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THE STUDY OF RISK FACTORS AND ANTIBIOTIC RESISTANCE PROFILE OF SURGICAL SITE INFECTIONS CAUSED BY *STAPHYLOCOCCUS AUREUS* WITH SPECIAL REFERENCE TO METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN A TERTIARY CARE HOSPITAL IN CENTRAL INDIA.

Dr. Swati Satish Kale*

Asst. Professor, Department of Microbiology, Government Medical College, Nagpur.

***Corresponding Author: Dr. Swati Satish Kale**
Asst. Professor, Department of Microbiology, Government Medical College, Nagpur.

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ABSTRACT
Background: Surgical site infection (SSI) is one of the most common and serious complications following surgery. The incidence of the infected surgical wounds may be influenced by factors such as pre-operative care, the theatre environment, post-operative care and the type of surgery. *S.aureus* is the commonest cause of SSI and other nosocomial infections. The incidence of Methicillin Resistant *S.aureus* (MRSA) in various studies from India ranges from 30-70%. **Material and methods:** *Staphylococcus aureus* isolates obtained from various pus and wound swabs of surgical site infection patients received in Microbiology Diagnostic Laboratory at Government Medical College, Nagpur for the microbiological investigations were selected for the study. All samples were inoculated on Sheep blood agar and MacConkey's medium and their antibiotic susceptibility was performed. Also the risk factors for SSI were determined accordingly. **Results:** A total of 158 pus samples were included in the study. Out of these, 48 *Staphylococcus aureus* clinical isolates were isolated from specimens of patients with SSI (30%).Out of these isolates, 28(58.33%) were MRSA and rest of them were MSSA(Methicillin Sensitive *S.aureus*). **Conclusion:** Due to the increased morbidity and mortality which are associated with *S.aureus*, an early detection and intervention is a prerequisite in surgical patients. Infection control guidelines for SSIs play a key role in reducing such infections.

KEYWORDS: Surgical site infection (SSI), *Staphylococcus aureus*, Methicillin Resistant *S.aureus* (MRSA).

INTRODUCTION

Surgical site infection (SSI) is one of the most common and serious complications following surgery. The occurrence of SSI varies according to the type of operation, wound cleanliness, and the operative field.[1-3]

Surgical infections which are acquired in the hospital, are recognized to be associated with an extended length of hospital stay, pain, discomfort and sometimes prolonged or permanent disability. These infections are usually caused by the exogenous and endogenous microorganisms that enter the operative wound during the course of the surgery.[1]

The incidence of the infected surgical wounds may be influenced by factors such as pre-operative care, the theatre environment, post operative care and the type of surgery. *S.aureus* is the commonest cause of SSI and other nosocomial infections.

S.aureus was once susceptible to Penicillin but widely resistant organisms soon emerged. The introduction of Methicillin initially solved the problem, but later, strains which were resistant to Methicillin developed. Thus, an increased number of resistant strains have been seen worldwide.[2,3] The clinical significance of Methicillin resistance further rises due to development of other resistance mechanism such as vancomycin resistance, vancomycin Intermediate and glycopeptide intermediate *Staphylococcus aureus.* The incidence of methicillin resistant *S.aureus* (MRSA) in various studies from India ranges from 30-70%.[4,5]

Therefore our study was done to determine the prevalence of *S.aureus* and Methicillin-Resistant *S.aureus* (MRSA) in surgical sites.

AIM AND OBJECTIVES

1) To study the prevalence of surgical site infection (SSI) caused by. *S.aureus* in Government Medical College, Nagpur during from January 2015 to October 2017 in the Microbiology Diagnostic Laboratory of Government Medical College, Nagpur.

2) To study the prevalence of MRSA in SSI caused by. *S.aureus.*
3) To study the risk factors causing SSI caused by. *S.aureus.*.
4) To study the antimicrobial susceptibility pattern of *S.aureus* causing surgical site infection (SSI).
5) To study the antimicrobial susceptibility pattern of MRSA Methicillin Resistant *Staphylococcus aureus* causing Surgical site infection (SSI).

