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SYSTEMATIC REVIEW ON MICROFLORA AND THEIR IMPLICATION IN THE FAILURE OF ROOT CANAL TREATMENT.

Dr. Krishna Prasada L[1], Dr Banashree Bhagawati[2]

[1]Department of Conservative and Endodontics, K.V.G Dental College and Hospital, Kurunjibagh, Sullia -574327.

[2]Department of Conservative Dentistry and Endodontics, K.V.G Dental College and Hospital, Kurunjibagh, Sullia -574327.

ABSTRACT

INTRODUCTION: Endodontic treatment is fairly predictable in nature with reported success rates up to 86–98%. The microbiota associated with failed cases differs markedly from that reported in untreated teeth (primary root canal infection).The current review aims to compile all the current studies concerning organisms that causes endodontic failure.**METHOD:** In this systemic review databases search on IRIS, Scopus, MEDLINE,Pubmed, google scholar, Cochrane library and web of science was conducted using specific inclusion and exclusion criteria. Among 1000 studies, only 11 met the inclusion criteria and were included in the review for further analysis. **RESULTS:**The 11 studies from 2000 to 2023 included in this analysis revealed that the E.Faecelis had been reported majority of the studies along with various other organisms such as Streptococcus, Peptostreptococcus,Actinomyces ,P. alactolyticus ,Propionibacterium propionicum etc. **CONCLUSION:**Enterococcus faecalis is identified as the main microorganism associated with endodontic failure, but recent studies have also isolated other major bacterias such as Streptococcus, Peptostreptococcus, Actinomyces etc.

KEYWORDS: Endodontic Infections, failure, endodontic microbiota, Retreatment

INTRODUCTION

Endodontic treatment is fairly predictable in nature with reported success rates up to 86–98%. However, there has not been a consensus in the literature on a consistent definition of endodontic treatment's “success” criteria. Likewise “failure” has variable definitions and the persistence of microbiological infection stands out as a prominent causative factor in endodontic failure, as evidenced by the literature. The literature has well-established the crucial role played by bacteria in peri-radicular infection, and endodontic treatment is at a higher risk of failure if microorganisms persist in the canals at the time of root canal obturation.[1]

The success of root canal treatment depends on the complete eradication of microflora from the root canal system. Understanding the microbial ecology of the pulp-dentine complex is important for disease prevention and treatment[2].

Microorganisms can enter the root canal system through various routes, such as traumatic damage, failing restorations, and dentinal tubules[3]. The endodontic microflora differs from that seen in other sites in the oral cavity[4]. Some species, such as M. dentalis, P. endodontalis, and P. nigrescens, are closely associated with endodontic infection[5]. Enterococcus faecalis is a microorganism that can persist in the root canal system and is recognized as a pathogen in post-treatment endodontic infections[6]. Biomechanical procedures during treatment can affect the susceptibility of different species, with certain species being more resistant than others. Adequate treatment can drastically change the root canal environment and eliminate bacteria.

The microflora in root canals plays a crucial role in the success or failure of endodontic treatment.The microbiota associated with failed cases differs markedly from that reported in untreated teeth (primary root canal infection). Whereas the latter is typically a mixed infection, in which gram-negative anaerobic rods are dominant, the former is usually composed of one or a few bacterial species, generally gram-positive bacteria, with no apparent predominance of facultative or anaerobes. Moller et al, after examining failed cases, reported a mean of 1.6 bacterial species per root canal. Anaerobic bacteria corresponded to 51% of the isolates. *Enterococcus faecalis* was found in 29% of the cases. Sundqvist *et al.* observed a mean of 1.3 bacterial species per canal and 42% of the recovered strains were anaerobic bacteria. *E. faecalis* was detected in 38% of the infected root canals. Whilst this facultative bacteria is restricted to a few cases of primary root canal infections, usually in low numbers, it is frequently isolated from secondary and/or persistent root canal infections, usually as a single species of microorganism.[7]

Understanding the microbial composition of root canals and its implications for treatment success is thus essential for developing effective strategies to prevent and manage endodontic failures. By conducting this systematic review and meta-analysis, we are gathering and analyzing data on the microbial composition of root canals from multiple studies to provide a comprehensive overview of the microflora and its impact on the success of root canal treatment.

