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A Prevalence and Antibiogram of Methicillin Sensitive and Methicillin Resistant *Staphylococcus aureus* in a Rural and Urban Setup

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To compare the prevalence of Methicillin Sensitive *Staphylococcus aureus* (MSSA) and Methicillin Resistant *Staphylococcus aureus* (MRSA) at a rural and urban setup and their susceptibility patterns to various antimicrobial agents. A Total of 8611 pus samples from both rural based tertiary care centre and urban based tertiary care centre were analysed. Samples with confirmed *Staphylococcus aureus* were subsequently tested for methicillin sensitivity and resistance using cefoxitin disc (30 μg) and antibiotic susceptibility pattern was determined. The data was analysed statistically. The prevalence or MSSA and MRSA in rural setup were 46% and 54% respectively. The prevalence or MSSA and MRSA in urban setup were 52.9% and 47.1% respectively. Isolation of MRSA is more in adults of more than 50 years of age (58.08%) in rural setup. The MSSA were sensitive to cloxacillin, amikacin, tetracycline, cephelexin, linezolid and vancomycin compared to MRSA in both the setups. The present study showed MRSA were more prevalent than MSSA in rural setup and MSSA were more than MRSA in urban set up. There was high resistance to both the groups, indicating the need for developing appropriate antibiotic policy and limiting the use of powerful antibiotics in the rural setup.

Key words: Methicillin Sensitive *Staphylococcus aureus* (MSSA), Methicillin Resistant *Staphylococcus aureus* (MRSA), Rural, Urban, Antimicrobial susceptibility.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium responsible for several difficult-to-treat infections in humans. It is also called oxacillin-resistant *Staphylococcus aureus* (ORSA)[1] A population-based study of the incidence of MRSA infections in San Francisco during 2004–05 demonstrated that nearly 1 in 300 residents suffered from such an infection in the course of a year and that greater than 85% of these infections occurred outside of the healthcare setting[2] In a meta-analysis of 31 studies, Cosgrove *et al.*,[3] concluded that MRSA bacteremia is associated with increased mortality as compared with MSSA bacteremia.[4] In addition, Wyllie *et al.* report a death rate of 34% within 30 days among patients infected with MRSA, a rate similar to the death rate of 27% seen among MSSA-infected patients[5] .MRSA began as a hospital-acquired infection, but has developed limited endemic status and is now sometimes community-acquired. The

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terms HA-MRSA (healthcare-associated MRSA) and CA-MRSA (community-associated MRSA) reflect this distinction. Diagnostic microbiology laboratories and reference laboratories are key for identifying outbreaks of MRSA. In the "search and destroy" strategy that was employed by all UK hospitals until the mid-1990s, all patients with MRSA were immediately isolated, and all staff were screened for MRSA and were prevented from working until they had completed a course of eradication therapy that was proven to work. Loss of control occurs because colonised patients are discharged back into the community and then readmitted, when the number of colonised patients in the community reaches a certain threshold, the "search and destroy" strategy is overwhelmed[6] Methicillin resistant *S. aureus* (MRSA) is now endemic in India. The incidence of MRSA varies from 25 per cent in western part of India[7] to 50 per cent in South India[8]

MATERIAL AND METHODS

It is a prospective cross sectional study for a period of one year from 1st. August 2013 to 31st. July 2014 at tertiary care centre of Srikakulam district (rural setup) and Visakhapatnam district (urban setup). A total of 8611 pus samples were analyzed, 3280 pus samples were from rural set up and 5331 from urban setups. All the samples were aseptically handled and processed.

Gram's Staining was done for all the samples and the likely organisms were determined. Then the pus samples were inoculated onto Blood agar, Mac Conkey agar & selective media (Mannitol salt agar) obtained from Hi-Media Laboratories Pvt. Ltd, Mumbai and incubated at 37°C for 24 hours. The suspected colonies of *Staphylococci* were taken and Gram's Staining was done, all the Gram–positive cocci in clusters were further confirmed using a battery of standard biochemical reactions including the production of bound and free coagulase enzymes using slide and tube coagulase tests based on standard methods. A known coagulase positive strain *Staphylococcus aureus* ATTC-25923 included as control. All the confirmed *Staphylococcus aureus* strains were subsequently tested for Methicillin resistance based on recommendations of Clinical Laboratory Standard Institute (CLSI). cefoxitin disc (30µg)[29] obtained from Hi-Media Laboratories Pvt. Ltd Mumbai was used to isolate MRSA and MSSA. The isolates were considered Methicillin resistant *Staphylococcus aureus* (MRSA) if the zone of inhibition was 22 mm or less, and zone > than 22 mm was considered as Methicillin sensitive *Staphylococcus aureus* (MSSA).

