Dossier dermatology

ANTIMICROBIAL PEPTIDES:
a new approach

ABSTRACT
ANTIMICROBIAL PEPTIDES
THERAPEUTIC NEWS

A new and innovative approach in veterinary dermatology and otology

Emmanuel Bensignor
Specialist in dermatology, Dip ECVD, DESV Dermatology

Sébastien Viaud
Specialist in dermatology, Dip ECVD
Bacterial infections are one of the main causes for dermatology and otology consultations in dogs and cats\(^1\). The bacteria most commonly seen in veterinary dermatology is *Staphylococcus pseudintermedius*, which is found in its natural state on the skin and in the digestive tract of most dogs\(^1\) (see photo 1). Different factors affect these bacteria, which multiply and infect the skin causing more or less severe bacterial pyodermatitis or otitis.

Medical advances and the development of new antibiotic molecules have, in the past, significantly reduced the mortality and morbidity associated with bacterial infections. Nevertheless, two new phenomena threaten this certainty: the emergence and the increasingly more common transmission of antibiotic resistant bacteria and the near absence of any new antibiotic molecules being developed over the past 10 years. Consequently, infections that were previously simple to cure have become ever more difficult, if not impossible, to treat\(^2\).

This is a problem on a European and global scale\(^3\), and its significance in the veterinary world is not limited only to the health of the animal: food and direct contact with animals can be a mode of transmission of antibiotic resistance from animal to human. Over the last 10 years, there has been an ever-growing awareness concerning the selection of bacteria resistant to antibiotics in domestic animals and the possible effects on human health. This is partly due to an increase in antibiotic prescriptions for animals, also when they are not always necessary, or even to poor antibiotic prescription habits in veterinary medicine.

Given this problem, the need to develop new antimicrobial agents has increased and significant efforts have been made by the pharmaceutical industry to find different and effective approaches that can be used as an alternative to antibiotics.

A promising option, to this day, is the development of antimicrobial peptides (AMP).
RESISTANCES IN DERMATOLOGY

STAPHYLOCOCCI RESISTANT TO ANTIBIOTICS: a current problem

Until recently, staphylococci were considered easy to eradicate, because they were sensitive to most antibiotics used routinely in dermatology. However, for the past 10 years, there have been many reports on the development of resistant strains. In addition, anecdotal evidence in canine dermatology/otology reveals other resistant species that also deserve our attention, in particular gram-negative bacteria (Pseudomonas and Proteus) in the case of otitis (see photo 2). The emergence, or rather the selection, of bacterial species resistant to antibiotics has been a subject of major concern in medicine for the past several years. Among the different types of acquisition and transmission of resistance factors, methicillin resistance due to PBP2a, a penicillin binding protein produced by the staphylococci, which enables bacteria to survive in the presence of the antibiotic, is one of the most widely studied and most problematic in clinical medicine. PBP2a is produced due to the bacteria’s acquisition of the mecA gene, which is spread by SCCmec ("Staphylococcal Cassette Chromosome mec"), a mobile genetic element that is integrated in the bacterial chromosome. For S. aureus, five types of SCCmec (I to V) have been identified, differing in size and genetic composition. PCR can help determine the presence of the mecA gene and the type of SCCmec. To this day, the identified methicillin-resistant staphylococci in dogs belong to the S. aureus species (MRSA, for methicillin-resistant S. aureus), S. pseudintermedius (MRSP, for methicillin-resistant S. pseudintermedius), S. schleiferi subsp. coagulans and to other species of coagulase negative staphylococci. Moreover, it seems that these strains have a tendency towards multiple resistance: indeed, multiple resistances to antibiotics, particularly to fluoroquinolones, macrolides, aminoglycosides and tetracyclines have become increasingly more common.

