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Antimicrobial susceptibility profiles of bacterial strains isolated from chronic apical periodontitis

Anca Diana Cristea[1], Luminita Marutescu[2,*], Mariana Carmen Chifiriuc[2], Veronica Lazar[2], Ioana Suciu[1], Andrei Iliescu[1]

ABSTRACT

The aim of the present study is to investigate the microbiota of root filled teeth with apical periodontitis and to determine the antibiotic susceptibility patterns of microbial strains isolated from twenty nine apical lesion samples taken from patients with fibrous chronic apical lesions. The present study has highlighted the polymicrobial nature of the root canal infections and the importance of facultatively anaerobic, Gram positive bacteria found in 90.74% of the isolated microorganims. The present study has shown an increased resistance of microorganisms to conventional antibiotics, which included penicillin, erythromycin and amoxicillin, as well as tetracyclines, although the last ones have been used sparingly in the past decade. The results of antibiotic sensitivity obtained in this study underline the importance of the microbiological diagnosis and antibiotic susceptibility testing in the choice of therapeutic agents used for the treatment of chronic apical lesions. Further studies with clinical correlation of effectiveness of these antibiotics and cultures taken after administration of antibiotics as well as recording of disappearance of symptoms are also recommended.

Keywords: *Root canal infections, Resistance, Facultative anaerobes, Enterococcus sp., Streptococcus sp., Actinomyces sp.*

1. INTRODUCTION

For the past 80 years, antibiotic therapy has played a major role in the treatment of bacterial infectious diseases. Since the discovery of penicillin in 1928 by Fleming and sulfanilamide in 1934 by Domagk, the entire world has benefited from one of the greatest medical advancements in history. The discovery of safe, systemic antibiotics has been a major factor in the control of infectious diseases and, as such, has increased life expectancy and the quality of life for millions of people. To avoid the deleterious effects of needless antibiotics on patients and the environment, the most important initial decision is whether use or not antibiotics. It has been estimated that up to 60% of human infections resolve by host defenses alone following removal of the cause of the infection without antibiotic intervention. Endodontic disease is infectious. Microorganisms cause virtually all pathoses of the pulp and periapical tissues. There is ample evidence to support that opportunistic normal oral microbiata colonize in a symbiotic relationship with the host, resulting in endodontic infections. The majority of endodontic infections do not require systemic antibiotic therapy when the cause of the infection has been properly managed (complete debridement of the pulp space and proper obturation and sealing of the pulp space from the oral environment) [1].

The rational choice and use of antimicrobial agents begins with the knowledge of the microorganisms most likely responsible for common dental infections of pulpal origin. The bacterial

[1] UMF Carol Davila, Bucharest, Romania
[2] Faculty of Biology, University of Bucharest, Romania

microbiota found in endodontic infections is indigenous, mixed (Gram-positive and Gram-negative) and predominantely anaerobic. Several species have been implicated with acute apical abscesses. These species include dark-pigmented bacteria (*Prevotella* and *Porphyromonas*), eubacteria, fusobacteria and *Actinomyces* [1].

Studies regarding the susceptibility of bacteria recovered from acute apical abscesses to five commonly used antibiotics in dentistry apical abscesses led to the following conclusions: pen-V-K is the antibiotic of choice for endodontic infections due to its effectiveness in polymicrobial infections, its relative narrow spectrum of activity against bacteria most commonly found in endodontic infections, its low toxicity and low cost. Clindamycin is the antibiotic of choice for patients allergic to penicillins. While amoxicillin and augmentin (amoxicillin plus clavulanate) demonstrated a higher antibacterial effectiveness than Pen-V-K, due to the broader antibacterial spectrum of amoxicillin and the increased cost of augmentin, the authors recommended that amoxicillin/augmentin be reserved for unresolved infections and patients who are immunocompromised. Metronidazol demonstrated the greatest amount of bacterial resistance and is only effective against anaerobes. Therefore, it should not be used alone for the treatment of endodontic infections [2].

Evidence exists that antibiotic resistance has increased in the oral microbiota over the last 10-20 years. The prevalence of β-lactamase producing microorganisms has been examined in different ecological niches of the oral cavity. Studies conducted 25 years ago demonstrate low levels of β-lactamase producing bacteria. During the last 10-15 years, a high level of β-lactamase producing bacteria (53-74%) has been discovered in periodontal pathology, while 38.5 % is discovered in samples of acute purulent infections of the oral cavity. Different microbial species, especially Gram-negative microorganisms, have become resistant to penicillin. Recent use of β-lactam antibiotics has a tendency to increase the emergence of β-lactamase- producing bacteria [3]. The aim of the present study is to investigate the microbiota of root filled teeth with apical periodontitis and to determine their antibiotic susceptibility patterns.