Further, the antibiotic susceptibility pattern of Methicillin sensitive and resistant *Staphylococcus aureus* strains was determined on the day of their isolation by disc diffusion method on Muller-Hinton agar using the criteria of standard zone sizes of inhibition to define sensitivity or resistance to different antimicrobials[9]. The panel of antibiotics used was penicillin, cloxacillin, erythromycin, azithromycin, amikacin, gentamicin, ciprofloxacin, gatifloxacin, tetracycline, cephalexine, cefuroxime, cefoperazone, piperacillin+tazo, linezolid and vancomycin. *Staphylococcus aureus* ATCC 29213 was used as control strain for the standardization of antibiotic susceptibility.

Statistical analysis

Z test and Chi square test was conducted to know the variability in sensitivity patterns to various antibiotics in MRSA and MSSA in both the setups.

RESULTS

Isolation of MSSA and MRSA in rural and urban setup

A total number of pus samples analyzed were 8611, 3280 (38.09%) from rural setup and 5331 (61.90%) from urban setup. Isolates of *Staphylococci aureus* in the rural setup were 441(13.44%) and 1052 (32.1%) were other coagulase negative Staphylococci. In the urban setup 563 (10.56%) were *Staphylococcus aureus* and 1185 (26.4%) were other coagulase negative *Staphylococci*. In the rural setup MSSA constituted 203 (46%) and MRSA 238 (54%) where as in urban setup 298 (53%) of isolates were MSSA and 265(47%) MRSA [Table-1]

Age and Sex distribution of MSSA and MRSA in rural and urban setup

Isolation rate of MSSA was more in males and MRSA more in females in both rural and urban setup [Table-2]. Isolation rate of MSSA and MRSA in rural areas was similar in children and adults

with predominance of MSSA in children and MRSA in adults in urban area. In adults both in rural and urban setup isolation rate of MSSA and MRSA in group <30 years and >30 years was almost similar. Isolation of MRSA was more in >50 years (58.08%) in rural setup. [Table-3]

Antimicrobial susceptibility in MSSA

The drugs showing sensitivity in both rural and urban setup were: cloxacillin (61.7%, 57.9%), amikacin (78.7%, 65.31%), tetracycline (91.5%, 81.4%), linozolid (98.7%, 97.6%) and vancomycin (90.1%, 88.6%). Antimicrobial susceptibility in MRSA: Sensitive drugs the drugs showing sensitivity in both rural and urban setup were Amikacin (63.3%, 51.6%), tetracycline (89.5%, 71.9 %), linozolid (97.2%, 96.7%) and vancomycin (76.7%, 75.9%). [Table-4]

DISCUSSION

Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates came into existence soon after the introduction of methicillin. Historically, MRSA isolates have been associated with nosocomial infections and rapidly developed resistance to multiple drug classes. However, in recent years, different strains with unique phenotypes have emerged in the community, and the reservoir of community-associated MRSA is rapidly expanding. Community-associated pathogens are likely to cause life-threatening systemic infections, especially in children and elderly individuals. The epidemiological and microbiological differences between community-associated and nosocomial MRSA infections necessitate different strategies to prevent antibiotic resistance[10].

In the present study prevalence of MSSA was 46% and MRSA 54% in rural setup and MSSA 53%, MRSA 47% in urban setup. Studies obtained from other workers in India showed rise in prevalence of MRSA compared to MSSA; Bandaru N Rao *et al.*[11] (52%), Chaudary A *et al.*[12] (52.8%), Majumder D *et al.*[13] (52.9%), Anupurba S *et al.*[14] (54.8%), Dar JA *et al.*[15] (54.85%), Singh S *et al.*[16](57.14%), LeBlanc DM *et al.*[17] (61%), Yilmaz S *et al.*[18](61.1%), Borg M *et al.*[19](65%), Mistry RD *et al.*[20] (66%) and Maninder Kaur *et al.*[21] (34.1%).

Rijal KR *et al.*[22] in their study the prevalence of MRSA among school children between 5-17 years was found to be 56%.In the present study the prevalence of MRSA among children was 54% in rural setup and 46% in urban setup.The results were comparable.

