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# In vitro activity of non-antibiotic drugs against *Staphylococcus aureus* clinical strains

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ABSTRACT

*Objectives:* We hypothesised that one or more of the non-antibiotic candidates selected for this study would demonstrate antibiotic activity against *Staphylococcus aureus*.
*Methods:* We determined minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) for non-antibiotic drugs (amlodipine, azelastine, ebselen and sertraline) against five clinical *S. aureus* isolates and one quality control strain using the Microplate Alamar Blue Assay (MABA). Our research group selected clinical isolates obtained from nasal and wound swab cultures of patients with skin and soft-tissue infections who were seen at primary care clinics in the South Texas Ambulatory Research Network (STARNet).
*Results:* Three of the non-antibiotic drugs had identical MICs for all isolates: amlodipine, 64 $\mu$g/mL; azelastine, 200 $\mu$g/mL; and sertraline, 20 $\mu$g/mL. MICs for ebselen were 0.25 $\mu$g/mL (SA-29213, A1019 and J1019), 0.5 $\mu$g/mL (A32 and B60) and 1 $\mu$g/mL (B72). MBCs for amlodipine, azelastine and sertraline were within one dilution of their MICs, indicating bactericidal activity for all test isolates. Ebselen MBCs were one to two dilutions higher in most isolates, also indicating bactericidal activity for all test isolates.
*Conclusion:* In summary, all four non-antibiotics demonstrated in vitro activity to varying degrees against *S. aureus* clinical isolates. Ebselen was the most potent of the four non-antibiotics tested.


## 1. Introduction

For decades, drug discovery and development was largely guided by a 'one drug–one target' approach, also known as a 'lock and key' model, which focused on the identification of a single drug that selectively binds to one single target while avoiding any 'off-targets' that could produce undesirable or adverse effects. Until recently, this was the prevailing principle of drug design but it has since shifted from a 'one drug–one target' model to a 'one drug–multiple targets' model, otherwise known as 'polypharmacology' [1,2]. While it is true that the interaction of a drug with its off-targets can lead to adverse effects in certain cases, it is also this very concept that creates opportunities to find new treatments. Drug repurposing is the discovery of unknown off-targets of existing drugs. Many of the repurposed success stories occurred through the serendipitous discovery of these off-targets. Now, however, the same success can be achieved through a more deliberate, systematic approach.

After a near 40-year innovation gap in antibiotic drug discovery, and with antibiotic resistance rates persistently on the rise, it is no surprise that drug repurposing is being pursued as another option for developing antimicrobials. *Staphylococcus aureus*, for example, remains one of the most burdensome antibiotic-resistant infectious pathogens in hospital and community settings. Large library collections of US Food and Drug Administration (FDA)-approved non-antibiotic drugs and other small molecules (proven safe but not approved) are now available for investigators to perform high-throughput screening. Several drug classes have shown evidence of possessing antibacterial properties. These include calcium chan-

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**Table 1**
Whole-genome sequencing (WGS) and antibiotic susceptibilities [a] for clinical isolates

| Isolate | Resistance gene(s) [b] | MIC ($\mu$g/mL) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | OXA | CLI | ERY | GEN | SXT | DOX | TET | VAN | CIP |
| SA-29213 | None | ≤0.5 | ≤0.25 | ≤0.5 | ≤0.5 | ≤10 | ≤0.5 | ≤4 | ≤0.5 | ≤0.5 |
| A1019 | *mecA* | ≥4 | ≤0.25 | ≤0.5 | ≤0.5 | ≤10 | ≤2 | ≤4 | ≤0.5 | ≤0.5 |
| A32 | *mecA, ermC, tetK, gyrA*(S84L) | ≥4 | ≥4 | ≥8 | ≤0.5 | 20 | ≤2 | ≥16 | 1 | ≥8 |
| B60 | *gyrA*(S84L), *norA* | ≤0.5 | ≤0.25 | ≤0.5 | ≤0.5 | ≤10 | ≤2 | ≤4 | ≤0.5 | ≥8 |
| B72 | *ermC, tetK* | ≤0.5 | ≥4 | ≥8 | ≤0.5 | >320 | 4 | ≥16 | 1 | ≤0.5 |
| J1019 | *norA* | ≤0.5 | ≤0.25 | ≤0.5 | ≤0.5 | ≤10 | ≤2 | ≤4 | ≤0.5 | ≤0.5 |

