


## REVIEW ARTICLE

# Drug repurposing screens and synergistic drug-combinations for infectious diseases

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Infectious diseases account for nearly one fifth of the worldwide death toll every year. The continuous increase of drug-resistant pathogens is a big challenge for treatment of infectious diseases. In addition, outbreaks of infections and new pathogens are potential threats to public health. Lack of effective treatments for drug-resistant bacteria and recent outbreaks of Ebola and Zika viral infections have become a global public health concern. The number of newly approved antibiotics has decreased significantly in the last two decades compared with previous decades. In parallel with this, is an increase in the number of drug-resistant bacteria. For these threats and challenges to be countered, new strategies and technology platforms are critically needed. Drug repurposing has emerged as an alternative approach for rapid identification of effective therapeutics to treat the infectious diseases. For treatment of severe infections, synergistic drug combinations using approved drugs identified from drug repurposing screens is a useful option which may overcome the problem of weak activity of individual drugs. Collaborative efforts including government, academic researchers and private drug industry can facilitate the translational research to produce more effective new therapeutic agents such as narrow spectrum antibiotics against drug-resistant bacteria for these global challenges.

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### Abbreviations

$C_{\max}$ , maximum drug concentration recorded in human blood or plasma; FDA, Food and Drug Administration;  $IC_{90}$ , the drug concentration required for 90% inhibition; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*

## Introduction

Since the discovery and application of antibiotics and vaccines, the mortality rate across the world has been dramatically reduced. However, infectious diseases still caused approximately 20% of deaths in 2010 (Lozano *et al.*, 2012) and remain as a medical challenge to physicians and health organizations. The emergence and re-emergence of infections caused by HIV, Ebola virus and Zika virus has put great pressure on the development of vaccines and new specific therapeutics. The rapid appearances of drug-resistant pathogens such as drug-resistant bacteria, fungi, parasites and viruses have been widely reported (Snitkin *et al.*, 2012; Ashley *et al.*, 2014; McCarthy, 2016; Takeda *et al.*, 2017).

Development of new therapeutic agents and vaccines usually takes a long time and requires immense resources. Vaccine development typically takes 10 to 15 years. The Vaccine Adverse Event Reporting System receives approximately 30 000 reports annually, in which 10–15% are classified as serious medical events. Effective vaccines are still not available for many infectious diseases such as malaria, HIV, Ebola virus and Zika virus. The traditional process of development for a new low MW drug usually requires an average of 10 to 12 years and costs hundreds of millions of dollars (Sun *et al.*, 2016a). Development of new broad spectrum antibiotics is increasingly difficult. Thus, alternative approaches, such as drug repurposing, are needed to meet the challenges of outbreaks and the emergence of drug-resistant infectious diseases.

## Infectious diseases

Based on the identity of a pathogen, infectious diseases can be categorized into four major classes: bacterial infections, fungal infections, viral infections and protozoan infections. Many of these infections still do not have effective therapeutic agents. Another major challenge in infectious diseases is the rising incidence of drug resistance of these pathogens. In the last 5 years, there were several outbreaks of severe infectious diseases, including these caused by the carbapenem-resistant *Klebsiella pneumoniae* (Snitkin *et al.*, 2012), *Exserohilum rostratum* in contaminated methylprednisolone solutions (Kainer *et al.*, 2012), Ebola virus (Carroll *et al.*, 2015), Zika virus (Heymann *et al.*, 2016; Kreuels *et al.*, 2014) and the emerging artemisinin-resistant malaria (Ariey *et al.*, 2014).

The speed of developing new therapies for drug-resistant pathogens has not kept up with the evolution of drug resistance by these pathogens. Currently, 194 low MW drugs and 10 biological agents are on the list of Food and Drug Administration (FDA)-approved drugs available for systemic use to treat infectious diseases (Santos *et al.*, 2017). In addition, a total of 79 approved vaccine products are available for the prevention of infectious diseases (available from <http://www.fda.gov/BiologicsBloodVaccines/>) (online accessed: 30<sup>th</sup> January 2017).

## Discovery and development of new antibiotics – issues and new approaches

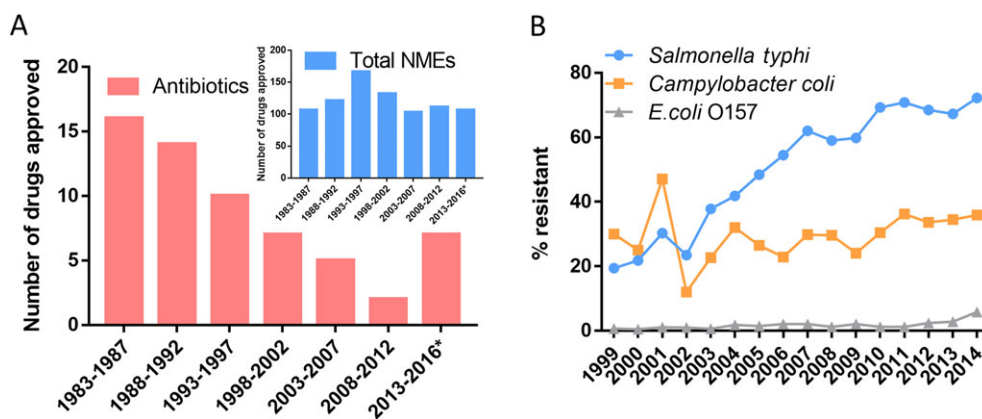
Most antibiotics were developed in the 1960s and 1970s by screening natural products and chemicals derived from

