The Impact of Dogs Oral Microbiota on Human Health: A Review

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The dog's mouth contains many bacteria, with more than 600 types present. Therefore, it's clear that dog saliva is not inherently cleaner than human saliva. Bacterial infections transmitted from dogs to humans, known as zoonotic infections, pose a significant public health concern. These infections can be transmitted from dogs to humans through direct contact, contaminated surfaces, or inhaling airborne particles. Understanding the risks associated with dog-related bacterial infections and implementing effective prevention and control measures is crucial for safeguarding human health. This review shows an overview of the dangers of dog-related bacterial infections and effective prevention and control measures essential for protecting human health. Also, Different practical considerations and methods are discussed in light of available literature and the authors' practicable experience.

Keywords: commensalism; Microorganisms; Microbiota; Microbiome; Saliva; Zoonotic infection.

The oral microbiota of domestic dogs plays a crucial role in their overall health, impacting oral hygiene and potentially influencing systemic well-being. Microbiota comprise diverse multi-species assemblies of microorganisms that coexist within a shared environment[1]. Whipps and colleagues initially coined the term 'microbiome' in 1988, focusing on microbial communities inhabiting well-defined habitats characterized by distinct physio-chemical properties [2]. This definition marked a significant advancement in our understanding of microbial communities, emphasizing their specific properties, functions, and interactions with their surroundings, forming unique ecological niches. Nevertheless, numerous alternative microbiome definitions have emerged over the years [3] .One of the most widely cited contemporary definitions describes the microbiome as a community of commensal, symbiotic, and pathogenic microorganisms within a given body space or environment [3](Table 1). Marchesi and Ravel's definition emphasizes the genomes, gene expression patterns, proteomes, and prevailing biotic and abiotic conditions in a particular environment [4] Within microbiomes, microorganisms engage in various interactions, which have varied effects on microbial fitness, population dynamics, and functional capabilities within the microbiome [5]. These interactions can occur between microorganisms of the same species

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or different species, genera, families, and domains of life. The patterns of interaction within these networks can be positive, such as mutualism, synergism, commensalism, negative, such as redation, parasitism, antagonism, competition, or neutral, where no observable interaction is evident [6].

Microorganisms are living things that are so small that they are invisible to the naked eye and exist all around us. They live in water, soil, and air. Millions of these microorganisms are also present in human body which are called human microbiota. Some microorganisms make us sick, while others are important for our health. Indeed, bacteria, viruses, fungi, and protozoa are all common types of microorganisms, but they differ significantly in terms of structure, function, and biology . These are tiny creatures that cause diseases such as toxoplasmosis and malaria. Bacteria are unicellular organisms. Some bacteria require oxygen to survive, while others do not. Some people prefer heat, while others prefer cold environments. Well-known examples of bacteria are salmonella and staphylococcus [7]. Most bacteria are harmless to humans, but many live in and within our bodies that have a role in maintaining our health. For example, lactic acid bacteria in the intestines help digest food. Other bacteria support the immune system by fighting germs. Less than 1% of all bacteria cause illness, but this is only an approximation as there is no exact number[8]. For example, tuberculosis is caused by bacteria [7]. Bacterial infections can be managed with antibiotics, which are medications designed to either eliminate bacteria or inhibit their proliferation. It's important to note that numerous conditions, including diarrhea, colds, and tonsillitis, can be attributed to bacteria, but viruses more commonly cause them. Antibiotics are ineffective against viruses[9]. Consequently, it is not advisable to initiate antibiotic treatment prematurely if you only suspect a bacterial infection[10]. The genetic information within this casing is essential for the virus to replicate. Many viruses can lead to illnesses, some causing mild conditions like the common cold, while others can result in severe diseases such as AIDS. Additional viral infections include the flu, measles, and viral hepatitis. Viruses infiltrate healthy cells and reproduce within them, relying on these host cells for replication. Not all viruses provoke symptoms; in many cases, the body successfully defends itself against the invaders, as seen with conditions like herpes, which can manifest as blisters in specific individuals when the immune system is compromised. Fighting viruses with drugs can be challenging, but vaccination can prepare the immune system to combat specific viruses. Fungi are highly adaptable and can thrive in diverse environments. Common fungi include yeasts, molds, and edible mushrooms like button mushrooms. Similar to bacteria, some fungi naturally inhabit the skin and body, but they can also be responsible for diseases known as mycoses. Examples of these include athlete's foot and fungal nail infections. Fungal infections can lead to inflammation in the lungs, oral mucous membranes, and reproductive organs such as genital or vaginal candidiasis is a condition that causes vaginitis, commonly known as a yeast infection. When a yeast infection occurs, it leads to inflammation, accompanied by symptoms such as itching (pruritus), a thick white or yellow discharge, and an odor, and they can pose a serious threat to individuals with weakened immune systems [10]. On the other hand, humans have also experienced advantages from the favorable attributes of certain mushrooms. The credit for the invention of penicillin goes to a particular mold employed in manufacturing this antibiotic.

