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Microbial profile of root canals of pulpally infected teeth in Ghanaians.

Perfil microbiano de los conductos radiculares de dientes con infección pulpar en ghaneses.

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Abstract: **Introduction:** Pulpal and periapical infections are initiated by microorganisms when they gain access to the dental pulp. The success of root canal treatment principally depends on the eradication of the microorganisms in the root canal system. The aim of this study was to evaluate the viable microbial profile of root canals with various stages of infection in Ghanaians. **Material and Methods:** 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 and subjected to 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 different microbial genera. Out of the 259 microbial species isolated, only two were *Candida albicans*, a fungi; 257 (99.2%) were bacterial isolates belonging to 19 genera. The 19 genera encompassed 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*, *Streptococcus mitis*, *Streptococcus mutans and Streptococcus 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 infected root canals are polymicrobial in nature. The determination of the microbial profile aids in understanding the pathogenesis of pulpal and periradicular infections and helps in choosing effective antimicrobial irrigation and medicament for root canal treatment.

Keywords: *endodontics; primary periapical periodontitis; microbiota; bacteria; Streptococcus; spectrometry, mass, matrix-assisted laser desorption-ionization*

Resumen: **Introducción:** Las infecciones pulpares y periapicales son iniciadas por microorganismos cuando estos acceden a la pulpa dental. El éxito del tratamiento del conducto radicular depende principalmente de la erradicación de los microorganismos en el sistema del conducto radicular. El objetivo de este estudio fue evaluar el perfil microbiano viable de los conductos radiculares con diversas etapas de infección, en ghaneses. **Material y Métodos:** Se reclutaron

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cuarenta y cuatro pacientes consecutivos (sesenta dientes), remitidos a la Clínica de Odontología Restauradora que requerían tratamiento de conducto. Se recogieron muestras del conducto radicular de los dientes con puntas de papel estériles. Las muestras se procesaron y se sometieron a análisis e identificación microbianos utilizando espectrometría de masa desorción/ionización láser asistida por una matriz con detección de masas por tiempo de vuelo (MALDI-TOF). **Resultados:** Se recuperaron un total de 259 aislamientos de los 60 conductos radiculares infectados, pertenecientes a veinte géneros microbianos diferentes. De las 259 especies microbianas aisladas, solo dos fueron *Candida albicans*, un hongo; 257 (99,2%) fueron aislados bacterianos pertenecientes a 19 géneros. Los 19 géneros abarcan 53 especies bacterianas, de las cuales 26 (49,1%) se identificaron como anaerobias facultativas, 15 (28,3%) como anaerobias obligadas y 12 (22,6%) aerobias. Las especies de *Streptococcus (Streptococcus oralis*, *Streptococcus mitis*, *Streptococcus mutans* y *Streptococcus constellatus)* fueron los aislamientos más predominantes, seguidas de *Prevotella* sp, *Actinomyces* sp, *Enterococcus faecalis* y *Rothia* sp, respectivamente. **Conclusion:** Los hallazgos de este estudio muestran que los conductos radiculares infectados son de naturaleza polimicrobiana. La determinación del perfil microbiano ayuda a comprender la patogenia de las infecciones pulpares y perirradiculares y ayuda a elegir una irrigación antimicrobiana eficaz y un medicamento adecuado para el tratamiento del conducto radicular.

Palabras Clave: *endodoncia; periodontitis periapical primaria; microbiota; bacterias; Streptococcus; espectrometría de masa por láser de matriz asistida de ionización desorción*

INTRODUCTION.

Primary root canal infections occur when microorganisms gain access to and colonize the pulpal tissue, impairing its function.[1] Inappropriate coronal seal, microleakage, failure in the chemical and mechanical preparation of the root canal, limitations and lack of proper quality of the canal filling material can result in a secondary infection that could cause apical periodontitis leading to failure of endodontic treatment.[2] Persistent infection is usually characterized by the presence of an apical periodontitis or radiographic lesion.[3]

Primary root canal infections are polymicrobial in nature and have been found to consist mainly of gram-negative anaerobic bacteria;[4] with an average of 4–7 intracanal species. Pourhajibagher *et al.*,[5] isolated 46.3% strict anaerobes, 37.1% facultative anaerobes, 10.5% microaerophilic, and 5.9% aerobic microorganisms in teeth with primary endodontic infections.

