Canine oral microbiota: A source of potentially pathogenic polyresistant bacteria

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ABSTRACT

Introduction: The current rise in polyresistant bacterial strains is due to self-medication, failure to comply the treatment, incorrect prescription of antibiotics by the physician, and even the abuse of these in agriculture industries. In this research, we suggest another possible source. Humans, being in coexistence with dogs and in contact with their saliva, could have been exposed to polyresistant microorganisms that are potential pathogens to. Identify, isolate and analyze these possible microorganisms were the main objectives of this study. Materials and methods: Oral samples (n=28) from domestic dogs were taken and cultured. Bacterial colonies (n = 160) were obtained and subjected to identification and antimicrobial sensitivity tests. Results: From 160 isolated colonies, the most prevalent species was Staphylococcus haemolyticus. Other bacteria such as Enterococcus faecium, Escherichia coli, Proteus mirabilis, and Pseudomonas aeruginosa were also found in a lesser proportion. There was an increased resistance of the bacteria against cell wall synthesis antibiotics. The resistance towards vancomycin was the highest, followed by cefalotin and cefixime. In contrast, all bacteria were sensible to imipenem. Conclusion: The resistance observed against protein synthesis inhibitors showed a high resistance towards erythromycin and clarithromycin but a high sensibility to amikacin and gentamicin. In this study several human pathogens that are the cause of infectious diseases were identified in the oral microbiota of dogs. Furthermore, another risk of polyresistant bacteria transmission is proposed with the determination of each bacterial resistance.

Key words: antimicrobial resistance; antibiotics; oral microbiota; canines.

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RESUMEN

Introducción: La reciente aparición de cepas de bacterias polirresistentes puede deberse a la automedicación, falla en el seguimiento del tratamiento, prescripción incorrecta de antibióticos por el médico, así como su sobreuso en la industria agrícola. En este artículo se sugiere otra posible fuente. Los humanos, al estar en estrecho contacto con los perros y con su saliva, pueden estar expuestos a microorganismos polirresistentes que son potencialmente patogénicos en los humanos. Objetivo: La identificación, aislamiento y análisis de dichos posibles microorganismos. Materiales y métodos: Se tomaron muestras de saliva de 28 perros domésticos para posteriormente ser cultivadas. Se obtuvieron colonias bacterianas (n = 160) para su identificación y sensibilidad antimicrobiana. Resultados: De las 160 colonias aisladas, la especie con mayor prevalencia fue Staphylococcus haemolyticus. Otras bacterias como Enterococcus faecium, Escherichia coli, Proteus mirabilis y Pseudomonas aeruginosa se encontraron en una menor proporción. Se observó un incremento en la resistencia hacia los antibióticos que inhiben la síntesis de pared celular, siendo la vancomicina el antibiótico con mayor resistencia bacteriana, seguido de cefalotina y cefixima. En contraste, todos los cultivos bacterianos fueron sensibles a imipenem. Conclusión: La resistencia observada a macrólidos y aminoglucósidos fue muy variable, con una alta resistencia hacia clariromicina, y una alta sensibilidad a amikacina y gentamicina. Varios patógenos que causan enfermedades infecciosas en humanos fueron encontrados en la microbiota oral de los perros. Esto, junto con la determinación de sus resistencias, representa una posible forma de transmisión de bacterias polirresistentes hacia los humanos.

Palabras clave: resistencia; antibióticos; microbiota oral; caninos.

INTRODUCTION

Bacterial resistance has increased in the last years, making treatments and therapeutic decisions more complex and difficult. Second- and third-line treatments are more commonly used to treat infections by exogenous pathogens. The need for understanding routes of antimicrobial resistance transmission must be studied. One important route is present between humans and animals by direct contact or through the food chain. It has been identified that gram-negative bacteria can develop transmission mechanisms of antimicrobial resistance between commensal bacteria and pathogenic bacteria, even between different species. As an example, nonpathogenic commensal species, like E. coli strains, can be resistant to antimicrobials. These species may transfer antimicrobial resistance genes to actual pathogenic species by horizontal gene transfer using plasmids, transposons, and integrons that carry these genes.

