Evaluation of Subgingival Bacteria in the Dog and Susceptibility to Commonly Used Antibiotics

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Summary:

The aim of the present investigation was to evaluate the subgingival aerobic and anaerobic flora of 13 dogs with periodontal disease and the susceptibility of these bacteria to antibiotics currently approved in Italy for treatment of canine infections. Of the anaerobic bacteria, Bacteroides fragilis was most frequently isolated, followed by Peptostreptococcus + Porphyromonas gingivalis and Prevotella intermedia. Of the aerobic bacteria, α-hemolytic Streptococcus was most frequently isolated, often associated with Escherichia coli or Pasteurella multocida. Resistance of anaerobic and aerobic bacteria to various antibiotics was generally high. Anaerobic bacteria appeared to be susceptible to amoxicillin + clavulanic acid, doxycycline, and erythromycin; aerobic bacteria appeared to be susceptible to amoxicillin + clavulanic acid, erythromycin, gentamycin, and sulfa-trimethoprim. Bacteroides fragilis was resistant to all of the antibiotics tested. The emerging worldwide problem of bacterial resistance to antibiotics resulting from overuse and misuse of antibiotics is discussed. ***J Vet Dent 23 (4); #$% - ^&*, 2006***

Introduction

Periodontal disease is an infectious condition of the tooth supporting tissues (gingiva, periodontal ligament, alveolar bone, and cementum) and is considered to be the most common disease in companion animals. The accumulation of plaque on tooth surfaces is responsible for the development of gingivitis and periodontitis.[1]

Gingivitis is inflammation of the gingiva and is reversible, if plaque is removed by home or professional oral hygiene procedures.[2] In addition to toxins and tissue-destructive enzymes produced by periodontopathogenic bacteria, the host's response to plaque leads to the release of agents from damaged neutrophils that can cause injury to the body's own tissues.[1] Inflammation may spread along the periodontal space and ultimately progresses to periodontitis, which is diagnosed as loss of attachment (gingival recession, resorption of alveolar bone, and formation of periodontal pockets). The periapical region of the tooth root may become affected, leading to retrograde pulpal infection. Thus, endodontic disease can occur as a result of severe periodontal disease. Eventually the tooth becomes mobile and is lost due to spontaneous exfoliation or professional extraction.[1]

Gingivitis and periodontitis are referred to as 'bacterial infections', but several hundred bacterial species have been identified to date in normal and diseased mouths of cats and dogs.[3-21] With maturation of plaque in subgingival areas and progression from a healthy periodontium to gingivitis and periodontitis, there is a shift from a gram-positive oriented, aerobic facultative flora to a predominantly gram-negative, anaerobic flora.[22] Periodontopathogens are bacteria that cause gingivitis and periodontitis. A catalase-positive form of the gram negative *Porphyromonas gingivalis* is considered to be the key periodontopathogen in cats and dogs[15,23-25] and is recognized as *P. gulae*.[26] Other canine and feline *Porphyromonas* organisms include *P. assacharolytica*, *P. cangingivalis*, *P. canoris*, *P. cansulci*, *P. endodontalis*, *P. circumdentaria*, *P. crevioricanis*, *P. salivosa*, *P. denticanis*, and *P. gingivicanis*.[11,27-30] Additional black-pigmented anaerobic bacteria associated with periodontal disease include *Prevotella intermedia*[23] and *Bacteroides* spp.[17,18,31,32] Pathogen-related oral spirochetes also are considered to play an important role in periodontal disease, but cultivation studies in cats and dogs have only been reported sparsely.[8,32-35]

Previous studies showed that amoxicillin + clavulanic acid and clindamycin had high *in vitro* susceptibility against anaerobes and enrofloxacin high *in vitro* susceptibility against aerobes from subgingival plaque samples in cats and dogs.[36,37] Although periodontal disease is caused by bacteria, antibiotic therapy should not be the primary treatment strategy.[38,39] Unfortunately, there is a tendency among veterinarians to use antibiotics as part of the management of any animal with periodontal disease or other oral condition. Resistance of plaque bacteria to antibiotics has clearly been demonstrated in humans,[40-43] and a similar pattern of bacterial resistance development may be present in cats and dogs.

The aim of the present investigation was to evaluate the subgingival flora (aerobic and anaerobic bacteria) of dogs with periodontal disease and the susceptibility of these bacteria to antibiotics currently approved in Italy for treatment of canine infections. Furthermore, the emerging problem of bacterial resistance to antibiotics in human and veterinary medicine is reviewed.

