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Review

Microbiological dental diseases: still waiting for advances in treatment

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Abstract

Diseases of microbial origin occur in different body sites including mouth and are described since the early times. Despite the risks and significant number of systemic diseases related to microbiological dental diseases (*eg.* endocarditis, respiratory infections, cardiovascular diseases, brain abscesses and oral and gastrointestinal cancers), the oral microbiota are not deeply explored or studied compared to others involved in diseases of global importance. In this article we briefly review the topics of oral microbiology including types of oral bacteria and the importance of microbial resistance for the effective oral treatments to remind their importance and the current demand for new treatments.

Keywords: Microbiology, oral bacteria, microbiota, dental, resistance.

INTRODUCTION

Mankind has been affected by diseases of microbial origin in different body sites including mouth since the early times. Therefore several molecules with antimicrobial profile have been identified over time using different technologies. Some of them are produced by living organisms, called antibiotics, other synthesized in the laboratory, named chemotherapics (Darveau et al., 2012).

The use of substances with antibiotic profile for the treatment of bacterial infections is not a new strategy, as in China, 2500 years ago; some of them have been used for treating plants against anthrax (Stingu et al., 2012). In 1928, the discovery of penicillin by Alexander Fleming was a turning point in the history of medicine despite of the detection of penicillin resistant microorganisms later on. Interestingly, in an interview for BBC radio in London in 1945, Alexander Flemming already predicted that the treatment would be disappointing if the penicillin was not properly used against microbes both in time and dose (Flemming, 1980, MLA, 2012).

Microbial resistance has become a global problem with medical, economic and public health importance with world different distribution (Ram et al., 2011). The inappropriate use of antimicrobials is a worldwide concern, and requires not only the constant development of new drugs, but also a process of re-education of the use of antibiotic therapy, against we should not ignore the oral infections (Barie, 2012).

The bacterial resistance is the acquired or inherited capacity that allows a microorganism to survive in the presence of some antibiotics. Thus, the resistant bacteria are those that do not suffer influence of the drug regardless of its concentration (Dane, 2011). Currently, special attention has been given to the increased resistance of various pathogens to conventional antibiotics.

In recent years, research studies in the pharmaceutical multinationals or university research centers tried to investigate new antibiotic drugs by: a) testing new drugs, similar or not to the existing ones to neutralize the mechanisms of bacterial resistance, b)

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developing antibiotics that act by means of new mechanisms of action, and c) identifying mechanisms that leads to therapeutic failure in treatment with antibiotics (Barie, 2012, Kang et al., 2012, Kirst, 2012).

In the search of molecules that can act on pathogenic microorganisms metabolism and structure, new drugs have been synthesized in a modified way to differentiate their action, also trying to reduce the toxic profile against the human body. The great advantage of antibiotics (molecule totally or partially produced by living organisms) and chemotherapies (synthesized in laboratories) with selective toxicity is that it allows the use for treating systemic infections. The mechanism of antimicrobials selectivity relies in some features such as the presence of the target only in the microorganism so host cells are not affected (Schmalstieg et al., 2012).

Several molecules with antibiotic profile can be identified in the literature, but few have medicinal value or commercial application. Some are commercially used for purposes other than the treatment of diseases, such as supplement in animal feed or as tools for pharmacological studies. Many antibiotics are also toxic to humans or may not have advantages over those already in use (Dane 2011). Thus the demand for the discovery of new more efficient and safer antibiotics prototypes of different origins (plant, animal or synthetic) for the treatment of infections caused by multidrug-resistant strains are still of great interest (WHO, 2012).

Microorganisms of dental importance

The pathogenicity of bacteria is determined by the development of mechanisms that increases infection capacity and help to avoid the host immune system (Geli et al., 2012). The pathogenic bacteria are classified into (1) primary, which cause infections in healthy individuals and (2) opportunistic, acting in individuals with compromised immune systems (Bowen et al., 2011).

The oral microbiota consists of a wide range of bacteria, fungi, viruses and even, sometimes, protozoa species (Figure 1). This diversity is due to the fact that the mouth is composed of different habitats that provide a range of different nutrients. In addition, in dental plaque, different gradients allow different parameters of ecological importance such as pH and oxygen tension, providing suitable conditions for coexisting microorganisms growth (Silva et al., 2012).