The highest isolation of multiresistant bacteria in animals is found in pets, followed by farm animals and the lowest in wild animals. This could be due to the daily close contact between the owner and the pet. The oral microbiota of dogs is one of the microsystems that is in direct contact with humans. It is known that the transmission of normal canine microbiota and periodontal pathogenic bacteria to humans is possible by daily contact as dogs tend to lick their owners. The identification of the same species in both dog and owner has been described. Bacteria of the genus Porphyromonas, Prevotella, and Tannerella have been the most frequently reported in such cases.

The canine oral microbiota mainly consists of bacteria from the Firmicutes and Bacteroidetes phyla. Current studies identify the oral pathogens present in dogs, establishing that the bacteria is a mixture of aerobic and anaerobic microorganisms that usually include Pasteurella canis, Streptococcus spp. Staphylococcus spp., Fusobacterium spp., Bacteroides spp., and Capnocytophaga canimorsus. Other studies have shown different species such as Porphyromonas cangingivalis, Porphyromonas gulae, Actinomyces canis, and Neisseria weaveri including the genus Fusobacterium spp. These species normally differ from those present in human oral microbiota.

Periodontal diseases as gingivitis and periodontitis are extremely frequent in dogs. In these diseases, an alteration occurs in the composition of the normal microbiota by the colonization of several pathogenic microorganisms. Some of them are strict anaerobic and facultative anaerobic bacteria, mainly of the genera Streptococcus spp., Staphylococcus spp., Enterobacteriaceae spp., Corynebacterium spp., Clostridium spp, and the species Eikenella corrodens and Pasteurella multocida.

In recent years, there has been an increase in the number of infections caused by different polirresistant bacteria affecting humans. Nevertheless, there are scarce studies on the profile of antimicrobial resistance in the canine oral microbiota as it has been somewhat ignored as a possible source. The present study analyzed the antimicrobial resistance of aerobic bacteria in the oral microbiota of domestic dogs whose owners live in middle- and upper-class areas in Mexico City.
MATERIAL AND METHODS

Sample collection
The group consisted of 28 male dogs (age 1−2 years, weight 30−40 kg, and height 60−65 cm). All dogs presented a good state of health when examined and auscultated. This reduced variables that could interfere with the study. Using sterile swabs, saliva samples were collected from the dogs in middle- and upper-class households in Mexico City (n=160). The swab was taken from the internal part of the cheeks of the oral cavity, gums, and teeth. Samples were immediately incubated in BHI (Brain heart infusion) (Becton Dickinson Dr, Franklin Lakes, NJ, USA) at 37 °C/24 h. All the samples were processed in the microbiology laboratory of the Health Science School at Anahuac University.

Bacterial Isolation
After incubation, blood agar was used to culture 10 μl BHI medium (Becton Dickinson Dr, Franklin Lakes, NJ, USA) and the mixture was incubated at 37 ºC for 24 h. The plates were examined and each morphotype was counted and subculture to obtain pure cultures. The pure colonies obtained were re-cultured in blood agar (n = 140) (Becton Dickinson Dr, Franklin Lakes, NJ, USA) at 37 °C/24 h.

Biochemical tests for bacterial identification
Gram staining, oxidase and indole test, and the biochemical tests described in the identification kit (Carbohydrate fermentation, citric acid, malonate utilization, and esculin hydrolysis, among others) were performed on the colonies obtained from blood agar (n = 140).

The micro and macro morphology of the bacteria were assessed by gram staining.BD BBL™ Gram Stain Kit (Becton Dickinson Dr, Franklin Lakes, NJ, USA). BBL™ Dryslide™ Oxidase (Becton Dickinson Dr, Franklin Lakes, NJ, USA) and BBL™ Dryslide™ Indole (Becton Dickinson Dr, Franklin Lakes, NJ, USA) were used for oxidase and indole testing.

The biochemical identification was performed using the BBL™ Crystal™ ID Kit (Becton Dickinson Dr, Franklin Lakes, NJ, USA), BBL™ Crystal™ MIND software, and the BBL™ Crystal ™ Autoreader (Becton Dickinson Dr, Franklin Lakes, NJ USA). The biochemical tests were carried out on the colonies obtained from the blood agar. Inoculum broth was adjusted to 0.5 McFarland standard (expected 3.0 x 10⁸ CFU/mL) and incubated without CO₂, 40–60% humidity, at 37 °C for 18 h. The 0.5 McFarland standard reading was made by nephelometry using the CrystalSpec™ Nephelometer (Becton Dickinson Dr, Franklin Lakes, NJ, USA).

