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Original Article

Antimicrobial effect of probiotics on bacterial species from dental plaque

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Abstract

Introduction: The antimicrobial role of probiotic *Lactobacillus casei* subspecies *casei* DG (*L. casei* DG) and of the mix culture of probiotic *Lactobacillus acidophilus* LA-5 and *Bifidobacterium* BB-12 was tested on species of *Staphylococcus*, *Streptococcus*, *Pasteurella*, and *Neisseria* genera from supragingival sites from dogs with dental disease of different breed, age, sex, weight, and diet. The research was conducted on these four genera because of their importance in zoonotic infections after dog bites.

Methodology: Species from *Staphylococcus*, *Streptococcus*, *Pasteurella*, and *Neisseria* genera were isolated and identified. To test the antimicrobial efficacy of *L. casei* DG and the mixed culture of probiotic *L. acidophilus* LA-5 and *Bifidobacterium bifidum* BB-12 on the pathogenic species, the agar overlay method was used.

Results: *L. casei* DG had a bactericidal effect on all analyzed species isolated from *Staphylococcus*, *Streptococcus*, *Pasteurella*, and *Neisseria* genera after 24 hours of incubation. The mixed probiotic culture made up of *L. acidophilus* LA-5 and *Bifidobacterium* BB-12 species had no bactericidal effect on the species of *Staphylococcus* and *Streptococcus* genera, which were resistant. However, it had a bacteriostatic effect on several species of *Pasteurella* and *Neisseria* genera.

Conclusions: This work highlights the antimicrobial potential of probiotics *in vitro*, demonstrating that the probiotic *L. casei* DG has a bactericidal effect on all analyzed species isolated from dental plaque and that the mix culture of probiotic *L. acidophilus* LA-5 and *Bifidobacterium* BB-12 has only a bacteriostatic effect.

Key words: Antimicrobial, biofilm, dental plaque, oral cavity, probiotics.

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Introduction

The role of microbial biofilms in oral pathology is a subject of interest for both researchers and clinicians seeking to establish the methods for prevention and treatment of diseases of the oral ecosystem. The presence of pathogenic microorganisms in the oral cavity is one of the main causes of systemic diseases. Since the mid-1960s, scientists in general and dentists in particular have studied the nature of dental plaque and its role in local and systemic pathology [1].

Research in recent decades has led to the recognition of dental plaque biofilm as a well-organized microbial community attached to the tooth surface and as the main cause of the pathological process [2,3]. Currently, the scientific community has shown a great interest in the process of biofilm formation, from oral cavities in humans and animals, because its understanding involves opening new horizons on the pathogenic properties of biofilms [4].

The frequent use of antibiotics in microbial therapy has led to the installation of microorganisms' resistance to their action, which required the discovery of new alternative methods to prevent and control the infectious processes of these products.

Probiotics are live microorganisms, mainly bacteria, that, given in adequate quantities, have a healthy benefit on the host [5,6]. The probiotic microorganisms that are often used for antimicrobial therapy are *Lactobaccillus*, *Bifidobacterium*, and *Streptococcus* [7,8].

In the oral cavity, probiotics hinder the formation of dental plaque, the biofilm build-up on teeth, by

blocking the attachment of microorganism to the surface of teeth. Furthermore, they compete with the bacteria of the oral cavity for nutritive sources, produce chemical substances that lead to the inhibition of the development of pathogenic bacteria, facilitate and adjust the local specific and unspecific immune response, as well as provide other non-immunologic defense mechanisms [7,9-11]. Currently, studies concerning the effect of probiotics on preventing and fighting the formation of dental plaque, of biofilms in the oral cavity, are insufficiently described. Even so, the use of probiotics is a key element in the success of therapy concerning mouth-related conditions [2,12].

According to the National Institute for Infectious Diseases, Bucharest, Romania, in 2014, in Bucharest, 7,907 people received prophylactic treatments after being bitten by dogs [13]. Based on the study of Talan *et al*. [14], the most frequent aerobic bacteria found in wounds caused by dog bites were *Pasteurella*, *Streptococcus*, *Staphylococcus*, *Neisseria*, *Corynebacterium*, *Moraxella*, *Enterococcus*, and *Bacillus*.