Microbiota role in human health

The term "microbiome" typically refers to the diverse community of microorganisms that live in or on the skin,oral, and gut of humans and animals [7]. It forms a complex ecosystem containing trillions of commensal, and even pathogenic microbes. The external environment, diet, and lifestyle are the most critical factors influencing the composition and vitality of the microbiome. Recent research has demonstrated that the microbiome plays a substantial role in influencing both health and disease. The dynamics of the microbiome encompass their numbers, intricate design, variability, and adaptability for survival. All these factors are involved in developing and treating many health diseases. Conditions ranging from severe ailments like cancer, metabolic disorders, and cardiovascular diseases to even psychiatric disorders such as schizophrenia are directly or indirectly impacted by the microbiome[7,11]. This complex ecosystem comprises diverse microorganisms, including bacteria, archaea, fungi, protozoa, and even viruses.[12]. At least 800 types of bacteria have been isolated from the intestines of healthy people. Additionally, the intestinal viral pool members have recently been identified in the intestine. The virome primarily comprises bacteriophages, plant viruses, and various giant viruses. In contrast to bacteria resident in the intestine, most still need to be recognized due to the technical difficulties in detecting and isolating saprophytic enteric virus. The microbiome's composition is dynamic and continuously changes in response to factors such as nutritional status, environmental factors, lifestyle, health conditions and diseases, and medications such as antibiotics [7,12]. The influence of infectious diseases on microbial dysbiosis and different strategies for its treatment and control are shown in (Fig.1).

The impact of the microbiome on our health and disease states has many directions. They can cause or alter metabolic, auto-immunogenic ,and neurodegenerative diseases [13]. Additionally, they modulate host immune responses and modulate drug interactions. Comprehending their influence on our well-being, it's crucial to recognize that just 10% of the total cells in the human body are of human origin. In contrast, the microbiome constitutes the remaining 90% of our cells, and likewise, only 1% of our body's distinct genes stem from humans. The remaining 99% are attributed to our gut and oral microbiomes. This collectively constitutes the human microbiome, often regarded as our secondary genome[12-14].

Transmission of bacterial zoonotic infections to human by domestic dogs

Zoonotic infections are diseases that can be transmitted between animals and humans. Dogs can act as carriers of various bacteria that have the potential to cause infections in humans. These infections can be transmitted through direct contact with infected dogs, their bodily fluids, or contaminated objects. One common bacterial zoonotic infection transmitted by dogs is Campylobacteriosis. This infection is caused by the bacterium *Campylobacter,* which is commonly found in the intestines of dogs. Humans can become infected by coming into contact with contaminated dog feces or through

