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Original article

Topical erythritol combined with L-ascorbyl-2-phosphate inhibits staphylococcal growth and alleviates staphylococcal overgrowth in skin lesions of canine superficial pyoderma

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Abstract

Erythritol (ERT) and L-ascorbyl-2-phosphate (APS) are bacteriostatic, but their effects on staphylococcal skin infections remain unknown. We aimed to determine whether ERT combined with APS inhibits the growth of staphylococci that are commonly isolated from pyoderma skin lesions in dogs. We investigated the individual and combined effects of ERT and APS on the growth of *Staphylococcus pseudintermedius*, *S. schleiferi*, and *S. aureus* using turbidity assays *in vitro.* Skin lesions from 10 dogs with superficial pyoderma were topically treated with 5% ERT and 0.1% APS for 28 days, and swabbed skin samples were then analyzed using 16S rRNA amplicon sequencing and quantitative real-time PCR (qPCR). Results showed that ERT inhibited *S. pseudintermedius* growth regardless of harboring the *mecA* gene, and APS increased the inhibitory effects of ERT against *S. pseudintermedius*, *S. schleiferi*, and *S. aureus in vitro*. Moreover, combined ERT and APS decreased the prevalence of staphylococci on canine skin lesions at the genus level. The combination slightly increased the α-diversity but did not affect the β-diversity of the microbiota. The qPCR results revealed that the combination significantly decreased *S. pseudintermedius* and *S. schleiferi* in skin lesions. Topical administration of EPS combined with APS can prevent staphylococcal colonization on the surface of mammalian skin. The results of this study may provide an alternative to systemic antibiotics for treating superficial pyoderma on mammalian skin surfaces.

Keywords: dog, erythritol, L-ascorbyl-2-phosphate, pyoderma, skin microbiota, staphylococci

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Introduction

Staphylococci are not only commensal bacteria found on the skin and in nasal cavities but also opportunistic pathogens responsible for pyoderma in mammals. For example, *Staphylococcus aureus* causes impetigo, folliculitis, and furunculosis in humans (Kang et al. 2019) as well as in domestic animals such as horses, cattle, sheep, and goats (Scott 2007). In addition, *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi* are frequently isolated from skin lesions of dogs with pyoderma (Kawatami et al. 2010).

In addition to cutaneous infections, cutaneous dysbiosis and *S. aureus* colonization might contribute to disease flares in human atopic dermatitis (AD) (Kong et al. 2012). Canine AD is characterized by dysbiosis and *S. pseudintermedius* colonization on skin lesions (Bradley et al. 2016). Therefore, we postulated that dogs could serve as spontaneous models of staphylococcal skin diseases in humans.

Sugar alcohols form when carbonyl groups of aldoses or ketoses are reduced. They have been used to inhibit the growth of oral and cutaneous bacteria because they exert bacteriostatic effects. For example, xylitol (XYL) inhibits staphylococcal growth in human AD lesions (Akiyama et al. 2002). Erythritol (ERT) is an alcohol with bacteriostatic effects on oral bacteria associated with periodontal disease and tooth decay (Nakakuki 2003). Furthermore, ERT is bacteriostatic against *Staphylococcus* species that cause human axillary odor and inhibits *S. aureus* biofilm formation when combined with chlorhexidine (Fujii et al. 2022). It is also safe for treating dogs (Dean et al. 1996). Sodium suppresses inflammatory and oxidative reactions induced by UVB stimulation in the skin (Nayama et al. 1999). Furthermore, it improves the solubility of the vitamin C derivative, L-ascorbyl-2-phosphate (APS), which is used to treat human acne vulgaris associated with *Cutibacterium acnes* (Ikeno et al. 2015).

Antibiotics such as first-generation cephalexin and amoxicillin are used to treat pyoderma. On the other hand, since the risk of developing multidrug-resistant bacteria exists with antibiotics, topical antibacterial agents such as chlorhexidine, benzoyl peroxide, and ethyl lactate have been used for decades (Domenico 2023), which are less prone to developing resistant bacteria. However, there is concern that these antimicrobials may adversely affect the skin microbiota and impair skin barrier function due to their high bactericidal potency.

These findings suggest that ERT combined with APS could exert bacteriostatic effects against staphylococci in infected skin, but this remains unknown. Dogs can be useful models of human skin diseases such as AD (Murray et al. 2016). Therefore, we investigated the effects of ERT and APS alone and together on staphylococcal infection, growth, and colonization in canine models of spontaneous superficial pyoderma *in vitro* and *in vivo*.

