Antibiotic susceptibility in anaerobic bacteria which are most frequently isolated from infected root canals.

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Background: This paper reports the antibiotic susceptibilities of 73 anaerobic bacterial species isolated from 58 infected teeth.

Methods: The bacterial species were collected from the mechanically opened root canals with sterile paper points, plated under anaerobic conditions, and isolates were identified based on biochemical reactions and carbohydrate fermentations. Antibiotic susceptibilities were performed by broth dilution method to 11 antimicrobial agents.

Results: The most frequently isolated bacteria were found to be anaerobic Streptococci (30.1%) and Prevotella sp. (26%). Azithromycin was found to be the most effective antibiotic only for Gram-negative rods, but amoxicillin + clavunate was overall the most effective for all isolates.

Conclusion: Amoxicillin + clavunate is recommended as the first choice in the treatment of acute dental abscesses which take origin from infected root canal flora. Ann Med Sci 1998; 7: 35-39

Keywords: Dental abscess, Amoxicillin + clavulanic acid, Endodontics.

Dental abscesses locate out of root, but originate from infected root canal(s). Conventionally, they can be healed by root canal treatment or endodontic surgery. Besides this, it may be necessary to give an antibiotic particularly in acute phase. This prevents the dissemination of bacteria and extension of acute abscess.

The ideal approach to treatment of acute abscesses is to make culture and antibiotic susceptibility test on the pathogen microorganism(s) which is isolated from the infected root canal.

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However, until the date when the results of tests are obtained, immediate antibiotic therapy may be given blindly in order to control infection earlier. A restricted group of bacteria can colonize in infected root canal, because ecological determinants such as low oxygen tension, ceased blood circulation and a low reduction potential (Eh) of infected root tissues are usually specific. Also, well defined commensal and antagonistic mechanisms operate in infected canal flora.[1] Bacterial population consist of 12-14 genera of the oral flora[1,2], and more than 90% of bacteria are anaerobic.[1,2,3]

In the first phase of infection, the low content of carbohydrates in serum are fermented to acids and alcohols in

the pulp chamber by saccharolytic and facultative bacteria. In the second phase, glycoproteins are the main energy source. *Prevotella* spp. increase, but facultative anaerobes decrease in this stage. In the third phase, protein degradation and extensive amino acid fermentation takes place.*Peptostreptococcus* spp., *Eubacterium* spp., *Fusobacterium nucleatum*, *Porphyromonas* spp., *Propionibacterium propionicum, Wolinella* spp., and *Selenomonas* spp. are the species occurring in this terminal phase.[1] During this phase, certain relationships are established between bacteria present[1,2], and the proportion of a strain to the total flora is specific for each infection. Sometimes, their proportions show fluctuations due to individual factors.[2,4]

Whether or not a particular antibiotic can mostly eliminate the third phase bacteria which are usually present in acute dental abscesses is important, clinically. The main goal of this study is to predict the most effective antibiotic(s) for early control of an acute dental infection.

MATERIALS AND METHODS:

Fifty-eight infected teeth of forty-six patients (male, 21; female, 25) were included in this study. Their mean age was 33 ± 11.4 and the median, 31. The duration of root canal infection was at least one year. Twenty-one teeth had a fistula. Before this investigation, 17 teeth had at least one root canal treatment. Five of the teeth had 3 roots; 7, 2 roots and 46, 1 root. None of the subjects had used systemic or local antimicrobic agent during the preceding 4 days.

For microbiological sampling, a rubber dam was used. The access cavity was opened with a sterile diamond burr. Povidone-iode (10%) and ethanol (70%) were successively applied to the tooth surfaces for two minutes. The root canal was enlarged with a sterile file (No.30). A sterilized absorbent paper-point (No.25) was introduced into the main canal, kept for 30 seconds and placed in 2 ml of freshly prepared Peptone-Yeast-extract-Glucose (PYG) broth. PYG broth contains trypticase (5 g), peptone (5 g), yeast extract (10 g), glucose (0.5 g), hemin (5 mg), Vit K_1 (1 ml of %1 solution), *L*-cystine (400 mg), per liter. The sample was vortexed and immediately transferred to two agar plates. The anaerobe blood agar plate (AnaBAP) was incubated in an anaerobic atmosphere (mixed gas: 5% H2, 5% CO2, 90% N2) and the sheep blood agar plate was incubated aerobically. AnaBAP contains TSA(BBL) (15 g), phytone (BBL) (5 g), yeast extract (5 g), agar (5 g), hemin (5 mg), Vit K_1 (1 ml of %1 solution), *L*-cystine (400 mg), per liter.

