Combating multidrug-resistant Gram-negative bacterial infections

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Introduction: Multidrug-resistant (MDR) bacterial infections, especially those caused by Gram-negative pathogens, have emerged as one of the world’s greatest health threats. The development of novel antibiotics to treat MDR Gram-negative bacteria has, however, stagnated over the last half century.

Areas covered: This review provides an overview of recent R&D activities in the search for novel antibiotics against MDR Gram-negatives. It provides emphasis in three key areas. First, the article looks at new analogs of existing antibiotic molecules such as β-lactams, tetracyclines, and aminoglycoside as well as agents against novel bacterial targets such as aminoacyl–tRNA synthetase and peptide deformylase. Second, it also examines alternative strategies to conventional approaches including cationic antimicrobial peptides, siderophores, efflux pump inhibitors, therapeutic antibodies, and renewed interest in abandoned treatments or those with limited indications. Third, the authors aim to provide an update on the current clinical development status for each drug candidate.

Expert opinion: The traditional analog approach is insufficient to meet the formidable challenge brought forth by MDR superbugs. With the disappointing results of the genomics approach for delivering novel targets and drug candidates, alternative strategies to permeate the bacterial cell membrane, enhance influx, disrupt efflux, and target specific pathogens via therapeutic antibodies are attractive and promising. Coupled with incentivized business models, governmental policies, and a clarified regulatory pathway, it is hoped that the antibiotic pipeline will be filled with an effective armamentarium to safeguard global health.

Keywords: alternative strategies, antibiotics, Gram-negative bacteria, multidrug resistance


1. Introduction

Multidrug-resistant (MDR) bacterial infections, especially those caused by Gram-negative pathogens, have emerged as one of the world’s greatest health threats [1]. These MDR bacteria have continued to evolve at an alarming rate and confer resistance to most or all currently available antibacterial treatments. The “ESKAPE” pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.) are considered to be clinical super-challenges in the 21st century [2-4]. Unfortunately, this increase in resistant bacteria has not been accompanied by an increase in the discovery of novel antibiotics. Worse still, there has been an exodus of pharmaceutical companies from antibiotic development because of unfavorable economic factors and regulatory challenges in gaining approvals [5,6].

Therefore, it is not surprising that recent decades have shown not only a steady decline in the number of approved antimicrobial agents but also a discovery void [7], in contrast to the “Golden Age” of antimicrobial research and development, beginning from Fleming’s discovery of penicillin in 1929 to the introduction of nalidixic acid in 1962, during which time more than 20 novel classes of antibiotics were discovered, developed, and marketed [8,9]. Since then, no new classes of
Antibacterial drugs have been approved to treat Gram-negative bacterial infections, while only two new classes of antibiotics have been introduced for the treatment of bacterial infections due to Gram-positive pathogens: oxazolidinones and lipopeptides--which have been isolated which confer resistance to virtually all existing classes of antibiotics, such as cephalosporins, carbapenems, and monobactams, account for most clinical development candidates including ceftolozane (CXA-101), avibactam (NXL-104), plazomicin (ACHN-490), and eravacycline (TP-434).

Clinical development of inhibitors targeting aminoglycosyl-tRNA synthetases and PDF are challenging but warrant further investigation.

Alternative strategies to conventional approaches such as how to bring antibiotics into bacterial cells and keep them inside these cells, including cationic antimicrobial peptides, peptidomimetics, and foldamers, siderophores, efflux pump inhibitors as well as therapeutic antibodies, are attractive and promising.

There has been renewed interest in treatments that have been either abandoned or approved only for limited indications, such as temocillin, fosfomycin, and phage therapy.

The GAIN Act enacted by the US Congress to incentivize the pharmaceutical industry for the development of products to treat, prevent, detect, and diagnose antibiotic-resistant infections is also discussed.

2. Background

2.1 Shortage of new antibiotics in development against multidrug-resistant gram-negatives based on existing molecular classes

The current global antibiotic pipeline is in no way even close to fulfilling the IDSA’s 10 by 20 near-term goal. Most current antibacterial research and development activities have been focused on novel analogs of existing classes of molecules, despite the potential of cross-resistance. The β-lactams such as cephalosporins, carbapenems, and monobactams continue to be one of the most prolific classes, along with tetracyclines and aminoglycosides.

β-Lactamases produced by Gram-negative pathogens, such as plasmid-encoded extended-spectrum β-lactamases (ESBLs) and carbapenemases such as the KPC family along with the VIM, IMP and metallo-β-lactamases (MBLs or class B), are the major resistance mechanisms in the degradation of the β-lactam ring. Combinations of ESBLs and carbapenemases have been isolated which confer resistance to virtually all β-lactam antibiotics. Nevertheless, carbapenems are relatively stable to hydrolysis by many ESBLs and are particularly active against MDR Gram-negative bacteria. Following the discontinuation of tompenem (CS-023) (Figure 2) from Phase II clinical trials by Roche in 2007, razupenem (PZ-601) (Figure 2) was also discontinued by Novartis in 2010 due to a high rate of adverse events observed. Tebipenem...
Figure 1. Antibiotics approved since 2000.
Adapted from [127] with permission of the Chinese Pharmacological Society.
(Figure 2), currently the only carbapenem in clinical trials, demonstrated good activity against *K. pneumoniae*, *Escherichia coli*, and *Acinetobacter* spp. but was inactive against MRSA and *P. aeruginosa* [22]. Tebipenem pivoxil, a prodrug that is the first oral carbapenem in development in Japan, is well absorbed in the intestine, with a half-life of 0.3 – 0.5 h in humans with otolaryngological infections [23]. It also has potential in the treatment of pneumonia in children. ME1036 (Figure 2) is a novel broad-spectrum parental carbapenem demonstrating preclinical activity against both MDR...
Gram-positive and -negative pathogens including MRSA and ESBL-producing Enterobacteriaceae [24].

