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Comparative Evaluation Of Clindamycin And Tetracycline On Microbial Decontamination Of Autogenous Bone Graft

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Abstract
Background*: Autogenous bone particles can be obtained with bone collectors during implant osteotomy; however, the collected bone particles contain oral microorganisms that may cause infectious complications.*
Materials and Methods: *Twenty bone samples were procured from a patient undergoing the implant procedure, using a bone collector with low-speed drilling and a standard bone collector from the implant site. The collected autogenous bone grafts were equally divided into 3 groups by weight. Group 1 (control group): Samples were not treated with any antimicrobial agent; Group 2: Clindamycin solution was used to immerse the bone sample for 3 min at a concentration of 20 µg/mL; Group 3: Tetracycline solution was used for the immersion of the bone sample for 3 min at a concentration of 50 mg/mL. A stringent aspiration protocol, prophylactic antibiotics, and preoperative chlorhexidine oral rinse were used to collect autogenous bone grafts from the patient. Each sample was placed in thioglycolate broth. After being vortexed for 30 seconds to homogenize the solutions, all of the samples were serially diluted (1:5, 1:10, 1:100, 1:1000). Thereafter, samples were streaked on petri dishes containing MacConkey, blood, and nutrient agar. The samples were kept in an anaerobic jar with 85% nitrogen and 15% CO_2 for 24-48 hours at 35-37°C for anaerobic culture. Gram staining was used to identify the organisms under a microscope, and colony-forming units per mL (CFU/mL) were calculated.*
Results*: One-way analysis of variance revealed that the control group (Group-1) had the highest CFU/mL count, while tetracycline (Group-3) had the lowest values, and multiple pairwise comparisons showed that there was no significant difference in the overall CFU/ml between the tetracycline and clindamycin groups.*
Conclusion: *Bacterial contamination was effectively minimized by immersion in acceptable concentrations of both clindamycin (20 µg/mL) and tetracycline (50 mg/mL) for 3 min.*
Key Word: *Dental Implant; Stringent Aspiration Protocol; Bacterial Decontamination; Clindamycin; Tetracycline.*

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I. Introduction

A poorly treated decay, genetic alteration of enamel or dentin, and trauma are sufficient to cause tooth loss. Thankfully, there are usually tooth replacement options available, including dentures, dental implants, and bridges but the local conditions, the state of the teeth, and the cost of the treatment all influence the solution. The increasing mean age of the population, and consequently, edentulism, necessitates prosthesis demand. Owing to the drawbacks of the removable partial denture and fixed partial denture, the implant as a treatment option has become popular.[1,2] Bone grafts are widely and routinely used to reconstruct defects, particularly in dental implants. However, it is problematic because the retrieved bone may be contaminated by microorganisms. Even when all the precautions are taken, bone grafts can get contaminated by common bacterial flora, regardless of whether they are harvested as a single piece or gathered as bone fragments.[3,4]

In surgical procedures like dental implant surgery, sinus lifting, filling cyst cavities, or augmentation procedures combined with the use of allogenic or alloplastic graft materials, particles collected by bone filters—collectors made of a filter placed in the surgical suction device—may be a better way to obtain bone grafts

(Figure 1). From the collected bone fragments, particulate autogenous bone is readily extracted. A strict aspiration strategy, antibiotic prophylaxis, and preoperative oral cavity cleaning with a CHX mouthwash are some of the methods that have been proposed to lower the risk of bone contamination. Local administration of antimicrobial drugs was suggested since the serum concentration of oral antibiotics is insufficient for antimicrobial effects on the reconstructed regions due to a temporary decrease in the blood flow to the surgical site. To ensure that the drug doesn't interfere with the cells' capacity to form new bone, it is essential to take it at the appropriate dosage and for the appropriate length of time.[4,5]

Figure 1: - Bone collector

II. Material And Methods

This comparative study was carried out on patients of Department of prosthodontics, crown and bridge & implantology at Darshan dental college and Hospital, Loyara, Udaipur, Rajasthan from February 2023 to January 2025. A total 20 adult subjects (both male and females) of aged ≥ 18, years were for in this study.