In the present study, we highlight the antimicrobial role of probiotic *L. casei* subsp. *casei* DG (*L. casei* DG) and the mix culture of probiotic *L. acidophilus* LA-5 and *Bifidobacterium* BB-12 on *Staphylococcus*, *Streptococcus*, *Pasteurella*, and *Neisseria* species from supragingival sites in dogs.

Methodology

Isolation, identification, and antibiotic resistance

This study focused on isolating bacterial species from four genera (*Staphylococcus*, *Streptococcus*, *Pasteurella*, and *Neisseria*) from dogs with dental diseases because of their importance in the transmission of bacterial zoonosis from dog bites to humans. The research was conducted on samples from 33 dogs with dental diseases, of different breed, age, sex, and diet. Sampling was carried out mainly from incisors, canines, premolars, and superior molars from supragingival sites for microbiological examination, from those presenting dental plaque.

Initially, the collected samples were inoculated on blood agar plates (sheep's blood) for *Staphylococcus*, *Pasteurella*, and *Neisseria* and on Edwards (Oxoid, Hampshire, UK) selective medium for *Streptococcus*, for the purpose of carrying out cultural and biochemical examinations to confirm bacterial genus. After isolation, the strains were stored in the freezer, in cryotubes in brain-heart infusion (BHI) broth (Oxoid) and glycerol at -50°C for further processing.

The identification of the species from *Staphylococcus* and *Streptococcus* genera was carried out using Vitek-2 equipment (BioMérieux, Craponne, France) and for the species of *Pasteurella* and *Neisseria* genera by 16S rDNA polymerase chain reaction (PCR) and sequencing.

To establish the minimum inhibitory concentrations of different antimicrobial substances for *Staphylococcus* and *Streptococcus* species, testing was performed using Vitek-2 equipment and Gram-positive antimicrobial susceptibility testing cards specific for these species.

Staphylococcus species were tested for penicillins (benzylpenicillin, ampicillin/sulbactam, oxacillin), a carbapenem (imipenem), aminoglycosides (gentamicin, kanamycin), fluoroquinolones (enrofloxacin, marbofloxacin), a macrolide (erythromycin), mupirocin, a lincosamide (clindamycin), nitrofurantoin, tetracycline, chloramphenicol, fusidic acid, rifampicin, and trimethoprim/sulfamethoxazole.

The sensitivity to antimicrobial substances of *Streptococcus* species was tested for penicillins (ampicillin, ampicillin/sulbactam, and oxacillin), a carbapenem (imipenem), an aminoglycoside (gentamicin), fluoroquinolones (enrofloxacin, marbofloxacin), a macrolide (erythromycin), a lincosamide (clindamycin), a glycopeptide (vancomycin), tetracycline, chloramphenicol, nitrofurantoin, and trimethoprim/sulfamethoxazole.

The sensitivity to the action of antimicrobial substances for the species of the genera *Pasteurella* and *Neisseria*, identified by the PCR method, was tested by the disk diffusion technique. The sensitivity of the species of the genus *Pasteurella* and *Neisseria* was tested against the following antibiotics routinely used in the laboratory: penicillins (doxycycline, amoxicillin, ampicillin, penicillin G), aminoglycosides (gentamicin, kanamycin), second-generation fluoroquinolones (ciprofloxacin, norfloxacin), flumequinorom, cefazolin, polymyxin B, and tetracyclines.

Biofilm formation

After isolation and identification, these species were tested for biofilm formation capacity *in vitro* in microtiter plates using the technique described by Djordjecvic *et al*. [15]. Briefly, the isolated bacterial species were cultured on BHI agar (37°C, 24 hours) and were used to make an inoculum that matched the 0.5 McFarland standard. This suspension was then diluted 1:30 in BHI broth. After these several dilutions, from each dilution with the tested bacterial species, 150 µL was added to each well, in eight wells per species. The

microtiter plates were incubated at 37°C for 72 hours. After incubation, the broth was removed from each well and the wells were washed twice with 160 µL 0.9% saline solution in order to remove planktonic cells. Crystal violet staining was performed adding 160 µL crystal violet 0.1% solution to each well and incubating the plates for 10 minutes at room temperature. The stain was then removed and the wells were washed twice with 170 µL of saline solution 0.9%. Ethanol 96% was added (170 µL) to each well for distaining for 30 minutes, and then the OD (optical density) was measured at 540 nm using an enzyme-linked immunosorbent assay (ELISA) reader.

