Surgical Site Infection by Methicillin Resistant *Staphylococcus aureus*– on Decline?

SUSMITA BHATTACHARYA[1], KUHU PAL[2], SONIA JAIN[3], SHIV SEKHAR CHATTERJEE[4], JAYASHREE KONAR[5]

ABSTRACT

Introduction: Surgical Site Infection (SSI) is the most common healthcare associated infection that could be averted by antibiotics prophylaxis against the probable offending organisms. As *Staphylococcus aureus* has been playing a substantial role in the aetiology of SSIs, Methicillin Resistant *Staphylococcus aureus* (MRSA) happens to be a problem while dealing with the postoperative wound infection.

Aim: To determine the prevalence of SSI caused by MRSA and the antibiotic sensitivity pattern of MRSA.

Materials and Methods: A cross-sectional study was conducted at Nil Ratan Sircar Medical College, Kolkata, West Bengal from July 2009 to December 2012. A total of 19,359 surgical procedures were done of which 3003 culture positive SSIs have been documented. The clinical samples were collected from patients of both sexes and all ages suspected to be suffering from SSI from different specialities. Samples were processed according to CLSI, 2007 guidelines. The isolated strains of *Staphylococcus aureus* were screened for MRSA by detection of resistance to Cefoxitin disc (zone of inhibition was ≤21 mm) and slidex staph latex agglutination tests were done on cefoxitin resistant strains to spot phenotypic expression of *mec A* gene. Then PCR was performed for detection of *mecA* gene. Antibiotic sensitivity test was done following Kirby Bauer technique.

Results: In this 3½ year study, 1049 *Staphylococcus aureus* (34.93%) were reported from 3003 cases of SSI followed by *Escherichia coli* (20.34%), *Klebsiella* spp. (18.08%), *Pseudomonas* spp. (7.99%), *Acinetobacter* spp. (7.49%) respectively. Among the *Staphylococcus aureus,* 267 strains were derived as MRSA (25.45%). MRSA were isolated from 167 (62.54%) male patients and 100 (37.45%) female patients having surgical site infections. Inpatients and outpatients distribution of MRSA were 235 (88.01%) and 32 (11.98%) respectively. Majority of the MRSA cases were reported from Surgery (12.49%) and Orthopaedics (11.85%) departments in the age group above 75 years (15.63%). The MRSA strains have been found to be 100% sensitive to linezolid and tigecycline followed by fucidin (92.51%), mupirocin (88.39%), levofloxacin (75.66%) and doxycycline (72.28%). No vancomycin resistant strains were detected, but 3 strains (1.12%) were found to be intermediately susceptible to it (VISA). Incidence of MRSA in SSI has been decreased by 15.17 % in 2012 in comparison to 2009. PCR revealed *mecA* gene was present in 96.25% of cefoxitin resistant *Staphylococcus aureus* strains.

Conclusion: *Staphylococcus aureus* being the predominant organism causing SSIs, MRSA needs the attention for its resistance to commonly used antibiotics in the hospital like penicillin, cephalosporin group of drugs. Regular monitoring of the MRSA, involved in the SSI of a particular setup is the basic requirement to trim down the incidence of the postoperative wound infections by proper antibiotic prophylaxis.

INTRODUCTION

Surgical intervention may be coupled with surgical site infection (SSI) as reported in 5% of the surgical procedures [1]. SSI may extend from easily manageable state to serious life threatening condition [1]. It still remains one of the most important healthcare associated infections causing pain, prolonged hospital stay with increased expenditure, cosmetically unacceptable scars, thus, leading to the miserable condition of the patients [2]. This entire sequel could be prevented by systemic antibiotic prophylaxis as the bacteria implicated in SSIs include those, which are conceded by the patients themselves (endogenous flora), or those that might be instigated in the operating room (exogenous flora) [3]. Infection caused by microorganisms from an external source following surgery is less frequent than the endogenous one [4].

Staphylococcus aureus is one of the most common microorganisms involved in SSI. It accounts for 20% of SSI in general hospitals [5]. A combination of nasal colonization and immuno-evasive strategies of *S.aureus* prompted it to be a major pathogen responsible for healthcare associated infection [6,7].

After emergence of MRSA in the United Kingdom in 1961 [8], MRSA has become a hospital superbug throughout the world [9].

MRSA is now responsible for 30% or more of all serious infections and is always not very easy to deal with [10,11]. The prolonged stay in hospital, arbitrary use of antibiotics, lack of awareness, over the counter dispensing of antibiotics etc. are the potential predisposing factors for emergence of MRSA [11].

