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ARTICLE

Drug repurposing in the context of common bacterial pathogens: insights from an *in vitro* study

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ABSTRACT The clinical problem of multidrug resistance (MDR) in bacteria is due to the lack of novel antibiotics in development and the dwindling pipeline of drugs receiving market authorization. Repurposing of non-antibiotic pharmacological agents may be an attractive pathway to provide new antimicrobial drugs. The aim of the present study was to ascertain the antibacterial and adjuvant properties of a wide range of pharmaceuticals against antibiotic-susceptible and drug-resistant bacteria. Sixty-five (n = 65) pharmacological agents were included in our experiments. For Gram-positive bacteria, *Staphylococcus aureus* ATCC 43300 (methicillin-resistant), *S. epidermidis* ATCC 12228, *Streptococcus pyogenes* ATCC 12384 and *Enterococcus faecalis* ATCC 29212 were used, while for Gram-negative bacteria, *Enterobacter cloacae* ATCC 13047 (extended-spectrum β-lactamase-positive), *Klebsiella pneumoniae* ATCC 49619, *Serratia marcescens* ATCC 29632 and *Pseudomonas aeruginosa* ATCC 27853 were included as representative strains. The minimum inhibitory concentrations (MICs) of the tested compounds were determined using the standard broth microdilution method, while a MIC reduction assay was included to ascertain the effect of the tested compounds on the MICs of standard antibiotics (ceftriaxone, ciprofloxacin and gentamicin). Seventeen and twelve drug molecules tested showed measurable antibacterial activities (MIC: 32-512 μg/mL) against Gram-positive and Gram-negative bacteria, respectively. Several compounds decreased the MICs of ciprofloxacin and gentamicin. Although there are increasing number of studies in this field, there are still significant gaps in the evidence to the potential use of non-antibiotic drugs in antimicrobial drug repurposing. **Acta Biol Szeged 66(2):140-149 (2022)**

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Introduction

Antimicrobial resistance – an umbrella term, encompassing the development of therapeutic resistance in bacteria, fungi, viruses and parasites – has emerged as one of the critical public health issues of the 21st century, leaving clinicians in a precarious situation with limited (and often toxic) therapeutic options to manage their patients' condition (Janz et al. 2022; Laxminarayan et al. 2013; World Health Organization 2014). The dangers of the emergence and global spread of multidrug resistant (MDR) bacteria were highlighted by both academia, pharmaceutical companies, policymakers and other stakeholders worldwide (Hosseini et al. 2021; Li et al. 2022; Maisch et al. 2022; Mshana et al. 2021; van Duin and Paterson 2020). According to the estimations of the Global Burden of Disease (GBD) database, 33 main bacterial pathogens (both susceptible and MDR) are responsible for 13.6% of deaths (7.7 million; 95% UI: 5.7-10.2 million) globally,

out of which, five principal pathogens (i.e. *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pneumoniae*) were the responsible to >50% of deaths among the investigated bacteria (GBD 2019 Antimicrobial Resistance Collaborators 2022). It is expected that the pandemic caused by the novel coronavirus (SARS-CoV-2) will further exacerbate the MDR situation in bacteria, due to the widespread use of antibiotics both for prophylactic and therapeutic purposes (Rizvi and Ahammad 2022). The lack of novel antibiotics in development and the dwindling pipeline of drugs receiving market authorization are some of the hallmarks of the clinical problem (Dutescu and Miller 2021); thus, the necessity to search for novel compounds with antibacterial activity via synthetic chemistry (Donadu et al. 2018), natural sources (Nacsa-Farkas et al. 2014; Spengler et al. 2022), *in silico* prediction and subtractive genomics (Ashraf et al. 2022) and various biotechnological approaches (Al Farraj et al. 2020) has intensified. Unfortunately, the research and development (R&D) of novel pharmaceuticals is a long and cost-intensive process; due to the significantly high attrition rate and the relatively low returns-on-investment (compared to drugs used for the management of chronic, non-communicable diseases), many pharmaceutical companies have abandoned their antimicrobial R&D programs altogether (Klug et al. 2021; Paul et al. 2010).

