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Research paper

Evaluation of microbial leakage in three different implant abutment connections using a quantitative analysis of presence of staphylococci bacteria an in vitro study

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Abstract

Dental implants have become a mainstay in providing fixed/semi fixed restorations successfully in the past few decades. One crucial component of dental implant which immensely contributes to the osseointegration and long term success of the restoration is the implant abutment connection (IAC). The IAC houses a microgap that can cause leakage of microorganisms which would then act as a bacterial reservoir.
We performed a laboratory study to evaluate the microbial leakage occurring within the IAC between three different popularly used implants. The samples were divided into three groups based on their IAC – Conelog connection (Biohorizon), Conexa connection (B and B) and Internal Octagon (Osstem). The implant analog – abutment assemblies to be studied were immersed in a peptone broth containing Staphylococci bacteria. After an incubation period of 14 days, these assemblies were disassembled and a swab was taken from the inner surface of the implant analog and abutment. This swab was plated and after 48 hours, colonies of Staphylococci were counted. From the study, it was clear that microbial leakage had taken place in all the groups.
This will help us to guide the decision of which is the most suitable implant to be used to provide a successful long term prosthesis to the patient.

Keywords: *Use about five key words or phrases in alphabetical order, Separated by Semicolon.*

1. Introduction

Dental implants have been shown to be a very successful treatment modality in the treatment of partial or complete edentulism. Rapid progress in research and development of dental implant technologies have given rise to a variety of innovative implant designs, predictable surgical protocols and various treatment options which help us to choose a suitable approach to treat a particular case. Two stage implants consist of an abutment which connects to the underlying fixture by the means of a geometric(frictional) connection and an abutment screw. When this implant abutment system is subjected to occlusal loading, the adaptation of the abutment to the implant connection may be affected leading to the development of microscopic gaps in the implant abutment connection. This micogap can cause leakage of microorganisms which would then act as a bacteriological reservoir. This gap is located at the level of the alveolar crest. This could lead to unfavourable biological consequences such as inflammation and infection leading to peri implantitis and increased crestal bone loss which could ultimately lead to implant failure.
This problem was seen more in external connection implants which have now been replaced by internal hexagon implants. These internal hexagon implants have made the concept of platform switching possible. Platform switching is based on medialisation of the IAC such that the microgap formation occurs towards the centre of the implant platform rather that adjacent to the alveolar crest.
Prevention of microbial leakage at the implant - abutment connection is a major challenge for the construction of modern two-stage implant systems in order to minimize inflammatory reactions and to maximize bone stability at the implant neck. Implant manufacturers aim to reduce this microleakage by increasing the stability of the implant abutment connection. Therefore, reducing micromobility of this connection by constructing a mechanically tight junction with a high level of precision is paramount for the longevity of the implant restoration
Materials and Methods

- 9 fixture analogs with Conelog connection of diameter 4 mm and length 7 mm from Biohorizon with corresponding abutments.
- 9 fixture analogs with Internal octagon connection of diameter 4 mm and length 7 mm from Osstem with corresponding abutments.
- 9 fixture analogs with Conexa connection of diameter 4 mm and length 7 mm from B and B with corresponding abutments.

The study was divided into the following steps:

a) Preparation of samples
b) Preparation of culture test specimen

c) Evaluation and verification of bacterial leakage

a) Preparation of samples

The implants to be studied were divided according to their connection system. They were divided into 3 groups, each group containing 9 implant analog abutment assemblies of the same geometry.

GROUP A: 9 implant analog - abutment assemblies with an Internal octagon connection (Osstem) were evaluated for bacterial leakage into the inner part of the implant analog

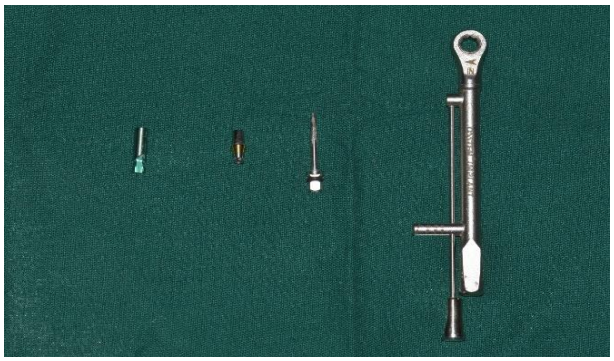


Fig. 1: Implant Analog, Abutment, Hex Driver and Torque Ratchet for Osstem.

