Vancomycin (100%), Rifampicin (100%), Amoxicillin (77.77%) and Amikacin (61.19%). **Conclusion:** In conclusion, vancomycin could be considered for treatment to eradicate the MRSA.

Key words: HAMRSA, CAMRSA, Antibiotics profiles, CCMCH, Durg

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INTRODUCTION

The emergence of a community pathogen depends on its ability to survive in different environments and to interact successfully with the host. *Staphylococcus aureus* is one of the successful and adaptable human pathogens.[1] *Staphylococcus aureus* has a broad pathogenic potential causing a wide range of community acquired as well as nosocomial infections.[2]

The organism has been found to be the most common bacterial agent recovered from blood stream infections, skin and soft tissue infections, pneumonia and hospital – acquired post -operative wound infections.[3] *Staphylococcus aureus* infections are associated with morbidity in hospitals and community.[4]

Before the availability of antibiotics, invasive staphylococcal disease was often fatal, and with the introduction of penicillin in early 1940s dramatically improved survival. Although penicillinase- producing strains soon emerged, methicillin and other penicillinase – stable β- lactam agents filled the breach.[5]

Methicillin resistant *Staphylococcus aureus* infection was initially reported in 1961, the same year in which methicillin (a penicillinase – resistant semi synthetic penicillin) was introduced. In1982, a "community – acquired outbreak" of MRSA, outside of a hospital, was first reported in intravenous drug users in Detroit, Michigan. During the 1990s, numerous outbreak of community acquired MRSA (CAMRSA). Indeed, the initial publication in the medical

Address for correspondence:
Dr. Animesh Panda, Assistant Professor of Microbiology, CCM Medical College and Hospital, Durg, (C.G.)
Phone: 7898968485, **E-mail:** animeshpanda48@yahoo.com

literature describing CAMRSA skin infections as "an emerging epidemic".[6]

With all the above facts in mind, the present study was undertaken to find out the prevalence of Methicillin resistance among *Staphylococcus aureus* isolates and also to find out drug resistance patterns among them.

MATERIALS AND METHODS

A total of two hundred and five different samples from different clinical specialties like surgery, orthopedics, pediatrics, gynecology and obstetrics, medicine, ENT departments, were collected and processed. This study was carried out between the period July 2014 to September 2015. All clinical specimens such as urine, pus, sputum/throat swab, blood, pleural fluid, catheter tip, tracheal and nasal swabs and vaginal swabs were collected for *Staphylococcus aureus* screening. All the samples were aseptically handled and processed.[7]

All the laboratory findings were included in standard proforma.

Methods of collection of samples

All the specimens for microbiology were collected only in appropriate sterile containers, aseptically.

Laboratory procedures

Specimens were brought to the laboratory immediately and processed within two hours of collection.

Gram's stain

The morphological types were done for all the samples based on the Gram's staining method to determine the likely organisms present.[7] Smears were made from the all clinical samples except blood, heat fixed and stained by Gram's stain. Smears were examined for, presence of Gram-positive cocci in cluster, that means GRAPES like appearance.

Culture

Subsequently, the clinical specimens, like sputum and pus were inoculated on to nutrient agar, blood agar plates (aerobic with 5% CO_2), MacConkey's agar, Robertson cooked meat medium, Glucose broth and some special media such as Manitol salt agar and milk salt agar, which were incubated at 37°C for 24 hours aerobically. Subculture from liquid media on to solid media was done after 24 hours of incubation.[7] Ultimately, on nutrient agar colonies produce golden yellow pigment, colonies on blood agar were hemolytic and on MacConkey's medium colonies were smaller and pink colors due to lactose fermentation.

Urine

Urine samples were inoculated with standard calibrated loops for determination of significant bacteriuria on blood agar and MacConkey's agar.

Blood

BHI broth of blood culture was inoculated on to blood agar and MacConkey's agar after 48 hours of incubation. If there was no growth, again BHI broth incubated and sub cultured successively with three successive subcultures. If there was growth, inoculated on blood and MacConkey's agar.

