1-17-2007

Antimicrobial activity of cathelicidins BMAP28, SMAP28, SMAP29, and PMAP23 against Pasteurella multocida is more broad-spectrum than host species specific

Kim A. Brogden
University of Iowa

Gwen E. Nordholm
United States Department of Agriculture

Mark R. Ackermann
Iowa State University, mackerma@iastate.edu

Antimicrobial activity of cathelicidins BMAP28, SMAP28, SMAP29, and PMAP23 against Pasteurella multocida is more broad-spectrum than host species specific

Abstract
The antimicrobial activity of linear, cationic α-helical peptides from cattle (BMAP28), sheep (SMAP28 and SMAP29), and pigs (PMAP23) were assessed to determine if activity was selective for *Pasteurella multocida* from a particular animal species or broad-spectrum against all *P. multocida* tested. The antimicrobial activities of synthetic peptides were determined for *P. multocida* isolated from cattle (10 isolates), sheep (10 isolates), and pigs (10 isolates) in a broth microdilution assay. All thirty isolates of *P. multocida* were susceptible to BMAP28 (MICs and MBCs, 1.0–1.9 μM); SMAP28 and SMAP29 (MICs and MBCs, 0.2–0.7 μM); and PMAP23 (MICs and MBCs, 4.3 to ≥6.8 μM). Overall, the results of this study suggest that synthesized cathelicidins from cattle, sheep, and pigs had broad-spectrum antimicrobial activity against all *P. multocida*.

Keywords
antimicrobial peptides, innate defense, Cathelicidins, Pasteurella multocida, cattle, sheep, pigs

Disciplines
Veterinary Pathology and Pathobiology

Comments
This article is from *Veterinary Microbiology* 119, no. 1 (January 2007): 76–81, doi:10.1016/j.vetmic.2006.08.005.

Available online at www.sciencedirect.com
ScienceDirect
Veterinary Microbiology 119 (2007) 76–81
veterinary microbiology
www.elsevier.com/locate/vetmic

Short communication

Antimicrobial activity of cathelicidins BMAP28, SMAP28, SMAP29, and PMAP23 against *Pasteurella multocida* is more broad-spectrum than host species specific

Kim A. Brogden [a,*], Gwen Nordholm [b], Mark Ackermann [c]

[a] *Department of Periodontics, Dows Institute for Dental Research, College of Dentistry, The University of Iowa, Iowa City, IA 52242, USA*
[b] *Respiratory Diseases of Livestock Research Unit, USDA, Agricultural Research Service, National Animal Disease Center, Ames, IA 50010, USA*
[c] *Department of Veterinary Pathology, Iowa State University, Ames, IA 50011, USA*

Received 8 June 2006; received in revised form 22 July 2006; accepted 1 August 2006

Abstract

The antimicrobial activity of linear, cationic α-helical peptides from cattle (BMAP28), sheep (SMAP28 and SMAP29), and pigs (PMAP23) were assessed to determine if activity was selective for *Pasteurella multocida* from a particular animal species or broad-spectrum against all *P. multocida* tested. The antimicrobial activities of synthetic peptides were determined for *P. multocida* isolated from cattle (10 isolates), sheep (10 isolates), and pigs (10 isolates) in a broth microdilution assay. All thirty isolates of *P. multocida* were susceptible to BMAP28 (MICs and MBCs, 1.0–1.9 μM); SMAP28 and SMAP29 (MICs and MBCs, 0.2–0.7 μM); and PMAP23 (MICs and MBCs, 4.3 to ≥6.8 μM). Overall, the results of this study suggest that synthesized cathelicidins from cattle, sheep, and pigs had broad-spectrum antimicrobial activity against all *P. multocida*.

