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Bacterial Load on Suture Material after Surgical Removal of Third Molars – A Comparative Clinical Studies between Silk Suture Vs Antibacterial Suture

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Abstract
Oral cavity harbors a complex network of microorganisms which are in steady state of equilibrium with another microflora. Surgical removal of third molar is a very common procedure in oral surgery and suturing of surgical site is a crucial component determining success of wound healing. These suture materials sometime act a nidus of infection because of potential adherence of bacteria to its rough surfaces which may lead to surgical site infection. AIM- The aim of this study was to compare bacterial load on normal silk suture over antibacterial suture following third molar removal in 50 healthy individuals free of any systemic and local pathology. MATERIALS AND METHODS – A microbiological analysis using culture sensitivity test of distal most suture was evaluated after 7 days of procedure.
RESULT - In relation to the colony count silk group showed higher number of colonization with a median of 80,000 cfu/ml. Relatively on the other side, antibacterial showed significant decrease in number of colonization with a median of 11,000 cfu/ml (p value < 0.0005). CONCLUSION – Antibacterial sutures group showed statistically significant reduction in bacterial count and can be possible alternative in patients who are unable to maintain good oral hygiene.

Keywords – *Surgical-site infection, microflora,antibacterial,colony forming unit*

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I. Introduction

Sutures have been around for thousands of years and are used to hold wound together until the healing process is complete. It was described as far back 3000 BC in ancient Egyptian literature[1]. They are the most implanted biomaterials in the human body forming an integral part of the surgical operation.

In the last few decades, several improvements of the suture materials have been introduced to enhance physical, chemical and biomechanical properties. Sutures are an integral part of surgical operations. They sometimes behave like foreign bodies. It can also contribute to the growth and multiplication of bacteria in areas which are prone to bacterial colonization like the oral cavity.

Indeed, many distressing complications such as infection, wound disruption and chronic sinus formation occur in a sutured wound. Previous studies indicate that suture materials vary in their propensity to produce bacterial infection in surgical wounds. The physical configuration of the suture thread has been suggested to be an important factor in determining its susceptibility to surgical infection. Thus, multifilament suture has been known for their compliance leading to secure and compact knots[2]. However, their intrinsic surface roughness and capillarity increase the potential of wound infection. Thus, sutures in multifilament form result in higher wound infection than the same sutures in monofilament form.

To solve this problem, many researchers have proposed various methods to develop antimicrobial non-absorbable multifilament sutures by using antimicrobial agents, compounds that have the ability to kill or inhibit the growth of microbes, thus preventing infection[3]. These include: antibiotics that are capable of inhibiting the life processes of all foreign organisms and antibacterial that kill and prevent the growth of bacteria. Previous research has shown that the antimicrobial activity in sutures can be achieved by blending or incorporating volatile or non-volatile antimicrobial agent while processing, coating or graft polymerization followed by immobilization of antimicrobial agents onto the suture surface[2,3]. Coating has been the most common technique used for applying the antibacterial agents on the textile surface.

In 2004, Ethicon Inc. developed and marketed the first antibacterial sutures on the market called Vicryl Plus, Monocryl Plus and PDS II Plus. These absorbable sutures have been coated with Triclosan and have an antibacterial effect against Staphylococcus Aureus, Staphylococcus Epidermidis, Escherichia coli and Klebsiella pneumoniae. Following the commercialization of Vicryl plus suture by Ethicon Inc., several works

have been conducted and confirmed the effectiveness of this suture. Alonso et al. and Rothenburger et al.[7] have also proved the antibacterial effect of this suture against Staphylococcus aureus and Staphylococcusepidermidis and Marzo et al. have shown a decrease of infection with Pseudomonas aeruginosa germs[4]. The success of these sutures have been also confirmed by a statistical survey, proving that the use of antibacterial sutures leads to reduction in the infection frequency. With this goal, in this study we compared normal Silk suture and ETHICON plus Antibacterial suture after surgical removal of lower third molars.

II. Objectives

This study was conducted to testthe efficacy of antibacterial sutures over silk sutures in reducing bacterial count and undergoing surgical removal of the lower third molar and also to compare bacterial colony growth on both suture materials post-operatively.