2. EXPERIMENTAL SECTION

2.1. Clinical material and sampling procedure. Twenty nine apical lesion samples were taken from patients (15 men and 14 women), older than 20 years of age, a dentistry clinic in Bucharest for current dental evaluation and treatment. All analyzed teeths (14 mandibular molars, 2 mandibular premolars, 3 upper premolars, 7 maxillary molars, 3 incisors) presented fibrous chronic apical lesions that could be detected on individual radiographs, and the endodontic retreatment was not possible. Patients signed an informed consent about the medical procedures. Using aseptic techniques and sterile instruments, bacterial samples of the root canals were taken and placed into a 2-mL centrifuge tube containing 1.5 mL thioglicolat medium.

2.2. Microbial identification. The samples were inoculated onto nonselective bloodagar plates and incubated in aerobic and anaerobic conditions. The microbial isolates obtained were presumptively identified based on colony morphology, Gram staining and conventional biochemical tests (catalase and oxidase). Colonies were then identified by using the commercial biochemical kits API (bioMérieux, France): API Staph, API Strep, API 20E, API NE, API 20A.

2.3. Antimicrobial susceptibility tests. The antibiotic susceptibility testing was performed by Kirby-Bauer standard disk diffusion method, using the following panels of antimicrobial disks recommended by CLSI, 2013. The tested antibiotics were: penicillin (P), ampicillin (AM), amoxicillin (AMX), piperacillin (PIP), amoxicillin-clavulanic acid (AMC), ticarcillin-tazobactam (TIM), piperacillin-tazobactam (TZP), ampicillin-sulbactam (AMS), cefotaxime (CTX), ceftriaxone (CRO), ceftazidime (CAZ), cefoxitin (FOX), oxacillin (OX), cefepime (FEP), sulfamethoxazole-

trimethoprim (SXT), ciprofloxacin (CIP), tetracycline (TE), ticarcillin (TIC), meropenem (MEM), ertapenem (ETP), aztreonam (ATM), gentamycin (GE), amikacin (AN), tobramycin (NN), clindamycin (DA), linezolid (LZD), tigecycline (TGC). The results were recorded after 24 h incubation at 37ºC.

3. RESULTS SECTION

Microbial analysis of the twenty nine root canal samples taken from 29 adult patient with chronic apical lesions led to isolation of fifty four microbial strains belonging to twenty eight bacterial species. All the specimens yielded microbial growth. The majority of the strains were facultative anaeobes. Gram positive bacteria belonging to *Streptococcus, Actinomyces, Gemella, Enterococcus, Eubacterium, Aerococcus, Bifidobacterium, Lactococcus* and *Propionibacterium* genera constituted 90.74% of the isolated microorganims.

Apical periodontitis has a polymicrobial etiology, and the bacterial community profiles significantly vary from subject to subject [4, 5, 6]. Because of these characteristics, endodontic infections should be ideally treated by using a broad-spectrum, nonspecific antimicrobial strategy, which has the potential to reach the most possible members of the endodontic bacterial communities.

Antibiotics are not generally used to treat chronic infections, such as apical periodontitis, in root filled teeth. Chronic alveolar infections are associated with pulpless teeth which have no blood supply reaching the pulp space. Following the systemic administration of an antibiotic, the concentration reaching the root canal is negligible and unlikely to inhibit bacterial growth.

Therefore, systemic antibiotic therapy is neither indicated nor likely to be beneficial [7]. Prophylactic use of antibiotics is, of course, another matter. Prophylactic use can be indicated if patients are considered at risk of infective endocarditis during endodontic treatment [7, 8]. In such cases, therapy should be directed primarily against the most important pathogens present.

However, it is important to emphasize that, because of ecological changes in an acute situation, the microbiota will change. Poymicrobial infections and obligate anaerobes are frequently found in canals of symptomatic root filled teeth [9]. Therefore, bacteria other than enterococci will often be the main target of the antibiotics in the acute infection.

The present study has shown an increased resistance of microorganisms to conventional antibiotics, which included penicillin, erythromycin and amoxicillin. This is in with the statement of Wood R who had reviewed the antibiotic sensitivity pattern of pathogenic microorganisms over a span of 20 years (1966 – 1986). He stated that there is a continuous decline in the sensitivities of the bacteria isolated to the most of the antibiotics used in dental practice. Slowly and persistently resistant strains of all types of microorganisms encountered in dental practice are emerging. Penicillin, erythromycin and amoxicillin having been prescribed very frequently in dental practice, thus the resistant strains have emerged as depicted in the present study. Even though tetracyclines have been used sparingly in the past decade due to resistance shown by various microorganisms in the past and emergence of newer broad spectrum antibiotics, in the present study only 33.33% sensitivity has been observed.