Keywords: Antimicrobial peptides; Innate defense; Cathelicidins; *Pasteurella multocida*; Cattle; Sheep; Pigs

1. Introduction

Pasteurella multocida is a Gram-negative, facultative anaerobic, coccobacillus. It occurs as a commensal in the nasopharynx of apparently healthy animals. It can also be a primary or secondary pathogen in disease processes of domestic and feral mammals and birds (Rimler and Rhoades, 1989). As a commensal, *P. multocida* can become host-adapted and isolates from different animal species display unique somatic serotypes (Blackburn et al., 1975; Rimler et al., 1984); physiologic characteristics and biotypes (Heddleston, 1976); and capsular morphologies and capsular types (Rimler and Rhoades, 1989). Under some circumstances, interspecies transmission of *P. multocida* is thought to occur (Davies et al., 2004).

* Corresponding author. Tel.: +1 319 335 8077; fax: +1 319 335 8895.
E-mail address: kim-brogden@uiowa.edu (K.A. Brogden).

0378-1135/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2006.08.005

On oronasal mucosal surfaces of mammals, *P. multocida* can induce low grade chronic infections resulting in mucosal inflammation associated with neutrophil infiltrates. For example, gnotobiotic pigs infected intranasally by a toxigenic strain of *P. multocida* type D became colonized (Ackermann et al., 1991). Tonsillar crypt epithelium became thickened and the tonsil lumen contained intraepithelial leukocytes, neutrophils, and macrophages. At 14 days post-infection, lumens contained mucus, desquaminated cells, degranulated neutrophils, and debris. Occasionally, these organisms reach the lower respiratory tract in animals and induce pneumonia (Brockmeier et al., 2001; Brogden et al., 1998; Purdy et al., 1997).

Almost nothing is known about the host innate immune factors produced on oronasal mucosal surfaces that discourage carriage of *P. multocida* or the bacterial factors that allow them to resist elimination by host factors. Likely, the chronic inflammatory response containing neutrophils and their granular contents play a role. Neutrophil granular contents contain antimicrobial proteins and peptides, including bovine myeloid antimicrobial peptides (BMAP), sheep myeloid antimicrobial peptides (SMAP), and porcine myeloid antimicrobial peptides (PMAP) (Gennaro et al., 1998) and may control *P. multocida* colonized in the nasopharynx of the respective host species. The Pasteurellae culture collection at the National Animal Disease Center in Ames, IA, USA allowed us the unique opportunity to assess whether synthesized BMAP28, SMAP28, SMAP29, and PMAP23 had selective activity against *P. multocida* isolated from cattle, sheep, and pigs, respectively or broad-spectrum of activity against all *P. multocida*. In this study, we assessed the antimicrobial activities of these peptides for *P. multocida* isolated from cattle, sheep, and pigs in a broth microdilution assay.

2. Materials and methods

2.1. Organisms

Isolates of *P. multocida* from natural cases of pasteurellosis in cattle are generally capsular type A (Davies et al., 2004), isolates from sheep are capsular types A, D, and F (Davies et al., 2003b), and isolates from pigs are capsular types A and D (Davies et al., 2003a). Cattle isolates are somatic serotypes 3; 3,4; 3,12; and 3,4,12; sheep isolates are somatic serotypes 3 and 3,4; and pig isolates are somatic serotypes 3; 4; and 4,12 (Blackburn et al., 1975). In this study we selected 30 isolates from the *P. multocida* culture collection at the USDA, ARS, National Animal Disease Center, Ames, IA, USA that were as representative as possible of these capsular and somatic serotypes (Table 1); 10 isolates were from cattle, 10 from sheep, and 10 from pigs. *Escherichia*

Table 1
Origin and characteristics of *Pasteurella multocida* from cattle, sheep, and pigs