Probiotics

The probiotic strains used in this study were *L. casei* DG isolated from the human-use commercial probiotic Enterolactis (in a drinkable solution), and the mixed culture of *L. acidophilus* LA-5 and *Bifidobacterium* BB-12, which was isolated from the human-use commercial product named Eubiotic (in capsules). *L. casei* DG is a patented strain by Sofar and registered with the Pasteur Institute of Paris [16]. *L. acidophilus* LA-5 is part of the Chr. Hansen patented cultures collection and is known under the name of *L. acidophilus*. The strain has been used since 1979 as a food supplement, and no side effects as consequence of human consumption have been reported. *Bifidobacterium* BB-12 is a patented probiotic culture, from the Chr. Hansen cultures collection, known by the name of *B. animalis* subsp. *lactis*. It has been used since 1985 as a food supplement, and no side effects as consequence of human consumption have been reported [17].

The antimicrobial activity of probiotics on pathogenic bacterial species was tested using the agar overlay method described by Karska-Wysocki *et al*. [18], with some modifications.

As a first step, the bacterium *L. casei* DG was isolated from the human-use probiotic and was grown on de Man, Rogosa, Sharpe (MRS) agar medium (Biokar Diagnostics, Allonne, France). The Petri plates were incubated anaerobically in GasPak jars at 37°C for 72 hours. After 72 hours of incubation, a bacterial suspension of *L. casei* DG was prepared in BHI broth. The turbidity of the broth culture was adjusted to match that of the 0.5 McFarland standard. Subsequently, the prepared probiotic suspension was individually inoculated onto MRS plates by swabbing a 2x2 cm area in the center of each plate. The plates were incubated anaerobically at 37°C for 72 hours.

The pathogenic species of *Staphylococcus*, *Streptococcus*, *Pasteurella*, and *Neisseria* genera from stock cultures (kept at -50°C) were cultivated on BHI agar 24 hours before the end of the incubation period of the MRS agar inoculated with *L. casei* DG. The stock cultures were swabbed on BHI agar and incubated aerobically at 37°C for 24 hours. The next day, a suspension of the pathogenic bacteria was prepared with the adjustment of the turbidity to the 0.5 McFarland standard. The plates with MRS agar were then overlaid with 10 mL of molten and cooled BHI agar previously inoculated with 1 mL of the prepared suspension of the pathogen cultures. The agar was allowed to solidify, and the plates were incubated aerobically at 37ºC for 24 hours. The plates were then examined for the presence of growth or inhibition. To further check whether the pathogens were inhibited or killed, the growth inhibition zone was swabbed. The swab was then inoculated into BHI broth and incubated aerobically at 37°C for 24 hours. The broth tubes were then checked for growth. The experiments were repeated three times.

To test the antimicrobial efficacy of the mixed culture of probiotic *L. acidophilus* LA-5 and *Bifidobacterium bifidum* BB-12 for pathogenic species of *Staphylococcus*, *Streptococcus*, *Pasteurella*, and *Neisseria* genera, the same procedure was done and the agar overlay method was used.

Data were analyzed using Student's *t*-test and correlation was done using Excel for Microsoft Office software. P values less than 0.05 were considered statistically significant.

Results

Bacterioscopic and bacteriological examination results led to the identification of species of *Staphylococcus*, *Streptococcus*, *Pasteurella*, and *Neisseria* genera.

The identified species of *Staphylococcus* and *Streptococcus* by Vitek-2 equipment were *S. warneri* (two species: C1 and C17b), *S. intermedius* (seven species: C18c, C19a, C20a, C24b, C25b, C31c, C33c), *S. epidermidis* (one species: C32c), *Strep. canis* (three species: C28c, C28ae, C30ae), *Strep. ovis* (two species: C20, C27), *Strep. sanguinis* (two species: C11 and C13), and *Strep. suis* (three species: C16, C22, C23).

Pasteurella and *Neisseria* species identified by 16S rDNA PCR technique were *P. stomatis* (two species: C2, C23b), *P. canis* (two species: C5, C18b), *P. multocida* (one species: C12a), *N. 3087* (two species: C9, C11a), *N. canis* (two species: C13a, C14b), *N.*

animaloris (one species: C18a), and *N. zoodegmatis* (one species: C26b).

