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In vitro antimicrobial activity of a gel containing antimicrobial peptide AMP2041, chlorhexidine digluconate and Tris-EDTA on clinical isolates of *Pseudomonas aeruginosa* from canine otitis

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Background – *Pseudomonas aeruginosa* (PA) may cause suppurative otitis externa with severe inflammation and ulceration in dogs. Multidrug resistance is commonly reported for this organism, creating a difficult therapeutic challenge.

Objective – The aim of this study was to evaluate the *in vitro* antimicrobial activity of a gel containing 0.5 μg/mL of antimicrobial peptide AMP2041, 0.07% chlorhexidine digluconate (CLX), 0.4% Tris and 0.1% EDTA on 30 clinical isolates of PA from canine otitis externa.

Materials and Methods – Antimicrobial activity was evaluated through minimal bactericidal concentration (MBC). Standardized bacterial suspensions were incubated with different concentrations of the gel at 37°C for 30 min and plated for colony forming unit (CFU) counts. Time-to-kill kinetics were evaluated with the undiluted product and at MBC for each PA strain at 30 s, 1, 5, 10, 15, 30 min, 24 and 48 h.

Results – The MBC was 1:64 for two of 30 strains, 1:128 for 15 of 30 strains and 1:256 for 13 of 30 strains. The geometric mean was 1:165, equivalent to a concentration of 0.003 μg/mL AMP2041 + 0.0004% CLX + 0.0024% Tris + 0.0006% EDTA. Time-to-kill assays with the undiluted product showed complete bactericidal effect within 30 s for all isolates, whereas at the MBC this effect was reached within 5 min for 20 of 30 isolates and within 30 min for all isolates. Bactericidal activity was maintained after 48 h for all isolates.

Conclusion – This gel has shown rapid, complete and long-lasting activity against a panel of 30 PA isolates from cases of canine otitis externa.

Introduction

Canine otitis externa is a common condition encountered in small animal medicine. Otitis is believed to be multifactorial and treatment can be difficult in some cases.[1–3] Common organisms isolated from dogs with otitis externa include *Staphylococcus* spp., *Pseudomonas* spp., *Proteus* spp., *Streptococcus* spp., *Escherichia coli, Klebsiella* spp., *Bacteroides* spp., *Pasteurella* spp. and *Malassezia* spp. *Pseudomonas aeruginosa* (PA) is an aerobic, Gram-negative bacillus which is associated with chronic otitis externa and otitis media, often leading to ulceration and inflammation within the ear canal. It is not considered part of the normal otic microflora,[4,5] although data are available which support its low prevalence in healthy ears.[6,7] Members of the genus *Pseudomonas* display a wide spectrum of innate resistance to several classes of antimicrobials[8] such as fluoroquinolones and aminoglycosides, in part because of the increasing frequency by which antimicrobials are prescribed.[9] Incomplete antimicrobial therapy readily selects for multidrug-resistant PA strains by mechanisms specific to each drug. Resistance to individual chemotherapeutic agents can be acquired via chromosomal mutations and/or lateral gene transfer.[10] Additionally, induction of a specific multidrug efflux system is observed upon exposure to sub-inhibitory concentrations of disinfectants such as chlorhexidine and benzalkonium.[11] Therefore, there is a need for more effective and convenient medical treatments.[12,13]

Antimicrobial peptides (AMP) could represent a promising alternative to conventional antimicrobials due to their wide activity and their low propensity to induce bacterial resistance.[14] AMPs belong to a broad family of cationic peptides that exert their bactericidal activity by destabilizing the bacterial membrane or increasing its permeability.[15] In the present study, *in vitro* antimicrobial activity of a commercial otic gel (Peptivet® oto gel ICF S.r.l.;

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Cremona, Italy) which contains the novel antimicrobial peptide AMP2041, was tested against 30 canine otitis clinical isolates of PA and the *P. aeruginosa* ATCC27843 reference strain.