AIM

To evaluate the microflora and their implication in failure of root canal treatment.

METHODOLOGY

Research Protocol

This systematic review is conducted in accordance with Joanna briggs institute (JBI) critical appraisal checklist for systematic reviews and reported as per preferred reporting items for systematic reviews and meta analysis (PRISMA) guidelines.

Literature search and screening strategy

A comprehensive search of scientific papers published between 2000-2023 using single/combination of key words in the following databases IRIS, Scopus, MEDLINE- Pubmed, google scholar, Cochrane library and web of science was conducted.

Search strategy

Search strategy was according to the following MESH type keywords

Endodontic Infections, failure, endodontic microbiota, Retreatment

Criteria for considering studies for this review

INCLUSION CRITERIA

Potentially relevant papers found from the reference lists of related research were hand searched..All relevant titles, abstracts published in English over the past 23 years (2000-2023) were identified and retrieved by the two authors. Studies done after 2000 investigating the bacterial associations with persistent endodontic lesions. Articles that described the different types of microorganisms in the root canal system .Articles published, “full text” articles, journal articles with an “impact factor” greater than 1.Clinical studies only in English language were included.

.EXCLUSION CRITERIA

Case reports , **systematic reviews** and articles other than randomize control trial and article with publication date prior to 2000 were excluded.

STUDY SELECTION

Initial search studies yielded 1000 abstracts/ titles since multiple databases were individually searched,& remaining abstracts/ titles were identified as duplicates and were excluded. A total of 102 abstracts / titles were excluded because they were literature review/ expert opinions and due to language restrictions or because they did not fulfil the inclusion/exclusion criteria. A total of 67 abstracts/ titles qualified for full text evaluation. At this level, these 67 articles/ titles were considered potentially eligible and sought for further assessment, and full-length articles were retrieved. These full-length articles were independently reviewed by three authors with expertise in the content area to establish whether the studies met inclusion criteria. 38 articles were excluded unanimously through the decision of the reviewers. Disagreements amongst the reviewers were resolved by discussion and wherever discussion was considered as final. Eventually a total of 11 articles were sought for qualitative synthesis in this systematic review. The title of the article, name of the journal, and authors with respective affiliations were masked and circulated among a panel of five reviewers. Each manuscript was analysed for methodological quality according to a prepared checklist based on CONSORT AND STROBE guidelines respectively.

