Antibiotics of the future are prone to resistance in Gram-negative pathogens

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INTRODUCTION

- Many pharmaceutical companies halted antibiotic research due to the unpredictable success of new drugs amid rising MDR bacteria.
- Predicting resistance evolution early in drug discovery is crucial for developing durable antibiotics.
- We combined laboratory evolution and functional metagenomics to study resistance emergence to 13 post-2017 and developing antibiotics (‘recent’), compared to 6 in current clinical use (‘control’).
- Laboratory evolution revealed clinically relevant resistance within 60 days of antibiotic exposure in Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii and Pseudomonas aeruginosa.
- **OUR MAIN GOAL WAS TO COMPARE THE RESISTANCE PROFILES OF ‘RECENT’ ANTIBIOTICS WITH ANTIBIOTICS ESTABLISHED FOR CLINICAL USE (‘CONTROL’).**

ANTIBIOTIC	ABBREVIATION	ANTIBIOTIC CLASS	GENERATION
Omadacycline	OMA	Tetracyclines	Recent
Eravacyline	ERA		Recent
Doxycycline	DOX		Control
Ceftobiprole	CTO	Cephalosporines	Recent
Cefiderocol	CID		Recent
Cefepime	CEP		Control
Delafloxacin	DEL	Topoismerase inhibitors	Recent
Gepotidacin	GEP		Recent
Zoliflodacin	ZOL		Recent
Moxifloxacin	MOX		Control
Apramycin	APR	Aminoglycosides	Recent
Gentamicin	GEN		Control
Sulopenem	SUL	Carbapenems	Recent
Meropenem	MER		Control
Tridecaptin M152-P3	TRD	Membrane-targeting	Recent
POL-7306	POL		Recent
SCH-79797	SCH		Recent
SPR-206	SPR		Recent
Polymyxin B	PMB		Control

Table 1 - Antibiotics used in the study.

OUR FRAMEWORK TO PREDICT ANTIBIOTIC RESISTANCE

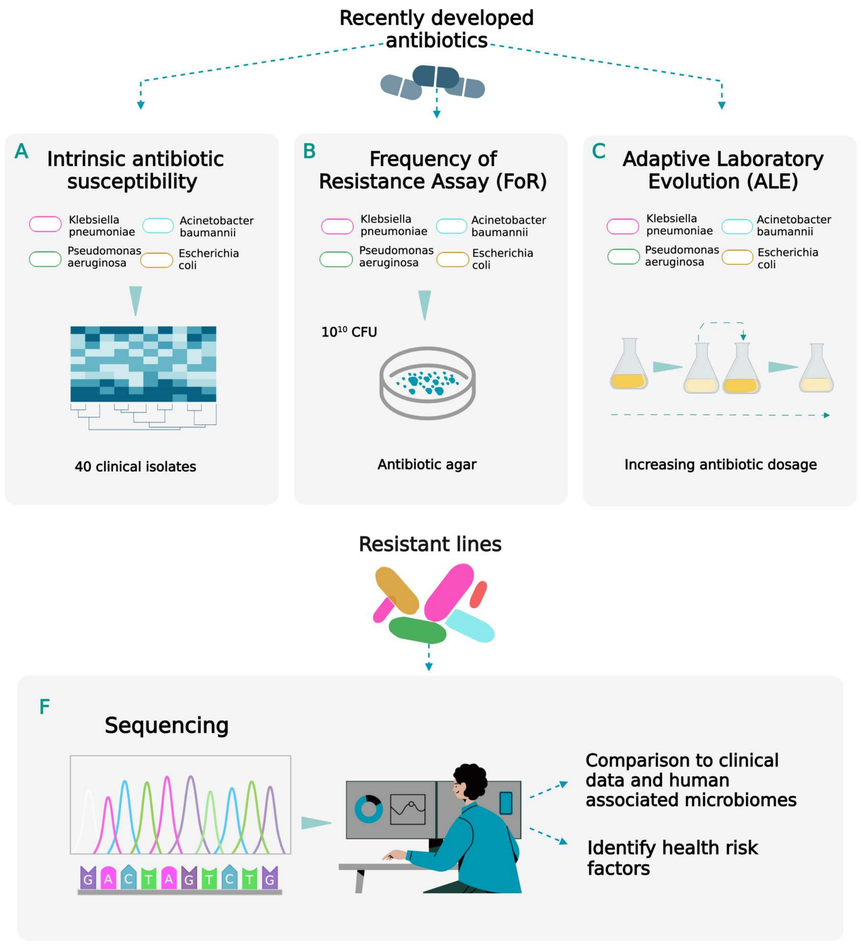

Figure 1 - Applied methodology.