semi-synthesis with phenotypic screening methods (Power, 2006). Drug-resistant bacteria quickly emerged because of the extensive uses of antibiotics against various infections, especially the overuse and misuse of broad spectrum antibiotics (Granizo *et al.*, 2000; Woodford *et al.*, 2014). Antibiotics become less effective for treatment of infections due to the increase in drug-resistant bacteria. With the advance of molecular biology and bacterial genome analysis, target-based drug discovery developed into a major path for antibiotic drug discovery in the 1990s (Broughton and Queener, 1991). High-throughput screening of the bacterial targets was carried out in many companies. A number of lead compounds were identified and optimized. However, a decade-long effort did not produce the expected results. Only a few compounds derived from target-based screening campaigns advanced to late-stage development. One of the reasons for this failure was the inability of these lead compounds to cross the bacterial cell wall. A second reason was that the narrow spectrum of the antibactericidal activities of these lead compounds did not meet the requirement for further development (Fan *et al.*, 2002; Jarvest *et al.*, 2002; Payne *et al.*, 2002). The number of antibiotics approved by the FDA has steadily decreased in the last two decades, while the total number of new molecular entities has remained about the same (Figure 1A).

Classically, antibiotics inhibit bacterial growth and kill bacteria via inhibition of a key enzyme or an essential process in the bacterial life cycle. The five main bacterial processes that are involved in the mechanisms of action for antibiotics include cell wall synthesis, protein synthesis, DNA synthesis, DNA-directed RNA polymerase and essential metabolic enzymes (Coates *et al.*, 2002). Based on the selectivity against different types of bacteria, antibiotics are divided into broad-spectrum antibiotics that suppress a wide range of bacteria including both Gram-positive and Gram-negative and narrow-spectrum antibiotics that are only active against small groups of bacteria, such as Gram-negative or Gram-positive bacteria.

Drug-resistant bacteria have developed a wide range of mechanisms to alter their susceptibility to antibiotics. Reduction of drug entry, decrease of intracellular drug concentrations by increasing efflux, inactivation/modification of drugs, bypass of metabolic pathways and alteration of drug binding sites are mechanisms commonly involved in the drug resistance of bacteria (Lewis, 2013). New drugs have been developed to overcome some specific drug-resistant mechanisms. For example, clavulanic acid (Wise *et al.*, 1978), sulbactam (Retsema *et al.*, 1980), tazobactam (Jacobs *et al.*, 1986) and avibactam (Stachyra *et al.*, 2009) are the  $\beta$ -lactamase inhibitors that are used in combinations with  $\beta$ -lactam antibiotics to overcome the resistance of  $\beta$ -lactamase-producing bacteria.

The pharmaceutical industry has primarily focused on the development of broad spectrum antibiotics in the last two decades and abandoned narrow spectrum lead compounds. The main reason for only developing broad spectrum antibiotics is the financial return on investment from such new drugs. The new broad spectrum antibiotics can be used more frequently in clinics, as they have more indications, and are suitable for early intervention in infections. A critical fiscal



**Figure 1**

The decline of new antibiotics and rise of drug-resistant bacteria. (A) The number of US FDA-approved new antibiotics fell from 16 between 1983 and 1987 to 2 between 2008 and 2012. In 2012, the Generating Antibiotic Incentives (Durand *et al.*, 2016) was signed into law, which may have contributed to the increase of approved antibiotics between 2013 and 2016. The period \*2013–2016 covers 4 years. Total new molecular entities (NMEs) are above 100 during every 5-year span between 1983 and 2016. (B) Increase of drug-resistant *Salmonella typhi*, *Campylobacter coli* and *E. coli* O157 from 1999 to 2014 in the US. Data are from Centers for Disease Control and Prevention in the United States.

goal of drug development is to find ‘blockbuster drugs’ or new therapies that earn at least \$1 billion in annual return.

Orphan drugs, often developed for rare diseases that affect less than 200 000 people in the U.S., offer less financial rewards than blockbuster drugs. With the limited patient population, it is more difficult to recover the cost for drug development from pharmaceutical sales. Narrow spectrum antibiotics face the same hurdles: antibiotics indicated for a small group of bacteria usually do not offer a big market share. Hence, the monetary incentive to develop this type of drug is too low to be profitable.