Trinems or tribactams are a new class of carbapenems with a fused tricyclic β-lactam backbone. The first compound in this series that reached clinical trials was sanfetrinem (Figure 2), which has high stability to many clinically important β-lactamases and to human renal dehydropeptidase I, resulting in a broad spectrum of activity against both Gram-positive and Gram-negative bacteria including Enterobacteriaceae spp. However, it exhibited no activity against non-fermentative P. aeruginosa. Sanfetrinem does not have an obvious pattern of cross-resistance with other β-lactams versus Acinetobacter spp. isolates; it can enter phagocytes and thus has the potential to kill intracellular pathogens [25]. Even though sanfetrinem cilexetil development ended in 2009 after Phase II clinical trials, further exploration of additional chemical structures of trinems is warranted.

Ceftolozane (CX-101) (Figure 2) is a novel, rapidly bactericidal cephalosporin of particular interest for the treatment of MDR Gram-negative infections, especially when combined with β-lactamase inhibitors [26]. Ceftolozane has potent activity against P. aeruginosa, which is not diminished by AmpC overexpression. Through increased binding to penicillin-binding protein 3 (PBP3), it maintains activity against organisms with porin deficiencies and efflux pumps. Its combination with the well-known β-lactamase inhibitor tazobactam demonstrated better in vitro activity than currently available β-lactam/β-lactamase inhibitor combinations against ESBL-producing E. coli and K. pneumoniae [27]. An intravenously administered combination (CX-A201) consisting of ceftolozane and tazobactam was advanced into Phase III trials in 2011 for the treatment of complicated UTIs and complicated intra-abdominal infections. The study results have yet to be reported.

2.1.2 β-Lactamase inhibitors

There are several potential β-lactamase inhibitors under investigation in different stages of preclinical development and clinical trials. These molecules are able to inhibit various classes of enzymes and restore the activity of penicillins, cephalosporins, or carbapenems against MDR strains [28]. BLI-489 (Figure 2) represents a novel imidazole-substituted 6-methylidene-penem which has demonstrated excellent in vitro inhibition of class A (e.g., TEM-1), class C (e.g., AmpC), and class D enzymes with significantly higher activity compared with tazobactam. The combination of BLI-489 with piperacillin not only enhances the activity of the latter against P. aeruginosa and β-lactamase-producing Enterobacteriaceae, but has also demonstrated in vitro a low probability of spontaneous resistance development for a panel of Gram-negative strains encoding various types of β-lactamase enzymes, with the exception of P. aeruginosa [29].

Oxapenems, including AM-112, AM-113, AM-114, and AM-115 (Figure 2), present a broad spectrum of activity against class A (including ESBLs), C, and D enzymes [29]. AM-114 and AM-115 displayed potent activity against class A enzymes (IC50 of 0.002 and 0.063 µM), comparable to that of clavulanic acid, while their activities against class C and D enzymes were superior to that of clavulanic acid. An enhanced activity of oxapenems in combination with cefazidime was reported against P. aeruginosa strains and MRSA [30]. AM-112 also has affinity for the PBPs of E. coli and appears to bind to PBP2 as an initial target; its combination with cefazidime was more effective than cefazidime alone against a strain of E. coli containing TEM-1 and CTX-M-1 β-lactamases [31]. However, there have not been further studies published on the oxapenem class of molecules since 2004.

LK-157 (Figure 2) is a trinem that has potent inhibitory activity against class A ESBLs and class C β-lactamases [32]. LK-157 exhibited IC50 values against TEM-1 (0.055 µM) and SHV-1 (0.151 µM) in the range of those of clavulanic acid and tazobactam, whereas its inhibitory activity (IC50 = 0.062 µM) against AmpC was >2000-fold more potent than clavulanic acid and approximately 28-fold more active than tazobactam. Combinations with various antibiotics could restore the activity against ESBLs, but not CTX-M- and KPC-producing strains. LK-157 is a good substrate for the BcII MBL produced by some strains of Bacillus cereus [33] and has very poor intestinal absorption, making it unsuitable for oral administration. Several ester prodrugs have since been designed to enhance the rate of diffusion through the intestinal membrane [34].

Avibactam (NXL-104) (Figure 2), an unusual strained bicyclic urea bearing an aminoxyl sulfone, is the most promising non-β-lactam that inhibits β-lactamase through the formation of a stable covalent carbamoyl linkage [35]. Devoid of any significant intrinsic antimicrobial activity, avibactam inhibits class A (including ESBL and KPC) and class C enzymes [36,37]. Its combination with various cephalosporins and carbapenems exhibited broad-spectrum activity against ESBL-producing E. coli and K. pneumoniae along with AmpC-hyperproducing E. coli and AmpC/ESBL-co-expressing E. coli. Remarkably, avibactam restored the susceptibility of all isolates tested to cephalosporins [38]. In addition, stable mutational resistance to the combination of cefaroline and avibactam was difficult to select and was often associated with mechanisms likely to be counter-selected in vivo [39]. Although avibactam does not inhibit MBPs, it can overcome the cefazidime resistance engendered by AmpC enzymes in P. aeruginosa [40]. Avibactam is currently under Phase II clinical development in combination with cefazidime and cefaroline fosamil for the treatment of complicated urinary tract and intra-abdominal infections.

Finally, MK-7655 (Figure 2), a structural congener of avibactam with limited stability in the presence of base or nucleophiles [41], is a novel, potent, and covalent β-lactamase inhibitor and has demonstrated potent inhibition of class A and class C β-lactamases. Combination of MK-7655 with imipenem considerably reduced imipenem MICs against a variety of imipenem-resistant isolates of AmpC- and
KPC-producing *K. pneumoniae* and *P. aeruginosa* [42,43]. MK-7655 is currently under clinical development.