However Gomes *et al.*,[6] reported 70% isolates which were either strict anaerobes or micro-aerophilic. Some studies have shown that obligate anaerobic bacteria in root canal infections comprise 90% of the bacterial species that were isolated.[4,7,8] Identifying the microorganisms involved in the pathogenesis of pulpal and periradicular infections helps in the selection of effective antimicrobial irrigation and medicament for root canal treatment.

This will improve treatment strategies to control root canal infections, by eliminating pathogenic agents, and to prevent reinfection and periapical lesions.[9]

Several factors, such as geographic location, socioeconomic status, dietary habits, and oral hygiene status affect the type and frequency of microbial agents involved in the pathogenesis of root canal infections.[5] In sub-Saharan African countries, where the diet consists mainly of carbohydrates and where patients seek dental care late when teeth are already cavitated, it is necessary to document the microbial profile of infected root canal in these settings.

MALDI-TOF mass spectrometry (MS) has several strengths compared to other diagnostic tools such as polymerase chain reaction (PCR) assays. Once the mass spectrometer and the corresponding databases have been made available in a laboratory, individual pathogen identification is inexpensive, and the sample

preparation procedure is neither highly technique sensitive nor requires complex additional laboratory infrastructure. It has high diagnostic accuracy, robustness, reliability, and rapid turnaround time and is considerably immune to contamination.[10] Bruker (the manufacturer) updates the MALDI-TOF MS database regularly and it contains the highest number of known oral microorganisms.

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

MATERIALS AND METHODS.

Case selection

Forty-four consecutive patients, representing 60 teeth, who were referred to the Restorative Dentistry Clinic for root canal treatment, were selected. The diagnoses of irreversible pulpitis, necrotic pulp, apical periodontitis, and apical abscess 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 four weeks prior to presentation.
- Teeth without active periodontal disease.
- Teeth with single root and 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 would affect healing or require antibiotic treatment.
- Antibiotics prior to presentation.
- Teeth with secondary root canal infection requiring retreatment.

Ten healthy teeth were used as controls. These teeth required elective root canal treatment as a result of traumatic or iatrogenic exposure of the pulp. Before and after root canal samples were taken for microbial analysis, using the sample procedure described below.

Ethical considerations

Ethical approval was obtained from the Ethical and Protocol Review Committee of the College of Health Sciences of the University of Ghana (Reference Number: CHS-Et/M.1-P 4.9/ 2016-2017).

The study was explained to the participants, and informed consent was obtained.

Sample collection

Consecutive patients who were referred to the clinic for treatment were recruited. Bacterial sampling and microbial identification were conducted, using a modified procedure described in earlier studies (Pourhajibagher *et al.*,[5] 2017). 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 (Figure 1) 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 the samples were collected while ensuring that the paper points did not touch any surface other than the root canal. For each tooth, three sterile paper points were in-serted into the canal, giving about 30–60 seconds for the paper point to be soaked before being immediately transferred into vials containing 2 ml of phosphate buffered saline (PBS).

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Strict sampling and transport conditions are essential for anaerobic bacteria to grow. Some bacteria in the oral cavity are very fastidious and difficult to culture, so a laboratory setup was created at the chair side of the dental clinic to begin processing the specimen immediately. The processing of the samples were done under strict anaerobic conditions. The processed specimen was plated and kept in anaerobic jar and transported in a mobile incubator to the laboratory within two hours; and finally placed in an incubator at 37°C for 18 - 24 hours for further processing.

Isolation and detection of species

The samples (paper points) placed in 2 ml of PBS were vortexed in a vortex mixer (Eltek VM 301) set at 45 seconds. 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 then spread with a sterile bent plastic rod.

