

Antibiotic Susceptibility of Periodontal Microorganisms

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Abstract: This literature reviewed antibiotic susceptibility of periodontal microorganisms. Information about disease causing periodontal pathogens, concepts of bacterial etiology of periodontal diseases, various antibiotic susceptibility tests and antibiotic susceptibility of periodontal organisms and its mechanism of action was demonstrated. Periodontitis is a bacterial disease that can be treated with systemic antibiotics, to provide the patient with an appropriate antibiotic therapy, antibiotic susceptibility testing is performed to guide the clinician in decision making. Susceptibility testing has long been recognized as an important tool in determining the effect of an antimicrobial agent on pathogenic bacteria.

Keywords: Periodontal pathogens, periodontal disease, antibiotic susceptibility, antibiotic therapy.

INTRODUCTION

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation and recession [1]. The clinical signs of periodontitis include changes in the morphology of gingival tissues, bleeding on probing as well as periodontal pocket formation. This provides an ideal environment for the growth and proliferation of anaerobic pathogenic bacteria [2]. Microbial dental plaque has long been recognized as the initiator of periodontal disease [3].

The clinically most important cultivable periodontal bacterial species occurring at sites of periodontal disease activity are *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia* [4].

Treatment of periodontitis involves reduction of the total periodontal bacteria load by supragingival and subgingival mechanical debridement. However, bacterial deposits in the depth of the pockets are often difficult to remove and may be responsible for a poor treatment outcome [4]. Therefore, antibiotic treatment can be indicated for certain cases. Antibiotic treatment of periodontitis aims at eradicating or controlling specific pathogens [5].

Several systemic antimicrobials as an adjunct to scaling and root planning have proven effective, such as metronidazole and the combination of metronidazole and amoxicillin. To provide the patient with an appropriate antibiotic therapy, it is critical to know the susceptibility profiles of clinically relevant oral pathogens [4].

Susceptibility testing has long been recognized as an important tool in determining the effect of an antimicrobial agent on pathogenic bacteria [6]. Generically, in order for an antimicrobial agent to be useful, the pathogen must be known, it must be

susceptible to the drug, it should not readily develop resistance to the drug, and it must be exposed to effective concentrations of the drug for an adequate length of time. Also, the drug should demonstrate little or no side effects. Although important progress has been achieved through recent scientific efforts, more information is needed in order to optimize the effectiveness of anti-microbial therapy in Periodontics [5].

The periodontal pathogens were considered to be susceptible, if the concentration of the antibiotic agent actually achievable in gingival crevicular fluid following recommended dosage was higher than the minimal inhibitory concentration. The susceptibility of subgingival microorganisms to a certain antibiotic depends on the minimal inhibitory concentration required for this microorganism on the one hand and on the concentration of the drug achievable in the infected tissues on the other hand [7]. Antibiotic susceptibility of the test bacteria to amoxicillin, clindamycin, azithromycin, metronidazole and tetracycline was determined by Etest [4].

Ideally, for antimicrobial agents to be used effectively in periodontal therapy, it is equally important for the clinician to know which antibiotics are effective against the etiologic agents likely to be present, the dosages necessary to achieve and maintain therapeutic levels and the effect that the antibiotic will have on the entire microbiota associated with the diseased site [6]. Oral pathogens can be disseminated from the oral cavity to other body sites, causing distant infections such as brain and lung abscesses. Treatment of these infections also requires knowledge of the antibiotic susceptibility profiles of oral anaerobes [4].

Hence an attempt is made to review the antibiotic susceptibility of various periodontal microorganisms.

DISEASE CAUSING PERIODONTAL PATHOGENS [8]

The periodontal pathogens that have been implicated in disease processes include porphyromonas gingivalis, Actinobacillus actinomycetamcomitans, Treponema denticola, Bacteroides forsythia, fusobacterium nucleatum, prevotella intermedia, campylobacter rectus, peptostreptococcus micros and Eikenella corrodens.