Most oral infectious processes are polymicrobial, and the anaerobic bacteria are the most frequent etiopathogenic agents (Wang et al., 2012). Among them we can cite :

- *Streptococcus mutans,* which is of great interest due to its role in the etiology of dental caries. Its name is due to the fact that these microorganisms may change their cocco morphology to short rods or cocci-like bacilli. *S. mutans* was originally isolated by Clarke (1924) from a human tooth caries and then obtained from endocarditis affected tissue with bacterial growth in the damaged heart valves, revealing the risk of co-infection of the tissue (Jurela et al., 2012).

- *Lactobacillus*: They are Gram-positive bacteria usually isolated from the oral cavity. They comprise, in general, less than 1% of the total cultivable microflora but in advanced carious lesions in enamel or on the root surface, the proportions and prevalence of these microorganisms increase. Several species have been identified producing lactate or ethyl lactate from glucose and the most common species reported are *L. casei, L. fermentum and L. acidophilus and L. salivarium, L. plantarum, L. brevis, L. cellobiosus and L. buchneri* (Kuvatanasuchati et al., 2012).

- *Eikenella corrodens*: Gram-negative facultative anaerobic bacilli that was isolated from a variety of oral infections, endocarditis and abscesses. It has been implicated in periodontal disease and their colonies characteristically dott the surface of the plate inoculation (Casarin et al., 2012).

- *Actinomycetemcomitans*: It was described by Klinger (1912) isolated as a coccus in lesions of human actinomycosis. This bacterium has been reclassified as *Actinobacillus actinomycetemcomitans* by Topley and Wilson (1929) and as *Haemophilus actinomycetemcomitans* by Potts et al. (1985). This species is associated with localized aggressive periodontitis and are currently classified as *Aggregatibacter actinomycetemcomitans* from the analysis of DNA sequence (Pejda et al., 2012).

Actinobacillus actinomycetemcomitans is implicated in the etiology of particularly aggressive forms of periodontal disease in adolescents (localized juvenile periodontitis). This strain has been described as being microaerophilic, facultative anaerobic, with reference for aerobic atmosphere enriched with 5-10% CO 2. *A. actinomycetemcomitans* is also an opportunistic pathogen isolated from of infective endocarditis cases, brain and subcutaneous abscesses, osteomyelitis, and periodontal disease (Marsh and Martin, 2005).

- *Fungi*: they are a small part of the oral microbiota mainly comprised by *Candida* yeast species. *Candida albicans* is by far the most common oral species, but others have been isolated including *C. glabrata, C. tropicalis, C. krusei, C. parapsilosis and C. guilliermondii, Rhodotorula* and as well as *Saccharomyces* spp. (Yoshioka et al., 2012).

Microbial resistance and dental antibiotic

Bacteria can be or become resistant to certain antibiotics. They may present two types of resistance:

a) Natural resistance, also called intrinsic, characterized by the absence of the structure or the binding site or pathway target of the antimicrobial. This

