



MICROBIOLOGICAL PROFILE OF EXPLANT CULTURES FROM INFECTED AND NON-INFECTED SURGICAL PATIENTS AND CORRELATION WITH SURGICAL SITE INFECTION

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ABSTRACT **INTRODUCTION** Surgical site infections (SSI) are one of the terrifying nosocomial infections that affecting surgical patients and cause a compromise to the quality of health care provided in the hospital. Nearly 5% of people who undergo surgeries might develop SSI, which can have serious consequences and even be deadly. **AIM:** To identify the microbiological profile of explant culture from suture materials and its correlation in development of SSI in the same patient. **METHODOLOGY:** Infected and non-infected suture materials that were removed aseptically from the patients were collected and transported to the Microbiology laboratory in sterile BHI broth. With the isolated colonies obtained. Identification was performed by standard microbiological technique and antimicrobial susceptibility testing was done. The culture reports of the swab collected from the pus/ exudate from the surgical site and the suture were compared. This was a prospective cross sectional study for a duration of 6 months. **RESULTS:** A sum of 190 isolates were obtained from 182 suture samples. 156(86%) suture materials had growth [monomicrobial (70%) polymicrobial growth (16%)] while 26(14%) had no growth. 7 different suture materials were employed in this study. Isolated organisms were gram positive bacilli (n=20), gram positive cocci (n=82), gram negative bacilli (n=87) and fungi (n=1). *Escherichia coli*, *Klebsiella pneumoniae* (n=19) and *CONS* (n=71) were commonly isolated gram negative organisms. The least common organism grown colonizing the suture material was *Klebsiella oxytoca*, *Citrobacter* species, *Candida* species, *Enterococcus faecalis* and *Enterococcus* species (n=1, 0.5%). Isolates like *Escherichia coli* (n=5), *Klebsiella pneumoniae* (n=2) and *Acinetobacter* species, *Staphylococcus aureus* (n=1) were found to have multiple drug resistance in the suture thread culture. SSI was seen eight cases (4.3%) and in two of them, the microorganism isolated from the surgical site and suture material was identical. **DISCUSSION** Out of 190 isolates in this study, 96 isolates (*CONS*-71, *Streptococcus*-4, *Diphtheroids*-2, *Aerobic spore bearers*-18, *Candida*-1) were members of normal flora of the skin which shows 50.5% of the suture materials were colonized by normal flora. The current study shows that ability of bacteria to adhere varies between different suture materials. Overall, the adhesion of bacteria to 3-0 Ethilon (10.4%) was found to be lower compared to 3-0 vicryl No 1,3 Prolene, 2-0 Ethilon and Silk. Suture thread must be taken out as soon as possible since opportunistic infections could result from the skin's regular flora.

KEYWORDS :

INTRODUCTION:

Surgical site infection is a type of hospital acquired infection where proliferation of microorganisms in the body occurs due to interactions of the host and microbes resulting in disease. One of the terrifying nosocomial infections that affects surgical patients is Surgical Site Infection (SSI)^(1,2,3). In the current situation nearly 5% of people who undergo surgery develop SSIs, resulting in serious consequences even death. The hospital finances are put under a lot of stress managing SSIs⁽¹⁾. Prior to surgery, the patient had to go through three crucial phases, including the pre-surgical, surgical, and post-surgical phases which involves host and microbial factors which might pose heavy risks of obtaining SSI⁽²⁾.

The risk factors for the development of SSI include a lot of host and microbial factors. Although there is no definite data on the number of SSIs linked to sutures, it is plausible to believe that a significant number of SSIs occur at locations harboring suture materials, and that the suture thread acts as a nidus for wound contamination and adherence of microbes. So, suture may be the one of the factors that leads to infection mechanism / colonization⁽³⁾. Sutures are of different types, including absorbable and non-absorbable types. Non absorbable sutures have high tensile capacity and will not be broken down by the body's natural healing process⁽⁴⁾. The biofilm development on suture materials is observed as seen on "implant devices."⁽⁵⁾ In this study, the microbiological profile of non-absorbable suture material is collected from infected and non-infected surgical patients and its correlation to development of SSI in the same individuals were noted.