Among the pathogens, the prominent microorganisms in localized aggressive periodontitis includes Actinobacillus actinomycetamcomitans, p. gingivalis, E. corrodens, c. rectus, F. nucleatum, Capnocytophaga spp and spirochetes and bacterial etiology in chronic periodontitis includes p. gingivalis, B. forsythia, p. intermedia, c. rectus, E. corrodens, F. nucleatum, A.actinomycetamcomitans, p. micros and T. denticola.

Concepts of bacterial aetiology of periodontal diseases [9]

Accumulation of bacteria on hard oral surfaces is considered the primary cause of gingivitis periodontitis. Removal of plaque leads to the disappearance of the clinical signs of gingival inflammation [16, 17]. Therefore, regular mechanical removal of all bacterial plaque from nonshedding oral surfaces is considered the primary means to prevent and stop the progression of periodontal disease.

Several hundred bacterial taxa have been identified in samples from human plaque but only relatively few are regularly found in high numbers in periodontitis lesions. Possible pathogens have been suggested among these organisms based on their animal pathogenicity and the demonstration of virulence factors. Certain species have attracted particular attention because longitudinal and retrospective studies indicated an increased risk of periodontal breakdown in positive sites and because results of treatment were better if the organisms could not be detected any more at follow-up [18-22].

Evaluation of antimicrobial agents for periodontal therapy [9]

In the large range of antimicrobial agents, a limited number have been tested thoroughly for systemic use in periodontal therapy. The choice of antibiotics was initially based on empirical evidence and included mainly penicillins.

Among the penicillin's, amoxicillin has been favored for treatment of periodontal diseases because it possesses considerable activity against periodontal pathogens at levels that occur in gingival fluid, with the exception of peptostreptococcus. Tetracycline-HCl became popular in the 1970s due to its broad spectrum antimicrobial activity and low toxicity. Clindamycin, tetracyclines, erythromycin have a broad spectrum of activity and are bactericidal. Metronidazole is known to convert into a reactive reduced form and affects specifically the obligately anaerobic part of the flora.

MICROBIAL TESTING [9]

Microbiology testing should be performed after completion of conventional mechanical therapy to assess the need for additional antibiotic treatment. Microbiology testing may be repeated at 1 to 3 months after the antimicrobial therapy to verify the elimination or marked suppression of the pathogens and to screen for possible superinfecting organisms [12].

ANTIBIOTIC SUSCEPTIBILITY [10]

The term susceptible means that the microorganism is inhibited by a concentration of antimicrobial agent that can be attained in blood with the normally recommended dose of the antimicrobial agent and implies that an infection caused by this microorganism may be appropriately treated with the antimicrobial agent. Antibiotic susceptibility has become a very essential step for properly treating infectious diseases and monitoring antimicrobial resistance in various pathogens. Oral bacteria are susceptible to many antibiotics.

Unfortunately there is no single antibiotic at concentration achieved in body fluids that inhibits all putative periodontal pathogens. Antibiotic susceptibility testing is strongly recommended, such a test provides valuable information regarding the periodontal pathogens present and their predicted response to different antibiotics, and it can be a decisive factor in the selection and clinical success of prescribed anti-infective drug regimens. Criteria of susceptibility to a particular antibiotic was based on whether or not a strain was inhibited by concentrations equivalent to those achieved and maintained either in the gingival crevicular fluid, when known or in the blood following recommended oral dosages.

SUSCEPTIBILITY TESTS [11]

Different techniques have been used to establish the antimicrobial susceptibility of periodontal pathogens.

- Dilution tests
- Diffusion tests
- E-test

DILUTION TESTS

A quantitative method for measuring the susceptibility of an antimicrobial agent is the minimal inhibitory concentration (MIC) test, which determines the lowest concentration of an antimicrobial agent that will inhibit visible growth in vitro. In an MIC test, a series of dilutions of an antimicrobial agent is prepared in broth and inoculated with the standard inoculum size of the test organism. After overnight incubation at an appropriate temperature (usually 37°C), the highest dilution in which there is no visible growth is recorded as the MIC.