As a result of the disappointment in producing new antibiotics, many pharmaceutical companies decreased their attempts to discover new antibiotic drugs in the early 2000s. This trend of reduced effort in antibiotic drug discovery by the industry continues, while the prevalence of drug-resistant bacteria such as salmonellae increase every year, although some others such as campylobacters and *Escherichia coli* did not change significantly (Figure 1B). Development of new drugs requires a significant amount of resources and time. Waiting to act is dangerous; the crisis of infections by drug-resistant bacteria is an emerging threat to public health. Hence, new strategies and technologies for antibiotic development and treatment of infectious diseases are critically needed. To inspire the development of new anti-infective treatments, the FDA Office of Orphan Products Development provides incentives (including fiscal ones) for sponsors to develop drugs for limited patient populations.

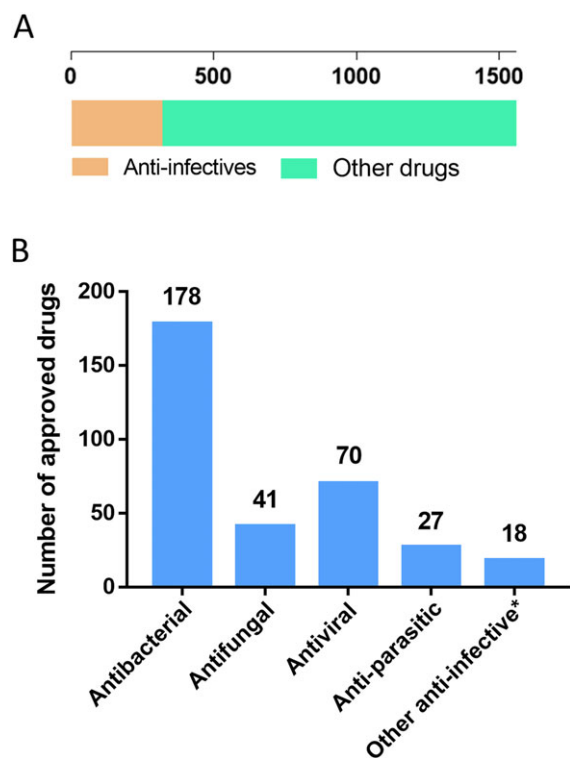
Phenotypic screening has re-emerged as an alternative approach for drug discovery in recent years (Zheng *et al.*, 2013). In contrast to mechanism-based drug discovery, phenotypic screening enables identification of active compounds that function by killing bacteria or inhibiting bacterial growth. For antibiotic drug development, specific strains of drug-resistant bacteria can be used in the primary compound screens, employing a phenotypic growth assay to identify new bactericidal compounds. The spectrum of initially identified active compounds can be determined quickly in the

follow-up confirmation experiments by screening additional strains of bacteria. The mechanisms of action of the identified active compounds are typically unknown after the phenotypic screen. If the newly identified compounds are approved drugs, the known functions of these drugs may provide some useful clues for the study of the mechanism of action. Phenotypic screening has not been extensively used in high-throughput screens against large collections of compounds for antibiotic drug discovery as the mechanism-based drug screening was the main approach in last two decades. The discovery of new bacterial genes and resistance plasmids further fuelled this target-based drug discovery effort, as well as the completion of bacterial genome mapping in the middle of 1990s (Kunst *et al.*, 1997). The phenotypic approach measures an actual biological response. Hence, phenotypic screens are more useful for identifying lead compounds with selective and narrow spectrums that target specific drug-resistant bacteria. A combination of phenotypic screening using patient-derived bacterial samples and drug repurposing could potentially identify new therapeutic agents to treat infections caused by drug-resistant bacteria.

## Drug repurposing

Drug repurposing of approved drugs provides an alternative method for rapid identification of new therapeutic agents to treat infections with drug-resistant bacteria and other emerging infectious diseases. The data for human pharmacokinetics and drug safety, as well as the preclinical results, are readily available for approved drugs. In the traditional drug development process, approximately one third of investigational drugs failed in clinical trials due to unexpected human toxicity and another one third failed due to lack of efficacy (Petrova, 2014). Repurposing approved drugs should avoid attrition in clinical trials due to drug toxicity and unfavourable issues in pharmacokinetics. The approved drugs found in drug repurposing screens can be advanced to

clinical trials or treatments quickly without prolonged pre-clinical study and a phase I clinical trial. A new indication of an FDA-approved drug qualifies the existing drug for a line extension. Currently, approximately 1500 US FDA-approved drugs are available for the treatments of a variety of diseases (Figure 2A). We conducted a pharmacological function search for each drug in Medical Subject Headings and other literature in December 2016. The majority of approved drugs are those for non-infective indications. Among the approved drugs, 310 showed anti-infective activities comprising 178 antibacterial agents, 41 antifungal agents, 70 antiviral agents, 27 anti-parasitic agents and 18 other anti-infective agents (anthelmintic and antiprotozoal) (Figure 2B). At the National Center for Advancing Translational Sciences, the approved drug collection has been expanded to a larger collection: the NCGC Pharmaceutical Collection (NPC) (Huang *et al.*, 2011). The NPC consists of approximately 2750 active low MW compounds including human drugs and animal drugs as well as investigational drugs being used in clinical trials. This collection will be updated periodically by the addition of newly approved drugs. While known antibiotics



