# A STUDY ON SURGICAL WOUND INFECTIONS CAUSED BY STAPHYLOCOCCUS SPECIES WITH SPECIAL REFERENCE TO METHICILLIN RESISTANT STAPHYLOCOCCI

**Gaurav Dalela [1], Jitendra Ahuja [2*], Nilofar Khayyam [3], Arvind Sharma [4]**

1. Associate Professor, Department of Microbiology, RUHS College of Medical Sciences, Jaipur
2. Associate Professor, Department of Biochemistry, RUHS College of Medical Sciences, Jaipur
3. Assistant Professor, Department of Microbiology, RUHS College of Medical Sciences, Jaipur
4. Assistant Professor, Department of Community Medicine, RUHS College of Medical Sciences, Jaipur

***Email id of corresponding author- drjitendraahuja@yahoo.co.in**

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**ABSTRACT**

**Background:** Surgical site infections (SSI's) are acquired in hospitals, a leading cause of nosocomial infection, a common complication of operative procedures, associated with prolong stay in hospital, lack of antisepsis measures, identified by pain at the site, discomfort, discharge of pus and disability caused by either exogenous or endogenous or both organism which may include commonly bacteria and fungi. **Aims and Objective:** To study the prevalence of Methicillin-Resistant Staphylococcus aureus (MRSA) in surgical sites associated with nosocomial infection at Jhalawar. **Material and Methods:** This study was conducted in the Department of Microbiology at Jhalawar Medical College and Hospital, Jhalawar from January 2013 to December 2013. The study population included two hundred thirty seven patients suffering from surgical site infections, between the ages of 15 to 73 years of age. **Results:** Of the 237 pus samples, 171 (72.15%) showed growth of aerobic bacteria. The most common organism was Staphylococcus aureus, with 57 (33.3%) isolates, of these 21 (36.84%) were Methicillin Resistant Staphylococcus Aureus (MRSA).**Conclusions:** Methicillin Resistant Staphylococcus aureus, common agent causing SSI's, pose a great problem in treatment of SSI's. Routine screening of MRSA should be done in Staphylococcus aureus and other Staphylococcus species.

**Key Words:** Surgical Site Infections, Staphylococcus aureus, Resistance, MRSA

## INTRODUCTION

Surgical site infections (SSI's) are acquired in hospitals, a leading cause of nosocomial infection, a common complication of operative procedures, associated with prolong stay in hospital, lack of antisepsis measures, identified by pain at the site, discomfort, discharge of pus and disability caused by either exogenous or endogenous or both organism which may include commonly bacteria and fungi. It is influenced by the environment of the hospital setting where the patient is operated or admitted, type of the surgery, post operative care and hospital infection control protocol which include mainly asepsis maintenance.**(1)**

The most common pathogenic bacteria indulge in surgical site infection is Staphylococcus

aureus, Commensal flora in nose and transmitted due to hand hygiene, clothes of staff and other healthcare tools but now a day Coagulase negative Staphylococci is also gaining importance. Other bacteria includes Pseudomonas species, Escherichia coil, Klebsiella sp., Enterobactre sp., Citrobacter sp., Proteus sp., Acineobacter sp., Enterococcus sp. etc. initially Staphylococcus aureus was susceptible to penicillin but due to misuse of antibiotics, it is resistant to most of the penicillin group world wide due to Methicillin resistance. **(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 production of *erm* gene also shows the resistance mechanism for macrolides.

Methicillin resistant Staphylococci sometimes show sensitivity for β-lactam antibiotics such as cephalosporins but they found to be clinically ineffective, so therapeutic options for this resistance is limited.**(3)** The incidence of methicillin resistant Staphylococci (MRS) in various studies from India ranges from 30-70%. **(4, 5)** Therefore our study was done to determine the prevalence of Methicillin-Resistant Staphylococci (MRS) in surgical sites associated with nosocomial infection at Jhalawar, as no previous study was undertaken in this region to show the prevalence.

## MATERIAL AND METHODS

### Study population

This study was conducted in the Department of Microbiology at Jhalawar Medical College and Hospital, Jhalawar from January 2013 to December 2013. The study population included two hundred thirty seven patients suffering from surgical site infections form surgical ward, orthopedic ward, Obstetric ward and gynecology ward, between 15 to 73 years of age.

### 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).**(6)**

### Exclusion criteria

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

### Method

Pus samples were collected from each patient with the help of two sterile swabs under strict aseptic precautions, one was used for smear preparation and the other was used for culture along with antibiotic sensitivity testing and processed immediately as soon as possible.