The optimum dosing strategy for a concentration dependent drug may be a single dose that results in a peak- serum concentration 8 to 10 times above the MIC of a pathogen. Doubling the concentration of a concentration- dependent drug in vitro will kill the same number of organisms in half the time.

The MIC test can be used to determine an antimicrobial agent's minimal bactericidal concentration (MBC), which is the lowest concentration that kills the inoculated bacteria. The MBC is determined by sub culturing aliquots of each dilution onto fresh medium without the antimicrobial agent and incubating overnight. The antimicrobial agent is considered bactericidal when the MBC is equal to or less than fourfold higher than the MIC. In general, dilution tests are recommended for slow-growing organisms, including strictly anaerobic oral bacteria.

DIFFUSION TESTS

Diffusion tests are performed with filter paper disks or tablets containing the antimicrobial agent. A plate is seeded over the entire surface with a bacterial isolate and disks are placed on the surface of the agar plate. After an appropriate period of incubation, the plate is examined for zones of growth inhibition around each antibiotic disk. The amount of antibiotic in each disk is related to achievable zones for different antibiotic disks vary. The larger the inhibition zone, the more susceptible the isolate.

These inhibition zones are compared with the inhibition zone of a reference organism, and the test isolate's susceptibility is expressed as either susceptible (S), intermediate (I), or resistant (R). An 'I' indicated that the antibiotic is less susceptible than the norm and that it may not be the first drug of choice or that the doses should be increased.

The size of the inhibition zone is determined by the inoculum size of the organism, the antibiotic concentration in the disk, and the incubation time. For a number of bacterial pathogens, the relationship between inhibition zones and the MIC has been established. The MIC of an unknown isolate can be read from a regression line after the inhibition zone has been determined. This technique, called the Kirby-Bauer method, can only be used when the standard techniques are strictly followed.

E TEST

Epsilon meter test method is a new technique for antimicrobial susceptibility testing for periodontal pathogens. This test has been developed to provide a direct quantification of antimicrobial susceptibility of microorganisms. Strips containing different concentration of an anti-microbial agent which can be placed directly on the agar plate. After 7 days of incubation in anaerobic condition the concentration of drug that inhibits 90% of bacterial growth in-vitro minimal inhibitory concentration could be easily read from the strip.

Antibiotic susceptibility of periodontal organisms and its mechanism of action [8-11]

Metronidazole

It is synthetic nitro imidazole patterned after a naturally occurring anti parasitic substance that was isolated from *Streptomyces* species in 1955. It is bactericidal antimicrobial, most effective against obligate anaerobic gram-negative bacteria and also active against obligate anaerobic cocci.

Disrupts DNA synthesis leading to cell death. Selectively kills bacteria associated with periodontal disease, due to its ability to kill anaerobic bacteria has led to its use in periodontal therapy. However, metronidazole may not affect some strains of *P. micros.* *A. actinomycetamcomitans*, *E. Corrodens* and *Capnocytophaga* are facultatively anaerobic bacteria which demonstrate low in vitro metronidazole susceptibility. It penetrates all bacterial cells equally well. Susceptible bacteria includes

- *Fusobacterium*, *Bacteroides*.
- *Peptostreptococcus*.
- *Treponema*, *campylobacter* & *Veillonella*.

Abu-Fanas *et al.*, [23] tested the susceptibility to different antibiotics of 61 Gram- negative rods isolated from deep periodontal pockets, including *P.gingivalis*, *C. gracilis*. *A. actinomycetemcomitans*, which is not an obligate anaerobe is inherently resistant to metronidazole. Feres *et al.*, [24], in a study with 20 chronic periodontitis, observed that the most prevalent resistant species in the metronidazole- treated group were *A. naeslundii*, *s. oralis*, *Actinomyces odontolyticus* and *Sanguis*. Due to the relatively lower rates of bacterial resistance to metronidazole and to its high activity against the Gram-negative anaerobic

bacilli, which are associated with periodontal diseases, this seems to be a promising drug for treating periodontitis.

