Microbial Profile of Root Canals of Pulpally Infected Teeth In Ghanaians

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Abstract

Background

Pulpal and periapical infections are initiated by microorganisms when they gain access into the dental pulp. The success of root canal treatment principally depends on the eradication of the micro-organisms in the root canal system. The aim of the study was to determine the microbial agents of infected root canals in Ghanaians patients.

Methodology

Forty four consecutive patients with sixty teeth referred to the Restorative Dentistry Clinic requiring root canal treatment were recruited. Root canal samples were collected from the teeth with sterile paper points. The samples were processed at the laboratory setup created at the chairside, subjected for microbial analysis and identification using Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS).

Results

A total of 259 isolates were recovered from the 60 infected root canals, belonging to twenty (20) different microbial genera. Out of the 259 microbial species isolated, only two (2) were candida albicans, a fungi; 257(99.2%) were bacterial isolates belonging to 19 genera. The 19 genera had 53 bacterial species, out of which 26(49.1%) were identified as facultative anaerobes, 15(28.3 %.) as obligate anaerobes and 12(22.6%) were aerobes. Streptococcus species (*Streptococcus oralis, S.mitis, S. mutans* and *S. constellatus*) were the most predominant isolates, followed by *Prevotella sp, Actinomyces sp, Enterococcus faecalis and Rothia sp* respectively.

Conclusion

The findings of this study show that, primary root canal infections are polymicrobial with facultative anaerobes been predominant. The determination of the microbial profile aids in understanding the pathogenesis of pulpal and periradicular infections and help in choosing effective antimicrobial irrigation and medicament for root canal treatment.

Introduction

Primary root canal infections occur when microorganisms gain access to, and colonize the pulpal tissue, impairing its function. [1] **S**econdary infection of root canals indicate a failure of endodontic treatment, especially due to the persistence of microbial infection in the root canal system.[2]

Primary root canal infections are polymicrobial in nature and have been found to consist mainly of gram-negative anaerobic bacteria [3]; with an average of 4–7 intra-canal species. [3,4,[5] isolated 46.3% strict

anaerobes, 37.1% facultative anaerobes, 10.5% microaerophilic, and 5.9% aerobic microorganisms in teeth with primary endodontic infections.

Some studies have shown obligatory anaerobic bacteria in root canal infections, which comprise 90% of all bacterial species that were isolated.[6,4]

Identifying the microorganisms involved in the pathogenesis of pulpal and periradicular infections, help in choosing effective antimicrobial irrigation and medicament for root canal treatment. This will improve the treatment strategies to control root canal infections by eliminating the pathogenic agents, as well as to prevent reinfection and periapical lesions.[7]

Several factors, such as geographic location, socioeconomic status, dietary habits, and oral hygiene status affects the type and frequency of the microbial agents involved in the pathogenesis of root canal infections. [5]

In sub-saharan african countries wheediet consist mainly of carbohydrates and patients seek dental care late when teeth are cavitated;it isimportant to is isolate and identify the

MALDI-TOF MS has several strengths compared to other diagnostic tools, such as polymerase chain reaction (PCR) assays. Once the mass spectrometer and the corresponding databases are available in a laboratory, individual pathogen identification is inexpensive, and the sample preparation procedure isn't higly technique sensitive nor require complex additional laboratory infrastructure. It has high diagnostic accuracy, robustness, reliability and rapid turn-around time and is considerably less prone to contamination.[8]

Currently, limited data is available on the types of microorganisms involved in root canal infections in the sub region. To the best of our knowledge, no study in Ghana has identified the microbes in root canal systems. The aim of the study was to evaluate the microbial profile of root canals with various stages of infections in Ghanaians. This will help to choose the appropriate root canal medicament to help eliminate them during treatment

Materials And Methods

Case selection

The data was collected between October 2016 and July 2017.Forty four consecutive patients with sixty teeth referred to the Restorative Dentistry Clinic requiring root canal treatment were selected. The diagnosis of the teeth were irreversible pulpitis, necrotic pulps, apical periodontitis and apical abscesses were used for this study.

