01521

In vitro synergy screens of FDA-approved drugs combined with last-line antibiotics reveal new bactericidal combinations against *Klebsiella pneumoniae*

05. New antibacterial agents, PK/PD & Stewardship

5a. Mechanisms of action, new compounds, preclinical data & pharmacology of antibacterial agents

Marta Gómara-Lomero [1], José Antonio Aínsa [1, 2], Santiago Ramón-García [1, 2, 3]

[1]Mycobacterial Genetics Group. Department of Microbiology, Paediatrics, Radiology and Public Health. Faculty of Medicine. University of Zaragoza - Zaragoza (Spain), [2]CIBER Respiratory Diseases, Carlos III Health Institute - Madrid (Spain), [3]Research & Development Agency of Aragon (ARAID) Foundation (Spain)

Background Treatment of infections caused by multi-drug resistant (MDR) enterobacteria remains challenging due to the limited therapeutic options. Drug repurposing could accelerate the development of urgently needed successful interventions.This work aimed to identify and characterize novel drug combinations against Klebsiella pneumoniae based on the concepts of synergy and drug repurposing.

Methods A semi high-throughput synergy screen (sHTSS) was optimized for K. pneumoniae to identify synergistic partners that would enhance the activity of three Primary Compounds (PCs), tigecycline, colistin and fosfomycin (last-line antibiotics against MDR Enterobacteriaceae). An FDA-library containing 1,430 clinically approved drugs was screened against these PCs.Synergy was defined as previously described (PMID: 21576426), based on the increment of the inhibition zones in presence of the PC (Figure 1). Selected hits were further validated by checkerboard (CBA) and time-kill (TKA) assays. A priority list of potential favourable combinations among the three PCs and FDA compounds was generated based on synergy, bactericidal activity and pharmacokinetic and pharmacodynamics properties analyses of the pairwise combinations.

Results sHTSS yielded 37, 31 and 41 hits showing synergy with tigecycline, colistin and fosfomycin, respectively. We found hits able to enhance the activity of more than one PC. Hit classification by their therapeutic use revealed most hits (75%) were known antibiotics (Figure 2). Non-antibiotic compounds included other anti-infective agents (7%), antineoplastics (7%) or antipsychotics (3%). Overall, 15.09% and 65.85% of hits were further confirmed by CBA and TKA respectively, indicating that TKA is better predictor of drug interactions than CBA. Accordingly, TKA were used for synergy classification by determining the bactericidal activities at 8, 24 and 48 hours. Twenty-seven combinations were validated by TKA showing synergistic activity against K. pneumoniae at the terminal endpoint of 48 hours, a proxy for

culture sterilization (Figure 3). We identified seven novel combinations with non-antibiotic drugs.

Conclusions sHTSS paired to TKA was validated as a useful tool for the identification of novel synergistic combinations involving drug repurposing against K. pneumoniae.Novel combinations showed effective eradication results against K. pneumoniae, and further pre-clinical studies might support their translational potential.

Figure 1.

Figure 1. Visual representation of a semi-High-throughput Synergy Screen (sHTSS) plate analysis. FDA compounds were pin-spotted onto a soft agar lawn of *K. pneumoniae* in the absence (control plate) or in the presence of sub-inhibitory concentrations of fosfomycin (16 and 32 mg/L; 1/8xMIC and 1/4xMIC, respectively). Compounds whose zones of inhibition were larger in the presence of fosfomycin than in plates without fosfomycin (examples A, B or C) were selected as hits for further validation. Ø, inhibition zone diameter; Red arrows, inhibition zone diameter; r, inhibition zone radius (when diameter could not be determined); $MIC_{FOSFOMYCIN}$ = 128 mg/L.