Biochemical tests

The colonies of Gram positive cocci in clusters were isolated on the basis of colony morphology, cultural characters, Gram's staining, catalase test, oxidase test and also by some biochemical tests, like urease test. All strains were further tested for the production of free coagulase enzyme using tube coagulase and bound coagulase enzyme using slide coagulase test based on standard methods. Also identified by using Hugh Leifson's oxidative fermentation test.[7]

Antimicrobial susceptibility test

All the confirmed *staphylococcus aureus* strains from different clinical samples, were subsequently tested for methicillin resistance based on Kirby-Bauer (1966) disc diffusion method according to NCCLS guidelines, using oxacillin discs (1 μg) obtained from Hi-Media laboratories, in Mueller-Hinton agar with 4% NaCl. The plates were incubated at 30°C for 24-48 hours.[7,8]

MRSA testing

The isolates were considered methicillin resistant if the zone of inhibition for oxacillin was 10 mm or less. Further, the antibiotic susceptibility pattern of methicillin resistant *Staphylococcus aureus* strains was determined on the day of their isolation by the modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar using the criteria of standard zone sizes of inhibition to define sensitivity or resistance to different antimicrobials. The antibiotics used were Penicillin-G (10 units), ampicillin (10 μg), cephotaxime (30 μg), erythromycin (15 μg), gentamycin (10 μg), amikacin (30 μg), ciprofloxacin (5μg), co-trimoxazole (25 μg), vancomycin (30 μg), Rifampicin (5 mcg) etc.

The readings were noted after the incubation and the sensitivity and resistance pattern of each isolates were recorded.[9]

Finally, the data were recorded and analyzed at the completion of the study. All the readymade media and antibiotics disc were procured from Hi-Media laboratories Pvt Ltd (Mumbai).

RESULTS

A total of 205 isolates from different clinical specimens were studied. This study was carried from June 2014 to September 2015 out in the Department of Microbiology, CCMMCandH, Kachandur.

The patients admitted to different clinical wards like, surgical ward, ENT, ICU, orthopedics, gynecology, ophthalmology, pediatrics, etc. formed the study group.

Table 1 shows the distribution of cases from different clinical wards, majority were from surgical wards 34.14% (70), in that MRSA was 35.71% (25) and MSSA was 64.28% (45) followed by medicine wards 21.95% (45), in that MRSA and MSSA were 33.33% (15) and 66.66% (30), respectively OBG wards 14.63% (30), in that MRSA and MSSA were 23.33% (07) and 76.66% (23), orthopedics wards 12.19% (25), in that MRSA and MSSA were 48% (12) and 52% (13), pediatrics wards 04.87% (10), in that MRSA and MSSA were 40% (04) and 60% (06), urology wards 3.90% (08), in that MRSA and MSSA were 37.5% (03) and 62.5% (05), gynecology wards 2.43% (05), in that MRSA and MSSA were 60% (03) and 40% (02), ENT wards 1.95% (04), in that MRSA and MSSA were 75% (03) and 25% (01), ICU 1.46% (03), in that MRSA are 100% (03), DVL 1.46% (03), in that MRSA and MSSA were 33.33% (01) and 66.66% (02), and 0.97% (02), in that MSSA 100% (02) were from ophthalmology wards respectively.

Table 2 shows 47 MRSA isolates. These cases are of 47 out of 105 men comprising 44.76%, whereas in case of females it was 29% i.e. 29 out of 100 cases. MRSA percentage of males was higher than the MRSA percentage of female. This therefore proves that men were more prone to acquire the infection by MRSA strain.

Table 3 shows the percentage (%) of MRSA among the various age groups are as follows, 0-10 yrs: 35.71%, 11-20 yrs: 28.57%, 21-30 yrs: 21.73%, 31-40 yrs: 48.38%, 41-50 yrs: 35.71%, 51-60 yrs: 45.23%, 61-70 yrs: 33.33% and >70 yrs: 58.33%.

Out of the 76 MRSA strains isolated, 45.23% (19), 33.33% (06) and 58.33% (07) were from patients over the age of 51-60, 61-70 and >70 yrs. Among these most of the MRSA strain were from the men over the age of 61-70 and >70 yrs. This proves that old age was a definite risk factor concerning MRSA infections.

Out of 46 MRSA isolates, 45.23% (19) were from patients in the age group between 51 – 60 years. Followed by 21.73% (10) in 21–30 years.