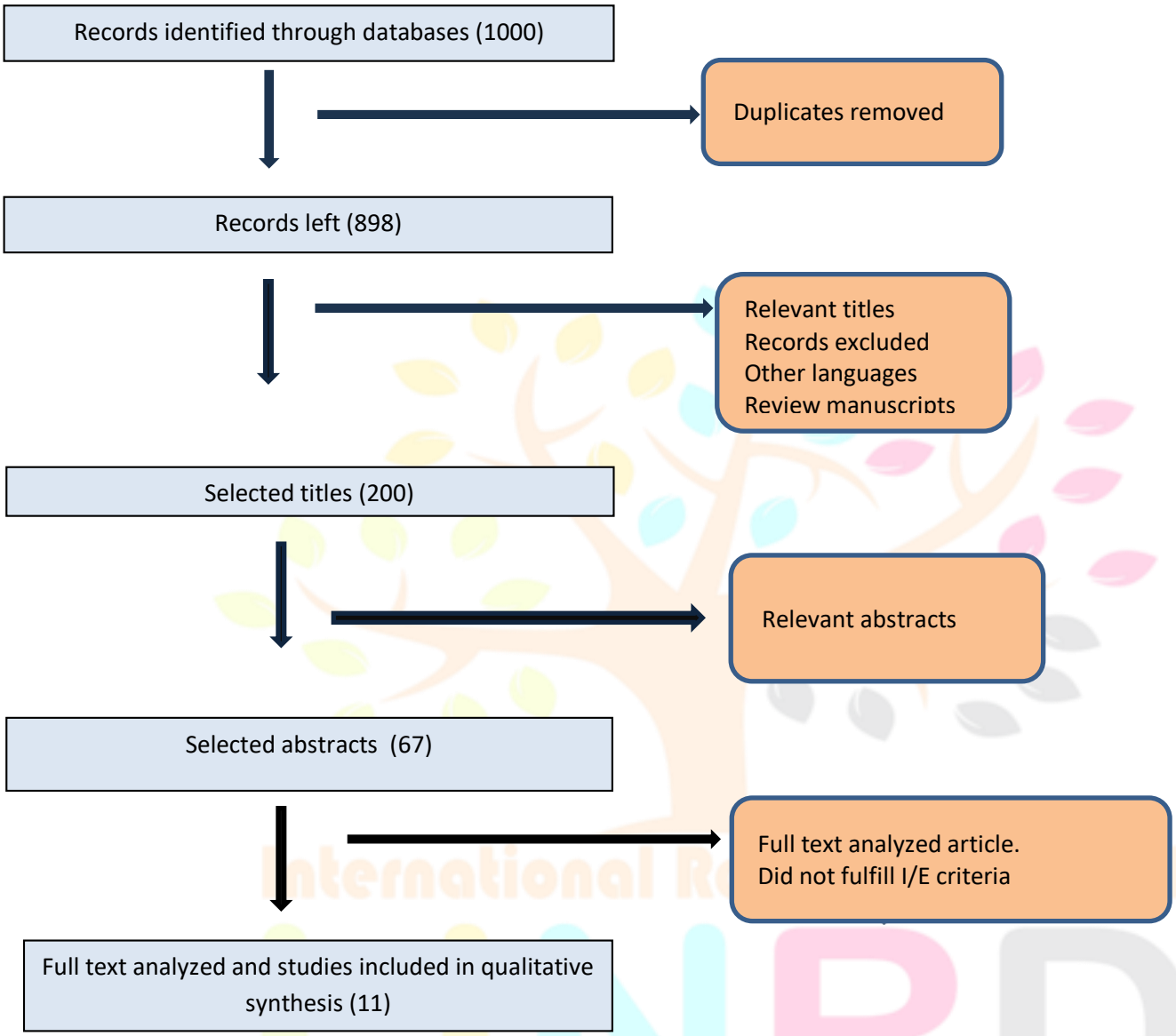
Data extraction

Data extraction was performed manually and data were entered into a structured Microsoft excel sheet. The characteristics of the study were extracted independently by the same investigators and recorded. The data were compared for accuracy and any discrepancies were discussed and resolved by consensus.

Risk of bias assessment

The risk of bias assessment was assessed using robins-I tool for non-randomised clinical trial and Cochrane collaboration's tool for randomised control trial.

