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Multidrug drug resistance in *Staphylococcus aureus* isolates from Clinical Specimens in Northern India

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***Staphylococcus aureus* is a well-known human pathogen that is primarily associated with nosocomial infections causing variety of diseases with increasing resistance to antibiotics. The goal of this study was to determine the prevalence and multi drug resistance patterns of both methicillin resistant *S. aureus* (MRSA) and methicillin susceptible *S. aureus* (MSSA) in the studied population. Out of 60 *S. aureus* isolated, 27 (45.00%) were found to be methicillin resistant, while 33 (57.14%) were methicillin susceptible. The incidence of MRSA in different samples was recorded with maximum in pus (55.55%), followed by wound swabs (50.00%), urine (45.95%) ear swab and blood samples (25.00%) each. MRSA isolates showed marked antibiotic resistance (over 70.00%) against oxacillin, penicillin, nalidixic acid, ampicillin, amoxicillin, and cefpodoxime. All MRSA isolates were foundto bemultidrug resistant with 14.81% isolates showing resistant to 14 drugs. The MSSA isolates tested were also highly resistant to penicillin (81.00%), ampicillin (90.00%), and amoxicillin (88.00%) with 6.06% isolates showing multi drug resistance to maximum of 11 antibiotics tested.**

Key words: Multi drug resistance, study, *Staphylococcus aureus*, methicillin resistant *S. aureus* (MRSA), methicillin susceptible *S. aureus* (MSSA), clinical specimens, Northern India.

INTRODUCTION

Multi drug resistance is very common in *Staphylococcus aureus* clinical isolates worldwide, particularly in developing countries. *S. aureus* is a causative agent of wide variety of diseases. It is also involved in causing urinary tract infections (UTI) (Akerele and Ahonkhai, 2000). Methicillin-resistant *S. aureus* (MRSA) has established itself to be one of the more often wide spread and stable nosocomial pathogens of the late 20th century (Nimmo et al., 2000). MRSA is relatively ubiquitous and is the cause of many community, endemic and epidemic nosocomial colonization and infections. They cause surgical site infections, bacteraemia, lower respiratory tract infections, urinary tract infections, etc. (Duguid et al., 1978). MRSA is of concern not only, because of its resistance to methicillin, but it is also resistant to many other antibiotics (Vidhani et al., 2001). It is a very common pathogen in hospital admitted patients globally and now become a challenge for clinicians to curing infections caused by *S. aureus*; as common public infections are increasing in various regions and countries

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(Layton et al., 1995).

In Africa, US, Southern European countries, Asia and South America, MRSA prevalence has been recorded in a varied trend as high in some countries and low in others (Bell et al., 2002). National Nosocomial Infection Surveillance System (NNIS) reported that 50.00% of hospital acquired infections in ICUs in the USA are due to MRSA (Salgado et al., 2003). MRSA is now endemic in India. The incidence of MRSA varies according to the region, lower in west (Patel et al., 2010) and higher in South India (Gopalakrishnan and Sureshkumar, 2010). MRSA prevalence is rapidly increasing with the time in India. In a study conducted in Indore, the MRSA has been increased significantly from 1992 to 1999 (Verma et al., 2000). Similar findings were also observed in Uttar Pradesh where the incidence of MRSA was significantly increased from 1988 to 2008 (Mathur et. al., 1994; Tiwari et al., 2008).

Infection with MRSA strains, which are resistant to wide range of antibiotics, is directly involve with producing diseases and death (Capitano et al., 2003). Most of the MRSA strains are multi-drug resistant; however, many of the β-lactams are ineffective against a significant proportion of *S. aureus* isolates (Guignard et al., 2005; Llarrull et al., 2009). Resistance in MRSA is related to a chromosomal *mecA* gene that is responsible for the production of an abnormal penicillin binding protein called PBP2a or PBP21. Penicillin-binding proteins are membrane-bound enzymes, which targets for all β-lactam antibiotics (Weems, 2001). The *mecA* gene complex also has insertion sites for transposons and plasmids that facilitate acquisition of resistance to other antibiotics. Thus, S*. aureus* is commonly resistant to non β-lactam antibiotics, such as erythromycin, clindamycin, gentamicin, co-trimoxazole and ciprofloxacin (Chambers, 1979; Chambers, 2001). The emergence of multi drug resistant MRSA is a serious and ongoing concern. The ultimate goal of our research work is to determine the prevalence of methicillin-resistant *S. aureus* from clinical specimens and to suggest an appropriate and potent drug for treatment to the patients suffering from MRSA in Northern India.