In contrast to classes A, C, and D β-lactamas that employ a nucleophilic serine residue at the active site, against which several serine-active β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam have been in use for many years in combination with classical β-lactam antibiotics, no clinically useful inhibitor of MBLs has yet been discovered [29]. The MBLs have one or two zinc ions in their active sites and are members of the metallo-enzyme superfamily that has human homologs such as angiotensin-converting enzyme. Thus, the ideal MBL inhibitors must be highly selective without activities toward human proteins. Several chemical families have already been studied as potential MBL inhibitors. Unfortunately, they remain impractical to use as therapeutic agents. However, after the recent spread of the New Delhi MBL (NDM-1) enzymes among *E. coli* and *K. pneumoniae*, the emergence of MBL-mediated resistance among *Enterobacteriaceae*, in addition to non-fermenting rods such as *P. aeruginosa* and *A. baumannii*, has become a serious public health concern, causing the acid for compounds that can counter the activity of MBLs to become even more crucial. Nonetheless, ME1071 (Figure 2) reduced the MICs of carbapenems for *Enterobacteriaceae* and *Acinetobacter* spp. with NDM-1 enzyme [44], though synergy was weaker than for bacteria with IMP and VIM metallo-enzymes, correlating with the weaker affinity of ME1071 for NDM-1 than IMP-1 and VIM-2 [45].

There is no doubt that β-lactamases will continue to adapt and that novel combinations of various β-lactamases will emerge. The highly active β-lactamase inhibitors may be only partially protective for a limited period of time [46].

### 2.1.3 Aminoglycosides

There have been several attempts to alter the aminoglycoside system to overcome the aminoglycoside-modifying enzymes [47]. Plazomicin (ACHN-490) (Figure 3) is a novel aminoglycoside synthetically derived from sisomicin by appending a hydroxyaminobutyric acid substituent at position 1 and a hydroxyethyl substituent at position 6’. Plazomicin inhibits bacterial protein synthesis and exhibits dose-dependent bactericidal activity, with enhanced activity against many MDR Gram-negative bacteria and MRSA [48,49]. It is not affected by any of the known aminoglycoside-modifying enzymes, except for AAC (2’)-Ia, -Ib, and -Ic (only found in *Providencia* spp.). Methylation of 16S ribosomal RNA, however, confers MICs of >8 µg/ml for plazomicin, as well as high-level resistance to all parenterally administered aminoglycosides that are currently in clinical use. Plazomicin has shown better activity than currently used aminoglycosides against carbapenem-resistant *Enterobacteriaceae* and *A. baumannii*. Against *E. coli* and *K. pneumoniae*, plazomicin was four times more active than amikacin; its activity against *P. aeruginosa* was similar to that of amikacin. However, it was not able to overcome NDM-1-mediated resistance [50]. The advantage of plazomicin is more profound against carbapenemase and/or ESBL-producing isolates [51]. The compound is currently under Phase II clinical development for the treatment of complicated UTIs and acute pyelonephritis as a single agent.

### 2.1.4 Tetracyclines

Ervacycline (TP-434) (Figure 3) is a novel fluoroacyl containing the tetracyclic core scaffold common to other tetracycline antibiotics, with two unique modifications: a fluorine atom at position C-7 and a pyrrolidinocetamido group at C-9 on the tetracyclic D ring [52]. Ervacycline binds bacterial ribosomes and inhibits protein synthesis with superior potency compared to that of legacy tetracyclines [53]. It has shown *in vitro* and *in vivo* activity against MDR Gram-negatives, except for *P. aeruginosa*; this activity is largely unaffected by the common tetracycline resistance mechanisms, including tetracycline-specific efflux and ribosomal protection. An intravenous formulation is currently in a Phase II clinical study to assess the treatment of community-acquired complicated intra-abdominal infections. An oral formulation is planned to be the intravenous switch for empiric treatment of severe and life-threatening bacterial infections.

### 2.2 Disappointing genomics approach to novel antibacterial agents

Since the deciphering of the complete genome of *Haemophilus influenzae* in 1995 [54], the genomics approach has been fruitless in identifying novel targets that lead to new drug candidates. GSK embarked on a target-based drug research program and spent 7 years (1995 – 2001) evaluating more than 300 genes for their potential as targets for novel antibacterials, identifying that more than 160 of them are genetically essential, and completing 70 high-throughput screening campaigns of individual targets with libraries containing between 260,000 and 530,000 molecules [55]. A total of five drug candidates were generated, none of which, however, has subsequently passed clinical trials to become licensed.

### 2.2.1 Aminoacyl-transfer RNA synthetase inhibitors

Aminoacyl-transfer RNA (tRNA) synthetases, which catalyze the attachment of the correct amino acid to its corresponding tRNA during translation of the genetic code, represent novel antimicrobial drug targets. GSK2251052 (AN3365) (Figure 3) [56,57], a unique boron-based leucyl-tRNA synthetase inhibitor, was discovered by Anacor based on the crystal structures of tRNA 

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terminate all the clinical programs on GSK2251052 and return the rights back to Anacor.

2.2.2 Peptide deformylase inhibitors
Peptide deformylase (PDF), essential to the growth of bacteria, represents another novel and attractive target for new antibacterial agents [59]. Protein synthesis in both prokaryotic and eukaryotic cells is typically initiated by ribosomal binding to N-formyl methionine transfer RNA (tRNA^{fMet}). Consequently, all N-terminal peptides start systematically with a formyl methionine. To complete protein synthesis and produce mature proteins, however, the formyl group must be removed by PDF at a very early stage of the processing, resulting in > 99% of the nascent polypeptides

Figure 3. Experimental aminoglycoside, tetracycline, and inhibitors of leucyl-tRNA synthetase and peptide deformylase.
without an N-formyl group, > 50% of which undergo further excision of the resulting methionine by methionine aminopeptidase. Bacterial PDF is encoded by the def gene, which is present in all pathogenic bacteria and does not share a functionally equivalent gene in mammalian cells. The enzyme with three highly conserved catalytic domains belongs to the matrix metallo-protease family. Blockade of bacterial PDF causes inhibition of protein synthesis.