The anaerobic culture medium was comprised of anaerobe basal agar supplemented with 5% defibrinated sheep blood plates, kept in an anaerobic jar (Becton Dickinson) with an anaerobic generating kit containing 85% N_2, 10% H_2 and 5% CO_2, (Oxoid Unipath Ltd., Basingstoke, Hampshire, UK) at 37°C for 48–72 hours. All the incubation plates were examined daily for growth. The incubation period lasted for 7 days but the plates that displayed extensive growth by 48-72 hours were opened and inspected. For aerobic culture, specimens were inoculated on sheep blood agar, selective media MacConkey agar (Oxoid Unipath Ltd., Basingstoke, Hampshire, UK) and UriSelect agar (Bio-Rad) by the streak plate method, kept in a mobile incubator, transported to the laboratory and finally placed in an incubator at 37°C for 18 to 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 subcultured and identified. (Figure 2A and Figure 2B)

Microbial Identification

We identified all the bacteria isolated from this study with a Bruker Biotyper Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometer (MALDI-TOF MS) system (Bruker Daltonics GmbH, Leipzig, Germany).

This system includes the Microflex LT/SH MS instrument and two software programs: Flex Control for the acquisition of protein spectra and Biotyper real-time classification (RTC) for automated spectral analysis. Pure cultures from both anaerobic and aerobic growth were overlaid on the MALDI matrix and iden-tified by the Microflex LT/SH MS instrument. (Figure 3A and Figure 3B)

RESULTS.

Forty-four participants requiring root canal treatment were recruited for the study, and 60 teeth were examined. The ages of the participants ranged between 20 and 75 years with a mean age

Figure 1. Pictures of some the teeth used for the study.

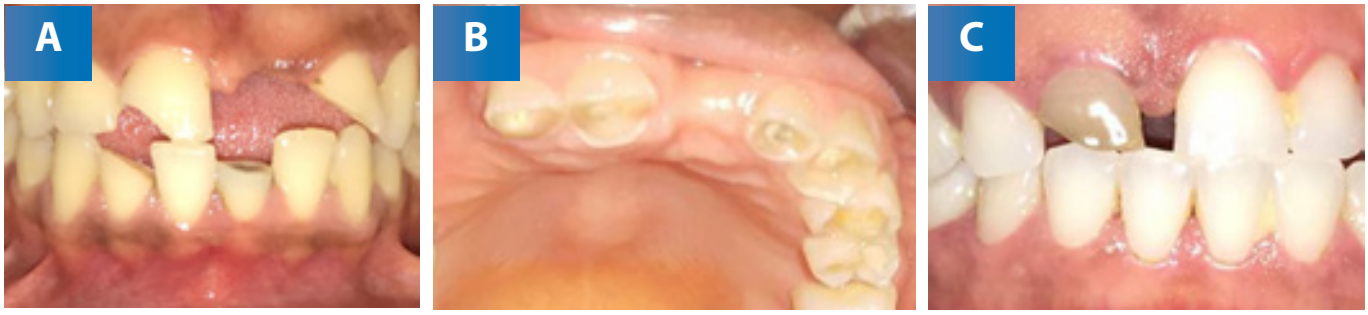

Figure 2. Aerobic and Anaerobic incubated plates displaying typical growth.