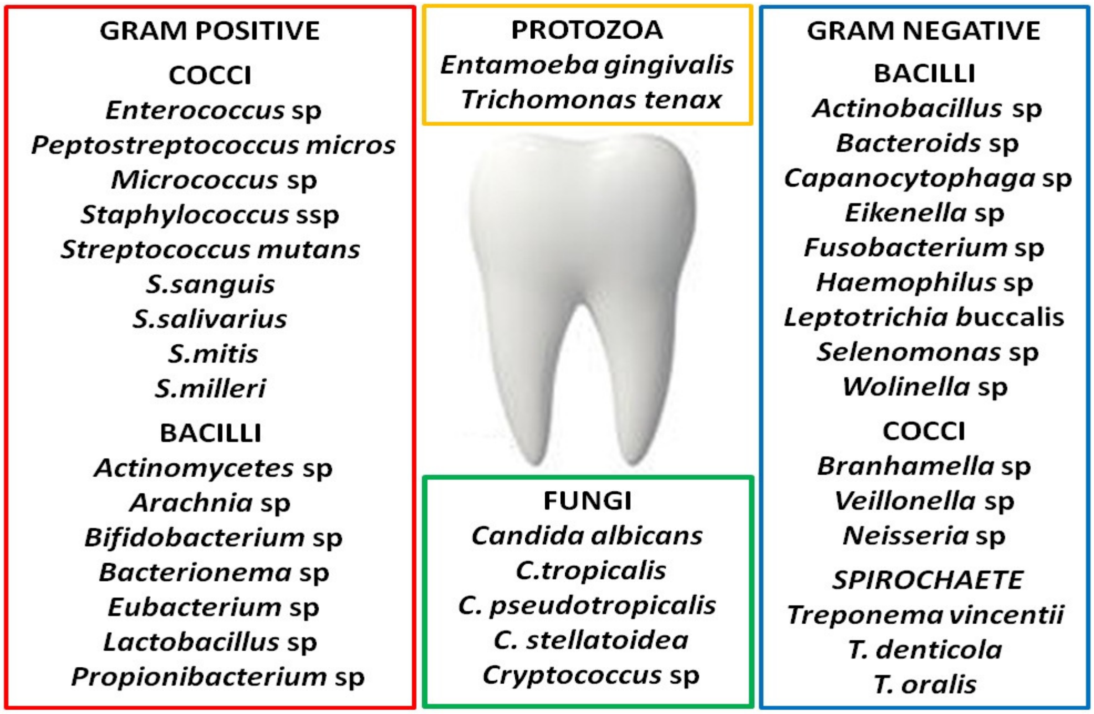

Figure 1. Some of the species present in oral microbiota including bacteria, fungi and even, sometimes, protozoa species.

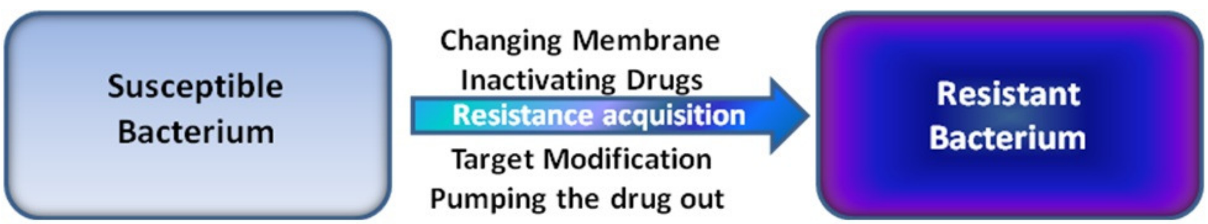

Figure 2. The main mechanisms of resistance.

resistance is typical of some species, and is transmitted vertically to the daughter cells with a hereditary character. For example, *Eikenella corrodens* is resistant to clindamycin and most *Fusobacterium* spp. are resistant to erythromycin (Roberts et al., 2010).

b) Acquired resistance: which emerge in the bacterium through spontaneous mutation, selection, or gene transfer (plasmids or transposons) (Otto 2012).

The main mechanisms of resistance, we may cite (Figure 2): (1) Drugproofing membrane: many Gram-negative bacteria resistant to penicillin G changed the characteristics of its own membrane making them impermeable to the drug. Changes in penicillin-binding proteins were also observed (Otto 2012). 2) Inactivation: many drugs are inactivated by enzymes produced by microorganisms (e.g. penicillinase, a Beta-lactamase cleaves the beta-lactam ring, inactivating penicillin). Other drugs may be inactivated due to changes catalyzed by these enzymes, such as the addition of chemical groups through reaction of phosphorylation or acetylation of antibiotics (Freitas, 1989).(3) Modification of the enzyme or the target structure including: (i) changes in the ribosome (23S rRNA), as in the case of resistance to erythromycin and chloramphenicol (ii) the enzyme, as in antibiotics that act on the metabolism, or (iii) the use of alternative metabolic pathways (Freitas, 1989). (4) pumping to the extracellular medium, efflux of drug out of the bacterial cell, such as the tetracycline resistance in *Enterobacteria* (Otto 2012).

