Networking the Way towards Antimicrobial Combination Therapies

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Abstract. The exploration of new antimicrobial combinations is a pressing concern for Clinical Microbiology due to the growing number of resistant strains emerging in healthcare settings and in the general community. Researchers are screening agents with alternative modes of action and interest is rising for the potential of antimicrobial peptides (AMPs). This work presents the first ever network reconstruction of AMP combinations reported in the literature fighting *Pseudomonas aeruginosa* infections. The network, containing 193 combinations of AMPs with 39 AMPs and 154 traditional antibiotics, is expected to help in the design of new studies, notably by unveiling different mechanisms of action and helping in the prediction of new combinations and synergisms. The challenges faced in the attempted text-mining approaches and other considerations regarding the manual curation of the data are pointed out, reflecting about the future automation of this type of reconstruction as means to widen the scope of analysis.

Keywords: Antimicrobial peptides, drug synergism, interaction network, *Pseudomonas aeruginosa* infections.

1 Introduction

Clinical Microbiology is currently facing major challenges regarding the discovery and/or design of new antimicrobial agents and the development of novel antimicrobial strategies. Drug and even multi-drug resistant (MDR) strains are emerging with increasing frequency, and rendering ineffective many conventional antibiotic treatments. Therefore, research is focused on finding alternatives to keep new resistance from developing and to prevent the resistance that already exists from spreading, either by discovering biomolecules with antimicrobial potential and different mode of action, or by combining agents and potentiating their efficacy. Notably, there is a growing interest in the use of antimicrobial combinations as a strategy to increase the antimicrobial spectrum, prevent the emergence of resistance, reduce toxicity and side

J. Sáez-Rodríguez et al. (eds.), *8th International Conference on Practical Appl. of Comput. Biol. & Bioinform. (PACBB 2014)*, Advances in Intelligent Systems and Computing 294,
DOI: 10.1007/978-3-319-07581-5_24,

effects and provide synergistic activity. In fact, synergy testing has been encouraged to guide clinical treatments for MDR strains, namely *Pseudomonas aeruginosa* associated pulmonary exacerbation [1].

Although combinations may be accomplished by using traditional antimicrobials, the most promising strategy at the moment is the use of novel antimicrobials with new mechanisms of actions, either combined with each other or with traditional compounds. Notably, antimicrobial peptides (AMPs) are short-length peptides (between 15 and 30 amino acids) that exert activity against a broad spectrum of microorganisms, such as Gram-negative and Gram-positive bacteria (including drug-resistant strains), and are effective both in planktonic and biofilm scenarios [2]. These peptides have been recognized as promising candidates to replace classical antibiotics due to their multiple mechanisms of action and low specificity in terms of molecular targets, which reduces the chance of acquired resistance [3]. Besides, AMPs can influence processes which support antimicrobial action, like cytokine release, chemotaxis, antigen presentation, angiogenesis and wound healing [4].

Recent advances in large-scale experimental technologies have resulted in an accumulation of data that reflect the interplay between biomolecules on a global scale. Bioinformatics approaches, such as network reconstruction, can help in profiling and interpreting the activity of AMPs and thus, in exploiting their potential as antimicrobial drugs. Networks can be used to map the interaction data outputted by combination studies, can be explored to unveil new interactions at the global scale, and also to classify new drugs by their mechanism of action [5]. In particular, the investigation of antimicrobial combinations has been supported by network models that have demonstrated that the partial inhibition of few cell targets can be more efficient than the complete inhibition of a single target [6].

Pharmacological networks can be constructed and integrated from heterogeneous and complementary sources of chemical, biomolecular and clinical information, but most of the information related to drug combinations is scattered over scientific literature. Manual curation is effort and time consuming, and virtually unfeasible if a systematic and up-to-date screening is desired. In this regard, some works have introduced text mining approaches to mine drug information. Most of these works focus on drug-drug interactions (DDIs), which are related to adverse events of combinations of available drugs, usually targeting the same gene or pathway [7].

The aim of this work was to reconstruct the first ever AMP interaction network. Currently, several databases collect AMP related data, namely sources, targets and minimum inhibitory concentrations [4]. However, it is difficult to find information about AMP interactions in these databases. Scientific literature is the primary source of data and thus, the use of text mining tools was investigated to alleviate manual curation. The application of AMP-based therapies to the treatment of infections caused by the bacterium *P. aeruginosa*, one of the most studied pathogenic microorganisms, was chosen as proof-of-concept to the development of a more systematic reconstruction framework.

