CLINICAL INVESTIGATION

Development of antibiotics for Gram-negatives: where now?

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The incidence of infections due to multidrug-resistance pathogens, such as *Enterobacteriaceae*, *Pseudomonas aeruginosa* or *Acinetobacter* spp. has been increasing. The paucity of antimicrobials active against multidrug-resistance strains are an important challenge. Novel anti-Gram-negative agents from old antimicrobial classes include β-lactamase inhibitors, cephalosporins, carbapenems, aminoglycosides, polymyxin analogs, tetracycline and monobactams. Among them, β-lactamase inhibitors seem the most promising as they might restore the activity of already known β-lactams against β-lactamase-producing strains. New classes of antimicrobials include *bis*-indoles, boron-containing antibacterial protein-synthesis inhibitors, outer membrane synthesis inhibitors, antimicrobial peptides and antibiotics targeting novel sites of the 50S ribosomal subunit. Although promising, they are still far from being introduced into clinical practice. Therefore, optimizing the use of current antibiotics and infection control policies are mandatory.

Keywords: antimicrobial peptides • ESKAPE • extended-spectrum β-lactamases • metallo-β-lactamases • new β-lactamase inhibitors • new cephalosporins

Matteo Bassetti[†1], Francesca Ginocchio[1], Daniele Roberto Giacobbe[1] & Malgorzata Mikulska[1]

[1]Division of Infectious Diseases, San Martino Hospital & University of Genoa, L.go R.Benzi 10, 16132 Genova, Italy
[†]Author for correspondence:
Tel.: +39 010 555 5132
Fax: +39 010 353 7680
E-mail: matteo.bassetti@hsanmartino.it

Multidrug resistance (MDR) is defined as nonsusceptibility to one or more antimicrobials in three or more antimicrobial classes, while strains nonsusceptible to all antimicrobials, including polymyxins and tigecycline, are classified as extreme drug-resistant strains [1]. MDR Gram-negative bacteria pose a serious and rapidly emerging threat to patients in healthcare settings and are especially common and problematic in some intensive care units [2]. Both in Europe and the USA, a significant increase in prevalence of resistant strains has been reported, as outlined in Figures 1 & 2, respectively [1,3,4]. This phenomenon of growing resistance has been resumed with the word 'ESKAPE', to explain the more frequent MDR microorganisms, including both Gram-positive (*Enterococcus faecium* and *Staphylococcus aureus*) and Gram-negative bacteria (*Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.) [5]. In addition, MDR *Escherichia coli* is another important pathogen causing not only healthcare-associated infections, but also community-acquired [6]. Moreover, MDR has a significant impact on mortality, hospital length of stay and hospital costs [7].

Numerous classes of antimicrobials are currently available for physicians to treat patients with Gram-negative infections, although the pace of antibiotic drug development has slowed during the last decade (Figure 3), and the pharmaceutical pipeline of antibiotics active against MDR Gram-negative is very limited. During the last few years, research has focused on methicillin-resistant *S. aureus* (MRSA), while multiple mechanisms of resistance of Gram-negative bacteria make them a more difficult target for drug development than Gram-positives [8]. Therefore, clinicians are forced to rediscover older drugs, such as polymyxins and fosfomycin, and to optimize the use of already existing molecules.

FUTURE SCIENCE part of fsg

Numerous agencies and societies have tried to draw attention to this significant lack of new antibiotics for MDR Gram-negative pathogens and since 2004 repeated calls for reinvigorating pharmaceutical investment in antibiotic research and development have been made by the Infectious Diseases Society of America (IDSA) and the US FDA [9]. Recently, IDSA supported an initiative of developing ten new systemic antibacterial drugs through the discovery of new drug classes, as well as exploring possible new molecules from already existing classes of antibiotics, the '10×'20' initiative [10].

The profile of antibiotic resistance to currently used antimicrobial agents and new anti-Gram-negative agents will be discussed.

Importance of MDR Gram-negative bacteria in clinical practice

The urgent need for new antibiotics active against resistant Gram-negatives is fuelled by an increase of the incidence, morbidity and mortality in infections caused by these pathogens.

Enterobacteriaceae

Extended-spectrum β-lactamases (ESBL)-producing *E. coli* and *Klebsiella* spp. are common in the healthcare setting, but pandemic clones, such as *E. coli* ST131, also cause community-acquired infections [6]. In addition, *Enterobacter* spp., which are frequently responsible for healthcare-acquired infections, are commonly resistant to multiple antibacterials (it was found that in 5206 strains, resistance to ceftazidime was 22%, to aztreonam 19%, to piperacillin/tazobactam 10% and to ciprofloxacin it was 11%) [11]. Moreover, carbapenem-resistant *Enterobacteriaceae* are increasingly recognized as a cause of sporadic infections and outbreaks worldwide [12–14]. In all these bacteria, the presence of MDR might have an important influence on mortality. For example, in a meta-analysis of 16 studies including 1682 infections, bacteremias caused by ESBL-producing pathogens were significantly associated with delayed initiation of effective therapy and an almost twofold increase in crude mortality [15].

