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In vitro antimicrobial activity of cold atmospheric microwave plasma against bacteria causing canine skin and ear infections

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Background – Cold atmospheric plasma (CAP) is a new generation medical therapeutic option for bacterial infections. CAP causes physical cell wall rupture and DNA damage, therefore making it highly useful in the treatment of various conditions such as skin infections.

Hypothesis/objectives – The antimicrobial activity of cold atmospheric microwave plasma (CAMP) against major strains in canine skin infections was tested and the difference in antimicrobial activity between the antibiotic-resistant and antibiotic-susceptible strains of *Staphylococcus pseudintermedius* was evaluated.

Methods and materials – American Type Culture Collection (ATCC) strains (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*) and clinical isolates identified as methicillin-resistant *S. pseudintermedius* (n = 27) and methicillin-susceptible *S. pseudintermedius* (n = 13) were exposed to CAMP for 10 s, 30 s and 60 s. Afterwards, the bacterial survival rate was confirmed.

Results – Gram-negative bacteria (*P. aeruginosa* and *E. coli*) were more susceptible than Gram-positive bacteria *(S. aureus* and *S. pseudintermedius*) for the same duration of CAMP exposure. Only the Gram-negative bacteria were completely killed after 60 s exposure. In *S. pseudintermedius* isolates, CAMP exposure had similar antibacterial effects regardless of antibiotic resistance.

Conclusions and clinical importance – CAMP has sufficient antimicrobial activity against major bacterial strains that cause pyoderma and otitis externa in dogs, and may be an alternative therapeutic option for *S. pseudintermedius* skin infections, for which antibiotics often are ineffective because of antimicrobial resistance in clinical veterinary medicine.

Introduction

The major bacteria that cause canine pyoderma and otitis are *Staphylococcus* spp. (Gram-positive bacteria), and *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus spp.* (Gram-negative bacteria).[1,2] Of these, *Staphylococcus pseudintermedius* causes the most skin infections in dogs; in particular, methicillin-resistant *S. pseudintermedius* (MRSP) causes difficulties in using appropriate antibiotics in the treatment of infectious diseases.[3,4] Owing to restrictions in using antibiotics, alternative therapeutic options are needed, one of which is cold atmospheric plasma (CAP).[5–8]

Plasma is a substance generated when gas flow, such as compressed air, argon, helium or nitrogen, passes through an electric field and become ionised. Nonthermal plasma, which is generated at room temperature, is composed of radicals, charged particles – except for neutral electrons that are generated at high temperature – and ultraviolet photons.[9,10] Nonthermal plasma, which is produced at low pressure, is convenient and economically effective; it is used in human medicine and is called CAP. CAP is effective in removing micro-organisms.[10–12] Through the action of reactive oxygen species and reactive nitrogen species released during plasma generation, CAP damages the bacterial cell wall and causes high oxidative stress and cell membrane rupture. CAP reacts with bacterial DNA to cause bacterial death.[5,11] In addition, CAP stimulates several cytokine factors to promote anti-inflammatory action, tissue healing and microcirculation stimulation.[13,14] In human medicine, CAP is used to heal wounds and infected areas, and its various dermatological applications are being studied.[13,15,16] CAP is divided into several types according to the production method. Among them, microwave discharge plasma, also called cold atmospheric microwave plasma (CAMP), is capable of generating plasma at a lower temperature and at a more reasonable price.[5,8,10]

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Hee-Jung Jin and Cheol-Yong Hwang contributed equally to this work.

This study aimed to evaluate the *in vitro* bactericidal activity of a CAMP type device (IonMedical Inc.; Seongnam, Republic of Korea) developed for use in veterinary medicine and the degree of efficacy with regards to the methicillin-resistance status of *S. pseudintermedius*.

Methods and materials

Bacterial strains

Field strains of *S. pseudintermedius* were obtained from the skin of 24 dogs with pyoderma and ears of 16 dogs with otitis externa (OE) at Konkuk Veterinary Medical Teaching Hospital from 2014 to 2015. The identification of *S. pseudintermedius* was performed using a Vitek 2 system (bioMérieux; Hazelwood, MO, USA) and the genetic diversity of the isolates was confirmed with several assays including multilocus sequence typing (MLST) in our previous study (see Figure S1 in Supporting information).[3] The strains were stored in Tryptic Soy Broth (Thermo Fisher Scientific Oxoid Ltd; Basingstoke, UK) with 50% glycerol at −70°C before testing. We defined methicillin resistance in *S. pseudintermedius* using the 1 μg oxacillin disk (Thermo Fisher Scientific Oxoid Ltd) agar diffusion method.[17] After a 24 h incubation at 37°C, the inhibition zones were determined and methicillin resistance was confirmed according to the Clinical Laboratory Standards Institute (CLSI) guidelines for animals (VET08).[18] For confirming the identification of methicillin resistance, PCR using the targeting *mecA* gene was performed for molecular identification of *S. pseudintermedius* isolates.[19] As standard strains, this study also used other pathogenic bacterial strains purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA): *P. aeruginosa* (ATCC 1750), *E. coli* (ATCC 29513) and *S. aureus* (ATCC 29154).