When tested for resistance to antimicrobial substances, *Staphylococcus* species were resistant to benzylpenicillin, tetracycline, enrofloxacin, erythromycin, clindamycin, kanamycin, ampicillin/sulbactam, oxacillin, gentamicin trimethoprim/sulfamethoxazole, marbofloxacin, vancomycin, and imipenem. *S. intermedius* was the most resistant species to all tested antibiotics (Table 1).

Streptococcus species were resistant to ampicillin, clindamycin, trimethoprim/sulfamethoxazole, erythromycin, and vancomycin. Of these, *Strep. canis* was resistant to most antimicrobial substances tested (Table 1). Comparing these findings, there are interspecific and intraspecific differences between the antimicrobial resistances of these species.

Table 1. Antibiotic resistance of *Staphylococcus* and *Streptococcus* isolated species.

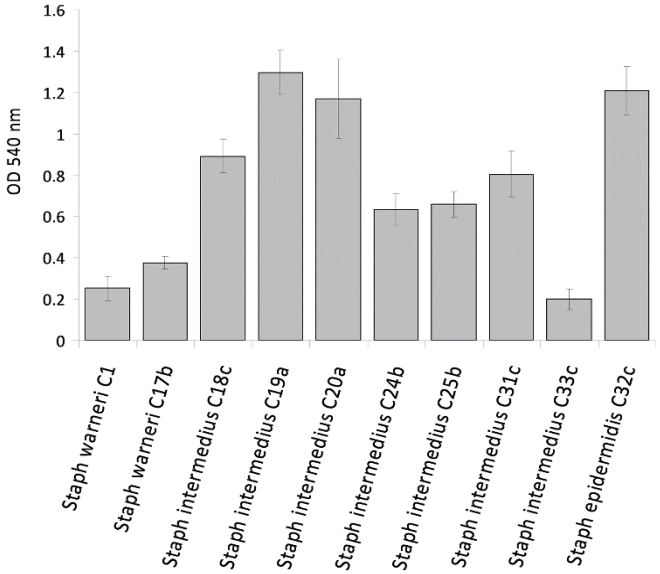
Species	Antibiotic	MIC	Species	Antibiotic	MIC
Staph. warneri **C1**	Benzylpenicillin	≥ 0.5	*Strep. canis* **C28c**	Ampicillin	≥ 32
	Enrofloxacin	≤ 0.5		Erythromycin	≥ 8
	Erythromycin	≥ 8		Clindamycin	≥ 8
	Clindamycin	≤ 0.25	*Strep. canis* **C28ae**	Ampicillin	16
Staph. warneri **C17b**	Benzylpenicillin	≥ 0.5		Erythromycin	≥ 8
	Enrofloxacin	≤ 0.5		Clindamycin	≥ 8
Staph. intermedius **C18c**	Benzylpenicillin	≥ 0.5		Trimethoprim	≤ 10
Staph. intermedius **C19a**	Benzylpenicillin	≥ 0.5		Sulfametoxazole	≤ 10
	Tetracycline	≥ 16	*Strep. canis* **C30ae**	Ampicillin	16
Staph. intermedius **C20a**	Benzylpenicillin	≥ 0.5		Erythromycin	≥ 8
	Kanamycin	≥ 64		Clindamycin	≥ 8
	Erytromycin	≥ 8		Vancomycin	≥ 32
	Clindamycin	≤ 0.25		Trimethoprim	≤ 10
	Tetracycline	≥ 16		Sulfametoxazole	≤ 10
Staph. intermedius **C24b**	Benzylpenicillin	≥ 0.5	*Strep. ovis* **C20**	Ampicillin	8
	Gentamicin	8		Erythromycin	≥ 8
	Kanamycin	≥ 64		Clindamycin	≥ 8
Staph. intermedius **C25b**	Benzylpenicillin	≥ 0.5		Trimethoprim	≤ 10
	Ampicillin	≥ 32		Sulfametoxazole	≤ 10
	Sulbactam	≥ 32	*Strep ovis* **C27**	Ampicillin	≤ 2
	Oxacillin	≥ 4		Clindamycin	≤ 0.25
	Gentamicin	4		Trimethoprim	≤ 10
	Kanamycin	≥ 64		Sulfametoxazole	≤ 10
	Enrofloxacin	≥ 4	*Strep. sanguinis* **C11**	Ampicillin	≤ 2
	Marbofloxacin	≥ 4		Erythromycin	≥ 8
	Erythromycin	≥ 8		Clindamycin	≤ 0.25
	Clinfamycin	≥ 8		Trimethoprim	≤ 10
	Tetracycline	≥ 16		Sulfametoxazole	≤ 10
	Trimetoprim	≥ 320	*Strep. sanguinis* **C13**	Ampicillin	≤ 2
	Sulfametoxazole	≥ 320		Clindamycin	≤ 0.25
Staph. intermedius **C31c**	Benzylpenicillin	0.25		Trimethoprim	≤ 10
	Tetracycline	≥ 16		Sulfametoxazole	≤ 10
Staph. intermedius **C33c**	Benzylpenicillin	≥ 0.5	*Strep. suis* **C16**	Ampicillin	≤ 2
	Vancomycin	≥ 32		Clindamycin	≤ 0.25
	Tetracycline	≥ 16		Trimethoprim	≤ 10
Staph. epidermidis **C32c**	Benzylpenicillin	≥ 0.5		Sulfametoxazole	≤ 10
	Ampicillin	≤ 2	*Strep. suis* **C22**	Ampicillin	≤ 2
	Sulbactam	≤ 2		Clindamycin	≤ 0.25
	Oxacillin	≥ 4		Trimethoprim	≤ 10
	Imipenem	≤ 1		Sulfametoxazole	≤ 10
	Enrofloxacin	≤ 0.5	*Strep. suis* **C23**	Ampicillin	≤ 2
	Trimetoprim	160		Clindamycin	≤ 0.25
	Sulfametoxazole	160		Trimethoprim	≤ 10
				Sulfametoxazole	≤ 10

