



Bacterial Profile of Root Canal Infections among Libyan Patients at Specialized Center. Benghazi, Libya

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ABSTRACT

Background and objective: the purpose of this study was to assess endodontic bacterial infections of permanent teeth, the sensitivity of isolated bacteria to antibiotics, efficiency of different intracanal medication and comparable root canal therapy approaches as well as the presence of bacteria at the final therapy step. Materials and methods: This was a prospective study of one hundred and twenty-two patients, both genders, aging from 15 to 70 years old, presented with single-rooted teeth that needed endodontic therapy due to root canal infection were chosen. After gaining access to the root canals surgically, the root canals were embedded with sterile paper points, which were then placed in transport medium and shipped to a microbiology lab, by using the culture technique, bacteria were isolated and identified. Statistical analysis was performed with a level of significance of (0.05), by the usage of SPSS. Results: Staphylococcus aureus and Streptococci spp. were the two most common microbial species. However, Diptheriod spp and Lactobacilli spp. were also often isolated. From a total of 100 samples taken, 63 cases had bacterial growth in the most recent therapeutic sequence were found, while 37 cases revealed no growth. Conclusion: Based on the finding, Gram positive cocci were more prevalent. Females are more common to root canal infections than males. The no-growth samples were correlated with the usage of the operators to calcium hydroxide, 0.6% chlorhexidine and metapex as intracanal medications, step-back technique as well as the years of experience of the operator were between 20 to 24 years.

Keywords: Endodontic bacterial pathogens, Root canal infections, Intracanal medications and therapy techniques.

1. Introduction

Bacterial root canal infections are the primary cause of the most prevalent disorders that affect the dental pulp and periapical tissues, whereas root canal infection could be caused by fungi as well as some viruses.⁽¹⁾ Since knowledge is applied to determine the type of therapy needed for these specific patients, it is crucial for the dentist to understand the pathophysiology of infections at these sites.⁽¹⁾

In a normal healthy status the pulp is free from any microorganisms, but there are numerous ways for bacteria to get into the pulpal cavity: Cavity preparation, Carious invasion, Restorations leakage, from the periodontal tissue, internal as well as external resorption and Anachoresis.^(1,2,3)

Bacterial radicular pathosis could be caused by Obligate Anaerobes such as *Peptostreptococci*, *Propionibacterium*, *Actinomyces*, *Eubacterium*, *Clactobacilli*, *Veillonella parvula*, *Bacteriodes* and *Fusobacterium*. Facultative anaerobes as *Streptococci*, *Staphylococci*, it could be caused by Microaerophilic as *Lactobacilli* as well as Facultative aerobes as *Diptheriods*.^(1,2,4,5)

There are three types of root canal infections which are primary root canal infections, secondary root canal infections and persistent root canal infections, at the first stage of pulpitis micro flora is simple, it occurs due to caries-related bacteria, analogous to advanced stages of pulpitis, pathogens become proteolytic bacteria Gram negative, this is because of the presence of essential nutrients within the pulp chamber, low PH as well as absence of oxygen, as a result of progression of the root canal infection from pulp chamber to periradicular region through periodontium leading to development of extraradicular infections.^(1,2,6)

Root canal therapy purpose is to complete removal of infected pulp, also biomechanical of root canals, for cleaning as well as disinfection of root canal system to promote healing of surrounding tooth tissue and preserve tooth function.^(3,4)

Analogous to roles of intracanal medications are for pain relief, infection control, root canal system debridement, treatment prevention and management.^(4,6,7) The ramifications of different types of intracanal drugs used in root canal treatment, are 5.25% Sodium hypochlorite (NaOCl), 0.9% Saline, 4% Iodine, 0.2-2% Chlorhexidine (CHX), Tetracycline as an irrigating solutions, meanwhile 17% Diethylenediamine tetraacetate in disodium (EDTA) is used to prepare root canals mechanically removed bacteria, further more as dressing 2-4% Camphorated parachlorophenol (CMCP), Metapex is Calcium Hydroxide with Iodoform has stronger radiopacity and more antibacterial activity against germs and Calcium hydroxide, its highest PH and calcium release activity.^(7,8,9,10)

Failure of a root canal treatment has extensive health, psychological and economic ramifications, Reducing the percentage of endodontic failure could have both individual and societal advantages that have large impact among such patients.^(11,12)

The objectives of this study was to assess the endodontic bacterial microbiota of permanent teeth with root canal infections, antibiotic sensitivity of isolated bacteria, antibiotic resistance of confined bacteria, detection of bacteria at the last therapy session, the clinical efficacy of intracanal medication and effect of different root canal biomechanical techniques in root canal therapy.

2. Materials and Methods

A Prospective study of Libyan patients required root canal therapy, The project was approved by the scientific committee in research of the specialized center for Dental treatment and education (SDC), Benghazi, prior to the execution of the procedures. This research had taken a term of 9 months from August 2019 to April 2020.

2.1. Patient selection

Both personal, dental and medical history were taken from participants who were referred to the department of endodontics, in the same time dental examination and Intraoral periapical radiographies were done, in order to conform the diagnosis with root canal infections, on account of 150,122 cases were selected in the conferred study. In general those Libyan patients were presented with Primary single rooted root canal infections both genders, their age ranged between 15-70 years old, each patient had given a coded number.

2.2. Operators involved in this study

A sum of 24 operators participated in the study, all were residents at department endodontics, two different techniques had been used by them the step-back techniques as well as crown-down techniques, their years of experience were between 9 to 25 years.

According to operators, they had prescribed systemic antibiotics to several cases due to massive infections, in opposition to there wasn't any patient required antibiotic prophylaxis before treatment.