The diagnosis was made with the aid of History, Clinical examination, response to pulp sensitivity tests, and review of radiographs.

Inclusion criteria

- Fully formed permanent teeth requiring non-surgical endodontic treatment
- No antibiotic treatment at least 4weeks prior to presentation
- Teeth without active periodontal disease
- Teeth with single root and single with radiographic evidence of patent canal
- Teeth that can be adequately isolated temporized and restored after the root canal treatment

Exclusion criteria

- Presence of systemic conditions such as diabetes, heart conditions, immunosuppressive conditions, autoimmune conditions etc. which will affect healing or require antibiotic treatment
- Antibiotics prior to presentation
- Teeth with secondary root canal infection requiring retreatment

Ten healthy teeth were used as control. These teeth required elective root canal treatment. Root canal samples were taken for microbial analysis.

Ethical considerations

Ethical approval was obtained from the Ethical and Protocol Review Committee of the College of Health Sciences of the University of Ghana. The study was explained to the participants and informed consent was obtained.

Sample collection

Consecutive patients referred to the clinic for treatment were recruited. Bacterial sampling procedure and microbial identification was conducted as using a modified procedure described in earlier studies. [5] Oral prophylaxis was carried out to remove plaque and calculus. All carious lesions and/or defective coronal restorations were then removed and the tooth restored. The tooth was cleaned with pumice and disinfected with methylated spirit (95% ethyl alcohol and 5% methyl alcohol). A rubber dam was fitted and methylated spirit used again. The procedure was done under aseptic conditions. Before entering the pulp chamber the access was disinfected again with methylated spirit. After accessing the pulp chamber, the patency of the root canal was established with minimal instrumentation when necessary and then the microbial sample was taken.

For each sample, a new sterile pouch (Henry Schien) was opened, and samples collected while ensuring that the paper points did not touch any other surface other than the root canal. For each tooth, three sterile paper points were inserted into the canal, for about 30sec-1min for the paper point to be soaked, it

was immediately transferred into a vial containing 2mls of Phosphate buffered saline (PBS).The roots canals were then debrided and shaped using protaper hand files using selected concentrations of sodium hypochlorite as irrigant.

The canal was deemed fully clean when the file snugged at the full working length and no debris found at the tip of the file when removed and viewed. A second microbial sample (S_2) was taken from the root canal with sterile paper point as described above for microbial analysis.

A laboratory setup was created at the chair side of the dental clinic to begin processing the specimen to avoid delay.

Isolating and detection of species

The samples (paper points) placed in 2mL of Phosphate Buffer Solution (PBS) were vortexed in a vortex mixer (Eltek VM 301) set at 45seconds. Ten -fold serial dilutions of the bacterial suspensions were prepared in Buffered Peptone Water.

Non-selective enriched Anaerobe basal blood agar (ABBA) primary isolation plates were inoculated with 0.1 ml dilution aliquots, and spread with a sterile bent plastic rod. The anaerobic culture medium comprised of Anaerobe basal agar supplemented with 5% defibrinated sheep blood plates and kept in anaerobic Jar (Becton Dickinson) with anaerobic generating kit containing 85%N_2, 10% H,2 and 5% CO_2, (Oxoid Unipath Ltd., Basingstoke, Hampshire, UK) at 37^{o}C for 48-72 hours. All the incubation plates were examined daily for growth. The anaerobic jar was opened after 48 hours and inspected for colonies.

For aerobic culture, specimens were inoculated on Sheep Blood agar and selective media Mac Conkey agar (Oxoid Unipath Ltd., Basingstoke, Hampshire, UK) and Uri Select agar (Bio-Rad) by streak plate method and kept in mobile incubator and transported to the laboratory and finally placed in an incubator at 37^{o}C for 18 - 24 hours in air. All the procedures for the identification of these microorganisms were conducted according to the CLSI (Clinical and Laboratory Standard Institute) guidelines. After incubation, each plate was examined and the different colonies were sub cultured and identified.