Study Design: Original Research Study

Study Location: Department of Prosthodontics, Crown and Bridge & Implantology at Darshan Dental College and Hospital, Loyara, Udaipur, Rajasthan

Study Duration: February 2023 to January 2025.

Sample size: 20 patients.

Subjects & selection method: The study population was drawn from patients who presented at Darshan Dental College and Hospital, with chief complaint of missing mandibular molar and wanted to replace them with fixed restorations from February 2023 to January 2025.

Inclusion criteria:
1. Either sex
2. Aged ≥ 18 years,
3. No recent history of viral or bacterial disease.
4. Edentulous area in the posterior mandibular region for implant placement.

Exclusion criteria:
1. Systemic disease
2. Periodontitis
3. Poor oral hygiene.
4. History of bone augmentation at the site of the implant.
5. Allergy to various drugs.

Procedure methodology:

The patient received 1 g of amoxicillin as an oral prophylactic antibiotic and 10 ml of 0.2% chlorohexidine mouthwash to rinse for 2 min. Local Anaesthesia (0.2% lignocaine with adrenaline) was administered to the involved site. To view the underlying bone, a crestal incision was performed and a full-thickness flap was raised after the subjective and objective symptoms of local anesthesia were confirmed. Osteotomy site preparation was performed using sequential drilling with 0.9% saline solution used as an irrigant and bone collection from the drill threads was performed using low-speed drilling (Figure 2). The stringent aspiration protocol was followed, where a separate bone collector with a suction tip was used to collect bone directly from the surgical site and control of salivary flow was achieved using separate suction. Once the final

osteotomy site was prepared, the implant was placed, the cover screw was secured, and the sutures were placed. The same surgical protocol was followed for all 20 samples. The collected bone sample was placed in a sterile container with 1 ml of 0.9% saline solution and taken to the laboratory for microbial analysis.

Figure 2: - (a) Bone collected from sequential drilling; (b) Collected bone sample in sterile container

Laboratory procedure:

The collected sample from each patient was then divided into three equal portions by weight (Figure 3),

Group 1: Sample without antimicrobial treatment.

Group 2: Bone samples were placed in 1 ml of clindamycin solution (20 µg/mL) for 3 min.

Group 3: Bone sample was placed in 1 ml of tetracycline solution (50 mg/mL) for 3 min.

Each sample was then immersed in thioglycolate broth. All the samples were then vortexed for about 30 seconds to homogenize the solutions and then diluted serially (1:5, 1:10, 1:100, 1:1000). The samples were then streaked onto petri dishes containing Nutrient agar, Blood agar, and MacConkey agar. To obtain anaerobic cultures, samples were placed in an anaerobic jar (85% nitrogen and 15% CO_2) for 24-48 hours at 35-37º.The organisms were identified by Gram staining under a microscope, and the colony-forming units per milliliter (CFU/mL) were determined.

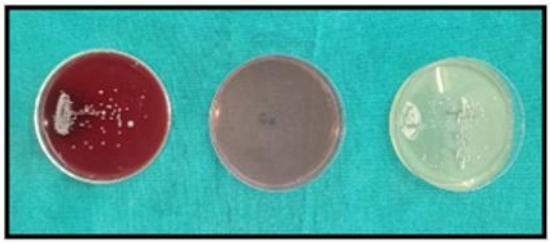


Figure 3: - Collected bone sample divided into 3 groups

Statistical analysis

Data was analyzed using the IBM SPSS version 2.0 software (IBM Corp., Armonk, NY, USA). Descriptive statistics, one-way analysis of variance with Tukey's post-hoc tests, and paired t-tests were performed to analyze the study data. The data are presented as bar charts. One-way analysis of variance showed that the control group (Group-1) demonstrated the highest CFU/mL count, whereas the tetracycline group (Group-3) showed the lowest values, followed by clindamycin in group (Group-2). These differences were statistically significant, as analyzed using one-way analysis of variance with a p-value less than 0.05. Multiple pairwise comparisons showed no significant differences in the overall CFU/ml between the tetracycline and clindamycin groups. A paired t-test was used to compare the gram-positive and gram-negative CFUs/ml within each of the three study groups. It was revealed that in all three groups, gram-negative CFUs were higher than gram-positive CFUs. However, these differences were not statistically significant in any of the three groups.