Antimicrobial susceptibility test
The antimicrobial susceptibility was tested by the Kirby–Bauer test under the protocol of the Clinical and Laboratory Standards Institute. Pure colonies (2−4) were taken from the blood agar and re-suspended in BBL™ Crystal™ Inoculum Broth (IB). Then, they were adjusted to 0.5 McFarland standard (Expected 1.5 x 10⁷ CFU/mL). A bacterial suspension was cultured in BD® BBL Müller Hinton medium (150 x 15 mm). The antibiotics used were cell wall synthesis inhibitors, aminoglycosides, macrolides, and DNA inhibitors: amoxicillin/clavulanic acid (AmC-30), piperacillin (PIP-100), piperacillin/tazobactam (TZP-110), ampicillin 10 (AM-10), cephalotin (CF-30), cefazolin (CZ-30), cefaclor (CEC-30), cefuroxime (CXM-30), cefixime (CFM-5), meropenem (MER-10), imipenem (IPM-10), vancomycin (Va-30), amikacin (AN-30), gentamicin (GM-10), kanamycin (K-30), spectinomycin (SPT-100), tetracycline (Te-30), erythromycin (E-15), azithromycin (AZM-15), clarithromycin (CLR-15), clindamycin (CC-2), chloramphenicol (C-30), nalidixic acid (NA-30), ciprofloxacin (CIP-5), and gatifloxacin (GAT-5). The medium and antibiotics employed were purchased from Becton Dickinson (Becton Dickinson Dr, Franklin Lakes, NJ, USA).

RESULTS

Bacterial identification
By using biochemical assays, we performed the isolation and identification of each bacterial culture obtained from the oral swab samples (n = 160). 20 Swab samples were cultured but there was no bacterial growth. The remaining pure samples were then evaluated (n = 140). In this study, Staphylococcus haemolyticus was the most frequently isolated species (20.7%) (Figure 1), followed by Enterococcus faecium and Escherichia coli (15.7% both). Proteus vulgaris and Pseudomonas aeruginosa were found in 10.7% of the samples studied. Other bacteria identified and isolated were Providencia rettgeri, Enterococcus durans, Bacillus cereus, Staphylococcus simulans, and Gemella morbillorum, each with 5% frequency (Figure 1). The remaining 1.5% corresponds to unidentified bacteria.
Antimicrobial susceptibility to inhibitors of bacterial cell wall synthesis

In this study, we tested each group of the most commonly prescribed antibiotics worldwide such as semi-synthetic penicillins with and without β-lactamase inhibitors, protein synthesis inhibitors antimicrobials, and DNA replication targeting antibiotics. First, we tested two semisynthetic penicillins not combined with β-lactamase inhibitors for bacterial resistance. We found a marked resistance for ampicillin (47.1%) and piperacillin (26.4%) (Figure 2). The susceptibility to antibiotics was also tested in combination with β-lactamase inhibitors. Resistance to amoxicillin/clavulanic acid was 42.1% and 10.7% for piperacillin/tazobactam (Figure 2). In this study, 62.9% of all the bacterial isolates were resistant to cephalothin and 42.1% were resistant to cefazolin, which are first-generation cephalosporins (Figure 2). Analyzing resistance to second-generation cephalosporins, 26.4% of the bacteria were resistant to cefaclor and 42.1% to cefuroxime. Resistance against third-generation cephalosporin cefixime was 63.6% and vs ceftizoxime, 47.1% (Figure 2).

In this study, resistance to carbapenems was low, 15.7% of isolated cultures were resistant to meropenem, unlike imipenem no resistance was observed. From the bacteria identified, 84.3% showed resistance to vancomycin (Figure 2).

Antimicrobial susceptibility to protein synthesis inhibitors

The antibiotic resistance against macrolides was 62.9% for erythromycin, 47.1% for azithromycin, and 68.6% for clarithromycin (Figure 3). Resistance to aminoglycosides like amikacin and gentamicin was 15.7%. Resistance to kanamycin and spectinomycin was 31.4 and 68.6%, respectively (Figure 3). Resistance to chloramphenicol, clindamycin, and tetracycline was 0.7, 73.6, and 37.1%, respectively (Figure 3).