Actinomycin and macroactin N (Figure 3) are two naturally occurring PDF inhibitors with IC50 values in the µM range and have served as templates for the design of more potent PDF inhibitors [60,61]. Three pseudopeptidic hydroxamic acids (N-formyl-N-hydroxylamines), the same functional group as in actinomycin, have been advanced to clinical studies. Intravenous BB-83698 and oral LBM-415 (Figure 3) were in Phase I trials for some time, while oral GSK1322322 (Figure 3) is in Phase II clinical trials for treatment of acute bacterial skin and skin structure infections when given as 1500 mg twice daily (BID) for 10 days in comparison with 600 mg BID linezolid for 10 days. While Phase I single-dose-escalation studies in healthy volunteers [62,63] appeared to be well tolerated for both BB-83698 and GSK1322322, the unexpected finding of methemoglobinemia (reversible cyanosis and low oxygen saturation) was reported for LBM-415 at the highest dose of 1000 mg thrice daily (TID) on Day 11 [64]. Repeat oral doses of GSK1322322 (500 – 1500 mg) in healthy adult and elderly volunteers for 10 days was well tolerated with all adverse events being mild to moderate in intensity [65]. There was no age effect or diurnal variation in the GSK1322322 pharmacokinetic profile. All three of these PDF inhibitors have demonstrated antibacterial activity against the key community-acquired pathogens, including MRSA; however, none has been reported as having significant activity against other MDR “ESKAPE” pathogens.

A new series of peptide- and nonpeptide-based PDF inhibitors have been designed and evaluated both in vitro on purified enzymes and in living bacteria. Several of these compounds inhibited bacterial PDF efficiently with IC50 values in the nM range; however, most of them showed variable antibacterial activities against Gram-positive bacteria and limited activities against Gram-negative bacteria such as E. coli, Enterobacteriaceae, or P. aeruginosa [66]. This limited potency against Gram-negative bacteria is due to the presence of a strong low-permeability barrier of the outer membrane combined with the expression of efflux pumps, e.g., AcrAB-ToIC, which decreases the susceptibility to various PDF inhibitors. In addition to the mechanical barrier, bacterial pathogens have developed two mechanisms to confer resistance to PDF inhibitors. One is amino acid substitutions within the target protein (Def) and the other is the formylation bypass which results from mutational loss of methionyl tRNA formyltransferase or of the folD gene. Sub-inhibitory concentration of polymyxin B has been reported to potentialize the activity of PDF inhibitors against Gram-negative bacteria such as E. coli, K. pneumoniae, and P. aeruginosa. The increase in bacterial susceptibility is associated with the destabilization of the outer membrane by polymyxin B, resulting in the increase of the entry of the inhibitors.

2.3 Alternatives to conventional antibiotics

Because of shortcomings in the conventional and genomic approaches to the discovery of novel antibacterials against Gram-negatives, much effort has recently been directed to alternative strategies to tackle two main challenges of resistance: how to bring drugs into and keep them inside the cell.

2.3.1 Cationic antimicrobial peptides

Cationic antimicrobial peptides that are generally short (containing 8 – 50 amino acids) and are composed of a mixture of hydrophobic and cationic residues exist in many forms of life, including insects, crustaceans, and mammals, and have been shown to function as modulators of innate immunity within the host organism. Over the last two decades, the study of cationic antimicrobial peptides has produced a rich source of potential candidates for the treatment of a broad spectrum of both Gram-positive and Gram-negative bacteria [67-70]. These peptides have demonstrated high antimicrobial potency both in vitro and in vivo. Unlike conventional antibiotics, the cationic antibacterial peptides are known to be less vulnerable to resistance and the mode of killing is extremely rapid via novel mechanisms of action that can involve different and multiple bacterial cellular targets. They can interact with, perforate, and translocate across the cytoplasmic membrane and affect the cytoplasmic processes including inhibition of macromolecular synthesis (e.g., enzymes) and cell division, or stimulation of autolysis. In addition, these peptides may have the ability to neutralize sepsis/endotoxemia and to stimulate innate immunity while simultaneously dampening the potentially harmful inflammatory response. Two cationic antibacterial peptides, omiganan (MX-226) and pexiganan (MSI-78), have shown efficacy in Phase III clinical trials, but neither of them has been approved for clinical use. Pexiganan, a synthetic analog to the frog magainins with 22 amino acids, was developed for the topical treatment of diabetic foot ulcers [71], whereas omiganan, a short peptide with 12 amino acids, was investigated for the prevention of catheter-related infection and colonization [72,73].

Despite these desirable properties, however, issues such as the susceptibility of peptides to proteolysis, unknown toxicities possibly associated with aggregation, limited tissue distribution, and poor systemic bioavailability have hampered progress toward the clinical use of the cationic antimicrobial peptides. In addition, many Gram-positive and -negative pathogens have developed multiple mechanisms to resist antimicrobial peptides [74]. Innovative solutions to these problems are necessary to provide a sturdy platform for the clinical development of these promising chemical agents. Using peptide array technology and in silico quantitative
structure–activity relationship modeling of artificial neural networks, Hancock et al. have designed and predicted the biological activity of 100,000 potent antimicrobial peptide candidates with 9 – 12 amino acids [75]. The best peptides, such as HHC10 (KRWKWKIRW-NH₂) and HHC36 (KRWKWKWWRR-NH₂), representing the top quartile of the predicted activities, have been synthesized, demonstrating both in vitro and in vivo effectiveness against MDR “ESKAPE” pathogens with activities that were equal to or better than frequently used conventional antibiotics and more effective than the most advanced clinical candidate antimicrobial peptides. Short peptides containing a simple sequence of Ile and Lys repeats, such as G(IKK)_nI-NH₂, have been designed and have shown potent bactericidal activity against both Gram-positive and -negative bacteria [76]. Unlike the proteins and other peptides produced by ribosomal synthesis which are often difficult to obtain in sufficient quantity and exhibit high manufacturing costs, these short peptides can be made by chemical synthesis or via recombinant technology in a cost-effective manner in large quantities [77].