Antimicrobial susceptibility testing

In order to determine the resistance of MRSP and methicillin-susceptible *S. pseudintermedius* (MSSP) strains to antimicrobial agents, each strain was subjected to an antibiotic susceptibility test with the agar disk diffusion method on Mueller–Hinton Agar (MHA; BD Diagnostic Systems; Sparks, MD, USA). The antimicrobial agents used (Thermo Fisher Scientific Oxoid Ltd) were (in μg/disk): penicillin (10); chloramphenicol (30); clindamycin (2); erythromycin (15); gentamicin (10); tetracycline (30); trimethoprim-sulfamethoxazole (25); and rifampicin (5). After 24 h incubation at 37°C, the diameters of the inhibition zones around the discs were measured and antibiotic resistance was interpreted according to the VET08.[18] The isolated strains were divided into sensitive or resistant to the drug; intermediate susceptibility was regarded as resistant.

Cold atmospheric microwave plasma

In all experiments, CAMP was used with nonionised argon gas. The plasma used in the experiment was generated in the device shown in Figure 1. Upon exposure to microwave energy (30–50W, 2,450 MHz) in the tube, the argon gas existing as a carrier gas was ionised in the tube. As a result, plasma was generated and emitted through the nozzle. The rate of argon gas flow into the tube could be adjusted 10–20 L/min, and the microwave energy could be adjusted to a power of 30 W or 50 W. The amount of plasma emitted changed according to the power consumed and gas velocity. The length of the plasma jet emitted also was affected (range 3–15 mm). The surface temperature of plasma was maintained below 40°C.

Plasma treatment and determination of antimicrobial effect using colony-forming units

Each isolate and standard bacteria strain in Mueller–Hinton Broth (MHB; BD Diagnostic Systems) was grown in a 120 rpm shaking incubator at 37°C for 24 h, and then diluted to 0.5 Mcfarland. The final inoculum concentration was $1–5 \times 10^4$ colony-forming units (CFU)/mL and 10 μL of bacterial suspension was inoculated into MHA made in six-well cell culture plates (each area, 9.6 cm^2). After inoculation, the inoculum was streaked on the plate with an inoculation loop and needle. The rate of gas flow out of the CAMP was set at 15 L/min, the microwave energy at 2,450 MHz, 3.5 kV, and power consumption at 30 W or 50 W. The distance from the plasma surface to the plate was the length of the jet, and the plasma took 10 s to pass each agar plate surface. The plates were exposed to the plasma for 10 s, 30 s and 60 s. The flow of nonionised argon gas and unexposed plasma were used for control condition. After CAMP exposure and an additional 24–48 h of growth at 37°C, the bacterial survival rate was measured after confirming the number of CFU. We conducted a minimum of three independent tests to measure the survival rates.

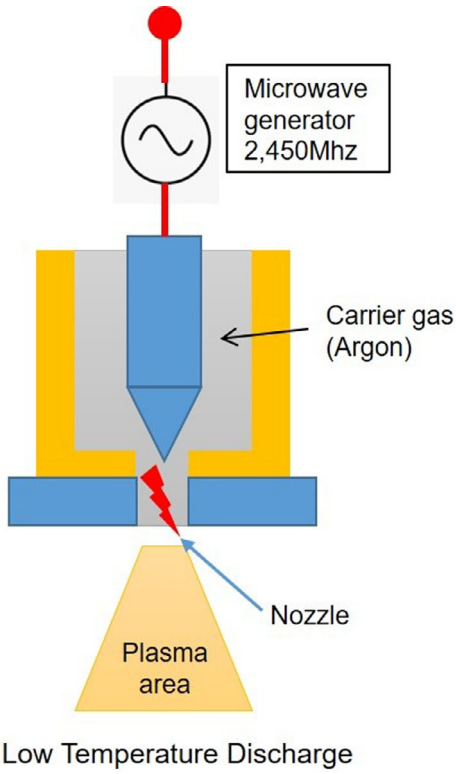

Figure 1. Schematic diagram of a device that generates cold atmospheric microwave plasma (CAMP).