Drug repurposing (pharmaceutical repositioning or reprofiling, therapeutic switching) is an alternative drug development strategy – which has received substantial attention in the recent 20-30 years – during which pharmaceuticals with existing indications are being marketed for additional clinical uses, based on their previously unexplored pharmacological properties (Pushpakom et al. 2019). Successful examples for drug repurposing include the use of acetylsalicylic acid (as an inhibitor of platelet aggregation), sildenafil (to treat erectile dysfunction), thalidomide (currently used for the treatment of multiple myeloma and leprosy) (Miró-Canturri et al. 2019). Repurposing of pharmaceuticals may be an attractive pathway, as the basic physico-chemical and therapeutic properties (pharmacokinetics, toxicology, dosing) of these molecules have already been established in costly clinical trials during their initial drug authorization process (Krishnamurty et al. 2022). Non-antibiotic pharmacological agents may be relevant as reprofiled drugs in numerous ways: on one hand, they may possess intrinsic antibiotic activity in clinically applicable doses, on the other hand (and more frequently), these compounds act as potentiators or adjuvants to sensitize or re-sensitize bacteria against existing antimicrobials (Seukep et al. 2022; Szerencsés et al. 2019; Yang et al. 2022). Possible mechanisms include the inhibition of bacterial efflux pumps, biofilm-formation or quorum sensing, depletion of ATP and disruption of bacterial metabolic processes, destabilization of the cell membrane, increasing permeability, generation of reactive oxygen species (ROS) and DNA-damage among others (Hegazy et al. 2020; Usai et al. 2019). The antibacterial activity and adjuvant properties of various central nervous system medications have already been described (Krzyek et al., 2019); for example, many experimental studies noted DNA-intercalation and efflux pump inhibition by phenothiazines used as antipsychotic medications (most notably chlorpromazine and thioridazine) (Aguilar-Vega et al. 2021; Amaral et al. 2004). Reserpine and verapamil – two representatives of drugs used in cardiovascular illnesses – are also well-known antibiotic potentiators (Gupta et al. 2015; Seukep et al. 2022). Moreover, a plethora of antineoplastic drugs show promising antibacterial activity through a wide variety of mechanisms, such as streptozotocin (via DNA-damage) (Soo et al. 2017), the anthracyclines doxorubicin and bleomycin (DNA-intercalation, ROS-generation, depletion of Fe^{2+} ions) (Lagadinou et al. 2020), docetaxel (inhibition of biofilm-formation) (Kaur et al. 2022), kinase inhibitors, such as imatinib and crizotinib (reduction in ATP-synthesis) (Dragoi et al. 2013; Zheng et al. 2022), and the selective estrogen receptor modulators (SERMs) tamoxifen and raloxifen (Hussein et al. 2017; Sforgliarini et al. 2022; Sui et al. 2012).

In a preliminary *in vitro* study, twenty non-antibiotic pharmacological agents were assessed for their antibacterial and adjuvant properties on methicillin-sensitive *S. aureus* (MSSA) and *Escherichia coli*; our experiments showed that eight structurally and pharmacologically distinct compounds (atorvastatin, celecoxib, clotrimazole, diclofenac-epolamine, ivermectin, lidocaine, mebendazole and terbinafine) showed antibacterial effects, (Gajdács 2020a). Additionally, it has also been established that several of these drugs also had the potential to negatively affect the viability of pathogenic bacteria in urine samples during urinalysis (Gajdács 2020b). The aim of the present study was to provide additional laboratory evidence in this field, by screening for the antibacterial and adjuvant properties of a wider range of pharmaceuticals against antibiotic-susceptible and drug-resistant bacteria, which are common etiological agents in human infections.

Materials and methods

Culture media

During the experiments, the following culture media were used: cation-adjusted Mueller-Hinton (CA-MH) broth (Bio-Rad, Hercules, CA, USA), tryptic soy broth (TSB) (Sigma-Aldrich, St. Louis, MO, USA), Luria-Bertani (LB)

broth (Bio-Rad, Hercules, CA, USA), eosine-methylene blue (EMB) agar (bioMérieux, Marcy-l'Étoile, France) and 5% sheep blood agar (bioMérieux, Marcy-l'Étoile, France) (Gajdács 2020a).