### 2.3.2 Peptidomimetics and foldamers

To overcome the undesired properties of cationic antimicrobial peptides shown above, small synthetic peptide mimics and foldamers have been designed to adopt amphiphilic conformations and have exhibited potent antimicrobial activity while being nontoxic to host cells [78,79]. Several such compounds are currently in clinical trials. Brilacidin (PMX-30063) (Figure 4), a defensin-mimetic, is de novo designed to selectively target and bind to bacterial cell membranes that have more negatively charged groups on the outer surface than mammalian cells and that also lack cholesterol, an essential component of all mammalian membranes. Brilacidin has shown rapid bactericidal activity against both Gram-positive and -negative pathogens including MRSA, drug-resistant Enterococcus, E. coli, and NDM-1 drug-resistant K. pneumonia. A Phase II clinical trial has been completed in patients with acute bacterial skin and skin structure infections. In this study, brilacidin demonstrated clinical response rates comparable to those of daptomycin and was shown to be safe and generally well tolerated. Triaryl scaffolds (Figure 4) [80] and meta-phenylene ethynylenes (Figure 4) [81] have also been designed to mimic antimicrobial peptides. The overall hydrophobicity appeared to have a more significant impact on antimicrobial and hemolytic activity than the conformational stiffness. These mimetics are in preclinical development.

LTX-109 (Figure 4) is a synthetic tripeptide antimicrobial containing a central 2,5,7-tri(tert-butyl)tryptophan residue flanked by two arginines and a C-terminal phenylene modification [82]; it lyses membranes, causing ultra-rapid membrane disruption, and displays a broad spectrum of activity against MRSA, vancomycin-resistant enterococci (VRE), and MDR P. aeruginosa isolates [83]. LTX-109 has exhibited a dose-dependent bactericidal effect in a mouse model of skin infection and is currently in Phase II clinical trials for topical treatment of MDR bacterial infections.

### 2.3.3 Siderophores and sideromycins

Iron is one of the major limiting factors and essential nutrients of microbial life. Therefore, iron assimilation is vital to cellular homeostasis. However, free iron in biological solutions at pH 7.0 is present as the oxidized Fe³⁺ form at very low concentrations (10⁻⁹ M or less), insufficient for bacterial growth. In mammals, most iron is bound by or incorporated into proteins (primarily ferritin, transferrin, lactoferrin, and hemoglobin) with high affinity, which reduces the bioavailability of iron to infecting bacteria and is an important component of innate immunity against bacterial infection. To compete for iron resources, microorganisms synthesize and excrete siderophores, ferric ion-selective chelating agents with K_d values typically in the range 10²⁻² – 10⁻⁹, to accumulate, mobilize, and transport iron for metabolism. Siderophores also play a critical role in the expression of virulence and development of biofilms by different microbes. Even though the routes used by bacteria to assimilate iron from host and environmental settings have been the subject of intense study for decades, no antibiotics have been successfully approved to interrupt this important process [84].

Talactoferrin is a recombinant human lactoferrin that is a member of the transferrin family of iron-binding glycoproteins and has shown multiple known biological activities including iron homeostasis, organ morphogenesis, host defense against infection, anti-inflammation, and anticancer activity [85]. Lactoferrin has broad-spectrum antimicrobial activity against bacteria, fungi, viruses, and protozoa. Its antimicrobial activity stems from two distinct effects: i) its iron-sequestering ability, which can be negated by saturation with iron and ii) its iron-independent killing due to a direct interaction with the microbial surface resulting in cell lysis [86]. Unfortunately, Phase II/III clinical trials of oral talactoferrin in severe sepsis were halted early due to 28-day all-cause mortality being higher than that in the placebo arm. In addition, a Phase III trial in patients with non-small-cell lung cancer did not meet its primary endpoint of improving overall survival compared to the placebo group.

hLF1-11 is a synthetic peptide comprising the first 11 N-terminal residues of human lactoferrin. It displays in vitro antimicrobial activity against MRSA, MDR A. baumannii, and invasive fluconazole-resistant Candida albicans only at subphysiological salt concentrations; however, its in vivo activity against these pathogens in neutropenic mice was more prominent [87]. The mechanisms of action of hLF1-11 include enhancing cytokine and chemokine production of monocytes in response to microbial stimuli, inhibiting myeloperoxidase and directing the GM-CSF-driven monocyte–macrophage differentiation toward an IL-10-producing macrophage subset that shows increased responsiveness toward microbial stimuli and enhanced phagocytosis and intracellular killing of pathogens [88]. A Phase I clinical trial in healthy human volunteers...
showed that repeated intravenous administrations of 5 mg hLF1-11 were well tolerated [89].

Some organisms have also developed the capacity of producing dual-function chemical warfare agents that couple siderophores with antibiotics, the sideromycins [90,91]. These molecules use the siderophore as bait to actively transport the antibiotic moiety into bacterial cells, resulting in the “Trojan Horse” effect, which increases the antibiotic efficiency by at least 100-fold when compared to those without an active transport mechanism. This approach is particularly relevant for Gram-negative bacteria, which possess an extra outer membrane posing a significant permeability barrier to antibiotic molecules [92]. Salmycin A and albomycin A1 are two natural sideromycins. There has also been marked activity in the development of synthetic molecules conjoining siderophores with existing antibacterials.