OXA, oxacillin; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; SXT, trimethoprim/sulfamethoxazole; DOX, doxycycline; TET, tetracycline; VAN, vancomycin; CIP, ciprofloxacin.
[a] From VITEK®2 automated system; shaded areas indicate resistance.
[b] WGS data were used to identify antimicrobial resistance genes using ARG-ANNOT (Antibiotic Resistance Gene-ANNOTation) database.

nel blockers [3], antihistamines [4,5] and psychotropic medications such as selective serotonin reuptake inhibitors [6,7]. In addition, another group of drugs, which possesses a unique mechanism of disrupting the thioredoxin system of microbes (auranofin, ebselen, PX-12), has been making headlines recently [8–10] due to increasing potential to be used as antibacterial agents. Interestingly, each of the drugs discussed here are either approved or under development for non-anti-infective indications. Because of our interest in drug repurposing for the development of antibiotics, we chose one drug from each of these classes to investigate against *S. aureus*. We aimed to assess the in vitro activity of four non-antibiotic drugs, namely amlodipine, azelastine, ebselen and sertraline, against *S. aureus* using the Microplate Alamar Blue Assay (MABA).

## 2. Central hypothesis, specific aim and objectives

### 2.1. Central hypothesis

Drug repurposing, or identifying new uses for existing drugs, has emerged as an alternative to traditional drug discovery processes involving de novo synthesis. Drugs that are currently approved or under development for non-antibiotic indications may possess antibiotic properties and therefore may have repurposing potential, either alone or in combination with an antibiotic. The objective of the proposed research was to utilise novel screening tools to characterise the antibiotic effects of select non-antibiotic drugs against *S. aureus* clinical isolates. We hypothesised that one or more of the non-antibiotic candidates selected for this study would demonstrate antibiotic activity against *S. aureus*.

### 2.2. Specific aim and objectives

The aim of this study was to quantify the in vitro antibiotic activity of each non-antibiotic drug against *S. aureus* clinical strains with known resistance genes.

Objective 1.1: To determine minimum inhibitory concentrations (MICs) for non-antibiotic drugs (amlodipine, azelastine, ebselen and sertraline) against *S. aureus* using the MABA.

Objective 1.2: To determine minimum bactericidal concentrations (MBCs) for non-antibiotic drugs (amlodipine, azelastine, ebselen and sertraline) against *S. aureus* using the MABA.

## 3. Methods and rationale

### 3.1. Staphylococcus aureus isolates

Non-antibiotic MICs and MBCs were determined for five *S. aureus* clinical isolates and one quality control strain (*S. aureus* ATCC 29213). Clinical isolates were selected from a previous clinical and epidemiological study conducted by our group [11]. These isolates were obtained from nasal and wound swab cultures of patients with skin and soft-tissue infections (SSTIs) who were seen at primary care clinics in the South Texas Ambulatory Research Network (STARNet). As previously described [11], samples were plated onto prefilled tryptic soy agar plates (Hardy Diagnostics, Santa Maria, CA, USA) and were incubated at 35–37°C for 24 h. Cefoxitin screening tests were used for the identification and isolation of methicillin-resistant *S. aureus* (MRSA) [11]. VITEK®2 AST-GP75 cards (bioMérieux, Durham, NC, USA) were used to determine the susceptibility of MRSA study isolates to antimicrobials [11]. Antimicrobial MICs were interpreted according to Clinical and Laboratory Standards Institute (CLSI) document M100-S12 (Table 1) [11]. Resistance genes were identified using whole-genome sequencing (WGS) (BioProject **PRJNA352260**) as described previously [11]. Each clinical isolate was genetically and phenotypically distinct from one another.