The significant increase in resistance to multiple antibiotics by the major human pathogens is of great concern (Bowen et al., 2011). From this perspective, the situation is disturbing by the drugs misuse and alarming considering the antibiotics use. Campaigns have been performed worldwide to controll the spread of antimicrobial resistance, through the knowledge of microbial resistance profile and adoption of preventive measures and control.

The antibiotics prescribed by dentists are often used by other health professionals to treat other infections unrelated to the oral cavity. For example, metronidazole, prescribed in some cases of aggressive periodontitis, is usually indicated as an adjunct in the treatment of giardiasis, amoebiasis and vaginitis. This may result in future natural selection for antimicrobial resistance (Barie, 2012).

In periodontology, which studies all tissues that are around the tooth, determining the patients sensitivity is recommended to determine the correct antibiotic to be used. However, in the absence of time to perform the appropriate and aggressive treatment against the presenting pathologies, the prescription of antibiotics is commonly performed without careful research. The professional mostly select the option that rapid relief the patient symptoms instead of performing a first bacterial sample collection and wait for the result to diagnose and treat the infection in a rational and best way (Casarin et al., 2012).

In the last decade, the most prescribed antibiotics in Periodontics such as penicillin and tetracycline, were frequently inefficient due to the significant increase of the antimicrobial resistance (Casarin et al., 2012). The case also includes the increasing elimination of susceptible bacteria and the emergence of resistant bacteria. Thus, it becomes clear how essential is the use of antibiograms to obtain the antimicrobial susceptibility profile and do not undertake to prescribe at random, choosing broad-spectrum antibiotics. Failing to perform the sensitivity, it is still recommended the prescription of antibiotics with proven effectiveness in the literature, following the recommended dosage (Kuvatanasuchati et al., 2012).

In the field of Periodontics, there is an increasing resistance to antibiotics used in periodontal treatment that still is ignored (Kuvatanasuchati et al., 2012). Two recent studies described the existence of resistance in the periodontal microbiota, while 300 strains were collected from patients with "Adult Periodontitis" and determined the susceptibility against seven antibiotics. The studies showed that the significant percentage of increased tetracycline resistance (from 18 to 31%) and amoxicillin (13 to 31%) for some subgingival bacteria (Casarin et al., 2012).

Testing antimicrobial profile: the search for new therapeutic prototypes

Sensitivity tests are appropriate for identifying the microorganisms susceptibility profile, when it is impractical to predict the sensitivity of this organism, even knowing their identification. In fact these tests are given more frequently when it is believed that the causative organism belongs to a species capable of offering resistance to antimicrobial agents commonly used.

Various laboratory methods can be used to measure the *in vitro* sensitivity of bacteria to antimicrobial agents. In many clinical microbiology laboratories, the disc-agar diffusion method are used to test the most common pathogens, allowing rapidly bacteria growing (CLSI, 2003).

In studies to identify new antimicrobial prototypes, classified strains have been used as templates or control as they can help to identify the initial profile of these compounds. The microbial classification seeks to establish a logical organization of the microorganisms based on their similarities and relationships (Marsh and Martin, 2005). The result of classification is the generation of internationally approved species. A species represents a collection of strains that share many common features, which differ considerably from other strains. Once a species has been recognized, then the model is nominated a strain that has the properties of representative species. The strains maintained in a model are national collections such as the American Type Culture Collection (ATCC) and the National Collection of Type Cultures (NCTC), which is located in the UK. These strains are commonly used in studies of new compounds, but still requires the use of strains of hospital origin and/or dentist to check the action of these compounds against multiresistant strains existing in the environment or community hospital.

The minimum inhibitory concentration (MIC) is determined by a sequence of drug decreasing concentrations in a broth, which is then inoculated with the bacteria. This assay identifies the first point that shows growth, and the previous point, which defines the MIC (Tortora et al., 2005). Each group and species of microorganisms has an individual susceptibility to the action of antibiotics known as MIC, so there is inhibition of bacterial growth. The MIC for each microorganism involved in the microbiology of infection is studied "in vitro", known and frequently changed due to the resistance mechanisms developed by bacteria (Silva et al., 2012).

