VETERINARY APPLICATIONS OF ANTIMICROBIAL PEPTIDES

The World Health Organisation lists antimicrobial resistance as one of the top ten threats to global health. Until recently, one of the key drivers of this problem was the routine use of antibiotics in agriculture to prevent disease in livestock and foodborne illness in consumers, and to enhance animal growth. To help slow the spread of resistant bacteria, regulators now prohibit most preventive use of antibiotics in agriculture. However, these restrictions are causing a number of problems in terms of animal health and product recalls due to bacterial contamination, resulting in significant financial losses for producers.

Amphoraxe is developing antimicrobial peptides (AMPs) as direct replacements for antibiotics. AMPs are short immune system proteins produced by all classes of life as a natural defense against infection with bacteria, fungi, and viruses. Categorically, AMPs are not "antibiotics". They have different structures, act faster and via different mechanisms, and do not induce resistance to the same degree. An AMP-based drug, colistin, has been used as a "last-resort" clinical treatment since 1959, and has also been widely used in agriculture. The first resistant strains were discovered only in 2011, whereas resistance to conventional antibiotics arises much more quickly - typically within 10-20 years, and often much faster[1].

Activity Test	Minimum Inhibitory Concentration (MIC) (ug/mL)													
Bacterial Isolate	P2	P2M	P3	P3M	P4	P4M	P5	P5M	P6	P6M	Ran-4	Ran-4M	LL37 (+ve)	Tp_P5 (-ve)
Gram negative														
E. coli ATCC 25922	16	4-8	-	64	32-64	16-32	4-16	4-16	8-16	4	2-4	8-16	8-16	-
ESBL *E. coli*	16	2-8	-	64	64	32	8-32	4-16	8-16	4	4-8	32-64	4-8	-
CPO *E. coli* NDM	16	4-8	-	128+	64-128	32-64	4-32	4-16	16-32	4-8	4-8	16-64	8-16	-
CPO *E. coli* KPC	16-32	8	-	64-128	32	16-32	4-16	4-16	8	4	4-8	16-32	8-16	-
S. enterica spp. Enteritidis	64	32-64	-	-	-	-	64-128	16-64	64-128	16-32	16-32	-	64+	-
S. enterica spp. Heidelberg	128	16-64	-	-	-	128+	32-128	16-64	64	16-32	16-32	128	-	-
Gram positive														
S. aureus ATCC 29213	-	64-128	-	-	-	-	4-8	2-8	128+	64-128	64-128	8-23	128+	-
MRSA	-	32-64	-	-	-	-	2-8	2-8	128+	64-128	64-128	8-16	-	-
Hemolysis Test	P2	P2M	P3	P3M	P4	P4M	P5	P5M	P6	P6M	Ran-4	Ran-4M	LL37 (+ve)	Tp_P5 (-ve)
HC_{50} (ug/mL) – 1X PBS	-	-	-	-	-	-	128	-	-	-	128	-	-	-
HC_{50} (ug/mL) – RPMI	-	64	-	-	-	-	8-64	64-128	-	-	16-64	-	-	-

The versatility of AMPs affords them a number of potential applications in veterinary and human medicine. Our initial veterinary target is poultry farming, where there is an urgent need for new solutions for infectious disease prevention and gut health optimization. At Amphoraxe we are initially working on two specific applications of our AMPs:

1. *In ovo* injectable prophylactic use, to reduce first week animal mortality from Avian Pathogenic *E. coli* and reduce the rate of colonisation by *Salmonella*, *Campylobacter*, and other microbes;
2. Feed additives, to prevent infection with a range of microbial species throughout the lifespan.

Our partner academic lab has discovered, optimised, and validated over 100 novel candidate AMPs from the genome and transcriptome of the North American bullfrog[2]. The efficacy of novel AMPs has been validated *in vitro* against a range of relevant bacterial pathogens, including various strains of *E. coli* and *Salmonella* (see Table, above). Furthermore, the pipeline's integrated machine learning tool has been demonstrated to have the capacity to selectively mutate amino acid residues of an AMP to improve antimicrobial action, safety, stability, manufacturing costs, and other desirable characteristics. Nine out of 12 designed mutant peptides tested showed an increase in activity, or become active when the native peptides were found to be inactive (see Table).

Preliminary toxicity data using porcine red blood cells demonstrate that the majority of these novel AMPs are non-toxic to animal cells in hemolysis assays. Hemolytic activity was only observed for two peptides, one of which was rendered non-toxic by implementing a sequence modification suggested by our optimisation pipeline.

Conventional AMP discovery pipelines are based on expensive and specialised proteomics protocols. One of the key innovations of the pipeline used to discover the AMPs described above was to use genomics and transcriptomics data[3]. The pipeline is three orders of magnitude faster and cheaper than proteomics approaches, due to reduced analysis time and computational resources, with no biological sample preparation or processing expenses. It is therefore a validated pipeline for the ongoing discovery, optimization, and validation of novel AMPs.

We are seeking co-development partnerships to further the progress of our lead peptides. Please contact **info@amphoraxe.ca** for more information.

1. Ventola, C. L. The antibiotic resistance crisis: part 1: causes and threats. *PT* **40**, 277–283 (2015).
2. Helbing, C. C. *et al.* Antimicrobial peptides from Rana [Lithobates] catesbeiana: Gene structure and bioinformatic identification of novel forms from tadpoles. *Scientific Reports* **9**, 1529 (2019).
3. Li, C. *et al.* AMPlify: attentive deep learning model for discovery of novel antimicrobial peptides effective against WHO priority pathogens. *bioRxiv* **155705**, (2020).

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