### 3.2. Selection of non-antibiotic drug candidates

Several drugs were initially considered for this study. Candidates in each drug class (Table 2) were evaluated and selected based on several criteria as follows.

- Safety and tolerability: drug candidates had to have proven safety and tolerability data in humans. FDA approval was also preferred.
- Dosage forms/routes: we also considered dosage forms and routes of administration, with oral, inhalation or intranasal routes being preferred over parenteral medications.
- Different drug classes: we selected drugs that represented various therapeutic classes (one from each class, rather than all from one class).
- Antibiotic activity: we sought drugs for which antibiotic activity has been demonstrated previously, but with limited data. If this activity appeared among several drugs within a class or a generalised 'class effect', the drug that demonstrated the greatest potency was selected.
- Efflux pump inhibition: we also considered whether a drug had previously been shown to inhibit P-glycoprotein efflux pumps, since this has previously been correlated with intrinsic antibiotic activity [14].

After consideration of all of the above factors, we chose one drug from each of the following classes: calcium channel blockers (amlodipine); selective serotonin reuptake inhibitors (sertraline); and antihistamines (azelastine). In addition to the three non-antibiotics, we included ebselen, a unique organoselenium compound with antioxidant and anti-inflammatory properties that has demonstrated biological activity for a variety of targets [15,16]. Although not yet FDA-approved, ebselen has been shown to be safe in humans and is being evaluated in phase II trials for bipolar

**Table 2**
Non-antibiotic drugs [12,13,17,23,24,26,28,37]

| Generic drug name | Drug class or primary indication | FDA approved | Mean peak plasma concentration | Concentrations for MIC determination ($\mu$g/mL) |
|---|---|---|---|---|
| Amlodipine | $Ca^{+}$ channel blocker | Yes | 6–14 ng/mL | 12.5–256 |
| Azelastine | Antihistamine | Yes | 200 pg/mL | 12.5–400 |
| Ebselen | Antioxidant | No | 30–83 ng/mL [a] | 0.12–4 |
| Sertraline | SSRI | Yes | 100–500 ng/mL | 2.5–160 |

FDA, US Food and Drug Administration; MIC, minimum inhibitory concentration; SSRI, selective serotonin reuptake inhibitor.
[a] Based on 200–1600 mg/day orally during a phase I clinical trial [37].

disorder as well as for the treatment and prevention of hearing loss [17,18]. In vitro studies have also observed antimicrobial activity for a wide range of infectious pathogens, including *S. aureus* [19,20].

### 3.3. Non-antibiotic drugs: solubility and storage

Standard laboratory-grade powders were obtained for the following non-antibiotic drugs: amlodipine besylate (Tocris, Minneapolis, MN, USA); ebselen (Cayman Chemical, Ann Arbor, MI, USA); azelastine (Selleck Chemicals, Houston, TX, USA); and sertraline (Selleck Chemicals). Stock solutions were prepared according to the manufacturer's instructions and were stored at –80°C. Azelastine was solubilised in distilled water (up to 35 mg/mL), while amlodipine, ebselen and sertraline were solubilised in dimethyl sulfoxide (DMSO). Each drug was further diluted with cation-adjusted Mueller–Hinton broth in 96-well microtitre plates for the MABA. The highest concentration of DMSO remaining in microtitre wells did not exceed 2% (v/v).