Statistical methods

Prism 5 (GraphPad Software Inc.; San Diego, CA, USA) was used for all statistical comparisons in this study. Comparison of differences in the survival rates according to plasma exposure time, one-way ANOVA was performed. *P*-values of <0.05 were considered significant.

Results

Bacterial isolates and antimicrobial susceptibility tests

Antimicrobial susceptibility tests were conducted with 40 clinical *S. pseudintermedius* isolates. According to the presence of the *mecA* gene and oxacillin resistance, 27 (67.5%) of the strains were MRSP and 13 (32.5%) were MSSP. The agar disk diffusion method was used to characterise the antimicrobial resistance patterns of the isolates. The resistance profiles of MRSP and MSSP strains to the antimicrobial agents are shown in Table 1. Three isolates (7.5%) were susceptible to all antibiotics, and one isolate (2.5%) was resistant to only one antibiotic. Other 36 isolates (90%) were confirmed to have

multidrug resistance at least three antibiotic classes. Multidrug resistance was detected in 22 (100%) MRSP strains and 13 (76.5%) MSSP strains, and high resistance to penicillin and tetracycline was identified among both strains.

Bactericidal effect of cold atmospheric microwave plasma *in vitro*

First, standard bacterial strains were evaluated for the effectiveness of CAMP (Figure 2). The plasma intensity was tested at two power levels (30 W and 50 W). The negative control without plasma exposure was assumed to have a survival rate of 100%, for convenience. At a plasma intensity of 30 W, when irradiated for 10 s, 30 s and 60 s, the survival rates of *P. aeruginosa* were 22.9%, 8.1% and 0.7%, respectively, and those of *E. coli* were 30.1%, 10% and 0.8%, respectively; however, the survival rates of *S. aureus* were 65%, 28.1% and 9.9%, respectively, which were similar to those of the methicillin-resistant standard strain. At 50 W, the survival rate of *P. aeruginosa* was reduced to 5.7% with only 10 s of irradiation, and the organism was completely killed after 60 s. All *E. coli* organisms also were confirmed to have been completely killed after irradiation for 60 s. In the case of *S. aureus*, the survival rate was 50.3% when exposed for 10 s, and the survival rate was 2.8% after 60 s of irradiation; thus, it was not completely killed. When an additional 120 s were given, the bacteria was confirmed to have completely died. In all strains, there was no significant difference in nonionised gas flow as positive control with nonplasma.

Figure 3 shows the comparison of the antimicrobial activity between MRSP and MSSP, which were clinical isolates. For both MRSP and MSSP, the survival rates decreased as plasma exposure time increased. Among the MRSP isolates, survival rates were confirmed to be 51.96% $\pm$ 37.37% after 10 s of exposure, 21.40% $\pm$ 16.10% after 30 s and 4.79% $\pm$ 4.79% after 60 s of exposure. In MSSP isolates, the survival rates were confirmed to be 44.59% $\pm$ 11.26% after 10 s, 20.33% $\pm$ 6.10% after 30 s and 6.08% $\pm$ 2.64% after 60 s of exposure ($P < 0.005$). CAMP exposure thus had a bactericidal effect, which occurred regardless of antimicrobial resistance.

Discussion

Among the mechanical methods that generate plasma, those most currently commercialised in the medical industry are the dielectric barrier discharge (DBD) system, in which gas flows between two electrodes and produces voltage to instantly generate plasma. With DBD, the temperature of the gas is instantaneously raised to 150°C, and a lot of power is consumed because of the high voltage required.[8,20] The microwave discharge system that generates CAP (CAMP) has the advantage of being easy to use because no electrode is necessary to generate plasma and particles are formed in a torch-like manner.[7,8,10] Moreover, it promotes bacterial death more quickly because it generates plasma directly. The device used in this study has maximum efficiency in generating plasma with a surface temperature <40°C and its power consumption (only 50 W) is lower than that of other commercial microwave discharge plasma machines.[8]