TETRACYCLINES

They are broad spectrum antibiotics effective against aerobic and anaerobic Gram-positive and Gram-negative bacteria. They have been widely used in the treatment of periodontal diseases. The most commonly used drugs are doxycycline, minocycline. They inhibit bacterial protein synthesis by binding to the 30S bacterial ribosome and preventing the access of amino acyl t RNA to the acceptor site on the m RNA-ribosome complex. This interrupts the formation of initiation complex required for amino acid protein synthesis.

Tetracycline exhibited poor activity against the oral pathogenic bacteria. Conversely, the new tetracycline derivatives, minocycline and doxycycline with a break-point of 8/ mL, expresses very pronounced antimicrobial activity and could inhibit over 95% of the isolated species, including *P. gingivalis*, *E. corrodens*, *P. intermedia*, *F. nucleatum* and *P. micra*.

Abu Fanas *et al.*, reported an increase in the MIC values of tetracycline for subgingival isolates of *P. gingivalis*, *P. intermedia* and *F. nucleatum* in a group of subjects that received 250 mg tetracycline 4 times daily for 2 weeks. Kornman and Karl evaluated 10 patients with periodontal disease who had been taking 250 mg of tetracycline daily for 2-7 years; they reported that up to 77 % of the cultivable subgingival microbiota was resistant to tetracycline at 1 -g/ml and Gram-negative rods constituted 58% of the microbiota. Rodrigues *et al.*, [25] longitudinally evaluated the tetracycline resistance patterns of the subgingival microbiota of periodontitis subjects treated with systemic or local antibiotic plus scaling and root planning. The predominant tetracycline-resistant species included *P. intermedia*, *Veillonella parvula*, *P. micra* and *A. actinomycetemcomitans*. Tetracycline-HCL and tetracycline derivatives demonstrate high in vitro activity against most periodontal pathogens, including *A. actinomycetemcomitans*, *B. gingivalis* and *B. intermedius*.

PENICILLINS

Penicillin G, penicillin V and the penicillinase-resistant penicillin derivatives are considered narrow-spectrum antibiotics because at usual doses they mainly affect gram-positive aerobic and facultative microorganisms, some anaerobes and spirochetes and inhibit bacterial cell-wall synthesis. The remaining penicillin derivatives exhibit an extended spectrum of antibacterial activity including many gram negative bacilli.

The penicillins are effective against many anaerobic microorganisms, including most of the

anaerobes found in the oral cavity that have been associated with dental and periodontal diseases, both acute and chronic : fusobacterium, peptostreptococcus, spirochetes, *Actinomyces*, *Eubacteria*, *campylobacter*, *prevotella*, *Bacteroides*, *porphyromonas* and *Capnocytophaga*. Certain periodontal infections can be caused by both gram-negative and gram-negative organisms for which an antimicrobial agent with a more extended antibacterial spectrum than penicillin V might be the agent of choice.

Amoxicillin is a bactericidal drug that inhibits the synthesis of bacterial cell walls and results in cellular disruption due to high osmotic pressure, it is effective against some subgingival bacterial species such as *P. micra* and *A. actinomycetemcomitans*, it also has enhanced tissue penetration and good activity against gram negatives. Penicillins as well as other beta-lactam antibiotics are bactericidal drugs, they kill susceptible bacteria by inhibiting the synthesis of the bacterial peptidoglycan cell wall. A very small proportion of the subgingival microbiota is resistant to penicillins.

Sutter *et al.*, [26] reported that among 193 assorted oral isolates, only 2% were resistant to penicillin G at a concentration of 2U/ML. Kinder *et al.*, [27] also showed that less than 3% of the subgingival micro-organisms are associated with adult periodontitis were resistant to penicillin. The predominant resistant subgingival isolates recovered in this investigation consisted of *Bacteroides*, *Veillonella*, *Haemophilus*, *Eikenella*, *Capnocytophaga* and *streptococcus* species.

