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*RESEARCH ARTICLE*

# ASSESSING MICROBIAL CONTAMINATION OF AS RECEIVED ORTHODONTIC BRACKETS AND WIRES- AN IN VITRO STUDY.

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## *Abstract*

**Objective:** To test the null hypothesis that orthodontic brackets and wires as supplied by manufacturers do not have microbial contamination and are ready to be inserted directly intraorally.

**Materials and Methods:** Two different types of orthodontic materials were used straight from the manufactures package (20 different stainless steel rectangular wires and 20 orthodontic brackets) without any additional sterilizing step. Three separate microbial tests were performed to check the contamination of the bacteria. The tests were DBTT (Direct Bacterial Turbidity Test), OST (Optical Screening Test) & Gram Staining, and PCR (Polymerised Chain Reaction) to isolate bacterial colonies and identify them.

**Results:** Of these materials tested, three genera of bacteria, implicated as opportunistic pathogens, namely, Staphylococcus aureus, Staphylococcus epidermidis and E. coli were recovered from the orthodontic brackets and wires.

**Conclusions:** Our data showing microbial contamination on orthodontic materials highlights the need of sterilization before clinical use. We also suggest that manufacturer s should declare the sterility state of orthodontic materials on their packaging or instruction s stating the need for sterilization.



## Introduction:-

The oral microbiota in the human oral cavity contains a number of different habitats and at least 400 to 700 different types of bacteria. This microbiota hosts a complex and dynamic microbial community that is responsible for two major and highly prevalent infectious diseases: caries and periodontal disease.[1]

When changes occur in the oral cavity, the microbiota also change, causing a loss of balance and increasing the possibility of disease development.[2]

Orthodontic treatment, via the use of fixed or removable appliances,[3] causes specific alterations in the oral cavity, including pH reduction, increased accumulation of dental biofilm,[4] and increased levels of microorganisms in saliva

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and biofilm.[5,6] As a result, diseases can be transmitted through direct contact with contaminated instruments or materials, either when used straight from the manufacturer's packaging or when used in more than one patient without proper disinfection or sterilization.[7]

Orthodontic brackets and wires are used in orthodontic treatment to achieve the movement of teeth. They come in contact with intact mucous membranes. Sometimes they lacerate the mucosa and cause injury. The design of the wires and brackets present potential areas for bacterial adherence.

International regulatory agencies have recommended the use of the Spaulding classification system for inanimate objects to evaluate their potential risk of disease transmission and infection.[10,11] This classification system is used by different centers of epidemiologists, microbiologists, and professional medical organizations aiming to determine the degree of disinfection or sterilization required for various medical devices and classifies objects as critical, semi-critical, or noncritical.

According to this classification, orthodontic materials, including brackets, are considered semi-critical because of the associated risk of infection (direct contact with the oral mucosa), and should therefore be sterilized before use. Semi-critical objects are those that have contact with mucous membranes only, preventing the invasion of subepithelial tissues, and should be sterilized.[10,11]

Purmal et al.[12] evaluated four different orthodontic buccal tubes used straight from the manufacturer's packaging and found aerobic bacterial contamination. The microorganisms isolated from the samples were Micrococcus luteus, Acinetobacter calcoaceticus, and Staphylococcus haemolyticus, microorganisms with a contamination potential that can pose risks to patients' health. Those authors recommended that the materials should be sterilized before use.[12]

Based on these findings and the scarcity of studies in the field, and taking into consideration the patient safety risks involved and the fact that materials that will be used in the oral cavity must be free of contamination, the objective of this study was to test the null hypothesis that orthodontic brackets and wires as supplied by manufacturers do not present microbial contamination. To authenticate the study, microbiological and molecular tests like DBTT (Direct Bacterial Turbidity Test), OST (Optical Screening Test) & Gram Staining, and PCR (Polymerised Chain Reaction) were used to detect the presence of bacterial growth, analyse the types of bacteria present, and identify bacteria.

## Materials and Methods:-

Two different types of orthodontic materials were used straight from the manufactures package (20 different stainless steel rectangular wires and 20 orthodontic brackets) without any additional sterilizing step.

Three separate microbial tests were performed to check the contamination of the bacteria. The tests were DBTT (Direct Bacterial Turbidity Test), OST (Optical Screening Test) & Gram Staining, and PCR (Polymerised Chain Reaction) to isolate bacterial colonies and identify them.

## Results:-

The samples were divided into three major categories. 1: control group(sterilized material), 2: Orthodontic Brackets(As Received from the manufacturer), 3: Orthodontic wires (As Received from the manufacturer) . The samples were subjected to sequential microbial tests in order to confirm the presence of particular species.