Previous studies confirmed that Gram-negative bacteria are more susceptible to CAP than Gram-positive bacteria.[6,8,12] CAMP generated in this study exhibited the same antibacterial effect: when exposed to CAMP for 1 min, all strains of Gram-negative bacteria (*P. aeruginosa, E. coli*) were killed even at low power (30 W), and the Gram-positive bacteria (*S. aureus*, *S. pseudintermedius*) showed survival rates of 9.05% at 30 W and 3.44% at 50 W. This result was attributable to the difference in thickness of the bacterial cell wall, as observed in previous studies.[12,21] The peptidoglycan in the Gram-positive bacteria was approximately 15–80 nm thick, whereas that in the Gram-negative bacteria was 1.5–15 nm.[22] Considering the varied thickness of these peptidoglycans, the degree of cell lysis caused by plasma exposure also varied, indicating the result of differences in depolarisation of the cell membrane and integrity.[12]

In the case of *S. pseudintermedius*, the experiment was performed by dividing it into MRSP and MSSP strains. When exposed to CAMP, the two types of strains showed no significant differences: the average survival rate on both strains was 47.12% when treated for 10 s, 18.61% for 30 s and 4.71% for 60 s. Neither MRSP nor MSSP strains showed a significant difference compared with the untreated group which was treated with argon gas only for 60 s. Also the CAMP had the same antimicrobial efficacy considering their genetic diversity had already been confirmed in the previous study with MLST.[3] The bactericidal effect was not attributable to temperature and argon gas also was considered to have no antibacterial effect, because the temperature of the surface generated during plasma exposure did not exceed 40°C. Plasma physically causes bacterial cell lysis;

Table 1. Antimicrobial resistance profiles of methicillin-resistant and -susceptible *Staphylococcus aureus* (MRSP and MSSP) isolates from dogs with pyoderma and otitis externa

		Number of resistant strains (%)								
Isolates	n	P	OX	GEN	ERY	CLI	TET	SXT	CHL	RI
MRSP	27	27 (100)	27 (100)	24 (88.9)	22 (81.5)	23 (85.2)	25 (92.6)	20 (74.1)	15 (55.6)	2 (7.4)
MSSP	13	9 (69.2)	0	8 (61.5)	8 (61.5)	8 (61.5)	9 (69.2)	4 (30.8)	6 (46.2)	0

P, penicillin; OX, oxacillin; GEN, gentamicin; ERY, erythromycin; DA, clindamycin; TE, tetracycline; SXT, trimethoprim-sulfamexothazole; CHL, chloramphenicol; RI, rifampicin.