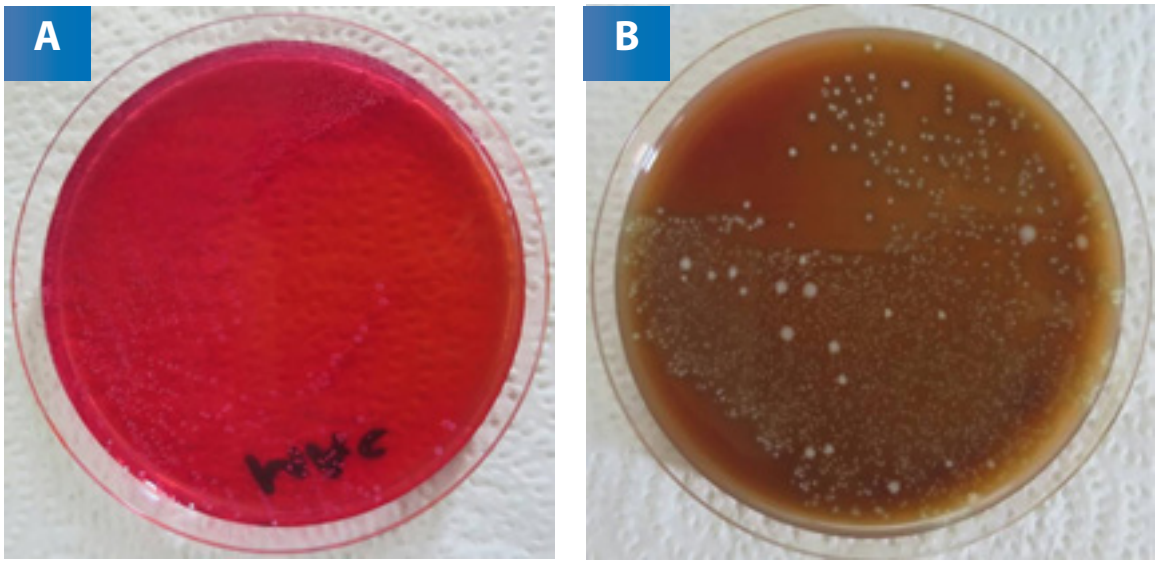

Figure 3. MALDI-TOF mass spectrometry machine and software.

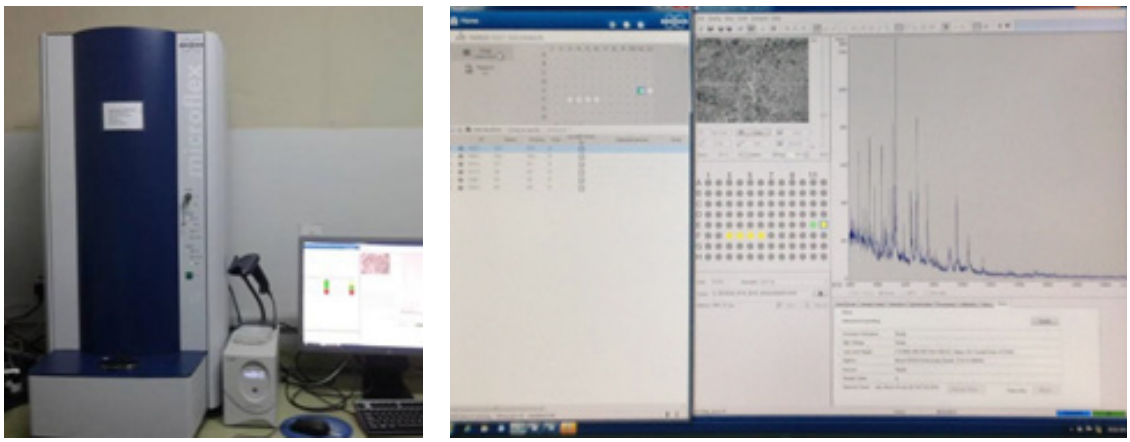

Table 1. Number of microbial species isolated per tooth.

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**

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Table 2. Profile of and number of microorganisms isolates recovered from infected root canals.

Microorganism	Gram stain	Oxygen Tolerance	Number of isolates
Escherichia	–	Aerobe	2
Rothia	+	Aerobe	11
Rhodococcus	+	Aerobe	3
Corynebacterium	–	Aerobe	1
Micrococcaceae	+	Aerobe	1
Fusobacterium	–	Anaerobe	6
Veillonella	–	Anaerobe	5
Prevotella	–	Anaerobe	19
Cutibacterium	+	Anaerobe	2
Slackia exigua	+	Anaerobe	3
Actinomyces	+	Anaerobe	16
Parvimonas	+	Anaerobe	2
Enterococcus	+	Facultative Aerobe	16
Streptococcus	+	Facultative Aerobe	146
Pseudomonas	–	Facultative Aerobe	1
Enterobacter	–	Facultative Aerobe	8
Staphylococcus	+	Facultative Aerobe	8
Neisseria	–	Facultative Aerobe	6
Lactobacillus	+	Facultative Anaerobe	1
Candida albicans	Not Applicable	Fungi	2

Table 3. Microbial species isolated from the infected root canals according to culture technique employed.