FLOWCHART OF DATA SEGREGATION



RESULTS & DISCUSSION

	AUTHORS	YEAR	ANALYSIS METHODS	Sample size	MAIN CONCLUSIONS
1.	Ericka Tavares Pinheiro	2003	polymerase chain reaction.	30	Microbial flora in canals after endodontic failure comprised predominantly facultative anaerobes and gram-positive species. The bacterial genera most frequently recovered were Enterococcus, Streptococcus, Peptostreptococcus and Actinomyces. -E.faecalis was the species most frequently isolated
2.	José F Siqueira	2004	polymerase chain reaction.	22	*E faecalis* was the most prevalent species, followed by 4 other anaerobic species: *P alactolyticus*, *P.propionicum*, *D pneumosintes*, and *F alocis*. All examined samples harbored at least 1 of the following gram-positive bacterial species: *E faecalis*, *P alactolyticus*, or *P propionicum*.
3.	M. Sakamoto	2008	Molecular analysis	9	Other bacteria such as Fusobacterium nucleatum, Dialister pneumosintes, Tannerella forsythia, Pseudoramibacter alactolyticus, Propionibacterium propionicum, Filifactor alocis, Parvimonas micra, Solobacterium moore, and Dialister invisus were also prevalent.
4.	Nóbrega et al	2013	Nested-PCR technique	40	Treponemas were detected in 56.5% of the samples analyzed . Individual root canals yielded a maximum of 6 target Treponema species. T. denticola (30.8%) and T. maltophilum (30.8%) were the most frequently detected species followed by T. medium (20.5%), T. socranskii (20.5%), T. pectinovorum (17.9%) and T. vicentii (17.9%).
5.	Aysin Dumani et al	2012	polymerase chain reaction.	114	E.faecalis was identified (10%) of 114 retreated canals **C. albicans** were found in (11%) retreated canals
6.	Cristiana Francescutti Murad et al	2014	Checkerboard DNA-DNA hybridization technique	36	The study investigated the microbiota of failed root canal cases and found Enterococcus faecium and Staphylococcus epidermidis to be the most prevalent species. Teeth with persistent endodontic infections have a mixed and complex microbiota, with Enterococcus faecium and Streptococcus

					epidermidis as the most prevalent species.
7.	Jian-Fang et al	2016	PCR		The study found that the detection rate of bacteria in root canal infection after treatment failure was 93.33%. - Root canal infections may or may not have symptoms. - **Actinomycetes and Enterococcus faecalis** are the main microorganisms involved.
8.	Monika Łysakowska et al	2016	(ATB system)	37	The most prevalent microorganism in failed root canal cases was **Enterococcus faecalis.** **Enterococci and streptococci** were the most commonly isolated bacteria from root canals, and the resistance of enterococci to many antimicrobials may pose a problem in their eradication.
9.	R. S. Pereira et al	2017	polymerase chain reaction	30	The bacterial population in both the root ends and the periradicular tissues consisted of, in decreasing order of prevalence: F. nucleatum (71.6%) > D. pneumosintes (58.3%) > T. forsythia (48.3%) > A. actinomycetemcomitans (25%) > T. denticola (16.6%) > P. intermedia (15%) > P. gingivalis (15%) > E. faecalis (11.6%) > P. endodontalis (10%) > Prevotella nigrescens (1.6%).
10.	Maryam Pou rhajibagher	2017	PCR		Presence of Fungal species in Secondary/persistent endodontic infection endodontic infections could be the cause of failure of endodontic treatment **C. albicans** were found in 42.86% cases in this study
11.	Jelena Nešković et al	2018	**polymerase chain reaction**	**30**	Presence of periapical lesions affects microbiological status. Isolated bacteria mainly belonged to E. faecalis (66.6%) followed by P.intermedia (60%), P. micros (46.6%), P. endodontalis (26.6%) and A. actinomycetemcomitans (10%) - Most frequently identified bacteria: **E. faecalis, P. intermedia, P. micros**

RESULT

A total of 11 studies from 2000 to 2023 were included in this review.analysis of this paper revealed that the E.Faecelis been reported majority of the studies followed by various other organisms such as Streptococcus, Peptostreptococcus,Actinomyces ,P. alactolyticus ,Propionibacterium propionicum, Dialister pneumosintes, Filifactor alocis, Candida albicans, F.nucleatum,Tannerella forsythia, Pseudoramibacter alactolyticus,P.micra, Staphylococcus warneri, T. denticola,T. maltophilum,Eubacterium saburreum,P. intermedia, P.endodontalis, P. gingivalis Leptotrichia buccalis.Also minorities reported the occurrence of Solobacterium moore, Dialister invisus Capnocytophaga sputigena Conversely, Treponema socranskii , T. pectinovorum ,T. vicentii,Fusobacterium periodonticum , Capnocytophaga gingivalis and Spiroplasma ixodetis etc (table -1).