From the operators point of view, they had used 5.25% Sodium hypochlorite, 0.9% Saline, as intracanal irrigation solutions, uniformly 65% Camphorated parachlorophenol were occupied as a dressing within root canal and temporary restoration in between therapy secession in all cases, however in some cases they had applied Calcium hydroxide, Metapex, 17% Ethylenediaminetetraacetic acid, Tetracycline as well as 0.6% Chlorhexidine as intracanal medicaments.

Correspondingly to dentists involved in this study had used conventional file instrumentation and rotary instrument devices.

2.3. Sampling and Bacterial identification

During the first session of root canal therapy a sample had been taken from each patient, to get an aseptic microbiological sample from a root canal, the tooth must first be isolated with a rubber dam, and the rubber dam and tooth surface must be cleaned. by the usage of a disinfectant such as sodium hypochlorite or another to rubber dam. To achieve entry, sterile burs and tools must be utilized. after the operator completed the crown surgical access, cleaning, and shaping of the root canal, a sterile paper point was inserted for 30 seconds to collect the material from the root canal, when the paper point was removed, these samples were placed into a transport medium, before being delivered to the microbiology laboratory, the tubes holding the transport medium were labelled with the **B** which represents before therapy as well as patient's coded number and an individual record was filled out with details about each patient. All samples were placed within an Carbon dioxide jar with a 10% hydrogen and 6% carbon dioxide in nitrogen environment for twenty-four hours at 37°C, then these samples were examined if it had growth or no growth, If it had grown, it had been subcultivated, on the following culture mediums, blood agar and MacConkey were kept at 37°C for Twenty-four hours in Carbon dioxide jar. Based on colony morphology on media growth, Gram staining, Catalase test, Coagulase test, hemolysis on blood agar, the identification of bacterial pathogens were carried out according to Monica's manual of systemic bacteriology and Streptocard enzyme latex test by the usage of Streptocard enzyme latex test kit from Oxoid, England, all isolates were confirmed. Utilizing the Kirby-Bauer Method's diffusion technique, the isolated, identified organisms were tested against several antibiotics.

2.4. Post-Biomechanical therapy samples

Out of 122 patients contributed at the last therapy session, 22 subjects had been excluded due to defective temporary restorations, some operators had done the obturation step without taking a sample and few patients didn't come back to the last therapy session, as done in the first therapy session by the aseptic techniques as well as using the rubbers dam, A second paper point was applied to the root canal at the end of the therapy, and it was then placed in a tube with transport media, those tubes were labelled with A which illustrates after as well as the patient's code, transported to microbiology laboratory, these samples were incubated at 37°C for 24 hours in Carbon dioxide jar and then each sample was subcultured on Blood agar plates, before being incubated in Carbon dioxide jar at 37°C for an additional 24 hours period, to the same extent even one colony were counted on the examined plates.

2.5. Statistical analysis

The statistical package SPSS version-20 (Property of IBM, Chicago) was used to evaluate all of the data, with a 95% confidence interval and a significant level of acceptance of *P*-value 0.05, meanwhile Chi square test was carried out to perform group comparison in the study that was given. Age was also separated into five age groups, descriptive statistics were applied, The mean was calculated for each age variable, along with frequencies, percentages and ranges.

3. Results

These results were based on individual's medical and dental history, clinical examination, data from operators as well as bacteriological analyzes of specimens taken from root canals at first session and last treatment session right formerly to obturation step.

3.1. Demographic data and Medical History

Higher incidence were Females 72.14%, followed by Males were 27.86%, additionally 16.3% Male cases were smokers, whereas average age of cases was 40 years, as shown in Table-1

Table-1: Demographic Data and Medical History

History		Prevalence(%)	X ²	P-square	Significance	
Personal data	Gender	Females	88(72.14%)	25.705 ^a	<0.00001	Significant
		Males	34(27.86%)			
	Age group	15-25	16(13.3%)			
		25-35	28(23%)			
		35-45	39(32%)			
		45-55	20(16.35%)			
		Above 55	19(15.35%)			
Medical History	Diabetes	Healthy	72(59.1%)	41.131 ^a	<0.0001	Significant
		Type-I	15(12.3%)			
		Type-II	35(28.6%)			
	Hypertension	Hypertensive	29(23.77%)	42.492 ^a	<0.0001	Significant
		Non-hypertensive	93(76.23%)			
	Smoking	Non-smokers	102(83.7%)	54.223 ^a	<0.00001	Significant
		Smokers	20(16.3%)			
Total		122(100%)				

3.2. Results from dental and clinical examination

By comparison distribution of teeth with root canal infections in the study, The most frequently infected teeth were the Maxillary first premolar, While the Mandibular central incisor as well as the Mandibular lateral incisor which had one case each, (**Table-2**).

Table-2: Dental history and Clinical examination

Dental history and Clinical examination		Frequency		Chi square	P-value	Statistic significance
		N	%			
Clinical symptoms	Asymptomatic	40	32.7	25.705 ^a	<0.0001	Significant
	Symptomatic	82	67.3			
Periodontal condition	Normal periodontium	83	68.11	70.492 ^a	<0.0001	Significant
	Chronic gingivitis	25	20.49			
	Mild generalized Chronic periodontitis	14	11.4			
Diagnosed teeth	Maxillary central incisor	7	5.7	104.393 ^a	<0.00001	Significant
	Maxillary lateral incisor	4	3.27			
	Maxillary canine	6	4.91			
	Maxillary first premolar	35	28.6			
	Maxillary second premolar	20	16.3			
	Mandibular central incisor	1	0.81			
	Mandibular lateral incisor	1	0.81			
	Mandibular canine	3	2.4			
	Mandibular first premolar	25	20.4			
	Mandibular second premolar	20	16.3			
Total no.of cases		122	100			

3.3.Results from Endodontists in this study

According to the operator's techniques used in this study, which included the use of the Crown-down technique by 87% and the step-back technique by 13%,In the same fashion, They had used ordinary files in 95.91% and rotary instrutments in 4.09%.