When comparing the bactericidal activity, amoxicillin/clavulanate was more effective than amoxicillin alone in susceptible strains of *P. intermedia*, *P. micra* and *Eikenella corrodens*. Both the MIC 50 and MIC 90 of amoxicillin/ clavulanate were 2 to 4 times lower than those of amoxicillin and ampicillin, respectively, but there were some strains of *E. corrodens*, *F. nucleatum* and *P. micra* that were resistant to amoxicillin/ clavulanate.

CLINDAMYCIN

Clindamycin is a semisynthetic derivative of lincomycin, produced by exchange of the hydroxyl group with a chlorine atom at C₇ of the lincomycin molecule. The mechanism of action of clindamycin is similar to that of erythromycin and chloramphenicol. Clindamycin binds to the 50 S subunit of bacterial ribosomes, there by inhibiting protein synthesis. At low concentrations, clindamycin exhibits bacteriostatic activity, however, bactericidal action against a number of susceptible microorganisms occur at concentrations readily achieved in vivo.

The antibacterial spectrum of clindamycin corresponds to that of erythromycin, with the following exceptions. Clindamycin has better activity against

most strains of *S.aureus*, it is more active against most gram-positive and gram-negative anaerobes.

P. gingivalis is susceptible to clindamycin. The drug should be considered a third choice for treatment of oral dental infections caused by susceptible gram-positive cocci or gram-positive or gram-negative anaerobes, useful when penicillins and macrolides cannot be used or are ineffective.

Clindamycin displayed a relatively high level of *in vitro* antimicrobial activity against subgingival *S. constellatus* and *S. intermedius*. Less effective against facultative pathogens (*A. actinomycetemcomitans* and *Eikenella*).

CIPROFLOXACIN

Ciprofloxacin is active against enteric rods, pseudomonads, staphylococci, *Actinobacillus actinomycetemcomitans* and other periodontal microorganisms. Since it demonstrates minimal effect on streptococcus species, which are associated with periodontal health, its administration may facilitate the establishment of microflora associated with periodontal health. At present, ciprofloxacin is the only antibiotic in periodontal therapy to which all strains of *A. actinomycetemcomitans* are susceptible.

MACROLIDE

The first described macrolide was erythromycin that has been available for clinical use. Azithromycin and clarithromycin are semi-synthetic macrolides similar in structure to erythromycin. Macrolides contain a lactone ring to which sugars are attached which binds to bacterial ribosomes and disrupts protein synthesis.

Azithromycin is found to be effective against anaerobes and gram-negative bacilli, it is also highly active against many periodontal pathogens although some *Enterococcus*, *staphylococcus*, *Eikenella corrodens*, *fusobacterium nucleatum* and *peptostreptococcus* strains may exhibit resistance. Erythromycin is effective against gram positive bacteria and spirochetes, but not against most gram-negative organisms. It is often used as an alternative to penicillin for those patients who are allergic to this antibiotic. Clarithromycin is two to four times more active than erythromycin against most streptococci and staphylococci whereas azithromycin is two to four times less active than erythromycin against these bacteria.

COMBINATION THERAPY

Periodontal infections contain a wide diversity of bacteria; hence, no single antibiotic can be effective against all putative pathogens. This mixed infection can include a variety of aerobic, microaerophilic and anaerobic bacteria, both gram negative and gram

positive. This scenario makes it mandatory to use more than one antibiotic, either serially or in combination.

Combination antibiotic therapy may help to broaden the antimicrobial range of the therapeutic regimen beyond that attained by single antibiotics to prevent or preclude the emergence of bacterial resistance by using agents with overlapping antimicrobial spectra, and to lower the dose of the single agents, exploiting possible synergistic action against target organisms. Disadvantages of combination drug therapy are increased adverse reactions and antagonistic drug interactions with improperly selected antibiotics. It is important to emphasize that some antibiotics in combination can lead to a reduction rather than an increase in their antimicrobial activity.