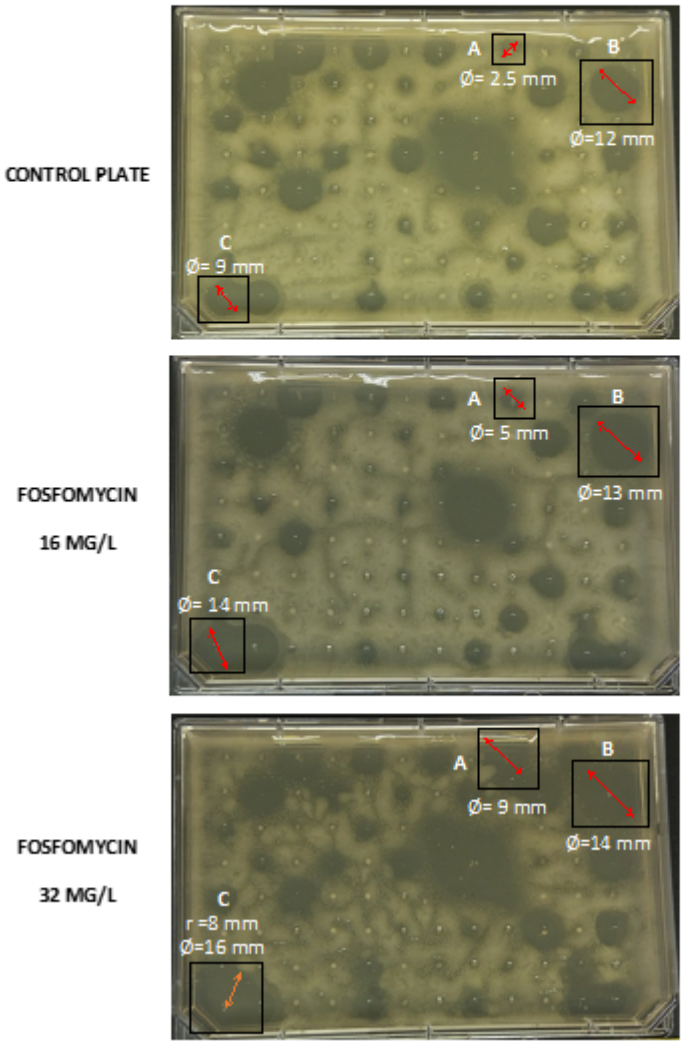


Figure 2.

Figure 2. FDA compounds with favorable interactions with tigecycline, colistin and fosfomycin identified by sHTSS and classified by their therapeutic use. Other anti-infective agents include antiparasitic, antiseptic and antiviral agents. Duplicate hits were removed from analysis. sHTSS, semi-high throughput synergy screening.