MRSAs

Isolation of MRSAs has been reported in dogs for many years. These bacteria are seen in cases of pyoderma but also in healthy dogs, in carriage sites (nose, mouth, anus, nasal mucous membrane). It seems that these strains are acquired from humans, as several studies have shown transmission is possible between the owner and their pet. MRSAs are generally carried and not apparent in animals, but they can also be responsible for infections. The carriage rates vary depending on the studies, but they may be higher in some veterinary clinics, which implies possible contamination from one animal to the next. MRSAs appear to be more frequently implicated in stubborn cases of deep pyoderma (see photo 3 and Table 1).
The risk factors for MRSA acquisition are still debated, but it seems that the prior use of antibiotics (particularly beta-lactam antibiotics and especially fluoroquinolones) and the insertion of venous catheters are implicated in many cases (see box below).

### WHEN TO SUSPECT A METHICILLIN-RESISTANT BACTERIAL INFECTION?

- Post-operative or traumatic infections (non-healing wounds)
- Infection at site of implant insertion (catheters, protheses, surgical material) (see photo 4)
- Pyoderma not responding to treatment
- Previous MRSA or MRSP infection
- Owners who are MRSA carriers
- Proven presence of MRSA in the environment

### EXAMPLES OF STUDIES ON THE PREVALENCE OF MRSA COLONIZATION IN DOGS

<table>
<thead>
<tr>
<th>POPULATION</th>
<th>COUNTRY</th>
<th>PREVALENCE</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Community of all dogs</td>
<td>United Kingdom</td>
<td>0%</td>
<td>Baptiste et al. 2005</td>
</tr>
<tr>
<td></td>
<td>United Kingdom</td>
<td>0.4%</td>
<td>Rich and Roberts 2006</td>
</tr>
<tr>
<td></td>
<td>Hong Kong</td>
<td>0.7%</td>
<td>Boost et al. 2007</td>
</tr>
<tr>
<td></td>
<td>Slovenia</td>
<td>0%</td>
<td>Vengust et al. 2006</td>
</tr>
<tr>
<td></td>
<td>United States</td>
<td>4%</td>
<td>Kottler et al. 2008</td>
</tr>
<tr>
<td></td>
<td>United States</td>
<td>0%</td>
<td>Griffeth et al. 2008</td>
</tr>
<tr>
<td>Dogs seen in a veterinary clinic</td>
<td>Denmark</td>
<td>0%</td>
<td>Bagcigil et al. 2007</td>
</tr>
<tr>
<td></td>
<td>Canada</td>
<td>0.5%</td>
<td>Hanselman et al. 2007</td>
</tr>
<tr>
<td>Hospitalised dogs</td>
<td>United Kingdom</td>
<td>9%</td>
<td>Loeffler et al. 2005</td>
</tr>
<tr>
<td></td>
<td>Canada</td>
<td>0%</td>
<td>Lefebvre et al. 2006</td>
</tr>
</tbody>
</table>
**ZOONOTIC RISKS**

The zoonotic risks seem low but are not void. Transmission from animal to human (and vice versa) is possible by direct contact, dust, aerosol and food. Rather than a strict zoonotic risk, the reality we face is the transmission of antibiotic resistance genes to dogs and then onwards by dogs. The phenomenon is all the more worrying in that some clones found in humans could adapt to dogs.

**MRSPs**

Methicillin-resistant strains of *S. pseudintermedius* are becoming more regularly reported in canine dermatology. Various epidemiological studies describe a regular increase in the isolation of these strains in cases of infection (from 5% up to 46% of isolations), but also an increase in carriage in healthy individuals (up to 4.5% in dogs, namely close of 10% of isolated strains). The mecA gene was regularly isolated from strains of *S. pseudintermedius* both in North America and more recently in Europe. Furthermore, it seems that the MRSPs isolated in North America genetically differ from those found in Europe. The increase, by horizontal transfer of resistance genes, in the incidence of these strains is proven and worrying. The clinical aspects are fairly similar to "classical" pyoderma cases and this type of bacteria should be considered in the presence of recurring infections.

**METHICILLIN-RESISTANT, COAGULASE NEGATIVE STAPHYLOCOCCI**

These species appear to be on the increase and methicillin-resistant strains are regularly identified among some of these species. Recent French data reported more than 25% of resistant strains.