**Figure 2**

Number of FDA-approved drugs with anti-infective activities. (A) 310 low MW drugs that have anti-infective activities. These compounds were curated from a total of 1578 US FDA-approved drugs by December 2016. (B) Anti-infective activities include antibacterial (antibiotics), antifungal, antiviral, anti-parasitic and other anti-infective (anthelmintic and antiprotozoal) agents. The anti-infective activities were curated from drug@fda, <http://www.accessdata.fda.gov/>, MeSH, Pubmed, NCATS Pharmaceutical Collection: <https://tripod.nih.gov/npc/> and <https://pubchem.ncbi.nlm.nih.gov/> (Huang *et al.*, 2011; Santos *et al.*, 2017). Note that a drug with multiple indications is counted as one unique low MW drug.

previously indicated for other bacteria can be directly applied for treatments of newly identified bacterial infections, a clinical trial is usually needed for treatment of infectious diseases with drugs approved for non-infective indications.

Due to commercial concerns, the pharmaceutical industry historically has lacked an interest in repurposing off-patent old drugs and/or exploring applications of approved drugs for unpredicted outbreaks of infectious diseases, such as the outbreak of Ebola virus. Therefore, drug repurposing for treatment of infectious diseases benefits from funding support through governments and foundations, as well as the collaborations between academic institutions and private industry.

Some successes have been achieved by repurposing anti-infective drugs for treatment of infectious diseases (Table 1). **Enoxacin**, a broad-spectrum fluoroquinolone antibacterial agent approved for treatment of urinary tract infections and gonorrhoea, showed antifungal activity in both a *Caenorhabditis elegans* assay and a murine model of candidiasis (Breger *et al.*, 2007). Delamanid, a drug for tuberculosis, exhibited activity against visceral leishmaniasis (Patterson *et al.*, 2016). More recently, **niclosamide**, an anti-worm medicine, showed potent activity against the Zika virus (Xu *et al.*, 2016).

Drugs that are not originally approved to treat an infectious disease have also been reported to inhibit infections caused by various pathogens. **Auranofin**, a gold-containing compound used for the treatment of rheumatoid arthritis, has been repurposed for several pathogens. The mechanism of action employed by auranofin is the inhibition of host or pathogen's thioredoxin reductases (Figure 3). It showed good activities against multidrug-resistant bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and *K. pneumoniae* (Harbut *et al.*, 2015; Sun *et al.*, 2016b). Auranofin also exhibited activities against other diseases including HIV/AIDS (Chirullo *et al.*, 2013), and some parasitic diseases (Debnath *et al.*, 2012), as well as Alzheimer's disease, Parkinson's disease (Madeira *et al.*, 2013; Madeira *et al.*, 2014) and cancer (Fiskus *et al.*, 2014). Notably, auranofin was evaluated in human clinical studies for gastrointestinal protozoa, HIV, and cancer. Additionally, **loperamide**, a diarrhoea drug, was repurposed against *Salmonella enterica* (Ejim *et al.*, 2011). The breast cancer drug **tamoxifen** showed efficacy in a murine model of cryptococcosis (Butts *et al.*, 2014). Chlorcyclizine, an old antihistamine, was repurposed for the treatment of infections by the hepatitis C virus (He *et al.*, 2015).