Final Considerations

The human mouth can maintain approximately 500 species of cultivable and uncultivable bacteria (Paster et al., 2001, Al-hebshi Al-HEBSHI et al., 2006). Over 100 species may be present in the oral cavity as commensals. Among these guests, some are opportunistic microorganisms implicated in the pathogenesis of gingivitis, periodontal disease and tooth decay (Rams et al., 2011). For example, *Porphyromonas gingivalis* and *Forsythia tannerella* are associated with periodontitis (Silva et al., 2012) whereas *Streptococcus* *mutans, S. sobrinus* (NAPIMOGA et al., 2005), non-mutans streptococci, *Actinomyces spp.* and *Bifidobacterium spp.* are related to caries (Beighton, 2005).

The role of these microorganisms has been established not only in the etiology of periodontitis and dental caries, which are among the most prevalent diseases in the world, (Stingu et al.,2012), but also in a significant number of systemic diseases such as endocarditis (Barrau et al., 2004; Silva et al., 2012), respiratory infections (Mojon and Bourbeau, 2003, Scannapieco et al., 2000), cardiovascular disease (Okuda et al., 2004, Meyerdh, 1998), brain abscesses (Corson et al., 2001), oral and gastrointestinal cancers (Ahn et al., 2012) .

Oral bacteria resistant to penicillin, metronidazole, tetracycline and macrolides have been reported in the literature by researchers from different countries also identified outside the oral cavity (Roberts, 2010). In an attempt to deal with the global problem of antibiotic resistance, a number of strategies such as reducing the use of antibiotics and new antibiotic options to either synthetic or natural origin has been proposed in the literature, but with restrictions. Thus, facing the major problem currently with the development of multi-resistant microorganisms to antibiotic drugs, we need new options in addition to changes in the way of professionals use the antibiotics to minimize this problem. Thus, new antibiotics more effective and safer should be developed to combat these infections in the general population and also undertake and especially immunocompromised patients.

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REFERENCES

Ahn J, Chen CY, Hayes RB (2012) Oral microbiome and oral and gastrointestinal cancer risk. Cancer Causes Control. Mar;23(3):399-404.

Al-hebshi N, Al-haroni M, Skaug N (2006). In vitro antimicrobial and resistance-modifying activities of aqueous crude khat extracts against oral microorganisms. Archives of oral biology. Mar;51(3):183-8.

Alvarez ML, Zarelli VE, Pappano NB, Debattista NB (2004). Bacteriostatic action of synthetic polyhydroxylated chalcones against *Escherichia coli*. Biocell Apr; 28(1):31-4.

Andrighetti-Fröhner CR, de Oliveira KN, Gaspar-Silva D, Pacheco LK, Joussef AC, Steindel M, Simões CM, de Souza AM, Magalhaes UO, Afonso IF, Rodrigues CR, Nunes RJ, Castro HC (2009). Synthesis, biological evaluation and SAR of sulfonamide 4-methoxychalcone derivatives with potential antileishmanial activity. Eur. J. Med. Chem. Feb; 44(2):755-63.

Barie PS (2012). Multidrug-resistant organisms and antibiotic management. Surg. Clin. North Am. Apr; 92(2):345-91.

Barrau K, Boulamery A, Imbert G, Casalta JP, Habib G, Messana T, Bonnet JL, Rubinstein E, Raoult D (2004). Causative organisms of infective endocarditis according to host status. Clin. Microbiol. Infect. Apr; 10(4):302-8.

Beighton D. The complex oral microflora of high-risk individuals and groups and its role in the caries process. Community Dent Oral Epidemiol 2005. Aug;33(4):248-55.

Bowen WH, Koo H (2011). Biology of *Streptococcus mutans*-derived glucosyltransferases: role in extracellular matrix formation of cariogenic biofilms. Caries Res. 45(1):69-86.

Casarin RC, Barbagallo A, Meulman T, Santos VR, Sallum EA, Nociti FH, Duarte PM, Casati MZ, Gonçalves RB (2012). Subgingival biodiversity in subjects with uncontrolled type-2 diabetes and chronic periodontitis. J Periodontal Res. Jul 4. doi: 10.1111/j.1600-0765.2012.01498.x.