Inclusion and exclusion criteria

Inclusion criteria includes healthy patients (ASA I or ASA II) of both sexes aged between 16-45 years diagnosed with partially or completely impacted lower third molars. While, exclusion criteria included patients with systemic disease (immune- depression, active infection, diabetes mellitus, hemorrhagic diseases),pregnancy, Peri coronal pathology associated with tooth of interest, drug abusers, patient with moderate alcohol consumption, Patients who have taken antibiotic in last three months.

III. Materials And Methods

A prospective-double blinded clinical study was designed and the sample size of 50 was calculated based on G power software where both participants and sample researcher would be blinded. Patients were segregated equally into two groups Group A (control group) and Group B(test group) twenty five each with the help of simple random sampling method. All patients undergoing removal of third molars received oral prophylaxis and antibiotic prophylaxis of 1 gm Amoxicillin, 2 hours before surgery and post op instructions consisting of tooth brushing and cleaning of surgical wound with physiological saline rinse three times a day. The local anaesthetic used was 2%lignocaine with adrenaline 1:80000. At least four simple interrupted suture 3/0 was used, normal silk suture in Group A patients and ETHICON antibacterial suture in Group B patients. Both the groups received same postoperative medicine that consisted of tab IMOL(ibuprofen +paracetamol) and Ranitidine 150 mg for five days in both the groups. The clinical variables will be the presence of bleeding and surgical wound suppuration upon removing the sutures 7 days after surgery.

SAMPLE PROCESSING

One suture knot of 1cm from the most distal side of operated site was removed after 7 days post operatively in each patient. Each suture sample was collected in 1ml of Normal saline medium and was analysed in microbiology laboratory.

After receiving the sample, the sample was thoroughly mixed in Vortex mixture (Fig.1). 10 ml of vortex sample mixture was inoculated in appropriate culture media. In our study culture media used was MacConkey's Agar, Brain Heart infusion Agar, Sabouraud Dextrose Agar and Blood Agar(Fig . Inoculation of clinical sample was done by Streak plating technique culture(Fig.3,4,5 and6) plate and was incubated for 48 hours for effective growth of microorganisms. Following the incubation process, the colonies on each plate were counted per colony forming units (cfu/cm/ml).(Fig 2) .Calculation of the differences in total count of microorganisms isolated from both type of suture material will be carried out using chi square test.

IV. Result

The study sample consisted of 28 men and 22 women,aged between 18 and 40 years, with a mean age of 26 years (standard deviation (SD) of 4.77). suture. The mean microorganisms count after 3 days was considerably lower with the antibacterial suture. According to these results, there was mean bacterial reduction of 87.3 %. (Table 1 ,Table 2 and Table 3) .

In relation to the colony count,Group A (silk) showed higher number of colonization with a median of 80,000 cfu/ml (Graph 1). Relatively on the other side, Group B (Ethicon) showed significant decrease in number of colonization with a median of 11,000 cfu/ml (Graph 1). Among the most frequently isolated species, mention must be made of Streptococcus viridians group (S. mitis, S. oralis, S. salivarius, S. parasanguis, S. sanguinis, S. anginosus and S. intermedius) Coagulase-Negative Staphylococcus, Pepto streptococcus spp., Lactobacillus spp. and Enterococcus faecalis. In general, Monocryl Plus yielded a lower count for almost all the isolated species with most frequently isolated was viridians group of streptococci species. However, there was no statistical significance of isolated organisms between two groups. However, isolated pathogenic organism like Staphylococcus Aureus, Pepto streptococci and E. coli was only grown in Group A sample media.

Although there were 5 cases of complications, 4 in Group A and 1 in Group B but it was beyond the scope of this study to clinically correlate the occurrence of complications with bacterial load on suture material.

V. Discussion

The bacteria that cause infection are most commonly part of theindigenous bacteria that normally live on or in the host. Odontogenic infections are no exception because the bacteria that cause odontogenic infections are part of the normal oral flora: those that comprise the bacteria of plaque, those found on mucosal surfaces, and those found in the gingival sulcus[6]. These bacteria are primarily aerobic gram-positive cocci, anaerobic gram-positive cocci, and anaerobic gram-negative rods. These bacteria cause a variety of common diseases such as dental caries, gingivitis, and periodontitis.

Many carefully performed microbiologic studies of odontogenicinfections have demonstrated the microbiologic composition ofthese infections. Several important factors must be noted. First, almost all odontogenic infections are caused by multiple bacteria.

