ANTIMICROBIAL PEPTIDES AS THERAPIES FOR MULTI-DRUG RESISTANT INFECTIONS

Most of the antimicrobial drugs used today are based on small molecule antibiotic compounds, which have lost or are losing effectiveness against bacteria that cause life-threatening infections. The search for novel antibiotics using conventional compound screening methods has yielded only 12 new approved drugs since 2000. All are iterations of existing drugs; no new class of antibiotics has been developed since daptomycin in 1986. Alternative approaches are needed to address the global crisis of antimicrobial resistance.

Amphoraxe is developing novel antimicrobial peptides (AMPs). AMPs are short immune system proteins, produced by all classes of life as a natural defense against bacterial, yeast, and viral infections. AMPs often play multiple roles against infections, acting directly on the infectious agents and/or modulating the innate or adaptive immune systems. AMPs and small molecule antibiotics have different structures, mechanisms, and metabolization. Categorically, AMPs are not "antibiotics". They act faster, do not damage microbial DNA, 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	-	-	-

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 these novel AMPs has been validated *in vitro* against a range of bacterial pathogens on the World Health Organization's priority list, including drug-resistant strains/serovars of *E. coli*, *S. aureus*, and *Salmonella*. Promisingly, many of the discovered AMPs have demonstrated significant activity against all isolates tested, irrespective of species, strain/serotype, and resistance status (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. 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 the 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 is its use of genomics and transcriptomics data[3]. The pipeline is three orders of magnitude higher in throughput and lower in cost than proteomics approaches, due to reduced analysis time and computational resources, with no biological sample preparation or processing expenses. The pipeline has therefore been validated for the ongoing discovery, optimization, and validation of novel AMPs, to hit the moving target of antimicrobial resistance.

We are seeking co-development partnerships to further the progress of the current lead peptides. Since AMPs have multiple mechanisms of action, there are also opportunities to co-develop diverse combination approaches. For example, some AMPs can sensitise bacteria to conventional antibiotics and other categories of antimicrobials, and enhance the efficacy of host immune system-based approaches including vaccines and immunotherapy [4]. 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).
4. Casciaro, B., Cappiello, F., Verrusio, W., Cacciafesta, M. & Mangoni, M. L. Antimicrobial Peptides and their Multiple Effects at Sub-Inhibitory Concentrations. *Current Topics in Medicinal Chemistry* **20**, 1264–1273 (2020).

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