ROOT CANAL INFECTIONS AND COAGULASE NEGATIVE STAPHYLOCOCCUS (CoNS)

Type of Manuscript: Research Article
Running Title: Root canal infections and CoNS

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Number of words: 4213

Abstract:

Aim: To identify the number of cases associated with Coagulase negative staphylococcus CoNS in root canal infections

Background: Root canal infections have multiple aetiology which include obligate aerobes, facultative anaerobes, microaerophiles and aerobes. Recent studies have shown Coagulase negative staphylococcus is being associated in many clinical isolates. So, this study aids in eliciting the number of cases associated with CoNS in root canal infections.

Materials and method: This study is done in patients who visited the root canal clinic and referred for root canal treatment were selected. Samples were collected from 20 patients who were selected randomly. After chemomechanical preparation was completed, a paper point was inserted into the canal and left for 2 minutes for absorption from the lateral canals. Then, it was transferred to a cuvette containing sterile saline. The cuvette was agitated vigorously for 10 minutes, for uncoating and a uniform suspension was made. From that, 50μL of the suspension was transferred by a micropipette using sterile tips onto the blood agar plates and then were incubated at 37°C for 24 hours. After incubation, the plates were checked for the presence of CoNS by adapting the standard microbiological protocol.

Results: Among the 20 samples collected, CoNS were present in 85% of the cases

Conclusion: root canal infections have multiple aetiologies. Among them, the presence of coagulase negative staphylococcus is found to be significant as it has the ability to elicit drug resistance against many antibiotics and intra canal medicaments. Therefore, this study is done to emphasise the presence of CONS in root canal infections and also to plan the treatment strategy to eliminate them to improve the success rate of the treatment.

Keywords: Root canal infections, Coagulase negative staphylococcus, samples, study, CONS.

INTRODUCTION:

It is commonly known that bacteria initiate root canal and periapical infections[1,2]. The cause behind these infections is the colonization of microorganisms. The success rate of any root canal treatment depends upon the reduction of the number of root canal microorganisms[3]. It has become possible to preserve teeth that are damaged by deep caries to an extent, by root canal therapy. This has gained a lot of popularity due to the success rate of the root canal therapies. Moreover, root canal infection is the major aetiological agent of apical periodontitis[4]. Many chemical and physical factors can elicit periapical inflammation. Recent studies suggest that microorganisms are the cause for the enhancement of different forms of apical periodontitis[5,6]. The untreated canals are called primary infected root canals, into which the micro-organisms have gained entry to elicit colonization in the pulpal tissue, resulting in necrosis5. Secondary root canal infection initiates due to the root canal therapy or treatment failure due to the persisting bacterial infections in the root canal system even after chemomechanical preparation and intracanal medication[7,8]. Recent studies demonstrated that in primary root canal infections, there is evidence of polymicrobial flora with an average of 4-7 intra-canal species, among which Gram-negative anaerobic species seem to be the most common. Many studies also show obligate
anaerobic bacteria in root canal infections, which constitute 90% of the bacterial species. Studies have also demonstrated the presence of facultative anaerobic bacteria in the pulp. The bacteria present in many unsuccessful restorations include gram-positive facultative aerobics, especially *Enterococcus faecalis* [9,10].

The reason for root canal failures mainly revolves around the occurrence of errors during root canal treatment which prevent the control and eradication of intra canal root canal infection. These errors include broken instruments, perforations, and overfilling, underfilling, ledges. However, procedural errors may not be the main reason behind the abolishment of the root canal treatment unless an infection is present. But the cause can also be due to various microorganisms which persist in the root canal system even after the procedure is completed [11, 12]. Environmental factors also function in the root canal system during treatment, thereby allowing the survival of certain microorganisms even after the procedure is completed. Supposing the root canal filling fails to provide a complete seal, seepage of tissue fluids can elicit substrate for bacterial growth. Several regulatory systems aid in the essentiality of the bacteria’s ability to withstand nutrition depletion. These systems are controlled by the transcription of genes which are activated under starvation [13].