MATERIAL AND METHODS

A hospital based cross sectional study was carried out from January 2015 to October 2017 in the microbiology diagnostic laboratory of Government Medical College, Nagpur. The study was approved by the Institutional Ethical Committee.

Staphylococcus aureus isolates obtained from various pus and wound swabs of surgical site infection patients received in Microbiology Diagnostic Laboratory at Government Medical College, Nagpur for the microbiological investigations were selected for the study.

The quality control and rejection criteria for the in-appropriate specimen were followed as per the standard guidelines.[6]

Specimens were processed within 2 hours of collection by the standard microbiological techniques.[6]

Sheep blood agar and MacConkey's medium were used for inoculation of all specimens. The plates were then incubated at 35±2^0 Celsius for 18-24 hours in aerobic atmosphere.[6]

Gram positive cocci uniform in size, appearing characteristically in groups mostly, but also seen singly and in pairs were further identified by the scheme described for the identification of the gram positive cocci arranged in clusters using following tests like catalase test, modified oxidase test, furazolidone susceptibility test, coagulase test (slide and tube coagulase), mannitol sugar fermentation test.[6]

Antibiotic discs

Commercially available antibiotic discs (Hi-media laboratories Pvt. Ltd. Mumbai) with proper diameter and potency were used. All the strains were tested for their sensitivity to antimicrobial drugs using recommended CLSI guidelines (2017) combined with institutional antibiotic policy and hospital formulary practices for the purpose of reporting to the clinician.[7]

MRSA detection

In this study, MRSA detection was performed by cefoxitin disk diffusion method.

Cefoxitin disk diffusion testing

All the *S. aureus* isolates were subjected to cefoxitin disk diffusion test using a 30 µg disk. A 0.5 McFarland standard suspension of the isolate was prepared and lawn culture done on Mueller–Hinton agar plates with 4% NaCl.

Plates were incubated at 37^0 C for 18 hour and zone diameters were measured.[8,9]

Quality Control: *Staphylococcus aureus* ATCC 25923 (methicillin susceptible) and *Staphylococcus aureus* ATCC 43300 (methicillin resistant) were used as the control strains.

Interpretive Criteria (in mm) for Cefoxitin Disk Diffusion Test		
	Susceptible	Resistant
Staph. aureus	≥ 22	≤ 21

Surgical site infection criteria

1) Inclusion criteria

All the patients having history of any operation with history of Diabetes or peripheral vascular disease (PVD), presenting with surgical wound, having pus or serous or seropurulent discharge with or without sign of sepsis was considered as surgical site infections (SSI's).[10]

2) Exclusion criteria

Wound with cellulitis and no discharge were not included in the study.

Statistical analysis: The statistical analysis was performed by using the Chi-square test and a *p* value of less than 0.05 was considered as statistically significant.

Definition of SSI

SSI was defined as any episode of clinical symptoms of infection following surgery, or when SSI was diagnosed by the surgeon. Antibiotic use and surgical removal of an implant were at the surgeon's discretion. Early and late SSI were defined by whether the onset of symptoms occurred within 30 days or more than 30 days after surgery, respectively.

The various co-morbidities (diabetes and hypertension etc) of patients diagnosed with SSI were assessed accordingly.

RESULTS

A total of 158 pus samples were included in the study.

Out of these, 48 *Staphylococcus aureus* clinical isolates were isolated from specimens of patients with SSI(30%).

Table 1: Detection of Methicillin resistant *S. aureus* (MRSA) by cefoxitin (30ug) disk using Kirby Bauer method (n=48).

Cefoxitin (30ug) disk diffusion	Resistant
MRSA	28(58.33%)
MSSA	20(41.66%)
Total (n)	48(100%)

Table no 2: Sex wise distribution of *S.aureus* (n=48).