Table-3: Data from operators during therapy

		Relevance (%)	Chi-square	P-value	Significance
Systemic Antibiotics	Non-prescribed	83(68)	32.760 ^a	<0.0001	Significant
	Amoxicillin	8(6.5)			
	Ampicillin	10(8.1)			
	Augmentin	12(9.8)			
	Doxycycline	0			
	Metronidzole	9(7.37)			
Techniques	Crown-down	19(15.57)	12.565 ^a	<0.00001	Significant
	Step back	5(4.09)			
Instruments	Files	110(90.1)	88.656 ^a	<0.0001	Significant
	Rotary instrument	12(9.8)			
Intracanal	Saline(0.9%)	122(100)		<0.00001	Significant

medications	Sodium hypochlorite(5.25%)	122(100)
	Camphorated parachtorophenol(65%)	122(100)
	Calcium hydroxide	10(8.1)
	Chlourohexdine (0.6%)	10(8.1)
	METAPEX	15(12.2)
	ETDA(17%)	29(23.7)
	Iodine	0
	Tetracycline	10(8.1)
Total	122(100)	

3.4.Results from bacterial isolated from root canals

Regarding the specimens taken at the first therapy session, one hundred-twenty one had growth(99.19%) and only one had no-growth (0.81%) as well as all isolates were Gram positive bacteria.

The majority of isolates were found to be *Staphylococcus aureus* ,rare by *Diphtheriod* spp. It appears that most common bacteria that cases root canal infections in non-diabetic patients were *Streptococci* spp.,infrequent by *Diphtrioid* spp.,on the contrary to diabetic patients the common bacteria in were *Staphylococcus aureus* and out of common *Enterococcus faecalis*, (Table-4,Figure-1), from the correlation of individuals history ,clinical examination also patient's codes all had *Staphylococcus aureus* within root canals also this bacterium had been showed among all smoker cases and pateints with periodontial diseases in this study.

Table 4: Relevance of Bacterial root canal infections

Bacterium	Incidence (%)		Total (%)	Chi-square	P-value	Statistic significances
	Non-diabetic	Diabetics				
<i>Staphylococcus aureus</i>	17(13.9)	22(18)	39(31.9)			
<i>Streptococci</i> spp.	Group-A	1(0.8)	0	32.000 ^a	<0.00001	Significant
	Group-B	0	0			
	Group-C	4(3)	1(0.8)			
	Group-D	9(8.04)	4(3.16)			
	Group-F	7(5.6)	1(0.8)			
	Group-G	5(4)	1(0.8)			
<i>Lactobacilli</i> spp.	14(11.4)	10(7.6)	24(19)			
<i>Enterococcus faecalis</i>	11(9.01)	5(4.99)	16(14)			
<i>Diphtheriod</i> spp.	3(2.45)	6(4.85)	9(7.3)			
No-growth	1(0.8)	0	1(0.8)			
Total	72(59)	50(41)	122(100)	52.000^a	<0.0001	Significant
Chi-square	35.333^a	19.400^a				
P-value	<0.00001	<0.000656				
Significance	Significant	Significant				

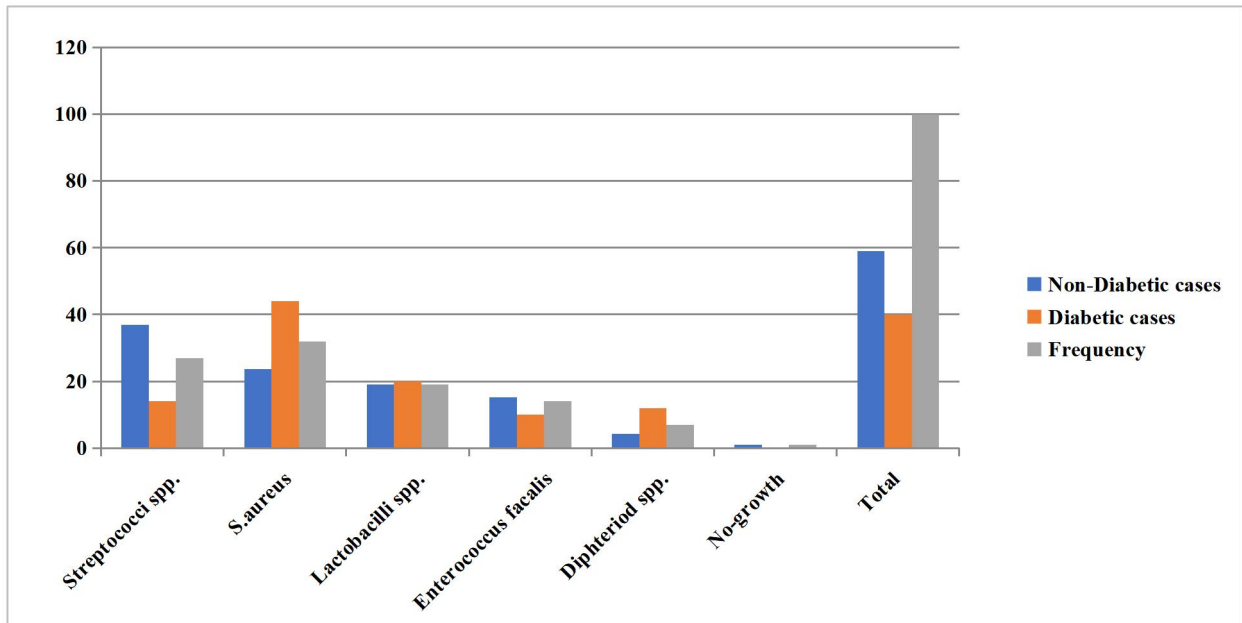


Figure-one: The prevalence Bacterial endodontic pathogens among one-hundred and twenty-two patients with root canal infections in percentage.