Materials and methods

Bacterial strains

Clinical isolates were obtained from cases of canine otitis externa during the period 2012–2015. All dogs were examined and sampled at the Parma University Veterinary Teaching Hospital. Otitis externa was diagnosed using cytology and cultural examination. Samples were collected from the horizontal canal with sterile swabs. Cytological examination showed large numbers of extra- and intracytoplasmic rods and highly degenerated neutrophils. In all cases, otoscopic examination showed presence of purulent exudate and intact tympanic membranes. Each swab was streaked to tryptose agar containing 5% bovine erythrocytes and McConkey agar plates (BD Difco; Sparks, MD, USA) in order to isolate PA. Isolates were identified to the species level using standard microbiological procedures, Gram staining and biochemical tests (API system, bioMerieux; Marcy l'Etoile, France). Thirty clinical isolates of PA, all derived from pure cultures, were collected. Isolates were stored on preservation beads (Oxoid, UK) at −80°C, then sub-cultured in Mueller Hinton Broth (BD Difco) and plated to tryptose agar/5% bovine erythrocytes prior to testing. ATCC *P. aeruginosa* reference strain 27853 was tested as a control.

In vitro susceptibility testing

Peptivet® oto gel is a commercial otological product containing 0.5 μg/mL of antimicrobial peptide AMP2041, 0.07% chlorhexidine digluconate, 0.4%Tris and 0.1% EDTA. The antimicrobial activity of AMP2041 has been reported previously[16,17] on different bacterial and fungal species and in particular, on different PA clinical isolates and ATCC strain 27853. In the present study, the antimicrobial activity of the undiluted and diluted product was evaluated. Two-fold dilutions of Peptivet® oto gel were prepared in the range 1:2–1:256 with phosphate buffer (PB) 10 mM, pH 7. The minimum bactericidal concentration (MBC) for the reference strain and each clinical isolate was evaluated by broth microdilution assay, based on CLSI guidelines.[18] Briefly, for each strain the log-growth phase was reached while incubating bacteria in Mueller Hinton Broth at 37°C in a shaker at 225 r.p.m for 3–4 h. To standardize the inoculum, after pelleting at 1,000 ***g*** for 20 min, the bacterial suspension was adjusted in PB to obtain an optical density (OD) value at 600 nm in a 1 cm light path cuvette in the range 0.08–0.13, approximately equivalent to a 10^8 CFU/mL suspension. This suspension was further diluted 1:100 in PB. Fifty microlitres of the bacterial suspension containing 10^6 CFU/mL were inoculated into each well, to obtain a final concentration of 5×10^5 CFU/mL.

MBC evaluation

In order to evaluate the MBC for each strain, fifty microlitres of the diluted product were added to each well containing 50 μL of 10^6 CFU/mL bacterial suspension and incubated for 30 min at 37°C, after which 20 μL of each dilution was plated onto tryptose agar containing 5% bovine erythrocytes and incubated for 24 h at 37°C for CFU count. The MBC was defined as the lowest concentration that killed >99.9% of bacteria. For each strain, the standardized bacterial suspension was exposed to the MBC value of the product and incubated at 37°C for up to 48 h. Aliquots of 20 μL were withdrawn at fixed intervals (30 s, 1, 5, 10, 15 and 30 min, 24 and 48 h), and spread onto blood agar plates. After overnight incubation at 37°C, the CFU were counted and the inhibition percentages calculated compared to growth of the control for each isolate. All of the experiments were performed in triplicate. The persistence of antimicrobial activity of Peptivet® oto gel over time was evaluated for all strains by adding a fresh bacterial suspension to wells which underwent 24 and 48 h of incubation in the MBC evaluation assay.

Results

Minimal bactericidal concentration values for each clinical isolate and for the reference strain are shown in Table 1. For ATCC strain 27853, the MBC after 30 min contact was obtained at the 1:64 dilution (corresponding to 0.008 μg/mL AMP2041, 0.001% CLX, 0.006% Tris, 0.002% EDTA). For the clinical strains the MBC was 1:64 for two of 30 strains, 1:128 (corresponding to 0.004 μg/mL AMP2041, 0.0005% CLX, 0.003% Tris, 0.001% EDTA) for 15 of 30 strains and 1:256 (corresponding to 0.002 μg/mL AMP2041, 0.00025% CLX, 0.0015% Tris, 0.0005% EDTA) for 13 of 30 strains. The geometric mean was calculated to be 1:165, which is equivalent to a concentration of 0.0004% CLX + 0.0024%Tris + 0.0006% EDTA + 0.003 μg/mL AMP2041. The mean MBC for clinical isolates was therefore lower than the value for the ATCC 27853 strain.

Results for the time-to-kill assay are reported in Table 1 and Figure 1. Time-to-kill assays performed with the undiluted product showed complete bactericidal effect for each clinical isolate and for the reference PA strain within 30 s (data not shown). Time-to-kill assays performed at each isolate's specific MBC value showed that complete bactericidal activity was reached within 5 min for 20 of 30 isolates, and within 30 min for all clinical isolates and the reference strain. An extended bactericidal activity of Peptivet® oto gel after 24 and 48 h of incubation in the MBC evaluation assay was observed (data not shown).

