The *in vitro* and the *in silico* power couple: facilitating the discovery of novel anti–infective strategies based on antimicrobial peptides and quorum sensing inhibitors

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The persistent growth of antibiotic-resistance and the resilience of biofilm-related infections is pressing researchers to develop novel strategies to control infectious diseases. New antimicrobial strategies, namely concerning the use of i) antimicrobial peptides (AMP) (natural compounds with alternative mechanisms of action), ii) quorum-sensing inhibitors (QSI) (destabilisers of key communication mechanisms that regulate virulence and biofilm formation); and iii) antimicrobial combinations (can lower effective concentrations and achieve synergy), can lead to more effective therapeutics for this ever-growing, world-wide problem.

This work presents a two-fold approach regarding these strategies, namely i) comprehensive *in silico* characterisation of existing experimental results on AMP combinations and QSI through bioinformatics, and ii) *in vitro* (laboratorial) study of novel AMP and QSI combinations.

The *in silico* approach outputted a semi-automated curation workflow [1] that supported the mining of scientific literature on AMP combinations and QSI, enabling the reconstruction of antimicrobial networks that allowed the creation of two public databases. The first (http://sing.ei.uvigo.es/antimicrobialCombination/) contains information on AMP combinations against major pathogenic bacteria/fungi. Records describe species, strains, combination effects, methodologies, mode of growth, and expert observations. The second (http://pcquorum.org) contains QSI information on *Pseudomonas aeruginosa*, capturing the effects over QS genes, QS signals and virulence factors/mechanisms. These drug-QS interactions are contextualised by details on the experimental methods, drugs, QS entities and strains.

In the *in vitro* approach, colistin was combined with the AMP temporin-A, citropin-1.1 and tachyplesin-I (linear analogue) and tested to prevent (prophylaxis) or treat (therapeutics) planktonic and biofilm cultures of *P. aeruginosa* and *Staphylococcus aureus*. These tests included single- and double-species biofilms, encompassing six strains (two MDR). Results showed synergy and additiveness for both bacteria, even for MDR double-species biofilms. The most effective combinations, however, were toxic, but future work will tackle this issue [2]. Current work is testing AMP combinations with QSI, such as Azithromycin, which is the top QSI in the PCQuorum database, against biofilms.

The outcomes derived from both approaches were complementary: the databases aided in the AMP combinations and species selection to be tested *in vitro*, which in turn outputted valuable information to be added to the databases, thus bridging the gap between the two approaches. Globally, the use of the two-fold approach (*in silico+in vitro*) allowed not only the creation of important resources for fellow researchers, but also pointed out AMP combinations that were deemed promising in the treatment of double-species biofilms of relevant pathogens.

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[2] Jorge, P, Grzywacz, D, Kamysz, W, Lourenço, A & Pereira, MO, Searching for new strategies against biofilm infections: colistin-AMP combinations against *Pseudomonas aeruginosa* and *Staphylococcus aureus* single- and double-species biofilms, *PLOS ONE* 12(3), e0174654, 2017.