SPECIES SPECIFIC RESISTANCE EVOLVES RAPIDLY TO MOST STUDIED ANTIBIOTCS

- Resistance evolved in 120 generations (60 days) of laboratory evolution.
- Clinically relevant level of resistance evolved in 87% of all studied populations.
- On average, recent and control antibiotics were equally prone to bacterial resistance (paired t-test, one-sided, P = 0.37).
- There was a highly significant variability in resistance evolution across antibiotic–strain combinations. (Kruskal–Wallis, P < 0.00001)
- **In contrast, antibiotics that simultaneously target membrane integrity and block another cellular pathway display reduced resistance development.**

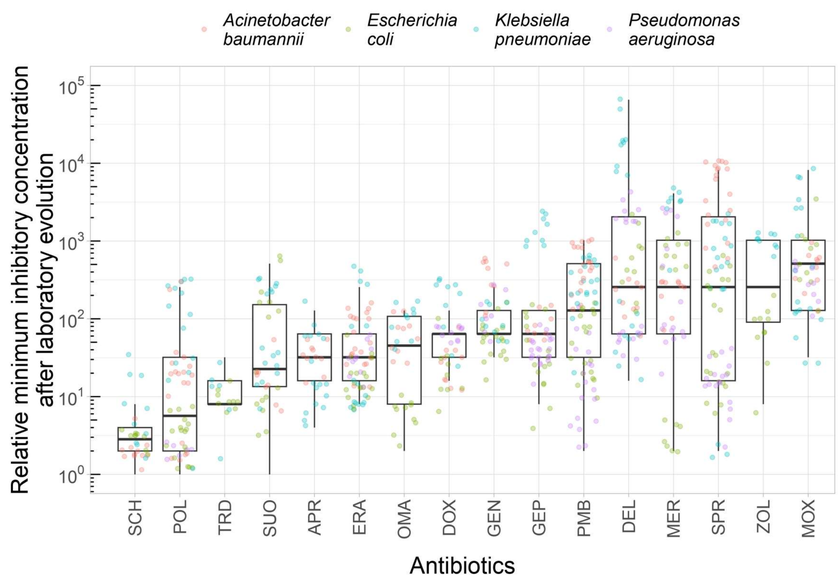

Figure 2 - Relative MIC of laboratory-evolved lines across all antibiotic–treatments.

OVERLAP IN MUTATED GENES DESPITE VARYING ANTIBIOTIC TREATMENTS

- 604 mutated protein-coding genes were detected, 193 of which were mutated in at least 2 independently evolved lines per species.
- Of all parallel-mutated genes, 69.4% carried mutations in lines adapted to different antibiotics.
- These results indicate that despite differences in antibiotic treatments, there is considerable overlap in the set of mutated genes.

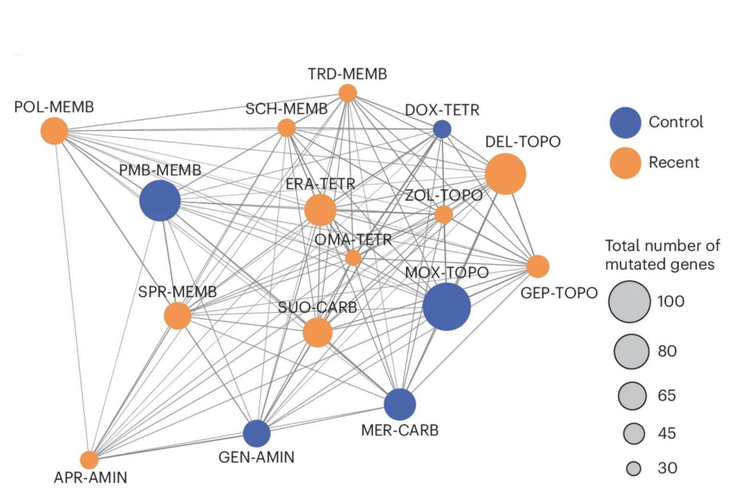

Figure 3 - Mutation profile similarity across antibiotic treatments.