Enterococci possess a vast array of mechanisms that confer antibiotic resistance to a range of antibiotics including penicillin, the drug of choice [10, 11]. These microorganisms show intrinsic resistance to certain antibiotics such as cephalosporins, clindamycin and aminoglycosides [12, 13]. In addition to these intrinsic resistances, enterococci have acquired genetic determinants that confer resistance to many classes of antimicrobials, including tetracycline, erythromycin, chloramphenicol, and, most recently, vancomycin [11, 12, 13, 14]. Clinical isolates of *E. faecalis* recovered from root canal infections demonstrate antimicrobial resistance to conventional treatment regimens

recommended for dental procedures. Some researchers [15] have described enterococcal isolates resistant to benzylpenicillin, ampicillin, clindamycin, metronidazole and tetracycline; whilst others [16] have discovered strains that are resistant to cephalosporins. Previous studies [17] have found *E. faecalis* strains which show resistance to azithromycin and erythromycin. In the present study, high resistance rates were registered for *Enterococcus* sp. strains for erithromycin, ampicillin and ciprofloxacin (Figure 1).

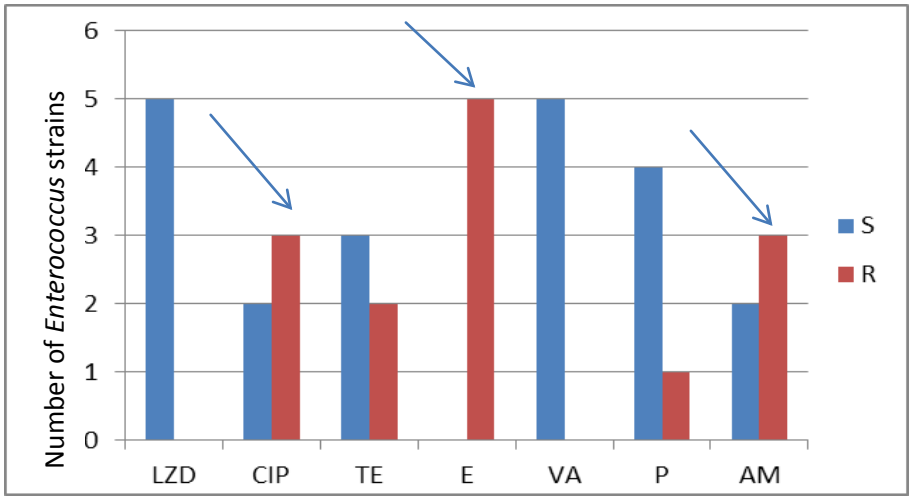

Figure 1: Graphic representation of antbiotic susceptibility profiles of the *Enterococcus* sp. strains isolated from chronic apical periodontitis

Clinical isolates of *Streptococcus sp.* recovered from root canal specimens exhibited multidrug resistance profiles, being resistant to more than 3 classses of antibiotics: macrolide, lincosamide, cephalosporines, glicopeptides, tetracyclines and penicllins (Figure 2).

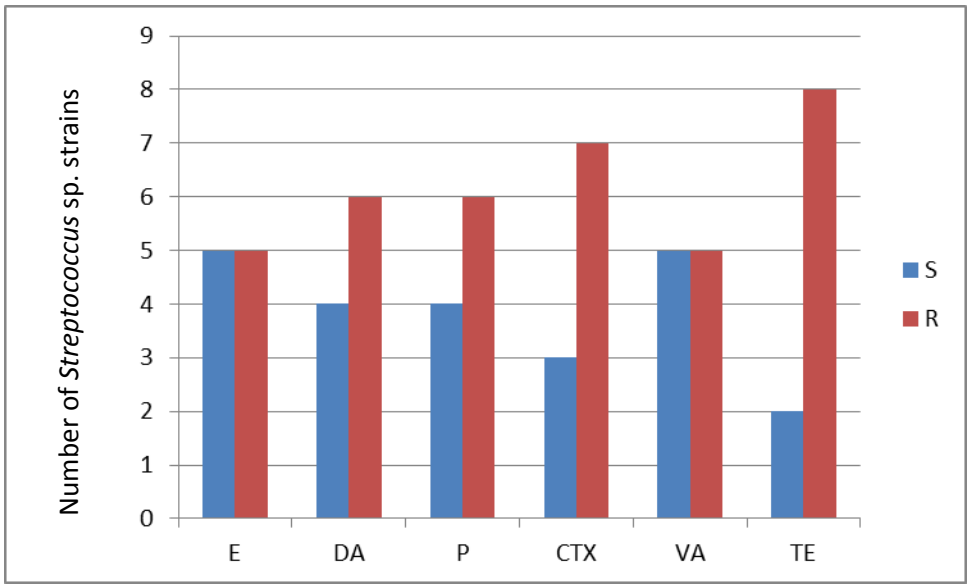

Figure 2: Graphic representation of antbiotic susceptibility profiles of the *Streptococcus* sp. strains isolated from chronic apical periodontitis.