METHODOLOGY:

This was a prospective cross-sectional study conducted after obtaining, Institute ethics committee approval for a period of six months at KMCH IHSR. The segments of suture materials that were

removed from the patients during their suture removal date or reoperation were collected from various surgical departments like OBG, General Surgery, Orthopedics, ENT, Dentistry and Dermatology. The designation of SSI as superficial/ deep or organ space was adopted by the criteria defined set by NHSN (National Healthcare Safety Network) of CDC (Centers for Disease Control and Prevention). Since the prevalence of SSI due to suture materials is negligible (<5%), all the patients who underwent surgery and came back for suture removal were included in the study. All suture materials that were removed aseptically, collected and transported to the Microbiology division of central laboratory services at KMCH Institute of Health Science and Research (KMCH IHSR) in sterile BHI broth, vortexed and incubated at 37^o overnight. Subsequently the broth was plated on Blood agar and Macconkey agar followed by incubation overnight at 37^o. The isolated colonies obtained were then inoculated into biochemical reactions for identification of organisms and antimicrobial susceptibility testing (AST) was done by Kirby-Bauer method⁽⁶⁾. Using a ruler, the zone of inhibition was calculated. The AST result was divided into three categories: susceptible(S), intermediate(I), and resistant(R). Panel of antibiotics that used for Gram positive cocci and Gram negative bacilli are listed below⁽⁷⁾.

For Gram positive cocci - Erythromycin (15µg), Penicillin (10units), Clindamycin (2µg), Cotrimoxazole (25µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Cefoxitin (30µg), Vancomycin (30µg), Linezolid (30µg), High level gentamicin (120 µg), Ampicillin (10µg). For Gram negative bacilli - Amikacin (30µg), Gentamicin (10µg), Cefuroxime (30µg), Ceftazidime (30µg), Piperacilin tazobactam (100/10 µg), Ciprofloxacin (5µg), Meropenem (10µg), Imipenem (10µg).

The organisms that grew in from the swab / pus taken from the site of suture following surgery and the organisms that grew from the suture material were correlated.

RESULT:

During the study period, 182 samples (suture materials) were collected from various surgeries conducted at KMCH Medical College Hospital, Coimbatore during the study period. Out of the 182 samples processed, 156 (86%) suture materials had growth, while 26 (14%) had no growth in their suture culture as shown in Figure 1. A total of 190 different organisms were isolated from 182 suture materials.

**Fig 1: Percentage of culture positive samples**

Most of the samples were from female patients 122 (67%) with the mean age of 37.02 followed by male patients 60 (33%) with a mean age of 43.51. The average day of suture removal post surgery was 12.02 days (minimum 3 days to maximum 36 days). Monomicrobial growth was seen in 127 samples while 29 samples showed polymicrobial growth (Table 1)

Table 1: Age wise distribution of patients and the various morphotypes

Age (years)	Morphotypes (n)			Total
	Monomicrobial	Polymicrobial	Sterile	
5-20	6	4	3	13
21-30	40	10	7	57
31-40	23	3	10	36
41-50	23	4	1	28
51-60	20	6	4	30
61-70	10	2	1	13
71-90	5	0	0	5
Total	127	29	26	182

Among the 190 microorganisms isolated from 182 suture samples, 82 were gram positive cocci, 20 were gram positive bacilli, 87 were gram negative bacilli and 1 were fungus (Table 2&3) shows the number of gram negative and gram positive bacteria isolated from the suture material. Gram negative and Gram positive microorganisms commonly isolated include *Escherichia coli*, *Klebsiella pneumoniae* (n=19) and *CONS* (n=71) respectively.

Table 2: Characterization of Gram Negative Isolates

Isolates	No. of Isolates	Percentage (%)
<i>Acinetobacter</i> species	16	8.4
<i>Citrobacter</i> species	1	0.5
<i>Escherichia coli</i>	19	10
<i>Enterobacter</i> species	5	2.6
<i>Klebsiella oxytoca</i>	1	0.5
<i>Klebsiella pneumoniae</i>	19	10
<i>Pseudomonas aeruginosa</i>	16	8.4
<i>Pseudomonas</i> species	10	5.2

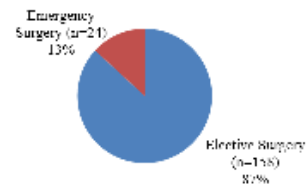
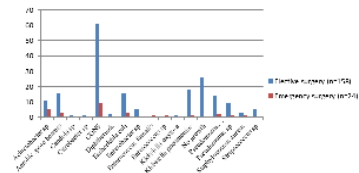
Table 3: Characterization of Gram Positive Isolates

Isolates	No. of Isolates	Percentage (%)
Aerobic spore bearers	18	9.4
CONS	71	37.3
Diphtheriods	2	1.0
<i>Enterococcus faecalis</i>	1	0.5
<i>Enterococcus</i> species	1	0.5
<i>Staphylococcus aureus</i>	4	2.1
<i>Streptococcus</i> species	5	2.6

