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Antibiogroup Resistant Pattern of Anaerobes Isolated from Root Canals Associated with Periapical Lesion

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Abstract: The dynamics of root canal system infections has been studied along the years. It has been established that bacteria and their by-products present in root canals are directly related to the development and maintenance of periapical lesion. The research to date found that a mixed population of facultative and obligate anaerobes as one of the aetiologic agents for periapical pathogenesis. With the possibility that resistant profiles may be emerging, it is prudent to characterize the antibiotic susceptibility pattern of these microorganisms involved in the periapical lesion of root canals. Thus, the objectives of this study are to isolate and identify the types of anaerobes present in the asymptomatic untreated root canal associated with periapical lesion. Its aim is also to determine the antibiotic susceptibility and resistant pattern of the isolated anaerobes. Therefore, the research methodology involved bacterial sampling, isolation and identification of the anaerobes from root canals associated with periapical lesion. The antibiotic susceptibility Kirby-Bauer test was carried out with Ampicillin (10 μg), Azithromycin (15 μg), Augmentin (30 μg), Metronidazole (5 μg) and Amoxicillin (10 μg). In addition, the antibiotic Minimal Inhibitory Concentration (MIC) of all five antibiotics was also carried out. The study has successfully isolated *Actinomyces naeslundii*, *Prevotella intermedia*, *Porphyromonas asaccharolytica* and *Peptostreptococcus micros*. It appears that metronidazole was 100% effective towards all isolated species. Azitromycin and Augmentin have exhibited high antibacterial effect (67%) towards the isolated anaerobes. Ampicillin and amoxicillin were the two least effective (40%) compared to the other three tested antibiotics in this study. This study has obtained two antibiogroup resistant patterns namely A_{mp} A_{mox} and A_{mp} A_{zith} A_{ug} A_{mox} from the isolated anaerobes of the root canal associated with periapical lesion in Malaysia.

Key words: Anaerobes, root canal, periapical lesion, antibiotic susceptibility

INTRODUCTION

More than 300 species of bacteria today are recognized as normal inhabitants of the oral cavity (Moore, 1987). It has been reported that endodontic infections are directly associated with invasion of complex microorganisms (Wasfy *et al*., 1992) with approximately 150 bacterial species (Debelian *et al*., 1994; Gulabivala *et al*., 1997). The predominant bacteria of the root canal have been found to be gram-negative anaerobic rods and cocci, although, initially gram positive bacteria was thought to be responsible for endodontic disease (Sundqvist, 1994). Microorganisms from endodontic infections in root canals can be eliminated by mechanical instrumentation, associated with the use of irrigant solution and an intracanal dressing. However, when systemic involvement occurs antibiotic therapy can be indicated as a co-adjunct of instrumentation and drainage of periapical tissues.

The discovery of antibiotics has brought great benefit to mankind and animals. However, lately multiresistant strains of bacterial species towards various antibiotics have been reported, due to the widespread use of chemotherapeutic agents. Therefore, drug resistant bacterial strains have appeared and spread all over the world.

The objectives of this study are to isolate and identify types of microorganisms present in the asymptomatic untreated root canal associated with periapical lesion via cultivation on selected agar medium. The study is also aimed to determine the antibiotic susceptibility and resistant pattern of the isolated anaerobes from the root canal, which is associated with periapical lesion.

MATERIALS AND METHODS

Sampling inclusion and exclusion criteria: Patients with teeth scheduled for non-surgical root canal treatment,

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which were associated with radiographic evidence of periapical disease has been selected for this study. Those teeth that could not be isolated satisfactorily were excluded. Therefore, intact teeth were the preferred choice. The selected patients were not on any antibiotics treatment for the past 6 months.

Sampling procedure on root canals: The selected teeth were anaesthetized and isolated with rubber dam. The tooth surfaces, rubber dam and clamp were swabbed with 5.2% sodium chloride (NaOCl) for 1 min and subsequently inactivated with saline or distilled water. The access cavity was carefully prepared by using sterile burs. Decontamination procedures were repeated after the access cavity was made. Extra care was taken to not allowing disinfectant to enter the pulp chamber. A sterile instrument was then introduced into the root canals to the apical foremen (determined with apex locator) and the walls were gently filed with a sterile ISO size 10 flexo-file to release debris. If the root canal was found to be dry, Phosphate Buffered Saline (PBS) solution will be syringed into the canal. Three sterile paper points were used to sample the root canal by leaving each paper point in the root canal for 30 sec. The paper points were then quickly placed into 1 mL Reduced Transport Fluid (RTF) vials. The vials were transported to the microbiology laboratory in an anaerobic jar for culturing as soon as possible preferably within 1 h of placement.

Identification of culturable clinical isolates: Samples were dispersed by vortexing for 30 sec at maximal setting and 20 μL aliquots of serial 10-fold dilutions in pre-reduced anaerobic fluid were plated onto fastidious anaerobe agar plates supplemented with 5% blood. Plates were then incubated for a maximum of 10 days and examined daily for evidence of bacterial growth. Each different colony type was subcultured for purity and identification. Isolates were identified using gram staining, standard biochemical methods and API identification system (Bio Merieux). In addition, the Colonies Forming Units (CFU) of each species isolated were determined.

