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In Vitro Screening of an FDA-Approved Library Against ESKAPE Pathogens

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Abstract

Bacterial resistance to conventional antibiotics is an increasingly serious threat to public health worldwide that requires immediate exploration and the development of novel antimicrobial compounds. Drug repurposing is an inexpensive and untapped source of new antimicrobial leads, and it holds many attractive features warranting further attention for antimicrobial drug discovery. In an effort to repurpose drugs and explore new leads in the field of antimicrobial drug discovery, we performed a whole-cell screening assay of 1,600 Food and Drug Administration (FDA) approved drugs against *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter cloacae* (ESKAPE) pathogens. The in vitro screening identified 49 non-antimicrobial drugs that were active against at least one species of ESKAPE pathogen. Although some of these drugs were known to have antibacterial activity, many have never been reported before. In particular, sulfonamide-containing structures represent a novel drug scaffold that should be investigated further. The characteristics of these drugs as antimicrobial agents may offer a safe, effective, and quick supplement to current approaches to treating bacterial infections.

Keywords

Repurposing; antibiotics; ESKAPE; antimicrobial; repositioning

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

INTRODUCTION

Antimicrobial resistance is a serious threat to global public health in every geographic region, requiring immediate global action. Bacterial resistance to antibiotics emerges rapidly and can disseminate globally following its initial recognition [1]. Infections with resistant pathogens are difficult to treat, requiring costly and sometimes toxic alternatives. Furthermore, patients with resistant infections require longer hospital stays and are often much more likely to die [2, 3]. Despite the emergence and global spread of highly resistant organisms such as *Enterococcus*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudo-monas aeruginosa*, and *Enterobacter* (ESKAPE) pathogens, the discovery and development of new antibacterials has slowed to an unacceptable level. This highlights the urgent need for the identification and development of novel antibacterial agents and their expedited introduction to the clinic in order to address this public health crisis.

Drug repurposing has many attractive features that merit further attention from the antimicrobial drug discovery sector. These drugs have already been tested in human patients: their safety, efficacy, pharmacokinetic parameters, and toxicity profiles have been extensively studied. Therefore, this permits a better understanding of the overall pharmacology of the drug, potential routes of administration, and the best way to establish an appropriate dosing regimen. In addition, because patients' extensive clinical data have already been acquired, repurposing existing approved drugs permits companies to bypass much of the early stage clinical trials required for new drugs, thus reducing the cost and time associated with bringing a drug to the marketplace [4–9].

Given the need for novel antimicrobials and many appeals of drug repurposing, we performed a whole-cell screening assay to identify Food and Drug Administration (FDA) approved non-antimicrobial drugs with antimicrobial activity toward the ESKAPE pathogens [10]. The screening of a library containing 727 FDA approved drugs and small molecules has produced promising results and has proven to be successful for the identification of novel antimicrobial drugs and new leads for drug development. However, additional approved drugs must be screened to identify their antibacterial spectrum of activity and to elucidate potential clinical applications.

In this study, we screened a library of 1,600 FDA approved drugs and clinical molecules (clinically safe but without proven use) against ESKAPE pathogens. This study has identified drugs with antimicrobial activity that have not been described before. Approved non-antimicrobial drugs with antimicrobial activity may represent an untapped reservoir of novel antimicrobial leads that will definitely guide the future development of new antimicrobial agents; these drugs will also speed up the development of new antibiotics, saving years of expensive research.

MATERIALS AND METHODS

Bacterial Strains and Reagents

ESKAPE pathogens used for the initial screening include vancomycin-resistant *E. faecium* ATCC 700221 (VRE), MRSA USA300, *K. pneumoniae* ATCC BAA-1706, multi-drug-resistant *A. baumannii* ATCC BAA-1605, *P. aeruginosa* ATCC 15442, and *E. cloacae* ATCC BAA-1143 (Table 1). Cation-adjusted Mueller-Hinton broth (CA-MHB), Mueller-Hinton broth (MHB), and Phosphate Buffer Saline (PBS) were purchased from Sigma-Aldrich (St. Louis, MO), while Trypticase soy broth (TSB), Trypticase soy agar (TSA), and brain heart infusion broth (BHI) were purchased from Becton, Dickinson and Company (Cockeysville, MD).