After five days, the colonies on both plates were evaluated. The anaerobic growth was more abundant than the aerobic. The organisms which were most predominant on AnaBAP but were not present on the aerobic blood agar plate, were purified. They were identified according to the Gram stain, indole reaction, fluorescence, pigmentation, hemolysis and carbohydrate fermentation profile.[5,6,7] A total of 73 anaerobic strains were collected (Table.1). Antibiotic susceptibility tests were performed using the broth-dilution method (Wilkins & Thiel, 1973)[4]. During the estimation of the antibiotic concentrations for susceptibility tests, their penetration capabilities into bone tissue and length of half-life were considered. Their final concentrations are summarized in the Table.II. Optic densities of species were read at 512 nm with a spectrophotometer (Spectronic 20D, Bausch&Lombs, USA).

Table.I Seventy-three anaerobic and microaerophilic species were isolated from 46 infected root teeth. Most frequent species was found to be *Peptostreptococci* spp. and *Prevotella* species

Bacterium	Number of strains isolated	% incidence at genus level	Most effective antibiotics
Anaerobic streptococci		30.1	AmC, E
Pepotpstreptococcus micros	9		
P. asaccharolyticus	5		
P. productus	3		
P. tetradius	1		
Streptococcus intermedius	3		
S. parvulus	1		
Prevotella sp.		26	AZM, AmC
P. denticola	6		
P. buccae	5		
P. intermedia	4		
P. melaninogenica	3		
P. bivia	1		
Lactobacillus sp.		10.3	E, AmC
L. casei	3		
L. yamashiensis	1		
L. plantorum	1		
Actinomyces sp.		5.5	AmC, CC
A. israelii	2		
A. viscosus	1		
A. bovis	1		
Eubacterium sp.		4.1	AmC, E
E. nodatum	2		
E. alactolyticum	1		
Mistuokella multiacidus	3	4.1	AZM
Porphyromonas asaccharolytica	3	4.1	AZM, SCF
Capnocytophaga sp.		4.1	E, AZM
C. ochracea	2		
C. gingivalis	1		
Selenomonas sputigena	2	2.7	AZM, AmC
Bifidobacterium dentium	2	2.7	CXM, E
Fusobacterium sp.		2.7	CC, CXM
F. nucleatum	1		
F. symbiosum	1		
Dichelobacter nodosus	2	2.7	AZM
Propionibacterium propionicum	1	1.4	CC
Bacteroides gracilis	1	1.4	AZM
unidentified	1	1.4	AZM

RESULTS:

The identities and prevalences of the microorganisms recovered from the canals are listed in Table.I. One anaerobic strain could not be identified. It was a nonmotile, Gram-negative, small rod in chains whose colony was

non-pigmented and hemolytic. Lactose, trehalose, cellobiose, *D*-xylose, maltose fermentation, aesculin hydrolysis and hydrogen sulphide production were negative; *D*-mannitol, sucrose and arabinose fermentation were positive. It was considered to be *Bacteroides gracilis* or *Dichelobacter nodosus*.

Table.II Antibiotics, their concentrations and percentage of sensitive strains in total isolated bacteria. The tested strains were accepted as being sensitive if their optic density were greater than 50.

Antibiotics	**Concentration (µg/ml)**	**% sensitive of total isolates**
Trimethoprim + sulfamethoxazole (SXT)	8	25
Piperacillin (PIP)	33	20.7
Sulbactam + cefoperazone (SCF)	25	29.7
Erythromycin (E)	5	46.5
Tetracycline (Te)	10	26
Azithromycin (AZM)	5	44.8
Amoxicillin + clavulanic acid (AmC)	3.3	65
Ciprofloxacin (CIP)	1.6	5.1
Clindamycin (CC)	3.3	46.5
Netilmicin (NET)	10	39.4
Cefuroxime (CXM)	10	38.3

Material was taken from one tooth of 40 subjects while materials were taken from more than one tooth of 6 subjects. A similar distribution of flora was detected in the samples from different teeth of the same subject.