Methicillin resistance is due to the acquisition of *mecA* gene which encodes a unique penicillin-binding protein, designated PBP 2′ or PBP 2a. This reduces affinity for β-lactams and allows effective cell wall synthesis even in the presence of penicillins including anti-staphylococcal penicillins, as well as cephalosporins and carbapenems [12]. Therefore, the choice of drugs becomes limited to combat the MRSA strains.

The proportion of SSIs due to *S. aureus* increased from 16.6% to 30.9% during the period from 1992 to 2002, when the number of MRSA isolates also raised from 9.2% to 49.3% [13]. The 90 days postoperative mortality has been reported as 6.7% and 20.7% for SSI patients with methicillin susceptible *S. aureus* (MSSA) and MRSA, respectively [14]. So, rational and restricted use of antibiotic is the essence of the day to confine the upsurge of this deadly MRSA strains. However, there are reports confirming that prophylactic administration of antibiotics in preoperative period (<

60 minutes before incision) significantly reduce SSIs after clean as well as contaminated operations [1,15]. Selection of antibiotics should cover the organisms that are expected to be encountered in SSI, which may be guided by the local data available.

On this background the study was conducted to determine the prevalence of MRSA in SSI and also to determine antibiotic susceptibility pattern of MRSA strains that may ultimately help the infection control team to plan the preoperative antibiotic policy.

MATERIALS AND METHODS

A cross-sectional study was conducted for a period of $3\frac{1}{2}$ years (July, 2009 to December, 2012) at Nil Ratan Sircar Medical College, Kolkata, West Bengal after getting approval from the Institutional Ethics Committee.

Samples were taken from the cases of SSIs, selected according to the CDC, HICPAC guidelines, 1999 [5]. Any infection involving skin and subcutaneous tissue arising around the incision within 30 days of a clean surgery is defined as SSI. Discharges from stitch abscesses, infection of an episiotomy or neonatal circumcision site, infected burn wounds, incisional wound that extend into the fascial and muscle layers and contaminated and dirty surgeries were excluded from the study.

A total of 19,359 surgical procedures were done of which 3003 culture positive SSIs were noted. The clinical samples were collected from patients of both sexes and all ages (starting from one year of age – 6 groups were made taking subsequent 15 years in each group), suspected to be suffering from surgical site infection from different specialities like Surgery, Orthopaedics, Gynaecology & Obstetrics, Urology, Paediatric Surgery and Cardiovascular surgery. Samples were also collected from patients with infected wounds who underwent minor surgical procedures like tracheostomy, venesection, peritoneal dialysis etc. of different wards like Medicine, Paediatrics, Haematology and Chest. The area around the wound was cleaned with 70% ethyl alcohol followed by normal saline and exudates were collected from the wound with a sterile inoculating loop or with a sterile swab stick soaked in normal saline or sometimes by aspirating with a sterile syringe and needle.

The samples were inoculated on Blood agar, MacConkey's agar and Mannitol salt agar. The plates were incubated aerobically at 37°C overnight. If growth failed to appear, incubation was continued up to 48 hours. The colonies suggestive of Staphylococcus aureus were identified by standard procedures (Gram staining, catalase test, slide coagulase and tube coagulase test, phosphatase test etc.,) [16]. Tube coagulase was taken as the main criteria for identification of Staphylococcus aureus [16].

Antibiotic susceptibility testing was done by Kirby Bauer method [17] following Clinical and Laboratory Standards Institute (CLSI) guidelines [17] using commercially available cefoxitin (30μg) disc (HiMedia) and the results were compared with Staphylococcus aureus ATCC 25923 and MRSA ATCC 43300 control strains. The other antibiotic discs used were vancomycin (30 μg), oxacillin (1μg), ciprofloxacin (5μg), netilmycin (30 μg), linezolid (30μg), gentamycin (10 μg), clarithromycin (15μg), erythromycin (15 μg), levofloxacin (5μg), clindamycin (2μg), fucidin (10 μg), mupirocin (5 μg), co-trimoxazole (25 μg) and azithromycin (15 μg). All Staphylococcus aureus strains were screened for MRSA by detection of resistance to Cefoxitin disc (zone of inhibition was ≤21 mm) following the CLSI guidelines [17]. Cefoxitin resistant strains were further subjected to Slidex staph latex agglutination tests (BioMerieux) to determine the phenotypic expression of mecA gene [18,19]. PCR was done to detect mecA for genotyping [20]. DNA extraction was done on all the isolates as described in Phenol-Chloroform Method with minor modification [21,22]. The DNA fragments of 606 bp were amplified from mecA gene using specific primers, mecA F 5'AGTTGTAGTTGTCGGGTTT3', mecA R

5'AGTGGAACGAAGGTATCATC3' [20,23]. The conditions for PCR were denaturation at 94°C for 5 minutes, followed by 34 cycles of an initial denaturation at 94°C for 1 min, annealing at 54°C for 1.5 mins, and extension at 72°C for 1 minute and final extension step at 72°C for 10 minutes [20]. The amplification was carried out in Thermal cycler (Biometra, Germany). The amplification product (10μl) was analysed by electrophoresis on 1% agarose gel and visualized with ultraviolet light after staining with ethidium bromide [22].