Antimicrobial susceptibility to DNA replication inhibitors

Finally, the Kirby–Bauer test showed bacterial culture resistance against the following quinolones: nalidixic acid (57.9%), ciprofloxacin (21.4%), and gatifloxacin (5.0%) (Figure 4).

DISCUSSION

In this study, we aimed to identify and describe polyresistant and potentially pathogenic bacteria from saliva samples belonging to healthy canine oral microbiota. The most prevalent species found in this study was Staphylococcus haemolyticus. This bacterium is a member of coagulase-negative Staphylococcus (CNS). This group consists of several species other than Staphylococcus aureus that have become increasingly important as opportunistic pathogens in health centers around the world. Among the species in CNS, Staphylococcus haemolyticus is recognized as an emerging and important human pathogen. It has been associated with severe infections, such as endocarditis, urinary tract infections, septicemia, peritonitis, wounds, bones and joint.12

Figure 1. Identification of bacterial species from pure colonies (n = 140).
Figure 2. Antimicrobial susceptibility to inhibitors of bacterial cell wall synthesis. Ampicillin (AM-10), piperacillin (PIP-100), amoxicillin/clavulanic acid (AmC-30), piperacillin/tazobactam (TZP-110), cephalothin (CF-30), cefazolin (C2-30), cefaclor (CX-30), cefuroxime (CXM-30), cefixime (E-15), cefizoxime (ZOX-30), meropenem (MEM-10), imipenem (IPM-10), vancomycin (Va-30). The highest resistance observed was against Vancomycin, first class cephalosporin, cephalothin, and the third class cephalosporin; cefixime.

Figure 3. Antimicrobial susceptibility to protein synthesis inhibitors. Erythromycin (E-15), azithromycin (AZM-15), clarithromycin (CLR-15), amikacin (AN-30), gentamicin (GM-10), kanamycin (K-30), spectinomycin (SPT-100), chloramphenicol (C-30), clindamycin (CC-2), tetracycline (Te-30). High resistance against macrolides is shown while broad-spectrum antibiotics (gentamicin) targeting gram-negative bacteria have higher rates of susceptibility.
This bacterial isolation contrast with the results showed by Yamasaki et al. where *Tannerella forsythia* and *Porphyromonas gulae* were the most commonly isolated bacteria in the oral microbiota of dogs.13

We also identified *E. faecium* in a great proportion. The genus *Enterococcus* belongs to the *Enterococcaceae*, a family of gram-positive, catalase-negative cocci. *Enterococcus* has been recognized as the third or fourth most important cause of nosocomial infections worldwide. It is also responsible for urinary tract infections and bacteremia, being the second and third most important cause, respectively.14 The third most isolated bacteria were *Escherichia coli*; this is one of the most widely distributed and known bacteria in the world. It is associated with multiple nosocomial and community-acquired infections such as urinary tract infections, diarrhea, and even bacteremia and sepsis.

We also identified *Proteus Mirabilis* in a great quantity. The genus *Proteus* consists of gram-negative bacilli that belong to the *Enterobacteriaceae* family. Strains of *Proteus* have been isolated from the intestinal tract of various mammals including human beings, as well as houses and hospitals. This bacterium is considered one of the main causes of urinary tract infections acquired both in the community and hospitals.15

An important pathogen isolated from the oral microbiota of dogs was *Pseudomonas aeruginosa*. It is considered the leading cause of nosocomial infections and is associated with several diseases including endocarditis, pneumonia, otitis, and bacteremia, as well as gastrointestinal, osteoarticular, ocular, skin and soft tissue infections, and bacteremia.16 Due to its presence in hospital settings, this bacterium has also been associated with multiresistance, which makes it one of the hardest pathogens to treat.

Following the identification, *Providencia rettgeri* is a gram-negative bacillus belonging to the *Enterobacteriaceae* family. It is associated with soft tissue infections, infections caused by contaminated urinary catheters, and bacteremia.17 We also isolated *Bacillus cereus*, a gram-positive bacillus found in different foods, soil, and water. It produces food poisoning associated to rice intake and produces diarrhea accompanied by severe cramps and profuse vomiting.