Bacterial strains

In the present study, *S. aureus* ATCC 43300 (methicillin-resistant, MRSA), *S. epidermidis* ATCC 12228, *S. pyogenes* ATCC 12384 and *Enterococcus faecalis* ATCC 29212 were used as representative Gram-positive strains, while *Enterobacter cloacae* ATCC 13047 (extended-spectrum β-lactamase [ESBL] positive (Chen et al. 2020), *K. pneumoniae* ATCC 49619, *Serratia marcescens* ATCC 29632 and *P. aeruginosa* ATCC 27853 were included as representative Gram-negative bacteria. For time periods shorter than 1 month, bacterial strains were maintained on 5% sheep blood agar (in case of Gram-positives) or EMB agar (in case of Gram-negatives) with continuous passage. For longer periods, bacterial strains were kept in a -80 °C freezer, in a 1:4 mixture of 85% glycerol and liquid LB medium.

Pharmacological agents and reagents used for microbiological studies

Sixty-five (n = 65) pharmacological agents, including a wide variety of mechanisms of action and chemical compositions were tested in our study: **1. non-steroidal anti-inflammatory drugs (NSAIDs):** acetylsalicylic acid (Sigma-Aldrich, St. Louis, MO, USA), celecoxib (Pfizer Hungary, Budapest, Hungary), diclofenac (Sigma-Aldrich, St. Louis, MO, USA), etodolac (Sigma-Aldrich, St. Louis, MO, USA), indomethacin (Sanofi, Paris, France), metamizole-sodium (Sanofi, Paris, France), nimesulid (CSC Pharmaceuticals, Mumbai, India), paracetamol (Sigma-Aldrich, St. Louis, MO, USA), **2. antifungal, antiviral or antiparasitic medications:** acyclovir (Teva Pharmaceuticals, Petah Tikva, Israel), amantadine (Sigma-Aldrich, St. Louis, MO, USA), cidofovir (Sigma-Aldrich, St. Louis, MO, USA), clotrimazole (Teva Pharmaceuticals, Petah Tikva, Israel), fluconazole (Sigma-Aldrich, St. Louis, MO, USA), ivermectin (Sigma-Aldrich, St. Louis, MO, USA), mebendazole (Richter Pharmaceuticals, Budapest, Hungary), terbinafine (GlaxoSmithKline Hungary Ltd., Budapest, Hungary), zidovudine (Sigma-Aldrich, St. Louis, MO, USA), **3. cardiovascular drugs:** atorvastatin (Sigma-Aldrich, St. Louis, MO, USA), enalapril maleate (Sigma-Aldrich, St. Louis, MO, USA), metoprolol succinate (Sigma-Aldrich, St. Louis, MO, USA), prazosin (Sigma-Aldrich, St. Louis, MO, USA), propafenone (Sigma-Aldrich, St. Louis, MO, USA), simvastatin (Sigma-Aldrich, St. Louis, MO, USA), valsartan (Sigma-Aldrich, St. Louis, MO, USA), verapamil (Teva Pharmaceuticals, Petah Tikva, Israel), **4. antihistamines, decongestants, mucolytics and antitussives:** acetyl-cysteine (Teva Pharmaceuticals, Petah Tikva, Israel), ambroxol (Teva Pharmaceuticals, Petah Tikva, Israel), azelastine (Sigma-Aldrich, St. Louis, MO, USA), cetirizine (Sigma-Aldrich, St. Louis, MO, USA), guaifenesin (Sigma-Aldrich, St. Louis, MO, USA), salbutamol (Sigma-Aldrich, St. Louis, MO, USA), xylometazoline (Sigma-Aldrich, St. Louis, MO, USA), **5. central nervous system medications:** amitriptyline (Sigma-Aldrich, St. Louis, MO, USA), carbamazepine (Sigma-Aldrich, St. Louis, MO, USA), fluoxetine (Sigma-Aldrich, St. Louis, MO, USA), gabapentin (Sigma-Aldrich, St. Louis, MO, USA), imipramine (Sigma-Aldrich, St. Louis, MO, USA), phenelzine (Sigma-Aldrich, St. Louis, MO, USA), risperidone (Sigma-Aldrich, St. Louis, MO, USA), sertraline (Teva Pharmaceuticals, Petah Tikva, Israel), valproic acid (Sanofi, Paris, France), **6. antitumor medications:** 5-fluorouracil (Teva Pharmaceuticals, Petah Tikva, Israel), cyclophosphamide (Baxter; Deerfield, IL, United States), gemcitabine (Sigma-Aldrich, St. Louis, MO, USA), methotrexate (Ebewe Pharma, Unterach am Attersee, Austria), topotecan (Teva Pharmaceuticals, Petah Tikva, Israel), vincristine (Teva Pharmaceuticals, Petah Tikva, Israel), **7. other pharmacological agents:** allopurinol (Sigma-Aldrich, St. Louis, MO, USA), atracurium (Sigma-Aldrich, St. Louis, MO, USA), caffeine (Sigma-Aldrich, St. Louis, MO, USA), chlorzoxazone (Sigma-Aldrich, St. Louis, MO, USA), famotidine (Sigma-Aldrich, St. Louis, MO, USA), lidocaine (Sigma-Aldrich, St. Louis, MO, USA), metformin (Sigma-Aldrich, St. Louis, MO, USA), probenecid (Sigma-Aldrich, St. Louis, MO, USA), prilocaine (Sigma-Aldrich, St. Louis, MO, USA), sitagliptin (Sigma-Aldrich, St. Louis, MO, USA), suxamethonium (Sigma-Aldrich, St. Louis, MO, USA), **8. vitamins and antioxidants:** Vitamin B_1 (EGIS Pharmaceuticals, Budapest, Hungary), Vitamin B_6 (EGIS Pharmaceuticals, Budapest, Hungary), Vitamin B_{12} (EGIS Pharmaceuticals, Budapest, Hungary), Vitamin C (EGIS Pharmaceuticals, Budapest, Hungary), Vitamin D (EGIS Pharmaceuticals, Budapest, Hungary), Vitamin E (EGIS Pharmaceuticals, Budapest, Hungary), Vitamin K (EGIS Pharmaceuticals, Budapest, Hungary). The pharmaceutical compounds were selected on the basis of being used for the treatment of illnesses with high global prevalence, or on the basis of their availability as over-the-counter (OTC) medications. Pharmaceutical compounds were dissolved in dimethyl sulfoxide (DMSO), with the concentration of DMSO being <1 V/V% in all experiments. All solutions were prepared on the day of the assay.