Culture	Origin	Species	Serotype	Site of isolation
P-5294	Texas	Cattle	A:3[a]	Not known[b]
P-5024	Idaho	Cattle	A:3	Lung
P-4922	Wisconsin	Cattle	A:3	Not known
P-5219	Texas	Cattle	A:3,4	Not known
P-4691	Virginia	Cattle	A:3,4	Not known
P-4664	Iowa	Cattle	A:3,4	Not known
P-5032	Idaho	Cattle	A:3,12	Nose
P-5238	Wisconsin	Cattle	A:3,4,12	Not known
P-5368	Idaho	Cattle	A:12	Not known
P-5649	California	Cattle	F:3,4	Not known
P-5380	Idaho	Sheep	A:–	Not known
P-2295	Texas	Sheep	A:3	Lung
P-2294	Texas	Sheep	A:3	Lung
P-5082	Washington	Sheep	A:3,4	Not known
P-2299	Texas	Sheep	A:3,12	Lung
P-5039	Iowa	Sheep	D:3	Not known
P-1028	Maryland	Sheep	D:–	Not known
P-5183	Washington	Sheep	F:–	Not known
P-5370	Idaho	Sheep	A:6,12	Not known
P-1773	Pennsylvania	Sheep	A–:–	Not known
P-5522	Canada	Pigs	A:3	Not known
P-4289	Arkansas	Pigs	A:3,12	Not known
P-5240	Wisconsin	Pigs	A:–	Not known
P-1293	Iowa	Pigs	D:3	Not known
P-2279	Missouri	Pigs	D:3	Not known
P-4290	Arkansas	Pigs	D:3,12	Not known
P-5715[c]	Canada	Pigs	D:12	Not Known
P-4986	Iowa	Pigs	D:12	Not known
P-4275[c]	Arkansas	Pigs	D:12	Nose
P-1683[c]	Iowa	Pigs	–:–	Not Known

[a] Designated as capsular:somatic serotype. A dash (–) means that neither the capsular or somatic serotype, or both, were not known.

[b] This information was not available. It was not listed in the official records received from the diagnostic laboratory at the time of request for capsular and somatic serotyping.

[c] Porcine strains P-5715, P-4275, and P-1683 were toxigenic and produced a potent, intracellular, mitogenic toxin known as *P. multocida* toxin.

Table 2
Amino acid sequences of 4 cathelicidins from cattle, sheep, and pigs

Animal/Cathelicidin	Sequence	Reference
Cattle		
BMAP28	GGLRSLGRKILRAWKKYGPIIVPIIRIG	Skerlavaj et al. (1996)
Sheep		
SMAP28[a]	RGLRRLGRKIAHGVKKYGPTVLRIIRIA-NH_2	
SMAP29	RGLRRLGRKIAHGVKKYGPTVLRIIRIAG	Bagella et al. (1995), Brogden et al. (2001), Mahoney et al. (1995), Skerlavaj et al. (1999) and Travis et al. (2000)
Pigs		
PMAP23	RIIDLLWRVRRPQKPKFVTVWVR	Storici et al. (1994), Tossi et al. (1995) and Zanetti et al. (1994)

[a] SAMP29 (SC5-1) was the peptide originally deduced from the nucleotide sequence (Mahoney et al., 1995). SMAP28 was the actual peptide isolated from ovine leukocytes (Kalfa et al., 2001). The C-terminal glycine appears to be post-translationally modified into a –NH_2 group.

coli ATCC 12795 was used as a susceptible control organism. *P. multocida* cultures in this collection have been maintained in a lyophilized state since their initial isolation to reduce any phenotypic changes that might occur as a result of repeated subculture. For this study, lyophilized cultures were reconstituted with tryptose broth, plated on dextrose starch agar, and incubated overnight at 37 °C. Single colonies were picked and transferred to Mueller Hinton broth (MHB). After incubation for 24 h at 37 °C, the cultures were adjusted to an optical density of 0.108 at 600 nm in the spectrophotometer and diluted 10^{-3}-fold to contain 1.0×10^5 CFU ml^{-1}.