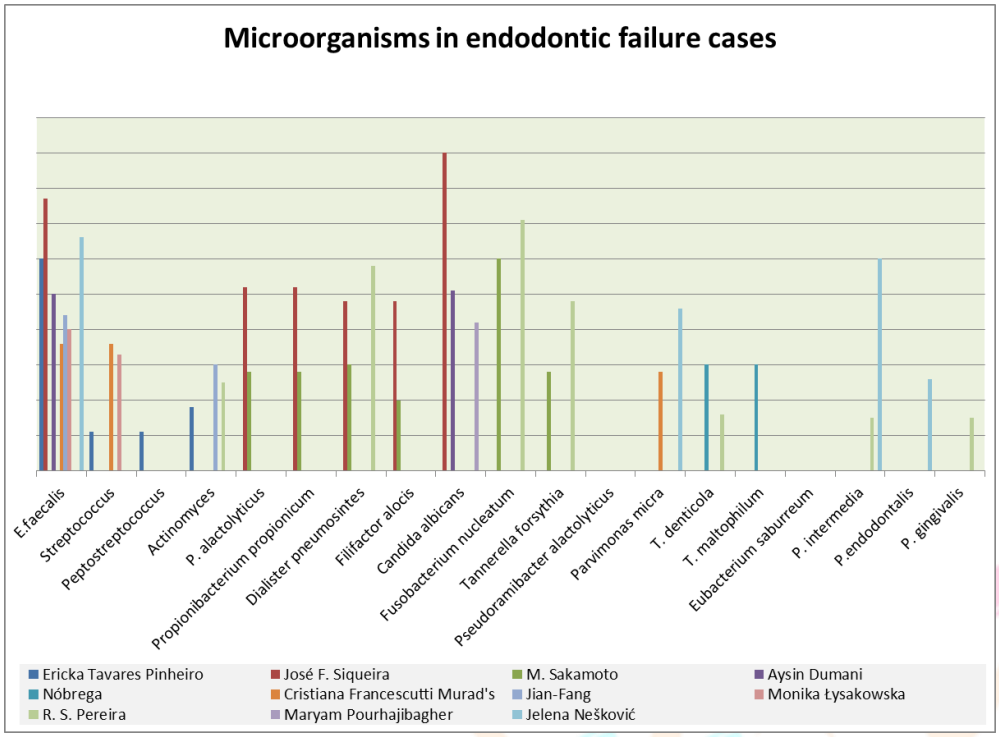


TABLE -1

The different analysis methodologies employed in all the 11 studies is represented in the pie diagram underneath. (Diagram 2) .Correlation between the presence of certain symptoms after the initial endodontic treatment and findings of specific bacterial species in root canal are shown in Table 3. E. faecalis and P. intermedia were identified in all patients experiencing pain, teeth that were sensitive to percussion, and pain upon biting. Additionally, these microorganisms were found in 50% of the samples collected from the root canal with sinus tract and swelling.