The polymicrobial nature of these infections makes it important that the clinician understand the variety of bacteria that are likely to cause infection[9]. In most odontogenic infections, the laboratory can identify an average of five species of bacteria. It is not unusual to identify as many as eight different species in a given infection. On rare occasions, a single species may be isolated. New molecular methods, which identify the infecting species by their genetic makeup, have allowed scientists to identify greater numbers and a whole new rangeof species[4], including unculturable pathogens, not previously associated with these infections.

Surgical site infection (SSI) is the third most common cause of nosocomial infections, and the most among surgical patients[2]. Two-thirds of all cases of SSI appear in the zone of the incision. This probability is even greater in the presence of suture material . It has been estimated that with conventional sutures (such as the natural black silk), barely 100 cfu would be needed to induce SSI[2,3] . Many methods have been studied to decrease the incidence of surgical site infection, although some are uncontrollable others can be controlled. One of these methods is the use of sutures coated with triclosan. In 2002, the United States Food and Drug Administration (FDA) authorized the use of polyglactin 910 coated with triclosan (Vicryl® Plus, Antibacterial suture).[3,5] Most studies conducted with sutures of this kind report a decrease in the amount of microorganisms sticked to their surface. However, Venema*etal*. [14], in an *in vitro* study with Vicryl® Plus suture, recorded no bacterial inhibition zone around the suture with either *Streptococcus sanguis*PK1889 or microorganisms from a human saliva sample. In contrast, animal studies have obtained favourable results. Storch *et al*. (12) reported a reduction of 96.7% with Vicryl® Plus suture after 48 hours in strains of *S.aureus*. Ming *et al*. [8], in a similar study but using Monocryl® Plus suture, recorded a bacterial reduction in the order of 3.4 log and 2 log in strains of *S.aureus*and *E. coli*, respectively. Gómez Alonso *et al*. (11) in turn obtained a reduction of about 87% with Vicryl® Plus suture previously infected with *S. epidermidis* and *E. coli*. Lastly, Marco *et al*. [4], in a study using rats, reported a 66% reduction in cultures positive for *S.epidermidis*. This is the first human study to date of the antibacterial action of Monocryl® Plus monofilament suture based on a quantitative and qualitative analysis of the microorganisms. The sutures provide the support necessary to maintain wound-edge approximation during the critical healing period (5-7 days after surgery) due to the high initial breaking strength, pass smoothly through fascia to minimize tissue trauma as consequence of its monofilament design and polymer properties that minimize drag force and elicit only a slight tissue reaction during absorption[13]. Furthermore, protect against colonization of the suture by organisms commonly associated with SSIs. In our study colonization rate was 83 percent lower than with silk suture after 7 days.

Triclosan is an antiseptic component with bacteriostatic action. At low concentrations, inhibits the growth of many nonsporulating gram-positive and gram-negative bacterial species. The amount added to these sutures reaches 1.5 μg/cm, and the range of minimum inhibitor concentrations (MICs) against the microorganisms that inhabit the oral cavity is 0.00178 μg/ml . In our study, the presence of triclosan in Monocryl® Plus was associated to a significant reduction of most microorganisms isolated after 7 days. The opposite effect was recorded with silk suture ,a mean of 83600 cfu/cm/ml was present at the end of day 7,while only mean of 11000 cfu/cm/ml (Graph 1 and Table 3) was present in Antibacterial suture.

However, isolated bacteria from silk suture were more diverse in contrast to antibacterial suture that was limited predominantly to viridians group of streptococcus and coagulase negative staphylococcus (Fig.7).

Differences in bleeding in our study were not significant, though either the effects of the remaining traces of triclosan or the lesser bacterial aggregation associated with the use of Monocryl plus caused the inflammatory reaction to be less pronounced with the antibacterial suture material after 7 days. No significant differences were recorded in the level of pain experienced by the patients with the two suture materials. However, postoperatively the incidence of complications was greater with silk suture as compared to Antibiotic group, but whether these suture has any role in incidence of infection was beyond the scope of this study.

The main drawback of this study is that study and controlcases were not performed on the same patients as standardizing patient oral biological flora would have given better outcomes. This method was not taken into consideration as bilateral extractions are unlikely to be accepted by patients under local anaesthesia on the same day.For this reason it would be advisable to carry out a clinical study with a tissue biopsy and to do further histopathological study at cellular level in order to determine whether antibacterial sutures effectively contribute to lessen surgical site infections in patients subjected to lower third molar extractions.