The pus samples were inoculated on Blood agar, MacConkey agar and nutrient agar and were incubated at 37$^{o}$C for 18-24 hours in 5 – 10% $CO_2$ concentration. After incubation, the plates were examined for the colony characteristics specific to individual microorganism and the colonies suggestive of Staphylococcus aureus were identified by Gram staining, Catalase test, both slide and tube Coagulase test and mannitol fermentation.

Antimicrobial susceptibility testing was done by disc diffusion method using modified Kirby-Bauer method. The antimicrobial agents used for sensitivity testing of Staohylocccci were: Amoxycllin 30 µg, erythrtomycin 15 µg, ciprofloxacin 5 µg,, Oxacillin 1 µg, doxycycline µg, cotrimoxazole 25 µg, cefoxitin 30 µg,

vancomycin 30 µg, teicoplanin 30 µg, amoxicillin + clavulanate 30/10 µg, clindamycin µg, Linezolid µg, Amikacin 30 µg (HiMedia Laboratories Pvt. Ltd). After incubation period the zone of inhibition was measured using Zone diameter measuring scale using zone size specific for Sensitive, Intermediate or Resistant as per Clinical and Laboratory Standard Institute (CLSI) guidelines.**(7)**

**The Oxacillin E test**: The MICs of oxacillin were determined by the E-test (AB Biodisk, Solna, Sweden), according to the instructions of the manufacturer [**AB BIODISK. E-test oxacillin package insert. Solna, Sweden: ABBIODISK; 2013**]. The plates were inoculated by swabbing the surfaces with a 0.5 McFarland's standard bacterial suspension on the MHA medium which was supplemented with 2% NaCl to enhance detection of resistance. The E-test strips were placed on the medium, and the plates were then incubated at 35°C for 24 hrs. The results were analyzed on the basis of the CLSI guidelines. **(7)**

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

**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.

## RESULTS

Of the 237 pus samples, 171 (72.15%) showed growth of aerobic bacteria. The most common organism was Staphylococcus aureus, with 57 (33.3%) isolates, of these 21 (36.84%) were Methicillin Resistant Staphylococcus Aureus (MRSA).

**Table 1: Comparison of antibiotic resistance pattern in both MRSA producers and non MRSA producers**

| Drug | MRSA (n=21) (%) | Non-MRSA (n=36) (%) |
|---|---|---|
| **Amoxycllin 30 µg** | 21 | 21 |
| **amoxicillin + clavulanate 30/10 µg** | 20 | 17 |
| **Amikacin 30 µg** | 13 | 9 |
| **cotrimoxazole 25 µg** | 21 | 18 |
| **cefoxitin 30 µg** | 21 | 0 |
| **clindamycin 10 µg** | 16 | 3 |
| **ciprofloxacin 5 µg** | 21 | 15 |
| **doxycycline 30 µg** | 19 | 16 |
| **erythromycin 15 µg** | 21 | 11 |
| **Linezolid 10 µg** | 0 | 0 |
| **Oxacillin 1 µg** | 17 | 8 |
| **Teicoplanin 30 µg** | 0 | 0 |
| **Vancomycin 30 µg** | 0 | 0 |

## MINIMUM INHIBITORY CONCENTRATION (MIC)

Out of 25 oxacillin resistant strains and only four cefoxitin resistant strains, 21 isolates which were resistant to cefoxitin confirmed as MRSA strains by oxacillin e-test showing highly significant correlation of MRSA with cefoxitin disc in disc diffusion method (p value <0.001). Other eight strains which were resistant or sensitive to oxacillin were Oxacillin sensitive with Oxacillin E-test.

The minimum inhibitory concentration of Oxacillin for 21 isolates which were resistant to

cefoxitin by disc diffusion, showed an MIC of > 8 mcg/ml.

## DISCUSSION

Identification of *mec*A gene is the most reliable method of detecting MRSA isolates, however not all laboratories can include molecular biology techniques in their routine clinical practice. So it is essential that phenotypic techniques able to detect MRSA isolates in a rapid and accurate manner are made available, in order to ensure correct antibiotic treatment and to avoid the spread of MRSA isolates in the hospital environment. This study confirms that those antibiotics able to induce expression of methicillin resistance, e.g. cefoxitin, is the most appropriate drug for detecting MRSA isolates, as in our study confirmation of MRSA isolates done by oxacillin E-test which gave correlative study with cefoxitin but oxacillin disc showed false positivity. The *mec*A-positive isolates were detected with the cefoxitin disc (30 μg) in predicting oxacillin resistance has been reported.**(8, 9, 10)** It is suggested that no special medium or incubation temperature is required with cefoxitin **(9)** so it can be used as better indicator for detection of MRSA.