MIC: minimum inhibitory concentration (μg/mL).

Interspecific and intraspecific differences were also observed among *Pasteurella* and *Neisseria* species. *P. stomatis* (C2) was sensitive to all the antibiotics and only one was resistant to penicillin G (C23b). The same resistance was seen for *P. canis*: one species (C5) was sensitive to all antimicrobial substances, and one species (C18b) was resistant to penicillin G. *P. multicida* (C12a) was resistant to penicillin G and kanamycin. *N. animaloris* (C18a) and *N. zoodegmatis* (C26b) were resistant to penicillin G. One species of *N. canis* (C13a) was resistant to penicillin G, and the other one (C14b) to kanamycin. Both species of *N. 3087* (C9, C11a) were resistant to penicillin G and kanamycin. The resistance to penicillin G and kanamycin was seen at 10 UI and 5 µg. respectively.

Results of the microtiter plate biofilm formation assay (Figures 1, 2, and 3) showed that there were differences in the ability of the isolated strains to form biofilm *in vitro*. The bacterial species that formed biofilm with high density were *S. intermedius* (C19a, C20a), *S. epidermidis* (C32c), *Strep. suis* (C23), *Strep. canis (*C28ae), *P. stomatis* (C23b, C2), and *N. 3087* (C11a). The species that formed biofilm with medium density were *S. intermedius* (C18c, C31c, C25b, C24b), *Strep. canis* (C30ae), *Strep. ovis* (C20), *Strep. canis* (C28c), *Strep. suis* (C22), *Strep. sanguinis* (C13), *Strep. suis* (C16), *Strep. sanguinis* (C11), *Strep. ovis* (C27), *P. canis* (C5, C18b), *N. canis* (C13a) *N. animaloris* (C18a), and *N. zoodegmatis* (C26b). The species that formed the lowest density biofilm were *S. warneri* (C17b, C1), *S. intermedius* (C33c), *P. multocida* (C12a), and *N. canis* (C14b).

Figure 1. Biofilm formation of *Staphylococcus* species (OD: optical density).



A weak positive correlation was found between the number of antibiotics to which the tested species were resistant and the OD of the formed biofilm (r = 0.086 for *Staphylococcus* strains and r = 0.127 for *Streptococcus* strains).