Minimum inhibitory concentrations of tested compounds

The antibacterial activity of the tested compounds (expressed as minimum inhibitory concentration [MIC] values) were determined using the standard broth mi-

crodilution method, based on the recommendations of the Clinical and Laboratory Standards Institute (CLSI; M07-A11) (CLSI 2018). The experiments were carried out in 96-well polystyrene microtiter plates using CA-MH broth. The concentrations of the tested compounds were ranging between 1-512 μg/mL, the two-fold serial dilutions were made starting in the third (C) row of the microtiter plates. During the experiments for *Staphylococcus aureus* ATCC 43300 (methicillin-resistant, MRSA) and *S. epidermidis* ATCC 12228, the CA-MB broth was also supplemented with 2% NaCl, in light with the recommendations of the CLSI (CLSI 2018). The plates were then incubated at 37 °C in an air thermostat; MIC values were recorded after 16-18 h of incubation; the results were determined by visual inspection. All experiments were carried out in triplicate.

MIC reduction assay

To test the effects of the non-antibiotic pharmaceuticals on the MICs of standard antibiotics, a MIC reduction assay was carried out, as described previously (Weaver et al. 2014). In these assays, *S. aureus* ATCC 43300 (MRSA) and *E. cloacae* ATCC 13047 (ESBL-positive) were selected as test organisms. Ceftriaxone, ciprofloxacin and gentamicin (all purchased from Sigma-Aldrich, St. Louis, MO, USA) were used as reference antibiotics. The addition of the pharmaceutical compounds in fixed concentrations as adjuvants (MIC/4 in cases were the MIC values were ≤ 256 μg/mL, and 128 μg/mL where MICs were higher than 256 μg/mL) was done in all wells, with the exception for medium control and cell control wells (Gajdács 2020a). Inoculation of the plates and their incubation was performed, according to the standard broth microdilution procedure, described previously. The MIC values after the treatment with the adjuvant compounds (compared to the MICs of the antibiotics alone) were determined by visual inspection. All experiments were carried out in triplicate.

Ethical considerations

Not applicable.

