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# Comparision of Biofilm Production among MRSA Strains Isolated from Surgical Site Infection

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**ABSTRACT**
*Surgical Site Infection (SSI) remains as the major reason for morbidity and mortality in patients undergoing surgery, even with the advances in the surgical techniques, better understanding of the pathogenesis of wound infections & wound healing. The management of postoperative infection is further complicated by, formation of virulence factors like biofilm along with MRSA by the organism.To study the prevalent of organism causing SSI virulence factors like biofilm & slime formation and genotypic characterization of MRSA genes using PCR. To detect the biofilm formation gene (ica A & ica D) of Staphylococcus aureus using PCR. Pus swabs and pus aspirates were collected from 62 suspected cases of SSI from patient admitted to obstetrics and gynaecology ward in MMCH&RI. The Samples were processed according to standard Clinical Laboratory Standard Institute guidelines (CLSI).The predominant isolate was Staphylococcus aureus. The antibiotic sensitivity testing was done by, Kirby Bauer disc diffusion method. The slime production was screened by modified Congo red agar method. Prevention of SSI and control measures will not only help in reducing the incidence of SSI but also can reduce the burden on the patient. So, this study was done in our hospital to find out the predominant organism and to study their virulence factor, the resistance pattern of the organism and their control measures.*
**Keywords:** *Staphylococcus aureus, biofilm, PCR.*

## INTRODUCTION

Surgical Site Infection (SSI) is the third most commonly reported Hospital Acquired Infection (HAI)[(1)]. SSI is defined as the infection occurring within 30 days of surgery or within a year in the case of implants left in the place of surgery, according to Centre for Disease Control and prevention (CDC).[(2,3)]

SSI incidence in various hospitals varies from 10-25% in India. The WHO has reported, 2 million cases of hospital acquired infection annually worldwide and described it as the major infectious disease and huge economical burden. SSI increases the postoperative hospital stay by 5 – 20 days per infections and substantially increases the economic burden. *Staphylococcus aureus* is the most predominant isolate followed by *Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli, Klebsiella* species, and *Enterococcus* species respectively[(4,5,6)].

A biofilm can be defined as a microbially derived sessile community, typified by cells that are attached to a substratum, interface, or to each other, are embedded in a matrix of extracellular polymeric substance, and exhibit an altered phenotype with regard to growth, gene expression and protein production.

Biofilm formation is a complex mechanism with four phases,

a) Attachment
b) Accumulation
c) Maturation &
d) Dispersion[7,8]

Biofilms are commonly associated with in dwelling medical devices like, prosthesis, stents, ventilators, intravenous catheters, invasive blood pressure units, infusion pumps, pacemakers, stitch materials, cardiac defibrillators, mechanical heart valves, cosmetic surgical implants etc.,

a. Exopolysaccharide PIA shields the organism from neutrophil phagocytosis, thus significantly contributing to biofilm resistance from elimination by innate host defense.
b. Prevention of the antibacterial substance from reaching its target, e.g. by limited diffusion or repulsion.

Role of biofim in infection:

c. The specific physiology of a biofilm, which limits the efficacy of antibiotics, mainly of those that target active cell processes and may also include specific subpopulations of resistant cells Delayed known as persisters.

The management of postoperative infection is further complicated by, formation of biofilm along with Methicillin Resistance *Staphylococcus aureus* (MRSA) by the microorganism. A working knowledge on factors causing SSIs, pathogenesis, etiology, virulence and resistance pattern will help in rationalizing, the use of appropriate antibiotics with proper timing & dosage for surgical prophylaxis.

Polymerase Chain Reaction (PCR) used for genotypic characterization of antimicrobial resistance using mecA gene and virulence factors like biofilm formation detected using icaA and icaD genes in *Staphylococcus aureus*.

SSI is an index of health care system in the hospital. Nowadays there is increase in the incidence of nosocomial infections and its resistance pattern. Hence SSI is now a topmost agenda for the infection control team. Prevention of SSI and control measures will not only help in reducing the incidence of SSI but also can reduce the burden on the patient [9,10,11,12].