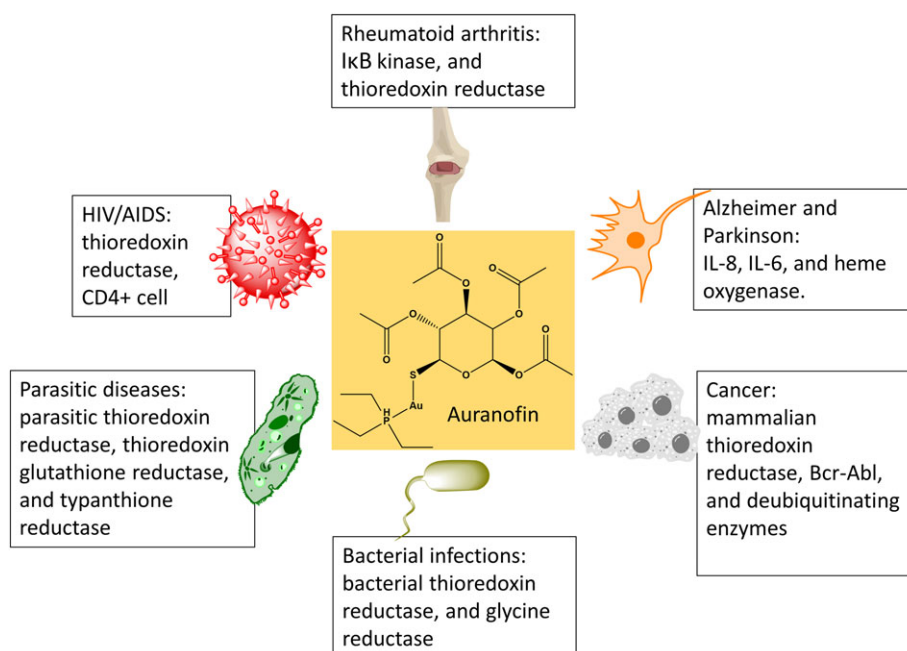
## Assays for drug repurposing screens

Although a mechanism-based assay can be used for drug repurposing screens, phenotypic screening of intact pathogens with the approved drug collection is more physiologically relevant for drug repurposing. The active compounds identified from phenotypic screening with bacterial strains can be tested directly in the animal models or in clinical trials. A number of cell viability assays are available for phenotypic screening of bacteria including absorbance growth assays (Highlander, 1997), ATP content assays (Sun *et al.*, 2016b) and resazurin reduction assays (Foerster *et al.*, 2017). These assays are robust and amenable to high-throughput

**Table 1**

Four classes of pathogens and examples of repurposed drugs

Pathogen	Examples of repurposed drugs/approved indication	Repurposed indication/model	Reference
Bacteria	Auranofin/rheumatoid arthritis	MRSA/ <i>in vitro</i> and mouse model	Proc Natl Acad Sci U S A. 2015 Apr 7; 112(14): 4453–4458
Bacteria	Loperamide/diarrhoea	<i>Salmonella enterica</i> / <i>in vitro</i> and mouse model	Nat Chem Biol. 2011 Jun 7; (6): 348–50
Parasite	Delamanid <sup>a</sup> /multidrug resistant tuberculosis	Visceral leishmaniasis/ <i>in vitro</i> and mouse model	Elife. 2016 May 24; 5. pii: e09744
Parasite	Auranofin/rheumatoid arthritis	Amebiasis/ <i>in vitro</i> , mouse model and phase IIa clinical trials	Nat Med. 2012 Jun 18; (6): 956–60. ClinicalTrials.gov Identifier: NCT02736968
Fungi	Tamoxifen/breast cancer	<i>Cryptococcus</i> / <i>in vitro</i> and mouse model	MBio. 2014 Feb 11; 5(1): e00765d-13
Fungi	Enoxacin/bacterial infection	Candidiasis/ <i>in vitro</i> and mouse model	PLoS Pathog. 2007 Feb 3; (2): e18
Virus	Nicosamide/tapeworm infection	Zika virus/ <i>in vitro</i> and brain organoids	Nat Med. 2016 Oct 22; (10): 1101–1107
Virus	Chlorcyclizine/allergy	Hepatitis C virus/ <i>in vitro</i> , mouse model and phase I clinical trials	Sci Transl Med. 2015 Apr 8; 7(282): 282ra49. ClinicalTrials.gov Identifier: NCT02118012

<sup>a</sup>Delamanid was approved in Europe, Japan and South Korea.**Figure 3**

Multiple indications of the rheumatoid arthritis drug, auranofin and the corresponding mechanisms of action. Auranofin was approved by US FDA for the treatment of rheumatoid arthritis. Auranofin was shown to be active in *in vitro* and/or preclinical models of HIV/AIDS, parasitic diseases, bacterial infections, Alzheimer's disease, Parkinson's diseases and cancer.

screening of large compound collections and hundreds of drug combinations. The  $IC_{50}$  and  $IC_{90}$  values of the compounds (the drug concentration required for 50% or 90%

inhibition) can be determined in these assays readily. A small amount of the final top lead compounds or drug combinations can be confirmed in the classical broth

dilution assays with low throughput in which the minimum inhibitory concentration (MIC) of confirmed compounds is determined. Generally, the  $IC_{90}$  values have correlated well with MICs (Munck *et al.*, 2014; Sun *et al.*, 2016b).

One of the main drawbacks for drug repurposing is that the new activity identified for an approved drug is usually not potent enough for the intended clinical application (Sun *et al.*, 2016a). For example, the repurposed drug is not therapeutically effective at its approved dose due to the limited human plasma concentrations. A higher dosage of repurposed drug is needed for the new indication, which can lead to undesired toxicity. From the perspective of clinical pharmacology, each drug is effective and safe in the approved drug dosage that allows a steady drug concentration in human plasma. All drugs can be toxic or cause severe adverse effects if drug dosage is too high and plasma drug concentration is above the safety threshold. Drug potencies ( $EC_{50}$  or  $IC_{50}$  values) can be obtained in drug repurposing screens, while the pharmacokinetic parameters of approved drugs,  $C_{max}$  (maximum drug concentration recorded in human blood or plasma), can be found in published papers (Schulz and Schmoldt, 2003) or databases, such as DailyMed (<https://dailymed.nlm.nih.gov/dailymed/index.cfm>) and NDAs at [drugs@FDA](mailto:drugs@FDA) (<http://www.accessdata.fda.gov/scripts/cder/daf/>).