Materials and Methods

Thirteen client-owned dogs with various degrees of periodontal disease (ranging from gingivitis to periodontitis as assessed by means of periodontal probing) were included in this study. There were three Yorkshire terriers, one German shepherd, one poodle, and eight mixed-breed dogs. Two dogs received sporadic oral hygiene at home. A professional scaling and polishing had been performed on all dogs 6-months prior to sample collection. Antibiotics had been given to most of the dogs in the past for conditions other than periodontal disease. However, no attempt was made to assess details of antibiotic history because owners were not able to verify names of antibiotics used, dates and routes of administration, duration of therapies, etc. Dogs had not received antibiotic therapy for at least 2-weeks before bacterial sampling. ???

Sample collection was performed under general anesthesia at the right maxillary canine tooth (104) and the right maxillary fourth premolar tooth (108) since both teeth had been excluded from prior periodontal probing. A sterile endodontic paper point was inserted into the depth of the gingival sulcus or periodontal pocket at buccal aspects of the teeth. The paper point was removed after a few seconds and placed into tubes containing a transport liquid media (thioglycollate broth)[a] for growth of aerobic and anaerobic bacteria. The samples were immediately transported to the reference laboratory and were vortexed to allow the detachment of microbial cells. Two dilutions (Log_{10}) were made for all samples that were plated on *Tryptic Soy Agar* plates with 5 % sheep blood[b] for aerobic bacteria and on *Brucella Agar*[c] for anaerobic bacteria. The plates were incubated aerobically for 24 to 48-hours at 37°C, and anaerobically[d] for 48 to 72-hours at 37°C.

The anaerobic flora was identified by growth on *Brucella Agar*[e], a medium containing Vit K and haemin. Gram's staining, Schaffer & Fulton's staining for spores, and API System 20A[f] were used as biochemical reference methods.

Aerobic bacteria were identified by their macroscopic (*e.g.*, morphology of colonies, presence of hemolysis) and microscopic (using Gram-staining) characteristics; moreover, biochemical tests were performed using macro- or micromethods[g]. For the identification of *Streptococcus* strains, the presence of hemolysis (α partial or β total) and the growth on *Mitis Salivarius Agar*[h], a medium for the isolation and identification of the streptococci of the oral cavity, were evaluated. For identification of *Pasteurella multocida*, the lack of growth on *Mac Conkey Agar*[i] was evaluated.

Evaluation of microbial sensitivity/resistance to antibiotics was performed using the Kirby-Bauer reference method or the agar disk diffusion test. A bacterial suspension, performed in saline buffer (0.9 % NaCl), was delivered onto a Mueller-Hinton plate, and then the disks containing different antibiotic molecules were placed on the plate. After incubation at 37°C under aerobic or anaerobic atmosphere for 24 to 48-hours, the susceptibility of each microorganism was recorded to the following antibiotics: amikacin, amoxicillin + clavulanic acid, doxycycline, erythromycin, gentamycin, kanamycin, metronidazole (only for anaerobes), and sulfa-trimethoprim.[k]

Table 1

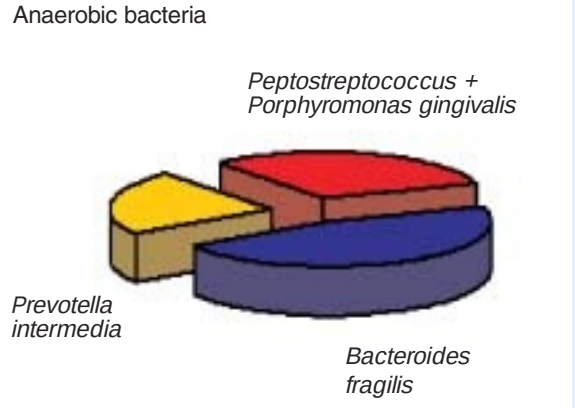
Signalment of the 13 dogs enrolled in the study.

Variable		n	%
Sex	Male	11	84.6
	Female	2	15.4
Age	< 5 years	3	23.1
	5-10 years	7	53.8
	> 10 years	3	23.1
Weight	< 12 kg	8	61.5
	12+ kg	5	38.5
Diet	Mixed	10	76.9
	Dry	3	23.1

Table 2

Isolation of anaerobic bacteria.