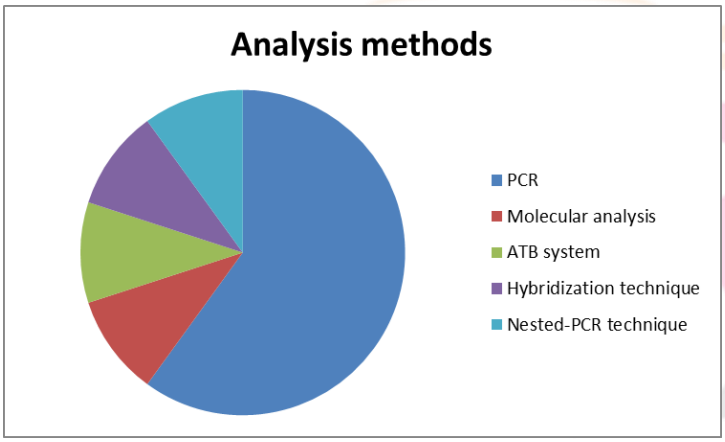


DIAGRAM-2

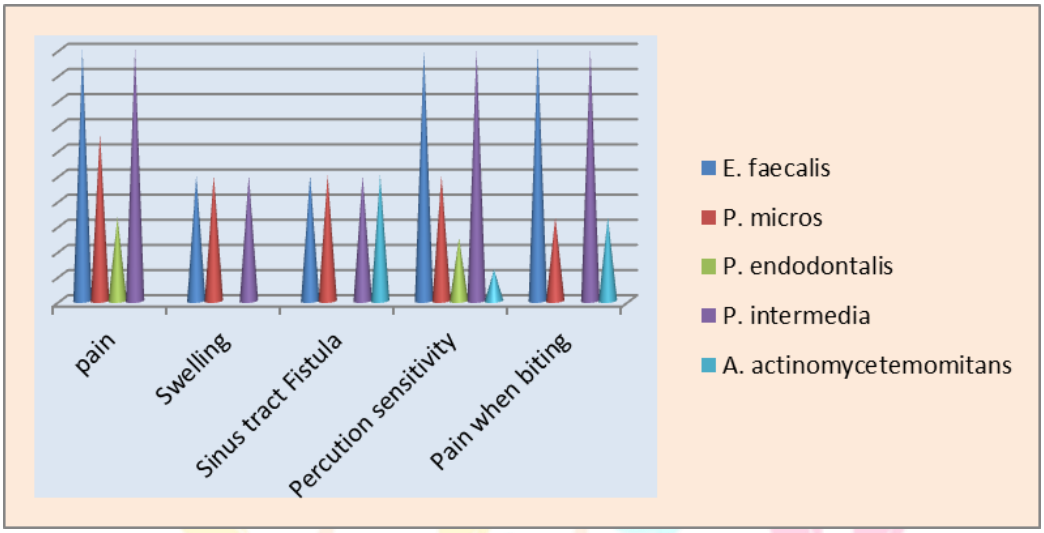


TABLE -3 Correlation between the presence of certain symptoms after the initial endodontic treatment and findings of specific bacterial species in root canal

DISCUSSION

Endodontic treatment failure is often associated with the presence of bacterial biofilms in the root canal system [8] . Biofilms are complex microbial communities that form on the root canal walls that are difficult to eliminate .These biofilms can harbor numerous microorganisms with varying ecological requirements and pathological potential. In addiction, these coordinated functional communities offer bacteria protection against other competitive microorganisms, antimicrobial agents and host defenses, increasing therefore its pathogenicity. Conventional endodontic treatment may not be able to reach and eliminate biofilms in inaccessible areas, such as the external surfaces of the root apex . The presence of bacterial biofilms in these areas can lead to persistent symptoms and treatment failure[9]

Various investigations have examined the composition of biofilms in root canals of teeth afflicted with apical periodontitis subsequent to endodontic therapy, yielding incongruous findings with respect to the preponderant pathogen in failed cases.

In 2003, Ericka Tavares Pinheiro reached a conclusion providing valuable insights into the microbial diversity present in these canals following endodontic failure. A total of 55 bacterial species were isolated, 80% were gram-positives and 58% facultative anaerobic microorganisms. The bacterial genera that were most commonly observed were Enterococcus, Streptococcus, Peptostreptococcus, and Actinomyces E.faecalis was the species most frequently isolated. [8]José F. Siqueira highlighted that statistically, the most prevalent microorganism found in previously filled root canals is E. faecalis, accounting for 77%. Following this, Pseudoramibacter alactolyticus and Propionibacterium propionicum were both found in 52% of cases, with Dialister pneumosintes and Filifactor alocis coming in at 48%. Candida albicans was found in only 9% of all samples.[10]

Subsequently M. Sakamoto conducted a study that resulted in the conclusion that multiple other bacterial species, namely Fusobacterium nucleatum, Dialister pneumosintes, Tannerella forsythia, Pseudoramibacter alactolyticus, Propionibacterium propionicum, Filifactor alocis, Parvimonas micra, Solobacterium moore, and Dialister invisus, were found to be prevalent alongside e.feacelis. This discovery was made through the utilization of 16S ribosomal

