Chapter

Drugs Repurposing for Multi-Drug Resistant Bacterial Infections

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Abstract

Different institutions recognized that antimicrobial resistance is a global health threat that has compounded by the reduction in the discovery and development of new antimicrobial agents. Therefore, the development of new antimicrobial therapeutic strategies requires immediate attention to avoid the 10 million deaths predicted to occur by 2050 as a result of multidrug-resistant (MDR) bacteria. Despite the great interest in the development of repurposing drugs, only few repurposing drugs are under clinical development against Gram-negative critical-priority pathogens. In this chapter, we aim: (i) to discuss the therapeutic potential of the repurposing drugs for treating MDR bacterial infections, (ii) to summarize their mechanism of action, and (iii) to provide an overview for their preclinical and clinical development against these critical-priority pathogens.

Keywords: repurposing drug, infection, bacteria, nosocomial, clinical

1. Introduction

Antimicrobial resistance poses a well-recognized global health threat due to the global dissemination of bacteria resistants to multiple antibiotic classes. This situation is deemed a global priority by the World Health Organization and the European Centre for Disease Prevention and Control [1, 2]. Currently, global deaths due to antimicrobial resistance are more than 70,000 in USA and in Europe together [3, 4]. Therefore, the development of new antimicrobial therapeutic strategies requires immediate attention to avoid the high number of deaths predicted to occur in the future as a result of multidrug-resistant (MDR) bacteria [5]. It is clear that effective solutions such as the establishment of antimicrobial stewardship programs to optimize the use of existing antibiotics, the promotion of novel rapid diagnostics to curtail the unnecessary use of antimicrobial agents; the promotion, development, and use of vaccines and novel antibiotic classes are urgently needed [5]. However, the increased prevalence of infections by MDR bacteria and the scarcity of novel antibiotic families that are under clinical development could warrant the development of new antimicrobial therapeutic strategies for use alone or together with one of the scarce but clinically relevant antibiotics.

Repurposing drugs have been gained renewed interest in the last decade as reflected by several recent studies [6–9]. Since then, 4% of the 407 preclinical

antibiotic projects from 314 institutions are related with repurposing drugs evaluated against bacterial infections [10]. Further evidence of the increased interest in these drugs class is that the development process for repurposed drugs benefits from a large body of available knowledge and reduces the time and cost of development [9]. The majority of repurposed drugs developed to treat bacterial infections are approved or in advanced clinical stages as anticancer drugs, anti-inflammatory/immunomodulatory drugs, antipsychotic and antidepressant drugs, statins and iron-storage drugs [9]. The large difference between the numerous drugs approved or in development for oncologic indications and the small number of new antibiotics is surprising given that over the past decades antimicrobial resistance has emerged as an important public health with high associated mortality, and in 2050 antimicrobial resistance would result in 10 million more deaths than those caused by cancers [5]. Although multiple factors contribute to the scarcity of new antibiotics for bacterial infections, the success of repurposing drugs-based antibiotic therapy as an alternative approach can be reached. Repurposing drugs-based approaches could provide a viable alternative for the treatment of certain MDR bacterial infections. This could be especially important for certain infections caused by MDR Gram-negative infections such as *Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacterales* carbapenems-resistants, for which current antimicrobial treatments are not active. The WHO has classified as critical priority these pathogens for research and development of new antibiotics [1].

In this chapter, we focus on the current state of knowledge regarding the potential benefits and disadvantages of repurposing drugs treatments for MDR Gram-negative infections. We outline the advances to-date in their preclinical and clinical development as antimicrobial agents. To this end, we introduce in Pubmed database different key words such as repurposing drug, repositioning, antimicrobial and/or antibacterial in order to find published literature about the repurposing drugs for treatment of bacterial infections.

2. Therapeutic potential of repurposing drugs

There is a widely acknowledged that repurposing drugs could address the global increase in antimicrobial resistance and especially the treatment of MDR Gram-negative bacteria. This could be due by the fact that repurposing drugs might exhibit some advantageous characteristics. Repurposing drugs target some genes and surface proteins that are not targets of currently used antimicrobials [9]. Of note, it is unlikely that their antibacterial activities can be disturbed by existing antimicrobial resistance mechanisms. The most of repurposing drugs increased the permeability and damaged the bacterial membrane without killing the bacteria. They enhanced the activity of the current antibiotics [9]. Furthermore, repurposing drugs are drugs approved by the Federal Drug Administration (FDA), information about their pharmacological characteristics (both safety and pharmacokinetic) in preclinical and clinical trials is widely available. Therefore, the time and economic costs associated with the repurposing of these drugs for other therapeutic applications such as the treatment of bacterial infections will be minimized [11]. Finally, to our knowledge, it was not reported that repurposing drugs produce selective pressure on the human microbiome.