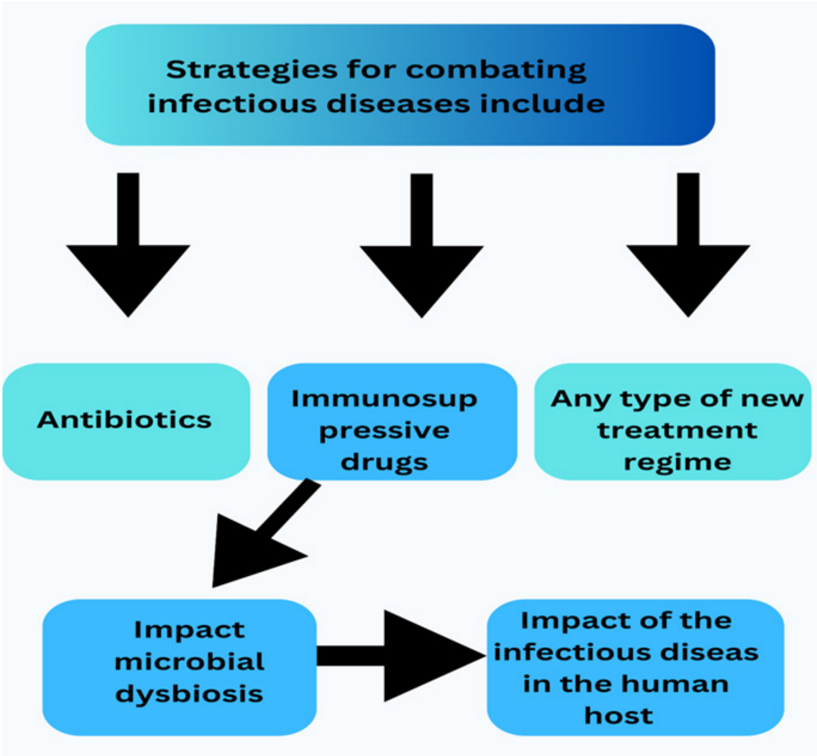

Fig. 1. Strategies for combating infectious disease

consumption of undercooked meat from infected animals. Symptoms of campylobacteria include diarrhea, abdominal pain, fever, and nausea [15,16]. Another bacterial zoonotic infection transmitted by dogs is Salmonellosis. Salmonella bacteria can be present in the intestines of healthy dogs and can be shed in their feces [17]. Humans can become infected by ingesting food or water contaminated with Salmonella or through direct contact with infected dogs. Symptoms of Salmonellosis include diarrhea, fever, abdominal cramps, and vomiting [14,18]. Leptospirosis is a bacterial zoonotic infection that can be transmitted to humans through contact with urine or other bodily fluids of infected dogs. The bacteria responsible for Leptospirosis are called Leptospira and can survive in water or moist environments for extended periods [19] . In humans, this infection can cause a wide range of symptoms, including high fever, headache, muscle aches, jaundice, and kidney failure [18]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is another bacterial infection that can be transmitted from dogs to humans, and it has developed resistance to many antibiotic [17,20]. Dogs can carry MRSA on their skin and in their nasal passages without showing any symptoms. Humans can become infected through direct contact with an infected dog or by touching contaminated objects. MRSA infections can cause skin and soft tissue infections, pneumonia, and bloodstream infections [17,20]. Lastly, dogs can also transmit *Escherichia* to humans. *E. coli* bacteria can be present in the intestines of dogs and can be shed in their feces. Humans can become infected by coming into contact with contaminated dog feces or through consumption of undercooked meat from infected animals. Symptoms of *E. coli* infection include diarrhea, abdominal pain, and fever [21].

The saliva of humans contains Streptococcus and Prevotella [22].However , oral diseases in dogs are more common, as they are in humans that cause extreme pain and may lead to tooth loss. Studying mouth microbial ecology is essential to demonstrating the etiology of oral diseases[23]A study found that the genera isolated from the dog's saliva were Streptococcus, Granulicatella, and Actinomyces [24]. Streptococcus are opportunistic pathogens commonly found to

Table 1. Comparison of the main type of microorganisms

Beneficial role	Characteristics	Microorganism	Prokaryotes
It helps digest food, protects against infections, and even helps maintain reproductive health	Unicellular, prokaryotic, anucleate, with cell membrane	Bacteria	
Improves plant health by promoting growth, inducing resistance and increasing tolerance to abiotic stresses.	Unicellular, prokaryotic, microscopic, without nucleus and with cell membrane.	Archeae	
Convert nutrients and make them available to plants.	Non-vascular, non-motile, heterotrophic organism. They may be unicellular or filamentous. reproduce using spores.	Fungi	Eukaryotes
They can target and kill cancer cells, act as gene and cell therapy tools to treat a variety of genetic diseases, and act as vaccines or agents for vaccine delivery.	They are small, have DNA or RNA genomes, and are obligate intracellular parasites. Viral capsids protect nucleic acids from the environment, and some viruses surround their capsids with a membrane envelope.	Viruses	

Table 2. Indicated the bacterial genus found in the dog's saliva according to previous literatures