RNA gene clone library analysis. The current findings revealed new candidate endodontic pathogens, including as-yet-uncultivated bacteria and taxa other than *E. faecalis*, which may participate in the mixed infections associated with post-treatment apical periodontitis.Aysin Dumani et al E.faecalis was identified (10%) of 114 retreated canals.**C. albicans** were found in (11%) retreated canals by polymerase chain reaction..[11]The study by Nóbrega et al 2013 revealed that a wide variety of Treponema species plays a role in persistent/secondary infection turning the root canal microbiota even more complex than previously described by endodontic literature. Individual root canals yielded a maximum of 6 target Treponema species. T. denticola (30.8%) and T. maltophilum (30.8%) were the most frequently detected species followed by T. medium (20.5%), T. socranskii (20.5%), T. pectinovorum (17.9%) and T. vicentii (17.9%). Positive association was verified between T. denticola and T. maltophilum such as T. medium (P<.05). T. lecithinolyticum was positively associated with intraradicular post (P<.05).[12]

Cristiana Francescutti Murad's study has determined that the microbiota found in teeth afflicted with persistent apical periodontitis is characterized by a diverse and intricate profile, with the most highly prevalent species being E. faecium and S. epidermidis. The most frequently occurring microbial species were Enterococcus faecium (36%), Streptococcus epidermidis (36%), Eubacterium saburreum (28%), Parvimonas micra (28%), Streptococcus sanguis (28%), Capnocytophaga sputigena (28%), Leptotrichia buccalis (28%) and Staphylococcus warneri (28%). Conversely, the occurrence of Treponema socranskii (3%), Fusobacterium periodonticum (3%), Capnocytophaga gingivalis (3%), and Spiroplasma ixodetis (3%) were relatively low.[13]

Jian-Fang et al in 2016 found that the detection rate of bacteria in root canal infection after treatment failure was 93.33%. Root canal infections may or may not have symptoms.[14]**Actinomycetes and Enterococcus faecalis** are the main microorganisms involved. Monika Łysakowska and her colleagues have ascertained that Enterococcus faecalis was the most frequently occurring microorganism in root canal cases that were deemed unsuccessful. It has been observed that enterococci and streptococci are the bacteria that are usually isolated from root canals. The resistance of enterococci to many antimicrobials may present a challenge in their elimination[15].R. S. Pereira et al. concluded that secondary and persistent infections are primarily polymicrobial. The bacterial population observed in both the root ends and periradicular tissues exhibited a prevalence ranking, in descending order, as follows: F. nucleatum (71.6%), D. pneumosintes (58.3%), T. forsythia (48.3%), A. actinomycetemcomitans (25%), T. denticola (16.6%), P. intermedia (15%), P. gingivalis (15%), E. faecalis (11.6%), P. endodontalis (10%), and Prevotella nigrescens (1.6%).[16] The fungal species are present in secondary and persistent endodontic infections, which may be responsible for the failure of endodontic treatment. As per the findings of a study, C. albicans were detected in 42.86% of cases. [17]

Jelena Nešković et al in 2018 Isolated bacteria mainly belonged to E. faecalis (66.6%) followed by P.intermedia (60%), P.micros (46.6%), P.endodontalis (26.6%) and A. actinomycetemcomitans (10%). Most frequently identified bacteria: E. faecalis, P. intermedia, P. micra.[18]

CONCLUSION

The failure of root canal treatment can be attributed to the persistence of microorganisms in the dentinal tubules and the inadequate seal of the root canal system. Enterococcus faecalis is identified as the main microorganism associated with endodontic failure, but recent studies have also isolated other major bacterias such as Streptococcus, Peptostreptococcus, Actinomyces. These microorganisms possess characteristics that enable them to survive disinfection measures, including biofilm formation, localization in hard-to-reach areas, synergism, and the

activation of survival genes .[19]Understanding the microbiota and their ability to resist disinfection measures is crucial for developing effective treatment regimens and preventive therapies for root canal infections .

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