Although clearing apart the limitations, this study clearly proved the superiority of antibacterial sutures over silk sutures in terms of reducing overall bacterial counts. From the clinical aspect, the antibacterial sutures should be considered in patients who has low immunity like diabetes, patients on low steroid therapy and patients who are unable to maintain good oral hygiene.

VI. Conclusion

There was a statistically significant difference in the bacterial load between both groups (p value-0.000), showing a marked reduction in antibacterial group.

The current study showed adequate clinical wound healing 7 days on suture removal after surgical extraction of impacted mandibular third molars in both the groups indicating that wound healing in healthy individuals is adequate irrespective of the types of sutures placed. The shortcoming is this study is that the tissue response to each type of suture was not studied.Although, the rate of post-operative complications cannot be correlated clinically with both types of sutures but we can safely say that antibiotic coated suture reduces the chance of local infection at the surgical site by bringing down the colony counts. Hence, antibiotic coated suture can be taken as consideration in medically compromised patients like diabetes where chances of surgical site infections are relatively higher than the healthy individuals.

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DECLARATION
The authors declare that they have no conflict of interest.

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Fig.1 Vortex mixture

Fig.2 Inoculation of sample by Streak plating technique culture.

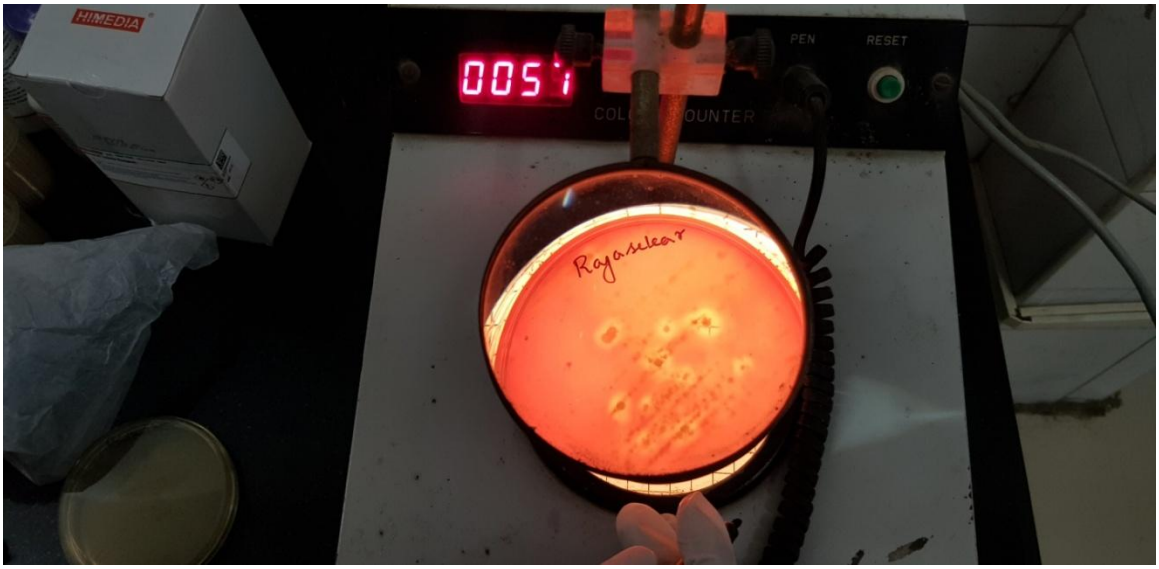


Fig.3 Colonies on each plate were calculated in colony forming unit/cm/ml.

