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Research Article

In vitro antimicrobial activity of a black currant oil based shampoo versus a chlorhexidine 4% shampoo on bacteria strains isolated from canine pyoderma: A comparative study

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Summary

Over the last few years, antimicrobial shampoo therapy has been increasingly used to treat skin infections in order to reduce systemic use of antibiotics. This study was aimed to compare the *in vitro* bactericidal effect of a black currant oil based shampoo (S1) to a chlorhexidine 4% shampoo (S2) against methicillin-sensitive *Staphylococcus pseudintermedius* (MSSP), methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), *Staphylococcus aureus* (SA), *Escherichia coli* (EC) and *Pseudomonas aeruginosa* (PA) isolates.

A collection of 50 bacterial strains from skin swabs of dogs with superficial recurrent pyoderma was selected: 10 MSSP, 10 MRSP, 10 SA, 10 EC and 10 PA. The two shampoos were blindly tested in duplicate with a microdilution plate method, with scalar concentrations from 1:2 to 1: 256. The MBC was performed for each dilution. A linear regression was used to detect a statistically significance between the two shampoos.

All isolates were completely killed at 1:2 up to 1:16 dilution of the two antiseptic products. At the 1:32 dilution the first bacterial growths were observed, in particular for 2 and 4 strains of MRSP by S1 and S2 respectively. The first lethal dilution for SA was at 1:64 for S1/S2 and only for S2 against SP. No significant difference was observed between the two shampoos according to the results of linear regression significant for: i) MRSP, PA and EC ($p < 0.05$); ii) MSSP and SA ($p < 0.1$).

This study showed that both black currant oil based shampoo and chlorhexidine 4% shampoo have a similar *in vitro* bactericidal activity.

More Information

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Introduction

Over the last decade, antimicrobial shampoo therapy has been increasingly used to treat skin infections [1,2]. This new approach is related to the increasing occurrence of antibiotic resistance in veterinary and human medicine. Thus, new strategies and topical treatments are required to address this issue[1-5]. Creams or ointments containing topical antibiotics may be applied on the skin as well as shampoos with antiseptics (e.g. chlorhexidine 2%-4%, benzoyl peroxide and ethyl lactate [6]). International guidelines suggest the use of topical antimicrobial shampoos and sprays for mild superficial, surface and/or focal infections [7,8]. This approach is particularly important if referred to multi-antibiotic resistant bacteria such as methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) and *Pseudomonas aeruginosa* (PA), as these two bacteria can be difficult to manage using systemic

antibiotics alone [9,10]. In fact, these bacteria may be resistant to all oral antibiotics, so the use of topical antimicrobial products, including antiseptics, is recommended to treat surface skin infections [11]. The active ingredients of topical treatments act primarily on the site of infection and the antimicrobial concentrations obtained may be more effective against resistant strains. Among antiseptics used in topic therapy benzoyl peroxide is reported to be an effective topical antimicrobial [12,13] and chlorhexidine is effective against staphylococci, *Pseudomonas* and *Malassezia* [14,15]. Black currant seed oil is derived from the seeds of *Ribes nigrum* and recently it has been given some attention as a source of PUFAs (Polyunsaturated Fatty Acids), in humans and dogs with AD [16]. It contains an unusually high amount of omega-3 and -6, linoleic and stearidonic acid, flavonoids, phenolic acids and proanthocyanidins. For these reasons it should be preferred in the treatment of inflammatory skin diseases owing to its physiological antiallergic, anticarcinogenic, antihypertensive, antiarthritic and antimicrobial activities [17].

This study was aimed to compare the *in vitro* bactericidal effect of a black currant oil based shampoo (S1) to a chlorhexidine 4% shampoo (S2) against methicillin-sensitive *S. pseudintermedius* (MSSP), methicillin-resistant *S. pseudintermedius* (MRSP), *S. aureus* (SA), *Escherichia coli* (EC) and *P. aeruginosa* (PA) isolates.