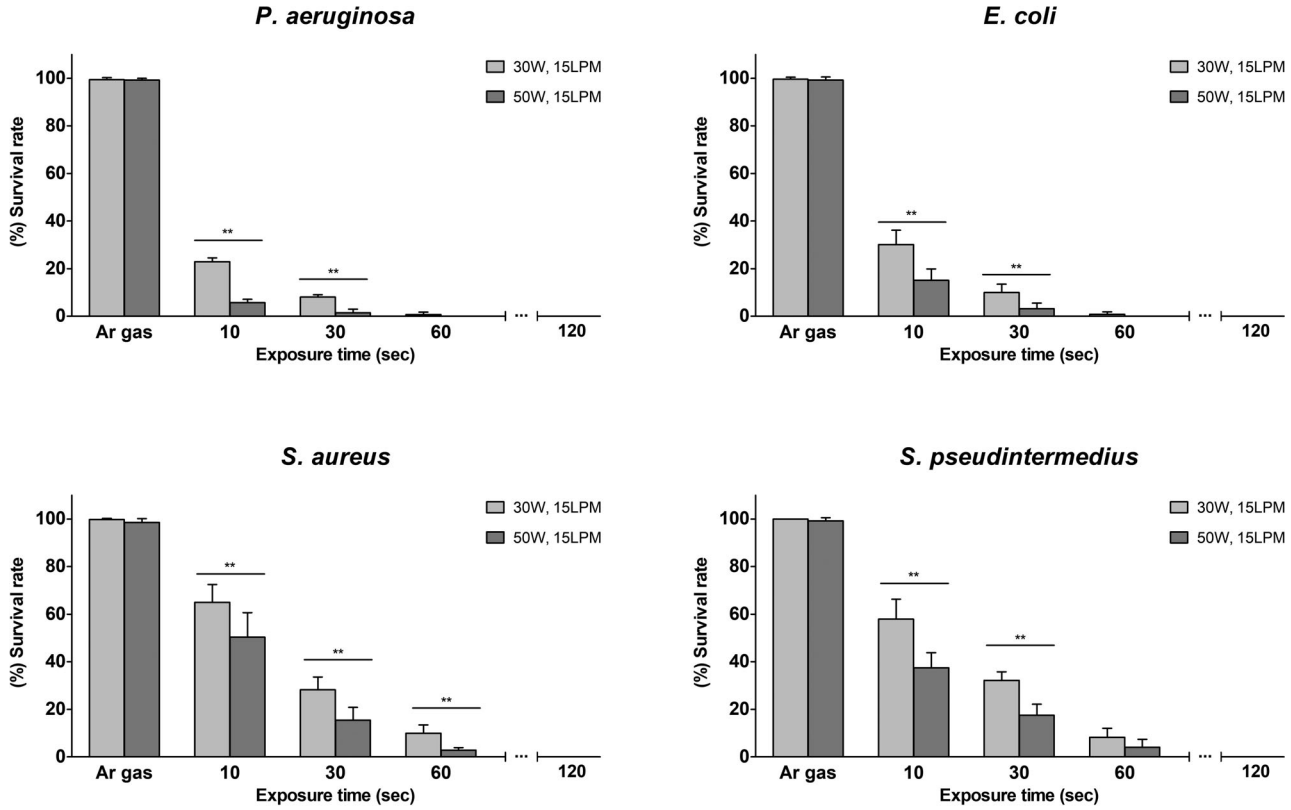

Figure 2. Cold atmospheric microwave plasma (CAMP) treatment of bacterial strains.
Each bacteria strain was grown on Mueller–Hinton agar for 24 ~ 48 h and exposed to CAMP for 10 s, 30 s and 60 s with argon feeding gas at 15 L/min, with 30 W or 50 W power. Argon gas flow only was used as positive control group. *Pseudomonas aeruginosa* was ATCC1750, *Escherichia coli* was ATCC 29513, *Staphylococcus aureus* was ATCC 29154 and *S. pseudintermedius* was a clinically isolated at methicillin-susceptible (MSSP) strain. **, $P < 0.005$; error bar ± SD (standard deviation) for triplicate analysis.

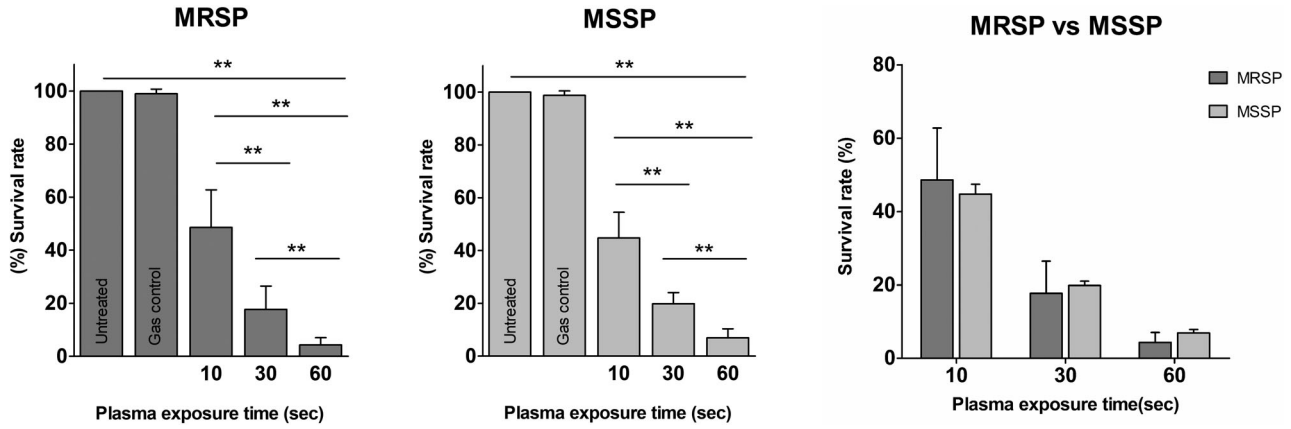

Figure 3. Cold atmospheric microwave plasma (CAMP) exposure to methicillin-resistant and -susceptible *Staphylococcus aureus* (MRSP and MSSP).
MRSP and MSSP were grown on Mueller–Hinton agar for 24 ~ 48 h and exposed to CAMP for 10 s, 30 s and 60 s with argon feeding gas at 15 L/min, with 50 W power. The untreated control group was set to 100% and argon gas flow only was used as positive control group. **, $P < 0.005$; error bar ± SD (standard deviation) for triplicate analysis.

therefore, we believe that it shows the same antibacterial effect even in highly antibiotic-resistant strains.