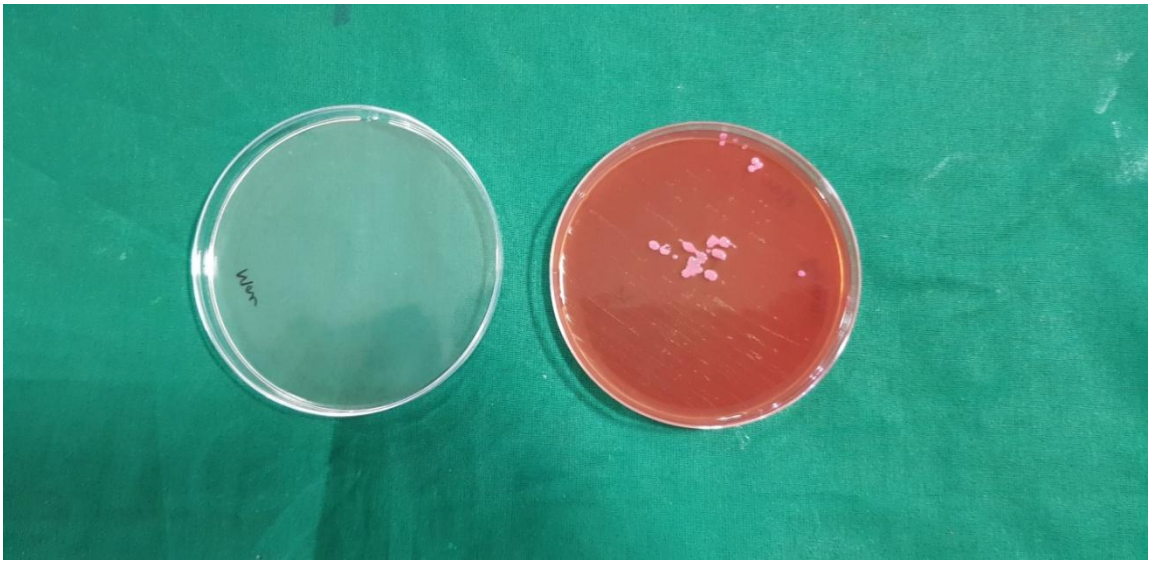


Fig 4. Inoculation of sample on Blood Agar

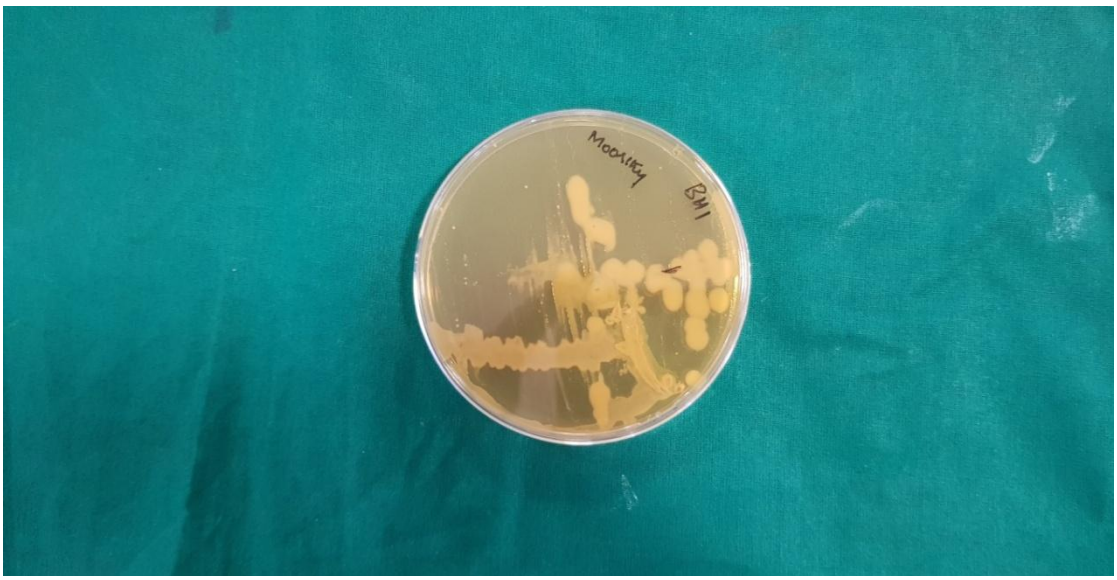


Fig. 5 Inoculation of sample of Sabouraud dextrose Agar