GROUP B: 9 implant analog - abutment assemblies with a Conexa connection (B and B) were evaluated for bacterial leakage into the inner part of the implant analog.

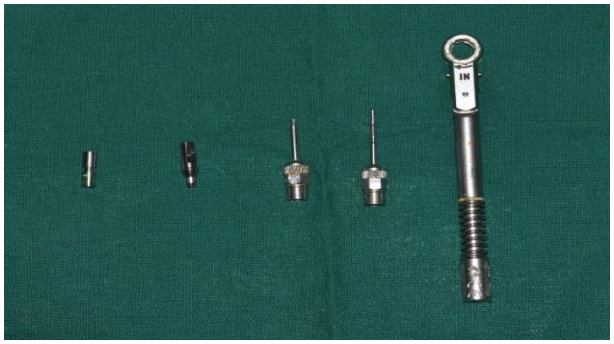


Fig. 2: Implant Analog, Abutment, Hex Driver, Specialised Extractor Tool and Torque Ratchet for B and B.

GROUP C: 9 Implant analog – abutment assemblies with a Conelog (Biohorizon) were evaluated for bacterial leakage in the inner part of the implant analog

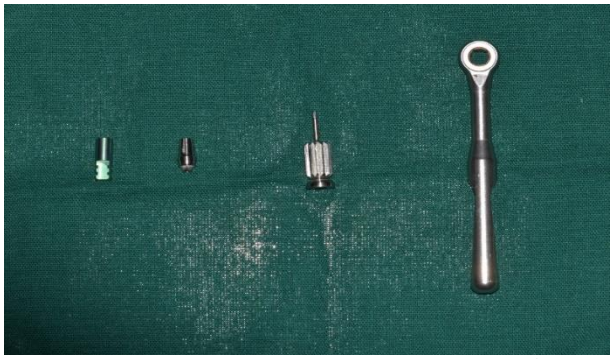


Fig. 3: Implant Analog, Abutment, Hex Driver and Torque Ratchet for Biohorizon.

The implant analogs were retrieved from their sterile packaging with the help of sterile pliers. The abutments were carefully connected to the implants and tightened using the respective torque ratchet according to the manufacturer's instructions (Figures 1,2 and 3) These implant analog – abutment assemblies were used as samples for testing the microbial leakage through their implant abutment connections. 9 such samples were made for each of the 3 groups

b) Preparation of culture test specimen

Test specimens of Staphylococcus aureus were prepared on blood agar plate using pus culture by incubating overnight at 37^0C. The culture was confirmed by coagulase test which gave positive results showing the presence of Staphylococcus aureus. An inoculating loop (Figure 4) was heated red hot on a Bunsen burner, allowed to air cool and then used to pick up bacterial colonies from a blood agar plate (Figure 5)

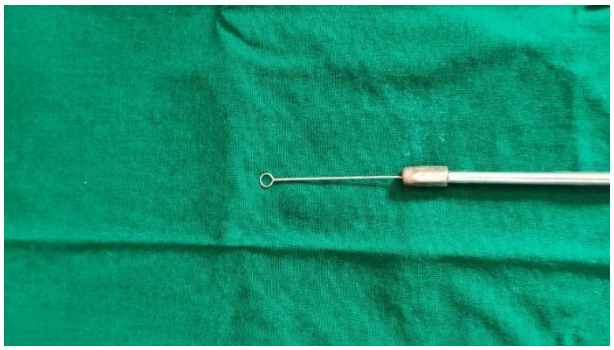


Fig. 4: Sterile Inoculating Loops for Picking Up Organisms and Immersing in Peptone Water.