Monobactam siderophore BAL19764 (Figure 5) was combined with the bridged monobactam BAL29880 (Figure 5) and clavulanate. BAL29880 is an inhibitor of AmpC enzymes while clavulanate inhibits ESBLs. This three-component combination (BAL30376) has exhibited activity against MBL-producing Enterobacteriaceae and some isolates of Burkholderia
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**Figure 5. Experimental siderophores.**

*B. cepacia* and carbapenemase-producing *A. baumannii*. However, BAL30376 was inactive against KPC-producing strains [93]. BAL30072 (Figure 5) is the only siderophore monobactam conjugate currently in Phase I clinical trials. BAL30072, a conjugate of dihydroxypyridone and tigemonam (Figure 5), possesses potent activity against a broad range of Gram-negative bacteria including MDR *P. aeruginosa*, *Acinetobacter* spp., *Klebsiella* spp., *Enterobacter* spp., and the *Burkholderia cepacia* complex [94-96]. BAL30072 is stable toward many types of β-lactamases and carbapenemases, is taken up very readily into bacteria via the essential nutrient uptake systems, and is able to circumvent resistance caused by changes in the outer membrane of Gram-negative bacteria. The compound has demonstrated bactericidal activity against both *Acinetobacter* spp. and *P. aeruginosa*, even those that produce MBLs, *Enterobacteriaceae* isolates with class A or B carbapenemases, and PBP-producing *E. coli*.

The Miller lab has been very active in this field and recently reported tris-catecholate siderophore-aminopenicillin (Figure 6) [97] and desferrioxamine B-ciprofloxacin conjugates (Figure 6) [98], as well as sideromycins of trihydroxamate siderophore with ciprofloxacin or a β-lactam carbacepholosporin, Lorabid (Figure 6) [99].

### 2.3.4 Efflux pump inhibitors

Efflux pumps, involved in the limitation of the intracellular (or periplasmic) concentration of all clinically used groups of antibiotics, are one of the key contributors in the emergence and dissemination of resistant Gram-negative bacteria.

Bacterial resistance due to efflux transporters, especially those belonging to the resistance-nodulation-division (RND) family, can cause Gram-negative bacteria to acquire additional mechanisms of resistance including mutation of antibiotic targets (e.g., mutation in gyrase) or the production of enzymes that degrade antibiotics (e.g., β-lactamases) and can also reinforce the effects of these acquired mechanisms [100]. Thus, numerous efforts have been undertaken to devise strategies to reverse or inhibit the action of efflux pumps so as to restore the normal intracellular concentrations of antibiotics used. Several naturally derived and synthetic compounds have been described to inhibit efflux pumps in various Gram-negative bacteria. Ideal efflux pump inhibitors should meet these criteria: i) to overcome the outer membrane barrier; ii) to enhance activity of other pump substrates; iii) to show no activity in mutants lacking the respective efflux pumps; iv) to increase accumulation and decrease extrusion of efflux pump substrates, and v) to not affect the proton gradient across the cytoplasmic membrane [101].

Pyridopyrimidine quaternary ammonium analog D13-9001 (Figure 7) is MexAB-OprM specific and exhibited potent *in vivo* efficacy against *P. aeruginosa* and a good safety profile in an acute toxicity assay [102]. Aryl-piperazines (Figure 7) have been reported to reverse MDR in *E. coli*, *Acinetobacter*, *Klebsiella*, and *Enterobacter* that overexpress RND-type efflux pumps, restoring susceptibility of commonly used antibiotics by increasing intracellular concentrations [103,104]. Quinazoline derivatives inhibit efflux pumps mediated by AcrAB-ToIC and MexAB-OprM and are potential chemosensitizers...
of antibiotic activity in MDR Enterobacter, K. pneumonia, and P. aeruginosa strains [105,106], whereas indole derivatives (Figure 7) were reported to be inhibitors of E. coli AcrAB-ToIC [107]. As of yet, no efflux pump inhibitor has been approved for clinical use. A bis(benzamidine) derivative, MP-601,205, was in Phase II clinical trials with ciprofloxacin for the treatment of pulmonary infections in cystic fibrosis patients, but was not pursued further because of tolerability issues [108].

2.3.5 Therapeutic antibodies

Plethoric preclinical studies suggest that anti-infectious antibodies have great potential to address the unmet medical needs associated with viral and bacterial infections,
particularly nosocomial infections. Palivizumab (Synagis) is, however, the only anti-infective monoclonal antibody (mAb) marketed for the prevention of severe Respiratory Syncytial Virus infections of neonates, but there is yet no approval for bacterial infections. Antibacterial antibodies generally target specific pathogens without affecting the commensal flora and can be divided into two categories: those that bind directly to the pathogen and cause phagocytosis and those that bind and neutralize toxins or other virulence factors so as to disarm the bacteria and allow the host a chance to clear the infection immunologically [109,110]. Due to their narrow specificity, antibacterial antibodies are usually intended for small markets and require rapid diagnosis.

Several antibacterial antibodies have been advanced to the clinical evaluation stage. A number of promising candidates, however, have failed late-stage clinical studies because of lack of efficacy, including polyclonal (Altastaph, Veronate) and monoclonal (Aurograb) antibody products against S. aureus infections. Pagibaximab, a chimeric mAb directed against lipoteichoic acid, a highly conserved glycolipid component of staphylococcal cell walls, is currently being evaluated for prevention of infection in low-birth-weight infants in Phase II clinical trials.

