

# *In vitro* activity of a commercial otological solution containing a novel antimicrobial peptide on 30 clinical isolates of *Pseudomonas aeruginosa* from canine otitis

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## INTRODUCTION

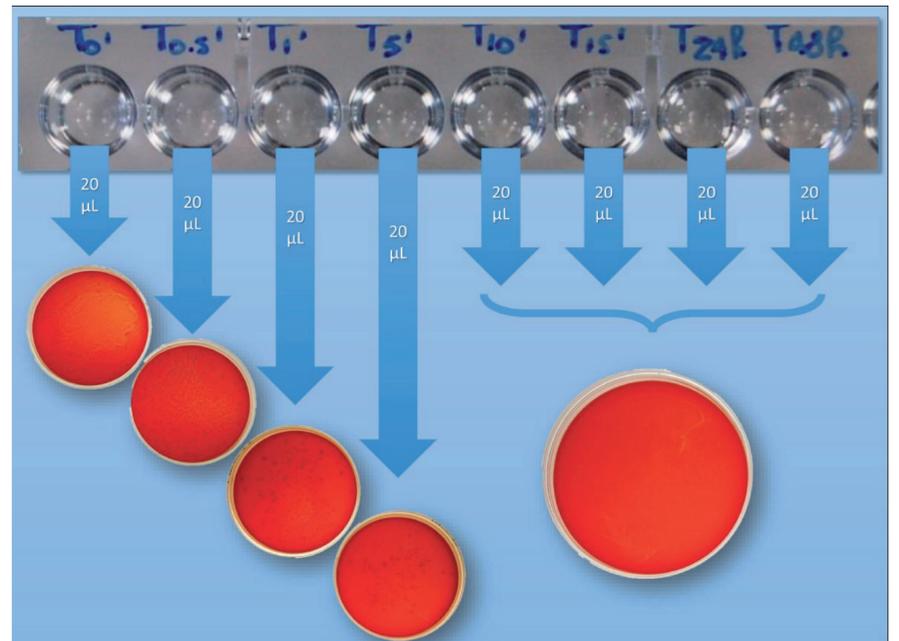
In dogs, *Pseudomonas aeruginosa* (PA) infections frequently present as an acute suppurative otitis, with severe inflammation, ulceration, discomfort and pain. This can be a therapeutic challenge that needs new effective treatment.

### Objective

Aim of this study was to evaluate the *in vitro* antimicrobial activity of a commercial otological solution (Peptivet<sup>®</sup> oto sol., ICF, Cremona, Italy) containing 0.02% chlorhexidine digluconate (CLX), 0.4% Tris, 0.1% EDTA and 0.5 µg/ml of the antimicrobial peptide AMP2041 on PA from canine otitis.

## MATERIALS AND METHODS

Minimal bactericidal concentrations (MBCs) were evaluated by broth microdilution. Standardized bacterial suspensions were incubated with different concentrations of the test solution at pH8 and 37°C for 30 min and then spread-plated for colony forming unit (CFU) counts. The dynamics of bacterial killing were evaluated with time-kill curves obtained with undiluted product and at MBC for each strain by aliquoting and plating 20 µl of the bacterial suspension for CFU counts at fixed intervals (30 sec, 1, 5, 10, 15 min, 24h and 48h).

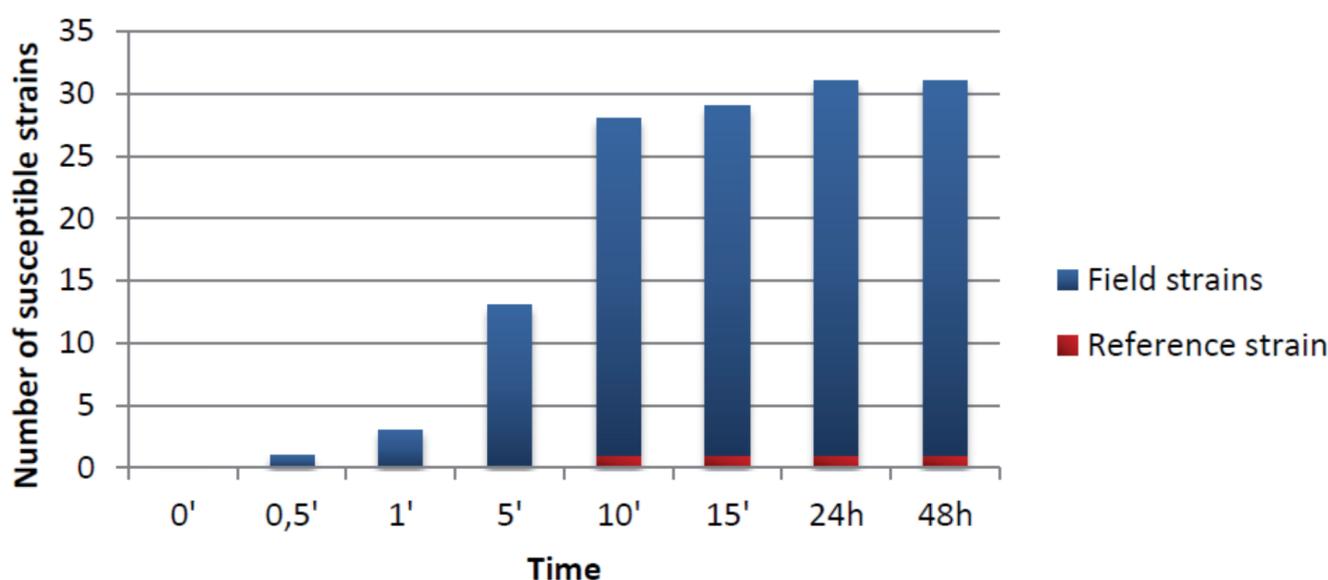


Complete bactericidal activity was observed after 48 hours for all strains of *Pseudomonas aeruginosa*

