Bismuth-based drugs sensitize *P. aeruginosa* to multiple antibiotics

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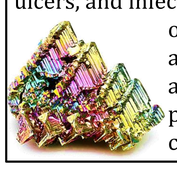
ABSTRACT

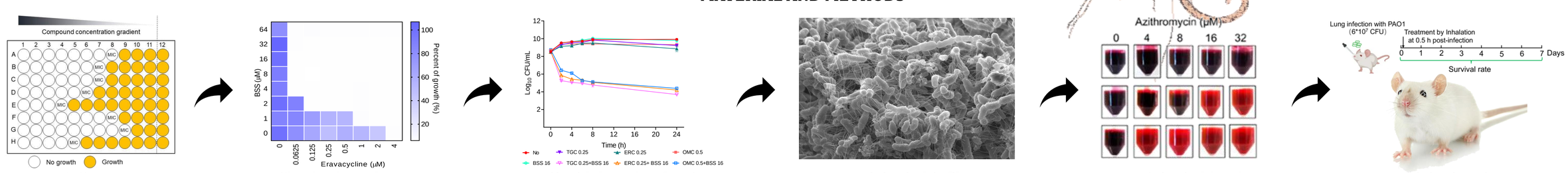
The emergence and rapid spread of multi-drug resistant (MDR) bacteria pose a serious threat to the global healthcare systems. New antibacterial substances and/or new treatment strategies to deal with the infections by MDR pathogens are urgently needed, especially against Gram-negative pathogens which remain largely the most challenging group. *Pseudomonas aeruginosa* (PA) is undoubtedly one of the most problematic Gram-negative pathogens. Its infections are difficult to treat as it doesn´t respond to commonly used antibiotics because of its high-level resistance to multiple antibiotic families. Synergistic combinations with already-in-use drugs have proven to be a powerful alternative in the treatment of antibiotic-resistant bacteria, allowing to extend both, the useful life of current antibiotics and their spectrum of action. Here, we screened 55 antimicrobial agents combined with 14 metal-based compounds against *P. aeruginosa*. Surprisingly, we found that, unlike other metals, bismuth-based compounds displayed strong and specific synergistic effects with a range of antibiotics families including macrolides, rifamycins, tetracyclines (for which PA is naturally resistant), or quinolones, even inhibiting the development of a high level of resistance to quinolones or tetracycline-related antibiotics. In addition, we proved that the bismuth compound enhanced the killing efficacy of the antibiotics in complex matrices as biofilms or in blood in an *ex vivo* PA bacteremia model. Finally, we demonstrated the activity of the combination *in vivo* in a lung PA mice infection model against a carbapenem resistance clinical strain. Our study provides some realistic treatment options for combining the FDA-approved bismuth-related drugs with multi antibiotics to combat infections caused by PA.

INTRODUCTION

PA is an opportunistic pathogen, which infects a wide range of hosts including plants, animals, and humans. In Spain and Europe, about 9-10% of the total infections are produced by PA being its presence even higher in the high-care units (13%). In humans, PA is the leading pathogen causing infections in vulnerable patients, for example, those with cystic fibrosis and other obstructive pulmonary diseases and in patients with permanent bladder catheters or who are burn wounds, diabetic foot ulcers, and infections occurring in otherwise healthy subjects, such as otitis media and keratitis. PA possesses a high level of intrinsic resistance to most antibiotics through restricted outer membrane permeability, efflux systems pumps, or the production of antibiotic-inactivating enzymes. To this must be added the extraordinary ability of this bacteria to survive antibiotic attack due to transient alterations in gene and/or protein expression in response to an environmental stimulus, as well as the formation of biofilms and the generation of persister cells. For all these reasons, PA is one of the bacterial pathogens that are considered priority targets for the development of novel antimicrobials by the WHO. Combinations of antibiotics and antibiotics or with non-antibiotic activity-enhancing compounds offer a productive strategy to address the widespread emergence of antibiotic-resistant strains. The combined use of two compounds to increase their potential/efficacy against pathogens is known as synergism and it is emerging as one of the most effective strategies in the fight against multi-resistant bacteria. In fact, currently, 9/43 of the therapeutic solutions in the pipeline are combinations of two known antibiotics. Here we explore the potential of bismuth-related drugs in combination with traditional antibiotics to fight PA infections from *in vitro* to *in vivo.*