Compounds and Library

The Pharmakon 1600 repositioning compound library was purchased from MicroSource Discovery Systems, Inc. (Gaylordsville, CT, USA). Compounds were delivered in microplates (10 mM, dissolved in DMSO) and kept at -80°C until use.

Screening Assay

The library was initially screened at a single concentration of 16 μM against ESKAPE pathogens to identify “hit” non-antibacterial drugs. Once the antimicrobial activity of the drugs was confirmed, the minimum inhibitory concentrations (MICs) were determined according to the Clinical and Laboratory Standards Institute (CLSI) [11].

RESULTS AND DISCUSSION

In this study, we were most interested in exploring non-antimicrobial drugs that show antimicrobial activity. Of the 1,600 drugs tested, we initially found 74 non-antimicrobial drugs with potential antimicrobial activity against at least one of the ESKAPE pathogens. Confirmation of antimicrobial activity in the initial screening, by MIC according to the guideline of CLSI, resulted in 49 confirmed hits (Tables 2 & 3). Although some of these drugs were known to have antibacterial activity, many had never been previously reported. Several drugs represented in the library showed potent antimicrobial activity when screened directly from the library, but when these drugs were ordered commercially, they did not show any activity. These drugs were excluded from this study due to their questionable identity.

Excluding drugs with cytotoxic properties, drugs that are hormone and hormone-like, and drugs labeled “Mandatory Hazard,” 26 of the drugs screened can be considered to have some value for medicinal chemists. However, the cytotoxic drugs with potent antimicrobial activity that have been excluded based on their expected genotoxicity profile or antineoplastic, may be considered to have a high clinical value in the cases of nosocomial infections in hospitalized tumor patients. In this regard, this study suggests floxuridine (CAS: 50-91-9) as a promising candidate with dual actions, since its MIC value against MRSA was found to be within the low nanomolar range (Table 2). It is well known that floxuridine is a prodrug that has to be converted *in vivo* into 5-fluorouracil or the

corresponding monophosphate form to exert its cytotoxic effect [12]. This piece of information is valuable from a drug design point of view, since medicinal chemists can structurally optimize floxuridine to prevent human in vivo activation while maintaining bacterial deoxyribonucleoside kinases specific activation [13]. In this case, the antibacterial effect will be extracted from its innate cytotoxic characteristics.

In terms of pharmacophores, five sulfonamide-containing structures were found to carry antibacterial properties against vancomycin-resistant *Enterococcus* (VRE): Acetazolamide, Chlorthalidone, Methazolamide, Torsemide, and Ethoxzolamide. The emergence of VRE has severely limited the utility of the antibiotics currently available to healthcare providers. The antimicrobial activity of these five drugs against VRE has never been reported and therefore warrants further exploration.

It was also notable that the tetrazolobiphenyls angiotensin II inhibitors (Olmesartan medoxomil, valsartan, and candesartan cilexetil) had reasonable antibiotic activity (Table 2); however, other members of this class revealed no antibacterial activity under the same experimental conditions.

The antihistaminic terfenadine and the antimuscarinic darifenacin each showed moderate antibacterial activities. Interestingly, both drugs share a common structural frame (diphenylmethane connected with cyclic amine). However, solifenacin succinate, the M3 antagonist, belongs to dihydroisoquinoline antimuscarinics; its chemical structure fits, with high similarity, with the previously identified pharmacophore. Structure-activity relationship (SAR) studies of terfenadine yielded 84 terfenadine-based analogues with improved activity against *S. aureus* [14]. Terfenadine exerts its antibacterial effect through the inhibition of the bacterial type II topoisomerases [14].

Clomiphene, a nonsteroidal selective estrogen receptor modulator and widely used fertility drug, has been reported recently as an inhibitor of bacterial cell wall synthesis in MRSA [15].

Finally, the antibacterial activities of phenol-containing drugs (benzbromarone, dioxybenzone, and nonivamide) might be attributed to the antiseptic properties of phenolic moiety.

Benzbromarone, a gout medication, has been found to inhibit *S. aureus* virulence, reduce abscess formation in mouse models, and provide protection against *S. aureus* infections. The scaffold of benzbromarone has been subjected to SAR studies; however, out of 24 analogues synthesized and tested, none displayed improved activity against *S. aureus*. The precise mechanism of antimicrobial activity of benzbromarone has not been elucidated yet [16].