P. aeruginosa is another pathogen extensively targeted for therapeutic antibody development. Panobacumab (KBPA101), a human IgM-mAb directed toward lipopolysaccharide of P. aeruginosa serotype O11, demonstrated in a Phase IIa trial an impressive 7% 28-day mortality rate in patients with ventilator-associated or hospital-acquired pneumonia, compared to the predicted 24% mortality rate [111]. KB001, a humanized pegylated Fab fragment inhibiting the type III secretion system PcrV of P. aeruginosa, is being evaluated in a pilot clinical study in mechanically ventilated patients for prevention of serious pneumonia caused by colonized P. aeruginosa. Only approximately 30% of the KB001-treated patients had experienced P. aeruginosa pneumonia and 46% were alive at Day 28 without P. aeruginosa infection, compared to 60 and 20%, respectively, in placebo recipients [112]. In addition, antibodies targeting Pseudomonas quorum sensing have been published [113].

Bacterial toxins are highly sensitive to antibody neutralization. Antibodies neutralizing Shiga toxins 1 and 2 have been developed for treatment of Shiga toxin-producing E. coli (STEC). Urotoxazumab, a humanized mAb directed against the Shiga-like toxin 2 (Stx2) produced by STEC, has been developed as a promising agent for the prevention of hemolytic-uremic syndrome. Safety and pharmacokinetics in healthy adults and STEC-infected pediatric patients showed that it was well tolerated and appeared to be effective in pediatric patients [114]. ShigamAbs, consisting of two chimeric mAbs (cαStx1, cαStx2), are designed to bind specifically and
exclusively to the Stx1 and Stx2 toxins secreted by STEC, thus not only able to address cases in which both Shigatoxins are present but also overcoming the inability of existing diagnostic technology to distinguish between the two toxins. The complex thus formed between ShigamAbs and toxins is absorbed and destroyed by the liver and spleen. Four Phase I clinical trials have been completed which demonstrated that ShigamAbs were well tolerated when administered both individually and in combination at various dose levels.

More broad-spectrum antibodies have been reported targeting the poly-N-acetylmuramic acid (PNAG) of bacterial polysaccharides [115,116]. This surface polysaccharide is expressed by a variety of serious MDR bacteria, including MRSA and ESBL-producing and carbapenemase-producing Enterobacteriaceae (e.g., E. coli and K. pneumonia), as well as less common pan-resistant A. baumannii. A fully human mAb (SAR279356, mAb F598) to PNAG has successfully completed a Phase I safety and pharmacokinetic dose-escalation trial in healthy adults and is currently undergoing a Phase II trial in mechanically ventilated patients.

2.3.6 Renewed interest in treatments that have been either abandoned or approved for limited indications

In addition to polymyxin antimicrobials [17,18], clinicians have also been attempting to revive and expand a few other antibiotics and therapies that have either been abandoned or approved only for limited indications to treat MDR bacterial infections. Examples include temocillin, fosfomycin, and phage therapy.

Temocillin (Figure 8) is the 6-α-methoxy derivative of ticarcillin, a β-lactam [117,118]. Developed and first marketed in the UK in the 1980s, temocillin was quickly abandoned due to the major drawbacks perceived at the time, namely a lack of activity against Gram-positive organisms and P. aeruginosa. Presence of the 6-α-methoxy moiety impairs binding of temocillin to PBPs, explaining its lack of activity against Gram-positive bacteria. Interestingly, the 6-α-methoxy moiety blocks the entry of a water molecule into the β-lactamase active site, thereby preventing activation of the β-lactam ring, and, therefore, temocillin has exhibited remarkable stability toward all classical and ESBLs including AmpC, TEM, SHV, and CTX-M enzymes. Though labile to the chromosomal MBL of Chryseobacterium meningosepticum, temocillin is relatively stable to several acquired metalloenzymes, whereas OXA-48 (Ampler class D) carbapenemase confers high-level resistance to temocillin. The MICs of temocillin for Enterobacteriaceae are between 2 and 32 µg/ml, with modes of 4 – 8 µg/ml and MIC₉₀ of 16 µg/ml. The MIC distributions of clinical isolates have remained stable without significant change since its introduction. Unlike cephalosporins, temocillin does not select derepressed mutants of AmpC-inducible species or overgrowth by C. difficile. It is envisaged that temocillin might be an alternative to carbapenems when combined with other agents to cover Gram-positives and Pseudomonas.

Fosfomycin is an epoxide-containing phosphonic acid, a unique chemical structure found in no other antimicrobial agents (Figure 8), which was first identified in Spain in 1969 from cultures of the Streptomyces species [119,120]. Fosfomycin has broad spectrum of activity against aerobic Gram-positive and Gram-negative pathogens, including MRSA, VRE, E. coli, K. pneumoniae, and P. aeruginosa, with the exception of A. baumannii. Oral fosfomycin (Monuril) at 3 g/day has been approved by the FDA for the treatment of uncomplicated UTI in adult women that is caused by E. coli and E. faecalis. Intravenous fosfomycin, available in five European countries (Spain, France, Germany, Austria, and Greece) but not in the US, administered at 12 – 16 g in 2 – 4 infusions, has been used to treat a variety of infections including meningitis, pneumonia, and pyelonephritis. Fosfomycin is an inhibitor of peptidoglycan assembly, thus disrupting cell-wall synthesis. Once it is taken up into the bacterial cell via active transport, i.e., the L-α-glycerophosphate transport and hexose phosphate uptake systems, fosfomycin competes with phosphoenolpyruvate to irreversibly inhibit the enzyme enolpyruvyl transferase that catalyzes the first step of peptidoglycan synthesis. Due to its unique mechanism of action, combinations of fosfomycin with other classes of antibiotics including β-lactams, aminoglycosides, and fluoroquinolones have demonstrated in vitro synergistic effects. Recent clinical studies showed potential benefits of intravenous fosfomycin in combination with other antibiotics in critically ill patients for the treatment of nosocomial infections due to MDR bacterial infections, especially carbapenem-resistant K. pneumoniae. However, the high frequency of mutational resistance to fosfomycin measured in vitro has casted uncertainty on its clinical utility as a systemic agent. Further randomized and controlled trials are needed to evaluate the efficacy of fosfomycin in combination with other antimicrobial agents, especially those that can prevent the selection of fosfomycin resistant strains, for the management of MDR bacterial infections.