MATERIALS AND METHODS

Samples collection

A total of 330 clinical specimens consisting of pus swabs, wound swabs, urine, ear swabs and blood samples were collected between January 2013 and December 2014 from the diagnostic centers of Lucknow in Era's Lucknow Medical College and Hospital, Lucknow, and Integral Institute of Medical Sciences and Research, Lucknow in Northern India.

Sterile cotton wool swabs moistened with Stuart's Transport Medium were used to collect the specimens and inoculated into freshly prepared slant of nutrient agar and mannitol salt agar. The clean-catch midstream urine samples were collected directly into sterile disposable universal bottles. The cultures were incubated at 37±1°C for 24 h (Kolawole and Shittu, 1997).

Isolation and identification of the isolates

The specimens collected were directly streak onto mannitol salt agar (Hi-Media). The plates were incubated at 37°C for 24 h. Plates were observed for smaller colonies that were yellow due to mannitol fermentation on mannitol salt agar medium. *S. aureus* colony was confirmed by Gram staining, catalase test, coagulase test, mannitol fermentation, gelatin liquefaction, and β-hemolysis on blood agar (Washington et al., 2006).

Determination of antimicrobial resistance

Pure isolates of identified *S. aureus* were subjected to antimicrobial susceptibility testing using the disc diffusion method as recommended by Kirby Bauer method according to the recommendations of Clinical Laboratory Standard Institute (CLSI, 2010), formerly National Committee for Clinical Laboratory Standards (NCCLS) (2002), using the following antibiotics discs obtained from Hi-Media Laboratories Pvt. Ltd, Mumbai: methicillin (MET) 5 µg, oxacillin (ox)1 µg, penicillin G (PEN) 10 IU, erythromycin (ERYTHRO) 5 µg, nalidixic acid (NA) 30 µg, kanamycin (KAN) 30 µg, nitrofurazone (NR) 100 µg, tetracycline (TET) 10 µg, polymyxin B (PB) 300 µg, ciprofloxacin (CIP) 5 µg, ampicillin (AMP) 10 µg, ofloxacin (OF) 5 µg, sulphadiazine (SZ) 300 µg, amoxicillin (AMX) 10 µg, and cefpodoxime (CPD) 30 µg. All isolates were grown in Brain Hearth Infusion broth (Biotech Laboratories, United Kingdom) and incubated at 37°C for 6 h until the turbidity of 0.5 McFarland standards was achieved. The isolates were then swabbed onto Muller Hinton Agar (Amershan, England) and allowed to dry for 15 min. The antibiotics discs were placed on the centre of the agar plates with the aid of sterile pointed tip forceps and incubated at 37°C for 24 h. The presence of a clear zone around the antibiotic disc is measured with meter rule. *S. aureus* strains were tested for methicillin resistance using the disc diffusion method (Bauer et al., 1966). *S. aureus* isolates are considered to be resistant to methicillin if the inhibition zones are <10 mm while susceptible if the zones of inhibitions were ≥ 10 mm.

Multiple antibiotic resistances (MAR) indexing

The MAR index profile was performed on both MRSA and MSSA isolates. The MAR index was calculated according to the formula: No. of antibiotics to which all isolates were resistant/No. of antibiotics tested × No. of isolates as recommended by Downing et al. (2011). Sampling site based MAR index was calculated by the same formula modified by the total number of isolates from a sampling site as described (Riaz et al., 2011).

Statistical analysis

Statistical analysis in the present study was done by using Microsoft Office Excel 2007, SPSS version 12 (Statistical Package for Social Sciences). t-test was performed to check the significance of the data by considering (p value=0.05).

RESULTS

Out of 330 samples studied, *S. aureus* was isolated from 60 samples (18.18%). The results showed that the highest prevalence of *S. aureus* was found in wound sample (25%) as compared to others. Others are ear swab and blood (20.00% each), pus swab (18.00%) and

Table 1. Occurrence rate of *S. aureus* (MRSA and MSSA) isolates from various clinical specimens.

Clinical samples	No. of samples	No. of positive *S. aureus* (%)	MRSA (%)	MSSA (%)
Pus swab	50	9 (18.00)	5 (55.55)	4 (44.44)
Wound	24	6 (25.00)	3 (50.00)	3 (50.00)
Urine	216	37 (17.13)	17 (45.95)	20 (54.05)
Ear swab	20	4 (20.00)	1 (25.00)	3 (75.00)
Blood	20	4 (20.00)	1 (25.00)	3 (75.00)
Total	330	60 (18.18)	27 (45.00)	33 (55.00)

Table 2. Antibiotic resistance profile of *Staphylococcus aureus* (MRSA) and (MSSA) isolates.

