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Isolation, identification and antibiotic susceptibility profiles in bacterial strains isolated from periodontal lesions

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ABSTRACT

The dental plaque biofilm is at origin of the pathogenic mechanisms of the periodontal lesions, resulting both from the direct degradative action and the indirect inflammatory lesions, mediated by microbial cells and their virulence factors. The purpose of this study was the isolation, identification and characterization of the antibiotic susceptibility profiles of bacterial strains isolated from patients with periodontal lesions. The identification of the 54 isolated bacterial strains was based on the examination of colonial, culture, microscopic and biochemical features. The obtained data show a large taxonomic diversity of the bacterial strains isolated from pacients with periodontal lesions (30 species), the most frequent being Actinomyces neslundii (12.96%), Pasteurella haemolytica (11.11%), Micrococcus sp. (7.4%) and Streptococcus intermedius (7.4%). The antibiotic susceptibility assay revealed different profiles and significant levels of antimicrobial resistance, reflecting the necessity to perform the microbiological analysis and the antibiotic susceptibility testing in order to select the optimal antimicrobial therapy for the treatment of the periodontal disease.

Key words: Periodontitis, Dental Plaque, Resistance

INTRODUCTION

Around 700 - 1000 bacterial species were described in the composition of dental plaque reaching densities of 10^8 bacterial cells/mg, many of them being uncultivable and associated in polymicrobial communities. The literature of the last decades has shown that almost all forms of the periodontal disease are consequences of the chronic, nonspecific or specific bacterial infections. Patients with chronic periodontitis showed a larger percentage (~ 85%) of Gram-negative anaerobic bacteria, like *Aggregatibacter actinomycetemcomitans*, a and b serotypes, *Campylobacter rectus*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythensis* and *Treponema denticola*. *P. gingivalis*, *T. forsythensis* and *T. denticola* associated with forms of chronic periodontitis [1, 2, 3]. Recent studies showed that Gram-positive microorganisms, such as *Peptostreptococcus* sp., *Filifactor* sp., *Megasphera* sp. and *Desulfobulbus* sp. also play an important role in the periodontal disease. *Staphylococcus aureus* strains were isolated in the periodontal pockets of non-smoker patients with aggressive periodontitis. *Pseudoramibacter* sp., *Bacteroidetes* sp., *Sphorocytophaga* sp., *Shuttleworthia* sp., *Dialister* sp., *Mogibacterium* sp., *Mycoplasma* sp., *Synergistes* sp., and *Acidaminococcaceae* sp., strains have also been isolated from patients with resistant forms of periodontitis. *Candida* species were identified in 15-21% of the healthy individuals, but also in patients with periodontitis. Epstein-Barr and CMV viruses were proven to be involved in the periodontal lesions pathology [4, 5].

The emergence and evolution of antibiotic resistance coupled with the technical difficulties of performing antibiotic susceptibility tests of periodontal pathogens, represent important cause for the therapeutic failure rates for this disease [6, 7]. One of the main reasons for the antibiotics ineffectiveness against the periodontopatogenic bacteria is that they grow in biofilms, becoming increasingly aggressive and difficult to destroy. The active antibiotic concentrations required to eradicate biofilms are very difficult to be achieved *in vivo*, especially when administered as local treatments, the biofilm penetration by biocides or antibiotics being strongly hindered [8, 9, 10].

The purpose of this study was the isolation, identification and characterization of the antibiotic susceptibility profiles of bacterial strains isolated from patients with periodontal lesions.

MATERIALS AND METHODS

Isolation and identification of bacterial strains

A number of 26 samples was collected from patients suffering from periodontal diseases, from the periodontal pockets and gingival sulcus. The samples were collected and delivered to laboratory in liquid thioglycollate medium, in order to preserve the anaerobic bacteria viability. The inocculated thioglycollate tubes were slightly vortexed and seeded on a Brucella agar medium, supplemented with a 5 % sheep blood. The inoculated plates were incubated at 37^{o}C, in anaerobic conditions (from 48 hours to 7 days). The microbial identification was performed by examining the culture and colonial characteristics, the morphology and Gram character, and by establishing the biochemical profiles, using API identification microsystems (bioMérieux, France) (API Staph, API Strep, API 20E, API NE, API 20A and CAUX).