## AIM AND OBJECTIVE

1. To study the prevalent of organism causing SSI virulence factors like biofilm & slime formation and genotypic characterization of MRSA genes using PCR.
2. To detect the biofilm formation gene (*ica A* & *ica D*) of *Staphylococcus aureus* using PCR.
3. To detect correlation between biofilm production & MRSA.

## MATERIALS AND METHODS

The study was conducted at Department of Microbiology, MMCH & RI, Enathur, Kanchipuram during the period of April -2014 to September -2015. After obtaining Institutional ethical clearance and consent from the patients, pus swabs / exudates / aspirates sample were collected from the patients who developed signs and symptoms of surgical site infection in the Obstetrics and Gynaecology Department.

**Inclusion criteria:**

- Clean and clean contaminated surgeries from Obstetrics and Gynaecology ward

**Exclusion criteria:-**

- Contaminated and dirty wounds
- Patient not coming for follow up till 30 days after surgery.

**Sample collection:**

When SSI was clinically suspected, the area around the surgical wound was cleaned with 70%

ethanol or with sterile saline and two pus swab specimens were collected from depth of the wound or aspirate collected from the wound and processed according to CLSI guidelines.

**Detection of slime production by modified Congo red agar (mCRA):**

Modified Congo red agar was prepared using Blood Base Agar – 2 (BAB-2) (40gms/L), Congo red dye (0.4gms/L) and glucose (10gms/L) respectively. Congo red was prepared as concentrated aqueous solution and autoclaved at $121^0$C for 15 minutes, separately from other medium constituents and was then added when the agar had cooled to $55^0$ C. plates were inoculated and incubated aerobically for 24-48 hours at $37^0$ C and subsequently 2-4 days at room temperature.

Black colonies with dry crystalline consistency were indicated as positive and red colonies considered as negative result. The experiment was performed in triplicate and repeated three times[10].

**Molecular characterization**

**Detection of biofilm genes by pcr:**

Detection of biofilm genes (*icaA* and *icaD)* were performed as described Mariana et al 2009. Bacterial DNA Extraction Kit was used for bacterial DNA extraction according to manufacturer's protocol (Xcleris Ltd).Briefly DNA was extracted from overnight broth culture and DNA was trapped in Column following the manufacturer's protocol. Eluted DNA was stored at -20˚C for further use. The genes regulated for Intracellular adhesion Locus icaA and icaD were detected by PCR[19].

**Primer sequences**

| Primer | Sequence | Temp (°C) | Product size (bp) |
|---|---|---|---|
| *icaA*-For<br>*icaA*-Rev | ACACTTGCTGGCGCAGTCAA<br>TCTGGAACCAACATCCAACA | 60 | 188 |
| *icaD*-For<br>*icaD*-Rev | ATGGTCAAGCCCAGACAGAG<br>AGTATTTTCAATGTTTAAAGCAA | 60 | 198 |
| *mec A*1 | 5'GTAGAAATGACTGAACGTC CGATAA | 60 | 310 bp |
| *mec A*2 | 5'CCAATTCCACATTGTTTCG GTCTAA | 60 | 310 bp |

**Detection of mec a genes by pcr:**

Detection of MRSA genes (mecA) were performed as described Anand et al 2009. Bacterial DNA Extraction Kit was used for bacterial DNA extraction according to manufact-urer's protocol (Xcleris Ltd).Briefly DNA was extracted from overnight broth culture and DNA was trapped in Column following the manufacturer's protocol. Eluted DNA was stored at -20˚C for further use. PCR amplification was done with specific gene primers and checked for the presence of mecA gene[4].

**RESULTS**

Out of 1240 cases operated in the Obstetrics and Gynaecology Department, 62 cases developed symptoms and signs of SSI.