Substantial progress has been made in the development of repurposed drugs against bacterial infections. Although some current compounds in the pipeline have exhibited promising results, existing pharmacokinetic characteristics limits the activity of many of them. It should be taken into account in the preclinical development of repurposing drugs the possible need for new formulations to increase

their bioavailability and absorption. This aspect is relevant for the development of anthelmintics. Extensive binding to plasma proteins has been reported for oxyclozanide and other salicylanilides, which currently limits their systemic and intravenous applications [12]. Of note, positive results have been seen with niclosamide derivative O-alkylamino-tethered, which has a potent antibacterial effect against carbapenemase producing and colistin resistant *Enterobacterales* isolates [13]. Inhalable nanosuspension and salt form of niclosamide, niclosamide ethanolamine, have presented better solubility profile and inhibited the *P. aeruginosa* quorum sensing (QS) [14, 15]. An additional relevant issue should be taken into account is that their administration route can be changed. ADMET tests should be performed before the development of these repurposing drugs in clinical trials. The choice of the route is a relevant aspect in serious infections in hospitalized patients such as ventilated-associated pneumonia who patients are intubated and other circumstances in which the oral route is not available.

3. Mechanisms of action of repurposing drugs against Gram-negative bacilli

Different agents are promising both in vitro and in vivo candidates to be repositioned as antimicrobial agents to treat infections caused by MDR Gram-negative bacilli. A variety of drugs with different mechanisms of action and targets have been selected including: DNA, RNA and proteins inhibitors [16–20], QS regulators [15, 17, 21–25], biofilm formation inhibitors and disruptors [26, 27], drugs that interact with cell membrane [28–30], drugs that interact with iron metabolism [31–35], and host immune system modulators [36–39]. These drugs and their mechanisms of action against critical-priority pathogens (*A. baumannii*, *P. aeruginosa* and *Enterobacterales*) are summarized in **Figure 1** and **Table 1**.

3.1 DNA, RNA and proteins inhibitors

Anticancer and anti-inflammatory drugs that interact with DNA, RNA and proteins have been reported. Mitomycin C, used in several types of carcinomas such

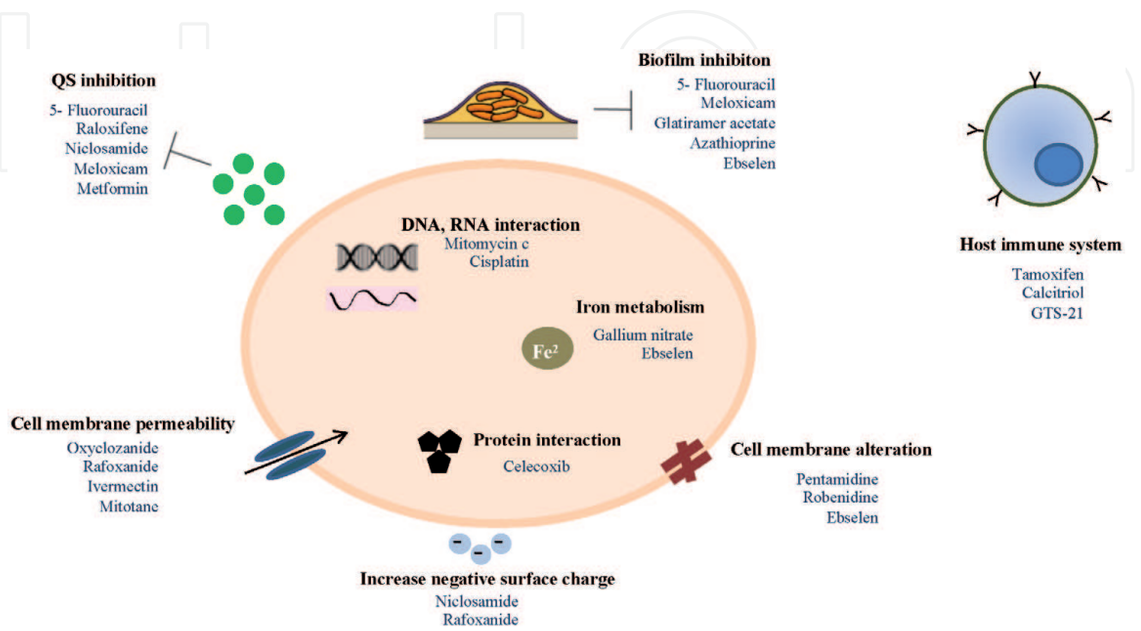

Figure 1.
Mechanisms of action of repurposed drugs against Gram-negative critical-priority pathogens.

Bacterial target	Repurposed drug	Clinical indications	Mechanism of action	Reference
DNA, RNA & proteins	Mitomycin c	Superficial vesical carcinoma	Binding to DNA during DNA synthesis and causes inhibition of its synthesis and function in *P. aeruginosa* and *E. coli*	[18]
	Cisplatin	Cancer	Upregulation of the *recA* gene of *P. aeruginosa,* which is known to be important for DNA repair	[20]
	Celecoxib	Inflammation	Inhibition of RNA, DNA, and protein synthesis in *S. aureus*	[19]
Quorum sensing	5-fluorouracil	Solid tumors	Inhibition of QS formation in *P. aeruginosa*	[23, 41]
	Raloxifene	Breast cancer	Binding to PhzB2 which is involved in the production of pyocyanin, a pigment related with virulence factor and QS signalling molecule in *P. aeruginosa*	[24]
	Niclosamide	Helminthiasis	Production of the QS signaling molecules N-3-oxododecanoyl-homeserine lactone and N-butanoyl-homoserine lactone in *P. aeruginosa*	[15, 25]
	Meloxicam	Inflammation	Interaction with active sites of the QS of *P. aeruginosa*	[26]
	Metformin	Diabetes	Inhibition of QS system by bind to LasR by hydrogen bonding and electrostatic interaction and to rhlR by hydrogen bonding in *P. aeruginosa*	[21]
Biofilm formation	5-Fluorouracil	Solid tumors	Regulation of different genes involved in the biofilm formation by *P. aeruginosa*	[41]
	Meloxicam	Inflammation	Decrease in the extracellular Psl, Pel, and alginate production by *P. aeruginosa*	[26, 27]
	Glatiramer acetate	Inflammation	Disruption of the biofilm formation by GNB	[42]
	Azathioprine	Crohn's disease	Inhibition of WspR, involved in the regulation of c-di-GMP known as a regulator of the bacterial biofilm formation by *P. aeruginosa* and *E. coli*	[43]
	Ebselen	Bipolar disorder and ischemic stroke	Inhibition of c-di-GMP in *P. aeruginosa*	[44, 45]

