Antimicrobial Susceptibility of Bacteria Detected from the Root Canal Infection (Before and After) Root-Filled Teeth: An in Vitro Study

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Abstract The aim of the study was to investigate the bacterial species from root canal infection (before and after) filled teeth and evaluate susceptibility to antibiotics, antimicrobial agents and plant extracted from ginger (*Zingiber officinale*). Thirty-three adult patients with symptoms of root canal infection was receiving at the outpatient clinic, Faculty of Dental Medicine, Al-Azhar University, Cairo, Egypt, 2014, diagnosed by a physician participated in the study. Forty- five bacterial samples were taken from root canals infection, 33 before and 12 after filled teeth. A total 126 bacterial isolates were isolated and identify from this samples. The predominant bacterial isolates were found *Enterococcus faecalis* 15%, followed by *Streptococcus mutans* 9.5%, *Streptococcus acidominimus* 8.7% and *Porphyrromonas gingivalis* 7.93%. The bacterial isolates highly sensitive to amoxicillin-clavulanic acid, vancomycin and erythromycin. About 36.50 % of the isolates were resistant to tetracycline. More than 50 % of all isolates were resistant to metronidazole. The sensitivity of bacterial isolates to antimicrobial agents revealed that sensitive 87.30% to potassium iodide 2.0 %, 73.01 % to calcium hydroxide 2 %, 69 % to chloridine 1% and 54% to sodium hypochlorite 0.5%. The in vitro antibacterial activity of *Zingiber officinale* was studied against bacterial isolates. The aqueous and ethanolic extracts of *Zingiber officinale* exhibited antibacterial activity against all bacterial isolates with MIC ranged from 0.5 to 1.3 mg/ml and 0.3 to 1.0 mg/ml respectively. In conclusions, amoxicillin-clavulanic acid and vancomycin most potent antibiotics and potassium iodide 2% most antimicrobial agents against bacterial isolates. *Zingiber officinale* extract has potential antimicrobial action against bacterial isolates.

Keywords: root canal infection, bacterial sp., antimicrobial susceptibility and *Zingiber officinale*

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1. Introduction

Oral diseases like dental caries, periodontitis, oral tissue lesions, infections and oral cavity cancers are major health problems worldwide [1]. Oral health influences general quality of life and poor oral health is linked to systemic conditions. In some countries dental diseases are the fourth most expensive diseases to treat. The oral cavity is colonized by 750 species of microbes and a number of these are implicated in oral diseases. [2]. Endodontic is largely concerned with the treatment of infections originating in the root canal system. Microorganisms are the primary etiological agent for the root canal system infections and periapical lesions [2,4]. Endodontic infections are polymicrobial involving a combination of Gram-positive, Gram-negative, facultative anaerobes and strict anaerobic bacteria [5]. The bacteria remaining in the root canal system after endodontic treatment cause secondary, or persistent, infections [6]. Although more than 300 species of bacteria have been isolated from the oral cavity, only a

limited number have been consistently isolated from endodontic infections [7]. These include species of the genera *Streptococcus*, *Fusobacterium*, *Prevotella*, *Porphyrromonas*, *Eubacterium*, *Peptostreptococcus*, *Bacteroides*, and *Lactobacillus* [7,8]. Baumgartner and Falkler 1991 found that *Actinomyces* species were amongst the most prominent bacteria cultured from the 10 root canals examined [9]. Hancock *et al.*, 2001 examined root filled teeth with persistent periapical radiolucencies, and found that as well as *Enterococcus*, other genera, viz. *Peptostreptococcus*, *Actinomyces*, and *Streptococcus*, predominated [10]. Additionally, *Enterococcus* has been isolated from 47% of root canals in which treatment had failed [11]. The role of systemic antibiotic therapy in endodontics is limited. Usually, it is applied when patients present with progressive, diffuse swelling and systemic signs of infection including fever, malaise, and lymphadenopathy. Systemic antibiotics may also be used as a prophylactic measure for medically compromised patients. The resistance of oral microflora to antibiotics has increased during the past decades, possibly because of