### 3.4. Selection of non-antibiotic drug concentrations

Non-antibiotic drug concentrations used for in vitro testing are shown in Table 2. These were similar to concentrations that were tested in a small number of prior reports that evaluated the in vitro antibacterial effects of non-antibiotics [21–23]. While the in vitro drug concentrations used in this study generally exceed those observed clinically, they might still be clinically useful. Drug concentrations are most commonly assessed from samples collected from plasma; however, plasma concentrations are not the only indicators of drug exposure. Additional factors such as route of administration, site of infection, and drug concentration at tissue sites must also considered. Sertraline concentrations in brain tissue, for example, can be 20- to 40-fold higher than in plasma [24,25]. Azelastine is an antihistamine that is available for use as a nasal spray. As one may expect, the drug concentrates in the nasal passages—not in plasma—after intranasal administration [26]. If azelastine has antibiotic activity, it is possible that the drug may impact *S. aureus* nasal colonisation or potentially other important bacterial flora. Furthermore, several alternative mechanisms of drug delivery currently exist, in which high concentrations of antibiotics are required for effectiveness. Examples include antibiotic lock therapy, aerosolised antibiotics, antibiotic-impregnated catheters and topical antibiotics.

Note that ebselen is not yet approved by the FDA. Peak plasma concentrations shown in Table 2 are from a phase I trial using oral doses up to 1600 mg/day. Additional trials are currently underway using similar oral doses, but it is unknown at this time whether this indication or dosing range will gain FDA approval.

### 3.5. Antibiotic susceptibility testing

#### 3.5.1. Determination of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs)

Standard broth microdilution procedures were followed according to CLSI methods, but with the addition of alamarBlue® dye (Invitrogen, Carlsbad, CA, USA). Bacterial cultures were inoculated in 96-well, flat-bottom microtitre plates containing serially diluted drugs at a final concentration of $\sim 10^5$ CFU/mL. To prevent evaporation, a volume of 200 $\mu$L of sterile water was added to outer-perimeter wells [18]. Control wells included Mueller–Hinton broth with alamarBlue® (background) and Mueller–Hinton broth with alamarBlue® plus bacteria (growth control). alamarBlue® was added at a volume equal to 10% of the total volume, while protecting from light exposure, prior to incubating for 16–20 h at 35 ± 2°C. MICs were defined as the lowest drug concentration that prevented a change in colour. In addition, 10 $\mu$L samples were withdrawn, primarily from growth control wells, and plated for colony counting. MICs were recorded and contents from wells for which there was no growth were plated onto Mueller–Hinton agar plates and incubated 18–24 h at 35 ± 2°C. The MBC was defined as the minimum drug concentration resulting in a ≥99.9% reduction (≥3 $\log_{10}$) in CFU/mL from the initial inoculum.

#### 3.5.2. Rationale for selection of antibiotic testing methods

An advantage of the broth microdilution technique compared with other susceptibility testing methods (i.e. disk diffusion, agar dilution) is that once MICs are obtained, they can be used to derive bactericidal concentrations with an additional step (see MBC determinations). The addition of a growth indicator, such as alamarBlue®, provides even more of an advantage. Resazurin, the active ingredient in alamarBlue®, is a cell-permeable non-toxic blue dye that undergoes an oxidation–reduction reaction after entering the cell, changing from non-fluorescent blue (oxidised form) to highly fluorescent, pink-coloured resorufin (reduced form). The extent of this colour conversion represents cell viability and can be assessed qualitatively by visual inspection of colour change.

### 3.6. Data analysis

MICs and MBCs were recorded for each non-antibiotic drug and bacterial isolate and were interpreted as follows: MBC/MIC ratio ≥4 indicated bacteriostatic activity; and MBC/MIC ratio ≤2 indicated bactericidal activity. Assays were performed in duplicate and were repeated at least once.

## 4. Results

Three of the non-antibiotic drugs had identical MICs for all isolates: amlodipine, 64 $\mu$g/mL; azelastine, 200 $\mu$g/mL; and sertraline, 20 $\mu$g/mL (Table 3). MICs for ebselen were 0.25 $\mu$g/mL (SA-29213, A1019 and J1019), 0.5 $\mu$g/mL (A32 and B60) and 1 $\mu$g/mL (B72). MBCs for amlodipine, azelastine and sertraline were within one dilution of their MICs, indicating bactericidal activity for all test isolates. Ebselen MBCs were one to two dilutions higher in most isolates, also indicating bactericidal activity for all test isolates.