Bacterial target	Repurposed drug	Clinical indications	Mechanism of action	Reference
Cell membrane	Niclosamide	Helminthiasis	Increase of the negative surface charge of *A. baumannii* and *K. pneumoniae*	[30]
	Oxyclozanide	Helminthiasis	Reduction of the membrane potential and increase of aminoglucosides accumulation in *P. aeruginosa.* Increase of the membrane permeability of *A. baumannii*, *P. aeruginosa* and *K. pneumoniae*	[28, 46]
	Rafoxanide	Helminthiasis	Increase of the negative surface charge of *A. baumannii* and *K. pneumoniae* Increase of the membrane permeability of *A. baumannii*, *P. aeruginosa* and *K. pneumoniae*	[47]
	Ivermectin	Helminthiasis	Increase of the membrane permeability of *A. baumannii*, *P. aeruginosa* and *K. pneumoniae*	[48]
	Mitotane	Cancer	Permeabilization of the outer membrane of *A. baumannii*, *P. aeruginosa* and *K. pneumoniae*	[49]
	Pentamidine	Protozoal infection	Alteration of the outer membrane of GNB, due to the interaction with membrane lipopolysaccharides	[29]
	Robenidine	Protozoal infection	Alteration of the cell membrane of GNB	[50]
	Ebselen	Bipolar disorder and ischemic stroke	Inhibition of the TonB-mediated physiology of *A. baumannii* and *E. coli*	[51]
Iron metabolism	Gallium Nitrate	Lymphoma and bladder cancer	Interferance with iron-dependent metabolic pathways in GNB	[31–33]
	Ebselen	Bipolar disorder and ischemic stroke	linhibition of TonB involved in iron acquisition by *A. baumannii* and *E. coli*	[51]
Host immune system	Tamoxifen	Breast cancer	Reduction in the migration of immune cells from bone marrow to blood through the reduction of MCP-1 and IL-18 in presence of *A. baumannii*, *P. aeruginosa* and *E. coli*	[36]
	Calcitriol		Enhancement of the killing activity of monocytes and macrophages towards *P. aeruginosa.*	[37]
	GTS-21	Inflammation	Enhancement of the macrophage function towards *P. aeruginosa* via inhibiting the release of nuclear protein high mobility group box-1 Reduction of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) release	[38, 39]

Table 1.
Repurposing drugs and their mechanisms of action.

as the superficial vesical carcinoma [40], has shown activity against *A. baumannii*, *P. aeruginosa* and *E. coli in vitro* and *in vivo* [16–18]. Mitomycin C binds to DNA during DNA synthesis and causes inhibition of its synthesis and function in *P. aeruginosa* and *E. coli* [18]. Cisplatin, approved for treatment of a number of cancers, was found to inhibit microbial cells growth [17, 20]. The mechanism of action of this drug has been attributed to the upregulation of the recA gene in *P. aeruginosa*, which is important for DNA repair, implicating that cisplatin could interfere with DNA replication [20]. Moreover, celecoxib, a non-steroidal anti-inflammatory drug (NSAID), has been tested in a *Caenorhabditis elegans* and in whole-animal *A. baumannii* and *P. aeruginosa* infection models. It was suggested that inhibit dose-dependently the DNA, RNA and protein synthesis as in *Staphylococcus aureus* [19].

3.2 Quorum sensing regulators

QS inhibition and regulation have been reported as antibacterial properties of anticancer, anthelminthic, anti-inflammatory and hypoglycemic drugs such as 5-fluorouracil, raloxifene, niclosamide, meloxicam and metformin. 5-fluorouracil is a potent drug indicated for the treatment of different types of solid tumors, that has shown antibacterial activity *in vitro* [17, 22, 23]. The antibacterial mechanism of this drug has been proposed as QS inhibitor [23, 41]. Also, 5-fluorouracil has dual inhibition mechanisms including functioning as an alternative substrate resulting in miscoding DNA and RNA, and inhibiting thymidylate synthase [22]. Moreover, the selective estrogen receptor modulator (SERM) raloxifene, used in the prevention of osteoporosis and invasive breast cancer in post-menopausal women, has presented activity against Gram-negative bacilli (GNB). Raloxifene binds to PhzB2 which is involved in the production of pyocyanin, a pigment related with both the virulence factor and the QS signaling molecule in *P. aeruginosa* [24]. Regarding the anthelmintic drugs, niclosamide, used for the treatment of helminthiasis, has been reported to inhibit QS in *P. aeruginosa* in *Galleria mellonella* model by hindering the cell's response and production of the QS signaling molecules as N-3-oxododecanoyl-homeserine lactone and N-butanoyl-homoserine lactone [15, 25]. Finally, meloxicam, a NSAID used to manage moderate-to-severe pain, and metformin, one of the most commonly prescribed oral hypoglycemic for treatment of type 2 diabetes, have been reported to interact with active sites and to inhibit the QS of *P. aeruginosa*, respectively [21, 26, 27]. Molecular docking study has shown that metformin could bind to LasR by hydrogen bonding and electrostatic interaction and to rhlR by hydrogen bonding only [21].