Among the MRSA strains, those found to be resistant to vancomycin (30 μg) by disc diffusion test (zone of inhibition≤ 14 mm) were further resorted to E test (BioMerieux) to determine the Minimum Inhibitory Concentration (MIC) for confirmation of their resistance status [24]. Isolates with a vancomycin MIC 4 to 8 μg ml−1 were identified as Vancomycin-intermediate Staphylococcus aureus (VISA), isolates with a vancomycin MIC >16 μg ml−1 were identified as Vancomycin-resistant Staphylococcus aureus (VRSA) [17].

Statistical analysis was done using the CDC Epi Info TM 7 Stat Calc software program. Chi square test/ Fisher Exact test were applied for comparison of categorical data. $p < 0.05$ was considered to be statistically significant.

RESULTS

In this 3½ year study, 15.51% SSIs have been documented. SSIs were predominantly due to Staphylococcus aureus (34.93%) followed by Escherichia coli (20.34%), Klebsiella spp. (18.08%), Pseudomonas spp. (7.99%), Acinetobacter spp. (7.49%), Enterococcus spp. (4.39%), Coagulase negative Staphylococcus spp (3.16%), Proteus spp. (1.73%), Citrobacter spp. (1.29%), Enterobacter spp. (0.26%), Providencia spp. (0.16%) and Morganella spp. (0.13%). Among the 1049 Staphylococcus aureus, 267 Methicillin resistant Staphylococcus aureus (MRSA) strains were observed. Prevalence of MRSA was (267 / 1049 x 100) 25.45%. MRSA were isolated from 167 (62.54%) male patients and 100 (37.45%) female patients having surgical site infection. Inpatients and outpatients distribution of MRSA were 235 (88.01%) and 32 (11.98%) respectively [Table/Fig-1]. As the age progresses there was more incidence of MRSA infecting the wounds which were statistically significant [Table/Fig-2].

While computing the distribution of MRSA in different specialities, Surgery and Orthopaedics departments accounted for most of the cases [Table/Fig-3].

*Doxycycline and tigecycline were not used in antibiogram of 73 children [Table/Fig-4].

#17 clinical isolates showed resistance to VA (vancomycin – 30 μg disc) by disc diffusion test. Of these, 3 were confirmed as VISA (1.12%) by the E-test showing an MIC range between 4-6 μg ml−1. For the other strains, vancomycin was ≤ 2 μg ml−1indicating Vancomycin sensitive strains. None of the isolates were resistant to Vancomycin by E test (MIC in the range of 16-64 μg ml−1).
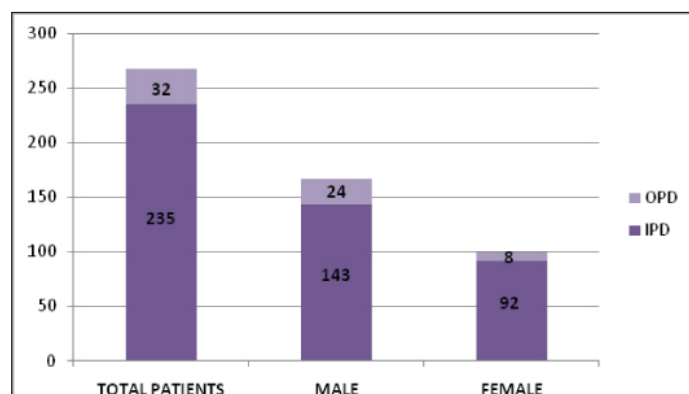
[Table/Fig-1]: Indoor and outdoor distribution of the male and female patients of SSI with MRSA.

Age distribution	MRSA (%) SSI	Non MRSA (%) SSI	Total SSI	
1-15 years	12(3.46)	335 (96.54)	347	Chi² = 23.346 with degree of freedom 5 and p-value <0.001
15-30 years	62(7.82)	731 (92.18)	793	
30-45 years	76(9.2)	750 (90.79)	826	
45-60 years	69(10.52)	587 (89.48)	656	
60-75 years	38(11.99)	279 (88.01)	317	
>75 years	10(15.63)	54 (84.37)	64	
Total	267(8.89)	2736(91.10)	3003	

[Table/Fig-2]: Age wise distribution of MRSA in SSI.

