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In vitro efficacy of a honey-based gel against canine clinical isolates of *Staphylococcus pseudintermedius* and *Malassezia pachydermatis*

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Background – *Staphylococcus pseudintermedius* and *Malassezia pachydermatis* are important agents in canine pyoderma and otitis.

Hypothesis/Objectives – Determine the *in vitro* efficacy of a honey-based gel (HBO) against meticillin-susceptible *S. pseudintermedius* (MSSP), meticillin-resistant *S. pseudintermedius* (MRSP) and *M. pachydermatis,* by minimum bactericidal concentration (MBC), minimum fungicidal concentration (MFC) and time-kill assay (TKA). Efficacy of the product's honey component (HO) also was evaluated.

Methods – Sixty *S. pseudintermedius* and 10 *M. pachydermatis* canine isolates were selected. All isolates were tested against serial dilutions of an HBO containing 40% HO (40%, 20%, 10%, 5% and 2.5% w/v) and HO alone (undiluted, 40%, 20%, 10%, 5% and 2.5% w/v). Microbroth assay followed by subculture was used to determine MBC and MFC. The same protocol was applied after product exposure to catalase. A well-diffusion assay for *S. pseudintermedius* was used to generate inhibition zones. A TKA for 10 isolates of *S. pseudintermedius* and 10 isolates of *M. pachydermatis* was performed.

Results – MBC was 20% w/v (5–20% w/v) for HBO and HO. HBO had lower MBC values when compared to HO ($P = 0.003$). No statistical difference was observed between MSSP/MRSP isolates (HBO $P = 0.757$, HO $P = 0.743$). Only HO was affected by catalase ($P = 0.015$). MFC for HBO was 10% w/v (5–10% w/v) and 40% w/v for HO (20–≥40% w/v). All isolates were killed after 4 h of exposure.

Conclusions and clinical importance – *Staphylococcus pseudintermedius* and *M. pachydermatis* are susceptible to the HBO and these results can be used for future clinical trials.

Introduction

Staphylococcus pseudintermedius (formerly *S. intermedius)* is the most common pathogen causing canine pyoderma.[1,2] Meticillin-resistant *S. pseudintermedius* (MRSP) was first reported in North America followed by emergence in Europe and Asia.[3–5] Treatment of pyoderma caused by MRSP can be challenging due to the limitations in antibiotic choices, because many of these isolates are also multidrug-resistant.[6] Guidelines have been published for the diagnosis and treatment of folliculitis which support the use of antibiotics and/or topical antibacterial therapy depending on several factors, which include extent of the lesions, and bacterial culture and susceptibility testing results.[6] Topical therapy can be used as a sole treatment or in combination with systemic antibiotics allowing a reduction in duration of antibiotherapy.[7,8] *Malassezia pachydermatis* is a nonlipid-dependent yeast that inhabits the skin and ears of the dog and an important aetiological agent of canine otitis externa.[9–11] Otitis caused by *Malassezia* is normally managed with topical therapy.[12–14]

Honey has been used from ancient times to treat several types of infected wounds including traumatic, venous and diabetic ulcers.[15,16] Recently, medical research has identified bactericidal, bacteriostatic, antiviral, antioxidant and anti-inflammatory activities of honey.[17–21] The antibacterial effect of honey is in part due to its hydrogen peroxide (H_2O_2) activity, which can be inhibited through conversion into water and oxygen by the presence of catalases.[22,23] Catalases are antioxidant enzymes normally present in tissues and chronic wounds and confer protection against oxidative damage.[24] L-Mesitran® Soft, (HBO, Triticum; Maastricht, the Netherlands) is a honey-based gel composed of 40% medical-grade honey (HO) which is marketed for the treatment of superficial and

Abbreviations: CLSI, Clinical Laboratory Standards Institute; DMSO, dimethyl sulfoxide/water; HBO, honey-based gel; HO, Honey; IP, Initial Population; MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration; MRSP, Meticillin-resistant *Staphylococcus pseudintermedius*; MSSP, Meticillin-sensitive *Staphylococcus pseudintermedius*; TCS, Triclosan; TKA, Time-kill assay.