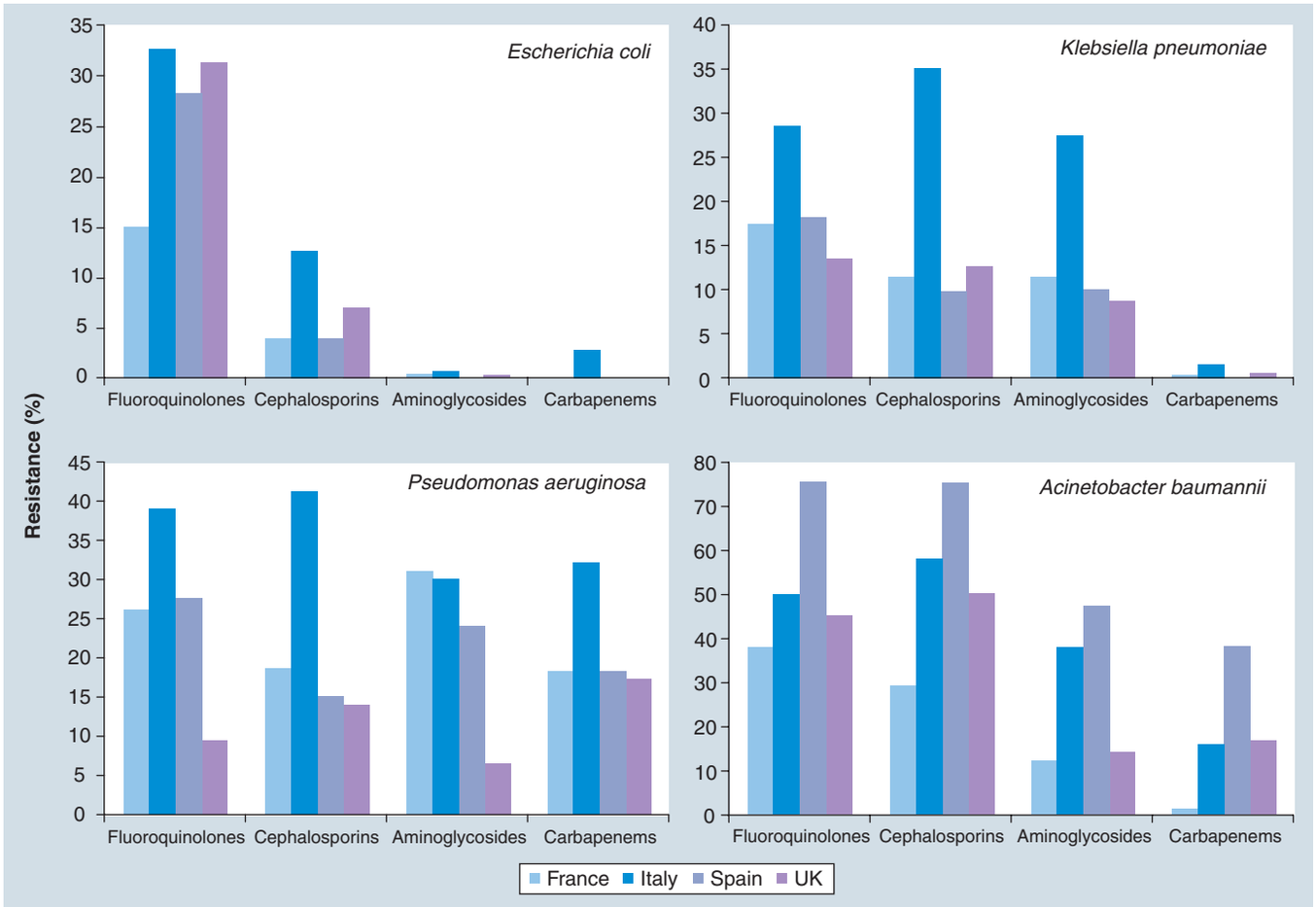

Figure 1. Resistance rates among Gram-negative bacteria in France, Italy, Spain and the UK.
Data taken from [2,3].