3.3 Biofilm formation inhibitors and disruptors

Compared with QS, much less drugs have been act on the biofilm formation. 5-fluorouracil has been revealed to regulate different genes involved in the biofilm formation by *P. aeruginosa* [41]. More specifically, meloxicam has been reported to inhibit biofilm formation of *P. aeruginosa* by decreasing the extracellular Psl, Pel and alginate production, three vital biofilm exopolysaccharides in this pathogen [26, 27]. Moreover, glatiramer acetate, a drug used in the treatment of multiple sclerosis, has also been shown to disrupt biofilm formation by GNB [42]. Finally, azathioprine, an immunosuppressive drug used for the treatment of Crohn's disease and other autoimmune diseases, has exhibited anti-biofilm activity against *P. aeruginosa* and *E. coli* through the inhibition of WspR [43]. WspR is a diguanylate cyclase involved in the regulation of a signal molecule called cyclic-di-GMP (c-di-GMP) known as a regulator of the bacterial biofilm formation [43]. The same mechanism of action has been used by ebselen to exhibit anti-biofilm activity

against *P. aeruginosa* [44, 45]. Ebselen, despite the fact that it is not an FDA-approved drug, it is being investigated in clinical trials for the treatment of bipolar disorder, hearing loss and tinnitus and ischemic stroke.

3.4 Interaction with cell membrane

Various anthelmintic, anticancer and antiprotozoal drugs such as niclosamide, oxyclozanide, rafoxanide, ivermectin, mitotane, pentamidine and robenidine have been reported to interact with the bacterial cell membrane. Three anthelmintic drugs in combination with colistin have shown activity against GNB by the regulation of electric charges. Niclosamide and rafoxanide were discovered to increase the negative surface charge of bacterial membrane in *A. baumannii* and *K. pneumoniae* clinical strains *in vitro* [30, 47]. In turn, oxyclozanide has enhanced the activity of additional tobramycin against *P. aeruginosa* by reducing the membrane potential and increasing tobramycin accumulation [28]. This increase in the negative surface charges allow to restore the activity of colistin in colistin-resistant (Col-R) *A. baumannii* and *K. pneumoniae*, and the activity tobramycin in tobramycin-resistant *P. aeruginosa* [30, 47]. Not only the regulation of electric charges has been reported as mechanism of action of anthelmintic drugs, but the increase of bacterial membrane permeabilization has also been reported. Oxyclozanide, rafoxanide and ivermectin have been shown to increase the membrane permeability of *A. baumannii*, *P. aeruginosa* and *K. pneumoniae*, especially in Col-R strains [46–48]. Moreover, Tran et al. have demonstrated that mitotane, a FDA-approved antineoplastic drug, in combination of polymyxin B lead mitotane to enter inside *A. baumannii*, *P. aeruginosa* and *K. pneumoniae* through the permeabilization of the outer membrane by polymyxin B [49]. Additionally, antiprotozoal drugs, pentamidine and robenidine, possess a mechanism of action that disturbs the outer membrane of GNB, due to the interaction with membrane lipopolysaccharides (LPS) [29, 50]. Finally, ebselen has also presented antibacterial effect against *A. baumannii* and *E. coli* by inhibiting the TonB-mediated physiology, which is involved in iron acquisition from host sources [51].

3.5 Interaction with iron metabolism

Antunes et al. have demonstrated that virulent bacteria are able to acquire iron in the blood and tissues [33]. Given the essential role of iron in bacterial physiology and pathogenicity, iron uptake and metabolism have become attractive targets for the development of new antibacterial agents [52, 53]. The ion gallium [Ga(III)], a ferric iron [Fe(III)] mimetic, has been shown to inhibit the growth of many bacterial species by interfering with iron-dependent metabolic pathways. Therefore, gallium drugs have gained special interest in the fight of MDR-GNB infections [31]. Gallium nitrate is an anticancer drug that was approved by the FDA for the treatment of cancer-associated hypercalcemia. Antibacterial properties of gallium nitrate have been previously reported against GNB infections, both *in vitro* and *in vivo* [31–35]. In addition, Ebselen as mentioned before has the characteristic to inhibit TonB in *A. baumannii* and *E. coli* [51].

3.6 Host immune system modulators

Also, some drugs that modulate host immune system have reported antibacterial activity against GNB. Tamoxifen, a SERM used for breast cancer treatment, can reduced the migration of immune cells from bone marrow to blood through the reduction of monocytes chemoattractant protein 1 (MCP-1) and IL-18 in a murine model of sepsis by *A. baumannii*, *P. aeruginosa* and *E. coli* [36]. Moreover, tamoxifen

has been shown to enhance the killing activity of macrophages and neutrophils against *A. baumannii* and *E. coli in vitro* [36]. Calcitriol, a bioactive form of vitamin D3 used to treat hypocalcemic conditions and renal osteodystrophy, has a similar mechanism of action which it has been described to enhance the killing activity of monocytes and macrophages towards *P. aeruginosa* [37]. Moreover, GTS-21, an anti-inflammatory drug, has presented therapeutic efficacy against *P. aeruginosa in vivo* by enhancing macrophage function via inhibiting the release of nuclear protein high mobility group box-1 (HMGB1) [39]. When GTS-21 is combined with M1 muscarinic acetylcholine receptor agonist and α7n-acetylcholine receptor agonist against *E. coli in vivo*, the blood concentrations of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 were reduced significantly [38].