Materials and Methods

Ethics

We followed the guidelines for good clinical practice and the Japanese National Guidelines for the Humane Treatment of Animals (Japanese Ministry of Health, Labour and Welfare Good Clinical Practice 2008). All owners provided written informed consent for their dogs to participate in the present study. Vet Derm Tokyo provided ethical approval for the study as a clinical research project [2021020].

Bacterial strains and inhibition of bacterial growth

Staphylococcus pseudintermedius JCM1751, *S. schleiferi* subsp. *coagulans* JCM7470, and *S. aureus* JCM8703 (Japan Collection of Microorganisms [JCM], Tsukuba, Japan), as well as 18 strains of *S. pseudintermedius* swabbed from the superficial pyoderma lesions of 11 dogs, were seeded in plates containing Luria-Bertani (LB) medium. Thereafter, single colonies were isolated, species were identified, and the *mecA* gene was determined using PCR and multiplex PCR.

The strains were cultured in LB liquid medium at 30°C for 6 h to serve as inocula. We then added 0.6 mL of 802 liquid medium (Fujifilm Wako, Osaka, Japan) comprising 1% Hipolypepton, 0.2% yeast extract, 0.1% $MgSO_4 \cdot 7H_2O$ (pH 7.0), 5, 10, or 15% (w/w) of each of the sugar alcohols ERT, XYL, sorbitol (SOR), or maltitol (MAL) (B Food Science Co. Ltd., Chita-Shi, Japan), and 0.02, 0.1, or 1% (w/w) APS to 96 Deep Well Plates (AxyGen Inc., Union City, NY, USA). Subsequently, each well was inoculated with 6 µL of each bacterial species and cultured at 30°C using an MBR-034P shaker (Taitec Corp., Koshigaya City, Japan) at 1,000 rpm for 17 h. Thereafter, 20 µL each of these cultures (n = 4) was suspended in 180 µL of water in 96-well flat-bottomed plates (4845-96F; Watson Bio Lab., Tokyo, Japan), and turbidity was measured at OD_{660} using a SpectraMax M2 microplate reader (Molecular Devices LLC., San Jose, CA, USA).

Inclusion and exclusion criteria for dogs with superficial pyoderma

This study included 10 dogs with atopic dermatitis (male, n=6; female, n=4) (3 Toy Poodles, Shih Tzu,

Table 1. Clinical scoring for canine superficial pyoderma.

Degree of skin lesions					
Lesions / Score	**0**	**1**	**2**	**3**	**4**
Erythema	None	Slight	Pale	Obvious	Bright
Papule/Pustule	None	Crusted pustule	Erythematous papule	Papule with exudates	Yellowish pustule
Crust	None	Slight	Scattered	Coalesced	Continuous
Alopecia	None	Slight	Mild, recognized in close proximity	Moderate, recognized from distances	Severe
Range of skin lesions					
Lesions / Score	**0**	**1**	**2**	**3**	**4**
Erythema	None	<1% of body surface	1-4% of body surface	5-9% of body surface	≥10% of body surface
Papule/Pustule	None	1-2	3-4	5-9	≥10%
Crust	None	Pin head-sized	Thumb tip-sized	Up to palm size in total dimension	More than palm size in total dimension
Alopecia	None	Covered by regenerated hairs only	1-4% of body surface	5-9% of body surface	≥10% of body surface

Clinical scoring for canine pyoderma was slightly modified from criteria published by Iwasaki et al. (2008) The severity and extent of the four types of skin lesions were scored on a scale of 0 to 4 and the multiplier was calculated as (type) × (severity) × (extent). The minimum score was 0 and the maximum score was 64. The body surface area for a 10-kg canine is approximately 0.46 m^2.