Sex	% of *S.aureus*	MSSA%	MRSA%
Males	27(56.25%)	15(75%)	12(42.85%)
Females	21(43.75%)	5(25%)	16(57.14%)
Total	48(100%)	20(100%)	28(100%)

The p-value is 0.026.Significant at $p < 0.05$

Table no 3: Type of presentation of SSI caused by *S.aureus.*

S.No	Type of SSI	% of *S.aureus*	MSSA%	MRSA%
1	Early(<30 days of surgery)	25(52.08%)	14(70%)	11(39.28%)
2	Late(>30 days of surgery)	23(47.91%)	6(30%)	17(60.71%)
3	TOTAL	48(100%)	20(100%)	28(100%)

The p-value is 0.03.Significant at $p < 0.05$

Table no 4: Type of surgery causing SSI caused by *S.aureus.*

S.No	Type of surgery	% of *S.aureus*	MSSA%	MRSA%
1	Clean	3(6.25%)	2(10%)	1(3.57%)
2	Clean contaminated	14(29.16%)	12(60%)	2(7.14%)
3	Dirty	31(64.58%)	6(30%)	25(89.28%)
4	TOTAL	48(100%)	20(100%)	28(100%)

The p-value is 0.000106.Significant at $p < 0.05$

Table no 5: Timing of surgery causing SSI caused by *S.aureus.*

S.No	Timing of surgery	% of *S.aureus*	MSSA%	MRSA%
1	Elective	17(35.41%)	8(40%)	9(32.14%)
2	Emergency	31(64.58%)	12(60%)	19(67.85%)
3	TOTAL	48(100%)	20(100%)	28(100%)

The p-value is 0.5747.Significant at $p < 0.05$

Table no 6: Risk factors associated with SSI caused by *S.aureus.*

S.No	Risk factors	% of *S.aureus*
1	Diabetes	12(25%)
2	Hypertension	4(8.33%)
3	Old age	14(29.16%)
4	Skin infections	2(4.16%)
5	Increased hospital duration	28(58.33%)
6	Smoking	9(18.75%)
7	Improper dressing/outside dressing done/improper handling of dressing	12(25%)
8	Hypo albulinaemia	4(8.33%)
10	Obesity	29(60.41%)
11	Post trauma	14(29.16%)
12	Prosthesis implanted	5(10.41%)
13	Other	3(6.25%)
	Total	48(100%)

Table 7: Specialty wise distribution of *Staphylococcus aureus* (n=48).

Sr. No	Ward	No. of *S. aureus (%)*	MSSA	MRSA
1	Surgery wards	26(54.16%)	11(55%)	15(53.57%)
3	Orthopaedic wards	14(29.16%)	5(25%)	9(32.14%)
4	Obstetrics and Gynaecology wards	6(12.5%)	3(15%)	3(10.71%)
7	ENT wards	2(4.16%)	1(5%)	1(3.57%)
8	OPD(Out-patient Department)	nil(%)	nil(%)	nil(%)
	Total	48(100%)	20(100%)	28(100%)

Table no 8: Antimicrobial susceptibility pattern of *S. aureus* by disk diffusion method (n=48).

Sr. No	Antibiotics	Sensitive no (%)MSSA	Sensitive no (%)MRSA
1	Penicillin G	NIL(%)	NIL(%)
2	Cotrimoxazole	16(80%)	16(57.14%)
3	Chloramphenicol	14(70%)	18(64.28%)
4	Ciprofloxacin	9(45%)	8(28.57%)
5	Ofloxacin	8(40%)	9(32.14%)
6	Gentamicin	15 (75%)	19(67.85%)
8	Tetracycline	14(70%)	18(64.28%)
9	Erythromycin	16(80%)	12(42.85%)
10	Pristinamycin	20(100%)	28(100%)
11	Linezolid	20(100%)	28(100%)
12	Vancomycin(E strip)	20(100%)	28(100%)