Other bacteria found was *Enterococcus durans*. It has been isolated from animal and human intestines, which, unlike other *Enterococci*, is rarely associated with infections in humans due to its low pathogenicity. However, it has been linked to a fatal case of aortic valve endocarditis.18 *Streptococcus simulans*, another CNS, is rarely identified as a possible cause of infections in humans. Out of the few reported cases, there is evidence that it produces soft tissue infection18 or septicemia.19 *Gemella morbillorum* has been isolated as a normal inhabitant of the respiratory, gastrointestinal, and genitourinary tract in humans. *Gemella morbillorum* has been reported to cause infections in the central nervous system, pneumonia, pleural empyema, mediastinitis, osteomyelitis, hepatic abscess, and peritonitis as well as cardiovascular infections.20

We found that most of the bacteria present in the oral cavity of the dogs were gram-negative, mostly aerobes and possible high pathogens also compatible with species long implicated in human infectious diseases. There are four main causes that have been associated with antimicrobial resistance in the clinical environment: self-medication, error

![Figure 4. Antimicrobial susceptibility to DNA replication inhibitors. Nalidixic acid (NA-30), ciprofloxacin (CIP-5), gatifloxacin (GAT-5). Nalidixic acid showed the highest resistance.](https://doi.org/10.36105/prua.2021v1n1.02)
in the medical prescription of antibiotics, lack of adherence to antimicrobial treatment, and the excessive use of antibiotics as growth promoters in the agriculture industry, in consequence generating food products with antibiotics and resistant bacteria.

In this investigation, we propose a possible fifth mechanism in which humans come into contact with potentially pathogenic polyresistant bacteria. This mechanism is domestic dogs and close contact with canine oral microbiota and saliva through licking and kissing. Infants and children would potentially have a greater contact with these pets and in result with the dog microbiota. There is also evidence that supports the connection between the bacteria in the oral cavity of dogs and their contact with the owners. Yamasaki et al. described how families co-share the oral microbiota with their pets. The high resistance noted in this study against β-lactams (ampicillin, amoxiclav) could be because most of the isolated bacteria were gram-negative and are naturally more resistant to these antibiotics. Additionally, the high resistance against vancomycin is due to the same factor; unlike gram-negative bacteria, gram-positive bacteria are highly susceptible to β-lactams because of its high penetrance into bacteria. Vancomycin is used principally against methicillin-resistant Staphylococcus aureus. There was also a marked resistance against cephalothin, cefixime, ceftizoxime, and cefazolin. These antibiotics are usually used to prevent wound infections in surgery or act as broad-spectrum drugs to inhibit bacterial growth. Resistance against these antibiotics was found in more than 40% of the bacteria. These results should be considered as a potential evidence of polyresistant bacteria in addition to the naturally resistant opportunistic microorganisms that infect the human. While there were many bacterial cultures susceptible to aminoglycosides, there was a high prevalence of bacteria resistant to azithromycin, clarithromycin and erythromycin. The resistance against macrolides could be an important factor to catalog these bacteria as polyresistant. The presence of polyresistant and potentially pathogenic bacteria in the canine oral microbiota reported in this study should be considered a potential hazard and a serious public health problem. We believe that information on the measures to be taken while in close and frequent contact with canines should be provided to pet owners and health professionals.

Future studies should focus on the correlation between clinical cases of nosocomial infections and those acquired in the community with possible exposure to these microorganisms. Furthermore, the limitation in this study is the lack of correlation between antibiotic resistance and isolated bacteria. This could encourage other studies to focus their research on this aspect. In addition, we must point out that pets are also susceptible to the colonization of polyresistant bacteria from humans, making this a vicious cycle. This study represents a new approach to describe the resistance profile of the oral canine microbiota.

**CONCLUSION**

The recent outbreak of infections caused by polyresistant bacteria is a major national and international health problem. There are several reasons for antibiotic resistance. This study proposes a potential, yet sometimes ignored, source of resistant bacteria. Based on the results obtained, contact with oral canine microbiota and saliva can be a risk factor to acquire infections from polyresistant and pathogenic bacteria.

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**CONFLICTS OF INTEREST**

The authors declare no conflict of interest.

**REFERENCES**