Table 4 shows a total of 205 clinical specimens were collected in the present study, the commonest samples were pus from wounds and wound swabs comprising 41.46% (85) followed by urine 26.82% (55), sputum 10.24%(21), high vaginal swab with 4.87% (10), blood 4.87% (10). The other samples were suction tip 2.92%(06), pleural fluid

Table 1: Department wise distribution of cases

Department	No. of cases	%	MRSA	%	MSSA	%
Surgery	70	34.14	25	35.71	45	64.28
Medicine	45	21.95	15	33.33	30	66.66
OBG	30	14.63	07	23.33	23	76.66
Pediatrics	10	04.87	04	40	06	60
ENT	04	01.95	03	75	01	25
Orthopedics	25	12.19	12	48	13	52
ICU	03	01.46	03	100	00	00
Urology	08	03.90	03	37.5	05	62.5
Ophthalmology	02	0.97	00	00	02	100
Gynecology	05	02.43	03	60	02	40
DVL	03	1.46	01	33.33	02	66.66
Total	205	100	76	37.07	129	62.92

Table 2: MRSA percentage among the different sex

Sex	No. of cases	No. of MRSA	%
Male	105	47	44.76
Female	100	29	29

Table 3: MRSA isolation among different age groups

Age in years	Total no. of cases	No. of MRSA	%
0-10	14	05	35.71
11-20	14	04	28.57
21-30	46	10	21.73
31-40	31	15	48.38
41-50	28	10	35.71
51-60	42	19	45.23
61-70	18	06	33.33
>70	12	07	58.33
Total	205	76	37.37

Table 4: Nature of clinical specimen

Specimen	Total no.	%	MRSA	%	MSSA	%
Pus/wound swab	85	41.46	32	37.64	53	62.35
Urine	55	26.82	20	36.36	35	63.63
Sputum	21	10.24	04	19.04	17	80.95
High vaginal swab	10	4.87	03	30	07	70
Blood	10	4.87	08	80	02	20
Suction tip	06	2.92	04	66.66	02	33.33
Pleural fluid	05	2.43	01	20	04	80
Bedsore	05	2.43	02	40	03	50
Cervical swab	02	0.97	02	100	00	00
Serous discharge	02	0.97	00	00	02	100
Throat swab	02	0.97	00	00	02	100
Conjunctival swab	01	0.48	00	00	01	100
Skin lesion	01	0.48	00	00	01	100
Total	205	100	76	37.07	129	62.92

2.43 % (05) and bedsore 2.43% (05). Rest of the samples, like conjunctival swab 0.48% (01), skin lesion 0.48% (01), cervical swab, throat swab, serous discharge were same, of 0.97% (02) respectively.

Table 5 shows the pattern of Staphylococcus aureus isolated.

Sixty four point thirty nine percent (132/205) resistance was noticed for penicillin followed by for Co-Trimoxazole 60% (123/205). In case of Erythromycin, Clindamycin and Gentamycine, the resistance percentage was 27.80% (57/205), 27% (54/200) and 44.39% (91/205) respectively. Resistance for Oxacillin was 35.12% (72/205). Least resistance was observed for Amikacin 16.74% (34/203).

Of the 55 urine isolates, 78.18% resistance was for Nalidixic Acid and 70.90% for Norfloxacin. Good sensitivity was noticed for Nitrofurantoin, which was 89.09%. It was further seen that all the isolates were susceptible to Rifampicin and Vancomycin.

Table 6 shows the explanation of the 76 MRSA isolates:- 89.47% resistance was noticed for penicillin-G. followed by Co-Trimoxazole (89.85 %). Majority were multidrug resistant. The resistance to Erythromycin, Clindamycin, Gentamycin and Ciprofloxacin was 68.42%, 75%, 50% and 67.12% respectively. All the MRSA strains were highly resistant to Nalidixic Acid (85.71%) and Norfloxacin (87.5%) and least resistance was observed for the Nitrofurantoin (22.72%).

The MRSA were seen to be highly sensitive to Vancomycin and Rifampicin which showed 100% sensitivity and also for Amoxicillin (77.77%), Amikacin (61.19%). Table 7 shows the analysis of MRSA study by different authors.

Table 5: Antibiotic susceptibility patterns

Antimicrobials	Total samples	Resistant	%	Sensitive	%
Penicillin –G (P)	205	132	64.39	73	35.60
Amoxicillin (Ac)	170	52	30.58	118	69.41
Co-Trimoxazole (Co)	205	123	60	82	40
Erythromycin (E)	205	57	27.80	148	72.19
Clindamycin (Cd)	200	54	27	146	73
Gentamycin (G)	205	91	44.39	114	55.60
Ciprofloxacin (Cf)	200	88	44	112	56
Amikacin (Ak)	203	34	16.74	169	83.25
Nitrofurantoin (Nf)	55	06	10.90	49	89.09
Nalidixic acid (Na)	55	43	78.18	12	21.81
Rifampicin (R)	205	00	00	205	100
Vancomycin (Va)	205	00	00	205	100
Oxacillin (Ox)	205	72	35.12	133	64.87
Teicoplanin (Te)	50	30	60	20	40
Norfloxacin (Nx)	55	39	70.90	16	29.09