**Table 1:** Incidence of culture positive and culture negative ssi (n=62)

| Isolates | Number of isolates | Percentage |
|---|---|---|
| Culture positive ssis | 53 | 85.5% |
| Culture negative ssis | 9 | 14.5% |
| Total | 62 | 100% |

**Table 2:** Distribution of gram positive and gram negative organism in ssis (n=62)

| Isolate | Number of isolates | Percentage |
|---|---|---|
| Gram positive cocci | 37 | 59.7% |
| Gram negative bacilli | 16 | 25.8% |
| Culture negative ssis | 9 | 14.5% |
| Total | 62 | 100% |

**Table 3:** Distribution of isolates among culture positive ssi (n=53)

| Isolate | Number of isolate | Percentage |
|---|---|---|
| *Staphylococcus aureus* | 35 | 66.03% |
| *Coagulase Negative Staphylococcus* | 2 | 3.8% |
| *Pseudomonas aeruginosa* | 7 | 13.2% |
| *Klebsiella pneumonia* | 5 | 9.43% |
| *Escherichia coli* | 4 | 7.54% |
| Total | 53 | 100 |

**Table 4:** Antibiotics susceptibility pattern of *staphylococcus aureus* by kirby bauer disc method; (n=35)

| Antimicrobial agent | Number of resistant isolate | Percentage of resistance (%) |
|---|---|---|
| Amikacin-AK(30µg) | 0 | 0 |
| Amoxyclav-AMC(20µg /10µg) | 14 | 40 |
| Ampicillin-A (10µg) | 21 | 60 |
| Cefoxitin-CX (30µg) | 7 | 20 |
| Ceftazidime-CAZ(30µg ) | 5 | 14.3 |
| Cefuroxime -CXM(30 µg) | 9 | 25.7 |

| | | |
|---|---|---|
| Ciprofloxacin-CIP(5µg) | 17 | 48.6 |
| Cotrimaxazole-COT (1.25 µg/23.75 µg) | 16 | 45.7 |
| Erythromycin-E (15 µg) | 8 | 22.86 |
| Gentamicin-GEN(10µg) | 0 | 0 |
| Linezolid-LZ (30 µg) | 0 | 0 |
| Netilmycin- (30 µg) | 0 | 0 |
| Oxacillin -OX(1 µg) | 6 | 17.1 |
| Tetracycline-TE (30 µg) | 8 | 22.86 |
| Vancomycin-VA (30 µg) | 0 | 0 |

The maximum resistance was reported in Ampicillin 21(60%) followed by Ciprofloxacin 17(48.6%). Cefoxitin resistance was reported in 7(20%) of *Staphylococcus aureus* isolates.

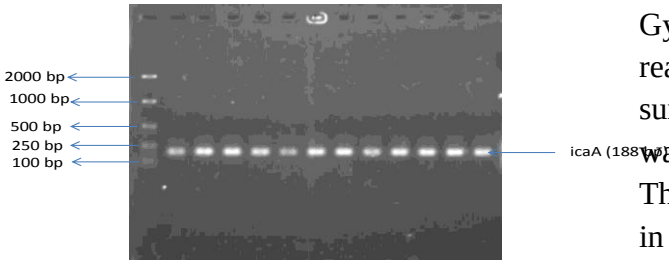
**Table 5:** showing slime production by modified congo red agar method

| Virulence: Slime production | Slime producers | Non producers | Total |
|---|---|---|---|
| *Staphylococcus aureus* | 12(34.3%) | 23(65.7%) | 35(100%) |

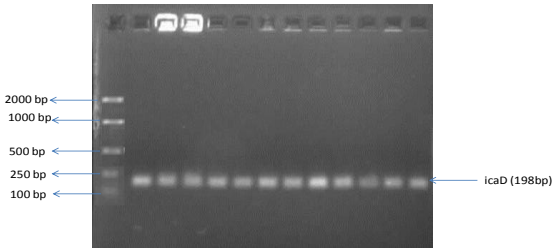
**Table 6:** Comparision between Methicillin Resistance *Staphylococcus aureu s(Mrsa), Methicillin Sensitive Staphylococcus aureus* (Mssa) & biofilm production

| Virulence: Biofilm & slimeproduction by pcr & mcra | Total no. Of isolates(n=35) | |
|---|---|---|
| | Mrsa (n=7) | Mssa (n=28) |
| Producer | 5(14.3%) | 7(20%) |
| Nonproducer | 2(5.7%) | 21(60%) |