2.2. Antimicrobial assay

A broth microdilution assay was used (Brogden et al., 2001; Turner et al., 1998; Wu and Hancock, 1999). Briefly, cathelicidins (Table 2) were synthesized as previously described (Brogden et al., 2001; Travis et al., 2000) and diluted in 140 mM NaCl (0.1–25.0 μM) in microtiter plates (Immulon 1 plates, ISC Bioexpress, Kaysville, Utah, USA). NaCl (140 mM) without peptide was added to control wells. MHB containing 1.0×10^5 CFU ml^{-1} −1 of *P. multocida* was added to wells containing peptides and 140 mM NaCl (positive growth control wells) and sterile MHB was added to wells containing 140 mM NaCl (negative growth control wells). After 24 and 48 h at 37 °C, the optical density of bacterial growth was determined (600 nm, Spectromax Microplate Reader, Molecular Devices Corp., Sunnyvale, CA, USA). The minimal inhibitory concentration (MIC) was defined as the lowest concentration of peptide that reduced the optical density of microbial growth by more than 50% of the positive growth control. Diluent from the preceding three wells with no optical density of microbial growth were then spotted onto trypticase soy agar with 5% defibrinated sheep blood (blood agar). The minimal bactericidal concentration (MBC) was defined as the lowest concentration of peptide that reduced viable growth when 50 μl of the well contents were plated on blood agar and incubated at 37 °C for 24 h.

3. Results

BMAP28, SMAP28, SMAP29, and PMAP23 were all active against the control strain of *E. coli* and MICs and MBCs ranged from 0.1 to 0.4 μM (Table 3). All thirty isolates of *P. multocida* were susceptible to BMAP28 (MICs and MBCs, 3.0–6.0 μg ml^{-1}), SMAP28 (MICs and MBCs, 0.3–0.4 μM), and SMAP29 (MICs and MBCs, 0.2–0.7 μM). All thirty isolates of *P. multocida* were susceptible to PMAP23 (MICs and MBCs, 4.3 to >6.8 μM) but MICs were approximately 20–40 times higher. Little variability was seen among the MIC and MBC values of SMAP28 and SMAP29 for all thirty isolates of *P. multocida*. A larger variability was seen among the MIC and MBC values of BMAP28 and PMAP23.

Table 3
Antimicrobial activities of SMAP28, SMAP29, PMAP23, and BMAP28 for *P. multocida* from cattle, sheep, and pigs[a]

	E. coli ATCC 12795		Ten strains of *P. multocida* from cattle		Ten strains of *P. multocida* from sheep		Ten strains of *P. multocida* from pigs	
	MIC[b]	MBC[b]	MIC	MBC	MIC	MBC	MIC	MBC
BMAP28	0.2 ± 0.0[c]	0.2 ± 0.0	1.0 ± 0.6[d]	1.4 ± 0.6	1.0 ± 0.2	1.9 ± 0.2	1.0 ± 0.2	1.8 ± 0.3
SMAP28	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
SMAP29	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.6 ± 0.2	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	0.7 ± 0.3
PMAP23	0.4 ± 0.0	0.4 ± 0.0	≥6.8	≥6.8	4.3 ± 1.2	5.6 ± 1.2	>6.8	>6.8

[a] The susceptibilities of other veterinary pathogens to some of these peptides were much more variable (Kalfa et al., 2001).
[b] The minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) of the peptide.
[c] μM mean + S.E. of three replications ($N = 3$) for *E. coli* MIC and MBC.
[d] μM mean + S.E. of three replications of 10 strains ($N = 30$) for *P. multocida* MIC and MBC.

4. Discussion

P. multocida is a commensal organism in the oronasal passages of cattle, sheep, and pigs. It can be isolated in lower numbers from the mucosal surfaces of apparently healthy animals and in higher concentrations from nasal secretions and pulmonary tissues of animals with polymicrobial upper respiratory disease and pneumonia (Brogden, 2002). In this study, we determined the effect to which *P. multocida* was susceptible to myeloid antimicrobial peptides and whether antimicrobial activity was host-species specific or broad-spectrum. In the context of host–parasite interactions, it is tempting to speculate that the host contains commensal (host-adapted) microorganisms that are more resistant to innate antimicrobial peptides than similar transient organisms acquired from other animal species. Such an observation can explain the carriage state or mucosal inflammatory response seen in animals chronically colonized with *P. multocida*. To the contrary, *P. multocida* isolates from cattle, sheep, and pigs were all susceptible to the four cathelicidins from the same species.

