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Occurrence of Antimicrobial Sensitivity Pattern for Methicillin Resistant *Staphylococcus aureus* and Methicillin Resistant Coagulase Negative *Staphylococcus* Isolated from Various Clinical Samples in a Tertiary Care Hospital, Jaipur, India

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A B S T R A C T

Methecillin resistant *Staphylococcus* (MRS) is problematic, as the therapeutic outcome of MRS infection is much worse than those caused by methicillin sensitive strain. This study was conducted to determine the occurrence and antimicrobial sensitivity pattern of MRSA and MRCoNS isolated from different clinical samples. MRSA and MRCoNS were identified among 215 *Staphylococcus* isolates, isolated from various clinical samples. All isolates were identified as per CLSI guideline and AST pattern was determined by Kirby Bauer disc diffusion method. A total of 215 *Staphylococcus* isolates were prolcessed of which 122(56.74%) were coagulase positive *Staphylococcus aureus* and 93 (43.26%) isolates were Coagulase negative *Staphylococcus*. Among 122 coagulase positive *Staphylococcus aureus* 64(52.46%) were MRSA, whereas among 93 CoNS, 36 (38.7%) were MRCoNS. Among the MRSA and MRCoNS isolates maximum resistance was seen with Penicillin-G which was 90.62% and 88.88% and both MRSA and MRCoNS was least with Vancomycin and Linezolid (100%). The regular surveillance of MRSA and MRCoNS will be useful for select an appropriate antibiotic and for limiting use of powerful antibiotic like Vancomycin as initial treatment and life threatening staphylococcal infection.

Introduction

Staphylococci are Gram-positive cocci, arranged in grape-like clusters. They are non-motile, non- sporing, occasionally capsulated and are facultative anaerobes that grow better under aerobic than anaerobic conditions. *Staphylococci* are classified as coagulase positive *Staphylococcus aureus* and coagulase negative *Staphylococci* (CoNS) (Collee *et al.*, 1996). *Staphylococcus aureus* has been renowned as an important cause of human disease for more than 100 years (Lowy *et al.*, 1998) *Staphylococcus aureus* is recognized as a cause of a wide range of infections, ranging from minor skin infections and chronic bone infections to devastating septicemia and endocarditis (Chambers *et al.*, 2005) Significant events in the evolution of *S. aureus* have included the development of methicillin

resistance, now a problem for many hospitals around the world. The recent emergence of community strains of *S. aureus* that are not only methicillin resistant but also harbor genes associate with increased virulence has become a therapeutic challenge (Chambers *et al.,* 2001, Vandenesch *et al.,* 2003). Both endemic and epidemic MRSA infections occur globally as infected and colonized patients in hospitals mediate the dissemination of these isolates and hospital staff assists further transmission (McDonald *et al.,* 1997).

Most of the *S. aureus* infections caused by Methicillin sensitive *S. aureus* (MSSA) that are usually susceptible to major class of anti-Staphylococcal antibiotics. But resistance to multiple antibiotics among the *Staphylococci* isolates in hospitals has been recognized as one of the major challenges in hospital infection control (Majumder *et al.,* 2001).

Coagulase – negative *Staphylococci* (CoNS) is a group of opportunistic pathogen causing wide spectrum of disease in humans. Its association with increased number of hospital acquired infections has been documented (Koksal *et al.,* 2009; Sohn *et al.,* 2001).

Until recently, Methicillin Resistant *Staphylococci* (both MRSA and MRCoNS) were predominantly nosocomial pathogens causing hospital acquired infections (Majumder *et al.,* 2001) but Methicillin resistant Staphylococcal (MRS) strains are now being increasingly isolated from community acquired as well (Basak *et al.,* 2010, Naimi *et al.,* 2003). MRSA and MRCoNS carry multiple antimicrobial resistance determinants conferring resistance to beta lactams (penicillin, cephalosporing and carbapenems) and non-beta - lacatam antibiotics (macrolides, amino glycosides, fluoroquinolones and lincosamides). Multiple drug resistance make them difficult to treat and limiting treatment options to glycopeptides antibiotics like vancomycin and teicoplanin (Mehdinejad *et al.,* 2008; Shorman *et al.,* 2008). MRS infections represent a burden for both patients and healthcare system because of their associated high morbidity, mortality and increased hospitalization coast. Hence this study is designed to trace the resistance trends of *Staphylococci* with special reference to methicillin resistance in a tertiary care hospital, Jaipur, Rajasthan.

Materials and Methods

Study design

The study was conducted in Department of Microbiology, NIMS Medical College and Hospital, Jaipur from December 2016 to May 2017.