Microbial Identification

All the bacteria isolated from this study were identified with Bruker Biotype Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometer (MALDI-TOF MS)system. (Bruker Daltonics GmbH, Leipzig, Germany). The Bruker Biotype MALDI-TOF MS system includes the Microflex LT/SH MSinstrument and two (2) software programs: FlexControl for acquisition of protein spectra and Biotyper real-time classification (RTC) for automated spectral analysis. Pure cultures from both anaerobic and aerobic were overlaid on the MALDI matrix and identified by the Microflex LT/SH MS instrument.

Results

A total of 44 participants with 60 teeth requiring root canal treatment were recruited for the study. Their ages ranged from 20 to 75 years with a mean age of 40.3 ± 14.9years.

Out of the 60 teeth sampled, 16 (26.7 %) were diagnosed with symptomatic apical periodontitis and 14 (23.3%) asymptomatic apical periodontitis. Thirteen (21.7%) of the teeth were diagnosed with irreversible pulpitis, 10 (16.7%) diagnosed with pulpal necrosis, 4(6.7% had acute apical abscess and 3(5%) had chronic apical abscess.

No microorganisms were cultured from the control teeth. There were positive cultures for all the sixty teeth. The average number of bacterial species per tooth was 4.85 ± 1.41. Most teeth (n=26, 43.3%) had five bacterial species isolated from them, followed by 17 (28.3%) teeth with 4 bacterial species isolated from them. Table 1 shows the number of bacteria isolated per tooth.

Table 1
Number of microbial species isolated per tooth

Number of bacterial species isolated per tooth	Number of teeth(n)	Percent (%)
2	3	5.0
3	3	5.0
4	17	28.3
5	26	43.3
6	5	8.3
7	2	3.3
8	2	3.3
9	2	3.3
Total	60	100.0

Microorganisms from twenty genera were isolated from the infected root canals. The genera of the organisms, their gram staining, oxygen tolerance and numbers isolated are shown in table 2 below.

Table 2
Profile of microorganisms isolated from infected root canals

Bacteria	Gram stain	Oxygen Tolerance	Number isolated
Escherichia	–	Aerobe	2
Rothia	+	Aerobe	11
Rhodococcus	+	Aerobe	3
Corynebacterium	–	Aerobe	1
Micrococcaceae	+	Aerobe	1
Fusobacterium	–	Anaerobe	6
Veillonella	–	Anaerobe	5
Prevotella	–	Anaerobe	19
Proprionibacterium	+	Anaerobe	2
Slackia exigua	+	Anaerobe	3
Actinomyces	+	Anaerobe	16
Parvimonas	+	Anaerobe	2
Enterococcus	+	FA/Aerobe	16
Streptococcus	+	FA/Aerobe	146
Pseudomonas	–	FA/Aerobe	1
Enterobacter	–	FA/Aerobe	8
Staphylococcus	+	FA/Aerobe	8
Neisseria	–	FA/Aerobe	6
Lactobacillus	+	FA/Anaerobe	1
Candida albicans		Fungi	2

Five organisms were aerobic, seven each were obligate anaerobic and facultative anaerobic/aerobic. Candida albicans, a fungus was also isolated. Ten of the bacteria genera isolated were gram positive, the remaining bacteria were gram negative. The prevalence of bacteria and fungi found in the root canals is shown in Table 3.

Table 3
Microbial species isolated from the infected root canals.