2 Materials and Methods

PubMed was searched for papers on synergistic interactions including AMPs. Specifically, we required the presence of any variant of the term synergism, using synergis* (where the * is a wildcard), following the query "antimicrobial peptide *Pseudomonas aeruginosa*". Then, we manually curated the interactions described in the papers yielded by the search, with at least on AMP as one of the combined antimicrobials. These interactions were represented in a network where nodes identify antimicrobial agents and edges encode the interactions among agents. Edge labels encode information on experimental evidence, such as *P. aeruginosa* strain(s), mode of growth (planktonic, biofilm and in vivo), method of combination analysis (determination of the fractional inhibitory concentration (FIC), time-kill assay, among others), and the PMID and URL of the publication.

Besides producing a high-quality reconstruction of known AMP interactions, this manual curation provided a "gold standard" for text mining. Notably, authors were able to identify the main elements of information to be collected as well as challenges in the interpretation of texts. The outputs of two public text mining tools were then evaluated – Chilibot [8] and PubTator [9].

3 Results and Discussion

3.1 AMP Literature Curation

From a total of 203 papers resulting from our PubMed search, 132 papers were manually curated. Some papers were excluded (37 %) on the basis of their relevance to the topic. Notably, these papers do not mention AMPs, *P. aeruginosa* as target and/or cover for antitumor and food preservation areas. Review works were also excluded (9.1 %), since most would represent repeated data from the other curated papers whilst they do not provide necessary details on the experiments. Then, some interesting evidences arose from the analysis of the information retrieved from the literature (Table 1).

Regarding the mode of bacterial growth, 91 % of the studies focused on the use of combinations on planktonic cells, and, surprisingly, only 3 % of the studies covered the biofilm mode of growth. Biofilms are recognized as one of the main causes of several infections in humans [10], being highly related to nosocomial and chronic infections, and more resistant to treatment [2]. As such, more studies should be devoted to AMP combinations towards biofilm treatment.

Most of the combinations (80 %) involved one AMP combined with another compound, mainly traditional antibiotics. This is linked to the rational that AMPs can enhance the activity of antibiotics, which act upon intracellular targets, by disrupting the cell membrane and facilitating the access within the cell. In terms of the bacterial strains most used, these studies focused on the reference strain *P. aeruginosa* ATCC 27853, *P. aeruginosa* PAO1, and clinical isolates. Finally, the determination of the fractional inhibitory concentration (FIC) and the time-kill assays stand out as the most common methods for synergy assessment, which is in concordance with recent reviews of the field [1].

Table 1. Statistics on the manual curation process. Legend: * - not in the network; ** - info retrieved from abstract; could be on the network.

		No. of papers (%)	*No. of combinations (%)*
Total curated		132 (100)	-
Off topic*		49 (37)	-
Reviews*		12 (9.1)	-
Manuscript not available/different language**		16 (12)	-
Total in the network		**56 (100)**	**193 (100)**
Mode of growth	Biofilm	2 (3.6)	3 (1.6)
	Planktonic	51 (91)	187 (97)
	In vivo	7 (13)	8 (4.1)
	Unknown	2 (3.6)	3 (1.6)
Combination with	AMPs	18 (32)	39 (20)
	Antibiotics and others	48 (86)	154 (80)

Regarding the text mining approaches, the success of the tools tested in automatically curate AMP knowledge was poor. Chilibot limits the search to a maximum of 50 terms at a time. So, it is not possible to execute a systematic screening of interactions among all known AMPs (using AMP database records as input, for example). In turn, PubTator does not recognize most AMPs as chemical entities. Moreover, authors became aware that full-text curation should be a requirement for this line of reconstruction. Most abstracts do not cover all combinations tested, focusing only on the best outcomes, and a great part do not give information about strains and methodology.

3.2 Network Topology

The constructed network (Figure 1) is represented as a non-oriented graph, containing 121 nodes, representing AMPs and other antimicrobial compounds, and 193 edges, each correspondent to a combination. The network is non-homogeneous, with an interior containing highly connected drugs and an exterior comprised of some drugs with low interactions. Each node is linked to an average of 3 nodes, which means that each AMP was combined with an average of 3 antimicrobials.

The network is dominated by a small number of highly connected nodes. The colors on the nodes on Figure 1 correspond to their degree of connectivity, i.e. the number of nodes to which they are directly connected, ranging from the highly connected nodes (red) to nodes with only one connection (green). The most highly connected node (degree = 49), hence the most used compound in studies concerning AMP combinations, is colistin. Colistin (polymyxin E) is an AMP currently used as a last resource treatment for *P. aeruginosa* infections in the respiratory tract of cystic fibrosis patients, as well as other MDR Gram negative bacterial infections [11]. The use of colistin combined with other antimicrobial could lower its dosage and, thus, the associated toxicity. Colistin is followed in connectivity by polymyxin B (degree = 19), which belongs to the same family of AMPs, which indicates a preference for polymyxins in combination studies towards *P. aeruginosa*.