Nonfermenting rods

Pseudomonas aeruginosa and *Acinetobacter* harbor frequent resistance to multiple antibiotics. A recent US study reported that in 2008 as much as 17% of *P. aeruginosa* and 74% of *A. baumannii* strains were MDR [1]. Indeed, the incidence of infections due to MDR *Acinetobacter* spp. continues to increase globally, in particular, carbapenem-resistance increased from 9% in 1995 to 40% in 2004 [16]. Inappropriate empirical therapy has been associated with increased mortality in *P. aeruginosa* infections, and the risk of using an inactive antibiotic is higher if MDR strains are present. Thus, worse clinical outcomes and higher costs of prolonged hospitalization might be associated with MDR infections [17].

Mechanism of resistance to currently used antimicrobial agents in MDR Gram-negative bacteria

Numerous mechanisms of drug resistance have developed in Gram-negative bacteria against available antimicrobials, and they include β-lactamases, efflux pumps, porin mutations and binding-site mutations. Moreover, the rapid emergence of resistance in human and veterinary medicine is partly caused by the horizontal transfer of clusters of genes conferring combined resistance to multiple drugs.

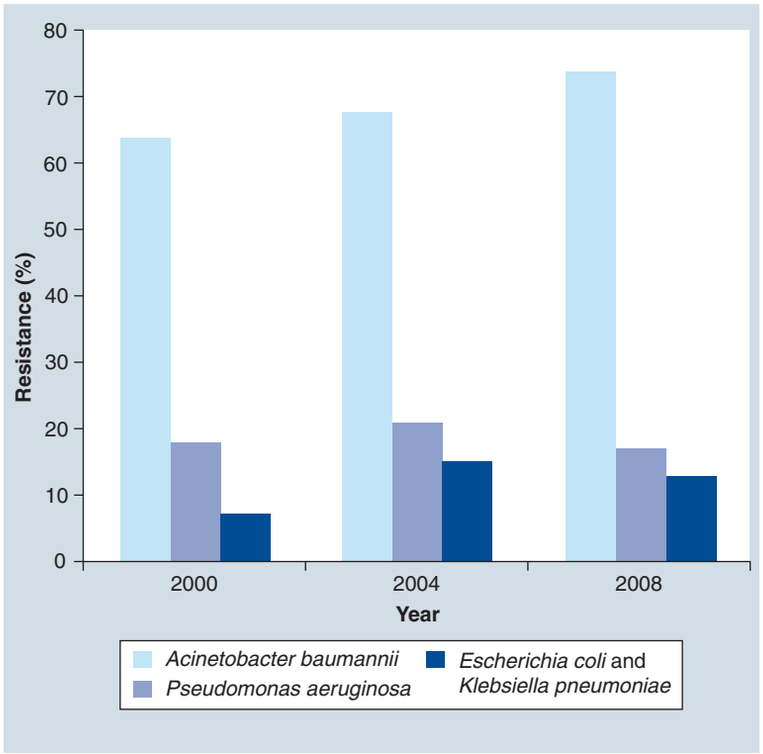

Figure 2. Resistance rates among Gram-negative bacteria in the USA. Data taken from [1].

Resistance to β-lactams

β-lactamase-mediated resistance is the most important and efficient method of resistance to β-lactams in Gram-negative bacteria. The origin of β-lactamases development is presumably ancient and has evolved to fight natural β-lactams, produced by bacteria such as *Streptomyces* or *Lysobacter,* or filamentous fungi, such as *Penicillium* or *Acremonium* [18]. However, resistance has been heavily influenced over the years by the widespread administration of antibiotics in clinical practice. For example, the rapid increase in resistance to widely used ampicillin in the early 1960s turned out to be due to a plasmid-mediated β-lactamase, one of the first described in Gram-negative bacteria, known as TEM (the TEM 1 enzyme was originally found in *E. coli* isolated from a patient named Temoniera, hence named as TEM) [19]. The further selection of resistant mutants led to the appearance of ESBLs that now compromise the use of third-generation cephalosporins. In the 1990s, pharmaceutical industries introduced carbapenems, which are extremely stable to degradation by β-lactamases. However, a variety