3.5. Antibiotic susceptibility and resistances of isolates in research

The ramifications of antibiotics, tetracycline had the highest susceptibility and penicillin had higher incidences of resistance in patients with root canal infection, (table-5, figure-5) According to the findings, the younger and older age categories where the penicillin-sensitive individuals were found as displayed in figure-6.

Table-4: Prevalence of Antibiotic susceptibility and resistance in study

Antibiotic	Sensitive		Resistance	
	N	%	N	%
Penicillin	16	13.1	106	86.88
Amoxycillin	24	19.67	98	80.3
Ampicillin	39	31.9	83	68.1
Augmentin	42	34.4	80	65.6
Erythromycin	63	51.6	59	48.36
Tetracycline	82	67.21	40	32.78
Total	121	100	121	100
Chi-square	38.282 ^a		74.362 ^a	
P-value	<0.00001		<0.0001	
Statistic significances	Significant		Significant	

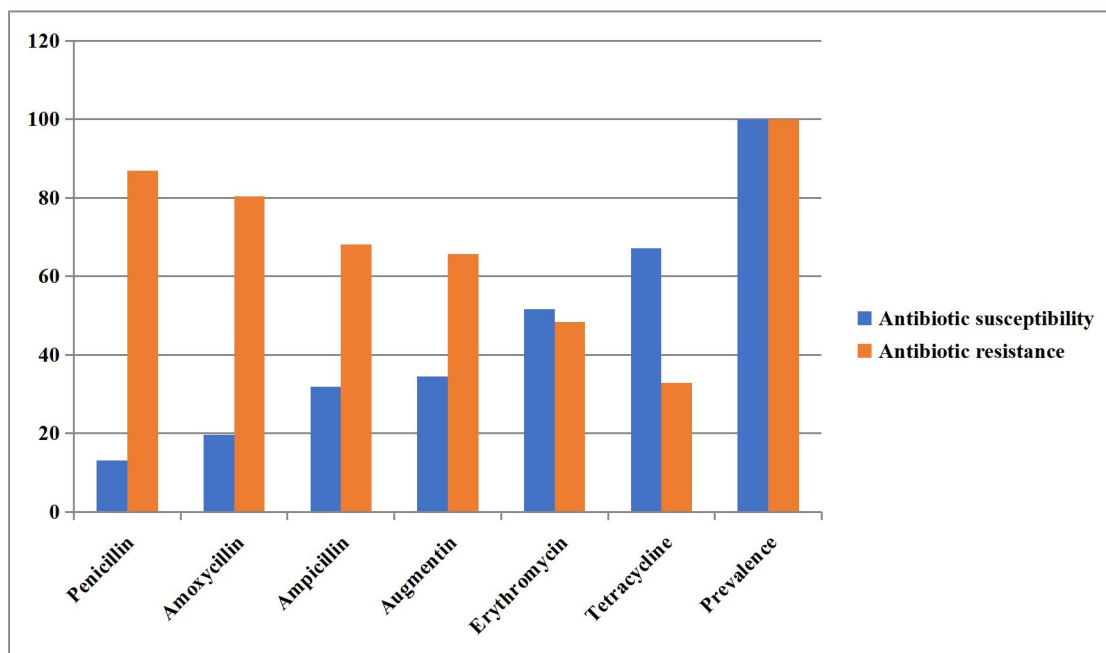


Figure-two: Antibiotic susceptibility and resistance in one-hundred and twenty-two patients participated in the study according to percentage.

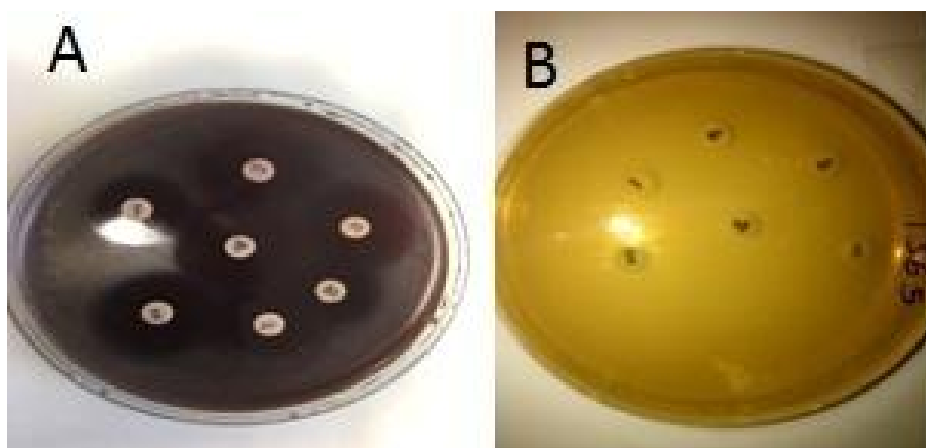


Figure- 6 A:Antibiotic susceptibility of *Streptococci* from a seventeen years old Female Libyan patient. **Figure-6 B:** Antibiotic resistances of *Staphylococcus aureus* from thirty-nine years old male Libyan patient.

3.6.Results from Pre- obturation samples

On account of 100 specimens 63% had growth,while 37% had no- growth,(Chi- square = 6.760^a,*P*-value was <0.00001), synonymous to these results 37 cases will have good prognosis,but 63 cases will have poor prognosis from outcomes of therapy.