## 5. Discussion and conclusion

We quantified the antibiotic effects of four non-antibiotic drugs (amlodipine, azelastine, ebselen and sertraline) against *S. aureus*

**Table 3**
Non-antibiotic susceptibilities (in $\mu$g/mL)

| Isolate | Amlodipine | | Azelastine | | Sertraline | | Ebselen | |
|---|---|---|---|---|---|---|---|---|
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| SA-29213 | 64 | 128 | 200 | 200 | 20 | 20 | 0.25 | 0.5 |
| A1019 | 64 | 64 | 200 | 200 | 20 | 20 | 0.25 | 0.5 |
| A32 | 64 | 64 | 200 | 200 | 20 | 40 | 0.5 | 1 |
| B72 | 64 | 64 | 200 | 200 | 20 | 40 | 1 | 1 |
| B60 | 64 | 128 | 200 | 200 | 20 | 40 | 0.5 | 1 |
| J1019 | 64 | 64 | 200 | 200 | 20 | 40 | 0.25 | 0.5 |

MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.

clinical strains using the MABA. All four non-antibiotics demonstrated in vitro activity to varying degrees against *S. aureus* clinical isolates. Ebselen was the most potent of the four non-antibiotics tested, with MICs between 0.25 $\mu$g/mL and 1 $\mu$g/mL and MBC/MIC ratios of 1–2. This range is consistent with what prior investigations have observed against *S. aureus* [16,27]. In fact, ebselen has demonstrated bactericidal activity various multidrug-resistant strains of *S. aureus*, including vancomycin-intermediate and vancomycin-resistant strains [21]. Additionally, ebselen has been shown to inhibit toxin production as well as biofilm formation [19–21].

Concentrations of non-antibiotics used in this study are higher than average plasma concentrations reported in humans. This does not, by default, rule out their clinical utility since plasma concentration is not the sole metric for assessing adequate drug exposure, even if it is the most practical. Sertraline plasma concentrations, for example, range from 0.1–0.5 $\mu$g/mL depending on the dose [28]. Sertraline MICs for *Cryptococcus neoformans*, a pathogen known to cause meningitis in acquired immune deficiency syndrome (AIDS) patients, range between 1 $\mu$g/mL and 10 $\mu$g/mL [29,30]. Although concentrations in this range are not achieved in blood with approved doses, sertraline may be 20- to 40-fold higher in brain tissue, which, in the case of cryptococcal meningitis, is the site of infection. Furthermore, the standard of care for treating infections from *Cryptococcus* spp. and other difficult-to-treat infections is to use a combination of antimicrobials [31–33].

Amlodipine MICs were also considerably higher than mean plasma concentrations (32 $\mu$g/mL vs. 14 ng/mL). However, prior studies have suggested that doses required to effectively treat an infection in vivo may be significantly lower than doses used in vitro to inhibit bacterial growth [23]. Amlodipine also appears to have an anti-inflammatory effect, which is something that cannot be reflected in the susceptibility test results. Dutta et al. observed a downregulation of expression of the inflammatory cytokines interferon-$\gamma$, interleukin-1$\beta$ and tumour necrosis factor-$\alpha$ in mice treated with amlodipine compared with non-treated mice [34].

With ebselen not yet approved by the FDA, there has been some uncertainty as to its pharmacokinetics and dosing. Ebselen doses of 50 mg/kg given orally and 1 mg/kg/h by intravenous infusion produced peak plasma concentrations up to 15 $\mu$g/mL and 12 $\mu$g/mL, respectively, in rat models [35,36]. Ebselen MICs would then fall within a clinically achievable range, as concluded by previous investigators [21,27]. However, a phase I study of ebselen at doses up to 1600 mg orally per day performed in healthy volunteers found that peak plasma concentrations ranged between 30 ng/mL and 83 ng/mL [37]. A follow-up phase II study determining the safety and efficacy of ebselen given at 200, 400 or 600 mg twice daily for the prevention of noise-induced hearing loss has been published [17]. Additional trials are currently underway using similar oral doses, but it is unknown at this time whether this indication or dosage form will obtain FDA approval. Sound Pharmaceuticals, Inc. has been developing ebselen for several years for the treatment and prevention of noise-induced hearing loss. Given the many biological targets of ebselen, however, eventual pursuit of an unrelated indication and altered dosing regimen would not be surprising.