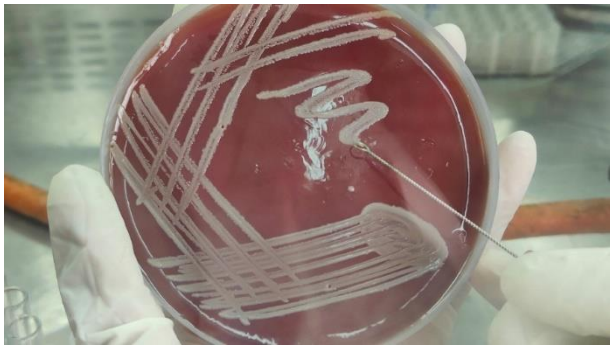


Fig. 5: Picking Up Organisms to Be Immersed in Peptone Water.

This inoculating loop with bacterial colonies was inserted in sterile test tubes filled with 4 ml of peptone water at room temperature and compared to a 0.5 McFarland standard to adjust the turbidity of bacterial suspension. The assembled implant analog-abutment samples were then completely immersed in 4 ml of bacterial suspension and was placed in an incubator at 37^0C for 14 days. All the 9 samples of Group A, Group B and Group C were subjected to the test in a similar manner (Figures 6, 7 and 8).

Fig. 6: Test Samples 1-9.

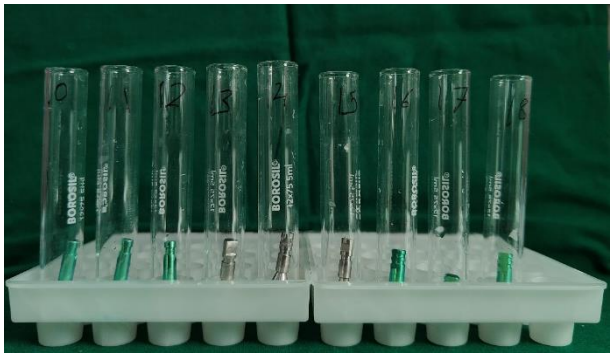


Fig. 7: Test Samples 10-18.

Fig. 8: Test Samples 19-27.

c) Evaluation and verification of Bacterial leakage

After 14 days of incubation, the samples were removed from the test tube using sterile pliers and wiped with 70% alcohol using a cotton ball and dried with sterile gauze. This procedure ensured the sterility of the outer surface of the samples without affecting bacterial viability on the inside. Next the specimens were carefully disassembled using respective hex drivers and the inner surface of the implant analogs were sampled for bacterial contamination using sterile paper points (Figures 9, 10 and 11).

Fig. 9: Paper Point Swab Obtained from Samples 1- 9.

Fig. 10: Paper Point Swab Obtained from Samples 9-18.

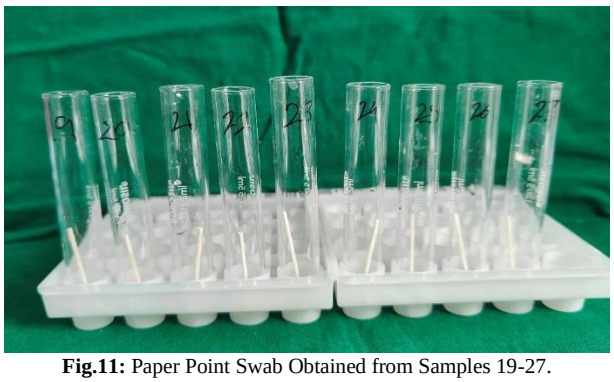


Fig.11: Paper Point Swab Obtained from Samples 19-27.

These paper points were streaked on blood agar plates and those plates were then incubated at 37^0C for 48 hours.

After incubation of the blood agar plates at 37^0C for 48 hours, the growth of colonies in the culture medium was evaluated. From the blood agar plates which showed the growth of organisms (Figures 12, 13 and 14), colonies of bacteria were picked up using inoculating loops which were heated red hot on a Bunsen burner and air cooled and then placed on sterile glass slides for gram staining

Fig. 12: Growth of Staphylococcus Colonies After 24h on Blood Agar Plates from Samples 1- 9.