Antibiotic susceptibility test: Antibiotic susceptibilities on selected antibiotics of Ampicillin (Amp) (10 μg), Azithromycin (Azth) (15 μg), Augmentin (Aug) (30 μg), Metronidazole (Mz) (5 μg) and Amoxicillin (Amox) (10 μg) were performed using the Kirby-Bauer test. The tests culture suspensions were swab evenly onto ISO-sensitest agar media suing a sterile cotton swab and allowed to dry for 5-10 min. Using a fine pointed forceps, antibiotic discs were placed onto the agar firmly. All plates were incubated in anaerobic environment at 37°C for 48 h. Sensitivity response of the bacteria was observed as inhibition zone around the discs. All tests were repeated 3 times to ensure results accuracy.

Antibiotic Minimal Inhibitory Concentration (MIC) test: Cultures were prepared to a density of a McFarland 0.5 turbidity standard. About 2 μL of the bacterial suspension was dropped onto the surface of Mueller Hinton antibiotic agar plates. All plates were incubated in anaerobic environment at 37°C for 48 h. MIC was recorded as the lowest antibiotic concentration with no visible growth.

RESULTS AND DISCUSSION

The study has isolated four types of anaerobes from the root canal, which was associated with periapical lesion. Two species of Gram negative rod, namely *Prevotella intermedia* and *Porphyromonas asaccharolytica* and two species of gram positive that is *Peptostreptococcus micros*, which is a facultative anaerobe and *Actinomyces naeslundii* (Table 1). The results showed agreement with Debelian *et al.* (1994), which mentioned that these four species were among the frequently isolated bacteria from endodontic infections.

Murat-Aydyn *et al.* (1998) have isolated *Peptostreptococcus* and *Prevotella* sp. frequently form infected root canal. Similarly in this study, *Peptostreptococcus micros*, *Prevotella intermedia* and *Actinomyces naeslundii* has been frequently isolated. Although, Sundqvist (1992) found *Fusobacterium* sp. to be the most prevalent species in infected root canal, other researcher have reported different findings. Wasfy *et al.* (1992) found *Eubacterium* sp., where as Brook *et al.* (1991) reported *Bacteroides* sp. to be the most predominant bacterial isolated from infected root canal. Murat-Aydyn *et al.* (1998) have suggested that the prevalence of bacterial species in infected root canal may be different from various reports. However, the types of bacteria remain the same because their prevalence depends greatly on the methods of isolation, infection phase and individual local/systemic factors of each sampled subjects. This could probably due to the common ecological determinants of the infected root canal of the oral cavity.

However, *Peptostreptococcus micros*, which is an anaerobic streptococci has shown to be the highest population isolated considering from the Colony Forming Unit (CFU) obtained.

Murat-Aydyn *et al.* (1998) has suggested that a certain group of bacteria is able to colonize in infected root canal, due to the various ecological determinants such as low oxygen tension, a low reduction potential

Table 1: Isolated anaerobic microorganisms species from root canal associated with periapical lesion

	Identified bacteria (CFU mL^{-1})			
Sample No.	*Peptostreptococcus micros*	*Prevotella intermedia*	*Porphyromonas asaccharolytica*	*Actinomyces naeslundii*
1	-	-	-	-
2	-	-	-	-
3	0.5×10^3	5.0×10^3		
4	-	-	1.2×10^3	2.0×10^3
5	-	-	100.0×10^3	32.0×10^3
6	-	1.2×10^3	-	2.0×10^3
7	20.0×10^3	-	-	1.0×10^3
8	20.0×10^3	-	-	-
9	30.0×10^3	15×10^3	-	-
10	-	24.0×10^3	-	24.0×10^3

(Eh) or ceased blood circulation of the infected root tissues. The various types of bacteria present in root canals could be due to the many types of nutrients supplies obtained. For example, carbohydrates in serum will be fermented by saccharolytic and facultative bacteria to acids and alcohol. In addition, the presence of glycoproteins will give rise to *Prevotella intermedia* and caused the decreased of facultative anaerobes, which is in agreement to this study. In addition, the degradation of protein and extensive fermentation of amino acid will encourage the growth of *Porphyromonas* sp., *Propionibacterium propionicum*, etc. (Murat-Aydyn *et al*., 1998).

All isolated species were tested for the antibiotic susceptibility towards five different antibiotics using the disc diffusion method of Kirby-Bauer.

All strains were sensitive towards metronidazole (Fig. 1), probably because metronidazole has a wide bactericidal spectrum against oral anaerobes. The redox enzyme pyruvate-ferredoxin oxidoreductase in anaerobic oral organisms is responsible to chemically convert and reduce the nitro group of metronidazole to its active form, which is responsible to disrupt the DNA helical structure and caused the inhibition of nucleic acid synthesis.