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acute wounds, superficial and partial thickness burns, chronic wounds, acute and postoperative surgical wounds.[25] Preliminary veterinary clinical data of cases treated with HBO suggests efficacy in the treatment of canine intertrigo and otitis; *in vitro* documentation of antimicrobial activity is limited.[26,27]

The objectives of the present study were to evaluate the *in vitro* bactericidal efficacy of HBO against *S. pseudintermedius* isolates from canine pyoderma and to compare susceptibility of both meticillin-susceptible and meticillin-resistant isolates. Additionally, we assessed the *in vitro* antifungal properties of HBO against *M. pachydermatis* using canine clinical isolates from cases of *Malassezia* otitis.

Materials and methods

Microbial isolation and identification

Sixty *S. pseudintermedius* isolates were collected from dogs with pyoderma. PCR was used for speciation and detection of the *mecA* gene using a published method.[28] Oxacillin susceptibility was determined by the Kirby–Bauer technique following the Clinical Laboratory Standards Institute (CLSI) guidelines.[29] The isolates were divided into MRSP (30 of 60) and meticillin-sensitive *S. pseudintermedius* (MSSP) (30 of 60). Isolates were stored at -80°C in a mixture of glycerol 30% (Scharlab S.L.; Barcelona, Spain) and nutrient broth until further analysis. Ten *M. pachydermatis* isolates were collected from canine ears with a diagnosis of *Malassezia* otitis. The isolates were identified based on colony macroscopic characteristics and microscopic cell characteristics after Gram staining. The isolates were stored at −20°C containing 10% glycerol in milk broth until further analysis. All media used were supplied by Oxoid (Oxoid; Hampshire, UK) unless stated otherwise.

Product preparation

The tested products were HBO and HO and provided by the manufacturer. HBO is composed of 40% HO after gamma-sterilization. Other components of HBO include medical-grade hypoallergenic lanolin, propylene glycol, polyethylene glycol 4000, and vitamins C and E. HBO was tested in undiluted form, followed by serial dilutions in nutrient broth,[30–32] which resulted in final concentrations of 20%, 10%, 5% and 2.5% w/v of HO content respectively. HO was tested undiluted and diluted to 40% w/v in nutrient broth in order to match the concentration present in HBO. Further serial dilutions at 20%, 10%, 5% and 2.5% w/v were prepared. All solutions were prepared shortly before testing to ensure H_2O_2 activity. HO was handled aseptically in dark containers in order to prevent degradation of peroxide activity due to light exposure. Products were initially tested for sterility by culturing 10 μL of undiluted product on 5% Sheep Blood agar and MacConkey agar during 24 h at 37°C under aerobic conditions.

Synthesized honey (LH) was used as a control to mimic the high osmolality and acidity of the honey.[33] LH was prepared by mixing 1.5 g sucrose, 7.5 g maltose, 40.5 g D-fructose and 33.5 g D-glucose (Sigma-Aldrich; St Louis, MO, USA) in 17 mL sterile deionized water. The solution was dissolved by briefly heating at 56°C in a water bath and autoclaved at 120°C for 20 min.[30] The concentrations of LH used in the experiment were undiluted, 40%, 20%, 10%, 5% and 2.5% w/v.

Triclosan (TCS) was used as a positive control against *S. pseudintermedius*. An initial stock solution of TCS (Irgasan, Sigma-Aldrich) was prepared in 40% dimethyl sulfoxide/water (DMSO 90%, Neogen; Lexington, KY, USA) with a concentration of 1 g/L. The solution was further diluted in nutrient broth (0.32–0.0003% w/v). Clotrimazole (CS, Canesten 10 g/L solution, Bayer Portugal SA; Barcelona, Spain) was used as a positive control against *M. pachydermatis* after dilution in Sabouraud's broth (0.5−0.0078% w/v).

Well-diffusion assay for *S. pseudintermedius*

Well-diffusion assay was carried out as described previously with minor modifications.[31,32,34] The isolates were diluted in saline to an optical density of 0.15 at 600 nm [previously determined to be approximately 1 x 10^7 colony forming units per mL (cfu/mL)]. Muller–Hinton agar plates were inoculated with sterile cotton swabs, after being immersed in the bacterial suspensions and left to stand for 10 min. After inoculation, four wells were cut into the agar with an 8 mm biopsy punch and filled with 80 μL of each of the honey products. Plates were incubated overnight at 37°C. The diameter of the inhibition halos, including the diameter of the well, was measured using a ruler.