the empiric use of antibiotics for a variety of pathologies [12]. Generally, facultative anaerobes are less susceptible to anti-microbial measures than are anaerobes and are therefore more likely to survive root canal treatment unless cleaning and shaping procedures are of the highest standard. The treatment resistance of enterococci in the root canal has long been recognized [13]. Significantly, calcium hydroxide has been shown to be ineffective in killing *E. faecalis* in root canals [14] and this is predictable because this microorganism is able to grow at high pH [15]. Sodium hypochlorite is also effective in aiding the mechanical flushing of debris from root canals. McComb and Smith 1975 showed that irrigation with 6% sodium hypochlorite resulted in canals that were almost completely free of debris [16]. This compound dissolves organic matter [16] and has a broad spectrum of antimicrobial activity. Thus, sodium hypochlorite effectively eliminates microbes from root canals [18] and also kills bacteria within open dentinal tubules [19]. Potassium iodide (KI) root canal disinfectant when used as 2% solution, has acceptable antimicrobial properties, more pleasant odor, taste and lower toxicity when compared with sodium hypochlorite [20,21,22]. Safavi *et al.* demonstrated the efficacy of KI irrigant in eliminating *E. faecalis* when used for 10 minute in infected dentinal tubules following sodium hypochlorite irrigation [23]. Chlorhexidine is the most frequently widely used antimicrobial agents for treatment of root canal infection [24]. The plant is reported to have antibacterial, anti-oxidant, antiprotozoal, anti-fungal, anti-emetic, anti-rhinoviral, anti-inflammatory, anti-insecticidal activity. Reported pharmacological activities of *Zingiber officinale* include antipyretic, analgesic and antibacterial in addition to hypersensitive effects [25]. Thus, the aim of this study was to evaluate, in vitro, antimicrobial susceptibility to (antibiotics, sodium hypochlorite, calcium hydroxide and potassium iodide, chlorhexidine and *Zingiber officinale*) and viability of some endodontic organisms associated with refractory root canal infections.

2. Material and Methods

2.1. Patient Selection

Thirty-three adult patients with symptoms of root canal infection was receiving at the outpatient clinic, Faculty of Dental Medicine, Al-Azhar University, Cairo, Egypt, 2014, diagnosed by a physician participated in the study. Information about the patients' use of antimicrobial drugs was collected before the sampling procedure.

2.2. Bacterial Sampling

Bacterial samples were obtained from the root canal by using sterile paper points which introduced to the predetermined working length and held in place for 60 seconds to absorb the contents of the canal. Afterwards, the paper points were immediately transferred to Reduced Transport Fluid (RTF) media with aseptic precautions. Forty five samples were collected from patient, thirty three samples before treatment and twelve samples after treatment (filled teeth).

2.3. Isolation and Characterization of Bacterial Species

The samples were collected immediately streaked on (blood agar, chocolate agar, Brain Heart Infusion (BHI) and thioglycolate agar) and incubated aerobic and anaerobic by using anaerobic jars at 37°C for 24, 48 and 72h. The bacterial isolates were identified using cultural, morphological, physiology and biochemical characteristics according to methods described by [26,27,28].

2.4. Antibiotics Susceptibility

The antibiotic susceptibility of all bacterial isolates was measured. The following antibiotics were tested: amoxicillin-clavulanic acid (30µg), ampicillin (10µg), penicillin (10µg), metronidazole (30µg), tetracycline (30µg), vancomycin (30µg), amoxicillin (25µg) and erythromycin (10µg). The antibiotics susceptibilities of the isolates were determined according to Clinical and Laboratory Standards Institute's guidelines National Committee for Clinical Laboratory Standards 2010 [29].

2.5. Susceptibility of Bacterial Isolates to Antimicrobial Agents

All isolates were also tested to determine susceptibility to antimicrobial agents including, 0.5% sodium hypochlorite, 2% potassium iodide, 2% calcium hydroxide, and 1% chlorhexidine by using agar dilution method. Growth meaning resistance and if there is no growth meaning sensitive.