Departments	MRSA (%) SSI	Non MRSA (%) SSI	Total SSI	
Surgery	134 (12.49)	939 (87.51)	1073	Chi² = 51.072 with degree of freedom 5 and p-value <0.001
Orthopedics	57 (11.85)	424 (88.15)	481	
Gynaecology	36 (6.93)	483 (93.07)	519	
Urology	15 (3.67)	347 (96.33)	362	
Pediatrics surgery	05 (2.25)	217 (97.74)	222	
Others	20 (5.7)	326 (94.23)	346	
	267	2736	3003	

[Table/Fig-3]: Department wise distribution of MRSA in SSI.

Antibiotics	Strain Total (N)		Resistance n %		Sensitivity n %		Statistics
Clindamycin	MRSA	(267)	116	43.45%	151	56.55%	χ² 8.334 df1,p=0.004
	MSSA	(782)	261	33.38%	521	66.62%	
Cefoxitin	MRSA	(267)	267	100.00%	0	0.00 %	Not applicable
	MSSA	(782)	1	0.13%	781	99.87%	
Co-trimoxazole	MRSA	(267)	192	71.91%	75	28.09%	χ² 85.826 df 1, p=0.000
	MSSA	(782)	304	38.87%	478	61.13%	
Clarithromycin	MRSA	(267)	180	67.42%	87	32.58%	χ² 78.824 df 1, p=0.000
	MSSA	(782)	275	35.17%	507	64.83%	
Doxycycline*	MRSA	(267)	74	27.72%	193	72.28%	χ² 112.195 df 1,p=0.000
	MSSA	(709)	29	4.09%	680	95.95%	
Fucidin	MRSA	(267)	20	7.49%	247	92.51%	χ² 55.77 df 1, p=0.0001
	MSSA	(782)	0	0.00%	782	0.00%	
Gentamycin	MRSA	(267)	219	82.02%	48	17.98%	χ² 165.726 df 1, p=0.000
	MSSA	(782)	283	36.19%	499	63.81%	
Levofloxacin	MRSA	(267)	65	24.34%	202	75.66%	χ² 18.156 df 1, p=0.000
	MSSA	(782)	102	13.04%	680	86.96%	
Linezolid	MRSA	(267)	0	0.00%	267	100%	Not applicable
	MSSA	(782)	0	0.00%	782	100%	
Mupirocin	MRSA	(267)	31	11.61%	236	88.39%	χ² 89.554 df 1, p=0.000
	MSSA	(782)	0	0.00%	782	100%	
Netilmycin	MRSA	(267)	97	36.33%	170	63.67%	χ² 104.830 df 1, p=0.000
	MSSA	(782)	73	9.34%	709	90.66%	
Oxacillin	MRSA	(267)	246	92.13%	21	7.87%	χ² 848.125 df 1, p=0.000
	MSSA	(782)	18	2.30%	764	97.70%	
Tigecycline*	MRSA	(267)	0	0.00%	267	100%	Not Applicable
	MSSA	(709)	0	0.00%	709	100%	
Vancomycin.#	MRSA	(267)	3	1.12%	264	98.88%	χ² 5.312 df 1, p=0.021
	MSSA	(782)	0	0.00%	782	100%	

[Table/Fig-4]: Antibiotic resistance pattern of *Staphylococcus aureus* (MRSA and MSSA) isolated from SSI.
* Doxycycline and Tigecycline were not used in antibiogram of children less than 8 years of age. (73 children).
# 17 clinical isolates were resistant to vancomycin 30 µg disc by the disc diffusion test of which 3 strains were confirmed as VISA by E test.

The MRSA strains have been found to be 100% sensitive to linezolid and tigecycline in this study. Other highly sensitive drugs were fucidin (92.51%), mupirocin (88.39%), levofloxacin (75.66%) and doxycycline (72.28%) [Table/Fig-4].

The isolated MRSA strains showed high degree of resistance towards clarithromycin, cotrimoxazole and gentamycin in comparison to MSSA strains. Among the 782 cases of MSSA, one was found to be resistant to cefoxitin but sensitive to oxacillin. The Slidex Staph latex agglutination test was also found to be negative with this strain. Therefore, it was considered as Methicillin sensitive *Staphylococcus aureus* (MSSA).

A decreasing trend of Staphylococcal SSI and MRSA has been found in this study which is statistically significant (χ^2 = 16.282, df 3, p =0.001) [Table/Fig-5].