BMAP28, SMAP28, SMAP29, and PMAP23 are potent antimicrobial peptides (Brogden et al., 2003). BMAP28 has a MIC of 0.2–8.0 μM for Gram-negative and Gram-positive bacteria (Benincasa et al., 2003; Gennaro and Zanetti, 2000; Skerlavaj et al., 1996); 0.1 to >10.2 μM for fungi; and 31.9 μM for protozoan parasites like *Cryptosporidium parvum* (Giacometti et al., 2003). Spirochaetes are susceptible but at much higher levels of peptide (Sambri et al., 2002). SMAP28 and SMAP29 have MICs of 0.04–16.0 μM for Gram-negative bacteria (Anderson et al., 2004; Gennaro and Zanetti, 2000; Kalfa et al., 2001; Skerlavaj et al., 1999); 0.08–1.0 μM for Gram-positive bacteria (Anderson et al., 2004; Gennaro and Zanetti, 2000; Skerlavaj et al., 1999); 0.3 to >32.0 μM for fungi (Anderson et al., 2004; Gennaro and Zanetti, 2000; Skerlavaj et al., 1999); and 3.1–30.7 μM for *C. parvum* (Giacometti et al., 2003). Again, spirochaetes are susceptible but at much higher levels of peptide (Sambri et al., 2002). Congeners of SMAP29 are also antimicrobial against bacteria (Kalfa et al., 2001) and fungi (Lee et al., 2002a). PMAP23 has a MIC of 2–16 μM for Gram-negative and Gram-positive bacteria (Storici et al., 1994; Zanetti et al., 1994); 2.5–20.0 μM for fungi (Lee et al., 2001; Lee et al., 2002b); and 25 μM for nematodes like *Caenorhabditis elegans* (Park et al., 2004). It is either an α-helical antimicrobial peptide (Lee et al., 2002b) or an antiparallel β-sheet peptide connected by a loop at the center (Zhang et al., 2000).

Overall, the results of this study suggest that BMAP28, SMAP28, SMAP29, and PMAP23 are potent antimicrobial peptides that do not have host-specific antimicrobial activities. Rather these peptides appeared to have broad-spectrum antimicrobial activities against *P. multocida* isolates from cattle, sheep, and pigs. However, we used an in vitro, broth-based assay, and we remain cautious that in vivo there may be host-specific antimicrobial activities. This may occur with peptides expressed by their own host in situ as well as when tested against a microorganism expressing whatever is appropriate for a given microenvironment and growing perhaps in a non-planktonic form.

Acknowledgment

We are grateful for the critical reading of the manuscript by Elizabeth A. Schmitt, Cairn Communications, MN.

References

Ackermann, M.R., Cheville, N.F., Gallagher, J.E., 1991. Colonization of the pharyngeal tonsil and respiratory tract of the gnotobiotic pig by a toxigenic strain of *Pasteurella multocida* type D. Vet. Pathol. 28, 267–274.

Anderson, R.C., Hancock, R.E., Yu, P.L., 2004. Antimicrobial activity and bacterial-membrane interaction of ovine-derived cathelicidins. Antimicrob. Agents Chemother. 48, 673–676.

Bagella, L., Scocchi, M., Zanetti, M., 1995. cDNA sequences of three sheep myeloid cathelicidins. FEBS Letters 376, 225–228.

Benincasa, M., Skerlavaj, B., Gennaro, R., Pellegrini, A., Zanetti, M., 2003. In vitro and in vivo antimicrobial activity of two alpha-helical cathelicidin peptides and of their synthetic analogs. Peptides 24, 1723–1731.

Blackburn, B.O., Heddleston, K.L., Pfow, C.J., 1975. *Pasteurella multocida* serotyping results (1971–1973). Avian Dis. 19, 353–356.