Shibadog, Boston Terrier, Pomeranian, Jack Russell Terrier, Shih Tzu, Maltese) with a median age of 6.5 (5.3±8.8) years diagnosed with superficial pyoderma and clinically scored according to the Guidelines for Clinical Trials of Antimicrobials in Canine Bacterial Pyoderma (Japanese Ministry of Health, Labour and Welfare Good Clinical Practice 2008) with slight modifications (Table 1). Briefly, the diagnosis was confirmed based on clinical signs of papules, erythema, scales, and epidermal collarettes alone or in various combinations and cytological findings of infiltrative neutrophils with extra- and intracellular cocci in skin lesions that were classified as erythema, papule/pustule, crust, and alopecia. Cytological findings were obtained by methanol solidification of a glass slide of the lesion, stained with Wright-Gimsa stain and Gram stain, and specimen examination. The severity and extent of the skin lesions were scored from 0 to 4, and a multiplier was calculated as (type) × (severity) × (extent). The score minima and maxima were 0 and 64, respectively. Exclusion criteria included ectoparasitic infestations with fleas, scabies, demodicosis, cheyletiellosis, pediculosis, and dermatophytic infections within 1 month; or treatment with antimicrobials, medicated shampoos, or anti-inflammatory or immunosuppressive drugs within 2 weeks before participating in the study. The fleas, ticks, lice, etc. were exterminated at least one month prior to the test to ensure that there were no infections other than pyoderma during the test.

Topical solution and microbiota sampling

A 1.5 mLtopical solution containing 5% (w/w) ERT (B Food Science Co., Ltd.) and 0.1% (w/w) APS (Showa Denko K.K., Tokyo, Japan) in purified water was sprayed over the lesions thrice daily for 28 days. Pyoderma lesions were photographed using a digital camera (Olympus, Tokyo, Japan). Typical pyoderma lesions, such as epidermal annuli, were sampled using epidermal microbiota collection kits (Healthcare Systems, Nagoya, Japan). One physician collected all swabbed skin samples at the same hospital throughout the study. Samples were taken from similar lesions before and 28 days after topical application.

Extraction of DNA and 16S rRNA amplicon sequencing

Samples suspended in 2 mL of storage solution were vortex-mixed and pipetted, after which the homogenates (0.4 mL) were shaken and crushed with 0.3 g of zirconium beads (1 mm) by centrifugation at 3,200 × *g* for 20 s in 2-mL vials. Nucleic acids in the supernatants (300 μL) were trapped in glycogen and precipitated with ethanol into 2-mL tubes. The precipitates were dissolved in Tris-EDTA (TE) buffer pH 8.0, and RNA primers for fragments arising during DNA replication were then removed by RNase (Nippon Gene, Tokyo, Japan) at 37°C for 30 min. Contaminating proteins were digested using Proteinase K (Fujifilm

Wako) at 65°C for 10 min. The digest was precipitated with ethanol, dissolved in TE buffer, and purified using NucleoSpin® gDNA Clean-up XS (Macherey-Nagel, Düren, Germany) for next-generation sequencing (NGS). A 16S rRNA library was constructed from purified DNA using the 16S (V3-V4) Metagenomic Library Construction Kit for NGS (Takara Bio Inc., Kusatsu, Japan). Thereafter, sequences were obtained using a 300PE Miseq (Illumina, San Diego, CA, USA). Overlapping paired-end reads were separately processed using the DADA2 pipeline in QIIME 2 (https://Qiime2.org). Unique ASVs were assigned taxonomy and aligned with the Silva 138 reference database at 99% sequence similarity.

Quantitative real-time PCR (qRT-PCR)

Copy numbers of *S. pseudintermedius* and *S. schleiferi* were quantified by real-time qPCR using *S. pseudintermedius* JCM 1751 and *S. schleiferi* JCM 7470, respectively, as references for standard curves. *Staphylococcus pseudintermedius* was amplified using the primers Pse-nucF and Pse-nucR, and *S. schleiferi* was amplified using the primers Sch-nucF and Sch-nucR as described previously (González--Domínguez et al. 2020). Amplification by qPCR proceeded using a Thermal Cycler Dice® Real-Time System III (Takara Bio Inc.) with TB Green FastqPCR Mix (Takara Bio Inc.) as described by the manufacturer under the following cycling conditions: 96°C for 30 s, then 45 cycles at 95°C for 5 s, 60°C for 20 s, and 72°C for 20 s. Reaction specificity was verified from the melting curves of the amplicons, and the number of bacteria/μg of purified DNA was calculated.

Bioinformatics and statistical analyses

The α-diversity of species distribution was calculated as the Shannon index using Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA), and β-diversity was determined by principal component analysis (PCA) and visualized as plots using SPSS v26.0 (SPSS Inc., Chicago, IL, USA). Differences in overall microbiota structure before and after treatment were assessed using the permutational multivariate analysis of variance (PERMANOVA) for Euclidean distance in R (https://www.r-project.org/; R Foundation for Statistical Computing, Vienna, Austria). Significance was analyzed by Tukey, Wilcoxon rank sum, and Mann-Whitney *U* tests using SPSS Statistics v26.0 (SPSS Inc.). Values with $p \leq 0.05$ were considered statistically significant.