In 2009, rate of MRSA was 30.48% followed by 29.77% in 2010, 25.47% in 2011 and 15.31% in 2012 with an overall MRSA rate of 25.45%. The study revealed presence of *mecA* gene in 257 strains (96.25 %) and absent in 10 (3.75%) strains.
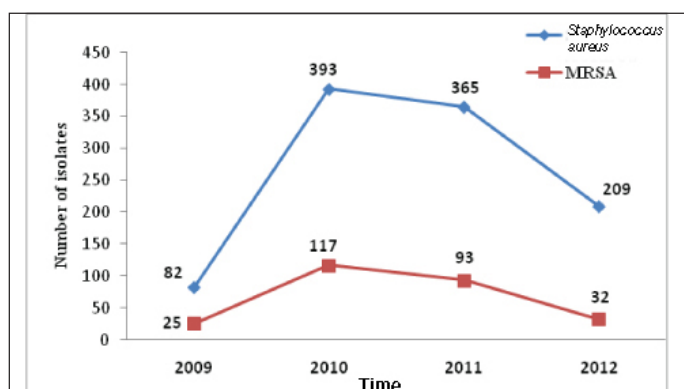
[Table/Fig-5]: Line chart showing decreasing trend of MRSA over the study period (2009-2012).

DISCUSSION

SSI is an important contributor of health care associated infections [25]. It is one of the preventable causes of nosocomial infections

[26]. Rate of SSIs in this study was 15.51% which was in tandem with a study in Uttarakhand (17.8%) [27]. But incidence of SSI varied from 2.5-41.9% [28]. Rate of SSI is an important indicator of quality of surgical procedures in a hospital and it is diverse in different set up.

Staphylococcus aureus comprised of more than 1/3rd of SSIs in this study, which was comparable to studies in Gwalior (34%) [29], Karnataka (31.3%) [4] and Uttarakhand (50.4%) [27]. Literature revealed that 80% of healthy individuals across the world harbour *Staphylococcus aureus* in their skin or anterior nares, and integrity of the skin if breached during any surgery could commonly cause skin and soft tissue infections with this organism [30]. All these factors have made up *S.aureus* as the most common organism causing SSIs [31]. Among the Gram negative organism causing SSI, *E.coli* and *Klebsiella* spp. were the major offenders followed by *Pseudomonas* spp. and *Acinetobacter* spp. in this study. *E.coli* was reported as the most common Gram negative organisms causing SSI in the studies from Uttarakhand [27] and Karnataka [4] also, but *Pseudomonas* spp. grew up as the second highest Gram negative organism responsible for SSIs in both the studies. Another study revealed *Pseudomonas* spp. (21%) as the most prevalent organism producing SSIs [29]. So there was a great variation among Gram negative organisms causing SSIs in different geographical areas and set up.

Among the isolated *Staphylococcus aureus* strains, 25.45% were MRSA which was quite similar to the studies done in Gwalior (27.96%) [29] and Karnataka (28.6%) [4]. Incidence of MRSA in SSI ranged from 15.7 % to 63.5% in studies conducted in India [27] and abroad [32]. A huge variation in incidence of MRSA might depend on pre & postoperative antibiotic policy and surveillance program prevailing in different set up. The incidence of MRSA in the male patients suffering from SSI has been found to be more than the female patients with the male female ratio of 1.67: 1 in this study. There were studies showing male predominance in SSI, though none commented on male: female distribution of MRSA in SSI [4,27].

In the present set up, most of the patients stayed for at least 5-7 days in the hospital during post- operative period in case of major surgeries and wound infection was reported before the patients were discharged from the hospital. Therefore, SSI with MRSA from indoor patients (88.01%) outnumbered the SSI with MRSA cases from outdoor in this study. But with the growing trend of same day surgery and lack of post discharge surveillance, actually contributed to the lesser number of MRSA infected SSIs in outdoor. Since a good number of SSIs might be apparent after discharge from the hospital, a possibility of under reporting could be the reason of paucity in the infected outpatient.

Distribution of SSI infected with MRSA was concentrated (72.28%) among patients aged more than 30 years in this study. Age wise distribution of MRSA cases was proved as statistically significant. Decreasing immunity, low healing power, amplified catabolic processes and existence of co-morbid illnesses, make the older age group more prone to SSIs [33]. But, there was a study reporting the maximum number of SSIs with *Staphylococcus*, in the age group of 21-40 years [29].

In this study, majority of SSIs with MRSA were from Surgery and Orthopaedics department followed by Obstetrics & Gynaecology which was comparable with a similar type of study in Karnataka [4]. This department wise distribution of MRSA cases was found to be significant.

