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Antimicrobial activity of 30 non-antibiotics alone and in combination with polymyxin B against multidrug-resistant Gram-negative bacteria

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Background: Increase of antimicrobial resistance is a major health problem worldwide. Discovery of new compounds with antibacterial activity or compounds that enhance activity of current antibiotics is urgently needed. In the absence of new antibiotics, we evaluated the in vitro activity of several established non-antibiotic compounds alone and in combination with polymyxin B (PolB) to determine its potential clinical applicability.

Material/methods: After reviewing literature, we selected 30 non-antibiotic drugs belonging to the following classes of drugs: antipsychotics (zuclopenthixol, promethazine, levomepromazine, Chlorpromazine, Haloperidol, Diazepam, Clonazepam), mucolytic agents (N-acetylcysteine), analgesics (Paracetamol, Ibuprofen, Diclofenac sodium, acetylsalicylic acid), antihypertensives (propranolol, verapamil, nifedipine, amlodipine), antiplatelets (clopidogrel), antidiarrheals (loperamide), statins (simvastatin, atorvastatin), pump inhibitors (esomeprazole, omeprazole), antimuscarinics (mebeverine), antidiabetic (glibenclamide), barbiturates (phenobarbital), local anaesthetics (lidocaine) and antidepressants (sertraline, imipramine, amitriptyline, citalopram). The MICs were determined by broth microdilution (ISO standard) against a panel of 20 multidrug resistant *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter baumanii* strains. Interaction of the non-antibiotics with PolB was first tested with an agar dilution-diffusion screening assay as follows. First, agar dilution MICs of PolB were determined for each strain. . Subsequently, non-antibiotics at 1280 mg/l were added to holes in agar, the agar containing the sub-MIC concentrations of PolB or saline control. The plates were inoculated on the surface with 0.5McFarland of each strain. After 18h of incubation, inhibition zones around each non-antibiotic on plates with and without PolB were measured. . Combinations showing inhibition of growth for multiple strains were then tested for

synergy by checkerboard assay and FIC index interpretation. Further characterization of interactions was performed by generating growth curves at sub-MIC concentrations of PolB and the non-antibiotics ($C_{non\text{-}Ab}$).

Results: Moderate antimicrobial activity was found for chlorpromazine, promethazine, clonazepam, loperamide, amitriptyline and sertraline for at least 13/20 strains with MICs 16-256 mg/l. The antimicrobial activity of sub-MIC concentrations of PolB was enhanced by chlorpromazine and promethazine for 9/9 and 6/9 strains respectively producing inhibition zones of 125-165 mm for chlorpromazine and 90-130 mm for promethazine. Subsequent checkerboard assays showed synergy/additive interactions when PolB was combined with loperamide (FICi=0.31-1, $C_{Non\text{-}Ab}$=4-32 mg/l, 10/10), chlorpromazine (FICi=0.31-0.5, CNon-Ab=0,125-32 mg/l, 6/6), promethazine (FICi=0.5-0.63, $C_{Non\text{-}Ab}$=1-32 mg/l, 6/6) and clonazepam (FICi 0.5-1, $C_{Non\text{-}Ab}$=0.06-32 mg/l, 6/6). No antagonistic effects were detected for any of the combinations. The growth curves revealed a delay of lag time (1.27-3.5 h of difference) and time to reach stationary phase (1.38-3.5 h) for several strains.

Conclusions: This study identifies chlorpromazine, promethazine, loperamide and clonazepam as enhancers of the antimicrobial activity of polymyxin B in vitro. Further studies should characterize the molecular mechanisms behind promising antimicrobial activity of these non-antibiotics as well as establish regimens that can be translated into the clinic.