Antibiotics	Susceptibility		P-value	
	MRSA (n=27) Resistance (%)	MSSA (n=33) Resistance (%)		
Oxacillin	25 (92.59)	4 (12.12)	<0.05	S
Penicillin-G	25 (92.59)	27 (81.81)	<0.05	S
Erythromycin	12 (44.44)	10 (30.30%)	<0.05	S
Nalidixic acid	19 (70.37)	10 (30.30)	<0.05	S
Kanamycin	18 (66.67)	9 (27.27)	<0.05	S
Nitrofurazone	7 (25.92)	7 (21.21)	>0.05	NS
Tetracycline	13 (48.15)	9 (27.27)	<0.05	S
Polymyxin-B	14 (51.85)	9 (27.27)	<0.05	S
Ciprofloxacin	14 (51.85)	9 (27.27)	<0.05	S
Ampicillin	27 (100)	30 (90.90)	<0.05	S
Ofloxacin	11 (40.74)	10 (30.30)	<0.05	S
Sulphadiazine	14 (51.85)	15 (45.45)	<0.05	S
Amoxicillin	2 7(100)	29 (87.88)	<0.05	S
Cefpodoxime	19 (70.37)	17 (51.51)	<0.05	S

P. value = Indicates statistically significance difference between methicillin resistance and sensitive strains. NS: Not significant, S: Significant.

urine (17.1%). Out of the total 60 strain of *S. aureus* isolated from different samples, 27 (45.00%) were found to be resistant to methicillin, while ear swab and blood samples had the least distribution for MRSA (Table 1).

The multidrug resistance data for all MRSA isolates was observed. All the MRSA isolates showed 100% resistance against ampicillin and amoxicillin. Maximum number of isolates (92.59%) showed resistance against oxacillin and penicillin-G; followed by 70.37, 66.67, 51.85, 48.15, 44.44%, 40.74 and 25.92% against nalidixic acid and cefpodoxime, kanamycin, ciprofloxacin, polymyxin B, sulphadiazine, tetracycline, erythromycin, ofloxacin and nitrofurazone, respectively (Table 2).

The multidrug resistance data for MSSA isolates was also observed. Maximum number of isolates (90.9%) showed resistance against ampicillin followed by 87.88, 81.88, 51.51, 45.50, 30.30, 27.27, 21.20 and 12.12% against amoxicillin, penicillin G, cefpodoxime, sulphadiazine, erythromycin, nalidixic acid, ofloxacin, kanamycin, tetracycline, polymyxin B, ciprofloxacin, nitrofurazone and oxacillin, respectively. A high occurrence rate of MSSA was recorded as compared to MRSA from clinical specimens but all the isolates of the MRSA showed a significantly higher multidrug resistance.

Multidrug resistance patterns in 27 MRSA and 33 MSSA isolates were also recorded. Both isolates of MRSA and MSSA showed a variety of resistance patterns. Besides four isolates of MRSA, each of the isolates demonstrated a single resistance pattern against different number of antibiotics. However, each of the MSSA isolates was observed with a unique and single multidrug resistance pattern. Out of the 27 MRSA isolates, 3.7% showed resistance to 7, 8, 9, 10, 11, 13, and 14 antibiotics at a time in different combinations, respectively, while 7.4% exhibited resistance to 8 and 14 antibiotics at a time in two different combinations, respectively. Of the 33 MSSA isolates, 3.03% showed resistance to 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 antibiotics at

Table 3. Antibiotic resistance patterns of 27 MRSA isolates.