Results

Antibacterial activity of non-antibiotic pharmaceutical compounds

The MIC values recorded for the non-antibiotic pharmaceutical compounds are presented in Table 1 and 2 for Gram-positive and Gram-negative bacteria, respectively. Out of the sixty-five pharmacological agents tested, n = 17 (namely amitriptyline, atorvastatin, atracurium, celecoxib, clotrimazole, diclofenac-epolamine, gemcitabine, gabapentin, fluconazole, ivermectin, ibuprofen, lidocaine, mebendazole, probenecid, sertraline, terbinafine and valproic acid), and n = 12 (namely amitriptyline, atorvastatin,

Table 1. Minimum inhibitory concentrations (MICs) of the tested pharmaceutical compounds on Gram-positive reference bacterial strains

	Minimal inhibitory concentrations (μg/mL)			
Compounds	*S. aureus* ATCC 43300 (MRSA)	*S. epidermidis* ATCC 12228	*S. pyogenes* ATCC 12384	*E. faecalis* ATCC 29213
Amitriptyline	>512	**256**	**256**	>512
Atorvastatin	**256**	**128**	**64**	**512**
Atracurium	>512	>512	**128**	>512
Celecoxib	**128**	**32**	**64**	**512**
Clotrimazole	>512	**64**	**64**	**512**
Diclofenac-epolamine	>512	**128**	**128**	>512
Gemcitabine	>512	**128**	**128**	**256**
Gabapentin	>512	>512	**128**	>512
Fluconazole	>512	**128**	**128**	>512
Ivermectin	**128**	**64**	**128**	>512
Ibuprofen	>512	**256**	**128**	>512
Lidocaine	>512	**256**	**128**	**512**
Mebendazole	**512**	**128**	**64**	**512**
Probenecid	>512	**128**	**128**	**512**
Sertraline	**512**	**128**	**128**	**512**
Terbinafine	**512**	**128**	**128**	>512
Valproic acid	**256**	**128**	**128**	**512**

Results in **boldface** represent MIC values ≤512 μg/mL

Table 1. Minimum inhibitory concentrations (MICs) of the tested pharmaceutical compounds on Gram-negative reference bacterial strains

	Minimal inhibitory concentrations (μg/mL)			
Compounds	*E. cloacae* ATCC 13047 (ESBL+)	*K. pneumoniae* ATCC 49619	*S. marcescens* ATCC 29632	*P. aeruginosa* ATCC 27853
Amitriptyline	**256**	**256**	**256**	>512
Atorvastatin	**512**	**512**	>512	>512
Atracurium	>512	>512	**128**	>512
Celecoxib	**512**	>512	>512	>512
Gabapentin	>512	**256**	>512	**256**
Ibuprofen	**256**	**256**	>512	>512
Lidocaine	**512**	**512**	**512**	>512
Mebendazole	**128**	**256**	>512	>512
Probenecid	>512	**256**	512	512
Sertraline	**512**	**128**	**512**	**256**
Valproic acid	**512**	**512**	>512	>512
Zidovudine	**256**	**256**	**512**	>512

Results in **boldface** represent MIC values ≤512 μg/mL

atracurium, celecoxib, gabapentin, ibuprofen, lidocaine, mebendazole, probenecid, sertraline, valproic acid and zidovudine) showed measurable antibacterial activities in the tested concentration range against Gram-positive and Gram-negative bacteria, respectively. Overall, n = 11 (namely amitriptyline, atorvastatin, atracurium, celecoxib, gabapentin, ibuprofen, lidocaine, mebendazole, probenecid, sertraline and valproic acid) compounds had relevant antibacterial properties against both bacterial groups. Compounds with MIC values >512 μg/mL were considered as inactive; these compounds were not included in Tables 1-2.

MIC reduction assays

The results of the MIC reduction assay involving *S. aureus* ATCC 43300 and *E. cloacae* ATCC 13047 are presented in Table 3. Eleven compounds (namely atorvastatin, celecoxib, clotrimazole, gemcitabine, ivermectin, lidocaine, sertraline, terbinafine, valproic acid valsartan and verapamil) had an adjuvant effect on MRSA, while six compounds (namely celecoxib, cetirizine, diclofenac-epolamine, sertraline, valsartan, verapamil, zidovudine) showed MIC-reducing properties against ESBL-producing *E. cloacae*; interestingly, some compounds had adjuvant effects without having intrinsic antibacterial properties. Overall, MICs of ciprofloxacin and gentamicin were reduced 2-4-fold by the non-antibiotic drugs, while none of the compounds affected methicillin-resistance or the production of ESBLs (as seen by the MICs of ceftriaxone) (Table 3).