**TABLE 2**: Examples of studies on the prevalence of MRSP colonization in dogs.

<table>
<thead>
<tr>
<th>POPULATION</th>
<th>COUNTRY</th>
<th>PREVALENCE</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy dogs</td>
<td>United States</td>
<td>2 %</td>
<td>Griffeth et al. 2008</td>
</tr>
<tr>
<td></td>
<td>Slovenia</td>
<td>1.5 %</td>
<td>Vengust et al. 2006</td>
</tr>
<tr>
<td>Dogs seen in a veterinary clinic</td>
<td>Canada</td>
<td>2 %</td>
<td>Hanselman et al. 2007</td>
</tr>
<tr>
<td></td>
<td>Japan</td>
<td>30 %</td>
<td>Sasaki et al. 2007</td>
</tr>
<tr>
<td>Dogs with pyoderma</td>
<td>United States</td>
<td>0 %</td>
<td>Medleau et al. 1986</td>
</tr>
<tr>
<td></td>
<td>United States</td>
<td>7 %</td>
<td>Griffeth et al. 2008</td>
</tr>
<tr>
<td></td>
<td>United States</td>
<td>3.5 %</td>
<td>Kania et al. 2004</td>
</tr>
</tbody>
</table>
AVOIDING THE EMERGENCE AND DEVELOPMENT OF RESISTANCES: A challenge to consider in everyday practice

It is therefore essential to avoid the risk of developing resistant bacteria on a daily basis, which involves a decrease in the use of antibiotics and hygiene measures: all animal licking should be avoided, particularly if skin wounds are present; hands should be washed between each consultation along with disinfection of tables and the environment; routine use of antiseptics should be considered as they are proven to be effective when used alone and could potentially decrease the duration of antibiotic therapy when used in combination with a systemic antimicrobial. In this context, there has been a growing number of studies over the last few years to establish alternatives to conventional antibiotic treatments: antibacterial potentiators, bacterial virulence inhibitors, bacteriophages, proteolytic enzymes, vaccination and so forth. Among these different approaches, resorting to antimicrobial peptides is a particularly interesting new alternative for avoiding or decreasing the use of antibiotics in clinical settings.

Photo 6: The presence of resistant staphylococci requires precautions for the owner and veterinarian.

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KEY POINTS according to (10)

- Methicillin-resistance is related to staphylococci production of the PBP2a protein, which gives them a low affinity for beta-lactam antibiotics and cephalosporins. It is due to the acquisition of the mecA gene, which is spread by a mobile genetic element called SCCmec.
- Methicillin-resistant staphylococci are responsible for recurring superficial or deep pyoderma and external otitis in dogs and cats.
- These strains are increasingly more common: in 2015, around 15% to 20% of strains received by the Resapath (French network of bacteriological monitoring) were MRSPs.
- Strains of methicillin-resistant *S. pseudintermedius* are resistant to beta-lactam antibiotics and to other classes of antibiotics.
- The risk of inter-species transmission, particularly between pets and humans, does exist.
- Bacteriology testing (and possibly an antibiogram) should be performed more routinely in cases of recurring pyoderma and external otitis.
- Rational use of antibiotics, alternative antibiotic therapy measures and strict hygiene measures are the best prophylaxis against the development of resistant germs.
ANTIMICROBIAL PEPTIDES: What are they?