III. Result

One-way analysis of variance showed that the control group (Group-1) had the highest CFU/mL count, whereas the tetracycline group (Group-3) had the lowest values, followed by the clindamycin group (Group-2). These differences were statistically significant, as analyzed using one-way analysis of variance with a p-value less than 0.05. Multiple pairwise comparisons showed no significant difference in overall CFU/ml between the tetracycline and clindamycin groups. A paired t test was used to compare the Gram positive and Gram negative CFUs/ml within each of the three study groups. It was revealed that in all the three groups, gram negative CFUs were observed to be higher than the Gram positive CFUs.

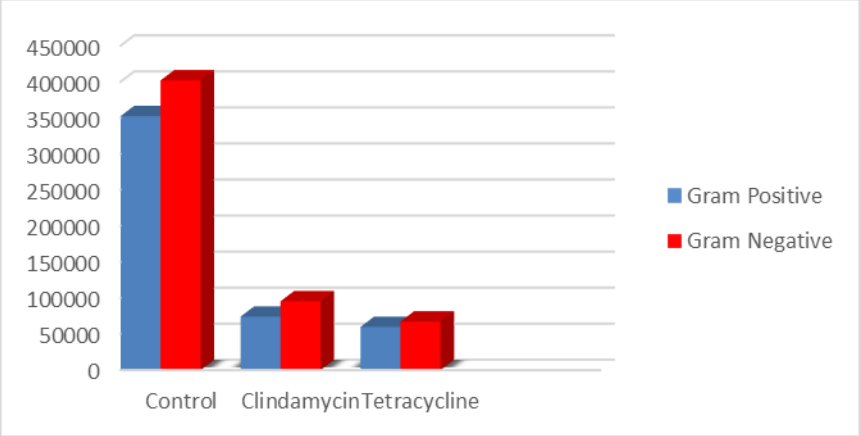
In the one-way of variance, for gram positive bacteria, the control, clindamycin, and tetracycline groups showed standard deviations of 128674.09, 26283.743, and 21150.003, respectively and for gram

negative bacteria showed a standard deviation of 125205.91, 34576.820, and 21793.334, respectively (Table 1). This demonstrated a statistically significant reduction in gram-positive and gram negative CFU/ml for the clindamycin and tetracycline groups as compared to the control group. (Graph 1)

Table 1: Comparison of Gram positive and Gram negative CFU/ml between the three groups

GROUP	N	Mean	Std. Deviation	95% confidence interval for mean		F value	P value
				Lower Bound	Upper Bound		
Gram Positive							
Control	20	352355.5	128674.09	288367.43	416343.68	83.013	<0.001*
Clindamycin	20	74088.89	26283.743	49299.02	70334.32		
Tetracycline	20	59816.67	21150.003	61018.29	87159.49		
Gram Negative							
Control	20	401911.11	125205.91	339647.68	464174.55	107.23	<0.001*
Clindamycin	20	95433.33	34576.820	78238.69	112627.98		
Tetracycline	20	67281.39	21793.334	56443.82	78118.96		

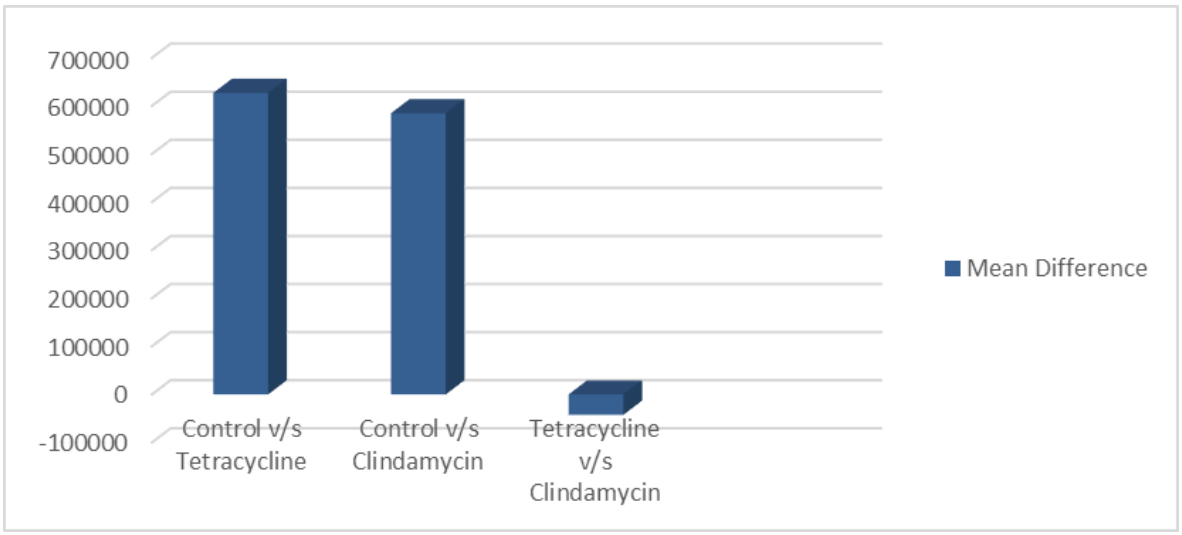
Graph 1: Comparison of Gram positive CFU/ml between the three study groups