Discussion

The strategy of drug repositioning – i.e. the pathway to use drugs authorized by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) in new, unrelated clinical indications – offers an attractive and cost-effective approach for the development of new antimicrobial drugs or sensitizing agents (Konreddy et al. 2019; Pushpakom et al. 2019; Xue et al. 2018). A plethora of *in vitro* and *in vivo* studies have reported the antibacterial effects on non-antibiotics drugs (or their enhanced antibacterial derivatives) against many bacterial strains, which offers hope for the viability of this drug development route (Foletto et al. 2021; Lagadinou et al. 2020). The exploration in the "chemical space" of approved pharmaceuticals (which includes around 6000-10000 compounds at present) through the high-throughput screening of molecular libraries against specific molecular targets of bacteria would further facilitate the drug repurposing agenda (Foerster et al. 2019; Yang et al. 2009). Additionally, pharmaceuticals with antibacterial effects may have potential relevance in other fields of medicine as well, e.g., during the use of intravenous preparations or infusions in anesthesiology and intensive care, where contamination of these medicines or the use of central lines may lead to severe bloodstream infections (Raad and Chaftari 2014). Thus, compounds that do not support the growth of potential contaminating bacteria may also possess innate antibacterial properties to be utilized (e.g., as a catheter lock). Such properties have been described for halothane (Bátai and Kerényi 1999), anesthetics (Bátai et al. 1999), atropine and glycopyrrolate (Ittzes et al. 2016), nitroglyc-

Table 3. Results of the MIC reduction assays using ceftriaxone, ciprofloxacin and gentamicin as reference antibiotics

	Minimal inhibitory concentrations (μg/mL)					
Compounds	*S. aureus* ATCC 43300 (MRSA)			*E. cloacae* ATCC 13047 (ESBL+)		
MICs of reference antibiotics (untreated):	**CXT: 64**	**CIP: 32**	**GEN: 0.25**	**CXT: 8**	**CIP: 0.03**	**GEN: 0.125**
Atorvastatin	64	**16**	**0.125**	8	0.03	0.125
Celecoxib	64	**16**	0.25	8	**0.0075**	**0.0625**
Cetirizine	64	32	0.25	8	**0.015**	0.125
Clotrimazole	64	**16**	**0.0625**	8	0.06	0.03
Diclofenac-epolamine	64	32	0.25	8	**0.0075**	0.125
Gemcitabine	64	**16**	**0.125**	8	0.03	0.125
Ivermectin	64	**16**	0.25	8	0.03	0.125
Lidocaine	64	32	**0.0625**	8	0.03	0.125
Sertraline	64	**8**	**0.0625**	8	**0.0075**	**0.0625**
Terbinafine	64	**16**	0.25	8	0.03	0.125
Valproic acid	64	32	**0.0625**	8	0.03	0.125
Valsartan	64	**16**	**0.0625**	8	**0.0075**	0.125
Verapamil	64	32	**0.125**	8	0.03	**0.0625**
Zidovudine	64	32	0.25	8	0.03	**0.0625**

CXT: ceftriaxone; CIP: ciprofloxacin; GEN: gentamicin; results in **boldface** represent cases when the MICs have decreased due to the effect of the adjuvants

erine (Chaftari et al. 2017) and amiodarone (Ittzes et al. 2020). In contrast, sugammadex (a γ-cyclodextrin-based agent used to reverse neuromuscular blockade) possesses no antimicrobial effects (Hanci et al. 2013).