Discussion

Peptivet® oto gel showed microbicidal activity against 30 canine otitis clinical isolates of PA and the PA ATCC27843 reference strain. The MBC was at least equal to 1:64, as obtained for the reference strain and for two of 30 clinical isolates, and the MBC geometric mean for the clinical strains was equal to 1:165. For all strains, complete bactericidal activity was reached within 30 min when Peptivet® oto gel was tested at MBC concentrations.

This study has shown that Peptivet® oto gel, which combines AMP2041 with chlorhexidine, produces very good *in vitro* microbicidal activity against clinical isolates of *P. aeruginosa*, even when highly diluted (0.003 μg/mL AMP2041 and 0.0004% chlorhexidine). Maintenance of activity at diluted concentrations is an important feature for antimicrobial agents used on the skin and in the ear canals.[19,20] A prior study of AMP2041 showed bactericidal effect against PA ATCC strain 27853 at an MBC value of 2.14 μg/mL after 2 h of contact time,[17] whereas reported MICs for chlorhexidine digluconate against PA range from 8 to >70 μg/mL.[21] Although Tris-EDTA alone is known to be bacteriostatic, its addition to topical products significantly lowers the MIC and MBC values for several antimicrobials.[22]

Antimicrobial peptides could represent a promising alternative to traditional antimicrobial drugs. A synergistic antimicrobial effect for AMP2041 with chlorhexidine has been hypothesized, due to their similar modes of action. Both compounds are strongly cationic in nature and bind strongly to bacteria, causing surface membrane damage.[17]

Table 1. Time-kill activity at minimum bactericidal concentration (MBC) values for 30 clinical isolates of *Pseudomonas aeruginosa* and for the ATCC27853 reference strain. Incubation times are from 0.5 to 30 min and at 24 and 48 h. Inhibition percentages are reported

Strain	MBC (dilution ratio necessary to kill >99.9% of the isolate)	Time							
		T0.5	T1	T5	T10	T15	T30	T24	T48
1	1:128	66.7%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
2	1:64	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
3	1:128	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
4	1:128	75.0%	96.5%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
5	1:128	91.7%	91.7%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
6	1:64	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
7	1:256	0.0%	0.0%	66.7%	93.3%	95.7%	100.0%	100.0%	100.0%
8	1:256	0.0%	33.3%	33.3%	66.7%	95.8%	100.0%	100.0%	100.0%
9	1:128	0.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
10	1:128	0.0%	58.3%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
11	1:256	33.3%	83.3%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
12	1:256	50.0%	83.3%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
13	1:128	83.3%	85.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
14	1:128	50.0%	90.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
15	1:128	16.7%	83.3%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
16	1:128	0.0%	83.3%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
17	1:256	0.0%	86.7%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
18	1:128	0.0%	50.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
19	1:256	0.0%	0.0%	0.0%	0.0%	83.3%	100.0%	100.0%	100.0%
20	1:256	0.0%	0.0%	0.0%	66.7%	83.3%	100.0%	100.0%	100.0%
21	1:128	16.7%	90.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
22	1:128	50.0%	83.3%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
23	1:128	0.0%	50.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
24	1:128	50.0%	66.7%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
25	1:256	0.0%	0.0%	0.0%	66.7%	91.7%	100.0%	100.0%	100.0%
26	1:256	50.0%	50.0%	70.0%	88.3%	95.0%	100.0%	100.0%	100.0%
27	1:256	0.0%	0.0%	0.0%	50.0%	91.7%	100.0%	100.0%	100.0%
28	1:256	0.0%	33.3%	50.0%	75.0%	91.7%	100.0%	100.0%	100.0%
29	1:256	0.0%	16.7%	16.7%	33.3%	75.0%	100.0%	100.0%	100.0%
30	1:256	0.0%	0.0%	0.0%	33.3%	66.7%	100.0%	100.0%	100.0%
ATCC27853	1:64	0.0%	50.0%	66.7%	80.0%	96.2%	100.0%	100.0%	100.0%

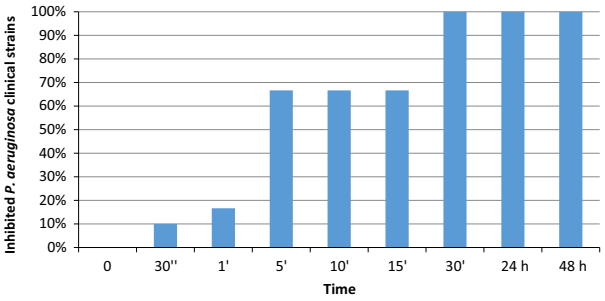

Figure 1. Time-kill activity at minimum bactericidal concentration (MBC) values for 30 clinical isolates of *Pseudomonas aeruginosa.* Inhibition percentages refer to the overall number of strains. 30'' 30 seconds, 1' 1 minute.