The study included those patients from whom *Staphylococci* have been isolated among different clinical samples submitted to Microbiology Laboratory for culture and sensitivity and excluded the specimens were *Staphylococci* isolates have been considered contamination due to Laboratory or skin flora.

Isolation and identification of clinical specimens

A total of 215 Coagulase positive and Coagulase negative *Staphylococci* isolates were obtained from various clinical specimens including pus, blood, urine, high vaginal swab (HVS), sputum, ET Secretions, stool, and catheter tip. These isolates were subjected to methicillin resistance screening using cefoxitin disc diffusion method.

The clinical specimens were inoculated on 5% sheep blood agar, MacConkey agar and incubated at 37^0 C aerobically for 24h. *S. aureus* was identified based on Gram's stain morphology, colony characteristics, and positive catalase and coagulase tests.

All the clinical specimens were collected from the patients, submitted to the microbiology laboratory for the sample processing according to standard protocols and the antimicrobial sensitivity was determined according to Clinical Laboratory Standard Institute (CLSI) guidelines.

Bactec culture bottles were used for the collection of blood and body fluids that are loaded in Bactec system according to the manufacturer instructions. On the detection of growth in the Bactec system, further sample processing was done.

Antimicrobial susceptibility testing

The antibiotic susceptibility pattern of all the confirmed *S. aureus* and CoNS were determined by modified Kirby-Bauer disc diffusion method against the following antibiotics as per CLSI guidelines 2017: Penicillin (10 µg), erythromycin (15 µg), clindamycin (2 µg), gentamycin (10 µg), vancomycin (30 µg), linezolid (15 µg), nitrofurentoin, norfloxacin and cefoxitin as per CLSI guideline.

Muller-Hinton agar used to perform all antimicrobial susceptibility tests, and the interpretation criteria were taken according to CLSI guideline.

Detection of MRSA and MR CoNS

Cefoxitin (30 µg) disc diffusion test (Himedia Mumbai)

The isolated samples were subjected to cefoxitin disc diffusion test by using 30 µg discs. A suspension, equivalent to 0.5 McFarland standard was prepared from each strain. Then, a swab was taken and dipped into the suspension and lawn culture was done on MHA plate after that plate was incubated at 37°C for 18-24 h and zone of inhibition was measured. An inhibition zone diameter of ≤21 mm was considered as cefoxitin resistant reported as methicillin-resistant and ≥22 mm was reported as cefoxitin sensitive indicating methicillin-sensitive.

Statistical analysis

The data were recorded and analyzed using Microsoft Excel (2007 Version). Results are presented in frequency (number) and percentage (%).

Results and Discussion

In our study, 215 *Staphylococcus* isolates were collected from various clinical samples among the IPDs, OPDs and ICUs patients in NIMS medical college and hospital, Jaipur, Rajasthan India.

The highest percentage of *Staphylococcus* isolates was obtained from Urine samples (51.2%), followed by pus (16.7%), Sputum (7.91%), ET Secretion (7.91%) etc (Table 1).

Out of 215 isolates 100 (46.51%) was coagulase positive *Staphylococcus aureus* and 115 (53.48%) was coagulase negative test CoNS (Table 2).

Out of 215 isolates 64 was MRSA followed by 58 isolates of MSSA, 36 isolates of MRCoNS and 57 isolates of MSCoNS. Among the 64 MRSA highest percentage was obtained from Urine 38(67.86%) followed by Pus 16(53.33%), ET Secretions 6(54.55%) and sputum 2(18.18%). Among the 36 MRCoNS highest percentage was obtained from Urine 35(35.19%) followed by Blood 5(45.45%), ET Secretion 4(66.67%) and Pus 3(50%).

Among isolates, maximum MRSA was found from 40-49 age group, MSSA from 30-39 age group, MRCoNS and MSCoNS were obtained from 20-29 age group (Table 4 and 5). From

total isolates male ratio is higher than female in MRSA (1:0.82) and MSSA (1:0.93) but the female ratio is higher than male in MRCoNS (0.8:1) and MSCoNS (0.78:1) (Table 6). Among the total isolates, MRSA and MRCoNS were highly resistant than MSSA and MSCoNS. Among the MRSA maximum resistant was seen with Penicillin-G (90.62%), followed by Erythromycin (87.5%), Clindamycin (82.81%), Norfloxacin (81.57%), Gentamycin (54.68%) and Nitrofuroitoin (7.89%). Among the MRCoNS maximum resistant was seen with Penicillin-G (88.88%), followed by Norfloxacin (84.21%), Erythromycin (80.55%), Clindamycin (75%), Gentamycin (38.88%), and Nitrofurontoin (5.26%). Lenezolid and Vancomycin were 100% sensitive with among the isolates (Tables 7–9).