FACULTATIVE ANAEROBES		AEROBES	
Bacterial Species	**Number Isolated**	**Bacterial Species**	**Number Isolated**
Gram positive cocci		**Gram positive cocci**	
Streptococcus	(103)	Streptococcus	(43)
S. oralis	22	*S.mutans*	12
S.mitis	10	*S. oralis*	12
S. constellatus	10	*S. mitis*	10
S. salivarius	9	*S. cristatus*	4
S. sanguinis	7	*S.constellatus*	3
S. mutans	6	*S. sanguinis*	2
S. parasanguinis	6	*Enterococcus faecalis*	(14)
S. anginosus	5	Staphylococcus	(8)
S. gordonii	5	*S. epidermidis*	4
S. infantis	5	*S. warneri*	1
S. sanguis	4	*S. saprophyticus*	1
S. cristatus	3	*S. haemolyticus*	2
S. pneumoniae	3	Micrococcaceae	
S. infantis	3	*M.luteus*	1
S. haemolyticus	2	Rhodococcus	
S. cristatus	1	*R. rhodochrous*	3
S. australis	1		
S.anginosus	1	**Gram negative diplococci**	
Enterococcus faecalis	2	Neisseria	(6)
Gram positive bacillus		*N. flavescens*	4
Corynebacterium amycolatum	1	*N. subflava*	2
		Gram negative rods	
Gram positive coccobacillus		*Escherichia coli*	2
Rothia species	(11)	*Pseudomonas stutzeri*	1

R. dentocariosa	6	Enterobacter	(8)
R. mucilaginosa	4	*E.cloecae*	7
R. aeria	1	*E. kobei*	1
Gram positive rod			
Actinomyces	(5)		
A. naeslundii	4		
A. radicidentis	1		

ANAEROBES			
Bacterial Species	**Number Isolated**	**Bacterial Species**	**Number Isolated**
Gram negative rods		**Gram negative cocci**	
Prevotella	(19)	*Veillonella parvula*	5
P.buccae	5	**Gram positive cocci**	
P. denticola	4	*Parvimonas micra*	2
P. intermedia	4	**Gram positive rods**	
P. marshii	2	Actinomyces	(11)
P. oralis	2	*Actinomyces meyeri*	3
P. loescheii	1	*Actinomyces odontolyticus ooodontolyicus*	8
P. oris	1	*Slackia exigua*	3
Fusobacterium	(6)	*Propionibacterium acnes*	2
F nucleatum	4	*Lactobacillus mucosae*	1
F. periodonticum	2		
FUNGI			
Candida albicans	2		

Of the 259 cultivable isolates, 257 were bacterial isolates belonging to 19 genera were identified.The other 2 were isolates of candida albicans, a fungus. Out of the 53 bacterial species isolated, 26 (49.1%) were identified as facultative anaerobes, 15 (28.3 %.) as obligate anaerobes and 12(22.6%) were aerobes. The species were mainly gram positive 37(69.8%).Out of the gram positive species (22, 59.5%) were cocci.

Relationship between bacteria species and the different clinical diagnosis

Gram positive cocci streptococcus species were the main organisms isolated in teeth with pulpal necrosis and irreversible pulpitis. Gram positive coccobacillus, *Rothia dentocariosa*, Gram negative rods, *Enterobacter cloacae, E.coli*, Prevotella species were isolated in teeth with pulpal necrosis.

Gram positive rods (*Actinomyces odonlyticus, Propionibacteria acnes*), Gram negative rods (*Provotella buccae, P. marshin, P. denticola, Pseudomonas stutzen, E.coli*) , *Veillonella parula* and *Candida albicans* were some of the organisms isolated in teeth with irreversible pulpitis.

Gram positive cocci dominated by strepotococcus species were the most predominate bacteria isolated in the teeth with apical periodontitis followed by gram negative black pigmented rods prevotella, *Veillonella parula*, *Actinomyces spp* and other anaerobes were also identified as shown in table 4 below.