Microbroth dilution assay

Staphylococcus pseudintermedius

Microbroth dilution assay was performed following CLSI guidelines.[29] Briefly, 96-well microtitre plates with round bottom wells (Deltalab S.L., Spain) containing 90 μL of progressive dilutions of the products, were inoculated with 10 μL of bacterial suspension (final inoculum 1–5 × 10^4 cfu/well) and incubated at 37°C for 20 h in aerobic atmosphere. Positive and negative controls were included on all plates/lines including: one well with broth and the micro-organism being tested; one well with honey product and broth; and one well with only nutrient broth. For minimum bactericidal concentration (MBC) determination, 10 μL of each well was subcultured in Muller–Hinton agar and incubated at 37°C for 24 h. Due to the colour and density of HBO, it was not possible to read the minimum inhibitory concentration results in microtitre plates; therefore, subculture agar plates were deemed necessary to allow the determination of MBC. Plates with no growth were recorded as representing bactericidal activity. The MBC was recorded as the lowest concentration where growth was not detected with the unaided eye. All experiments were performed in duplicate.

Malassezia pachydermatis

In order to determine the minimum fungicidal concentration (MFC), the isolates were tested using a similar protocol to that used for *S. pseudintermedius*. A 96-well microtitre plate, containing 90 μL progressive dilutions of the products, was inoculated with 10 μL of the cell suspension (final inoculum 1–5 × 10^5 cfu/well). The plates were incubated at 37°C for 24 h. MFC was determined by subculturing 10 μL of each well in Sabouraud's chloramphenicol agar followed by incubation at 37°C for three days. Positive and negative controls were included on all plates. The experiment was performed in duplicate.

Catalase-treatment of products

Activity of H_2O_2 against the *S. pseudintermedius* isolates was determined by treating both products with catalase. Diluted catalase (Bovine liver catalase, Sigma-Aldrich) was added (1,000 units/mL) to the dilutions of both products described previously and incubated for 1 h at 37°C. Microbroth assay was then repeated as described previously.

Time-kill assay

Time-kill assay (TKA) was based on the Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure.[35] The efficacy of undiluted HBO was assessed against 10 *S. pseudintermedius* (five MSSP and five MRSP) and 10 *M. pachydermatis* isolates. The time-kill protocol was performed at 1 h (T1), 4 h (T4), 8 h (T8), 12 h (T12) and 24 h (T24), with contact times determined based on previously published studies for honey.[36,37] In short, 10 μL of *S. pseudintermedius* or *M. pachydermatis* suspension was added to 1 mL of product, followed by mixing and vortexing for 1 min at 161 ***g*** and incubation at 37°C. At each designated time, 9 mL of sterile saline was added to each testing tube suspension, in order to neutralize the action of HBO. Micro-organism counts at each time point were determined by spreading 10 μL of the solution onto Muller–Hinton

agar, followed by incubation at 37°C for 24 h for *S. pseudintermedius*. For *M. pachydermatis* the inoculum was cultured in Sabouraud's chloramphenicol and incubated at 37°C for three days. Positive growth and negative controls were included. The Initial Population (IP) was determined by adding the same volume of inoculum suspension to a dilution blank containing the same volume as used for HBO testing followed by neutralization, culture and incubation as described before. The calculation of percentage of reduction was performed using the following formula: percentage reduction (PR) = (IP−*T*/IP) x 100 (IP number of viable micro-organisms in the initial population, *T* number of viable micro-organisms in HBO at each time point).

Statistical analysis

Data were analysed using Statistical Package for Social Sciences, v25 (IBM SPSS; Chicago, IL, USA) for Windows.

Results

Results for well-diffusion assay

Partial growth inhibition of *S. pseudintermedius* was observed with HBO at concentrations of 40% and 20%, and HO in pure form and at 40% (Table 1). No inhibition was seen for HBO or HO at lower concentrations or with the synthesized honey.