4. Repurposing drugs against MDR Gram-negative bacilli

There are currently multiple repurposing drugs in preclinical development for the treatment of infections by Gram-negative critical-priority pathogens. Few of them have been evaluated in early and late stage clinical trials. A summary of recent developments in repurposing drugs *in vitro*, in animal models and in clinical trials is presented below. The different clinical trials with repurposing drugs against these pathogens are listed in **Table 2**.

4.1 *Acinetobacter baumannii*

MDR *A. baumannii* is a well-recognized current global health threat that needs urgent effective solutions [1, 2]. Last-resort treatments such as colistin are no longer effective in an increasing number of cases, leading to a mortality rate of around 35–60% in hospitalized patients with ventilator-associated pneumonia [54, 55]. The number of antibiotics approved by the FDA cannot keep up with the pace at which resistance is acquired by *A. baumannii*. The therapeutic potential of different repurposing drugs against *A. baumannii* has been tested in preclinical models, most of them in combination with polymyxins (colistin and polymyxin B) or its prodrug colistimethate sodium (CMS).

Repurposing drug	Clinical indication	Target bacteria	New clinical indication	Clinical trial phase	Clinical trial identifier
GTS-21	Inflammation	*E. coli*	Endotoxemia	Interventional (Clinical trial)	NCT00783068
Sodium nitrite	Acute cyanide poisoning	*P. aeruginosa*	Cystic fibrosis (antimicrobial agent)	Phase I/II	NCT02694393
Sodium nitrite	Acute cyanide poisoning	*P. aeruginosa*	Cystic fibrosis (biofilm disruptor)	Phase II	NCT02295566
Gallium nitrate	Cancer-associated hypercalcemia	*P. aeruginosa*	Cystic fibrosis	Phase II	NCT02354859
Amitriptyline	Depression	*P. aeruginosa*	Cystic fibrosis	Phase II	NCT00515229
Atorvastatin	Hyper cholestremia	*P. aeruginosa*	bronchiectasis and infection	Phase IV	NCT01299194

Table 2.
List of repurposing drugs under clinical trial development against Gram-negative critical-priority pathogens.

4.1.1 Anticancer drugs

The antibacterial activity of anticancer drugs has been reported *in vitro* and *in vivo* non-vertebrate and vertebrate models by *A. baumannii*. Gallium nitrate has demonstrated an inhibitory effect on bacterial growth in a collection of 58 MDR clinical isolates of *A. baumannii in vitro* [33]. This antibacterial activity is maintained in *G. mellonella* model. The administration of this drug alone and in combination with colistin, at concentrations mimicking the human therapeutic dose of gallium nitrate used for cancer patients (28 μM), significantly increased the survival of larvae after infection by A. baumannii [33]. When a vertebrate model was used such as murine models of acute and chronic lung infections by *A. baumannii*, gallium nitrate has reduced lung injury and bacterial loads in tissues [32]. Moreover, the combination of mitomycin C with tobramycin and ciprofloxacin together has increased *in vitro* the activity of this anticancer drug against MDR clinical isolates of *A. baumannii* [17]. Whereas, mitotane combined with polymyxin B against polymyxin B-resistant *A. baumannii* has presented synergy with polymyxin B, increasing the activity of polymyxin B *in vitro* and in murine model of burn wound infection by reducing the bacterial load in wounds [49]. Another group of anticancer drugs developed to combat breast cancer is the SERMs. Tamoxifen has been reported to exhibit activity in the immunocompetent and neutropenic murine model of peritoneal sepsis by ATCC 17978 strain by decreasing the bacterial loads in spleen, lungs and blood and increasing the mice survival [36]. Tamoxifen metabolites (N-desmethyltamoxifen, 4-hydroxytamoxifen and endoxifen), produced after tamoxifen metabolizing by cytochrome P450 [56], have presented antibacterial activity in vitro with MIC50 and MIC90 of 8 and 16 mg/L, respectively, against a collection 100 MDR and pan-drug resistant (PDR) clinical isolates of *A. baumannii* [36].

4.1.2 Anthelmintic drugs

The potential activity of the anthelmintic drug has been also tested *in vitro* and in animal models by *A. baumannii*. Niclosamide, oxyclozanide, rafoxanide and ivermectin have been shown to potentiate the activity of colistin against clinical isolates of Col-R *A. baumannii in vitro* [30, 46–48, 57]. In the murine model of peritoneal sepsis by Col-R *A. baumannii* clinical isolate, rafoxanide plus CMS, a prodrug of colistin, compared with CMS alone increased mice survival to 53.8% and reduced bacterial loads in tissues and blood between 3 and 4 log10 cfu/g or mL, respectively [47]. Only, rafoxanide has exhibited antibacterial activity in monotherapy in this model of infection, but not *in vitro* [47].