DISCUSSION

Surgical site infection (SSI) is one of the most common and serious complications following surgery. The occurrence of SSI varies according to the type of operation, wound cleanliness, and the operative field. This complication can lead to prolonged hospitalization, which increases the cost of treatment.[1-3]

Surgical infections which are acquired in the hospital, are recognized to be associated with an extended length of hospital stay, pain, discomfort and sometimes prolonged or permanent disability.[11] Surgical site infections (SSIs) are common complications that follow all types of operative procedures.[11] These infections are usually caused by the exogenous and endogenous microorganisms that enter the operative wound during the course of the surgery.[11]

The incidence of the infected surgical wounds may be influenced by factors such as pre-operative care, the theatre environment, post operative care and the type of surgery.

The prevalence rate of surgical site wound infection ranges from 4 to 30% as seen in various studies from India shows that Methicillin resistance in *Staphylococcus aureus*, the most important species, can be prevented by its early detection.[12-22]

We tried to compare the results of SSI with number of MRSA and MSSA.

Several studies all over the world[12-16,18,23-29] have well established that the early detection of Methicillin resistance is very essential in the prognosis of infections which are caused by *S.aureus.*

It has been regularly noted that S. aureus continues to be the single most important bacterial species in the primary aetiology of surgical site infections since the past thirty years or so.[25]

As there is paucity of data in this area, so we conducted this study to estimate the prevalence of *Staphylococcus aureus* and Methicillin resistance in surgical site infections at Nagpur. A total of 158 pus samples were included in the study. Out of these, 48 *Staphylococcus aureus* clinical isolates were isolated from specimens of patients with SSI(30%) The higher rates of SSI could be due to government setting where mostly very low socio-economic status people are catered. They usually have poor nutritional status and lower hygienic conditions and this needs further confirmatory studies.

Out of the 48 *Staphylococcus aureus* clinical isolates, 28(58.33%) were MRSA and 20(41.66%) were MSSA.(table no 1).

A study by Weigelt *et al* in USA, found an incidence of 20.6% MRSA in SSIs.[21] Higher incidences of MRSA of 58.2% by Keith *et al* and 45% by Eagye *et al* have been documented.[17,20]

The incidence of Methicillin–resistant *Staphylococcus aureus* in our study was 21 (36.84%). We found that all the MRSA strains (100%) were sensitive to Vancomycin, Teicoplanin and Linezolid was of relevant clinical use in the antibiotic policy guidelines for hospitals.

Harbarth *et al* have observed that Methicillin resistant *Staphylococcus aureus* (MRSA) alone constituted 5.1% of the surgical site infections.[15]

Males and females were equally affected. There was no much difference in their rates of SSI.(table no 2)

There was also not much difference in the type of presentation of SSI caused by *S.aureus*, early(52.08%);.out of these, 14(70%) were MSSA and 11(39.28%) were MRSA.(table no 3). Late presentation *S.aureus*, comprised of 23(47.91%).Out of these, 6(30%) were MSSA and 17(60.71%) were MRSA. It can be seen that the percentages of late presentation of SSI was more with MRSA isolates. The reason for this could be prior self medication or inappropriate antibiotic use, unhygienic conditions, late presentation to institutions etc.

Clean surgeries are always associated with less chances of developing SSI due to lesser bacterial contamination, less burden of bacteria in and or on surrounding. Clean contaminated and dirty surgeries are often complicated by SSI due to increased bacterial burden during surgery e.g. gastrointestinal surgery where translocation of gut bacteria is always possible.

Post traumatic surgeries e.g. Road traffic accidents, gangrenous operations, amputations etc. always have high chances of developing SSI in near time.