DISTRIBUTION, STRUCTURE AND ROLES OF AMPs

A vast majority of living organisms produce antimicrobial peptides. These peptides (AMPs) are virtually the universal agents of the first line of immune system defence for all forms of life. Classically, AMPs are relatively short chains of amino acids (from 12 to 100 amino acids), positively charged (cationic), amphipathic (with both hydrophilic and lipophilic properties), and which have been isolated in unicellular microorganisms, insects and other invertebrates, plants, amphibians, fish and mammals, including humans. The basic structural characteristic of AMPs relies on their capacity to adopt a configuration in which groups of hydrophobic and cationic amino acids are organised into discrete sectors of the molecule. To this day, more than 1,700 AMP’s have been identified, which highlights their significance in the non-specific immune system, the main defence system for most living beings. Their main role in most cases is to kill invading and potentially pathogenic organisms. However, while these peptides are described as powerful antimicrobials, their direct antimicrobial activity is certainly inhibited in normal physiological conditions by endogenous proteases, some polyvalent anions, such as glycosaminoglycans, and a low local concentration of these same peptides. Moreover, these peptides are important effectors as modulators of the immune system. Expression of these AMPs can be constitutive or induced by infectious and/or inflammatory stimuli (pro-inflammatory cytokines, bacteria, and bacterial molecular constituents like lipopolysaccharides [LPS]). Indeed, they are capable of increasing the phagocytosis process, of stimulating prostaglandin release, of neutralising the septic properties of LPS and of strengthening the recruitment and accumulation of a large variety of other defence cells on inflammatory or infectious sites. They are also capable of encouraging angiogenesis and inducing the healing process. In addition, the peptides of mammals have shown to play an active part in the transition of a non-specific immune response towards an adaptive immune response due to their positive chemotactic activity for monocytes and T-lymphocytes in humans, but also due to their adjuvant and polarising properties on the development of dendritic cells.

Antimicrobial peptides would thus be veritable sentinels of adaptive immunity. Therefore, while these peptides may have direct effects on germs by destabilising or deteriorating the membranes of bacteria, viruses or potentially pathogenic fungi, they are found to be involved in orchestrating the process of immune responses and inflammatory responses. Moreover, the importance of their microbicide activity in host defences would seem to vary depending on the sites in a same organism, but also between different organisms. In multicellular animals, it would seem that they are routinely expressed and/or located on or in those cells or tissues of the body most exposed to infections, such as the mucous membranes and the skin.

CATHELICIDINS AND DEFENSINS: Predominant antimicrobial peptides among mammals

PEPTIDES OF BACTERIAL ORIGIN

Antimicrobial peptides of bacterial origin (bacteriocins) were the first to be isolated and characterised. They contribute to the survival of bacteria by killing other bacteria that would compete for feeding with them in the same environment.

Bacteriocins are a large and diverse group classified into two categories: peptides with lanthionine (an unusual amino acid) and those without lanthionine.

Among the antibiotics (with lanthionine), nisin produced by Lactococcus lactis is undoubtedly the most extensively studied since it has been used for more than 50 years as a food preservative with no resistance phenomena having been observed to this date. Nisine, in the form of impregnated wipes, used alone or in combination with oral antibiotic therapy, has also been studied in dogs for the treatment of bacterial pyoderma and surface bacterial proliferations with some success.
VERTEBRAE PEPTIDES

As for vertebrates, AMPs have been isolated in many species. This means that even in the presence of an adaptive immune system, these peptides play a significant part in the host defence system. Their direct microbicidal activity nevertheless varies in physiological conditions and stands out especially on sites with a greater concentration, for example in the granules of the phagocytic cells of the crypts of the small intestine\textsuperscript{21}.

• Cathelicidins are a large and diverse group of AMPs in vertebrates. They are characterised by a specific common N-terminal segment (cathelin domain), which is proteolytically cleaved resulting in the active mature form of the peptide, and a cationic peptide with variable structure at its C-terminal extremity. Most cathelicidins are therefore conserved in their inactive immature form, mainly in the granules of circulating immune cells. The granules of neutrophils, for example, are the main source of cathelicidins, found mainly in the mucous membranes of the mouth, lungs, and genitourinary system and in the keratinocytes in the event of inflammation, as is the case for human cathelicidin LL-37 (the only cathelicidin identified in humans)\textsuperscript{22}. Beyond their common N-terminal segment, the structural diversity within this large family of peptides reflects distinct and visible properties, undoubtedly with various microbicidal and immunomodulatory activities. Cathelicidins have been isolated in many species of mammals such as mice, rabbits, sheep, horses, cats, dogs and humans. Animal studies reveal the significance of cathelicidins as first choice players in the innate host defence. Thus, mice with a gene abnormality to their single cathelicidin are incapable of controlling infections due to group A streptococci\textsuperscript{23}.