Cases of chlorhexidine resistance have been reported over the past 30 years.[23] According to the Committee for Veterinary Medicinal Products of the European Agency for the Evaluation of Medicinal Products,[24] chlorhexidine is bactericidal above 100 μg/mL. In veterinary medicine products, chlorhexidine digluconate is present at concentrations starting at 1.5% w/v (15 mg/mL) up to 4% w/v (40 mg/mL) and can be used with a dilution factor of around 1 part to 30. However, it has been suggested that the exposure of bacteria to chlorhexidine residual concentrations under the MIC value could lead to an increase in bacteria resistance.[21] Chlorhexidine cytotoxicity has been observed both *in vitro* and *in vivo* after contact. Human erythrocyte and neutrophil lysis at chlorhexidine concentrations above 2% have been demonstrated *in vitro.*[25] Nevertheless, chlorhexidine 0.20%, used in the ear canal with a perforated tympanic membrane in dogs did not induce cochlear or vestibular neurotoxicity.[26] Therefore, chlorhexidine toxicity may be related to its concentration.[27]

On the basis of the data reported here, products which combine antimicrobial peptides with chlorhexidine present a potentially sound and novel approach to antiseptic treatment. The sustained killing time afforded by this combination may allow for less frequent application and improve owner compliance. The low concentration of each component drug may reduce the probability of toxicity, thereby enhancing tolerability. In conclusion, Peptivet® oto gel has a rapid and long-lasting *in vitro* activity against clinical isolates of *P. aeruginosa*. Randomized clinical trials are needed to prove its *in vivo* efficacy in the setting of canine *Pseudomonas* otitis.

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Résumé

Contexte – *Pseudomonas aeruginosa* (PA) peut entrainer une otite externe suppurée avec une sévère inflammation et des ulcérations chez le chien. Une multi-résistance est fréquemment rapportée pour cet organisme, créant un difficile défi thérapeutique.

Objectifs – Le but de cette étude était d'évaluer l'activité antimicrobienne *in vitro* d'un gel contenant 0,5 μg/mL du peptide antimicrobien AMP2041, 0.07%, de digluconate de chlorhexidine (CLX), 0.4% de Tris et 0.1% d'EDTA sur 30 souches issues d'otites canines externes.

Matériel et méthodes – L'activité antimicrobienne a été évaluée par MBC (concentration bactéricide minimale). Les suspensions bactériennes standardisées ont été incubées avec différentes concentrations du gel à 37°C pendant 30 min et mis en culture pour dénombrement des CFU (colony forming unit). Les cinétiques de temps pour tuer ont été évaluées avec le produit non dilué à MBC pour chaque souche de PA à 30s, 1, 5, 10, 15, 30 mon, 24 et 48h.

Résultats – La MBC était de 1 :64 pour deux des 30 souches, 1 :128 pour 15 des 30 souches et 1 :256 pour 13 des 30 souches. La moyenne géométrique était 1 :165, équivalente à la concentration de 0.003 μg/mL AMP2041 + 0.0004% CLX + 0.0024% Tris + 0.0006% EDTA. Les essais de temps de tuer avec le produit non-dilué ont montré un effet bactéricide complet dans les 30 s pour toutes les souches, tandis qu'au MBC cet effet était atteint en 5 min pour 20 des 30 souches et dans les 30 min pour toutes les souches. L'activité bactéricide était maintenue après 48h pour toutes les souches.

Conclusion – Ce gel a montré une activité rapide, complète et de longue action contre un panel de 30 souches de PA issues d'otites externes canines.

Resumen

Introducción – *Pseudomonas aeruginosa* (PA) puede causar otitis externa supurativa con inflamación y ulceración en perros. La resistencia a múltiples fármacos se ha diseminado para este organismo, creando un desafío terapéutico de difícil solución

Objetivo – El objetivo de este estudio fue evaluar la actividad antimicrobiana in vitro de un gel que contiene 0,5 mg / ml de péptido antimicrobiano AMP2041, 0,07% de digluconato de clorhexidina (CLX), 0.4% Tris y 0,1% de EDTA en 30 aislados clínicos de PA de otitis externa canina.