In addition to drug-repurposing, the pool of approved therapies—with their well-studied safety and pharmacokinetic profiles— can be used as a source for new lead compounds. Finding a new lead structure is the first significant chemistry milestone along the path to the discovery of small therapeutic molecules [17]. In the structure optimization stage, more substructures are typically tethered to the lead scaffold to ameliorate its selectivity or enhance its potency. This results in molecules with higher molecular weights, higher partition coefficient (logP) values, and more hydrogen-bond acceptor(s)/donor(s); this definitely deteriorates Lipinski's Rule of 5. Several lead-like properties have been published and suggested throughout the literature [18]; however, computational calculations [19] suggest what is called roughly the "Rule of 4": MWt NMT 460, logP between -4 and 4, rings NMT 4, hydrogen-bond donor NMT 5, and hydrogen-bond acceptors are NMT 9. Applying those rules to our list, 22 of our screened structures can be considered good leads for further chemical optimization.

In addition, the small molecular weights of some of these compounds suggest another avenue in drug discovery by including them in the fragments library. Fragment-based drug design is an emerging technique that aims to utilize small molecular weight molecules to occupy different parts within the binding pocket and then tether parts together to obtain more potent compounds. A set of rules has been proposed to identify fragment-like properties, called the "Rule of 3": [20] MW ≤ 300; clog P ≤ 3; Rotatable bonds ≤ 3; Hydrogen-bond donors ≤ 3; Hydrogen-bond acceptors ≤ 3; Polar surface area (PSA) ≤ 60 Å². In this regard, acetazolamide, vortioxetine, methazolamide, ethoxzolamide, ebselen, citicoline, and nonivamide can be added to an existing fragment library. Once the antibacterial molecular target is established, further substructures can easily be added in order to enhance their antibiotic properties without violating drug-like properties.

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Table 1

ESKAPE pathogens used in this study.

Strain Name		Isolation		Phenotypic Properties
Strain ID	Alternate Designation	Origin	Year	
<i>E. faecium</i> ATCC 700221	VRE	Human feces, Connecticut	-	Resistant to Vancomycin and Teicoplanin and used as Quality control strain for IDEXX products
<i>Staphylococcus aureus</i> NRS384	USA300-0114	United States	<i>Staphylococcus aureus</i> NRS384	USA300-0114
<i>Klebsiella pneumoniae</i> ATCC BAA 1706	AIS 2007023	-	-	Modified Hodge Test (HMT) negative control designation
<i>Acinetobacter baumannii</i> ATCC BAA-1605	-	Sputum of military personnel returning from Afghanistan entering a Canadian hospital	2006	Resistant to Ceftazidime, Gentamicin, Ticarcillin, Piperacillin, Aztreonam, Cefepime, Ciprofloxacin, Imipenem, and Meropenem. Sensitive to Amikacin and Tobramycin
<i>Pseudomonas aeruginosa</i> ATCC15442	PRD-10	Animal room water bottle	-	Quality control strain and for Testing antimicrobial agent
<i>Enterobacter cloacae</i> ATCC BAA-1134	Entb 55M	Derived from existing strain	1982	Control strain

Table 2

Structure, description and MIC of Pharmakon non-antimicrobial compounds against Gram positive bacteria.

No	name	Description	MIC (μM)	
			VRE	MRSA
1.	Sanguinarium chloride	antineoplastic, antiplaque agent	16	4
2.	Mitomycin	antineoplastic	4	16
3.	Acetazolamide	carbonic anhydrase inhibitor, diuretic, antiglaucoma	0.5	>16
4.	Chlorthalidone	diuretic, antihypertensive	8	>16
5.	Clomiphene	anti-estrogen	16	>16
6.	Daunorubicin	antineoplastic	16	16

No	name	Description	MIC (μM)	
			VRE	MRSA
7.	Fluorouracil	antineoplastic, pyrimidine antimetabolite	0.0156	0.5
8.	Methotrexate	antineoplastic, antirheumatic, folic acid antagonist	0.125	>16
9.	Streptozosin	antineoplastic, alkylating agent	4	8
10.	Tamoxifen citrate	estrogen antagonist, antineoplastic	16	>16
11.	Isotretinoin	antiacne, antineoplastic	16	>16
12.	Tretinoin	keratolytic, antiacne, antineoplastic	16	>16