Fig. 13: Growth of Staphylococcus Colonies After 24h on Blood Agar Plates from Samples 10-18.

Fig. 14: Growth of Staphylococcus Colonies After 24h on Blood Agar Plates from Samples 19-27.

The slides were then observed under a compound microscope (Figure 15). The microscopic appearance of Staphylococcus species are cocci in grape like clusters.

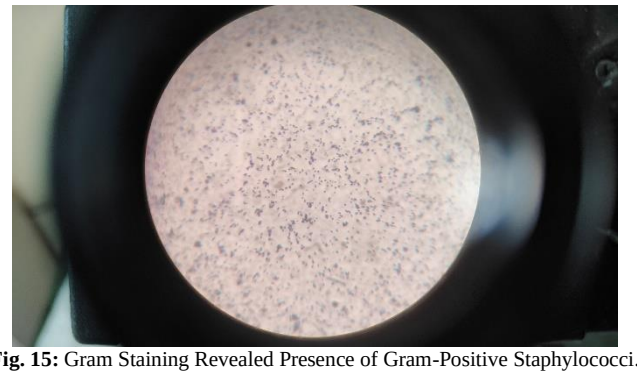


Fig. 15: Gram Staining Revealed Presence of Gram-Positive Staphylococci.

After microscopic examination, a coagulase test using plasma was carried out to verify and confirm the presence of Staphylococcus aureus in the inner part of the assembly.

2. Results

9 samples each of the Conexa, Conelog and Internal Octagonal connection implant analogs with the abutments were checked for microbial leakage. Occurrence of microbial leakage was individually calculated for each sample after a time period of 14 days. Presence of leakage into the implant abutment interface indicated the presence of micro gaps.

The observation for all the samples is tabulated as follows:

Table 1: Table Showing Microbial Leakage in Group A, Group B and Group C After 14 Days.

Sample	Group A (No. of colonies)	Group B (No. of colonies)	Group C (No. of colonies)
Sample 1	0	18	2
Sample 2	0	1	3
Sample 3	0	0	0
Sample 4	0	8	0
Sample 5	0	0	0
Sample 6	14	0	0
Sample 7	23	13	2
Sample 8	4	36	16
Sample 9	18	27	24

3. Statistical analysis

Table 1: Normality Testing Using Shapiro-Wilk Test

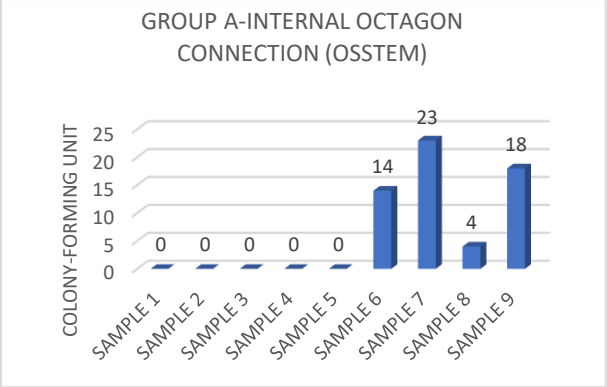
Tests of Normality	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
GROUP A (OSSTEM)	.317	9	.009	.748	9	.005
GROUP B (CONEXA)	.229	9	.190	.855	9	.085
GROUP C (CONELOG)	.379	9	.001	.669	9	.001
a. Lilliefors Significance Correction						

The result of the test of normality using Shapiro-Wilk test has been provided in Table 1.