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. **(11 – 21)**

As there is paucity of data in our area, so we conduct this study to estimate the prevalence of Staphylococcus aureus and Methicillin resistance in surgical site infections at Jhalawar. Our findings were not representative of whole of India as literacy, nutritional status and health care facilities of Jhalawar are not comparable to those in the cities of Rajasthan.

In our study, 237 pus samples which were collected from the patients with surgical site infections (SSI), the highest incidence of SSIs was seen in orthopedic cases (53.21%), followed by surgical (30.4%) and obstetrics and gynecology cases (16.96%) respectively as majority of the orthopedics patients were operated for the open reduction of fractures therefore contamination from the external environment can be a possible reason for this higher incidence of surgical site infections (SSI) in orthopedic cases.

Also, the number of male patients (62.44%) was higher than the female patients (37.56%) due to increased mobility in the males. In both the sexes, most of the patients belonged to the age group of 21-30 years.

In developing countries due to the relatively poor hygienic measures in the government hospitals, there was an increase incidence of SSI's and this needs further confirmatory studies.

The changing pattern could be due to the reported indiscriminate use of new/ many broad spectrum antibiotics, the increasing use of instrumentation, the long preoperative stay and coexisting infections at a remote body site.**(22, 23)**

Staphylococcus aureus was the most common pathogen found, 57 isolates (33.3%), which was found to be statistically highly significant (p value 0.001) as seen in the study conducted by Edwards et al, who reported a nearly equal incidence of S. aureus (30.3%), Surange et al and Subramanian et al have reported the incidence of S. aureus 34.2% and 30.9% respectively.**(12,24,25)** Kownhar et al have

shown 37% and Eagye et al in USA shown an incidence of 39% of Staphylococcus aureus.**(13,19)** A higher predominance of S.aureus (51.6%) was observed by Keith et al in older operative patients as there may be more nasal carriers of Staph. **(16)** aureus, low immunity status of older patients and poor hand hygiene of the hospital staff. Also there was concurrent occurrence of Staphylococcus aureus with Pseudomonas sp., Esch. coli, and Acinetobacter species.

In antibiotic sensitivity patterns, Vancomycin, Linezolid and Teicoplanin were the most sensitive (100%) antibiotics for Staphylococcus aureus. Among the first line antibiotics, the highest sensitivity was recorded with Amikacin (61.4%) and the least sensitivity (26.3%) was seen with Amoxycillin. Staph. aureus has been known to acquire resistance to most antibiotics including the penicillinase resistant ones like Oxacillin and Cefoxitin. A study by Weigelt et al in USA, found an incidence of 20.6% MRSA in SSIs. **(20)** Higher incidences of MRSA of 58.2% by Keith et al and 45% by Eagye et al have been documented.(16,19) 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. **(14)**

Hence, the prevention of SSIs is essential as a major clinical, political and therapeutic challenge.**(15)** Infection control measures should be opt such as the active surveillance of SSIs, implementation of infection control checklist, its compliance and training of healthcare workers, MRSA screening, clipping instead of shaving, adherence to pre- and peri-operative antibiotic prophylaxis, maintaining intra-operative temperature and blood glucose level as they are essential in order to prevent SSIs.**(18)** Guidelines and protocols for basic infection control practices such as hand washing and insertion of intravascular canulas and catheters should be widely available and adhered to it.**(18, 21)**

## CONCLUSIONS

Early detection and intervention of cases of surgical site infection (SSI) is a prerequisite in surgical patients to decrease the incidence of morbidity, mortality and wastage of health care resources although they cannot be completely eliminated, a reduction in the infection rate to a minimal level could have significant benefits.

Antimicrobial therapy should be designed in such a way that it will deliver an adequate drug concentration to the site of infection. Therefore governments should take steps in setting up national hospital antibiotic policy guidelines as per different regions of country, also prevent the transmission of MRSA infection through health care personnel. Hospitals should screen for MRSA among their staff and treat the hospital staffs.

## REFERENCES

1. Nichols RL. Current Strategies for Prevention of Surgical Site Infections: Curr Infect Dis Rep 2004; 6(6):426-34.
2. Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR. Guidelines for Prevention of