No. of antibiotics	Resistance pattern	No. of resistant isolates	%	MAR
7	METH, OX, PEN, KAN, AMP, AMOX, CPD	1	3.7	0.46
	METH, PEN, ERY, NA, AMP, AMOX, CPD	1	3.7	0.46
8	METH, OX, PEN, NA, KAN, PB, AMP, AMOX,	1	3.7	0.53
	METH, OX, PEN, NA, KAN, AMP, AMOX, CPD	2	7.4	1.06
	METH, OX, PEN, ERY, NA, KAN, AMP, AMOX	1	3.7	0.53
	METH, OX, PEN, NA, TET, AMP, AMOX, CPD	1	3.7	0.53
	METH, OX, PEN, KAN, CIP, AMP, OF, AMOX	1	3.7	0.53
	METH, OX, PEN, KAN, TET, AMP, OF, AMOX	1	3.7	0.53
9	METH, OX, PEN, NA PB, AMP, SZ , AMOX, CPD	1	3.7	0.6
	METH, OX, PEN, KAN, NR, TET AMP, AMOX, CPD	1	3.7	0.6
	METH, OX, PEN,, PB, AMP, CIP, SZ, AMOX, CPD	1	3.7	0.6
10	METH, OX, PEN, ERY, TET, PB, CIP, AMP, OF, AMOX	1	3.7	0.66
	METH, OX, PEN, NA, PB,CIP, AMP, OF, AMOX, CPD	1	3.7	0.66
	METH, OX, PEN, NA, KAN, PB, AMP, SZ, AMOX, CPD	1	3.7	0.66
	METH, OX, PEN,, NA, KAN, NR, AMP, OF, AMOX, CPD	1	3.7	0.66
	METH, OX, PEN, ERY, NA, KAN, PB, AMP, SZ, AMOX	1	3.7	0.66
	METH, ERY, NA, TET, CIP, AMP,OF SZ, AMOX, CPD	1	3.7	0.66
11	METH, OX, ERY , NA, KAN, NR, TET, CIP, AMP, SZ, AMOX	1	3.7	0.73
	METH, OX, PEN, KAN, NR, PB, CIP, AMP, SZ, AMOX, CPD	1	3.7	0.73
	METH, OX, PEN, KAN, NR, TET, CIP, AMP, SZ, AMOX, CPD	1	3.7	0.73
13	METH, OX, PEN, ERY, NA, TET, PB, CIP, AMP, OF, SZ, AMOX, CPD METH,	1	3.7	0.86
	OX, PEN, ERY, NA, KAN, TET, PB, CIP, AMP, SZ, AMOX, CPD	1	3.7	0.86
14	METH, OX, PEN, ERY, NA, NR, TET, PB, CIP, AMP, OF, SZ, AMOX, CPD	1	3.7	0.93
	METH, OX, PEN, ERY, NA, KAN,TET, PB, CIP, AMP, OF, SZ, AMOX, CPD	2	7.4	1.86
	METH, OX, PEN, ERY, NA, KAN, NR, TET, PB, CIP, AMP, OF, SZ, AMOX	1	3.7	0.93

a time in different combinations, respectively.

A varied trend of MAR index was also observed among the MRSA isolates with 3.7% of the isolates showing a MAR of 0.46 to 0.93 range against different number of antibiotics. Also MAR of 1.06 and 1.86 were recorded by 7.4% isolates. In the case of MSSA isolates, 3.03% isolates demonstrated 0.13 to 0.73 MAR range (Tables 3 and 4).

DISCUSSION

S. aureus is a well-known human pathogen that is primarily associated with nosocomial infections in human causing variety of diseases with increasing resistance to β lactam antibiotics. MRSA strains have become a global cause of concern, because of their continuous increasing resistance nature against anti-staphylococcal drugs (Mulla et al., 2007). In India, the methicillin resistant *S. aureus* strain's importance as a problem has been recognized relatively late (Rajaduraipandi et al., 2006). In India, the MRSA prevalence is not uniform and it varies in different parts of the country. A Delhi hospital report of 2001 showed that the prevalence rate of MRSA was 51.6%, whereas in the same hospital, a study was conducted in 2008 where a prevalence rate of 38.44% reported (Tiwari et al., 2008). Dar et al. (2006) in their study at Aligarh, India reported 35.1% of *S. aureus* isolates resistant to methicillin. Rajaduraipandi et al. (2006) in Tamil Nadu reported that 31.1% of *S. aureus* isolates from clinical samples were methicillin resistant. Sangeeta et al. (2013) in their study reported that the prevalence rate of MRSA strains was 42% in 2008 and 40% in 2009. 27 MRSA and 33 MSSA strains were

Table 4. Antibiotic resistance patterns of 33 MSSA isolates.