Results

Both ERT and APS inhibited S. pseudintermedius, S. schleiferi, and S. aureus growth in vitro

We investigated the effects of various sugar alcohols on the growth of *S. pseudintermedius* JCM 1751, *S. schleiferi* JCM 7470, and *S. aureus* JCM 8703 *in vitro* (Fig. 1a-c). The data in Fig. 1a have been submitted to BMC Veterinary Research under Submission ID 4f6b4bfb-a901-4a6c-b40a-b6ddd494120d. Our results indicated that ERT and XYL significantly inhibited the growth of these staphylococcal strains compared with that of the controls. Furthermore, staphylococcal proliferation *in vitro* was significantly inhibited by 5 and 10% ERT compared with XYL ($p \leq 0.001$ for both) but not by sorbitol or maltitol.

We isolated *S. pseudintermedius* with or without the *mecA* gene from clinical lesions of dogs with superficial pyoderma (Fig. 1d-f) and found that ERT significantly inhibited the growth of these bacteria *in vitro,* regardless of the *mecA* gene.

We also found that < 0.01% (w/w) APS inhibited the *in vitro* growth of the reference strains *S. pseudintermedius*, *S. schleiferi*, and *S. aureus* (Fig. 1g-i) compared with that of the controls. The growth of these strains *in vitro* was significantly inhibited by the combination of ERT and APS compared with ERT alone (Fig. 1g-i).

Topical administration of ERT plus APS alleviates clinical symptoms and inhibits staphylococcal growth in skin lesions of canine superficial pyoderma

After topical administration of ERT plus APS, superficial pyoderma lesions in the dogs improved (Fig. 2a and b), and clinical scores were significantly lower compared with those before administration (Fig. 2c). Changes in the microbiota in pyoderma lesions after topical ERT plus APS administration were analyzed using 16S amplicon sequencing and qPCR. The median Shannon index values increased after therapy, compared with those before therapy with ERT plus APS (Fig. 2d). The PCA plots of epidermal microbiota indicated no apparent differences between before and after treatment (p=0.186, R^2=0.097, Fig. 2e). However, the occupancy rate of *Staphylococcus* bacteria in pyoderma lesions significantly decreased after treatment, whereas those of other bacteria increased at the genus level (Table 2). Figure 3 shows changes in the relative abundance of microbiota across individuals. The qPCR results revealed that topical therapy significantly decreased copy numbers of *S. pseudintermedius* and *S. schleiferi* in skin lesions of superficial