While non-antibiotic MICs in this study may be too high to achieve in blood, concentrations may still be relevant at other tissue sites, even with minimal to no systemic exposure. Furthermore, non-systemic medication applications such as antibiotic lock therapy, aerosolised antibiotics and antibiotic-impregnated catheters actually require high concentrations in order to be effective [38–40]. Azelastine, an antihistamine available as a nasal spray, shows only negligible presence (pg/mL) in blood but can deliver a total daily dose of up to 274 $\mu$g/mL in each nostril [26]. We observed inhibitory and bactericidal activity at concentrations of 200 $\mu$g/mL against *S. aureus*. It is therefore plausible that the amount of azelastine exposure from the nasal spray could be enough to impact *S. aureus* nasal colonisation or potentially other important bacterial flora. This also raises an important question as to whether or not frequent use of such a medication could impact not only colonisation but also antibiotic resistance.

This was a proof-of-concept study using clinical isolates collected in primary care clinics from patients with SSTIs. Using real-world isolates made the study more translational. However, none of the clinical isolates were resistant to vancomycin or daptomycin, therefore we were unable to compare the non-antibiotic drugs against clinical isolates with those phenotypes. Furthermore, some clinicians believe that appropriate management of patients with SSTIs includes assessment and eradication of nasal colonisation; therefore, isolates both from nasal and wound swabs were studied. Ultimately, this study is most applicable to patients presenting to primary care clinics with SSTIs.

In summary, all four non-antibiotics demonstrated in vitro activity to varying degrees against *S. aureus* clinical isolates. Ebselen was the most potent of the four non-antibiotics tested, with MICs of 0.25–1 $\mu$g/mL and MBC/MIC ratios of 1–2. In fact, ebselen has demonstrated bactericidal activity various multidrug-resistant strains of *S. aureus*, including vancomycin-intermediate and vancomycin-resistant strains. In addition, ebselen has been shown to inhibit toxin production as well as biofilm formation. When viewed in the context of available literature, these data suggest that the non-antibiotic candidates selected for this study might have future potential as antibiotics.

## Competing interests

In the last 3 years, CRF's institutions have received grant money for CRF to perform research from AstraZeneca Pharmaceuticals. All other authors declare no competing interests. The views expressed in this article are those of the authors and do not necessarily represent the views of the Department of Veterans Affairs, the National Institutes of Health, or the authors' affiliated institutions.

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## Ethical approval

Not required.

## References

[1] Bolognesi ML. Polypharmacology in a single drug: multitarget drugs. Curr Med Chem 2013;20:1639–45.
[2] Medina-Franco JL, Giulianotti MA, Welmaker GS, Houghten RA. Shifting from the single to the multitarget paradigm in drug discovery. Drug Discov Today 2013;18:495–501.