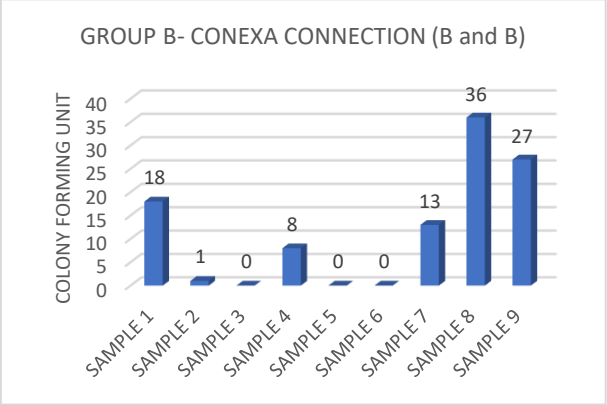
Table 2: Descriptive Statistics of Microbial Leakage Using the Three Implant-Abutment Interface Using Colony Forming Unit as A Parameter

	MINIMUM	MAXIMUM	MEAN	STANDARD DEVIATION
GROUP A-INTERNAL OCTAGON CONNECTION (OSSTEM)	0.00	23.00	6.556	9.20
GROUP B- CONEXA CONNECTION (B and B)	0.00	36.00	11.444	13.24
GROUP C- CONELOG (BIOHORIZON)	0.00	24.00	5.222	8.68

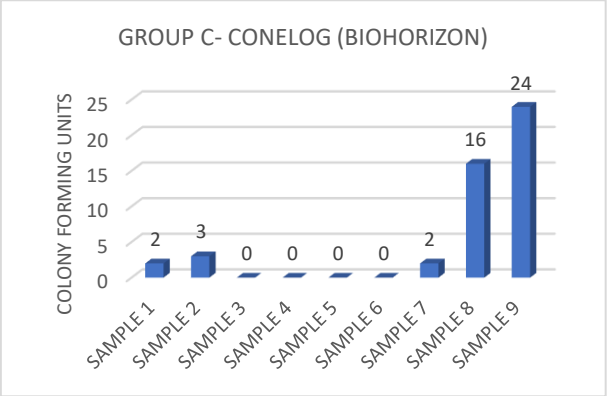
Table 2 provides the descriptive statistics related to the three implant-abutment interfaces.



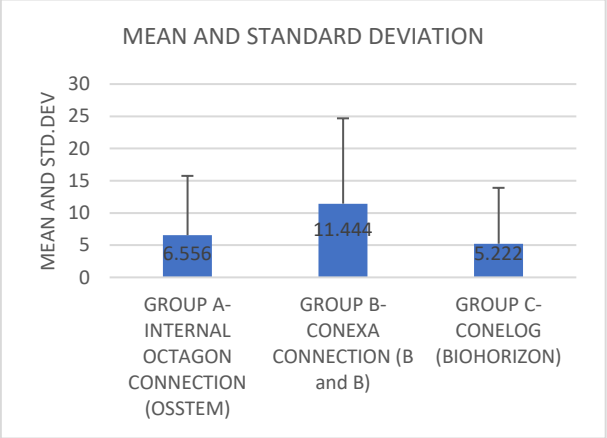
Graph 1: Distribution of Samples Along with Colony-Forming Units for Group A (OSSTEM).



Graph 2: Distribution of Samples Along with Colony-Forming Units for Group B (Conexa Connection).



Graph 3: Distribution of Samples Along with Colony-Forming Units for Group C (Conelog- Biohorizon).



Graph 4: Comparison of Mean and Standard Deviation of the Three Groups of Implant-Abutment Interface.

Table 3: Comparison of Microbial Leakage Between the Three Groups Using Kruskal-Wallis Test

	MEAN RANK	KRUSKAL-WALLIS H VALUE	p-VALUE
GROUP A-INTERNAL OCTAGON CONNECTION (OSSTEM)	12.89		
GROUP B- CONEXA CONNECTION (B and B)	16.11	1.048	0.592
GROUP C- CONELOG (BIOHORIZON)	13.00		

The comparison between the three groups (Group A vs Group B vs Group C) was conducted using the Kruskal-Wallis test as the data was not normally distributed and the results are represented in Table 3

Table 4: Post-Hoc Analysis for Comparison between Two Individual Groups Using Mann-Whitney U Test