Metronidazole and its hydroxymetabolite exert synergy *in vitro* against *A. actinomycetemcomitans* [28, 29]. Pavicic *et al.*, [30] found the metronidazole susceptibility of *A. actinomycetemcomitans* to be associated with the presence of nitro-reductases. Synergy also exists between metronidazole and amoxicillin as well as between the hydroxy-metabolite of metronidazole and amoxicillin and other β -lactam antibiotics [29]. The synergistic effect may be due to the ability of amoxicillin to enhance metronidazole uptake [30]. Ciprofloxacin and metronidazole also act synergistically against *A. actinomycetemcomitans* *in vitro* [29].

Metronidazole/ amoxicillin have also proven to be effective against non-oral pathogens [31, 32]. Metronidazole/ ciprofloxacin may be useful in the treatment of mixed periodontal infections involving anaerobic bacteria, *A. actinomycetemcomitans*. Since metronidazole / ciprofloxacin do not affect most gram-positive facultative bacteria, this combination of antimicrobials may facilitate recolonization of the pocket by facultative streptococci of low periodontopathic potential (Haffajee & Socransky).

DISCUSSION

In a study on antibiotic susceptibility of periodontal microorganisms by Walker CB *et al.*, [6] showed that the *in vitro* susceptibilities of bacterial isolates from periodontal lesions to eight antibiotics were relatively susceptible to the penicillin's and greater activity was generally noted with amoxicillin than with either penicillin or ampicillin with the exception of *Selenomonas sputigena* and *Peptostreptococcus*. Penicillin was effective at high concentrations but could not be recommended because organisms which are not inhibited by low concentrations are penicillinase producers.

Antibacterial activities obtained with minocycline were significantly higher than with tetracycline for *Actinobacillus actinomycetemcomitans* and *Streptococcus*. Clindamycin and metronidazole

both demonstrated excellent activity against the anaerobic Gram-negative rods but were less effective against some of the capnophilic and facultative organisms. *A. actinomycetemcomitans* was generally resistant to clindamycin but relatively susceptible to metronidazole. Erythromycin was considerably less active than the other antibiotics against the majority of the periodontal bacteria

Baker PJ *et al.*, [12] in his study on the activity of antibiotics on diverse human oral flora reported that the total cultivable oral flora was susceptible to the tetracycline's, minocycline, doxycycline, and oxytetracycline and to erythromycin. The gram-negative organisms involved in adult periodontitis were most susceptible to the tetracyclines, tyrothricin, carbenicillin and clindamycin, while those associated with localized juvenile periodontitis were susceptible to the tetracyclines or erythromycin and also confirmed that tetracycline or minocycline are likely to be good choices in the treatment or prevention of oral diseases. In contrary to the above Pajukanta R *et al.*, [13] showed that Erythromycin showed poor in vitro activity against *A. actinomycetemcomitans*.

Slots J *et al.*, in 1990 [14] assessed the occurrence of non-oral gram-negative facultatively anaerobic rods in advanced adult periodontitis and observed that all study strains demonstrated high in vitro susceptibility to ciprofloxacin, and concluded that Systemic ciprofloxacin appears to be capable of eradicating these potential pathogens from deep periodontal pockets. In favour of this Barbosa FC *et al.*, [15] showed that ciprofloxacin might be the antibiotic of choice to eradicate these pathogens from periodontal pockets.

CONCLUSION

The use of antibiotics must be based on susceptibility testing, instead of a unique adjunctive antimicrobial regimen. These results could impact periodontal treatment, mainly with regard to the selection of adjunctive systemic antibiotic. Antibiotic susceptibilities indicate that several different antimicrobial agents have well to excellent activity against many of the periodontal bacteria frequently associated with diseased sites. However, no one antibiotic emerges as being inhibitory for all organisms encountered or all that are suspected of being possible periodontopathogens.

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