Materiales y Métodos – La actividad antimicrobiana se evaluó mediante la concentración bactericida mínima (MBC). Suspensiones bacterianas estandarizadas se incubaron con diferentes concentraciones de gel a 37 ° C durante 30 min y se sembraron para evaluar la formación de unidades formadoras de colonia (CFU). La cinética del tiempo para destruir los organismos fue evaluada con el producto sin diluir y a la MBC para cada cepa de PA a los 30 s, 1, 5, 10, 15, 30 min, 24 y 48 h.

Resultados – La MBC fue 1:64 para dos cepas de 30 cepas, 1: 128 en 15 de 30 cepas y 1: 256 en 13 de 30 cepas. La media geométrica fue de 1: 165, equivalente a una concentración de 0,003 mg / ml AMP2041 + 0,0004% CLX + 0,0024% Tris + 0,0006% EDTA. Los ensayos del tiempo transcurrido hasta destruir los organismos con el producto sin diluir mostraron un efecto bactericida completo dentro de los 30 primeros segundos para todos los aislados, mientras que a la MBC se produjo este efecto dentro de 5 minutos para 20 de los 30 aislados y dentro de 30 minutos para todos los aislados. La actividad bactericida se mantuvo después de 48 h para todos los aislados.

Conclusión – Este gel ha mostrado una actividad rápida, completa y duradera frente a un panel de 30 aislados de PA de casos de otitis externa canina.

Zusammenfassung

Hintergrund – *Pseudomonas aeruginosa* (PA) kann eine suppurative Otitis externa mit starker Entzündung und Ulzerierung bei Hunden verursachen. Für diesen Organismus wird häufig eine Multi-Resistenz beschrieben, die eine therapeutische Herausforderung darstellt.

Ziel – Das Ziel dieser Studie war eine Evaluierung der *in vitro* antimikrobiellen Aktivität eines kommerziellen otologischen Gels, welches 0,5µg/mL eines antimikrobiellen Peptids AMP2041, 0,07% Chlorhexidin Digluconat (CLX), 0,4% Tris und 0,1% EDTA bei 30 klinischen Isolaten von PA von Otitis externa von Hunden.

Material und Methode – Die antimikrobielle Wirkung wurde anhand der minimalen bakteriziden Konzentration (MBC) evaluiert. Standardisierte Bakteriensuspensionen wurden mit verschiedenen Konzentrationen des Gels bei 37°C für 30 Minuten inkubiert und für Kolonie-bildende Einheiten (CFU) auf Platten ausgestrichen. Die „Time-to-Kill" Kinetik wurde mit dem unverdünnten Produkt evaluiert und mittels MBC für jeden PA Stamm bei 30s, 1, 5, 10, 15, 30 Minuten, sowie nach 24 und 48h.

Ergebnisse – Die MBC betrug 1:64 bei zwei der 30 Stämme, 1:128 bei 1 der 30 Stämme und 1:256 bei 13 der 30 Stämme. Der geometrische Durchschnitt lag bei 1:165, was einem Equivalent der Konzentration bei 0,003 µg/mL AMP2041 + 0,0004% CLX + 0,0024% Tris + 0,0006% EDTA entsprach. Die „Time-to-Kill" Assays mit dem unverdünnten Produkt zeigten innerhalb von 30 s für alle Isolate eine vollständige bakterizide Wirkung, während bei der MBC diese Wirkung erst nach 5 Minuten für 20 der 30 Isolate und innerhalb von 30 min für alle Isolate eintrat. Die bakterizide Wirkung konnte 48 h lang für alle Isolate aufrechterhalten werden.

Schlussfolgerung – Dieses Produkt zeigte eine rasche, gänzliche und langwirksame Aktivität gegenüber einer Auswahl von 30 PA Isolaten von Fällen mit caniner Otitis externa.