CLSI/NCCLS - Clinical and Laboratory Standards Institute (2002) - Standard M27-A2-Reference Method Of Testing In Broth Dilution To determine the Yeasts sensitivity of antifungal therapy: Approved Standard - Second Edition. Available in http://www.sbac.org.br/pt/pdfs/biblioteca /clsi_OPAS1M27-A2.pdf.

CLSI/NCCLS - Clinical and Laboratory Standards Institute (2004) - Norma M44-A— Method For Antifungal Disk Diffusion Susceptibility Testing Of Yeasts. Available in http://www.springer.com/cda/content/document/cda_downloaddocument/9781588298478-c1.pdf?SGWID=0-0-45-1266649-p173729607.

CLSI/NCCLS - Clinical and Laboratory Standards Institute (2005) Standard M2-A8-performance standards for antimicrobial susceptibility tests.15º Suplement Informative 2005.

Corson MA, Postlethwaite KP, Seymour RA (2001). Are dental infections a cause of brain abscess? Case report and review of the literature. Oral Dis. Jan; 7(1):61-5.

Dane A (2011). Active controlled studies in antibiotic drug development. Pharm Stat. Sep-Oct;10(5):454-60.

Darveau RP, Hajishengallis G, Curtis MA (2012). Porphyromonas gingivalis as a Potential Community Activist for Disease. J Dent Res. 0022034512453589, first published on July 6.

Fleming, A (1980). "On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenzae. 1929". Rev. Infect. Dis. 2 (1): 129–39

Geli P, Laxminarayan R, Dunne M, Smith DL (2012) " One-size-fits-all"? Optimizing treatment duration for bacterial infections. PLoS One.7(1):e29838.

Jurela A, Repic D, Pejda S, Juric H, Vidakovic R, Matic I, Bosnjak A (2012). The effect of two different bracket types on the salivary levels of *S mutans* and *S sobrinus* in the early phase of orthodontic treatment. Angle Orthod. 22765642.

Kang SJ, Kim DH, Mishig-Ochir T, Lee BJ (2012) Antimicrobial peptides: their physicochemical properties and therapeutic application. Arch. Pharm. Res. Mar; 35(3):409-13.

Kirst HA (2012) Recent derivatives from smaller classes of fermentation-derived antibacterials. Expert Opin Ther Pat. Jan; 22(1):15-35.

Kuvatanasuchati J, Chamroensaksri N, Tanasupawat S (2012). Phenotypic and genotypic characterization of Thai oral streptococci, lactobacilli and pediococci. Trop Biomed. Jun; 29(2):254-64.

Liu XL, Xu YJ, Go ML (2008). Functionalized chalcones with basic functionalities have antibacterial activity against drug sensitive Staphylococcus aureus. Eur. J. Med. Chem. Aug; 43(8):1681-7.

Lunardi F, Guzela M, Rodrigues AT, Corrêa R, Eger-Mangrich I, Steindel M, Grisard EC, Assreuy J, Calixto JB, Santos AR (2003). Trypanocidal and leishmanicidal properties of substitution-containing chalcones. Antimicrob Agents Chemother. Apr; 47(4):1449-51.

Marsh P. & Martin MV (2005). Microbiologia Oral. 4ª Edição. São Paulo: Ed. Livraria Santos.

Mojon P, Bourbeau J (2003). Respiratory infection: how important is oral health? Curr. Opin. Pulm. Med. May; 9(3):166-70.

MLA (2012). "Sir Alexander Fleming - Biography". Nobelprize.org. Accessed in 17 Jul 2012. Available in http://www.nobelprize.org/nobel_prizes/medicine/laureates/1945/fleming-bio.html

Napimoga MH, Höfling JF, Klein MI, Kamiya RU, Gonçalves RB (2005).Transmission, diversity and virulence factors of *Streptococcus mutans* genotypes. J Oral Sci. Jun;47(2):59-64.

NCCLS. (2003) Performance standards for antimicrobial disk susceptibility tests. Approved Standard-Eighth Edition. NCCLS Document M2-A8 2003.