Overall effectiveness of different types of intracanal medications used by the operators in one-hundred patients with root canal infections during therapy as compared with data taken from the operators, there were a correlation with the no-growth samples consistent to using the step-back technique as well as the usage of intracanal medication as Calcium hydroxide (dycal base), 0.6%Chlourohexdine gel and Metapex within the root canal as well as the operators years of experience from 22 to 25 years.

One of the cases in this study **Figure7-A** radiograph taken Before treatment, based on the finding there were widening in Lumina dura,diffused radiolucency at periradicular region and canine wasn't in normal anatomical ailment,the causative agent was *Staphylococcus aureus*,analogous to antibiotics,it was only susceptible to tetracycline,the operator had used it, in addition with metapex ,As shown in **figure7 -B** which was a follow-up Intraoral periapical radiograph, taken at almost nine months post-treatment, from the appearance of radiograph there was an improvement in the case, represented by reduction in size of Lumina dura,smaller in size well defined radiolucency at the periapical region.

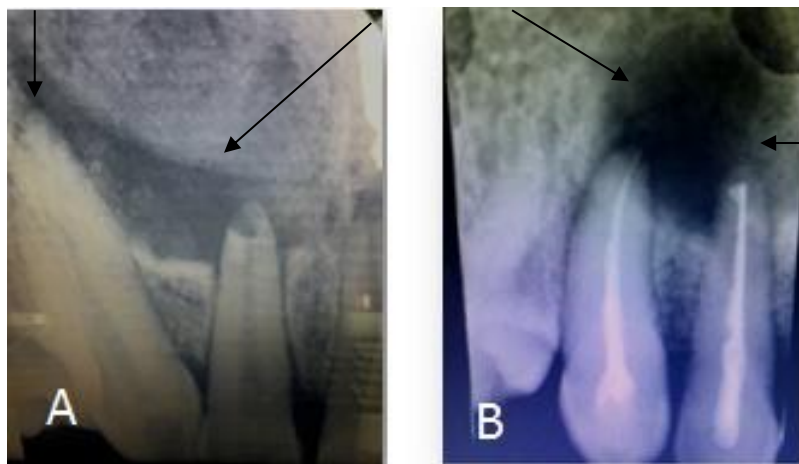


Figure -7(A,B): Intraoral periapical radiographs of one cases in presented study

4. Discussion

As known, the consequences of bacterial invasion will lead to root canal infections, where else from specialized center records, the endodontic resident dental staff had done more than 18000 root canals from the year of 2017 to the recent date, in average of 300 cases per month, these are consider as high incidence of root canal infections among the Benghazi's society. From the American association of endodontology annually, more than 15 million root canals are carried out in the United States.⁽¹³⁾

In comparison gender with root canal infections in this study, out of account one-hundred-twenty two, eighty-eight pateints were Females (72.14%) and thirty-four pateints were Males(27.86%),In opposition to Hollanda et al. had studied prevalence of endodontically treated in Brazilian's adults,from 1401 cases, Females were 61.9% meanwhile Males were 38.1%,⁽¹⁴⁾also Scavo et al. had studied this phenomena in Argentina ,found from eight hundred and sixty pateints, nine hundred and seventy-five teeth ,that Females were six hundred and thirty-five(65.13%) and Males were three hundred and forty(34.87%),⁽¹⁵⁾ furthermore Anderson et al. in there study that Females were higher tendencies to root canal infections thirty-one cases(77%),while Males were nine cases (22%),⁽¹⁶⁾meanwhile according to Bhavika et al. from four hundred and nineteen cases with root canal infections, Males pateints were three hundred and sixty(60%),Female pateints were two hundred and forty (40%),⁽¹⁷⁾an opposing view by Al-Awasi etal. their research had found from a total of 2161 subjects ,800 subjects were males and 1161 subjects were females.⁽¹⁸⁾

Root canal infections may happens at any age,either tooth has experienced a deep infections, or it may had go many treatments, according to data it pecks with two groups, frist age group were 15 to 25 years, in the same way in developmental counties such as Nigeria root canal therapies were between 8 to 54 years with an average age 28 years old,⁽¹⁹⁾ it may be due to advanced tooth carries, although the most common age of patients with root canal infection of this study were between 35 to 45years old 39 cases (32%), followed by 25 to 35 years old in 28 cases (23%), 45 to 55 years old in 20 cases (16.35%), old 16 case(13.5%) and the rarest was from 60 to 70 years old with only 3 cases ,uniformly to Hollanda et al. based on their finding from 1401 pateints, highest prevalence group were 46-60 years(47.6%),from 31-45 years were (28.4%),above 60 years were 20.14% and rarest were among less than 30 years (3.4%),⁽¹⁴⁾consistent with Vehkalahti et al. based on data of Private dental practice in Finland with five age groups from 20-90 years old, from years of 2012 to 2017 with the total of 183932 pateints, who requires root canal treatment were studied, results were average age were 53.6 years old in 2012 and 55.9 years old in 2017,where in both years 38% were aged from 50-64years,but in 2012 were 9.9% and 2017 in 8%,⁽²⁰⁾in a view of Ilić J. etal. studied distribution of age among patients with root canal infections,it involved 153 cases, their ages were 40-49 old were 42 cases(27.4%),18-29 old were 31 cases(20.3%),30-39 old were 29 cases(19%),50-59 old were 28 cases(18.3%) and above 60 old were 23 cases(15%).⁽²¹⁾ However Al-Awasi et al.2022 found the prevalence of root canal treatment were more among 41 to 45 years old.⁽¹⁸⁾