4.1.3 Anti-inflammatory drugs

As is the case with anthelmintic drugs, anti-inflammatory drugs have demonstrated antibacterial activity against *A. baumannii* in monotherapy and in combination with polmyxins *in vitro*. Glatiramer acetate has presented activity against reference strains and clinical bacteremic isolates of A. baumannii by disrupting the biofilm formation [42]. In addition, ebselen has presented antibacterial effect against *A. baumannii* by reducing their bacterial growth at MICs of 32 μM due to the inhibition of the siderephore TonB [51]. In combination with polymyxins, auranofin, a drug used for the treatment of rheumatoid arthritis, and celecoxib have exhibited synergy with polymyxin B and colistin against reference strains of *A. baumannii* respectively [19, 58].

4.1.4 Other drugs

Other drugs with different modes of action and clinical indications have been evaluated as antibacterial agents against *A. baumannii* in monotherapy and in combination with antibiotics. Simvastatin, used in the treatment of atherosclerotic cardiovascular disease and hypercholesterolemia, has exhibited antibacterial activity in combination with sub-inhibitory concentrations of colistin against a collection of clinical isolates of *A. baumannii*, reducing the MIC of simvastatin from >256 mg/L to a range between 8 and 32 mg/L [59]. Two antiprotozoal drugs have been also evaluated in monotherapy and in combination with antibiotics. Robenidine has presented bactericidal activity alone and in combination with polymyxin B nanopeptide against reference strains of *A. baumannii in vitro* [60]. Pentamidine, in turn, has present synergy with novobiocin, a drug used for Gram-positive cocci infections, in vitro and in murine sepsis model by a reference strain of *A. baumannii* [29].

4.2 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is one of the most relevant pathogens causing human opportunistic infections in immunocompromised patients and severe nosocomial infections [61–63]. Indeed, *P. aeruginosa* is the top pathogen causing ventilator-associated pneumonia and burn wound infections and is a major cause of nosocomial bacteremia [62–64]. An MDR pattern is commonly observed in *P. aeruginosa* clinical isolates, raising the threat of difficult-to-treat infections [65–67]. These MDR isolates are generally susceptible to polymyxins and resistant to imipenem and ceftazidime [68]. New beta-lactamases inhibitors, combined with existing antibiotic families, such as ceftazidime/avibactam, ceftolozane/tazobactam, and imipenem/relebactam, against specific carbapenemases, have recently been developed [69]. Compared with *A. baumannii*, much more work has been done regarding the development of repurposing drugs for *P. aeruginosa* in preclinical and clinical stages.

4.2.1 Anticancer drugs

Different studies have been performed on *P. aeruginosa* to evaluate the antibacterial effect of anticancer drugs. Regarding SERM drugs, raloxifene attenuated *in vitro* and in *C. elegans* model the virulence of *P. aeruginosa* by binding to PhzB2 which is involved in the production of pyocyanin [24]. Whereas, tamoxifen exhibit therapeutic efficacy in murine model of peritoneal sepsis by PAO1 strain by decreasing the bacterial loads in spleen, lungs and blood and increasing the mice survival [36]. In addition, cisplatin was found to inhibit microbial cells growth [17, 20] by the upregulation of the recA gene in *P. aeruginosa* [20]. 5-fluorouracil, in turn, has been used against a collection of 5850 mutants of the PA14 strain, revealing positive activity via the regulation of a large number of genes involved in QS and biofilm formation [41, 70]. In combination with antibiotics, two anticancer drugs have been tested. Mitomycin C and mitotante in combination with tobramycin-ciprofloxacin [17] and polymyxin B [49], respectively, have shown synergy against MDR clinical and polymyxin-resistant isolates of *P. aeruginosa*, respectively. Finally, gallium nitrate is one the most studied and advanced cancer drug in clinical development against *P. aeruginosa* infection with promising data. Gallium nitrate has demonstrated an inhibitory effect on bacterial growth in *P. aeruginosa* at concentrations >3.13 μM *in vitro* [71, 72]; although the presence of pyoverdine and proteases in human serum reduce the efficacy of gallium nitrate against *P. aeruginosa* [73]. At non-bactericidal concentrations, gallium nitrate can

affect the production of virulence factors of *P. aeruginosa* [71, 74]. In murine models of acute and chronic lung infections by *P. aeruginosa* gallium nitrate has reduced the lung injury and bacterial loads in tissues of animals [71]. At clinical stage, a phase II clinical trial has been started in 2016 evaluating the capacity of gallium nitrate to improve the pulmonary function in 60 patients with cystic fibrosis by *P. aeruginosa*. The results of this trial showed that treatment with gallium nitrate increase the forced expiratory volume in these patients [75].