## RESULTS

The MBC was 1:32 for 1/30 strains and 1:64 for 29/30 strains. The geometric average was 1:62.54, equivalent to a concentration of 0.0003% CLX/0.0064% Tris/0.0016% EDTA/0.008 µg/ml AMP2041. The time-kill assays with the undiluted product showed complete bactericidal effect within 5 min for all isolates, while at MBC this effect was reached within 10 min for 28/30 isolates. Complete bactericidal activity was observed after 48 hours for all strains.

## Time Kill assay at MBC values



**CONCLUSIONS:** The product shows fast, complete and long-lasting antimicrobial activity against a panel of 30 PA clinical isolates from canine otitis.

# *In vitro* antimicrobial activity of a commercial shampoo (Peptivet® shampoo) containing chlorhexidine, Tris-EDTA and a novel antimicrobial peptide (AMP2041)

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## INTRODUCTION

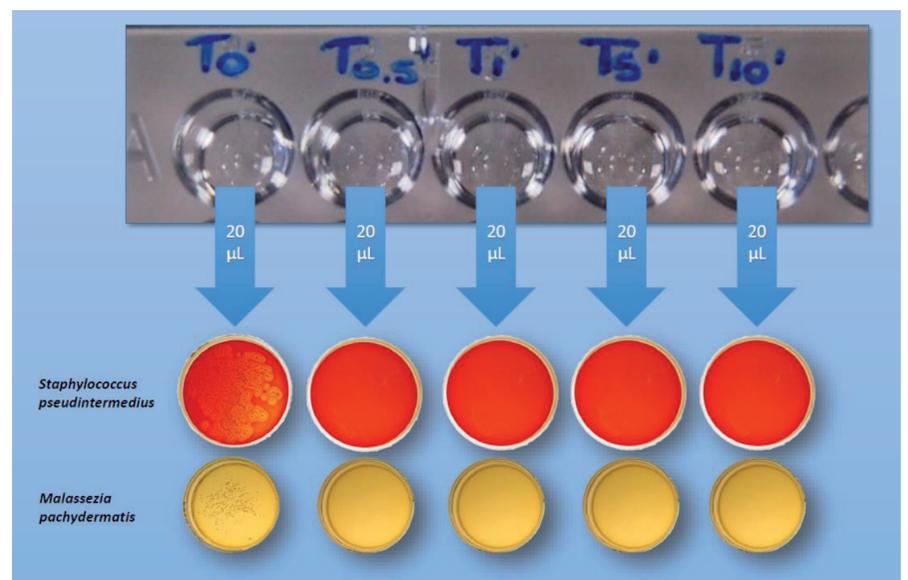
Cutaneous infections are frequently present in dogs due to bacteria and/or yeasts. The presence of new antimicrobials could help to reduce the use of antibiotics and antifungal drugs.

## Objective

Aim of this study was to evaluate the *in vitro* antimicrobial activity of a commercial shampoo (Peptivet® shampoo, ICF, Cremona, Italy) containing 0.08% chlorhexidine digluconate (CLX), Tris 1.6%, EDTA 0.4%, isopropyl alcohol 2.5% and 2 µg/ml of antimicrobial peptide AMP2041 on bacterial and fungal reference strains involved in canine cutaneous infections.

## MATERIALS AND METHODS

The dynamics of bacterial killing was evaluated with product diluted 1:4 with sterile water to simulate field conditions. The product so diluted has been incubated with the standardized suspensions of *E. coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Proteus mirabilis* ATCC14153, *Staphylococcus aureus* ATCC25923, MRSA ATCC43300, *Staphylococcus pseudintermedius* ATCC49444, *Streptococcus canis* ATCC20715, *Malassezia pachydermatis* ATCC14522 and *Candida albicans* ATCC10231. At fixed intervals (30 sec, 1, 5, 10 min) 20 µl of suspension were plated for CFU counts.



Time-kill assay obtained with the product diluted 1:4 showed complete microbicidal effect at 30 sec for *Staphylococcus pseudintermedius* and at 1 min for *Malassezia pachydermatis*

## RESULTS

Time-kill assay obtained with the product diluted 1:4 showed complete microbicidal effect at 30 sec for *E. coli*, *P. aeruginosa*, *S. aureus*, MRSA, *S. pseudintermedius*, *S. canis* and *Candida albicans* reference strains. The fungicidal effect was complete at 1 min for *Malassezia pachydermatis* ATCC14522, while the bactericidal effect for *Proteus mirabilis* ATCC14153 was complete within 5 min.