[3] Machado D, Pires D, Perdigao J, Couto I, Portugal I, Martins M, et al. Ion channel blockers as antimicrobial agents, efflux inhibitors, and enhancers of macrophage killing activity against drug resistant *Mycobacterium tuberculosis*. PLoS One 2016;11:e0149326.
[4] Perlmutter JI, Forbes LT, Krysan DJ, Ebsworth-Mojica K, Colquhoun JM, Wang JL, et al. Repurposing the antihistamine terfenadine for antimicrobial activity against *Staphylococcus aureus*. J Med Chem 2014;57:8540–62.
[5] El-Nakeeb MA, Abou-Shleib HM, Khalil AM, Omar HG, El-Halfawy OM. In vitro antibacterial activity of some antihistaminics belonging to different groups against multi-drug resistant clinical isolates. Braz J Microbiol 2011;42:980–91.
[6] Bhardwaj AK, Mohanty P. Bacterial efflux pumps involved in multidrug resistance and their inhibitors: rejuvinating the antimicrobial chemotherapy. Recent Pat Antiinfect Drug Discov 2012;7:73–89.
[7] Karine de Sousa A, Rocha JE, Goncalves de Souza T, Sampaio de Freitas T, Ribeiro-Filho J, Melo Coutinho HD. New roles of fluoxetine in pharmacology: antibacterial effect and modulation of antibiotic activity. Microb Pathog 2018;123:368–71.
[8] Thangamani S, Mohammad H, Younis W, Seleem MN. Drug repurposing for the treatment of staphylococcal infections. Curr Pharm Des 2015;21:2089–100.
[9] Sun LH. Scientists find way to disarm deadly bacteria without destroying the good ones in your gut. The Washington Post; 2015. https://www.washingtonpost.com/news/to-your-health/wp/2015/09/23/scientists-find-way-to-disarm-deadly-bacteria-without-destroying-the-good-ones-in-your-gut/?utm_term=.0328b1024dce [accessed 15 March 2021].
[10] Purdue scientist receives $1.6 million grant on repurposed drugs as treatment. Purdue University Research Foundation News; 2017. https://www.purdue.edu/newsroom/releases/2017/Q3/purdue-scientist-receives-1.6-million-grant-on-repurposed-drugs-as-treatment.html [accessed 15 March 2021].
[11] Lee GC, Long SW, Musser JM, Beres SB, Olsen RJ, Dallas SD, et al. Comparative whole genome sequencing of community-associated methicillin-resistant *Staphylococcus aureus* sequence type 8 from primary care clinics in a Texas community. Pharmacotherapy 2015;35:220–8.
[12] Abernethy DR. The pharmacokinetic profile of amlodipine. Am Heart J 1989;118:1100–3.
[13] Elliott HL, Meredith PA. The clinical consequences of the absorption, distribution, metabolism and excretion of amlodipine. Postgrad Med J 1991;67(Suppl 3):S20–3.
[14] Leitner I, Nemeth J, Feurstein T, Abrahim A, Matzneller P, Lagler H, et al. The third-generation P-glycoprotein inhibitor tariquidar may overcome bacterial multidrug resistance by increasing intracellular drug concentration. J Antimicrob Chemother 2011;66:834–9.
[15] Noguchi N. Ebselen, a useful tool for understanding cellular redox biology and a promising drug candidate for use in human diseases. Arch Biochem Biophys 2016;595:109–12.
[16] Thangamani S, Younis W, Seleem MN. Repurposing clinical molecule ebselen to combat drug resistant pathogens. PLoS One 2015;10:e0133877.
[17] Kil J, Lobarinas E, Spankovich C, Griffiths SK, Antonelli PJ, Lynch ED, et al. Safety and efficacy of ebselen for the prevention of noise-induced hearing loss: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet 2017;390:969–79.
[18] Azad GK, Tomar RS. Ebselen, a promising antioxidant drug: mechanisms of action and targets of biological pathways. Mol Biol Rep 2014;41:4865–79.
[19] AbdelKhalek A, Abutaleb NS, Mohammad H, Seleem MN. Repurposing ebselen for decolonization of vancomycin-resistant enterococci (VRE). PLoS One 2018;13:e0199710.