One solution to the problem of insufficient drug concentrations in human plasma is to utilize synergistic drug combinations, which will be discussed in the next section. Another remedy is to conduct extensive preclinical development and new clinical trials for the repurposing drug candidates in order to find new optimal dosing and formulation. Approved anti-infective agents identified from the repurposing screen may be used immediately to treat patients with severe infections for which they were not developed initially (Bassetti *et al.*, 2011). Conversely, non-anti-infective drugs such as antihypertensive and antihistamine agents, once found from drug repurposing screens, typically do need new clinical trials to demonstrate their safety and efficacy for the treatment of infections (He *et al.*, 2015).

## Synergistic drug combinations for infectious diseases

Drug combinations have been used for treatment of a variety of diseases including infectious diseases. There are several advantages of drug combinations. First, drug combinations expand the spectrum of antibiotics for a broader coverage of pathogens. This is important for severe infections where early and effective treatment is critical (Zilberberg *et al.*, 2014). Second, drug combinations are effective in overcoming drug resistance (Fleisher *et al.*, 1983; Houang *et al.*, 1984; Qin *et al.*, 2017). For example,  $\beta$ -lactams are effective against many sensitive bacterial strains but not the  $\beta$ -lactamase producing resistant bacteria which hydrolyzes the  $\beta$ -lactam antibiotics and inactivates them. Addition of a  $\beta$ -lactamase inhibitor to a  $\beta$ -lactam antibiotic in treatments effectively overcomes this type of drug resistance. Third, prudent use of drug combinations may reduce the development of antibiotic resistance (Levin, 2002; Mahamat *et al.*, 2007; Aldeyab *et al.*, 2008). Fourth, combinations of two or more drugs

may lead to a synergistic effect, which is achieved by different mechanisms of action. Examples include the combinations of streptomycin–penicillin (Plotz and Davis, 1962) and trimethoprim–sulfa drugs against *E. coli* (Nichols *et al.*, 2011) as well as the unexpected synergism between minocycline and non-antibiotics (Ejim *et al.*, 2011).

Synergistic drug combination is particularly useful for drug repurposing because many active compounds identified from phenotypic screens have weak activities and cannot be directly applied in humans as a single agent. In a recent screen, we found 25 approved drugs with activities against the drug-resistant *K. pneumoniae* (Sun *et al.*, 2016b). Many newly identified drugs have not been used for drug-resistant *K. pneumoniae* previously, and more than a half of them were not antibiotics. The potency of these 25 drugs was not high enough for the clinical use as a single agent due to the limited drug concentration in human plasma. A new drug combination screen led to identification of synergistic drug combinations against the drug-resistant *K. pneumoniae*. Seventeen three-drug combinations were effective against the drug-resistant pathogen at clinically reachable individual drug concentrations. Another group also reported the strong synergy between meropenem, piperacillin and tazobactam against MRSA (Gonzales *et al.*, 2015). The concentrations of individual drugs in the combinations are lower than the clinical susceptibility break points that are required for the clinical applications. Hence, treatment with drug combinations is an important consideration for the treatment of multidrug-resistant bacteria (Table 2A). In another example, 53 approved drugs were identified with activities against the Ebola virus in a drug repurposing screen (Kouznetsova *et al.*, 2014). Similarly, the activity of most of the 53 drugs was too weak to be used in patients with Ebola infection as a single agent. We then carried out a new screening of synergistic drug combinations with individual drug concentrations relevant to human plasma concentrations. Several three-drug combinations with the clinically relevant drug concentrations that effectively suppressed Ebola virus infection *in vitro* were identified (Sun *et al.*, 2017) (Table 2B and Figure 4).

Current treatment of bacterial infections commonly employs a broad-spectrum antibiotic agent until a pathogen can be isolated and identified and antimicrobial susceptibility testing is completed, a process which takes 3 to 4 days. The methods of antimicrobial susceptibility testing for clinical diagnosis include broth microdilution, agar dilution, rapid automated instrument methods, disk diffusion and gradient diffusion methods (Jorgensen and Ferraro, 2009) (Table 3). Limited numbers of antibiotics, approximately 25, can be tested with the current methods in clinical diagnostic laboratories. It is not possible to use these methods for phenotypic screening of approved drug collection, or even a set of 200 antibiotics. They are also not suitable for testing of optimal drug combinations from hundreds of drug combinations in two-drug and three-drug combination formats. Several new methods have been under investigation for antimicrobial susceptibility testing (Smith and Kirby, 2016b; Sun *et al.*, 2016b; van Belkum and Dunne, 2013) (Table 3). Improvement of the current antimicrobial susceptibility testing methods or invention of new methods is needed to meet the challenge of drug-resistant bacteria. Recent advances include the use of matrix-assisted laser desorption/ionization time of