Overall, MRSA strains were found to be more resistant than MSSA strains to all the antibiotics used and that was statistically significant except for vancomycin, linezolid and tigecycline. Almost similar results were observed in studies in Uttarakhand [27], Gwalior [29] and Karnataka [4] and in a multicentric study from India [34] where

vancomycin and linezolid showed 100% sensitivity to MRSA. But no data regarding use of tigecycline was documented in any of these studies. No vancomycin resistant *Staphylococcus aureus* (VRSA) was detected in this study but 3 (1.12%) isolates were stamped as VISA on the basis of MIC value in E-Test. In a study from Northern India, six (0.76%) VISA strains and two (0.25%) VRSA strains were reported [35]. But in a study from Congo, Africa 19% resistance was seen against vancomycin [32].

The increase in resistance to all group of antibiotics and reports of reduced susceptibility to the glycopeptides made it necessary to search for other alternative drugs for treatment of the MRSA strains [32,35,36]. Antibiogram was done using doxycycline (30µg) and tigecycline (15 µg) discs. They showed encouraging result with 100% sensitivity for tigecycline and 72.28% sensitivity to doxycycline. The sensitivity to tigecycline has been reported to be 100% against MRSA in other studies from India [37,38]. As they were not much used in clinical practice, these two drugs might act as important weapons against MRSA just like vancomycin and linezolid. In this study, newer antibiotics like quinupristin - dalfopristin and daptomycin were not used. Few reports from India had shown encouraging results with these antibiotics [39,40]. As more and more resistance is developing, future work can be directed by performing the in-vitro drug sensitivity testing with these drugs, against MRSA, in this part of India.

Slight fall in the rate of MRSA was noted from 2009 to 2010 (30.48% to 29.77%), followed by a significant fall in the rate during 2011 to 2012 (25.47% to 15.31%) with an overall prevalence of 25.45% during the study period. The fall in the prevalence from 2009-2010 to 2011-2012 was statistically significant (p-value-0.0028). Throughout the world where increasing incidence of MRSA is a threat to the health care system, this study reveals a paradoxical incidence. This has been possible because of effective control measures in the form of proper hand washing, barrier methods of nursing and use of appropriate disinfectants among the health care personnel attending to the patients undergoing surgery. Another important factor for the reduction of prevalence of MRSA is the heightened awareness among doctors and nursing staff about MRSA by thorough campaign, posters and seminars going on in this institute. Fall in prevalence was also observed in a study with strict enforcement of intervention strategy, following an outbreak of MRSA [41]. In our study *mecA* gene was present in 96.25% strains and absent in 3.75% strains. Similar report of *mecA* gene negative MRSA has also been reported by Kocazog et al., and Shorman et al., [42,43]. *mecA* gene negative MRSA was explained by β-lactamase hyperproduction by the isolates [42].

LIMITATION

1. Sensitivity of newer drugs like quinupristin - dalfupristin and daptomycin, against MRSA have not been demonstrated in this study.

2. Presence of *mecB* and *mecC* genes, in *mecA* negative MRSA strains have not been explored in this current research work.

CONCLUSION

Staphylococcus aureus played a predominant role in the aetiology of SSIs in this hospital, one fourth of which was due to MRSA strains. Mainstay of treatment for MRSA infections still depends on glycopeptides and linezolid, whereas doxycycline and tigecycline could be used as alternative drugs as revealed in this study. A decline in the rate of MRSA causing SSI in this study might be a ray of hope against the rising trend of this superbug. Compilation of local data on SSIs and feedback are the fundamental concern for the formulation of a proper guideline for peri-operative prophylaxis of antibiotics to mitigate the rate SSIs in the hospital. Post discharge surveillance must now be incorporated in the hospital policy to avoid under reporting of SSIs.

REFERENCES

[1] National Institute of Health and Clinical Excellence. Surgical site infection. Prevention and treatment of surgical site infection. Clinical Guideline 74. www.nice.org.uk/nicemedia/live/11743/42378/42378.pdf. Published October 2008. Accessed October 31, 2013.

[2] Bayat A, McGrouther DA, Ferguson MW. Skin scarring. *BMJ.* 2003;326(7380):88-92.

[3] World Health Organization. WHO Guidelines for Safe Surgery 2009: Safe Surgery Saves Lives. http://whqlibdoc.who.int/publications/2009/9789241598552_eng.pdf. Accessed October 31, 2013.

[4] Krishna S, Divya P, Shafiyabi S. Postoperative surgical wound infections with special reference to methicillin resistant *Staphylococcus aureus*: an experience from VIMS hospital, Ballari. *J Biosci Tech.* 2015;6(3):697-702.