2.6. Zingiber officinale Extracts

Ginger (*Zingiber officinale*) used in the present study were purchased from the local market of Cairo Egypt. Two types of extracts such as aqueous and ethanol extract from ginger were prepared separately. The fresh ginger rhizomes were washed, peeled, sliced and sun dried for seven days. After drying ginger slices were ground to fine powder separately using electric blender. 10g powder of ginger was soaked in 100 ml of distilled water and ethanol separately. The flasks were incubated at room temperature for 72 hours with shaking at 120 rpm. The crude extracts were centrifuged at 3000rpm for 10 minutes at 25°C. The ethanol extracts was evaporated at 50°C while the aqueous extracts were evaporated at 80°C in rotary evaporator. All dried extract samples were dissolved in distilled water separately to the final concentration of 80 mg/ml and centrifuged again at 10,000 rpm to remove the undissolved residues. The extract solutions were stored at 4°C. The controls ethanol and water were treated in similar fashion as described for extract preparation and checked for antimicrobial activity.

2.7. Antimicrobial Assay of Zingiber officinale

The antimicrobial assay was performed by disc diffusion method as described by Kirby-Bauer [30]. The (BHI) plates were inoculated separately with 10⁸CFU of each test bacterial isolates culture and evenly spread on entire surface of each plate. The sterile discs (5 mm diameter) were dipped aseptically in different extracts for one minute and placed over nutrient agar plates seeded with bacterial culture. The plates were left at ambient temperature for 15 minutes and then incubated at 37°C for 16 hours and observed for zone of inhibition. The diameter of inhibition zones was measured in millimeters.

2.8. Determination of Minimum Inhibitory Concentration (MIC)

The MIC of ginger extracts was determined by method described by Natta *et al* [31]. The extracts were diluted ranging from 2 mg/ml to 0.1 mg /ml and checked for MIC against bacterial isolates. Sterile discs were dipped in different dilutions of aqueous and ethanol extracts of ginger and placed over (BHI) agar plates seeded with 10^8 CFU of each bacterial culture separately. Plates were placed at 37°C for 16 hours. The zone of inhibition in each case was measured as the diameter of the clearing zones and results were recorded.

2.9. Identification Tests for Active Compounds

Different color reactions were carried out to detect the presence of certain chemical constituents or chemical group in the *Zingiber officinale* extract according to methods described by Jadon and Dixit [32].

3. Results

Forty five samples from root canal infection (33 before and 12 after) filled teeth were used in this study. A total 126 bacterial isolates were isolated from this samples (72 isolates before and 54 isolates after) filled teeth. The bacterial isolates were subject to scheme for characterization and identification according to morphological, physiology and biochemical tests. After identification, bacterial isolates were grouping as the following, *Streptococci* sp. 36 isolates 28.5% (22 isolates before and 14 after), *Enterococcus faecalis* 19 isolates 15% (9 isolates before and 10 after), anaerobes 60 isolates 47.6 (32 isolates

before and 28 after) and other this group 11 isolates 8.7 % (9 isolate before and 2 after). From results identification of bacterial isolates were found the predominate isolates *Enterococcus faecalis* 15% (frequency 9 isolates before and 10 after) filled teeth followed by *Streptococcus mutans* 9.5% (7 isolates before and 5 after), *Streptococcus acidominimus* 8.7 % (6 isolates before and 5 after) and *Porphyromonas gingivalis* 7.39 % (5 isolates before and 5 after). *Streptococcus salivarius*, *Staphylococcus haemolyticus*, *Bacillus subtilis*, *Actinomyces viscosus* and *Corynebacterium* sp. were isolated from root canal infection before, but not isolated after filled teeth. Figure 1, Figure 2. The antibiotics susceptibility of bacterial isolates, shown that most potent antibiotics were found amoxicillin-clavulanic acid, vancomycin, erythromycin recorded effective against isolates 96%, 94% and 56.13% respectively Table 1. About 56.34 % of the isolates were resistant to metronidazole, 36.50 % to tetracycline, 34.50% to ampicillin, 32.53 to penicillin and 28.56 % to amoxicillin. The sensitivity of bacterial isolates to antimicrobial agents summarized in Table 2 revealed that sensitive 87.30% from bacterial isolates to potassium iodide 2.0 %, 73.01 % calcium hydroxide 2 %, 69 % to chloridine 1% and 54% to sodium hypochlorite 0.5% Table 2. The in vitro antibacterial activity of the *Zingiber officinale* extract using the disk diffusion method was demonstrated that *Zingiber officinale* had most potent activity against bacterial sp. isolated from root canal infection. The results of MIC values are summarized in Figure 3 shown that MIC ranged from 0.3 to 1.0 mg/ml with alcohol extract and 0.5 to 1.3 mg/ml with aqueous extract. Phytochemical analysis of *Zingiber officinale* constituents revealed that having chemical compounds, saponin, alkaloids, terpenoid and flavonoids. The results of phytochemical analysis are summarized in Table 3.