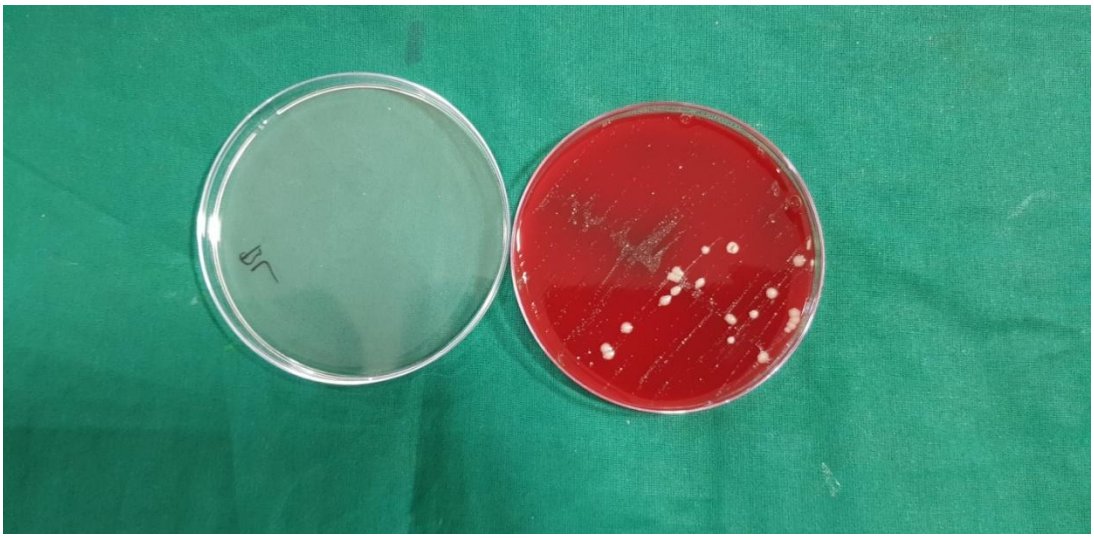


Fig. 6 Inoculation of sample on Brain Heart infusion Agar

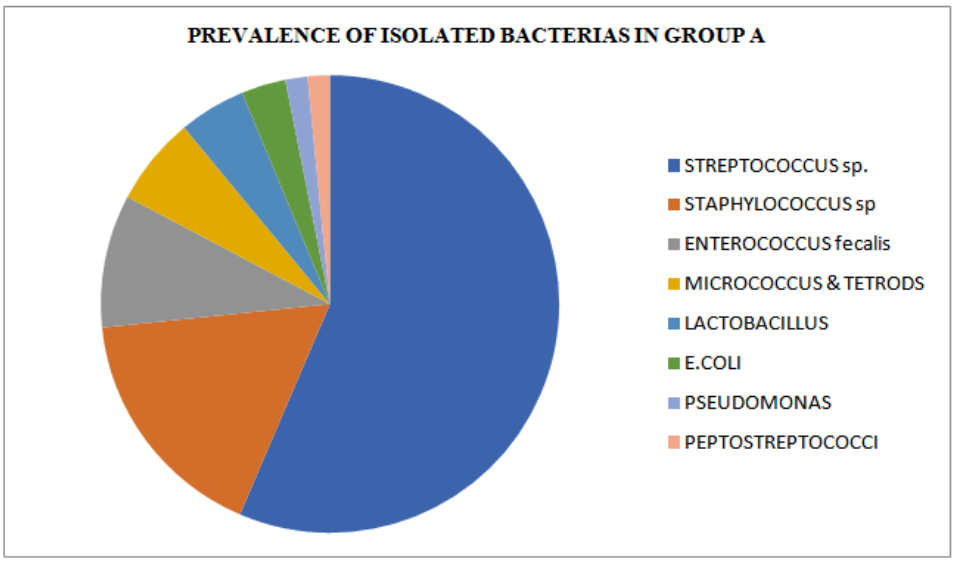
Table 1- Bacterial load in Group A Samples (Silk sutures)