Table 6: Susceptibility to individual antimicrobials in MRSAs isolated from different clinical specimens

Antimicrobials	Total no. of MRSA	Resistant	%	Sensitive	%
Penicillin –G (P)	76	68	89.47	08	10.52
Amoxicillin (Ac)	72	16	22.22	56	77.77
Co-Trimoxazole (Co)	69	62	89.85	07	10.14
Erythromycin (E)	76	52	68.42	24	31.57
Clindamycin (Cd)	76	57	75	19	25
Gentamycin (G)	76	38	50	38	50
Ciprofloxacin (Cf)	73	49	67.12	24	32.87
Amikacin (Ak)	67	26	38.80	41	61.19
Nitrofurantoin (Nf)	22	05	22.72	17	77.27
Nalidixic acid (Na)	21	18	85.71	03	14.28
Rifampicin (R)	76	00	00	76	100
Vancomycin (Va)	76	00	00	76	100
Oxacillin (Ox)	76	76	100	00	00
Teicoplanin (Te)	23	19	82.60	04	17.39
Norfloxacin (Nx)	24	21	87.5	03	12.5

DISCUSSION

The epidemiology of MRSA has continued to evolve since its first appearance more than three decades ago. Initially, there were sporadic reports of methicillin resistance amongst nosocomial *Staphylococcus aureus* isolates but later MRSA became a well-established hospital acquired pathogens with a few reports of community-acquired isolates.

MRSA strains in the hospitals are difficult to eradicate because usually they are multi drug resistant. Today MRSA is considered to be one among the most important nosocomial pathogens. MRSA strains have been responsible for outbreaks of nosocomial infections worldwide.

MRSA is a major nosocomial pathogen causing significant morbidity and mortality. The important reservoirs of MRSA in hospitals/institutions are infected or colonized patients and transient hand carriage on the hands of health care workers is the predominant mode for patient-to-patient transmission. In India, the significance of MRSA had been recognized relatively late and it emerged as a problem in

Table 7: Analysis of MRSA by different authors

Name of the author	Year of study	MRSA (%)
S.K. Mathur et al	1994	32.8
Pulimood T.B. et al	1996	24
Udaya Shankar et al	1997	32
Metha et al	1998	33
S. Vidhani et al	2001	51.6
Anupurba S. et al	2003	54.85
Qureshi et al	2004	83
Rajaduraipandi et al	2006	33.6
Gregory J. Morgan et al.	2006	59
Present study	2015	37.07

the 80s and in the 90s.Epidemic strains of these MRSA are usually also resistant to several other antibiotics. During the past 15 years, the appearance and world wide spread of many such clones has caused major therapeutic problems in many hospitals, as well as diversion of considerable resources to attempts at controlling their spread.

Risk factors that has been associated with MRSA acquisition include older age, prolonged hospitalization, prior antibiotic therapy, more severe underlying disease and degree of disability, surgical procedures, presence in an intensive care or burn unit, having a surgical wound infection, intravascular devices, mechanical ventilation, tracheostomy, pressure ulcers, or exposure to other infected or colonized individuals. Not only does antibiotic therapy predispose patients to colonization with MRSA, but it also increases the risk of invasive disease and infection. Other host factors associated with progression from colonization to infection include recent hospitalization, preceding surgical or wound debridement, and the number of invasive procedure.

Isolated 76 MRSA strains from different 205 clinical specimens respectively, that means, out of which the proportion of MRSA was found to be 76(37.07%) and the rest, MSSA was found 129(62.92%).

Since the past decade there were reports by number of workers, some of which are shown in Table (7).The prevalence rate in the present study is 37.07%, which is comparable with the other reports where, it ranged between 32.8% in 1994 [Mathur SK, et al.1994], 24% in 1996 [Pulimood TB., et al.1996],[10] 32% in 1997[MRSA surveillance study group,1997] and 51.6% in 2001 [Vidhani S., et al.].[11] Similar observation was made by Mehta, who in his study ion control of MRSA in a tertiary care centre, had reported an isolation rate of 33% from pus and wound swabs [Mehta AP., et al.,1998]. However, Qureshi from Pakistan reported a high isolation rate of up to 83% MRSA from pus [Qureshi AH, et al,2004].[12] This implies that the incidence of infection by MRSA isolates keeps changing every year and it is on a rise compared to last few years.[7]

Specimen wise distribution

In the present study, 20 of 55 (36.36%)isolates from urine, 32 of 85 (37.84%) isolates from pus, 8 of 10 (80%) isolates from blood and of the isolates from sputum 4 were (19.04%) positive for MRSA strains. 4 of 6 (66.66%) isolates from suction tip, 2of 2(100%)from cervical swab, 3 of 10 (30%)isolates from high vaginal swab and 2 0f 5 (40%)isolates from bedsore were MRSA strain (Table 3).