Table 2

(A) Top panel: examples of combinations of repurposed drugs against MRSA<sup>a</sup>. (B) Bottom panel: examples of combinations of repurposed drugs against Ebola virus-like particles<sup>e</sup>

Name	MIC <sup>b</sup> ( $\mu\text{g}\cdot\text{mL}^{-1}$ )	Clinical susceptibility breakpoints <sup>c</sup> ( $\mu\text{g}\cdot\text{mL}^{-1}$ )	Final concentration in combination <sup>d</sup> ( $\mu\text{g}\cdot\text{mL}^{-1}$ )
Meropenem	16	4–8	2
Piperacillin	64	4–8	2
Tazobactam	128	N/A	2
Name	IC <sub>50</sub> <sup>f</sup> ( $\mu\text{M}$ )	C <sub>max</sub> <sup>g</sup> ( $\mu\text{M}$ )	Final concentration in combination ( $\mu\text{M}$ )
Posaconazole	24.9	4.05	4
Toremifene	0.57	2.98	0.15
Mefloquine	6.45	5.84	2

<sup>a</sup>Data are from Nature Chemical Biology 11(11):855–61.

<sup>b</sup>MIC is minimum inhibitory concentration.

<sup>c</sup>Clinical susceptibility breakpoints for each drug alone against methicillin-susceptible *S. aureus*.

<sup>d</sup>Final drug concentration used in three-drug combinations.

<sup>e</sup>Data are from Antiviral Research 137 (2017) 165–172.

<sup>f</sup>IC<sub>50</sub> is the mean half-maximum inhibitory concentrations in single drug use.

<sup>g</sup>C<sub>max</sub> is the peak plasma or serum concentration in human.

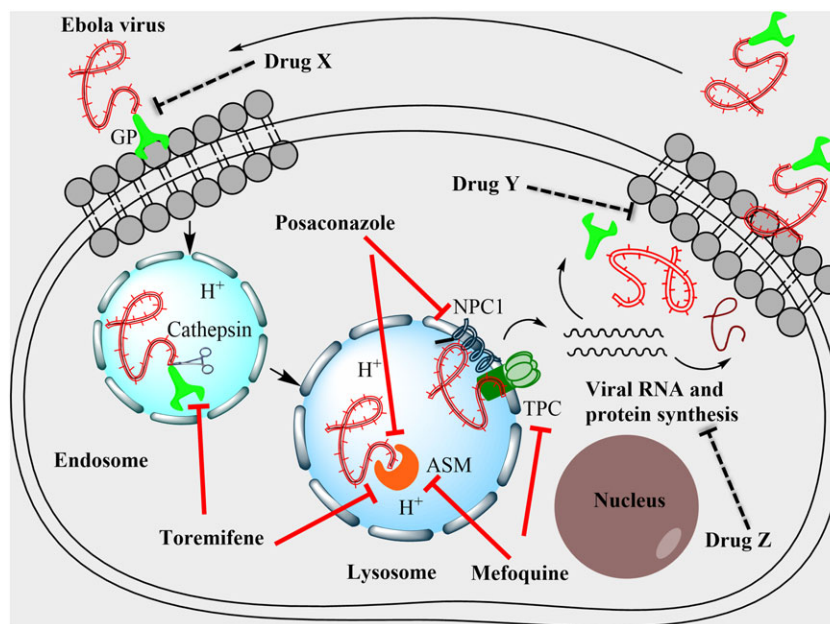


Figure 4

Ebola virus life cycle, host targets and repurposed drug candidates. Selected drugs are shown as an example of targeting host–pathogen system interactions to block Ebola virus infection. Note: ASM, acid sphingomyelinase; GP, glycoprotein; NPC1, niemann-Pick C1; TPC, two-pore channel.

flight MS and next generation sequencing that enables rapid identification of proteins and plasmids of clinically relevant multidrug-resistant bacteria in a real time and high-throughput fashion (Conlan *et al.*, 2014; Dekker and Frank, 2016; Youn *et al.*, 2016). The new and future generations of antimicrobial susceptibility testing methods should be able to rapidly screen hundreds of approved drugs in a concentration–response manner with individual compounds and with hundreds of drug combinations.