Antibiotic susceptibility testing

The antibiotic susceptibility testing was performed by Kirby-Bauer standard disk diffusion method, using panels of antimicrobial disks recommended by CLSI, 2013. The tested antibiotics were: penicillin, ampicillin, amoxicillin, piperacillin, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, piperacillin-tazobactam, ampicillin-sulbactam, cefotaxime, ceftriaxone, ceftazidime, cefoxitin, oxacillin, cefepime, sulfamethoxazole-trimethoprim, ciprofloxacin, tetracycline, imipenem, meropenem, ertapenem, aztreonam, gentamycin, amikacin, tobramycin, clindamycin, linezolid, tigecycline. The results were recorded after 24 h incubation at 37ºC.

RESULTS AND DISCUSSION

The microbiological analysis of the 26 samples led to the isolation of 54 bacterial strains, with a large diversity of morphological types. These results come into agreement with other literature data, showing that the periodontal disease microbiomes are more diverse in terms of community structure than the healthy microbiome which has a relatively lower taxonomic diversity, remaining relatively constant over time. This natural balance termed “microbial homeostasis” supports the innate and adaptive host defenses in excluding exogenous (and often pathogenic) microorganisms, and is responsible for the natural development of the normal physiology of the host [9].

The most frequently isolated species belonged to « good » Gram-positive *Actinobacteria* and *Micrococcaceae*, i.e.: *Actinomyces naeslundii* (12.96%), *Micrococcus* sp. (7.4%), but *Streptococcus intermedius* (7.4%) and the Gram-negative *Pasteurella haemolytica* (11.11%) were also present.

Dental plaque displays properties that are typical for biofilms, being structurally and functionally organized in polyspecific communities embedded in an extracellular matrix of exopolymers [9], characterized by an increased metabolic efficiency, pathogenic synergism, greater resistance to host stress factors and enhanced virulence [10]. The control of biofilm accumulation on teeth surfaces has been the cornerstone of periodontal disease prevention for decades, periodontitis being the result of both direct degradative action and indirect inflammatory lesions, mediated by the presence of microbial cells and of their virulence factors, affecting the periodontium integrity [11]. Taking into account that one of the major factors responsible for the transition from periodontal health to either gingivitis or periodontitis is the acquisition of certain species/ combinations of species [12], the diagnosis of dysbiosis that precedes the clinical manifestation of disease, could be a potential tool for the early diagnosis of periodontitis.