	MANN-WHITNEY U VALUE	Z-VALUE	p-VALUE
GROUP A-INTERNAL OCTAGON CONNECTION (OSSTEM) Vs GROUP B- CONEXA CONNECTION (B and B)	31.000	0.878	0.436
GROUP A-INTERNAL OCTAGON CONNECTION (OSSTEM) Vs GROUP C- CONELOG (BIOHORIZON)	40.000	0.047	0.962
GROUP B- CONEXA CONNECTION (B and B) Vs GROUP C- CONELOG (BIOHORIZON)	31.000	0.865	0.387

Since Kruskal-Wallis test was done to compare between the three groups, Mann-whitney U test was employed to compare between the two groups individually as non-parametric tests had to be used and the results of which has been shown in Table 4

4. Discussion

The implant abutment connection is one of the most crucial component of the implant restoration to fulfil the treatment objectives of prosthesis success and longevity. Any micromovements taking place at the implant and abutment junction should be eliminated and an ideal 'cold weld' must be established between the implant and abutment. Traditionally used external connection implants had a few major drawbacks such as lack of height to resist non axial loads, frequent screw loosening and component fracture.

Among the two-piece implant systems, the internal hexagonal connection—in which a portion of the abutment is incorporated in the implant body—is currently the most popular. The fact that this connection guarantees appropriate abutment seating, anti-rotational engagement, resistance to lateral stresses, and great aesthetic results most certainly accounts for this. According to reports, compared to the external connection, this connection is less conducive to fluid penetration. Different internal connections have been found to be more stable under loading conditions than external connections, which, when subjected to functional loading, encourages the abutment to move slightly and, as a result, causes bacterial leakage

Eventually, the popularity of Morse Taper and friction fit implants gave rise to multiple manufacturers introducing their proprietary connection designs with various claims of benefit. Thus, three popular connection geometries were chosen to be evaluated, namely, the internal hexagon, internal octagon and conical connections. Most connections in the market used today vary from 5° to 15° of internal taper. Thus, it was chosen to evaluate internal tapers of 5°,7.5° and 8° to represent various connections that feature similar taper.

5. Conelog connection (biohorizon)

Conelog® screw-line implants are equipped with a 7.5° internal taper and a conical connection for reliable transfer of force and torque and the three proven CAMLOG grooves for precision abutment positioning. Clearly perceptible tactile feedback lets the user know when the abutment is positioned correctly by the three grooves and apical external taper. The conelog connection allows for greater precision in the placement of the abutment, ensuring a more accurate and aesthetically pleasing result.

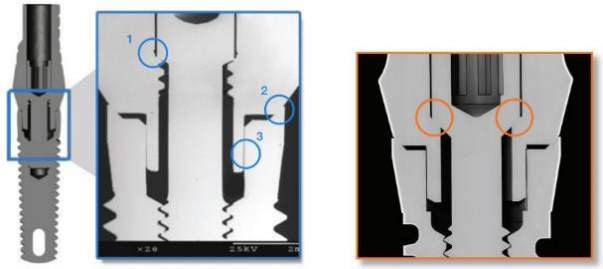


Fig. 16: Schematic Cross Section of the Biohorizon Implant.

6. Internal octagon (osstem)

Com-octa implants and abutments feature a connection that is and internal octagon and 8° internal taper. The octagonal design creates a more secure connection between the implant and the abutment, providing improved stability and reducing the risk of loosening over time.

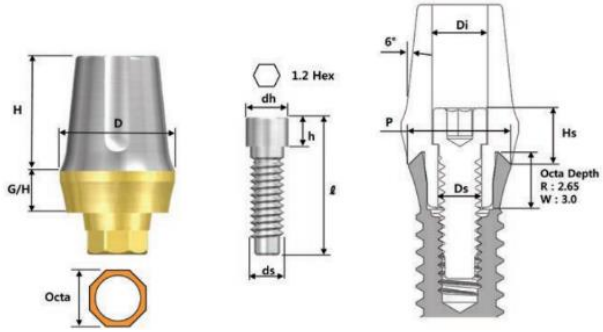


Fig. 17: Schematic Cross Section of the Osstem Implant.