Minimum bactericidal concentration results for *S. pseudintermedius*

For both HBO and HO, the MBC ranged between 5 and 20% w/v (Table 2). Sixteen isolates had a significantly lower MBC for HBO compared to HO ($P = 0.003$). No statistical difference was observed in MBC values between MSSP and MRSP isolates for any of the products tested (HBO, $P = 0.757$; HO, $P = 0.743$). Following incubation with catalase, there was no change in MBC values for HBO ($P = 0.072$). However, with HO, the MBC increased in 58 of 60 (97%) of the isolates ($P = 0.015$).

Minimum fungicidal concentration results for *M. pachydermatis*

The MFC for HBO ranged between 5 and 10% (Table 3). For HO, the MFC varied between 20 and 40%, apart from two isolates that had MFCs greater than 40%.

Time-kill assay

Exposure of *S. pseudintermedius* and *M. pachydermatis* to pure HBO decreased viability and none of the micro-organisms were able to survive after 4 h of exposure to the product (Table 4).

Discussion

This study documents the antibacterial effect of a honey-based product against *S. pseudintermedius,* the most common agent causing canine pyoderma.[1,38] This product also was effective against *M. pachydermatis* which, along with *S. pseudintermedius*, frequently causes canine otitis externa.[26] The present work also shows that MSSP and MRSP isolates are equally susceptible to HBO. Bactericidal effect was observed at 20% w/v and no difference was seen between MSSP and MRSP isolates. The results obtained for HBO are in agreement with a previous study performed with a small number of meticillin-susceptible and -resistant *Staphylococcus aureus* isolates of human origin.[39]

Our results suggest that HBO has a higher antibacterial activity when compared with HO. This is likely due to the presence of other components in the gel, such as medical-grade hypoallergenic lanolin, propylene glycol, polyethylene glycol 4000, and vitamins C and E. This work does not evaluate the antibacterial effect of each

Table 2. Minimum bactericidal concentration (MBC) of HBO and HO with the percentage of dead isolates of *Staphylococcus pseudintermedius* for each dilution

Product concentration	HBO	HO
2.5% w/v	0	0
5% w/v	12	13
10% w/v	83	62
20% w/v	5	25
40% w/v	100	100
Undiluted	N/A	100

HBO honey-based gel, HO medical-grade honey, N/A not applicable.

Table 3. Minimum fungicidal concentration (MFC) of HBO and HO with the percentage of dead isolates of *Malassezia pachydermatis* for each dilution

Product concentration	HBO	HO
2.5% w/v	0	0
5% w/v	20	0
10% w/v	80	0
20% w/v	100	40
40% w/v	100	40
Undiluted	N/A	20

HBO honey-based gel, HO medical-grade honey, N/A not applicable.

Table 1. Comparison of zones of inhibition of growth of canine Staphylococci isolates in honey-based gel (HBO) and medical-grade honey (HO)

Composition of honey product	Mean zone of inhibition (mm)	SD	*P*-value
HBO 40%	34.30	2.39	$P = 0.001$
HBO 20%	24.75	3.87	
HO undiluted	37.48	4.01	$P = 0.001$
HO 40%	30.85	2.2	
HBO 40%	34.30	2.39	$P = 0.5$
HO 40%	30.85	2.2	

SD standard deviation.

Table 4. Percentage of reduction in viability of isolates of *Staphylococcus pseudintermedius* and *Malassezia pachydermatis* at different contact times with undiluted honey-based gel (time-kill assay)

Undiluted honey-based gel	T1 (1 h)	T4 (4 h)	T8 (8 h)	T12 (12 h)	T24 (24 h)
S. pseudintermedius	98.24%	99.31%	100%	100%	100%
M. pachydermatis	99.57%	99.95%	100%	100%	100%

component, but it is likely that other components contribute to the enhanced antibacterial effect of the gel. Propylene glycol is widely used as an excipient, whereas it also has antibacterial activity against *S. aureus,*[40] *Streptococcus mutans, Enterococcus faecalis* and *Escherichia coli*.[41] Vitamin C is an antioxidant and can improve healing in partial-thickness burns when mixed with honey, vitamin E and polyethylene glycol 4000.[42]