Table 4
Various clinical diagnosis and their microbial profile

Microbial species	Clincal Diagnosis			
Gram positive cocci	**Symptomatic Apical Periodontitis**	**Asymptomatic Apical periodontitis**	**Acute Apical abscess**	**Chronic Apical Abscess**
Streptococcus				
S. oralis	+	+	+	+
S.mitis	+	+	+	+
S. constellatus	+	-	-	+
S. salivarius	+	+	-	-
S. sanguinis	+	+	-	-
S. mutans	+	+	+	-
S. parasanguinis	+	+	-	-
S. anginosus	+	+	+	-
S. gordonii	+	+	-	-
S. infantis	+	-	+	-
S. cristatus	-	+	-	-
S. pneumoniae	+	-	-	-
S. haemolyticus	+	-	-	-
Staphylococcus warnei	+	-	-	-
Staphylococcus epidermidis	+	+	-	-
Parvimonas micra	+	-	-	-
Enterococcus faecalis	+	+	-	+
Rhodococcus rhodochrous	-	-	+	-
Gram positive bacillus				
Corynebacterium amycolatum	-	+	-	-
Rothia *mucilaginosa*	+	-		
Gram positive rod				

Actinomyces spp				
Actinomyces odonlyticus	+	+	-	+
A.meyeri	+	+	-	+
A. naeslundii	+	-	+	-
A. radicidentis	-	+	-	-
Slackia exigua	+	-	-	
Propionibacteria acnes	-	+	-	-
Lactobacillus mucosae	-	+	-	-
Gram negative rods				
Prevotella spp				
P. buccae	+	+	-	-
P.intermedia	+	-	-	-
P. loescheii	-	+		
P. oralis	-	+	+	-
Fusobacterium.nucleatum	+	+	-	+
Enterobacter kobei	+	-		
E. cloacae	+	+	-	+
Gram negative cocci				
Veillonella parula	+	+	-	-
FUNGI				
Candida albicans	-	+	-	-

In both acute and chronic apical abscess mainly anaerobic species were isolated. In acute apical abscess, Rhodococcus rhodochrous, Actinomyces naeslundii and Prevotella oralis were some of the organisms recovered. While in chronic apical abscess, *Enterobacter cloacae, Enterococcus faecalis. Actinomyces odonlyticus, Actinomyces meyeri, Fusobacterium nucleatum* were also recovered

After treatment only two teeth had specie each of *Streptococcus mitis* and *S.oralis*

Discussion

The present study evaluated the microorganisms present in infected root canals. All teeth used in this study had primary root canal infections and had positive cultures in all the sixty teeth. Our study yielded

negative cultures for the 10 control teeth which were selected for elective surgery (0% positive cultures). Total absence of positive cultures from control teeth, demonstrates the efficiency of the sampling technique used, and affirms the assertion by Shuping et al [9]that healthy vital pulps are largely free of microorganisms.

Some in vivo culture studies have demonstrated that primary endodontic infections are characterized by a mixture of organisms dominated by anaerobic bacteria and composed of a mean number of 2.6 to 5.4 species per canal.[10,11]In this study, at least two bacterial species were isolated per tooth with the highest number per tooth being 9. The average number of bacterial species per tooth was 4.85 ± 1.41 species.

The 53 bacterial species isolated, were predominantly facultative anaerobes 26 (49.1%), followed by obligate anaerobes 15 (28.3%.) and 12(22.6%) aerobes. The species were mainly gram positive 37, (69.8%). Out of the gram positive species 22 (59.5%) were cocci. This is consistent with the findings of Ercan et al.[12] who found predominantly facultative anaerobes (52.7%) and gram positive (67.8%) microorganisms in their study. Some studies however found obligate anaerobes to be predominant. [6,4,13]

Facultative anaerobes and aerobic bacteria isolated in this study were similar to those mentioned in Lee et al's[14] study. The facultative anaerobes isolated included, *Enterococcus faecalis, Corynebacterium amycolatum*, *Rothia*,Actinomyces.

The aerobes identified included *Neisseria spp. Escherichia coli, Pseudomonas stutzeri, Enterobacter kobei, E.cloecae* which are Gram negative rods were also identified.