This study is different from other studies showing decontamination or antibacterial effect of previous plasma because we evaluated the antibiotic-resistant strain of *S. pseudintermedius* that was clinically isolated in dogs' skin infections (pyoderma and OE). In terms of the energy use and exposure time, the CAMP used in this study was more effective than that used for evaluating the results of the antimicrobial effect on *Staphylococcus* spp. in other types of plasma machines. Moreover, it is a very efficient method because it has similar antimicrobial effects even at 30 W to other microwave-type plasma machines, whose energy use is generally >80 W.[8,21,23] With the CAMP used in this study, the surface temperature is low and the device where plasma comes out is

a pen type; therefore, it might be more applicable to veterinary medicine, especially exposed skin. However, in the case of OE, the gas velocity required when CAMP is generated creates pressure and some noise. Therefore, an otological safety test is necessary because there is a possibility of damage to the tympanic membrane or other adverse effects on the auditory system. Additional experiments are necessary to determine its suitability for clinical use. When applied to tissues, it is not only antiseptic, but also enables tissue regeneration. Therefore, the effectiveness of the plasma–cell and plasma–tissue interactions must be evaluated.[24] Furthermore, for actual clinical application, an effective plasma dose should be established, skin irritation should be assessed, as well as skin hydration, and transepidermal water loss should be evaluated to see whether it affects the skin barrier function *in vivo*.

In this study, the antimicrobial efficacy of CAMP was verified against the bacteria, especially *S. pseudintermedius*; commonly found among the microbial causes of canine skin infection. Therefore, further study is needed to test the efficacy of CAMP on fungi such as dermatophytes and *Malassezia* spp., which also could cause skin infection. Notwithstanding these limitations, this study provides a basic confirmation of the antimicrobial activity of CAMP against clinical isolates in veterinary medicine. Based on this study, future CAMP tests are expected on other bacterial skin infections or other microbial skin infections.

In conclusion, the present study has demonstrated that CAMP has antibacterial effects *in vitro* against antimicrobial-resistant strains of *S. pseudintermedius*. Clinical studies are necessary to assess the safety and efficacy of *in vivo* use of CAMP against various infections in veterinary medicine.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1. Genetic diversity of *S. pseudintermedius* isolates used in this study by MLST.

Résumé

Contexte – Le CAP (Cold atmospheric plasma) est une nouvelle génération de traitement pour les infections bactériennes. CAP cause une rupture physique de la paroi et endommage l'ADN, le rendant ainsi hautement utile pour le traitement d'atteintes variées telles que les infections bactériennes.

Hypothèses/Objectifs – L'activité antimicrobienne de CAMP (cold atmospheric microwave plasma) contre des souches majeures des infections cutanées canines a été testée et la différence d'activité antimicrobienne entre les souches résistances ou sensibles aux antibiotiques de *Staphylococcus pseudintermedius* a été évaluée.

Matériels et méthodes – Les souches ATCC (American Type Culture Collection) (*Staphylococcus aureus*, *Pseudomonas aeruginosa* et *Escherichia coli*) et les souches cliniques isolées de *S. pseudintermedius* identifiées comme résistantes à la méticiline (n = 27) *S. pseudintermedius* sensibles à la méticiline (n = 13) ont été exposées à CAMP pendant 10 s, 30 s et 60 s. Ensuite, le taux de survie bactérien a été confirmé.

Résultats – Les bactéries Gram-négatives (*P. aeruginosa* et *E. coli*) étaient plus sensibles que les bactéries Gram-positives *(S. aureus* et *S. pseudintermedius*) pour la même durée d'exposition au CAMP. Seules les bactéries Gram-négatives étaient complètement tuées après 60s d'exposition. Dans les souches de *S. pseudintermedius*, l'exposition à CAMP avait des effets antibactériens similaires au regard de la résistance antibiotique.

Conclusions et importance clinique – Le CAMP a une activité antimicrobienne suffisante contre les souches bactériennes majeures causant pyodermite et otite externe chez le chien et peut être une option thérapeutique alternative pour les infections cutanées à *S. pseudintermedius* pour laquelle les antibiotiques sont souvent inefficaces en raison de résistances antimicrobiennes en médecine vétérinaire clinique.

Resumen

Introducción – el plasma atmosférico frío (CAP) es una opción terapéutica médica de nueva generación para las infecciones bacterianas. El CAP provoca la ruptura física de la pared celular y el daño al DNA, por lo que es muy útil en el tratamiento de diversas afecciones, como las infecciones de la piel.

Hipótesis/objetivos – se probó la actividad antimicrobiana del plasma de microondas atmosférico frío (CAMP) contra las principales cepas en las infecciones cutáneas caninas y se evaluó la diferencia en la actividad antimicrobiana entre las cepas resistentes y sensibles a los antibióticos de *Staphylococcus pseudintermedius*.