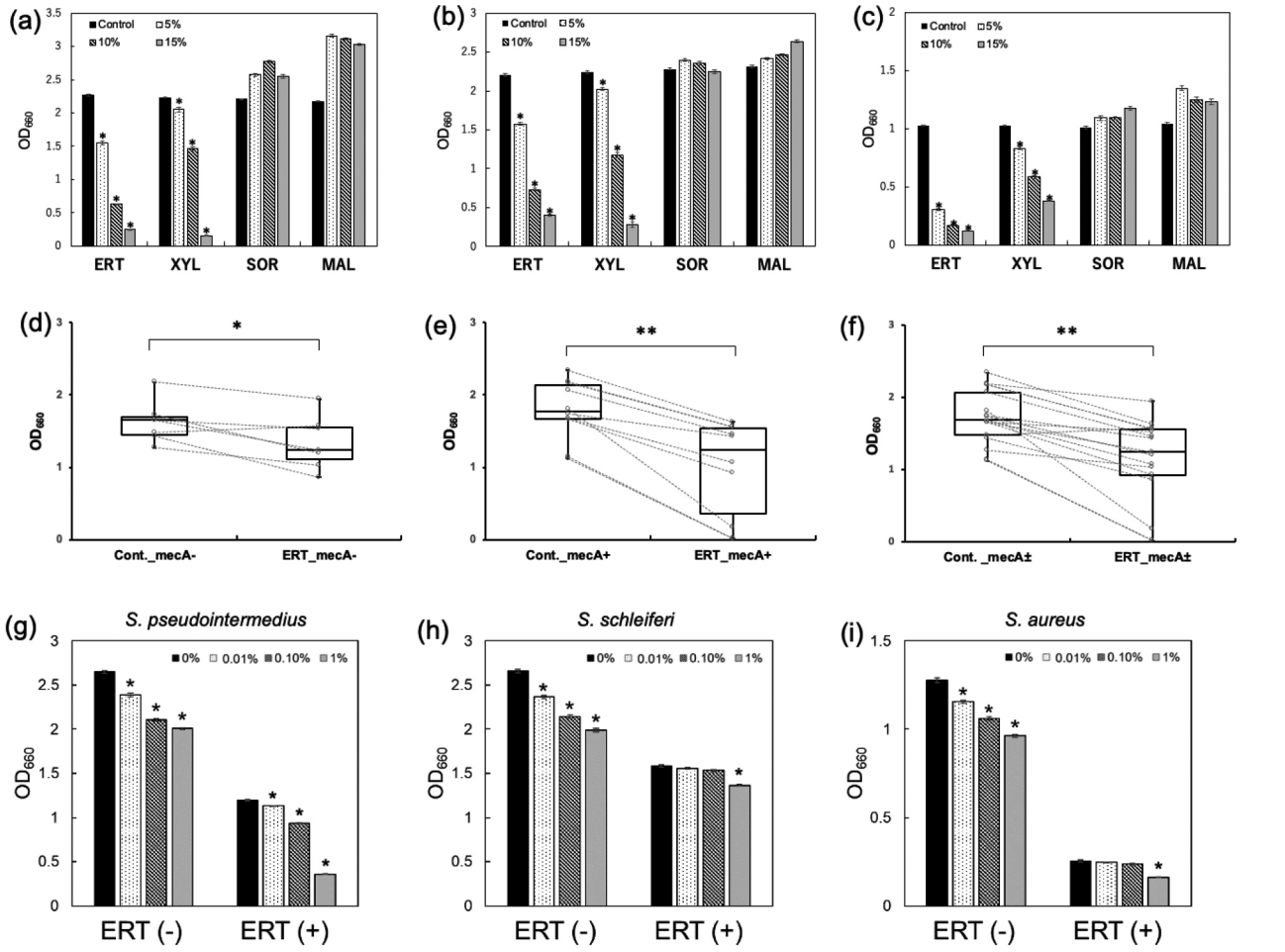

Fig. 1. Effects of erythritol (ERT), xylitol (XYL), sorbitol (SOL), and maltitol (MAL) on the *in vitro* growth of *Staphylococcus* strains. (**a**) *Staphylococcus pseudintermedius*, (**b**) *S. schleiferi*, and (**c**) *S. aureus* strains isolated from dogs with superficial pyoderma were analyzed using turbidity assays *in vitro* and compared with the reference strains. All strains exhibited significantly lower O_{D660} *vs*. controls (* $p<0.05$, Tukey's test; n=4). Eighteen strains of (**d**) *S. pseudintermedius*, including (**e**) 10 *mecA* (+) and (**f**) 8 *mecA* (-) strains, were incubated with 5% (w/w) ERT for 16 h, and turbidity was then assayed *in vitro.* * $p<0.05$, ** $p<0.001$, and ns (Tukey's test, n=4). *Staphylococcus. pseudintermedius* JCM 1751(**g**), *S. schleiferi* JCM 7470 (**h**), and *S. aureus* JCM 8703 (**i**) were incubated for 16 h with or without 5% (w/w) ERT and various concentrations (w/w) of L-ascorbyl-2-phosphate (APS), and turbidity was then assayed *in vitro*. Significantly lower OD_{660} in APS *vs.* controls without ERT or APS (* $p<0.05$, Tukey's test, n=4) and in ERT with APS *vs.* ERT alone ($p<0.05$, Tukey's test, n=4). ns, not significant.

Table 2. Metagenomic analysis of the microbiota of pyoderma lesions identified by 16S rRNA sequencing before and after 4 weeks of topical application of combined erythritol and L-ascorbyl-2-phosphate.