• Defensins are the second predominant group of AMPs in mammals\textsuperscript{21}. These cyclic peptides are divided into three sub-groups depending on the position of their disulphide linkages: α-defensins, β-defensins and θ-defensins. As with cathelicidins, defensins in vertebrates are synthesised in their pre-peptide form, which requires proteolytic maturation into active form. The α-defensins and β-defensins are extensively distributed among vertebrates, while the θ-defensins are rarer (in Old World monkeys only). The α-defensins and β-defensins are present especially in the granules of neutrophils, macrophages, NK cells, intestinal Paneth cells and in the epithelial cells in the skin and of the pulmonary and urogenital mucous membranes. \textit{In vitro} studies have shown that defensins generally have low bactericidal, fungicidal and virucidal action.

The microbicidal properties of α-defensins and β-defensins are inhibited by physiological saline concentrations\textsuperscript{24}. However, the strong concentrations of α-defensins, in particular, that can be found in some sites, and especially in the granules of phagocytic cells and in the intestinal crypts, are \textit{a priori} sufficient to express their microbicidal potential despite salt antagonism\textsuperscript{25}.

ANTIMICROBIAL PEPTIDES AND THE SKIN

Four β-defensins and one cathelicidin were isolated from human skin.

They had antimicrobial activities against bacteria, including methicillin-resistant \textit{Staphylococcus aureus} (MRSA), yeasts and viruses. Six β-defensins (cBD1-like, cBD2-like/122, cBD3-like, cBD102, cBD103 and cBD127) and one cathelicidin (cCath) were identified in the skin and mucous membranes of dogs\textsuperscript{26,27,28}. The cBD1-like, cBD2-like/122, cBD3-like, cBD103 and cCath had antimicrobial activity against MRSA, methicillin-sensitive and resistant \textit{Staphylococcus aureus}, \textit{Escherichia coli}, \textit{Proteus mirabilis}, \textit{Pseudomonas aeruginosa}, \textit{Malassezia pachydermatis} and \textit{Candida albicans}. The cBD103 appeared to be more effective against MRSA, methicillin-sensitive and resistant \textit{Staphylococcus aureus}, \textit{Escherichia coli}, \textit{Proteus mirabilis}, \textit{Pseudomonas aeruginosa}, \textit{Malassezia pachydermatis} and \textit{Candida albicans}. The cCath appeared to be more effective against \textit{Escherichia coli}\textsuperscript{29}. 

THE DIFFERENT MODES OF ACTION OF ANTIMICROBIAL PEPTIDES

AMPs target a basic difference between the constitution of membranes of germs and that of cells of multicellular organisms.

This process is presently better understood for the bacterial targets of AMPs. Indeed, bacterial membranes are organised in such a way that the outer layer of the cytoplasmic membrane presents with lipids and their negatively charged polar heads in the external environment. By contrast, the outer layer of the cytoplasmic membranes of multicellular organisms is composed of lipids with no notable charge. Most of the polar heads are part of the cytoplasmic membrane and move towards the cytoplasm.

A model proposes a mode of interaction between the AMPs and the bacterial cytoplasmic membrane, followed by structural modifications of the latter (displacement of phospholipids), its disruption and in some cases, AMPs entering the target bacterium. In addition, the presence of cholesterol molecules in the cytoplasmic membrane of multicellular organisms reduces the activity of AMPs due to the stabilisation they provide or quite simply their direct interactions with the AMPs. This theory of bacterial membrane patency was, for a relatively long time, the only mode of action considered for AMPs against bacteria. However, it is now clear that many peptides exert their microbicidal activity through alternative means, and that they could even act on several targets simultaneously.

Regardless, nearly all strategies depend on the interactions between the AMPs and the bacterial membrane: formation of temporary channels in the bacterial membrane, micellization or dissolution of the membrane, translocation through the membrane. With this last mode of action in particular, which does not constitute patency of the membrane, the AMPs would be capable of inhibiting DNA, protein, enzyme and/or bacterial wall synthesis once present in the bacterial cytoplasm.