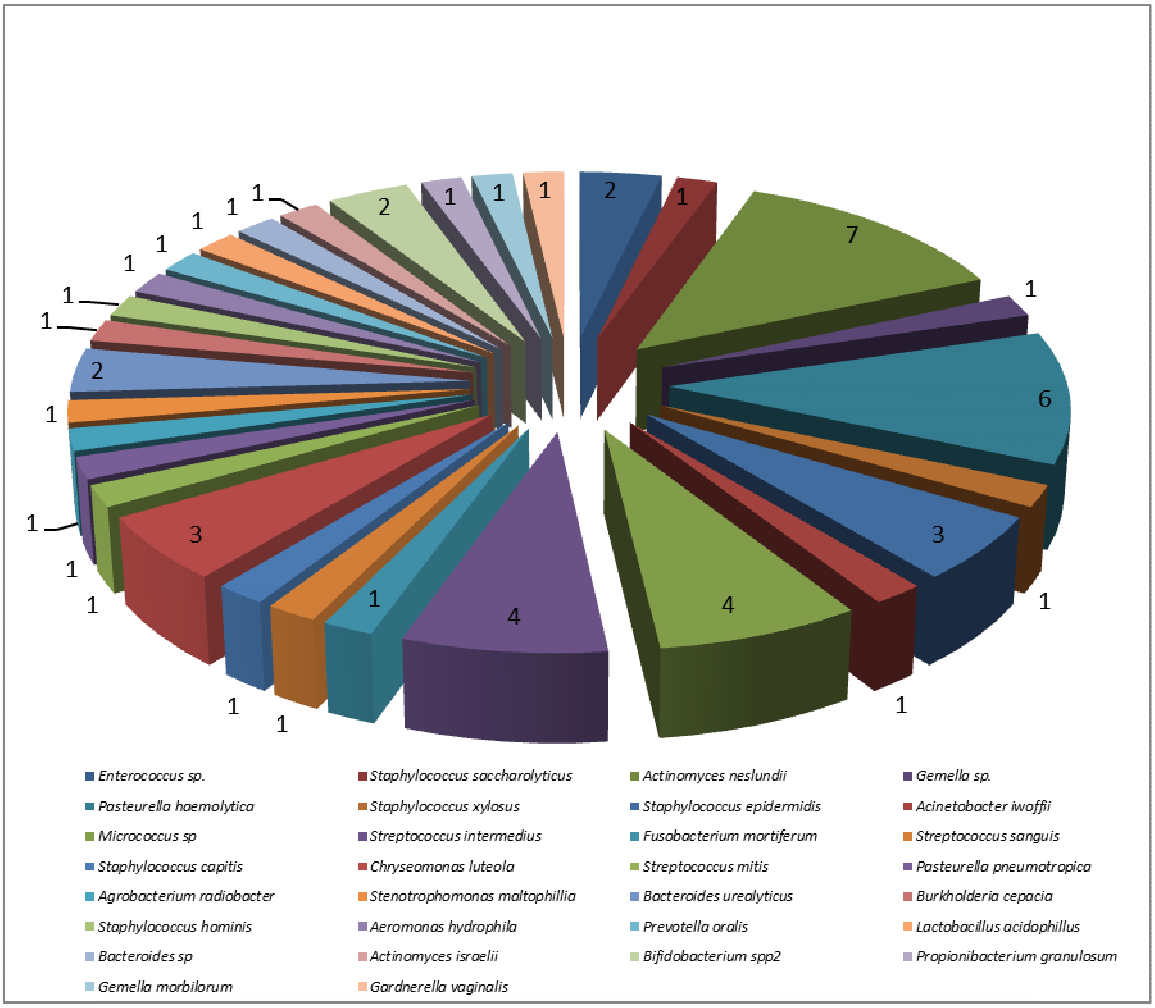

Fig. 1. The distribution of the isolated strains according to their taxonomic affiliation

Antibiotic susceptibility profiles

The antibiotics most commonly used in the periodontal treatment are penicillin, tetracycline, macrolides and metronidazole. The emergence of antibiotic-resistant bacteria has frequently been reported to be a direct result of antibiotic usage. The extensive use of antimicrobials both in the community and hospitals has accelerated the emergence of antibiotic-resistant organisms. Our study revealed a significant rate of resistance to macrolides, cephalosporins (four and third generation) and aminoglycosides among the tested microbial strains.

The antibiotic susceptibility patterns of Gram-positive cocci revealed high resistance rates to macrolides (including clindamycin) and also to the third and fourth generation cephalosporins for *Streptococcus* sp. strains (fig. 2), respectively to macrolides and aminoglycosides (gentamycin) for *Micrococcaceae* strains (fig. 2). The high resistance to macrolides might be explained by the use of tetracycline in the periodontal treatment. Studies regarding the effect on oral biofilms of a single tetracycline pulse is selecting for organisms that are resistant to multiple antibiotics, including erythromycin, as shown by the increase in the proportion of such isolates in the biofilms from 5% to 28% [13]. In our study, of the isolates resistant to tetracycline, 67% were also resistant to erythromycin.

The Gram-negative bacilli strains exhibited high resistance rates to beta-lactam antibiotics (i.e. third generation cephalosporins, monobactams) and aminoglycosides (i.e. tobramycin) (Fig. 2). It is established that enteric Gram-negative rods constitute less than 1% of total cultivable microbiota of subgingival samples [14]. However, administration of some antibiotics may give a selective advantage to many resistant species colonizing bacterial strains presented resistance to ampicillin and amoxicillin/clavulanic acid, drugs commonly used in treatment of oral infections or in the prophylaxis of systemic infection during dental therapy [15,16].

Aminoglycoside antibiotics are not usually recommended to infections in oral cavity, and are not used as chemotherapeutic in periodontal therapy. Both aminoglycoside and fluoroquinolones are employed for the empirical treatment of febrile neutropenic patients and in serious infections caused by aerobic and facultative Gram-negative

bacilli, including *Enterobacteriaceae* [17,18]. However, the occurrence of microbial resistance to aminoglycosides in commensal flora of periodontitis lesions alerts for the risk of systemic dissemination of resistant enterobacterial clones in hospitalized patients with periodontal diseases.