No. of antibiotics	Resistance pattern	No. of resistant isolates	Percentage	MAR
2	PEN, AM0X	1	3.03	0.13
	PEN, NA	1	3.03	0.13
	ERY, AMP	1	3.03	0.13
3	PEN, CIP, AMP	1	3.03	0.2
	PEN, AMP, AMOX	1	3.03	0.2
	AMP, SZ, AMOX	1	3.03	0.2
4	PEN, PB, AMP, AMOX,	1	3.03	0.26
	PEN, AMP, AMOX, CPD	1	3.03	0.26
	PEN, AMP, OF, AMOX	1	3.03	0.26
	PEN, CIP, AMP, AMOX	1	3.03	0.26
	PEN, KAN, OF, CPD	1	3.03	0.26
	NA, AMP, AMOX, CPD	1	3.03	0.26
5	PB, CIP, AMP, AMOX, CPD	1	3.03	0.33
	PEN, PB , AMP, AMOX, CPD	1	3.03	0.33
	PEN, NA, TET, AMP, AMOX	1	3.03	0.33
	PEN, NA , AMP, SZ ,AMOX	1	3.03	0.33
	PEN, KAN, PB, AMP, OF, AMOX	1	3.03	0.4
6	PEN, KAN, PB, AMP, SZ, AMOX	1	3.03	0.4
	OX, PEN, NA, AMP, OF, AMOX	1	3.03	0.4
	PB, CIP, AMP, SZ, AMOX, CPD	1	3.03	0.4
	E, NA, AMP, SZ, AMOX, CPD	1	3.03	0.4
7	OX, PEN, NR, TET, AMP, SZ, AMOX	1	3.03	0.46
	PEN, NR, TET, PB, AMP, AMOX, CPD	1	3.03	0.46
	PEN, KAN, AMP, OF, SZ, AMOX, CPD	1	3.03	0.46
8	PEN, ERY, TET, CIP, AMP, SZ, AMOX, CPD	1	3.03	0.53
	PEN, ERY, KAN, NR, TET, AMP, SZ, AMOX	1	3.03	0.53
9	PEN, ERY, KAN, AMP, OF, SZ, AMOX, CPD	1	3.03	0.6
	PEN, ERY, NA, KAN, NR, AMP, OF, AMOX, CPD	1	3.03	0.6
	OX, PEN, TET, CIP, AMP, OF, SZ, AMOX, CPD	1	3.03	0.6
10	PEN, ERY, NA, TET, PB, CIP, AMP, SZ, , AMOX, CPD	1	3.03	0.66
	PEN, ERY, KAN, NR, TET, CIP, AMP, SZ, AMOX, CPD	1	3.03	0.66
11	OX, PEN, ERY, NA, NR, PB, AMP, OF, SZ, AMOX, CPD	1	3.03	0.73
	PEN, ERY, KAN, NR, TET, CIP, AMP, OF, SZ, AMOX, CPD	1	3.03	0.73

isolated from 60 clinical samples. In our study, a lower prevalence rate of MRSA (45%) was recorded as compared to MSSA (55%) from all the clinical specimens tested. Our findings in relation to MRSA prevalence rate are quite similar with the other studies conducted in India where higher prevalence rate was recorded ranging from 40 to 60% (Anupurba et al., 2003, Tiwari and Sen, 2006; Muralidharan, 2009; Arora et al., 2010). A study was conducted to find the prevalence rate of MRSA in Eastern Uttar Pradesh and AIIMS in New Delhi and the prevalence rate of MRSA was 54.85 and 44%, respectively (Anupurba et al., 2003; Arti et al., 2008). Maximum

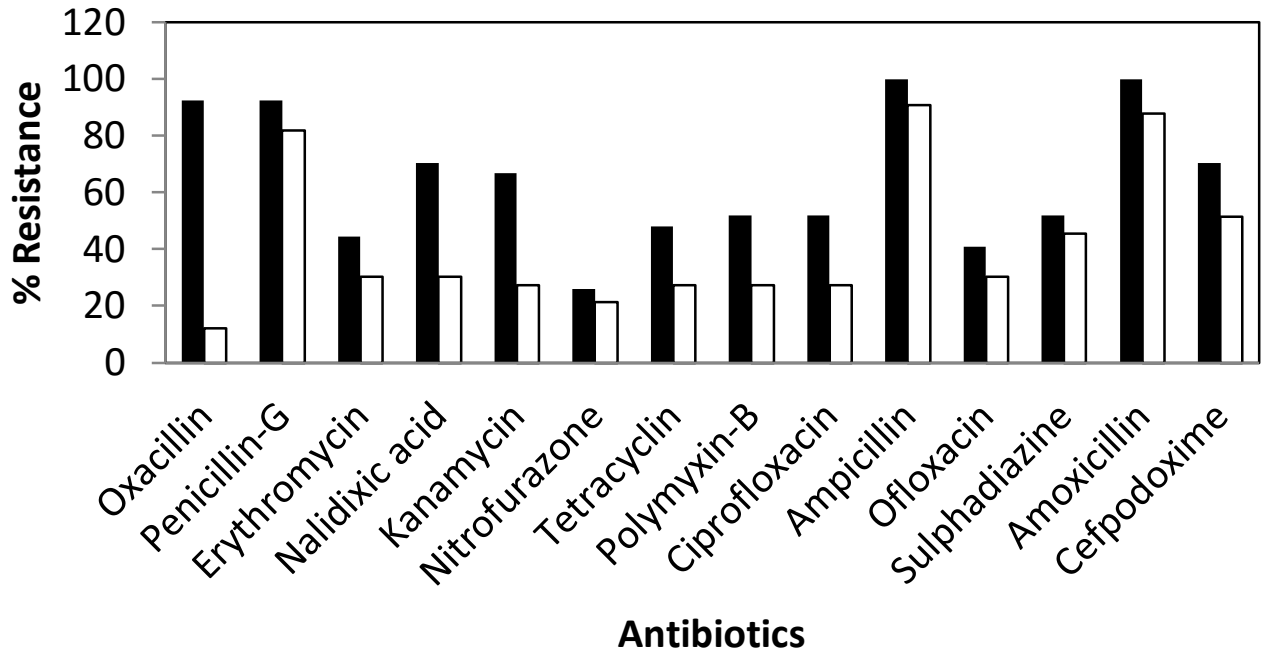

Figure 1. Antibiotic resistance profile of *S. aureus* (MRSA and MSSA) isolates.