In case of nitrogen starvation, ntr gene system aids in enabling bacteria that require ammonia as a nitrogen source to scavenge even small amounts of ammonia. Under high concentration of ammonia, the ntr gene system is interrupted. Aerobic respiration regulatory which includes the arcA and arcB genes, under low concentrations of molecular oxygen, is activated by some facultative bacteria. Under low glucose concentrations, some bacteria can activate the catabolite repressor system, under the influence of genes cya (adenylate cyclase) and crp (catabolite repressor protein) which induce enzyme synthesis for usage of other organic carbon sources. The microbiota attributed with failed root canal procedures differ from those of the untreated teeth. The latter in most cases is a type of mixed infection, whereby gram negative anaerobic rods are dominant. The former is usually composed of one or few bacterial species, such as gram-positive bacteria, with no facultatives or anaerobes. After failed cases, there was an estimation of 51% of anaerobic bacteria and 29% of *Enterococcus faecalis* of the cases[14,15,16]. Yeast-like organisms are also found in many of the obturated canals in which the treatment had failed. In other words, these organisms elicit drug resistance. Moreover, Candida spp. are also found to be resistant to commonly used root canal medicaments [17,18].

Among the multiple aetiologies demonstrated so far, Coagulase Negative Staphylococcus seems to also elicit drug resistance to a high degree which has been the reason behind many root canal failures in the modern day. Coagulase negative staphylococcus is one of the major nosocomial pathogens, with *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* being the most common species. Coagulase Negative Staphylococcus (CONS) also comes under the normal flora of human skin and mucosa. CONS infections are basically treated with glycopeptides, also including vancomycin but there has been an emerging concern regarding the resistance to these agents. Up to 90% of CONS strains are methicillin resistant with additional resistance to aminoglycosides and macrolides among nosocomial infections. These strains also elicited resistance to linezolid but at a less frequency rate[19,20,21]. Moreover, they also elicited resistance to penicillin. In the modern day, resistance elicited is around 91% in clinical isolates[22,23]. Henceforth, CONS colonies serve as a reservoir for the spread of resistance genes. So this study mainly focuses on the incidence of the colonies of Coagulase negative staphylococcus in root canal treatments to elicit awareness regarding the persistence and the drug resistance capacity of CONS. Previously our team investigators has a rich experience in working on various research projects across multiple disciplines[24-33].The above clinical trials and systematic reviews performed in the previous studies led to the idea for the current study which aims to associate the relationship between drug induced gingival enlargement and anti hypertensive drugs as well as to determine the predominant drug causing hypertension.

**MATERIALS AND METHODS:**

This study is conducted in patients who visited the root canal clinic and opted for root canal treatment. 20 patients were selected randomly for the study. Root canal treatment was given after radiological validation. Access opening was done without using any chemicals or irrigants or debridement.

Sterile paper-points were taken with the help of sterile forceps and were inserted into the root canal after access opening; and left for 2 minutes for absorption from the lateral canals. Then, the paperpoint sample was removed from the root canal and was introduced into a sterile cuvette containing sterile normal saline. This cuvette was agitated manually for 10 minutes for uncoating the bacteria.

Then, a uniform suspension of the root canal material was obtained. Then, from this, 50μL of the suspension was taken by a micropipette and transferred onto the blood agar plate and spread with a loop uniformly on the entire surface. Then, the blood agar plates were incubated in the incubator for 24 hours at 37°C aerobically. After 24 hours, the plates were taken from the incubator and the colonies were identified based on the morphological characters, haemolytic activity and preliminarily identified by Gram staining. The colonies of gram-positive cocci in clusters were subjected for coagulase test and confirmed by the negative reaction. Both slide test and tube test were done for confirmation. Some colonies elicited clumping processes on slide as well as clotting in the tube. However, even after coagulase test, certain colonies did not elicit a clumping factor. The colonies which did not elicit clumping or agglutination were identified to be Coagulase negative and the colonies which showed clumping appearance were found to be Coagulase positive. Further conformational test of the colonies was done by tube coagulase test. Total number of colonies and number of Coagulase Negative Staphylococcus colonies were calculated and tabulated.