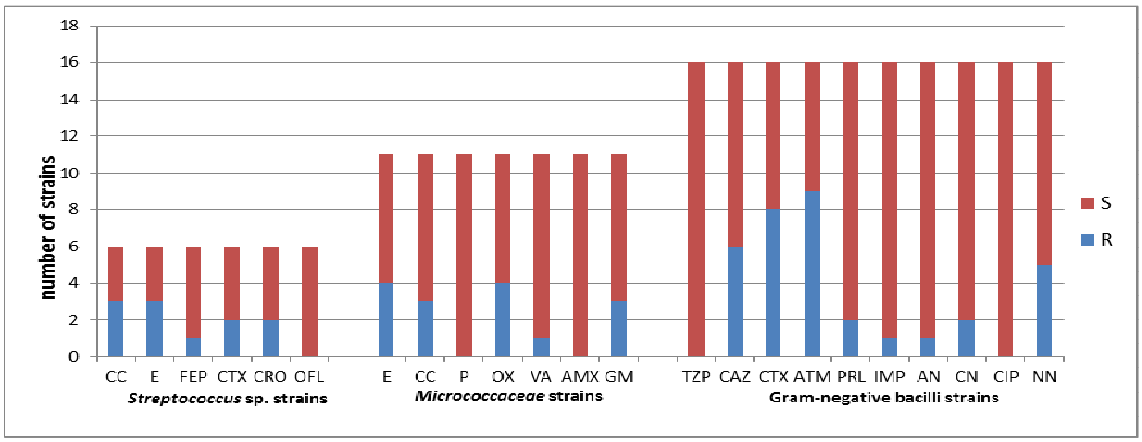

Fig. 2. The antibiotic susceptibility/resistance rates of the Gram-negative bacilli and Gram-positive cocci strains.

Taking into account that prevention of gingival inflammation might reduce the prevalence of mild to moderate periodontitis and that the mechanical plaque control procedures are insufficient in preventing gingival inflammation, the new field of perioceutics has emerged. Perioceutics are pharmacological agents specifically developed to aid in the management of periodontal diseases along with mechanical debridement.

Several antimicrobials have been tested as potential adjuvants to mechanical plaque control [19, 20, 21, 22]. Formulations containing chlorhexidine, mouth rinses containing essential oils and triclosan/copolymer dentifrices have been well documented for the antiplaque and antigingivitis clinical effects, due to their ability to penetrate the biofilm mass and kill the embedded bacteria, and to reach difficult-to-clean areas, such as the interproximal surfaces. These agents have also a positive track record of safety. The active progressive forms of periodontitis, which are often associated with the presence of specific bacterial species requires a systemic antibiotic therapy with tetracycline, penicillin, metronidazole or clindamycin. It has been recently proved that both the concentration in the crevicular liquid of systemically administered tetracycline, for therapeutic purposes, was more reduced than the plasma concentration and varied from one individual to another. On the other side, the periodontal lesions frequently contain a mixture of pathogenic bacteria and, as a result, a combined therapy is required for the treatment of these microbial infections (e.g., amoxicillin-metronidazole for the eradication of *A. actinomycetemcomitans* infections and of different anaerobic periodontal infections, and metronidazole-ciprofloxacin for the eradication of periodontal anaerobic bacteria, as well as of the Gram-negative enterobacteria and pseudomonades). Thus, the microbiological analysis and the antibiotic susceptibility testing should ideally be the base for the selection of an optimal antimicrobial therapy, due to the diversity of the periodontal microbiota with different antibiotic susceptibility patterns.

CONCLUSION

The obtained data reflect both a great taxonomic variety of microbial strains, isolated from the periodontal pocket or the gingival sulcus in patients with periodontal diseases, and significant levels of antimicrobial resistance to the antibiotics currently used in the treatment of the periodontal disease, reflecting the necessity to perform the microbiological analysis and the antibiotic susceptibility testing in order to select the optimal antimicrobial therapy.

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