On account of distribution of teeth with root canal infections in the study,The most frequently infected teeth were the Maxillary first premolar (28.6%), followed by the Mandibular first premolar(20.4%), the Maxillary second premolar (16.3%),the Mandibular second premolar (16.3%), the maxillary central incisors, the maxillary canines(4.91%),the Maxillary lateralincisors(3.27%),the Mandibular canine (2.4%), the Mandibular central incisor as well as the Mandibular lateral incisor which had (0.81%),contrary to Hollanda et al.had evaluated Endodontic therapy were more prevalent in Maxillary premolars and molars but Mandibular incisors had lower frequency,⁽¹⁴⁾ furthermore Scavo et al.as end result of their study, were Maxillary teeth (55.69%) and Mandibular teeth (44.30%),most teeth required endodontic treatment were Mandibular right frist molar(9.12%) then followed by Mandibular left frist molar (7.07%),⁽¹⁵⁾ however Bhavika et al. had detected that frist molar were (28.7%),second molar(16.2%),second premolar(15%),Central insicor (10.3%),Lateral insicor also second premolar(7.2%),Canine(5.5%) and rare in third molar in (3.8%),⁽¹⁷⁾but Al-Awasi etal. out of 2161 patients, Anterior teeth were in 498 pateints,Premolars were in 765 pateints and Molars were in 898 pateints.⁽¹⁸⁾

The antithesis of sysmptomic and asysmptomic cases in study were forty cases were asysmptomic (32.7%) and eighty- two cases were sysmptomic (67.3%),thus Rôças et al. had analysis symptomatic as well as asysmptomic of primary root canal infections among adult Norwegian pateints by usage of molecular microbiology techniques also studied the correlation between the history of pain and bacterial grouping, subsequently found the more virulent organism and their combinations will give arise of acute signs and symptoms of inflammation,⁽²²⁾furthermore Anderson et al. investigated

bacterial composition and structure in symptomatic and asymptomatic pain cases of root canal infections by using Pyrosequencing, fifty samples, 23 cases were asymptomatic cases and 17 cases were symptomatic cases from Sudanese patients with peridicular regions, the study had found a clear association of bacterial community with symptoms of pain.⁽¹⁶⁾

The results of this study shown from the samples taken from total of 122 samples from the first therapy session, 121 (99.19%) had growth and only one sample had no-growth (0.81%), several studies had proved that all root canal infections are caused by polymicrobial pathogens,^(6,11,23,24,25,26) but this study reveals root canal infections were caused by monomicrobial bacteria, whereas Tennert et al. had found monomicrobial infections were found for *Enterococcus faecalis* and *Actinomyces viscosus*. A secondary endodontic infection with an inadequate root canal filling and a slight pain sensation was found to have *Moraxella osloensis*. From teeth with chronic apical abscesses, a new bacterial composition including *Atopobium rimaie*, *Anaerococcus prevotii*, *Pseudoramibacter alactolyticus*, *Dialister invisus* and *Fusobacterium nucleatum* was discovered.⁽²⁷⁾ The distribution of the isolated bacteria in general according to their percentage were represented as following *Staphylococcus aureus* (31.9%), *Streptococci* spp. in (26.2%), *Lactobacilli* spp. in (19.6%) *Enterococcus faecalis* in (13%) and *Diphtheriod* spp. in (7.5%). Counter to Guimarães et al. had found the cause of root canal infections were absolute anaerobes Gram-negative bacilli, Gram-positive cocci and non-sporulating *Fusobacterium nucleatum*, The majority of the germs were Gram-positive bacilli, also one was identified as a facultative anaerobe because it showed no signs of growth. The most frequently isolated bacteria were *Streptococci* spp., *Staphylococci* spp., *Staphylococcus aureus* and two strictly anaerobe species of *Pseudomonas* spp.⁽²⁶⁾ In a same manner Peculien et al. Studies on root canal infections reveal a dynamic process with different bacterial species predominating at different phases.⁽²³⁾ Ter Steeg and Van der showed in experimental tests that the availability of nutrition, oxygen level, and the local PH within the root canal are the most significant elements driving this process, even if their main source of energy is carbohydrates. It is clear that when there is no direct interface with the oral cavity, the availability of carbohydrates in the root canal diminishes. Naturally, since there is no direct interface with the oral cavity, the supply of carbohydrates in the root canal decreases, which restricts the capacity of facultative anaerobes to proliferate,⁽²⁸⁾ Even though its pathogenic potential is yet unknown, the bacterium has repeatedly been detected in persistent intraradicular and extraradicular endodontic infections that do not respond to traditional endodontic treatment.⁽²⁴⁾ Among the recovered bacterial species in this study all were Gram positive bacteria,⁽²⁴⁾ Compared to Alencer et al. found that 83% were *Streptococci* spp.⁽²⁹⁾ Bacteria participating in persistent infections can be identified as those present in the canal at the time of filling, although it must be recognized that many of the species found still had no sufficient time to establish a real infection and will die after filling,^(30,31) Mean while in Non-diabetic patients in presented study were highest frequency of bacterial pathogens were *Streptococci* spp. were (37%), followed by *Staphylococcus aureus* were 23.6%, *Lactobacilli* spp. were (19%), *Enterococcus faecalis* were (15.2%) and *Diphtheriod* spp. were (4.2%), Sharma et al. reported both Gram positive and Gram negative bacteria were involved in root canal infections among diabetic patients,⁽³²⁾ as well as Bissong et al. shown in both diabetic and non-diabetics were *Streptococcus mutans*, *Lactobacilli* spp., *Candida* spp. and Gram negative aerobic bacteria plays a role in infections in diabetic patients,⁽³³⁾ while in diabetic patients in this study the most common bacteria were *Staphylococcus aureus* in (44%) of the cases, followed by *Lactobacilli* spp. in (20%) of the cases, *Streptococci* spp. in (14%) of the cases and *Enterococcus faecalis* in (10%) of the cases, on the contrary Munson et al. had found that in cases with a high number of appointments prior to sampling, enteric bacteria were also more frequently secluded, in the group of patients with enteric bacteria, 35% of the samples were taken at the tenth visit, *Enterococci* are more likely to survive chemotherapy, mechanical instruments, and root canal medications as well as that root canal which were unsealed at some point during the treatment, harbored enteric bacteria more frequently than the canal with an adequate seal between the appointments.⁽³⁴⁾