**RESULTS:**

<table>
<thead>
<tr>
<th>NO. OF SAMPLES</th>
<th>MEAN TOTAL COLONY FORMING UNITS</th>
<th>MEAN TOTAL CoNS COLONIES</th>
<th>PROPORTION OF CoNS IN %</th>
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### DISCUSSION:

In this study it is demonstrated that CoNS is found to be associated in 16.6% of the isolates from the root canal. Enterococci contributes to nearly 40% of the root canal failure cases. The presence of CoNS has to be explored for its role in root canal failure cases. Coagulase negative Staphylococcus seems to be attributed to a heterogeneous group and also a very versatile gram-positive bacteria. From the results, it was observed that, among organisms isolated from the samples, Coagulase Negative Staphylococcus colonies were seen in 85% of the cases. Henceforth, the inference from this suggests that suitable intra canal medicaments should be identified which will elicit in the eradication of CON5 colonies for a successful root canal treatment. This study focuses on the necessity of representation of more studies on CON5 ability to elicit drug resistance to many antibiotics.

There is only limited information available on differences in virulence and pathogenicity among CON5 species. A study demonstrated how more than half of the study isolates (CONS) were associated with clinical or nosocomial infections. An earlier study determined that 21% of CONS colonies were present to be significant among the total number of colonies when taken from sterile body sites. In most cases, nosocomial isolates of CONS were present in polymicrobial cultures. The other laboratory criteria oriented study isolates could not differentiate significant clinical isolates from colonizers. The most common species found were *S.epidermidis* and *S. lugdunensis*. *S.lugdunensis* accounts for about 55% in clinical isolates taken from pelvic girdle[34,35]. Previous studies have shown the presence of *S.lugdunensis* in acute cutaneous infections, breast and soft tissues.[36,37,38]. CONS also represent a regular part of the microbiota of skin and mucous membranes of humans and animals. They have a strong capacity to produce biofilms[39,40]. The actual number of cases of CONS transmission lineages are higher, as CONS outbreaks usually remain unidentified. *S.epidermidis* and *S.haemolyticus* strains elicit biofilm production associated with disease among preterm neonates[41]. The spread of multidrug- resistant endemic clonal CONS was also detected in neonatal ICUs and wards in hospitals. Within an eleven year period, there was an emergence of one molecular cluster as the major causative agent of CONS sepsis in Dutch neonatal ICU[42,43,44,45]. Moreover, due to various difficulties in the CONS differentiation in the pre molecular level, all the data related to the species must be dealt with carefully. So, this study demonstrates that CONS, although less virulent in nature, have the capability to elicit infections, especially when cofactors are already present. These cofactors include foreign bodies, immunosuppression[46]. So, one of the most important clinical aspects of CONS infections, is that its foreign body- related[47]. CONS strains obtained from patients with Toxic Shock Syndrome produced no strains, although, had the capacity to stimulate human monocytes to produce cytokines, which also represent some of the clinical symptoms. These strains also have the ability to carry enterotoxin genes[48]. Any implanted foreign body can be colonized by CONS colonies[49]. In many studies, CONS group had exhibited to be the common cause of peritonitis by about 30%[50,51,52,53]. Thus, this study focuses on emphasizing the fact that CONS is also present in root canal infections and also has the capability to cause root canal failures. In other words, more studies have yet to be conducted to focus on the incidence of CONS in root canal infections.

### CONCLUSION:

So, to conclude, root canal infections might have multiple aetiologies amongst which Coagulase negative Staphylococci seem to be the dominant entity among the polymicrobial presence. These colonies may lead to root canal treatment failure due to their capacity of multiple drug resistance. Henceforth, this study was conducted to determine the incidence of Coagulase Negative Staphylococcus colonies in root canal infections.

### REFERENCES:


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