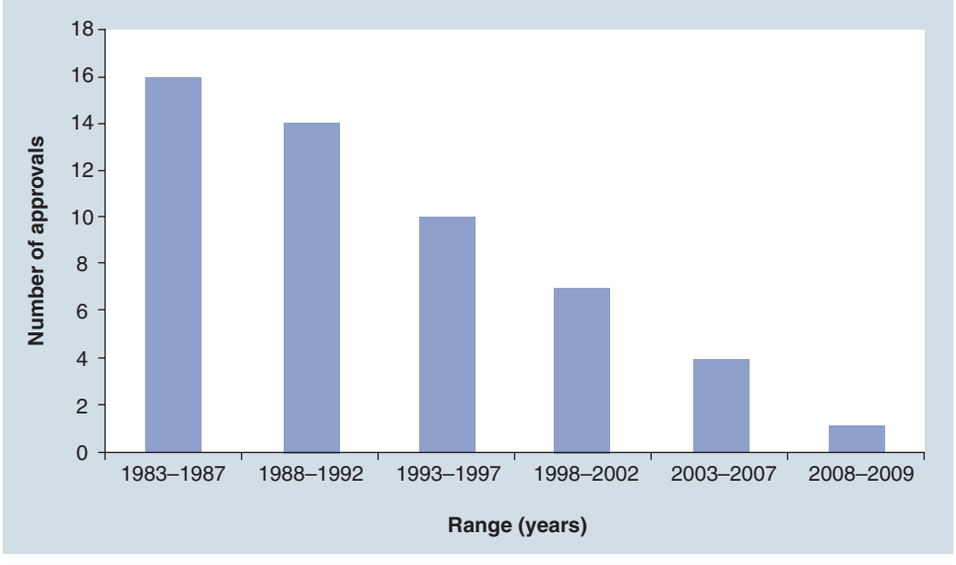

Figure 3. New antibacterial agents approved in the USA, 1983–2009 (as reported by Infectious Diseases Society of America's Antimicrobial Availability Task Force). Reproduced with permission from [202].

of β-lactamases capable of hydrolyzing these antibiotics, including IMP, VIM, *K. pneumoniae* carbapenemases (KPC) and OXA are increasingly seen in Gram-negative isolates [20]. Different classifications of β-lactamases have been proposed, but the Ambler classification is the most widely used and divides β-lactamases into four classes (A, B, C and D) based upon their amino acid sequences (Table 1) [21–23]. Briefly, class A enzymes are mostly plasmid-mediated penicillinases, such as those belonging to TEM and SHV subclasses. However, some of the evolved class A β-lactamases accept cephalosporins as substrates and are known as ESBLs, even though there are ESBL enzymes belonging to other classes as well. Class B enzymes are metallo-β-lactamases (MBL) with broad substrate specificity that includes not only penicillins and cephalosporins, but also carbapenems. Class C enzymes are primarily chromosomally encoded cephalosporinases and are often referred to as AmpC β-lactamases resistant to inhibition by commercially available β-lactamase inhibitors. Finally, class D β-lactamases have a substrate preference for oxacillin and are therefore called oxacillinases. Of note, this classification is based on molecular sequence of enzymes and the same antibiotic can be inactivated by β-lactamases from different classes. For example, carbapenems can be inactivated by enzymes belonging to class B (MBL), class A (KPC) and class D (OXA-23 and -48). This enzyme diversity is a crucial aspect of antimicrobial resistance. Recently, a new plasmidic MBL, the New Delhi MBL termed NDM-1, has been identified in *K. pneumoniae* and *E. coli* recovered from a Swedish patient who was admitted to a hospital in New Delhi, India [13]. Of particular concern is that NDM-1 enzymes were present in *E. coli,* a common cause of community-associated urinary tract infections (UTIs) and bloodstream infection in humans of all ages [6]. The NDM-1-producing bacteria are frequently resistant to many groups of antibiotics, including fluoroquinolones, aminoglycosides and β-lactams (especially carbapenems), remaining susceptible only to colistin and tigecycline [13]. Nevertheless, even these two agents might lose their activity.

Resistance to colistin

Colistin acts by binding to lipid A moiety of the bacterial lipopolysaccharide and subsequently disintegrating the bacterial membranes [24]. The chromosomal AmpC cephalosporinase, the outer membrane porin OprD and a multitude of efflux pumps are particularly relevant to confer resistance to colistin in *P. aeruginosa* strains [25]. A detailed review of various resistance mechanisms to polymyxins has been recently published [24].

Resistance to tigecycline

As far as resistance to tigecycline is concerned, low concentrations attained in the serum are probably the driving force for the development of resistance while on treatment, particularly when the MICs of the targeted pathogen exceed the Cmax of the drug, which is almost the rule for all targeted *A. baumannii* strains [26]. The genetic basis for the development of resistance have been investigated in molecular studies and efflux pumps seem to be the most important mechanism. Various efflux pumps have been reported in *E. coli, Enterobacter cloacae*, *K. pneumoniae* and *Acinetobacter calcoaceticus–baumannii* [27].