Materials and methods

Bacteria isolates and media

A collection of 50 bacteria strains obtained from skin swabs of different dog breeds with superficial recurrent pyoderma was selected. The bacteria strains were represented by 10 MSSP, 10 MRSP, 10 SA, 10 EC and 10 PA. The swabs were inoculated onto Tryptone Soya Agar plates with 5% sheep's blood, Mannitol Salt Agar and McConkey Agar (Oxoid®, Italy), incubated at 37 °C for 24h, before the final reading. Based on phenotypic identification standards, pure isolates were subjected to Gram staining, catalase and oxidase test. Biochemical-enzymatic identification was performed by API® galleries with apiweb database (bioMérieux®, France) and with an acceptable value ≥ 85% for species affinity. The staphylococci that were identified by the system as *Staphylococcus intermedius* are here referred to as *S. pseudintermedius* (SP) based on recent classifications of the related group [18].

To check methicillin-resistant strains Kirby-Bauer oxacillin diffusion test (ODD - 5 µg/mL) was performed, in accordance with the indications of the Clinical Laboratory and Standard Institute [19] and onto selective Brilliance MRSA2 Agar [20] (Oxoid®, Italy). Out of the 50 bacterial strains, 10 are MRSPs, 20 are MSSPs and MSSAs, and 20 bacterial strains (EC and PA) are non-MDR (Multi-Drug Resistant [21]) population.

In vitro susceptibility test

The two biocide shampoo products used in this study (S1 and S2) are shown in table 1, along with their active ingredients. The two shampoos were blindly tested in duplicate with a microdilution plate method [22,23]. The bacterial strains were isolated in pure culture and suspended in saline phosphate buffer (PBS; pH 7.2) up to turbidity equal to 0.5 McFarland, by means of densimeter (DEN-1B, Biosan® Sia, Latvia) and corresponding to a concentration of approximately 10^8 CFU/mL of bacteria. The bacterial suspension was then further diluted 1:100 and to obtain a final concentration of 10^6 CFU/mL it was used for serial dilutions in a 96-well sterile cell culture plate containing 100 µl of the shampoo to be tested, with scalar concentrations from 1:2 to 1: 256. Then, 100 µL of PBS without antiseptic were added to the bacterial suspension and served as positive control (PC), while shampoo alone was used as negative control (NC). After 30 min incubation at + 37 °C, an aliquot of 10 µl was taken from each well and plated onto Tryptone Soya Agar with 5% sheep's blood and finally incubated for a further 24 hours. The count of bacterial colony forming units (CFU) was therefore visually performed on a plate up to a maximum of 100 and then defined as "confluent" when the units were too numerous to be counted individually. The minimum bactericidal concentration [24] (MBC) was defined as the lowest concentration capable of inhibiting bacterial growth of at least 99.9% of the initial population [25] (absence of growth on plate) and was therefore evaluated.

Statistical analysis

A linear regression was used to detect statistically significance between the two shampoos tested, considering the number of bacterial colonies and shampoo dilutions.

Results

In all cases, the microbial isolates incubated in PC line resulted in confluent growth. No growth was seen in the NC wells containing shampoos alone.

The products showed bactericidal activity against all pathogens tested as shown in figure 1. MBCs differed substantially depending on the species of microorganism but usually varied by two-fourfold dilutions only among strains belonging to the same species. All isolates were completely killed at 1:2 up to 1:16 dilution of the antiseptic product (Table 2). At the 1:32 dilution the first bacterial growths were

Table 1: strains growth at progressive dilutions and corresponding CFU. Shampoos used in the study.

	Shampoo	Ingredients	Manufacturer's pH	pH as used
S1	Ribes Pet Ultra shampoo® (NBF Lines®, Milan, Italy)	Blackcurrant seed oil, ac.18β-glycirretic, octopirox, vegetable protein, Vit.E, selected ceramide complex, menthol	6,5	6,5
S2	Clorexyderm® solution 4% (Icf, Cremona, Italy),	Chlorhexidine digluconate 4%, Propylene glycol; Glycerin; Lanolin; Fatty alcohol ethoxylate	n.d.	n.d.

Table 2: strains growth at progressive dilutions and corresponding CFU.