In the present study, sixty-five pharmacological agents from a wide variety of clinical uses were screened for their antibacterial and adjuvant properties. Our findings were consistent with the results of the previous study (Gajdács 2020a), where the non-antibiotic pharmaceuticals were more effective against the tested Gram-positive bacteria, while their activity against Gram-negatives was more modest in both assays. Central nervous system drugs (antidepressants, anticonvulsants), NSAIDs (most notably celecoxib and ibuprofen), antifungals (clotrimazole, fluconazole, terbinafine), antiparasitic medications (ivermectin, mebendazole), and local and general anesthetics (lidocaine, atracurium) were commonly noted for their antibacterial effects in both studies (Gajdács 2020a); these results are in line with previous studies for NSAIDs (Chan et al. 2017), anesthetics (Johnson et al. 2008) and antifungals (Azevedo et al. 2015). When it comes to their adjuvant activities, numerous compounds were able to reduce the inhibitory concentration of ciprofloxacin and gentamicin 1-3-fold, however, they had no effect on ceftriaxone MICs, owing to specific resistance mechanisms of the MDR strains included in our experiments (MRSA, ESBL + *E. cloacae*). The synergistic relationship between ciprofloxacin and non-antibiotic drugs may be explained through similar mechanisms of action (interaction with bacterial DNA) or through inhibition to bacterial efflux pumps, which would otherwise extrude fluoroquinolones (Gajdács 2020a); Mahey et al. (2021) performed a comprehensive screening of non-antibiotics with efflux pump inhibitory properties, identifying raloxifene and pyrvinium as enhancers of ciprofloxacin against *S. aureus*. As numerous bacterial efflux pump superfamilies have wide substrate specificity, elimination of this resistance mechanism may lead to the sensitization towards available antibiotics (Nikaido and Pagés 2012). In line with our results, the effects of probenecid on the viability of *S. aureus* and *P. aeruginosa* were reported previously (Kamurai et al. 2020). Interestingly, ivermectin was only effective against Gram-positive bacteria, while zidovudine only showed antibacterial and adjuvant effect towards Gram-negative strains; this selectivity in the antimicrobial spectrum has been noted in previous publications (Ashraf et al. 2018; Thomson and Lamont 2019).

The antibacterial effects of carbamazepine have been studied in various *in vitro* studies (Nathiya et al. 2015); additionally, in a clinical study by Shewell et al. (2023) it was noted that this antiepileptic drug reaches clinically-relevant concentrations in vaginal secretions, which provides evidence to its possible use to treat cervicitis caused by *Neisseria gonorrhoeae*, a bacterial pathogen which has emerged as a serious public health issue in recent years. The antidepressant sertraline was shown to have a synergistic effect with essential oils and antibiotics currently in clinical use (Ayaz et al. 2015; Barbarossa et al. 2022). Similarly, amitriptyline was shown to have synergistic effect in 15 combinations with antibiotics, in

addition to facilitating cleavage of plasmid DNA (Machado et al. 2019). Previously used exclusively as an antiepileptic drug, there is increasing evidence accumulating about the pleiotropic effects of valproic acid (Singh et al. 2021); Nathiya and colleagues (2015) have demonstrated that valproic acid had MIC values in the ranges of 100-800 μg/mL in a selection of tested bacteria, while other tested drugs (e.g. lamotrigine, clonazepam) did not show detectable antibacterial effect. In contrast to our findings, Naftalin et al. (2017) showed that allopurinol enhanced the anti-tubercular activity of pyrazinamide, a first-choice drug in the treatment of *Mycobacterium tuberculosis* in a whole-blood model. Similarly, we did not demonstrate antibacterial or MIC-reducing properties in the vitamins included in our experiments; however, the findings of other studies showed that Vitamin A (Tintino et al. 2016), Vitamin C (Kwiecinska-Piróg et al. 2019), Vitamin D (Andrade et al. 2018), Vitamin E (Andrade et al. 2017), and Vitamin K (Andrade et al. 2017) may have adjuvant properties when co-administered with antibiotics, through mechanisms such as efflux pump inhibition and cell membrane depolarization. Overall, due to the increasingly limited treatment options to manage bacterial infections, drug repurposing may be applied successfully in antimicrobial research. Our study was to provide a snapshot into the relevance of various pharmaceuticals as antibacterial adjuvants, using basic laboratory methods in microbiology to assess their potency. Although there are increasing number of studies in this field, there are still significant gaps in the evidence to the potential use of non-antibiotic drugs; thus, systematic, high-throughput screening procedures should be applied on a plethora of bacterial targets or preferably, in more comprehensive *in vivo* animal infection models to establish the interactions between the pathogens, the immune system and the potential effects of these pharmaceutics to enhance the eradication of the microorganisms in "real-life" conditions.

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