Resistance to fluoroquinolones

Fluoroquinolones act by binding to DNA gyrase and topoisomerases IV [28]. Numerous bacteria have developed several resistance mechanism, both chromosomal and plasmidic. Chromosomal resistance include target mutations, such as GyrA/GyrB for DNA gyrase and ParC/ParE for topoisomerase IV and augmented expression of efflux pumps [29]. Among plasmidic-mediated resistance mechanisms are acetylation, efflux pumps and the production of fluoroquinolones-resistant proteins, which protect the quinolone targets from inhibition [30].

Resistance to aminoglycosides

Aminoglycosides kill bacteria by inhibiting protein synthesis as they bind to the 16S rRNA and by disrupting the integrity of bacterial cell membrane [31]. Bacterial resistance to aminoglycosides is driven by three major mechanisms: inactivating aminoglycoside-modifying enzymes, regulation of intracellular concentration by overexpression of efflux pumps and target modification [32].

Resistance to fosfomycin

Fosfomycin has a mechanism of antimicrobial action that involves the inhibition of an enzyme that catalyzes the first step in bacterial cell-wall synthesis within the cell [33]. The most commonly described mechanism of resistance to fosfomycin in *E. coli* is the overexpression of the plasmidic genes FomA and FomB, leading to phosphorylations of fosfomycin and fosfomycin monophosphate [34]. However, fosfomycin seems to be spared from the effect of various mechanisms of multiple resistance to antimicrobials owing to its unique chemical structure and mechanism of action. High levels of antimicrobial activity of fosfomycin have been reported in fluoroquinolone-resistant and ESBL-producing *Enterobacteriaceae* (97% of 1657 *E. coli* strains and 81% of 748 *K. pneumoniae* isolates producing ESBL were susceptible to fosfomycin) [24]. Fosfomycin could be an old, but important alternative for the treatment of UTIs caused by MDR pathogens, although further research is needed.

Spread of multiple-resistance genes

The rapid emergence of resistance to various antibiotics in human medicine and animal husbandry is partly due to horizontal transfer of resistance genes, which is a successful mechanism for the transmission and dissemination of MDR among bacterial pathogens [35]. The impact of horizontally transmitted genetic determinants in the evolution of resistance is particularly evident when resistance genes are physically associated in clusters and transferred together to the recipient cell [36]. Integrative and conjugative elements are a diverse group of mobile genetic elements found in both Gram-positive and Gram-negative bacteria, that can be responsible for horizontal transfer antimicrobial resistance [37]. These mechanisms of spread of resistance are not without serious clinical consequences, as indicated by the novel case of *K. pneumoniae* carbapenemases [38].

Table 1. Classification schemes for bacterial β-lactamases.

Molecular class (subclasses)	Bush–Jacoby group (2009)	Distinctive substrate(s)	Defining characteristic(s)	Representative enzyme(s)
A	2a	Penicillins	Greater hydrolysis of benzylpenicillin than cephalosporins	PC1
A	2b	Penicillins, early cephalosporins	Similar hydrolysis of benzylpenicillin and cephalosporins	TEM-1, TEM-2, SHV-1
A	2be	Extended-spectrum cephalosporins, monobactams	Increased hydrolysis of oxyimino-β-lactams (cefotaxime, ceftazidime, ceftriaxone, cefepime and aztreonam)	TEM-3, SHV-2, CTX-M-15, PER-1, VEB-1
A	2br	Penicillins	Resistance to clavulanic acid, sulbactam and tazobactam	TEM-30, SHV-10
A	2ber	Extended-spectrum cephalosporins, monobactams	Increased hydrolysis of oxyimino-β-lactams combined with resistance to clavulanic acid, sulbactam and tazobactam	TEM-50
A	2c	Carbenicillin	Increased hydrolysis of carbenicillin	PSE-1, CARB-3
A	2ce	Carbenicillin, cefepime	Increased hydrolysis of carbenicillin, cefepime and cefpirome	RTG-4
A	2e	Extended-spectrum cephalosporins	Hydrolyzes cephalosporins Inhibited by clavulanic acid but not aztreonam	CepA
A	2f	Carbapenems	Increased hydrolysis of carbapenems, oxyimino-β-lactams and cephamycins	KPC-2, IMI-1, SME-1
B (B1)	3a	Carbapenems	Broad-spectrum hydrolysis including carbapenems but not monobactams	IMP-1, VIM-1, CcrA, IND-1
B (B2)	3b	Carbapenems	Preferential hydrolysis of carbapenems	CphA, Sfh-1
B (B3)				L1, CAU-1, GOB-1, FEZ-1
C	1	Cephalosporins	Greater hydrolysis of cephalosporins than benzylpenicillin; hydrolyzes cephamycins	*Escherichia coli* AmpC, P99, ACT-1, CMY-2, FOX-1, MIR-1
C	1e	Cephalosporins	Increased hydrolysis of ceftazidime and often other oxyimino-β-lactams	GC1, CMY-37
D	2d	Cloxacillin	Increased hydrolysis of cloxacillin or oxacillin	OXA-1, OXA-10
D	2de	Extended-spectrum cephalosporins	Hydrolyzes cloxacillin or oxacillin and oxyimino-β-lactams	OXA-11, OXA-15
D	2df	Carbapenems	Hydrolyzes cloxacillin or oxacillin and carbapenems	OXA-23, OXA-48

Adapted from [22].