Table 1. Susceptibility of bacterial isolates to antibiotics

Antibiotics		Anaerobes N = 60 %	Streptococci N = 36 %	Enterococci N = 19 %	Other N = 11 %	Total N = 126 %
Penicillin	S	43.33	58.32	63.20	45.45	50.79
	I	21.60	22.20	0.0	0.0	16.66
	R	34.87	19.50	36.84	54.54	32.53
Amoxicillin	S	46.48	52.77	68.41	54.54	52.37
	I	24.90	19.50	5.26	9.09	19.04
	R	28.22	27.80	26.31	36.36	28.56
Ampicillin	S	38.20	47.21	63.20	45.45	45.23
	I	20.0	30.55	10.53	0.0	19.84
	R	41.85	22.22	26.31	54.54	34.91
Tetracycline	S	49.98	44.44	52.63	36.36	47.61
	I	18.32	13.9	10.53	18.18	15.87
	R	31.65	41.70	36.84	45.45	36.50
Amoxicillin-clavulanic acid	S	100	91.57	89.47	100	96.00
	R	0.0	8.31	10.52	0.0	4.00
Vancomycin	S	100	88.64	89.47	90.91	94.43
	R	----	11.08	10.52	9.09	5.55
Erythromycin	S	53.31	55.55	68.42	63.63	57.13
	I	18.32	8.31	0.0	0.0	11.11
	R	28.32	36.10	31.58	36.36	31.74
Metronidazole	S	68.10	0.0	0.0	0.0	32.53
	I	8.30	13.90	10.53	18.18	11.11
	R	23.33	86.09	89.48	81.82	56.34

Figure 1. Frequency of different bacterial species found in root canal infection before and after filled teeth

Figure 2. Frequency of Bacterial groups before and after filled teeth

Figure 3. Minimum inhibitory concentration (MIC) of Zingiber officinale extract against tested bacterial isolates

Table 2. Susceptibility of bacterial isolates to antimicrobial agents

antimicrobial agents		Anaerobes N = 60 %	Streptococci N = 36 %	Enterococci N = 19 %	Other N = 11 %	Total N = 126 %
Potassium iodide 2.0 %	S	88.00	86.00	79.00	100	87.30
	R	12.00	14.00	21.00	0.0	12.70
Calcium hydroxide 2.0%	S	76.63	72.00	63.00	72.72	73.01
	R	23.37	28.00	37.00	27.28	30.00
Sodium hypochlorite 0.5%	S	56.64	53.00	42.10	63.64	54.00
	R	43.36	47.00	57.90	36.36	46.00
Chlorhexidine 1.0 %	S	68.30	83.00	52.63	63.64	69.83
	R	31.70	17.00	47.37	36.36	30.15

Table 3. Phytochemical constituents of *Zingiber officinale* extract

Chemical tests	Results	Remarks
Alkaloid	+ve	Alkaloid present
Glycosides	-ve	No glycosides
Terpenoid	+ve	Terpenes present
Saponins	+ve	Saponins present
Flavonoid	+ve	Flavonoid present
Tannins	-ve	No tannins
Fehling test	-ve	No Reducing sugars