Okuda K, Kato T, Ishihara K (2004).Involvement of periodontopathic biofilm in vascular diseases. Oral Dis. Jan; 10(1):5-12.
Opletalová V (2000).Chalcones and their heterocyclic analogs as potential therapeutic agents in bacterial diseases. Ceska Slov. Farm. Nov; 49(6):278-84.
Opletalová V, Sedivý D (1999). Chalcones and their heterocyclic analogs as potential antifungal chemotherapeutic agents. Ceska Slov. Farm. Nov; 48(6):252-5.
Opletalová V, Jahodár L, Jun D, Opletal L (2003). Chalcones (1,3-diarylpropen-1-ones) and their analogs as potential therapeutic agents in cardiovascular system diseases. Ceska Slov. Farm. Jan; 52(1):12-9.
Otto M (2012). MRSA virulence and spread. Cell Microbiol. j.1462-5822.2012.01832.
Parisotto TM, King WF, Duque C, Mattos-Graner RO, Steiner-Oliveira C, Nobre-Dos-Santos M, Smith DJ (2011). Immunological and microbiologic changes during caries development in young children.Caries Res. 45(4):377-85.
Paster BJ, Boches SK, Galvin JL, Ericson RE, Lau CN, Levanos VA, Sahasrabudhe A, Dewhirst FE (2001). Bacterial diversity in human subgingival plaque. J. Bacteriol Jun;183(12):3770-83.
Pejda S, Varga ML, Milosevic SA, Mestrovic S, Slaj M, Repic D, Bosnjak A (2012). Clinical and microbiological parameters in patients with self-ligating and conventional brackets during early phase of orthodontic treatment. Angle Orthod. 22765511.
Rams TE, Dujardin S, Sautter JD, Degener JE, van Winkelhoff AJ (2011). Spiramycin resistance in human periodontitis microbiota. Anaerobe. Aug; 17(4):201-5.
Roberts AP, Mullany P (2010). Oral biofilms: a reservoir of transferable, bacterial, antimicrobial resistance.Expert Rev Anti Infect Ther. Dec; 8(12):1441-50.
Scannapieco FA, Wang B, Shiau HJ (2001). Oral bacteria and respiratory infection: effects on respiratory pathogen adhesion and persistence. Drug Resist. Update Dec; 6(1):78-86.
Schmalstieg AM, Srivastava S, Belkaya S, Deshpande D, Meek C, Leff R, van Oers NS, Gumbo T (2012). The Antibiotic-Resistance Arrow of Time: Efflux Pump Induction is a General First Step in the Evolution of Mycobacterial Drug-Resistance. Antimicrob Agents Chemother. 22751536.
Silva MS, Brandão DO, Chaves TP, Formiga Filho AL, Costa EM, Santos VL, Medeiros AC (2012). Study bioprospecting of medicinal plant extracts of the semiarid northeast: contribution to the control of oral microorganisms. Evid Based Complement Alternat Med. 2012;2012:681207.
Stingu CS, Schaumann R, Jentsch H, Eschrich K, Brosteanu O, Rodloff AC (2012). Association of periodontitis with increased colonization by *Prevotella nigrescens.* J. Investig. Clin. Dent. j.2041-1626.2012.001292.
Tanner AC, Paster BJ, Lu SC, Kanasi E, Kent R Jr, Van Dyke T, Sonis ST (2006). Subgingival and tongue microbiota during early periodontitis. J Dent Res. Apr; 85(4):318-23.
Wang J, Jiang Y, Chen W, Zhu C, Liang J (2012). Bacterial Flora and Extraradicular Biofilm Associated with the Apical Segment of Teeth with Post-treatment Apical Periodontitis. J Endod. ul; 38(7):954-9.
Washington J (2009). Principles of diagnosis. Available in: http://gsbs.utmb.edu/microbook/ ch010.htm Accessed in January 2009.
World Health Organization (WHO). Acessed in December 2008. Available in http://www.who. int/en.
Yoshioka H, Kuwa S, Nakamura K, Mataga I (2012). Virulence of *Candida dubliniensis* Using a Murine Experimental Oral Candiddiasis Model in Comparison with *Candida albicans*. Med Mycol J. 2012; 53(2).