No	name	Description	MIC (μ M)	
			VRE	MRSA
13.	Azacitidine	antineoplastic, pyrimidine antimetabolite	16	>16
14.	Quinestrol	estrogen	16	>16
15.	Pemetrexed	antineoplastic, thymidylate synthase inhibitor	0.0625	>16
16.	Vortioxetine	antidepressant	4	>16
17.	Floxuridine	antineoplastic, antimetabolite	0.0156	<0.0078
18.	Perhexiline	calcium channel blocker, coronary vasodilator	16	>16

No	name	Description	MIC (μ M)	
			VRE	MRSA
19.	Methazolamide	carbonic anhydrase inhibitor	4	>16
20.	Terfenadine	antihistaminic	16	16
21.	Prednicarbate	antiinflammatory, glucocorticoid	4	8
22.	Nateglinide	antidiabetic	16	>16
23.	Olmesartan medoxomil	angiotensin II inhibitor prodrug, antihypertensive	16	>16
24.	Valsartan	angiotensin II inhibitor, antihypertensive	16	>16

No	name	Description	MIC (μ M)	
			VRE	MRSA
25.	<p>Torsemide</p>	diuretic, inhibits Na/K/2Cl carrier system	16	>16
26.	<p>Ethoxzolamide</p>	carbonic anhydrase inhibitor, antiulcer, antiglaucoma	1	>16
27.	<p>Algestone acetophenide</p>	antiacne, progestin	16	>16
28.	<p>Ascorbyl palmitate</p>	antioxidant	8	>16
29.	<p>Darifenacin hydrobromide</p>	M3 muscarinic antagonist, bladder suppressant	16	>16
30.	<p>Toremifene citrate</p>	antineoplastic, antiestrogen	8	16

No	name	Description	MIC (μ M)	
			VRE	MRSA
31.	<p>Dactinomycin</p>	antineoplastic, intercalating agent	0.0625	0.5
32.	<p>Amiodarone hydrochloride</p> <p>HCl</p>	adrenergic agonist, coronary vasodilator, Ca channel blocker	8	>16
33.	<p>Aurothioglucose</p>	antirheumatic	16	>16
34.	<p>Dequalinium chloride</p>	antiseptic wound dressings and mouth infections	8	>16
35.	<p>Ebselen</p>	antioxidant, lipoxygenase inhibitor, inhibits oxidation of LDL	2	0.5
36.	<p>Doxifluridine</p>	antineoplastic, pyrimidine antimetabolite	1	2

No	name	Description	MIC (μ M)	
			VRE	MRSA
37.	Citiolone	hepatoprotectant, free radical scavenger	8	>16
38.	Nonivamide	analgesic (topical), depletes Substance P	4	>16
39.	Carmofur	antineoplastic	0.0312	4
40.	Hydroxyprogesterone	progestin	2	>16
41.	Diethylstilbestrol	estrogen	>16	16
42.	Menadione	prothrombogenic agent	>16	16

No	name	Description	MIC (μ M)	
			VRE	MRSA
43.	Bleomycin	antineoplastic	>16	4
44.	Teniposide	antineoplastic	>16	4
45.	Candesartan cilexetil	angiotensin 1 receptor antagonist	>16	16
46.	Solifenacin succinate	muscarinic M3 antagonist	>16	16
47.	Benzbromarone	uricosuric	>16	16

No	name	Description	MIC (μ M)	
			VRE	MRSA
48.	<div>Lindane </div>	topical treatment of lice and scabies	>16	16

Structures were downloaded from www.chemspider.com.

Table 3
Structure, description and MIC of Pharmakon non-antimicrobial compounds against Gram negative bacteria.

name	Description	MIC (μM)			
		<i>A. baumannii</i>	<i>E. cloacae</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>
<p>Bleomycin</p>	antineoplastic	8	0.25	0.5	>16
<p>Mitomycin</p>	antineoplastic	>16	8	>16	>16
<p>Oxymetazoline hydrochloride</p>	adrenergic agonist, nasal decongestant	>16	4	>16	>16

name	Description	MIC (µM)			
		<i>A. baumannii</i>	<i>E. cloacae</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>
Floxuridine	antineoplastic, antimetabolite	>16	8	8	>16

Structures were downloaded from www.chemspider.com