Table 2. Old and new β-lactamase inhibitors and specific activity against different classes of β-lactamases.

Inhibitor	Class A	Class B	Class C	Class D	US FDA status
Inhibitors with β-lactam structure					
Clavulanic acid	+	-	+	+	Approved
Tazobactam	+	-	+	+	Approved
Sulbactam	+	-	+	+	Approved
BLI-489	+	?	+	+	Phase I[†]
BAL 30376	?	+	+	?	Phase I[†]
LK-157	+	?	+	?	Preclinical
Oxapenems	+	?	+	+	Preclinical
Inhibitors without β-lactam structure					
NXL104	+	+	+	+	Phase I and II[†,‡]
ME1071	?	+	?	?	Phase I (Japan)[†]
MK7655	+	?	+	?	Phase I[†]

[†]Complete results not published. [‡]In combination with ceftaroline and ceftazidime, respectively.
+: Active; -: Nonactive; ?: Unknown.
Data taken from [39–55].

New antimicrobials

Novel antimicrobials with potential activity against Gram-negative bacteria include new β-lactamase inhibitors (Table 2), cephalosporins, carbapenems (Table 3) and single agents belonging to different classes (Table 4). The MIC values of some novel agents and their comparators are reported in Table 5.

New β-lactamase inhibitors

In the combination of β-lactam agents with β-lactamase inhibitors, the latter potentates the action of the former by protecting it from enzymatic hydrolysis. Currently used β-lactam/β-lactamase inhibitor compounds are highly active against class A enzymes and various ESBLs, while activity against class C and class D is poor and they are not active against class B β-lactamases [39]. Details of both old and new β-lactam inhibitors are outlined in Table 2. Several compounds are now under investigation as potential β-lactamase inhibitors, in different stages of preclinical and clinical trials, but the results of Phase I and II trials were not published in peer-reviewed journals. These compounds can be classified according to their molecular structure as β-lactams and non-β-lactams. Their main advantage over the older available β-lactamase inhibitors is conferred by the ability to inhibit class C and D enzymes. MIC of various currently

Table 3. Status and pharmacokinetic characteristics of new carbapenems.

Drug	Status	Dose	Administration	Half-life (h)	Activity against			
					Enterobacteriaceae	*Pseudomonas aeruginosa*	*Acinetobacter* spp.	MRSA
Ertapenem	FDA approved	1 g q.d.	iv.	4	+	-	-	-
Doripenem	FDA approved	500 mg t.i.d.	iv.	1	+	+	+	-
Panipenem	Approved in Japan, China and Korea	0.5/0.5 g b.i.d.	iv.	1.1–0.7	+	-	?	-
Biapenem	Phase II	300 mg b.i.d.	iv.	1.03	+	+	+	-
Tomopenem	Phase II	750–1500 mg t.i.d.	iv.	1.7	+	+	+	+
Razupenem	Phase II	?	iv.	?	+	+	?	+
Tebipenem	Phase II	4–6 mg/kg b.i.d.	Oral	?	+	-	?	-

+: Active; -: Nonactive; ?: Unknown; b.i.d.: Twice daily; iv.: Intravenously; MRSA: Methicillin-resistant *Staphylococcus aureus*; q.d.: Every day; t.i.d.: Three-times daily.
Data taken from [65–79].

Table 4. US FDA status and antimicrobial activity of novel antimicrobials against multidrug-resistant Gram-negative strains.

Drug	FDA status	Antimicrobial class	*In vitro* activity against MDR Gram-negative bacteria			
			Escherichia coli	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Acinetobacter baumannii*
ACHN-490	Phase II	Aminoglycosides	+	+	+	+
KB001	Phase II	Antibody fragment	-	-	+	-
CB-182804	Phase I	Polymyxins	+	+	+	+
AN3665	Phase I	Protein-synthesis inhibitors	+	+	+	+
TP-434	Phase I	Tetracyclines	+	+	+	+
MBX agents	Preclinical	*Bis*-indoles	?	+	?	?
BAL30072	Preclinical	Monobactams	+	+	+	+
CHIR-090	Preclinical	LpxC inhibitors	+	?	+	?