[20] Wall G, Chaturvedi AK, Wormley FL Jr, Wiederhold NP, Patterson HP, Patterson TF, et al. Screening a repurposing library for inhibitors of multidrug-resistant *Candida auris* identifies ebselen as a repositionable candidate for antifungal drug development. Antimicrob Agents Chemother 2018;62:e01084 -18.
[21] Thangamani S, Younis W, Seleem MN. Repurposing ebselen for treatment of multidrug-resistant staphylococcal infections. Sci Rep 2015;5:11596.
[22] Ayaz M, Subhan F, Ahmed J, Khan AU, Ullah F, Ullah I, et al. Sertraline enhances the activity of antimicrobial agents against pathogens of clinical relevance. J Biol Res (Thessalon) 2015;22:4.
[23] Mazumdar K, Asok Kumar K, Dutta NK. Potential role of the cardiovascular non-antibiotic (helper compound) amlodipine in the treatment of microbial infections: scope and hope for the future. Int J Antimicrob Agents 2010;36:295–302.
[24] Lewis RJ, Angier MK, Williamson KS, Johnson RD. Analysis of sertraline in postmortem fluids and tissues in 11 aviation accident victims. J Anal Toxicol 2013;37:208–16.
[25] Tremaine LM, Welch WM, Ronfeld RA. Metabolism and disposition of the 5-hydroxytryptamine uptake blocker sertraline in the rat and dog. Drug Metab Dispos 1989;17:542–50.
[26] Bernstein JA. Azelastine hydrochloride: a review of pharmacology, pharmacokinetics, clinical efficacy and tolerability. Curr Med Res Opin 2007;23:2441–52.
[27] Younis W, Thangamani S, Seleem MN. Repurposing non-antimicrobial drugs and clinical molecules to treat bacterial infections. Curr Pharm Des 2015;21:4106–11.
[28] Rhein J, Morawski BM, Hullsiek KH, Nabeta HW, Kiggundu R, Tugume L, et al. ASTRO-CM Study Team. Efficacy of adjunctive sertraline for the treatment of HIV-associated cryptococcal meningitis: an open-label dose-ranging study. Lancet Infect Dis 2016;16:809–18.
[29] Butts A, DiDone L, Koselny K, Baxter BK, Chabrier-Rosello Y, Wellington M, et al. A repurposing approach identifies off-patent drugs with fungicidal cryptococcal activity, a common structural chemotype, and pharmacological properties relevant to the treatment of cryptococcosis. Eukaryot Cell 2013;12:278–87.
[30] Zhai B, Wu C, Wang L, Sachs MS, Lin X. The antidepressant sertraline provides a promising therapeutic option for neurotropic cryptococcal infections. Antimicrob Agents Chemother 2012;56:3758–66.
[31] Whitney LC, Bicanic T. Treatment principles for *Candida* and *Cryptococcus*. Cold Spring Harb Perspect Med 2014;5:a024158.
[32] Bassetti M, Righi E. Multidrug-resistant bacteria: what is the threat? Hematology Am Soc Hematol Educ Program 2013;2013:428–32.
[33] Le T, Bayer AS. Combination antibiotic therapy for infective endocarditis. Clin Infect Dis 2003;36:615–21.
[34] Dutta NK, Mazumdar K, DasGupta A, Dastidar SG. In vitro and in vivo efficacies of amlodipine against *Listeria monocytogenes*. Eur J Clin Microbiol Infect Dis 2009;28:849–53.
[35] Imai H, Masayasu H, Dewar D, Graham DI, Macrae IM. Ebselen protects both gray and white matter in a rodent model of focal cerebral ischemia. Stroke 2001;32:2149–54.
[36] Masumoto H, Hashimoto K, Hakusui H, Takaichi M, Yokota T, Honda T, et al. Studies on the pharmacokinetics of ebselen in rats (1): absorption, distribution, metabolism and excretion after single oral administration. Drug Metab Pharmacokinet 1997;12:596–609.
[37] Lynch ED, Kil J. Development of ebselen, a glutathione peroxidase mimic, for the prevention and treatment of noise-induced hearing loss. Semin Hear 2009;30:47–55.
[38] Justo JA, Bookstaver PB. Antibiotic lock therapy: review of technique and logistical challenges. Infect Drug Resist 2014;7:343–63.
[39] Bhatia P, Sharma A, Gupta S, Vyas V, Kaloria N, Sethi P. Aerosolized antibiotics: for prophylaxis or for treatment? J Crit Care 2018;47:348.
[40] McAllister K, Chalmers JD. Old drugs for bad bugs—aerosolized antibiotics in ventilator-associated pneumonia. Crit Care Med 2015;43:697–8.