Anaerobic bacteria	Percent (%)
Peptostreptococcus + Porphyromonas gingivalis	30.8
Bacteroides fragilis	46.1
Prevotella intermedia	23.1
	100



Results

Results of variables of signalment (sex, age, weight, and diet) of the 13 dogs enrolled in the study are reported in Table 1. The majority of dogs were male (84.6 %), 5 to 10-years of age (53.8 %), < 12.0 kg (61.5 %), and eating a mixed (soft and dry) diet (76.9 %).

Of the anaerobic bacteria (Table 2), *Bacteroides fragilis* was most frequently isolated from subgingival samples, followed by *Peptostreptococcus + Porphyromonas gingivalis*, and *Prevotella intermedia*. Of the aerobic bacteria (Table 3), α-haemolytic *Streptococcus* was most frequently isolated, often associated with *Escherichia coli* or *Pasteurella multocida*.

Susceptibility of anaerobic and aerobic bacteria to various antibiotics is shown in Tables 4-8. Resistance of isolated bacteria to tested antibiotics was generally high. Anaerobic bacteria appeared to be susceptible to amoxicillin + clavulanic acid, doxycycline, and erythromycin, while aerobic bacteria appeared to be susceptible to amoxicillin + clavulanic acid, erythromycin, gentamycin, and sulfa-trimethoprim. *Bacteroides fragilis* was resistant to all of the antibiotics tested.

Discussion

The aim of the present investigation was to evaluate the subgingival aerobic and anaerobic bacterial flora of 13 dogs with

periodontal disease and the susceptibility of these bacteria to antibiotics currently approved in Italy for treatment of canine infections. Except for the high prevalence of *Bacteroides fragilis*, the predominant subgingival flora obtained in this study confirms results reported in previous studies.[15,17,18,23-25,31,32] Of the anaerobic bacteria, *Bacteroides fragilis* was most frequently isolated, followed by *Peptostreptococcus* + *Porphyromonas* gingivalis and *Prevotella intermedia*. The reason for the unusually high prevalence of *Bacteroides fragilis* is not clear. Differing results between isolation studies may be due to differences in study methodology, including sample population utilized and isolation techniques applied. Of the aerobic bacteria, α-haemolytic Streptococcus was most frequently isolated, often associated with *Escherichia coli* or *Pasteurella multocida*.

The fast growth rate, high concentration of cells, genetic processes of mutation and selection, and ability to exchange genes account for the extraordinary adaptation and evolution of bacteria.[44] For these reasons bacterial resistance to antibiotics may take place very rapidly in evolutionary time. Risk factors responsible for the emergence and spread of resistant bacteria include: (1) antibiotic use; (2) reservoirs for resistance; (3) medical advances; and (4) societal changes.[45] Antibiotics make conditions favorable for overgrowth of some bacteria, including those that possess mechanisms of drug resistance. If a resistant organism is present, antibiotics will create 'selective pressure' favoring the growth of that organism. A number of studies have demonstrated conclusively that the development of bacterial resistance to antibiotics is correlated with the level of antibiotic use.[46-48] Antibiotic resistance of nosocomial pathogens in hospitals, nursing homes, day-care centers, and animal facilities is increased by the transfer of individuals already colonized by resistant organisms from one location to another. Progress in the treatment of many diseases has led to an increased life span of humans and animals. Consequently, with advanced age, chronic disease or immunosuppression, individuals can be more susceptible to bacterial infections, resulting in greater use of antibiotics. Worldwide spread of bacterial resistance to antibiotics has occurred due to the increased mobility of today's society.[45]

Pet animal numbers have substantially increased in modern society, and attention is increasingly devoted to pet welfare. Antibiotics are frequently used in small animal practice, with heavy use of broad-spectrum agents such as amoxicillin + clavulanic acid, cephalosporins, and fluoroquinolones. The practice of antibiotic overuse and misuse in cats and dogs has contributed to the development of *Staphylococcus* spp., *Escherichia coli* and various other bacteria that are resistant to antibiotics.[49-53] The role of pets in the dissemination of bacterial resistance to antibiotics has been given relatively little attention when compared with that of food animals, and a marked contrast is evident between the current policies on antibiotic usage in companion and food animals. However, the possible transfer of resistant bacteria from cats and dogs to humans has recently been acknowledged as a potential threat to public health.[44,54]