Various studies shows^(35,36,37), the pulpal cavity and periodontium are correlated to each other due to transmission of lesions among these tissue structures as well as bacterial resemblance amid root canal infections and upgraded periodontitis, to have a favorable outcome of root canal therapy, both periodontal and endodontic treatment has to be done in such cases, correspondingly in this study all patients who had been diagnosed with periodontal disease had *Staphylococcus aureus* within the root canal as well as smoker patients, likewise Kamberi et al. studied prevalence of apical periodontitis and endodontic therapy in Kosovar adult population, had concluded it is a far-reaching disease and association of this disease with insufficient root canal therapy,⁽³⁸⁾ in a similar fashion Bhavika et al. had studied 600 patients, who needed endodontic treatment, they had healthy periodontal condition in 22.5%, Gingivitis 29%, acute apical periodontitis 35.7% and chronic apical periodontitis 11.3%,⁽¹⁷⁾ however in this research from the total of 122 patients 68.11% had a healthy periodontium, Chronic gingivitis 20.49% and mild generalized chronic periodontitis 11.4%, opposing to Bissong et al. had studied 265 cases, 38 of those cases were diagnosis with gingivitis and 39 cases had various forms of Periodontitis, Gram positive and Gram negative had been isolated from their root canals.⁽³³⁾

In the preserved study the operators prescribed systemic antibiotics to five patients out of 122 patients with root canal infections, this shows that the operators follow the guidelines of antibiotic prescription, in comparison Bansal et al. shown there were lack of knowledge of usage of antibiotics among dental practitioners and it's multiple factor problem,⁽³⁷⁾ also Marra et al. dentist still prescribe antibiotics for endodontic infections which requires only restorative measures,⁽³⁹⁾ although the antibiotic resistance in patients with root canal infections in the study were highest in penicillin in 106 cases, followed by amoxicillin in 98 cases, ampicillin in 83 cases, augmentin in 80 cases, erythromycin in 59 cases and tetracycline in 40 cases, these results indicate miss usage of antibiotics and easy accessibility to antibiotics by the patients in the study, the ramifications of age grouping and its relationship with antibiotic resistance, were most common in 30 to 50 years old and rare in 60 to 70 years old and 15 to 17 years old. Rana et al. The interaction between bacteria and endodontic disease must be understood by practitioners in order to create an appropriate treatment plan.⁽²⁾ Antibiotic medication as a supplement to dental care should be taken into consideration, particularly when the infection is spreading locally or systemically. Most aerobic and anaerobic bacteria can be treated with amoxicillin or penicillin, but some patients may have bacteria that are resistant to these medications. These patients should be treated with antibiotics that are effective against these bacteria, such as amoxicillin clavulanate, clindamycin, or a combination of metronidazole and amoxicillin or macrolides,^(39,40,41) although in this study shows ineffectiveness of these antibiotics against isolated bacterial strains, it may be due to easy access to these antibiotics within the Libyan population.

Comparable in performed study there were bacterial presence in last therapy session among patients had been diagnosed with root canal infections according to percentage, a total 100 samples analyzed showed 63% yield bacterial growth while 37% showed no-growth, the operators used 0.9% saline, 5.25% NaOCL and CMCP in all the cases between therapy sessions, mean while Vianna et al. they used 2% CHX gel and dycal as intracanal medicament for 10 days, after taking a samples at last therapy succession, they detected growth by the usage of culture technique,⁽⁴²⁾ as well as Sakamoto et al. similar in fashion used 2.5% NaOCL as irrigants as well as dycal, CMCP as intracanal medicament, they found growth at last therapy session,⁽⁴²⁾ although Peters et al. had used 5% NaOCL as irrigant, using microbiological method, they found growth at end of root canal treatment session, even through the usage of different intracanal medications.⁽⁴³⁾ Number of studies have revealed that chemomechanical preparation using NaOCL at different concentrations does not suffice to predictably render root canal free cultivable bacteria, about 40% to 60% of the root canal are still positive for bacterial presence.^(44,46,47)