Multiple pairwise comparisons showed a mean difference of 627168.611 between the control and tetracycline groups, mean difference of 584744.444 between the control and clindamycin groups, and mean difference of -42424.167 between the clindamycin and tetracycline groups (Table 2). This demonstrated that there was no statistically significant difference in the overall CFU/ml between the tetracycline and clindamycin groups.

Table 2: Multiple pairwise comparisons of overall CFU/ml between the three

Reference group	Comparison group	Mean difference	Std. error	P value
Control	Tetracycline	627168.611*	25753.286	<0.001*
Control	Clindamycin	584744.444*	25753.286	<0.001*
Tetracycline	Clindamycin	-42424.167	25753.286	.235



IV. Discussion

Due to the possibility of regeneration, autogenous bone is regarded as the "gold standard" for bone grafting techniques. But autogenous bone graft has various disadvantage like need for second surgical site and donor site morbidity. To reduce this downside particulate bone can be collected from implant site. However, it is alarming that the obtained bone may have been contaminated by microorganisms. Saliva and plaque in the oral cavity typically include 10^8 bacterias. Bone grafts, whether harvested as a single piece or collected as bone particles, can get contaminated with the common bacterial flora of the oral cavity even when all the principles are followed. Hence, decontamination of autogenous particulate bone graft is necessary.

It has been claimed that a significant barrier in harvesting bone graft via drilling is irrigation, which flushes bone fragments away while attempting to minimize excessive heat generation and necrosis. Studies have shown that irrigation can be reduced during osteotomy preparation. In implant site preparation, ***Manzano-Moreno et al.,***[3] ***Anitua et al.,***[7] ***Park et al.***[8] ***and Kim et al.***[9] showed that low-speed drilling (20-80 rpm) is a straightforward and safe way to collect bone graft material. The collected bone could be utilized for bone regeneration without compromising volume stability. Therefore, the bone particles collected via low-speed drilling in the present study favor new bone formation.

In this study, bone particles were collected using a standard bone collector (GDC). This is a tool with a filter-filled chamber. A surgical suction hose was attached to the bone collector in order to aspirate and gather the bone pieces.[10] According to ***Hashemi and Beshkar et al.***[11] bone particles collected using a bone filter had greater levels of contamination as compared to bone fragments acquired with a rongeur.

Young et. al.[12]***, Tezulas et al.***[13] *and* ***Esposito et. al***[14] has suggested that a preoperative chlorhexidine mouth rinse and prophylactic antibiotic should be used in conjunction with stringent aspiration protocol to reduce further the bacterial contamination of collected bone particles. Despite using all the protocols and following all the aseptic measures, the bone particulates showed the contamination of bacterial count (CFU/ml). Consequently, the use of antibacterial treatments to decontaminate the collected bone fragments was taken into consideration.