MATERIAL AND METHODS



RESULTS

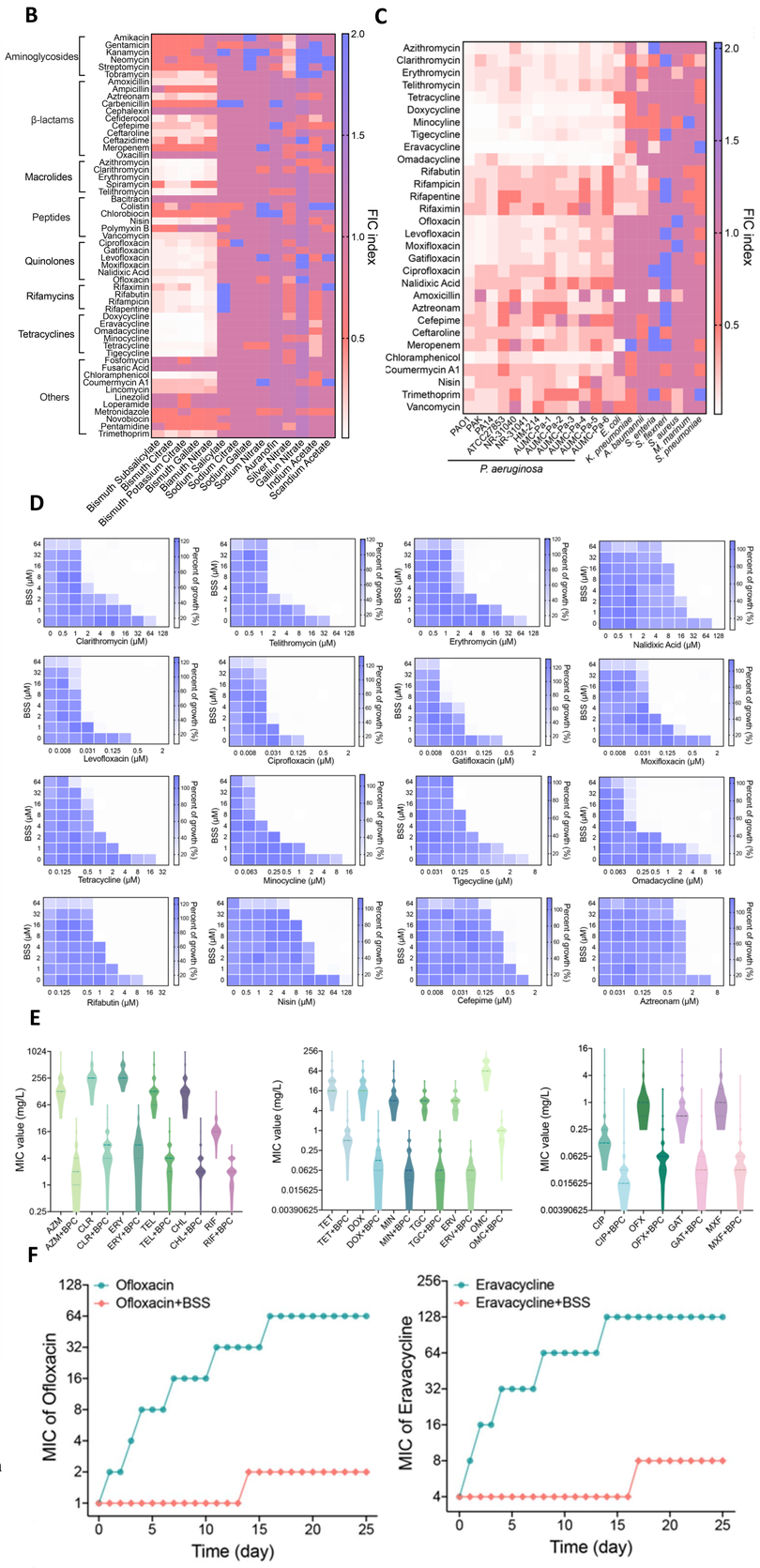
A

Antibiotics	MICa (μM)↓ MICb/MICbc (μM)→	Bismuth Subsalicylate 1024/16	FICI
Amikacin	1	0.5	0.516
Gentamicin	1	0.5	0.516
Kanamycin	64	32	0.516
Neomycin	2	1	0.516
Streptomycin	16	8	0.516
Tobramycin	2	0.5	0.266
Amoxicillin	>128	32	0.141
Ampicillin	>128	>128	1.016
Aztreonam	16	2	0.141
Carbenicillin	64	32	0.516
Cephalexin	>128	>128	1.016
Cefiderocol	0.5	0.0313	0.078
Cefepime	2	0.5	0.266
Ceftaroline	128	16	0.141
Ceftazidime	2	0.5	0.266
Meropenem	2	1	0.516
Oxacillin	>128	>128	1.016
Azithromycin	128	4	0.047
Clarithromycin	128	8	0.078
Erythromycin	128	8	0.078
Spiramycin	>128	128	0.516
Telithromycin	64	4	0.078
Ciprofloxacin	0.25	0.0156	0.078
Gatifloxacin	0.5	0.0156	0.063
Levofloxacin	0.5	0.0313	0.078
Moxifloxacin	2	0.125	0.078
Nalidixic Acid	>128	32	0.141