Brockmeier, S.L., Palmer, M.V., Bolin, S.R., Rimler, R.B., 2001. Effects of intranasal inoculation with *Bordetella bronchiseptica*, porcine reproductive and respiratory syndrome virus, or a combination of both organisms on subsequent infection with *Pasteurella multocida* in pigs. Am. J. Vet. Res. 62, 521–525.

Brogden, K.A., 2002. Polymicrobial diseases of animals and humans. In: Brogden, K.A., Guthmiller, J.M. (Eds.), Polymicrobial Diseases. ASM Press, Washington, DC, pp. 3–20.

Brogden, K.A., Ackermann, M., McCray, P.B., Tack, B.F., 2003. Antimicrobial peptides in animals and their role in host defences. Int. J. Antimicrob. Agents 22, 465–478.

Brogden, K.A., Kalfa, V.C., Ackermann, M.R., Palmquist, D.E., McCray Jr., P.B., Tack, B.F., 2001. The ovine cathelicidin SMAP29 kills ovine respiratory pathogens in vitro and in an ovine model of pulmonary infection. Antimicrob. Agents Chem. 45, 331–334.

Brogden, K.A., Lehmkuhl, H.D., Cutlip, R.C., 1998. *Pasteurella haemolytica* complicated respiratory infections in sheep and goats. Vet. Res. 29, 233–254.

Davies, R.L., MacCorquodale, R., Baillie, S., Caffrey, B., 2003a. Characterization and comparison of *Pasteurella multocida* strains associated with porcine pneumonia and atrophic rhinitis. J. Med. Microbiol. 52, 59–67.

Davies, R.L., MacCorquodale, R., Reilly, S., 2004. Characterisation of bovine strains of *Pasteurella multocida* and comparison with isolates of avian, ovine and porcine origin. Vet. Microbiol. 99, 145–158.

Davies, R.L., Watson, P.J., Caffrey, B., 2003b. Comparative analyses of *Pasteurella multocida* strains associated with the ovine respiratory and vaginal tracts. Vet. Rec. 152, 7–10.

Gennaro, R., Scocchi, M., Merluzzi, L., Zanetti, M., 1998. Biological characterization of a novel mammalian antimicrobial peptide. Biochim. Biophys. Acta 1425, 361–368.

Gennaro, R., Zanetti, M., 2000. Structural features and biological activities of the cathelicidin-derived antimicrobial peptides. Biopolymers 55, 31–49.

Giacometti, A., Cirioni, O., Del Prete, M.S., Skerlavaj, B., Circo, R., Zanetti, M., Scalise, G., 2003. In vitro effect on *Cryptosporidium parvum* of short-term exposure to cathelicidin peptides. J. Antimicrob. Chemother. 51, 843–847.

Heddleston, K.L., 1976. Physiologic characteristics of 1,268 cultures of *Pasteurella multocida*. Am. J. Vet. Res. 37, 745–747.

Kalfa, V.C., Jia, H.P., Kunkle, R.A., McCray Jr., P.B., Tack, B.F., Brogden, K.A., 2001. Congeners of SMAP29 kill ovine pathogens and induce ultrastructural damage in bacterial cells. Antimicrob. Agents Chem. 45, 3256–3261.

Lee, D.G., Kim, D.H., Park, Y., Kim, H.K., Kim, H.N., Shin, Y.K., Choi, C.H., Hahm, K.S., 2001. Fungicidal effect of antimicrobial peptide, PMAP-23, isolated from porcine myeloid against *Candida albicans*. Biochem. Biophys. Res. Commun. 282, 570–574.

Lee, D.G., Kim, P.I., Park, Y., Park, S.C., Woo, E.R., Hahm, K.S., 2002a. Antifungal mechanism of SMAP-29 (1–18) isolated from sheep myeloid mRNA against *Trichosporon beigelii*. Biochem. Biophys. Res. Commun. 295, 591–596.

Lee, D.G., Kim, P.I., Park, Y., Woo, E.R., Choi, J.S., Choi, C.H., Hahm, K.S., 2002b. Design of novel peptide analogs with potent fungicidal activity, based on PMAP-23 antimicrobial peptide isolated from porcine myeloid. Biochem. Biophys. Res. Commun. 293, 231–238.