[5] Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR. Guideline for prevention of surgical site infection. *American Journal of Infection Control.* 1999;27:97–134.

[6] Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clin. Microbiol.* 1997;10(3):505–20. PMC 172932. PMID 9227864.

[7] Cole AM, Tahk S, Oren A, Yoshioka D, Kim YH, Park A, et al. Determinants of *Staphylococcus aureus* nasal carriage. *Clin Diagn Lab Immunol.* 2001;8(6):1064–69.

[8] Jevons MP. Celbenin-resistant staphylococci. *Br Med J.* 1961;1:124-25.

[9] Timothi FJ. The *Staphylococcus aureus* "superbug". The Journal of Clinical Investigation. 2004;114:1693-96.

[10] Borriello Peter S, Murray R. Patrick, Funke Guido in Topley & Wilson's Microbiology and Microbial Infections, Bacteriology; Hodder Arnold. 2007; 10th ed. 2: 793 -4.

[11] Anupurba S, Sen MR, Nath G, Sharma BM, Gulati AK, Mohapatra TM. Prevalence of methicillin resistant *Staphylococcus aureus* in a Tertiary care Referral Hospital in Eastern UttarPradesh. *Indian J Med Microbiol.* 2003;21:49-51.

[12] Tsubakishita S, Kuwahara-Arai K, Sasaki T, et al. Origin and molecular evolution of the determinant of methicillin resistance in Staphylococci. *Antimicrob Agents Chemother.* 2010;54:4352-59.

[13] Jernigan J. Is the burden of *Staphylococcus aureus* among patients with surgical-site infections growing? *Infect Control Hosp Epidemiol.* 2004;25:457–60.

[14] Engemann JJ, Carmeli Y, Cosgrove SE, Fowler VG, Bronstein MZ, et al. Adverse clinical and economic outcomes attributable to methicillin resistance among patients with *Staphylococcus aureus* surgical site infection. *Clin Infect Dis.* 2003;36:592–98.

[15] Alexander JW, Solomkin JS, Edwards MJ. Updated recommendations for control of surgical site infections. *Ann Surg.* 2011;253(6):1082-93.

[16] Winn W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, et al. Eds. In: Koneman's Colour Atlas and TextBook of Diagnostic Microbiology. 6th ed. Lippincott, Williams and Wilkins; 2006:643-648.

[17] Performance standards for antimicrobial susceptibility testing; 17th informational supplement. CLSI M100-S17. CLSI 2007, Wayne, PA.27(1)

[18] Nakatomi Y, Sugiyama J. A rapid latex agglutination assay for the detection of penicillin-binding protein 2'. Microbiol Immunol. 1998;42:739–43.

[19] Griethuysen AV, Pouw M, Leeuwen NV, Heck M, Willemse P, Buiting A, et al. Rapid slide latex agglutination test for detection of methicillin resistance in *staphylococcus aureus. J Clin Microbiol.* 1999;37:2789–92.

[20] Khan AU, Sultan A, Tyagi A, Zahoor S, Akram M, Kaur S. Amplification of *mecA* gene in multi-drug resistant *staphylococcus aureus* strains from hospital personnel. *J Infect Dev Ctries.* 2007;1:289-95.

[21] Sambrook J, Russell DW. Commonly used Techniques in Molecular Cloning, Molecular Cloning, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 2001. Volume 3, 3rd edition, Appendix 8.

[22] Japoni A, Alborzi AV, Rasouli M, Pourabbas B. Modified DNA Extraction for Rapid PCR detection of methicillin-resistant staphylococci Iranian Biomedical Journal. 2004;8(3):161-65.

[23] Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Identification of Methicillin-resistant Strains of Staphylococci by polymerase chain reaction. J Clin Microbiol. 1991;29:2240-44.

[24] Etest application guide 16273B - en - 2012/07, Biomerieux SA, Chemin de l'Orme 69280 Marcy-l'Etoile– France

[25] Health care associated infections FACT SHEET – WHO. www.who.int/gpsc/country_work/gpsc_ccisc_fact_sheet_en.pdf.

[26] Spruce L. Back to Basics: Preventing Surgical Site Infections. *AORN J.* 2014;99(5):600–11.

[27] Negi V, Pal S, Juyal D, Sharma MK, Sharma N. Bacteriological profile of surgical site infections and their antibiogram: a study from resource constrained rural setting of uttarakhand state, india. *J Clin Diagn Res.* 2015;9(10):17-20.