## Perspectives

Currently, broad-spectrum antibiotics are usually used in clinical treatment of bacterial infections until a pathogen can be isolated/identified and an effective antibiotic agent is found. In many cases, the broad-spectrum antibiotics are used through the entire course of treatment. The overuse of broad-spectrum antibiotics actually contributes to development of drug resistance in pathogens as well as in many

**Table 3**

Antimicrobial susceptibility testing methods

	Name of methods	Reference
Current clinical diagnosis methods	Broth microdilution, agar dilution, rapid automated instrument methods, disk diffusion and gradient diffusion methods	Clin Infect Dis. 2009 Dec 1; 49(11): 1749–55
Investigational methods	Automated digital dispensing platform for at-will broth microdilution, automated ultra-high-throughput bacterial growth assay, matrix-assisted laser desorption ionization-time of flight MS, next generation sequencing and so on	J Clin Microbiol. 2016 Sep; 54(9): 2288–93. Emerg Microbes Infect. 2016 Nov; 5(11): e116. Expert Rev Mol Diagn. 2017 Mar; 17(3): 257–269. J Clin Microbiol. 2013 Jul; 51(7): 2018–24.

non-harmful or less pathogenic bacteria. To avoid this unnecessary generation of resistance, effective and narrow-spectrum antibiotics might be the first choice for treatment of infections if the pathogens can be diagnosed quickly with new methods such as the bacterial genome sequencing technology (Dekker and Frank, 2016).

Effective narrow-spectrum antibiotics can be a good choice for treatment of infections with drug-resistant bacteria. Although narrow-spectrum antibiotics may not have a big market initially, their usage can increase with an improvement in antimicrobial susceptibility testing and an application of drug combination therapy. Narrow-spectrum antibiotics or lead compounds can be found by phenotypic screens of approved drug collection and other compound collections against individual drug-resistant pathogens. The leads can then be optimized and developed through an accelerated drug development process. Because of the small market and high costs associated with the development of narrow-spectrum antibiotics, a collaborative consortium of government, academic institutes and private drug industry may be needed for such an effort. For example, the National Center for Advancing Translational Sciences of the National Institutes of Health in the United States has initiated a new drug repositioning model with three-way partnerships between public funders, the pharmaceutical industry and academic investigators (Frail *et al.*, 2015). Involvement of government funders facilitates translational research and 'de-risks' these drug development projects which have a small, unprofitable share of the market.

Currently, initial treatment of infectious diseases is almost always based on a preliminary clinical diagnosis of potential pathogens. The individual responses and the genetic background of patients to antimicrobial treatment are usually either not or less frequently considered. Variations in the genetic background of individuals contribute to adverse effects of drug treatment as well as the therapeutic effects. Varied patient responses to drug treatments may also be caused by the interaction of pathogens with the microbiome of patients (Schwab and Schaeffeler, 2012; Nirmal Kumar Ganguly, 2013; Chaudhry *et al.*, 2016). Therefore, a personalized treatment for infectious diseases with consideration of pharmacogenomics is a future direction for combating severe infections and infections with drug-resistant bacteria which may increase the therapeutic efficacy, reduce adverse effects and decrease the possibility of developing drug resistance.

To improve the current treatment methods and to establish new treatment approaches for infectious diseases, physicians will need new antibiotics and technologies. These include more choices of narrow-spectrum antimicrobials and better diagnostic methods for pathogens, genome sequencing and analysis tools, and rapid antimicrobial susceptibility testing methods with real-time and high-throughput capacity. In particular, the current methods of antimicrobial susceptibility testing in clinical diagnostics are based on methods developed over 40 years ago. Unsurprisingly, these approaches do not have enough throughput and capacity for compound screening and cannot accommodate the need for screening of synergistic drug combinations. Modernization of the methods of testing for antimicrobial susceptibility is needed to meet the challenges of treating of infectious diseases. The bacterial growth assay in a miniaturized format (384- or 1536-well plates) (van Belkum and Dunne, 2013; Smith and Kirby, 2016a; Sun *et al.*, 2016b) or a chip-based method can be developed and optimized for this purpose. Only a short time (8 - 10 h) is needed for determination of effective antimicrobial agents and effective drug combinations in the bacterial growth assay with an absorbance assay format (Sun *et al.*, 2016b). New methods for rapid diagnosis of pathogens (10 h or less) such as genome sequencing of pathogens are also needed (van Belkum and Dunne, 2013; Dekker and Frank, 2016). The data should be quickly analysed to reveal the nature of pathogens and the information of drug susceptibility for a particular pathogen. In addition, pathogens should be quickly isolated from patient samples to be used in a rapid antimicrobial susceptibility test.

## Conclusion

To treat the growing numbers of infections with drug-resistant bacteria, phenotypic screens of an approved drug collection as well as synergistic combinations are a useful approach for rapid identification of new therapeutics. This approach may also be useful for emerging outbreaks of infectious diseases such as Ebola and Zika virus for which vaccines and therapeutic agents are unavailable and unrealistic to be developed in a short period of time. Meanwhile, development of new narrow-spectrum and selective antimicrobials using the phenotypic screening approach is a feasible direction to combat increasing infections of drug-resistant



bacteria. Collaboration between government, academic institutes and private drug industry may be a solution for development of new anti-infective therapies.

### Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.*, 2015).

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## Conflict of interest

The authors declare no conflicts of interest.

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