Bacteria	Before	After
Staphylococcus	48.01 (9.25–63.42)	3.83 (0.26–12.92)*
Bacteroides	2.35 (0.60–4.36)	4.02 (2.97–6.86)
Fusobacterium	1.02 (0.62–1.38)	2.40 (0.85–4.94)
Actinomyces	0.92 (0.33–3.08)	1.51 (0.18–3.26)
Corynebacterium	1.29 (0.25–2.36)	3.98 (0.23–4.57)
Porphyromonas	1.77 (0.93–2.50)	0.60 (0.00–2.10)
Neisseria	1.21 (0.08–1.72)	0.20 (0.00–1.84)
Capnocytophaga	1.02 (0.01–1.40)	0.00 (0.00–1.44)
Cutibacterium	1.05 (0.18–1.71)	0.00 (0.00–0.58)
Others	40.08 (24.15–71.85)	68.53 (58.89–74.29)

Data are shown as medians (IQR 25-75%) of occupancy by bacterial genera among total bacteria. Bacteria with occupancy rates of ≥ 0.5% before or after treatment are statistically significant (* $p<0.01$, Mann-Whitney *U* tests, $n=10$). IQR, interquartile range.

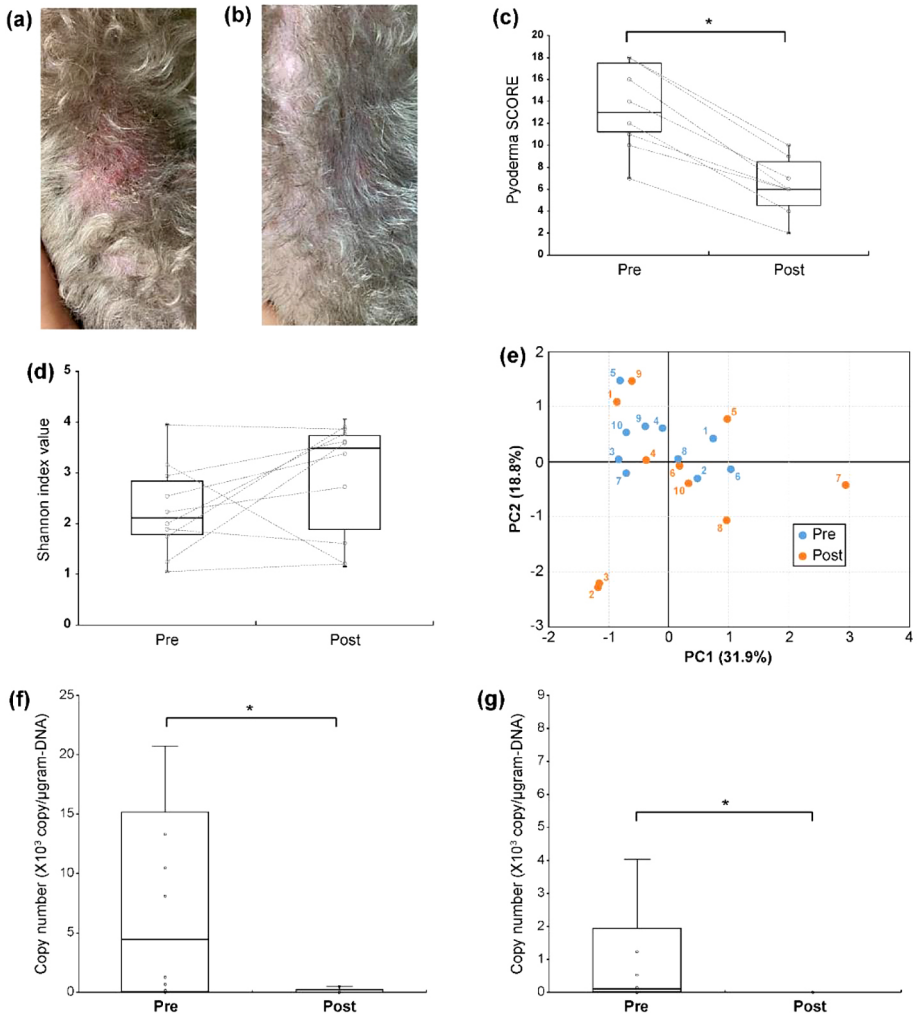

Fig. 2. Topical application of erythritol (ERT) plus L-ascorbyl-2-phosphate (APS) reduced clinical scores and copy numbers of *Staphylococcus pseudintermedius* and *S. schleiferi* in canine superficial pyoderma skin lesions. Clinical presentation of a skin lesion on a 6-year-old female toy poodle (**a**) before and (**b**) after treatment in torso. Methicillin-resistant *S. pseudintermedius* was detected in the skin lesion before topical ERT/APS application. Concomitant medications were not prescribed. (**c**) Clinical scores for canine superficial pyoderma before and after treatment are shown as medians with IQR (25-75%) of 10 dogs. Significant differences in dogs before and after treatment were observed (* $p<0.05$, Wilcoxon rank sum tests, n=10). (**d**) Shannon indexes before and after treatment. (**e**) Two-dimensional PCA analysis. Numbers 1-10 beside symbols are dog IDs. Copy numbers of (**f**) *S. pseudintermedius* and (**g**) *S. schleiferi* before and after topical therapy determined by qPCR. Values before and after treatment are shown as medians with IQR (* $p<0.05$; Wilcoxon signed-rank tests; n=10). ID, identity; IQR, interquartile range.

pyoderma (p=0.038 and p=0.028, respectively, Figs. 2f and g). No adverse events occurred during the clinical study *in vivo*.

Discussion