The antimicrobial effect of probiotics (*L. casei* DG and the mixed culture of *L. acidophilus* LA-5 and *Bifidobacterium bifidum* BB-12) were analyzed after 24 and 48 hours of incubation by measuring the halo created (radius in mm) from the probiotic periphery to the growth of the pathogenic strain. The antimicrobial

Figure 2. Biofilm formation of *Streptococcus* species (OD: optical density)

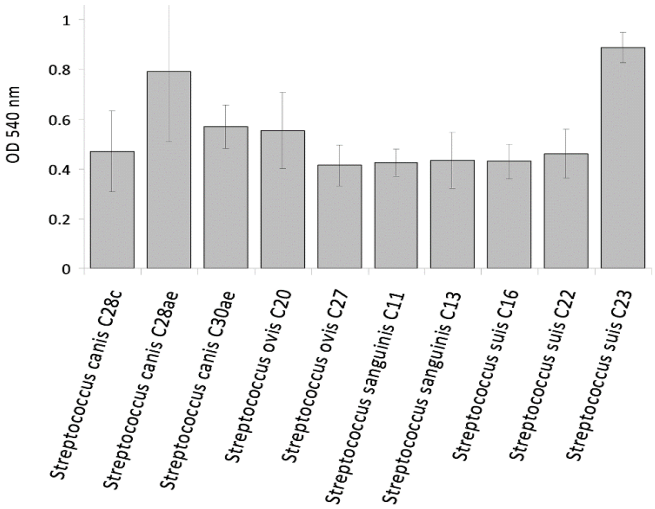
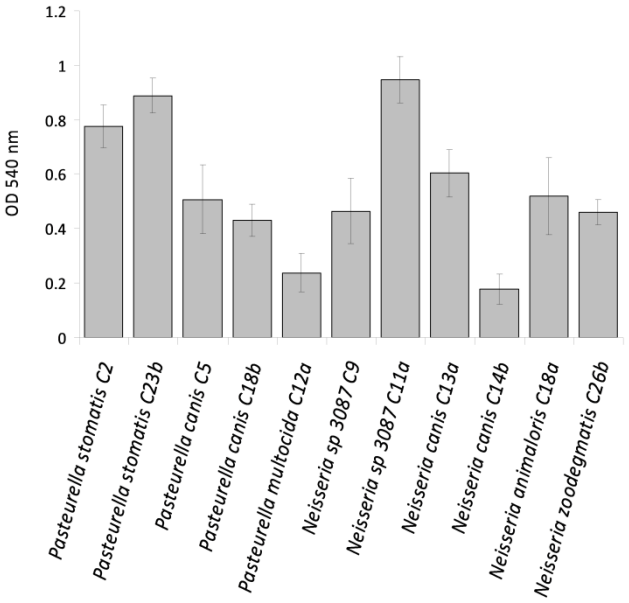


Figure 3. Biofilm formation of *Pasteurella* and *Neisseria* species (OD: optical density).



activity – the bactericidal or bacteriostatic effect of probiotics for each species – are shown in (Figure 4).

After measuring the halo created, its size was noted to have grown slightly during the incubation period, from 24 to 48 hours. After 48 hours, it was found that the probiotic containing *L. casei* DG had a bactericidal effect on all tested bacterial species.

The most conclusive strong bactericidal effect (18–20 mm halo radius) was seen against *S. intermedius* (C18c, C19a, C20a, C25b), *S. epidermidis* (C32c), *Strep. suis* (C22, C23, C16), *P. canis* (C5), *P. multocida* (C12a), and *N. canis* (C13a, C14a), which are known as high-pathogenic bacteria from the oral cavity. The lowest effect but still bactericidal (7–10 mm halo radius) was seen against *Strep. sanguinis* (C13), *Strep. ovis* (C27), *Strep. canis* (C28ae, C30ae, C28c), and *N.* 3087 (C9). For the other species, the bactericidal effect was medium and above medium (10–18 mm halo radius).