要約

背景 — Pseudomonas aeruginosa(PA)は、犬に重度の炎症と潰瘍を伴う化膿性外耳炎を引き起こす。本病原体の多剤耐性が一般的に報告されており、治療を困難にさせている。

目的 — 本研究の目的は、犬外耳炎患者より分離した30株のPAを用いて、市販の耳用ゲル(0.5 µg/mLの抗菌ペプチドAMP2041、0.05%クロルヘキシジングルコネート(CLX)、0.4%Tris、0.1%EDTAを含む)のvitroにおける抗菌効果を評価すること。

方法 — 抗菌効果は最小殺菌濃度(MBC)によって評価した。標準細菌浮遊液をそれぞれ異なる濃度のゲルとともに、37°Cで30分間培養し、コロニー形成単位(CFU)測定のために培地に塗布した。原液商品および各PA株のMBCを用いて、30 秒、1分、5分、10分、15分、30分、24時間、48時間培養し、殺菌時間動態(time-to-kill kinetics)を評価した。

結果 — MBCは30株中、2株において1:64、15株において1:128、13株において1:256であった。幾何平均は1:165で、これは0.003 µg/mlのAMP2041 + 0.0004% CLX + 0.0024% Tris + 0.0006% EDTAと等しい。原液商品を用いた殺菌時間動態では、すべての分離株において30分以内に完全な殺菌効果を示した。一方で、MBCを用いた場合、この効果は30株中20株で5分以内に発揮され、すべての分離株において30分以内に発揮された。すべての分離株において、殺菌効果は48時間持続した。

結論 — 本商品は犬の外耳炎症例から分離した30株のPAに対して、迅速で完全かつ長時間持続性の効果を示す。

摘要

背景 — 铜绿假单胞菌(PA)可能导致犬化脓性外耳炎,耳道伴有严重炎症和溃疡。这种微生物常见多重耐药,给治疗带来了巨大挑战。

目的 — 本次研究目的为评估一款商品化耳部药膏,针对外耳炎分离出的30株临床菌株,其体外抗微生物活性,耳药含有0.5 μg/mL的抗微生物肽AMP20410、0.07%葡萄糖酸氯已定、0.4% Tris、0.1% EDTA。

材料与方法 — 通过最小抑菌浓度(MBC)评估抗微生物活性。不同浓度的标准细菌悬浮液在37°C下孵化30分钟,并用集落形成单位(CFU)计数。每个PA菌株在30秒、1、5、10、15、30分钟、24和48小时,评估未稀释产品的杀伤时间动力学和MBC。

结果 — 30株菌株中有两株MBC为1:64,十五株为1:128,十三株为1:256。几何学平均值为1:165,等价浓度为0.003 μg/mL AMP2041 + 0.0004% CLX + 0.0024%Tris+ 0.0006% EDTA。评估未稀释产品杀伤时间,所有菌株30秒内有完全杀菌效果。所有菌株杀菌活性可维持到48h以后。

总结与临床意义 — 对犬外耳炎病例的30株PA,该产品显示出快速、完全、长时间的杀菌活性。

Resumo

Contexto – *Pseudomonas aeruginosa* (PA) pode causar otite externa supurativa com inflamação severa e ulceração em cães. Multirresistência é comumente relatada neste microrganismo, criando um difícil desafio terapêutico.

Objetivo – O objetivo deste estudo foi avaliar a atividade antimicrobiana *in vitro* de um gel contendo 0,5 μg/mL do peptídeo antimicrobiano AMP2041, digluconato de clorexidine (CLX) 0,07%, Tris 0,4% e EDTA 0,1% em 30 isolados clínicos de PA de otite externa de canina.

Materiais e métodos – Atividade antimicrobiana foi avaliada por concentração antimicrobiana mínima (CAM). Suspensões bacterianas padronizadas foram incubadas com concentrações diferentes do gel a 37°C por 30 minutos e plaqueado para contagem de unidades formadoras de colônia (UFC). A cinética de tempo de eliminação foram avaliadas com o produto não diluído e em CAM para cada cepa de PA em 30 s, 1, 5, 10, 15, 30 min, 24 e 48 horas,

Resultados – O CAM foi 1:64 para duas das 30 cepas, 1:128 para 15 de 30 cepas e 1:256 para 13 das 30 cepas. A média geométrica foi 1:165, equivalente à concentração de 0,003 μg/mL AMP2041 + CLX 0,0004% + Tris 0,0024% + EDTA 0,0006%. Ensaios de tempo de eliminação com o produto não diluído demonstrou efeito bactericida completo em 30 segundos para todos os isolados, enquanto no CAM, este efeito foi alcançado em cinco minutos para 20 dos 30 islados e em 30 minutos para todos os isolados. Atividade bactericida foi mantida após 48 horas para todos os isolados.

Conclusões – Este gel demonstrou atividade rápida, completa e duradoura contra um painel de 30 isolados de PA de casos de otite externa canina.