Bacterial Strains	Time (min)					Percentages of Reduction
	0'	0,5'	1'	5'	10'	
<i>Escherichia coli</i>	0	100	100	100	100	
<i>Pseudomonas aeruginosa</i>	0	100	100	100	100	
<i>Proteus mirabilis</i>	0	96,8	98,8	100	100	
<i>Staphylococcus aureus</i>	0	100	100	100	100	
MRSA	0	100	100	100	100	
<i>Staphylococcus pseudintermedius</i>	0	100	100	100	100	
<i>Streptococcus canis</i>	0	100	100	100	100	
<i>Malassezia pachydermatis</i>	0	99,4	100	100	100	
<i>Candida albicans</i>	0	100	100	100	100	

**CONCLUSIONS:** The product shows a very fast and complete antimicrobial activity against a panel of bacterial and fungal reference strains involved in canine cutaneous infections.

# *In vitro* antimicrobial activity of a commercial dermatologic solution (Peptivet® sol.) containing chlorhexidine digluconate, Tris-EDTA and a novel antimicrobial peptide (AMP2041)

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## INTRODUCTION

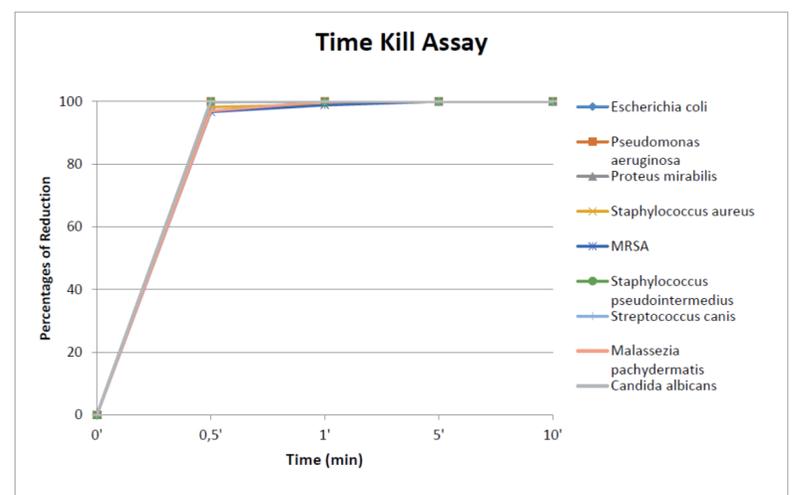
Cutaneous infections are frequently present in dogs due to bacteria and/or yeasts. The presence of new topical antimicrobials could help to reduce the use of systemic antibiotics and antifungal drugs.

### Objective

Aim of this study was to evaluate the *in vitro* antimicrobial activity of a commercial dermatologic solution (Peptivet® sol. ICF, Cremona, Italy) containing 0.02% chlorhexidine digluconate (CLX), Tris 0.4%, EDTA 0.1% and 0.5 µg/ml of antimicrobial peptide AMP2041 on bacterial and fungal reference strains involved in canine cutaneous infections.

## MATERIALS AND METHODS

The dynamics of bacterial killing was evaluated with time-kill curves obtained with undiluted product incubated with the standardized suspensions of *E. coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Proteus mirabilis* ATCC14153, *Staphylococcus aureus* ATCC25923, MRSA ATCC43300, *Staphylococcus pseudintermedius* ATCC49444, *Streptococcus canis* ATCC20715, *Malassezia pachydermatis* ATCC14522 and *Candida albicans* ATCC10231. At fixed intervals (30 sec, 1, 5, 10 min), 20 µl of suspension were plated for CFU counts.



## RESULTS

Time-kill assay obtained with the undiluted product showed complete microbicidal effect at 30 sec for *E. coli*, *P. aeruginosa*, *S. canis*, *S. pseudintermedius* and *Candida albicans* reference strains. The fungicidal effect was complete at 1 min. for *Malassezia pachydermatis* ATCC14522, while for *Proteus mirabilis*, *S. aureus*, and MRSA reference strains a complete bactericidal effect was reached within 5 min.

Bacterial Strains	Time (min)					Percentages of Reduction
	0'	0,5'	1'	5'	10'	
<i>Escherichia coli</i>	0	100	100	100	100	
<i>Pseudomonas aeruginosa</i>	0	100	100	100	100	
<i>Proteus mirabilis</i>	0	99,83	100	100	100	
<i>Staphylococcus aureus</i>	0	98,23	99	100	100	
MRSA	0	96,67	98,83	100	100	
<i>Staphylococcus pseudintermedius</i>	0	100	100	100	100	
<i>Streptococcus canis</i>	0	100	100	100	100	
<i>Malassezia pachydermatis</i>	0	97	100	100	100	
<i>Candida albicans</i>	0	100	100	100	100	

**CONCLUSIONS:** The undiluted product shows a very fast and complete antimicrobial activity against a panel of bacterial and fungal reference strains involved in canine cutaneous infections although clinical studies will be needed to evaluate the *in vivo* activity.