In this study, the broad spectrum antibiotics of Azithromycin (Azth) and Augmentin (Aug) were also effective towards 67% of the anaerobes, which were isolated from root canal. Azithromycin is an azalide and is a subclass of macrolide antibiotics. This antibiotic is effective in treating bacterial infection due to the ability to bind to the 50 S subunit of bacterial ribosome and causing the inhibition of mRNA translation. Augmentin is an antibacterial consisting of a combination of semisynthetic antibiotic of amoxicillin and clavulanate potassium (a β-lactamase inhibitor). It is a β-lactam that is structurally related to the penicillins and has the ability to inactivate a wide variety of β-lactamases by blocking the active sites of these enzymes. Many strains, which colonize infected root canals are able to produce β-lactamase (Murat-Aydyn *et al*., 1998) and therefore, making the antibacterial effect of augmentin very effective. In addition, augmentin is also said to be able not

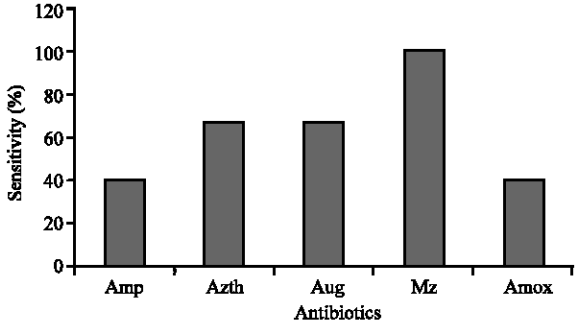

Fig. 1: The frequency of antibiotic sensitivity obtained

only against oral anaerobes such as *Prevotella* sp. but include facultative anaerobes such as *Peptostreptococcus* sp. and also able to reduce the development of drug resistant bacteria. Thus, augmentin that is of the combination of amoxicillin and clavulanate potassium acts as a wide spectrum antibiotic, which is reflected in these results. The study has also shown that a higher susceptibility percentage (67%) was obtained when augmentin (amoxicillin/clavulanate potassium) was used compared to using amoxicillin (40%). Ampicillin has shown to be able to exert antibacterial effect on 40% of the isolated species, which is similar to the effect shown by amoxicillin. This could be suggested by the antibiotic spectrum and level of activity for amoxicillin and ampicillin, which is equivalent. Ampicillin is a β-lactam antibiotic, which is able to penetrate gram positive and gram negative bacteria. It acts as a competitive inhibitor to transpeptidase enzyme, which is needed by bacteria to make bacterial cell walls. Therefore, ampicillin is able to inhibit the bacterial cell wall synthesis, which ultimately leads to cell lysis.

The discovery of penicillin in 1928 has made broad spectrum antibiotics been widely used against bacterial infection. Unfortunately, due to evolutionary stress and environmental pressure, some bacteria have developed various ways to ensure survival against antibiotics. This natural phenomenon is inevitable as bacteria are able to allow changes or mutation in the gene. Several studies have reported that the pattern of antibiotic usage could

greatly affect the development of resistant organisms (Alexander, 1994; Klevens *et al*., 2007). A multiresistant bacteria could even carries several resistance genes making infection difficult to be treated with antibitotics.

The study has shown that all isolated samples of *Prevotella intermedia* and *Porphyromonas asaccharolytica* were sensitive towards all five antibiotics tested. However, all isolated strains of *Peptostreptococcus micros* were resistant towards ampicillin and amoxicillin giving the antibiogroup resistant pattern to be A_{mp} A_{mox}. In contrast, multiple antibiotic resistant was discovered for *Actinomyces naeslundii* that has antibiogroup resistant pattern of A_{mp} A_{zith} A_{ug} A_{mox} due to all isolated strains being resistant to four antibiotics tested except metronidazole. The possible reason for the multi resistant shown by *Actinomyces naeslundii* in this study was due to the selection of antibiotics. Although, the antibiotics chosen were broad spectrum, however, these antibiotics were obviously effective on gram negative anaerobes, thus giving less antibacterial effect towards gram positive anaerobes. Nevertheless, there is a possibility of the strain carrying the resistant gene/s and further confirmation can only be concluded after analysis on the molecular biology aspect has been carried out.

CONCLUSION

The study has successfully isolated *Actinomyces naeslundii*, *Prevotella intermedia*, *Porphyromonas asaccharolytica* and *Peptostreptococcus micros*. It appears that metronidazole was 100% effective towards all isolated species. Azitromycin and Augmentin have exhibited high antibacterial effect (67%) towards the isolated anaerobes. Ampicillin and amoxicillin were the two least effective (40%) compared to the other three tested antibiotics in this study. This study has obtained two antibiogroup resistant patterns namely A_{mp} A_{mox} and A_{mp} A_{zith} A_{ug} A_{mox} from the isolated anaerobes of the root canal associated with periapical lesion in Malaysia.

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