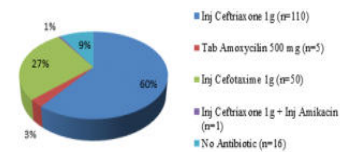
The vast majority of patients underwent elective surgery 158 (87%) and the rest were emergency surgeries 24 (13%). General surgery performed the most elective operation, followed by OBG and OBG had higher numbers of emergency cases. (Figure 2)

The suture material from both elective and emergency surgeries grew *Acinetobacter* species, *CONS*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas* species and *Staphylococcus aureus* as shown in the (Figure 3). *Enterococcus* species was exclusively seen in emergency procedures. All the suture

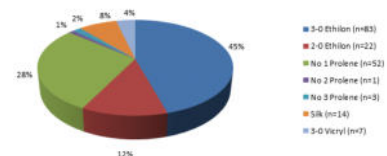
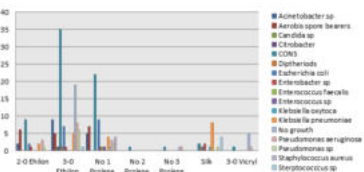
materials that showed no colonization was obtained from patients who underwent elective surgeries. The least common organism grown colonizing the suture material was *Candida* species, *Citrobacter* species, *Enterococcus faecalis*, *Enterococcus* species and *Klebsiella oxytoca* (n=1, 0.5%). In the study done by Owens et al, *Staphylococcus aureus*, *CONS*, *Enterococcus* species, and *Escherichia coli* were the most typically isolated pathogens, depending on the type of surgery (8).

**Fig 2: Percentage distribution of elective and emergency surgeries****Fig 3: Colonizers associated with elective and emergency surgeries**

Antibiotic prophylaxis reduces thread colonization at the end of surgery, bacterial adhesion, and the probability of reducing in infection. Preoperative antibiotics should be given at least 30 but no over than 60 minutes prior to the skin incision, as recommended in the literature (3,6). This protocol was followed at our institution, where 166 (91.2%) patients received preoperative antibiotics. (Figure 4) illustrates several antibiotics and their percentage.

**Fig 4: Various antibiotics given as pre surgical prophylaxis**

There were 31 different types of surgeries included in this study and among them 7 different suture materials were employed; specifics are presented in (Figures 5). The frequently performed surgery was LSCS (n=23, 12.6%) followed by hernioplasty (n=22, 12%). Absorbable sutures (n=7) that are removed are also part of this study as they are removed externally by physicians since the wounds have healed and patients claim on it. (Figure 6) displays the suture material's characteristics as well as the organisms that were isolated from it. Diphtherias (n=2), *Streptococcus* species (n=4), *CONS* (n=71), *Candida* species (n=1) are examples of normal flora species.

**Fig 5: Proportion of various suture materials used****Fig 6: Different types of Suture material and growth characteristics of organism**

Among 87 Gram negative isolates, *Pseudomonas aeruginosa* (n=16) were found to be sensitive to all antibiotics. *Enterococcus* species were

sensitive to Ciprofloxacin, Levofloxacin, Ampicillin, High level Gentamicin, Vancomycin, Linezolid and Erythromycin. All isolated *Staphylococcus aureus* (n=4) were MSSA. No MRSA was found in this study. We found that, sensitivity of Amikacin was 89%, Piperacillin tazobactam was 90%, Ciprofloxacin 83%, Ceftazidime was 77%, Gentamicin 85%, Cefuroxime was 51%, Meropenem was 94%, Imipenem was 97%, Erythromycin was 83%, Clindamycin was 75% and Penicillin, Cotrimoxazole, Levofloxacin, Ampicillin, Vancomycin, Linezolid, High level Gentamicin was 100% sensitive to all the isolates. *Escherichia coli* (n=5), *Klebsiella pneumoniae* (n=2), *Acinetobacter* species and *Staphylococcus aureus* (n=1) were found to have MDR in the suture thread culture. (Table 4) lists bacteria with multiple drug resistance and the drugs to which they are resistant. Three of the *Escherichia coli* and one *Klebsiella pneumoniae* strains is resistant to all six antibiotics used.