Métodos y materiales – cepas de la American Type Culture Collection (ATCC) (*Staphylococcus aureus, Pseudomonas aeruginosa y Escherichia coli)* y aislados clínicos identificados como *S. pseudintermedius* resistente a meticilina (n = 27) y *S. pseudintermedius* sensible a meticilina (n = 13) fueron expuestos a CAMP durante 10 s, 30 s y 60 s. Posteriormente, se valoró la tasa de supervivencia bacteriana.

Resultados – las bacterias Gram negativas (*P. aeruginosa y E. coli*) fueron más susceptibles que las bacterias Gram positivas (*S. aureus y S. pseudintermedius*) durante la misma duración de exposición a CAMP. Solo las bacterias Gram negativas murieron por completo después de 60 s de exposición. En los aislados de *S. pseudintermedius*, la exposición a CAMP tuvo efectos antibacterianos similares independientemente de la resistencia a los antibióticos.

Conclusiones e importancia clínica – CAMP tiene suficiente actividad antimicrobiana contra las principales cepas bacterianas que causan pioderma y otitis externa en perros, y puede ser una opción terapéutica alternativa para las infecciones cutáneas por *S. pseudintermedius*, para las cuales los antibióticos a menudo son ineficaces debido a la resistencia a los antimicrobianos en medicina veterinaria.

Zusammenfassung

Hintergrund – Kaltes atmosphärisches Plasma (CAP) zählt zu einer neuen Generation an medizinischen Behandlungsoptionen für bakterielle Infektionen. CAP verursacht eine physische Zellwandruptur und eine DNA Schädigung, was es in der Folge bei der Behandlung verschiedener Erkrankungen wie Hautinfektionen sehr wirkungsvoll macht.

Hypothese/Ziele – Die antimikrobielle Wirksamkeit von kaltem atmosphärischem Mikrowellenplasma (CAMP) wurde gegenüber wichtigen Stämmen bei Hautinfektionen des Hundes getestet und der Unterschied der antimikrobiellen Aktivität zwischen den Antibiotika-resistenten und den Antibiotika-empfindlichen Stämmen von *Staphylococcus pseudintermedius* evaluiert.

Methoden und Materialien – Stämme (*Staphylococcus aureus*, *Pseudomonas* aeruginosa und *Escherichia coli*) aus der American Type Culture Collection (ATCC) und klinische Isolate, die als Methicillin-resistente *S. pseudintermedius* (n = 27) und Methicillin-empfindliche *S. pseudintermedius* (n = 13) isoliert worden waren, wurden CAMP für 10 s, 30 s und 60 s ausgesetzt. Danach wurde die bakterielle Überlebensrate bestätigt.

Ergebnisse – Gram-negative Bakterien (*P. aeruginosa* und *E. coli*) waren empfindlicher als Gram-positive Bakterien (*S. aureus* und *S. pseudintermedius*) bei gleich langer Dauer der Exposition zu CAMP. Nur die

Gram-negativen Bakterien waren nach einer 60 s dauernden Exponierung völlig abgetötet. In *S. pseudintermedius* Isolaten zeigte die Exponierung unabhängig von der antibiotischen Resistenz zu CAMP eine ähnliche antibakterielle Wirkung.
Schlussfolgerungen und klinische Bedeutung – CAMP zeigt eine ausreichende antimikrobielle Aktivität gegenüber bakteriellen Stämmen, die eine Pyodermie und eine Otitis externa bei Hunden verursachen und könnte eine alternative therapeutische Option für Hautinfektionen durch *S. pseudintermedius* darstellen, für die Antibiotika aufgrund der antimikrobiellen Resistenz in der klinischen Veterinärmedizin oft unwirksam sind.