Table.1 Distribution of *Staphylococcus* isolates from various clinical samples

Clinical Specimens	No. of isolates	Percentage
Urine	110	51.2
Pus	36	16.7
Sputum	17	7.91
ET secretions	17	7.91
Blood	17	7.91
HVS	8	3.72
Ear Swab	6	2.79
Catheter tip	2	0.93
Stool	1	0.47
CSF	1	0.47
Total	215	100

Table.2 Isolation of Coagulase positive and Coagulase Negative *Staphylococcus* isolates from various clinical samples

Clinical Specimens	No. of *S. aureus*	%	No. of CoNS	%
Urine	56	45.9	54	58.1
Pus	30	24.6	6	6.45
Sputum	11	9.02	6	6.45
ET secretions	11	9.02	6	6.45
Blood	6	4.92	11	11.8
HVS	3	2.46	5	5.38
Ear Swab	3	2.46	3	3.23
Catheter tip	1	0.82	1	1.08
Stool	0	0	1	1.08
CSF	1	0.82	0	0
Total	122	100	93	100

Table.3 Isolation of Methicillin resistance and Methicillin sensitive *Staphylococcus aureus* isolates from various clinical samples

Clinical Specimens	No. of *S. aureus*	No. of MRSA	%	No. of MSSA	%
Urine	56	38	67.86	18	32.14
Pus	30	16	53.33	14	46.67
Sputum	11	02	18.18	09	81.82
ET secretions	11	06	54.55	05	45.45
Blood	6	01	16.67	05	83.33
HVS	3	01	33.33	02	66.67
Ear Swab	3	00	00	03	100
Catheter tip	1	00	00	01	100
Stool	0	00	00	00	00
CSF	1	00	00	01	100
Total	122	64	52.46	58	47.54

Table.4 Isolation of Methicillin resistance and Methicillin sensitive Coagulase negative *Staphylococcus* isolates from various clinical samples

Clinical Specimens	No. of CoNS	No. of MRCoNS	%	No. of MSCoNS	%
Urine	54	19	35.19	35	64.81
Pus	6	03	50	03	50
Sputum	6	01	16.67	05	83.33
ET secretions	6	04	66.67	02	33.33
Blood	11	05	45.45	06	54.55
HVS	5	02	40	03	60
Ear Swab	3	01	33.33	02	66.67
Catheter tip	1	00	00	01	100
Stool	1	01	100	00	00
CSF	0	00	00	00	00
Total	93	36	38.71	57	61.29

Table.5 Distribution of Staphylococcal isolates according to age group

Age Group	No. of MRSA	%	No. of MSSA	%	No. of MRCoNS	%	No. of MSCoNS	%
0-9	00	0	00	0	02	5.55	09	15.79
10-19	05	7.81	10	17.24	05	13.89	05	8.77
20-29	09	14.06	11	18.97	12	33.33	21	36.84
30-39	12	18.75	12	20.69	07	19.44	09	15.79
40-49	14	21.88	09	15.52	05	13.89	03	5.26
50-59	09	14.06	03	5.17	01	2.77	04	7.01
60-69	12	18.75	04	6.89	04	11.11	05	8.77
70-79	02	3.12	07	12.07	00	0	01	1.75
80-89	01	1.56	02	3.44	00	0	00	0
90-99	00	0	00	0	00	0	00	0
Total	64	100	58	100	36	100	57	100

Table.6 Distribution of Staphylococcal isolates according to sex

Organism	Male	Female	Ratio (M:F)
MRSA	35	29	1:0.82
MSSA	30	28	1:0.93
MRCoNS	16	20	0.8:1
MSCoNS	25	32	0.78:1

Table.7 Antimicrobial resistant pattern of MRSA isolates

Name of Antibiotic	No. of Sensitive Organism	%	Resistant	%
Penicillin-G	6	9.37	58	90.62
Clindamycin	11	17.18	53	82.81
Erythromycin	8	12.5	56	87.5
Linezolid	64	100	0	0
Vancomycin	64	100	0	0
Gentamycin	29	45.31	35	54.68
Norfloxacin	7	18.42	31	81.57
Nitrofurentoin	35	92.10	3	7.89