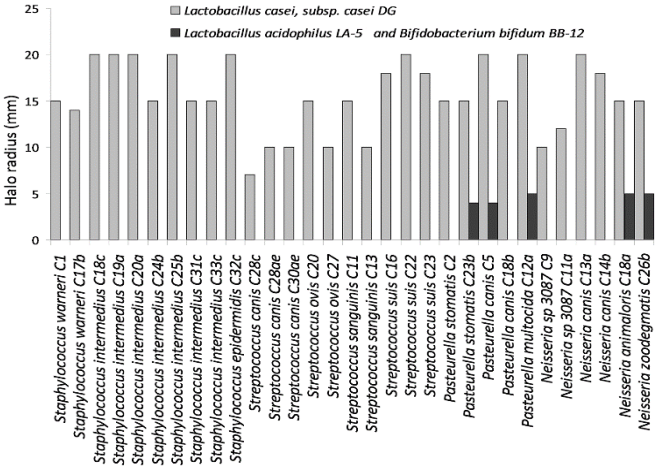
The mixed probiotics culture made up of *L. acidophilus* LA-5 and *Bifidobacterium* BB-12 species had no bactericidal effect on the species of *Staphylococcus* and *Streptococcus* genera, which proved to be resistant, but had a bacteriostatic effect on some species of *Pasteurella* and *Neisseria* genera: *P. stomatis* (C23b), *P. multocida* (C12a), *P. canis* (C5), *N. animaloris* (C18a), and *N. zoodegmatis* (C26b). Student's *t*-test was performed in order to determine if the antimicrobial activity of the two probiotics was different. Significant differences were observed between the antimicrobial effects of the probiotics tested ($p < 0.05$).

Discussion

This study of the antimicrobial role of probiotics to the isolated pathogenic species from the oral cavities of dogs on *Staphylococcus*, *Streptococcus*, *Pasteurella*, and *Neisseria* strains brings novelties to veterinary microbiology. The literature does not report many experiments relying on probiotics as an alternative to antimicrobial therapy against pathogenic bacteria existing in dogs' oral cavities.

It is well known that bacterial cells that grow within a biofilm often exhibit altered phenotypes, such as increased antibiotic resistance. The stable structural properties and proximity of the bacterial cells within the biofilm appears to be an excellent environment for horizontal gene transfer, which can lead to the spread of antibiotic resistance genes among the biofilm inhabitants [19]. Because of the high resistance of the bacterial species tested for antimicrobial resistance in this study, the importance of introducing a novel

Figure 4. Antimicrobial effect of tested probiotics (halo radius in mm).



therapy for therapeutic purposes in oral cavity diseases is highlighted.

The study was conducted on species from four bacterial genera (*Staphylococcus*, *Streptococcus*, *Pasteurella*, and *Neisseria*) because of their importance in the transmission of bacterial zoonosis from dog bites to humans. According to Talan *et al*. [14], some of the aerobic bacteria found in the oral cavities of dogs were isolated from wounds caused by dog bites. The frequency of aerobic bacteria isolated from 50 dog bite wounds was: *Pasteurella*, 50%; *Streptococcus*, 46%; *Staphylococcus*, 46%; *Neisseria*, 32%; *Corynebacterium*, 12%; *Moraxella*, 10%; *Enterococcus*, 10%; and *Bacillus*, 8%. The complications produced by inoculating aerobic and anaerobic bacteria after dog bites included abscesses (67%), purulent wounds (62%), and non-purulent wounds (13%). The aerobic bacteria were identified in non-purulent wounds complicated with infectious cellulitis and lymphangitis (67%), purulent wounds (34%), and abscesses (17%). The same study found that *S. intermedius* was the most frequently identified species from supragingival sites and it was the species with the highest pathogenicity within the *Staphylococcus* genus [14]. Our study confirmed the results obtained by Talan *et al*. [14], in whose study *S. intermedius* had the highest resistance to the antimicrobial substances tested and among the bacterial species that formed dense biofilms.

Regarding the use of antibiotics, Beever *et al*. [20] underlined the responsible use of antibacterial therapy in small animal practice as a consequence of increasing resistance to important antimicrobials that was identified over time in clinical isolates of the *S. intermedius* group from dogs and cats.