	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	
	MRSP	MRSP	MSSP	MSSP	SA	SA	PA	PA	EC	EC	CFU/ml
1:2	0	0	0	0	0	0	0	0	0	0	0
1:4	0	0	0	0	0	0	0	0	0	0	0
1:8	0	0	0	0	0	0	0	0	0	0	0
1:16	0	0	0	0	0	0	0	0	0	0	0
1:32	4	2	2	0	0	0	4	2	2	2	0-10
1:64	4	4	6	6	6	4	4	4	4	2	11-20
1:128	2	4	2	4	2	4	2	4	4	4	21-50
1:256					2	2				2	> 100

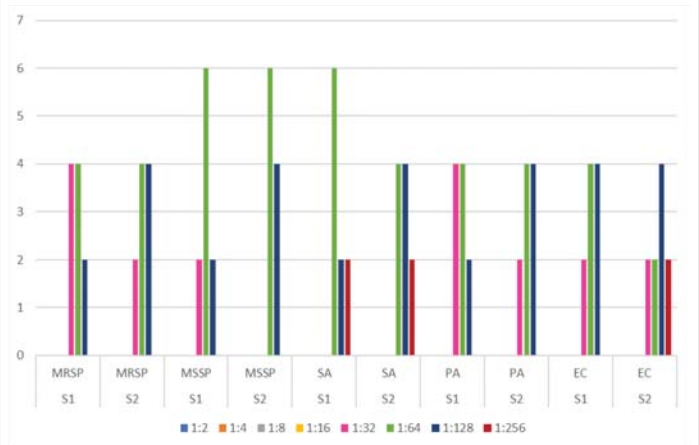

Figure 1: The products showed bactericidal activity against all pathogens tested.

observed, in particular for 2 and 4 strains of MRSP by S1 and S2 respectively. The first lethal dilution for SA was at 1:64 for S1/S2 and only for S2 against SP.

The CFU detected were homogeneous among the different bacterial species and in relation to the dilutions performed it was possible to identify progressive growth ranges up to the 1: 256 dilution with confluent appearance (Table 2).

No significant difference was observed between two shampoos. Only the dilution used was significant for the bacterial growth proliferation for MRSP, PA and EC ($p < 0.05$), MSSP and SA ($p < 0.1$).

Discussion

The black currant oil based shampoo turned out to have an active *in vitro* efficacy against the most common skin bacteria responsible for canine pyoderma. Furthermore, this study revealed that both black currant oil based shampoo and chlorhexidine 4% shampoo have a similar *in vitro* bactericidal activity. Previous research on similar formulations showed *in vitro* antimicrobial characteristics of products for skin use or otology [22-26]. These studies described the bactericidal capacity of known local antiseptics such as gluconate chlorhexidine in different concentrations, Tris-EDTA, isopropyl alcohol, benzoyl peroxide, often associated with low pH values of the disinfectant solution or other components with antimicrobial activity. Chlorhexidine 4% (S2), which can display mechanisms of resistance against Gram-negative bacteria and *S. aureus* [27], here showed killing activity at 1:32 and at 1:64 respectively. The latter dilution (1:64) is the same also for MSSP while S1 shows bactericidal activity at 1:64 only for SA. In all other cases S1 is active up to 1:32. Otherwise the two shampoos differ only in the number of strains killed at the same dilution (Table 2). The black currant oil based shampoo (S1) tested in this study has shown antibacterial and anti-inflammatory activities as well as skin protection and soothing effects, as it claims. However, the real bactericidal efficacy against the most common bacteria responsible for canine superficial pyoderma had never tested *in vitro* until now. Over the past decade, there has been an increasing interest in the use of topical natural products with antimicrobial activity both as a single or an adjuvant therapy for the treatment of superficial bacterial or fungal infections in dogs [4.28.29] . With regards to S1 it is possible to assert that black currant seed oil, as other studies have revealed, displays an *in vitro* antimicrobial activity [30,31]. Unfortunately, it was not possible to evaluate which is the component or which are the components with the specific antibacterial activity. Among the S1 molecules, the antiseptic action of pyroctolamine, thanks to its intrinsic broad-spectrum antibacterial activity towards Gram positive, Gram negative and mycotic germs [32], is well known. The 18 β-glycyrrhetinic acid, vegetable proteins, tocopherol and a complex of ceramides have instead an emollient and protective action against the skin barrier. No antibacterial activity has ever been described for these components. Thus, the authors hypothesize that it is the combination of the components which exhibits an *in vitro* antibacterial action capable of determining bacterial death within 30 minutes of its contact at dilutions four times higher than the starting product.

This study has confirmed how the black currant seed oil based shampoo can be considered an antiseptic with a local action. Furthermore, this product has an antibacterial *in vitro* activity comparable to that of chlorhexidine 4% [23,30] against the most common bacteria responsible for canine pyoderma. Further studies are however required to test the *in vivo* efficacy of this shampoo in controlling superficial skin infections.

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Conflict of interest

One of the authors is an NBF Lanes Consultant

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