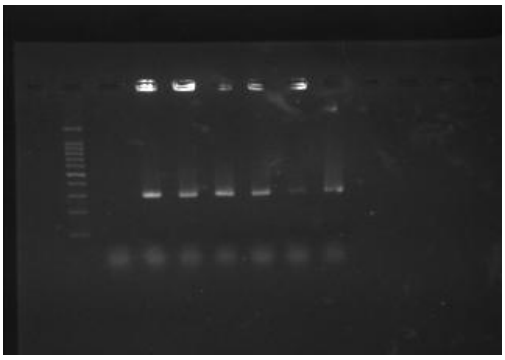
**Gel picture shows positive for *ica a* (188bp)**




**Gel picture shows positive for *ica d* (198 bp)**




**Gel picture shows positive for *mec a* (310bp)**



## DISCUSSION

The overall incidence of SSI in India varies from 4 to 30%. In our study 5% of SSIs were reported from Obstetrics and Gynaecology patients which was concordant with the previous study who has also reported 6.12% and 6% from Obstetrics and Gynaecology patients[1,13]. The incidence is slightly lower than 10% of SSIs in the patients undergoing surgery in Obstetrics and Gynaecology ward[17]. This may be attributed the reason that incidence vary among each hospital, surgeon operating and also varies among each ward present within the hospital.

The incidence of culture negative SSI was 14.5% in our study which was concordant with Saraswathi *et al* who has reported 13% were culture negative SSIs, which was much lower than Reddy et al who has reported 22.22% of culture negative SSIs. This may be attributed to reasons like poor wound healing due to anaemia, diabetes, chemotherapy, poor host defence or comorbidities and our study does not involve screening for anaerobic organism.

In our study the most common isolate was *Staphylococcus aureus* 35(66.03%) which correlates with Ramesh *et al* who has reported that the common isolate was *Staphylococcus aureus* in 19(66%) cases. Similarly Jyoti Sonawane *et al.*, reported that the predominant isolate was *Staphylococcus aureus* (29.2%) which was concordant with our study. Whereas the most common pathogen isolated was *Enterobacter* (33%) followed by *Staphylococcus aureus* (27%) in the study conducted by Priti Goyal *et al* which is not in accordance with our study, this may be attributed to the reason that each hospital has its own flora, varies with each patient & surgeon.

In our study *Staphylococcus aureus* showed highest resistance to Ampicillin 21(60%) followed by Ciprofloxacin 17(48.6%), Cotrimoxazole 16(45.7%) and Amoxyclav 14(40%) which was concordant with Jyoti sonawane *et al* who has reported highest resistance with penicillin 119(75.32%) followed by Cotrimoxazole 109(68.99%) and Ciprofloxacin 102(64.56%) respectively. In our study all the *Staphylococcus aureus* isolates were 100% sensitive to Vancomycin. In our study 20% MRSA were isolated from SSIs which is slightly lower than Jyoti Sonawane *et al* who isolated 27.8% MRSA in SSIs.

In our study 12 (34.3%) isolates were positive for slime production which was in accordance with Akinkunmi *et al* repoted in his study that 36% of isolates were slime producers.

All the isolates were subjected to PCR for detection of *ica A* and *ica D* genes, all were found to be positive. In our study slime production correlated with MRSA 5(14.3%) with significant p value of 0.0331, which was concordant with Suma Kulkarni *et al* who has reported slime production correlated with MRSA 10(26.32%), with significant p value of <0.001 respectively.

Early detection and intervention is a prerequisite in surgical patients due to increased morbidity and mortality associated with biofilm producing and drug resistant organisms. Although SSI cannot be completely eliminated, a reduction in the infection rate to a minimal level could have significant benefits, by reducing morbidity, mortality, economic burden and the wastage of health care resources.

To prevent the emergence of multidrug resistant bacteria, judicious use of antibiotics to treat the patients today and preservation of newer drugs for future generation should be adopted, whenever possible. Strict adherence to standardised infection control policies and antibiotic policy will decrease the SSIs due to hospital acquired multidrug resistant microorganisms.

A working knowledge of the prevalent organism, virulence and resistance profile will help the infection control practitioner and surgeon to treat the infection effectively at the earliest and also decreases economic burden due to SSI. It also prevents spread of multidrug resistant strains like MRSA which increases mortality and morbidity in patients undergoing surgery.

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