FACULTATIVE ANAEROBES		AEROBES		ANAEROBES	
Bacterial Species	Number Isolated	Bacterial Species	Number Isolated	Bacterial Species	Number Isolated
Streptococcus oralis	22	*Streptococcus mutans*	12	*Prevotella buccae*	5
Streptococcus mitis	10	*Streptococcus oralis*	12	*Prevotella denticola*	4
Streptococcus constellatus	10	*Streptococcus mitis*	10	*Prevotella intermedia*	4
Streptococcus salivarius	9	*Streptococcus cristatus*	4	*Prevotella marshii*	2
Streptococcus sanguinis	7	*Streptococcus constellatus*	3	*Prevotella oralis*	2
Streptococcus mutans	6	*Streptococcus sanguinis*	2	*Prevotella loescheii*	1
Streptococcus parasanguinis	6	*Enterococcus faecalis*	14	*Prevotella oris*	1
Streptococcus anginosus	5	*Staphylococcus epidermidis*	4	*Fusobacterium nucleatum*	4
Streptococcus gordonii	5	*Staphylococcus warneri*	1	*Fusobacterium periodonticum*	2
Streptococcus infantis	5	*Staphylococcus saprophyticus*	1	*Candida albicans**	2
Streptococcus sanguis	4	*Staphylococcus haemolyticus*	2	*Veillonella parvula*	5
Streptococcus cristatus	3	*Micrococcus luteus*	1	*Parvimonas micra*	2
Streptococcus pneumoniae	3	*Rhodococcus rhodochrous*	3	*Actinomyces meyeri*	3
Streptococcus infantis	3	*Neisseria flavescens*	4	*Actinomyces. odontolyticus*	8
Streptococcus haemolyticus	2	*Neisseria subflava*	2	*Slackia exigua*	3
Streptococcus cristatus	1	*Escherichia coli*	2	*Cutibacterium acnes*	2
Streptococcus australis	1	*Pseudomonas stutzeri*	1	*Lactobacillus mucosae*	1
Streptococcus anginosus	1	*Enterobacter cloacae*	7		
Enterococcus faecalis	2	*Enterobacter kobei*	1		
Corynebacterium amycolatum	1				
Rothia dentocariosa	6				
Rothia mucilaginosa	4				
Rothia aeria	1				
Actiomyces. naeslundii	4				
Actiomyces radicidentis	1				

***:**Fungi.

Table 4. Various clinical diagnosis and their microbial profile.

Microbial species	Clinical Diagnosis					
	IP	PN	SAP	AAP	AAA	CAA
Streptococcus oralis	+	+	+	+	+	+
Streptococcus mitis	+		+	+	+	+
Streptococcus constellatus	+	-	+	-	-	+
Streptococcus salivarius			+	+	-	-
Streptococcus sanguinis	+	+	+	+	-	-
Streptococcus mutans	+	+	+	+	+	-
Streptococcus parasanguinis	-	+	+	+	-	-
Streptococcus anginosus	-	+	+	+	+	-
Streptococcus australis	-	+				
Streptococcus gordonii	-	+	+	+	-	-
Streptococcus infantis	+	-	+	-	+	-
Streptococcus cristatus	+	-	-	+	-	-
Streptococcus pneumoniae	+	-	+	-	-	-
Staphylococcus haemolyticus	+	-	+	-	-	-
Staphylococcus epidermidis	-	-	+	+	-	-
Staphylococcus saprophyticus	+	-	-	-	-	-
Staphylococcus intermedius	+	-	-	-	-	-
Parvimonas micra	+		+	-	-	-
Enterococcus faecalis	+	+	+	+	+	+
Micrococcus luteus	-	+	-	-	-	
Rhodococcus rhodochrous	-		-	-	+	-
Veillonella parula	+	-	+	+	-	-
Neisseria flavescens	-	+	+	+	+	+
Neisseria subflava	-	-	-	+	-	+
Corynebacterium amycolatum	-	-	-	+	-	-
Rothia mucilaginosa	-		+	-		
Rothia dentocariosa	-	+				
Actinomyces odontolyticus	+	-	+	+	-	+
Actinomyces meyeri	-	-	+	+	-	+
Actinomyces naeslundii	-	-	+	-	+	-
Actinomyces radicidentis	-	-	-	+	-	-
Slackia exigua	-	-	+	-	-	-
Cutibacterium acnes	+		-	+	-	-
Lactobacillus mucosae	-		-	+	-	-
Prevotella buccae	+	-	+	+	-	-
Prevotella intermedia	-	+	+	-	-	-
Prevotella loescheii	-		-	+		
Prevotella oralis	-	+	-	+	+	-
Prevotella marshin	+	+	-	-	-	-
Prevotella denticola	+	+	-	-	-	-
Pseudomonas stutzeri	+	-	-	-	-	-
Escherichia coli	+	+	-	-	-	-
Enterobacter cloacae	-	+	-	-	-	-
*Candida albicans**	+		-	+	-	-

IP: Irreversible Pulpitis. **SAP:** Symptomatic Apical Periodontitis. **AAP:** Asymptomatic Apical periodontitis. **PN:** Pulpal Necrosis. **AAA:** Acute Apical abscess. **CCA:** Chronic Apical Abscess. *: Fungi.