The activity of H_2O_2 is one of the most important components in honey and can be inhibited by the presence of catalases.[22] Catalase enzymes convert H_2O_2 into water and oxygen.[23] Catalases are antioxidant enzymes that are part of the natural defence against oxidative damage and are often found in tissue and chronic wounds, potentially rendering H_2O_2 inactive.[24] Activity of H_2O_2 can be determined by testing the honey with catalase and observing a decrease in the bactericidal effect. This work shows that HBO's antibacterial effect is not dependent on H_2O_2. In contrast, HO alone was affected by catalase activity, resulting in an increase of the MBC. Our data suggest that during HBO preparation H_2O_2 is lost. It is known that H_2O_2 activity in honey decreases when honey is diluted,[22] or with time due to degradation.[43] A bactericidal effect independent of H_2O_2 is an advantage, because antibacterial activity will be less affected by the catalases present in wounds and fluids.[44]

The discrepancy between MBC and inhibition halos might be due to the denser and stickier texture of HBO compared to HO, which probably affected the diffusion of the product in the agar plate. A similar study demonstrated that microbroth assay results had greater sensitivity when compared with well- and disk-diffusion assays for manuka honey.[31]

Time-kill tests are commonly used for the determination of bactericidal and antifungal effects. They can easily demonstrate the antimicrobial or antifungal effect of a product and evaluate it over time.[45] Our results demonstrate the effectiveness of HBO in killing *S. pseudintermedius* and *M. pachydermatis* isolates as quickly as after 1 h of exposure. A complete bactericidal and antifungal effect for both micro-organisms was observed after 4 h of exposure time. The biocidal activity of HBO was tested using a biocidal activity assay in five *S. pseudintermedius* isolates collected from canine ears.[26] That study concluded that HBO had a biocidal activity but the time necessary for killing effect was not determined. Another study with a similar product demonstrated its effectiveness against *Candida albicans* (L-Mesitran® gel containing 30% of medical honey from Triticum). In the same study, yeast growth was reported within the first hour, followed by absent growth at 24 and 48 h. This study also reports its effectiveness in women with candidiasis vaginitis. A decrease or absence of the micro-organism and inflammatory cells was noticed microscopically after seven days of treatment.[46] To the best of the authors' knowledge, there are no time-kill studies using HBO against *S. pseudintermedius* and *M. pachydermatis*.

Several studies have demonstrated the bactericidal effect of honey in the treatment of chronic ulcers, wounds, partial-thickness burns and post-surgical infection sites in human medicine.[15,18,47] Studies in animals are much more limited; a pilot study reported the efficacy of the application of HBO for the treatment of 13 surface pyoderma lesions in dogs in which 85% healing occurred after 14 days. The only adverse effect was pruritus after application in two dogs.[27] Considering our results, the application of HBO in superficial and deep pyoderma lesions seems to be a viable treatment option, particularly in focal lesions and in cases where no other antibacterial option is available.

HBO revealed fungicidal activity in all *M. pachydermatis* isolates. The MFC observed was 10% which suggests that the product can be diluted and still maintain antifungal activity. The gel had been previously reported to be effective in the management of canine otitis due to *Malassezia*. In a clinical trial, 15 dogs with bacterial and/or yeast otitis were treated daily with application of the product for 21 days. At the end of the study, 90% of the dogs were deemed to be clinically cured and there was a decrease in the number of micro-organisms on cytological samples.[32] The proprietary honey content in the gel may not be the only ingredient active against *M. pachydermatis*. Propylene glycol is reported to be beneficial in the control of seborrhoeic dermatitis of the scalp due to *M. furfur*, formerly *Pityrosporum orbiculare* or *P. ovale*.[48] Ascorbic acid has an indirect antifungal activity by enhancing the activity of sesquiterpenoids against *M. furfur*.[49] Vitamin E is not an antimycotic agent, although one study reported low levels of vitamin E in individuals affected with seborrhoeic dermatitis due to *Pityrosporum* yeasts, when compared to normal controls.[50] To the best of the authors' knowledge, lanolin and polyethylene glycol have not been reported as antimycotics against *Malassezia* yeasts. Antifungal effects of lanolin, polyethylene glycol and vitamins, or the combination of all ingredients, cannot be ruled out in the present work.

The results of our study demonstrate that HBO is effective *in vitro* at killing *S. pseudintermedius* and *M. pachydermatis*; further studies are needed to describe the detailed mechanisms of action of the product against both pathogens with a larger number of isolates, as well as to further document the *in vivo* effectiveness of the product in the treatment of clinical cases of canine pyoderma and otitis externa.