4.2.2 Anthelmintic drugs

The anthelmintic drugs niclosamide, oxyclozanide, rafoxanide and ivermectin have been shown to restore the activity of colistin against a collection of Col-R *P. aeruginosa in vitro* [46–48, 57]. Not only in combination with colistin, oxyclozanide has presented synergy with tobramycin to destruct the biofilm formation, permeabilizing the cells membrane and depolarizing the membrane potential of *P. aeruginosa* strains resistant to tobramycin *in vitro* [28]. In the murine model of peritoneal sepsis by Col-R *P. aeruginosa* clinical isolate, rafoxanide plus CMS compared with CMS alone, increased mice survival to 73.3%, and reduced bacterial loads in tissues and blood between 3 and 5 log10 cfu/g or mL, respectively [47]. In monotherapy, niclosamide and rafoxanide have exhibited antibacterial activity against *P. aeruginosa*. One *in vitro* study has indicated that niclosamide presented an anti-virulent effect against *P. aeruginosa* via the inhibition of QS and virulence genes, reducing elastase and pyocyanin levels [15]. Two additional *in vivo* studies have reported that niclosamide and rafoxanide showed therapeutic efficacy in *G. mellonella* larvae and in murine peritoneal sepsis models by a reference strain and Col-R clinical isolate of *P. aeruginosa*, respectively [15, 47]. Nevertheless, the absorption of niclosamide is lower. To increase this absorption, formulation of niclosamide under nanosuspension has been performed and showed lower toxicity in a rat lung infection model involving *P. aeruginosa* [14].

4.2.3 Anti-inflammatory and immunosuppressive drugs

Similar with *A. baumannii*, anti-inflammatory and immunosuppressive drugs have presented antibacterial activities in monotherapy and in combination with antibiotics against *P. aeruginosa*. The activity of glatiramer acetate against reference and clinical isolates of *P. aeruginosa* from chronic respiratory infections in cystic fibrosis patients has been observed by disruption of the biofilm formation [42]. With the same mechanism of action, ebselen and azathioprine has exhibited activity against *P. aeruginosa* [43, 45]. In turns, celecoxib and betamethasone have presented synergy with colistin, and with ceftazidime, erythromycin and ofloxacin against *P. aeruginosa* in vitro, respectively [19, 76]. Similarly, meloxicam has been reported to be in vitro active alone and in combination with the sub-MIC of tetracycline, gentamicin, tobramycin, ciprofloxacin, ceftriaxone, ofloxacin, norfloxacin, ceftazidime against PAO1 strain, by inhibiting the biofilm formation [27]. Finally, GTS-21 has improved *P. aeruginosa* clearance in a murine model of ventilator-associated pneumonia and reduced acute lung injury by enhancing macrophage function [39].

4.2.4 Antidepressive drugs

Regarding the antidepressive drugs, amitriptyline has reduced the inflammation in the lung of cystic fibrosis mice and prevented infection by *P. aeruginosa* [77]. At clinical stage, a phase II clinical trial evaluating the effect of amitriptyline on the

improvement of lung function in 18 patients with cystic fibrosis patients showed that amitriptyline improves the lung function by increasing the forced expiratory volume and weight of these patients [78, 79].

4.2.5 Other drugs

Other drugs with different modes of action and clinical indications have been evaluated as antibacterial agents against *P. aeruginosa*. Metformin has been reported to inhibit QS, biofilm formation, and swimming and twitching motilities of PAO1 strain [21]. Calcitriol has enhanced the bactericidal activity against *P. aeruginosa*, modulating the activity of monocytes and macrophages to increase their bacterial killing [37]. Compared with *A. baumannii*, robenidine has been recently showed to present only synergy with polymyxin B nanpeptide against reference strains of *P. aeruginosa in vitro* [60]. Polymyxin B and colistin have been also combined with auronafin and simvastatin, respectively. Both drugs exhibited synergy with sub-inhibitory concentrations of polymyxin B and colistin against a collection of reference strains of *P. aeruginosa*, reducing the MIC of auronafin from >256 mg/L to 0.125–0.5 mg/L and the MIC of simvastatin from >256 mg/L to 16–32 mg/L [58, 59]. At clinical stage, a phase IV trial determining the role of atorvastatin, another statin, in patients with bronchiectasis and infection with *P. aeruginosa* showed that atorvastatin reduced systemic inflammation and improved quality of life of these patients [80, 81]. In addition, sodium nitrite, used for treatment of acute cyanide poisoning, has been shown in vitro to kill mucoid *P. aeruginosa* strains isolated from patients with cystic fibrosis, under anaerobic planktonic and biofilm conditions [82, 83]. Two early stage (I/II and II) clinical trials have been conducted to evaluate sodium nitrite as antimicrobial agent and as disrupter of biofilm formation in patients with cystic fibrosis by *P. aeruginosa* [84, 85]. The results from both studies have not yet published.

4.3 *Enterobacterales*

Escherichia coli and *Klebsiella pneumoniae* are of the most important pathogens in humans involved in different community and nosocomial infections, including bloodstream infections, urinary tract infections, intraabdominal infections and pneumonia [86–89]. The success of *E. coli* and *K. pneumoniae* as a community and nosocomial pathogens is attributed to their resistance to several antibiotic categories [86, 90]. Similar to *P. aeruginosa* the repurposing drugs developed today for *E. coli* and *K. pneumoniae* are in the preclinical and clinical stages of development.

4.3.1 Anticancer drugs

Anticancer drugs were developed against *E. coli* and *K. pneumoniae in vitro* and in animal models. Tamoxifen has been reported to exhibit activity in the immunocompetent and neutropenic murine model of peritoneal sepsis by *E. coli* ATCC 25922 strain by decreasing the bacterial loads in spleen, lungs and blood and increasing the mice survival [36]. Tamoxifen metabolites N-desmethyltamoxifen, 4-hydroxytamoxifen and endoxifen have presented antibacterial activity *in vitro* with MIC50 and MIC90 of 16 mg/L, against a 47 MDR clinical isolates of *E. coli* [36]. The activity of mitomycin C in monotherapy and in combination with tobramycin and ciprofloxacin together was increased against MDR clinical isolates of *E. coli* and *K. pneumoniae in vitro* [17, 18]. While, mitotane in combination with polymyxin B against polymyxin-resistant *K. pneumoniae* increased the activity of polymyxin B *in vitro* [49].