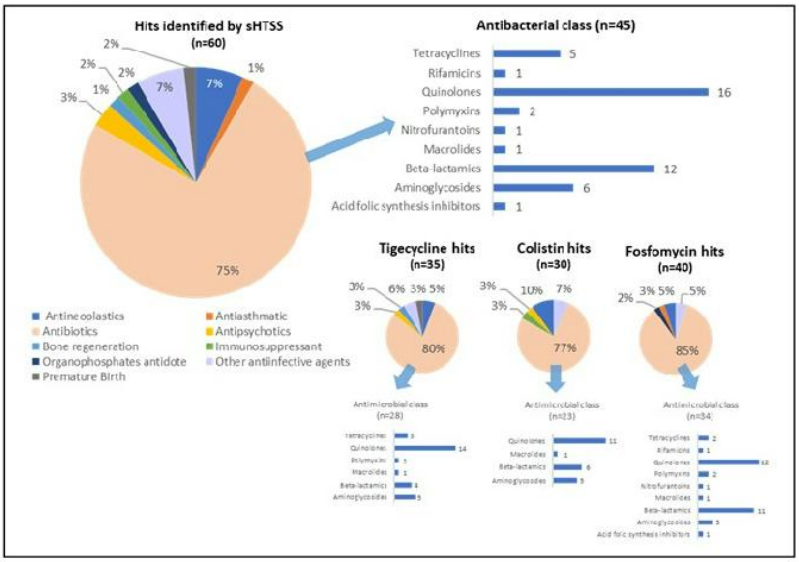
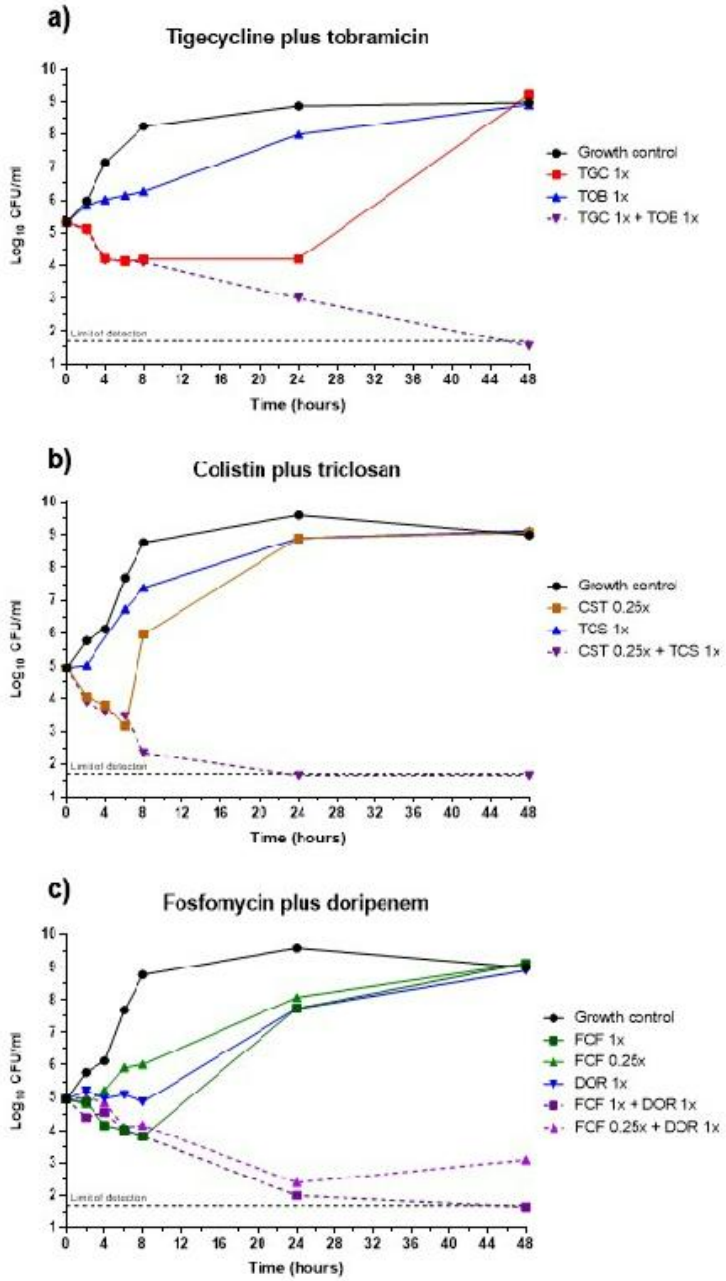


Figure 3.

Figure 3. Time-kill assays showing representative validated synergistic combinations of tigecycline, colistin and fosfomycin against *K. pneumoniae* ATCC 13883. **a)** Tigecycline (TGC) combined with tobramycin (TCB); **b)** Colistin (CST) in combination with triclosan (TCS); **c)** Fosfomycin (FOF) in combination with doripenem (DOR). The three combinations showed synergistic and bactericidal activity to the limit of detection at 48h of incubation, while drugs alone were not active at the end of the assay.
MIC_{CST} = 1 mg/L; MIC_{FOF} ≥128 mg/L; MIC_{TGC} = 0.5 mg/L; MIC_{TOB} = 0.125 mg/L; MIC_{TCS} = 0.25 mg/L; MIC_{DOR} = 0.03 mg/L



Keyword 1
drug repurposing
Keyword 2
synergy

Keyword 3
Klebsiella pneumoniae

Conflicts of interest

Do you have any conflicts of interest to declare?
I have the following potential conflict(s) of interest to report
Institutional grants/research supports
Other support
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