Table 4: Suture materials that showed MDR organisms

Gram Negative Isolate	Age/Sex	Diagnosis	Pre Surgical Antibiotic	Procedure	Type of Surgery	Suture material	AST pattern					
							AK	G	CIP	PIT	CAZ	CXM
<i>Escherichia coli</i>	58/F	Incisional Hernia	Inj Ceftriaxone - 1g	Open Mesh Repair	Elective	3-0 Ethilon	R	R	R	R	R	R
<i>Escherichia coli</i>	55/F	Ventral hernis	Inj Ceftriaxone - 1g	Open ventral hernia repair	Elective	2-0 Ethilon	R	R	R	R	R	R
<i>Escherichia coli</i>	24/F	G2P1L1/ Previous LSCS	Inj Cefotaxime - 1g	LSCS	Emergency	No 1 Prolene	R	R	R	R	R	R
<i>Escherichia coli</i>	24/F	G2P1, Breech in Labour	Inj Cefotaxime - 1g	LSCS with Sterilization	Emergency	No 1 Prolene	S	R	R	R	I	R
<i>Escherichia coli</i>	29/F	G2P2L2	Inj Cefotaxime - 1g	Puerperal Sterilization	Elective	No 1 Prolene	S	S	R	S	R	R
<i>Klebsiella pneumoniae</i>	25/M	Right Inguinal Hernia	Inj Ceftriaxone - 1g	Right Inguinal Hernioplasty	Elective	2-0 Ethilon	R	R	R	R	R	R
<i>Klebsiella pneumoniae</i>	50/M	Tooth pain	-	Dental extraction	Elective	Silk	S	S	R	S	R	R
<i>Acinetobacter</i> species	21/F	Primi at 37 weeks 2 days with failed induction	Inj Cefotaxime - 1g	LSCS	Emergency	No 1 Prolene	I	S	I	I	R	R
Gram Positive Isolate	Age/Sex	Diagnosis	Pre Surgical Antibiotic	Procedure	Type of Surgery	Suture material	AST pattern					
<i>Staphylococcus aureus</i>	45/F	AUB	Inj Cefotaxime - 1g	Total AH+BSO	Elective	No 1 Prolene	E	P	CIP	CD	CX	COT
							R	S	R	R	S	S

AK= Amikacin, G=Gentamicin, CIP= Ciprofloxacin, PIT= Piperacillin tazobactam, CAZ= Ceftazidime, CXM= Cefuroxime, E= Erythromycin, P=Penicillin, CD= Clindamycin, CX= Cefoxitin, COT= Cotrimoxazole, S= Sensitive, I=Intermediate, R=Resistant, LSCS = Lower Segment Cesarean Segment, AUB= Abnormal uterine bleeding, AH+BSO= Abdominal Hysterectomy+ Bilateral salphingo-oophorectomy)

In the present study there was secondary suturing done for four cases. The organisms obtained (CONS) from primary and secondary suture were similar in two cases. (Table5) shows the relationship between the organism that causes surgical site infection and the organism that was isolated from the suture material. There were eight cases of SSI and in two of them, the microorganism isolated from the surgical site and suture material were identical. The other six suspected by the clinician as surgical site infection were culture sterile or no growth.

Table 5: Incidence of SSI

Age/Sex	Diagnosis	Pre Surgical Antibiotic	Procedure	Surgery type	Suture material	Time interval*	Site	Suture
51/M	Chronic otitis media	Inj Ceftriaxone- 1g	Left Mastoidectomy	Elective	3-0 Ethilon	13	No Growth	CONS
24/F	Primi (failed Induction)	Inj Cefotaxime- 1g	LSCS	Emergency	No1 Prolene	7	Enterococcus faecalis	Enterococcus faecalis
49/M	Abscess over abdomen	Inj Cefotaxime- 1g	Incision and Drainage	Elective	3-0 ethilon	19	No Growth	<i>Klebsiella pneumoniae</i>
45/F	AUB	Inj Cefotaxime- 1g	Total AH +BSO	Elective	No1 Prolene	13	No Growth	MSSA
52/F	Adenomyosis	Inj Cefotaxime- 1g	Total AH +BSO	Elective	No1 Prolene	12	No growth	CONS
55/F	Ventral hernia	Inj Ceftriaxone- 1g	Open ventral hernia repair	Elective	2-0 Ethilon	15	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
65/F	Left breast carcinoma	Inj Ceftriaxone- 1g	Modified radical mastectomy	Elective	2-0 Ethilon	19	No growth	<i>Escherichia coli</i>
59/F	Infected Tibia IM nail- Insitu	Inj Ceftriaxone- 1g	Tibia IM nail removal	Elective	3-0 Ethilon	23	No growth	No growth