Laxmi Kant Khanal[23] analysed 600 samples of *Staphylococcus aureus* and found MRSA was higher among patients above 30 years of age. Greatest burden of MRSA infection occurred

Table 1. Isolation of MSSA & MRSA in rural and urban areas from pus samples of different etiology.

Pus Samples (No) (%)	Staphylococcus spp. (No) (%)	Staphylococcus aureus (No) (%)	MSSA (No) (%)	MRSA (No) (%)
Rural 3280 (100)	1052 (32.1)	441(13.44)	203 (46)	238 (54)
Urban 5331 (100)	1185 (27.4)	563 (10.56)	298 (53)	265 (47)

Table 2. MSSA and MRSA isolation rate in rural and urban setup among different Sexes in Rural area

Rural area	Total	MSSA (%)	MRSA (%)	P value
Male	245	113 (46.12)	132 (53.88)	> 0.05
Female	196	90 (45.92)	106 (54.08)	
Urban area				
	Total	MSSA (%)	MRSA (%)	P value
Male	304	161 (52.96)	143 (47.04)	> 0.05
Female	259	137 (52.89)	122 (47.11)	

Table 3. MSSA and MRSA isolation rate among different age groups in rural and urban setups

Rural area				
	Total	MSSA (%)	MRSA (%)	P value
Children	98	45 (45.92)	53 (54.08)	> 0.05
Adults	343	158 (46.06)	185 (53.94)	
Urban area				
	Total	MSSA (%)	MRSA (%)	P value
Children	98	53 (54.08)	45 (45.92)	< 0.05
Adults	465	245 (52.69)	220 (47.31)	
Adults				
Rural area				
	Total	MSSA (%)	MRSA (%)	P value
d" 30 years	168	78 (46.43)	90 (53.57)	> 0.05
31 to 50 years	137	68 (49.64)	69 (50.36)	
> 50 years	136	57 (41.91)	79 (58.09)	
Urban area				
	Total	MSSA (%)	MRSA (%)	P value
d" 30 years	204	108 (52.94)	96 (47.06)	> 0.05
31 to 50 years	178	93 (52.25)	85 (47.75)	
> 50 years	181	97 (53.59)	84 (46.41)	

Table 4. Sensitivity of various drugs to MSSA and MRSA in Rural and Urban setup in percentages

Name of the Drug	MSSA		MRSA	
	Rural	Urban	Rural	Urban
Penicillins				
Pencilln (P)	10.6	8	0	1.6
Cloxacillin (COX)	61.7	57.9	36.7	35.9
MACROLIDES				
Erythromycin (E)	25.5	25.1	13.3	17.2
Azithromycin (AZM)	34.5	49.1	13.3	32.8
AMINOGLYCOSIDES				
Amikacin (AK)	78.7	65.3	63.3	51.6
Gentamicin (G)	59.6	56.5	36.7	40.6
FLUOROQUINOLONES				
Ciprofloxacin (CIP)	27.7	36.3	20	29.7
Gatifloxacin (GAT)	21.3	29.1	10	18.8
TETRACYCLINE				
Tetracycline (TE)	91.5	81.4	89.5	71.9
CEPHALOSPORINS				
Cephalexin (CN)	53.2	51.6	20	28.1
Cefuroxim (CXM)	46.1	43.5	20	21.9
Cefoperazone (CPZ)	14.9	14.5	10	14.1
OXAZOLIDINONE				
Linezolid (LZ)	98.7	97.6	97.2	96.7
GLYCOPEPTIDE				
Vancomycin (VA)	90.1	88.6	76.7	75.9
COMBINED DRUGS				
Piperacillin with tazobactum (PIT)	51.1	48.9	48.4	36.3

among older individuals especially those older than 65 years. Half of all MRSA infection occurred in individuals aged 50 years and old. Sex distribution was equal. In the present study isolation of MRSA was more in females in both rural and urban setup. MRSA was more in the age group greater than 50 years(58.08%) in the rural setup.