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of 40.3 ± 14.9 years. The diagnoses of the 60 teeth were symptomatic apical periodontitis (n=16, 26.7%), asymptomatic apical periodontitis (n=14, 23.3%), irreversible pulpitis (n=13, 21.7%), pulpal necrosis (n=10, 16.7%), acute apical abscess (n=4, 6.7%), and chronic apical abscess (n=3, 5.0%).

No microorganisms were cultured from the control teeth, and all sixty teeth had positive cultures. The average number of bacterial species per tooth was 4.85 ± 1.41. Most of the teeth (n= 26, 43.3%) yielded five bacterial species isolated, which was followed by (n=17, 28.3%) teeth with four bacterial species, (Table 1). Shows the number of bacterial species isolated per tooth. Microorganisms from 20 genera were isolated from the infected root canals.

The details on the genera of the organisms, their Gram staining, oxygen tolerance, and the number of species isolated are given below. The isolated microorganisms included genera that are aerobic,[5] obligate anaerobic,[7] *facultative anaerobic/aerobic*,[7] and *Candida albicans*, a fungus. Ten of the isolated bacteria genera were Gram-positive, and the remaining bacteria were Gram-negative. The pre-valence of bacteria and fungi found in the root canals. (Table 2)

Out of the 259 cultured isolates, 257 bacterial isolates belonging to 19 genera were identified. The other two were *Candida albicans isolates*. Out of the 53 isolated bacterial species (n=26, 49.1%) were identified as facultative anaerobes (n=15, 28.3 %) were obligate anaerobes, and (n=12, 22.6%) were aerobes. The species were mainly Gram-positive (n=37, 69.8%). Out of the Gram-positive species (n=22, 59.5%) were cocci.

Relationship between bacterial species and the different clinical diagnoses

The Gram-positive cocci *Streptococcus* was the main genera isolated from the teeth with pulpal necrosis and irreversible pulpitis. Gram-positive *coccobacillus*, *Rothia dentocariosa*, Gram-negative rods, *Enterobacter cloacae*, *Escherichia coli*, and *Prevotella* species were isolated from the teeth with pulpal necrosis.

Gram-positive rods (Actinomyces odontolyticus, Cutibacterium acnes), Gram-negative rods (*Provotella buccae*, *Provotella marshin*, *Provotella denticola*, *Pseudomonas stutzen*, *Escherichia coli)*, *Veillonella parula*, and *Candida albicans* were some microorganisms isolated from teeth with irreversible pulpitis. Gram-positive cocci, predominantly *Streptococcus* species, were isolated from teeth with apical periodontitis. Gram-negative black pigmented rods *Prevotella*, *Veillonella parula*, *Actinomyces* spp, and other anaerobes were also isolated as described. (Table 4)

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 organisms recovered. While from a chronic apical abscess, *Enterobacter cloacae*, *Enterococcus faecalis*, *Actinomyces odonlyticus*, *Actinomyces meyeri* and *Fusobacterium nucleatum* were also recovered.

DISCUSSION.

The present study evaluated the microorganisms present in infected root canals, The microbial analysis was done using MALDI-TOF which is a relatively new and underexploited method in endodontics. All teeth used in this study had primary root canal infections and had positive cultures in all sixty teeth. Our study yielded negative cultures for the ten control teeth which were selected for elective surgery (0% positive cultures).