7. Conexa (B and B)

Conexa connection features a 5° internal taper and an internal hexagon. This connection also features a cold weld that occurs between the abutment and the implant instantaneously when the abutment is torqued onto the implant. This reduces the possibility of screw loosening and reduces the amount of microleakage. A specialised extractor tool is used if the abutment has to be removed from the implant.

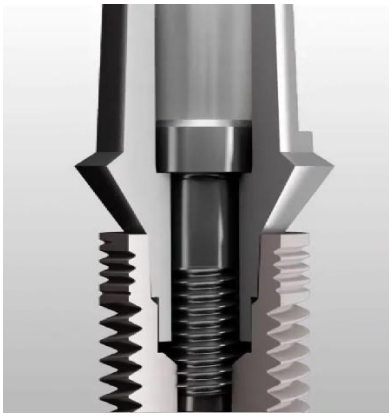


Fig. 18: Schematic Cross Section of the B and B Implant.

Oral bacteria include streptococci, lactobacilli, staphylococci, corynebacteria, and various anaerobes in particular bacteroides. Oral microflora are most commonly found in gingival crevices, coronal plaques, dorsum of the tongue, buccal mucosa and saliva. In a study by Ohara-Nemoto Y[13] the occurrence of staphylococci in the oral cavity was examined in healthy adults. The results showed that Staphylococcus

aureus was the most frequent species found in the oral cavity followed by Staphylococcus epidermidis and others. Since Staphylococcus aureus is a common resident of the oral microflora it was chosen in the study to check for bacterial leakage.

In the present study, the following inferences could be drawn from the results and statistical analysis. The result of the test of normality using Shapiro-Wilk test has been provided in Table 1. Since the p-value of 2 groups out of 3 was found to be less than 0.05, therefore, the data were not normally distributed. Non-parametric tests were used for comparative statistics between the three groups. Table 2 provides the descriptive statistics related to the three implant-abutment interfaces. The mean value of Internal octagon connection (OSSTEM) was found to be 6.556 with a standard deviation of 9.20. The distribution among samples for Internal Octagon connection is seen in Graph 1. Similarly, for Conexa connection (B and B), the mean value of the colony-forming unit was 11.444 and the standard deviation was 13.24 and it was the highest among the three groups. The distribution among samples for Conexa connection is seen in Graph 2. Lastly, for Conelog (Biohorizon), the mean ± standard deviation was found to be 5.222 ± 8.68. The distribution among samples for Conexa connection is seen in Graph 3. The comparison between the three groups (Group A vs Group B vs Group C) was conducted using the Kruskal-Wallis test as the data was not normally distributed and the results are represented in Table 3. Comparison of mean and standard deviation among the three groups is given in Graph 4. The p-value was found to be greater than 0.05 which was indicative of the fact that the mean difference between the three groups were not statistically significant. Therefore, no statistical inferences can be drawn on the basis of the colony-forming units in between the three-groups. Since Kruskal-Wallis test was done to compare between the three groups, Mann-whitney U test was employed to compare between the two groups individually as non-parametric tests had to be used and the results of which has been shown in Table 4. The comparison of mean differences between Group A vs Group B was not found to be statistically significant. Similarly, the other two comparisons (Group A vs Group C and Group B vs Group C) was found to be statistically insignificant as well

8. Conclusion

Within the limitations of an in vitro study, the following conclusions could be drawn:

1) Microbial leakage was seen through the implant analog – abutment interface in the Internal Octagonal connection
2) Microbial leakage was seen through the implant – analog abutment interface in the Conexa connection
3) Microbial Leakage was seen through implant – analog abutment interface in the Conelog connection
4) The Conelog connection implant showed the least amount of microbial leakage followed by the Conexa and the Internal Octagonal connection implants.

However, comparison between the amount of Microbial Leakage that was seen in the Internal Octagonal, Conexa and Conelog implant connection systems was statistically insignificant and the null hypothesis was accepted.

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