SAMPLE NO	COLONY COUNT (cfu/ml)	BACTERIA IDENTIFIED	POST OP COMPLICATIONS
1	1,00,000	ENTEROCOCCI STAPHYLOCOCCUS sp. (CONS) STREPTOCOCCUS sp. (Viridians)	
2	1,20,000	MICROCOCCI & TETRODS STAPHYLOCOCCUS sp. (CONS) ENTEROCOCCUS feacalis STREPTOCOCCI sp. (Viridians)	Wound gaping
3	80,000	STREPTOCOCCI sp. (Viridians)	
4	50,000	MICROCOCCI &TETRODS E.COLI PSEUDOMONAS sp. STREPTOCOCCI sp.	
5	1,00,000	STAPHYLOCOCCUS aureus STREPTOCOCCUS sp. (Viridians)	Moderate pain and trismus
6	1,30,000	STREPTOCOCCUS sp. (Viridians)	
7	1,20,000	STAPHYLOCOCCUS aureus MICROCOCCUS TETRADS ACENATOBACTER	Wound dehiscence
8	75,000	STREPTOCOCCUS sp. (viridians) STAPHYLOCOCCUS sp. (CONS)	
9	1,10,000	CANDIDA sp. STREPTOCOCCUS sp. (viridians) LACTOBACILLUS	
10	50,000	STREPTOCOCCUS sp. (viridians)	
11	70,000	STREPTOCOCCUS sp. (viridians)	
12	1,00,000	MICROCOCCUS& TETRODS ENTEROCOCCUS feacalis STAPHYLOCOCCUS (cons)	
13	1,00,000	STAPHYLOCOCCUS (cons) ENTEROCOCCI STREPTOCOCCUS sp. (viridians)	
14	1,00,000	STREPTOCOCCUS sp. (viridians)	
15	1,00,000	LACTOBACILLUS sp. STREPTOCOCCUS sp.(viridians)	
16	1,00,000	STREPTOCOCCUS sp. (viridians)	Dry socket
17	60,000	STREPTOCOCCUS sp. (viridians) STAPHYLOCOCCUS aureus	
18	50,000	STREPTOCOCCUS sp.(viridians) PSEUDOMONAS sp.	
19	1,00,000	STREPTOCOCCUS (viridians) E.COLI	
20	50,000	ENTEROCOCCUS fecalis	
21	50,000	STAPHYLOCOCCUS (cons) PEPTOSTREPTOCOCCI	
22	75,000	STREPTOCOCCI sp. (viridians)	
23	50,000	STREPTOCOCCUS sp.(viridians) ENTEROCOCCUS fecalis PSEUDOMONAS sp.	
24	75,000	STREPTOCOCCUS sp. (viridians)	
25	75,000	STREPTOCOCCUS sp.(viridians)	

Table 2- BACTERIAL LOAD IN GROUP B SAMPLES(MONOCRYL ETHICON PLUS)

SAMPLE NO	COLONY COUNT	BACERIA IDENTIFIED	POST OP COMPLICATIONS
1	8,000	STREPTOCOCCUS sp (viridians)	
2	15,000	STREPTOCOCCUS sp. (viridians)	
3	12,000	MICROCOCCI & TETRODS STREPTOCOCCUS (viridians) STAPHYLOCOCCUS (CONS)	
4	15,000	MICROCOCCI & TETRODS STREPTOCOCCUS sp.(viridians)	
5	12,000	STREPTOCOCCUS sp. (viridians) STAPHYLOCOCCUS sp. (cons)	
6	11,000	STREPTOCOCCUS sp. (viridians)	

		LACTOBACILLUS	
7	10,000	STREPTOCOCCUS sp. (viridians)	
8	8,000	STREPTOCOCCUS sp. (viridians)	
9	8,000	STREPTOCOCCUS sp. (viridians)	
10	40,000	STAPHLOCOCCUS sp. (cons) PEPTOSTREPTOCOCCI	Dry socket
11	8,000	STREPTOCOCCUS sp. (viridians)	
12	10,000	STREPTOCOCCUS sp. (viridians)	
13	14,000	ENTEROCOCCUS fecalis	
14	8,000	ENTEROCOCCUS fecalis	
15	8,000	STREPTOCOCCUS sp.(viridians)	
16	-	-	
17	14,000	STREPTOCOCCUS sp. (viridians)	
18	40,000	MICROCOCCI & TETRODS	
19	12,000	STREPTOCOCCUS sp.(viridians)	
20	8,000	MICROCOCCI & TETRODS	
21	6,000	STREPTOCOCCUS sp. (viridians)	
22	14,000	STREPTOCOCCUS sp. (viridians)	
23	30,000	LACTOBACILLUS	
24	20,000	STREPTOCOCCUS sp.(viridians) STAPHYLOCOCCUS sp. (cons)	
25	10,000	MICROCOCCI & TETRODS	