要約
背景 – 低温大気圧プラズマ (CAP) は、細菌感染症に対する新世代の治療法として注目されている。CAPは、物理的な細胞壁破壊とDNAの損傷を引き起こすため、皮膚感染症などの様々な症状の治療に非常に有用である。
仮説・目的 – 本研究の目的は、犬の皮膚感染症における主要な菌株に対する低温マイクロ波プラズマ (CAMP) の抗菌活性を試験し、*Staphylococcus pseudintermedius*の抗生物質耐性菌株および抗生物質感受性菌株間の抗菌活性の違いを評価することであった。
材料・方法 – American Type Culture Collection(ATCC) 株 (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*)と、メチシリン耐性*S. pseudintermedius*(n = 27)およびメチシリン感受性*S. pseudintermedius*(n = 13)と同定された臨床分離株をCAMPに10秒、30秒、60秒曝露し、その後、細菌の生存率を確認した。
結果 – グラム陰性菌 (*P. aeruginosa*および*E. coli*) は、同じCAMP曝露時間でもグラム陽性菌 (*S. aureus*および*S. pseudintermedius*) よりも感受性が高かった。60秒の曝露で完全に死滅したのはグラム陰性菌のみであった。*S. pseudintermedius*分離菌では、CAMP曝露は抗生物質耐性にかかわらず同様の抗菌効果を示した。
結論および臨床上の重要性 – CAMPは犬膿皮症および外耳炎の原因となる主要な細菌株に対して十分な抗菌活性を有しており、臨床獣医学では抗菌剤耐性のために抗生物質が効かないことが多い*S. pseudintermedius*皮膚感染症の代替治療法となる可能性がある。

摘要
背景 – 大气低温等离子体(CAP)是新一代细菌感染的药物治疗选择。CAP引起物理性细胞壁破裂和DNA损伤, 因此使其在皮肤感染等多种疾病的治疗中非常有用。
假设/目的 – 检测了大气低温微波等离子体(CAMP)对犬皮肤感染中主要菌株的抗菌活性, 并评价了假中间型葡萄球菌的抗生素耐药性和抗生素敏感性菌株之间的抗菌活性差异。
方法和材料 – 将美国典型培养物保藏中心(ATCC)菌株 (金黄色葡萄球菌、铜绿假单胞菌和大肠埃希菌) 和鉴定为耐甲氧西林假中间型链球菌(n = 27)和甲氧西林敏感假中间型链球菌(n = 13)的临床分离株暴露于CAMP 10s、30s和60s后, 证实了细菌存活率。
结果 – 在相同的CAMP暴露时间内, 革兰氏阴性菌 (铜绿假单胞菌和大肠埃希菌) 比革兰氏阳性菌 (金黄色葡萄球菌和假中间型链球菌) 更敏感。暴露60s后仅完全杀灭革兰氏阴性菌。在假中间型链球菌分离株中, 无论抗生素耐药性如何, CAMP暴露均具有相似的抗菌作用。
结论和临床重要性 – CAMP对引起犬脓皮病和外耳炎的主要细菌菌株具有足够的抗菌活性, 可能是假中间型葡萄球菌皮肤感染的替代治疗选择, 在临床兽药中, 由于抗菌药物耐药性, 抗生素通常无效。

Resumo
Contexto – O plasma atmosférico frio (PAF) é uma opção terapêutica de nova geração para infecções bacterianas. O PAF causa ruptura física da parede celular e danos ao DNA, sendo, portanto, altamente útil no tratamento de várias condições, como infecções de pele.
Hipótese/objetivos – A atividade antimicrobiana do plasma atmosférico frio de microondas (PAFM) contra as principais cepas em infecções cutâneas caninas foi testada e a diferença na atividade antimicrobiana entre as cepas de *Staphylococcus pseudintermedius* resistentes a antibióticos e suscetíveis a antibióticos foi avaliada.
Métodos e materiais – Cepas da *American Type Culture Collection* (ATCC) (*Staphylococcus aureus, Pseudomonas aeruginosa e Escherichia coli*) e isolados clínicos identificados como *S. pseudintermedius* resistente à meticilina (n = 27) e *S. pseudintermedius* sensível à meticilina (n = 13) foram expostos ao PAFM por 10 s, 30 s e 60 s. Posteriormente, a taxa de sobrevivência bacteriana foi confirmada.
Resultados – As bactérias Gram-negativas (*P. aeruginosa e E. coli*) foram mais suscetíveis do que as bactérias Gram-positivas (*S. aureus e S. pseudintermedius*) para a mesma duração da exposição PAFM. Apenas as bactérias Gram-negativas foram completamente mortas após 60 s de exposição. Nos isolados de *S. pseudintermedius*, a exposição ao PAFM teve efeitos antibacterianos semelhantes, independentemente da resistência aos antibióticos.
Conclusões e importância clínica – PAFM tem atividade antimicrobiana suficiente contra as principais cepas bacterianas que causam piodermite e otite externa em cães, e pode ser uma opção terapêutica alternativa para infecções cutâneas por *S. pseudintermedius*, para as quais os antibióticos muitas vezes são ineficazes devido à resistência antimicrobiana na medicina veterinária.