One of the biggest problems is inappropriate prescribing of antibiotics. There are many reasons for this, including demand from patients/patient owners, time pressure on physicians/veterinarians, and diagnostic uncertainty. Several recent studies showed that pediatricians prescribe antibiotics significantly more often, if they perceive parents expect them, and significantly less often, if they feel parents do not expect them.[55-58] The best way to combat this situation is to educate patients/patient owners and doctors/veterinarians to decrease both demand and over-prescribing. Unfortunately, there is a tendency to use antibiotics as part of the management of any animal with periodontal disease or other oral condition, although there is no apparent justification for this practice. Similar to dogs in the present study, resistance of plaque bacteria to antibiotics has clearly been demonstrated in

Table 3

Isolation of aerobic bacteria.

Aerobic bacteria	Percent (%)
α-*hemolytic Streptococcus + E. coli*	38
α-*hemolytic Streptococcus + P. multocida*	38
α-*hemolytic Streptococcus + E. coli + P. multocida*	8
α-*hemolytic Streptococcus + E. coli + S. intermedius*	8
α-*hemolytic Streptococcus S. intermedius + P. multocida*	8
	100

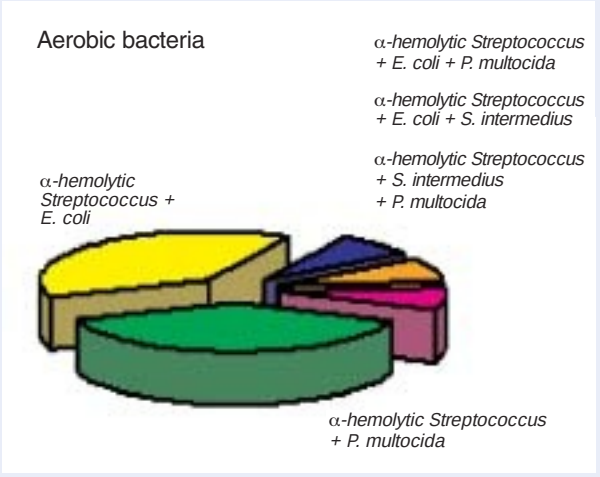


Table 4

Antibiotic activity versus *Prevotella intermedia.*

Antibiotic	Sensitivity (%)	Resistance (%)
Amikacin	-	100
Amoxicillin+Clavulanic acid	66.7	33.3
Doxycycline	33.3	66.7
Erythromycin	66.7	33.3
Gentamycin	-	100
Kanamycin	-	100
Metronidazole	-	100
Sulfa-trimethoprim	-	100

Table 5

Antibiotic activity versus *Bacteroides fragilis*.

Antibiotic	Sensitivity (%)	Resistance (%)
Amikacin	-	100
Amoxicillin+Clavulanic acid	-	100
Doxycycline	-	100
Erythromycin	-	100
Gentamycin	-	100
Kanamycin	-	100
Metronidazole	-	100
Sulfa-trimethoprim	-	100

Table 6

Antibiotic activity versus *Porphyromonas gingivalis + Peptostreptococcus*.

Antibiotic	Sensitivity (%)	Resistance (%)
Amikacin	25	75
Amoxicillin+Clavulanic acid	75	25
Doxycycline	100	-
Erythromycin	25	75
Gentamycin	25	75
Kanamycin	25	75
Metronidazole	25	75
Sulfa-trimethoprim	-	100

Table 7

Antibiotic activity versus α-haemolytic *Streptococcus + E. coli.*

Antibiotic	Sensitivity (%)	Resistance (%)
Amikacin	20	80
Amoxicillin+Clavulanic acid	40	60
Doxycycline	20	80
Erythromycin	20	80
Gentamycin	80	20
Kanamycin	20	80
Sulfa-trimethoprim	60	40

Table 8

Antibiotic activity versus α-haemolytic *Streptococcus + P. multocida*.

Antibiotic	Sensitivity (%)	Resistance (%)
Amikacin	-	100
Amoxicillin+Clavulanic acid	-	100
Doxycycline	-	100
Erythromycin	40	60
Gentamycin	-	100
Kanamycin	-	100
Sulfa-trimethoprim	-	100

humans.[40-43] It is therefore imperative to review periodontal treatment strategies and determine whether systemic antibiotics have a role to play in the management of periodontal disease.