4. Discussion

Oral diseases sources major health problems in the worldwide [1]. Microorganisms that are able to invade the pulp tissue and survive in a root canal system are the causative pathogens in primary endodontic infections. The bacteria remaining in the root canal system after endodontic treatment cause secondary, or persistent, infections [33]. Successful treatment of endodontic infections is strongly dependent on the eradication of microbial infection from the root canal system. Therefore, the absence or presence of bacteria in the root canals prior to obturation is useful information that can help evaluate treatment efficacy [34]. The present study aimed to investigate the bacterial profiles of root canal infection (before and after) filled teeth and susceptibility of bacterial isolates to antibiotics, antimicrobial agents and ginger (*Zingiber officinale*) extracted. The results of the microbiological analysis of this study revealed that bacteria were found in all infected root canals. Most of the bacterial isolates were isolated belong to *E. faecalis*, *Streptococci* and anaerobic bacteria. *E. faecalis* was found most frequently bacterial species before and after filled teeth. This finding agrees to a large extent with the findings of several studies, [35, 36, 37] which found that *E. faecalis* was associated with persistent endodontic infections. This is due to the fact that, Enterococci are able to survive for a long time with a minimum amount of nutrients.[38] In addition *E. faecalis* has the ability to invade the dentinal tubules, which can allow them to resist the chemomechanical preparation and intracanal medication such as calcium hydroxide [39]. The goal of the treatment of root canal infection is to control the spread of infection and provide symptom relief, which can usually be achieved without the use of antibiotics and other antimicrobial agents. Therefore, all bacterial isolates in this study were tested for susceptibilities to antibiotics, other antimicrobial agent and plant extract with the intention of determining the most effective drug for all bacterial isolated. The result obtained revealed that the most potent antibiotics against all bacterial isolates were found amoxicillin-clavulanic acid and vancomycin, while

metronidazole and tetracycline give high resistant ratio. This finding agrees to a large extent with the findings by Endo *et al.* [40]. Resistance of the bacteria found in endodontic infections to antibiotics has been reported [12,41] The evaluate potency of antimicrobial agents, revealed that, potassium iodide 2% exhibited most potent activity against bacterial isolates from root canal compare with another antimicrobial agents has been used in this study. Lin *et al.* was demonstrated that potassium iodide is a better chemical agent for disinfection than calcium hydroxide in shorter exposure times [42]. A clinical study on root-filled teeth with apical periodontitis (failed endodontic cases) demonstrated that a 5 minute final flush with potassium iodide following 2.5% sodium hypochlorite irrigation completely eradicated bacterial in all [43]. For the prevention of the emergence of resistant microorganisms commonly used plant extracts. The in vitro antibacterial activity of *Zingiber officinale* was studied against bacterial isolates. The aqueous and ethanolic extracts exhibited antibacterial activity against all bacterial isolates with MIC ranged from 0.5 to 1.3 mg/ml and 0.3 to 1.0 mg/ml respectively. Victor *et al.*, 1996 considered a strong response to exist when the extract produced an effect at concentration of 10 mg/ml or blow, therefore consider most active extracted from *Zingiber officinale* [44]. *Zingiber officinale* extracts has been reported to have antifungal and antibacterial activity in vitro [45, 46]. These results agree with studies by Gull *et al.* [47]. The *Zingiber officinale* extracts having chemical compounds such as saponin, alkaloids and flavonoids have been reported to have antifungal and antibacterial activities in-vitro (Barasch *et al* [48].

5. Conclusion

Based on the study results, amoxicillin-clavulanic acid and vancomycin were the most potent antibiotics and potassium iodide 2 % most antimicrobial agents in vitro, against bacterial sp. isolated from root canal infection, therefore can be used treatment root canal infection. *Zingiber officinale* extract has potential antimicrobial action against root canal pathogen. Such investigation on natural products to cure diseases may create an alternative source of promising medicines. This study might open the possibilities of finding new clinically effective herbal remedy for root canal infection.

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