Ofloxacin	1	0.0625	0.078
Bacitracin	>128	>128	1.016
Colistin	0.125	0.0625	0.516
Chlorobiocin	0.25	0.125	0.516
Nisin	128	8	0.078
Polymyxin B	0.125	0.0625	0.516
Vancomycin	>128	32	0.141
Rifaximin	8	2	0.266
Rifabutin	16	2	0.141
Rifampicin	16	2	0.141
Rifapentine	32	4	0.141
Doxycycline	16	0.0625	0.020
Eravacycline	4	0.0313	0.023
Omadacycline	16	0.25	0.031
Minocycline	16	0.125	0.023
Tetracycline	32	1	0.047
Tigecycline	8	0.125	0.031
Fosfomycin	>128	>128	1.016
Fusaric Acid	>128	>128	1.016
Chloramphenicol	128	2	0.031
Coumermycin A1	4	1	0.266
Lincomycin	>128	64	0.266
Linezolid	128	128	1.016
Loperamide	>128	>128	1.016
Metronidazole	>128	128	0.516
Novobiocin	>128	128	0.516
Pentamidine	>128	>128	1.016
Trimethoprim	>128	64	0.266

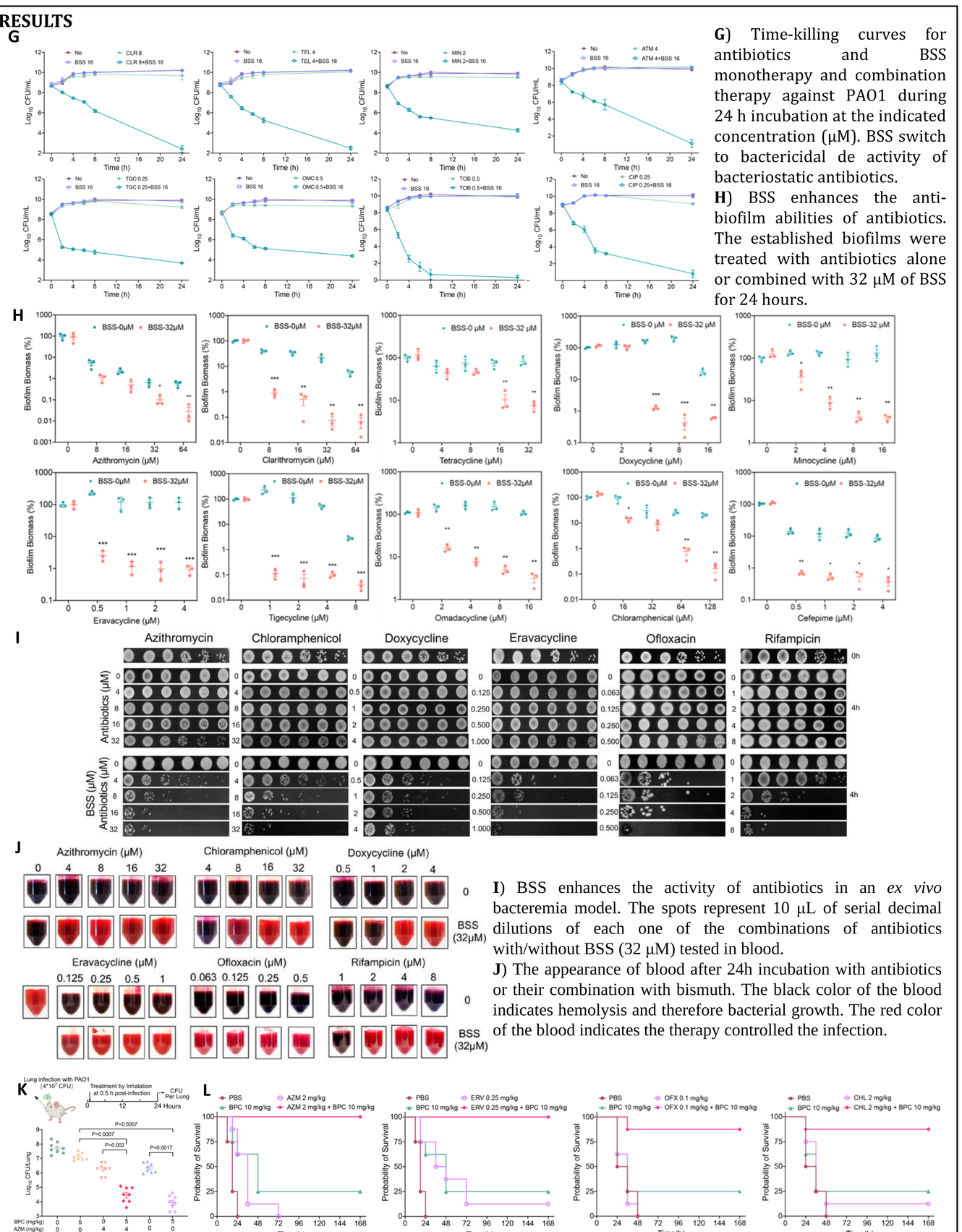
$$FIC_A=\frac{\text{MIC compound A in combination with B}}{\text{MIC compound A}}$$