Decontamination of the particulate bone graft was successfully accomplished by immersion of bone sample in antimicrobial agents like ***Pommer et al.***[15] *use* 1% chlorohexidine, ***Verdugo et al.***[16]**,** use of 10% Povidone-iodine, ***Sivolella et al.***[17] used 0.5% rifamycin but the decontamination was successfully accomplished by immersing bone samples in low dosage clindamycin according to ***Mohajerani et al***.[4] and ***Olvera-Huertas***[18]**.** Therefore, in present study, for achieving more effective decontamination with the least cytotoxic effects, a contact time as 3 minutes was followed to immerse collected bone particles in tetracycline or clindamycin solutions. Clindamycin was used at a concentration of 20 µg/ml and tetracycline at 50 mg/ml, which were considered effective and least cytotoxic, respectively.

In the present study, bone particles were collected and transported in a sterile container containing 0.9% saline solution. Numerous studies have recommended different media, such as normal saline solution,[19] 5% dextrose in water, "balanced salt solution," Collin's Terasaki solution, and assorted tissue culture, for the temporary storage of bone grafts during surgery.

In the current study, bacterial cultivation was performed using a variety of media, including nutrient, blood, and MacConkey agar. The general-purpose medium known as nutrient agar is typically used for routine culture, and it ensures the survival of microorganisms for a longer period of time. Blood agar (BA) is an enriched medium. It is used to cultivate a variety of pathogens, including the notoriously difficult-to-grow Haemophiles influenzae, Streptococcus pneumoniae, and Neisseria spp. MacConkey agar is a selective and differential medium that ensures the isolation and differentiation of non-fastidious gram-negative rods, particularly those belonging to Enterobacteriaceae familyceae and genus Pseudomonas. There was no evidence of Gram-negative anaerobic organisms in any of the samples, as patients with a history of periodontal disease were excluded from the study. Various micro-organisms detected in collected bone sample include (Table 3)

Table 3: Various microorganisms isolated from the collected bone after culture

Microorganism	Aerobic Species	Anaerobic Species
Gram Positive	*Staphylococcus hominis* *Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus saprophyticus* *Staphylococcus capitis* *Streptococcus pneumoniae* *Streptococcus oralis* *Streptococcus salivaris* *Streptococcus agalactiae* *Enterococcus sps.* *Staphylococcus hominis* *Staphylococcus aureus* *Staphylococcus epidermidis*	

	Staphylococcus saprophyticus	
Gram Negative	*Escherichia coli* *Klebsiella pneumoniae* *Klebsiella sps.* *Pseudomonas aeruginosa*	
Total Number	*18*	

No anaerobic bacteria were found in the bone samples as per the inclusion and exclusion criteria.

In all three groups, gram-negative CFUs were higher than gram-positive CFUs.These differences were statistically significant. Multiple pairwise comparisons revealed no significant difference between the tetracycline and clindamycin groups (Table-3). The control group (Group 1) showed the highest level of microbial contamination, suggesting that the prophylactic antibiotic, oral chlorohexidine rinse, and stringent aspiration protocol were not effectively effective in reducing microbial contamination, whereas the tetracycline group showed the least bacterial contamination. One-way analysis of variance revealed that these differences were statistically significant. The colony forming units per milliliter were measured to determine bacterial contamination levels. Colony-forming units (CFUs) were counted in each medium at the end of the incubation period in CO_2-rich or anaerobic environments.

In order to lessen contamination, the current study's data support immersing the collected bone particles in an antimicrobial solution. Though it was not statistically significant, tetracycline immersion has resulted in significant reduction of microbial load compared to clindamycin.

V. Conclusion

According to the study, the tetracycline group (Group-3) had the lowest CFU/mL counts, while the control group (Group-1) had the highest. The tetracycline and clindamycin groups did not significantly vary in CFU/mL, according to pairwise tests. Bacterial contamination was inevitable even when sterile and aseptic procedures were followed while obtaining intraoral autogenous bone grafts from implant sites. Based on the results, it was determined that antimicrobial treatment was essential because common precautions such as rigorous aspiration protocols, preoperative chlorhexidine mouthwash, and antibiotic prophylaxis were insufficient to prevent infection. However, the presence of bacteria was greatly decreased by soaking the graft material in either tetracycline (50 mg/mL) or clindamycin (20 µg/mL) for three minutes. The study's clinical implications include Tetracyclines and other antibiotics can aid in preventing the growth of biofilm on implant surfaces and graft materials. A lower bacterial load could improve new bone development and graft integration, improving the implant's primary stability.

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