+: Active; -: Nonactive; ?: Unknown; MDR: Multidrug resistant.
Data taken from [8,83–107]

used β-lactams, such as piperacillin or ceftazidime, is decreased when administered together with a novel β-lactam inhibitor, and these antibiotics reactivate against ESBL-producing strains. Moreover, the combined use of some new β-lactamase inhibitors with carbapenems makes the latter active against MBL-producing strains. Although no large clinical studies on usefulness of new β-lactam inhibitors have been performed, they seem particularly promising as therapeutic agents.

■ Inhibitors with β-lactam structure

Imidazole-substituted 6-methylidene-penem molecules

The unique structure of these compounds imparts potent activity against class A and C β-lactamases, such as AmpC, which is not observed with the currently used inhibitors. Several novel compounds demonstrated excellent *in vitro* inhibition of the TEM-1 and AmpC enzymes with significantly higher activity compared with tazobactam, which already has better class C activity than clavulanic acid [40,41]. *In vitro* and *in vivo* tests showed synergistic activity of these compounds when combined with piperacillin (90% of the tested strains were susceptible and a synergistic effect was observed in animal models) [40,42]. Among these agents, BLI-489 is the compound with the most promising clinical data. It has shown activity against class A, C and D enzymes, including ESBL and some ESBL-producing strains nonsusceptible to piperacillin/tazobactam were susceptible to piperacillin/BLI-489 [43,44].

Monobactam-based structure compounds

BAL 30376 is a β-lactamase inhibitor and is a combination of BAL 19764 (a siderophore monobactam), BAL 29880 (a bridged monobactam which is a class C inhibitor) and clavulanic acid [45]. BAL 30376 is active against various Gram-negative bacteria and MICs were observed in a range of at most 0.06–4 mg/l, including most carbapenem-resistant strains, while higher MICs were observed for a few strains of *Acinetobacter* spp., *Enterobacter* spp. and *P. aeruginosa* [44]. It was found active at 4 mg/l against MBL-producing strains and some isolates of *Burkholderia cepacia* and carbapenemase-producing *A. baumannii*; however, it was inactive against KPC-producing strains [45].

Trinems

LK-157 is a tricyclic carbapenem inhibitor of class A and C β-lactamases [46]. LK-157 decreased the MICs of aztreonam, ceftazidime and cefuroxime for *B. fragilis* and a wide range of β-lactamases-producing *Enterobacteriaceae* members. However, it was noted that LK-157 did not affect the MICs of aztreonam, ceftazidime or cefuroxime against CTX-M-producing strains [44]. In combination with various antibiotics, it restored the activity against ESBLs, except for CTX-M and KPC-producing strains [46].

Oxapenems

Four β-lactamase inhibitors, members of oxapenems, are being developed (AM-112–115), and they express activity against class A, C and D enzymes [47]. AM-114 and -115 displayed the most potent activity against class A, comparable to that of clavulanic acid. Activity against class C and class D enzymes was similar for all four agents and was superior to that of clavulanic acid. A synergistic activity of ceftazidime with the oxapenems was demonstrated against SHV- and TEM-producing *E. coli* [48]. Ceftazidime alone was also less effective than ceftazidime plus AM-112 against a strain

Table 5. MIC_{90} of some new agents and comparators against Gram-negative rods in different studies.