The first test done was the Direct Bacterial Turbidity Test (DBTT). The media used was BHI/Broth and the results of this tests showed (++) and (+) for two sets of orthodontic wires respectively and (+) for orthodontic brackets and no positive results for control group. (Figure 10)

The test followed by DBTT was the Optical Screening Test (OST) and Gram Staining. The purpose of this test was to quantify the isolated forms in the medium. The media used was BHI/Agar and the sample type was cultured broth. The results of this test showed 4 isolated forms (3 gram positive coccus, 1 gram negative bacilli) in one orthodontic wires group and 3 isolated forms (3 gram positive coccus) in second archwire group. 2 isolated forms (2 gram negative bacilli) in the orthodontic bracket group.

After the OST and G-staining, the test performed was Polymerised Chain Reaction (PCR). The purpose of this test was to identify the organisms by identifying the sequence of primers of the same. The results described three genera of bacteria, implicated as opportunistic pathogens, namely, Staphylococcus aureus (first orthodontic wire group and orthodontic brackets group), Staphylococcus epidermidis (first orthodontic wire group) and E. coli (second orthodontic wire group) were recovered from these orthodontic materials.

## Discussion:-

Infection by microorganisms is a concern for health care professionals in general and for dental practitioners in particular, and the dental literature has long pointed to the need to sterilize or disinfect any material before its use in the oral cavity. However, in orthodontic clinical practice, the use of orthodontic wires and brackets directly from manufacturer's pack is still routine. In this scenario, it becomes extremely important to assess the potential contamination of these materials so as to determine sterilization protocols and maintain the health of patients and dental practitioners working in this field.

In the present study, bacterial contamination was confirmed in orthodontic brackets and wires. Similarly, Purmal et al.[1] also reported biological contamination of the orthodontic buccal tubes evaluated in their study. Those authors suggested that the presence of the bacteria could result from unhygienic practices during material manufacturing and packaging.

According to Anhoury et al.,[3] in a healthy oral environment, the interplay between microorganisms and the host is complex and balanced. However, when orthodontic brackets, wires and bands are placed in the oral cavity, they can induce such changes as a decrease in pH and biofilm accumulation, especially when the materials used have not been previously sterilized.[3] The relationship between changes in the oral microbiota and the use of orthodontic materials has been confirmed by Naranjo et al.,[4] who found alterations in subgingival microbiota after the placement of orthodontic bands.[4]

In our study, bacterial contamination of the sample groups was suggested by microbial and biochemical tests and confirmed by DNA extraction and PCR. PCR was chosen because it is a highly specific and sensitive method and because it has been validated for this purpose.[12]

Genetic evaluation of our samples using PCR revealed contamination by S aureus in group 1 and S epidermidis in group 2. Looking at previous studies, Purmal et al.[1] found M luteus, S haemolyticus, and A calcoaceticus, whereas Hong et al.[5] found an increase in Streptococcus mutans in the saliva and biofilm after the placement or orthodontic brackets.[5]

With regard to the pathogenicity of microorganisms, Andrucioli et al.[13] had already underscored that high levels of oral microorganisms increase the risk not only of caries and periodontal disease but also of systemic complications.[12] According to Levinson and Jawetz[14] S epidermidis may cause endocarditis, a rare but possible complication of dental treatment that will only develop in the presence of bacteraemia.[14]

About 75% of bacterial infections caused by coagulase-negative Staphylococcus have S epidermidis as the causative agent,[15,16] which was also observed in our groups as well. These bacteria rarely cause suppuration, but they can infect orthopedic and cardiovascular prosthesis and cause diseases in immunosuppressed persons.

About the S aureus found in brackets in group 1, their pathogenic capacity is in the combined effect of extracellular factors and toxins, together with their invasive properties. Dissemination of S aureus can cause endocarditis, osteomyelitis, meningitis, or lung infection.[17] According to Oliveira et al.,[18] the presence of these respiratory pathogens in the biofilm can serve as a reservoir for microorganisms associated with nosocomial pneumonia.

Finally, considering that not all materials from manufacturers are free of contamination on the packaging, the null hypothesis was rejected. This suggests a concern for the health of patients and the need for better control of contamination in packages of brackets.

## Conclusion:-

In the present study, bacterial contamination was confirmed in orthodontic brackets and wires of the groups B and C (As received from the manufacturer). Of these materials tested, three genera of bacteria, implicated as opportunistic pathogens, namely, Staphylococcus aureus, Staphylococcus epidermidis and E. coli were recovered from the orthodontic brackets and wires.

Our data showing microbial contamination on orthodontic materials highlights the need of sterilization before clinical use. We also suggest that manufacturers should declare the sterility state of orthodontic materials on their packaging or instructions stating the need for sterilization. The clinicians should use a method of disinfection or sterilization before their use in the orthodontic clinic for security of patient health.

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