frequency of the MRSA isolated were from the pus samples, that is, 5 out of 9 (55.55%) followed by wound swab (50%), urine (45.95%), ear swab and blood samples (25%) as shown in Table 1. This is similar with studies by Anupurba et al. (2003) and Vidya et al. (2010). Maximum incidence of MRSA was recorded from pus, reported by Tiwari et al. (2009) and also by Anupurba et al. (2003). Our result differs with other studies where wound swabs were the main source (Rajaduraipandi et al., 2006). In this study, resistance nature of MRSA to different antibiotics was more than MSSA strains. The present study shows high resistance to ampicillin, amoxicillin, oxacillin, and penicillin. This result is similar with other studies (Tiwari et al., 2009; Vidya et al., 2010). MSSA isolates show higher sensitivity than MRSA strains to oxacillin (7.41% vs. 87.88%), penicillin (7.41% vs. 18.19%), erythromycin (55.56% vs. 69.7%), nalidixic acid (29.63% vs. 69.7%), kanamycin (33.33% vs. 72.73%), tetracycline (51.85% vs. 72.73%), chloramphenicol and polymyxin B (48.15% vs. 72.73%) and sulphadiazine (48.15% vs. 54.55%). Resistance to (ciprofloxacin) was (51.85%) in the present study while a study conducted by Saikia et al. (2009), reported the resistant rate of *S. aureus* with ciprofloxacin to be 87.5%. But in 2003, a study was conducted by Kumari et al. (2008) who reported that 32.6% of the *S. aureus* strains were resistant to ciprofloxacin. There is a difference between antibiogram of MRSA and MSSA isolates. Maximum resistance was seen with ampicillin and amoxicillin (Figure 1). This result is similar with that of Shamsadh et al. (2011). The highest level of resistance of *S. aureus* strain has been observed with ampicillin (100%), amoxicillin (100%) and both penicillin and oxacillin (92.59%), which is in accordance with the reports of Tiwari et al. (2009). More than 70% resistance was also seen with nalidixic acid and cefpodoxime (70.37%). The high frequency of resistance observed with kanamycin (67.7%), nalidixic acid and cefpodoxime (70.37%), penicillin (92.59%), ampicillin and amoxicillin (100%), could be attributed to their use in treatment of diseases in animals and humans. Resistant bacteria may transfer resistance genes to other bacteria and become important in the spread of antibiotic resistance. Indiscriminate use of antimicrobial agents and antibiotic sale behavior (for example, sale of antibiotics without prescription, sale of under dose and substituting brands) enhances the development of drug resistance (Indalo et al., 1997). In our findings, all the MRSA isolates showed high percentage of multidrug resistance against 7 to 14 antibiotics in 25 different patterns of combination (Tables 2 and 3). Similar trend of resistance was observed in MSSA isolates (Tables 2 and 4). A study was conducted by Majumder et al. (2001) from Assam which reported 23.2% of the MRSA isolated from clinical samples were found to be multidrug resistant. Higher percentage of multidrug resistant MRSA was also reported by Anupurba (Assadullah et al., 2003). Vidhani et al. (2001) in their study also reported a higher percentage of MRSA isolated from high risk patients admitted in burns and orthopedic units. So, the awareness should be created about the route of MRSA transmission in the hospital environment and the risk of its infections in the community.

CONCLUSION AND RECOMMENDATION

A high rate of MRSA prevalence in various clinical specimens and their multidrug resistance response was recorded against a variety of commonly used effective antibiotics. This rapid transmission and alarming increase of drug resistance in MRSA require a development of new antimicrobial drugs and their regulatory and safe use for future treatment in MRSA caused infections. Our findings may also help to explore the ways to mitigate the excess and unnecessary use of antibiotics and to manage the effective combinations of antibiotics to treat the MRSA infections.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Akerele J, Ahonkhai IA (2000). Urinary pathogen and antibacterial susceptibility. A retrospective study of private diagnostic laboratory in Benin City, Nigeria. J. Med. Lab. Sci. 9:47-48.

Anupurba S, Sen MR, Nath G, Sharma BM, Gulati AK, Mohapatra TM (2003). Prevalence of MRSA at a tertiary care Referral hospital in East Uttar Pradesh. Indian J. Med. Microbiol. 21:49-51.

Arora S, Devi P, Arora U and Devi V (2010). Prevalence of methicillin resistance *Staphylococcus aureus* in a tertiary care hospital in Northern India. J. Lab. Physicians 2(2):78-81.