If accumulation of plaque is prevented, periodontal disease does not develop.[2] Although this condition is caused by bacteria, antibiotic therapy is not considered the primary treatment strategy.[38,39] Instead, treatment of periodontal disease should be directed at mechanical removal or reduction of plaque and calculus accumulation, suppression of the tissue-destructive effects of the inflammatory response, surgical management of periodontal pockets, extraction of more severely affected teeth, and thorough debridement of extraction sites.[1] A controlled-release local antibiotic delivery system, reaching periodontopathogens deep within periodontal pockets, has been described in dogs.[59] Professional supra- and subgingival scaling, followed by daily tooth brushing, is the 'gold standard' for prevention of periodontal disease.[1] Home oral hygiene may be enhanced by offering products that support dietary abrasion or chemically suppress plaque and calculus accumulation.[60]

Bacteremia secondary to periodontal disease occurs daily in patients with periodontal disease, and it is normally rapidly cleared by the reticulo-endothelial system in the healthy patient.[61] Therefore, for the great majority of otherwise healthy cats and dogs presenting with periodontal disease and other oral conditions, systemic antibiotics are not indicated.[1,39] Bacteremia can be prevented or reduced in severity by rinsing the oral cavity

Table 9

American Veterinary Dental College (AVDC) Position Statement on the Use of Antibiotics in Veterinary Dentistry.

The AVDC endorses the use of systemic antibiotics in veterinary dentistry for treatment of some infectious conditions of the oral cavity. Although culture and susceptibility testing is rarely performed on individual patients that have an infection extending from/to the oral cavity, the selection of an appropriate antibiotic should be based on published data regarding susceptibility testing of the spectra of known oral pathogens. Patients that are scheduled for an oral procedure may benefit from pre-treatment with an appropriate antibiotic to improve the health of infected oral tissues. Bacteremia is a recognized sequela to dental scaling and other oral procedures. Healthy animals are able to overcome this bacteremia without the use of systemic antibiotics. However, use of a systemically administered antibiotic is recommended to reduce bacteremia for animals that are immune compromised, have underlying systemic disease (such as clinically-evident cardiac, hepatic, and renal diseases) and/or when severe oral infection is present. Antibiotics should never be considered a monotherapy for treatment of oral infections, and should not be used as preventive management of oral conditions. Adopted by the Board of Directors, April 2005

with dilute chlorhexidine gluconate (0.12 %) prior to commencing the oral procedure. Perioperative systemic antibiotics are indicated in: (1) debilitated and immunocompromised patients; (2) patients suffering from organ disease, endocrine disorders, cardiovascular disease, and severe local and/or systemic infections; and (3) patients having permanent implants and transplants. Unless there is a well-founded positive reason for their administration, systemic antibiotics should not be used.

Although there is a position statement on the use of antibiotics in veterinary dentistry provided by the American Veterinary Dental College (Table 9),[62] compulsory guidelines for prudent prescription patterns and use of antibiotics in small animals with periodontal disease or other oral conditions, which describe the minimum requirements to be followed by veterinarians, are not available. Key elements of these guidelines should be the use of antibiotics on the basis of an exact (preferentially microbiological) diagnosis, choice of the most suitable antibiotic (antibacterial spectrum as narrow as possible, margin of safety as high as possible, and good tissue penetration if necessary), restricted use of antibiotics with last resort character, and adherence to label instructions (no underdosing or prolongation of dosing interval, so-called "pulse dosing"). Any deviation from the guideline recommendations must be justified and recorded.[63,64]

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[a] Thioglycollate Broth, Oxoid Ltd, Basingstoke, UK

[b] Trypric Soy Agar plus 5 % Sheep Blood Plate, Oxoid Ltd, Basingstoke, UK

[c] Brucella Agar, Oxoid Ltd, Basingstoke, UK

[d] Gas Pak System, Becton Dickinson, Franklin Lakes, USA

[e] Brucella Agar, Oxoid Ltd, Basingstoke, UK

[f] API 20A System, BioMériéux, Lion, France

[g] API 20E and API 20NE Systems, BioMériéux, Lion, France

[h] Mitis Salivarius Agar, DIFCO, DID, Detroit, USA

[i] Mac Conkey Agar n°3, Oxoid Ltd, Basingstoke, UK

[j] Mueller-Hinton Agar, Oxoid Ltd, Basingstoke, UK

[k] All disks used for antimicrobial sensitivity tests produced by Oxoid Ltd, Basingstoke, UK

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