The present findings revealed that both ERT and XYL inhibited the growth of *S. pseudintermedius*, *S. schleiferi*, and *S. aureus in vitro*. Topical ERT reduces *Staphylococcus* sp. occupancy in the human axillary vault (Fujii et al. 2022). Combining ERT with chlorhexidine can remove biofilms and inhibit *S. aureus* colonization by air-polishing (Drago et al. 2014). Moreover, XYL inhibits *Staphylococcus* growth and biofilm formation in human AD lesions. These findings together implied that ERT and XYL directly inhibit the growth of human commensal staphylococci and reduce their occupancy in cutaneous microbiota. Therefore, both formulations can be safely and effectively applied to prevent bacterial skin diseases or AD in humans by inhibiting staphylococcal overgrowth. However, XYL causes hypoglycemia, seizures, and liver failure, and can be fatal to dogs (DuHadway et al. 2015). Therefore, even topical XYL cannot be used to prevent cutaneous staphylococcal overgrowth in dogs, whereas ERT can safely and effectively prevent staphylococcal overgrowth on the skin of humans and dogs.

The mechanism of *S. pseudintermedius*, *S. schleiferi*, and *S. aureus* growth inhibition by ERT remains

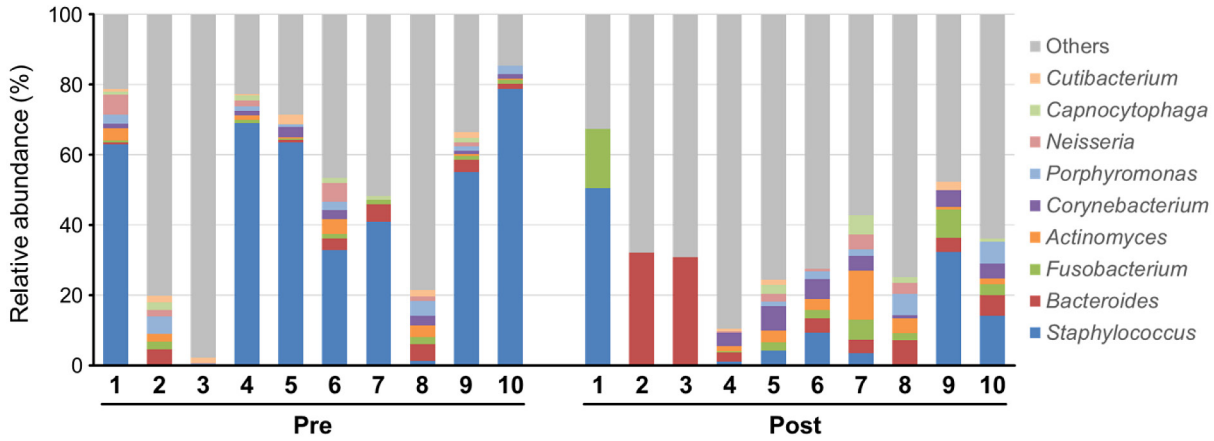

Fig 3. Metagenomic analysis of microbiota of pyoderma lesions identified by 16S rRNA sequencing pre-(week 0) and post-(week 4) ERT-APS topical treatment on individual basis.The relative abundance of each sampled genus is shown relative to all genera sampled. Bacteria with an occupancy rate of ≥ 0.5% pre- or post-treatment are included and bacteria with less than 0.5% are listed as "others." Numbers indicate individuals (1-10).

unclear. Xylitol inhibits the growth of *Staphylococcus mutans* by inhibiting glucose metabolism-related enzymes (Miyasawa-Hori et al. 2006). Erythritol RT suppresses the growth of *S. gordonii*, a resident of the oral cavity, and *Porphyromonas gingivalis*, which causes periodontal disease, by inhibiting metabolic pathways, such as nucleic acid synthesis and glycolysis pathways (Hashino et al. 2013). Based on these findings, we speculate that inhibited enzymes involved in glucose metabolism or nucleic acid synthesis might be the underlying mechanism(s) responsible for the ability of ERT to inhibit staphylococcal growth. Further investigation is needed to elucidate the mechanisms involved.