Assadullah S, Kakru DK, Thoker MA, Bhat FA, Hussain N, Shah A (2003). Emergence of low level vancomycin resistance in MRSA. Indian J. Med. Microbiol. 21:196-198.

Arti T, Arti K, Padma S (2008). Incidence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in Pus Samples at a tertiary Care Hospital, AIIMS, New Delhi. J. Indian Acad. Clin. Med. 9(1):33-35.

Bauer AW, Kirby WMN, Sherris JC (1966). Antibiotic susceptibility testing by standardized single disc method. Am. J. Clin. Pathol. 45:493-496.

Bell JM, Turnidge JD, Sentry Apac participants (2002). High prevalence of oxacillin – resistant *Staphylococcus aureus*isolates from hospitalized patients in Asia – pacific and South Africa results from SENTRY. Antimicrobial surveillance program, 1998-1999. Antimicrob. Agents Chemother. 46:897-881.

Capitano B, Leshem OA, Nightingale CH, Nicolau DP (2003). Cost effect of managing methicillin-resistant mortality associated with Methicillin resistant and methicillin susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. Clin. Infect. Dis. 36:53-59.

Chamber HF (1979). Methicillin resistance in Staphylococci: molecular and biochemical basis and clinical implication. Clin. Microbiol. Rev. 10:781-791.

Chambers HF (2001). The changing epidemiology *Staphylococcus aureus*. Emerg. Infect. Dis. 7:178-182.

Dar JA, Manzoor AT, Jamal AK, Asif A, Mohammed AK, Mohammed R, Khalid HB, Mohammad JD, Niyaz A, Shamim A (2006). Molecular epidemiology of clinical and carrier strains of methicillin resistant *Staphylococcus aureus* (MRSA) in the hospital settings of north India. Ann. Clin. Microbiol. Antimicrob. 5(1):22.

Diekema DJ, Pfaller M A, Schmitz FJ, Smayevsky J, Bell J, Jones RN, Beach M (2001). Survey of infections due to *Staphylococcus species*: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe and the Western pacific region for the SENTRY antimicrobial surveillance program, (1997-1999). Clin. Infect. Dis. 32:S114-S132.

Downing T, Imamura H, Decuypere S, Clark TG, Coombs GH, Cotton JA, Hilley JD, de Doncker S, Maes I, Mottram JC, Quail MA, Rijal S, Sanders M, Schönian G, Stark O, Sundar S, Vanaerschot M, Hertz-Fowler C, Dujardin JC, Berriman M (2011). Whole genome sequencing of multiple *Leishmaniadonovani* clinical isolates provides insights into population structure and mechanisms of drug resistance. Genome Res. 21:2143-215.

Duguid JP, Marmion BP, Swain RHA (1978). *Staphylococcus*: in Mackie and McCartney Medical Microbiology 13th Ed. Longman group Ltd. U.K. pp. 236-244.

Gopalakrishnan R, Sureshkumar D (2010). Changing trends in antimicrobial susceptibility and hospital acquired infections over an 8 year period in a tertiary care hospital in relation to introduction of an infection control programme. J. Assoc. Physicians India 58:25-31.

Guignard B, Entenza JM, Moreillon P (2005). Beta-lactams against methicillin-resistant *Staphylococcus aureus.* Curr. Opin. Pharmacol. 5:479-489.

Indalo AA (1997). Antibiotic sale behaviour in Nairobi, a contributing factor to antimicrobial drug resistance. East Afr. Med. J. 74:171-173.

Kolawole DO, Shittu AO (1997). Unusual recovery of animal staphylococci from septic wounds of hospital patients in Nigeria. Lett. Appl. Microbiol. 24:87-90.

Kumari N, Mohapatra TM, Singh YI (2008). Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in a Tertiary-Care Hospital in Eastern Nepal. J. Nepal Med. Assoc. 47(170):53-56.

Layton MC, Hierholzer WJ, Patterson JE (1995). The evolving epidemiology of methicillin-resistant *Staphylococcus aureus* at a university hospital. Infect. Control Hosp. Epidemiol. 16:12-17.

Llarrull LI, Fisher JF, Mobashery S (2009). Molecular Basis and Phenotype of Methicillin resistance in *Staphylococcus aureus* and Insights into New β-Lactams That Meet the Challenge. Antimicrob. Agents Chemother. 53(10):4051-4063.

Majumder D, Bordoloi JN, Phukan AC, Mahanta J (2001). Antimicrobial susceptibility pattern among methicillin resistant *Staphylococcus* isolates in Assam. Indian J Med Microbiol.19:138-40.