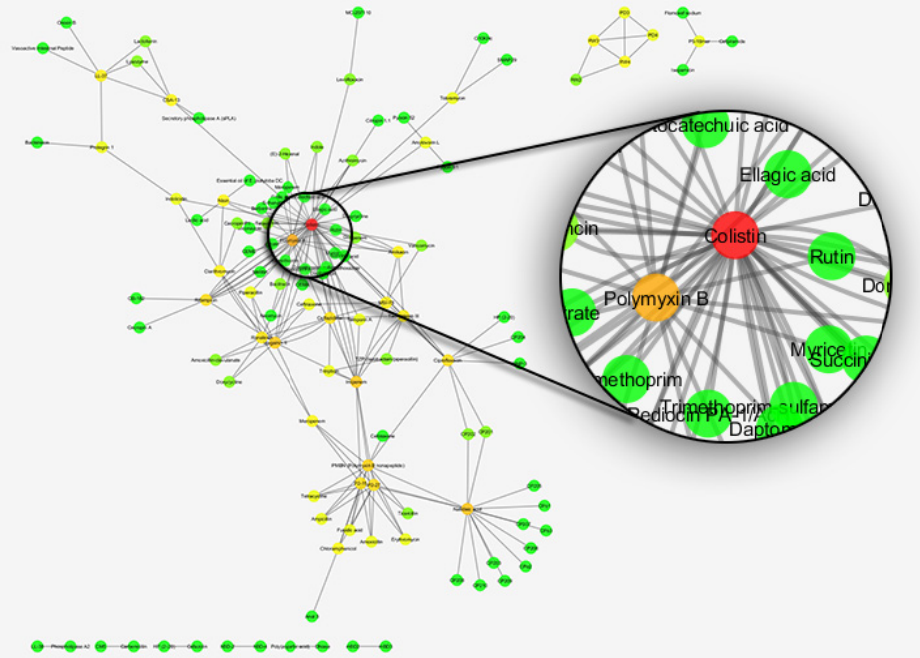

Fig. 1. General view of the AMP interaction network for *P. aeruginosa*, highlighting the two most connected AMPs – colistin and polymyxin B. Colors range from red (higher degree of connectivity) to green (lower degree of connectivity).

4 Conclusions

The increasing number of publications on the antimicrobial potential of drug combinations offers a wealth of information that can support interpretation of experimentally derived data and greatly enhance hypothesis generation. Drug interaction and functional networks are not simply new renditions of existing data: they provide the opportunity to gain insights into the impact of antimicrobial strategies in pathogenic systems.

We presented in this work the first reconstruction of an AMP combination network, specifically for the bacterial pathogen *P. aeruginosa*. Text-mining approaches that are currently available online came short in helping in the construction of the network. By recurring to the manual curation of PubMed articles, the authors were able to identify far more information that the one provided in the abstracts, which brings attention to the lack of systematization in data presentation. The resulting network currently contains 193 annotated combinations, with AMPs combined with 39 AMPs and 154 traditional antibiotics. The network is dominated by few highly connected AMPs, such as colistin and polymyxin B, characterizing them as current favorites in these studies. Future work will soon lead to a more complete network, encompassing also studies regarding antagonism. The annotation of this information is of interest, since antagonism is becoming a hot topic due to its possible role on the evolution and spread of drug resistance [5].

Acknowledgements. The authors thank the project PTDC/SAU-ESA/646091/2006/ FCOMP-01-0124-FEDER-007480FCT, the Strategic Project PEst-OE/EQB/ LA0023/2013, the Project "BioHealth - Biotechnology and Bioengineering approaches to improve health quality", NORTE-07-0124-FEDER-000027, co-funded by the Programa Operacional Regional do Norte (ON.2 – O Novo Norte), QREN, FEDER, the project "RECI/BBB-EBI/0179/2012 - Consolidating Research Expertise and Resources on Cellular and Molecular Biotechnology at CEB/IBB", FCOMP-01-0124-FEDER-027462, and the Agrupamento INBIOMED from DXPCTSUG-FEDER unha maneira de facer Europa (2012/273). The research leading to these results has received funding from the European Union's Seventh Framework Programme FP7/REGPOT-2012-2013.1 under grant agreement n° 316265, BIOCAPS. This document reflects only the author's views and the European Union is not liable for any use that may be made of the information contained herein. The authors also acknowledge the PhD Grant of Paula Jorge, Ref. SFRH/BD/88192/2012.

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