Mechanism of resistance is by drug inactivation by cellular transferase enzyme, even when the organisms are sensitive either alone or to multiple drugs. Maple *et al*[24] found sensitivty to gentamicin, tobramycin, netilmicin and amikacin to be less than 10%, Pulimood TB *et al.*[25] reported 14.5% sensitivity to gentamicin. Majumder D *et al*[13] reported 5.9% sensitivity to gentamicin and 79.5% by Rajaduraipandi K *et al.*[26] SA strains are also resistant to macrolides. Strains resistant to erythromycin are generally resistant to clarithromycin and azithromycin. Mechanism of resistance is target site alteration. Maple *et al*[24] in 1989 recorded 10% sensivity to erythromycin, and 83% to ciprofloxacin, Pulimood TB *et al.*[25] reported 10% sensitivity and Majumder D *et al.*[23] reported 77.2% sensitivity and 87.2% by Rajaduraipandi K *et al.*[26] All the above studies show that the MRSA isolates are often resistant to multiple antibiotics. Therefore treatment of infections by these organism and its eradication is difficult, and also use of beta lactam antibiotics in MRSA infections will increase antibiotic selection pressure. In the present study tetracycline, linezolid and vancomycin were found to be useful drugs in treating MRSA infections and similar findings were observed by Rajaduraipandi K *et al.*[26] with 100% sensitivity for linezolid and vancomycin. In the study by Rajesh Kamtikar *et al.*[27] highest resistance was seen with penicillin and least resistance to vancomycin and linezolid and alarming resistance to the routinely used antistaphylocoocal antibiotics like erythromycin(83.3%), cephalosporins group(50%) and gentamicin(58.3%). Rubeena Hafeez *et al.*[28] and Maninder Kaur *et al.*[21] recorded similar observations.

In the present study sensitive drugs for MSSA are cloxacillin, amikacin, teracyclines, linezolid and vancomycin. Using Z test there was no significant difference between MSSA in rural and urban areas in the sensitivity pattern to pencillin, cloxacillin, erythromycin, gentamicin, ciprofloxacin , gatifloxacin, cephalexine, cefuroxime, cefoperazone , piperacillin + tazobactum, linezolid and vancomycin. ($p>0.05$).There is a significant difference between MSSA in rural and urban areas in the sensitivity pattern to azithromycin, amikacin and tetracyline. ($p<0.05$)

In the present study MRSA are sensite to amikacin, tetracyclines, linezolid and vancomycin. Using Z test there is no significant difference between MRSA in Rural and Urban areas in the sensitivity pattern to pencillin, cloxacillin, erythromycin, amikacin, gentamicin, ciprofloxacin, gatifloxacin, cephalexine, cefuroxime, cefoperazone, piperacillin + tazobactum, linezolid and vancomycin ($p>0.05$), where as there is a significant difference between MRSA in rural and urban areas in the sensitivity pattern to azithromycin ($p<0.001$) and tetracyline ($p<0.01$).

There is no significant difference between MSSA & MRSA in rural area regarding sensitivity pattern to drugs like ciprofloxacin, tetracyline, cefoperazone, piperacillin + tazobactum and linezolid ($p>0.05$). there is a significant difference between MSSA & MRSA in rural area regarding sensitivity pattern to drugs like erythromycin, amikacin, gatifloxacin ($p<0.05$), vancomycin, gentamicin ($p<0.01$), pencillin, cloxacillin, azithromycin, cephalexine and cefuroxime ($p<0.001$).

There is no significant difference between MSSA & MRSA in urban area in their sensitivity pattern to erythromycin, ciprofloxacin, gatifloxacin, tetracyline, cefoperazone, piperacillin + tazobactum, and linezolid ($p>0.05$). There is a significant difference between MSSA and MRSA in urban area in their sensitivity pattern to pencillin, azithromycin, amikacin, gentamicin, vancomycin ($p<0.05$) cloxacillin ($p<0.01$) cephalexine and cefuroxime ($p<0.001$).

Hence regular surveillance of hospital associated infections and monitoring antibiotic sensitivity pattern of MSSA & MRSA and formulation of definite antibiotic policy may be helpful for reducing the incidence of MRSA infections. The degree of resistance or sensitivity of MRSA towards commonly used antibiotics recognized to be diverse from region to region. Tetracyclines

are more sensitive in both MSSA and MRSA group in the present study and can be used routinely. Vancomycin and Linezolide seems to be highly sensitive antimicrobial agents and may be used as the drugs of choice for treating multidrug resistant MRSA infections in life threating conditions. In India, a continuous surveillance on the antimicrobial susceptibility pattern and use of antibiotics judiciously by the clinicians is very much necessary to prevent antimicrobial resistance.

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