IS RESISTANCE INVOLVED IN ANTIMICROBIAL PEPTIDES?

Resistance acquisition by an initially sensitive strain is unlikely. Of course, innate resistances are present in some bacteria, such as *Morganella spp* and *Serratia spp*, whose membranes do not have sufficiently charged lipids to guarantee AMP-bacteria interaction.

“The low probability of the emergence of bacterial resistance to AMPs is quite simply due to the essential characteristic of their relationship, i.e. the specific structure of the bacterial membrane. Thus, in short, to acquire resistance against a peptide, the organisation or the actual composition of a bacterium’s cytoplasmic membrane would need to be revised.”

When considering protease production by bacteria against one or several types of AMPs, the necessary presence of a single and constant recognition site for these enzymes should be highlighted, while most AMPs originate from sequences of indefinable amino acids without a single epitope. Finally, multicellular organisms attack bacteria using several types of AMPs with different structures. Thus, the destruction of one type of peptide would not necessarily guarantee bacteria survival in the face of other types of AMPs in action.

The problem of the emergence of bacterial resistance to conventional antibiotics, and therefore the urgent need to develop new antibiotic strategies has naturally prompted the interest for the development of AMPs in the treatment arsenal of human medicine, and more recently, in veterinary medicine.
RESULTS OF STUDIES ON THE USE OF ANTIMICROBIAL PEPTIDES IN VETERINARY DERMATOLOGY AND OTOLOGY

FIRST APPROACH

A first approach consists of encouraging the synthesis of endogenous peptides by the organism. Few studies validating this approach have been published. One *in vitro* study showed that the use of a combination of plant extracts (*Peumus boldus* and *Spiraea ulmaria*) stimulated the expression of genes coding for the synthesis of beta-defensin (hBD3) and cathelicidin (hCath)\(^\text{32}\). *In vivo*, another study, presented as a poster assessed the same combination that proved more effective than a placebo for decreasing the number of bacteria on the skin of dogs with atopic dermatitis after 14 days’ application, with a significant decrease in the ratio of staphylococci to total bacteria and with no side effects (*p* = 0.037)\(^\text{33}\).

SECOND APPROACH

A second approach consists of using antimicrobial peptides directly on the skin. This is a technique that has been validated in medical dermatology, particularly for multi-resistant staphylococci; some synthetic peptides, in addition to their direct antimicrobial effect, have a capacity to decrease the synthesis of pro-inflammatory mediators but also destroy bacterial biofilms\(^\text{34}\).

In dogs, two *in vitro* studies were performed, mainly concerning the relevance of these synthetic peptides against staphylococci in cases of pyoderma or otitis, but also for *otitis externa*, particularly caused by *Pseudomonas*. One of them, AMP2041, was created from a STAMP peptides generator and has been the subject of efficacy and safety research, particularly targeting...
methylillin-resistant strains in bovines, pigs and dogs\textsuperscript{35}. A microbicidal effect was noted after 20 minutes incubation at 100% against *Pseudomonas aeruginosa* and at 90% against *Escherichia coli*. A decrease of more than 90% was noted against *S. aureus* after 120 minutes incubation. This peptide is characterised by a high solubility degree in aqueous environments (more than 95%), which guarantees ease of use in various pharmaceutical dosage forms, high stability due to disulphide bonds, specificity towards prokaryote cells, broad spectrum of activity against bacteria and fungi, and a synergistic action with conventional antibiotics. AMP2041 selectively acts on recognising the differences in charge and composition of cytoplasmic membranes of superior eukaryote cells and those of prokaryote cells and of fungi. The bacterial cell membranes are mainly composed of negatively charged phospholipids, such as phosphatidylserine, phosphatidylglycerol, and bis[phosphatidyl]glycerol. Conversely, the eukaryote cell membranes consist of a large number of compounds with a physiological neutral pH, such as cholesterol, phosphatidylcholine and sphingomyelin, and are therefore not attacked by peptides. AMP2041 activity manifests mainly by the disruption and destruction of bacterial membranes\textsuperscript{36}.