Age wise distribution

This present study shows that the MRSA strains affected all age groups, but almost half, that means (45.23%)of the patients were in the extremes of age group, that means [50 to 60 years],(younger than one or older than 50 years).

Sex wise distribution

In the present study shows that MRSA strains affected all the male and female sex groups, but the incidence of MRSA is more in the male sex groups, that means 47 of 105(44.76%).

Department wise distribution

The prevalence was highest in the gynecology ward, 3 of 5 (60%). followed by the urology, 3 of 8 (37.5%),medicine ward,15 of 45 (33.33%), surgical, 25 0f 70 (35.71%), the intensive care unit, 3 of 3(100%), orthopedics, 12 of 25 (48%) and so on. Approximately, two-third of cases represented infections and one-third represented colonization.

Clinical characteristics

In the present study the body sites that were most frequently affected by overt MRSA infection were surgical sites (most cases were Type II DM), the chest (pneumonia), and endovascular catheter sites (infections). The total mortality of patient of MRSA infections was high, as was the mortality attributable to MRSA infection. It was generally believe that MRSA strains were not more virulent than methicillin-susceptible *Staphylococcus aureus* (MSSA) strains.

Antimicrobial susceptibility

An important feature of MRSA is their propensity to spread and colonize debilitated patients. Since these strains tend to be multiple antibiotic-resistant, they pose a major difficulty in treating systemic infections. MRSA are more pathogenic than methicillin-sensitive *Staphylococcus aureus*, especially in the seriously ill and immunosuppressed patients. Both can cause a spectrum of illnesses ranging from minor skin infections to life-threatening complication like bacteremia and pneumonia.[13]

In the present study, the antibiotic sensitivity results showed that all MRSA isolates were significantly more resistant to various antibiotics. The resistance of MRSA to β-lactams like Penicillin was 89.47% while co-trimoxazole resistance was seen in 89.85%of the isolates, which was much higher than the resistance obtained in another study in 1999 to 2004(13.3%) and in 1997 to 1998(45.4%)from the different institution.

In our study the spectrum of antimicrobial resistance among MRSA, quinolones (Ciprofloxacin) was also found to be high i.e 67.12%. This co-relates with an earlier finding where it has been shown that the resistance to ciprofloxacin is steadily increasing from 39% in 1992 to

68% in 1996.In 1997 also a high incidence of ciprofloxacin resistance (95.8%) was reported.[14] Again the resistance to ciprofloxacin is decreasing from 59.1% in 1999-2004.

However, Pulimood had observed only 8% resistance of MRSA to gentamycin, as against 50% in our study. Gentamycin resistance is on rise since 1996.An increase of gentamycin o% before 1996 to 80% after 1996 has been reported. Qureshi had reported a gentamycin resistance of 97.8%, which is higher compared to our study.

But in the present study 75% of the strains were resistant to clindamycin, which is the same for erythromycin 68.42%. The resistant rate to amikacin and nalidixic acid were 38.80% and 85.71%.

All the MRSA isolates were found to be susceptible to vancomycin (100%) and rifampicin (100%), MRSA also showed high level of resistance to amoxicillin.

At last vancomycin seems to be the only antimicrobial agent which showed 100% sensitivity and may be used as the drug of choice for treating multidrug resistant MRSA infections. However, regular monitoring of vancomycin sensitivity and routine testing of other newer glycopeptides like teicoplanin should be carried out. Further, the regular surveillance of hospital associated infections including monitoring antibiotic sensitivity pattern of MRSA and formulation of definite antibiotic policy may be helpful for reducing the incidence of MRSA infection.[9]

CONCLUSION

The present study was undertaken to ascertain the prevalence of MRSA in wound infections from patients attending to CCM Medical College and Hospital, and to detect the antimicrobial susceptibility of these strain so as to select the drug of choice in management of infections caused by this organism.

In conclusion, the degree of resistance or sensitivity of MRSA towards commonly used antibiotics is recognized to be diverse from region to region and vancomycin was the only antibiotic found to give uniform sensitivity (100%).

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Authors Contribution:
SC and SB- Concept and design of the study, reviewed the literature, manuscript preparation and critical revision of the manuscript;
AP, SKE and DHC- Concept, collected data and review of literature and helped in preparing first draft of manuscript.

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