When there is direct connectivity between the endodontic treatment case and the oral cavity, such teeth are differentiated as being more resistant to endodontic treatment operations.⁽¹³⁾ Siren et al. research demonstrated that root canals with an inadequate seal between visits were more likely to harbour enteric germs than those with a root canal that had been unsealed at some point throughout the therapy. When teeth were left open throughout treatment, enteric bacteria were found in 55% of cases, but in the group where only non-enteric bacteria were found, 30% of the teeth had been open.⁽⁴⁷⁾ The retreatment procedures are less predictable than treatment of primary endodontic cases, this is due the specific more resistant species of microorganisms which can survive not only during antimicrobial treatment, but also root filling procedure.⁽²⁴⁾ Already been detected post instrumentation and post medication samples, Gram-positive bacteria are the most dominant.⁽²⁵⁾ Determination of the threshold of bacterial levels below which a favourable host response is expected can help establish a goal to focus on and has the potential to drive standardization of treatment protocol.⁽⁴⁴⁾ In other words, the best treatment protocols are those that reduce bacterial counts to levels below a known threshold, for want of a more reliable approach, results from culture studies are recommended as surrogate endpoints for long-term clinical outcome studies,^(45,46) despite the well-recognized limitations of culturing methods. Notwithstanding the well-known drawbacks of culturing techniques, results from culture studies are recommended as surrogate endpoints for long-term clinical outcome studies.⁽⁴⁷⁾ The optimal treatment procedures are those that lower bacterial counts to levels below a defined threshold.^(48,49,50) Likewise Tronstad et al. found there are a correlation between persistent bacteria and the treatment outcome, they have shown that occurrence of positive cultures projects poor prognosis,⁽⁵¹⁾ so the goal of endodontic treatment is to reduce bacterial detection by microbiological culture procedure to have a better prognosis, although in this study 37% will have good prognosis and 63% will have poor prognosis, analogous to Al-Awasi et al. on of possession of 2161 cases, 61.8% will have poor outcome as well as 38.2% will have good outcome.⁽¹⁸⁾

In the same way no-growth samples were correlated with the usage of the operators to calcium hydroxide, 0.6% chlorhexidine gel, metapex as intracanal medications and the usage of step-back technique, this study proves the ineffectiveness of 65% CMCP, 0.9% saline, 5.25% NaOCL as intracanal medication as well as crown-down technique in root canal therapy. Eriksen verified the success of sound treatment outcome is primary clinical goal, however the success rate of root canal treatment performed is in the order of 87% compared with 72% for treatment in general practice,⁽¹¹⁾ in this study no-growth samples were correlated with operators years of experience from 22 to 25 years.

Very few studies have evaluated the results of root canal therapy that was performed,^(52,53,54,55) success rate of repeat root canal therapy have been 50%, or less with little subsequent alteration in healing after one year,⁽⁵⁶⁾ but these are relate to discontinued techniques.⁽⁴⁶⁾ In recent review of the outcome of retreatment of the root canals, there was a near equal distribution of the cases between the three outcome groups: 35.7% healed successfully, 26.3% healed with uncertain results and 38% did not heal.^(56,57,58,59)

5. Conclusion and Future recommendations

The ability of root canal therapy to effectively remove bacteria depends on a mix of direct bactericidal and ecological effects. In this study the root canal infections were widespread in Libyans Females are more likely than males to get root canal infections. Non-diabetic patients were more than Diabetic patients with root canal infections, Female diabetic patients were more frequent than Male diabetic patients with root canal infections. Comparatively Type-Two diabetes mellitus were higher distribution than Type-one diabetes mellitus in this study. Analogous to patients who took part in the study had root canal infections most frequently between the ages of thirty and forty and less frequently between the ages of sixty and seventy. Meanwhile the Most prevalent teeth with root canal infections were the first premolars, followed by second premolar, canines and at least were the Mandibular incisors. On the other hand Root canal infections occurred in 67.3% of symptomatic patients and 32.7% of asymptomatic cases. In contrast Root canal infections were more among patients with healthy periodontium. Chronic gingivitis were more analogous to mild generalized chronic periodontitis within patients selected in the study. Synonymous root canal infection is caused by facultative anaerobic Gram-positive bacteria in all the patients involved in this study. In cases Smokers with root canal infections made up 16.3% of the male patients and *Staphylococcus aureus* were found in their root canals. Uniformly Patients with various forms of periodontal illnesses had *Staphylococcus aureus* has been identified in all root canals, whereas *Staphylococcus aureus* were more prevalence bacterium found within the root canal cavity in this study. as well as was shown to be the most prevalent bacterium in the study's diabetes individuals.

In view of Patients with root canal infections who are not diabetic frequently harbor *Streptococci*, despite the fact that *Enterococcus faecalis* strain was the most prevalent *Streptococcal* strain associated with root canal infections.

There were more penicillin-resistant strains of the isolated bacteria. In opposition to isolated Bacterium were less resistance to tetracycline and erythromycin due to it aren't use a lot within Libyan society, likewise first and last age groups of patients in this investigation were where penicillin sensitivity was found patients who have a higher level of antibiotic resistance because they misused the drugs and these patient-related characteristics.

It indicates easy accessibility to antibiotics among the Libyan population, to the same extent operators who took part in this study were knowledgeable about antibiotic prescription, they only gave prescriptions in circumstances where they were necessary.

Consistent with Last treatment samples 63% had growth by comparison to 37% had no-growth, it is a sign that 63% will have poor prognosis, although patients immune system plays important role in the outcome of therapy of root canal infections, contrarily Step-back technique is better than crown-down technique. Along the same lines, This study proves ineffectiveness of 5.25% sodium hypochlorite, 0.9% saline and 65% Camphorated para-chlorophenol in root canal therapy. The usage of Calcium hydroxide, 0.6% Chlorhexidine gel as well as Metapex within the root canal will have better outcome. Root canal therapy performed by dentist that had years of experience from 20 to 25 years had better prognosis.

Statement of patients consent

The authors attest that they have the necessary patients consent forms.

Patients understand that no names or initials will be published and that every effort will be made to keep their identities hidden, but anonymity cannot be guaranteed, by signing the consent form for the use of their personal information, intraoral periapical radiographs and clinical data in the study.

Operators consent

Twenty-four participants signed and submitted a consent form for research purposes, indicating their willingness to take part in the study.

Supporting and sponsoring financially

Non

Adherence to moral guidelines

The general manager of specialized center for dental treatment and education (SDC), Head of El Saleem Labourites had approved prior research ethical permission, patients and operators consent for publication had also been given.

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