The total absence of positive cultures from control teeth demonstrates the efficacy of the sampling technique used and affirms the assertion by Shuping *et al.*,[11] 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 of 2.6 to 5.4 species per canal.[12,13]

In this study, at least two bacterial species were isolated per tooth, with nine as the maximum species obtained per tooth. 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 aerobes (12, 22.6%). 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*.,[14] who found predominantly facultative anaerobes (52.7%) and Gram-positive (67.8%) micro-organisms in their study. Some studies however found obligate anaerobes to be predominant.[4,7,8] The discrepancy in our study might be due to the difference in sample collection. Immediate processing at the chair side and an anaerobic jar were used in this study instead of an anaerobic chamber as used in some of the other studies. Facultative anaerobes and aerobic bacteria isolated in this study were similar to those mentioned in Lee *et al*.,[15] study.

The facultative anaerobes isolated included: *Enterococcus faecalis*, *Corynebacterium amycolatum*, *Rothia* and *Actinomyces*. The aerobes identified included *Neisseria* spp, *Escherichia coli*, *Pseudomonas stutzeri*, *Enterobacter kobei* and *Enterobacter cloacae*, which are Gram-negative rods.

The number of bacterial species isolated in this study was largely consistent with the array of bacteria reported in the review by Narayanan *et al*.,[16] in which *Streptococcus mitis*, *Streptococcus oralis*, and *Streptococcus mutans* belonging to the viridans group were the most frequently isolated bacterial species.

Additional bacteria recovered in this study belonged to *Prevotella*, *Actinomyces* and *Rothia* species. These findings differ from those of Chávez de Paz *et al*.,[17] where *Streptococcus gordonii* and *Strepto-coccus oralis* were predominant in teeth with apical periodontitis.

Regarding the correlation between bacteria and type of infection, this study found that irreversible pulpitis contained mainly *Streptococcus* and *Prevotella* species. This is consistent with bacteria implicated and isolated in primary root canal infections as reported by Narayanan *et al*.[16] Teeth with diagnoses of apical abscesses (both acute and chronic) yielded mainly facultative anaerobes isolated.

The isolated species 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 favors facultative and strict anaerobes and allows them to thrive, which most likely explains the findings in this study and others.

These findings are comparable with those of Fowel *et al*.[18] that implicated facultative anaerobes belonging to the *Streptococcus anginosus* viridans group in a dental abscess. However, some studies have isolated *Staphylococcus aureus* frequently from acute dental abscesses, ranging from 0.7% to 15%.[19,20] The species isolated in chronic abscess in this study were Gram-positive cocci (*Streptococcus oralis*, *Streptococcus mitis*, and *Streptococcus constellatus*), *Enterococcus faecalis* (Gram-positive rod), *Actinomyces odontolyticus*, *Actinomyces meyeri* (Gram-negative rod), *Fusobacterium nucleatum*, and *Enterobacter cloacae*.

Facultative anaerobes, such as the viridans group *streptococci* and *Streptococcus anginosus*, and *strict anaerobes*, especially the *anaerobic cocci*, as well as *Prevotella* sp, and *Fusobacterium* species have been observed as the most common organisms isolated from a dentoalveolar abscess.[20] They have been found in 10% - 87% of dentoalveolar abscesses.[21,22] In this study, *Enterococcus faecalis* was isolated in almost all the diagnoses. This might be due to the delay in seeking dental care when teeth are already cavitated. *Enterococcus faecalis* being an opportunistic member of the oral microbiome, is present in the mouth from an early age.[23] Some of the teeth used in the study had long-standing carious lesions and cavitation which might have led to this phenomenon.

This study investigated the viable microbial profile; however bacteria can remain into a viable-but-not-culturable state. Adding culture-independent techniques using 16S rRNA gene sequencing would have been able to identify these bacterial species. Within the limitations of this study, we recommend

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further studies to determine the type of root canal irrigants and medications which will be effective for root canal infection. Advanced open-ended molecular techniques that will allow genome mapping of the entire spectrum of bacteria in a sample to provide a comprehensive characterization of the microbiota associated with endodontic infections to help institute the best treatment protocol is also recommended.

CONCLUSION.

Root canal infections in Ghanaians are polymicrobial with facultative anaerobes being predominant. The bacteria frequently isolated included *Streptococcus* spp., *Prevotella* spp., *Actinomyces* spp., *Enterococcus faecalis*, and *Rothia* spp.

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Thomas Akuetteh Ndanu: data analysis/inter-pretation, write up of the manuscript, and proof reading of the manuscript.

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