We showed that ERT combined with APS inhibited the growth of *S. pseudintermedius*, *S. schleiferi*, and *S. aureus in vitro* and that ERT alone can also inhibit staphylococcal growth. However, whether the inhibitory effects found in one study were due to chlorhexidine or ERT has not been specified.

The development of methicillin-resistant staphylococci harboring the methicillin resistance (*MecA*) gene is important in the treatment of canine skin diseases (Norström et al. 2013). Our results showed that ERT inhibited staphylococcal growth regardless of the presence or absence of this gene. This finding might have applications in the treatment of infections by multidrug-resistant *Staphylococcus*. Further detailed studies will be needed to determine the effect of ERT on the growth of drug-resistant bacteria.

The topical application of a mixture of 5% ERT and 0.1% APS improved clinical manifestations and decreased *Staphylococcus* occupancy on skin lesions of canine superficial pyoderma. This combination also exerts synergistic inhibition of S. *pseudintermedius* growth *in vitro*, with α- and β-diversities representing the diversity of bacterial flora and its differences between the two samples, respectively (Shannon, 1948). Microbiota analysis revealed that topical application did not affect cutaneous microbiota diversity in skin lesions but increased diversity in normal skin, although the difference did not reach statistical significance. These findings indicate that therapy using topical ERT with APS might inhibit staphylococcal growth and mildly affect the richness of the cutaneous microbiota in skin lesions of canine superficial pyoderma. Moreover, topical ERT with APS significantly decreased the copy number of *S. pseudintermedius* and *S. schleiferi*, which are primary isolates from skin lesions of canine superficial pyoderma.

Pyoderma is a typical staphylococcal infection, and spontaneous recovery is unlikely. It is usually secondary to underlying disease, such as allergies or endocrine disorders. Despite being a preliminary open study, we showed that topical ERT with APS inhibits the growth of staphylococci. Nevertheless, in the future, randomized, large-scale trials will be essential.

The effects of XYL and ERT have been investigated mostly on oral microbiota (Rafeek et al. 2019, Söderling and Pienihäkkinen 2020). For example, short-term XYL treatment reduces only mutans streptococci without affecting oral flora (Söderling et al. 2015). Furthermore, ERT selectively inhibits the abundant growth of *Staphylococcus* and *Corynebacterium* bacteria that cause odors in the axillary vault (Fujii et al. 2022). The present findings on the antibacterial effects of ERT against staphylococcal infections are in line with these results. We speculate that XYL and ERT maintain microbiota homeostasis by selectively inhibiting the growth of pathogenic bacteria with a high glycolytic activity that comprises a large proportion of lesion sites.

In addition to its anti-inflammatory and antioxidant effects (Woolery-Lloyd et al. 2010, Ikeno et al. 2015),

APS inhibited the growth of staphylococci. However, the underlying molecular mechanisms await elucidation. We suggest that future studies should assess the effects of ERT on staphylococci *in vitro*.

In conclusion, the topical administration of a solution of ERT plus APS inhibited staphylococcal growth and restored a normal cutaneous microenvironment in dogs with superficial pyoderma. Because the etiology and pathophysiology of superficial pyoderma are similar in humans, dogs, and livestock, this approach might also be applicable to prevent pyoderma caused by staphylococcal infections in these and other species. Future large-scale clinical trials should provide additional evidence to help elucidate the bacteriostatic mechanism(s) responsible for the ability of ERT plus APS to inhibit staphylococcal growth. The results of this study may provide an alternative to systemic antibiotics for treating superficial pyoderma on mammalian skin surfaces.

Acknowledgements

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Data Availability: The data that support the study findings are openly available in DDBJ BioProject at https://ddbj.nig.ac.jp/resource/bioproject/PRJDB13737, reference number PRJDB13737 (Submission ID: PSUB017721).

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