In this series, neither Gram-positive nor Gram-negative bacteria were dominant in a significant percentage. However, 49 (67.1%) of the bacteria were found to be rods (Gram-negative, 36; Gram-positive, 13). Gram-negative cocci were not recovered (e.g. *Veillonella* sp., *Acidaminococcus* sp.).

Anaerobic Streptococci (18 *Peptostreptococcus* sp., 4 strictly anaerobic *Streptococcus* sp.) were the most prevalent out of all isolates (30.1%) and of the Gram-positive bacterial population (59.4%). The *Prevotella* group was the most prevalent (52.7%) among the Gram-negative population but their incidence was 26% of all isolates (19 of 73). Seventeen facultative anaerobes were isolated (12 *Streptococcus* sp., 3 *Enterobacter* sp., 2 *Eikenella corrodens*) and they were more sensitive to amoxicillin + clavulanic acid than to other antibiotics ($p<0.05$), but they have not been reported in this paper.

Optic density measured for amoxicillin + clavulanic acid was found to be higher than other antibiotics for all bacteria isolated (64 ± 24.9; median, 69.2; mod, 93) (Fig.1 and Table.II). On the other hand, it was more effective on Gram-positive specimens than on Gram-negative bacteria. Although, azithromycin was the most effective antibiotic for all Gram-negative rods (59.2 ± 31.7; median, 66.6; mod, 70) including *Prevotella* sp. (Fig.1). Table.II shows which antibiotic is more potent on which bacterial isolates.

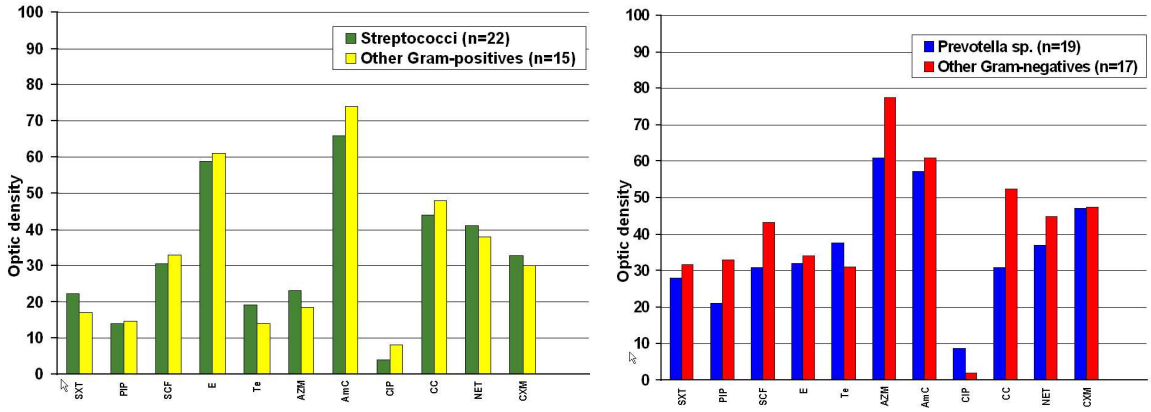

Fig.1 A, B amoxicillin + clavulanic acid was found to be higher than other antibiotics for all bacteria isolated (64 ± 24.9; median, 69.2; mod, 93)

DISCUSSION:

In this study, the bacterial species encountered, were mostly similar to those reported in the literature, but their frequencies were different. Sundqvist[2] found that Fusobacterium species were the most prevalent (48%) in infected root canals. Wasfy et al.[8] found that the most frequently isolated organisms were *Eubacterium* species (68%), Brook et al.[3] reported that, *Bacteroides* spp. were predominant (39.4%) isolates, but anaerobic cocci made up only 23%. John et al.[9] found that the prevalence of *Bacteroides* spp. was 41.5% and of anaerobic Gram-positive cocci, 30.5%. Gümrü et al.[10] showed that *Peptostreptococci* were dominant bacteria (29.2%). However, we found that Gram-positive anaerobic *Streptococci* occurred in the infected root canal with a prevalence of 30.1%; *Prevotella* spp., 26%; *Eubacterium* spp., 4.1% and only 2 *Fusobacteria*, 2.7%. The prevalence of bacterial species in infected root canal flora may differ from a report to another, while their types are remain similar, because, their prevalence depends mostly on methodology, phase of infection and individual local/systemic factors of subjects. It is interesting that, in the same mouth, two or more infected teeth shared similar bacterial flora. This suggests that, in a mouth, the ecological determinants of infected canals are common.

Concentrations of all antibiotics used were adjusted according to their diffusion capabilities into oral tissues. Clindamycin is more capable of concentrating in bone tissue and saliva than that in serum. For this reason, clindamycin was tested in a concentration of 3.3 µg/ml despite the fact that a standard concentration of 1.6 µg/ml is recommended.[5,10] Tetracycline was also used in concentration of 10 µg/ml instead of 4 µg/ml. The concentration of trimethoprim + sulfamethoxazole was limited to 8 µg/ml because it can briefly penetrate into bone tissue.

The amoxicillin + clavulanic acid group of antibiotics which consist of a penicillin derivate and a ß-lactamase inhibitor, was found to be more potent on all anaerobes isolated. It can be suggested that, ß-lactamase inhibition played a key role in this series. Because, many strains which can colonize infected root canals are able to produce ß-lactamase. This activity of one member of the flora is important during the antibiotic therapy, because a ß-

lactamase producing strain would protect not only itself from penicillin derivates but also the other bacteria.[9] This capability is most often seen in the *Prevotella* genus. Könönen et al.[11] showed that 168 of 226 (74.3%) *P. melaninogenica* strains produced ß-lactamase. Also, ß-lactamase producing organisms were recovered from 7 of the 21 (33%) specimens that were tested by Brook et al.[3], and from 15 of the 38 (39.4%) tested by Gümrü et al..[10] We found ten strains of *Prevotella* which were resistant to piperacillin (extended spectrum penicillin) but sensitive to amoxicillin + clavulanic acid. Liebana et al.[12] also, showed that many oral *Streptococci* which were dominant in the facultative flora in infected root canals can be inhibited with 2 µg/ml of penicillin derivates. It is, likely that, the amoxicillin + clavulanic acid can repress not only anaerobes, but also facultatives.

Pajukanta et al.[13] tested eightytwo *P. gingivalis* strains and all of them were inhibited by azithromycin at a concentration of 1 µg/ml or less. We found that, either *Prevotella* members or other Gram-negatives were more sensitive to azithromycin than to amoxicillin + clavulanic acid. However, azithromycin did not inhibit all of the Gram-positive bacteria.

On the other hand, when the total flora was taken as a base, clindamycin and erythromycin were more effective then either trimethoprim + sulfamethoxazole or azithromycin. Further, clindamycin is more penetrable into bone, but clindamycin may cause a predisposition to gastro-intestinal disorders when it was orally used. Clindamycin is a highly selective antibiotic and has a shorter half-life than either trimethoprim + sulfamethoxazole or azithromycin. However, the half-life of amoxicillin + clavulanic acid which has a less adverse effect, is longer than that of clindamycin but not of trimethoprim + sulfamethoxazole.

Ciprofloxacin was found as to be ineffective on many anaerobes. This result should not be surprising, because it is a DNA inhibitor and mostly effective on aerobic bacteria. Although, the use of certain antibiotic combinations seems to be a good idea, but unpredictable interactions between antibiotics as well as between antibiotics and the patient may occur and must be monitored if two or more systemic antibiotics are given to a patient. Sato et al.[14] suggested that the endodontic lesions may be sterilized by a mixture of ciprofloxacin, metronidazole and cefaclor in situ. However, multi-drug application may cause many adverse effects on oral microflora.[9,15] On the other hand, our results demonstrated that single drug therapy may be sufficient for many oral bacteria so that adverse effects can be kept minimal.

We concluded that the amoxicillin + clavulanic acid group of antibiotics should be the first choice for early control of acute dental abscesses.

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