$$FIC_B=\frac{\text{MIC compound B in combination with A}}{\text{MIC compound B}}$$

$$FICI=FIC_A+FIC_B \begin{cases} \text{FICI} \geq 4 \text{ Antagonism} \\ 4<\text{FICI}\geq \text{ Indiferent} \\ 1<\text{FICI}\geq 0.5 \text{ Additive} \\ \text{FICI}<0.5 \text{ Synergistic} \end{cases}$$



A) MIC reduction for different drugs in the presence of 16 μM of bismuth subsalicilate (BSS) and Fractional Inhibitory Concentration Index (FICI) calculation. **B**) The heat map of the FICI for the synergistic effects of 55 antimicrobial agents combined with five different bismuth drugs, their sodium counterpart salts, and five metal-based compounds against PAO1.**C**) The heat map of the FICI for the synergistic effects of BSS and antibiotics against different strains of PA and other pathogens. **D**) Representative heat plots of microdilution checkerboard assay for the combination of different antibiotics with BSS against PAO1. **E**) Susceptibility testing showing the combined effect of BSS (8 mg/L) and several antibiotics on 124 clinically isolated PA. **F**) The addition of BSS thwarts the evolution of ofloxacin and eravacycline resistance in PAO1.



G) Time-killing curves for antibiotics and BSS monotherapy and combination therapy against PAO1 during 24 h incubation at the indicated concentration (μM). BSS switch to bactericidal de activity of bacteriostatic antibiotics.

H) BSS enhances the anti-biofilm abilities of antibiotics. The established biofilms were treated with antibiotics alone or combined with 32 μM of BSS for 24 hours.

I) BSS enhances the activity of antibiotics in an *ex vivo* bacteremia model. The spots represent 10 μL of serial decimal dilutions of each one of the combinations of antibiotics with/without BSS (32 μM) tested in blood.

J) The appearance of blood after 24h incubation with antibiotics or their combination with bismuth. The black color of the blood indicates hemolysis and therefore bacterial growth. The red color of the blood indicates the therapy controlled the infection.

K) Bacterial load in the mouse lung infection model. The bacterial load of the lung infected with a sub-lethal dose of PA decreased significantly after a single dose of drug-combined treatments.

L) Survival curves showing efficacies in a murine acute pneumonia model. Increased survival rates of mice over 7 d challenging by a lethal dose of PA, treated with bismuth (10 mg/kg), antibiotics alone or in combination. AZM, azithromycin, ERV, eravacyclin, OFX, ofloxacin, CHL, chloramphenicol.

CONCLUSIONS

In this work, we conducted a detailed study on the synergistic effect of bismuth-based drugs and antibiotics against *Pseudomonas aeruginosa*. Here, we demonstrated the potential of repurposing the FDA-approved anti-*H. pylori* bismuth-related drugs to enhance multiple antibiotic efficacies against *P. aeruginosa* from *in vitro* to *in vivo*. We showed the potential of bismuth to impair the ability of *Pseudomonas* to develop *in vitro* resistance to antibiotics as tetracyclines or quinolones, as well as its capacity to induce a switch in the nature of the biocidal action of most of the tested antibiotics, changing from bacteriostatic to bactericidal and being even active in biofilms. Our results obtained in the *ex vivo* bacteremia model and in the *in vivo* lung infection mice model provides strong evidence for this strategy to enhance the antimicrobial activity of existing antibiotics against *P. aeruginosa* infection, extending the use of those ones not in clinical administration against this bacteria and supplying a source of alternative treatments against *Pseudomonas* while new drugs are developed.

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