Mahoney, M.M., Lee, A.Y., Brezinski-Caliguri, D.J., Huttner, K.M., 1995. Molecular analysis of the sheep cathelin family reveals a novel antimicrobial peptide. FEBS Letters 377, 519–522.

Park, Y., Jang, S.H., Lee, D.G., Hahm, K.S., 2004. Antinematodal effect of antimicrobial peptide, PMAP-23, isolated from porcine myeloid against *Caenorhabditis elegans*. J. Pept. Sci. 10, 304–311.

Purdy, C.W., Raleigh, R.H., Collins, J.K., Watts, J.L., Strauss, D.C., 1997. Serotyping and enzyme characterization of *Pasteurella haemolytica* and *Pasteurella multocida* isolates recovered from pneumonic lungs of stressed feeder calves. Curr. Microbiol. 34, 244–249.

Rimler, R.B., Rebers, P.A., Phillips, M., 1984. Lipopolysaccharides of the Heddleston serotypes of *Pasteurella multocida*. Am. J. Vet. Res. 45, 759–763.

Rimler, R.B., Rhoades, K.R., 1989. *Pasteurella multocida*. In: Adlam, C., Rutter, J.M. (Eds.), Pasteurella and Pasteurellosis. Academic Press, London, pp. 37–73.

Sambri, V., Marangoni, A., Giacani, L., Gennaro, R., Murgia, R., Cevenini, R., Cinco, M., 2002. Comparative in vitro activity of five cathelicidin-derived synthetic peptides against Leptospira, Borrelia and *Treponema pallidum*. J. Antimicrob. Chemother. 50, 895–902.

Skerlavaj, B., Benincasa, M., Risso, A., Zanetti, M., Gennaro, R., 1999. SMAP-29: a potent antibacterial and antifungal peptide from sheep leukocytes. FEBS Lett. 463, 58–62.

Skerlavaj, B., Gennaro, R., Bagella, L., Merluzzi, L., Risso, A., Zanetti, M., 1996. Biological characterization of two novel cathelicidin-derived peptides and identification of structural requirements for their antimicrobial and cell lytic activities. J. Biol. Chem. 271, 28375–28381.

Storici, P., Scocchi, M., Tossi, A., Gennaro, R., Zanetti, M., 1994. Chemical synthesis and biological activity of a novel antibacterial peptide deduced from a pig myeloid cDNA. FEBS Lett. 337, 303–307.

Tossi, A., Scocchi, M., Zanetti, M., Storici, P., Gennaro, R., 1995. PMAP-37, a novel antibacterial peptide from pig myeloid cells. cDNA cloning, chemical synthesis and activity. Eur. J. Biochem. 228, 941–946.

Travis, S.M., Anderson, N.N., Forsyth, W.R., Espiritu, C., Conway, B.D., Greenberg, E.P., McCray Jr., P.B., Lehrer, R.I., Welsh, M.J., Tack, B.F., 2000. Bactericidal activity of mammalian cathelicidin-derived peptides. Infect. Immun. 68, 2748–2755.

Turner, J., Cho, Y., Dinh, N.N., Waring, A.J., Lehrer, R.I., 1998. Activities of LL-37, a cathelin-associated antimicrobial peptide of human neutrophils. Antimicrob. Agents Chem. 42, 2206–2214.

Wu, M., Hancock, R.E., 1999. Interaction of the cyclic antimicrobial cationic peptide bactenecin with the outer and cytoplasmic membrane. J. Biol. Chem. 274, 29–35.

Zanetti, M., Storici, P., Tossi, A., Scocchi, M., Gennaro, R., 1994. Molecular cloning and chemical synthesis of a novel antibacterial peptide derived from pig myeloid cells. J. Biol. Chem. 269, 7855–7858.

Zhang, G., Ross, C.R., Blecha, F., 2000. Porcine antimicrobial peptides: new prospects for ancient molecules of host defense. Vet. Res. 31, 277–296.