[28] Malik S, Gupta A, Singh PK, Agarwal J, Singh M. Antibiogram of aerobic bacterial isolates from post- operative wound infections at a tertiary care hospital in India. *Journal of Infectious Diseases Antimicrobial Agents.* 2011;28:45-51.

[29] Ranjan KP, Ranjan N, Gandhi S. Surgical site infections with special reference to methicillin resistant *Staphylococcus aureus*: experience from a tertiary care referral hospital in North India. *Int J Res Med Sci.* 2013;1(2):108-11.

[30] David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clin Microbiol Rev.* 2010;23:616-87.

[31] Reichman DE, Greenberg JA. Reducing Surgical Site Infections: A Review. *Rev Obstet Gynecol.* 2009;2(4):212–21.

[32] Iyamba JM, Wambale JM, Lukukula CM, zaBalegaTakaisi-Kikuni N. High prevalence of methicillin resistant staphylococci strains isolated from surgical site infections in Kinshasa. *Pan Afr Med J.* 2014;18:322.

[33] Khan AKA, Rashed MR, Banu G. A Study on the Usage Pattern of antimicrobial agents for the prevention of surgical site infections in a tertiary care teaching hospital. *J Clin Diagn Res.* 2013;7(4):671-74.

[34] Joshi S, Ray P, Manchanda V, Bajaj J, Chitnis DS, Gautam V, et al. Methicillin resistant *Staphylococcus aureus* (MRSA) in India: Prevalence & susceptibility pattern. *Indian J Med Res.* 2013;137:363-69.

[35] Tiwari HK, Sen MR. Emergence of Vancomycin resistant *Staphylococcus aureus* (VRSA) from a tertiary care hospital from northern part of India. *Infect Dis.* 2006;6:156.

[36] Hiramatsu K, Katayama Y, Matsuo M, Sasaki T, Morimoto Y, Sekiguchi A, et al. Multi-drug-resistant *Staphylococcus aureus* and future chemotherapy. *Journal of Infection and Chemotherapy.* 2014;20(10):593–601.

[37] Mane M, Gangurde N. In vitro activity of Tigecycline against Methicillin resistant *Staphylococcus aureus* (MRSA) and Vancomycin resistant enterococci (VRE) as evaluated by disc diffusion method and E test. *International Journal of Collaborative Research on Internal Medicine and Public Health.* 2013;5(8):567-74.

[38] Tellis R, Rao S, Lobo A. An invitro study of Tigecycline susceptibility among multidrug resistant bacteria in a tertiary care hospital. *International Journal of Biomedical Research IJBR.* 2012;3(4):192-95.

[39] Kali A, Stephen S, Umadevi S, Kumar S. Detection of Quinupristin-Dalfopriston in methicillin resistant *Staphylococcus aureus* in South India. *Indian Journal of Pathology and Microbiology.* 2013;56(1):73-4.

[40] Kaur R, Gautam V, Ray P, Singh G, Singhal L, and Tiwari R. Daptomycin susceptibility of methicillin resistant *Staphylococcus aureus* (MRSA). *Indian J Med Res.* 2012;136(4):676–77.

[41] Bhattacharya S, Pal K, Barua JK, Jain S, Kundu PK, Niyogi SK. Outbreak of methicillin resistant *staphylococcus aureus* in neonatal intensive care unit in a tertiary care hospital in kolkata. *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS).* 2014;13(4):63-7.

[42] Kocazog S, Unal S. Practical use of PCR for rapid detection of methicillin resistance among Staphylococcal clinical isolates from Turkish Hospital. *J Clin Microbiol.* 1997;35:2188-89.

[43] Shorman MA, Atoom NM, Abuharfeil NM, Al Majali AM. Identification of methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin resistant coagulase-negative *Staphylococcus* (CoNS) in clinical settings. *American Journal of Infectious Diseases.* 2008;4(2):151-61.

PARTICULARS OF CONTRIBUTORS:
1. Professor, Department of Microbiology, NRS Medical College, Kolkata, West Bengal, India.
2. Associate Professor, Department of Microbiology, College of Medicine and JNM Hospital, Kalyani, Nadia, West Bengal, India.
3. PhD Student, Department of Microbiology, Medical College, Kolkata, West Bengal, India.
4. Assistant Professor, Department of Microbiology, NRS Medical College, Kolkata, West Bengal, India.
5. Demonstrator, Department of Microbiology, NRS Medical College, Kolkata, West Bengal, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Kuhu Pal,
Associate Professor, Department of Microbiology, College of Medicine and JNM Hospital, WBUHS,
Kalyani, Nadia-741235, West Bengal, India.
E-mail: kuhupal18@gmail.com