(*Time interval between date of surgery and suture removal)

AH+BSO – Abdominal hysterectomy+ Bilateral salphingo-oophorectomy AUB- Abnormal uterine bleeding

LSCS -Lower Segment Cesarean Segment)

DISCUSSION:

Contamination of the wound area, the virulence of the bacteria, and the immunological response of the host all affect the likelihood of SSI. The microorganism that promotes SSI typically originates endogenously from the skin or opened viscus of the patient. The exogenous sources of SSI are operating room, equipment or the surrounding environment. Rarely, SSI is brought on by the spread of pathogens from outdoor sources attaching to the device or other implantation left in the surgical site. To try to reduce the amount of germs at the surgical site, SSI infection prevention and control procedures are required⁽⁷⁾.

In order to determine the variations in the microorganism isolated from various types of surgery and the role of suture materials in SSI, we analyzed the role of the suture thread in 182 infected and non-infected patients who underwent both elective and emergency surgery with or without pre surgical antibiotics. As a result 86% shows growth and 14% were sterile in their suture culture.

The developments of SSI in elective cases were 87% and emergency surgeries were 13%. A similar observation was found in the study done by Shahane *et al*, where SSI has happened more frequently in elective procedures (7.9%) than in emergency surgeries (2.7%). This finding may come as a surprise, as emergency cases have been known to end up in SSI more frequently than elective cases. Furthermore, in study, there have been more elective cases (63%) than emergency cases during the course of the year (37%)⁽⁹⁾. There was no significant correlation between the development of SSI and antibiotics usage. Most of the cases which received pre surgical antibiotic prophylaxis showed no signs of SSI in this study.

Out of 190 isolates in this study, 96 isolates were members of normal flora of the skin. This indicates that almost 50.5% of the suture materials were colonized by normal flora of the skin. Infections at the surgical field are usually triggered by patients' own microorganisms^(2,3). Drug resistance was common, particularly among Enterobacteriaceae⁽¹⁰⁾. Resistance to antibiotics was higher among the gram negatives than gram positives in the present study. The gram negatives showed their maximum resistance against cephalosporins.

The current study shows that the ability of the bacteria to adhere differs for different suture materials. Overall, adhesion of bacteria to 3-0 Ethilon (10.4%) was found to be lower compared to 3-0 vicryl No 1,3 Prolene, 2-0 Ethilon and Silk. It was also noted that organisms isolated from surgical sites were correlated with organisms isolated from sutures. Two of the eight suspected cases of SSI showed growth with positive correlation, while the others were sterile. This demonstrates that a patient's environment, medical professionals, immunity all have a notable effect on their chance of developing SSI.

Pathogens that form biofilms are extremely difficult to eradicate, making them a common source of potentially fatal hospital-acquired infections^(5,11). Non absorbable sutures are almost as a lasting implant as any of the bigger implanted medical devices which are now known to be prone to biofilm infections and should be considered as a possible source of infectious problems and cause chronic SSI⁽¹²⁾. Fortunately, as per (12), no biofilm formation was observed in our SSI cases.

Wound hygiene is critical following surgery. The gold standard is to avoid handling wounds and dressings with bare hands using 'non-touch' techniques. For rinsing the wound, sterile saline should be used. The patient should take a shower and wash his or her body with soap 48 hours after surgery. It is not advised to use local antibacterial treatments to lessen the risk of infection⁽²⁾. The best strategy to prevent SSI is to remove the suture as soon as the incision heals and practice good wound cleanliness.

CONCLUSION

Our results dictate that, the first choice of suture materials between the present tested, whenever possible should be 3-0 Ethilon. If 3-0 Ethilon sutures cannot be used, the selection of the other tested suture materials with minimal effect on bacterial adherence need to be utilized based on the personal preference of the surgeon. But many sutures were found to house bacteria, any suture could be as a port of entry for infection, which in turn may compromise wound healing. It is thus advisable to minimize the duration of the presence of sutures on the patient, and planned removal as early as possible, according to the healing conditions of the surgical site.

LIMITATIONS OF THE STUDY

Due to the pandemic situation that prevailed during the study period, many patients included in the study opted to visit the medical services near to their place of stay, they were lost to follow up for the development of SSI within the appropriate timeframe of 30 days or 90 days.

ABBREVIATIONS

ASB- Aerobic spore bearers

CONS- Coagulase negative Staphylococcus

MSSA- Methicillin sensitive *Staphylococcus aureus*

MRSA- Methicillin resistant *Staphylococcus aureus*

SSI- Surgical site infection

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