Ref.	Sample	Phylum	Class	Family	Genus and species
24	Saliva	Bacillota	Bacilli	Streptococcaceae	*Streptococcus* spp.
		Bacillota	Bacilli	Carnobacteriaceae	*Granulicatella* spp.
		Actinomycetota	Actinomycetia	Actinomycetaceae	*Actinomyces* spp.
		Bacteroidota	Bacteroidia	Bacteroidaceae	*Bacteroides melaninogenicus*
27	Saliva	Actinomycetota	Actinomycetia	Corynebacteriaceae	*Corynebacterium ulcerans*
		Pseudomonadota	Betaproteobacteria	Neisseriaceae	*Neisseria* spp.
		Actinomycetota	Actinomycetia	Nocardiaceae	*Nocardia* spp.
		Microbacteriaceae	Actinomycetia	Microbacteriaceae	*Microbacterium* spp.
		Pseudomonadota	Gammaproteobacteria	Enterobacteriaceae	*Escherichia Proteus*
		Pseudomonadota	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas* spp.
		Bacillota	Bacilli	Lactobacillaceae	*Lactobacillus* spp.
		Actinomycetota	Actinomycetia	Brevibacteriaceae	*Brevibacterium* spp.
32	Saliva	Campylobacterota	Campylobacte-ria	Helicobacteraceae	*Helicobacter* spp.
37	Saliva	Bacillota	Clostridia	Clostridiaceae	*Clostridium* spp.

reside in intestinal, upper respiratory, genital, and lower urinary tracts but might cause septicemia or localized infection in dogs of all ages [25]. In addition, a higher abundance of *Bacteroides melaninogenicus* was found in dogs, which is related to periodontal infections [24]. Furthermore, a study found that the genera isolated from the saliva of the dog were *Brevibacterium, Kurthia, Lactobacillus, Arthrobacter, Pseudomonas, Escherichia, Proteus, Actinomyces, Microbacterium, Nocardia, Streptococcus, Neisseria and Corynebacterium* [26]. *Corynebacterium ulcerans* is known as zoonotic, which releases diphtheria toxin and leads to diphtheria-like illness in people [27]. This bacteria usually infects and prevalent among dogs that can transmit and infect humans [28]. Moreover, *C. ulcerans* is normally carried by wild animals such as game animals [29]. Previous studies have suggested that dogs were asymptomatic carriers of *C. ulcerans* but able to transmit it to humans and other dogs [28,30,31] .A study suggested that *Helicobacter* spp. was found in low abundance in the saliva of dogs in Brazil [32]. In dogs, the main species of Helicobacter are *H. canis ,H. heilmannii*, *H. felis, H. bizzozeronii* and *H. salomonis* [31,33]. In addition, *Porphyromonas cangingivalis* was higher in the dog's saliva [34]. *Porphyromonas* impacts human periodontal disease [35]. *Porphyromonas cangingivalis* is the most prevalent bacteria in dogs and can cause periodontitis [36] . A study conducted in cats and dogs found this species in their specimens includes *Fusobacterium necrophorum, Actinomyces, Bacteroides melaninogenicus, Bacteroides spp, Clostridium perfringens and Clostridium spp* [37] (Table 2).

Prevention and control measures

Good Hygiene Practices

Regularly washing hands with soap and water after handling dogs' waste or any contaminated items is essential. Hand sanitizers can be used when soap and water are not available. **Proper Waste Disposal**: Promptly removing and disposing of dog feces from the environment helps prevent the spread of bacterial infections. Using gloves or plastic bags for waste disposal is recommended. **Routine Veterinary Care**: Regular veterinary check-ups, vaccinations, and deworming of dogs are crucial for preventing bacterial infections. Vaccinations against zoonotic diseases, such as leptospirosis, should be administered as per veterinary recommendations. **Avoiding Contaminated Environments**: Limiting exposure to potentially contaminated environments, such as stagnant water sources or areas heavily soiled with dog feces, reduces the risk of infection transmission[38]. **Education and Awareness**: Educating dog owners about the risks of zoonotic infections and promoting responsible pet ownership practices can contribute to prevention efforts[16].

Advancement in Microbiota Technology

Gram stain

Gram staining, a microbiological method, is named after the Danish bacteriologist Hans Christian Gram, who pioneered its use in 1882 for primarily identifying pneumonia-causing microorganisms [39]. In the initial step of the procedure, either crystal violet or methylene blue is commonly employed as the primary dye [40]. Microorganisms that retain this primary dye and exhibit a purple appearance when viewed under a microscope are Gram-positive. On the other hand, microorganisms that do not have the primary dye show up as red when observed under a microscope and are classified as Gram-negative [40].