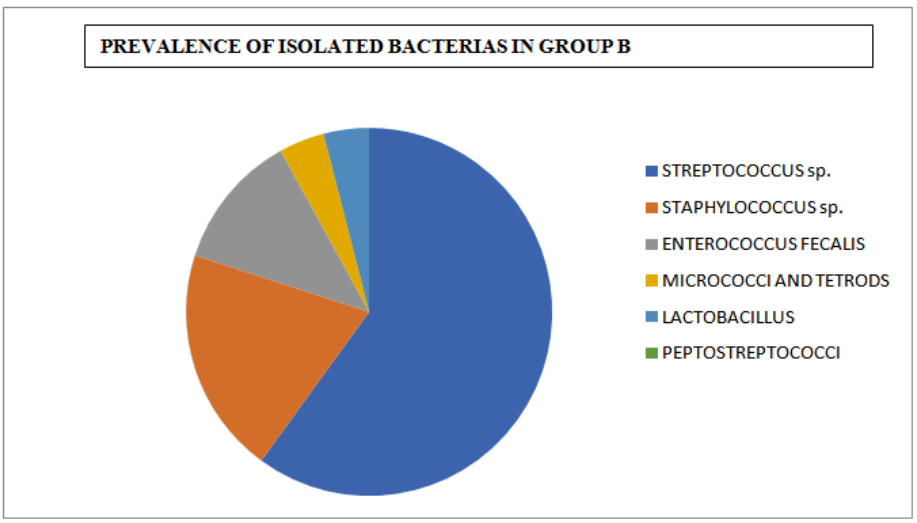
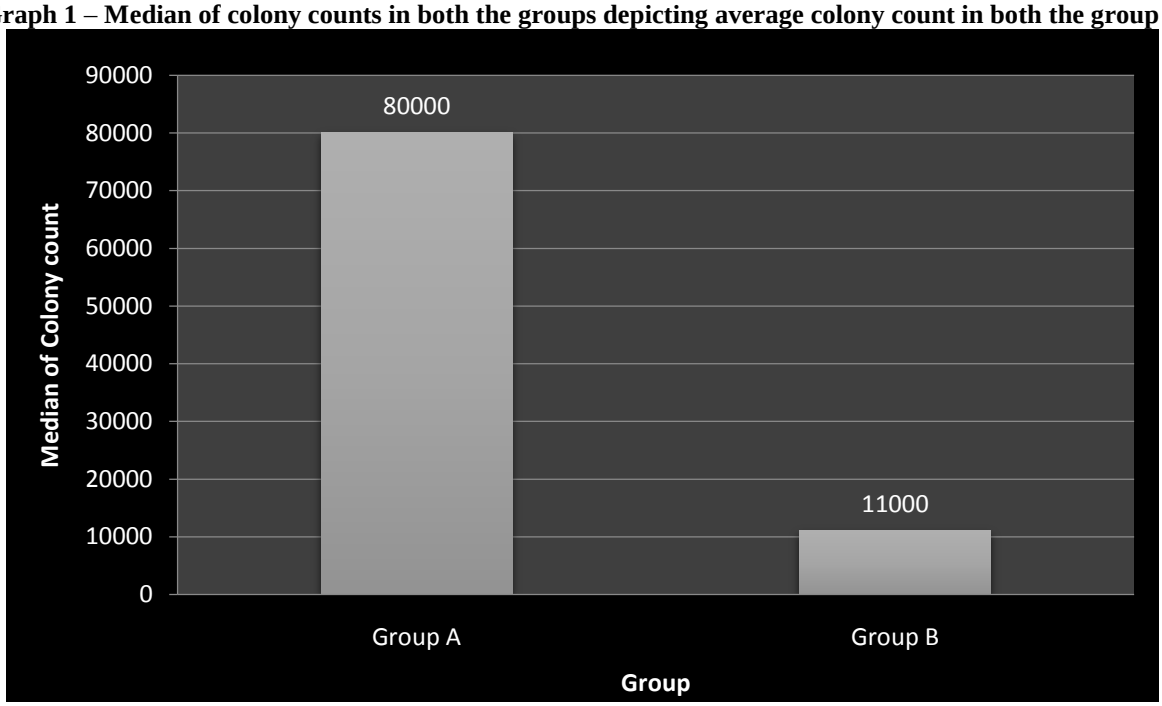


Fig. 7 Pie-chart representation of bacteria isolated in both the groups.

Table 3- Mean colony count in both the samples

Group	N	Mean	Std. Deviation	Median	IQR	P value
Group A	25	83600.0000	25393.56874	80000.0000	45000.00	0.000
Group B	25	13640.0000	9625.83330	11000.0000	6500.00	

Graph 1 – Median of colony counts in both the groups depicting average colony count in both the group



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