SSI, 1999,Criteria for defining SSIs. The Hospital Infection Control Practices Advisory Committee. Infect control Hosp Epidemiol 1999; 20; 4; 24.
3. Susan LK. Concepts in Antimicrobial therapy; TB of Diagnostic Microbiology: 3rd edition: WB Saunders. Editors; Mahon C.R, Manuselis G: 2007: 82.
4. Verma S, Joshi S, Chitnis V, Hemavani N, Chitnis D. Growing problems of Methicillin Resistant Staphylococci-Indian Scenario. Indian J Med Sci 2000; 54: 535-40.
5. Bratzler DW, Hunt DR. The surgical infection prevention and surgical care improvement projects: national initiatives to improve outcomes for patients having surgery. Clin Infect Dis 2006; 43: 322-30.
6. Oslon M, Connor O, Schwartz ML. A five year Prospective Study of 20193 Wounds at Minneapolis V a Medical Center. Ann Surg1984; 199: 253-9.
7. Wayne. Clinical and Laboratory Standards Institute / NCCLS Performance standards for Antimicrobial disc diffusion tests; Approved standards. 9th ed. CLSI Document M2-M9. Clinical and Laboratory Standards Institute; 2006; 4456.
8. Skov R, Smyth R, Clausen M et al. Evaluation of cefoxitin 30 μg disc on isosensitest agar for detection of methicillin resistant Staphylococcus aureus. J antimicrob chemother 2003; 52:204-7.
9. Felten A, Grandry B, Lagrange PH et al. Evaluation of three techniques for detection of low level methicillin resistant S. aureus (MRSA): a disc diffusion method with cefoxitin and moxalactum, the Vitek-2 system, and the MRSA screen latex agglutination test. J Clin Microbiol 2002; 40:2766-71.
10. Cauwelier B, Gordts B, Descheemaecker P et al. Evaluation of disc diffusion method with cefoxitin (30 μg) for detection of methicillin resistant Staphylococcus aureus. Eur J Clin Microbiol Infect Dis 2004; 23:389-92.
11. Agarwal SL: Study of postoperative wound infections: Ind.J Surg 1972:314-20.
12. Surange BN, Rai UK: Bacteriological Pattern and their sensitivity to antibiotics in Wound Infections. Indian J Pathol Microbiol: 1979; 22; 331-36.
13. Kownhar H, Shanker EM, Vignesh R, Sekar R, Velu V, Rao VA: High rate of isolation of S.aureus from Surgical Site Infections in an Indian Hospital; J Antimicrob Chemother 2008;61(3): 758-60.
14. Harbarth S, Fankhauser C, Schrenzel J, Christenson J, Gervaz P,Bandiera-Clerc C, Renzi G, Vernaz N, Sax H, Pittet D. Universal Screening for Methicillin-Resistant Staphylococcus aureus at Hospital Admission and Nosocomial Infection in Surgical Patients. JAMA.2008; 299: 1149-1157.
15. Lilani SP, Jangale N, Chowdhary A, Daver GB. Surgical site infection in clean and clean-contaminated cases. Indian J Med Microbiol 2005; 23: 249-52.
16. Keith SK, Deverick JA, Sloane R, Chen LF, Choi Y, Link K, Sexton DJ, Schmader KE: The impact of Surgical Site Infection on older operative patients: J Am Geriatr Soc 2009 ;57(1):46-54.
17. Rao AS, Harsha M. Postoperative wound infections. J Indian Med Assoc 1975; 64:90-3.

18. Chaudhary U, Aggarwal R. Extended spectrum b-lactamases (ESBL)-an emerging threat to clinical therapeutics. Ind J Med Microbiol 2004; 22(2): 75-80.
19. Eagye KJ, Kim A, Laohavaleeson S, Kuti JL, Nicolau DP: Surgical Site Infection: does inadequate antibiotic therapy affect patient outcomes?: Surgical Infect (Larchmt) 2009 ;10(4):323-31.
20. Weigelt JA, Lipsky BA, Tabak YP, Derby KG, Kim M, Gupta V: Sugical Site Infections: Causative pathogens and associated outcomes; Am J Infect Control 2010;38(20):112-20.
21. Anand KB, Agrawal P, Kumar S, Kapila K: Comparison of Cefoxitin Disc Diffusion Test, Oxacillin Screen Agar, and PCR For mecA Gene for Detection of MRSA: Indian J Med Microbiol 2009; 27(1): 27-9.
22. Udgaonkar, Bhavthanker A: Bacteriological study of Postoperative wound infections. JIMA; 1985; 83; 231-5.
23. Soleto L et al: Incidence of surgical- site infections and the validity of the National Nosocomial Infections Surveillance system risk index in a general surgical ward in Santa Cruz, Bolivia: Infect Control Hosp Epidemiol 2003;24(1):26-30.
24. Edwards LD: The Epidemiology of 2056 Remote Site Infections and 1966 Surgical Wound Infections occurring in 1865 patients. Annals Surgery 1976; 184; 758-66.
25. Subramanian KA, Prakash A, Shrinvas, Bhujwal RA. Post-operative wound infection. Ind J Surg 1973; 57-64.