RESISTANCE MUTATIONS TO RECENT ANTIBIOTICS ARE ALREADY PRESENT IN THE ENVIRONMENT

- We examined non-synonymous mutations from lab-evolved A. baumannii and E. coli in 15,185 and 20,786 natural isolates, estimating their environmental frequencies.
- For E. coli, up to 31.4% of the 245 laboratory-observed non-synonymous mutations were identified in at least 1 of the genomes from natural isolates, whereas for A. baumannii, 27.3% of 216 mutations were found in at least 1 natural isolate.
- No significant difference was found in the fraction of non-synonymous mutations shared by natural strains between control and recent antibiotics (binomial regression model, two-sided test, P = 0.206).

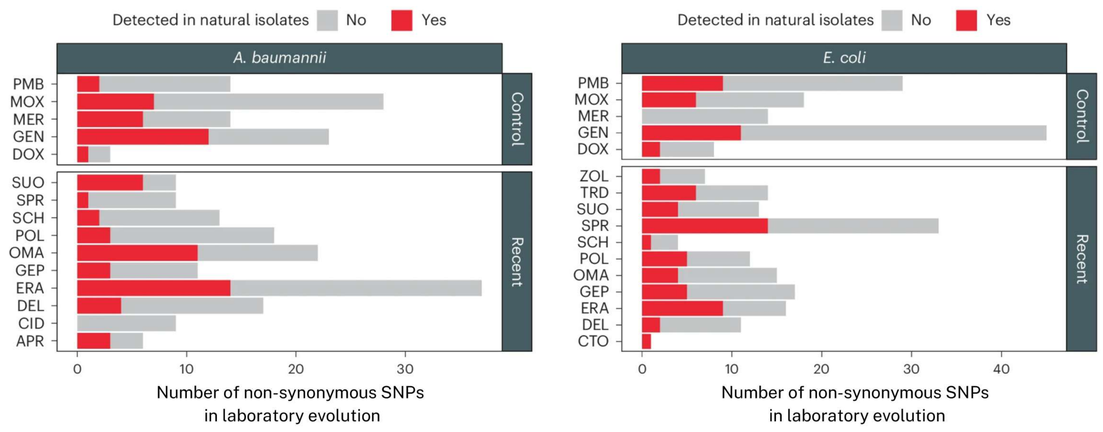

Figure 4 - Non-synonymous mutations shared by laboratory-evolved lines and natural isolates of A. baumannii. The bar plots show the number of non-synonymous mutations found in laboratory-evolved A. baumannii adapted to different antibiotics. Mutations also detected in the genomes of natural isolates of the same species are marked in red, whereas those that remained undetected are marked in grey.

CONCLUSIONS

- Antibiotic candidates in development are as susceptible to resistance evolution in Gram-negative ESKAPE pathogens as those in clinical use.
- The molecular mechanisms of resistance to recent antibiotics overlap with those found in commonly used ones.
- Resistance mechanisms to recent antibiotics are already present in natural populations of pathogens.
- Certain combinations of antibiotics and bacterial strains are less prone to developing resistance, emphasizing the potential of narrow-spectrum antibacterial therapies.
- POL-7306, SCH79797 and tridecaptin M152-P3, all membrane targeting antibiotic candidates with dual mode of action show limited susceptibility to resistance.

PUBLICATIONS

- Daruka, L., Czikkely, M.S., Szili, P. et al. ESKAPE pathogens rapidly develop resistance against antibiotics in development in vitro. Nat Microbiol 10, 313–331 (2025)
- Ana Martins et al., Antibiotic candidates for Gram-positive bacterial infections induce multidrug resistance. Sci. Transl. Med. 17, eadl2103 (2025)
- **Why POL-7306, SCH79797 and tridecaptin M152-P3 are not prone to resistance?**
Maharramov, E., Czikkely, M.S., Szili, P. et al. Exploring the principles behind antibiotics with limited resistance.
Nat Commun 16, 1842 (2025)