Table.8 Antimicrobial resistant pattern of MSSA isolates

Name of antibiotics	No. of sensitive organism	%	No. of resistant organism	%
Penicillin-G	22	37.93	36	62.06
Clindamycin	39	67.24	19	32.75
Erythromycin	30	51.72	28	48.27
Lenezolid	58	100	0	0
Vancomycin	58	100	0	0
Gentamycin	52	89.65	6	10.34
Norfloxacin	9	50	9	50
Nitrofurentoin	18	1000	0	100

Antimicrobial resistant pattern of MRCoNS isolates

Name of antibiotics	No. of sensitive organism	%	No. of resistant organism	%
Penicillin-G	4	11.12	32	88.88
Clindamycin	9	25	27	75
Erythromycin	7	19.44	29	80.55
Lenezolid	36	100	0	0
Vancomycin	36	100	0	0
Gentamycin	22	61.11	14	38.88
Norfloxacin	3	15.78	16	84.21
Nitrofurentoin	18	94.74	1	5.26

Table.9 Antimicrobial resistant pattern of MSCoNS isolates

Name of antibiotics	No. of sensitive organism	%	No. of resistant organism	%
Penicillin-G	22	38.59	35	61.40
Clindamycin	29	50.87	28	49.12
Erythromycin	24	42.10	33	57.89
Lenezolid	57	100	0	0
Vancomycin	57	100	0	0
Gentamycin	50	87.71	7	12.28
Norfloxacin	16	45.71	19	54.28
Nitrofurentoin	33	94.28	2	5.71

There is a growing concern about the rapid rise in resistance of *S. aureus* to antimicrobial agents. In India, the importance of MRSA as a problem has been recognized relatively late. The prevalence of MRSA varies in different parts of India and is not uniform. Reports from a Delhi hospital showed a prevalence rate of 51.6% in 2001, whereas it was reported as 38.44% in the same hospital in 2008 (Rajaduraipandi *et al.*, 2006). A recent study (Sangeeta Joshi *et al.*, 2003) found the prevalence to be 42% in 2008 and 40% in 2009. In a study at Aligarh, India (Dar JA *et al.*, 2006) it was shown that 35.1% of *S. aureus* and 22.5% of coagulates-negative staphylococcal isolates were resistant to methicillin.

In another study (Rajaduraipandi *et al.*, 2006) conducted in Tamil Nadu, out of 906 strains of *S. aureus* isolated from clinical samples, 250 (31.1%) were found to be methicillin resistant. Our study had MRSA prevalence is 52.46% and MRCoNS 38.71% and most of MRSA infection seen in Male patients 35 and most of MRCoNS infection seen in females about 20 numbers. This variation in prevalence may be because of several factors like healthcare facilities available in the particular hospital, implementation and monitoring of infection control committee, rationale antibiotic usage which varies from hospital to hospital.

Maximum samples of the MRSA isolated were from the urine samples i.e. 38(67.8%) followed by Pus, ET secretion and sputum samples as shown in table 3 and in MRCoNS isolates from Urine 54 (58%) followed by Blood (11.8%), Pus (6.45) shown in table 2. Some other Indian studies where they show throat swabs and wound swabs were the main source MRSA and MRCoNS infection (Anupurba *et al.*, 2003).

MRSA strains were more resistant to all antibiotics except for Linezolid and Vancomycin. The present study shows high resistance to Penicillin 58%, Erythromycin 56% and clindamycin 53%, this is in accordance with other studies (Saikia *et al.*, 2009; Kumari *et al.*, 2008). In case of MRCoNS were more resistant to all antibiotics except for Linezolid and Vancomycin and show resistance to other antibiotics like Penicillin 22%, Erythromycin 29% and clindamycin 27%. In urine samples for both organisms shows nitrofurantoin resistance is very low like MRSA only 3% and MRCoNS only 1% reported.

Most common reason for multi-drug resistant MRSA is indiscriminate use of antibiotics without drug sensitivity testing which may be due to due to lack of advanced laboratory facilities or negligence on the part of medical practitioners or patients poor economic status.

Coagulase-negative staphylococcus (CoNS) is a group of opportunistic pathogens causing wide spectrum of diseases in humans. Recently MRCoNS have been associated with increased number of infections in hospitalized patients (Koksal *et al.,* 2007; Shamsadh Begum *et al.,* 2011).

The present study showed a high level prevalence of MRSA and MRCoNS strains resistance against widely used antimicrobial agents which routinely used in microbiology lab. The regular surveillance of MRSA and MRCoNS will also be useful for selecting an appropriate antibiotic, to know the changing trends of antibiotic susceptibility pattern, for developing hospital antibiotic policy and for limiting the use of powerful antibiotics like Vancomycin as initial treatment and save it for the treatment of resistant and life-threatening staphylococcal infections.

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