Mathur SK, Singal S, Prasad KN, Kishore J, Ayyagiri A (1994). Prevalence of MRSA in tertiary care hospital. Indian J. Med. Microbiol. 12:96-101.

Mulla S, Patel M, Shah L, Vaghela G (2007). Study of antibiotic sensitivity pattern of methicillin-resistant *Staphylococcus aureus*. Indian J. Crit. Care Med. 11(2):99-101.

Muralidharan S (2009). Special article on methicillin resistant *Staphylococcus aureus*. J. Acad. Clin. Microbiol. 11:15-16.

National Committee for Clinical Laboratory Standards (NCCLS) (2002). Performance Standards for antimicrobial susceptibility testing. 8th Informational Supplement. M100S12. Villanova, Pa.

Nimmo GR, Schooneveldt J, O'Kane G, McCall B, Vickery A. (2000). Community acquisition of Gentamicin-sensitive Methicillin Resistant *Staphylococcus aureus* in Southeast Queensland, Austr. J. Clin. Microbiol. 38(11):3926-3931

Pai V, Rao VI, Rao SP (2010). Prevalence and antimicrobial susceptibility pattern of Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates at a tertiary care hospital in Mangalore, South Indian. J. Lab. Physicians 2(2):82-84.

Patel AK, Patel KK, Patel KR, Shah S, Dileep P (2010). Time trends in the epidemiology of microbial infections at a tertiary care center in

west India over last 5 years. J. Assoc. Physicians India 58:37-40.
CLSI (Clinical and Laboratory Standards Institute) (2010). Performance Standards for Antimicrobial Susceptibility Testing; 20th Informational Supplement, Clinical and Laboratory Standards Institute Wayne, PA: M100-S20: 30(1).
Rajaduraipandi K, Mani KR, Panneerselvam KM, Bhaskar M, Manikandan P (2006). Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus*: a multicentre study. Indian J. Med. Microbiol. 24:34-38.
Riaz S, Faisal M, Hasnain S (2011). Antibiotic susceptibility pattern and multiple antibiotic resistances (MAR) calculation of extended spectrum β- lactamase (ESBL) producing *Escherichia coli* and *Klebsiella* species in Pakistan. Afr. J. Biotechnol. 10(33):6325-6331.
Saikia L, Nath R Choudhary B, Sarkar M (2009). Prevalence and antimicrobial susceptibility pattern of methicillinresistant *Staphylococcus aureus* in Assam. Indian J. Crit. Care Med. 13:156-8.
Salgado CD, Farr BM, Calfee DP (2003). Community acquired methicillin resistant *Staphylococcus aureus*: A meta-analysis of prevalence and risk factors. Clin. Infect. Dis. 36(2):131-139.
Sangeeta J, Pallab R, Vikas M, Bajaj J, Chitnis DS, Gautam V(2013). Methicillin resistant *Staphylococcus aureus* (MRSA) in India: Prevalence & susceptibility pattern. Indian J. Med. Res. 137(2):363-369.
Shamsadh B, Anbumani N, Kalyani J, Mallika M (2011). Prevalence and antimicrobial susceptibility pattern of coagulase-negative Staphylococcus. Int. J. Med. Public Health 1(4):59-62.
Tiwari HK, Sen MR (2006). Emergence of vancomycin resistant *Staphylococcus aureus* (VRSA) from a tertiary care hospital from northern part of India. BMC Infect. Dis. 6:156.
Tiwari HK, Das AK, Sapkota D, Sivarajan K, Pahwa VK (2009). Methicillin resistant *Staphylococcus aureus*: Prevalence and antibiogramin a tertiary care hospital in western Nepal. J. Infect. Dev. Ctries. 3:681-684.
Tiwari HK, Sapkota D, Sen MR (2008). High prevalence of multidrug-resistant MRSA in a tertiary care hospital of northern India. Infect. Drug Resist. 1:57-61.
Tyagi A, Kapil A, Singh P (2008). Incidence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in Pus Samples at a tertiary Care Hospital, AIIMS, New Delhi. J. Indian Acad. Clin. Med. 9:33-35.
Verma S, Joshi S, Chitnis V, Hemwani N, Chitnis D (2000). Growing problem of methicillin resistant staphylococci - Indian scenario. Indian J. Med. Sci. 54:535-540.
Vidhani S, Mendiratta PL, Mathur MD (2001). Study of methicillin resistant *S. aureus* isolates from high risk patients. Indian J. Med Microbiol. 19(2):13-16.
Washington CW Jr, Stephen DA, William MJ, Elmer WK, Paul CS, Gary WP, Gail LW (2006). Gram positive cocci. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th edition. Philadelphia: Lippincott Williams & Williams 12:623-671.
Weems JJ (2001). The many faces of *Staphylococcus aureus* infections. Postgrad. Med. 110(4):24-36.