**EFFICACY IN PYODERMA**

*In vitro*, the relevance of an AMP2014 antimicrobial peptide, Tris-EDTA and chlorhexidine digluconate combination was assessed in two formulations: shampoo containing respective amounts of 2 µg/ml, 2% and 0.08% and solution containing respective amounts of 0.5 µg/ml, 0.5% and 0.02%\textsuperscript{37,38}. The shampoo was tested at a quarter dilution against various bacterial and fungal strains at fixed intervals (30 seconds, 1, 5 and 10 minutes).

A complete antimicrobial effect was noted after 30 seconds for *E. coli*, *P. aeruginosa*, *S. aureus*, methicillin-resistant *S. aureus*, *S. pseudintermedius*, *S. canis* and *C. albicans*. For *Malassezia pachydermatis*, the effect was obtained after 1 minute and after 5 minutes for *P. mirabilis*, which confirmed that the shampoo offers a rapid and complete antimicrobial effect against the main microbes responsible for infection in dogs.

For the solution, comparable results were observed (bactericidal/fungicide effect after 30 seconds for *E. coli*, *P. aeruginosa*, *S. pseudintermedius*, *S. canis* and *C. albicans*), after 1 minute for *P. mirabilis* and *M. pachydermatis* and after 5 minutes for *S. aureus* and methicillin-resistant *S. aureus* (see photo 9).

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**EFFICACY IN OTITIS**

*In vitro*, the assessment of a gel containing 0.5 µg/mL of the AMP2041 peptide, 0.07% chlorhexidine digluconate, 0.4% Tris and 0.1% EDTA was tested on 30 strains of *Pseudomonas* isolates from cases of otitis externa\textsuperscript{39}. The minimal bactericidal concentration and time-to-kill the bacteria activity were calculated with a series of dilutions system.
Peptivet® oto gel

- Improves the physiological state of the skin
- Counteracts irritation
- Restores the skin barrier

For further information infoamp2041@icfsrl.it

References

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

AMP2041:........ IT1418804 WO2014102596
CLX - tris - EDTA:....................EP1711158
AMP2041 - CLX - tris - EDTA:..WO2015198265
A bactericidal effect in less than 30 seconds was observed for all strains tested, which suggests potential efficacy in cases of *otitis externa*.

*In vivo*, a pilot study focused on the efficacy of the same antimicrobial peptides combined with the Tris-EDTA and chlorhexidine in cases of canine *otitis externa*, acute or recurrent, caused by bacteria *(see photo 10)* or *Malassezia*.

Treatment was applied every other day for two weeks. Criteria assessed included pruritus, pain, and the presence of exudate, erythema, and erosions/ulcerations with an OTIS3 validated score and semi-quantitative analysis of the number of bacteria and yeasts.

A clinical improvement of 48.8% was noted after one week; after two weeks, improvement of 84.8% was noted with 13/16 dogs cured and 3/16 dogs showing notable improvement. Cytological improvement was 40.2% by D7 and more than 71% for bacteria *(see photo 11)* and more than 77% for yeasts by D14.

Overall assessment by the owner and the veterinarian was more than 96% by D14.

**CONCLUSION**

In the current context of reasonable use of antibiotics, of the Ecoantibio programme that provides for a decrease in the amounts of antibiotics prescribed and delivered to animals, and of the sometimes alarming increase in cases of bacterial resistance in humans and animals, developing alternative solutions to antibiotic therapy is the subject of extensive research. The discovery and development of antimicrobial peptides is in line with this process. The *in vitro* and *in vivo* proof of their efficacy is now available and it is therefore logical that this new approach be integrated into the arsenal of therapies we have in our everyday practice.

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Photo 10: Bacterial otitis in a Cocker Spaniel dog.
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Photo 11: Rod-shaped bacteria with obvious holes in the bacterial membrane caused by AMP2041.
Copyright: Parma University
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