In our study also, 31(64.58%) of *S.aureus* were found in dirty surgeries. The percentage of MRSA was also highest in the dirty surgeries 25(89.28%). The statistical analysis was done by Chi square test and the p-value was statistically significant (0.000106).(table no 4)

The timing of surgery was also evaluated as a cause of SSI. Elective surgeries have a less risk of developing SSI due to detailed pre –operative check up, antibiotic prophylaxis, skin preparation etc and time is also available for routine evaluation. Emergency surgeries like intestinal obstruction, perforations, tracheostomies, RTA(road traffic accidents), emergency C-sections where the surgeon and anesthetist do not have much time to spare on pre operative check-ups, chances of breach in sterility can be a possibility. Total number of *S.aureus* isolated from emergency surgeries was found to be 31(64.58%).Out of these, 19(67.85%) were MRSA.(table no 5).

A number of risk factors increase the development of SSI. We studied diabetes, hypertension, obesity, smoking, increased hospital stay, unhygienic conditions etc. Among all these co-morbidities maximum number of patients were obese 29(60.41%), followed by increased hospital duration in 28(58.33%), post trauma in 14(29.16%) and diabetes in12(25%).All of these are known to increase risk of developing SSI.

Maximum number of isolates were from surgery wards 26(54.16%) and orthopaedic wards 14(29.16%). The reason could be that these are the busiest wards in hospitals catering to a large number of patients and also emergencies.

The antibiotic susceptibility pattern of these isolates was also studied to treat all these SSI successfully.

All isolates (both MRSA and MSSA) were uniformly susceptible to Vancomycin, Pristinamycin and Linezolid. Pristinamycin can be used in MRSA isolates as an alternative to Vancomycin and Linezolid. Cotrimoxazole showed 80% sensitivity which is also a good option to use in such isolates. Least sensitivity was seen to Penicillin (0%) and Ciprofloxacin(45%).(table no 8)

Naik *et al* also reported all *S.aureus* isolates of SSI to be uniformly susceptible to vancomycin and linezolid.[30]

Mostly the infection may originate from the patient's normal flora or that it may be derived from the hospital environment. Multidrug resistant *S.aureus* is commonly associated with the hospital environment. In the present study, 58.33%of isolates of the postoperative wound infections were due to MRSA and this indicates that the possible source of infection in these cases may be from the hospital environment. This fact needs to be kept in mind by the surgeons. Also, is well known that surgical site infections are also associated with significant increases in the length of hospital stay, additional costs, morbidity and mortality. The widespread availability and the use of antimicrobial agents for prophylaxis seem to have altered the surgical practice in the past twenty years.

It is highly desirable that prophylactic antibiotics should be administered within one hour before the operation and that they should be continued for only 24-72 hours post operatively, as a policy to avoid multidrug resistance. Inappropriate dosage and the duration of antibiotic use not only fail to reduce the infection, but also lead to an increase in multidrug resistant mutants due to selection pressures.[16,17] Hence, the prevention of SSIs is essential and this poses a major challenge in any healthcare system.[16] Infection control measures such as the active surveillance of SSIs, the implementation of a checklist, compliance observations and instruction/training of healthcare workers, as well as *Staphylococcus aureus* /MRSA screening, clipping instead of shaving, adherence to peri operative antibiotic prophylaxis, maintaining intra operative normothermia and blood glucose control are essential in order to prevent SSIs.[17-19]

Guidelines and protocols for basic infection control practices such as hand washing and written protocols for the safe insertion and maintenance of devices such as intravascular catheters, should be widely available and adhered to.[19-22]

CONCLUSION

Due to the increased morbidity and mortality which are associated with *S.aureus*, an early detection and intervention is a prerequisite in surgical patients. Although surgical wound infections cannot be completely eliminated, a reduction in the infection rate to a minimal level could have significant benefits, by reducing postoperative morbidity and mortality, and the wastage of health care resources. Infection control guidelines for SSIs play a key role in reducing such infections.

The hospital managements should ensure a regular, close clinical liaison between the surgical team, the microbiologist and the infection control team to provide proper quality surgical services.

Conflict of interest –Nil

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