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Résumé

Contexte – *Staphylococcus pseudintermedius* et *Malassezia pachydermatis* sont d'importants agents des pyodermites et otites canines.

Hypothèses/Objectifs – Déterminer l'efficacité *in vitro* d'un gel à base de miel (HBO) contre les MSSP (*S. pseudintermedius*), les MRSP (meticillin-resistant *S. pseudintermedius*) et *M. pachydermatis* par MBC (minimum bactericidal concentration), MFC (minimum fungicidal concentration) et TKA (time-kill assay). L'-ficacité d'un produit contenant du miel (HO) a également été évalué.

Méthodes – Soixante souches de *S. pseudintermedius* et 10 *M. pachydermatis* ont été sélectionnées. Toutes les souches ont été testées contre des dilutions sériques d'un HBO contenant 40% d'HO (40%, 20%, 10%, 5% et 2.5% w/v) et HO seul (non-dilué, 40%, 20%, 10%, 5% et 2.5% w/v). Les MBC et MFC ont été déterminées par microdilution en milieu liquide et sous-culture. Le même protocole a été appliqué après exposition des produits à la catalase. Un test de bonne diffusion pour *S. pseudintermedius* a été utilisé pour générer des zones d'inhibition. Un TKA pour 10 souches de *S. pseudintermedius* et 10 souches of *M. pachydermatis* a été réalisé.

Résultats – La MBC était 20% w/v (5–20% w/v) pour HBO et HO. HBO avait de plus faibles valeurs de MBC en comparaison à HO ($P = 0.003$). Aucune différence statistique n'a été observée entre les souches MSSP/MRSP (HBO $P = 0.757$, HO $P = 0.743$). Seul HO a été affecté par la catalase ($P = 0.015$). La MFC pour HBO était 10% w/v (5–10% w/v) et 40% w/v pour HO (20–≥40% w/v). Toutes les souches ont été tuées après 4h d'exposition.

Conclusions et importance clinique – *Staphylococcus pseudintermedius* et *M. pachydermatis* sont sensibles à un HBO et ces résultats peuvent être utilisés pour de futurs tests cliniques.

Resumen

Introducción – *Staphylococcus pseudintermedius* y *Malassezia pachydermatis* son agentes importantes en pioderma y la otitis canina.

Hipótesis/Objetivos – Determinar la eficacia *in vitro* de un gel a base de miel (HBO) contra *S. pseudintermedius* sensible a meticilina (MSSP), *S. pseudintermedius* resistente a meticilina (MRSP) y *M. pachydermatis*, por concentración bactericida mínima (MBC) , concentración mínima fungicida (MFC) y ensayo de tiempo de muerte (TKA). La eficacia del componente de miel del producto (HO) también se evaluó.

Métodos – Se seleccionaron 60 cepas caninas de *S. pseudintermedius* y 10 de *M. pachydermatis*. Todos los aislamientos se probaron contra diluciones seriadas de una HBO que contenía 40% HO (40%, 20%, 10%, 5% y 2,5% p/v) y HO solo (sin diluir, 40%, 20%, 10%, 5% y 2.5% p/v). El ensayo en microcaldo seguido de subcultivo se usó para determinar MBC y MFC. El mismo protocolo se aplicó después de la exposición del producto a la catalasa. Se usó un ensayo de difusión de *S. pseudintermedius* para generar zonas de inhibición. Se realizó una TKA para 10 aislamientos de *S. pseudintermedius* y 10 aislamientos de *M. pachydermatis*.

Resultados – la MBC fue del 20% p/v (5-20% p/v) para HBO y HO. HBO tenía valores más bajos de MBC en comparación con HO (P =0,003). No se observó diferencia estadística entre los aislados de MSSP/MRSP (HBO $P = 0,757$, HO $P = 0,743$). Solo HO fue afectado por catalasa ($P = 0,015$). MFC para HBO fue 10% p/v (5-10% p/v) y 40% p/v para HO (20-≥40% p/v). Todos los aislados murieron después de 4 h de exposición.

Conclusiones e importancia clínica – *Staphylococcus pseudintermedius* y *M. pachydermatis* son susceptibles a una HBO y estos resultados pueden usarse para futuros ensayos clínicos.