The use of broad spectrum, third generation cephalosporins, like cefotaxime in paediatrics and gynaecology has been much reported and recommended. But no report is available regarding their use in treatment of acute odontogenic infections. In the present study, 77.77% of the *Streptococcus* strains exibited resistance towards this antibiotic. Because of the ability of the actinomycotic organisms to establish extraradicularly, they can perpetuate the inflammation at the periapex, even after orthograde root canal treatment. Therefore, periapical actinomycosis is important in

endodontics [18, 19, 20, 21, 22]. *A. israelii* and *P. proprionicum* are consistently isolated and characterized from the periapical tissue of teeth which did not respond to proper conventional endodontic treatment [21, 22]. The properties that enable these bacteria to establish in the periapical tissues are not fully understood, but appear to involve their ability to build cohesive colonies that enable them to escape the host defense system [23]. In our study, *Actinomyces naeslundii* exhibited multiple drug resistance (Table 1). It is to be noticed that the majority of the anaerobic strains, other than *Enterococcus* sp. and *Streptococcus* sp. exhibited high resistance rates to aztreonam and gentamycin (table 1).

Table 1: Antibiotic susceptibility profiles of the isolated anaerobic strains, other than *Enterococcus* sp. and *Streptococcus* sp. (presented values are growth zone inhibition diameters expressed in mm)

Strain	AN	TIC	DA	TZP	CRO	ATM	CAZ	CN	CIP	AMC	ETP	FOX	TIM	MEM	TGC	P	CTX	PRL	SAM	AMX
Gemella morbillorum	35	29	34	39	28	R	25	32	35	22	30	27	42	39	36	26	24	23	43	50
Actinomyces israeli	19	20	27	34	20	R	14	26	24	10	27	27	32	34	28	16	19	20	37	37
Propioniumbacterium	30	39	32	47	36	R	29	28	35	32	34	34	48	37	24	28	33	32	39	44
Actinomyces sp.	23	23	28	30	35	R	28	22	32	17	37	34	36	40	30	15	35	18	28	28
Prevotella intermedia	23	23	27	31	36	R	35	24	33	12	32	33	35	42	43	33	32	30	R	R
Prevotella oralis	23	23	28	30	36	R	33	12	32	22	33	30	29	40	34	33	31	27	R	R
Lactococcus lactis ssp cremoris	12	27	35	36	42	R	33	11	27	25	35	21	37	40	28	23	42	29	38	50
Gemella morbillorum	R	21	28	40	30	R	24	R	18	22	31	24	30	34	23	20	33	27	34	38
Lactococcus lactis ssp cremoris	19	32	38	40	42	R	32	R	25	22	38	24	44	45	60	24	40	20	40	44
Actinomyces naeslundii	25	12	R	26	R	20	23	8	27	R	R	R	32	R	30	R	R	13	R	R
Gemella morbillorum	R	26	16	37	28	R	23	R	10	26	36	29	35	37	47	29	35	34	35	36
Lactococcus lactis ssp cremoris	R	27	R	35	26	R	24	R	10	R	29	22	31	33	24	26	31	28	33	35
Gemella morbillorum	25	15	32	34	20	R	15	25	30	R	25	24	35	37	28	32	25	22	28	15

4. CONCLUSIONS

The present study has highlighted the polymicrobial nature of root canal infections and the importance of facultative microorganisms in symptomatic non-vital teeth having pariapical pathosis. The results of antibiotic sensitivity obtained in this study offer an important indication in the choice of therapeutic agent for the treatment of chronic apical lesions. Further studies with clinical correlation of effectiveness of these antibiotics and cultures taken after administration of antibiotics as well as recording of disappearance of symptoms are recommended. Since their discovery eight decades ago, safe systemic antibiotics have revolutionized the treatment of infections, transforming once deadly diseases into manageable health problems. However, the growing phenomenon of bacterial resistance, caused by the use and abuse of antibiotics and the simultaneous decline in research and development of new antimicrobial drugs, is now threatening to take us back to the pre-antibiotic era. Without effective treatment and prevention of bacterial infections, we also risk rolling back important achievements of modern medicine such as major surgery, organ transplantation and cancer chemotherapy [24].

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