Bacteriophages or phages are bacteria-specific viruses, the natural predators of bacteria [121,122]. In fact, phage therapy to treat bacterial infections dates back to the early 1900s in the preantibiotic era, which had lost favor after the introduction of antibiotics. However, due to the emergence of widespread MDR bacterial infections, there has been renewed interest in phage therapy.
interest in phage therapy. Several clinical trials with phage therapy have been reported in recent years, which are more focused on topical use, for example, BFC-1 (a phage cocktail) for wound infections caused by *P. aeruginosa* or *S. aureus*, BioPhage-PA against *P. aeruginosa* ear infections and WPP-201 for venous leg ulcers. The challenges in developing phage therapy include the lack of efficacy and pharmacokinetic data, as few clinical trials were randomized with too small of a sample size. Phages are pathogen specific with very narrow specificity and thus phage therapy will require rapid diagnostics to identify the specific infecting agent(s) before determining what bacteriophage(s) can be administered. Even though the frequency of resistance has been reportedly low in vivo during phage therapy, high risk of resistance development has been observed in vitro. Phages can also be neutralized and inactivated by the host immune system. FDA in 2006 approved the use of bacteriophages in the prevention of *Listeria monocytogenes* contamination of meat and poultry, and yet no bacteriophages have cleared the regulatory hurdle for pharmaceutical use by the USA or European Medicines Agency. Therefore, the clinical potential of phage therapy in treating MDR bacterial infections remains to be confirmed.

### 3. Expert opinion

The input and output of the current global R&D activities are insufficient to develop new therapeutics which are desperately needed to combat MDR bacterial infections. A few antibacterial agents, such as ceftolozane (CXA-101), avibactam (NXL-104), plazomicin (ACHN-490), and eravacyn (TP-434), have exhibited encouraging activity in vitro, in animal models and in clinical studies. Effective inhibitors of MBLs are urgently needed to restrict the proliferation and global spread of NDM-1 bacteria. Alternative approaches to permeate bacterial cell membranes, enhance influx, disrupt efflux, and target specific pathogens by antibodies and bacteriophages, though challenging, are attractive and promising, among which are candidates such as brilacidin (PMX-30063), BAL30072, and mAb F598. However, there are a plethora of unexpected challenges that may arise and cannot always be solved which cause promising drugs to fail, such as tRNA<sup>16S</sup> synthetase inhibitor GSK2251052 (AN3365). PolyMedix, the company responsible for brilacidin (PMX-30063) development, has recently filed for Chapter 7 bankruptcy protection, which will undoubtedly slow down its clinical programs, casting dark clouds over the fate of brilacidin.

There has been growing enthusiasm in combination therapy for the treatment of Gram-negative bacterial infections in anticipation of achieving the following: i) broadening the empiric coverage; ii) improving clinical outcomes through the synergistic effects observed in vitro; iii) increasing the drug pressure to prevent or delay the development of resistance [123]. The full benefits of combination therapy, however, have yet to be proven by prospectively designed clinical studies that are adequately randomized and appropriately controlled, even though several meta-analyses of observational studies indicated certain benefits. Countering the limited benefit is the increased nephrotoxicity and ototoxicity that have been observed and well documented with combination therapy. Consequently, the empiric combination regimen should be justified according to the local epidemiology and the environmental exposure of individual patients and should be promptly narrowed or discontinued once the culture and susceptibility profile results are available.

Rapid diagnosis of drug resistance profiles from primary clinical samples, instead of cultures, will be a powerful tool to transition physicians from an empirical prescription practice to a more targeted and individualized antibiotic therapeutic approach [124]. To attract more capital investment and optimize the return on investment, various business models including partnerships between public, private, and governmental entities have been proposed [125]. The U.S. Congress has passed the Generating Antibiotic Incentives Now (GAIN) Act of 2011 to incentivize the pharmaceutical industry for the development of products to treat, prevent, detect, and diagnose antibiotic-resistant infections caused by MRSA, vancomycin-resistant *S. aureus*, VRE, MDR Gram-negative bacteria (including *Acinetobacter, Klebsiella, Pseudomonas*, and *E. coli* species), MDR tuberculosis, *C. difficile*, or any other infectious pathogens identified by the secretary of the U.S. Department of Health and Human Services (HHS). The incentives include a 5-year extension of market exclusivity for qualified infectious disease products (QIDPs), in addition to the applicable exclusivities (e.g., Hatch-Waxman 5-year new chemical entity exclusivity, 3-year new clinical studies exclusivity, 7-year orphan drug exclusivity, and 6-month pediatric exclusivity), as well as an additional 6 months of exclusivity for the QIDP if a companion diagnostic test is also approved for that drug. The GAIN Act also allows the QIDP sponsor to request written recommendations from the HHS secretary for nonclinical and clinical investigations necessary for approval of the drug. With the reauthorization of Prescription Drug User Fee Act for the fifth time, the regulatory pathway for QIDPs is more clear in terms of which drugs are eligible for priority review and fast-track approval by the FDA. The real impact of the GAIN Act remains to be seen [126].

Clearly, it is imperative that creative and practical strategies be pursued to fill the antibiotic pipeline with novel and effective drug candidates and to arm health practitioners with an adequate drug armamentarium to combat emerging drug-resistant pathogens.

### Declaration of interest

There is no declaration of interest with exception that authors are affiliated with the respective organizations.
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