Zusammenfassung

Hintergrund – *Staphylococcus pseudintermedius* und *Malassezia pachydermatis* sind wichtige Faktoren bei der caninen Pyodermie und Otitis.

Hypothese/Ziele – Die Feststellung der *in vitro* Wirksamkeit eines auf Honigbasis bestehenden Gels (HBO) gegen Methicillin-empfindliche *S. pseudintermedius* (MSSP), Methicillin-resistente *S. pseudintermedius* (MRSP) und *M. pachydermatis* mittels minimaler bakterieller Konzentration (MBC), minimaler fungizider Konzentration (MFC) und Time-Kill Assay (TKA). Die Wirksamkeit der Honigkomponente des Produkts (HO) wurde ebenfalls evaluiert.

Methoden – Sechzig *S. pseudintermedius* und 10 *M. pachydermatis* Isolate von Hunden wurden ausgewählt. Alle Isolate wurden mittels Serienverdünnung eines HBO enthaltenden 40% HO (40%, 20%, 10%, 5% und 2,5% w/v) und HO alleine (unverdünnt, 40%, 20%, 10%, 5% und 2,5% w/v) ausgetestet. Ein Mikrobouillon Assay, gefolgt von einer Subkultur wurde verwendet, um MBC und MFC zu bestimmen. Dasselbe Protokoll wurde angewendet, nachdem das Produkt einer Katalase ausgesetzt war. Ein Well-Diffusionstest für *S. pseudintermedius* wurde verwendet, um Inhibitionszonen zu schaffen. Ein TKA für 10 Isolate von *S. pseudintermedius* und 10 Isolate von *M. pachydermatis* wurde durchgeführt.

Ergebnisse – Die MBC betrug 20% w/v (5-20% w/v) für HBO und HO. Das HBO zeigte niedrigere MBC Werte im Vergleich zum HO ($P = 0{,}003$). Es konnte kein statistischer Unterschied zwischen MSSP/MRSP Isolaten (HBO $P = 0{,}757$, HO $P = 0{,}743$) beobachtet werden. Nur die HO wurde durch Katalase beeinflusst ($P = 0{,}015$). Die MFC für das HBO betrug 10% w/v (5-10% w/v) und 40% w/v für das HO (20-≥ 40% w/v). Alle Isolate waren nach einer 4 stündigen Exposition abgetötet.

Schlussfolgerungen und klinische Bedeutung – *Staphylococcus pseudintermedius* und *M. pachydermatis* sind für ein HBO empfänglich und diese Ergebnisse können in zukünftigen Versuchen angewendet werden.

要約

背景

Staphylococcus pseudintermedius及び Malassezia pachydermatisは、犬の膿皮症や耳炎における主要な病原体である。

仮説/目的 – 本研究の目的は、メチシリン感受性 S. pseudintermedius(MSSP)、メチシリン耐性 S. pseudintermedius(MRSP)および M. pachydermatisに対するハチミツゲル(HBO)のin vitroにおける効能を、最小殺菌濃度(MBC)、最小殺真菌濃度(MFC)および最小殺菌時間評価(TKA)を基に判定することである。また、製品中に含まれる蜂蜜成分(HO)の有効性も評価した。

方法 – 犬由来S. pseudintermedius 60株とM.pachydermatis 10株を供した。全株に対し、段階希釈した40%HO含有HBO(40%、20%、10%、5%、2.5%w / v)、段階希釈したHO(希釈なし、40%、20%、10%、5%、2.5% w / v)を適用した。 MBCおよびMFC値については、微量液体法後に継代培養を行い決定した。またカタラーゼ曝露後の製品についても同様の検討を行った。 S. pseudintermediusの阻止円形成についてはウェル拡散法を用いて評価した。TKAは S. pseudintermediusおよびM. pachydermatisそれぞれ10株について実施した。

結果 – MBCは、HBO群およびHO群のいずれも20%w / v(5-20%w / v) であった。 HBO 群におけるMBC値はHO群よりも低値を示した(P = 0.003)。 MSSP / MRSP分離株間で統計学的有意差は認められなかった(HBO P = 0.757、HO P = 0.743)。 HO群のみでカタラーゼによる影響が認められた(P = 0.015)。MFCは、HBO群では10%w / v(5˜10%w / v)であったのに対し、HO群では40%w / v(20˜40%w / v)であった。全ての分離株は、曝露後4時間で死滅した。