Biochemical tests

Here are some commonly used biochemical tests and their significance in determining phenotypic appearance:

The catalase test determines the presence of the enzyme catalase, which breaks down hydrogen peroxide into water and oxygen. It differentiates between catalase-positive organisms (bubbles of oxygen are produced) and catalase-negative organisms (no bubbles are produced). This test is beneficial in identifying certain bacteria [41].

Oxidase test detects the presence of cytochrome c oxidase, an enzyme involved in the electron transport chain. It is used to identify bacteria that possess this enzyme, such as Pseudomonas species. A color change indicates a positive result after adding a reagent containing a chromogenic substrate [42].

The indole test determines the ability of an organism to produce indole from tryptophan, an amino acid. It is commonly used to differentiate between different species of Enterobacteria [43].

The coagulase test is a diagnostic microbiological procedure employed to differentiate between coagulase-positive and coagulase-negative Staphylococcus species. In this test, the ability of the bacteria to produce the enzyme coagulase is assessed. Coagulase is an enzyme that has the capability to induce the clotting of blood plasma. [44].

The nitrate reduction test determines an organism's ability to reduce nitrate to nitrite or other nitrogenous compounds. It is used to differentiate between different species of bacteria, including members of the Enterobacteriaceae family. The test involves adding reagents to detect the presence of nitrite or further reducing nitrate to nitrogen gas.

These biochemical tests provide valuable information about an organism's metabolic capabilities and can aid in identifying and classifying various microorganisms [45].

Antibiotic tests

Antibiotic tests are laboratory procedures used to determine the effectiveness of antibiotics against specific bacteria or microorganisms. These tests are crucial in guiding healthcare professionals in selecting the most appropriate antibiotic treatment for bacterial infections. By assessing the susceptibility of bacteria to different antibiotics, these tests help ensure that patients receive the most effective and targeted treatment. There are several types of antibiotic tests commonly used in clinical settings:

Disk diffusion test

Also known as the Kirby-Bauer test, involves placing antibiotic-impregnated disks on a culture plate containing bacteria. The plate is then incubated, allowing the antibiotic to diffuse into the surrounding agar medium. The formation of a clear zone around the disk indicates that the antibiotic has inhibited bacterial growth, suggesting susceptibility [43].

The minimum inhibitory concentration (MIC) test

This method determines the lowest concentration of an antibiotic that inhibits bacterial growth. It involves preparing a series of antibiotic dilutions and inoculating them with a standardized amount of bacteria. After incubation, the tubes or wells showing no visible growth indicate the minimum concentration required to inhibit bacterial growth [46].

E-test

This method combines aspects of both disk diffusion and MIC tests. It uses strips containing a gradient of antibiotic concentrations placed on an agar plate inoculated with bacteria. The point at which the elliptical zone of inhibition intersects with the strip indicates the MIC value [47].

The broth dilution test

Similar to the MIC test, involves preparing serial dilutions of antibiotics in a liquid broth medium and inoculating them with bacteria. After incubation, the tubes showing no visible growth indicate the minimum inhibitory concentration [48].

Time-kill assay

This test assesses not only bacterial susceptibility but also the bactericidal activity of antibiotics over time. It involves exposing bacteria to different concentrations of antibiotics and periodically sampling to determine bacterial viability [49].

Next Generation Sequencing

The primary application of Next-Generation Sequencing (NGS) in microbiology lies in its ability to replace traditional methods of identifying pathogens based on their appearance, staining properties, and metabolic characteristics with a genetic definition of these pathogens. NGS has facilitated such microbiome explorations via 16SrRNA AND 18SrRNA and ITS analysis. The bacterial types identified in dogs significantly differ from those in humans, with only 16.4% of oral bacterial types shared between dogs and humans based on a 98.5% 16S rRNA[50].

CONCLUSION

Microbiota is the collection of microorganisms living in a particular environment, such as the dog's saliva. They play a crucial role in various ecological processes and significantly impact the health and functioning of their host organisms. Many bacteria (over 600 different types) are found in the dog's mouth. Bacteria in the oral cavities of dogs do have potential health risks for humans. Reinforcing that even their presence as normal flora in canine mouths can lead to multiple infections. Our study builds upon this knowledge by expanding our understanding of bacteria in dog saliva and raising awareness among pet owners and healthcare professionals regarding the potential transmission of pathogenic bacteria from dogs to humans, which can be detected using biochemical tests and next-generation sequencing. Further studies are necessary to assess these bacteria's pathogenicity and transmission dynamics and investigate additional potential pathogens in dog saliva.

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Conflict of Interest

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