4.3.2 Anthelmintic drugs

Four anthelmintic drugs, niclosamide, oxyclozanide, rafoxanide and ivermectin were shown to present synergy with colistin against Col-R *K. pneumoniae* [30, 46–48, 57]. Compared to Col-R isolates of *A. baumannii* and *P. aeruginosa* much lesser effect has been observed regarding the effect of these drugs in combination with colistin against Col-R isolates of *K. pneumoniae*. Additionally, in the murine model of peritoneal sepsis model by Col-R clinical isolate of *K. pneumoniae*, rafoxanide in monotherapy and in combination with CMS compared with control animals and with CMS alone, increased mouse survival to 50 and 67%, and reduced bacterial loads in tissues and blood between 2.5 and 3 log10 cfu/g or mL, and 2 and 3 log10 cfu/g or mL, respectively [47].

4.3.3 Anti-inflammatory drugs

In the case of anti-inflammatory drugs, two drugs have presented synergistic effect with antibiotics against *E. coli* and *K. pneumoniae*. The first one is celecoxib which has potentiated the activity of colistin against *E. coli* and *K. pneumoniae* [19]. In turn, betamethasone has presented synergy with ceftazidime and ofloxacin against some isolates of *E. coli* [76]. Similar to *A. baumannii* and *P. aeruginosa*, glatiramer acetate has presented antibacterial effect against reference strains of *E. coli* by disrupting the biofilm formation [42]. Moreover, ebselen has been shown to present antibacterial effect against *E. coli* by reducing their bacterial growth at MICs <128 μM due to the inhibition of TonB [51], and azathioprine has exhibited anti-biofilm activity against *E. coli* through the inhibition of WspR *in vitro* [43]. Finally, GTS-21 in combination with M1 muscarinic acetylcholine receptor agonist have been shown to reduce the mortality of mice in sepsis model by *E. coli* in 4 and 24 h [38]. At clinical stage, an interventional clinical trial on anti-inflammatory effects of oral administration of GTS-21 on the inflammatory response in 7 patients with endotoxemia by LPS of *E. coli* showed that GTS-21 reduced the levels of proinflammatory cytokines in the plasma of these patients [91, 92].

4.3.4 Other drugs

Other drugs with different modes of action and clinical indications have been evaluated as antibacterial agents in monotherapy and in combined therapy with a large list of antibiotics against *E. coli* and *K. pneumoniae in vitro* and in animal models. Amoxapine has been reported to present therapeutic efficacy in an experimental murine model of respiratory infection by *K. pneumoniae* [93]. In addition, pentamidine in combination with different antibiotics ([novobiocin, erythromycin and rifampin] and [amikacin, tobramycin, tigecycline and rifampin]) has presented synergistic activity *in vitro* against different clinical isolates of *E. coli* harboring *mcr-1* and *K. pneumoniae* producing carbapenemases, respectively [94]. In turn, robenidine has been recently showed to present only synergy with polymyxin B nanopeptide against reference strains of *K. pneumoniae in vitro* [60]. Finally, auronafin and simvastatin exhibited synergy with sub-inhibitory concentrations of polymyxin B and colistin against a collection of reference strains of *E. coli* and *K. pneumoniae in vitro*, reducing the MIC of auronafin from >256 mg/L to 0.25–1 mg/L and the MIC of simvastatin from >256 mg/L to 8–32 mg/L, respectively [58, 59].

5. Conclusions

The retreat of the pharmaceutical sector from new antibiotic development has exacerbated the challenge of widespread resistance and signals a critical need for innovation. Repurposing drugs are an increasingly common practice in the pharmaceutical industry where an already existing drug is applied in a new, previously unknown, way. This is advantageous mainly because these drugs are already cleared for human use and thus may skip straight to phase II clinical trials which presents considerably less risk and costs compared to developing new drugs. They could represent a promising approach to enrich the therapeutic arsenal against Gram-negative critical-priority pathogens.

Some drugs indicated for human and veterinary use have been developed in combination with antibiotics; almost of them with polymyxins. They have yielded promising data in preclinical studies, specifically those with activity against biofilm formation and quorum sensing. However, additional relevant issues are required such as new formulations to increase their bioavailability and ADMET tests if the administration route is changed. Other drugs indicated for human use who have showed good activity against these pathogens in preclinical studies can be tested in advanced clinical trials. Early and late stages clinical trials with four repurposing drugs to treat cystic fibrosis and bronchiectasis by *P. aeruginosa*, and endotexemia by *E. coli* have provided promising results. Nevertheless, further clinical studies with extended clinical indications are needed to address the urgent demand for new treatments targeting infections caused by Gram-negative critical-priority pathogens.

Acknowledgements

This study was supported by the Instituto de Salud Carlos III, Proyectos de Investigación en Salud (Grant No. PI19/01435). YS is supported by the Subprograma Miguel Servet Tipo I from the Ministerio de Economía y Competitividad of Spain (CP15/00132).

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