結論と臨床的重要性

Staphylococcus pseudintermediusおよびM.pachydermatisはHBOに感受性を示すため、本研究の結果は今後の臨床試験に応用可能である。

摘要

背景

假中间型葡萄球菌和马拉色菌是引起犬脓皮症和耳炎的重要原因。

假设/目的 — 通过最小杀菌浓度(MBC)、最小杀真菌浓度(MFC)和时间杀菌试验(TKA),确定蜂蜜成份凝胶(HBO)对甲氧西林敏感假中间型葡萄球菌(MSSP)、甲氧西林耐药假中间型葡萄球菌(MRSP)和厚皮马拉色菌,在体外试验中的抗菌功效。也同时评估含有蜂蜜成分产品(HO)的功效。

方法

自犬身上分离60株假中间型葡萄球菌和10株马拉色菌。连续稀释含有40%HO的HBO(40%、20%、10%、 — 5%和2.5%w/v)和单独HO(未稀释、40%、20%、10%、5%和2.5%w/v),将其作用于所有菌株的抗菌试验。微量肉汤试验随后继代培养,用于确定MBC和MFC。产品接触过氧化氢酶后,做同样的试验。对假中间型葡萄球菌进行打孔扩散试验,确定抑菌环。对10株假中间型葡萄球菌和10株马拉色菌进行时间杀菌试验。

结果 — HBO和HO的MBC是20%w/v(5–20% w/v)。与HO相比,HBO的MBC更低(P = 0.003)。MSSP和MRSP菌株组无统计学差异(HBO P = 0.757, HO P = 0.743)。只有HO对过氧化氢酶有反应(P = 0.015)。HBO、HO组的MFC分别是10% w/v (5–10% w/v)和40% w/v (20–≥40% w/v)。接触药物4h后,所有菌株均被杀死。

结论和临床意义
假中间型葡萄球菌和厚皮马拉色菌对HBO敏感,这些结果可以用于未来的临床试验。

Resumo
Contexto – *Staphylococcus pseudintermedius* e *Malassezia pachydermatis* são agentes infecciosos importantes nas piodermites e otites caninas.
Hipótese/Objetivos – Determinar a eficácia *in vitro* de um gel à base de mel (HBO) contra *S. pseudintermedius* suscetíveis à meticilina (MSSP), *S. pseudintermedius* resistentes à meticilina (MRSP) e *M. pachydermatis,* através de concentração bactericida mínima (CBM), concentração fungicida mínima (CFM) e ensaio de tempo para matar (ETM). A eficácia do composto de mel (HO) do produto também foi avaliada.
Métodos – Selecionou-se 60 isolados caninos de *S. pseudintermedius* e 10 de *M. pachydermatis*. Todos os isolados foram testados com variadas diluições de um HBO contendo 40% HO (40%, 20%, 10%, 5% e 2,5% p/v) e somente o HO (não diluído, 40%, 20%, 10%, 5% e 2,5% p/v). Utilizou-se um ensaio de microdiluição em caldo seguido de subcultura para determinar CBM e CFM. O mesmo protocolo foi aplicado após a exposição dos produtos à catalase. O teste de disco-difusão em ágar para *S. pseudintermedius* foi utilizado para gerar zonas de inibição. O ETM foi realizado para 10 isolados de *S. pseudintermedius* e 10 isolados de *M. pachydermatis*.
Resultados – A CBM foi 20% p/v (5-20% p/v) para HBO e HO. HBO obteve valores de CBM menores quando comparados com HO ($P = 0{,}003$). Não foram observadas diferenças estatísticas significativas entre os isolados MSSP/MRSP (HBO $P = 0{,}757$, HO $P = 0{,}743$). Apenas o HO foi afetado pela catalase ($P = 0{,}015$). A CFM para HBO foi 10% p/v (5–10% p/v) e 40% p/v para HO (20–≥40% p/v). Todos os isolados foram mortos após 4 h da exposição.
Conclusões e importância clínica – *Staphylococcus pseudintermedius* e *M. pachydermatis* são suscetíveis a um HBO e estes resultados podem ser usados para futuros ensaios clínicos.