The number of bacterial species isolated in this study was largely consistent with the array of bacteria in the review by Narayanan et al [15] in which *S mitis, S oralis and S.mutans* belonging to the viridans groups are the most frequently isolated bacterial species. Additional bacteria recovered in this study, belonged to Prevotella, Actinomyces and Rothia species respectively. These findings however differ from those of [16] where *S. gordonii and S oralis* were predominant in teeth with apical periodontitis

Regarding correlation between bacteria and type of infection, this study found that, irreversible pulpitis had mainly streptococcus species and provetella species. This is consistent with bacteria implicated and isolated in primary root canal infections as reported by Narayanan et al[15]

Teeth with diagnoses of apical abscesses (both acute and chronic) had mainly facultative anaerobes isolated. The species isolated were mainly *Streptococcus spp, Actinomyces naeslundii*, and *Prevotella oralis*, a black pigmented Gram negative rod.

The ecology of the root canal system changes with time and at increasing depths. With increasing bacterial population there is a reduction in available nutrition, as well as a decrease in the available

oxygen. This favours facultative and strict anaerobes and allows them to thrive, which most likely explains the findings in this study and others.

These findings compares with findings by Fowel et al.[17] which implicated facultative anaerobes belonging to the streptococcus viridans anginosus group in dental abscess. However some studies have isolated Staphylococcus aureus frequently from acute dental abscess, ranging from 0.7–15%.[18,19]

The species isolated in Chronic abscess in this study were Gram positive cocci (*Streptococcus oralis, Streptococcus mitis,Streptococcus constellatus,Strep constellatus) Enterococcus faecalis* (Gram positive rods)*Actinomyces odonlyticus,Actinomyces meyeri*(Gram negative rods)(*Fusobacterium nucleatum, and Enterobacter cloacae*).

Facultative anaerobes, such as the viridans group streptococci and the *Streptococcus anginosus* group, and strict anaerobes, especially anaerobic cocci, *Prevotella sp* and *Fusobacterium species* have been observed as the most common organisms isolated in dentoalveolar abscess.[19]They have been found in 10–87% of dentoalveolar abscesses. [20,21]

Conclusion

Root canal infections in Ghanaians are polymicrobial in nature with facultative anaerobes been predominant. Streptococcus was the most predominant genera isolated, followed by *Prevotella sps , Actinomyce sps*, *Enterococcus faecalis* and *Rothia sp.* The use of MALDI-TOF MS for identification and under-standing of these microorganisms associated with root canal infections and their clinical relevance will help in the development of appropriate treatment protocols for Ghanaians.

Recommendations

It is recommended that further studies be undertaken in order to monitor the changes in the microbial flora in the pulpally infected teeth and to institute the best treatment protocol; as different antibacterial agent work against different microbes.

Declarations

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Author Contribution

Contribution: Dr. Akua Boakyewaa Konadu was involved in the concept and design, data gathering, data analysis/interpretation literature review and write up of the manuscript and proof reading

Contribution: Prof Ebenezer Anno Nyako was involved in the concept and design, literature review,write up and proof reading of the manuscript.

Contribution: Dr. Patrick Caldicock Ampofo was involved in the concept and design, literature review, write up and proof reading of the manuscript.

Contribution: Dr Thomas Akuetteh Ndanu was involved in the data analysis/interpretation, write up of the manuscript, and proof reading of the manuscript

Contribution: Mr Moses Lorenzo Akyeh was involved in the design, data gathering, data analysis/interpretation write up of the manuscript, and proof reading of the manuscript.

Contribution: Prof Dorothy Yeboah-Manu was involved in the design, and data gathering and proof reading of the manuscript.

Approval by the Authors

The study was a product of honest and hard work by the authors. All the authors read the manuscript and approved that it should be submitted to the BMC Oral Health

Conflict of interest statement

This statement is to certify that the authors of this manuscript do not have any conflict of interest. There was also no funding for the article.

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