The species of *Streptococcus* genera are normally sensitive to the action of the penicillin group, but in this experiment, all species were resistant to ampicillin. *Strep. canis* showed the highest resistance to the tested antimicrobial substances, which included ampicillin, clindamycin, trimethoprim/sulfametoxazole, erythromycin, and vancomycin. These species are important opportunistic pathogens of cats and dogs, infecting a wide range of tissues [21,22]. Of concern are the accumulating reports of human infection (including numerous cases of dog-to-human transmission) [23,24] with clinical manifestations similar to those seen in cats and dogs. For example, descriptions of human cases include soft tissue infection, bacteremia, urinary infection, bone infection, pneumonia, and two reports of death from sepsis [22,25].

Green and Golstein [26] showed that species of *Pasteurella* and *Neisseria* genera have an acquired resistance to penicillin G and kanamycin because of the broad use of these antibiotics for treating local and general infections. These results were also confirmed in the present study after testing the antimicrobial resistance of these species.

The data in this study proved the diversity of species of *Staphylococcus*, *Streptococcus*, *Pasteurella*, and *Neisseria* genera and the broad variability of resistance phenotypes to different classes of antibiotics.

Research carried out within the framework of this experiment confirmed the beneficial effect of the probiotic bacteria in the oral cavity. The antimicrobial activity of probiotics is due to their ability to produce lactic and acetic acids that diminish the medium's pH. As well, the probiotic bacteria compete with the pathogenic strains for nutrient sources, releasing hydrogen peroxide and bacteriocins, whose action are similar to that of antibiotics [27]. The mechanism involved is that the undissociated form of the organic acid enters the bacterial cell and dissociates inside the cytoplasm. Lowering of the intracellular pH or the intercellular accumulation of the ionized form of the organic acid leads to death of the pathogen [28].

Research conducted by Comelli *et al*. [29] showed that *Lactococcus lactis* NCC2211 was able to modulate the growth of the oral bacteria, and in particular, to diminish the colonization of *Strep. oralis* OMZ607, *Veillonella dispar* OMZ493, *Actinomyces naeslundii* OMZ745, and the cariogenic *Strep. sobrinus* OMZ176. These findings confirmed the beneficial effects of probiotics on maintaining the microbial balance of the oral cavity through the destruction of pathogenic bacteria in dental plaque formation and tipping the balance in favor of its beneficial microorganisms. It can be emphasized that similar effects may be seen using the probiotics tested in the present study based on the antimicrobial effect on pathogenic isolates of *Staphylococcus*, *Streptococcus*, *Pasteurella*, and *Neisseria*. Further research can be done using more complex scenarios that involve anaerobic bacterial species with spore-forming capacity.

It was shown that the probiotic *L. casei* DG culture had a bactericidal effect on all tested species of *Staphylococcus*, *Streptococcus*, *Pasteurella*, and *Neisseria* genera. The most powerful bactericidal effect was against *S. intermedius*, *Strep. suis, Strep. canis*, *P. multocida*, *P. canis*, and *N. canis*.

The mixed probiotic culture made up of *L. acidophilus* LA-5 and *Bifidobacterium* BB-12 species had no bactericidal effect on the species of *Staphylococcus* and *Streptococcus* genera, which were resistant. However, it had a bacteriostatic effect on several species of *Pasteurella* and *Neisseria* genera (*P. stomatis*, *P. multocida*, *P. canis*, *N. animaloris*, and *N. zoodegmatis*). Ogawa *et al.* [30] showed that the cytotoxic effect of the undissociated lactic acid can be divided into two phases: the bacteriostatic phase (between 3.2–62 mm) and the bactericidal phase (over 62 mm). Thus, the bactericidal effect depends on lactic acid production and pH reduction effect, a fact that explains the difference in antimicrobial activity between the effective and less-effective species of *Lactobacillus* genera or other probiotics.

Conclusions

This work highlights the antimicrobial potential of probiotics *in vitro*. More specifically, it was demonstrated that the probiotic *L. casei* DG had a bactericidal effect on all multidrug-resistant analyzed species isolated from dental plaque. The mix culture of probiotic *L. acidophilus* LA-5 and *Bifidobacterium* BB-12 had a bacteriostatic effect.

Based on these findings, it can be suggested that the antimicrobial effect of these probiotics can inhibit the growth of the potential pathogen species from the oral cavity and implicitly the biofilm formation, thus reducing zoonotic infections after dog bites.

The results of the study allow us to suggest further research to introduce probiotics in veterinary preparations for use in the prevention and control of dental plaque formation and other dental diseases.

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