Bacteria (number of isolates)	MIC_{90} (range), μg/ml		Ref.
Novel β-lactams inhibitors			
	Piperacillin + tazobactam	**Piperacillin + BLI-489**	[43]
Escherichia coli (52)	2 (0.5–128)	2 (0.25–64)	
E. coli ESBL-A† (31)	>128 (1–>128)	16 (1–32)	
E. coli AmpC (17)	32 (2–64)	16 (1–16)	
Enterobacter cloacae (52)	>128 (0.5–>128)	16 (0.5–16)	
Klebsiella pneumoniae (54)	16 (1–>128)	8 (1–16)	
K. pneumoniae ESBL-A (36)	>128 (2–>128)	2–128 (32)	
K. pneumoniae AmpC (30)	>128 (8–>128)	>128 (4–>128)	
Acinetobacter spp. (30)	32 (≤0.12–>128)	16 (0.5–32)	
Pseudomonas aeruginosa (54)	>128 (4–>128)	64 (4–>128)	
	Meropenem	**Meropenem + ME1071**	[51]
MBL-producing *P. aeruginosa* (174)	>64 (0.5–>64)	>64 (0.25–>64)	
Non-MBL-producing *P. aeruginosa* (16)	64 (0.12–64)	64 (0.5–64)	
Novel carbapenems			
	Imipenem	**Tebipenem**	[77]
E. coli (42)	0.125 (≤0.063–0.25)	≤0.063 (≤0.063)	
K. pneumoniae (34)	≤0.063 (0.125–0.5)	0.25 (≤0.063–0.5)	
P. aeruginosa (53)	25 (0.39–25)	100 (3.13–>100)	
	Meropenem	**Tomopenem**	[72]
E. coli (25)	≤0.03 (≤0.03–0.25)	≤0.03 (≤0.03–0.12)	
K. pneumoniae (25)	≤0.03 (≤0.03–0.06)	0.06 (≤0.03–0.12)	
P. aeruginosa (100)	16 (0.06–>32)	4 (0.06–32)	
	Imipenem	**ME-1036**	[78]
E. coli (27)	0.125 (0.063–0.25)	0.125 (0.031–0.25)	
K. pneumoniae (26)	0.125 (0.063–0.25)	0.063 (0.031–0.125)	
P. aeruginosa (27)	32 (1–32)	1024 (256–1024)	
Novel monobactams			
	Meropenem	**BAL-30072**	[80]
P. aeruginosa (265)	16	4	
Acinetobacter spp. (40)	16	8	
Novel polymyxins			
	Colistin	**CB-182804**	[92]
E. coli (80)	0.5	2	
K. pneumoniae (81)	2	4	
P. aeruginosa (100)	2	2	
Acinetobacter spp. (81)	4	4	

†Class A extended-spectrum β-lactamases.

MBL: Metallo-β-lactamases; MIC_{90}: Minimum inhibitory concentration required to inhibit the growth of 90% of organisms.

Table 5. MIC_{90} of some new agents and comparators against Gram-negative rods in different studies (cont.).

Bacteria (number of isolates)	MIC_{90} (range), µg/ml		Ref.
***Bis*-indole agents**			
	Meropenem	**MBX 1196**	[96]
A. baumannii (30)	>8 (0.5–>8)	1 (0.06–1)	
Protein-synthesis inhibitors			
	Imipenem	**AN3365**	[98]
P. aeruginosa (101)	>64 (0.25–>64)	8 (1–16)	
Acinetobacter spp. (25)	>64 (8–>64)	16 (4–32)	

†Class A extended-spectrum β-lactamases.
MBL: Metallo-β-lactamases; MIC_{90}: Minimum inhibitory concentration required to inhibit the growth of 90% of organisms.

of *E. coli* containing TEM-1 and CTX-M-1 [49]. An enhanced activity of oxapenems in combination with ceftazidime was also noted against *Pseudomonas* strains and MRSA [48].

■ Inhibitors with no β-lactam structure

NXL104

NXL104 is a non-β-lactam compound that inhibits β-lactamases through the formation of a stable covalent carbamoyl linkage. It showed a four- to 8000-fold increase in the activity of ceftazidime and cefotaxime against CTX-M-producing *Enterobacteriaceae* [44]. NXL104 showed a stronger inhibition of P99 (class C) than tazobactam, while clavulanic acid was inactive. Moreover, the combination with NXL104 restored the activity of ceftazidime and cefotaxime against isolates producing the class A carbapenemases [50]. NXL104/ceftazidime combination is currently undergoing clinical trials: NXL104/ceftazidime plus metronidazole versus meropenem in complicated intra-abdominal infections (study completed, results not published yet) and NXL104/ceftazidime versus imipenem/cilastin in UTIs (trial ongoing).

Maleic acid derivates

ME1071, previously known as CP3242, is a inhibitor of MBL that competitively inhibits both IMP-1 and VIM-2. It significantly lowered the MICs of biapenem in a concentration-dependent manner against MBL-producing *P. aeruginosa* [51]. This effect was also observed for IMP or VIM producing *E. coli*, *Serratia marcescens*, *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* [44,51].

MK-7655

MK-7655 is active against class A and class C carbapenemases and has a good *in vitro* and *in vivo* activity in combination with imipenem [52–54]. A Phase I, randomized, double-blind, placebo-controlled study showed that MK-7655 was generally well tolerated following a single intravenous dose and it demonstrated a favorable pharmacokinetics profile when administered in combination with cilastin and imipenem [55].

New cephalosporins

New cephalosporins (ceftobiprole, ceftaroline and CXA-101) are very resistant to penicillinases and the first two are also active against MRSA. Although they have some activity against Gram-negative bacteria, there is no evidence of an enhanced activity against MDR strains when compared with older cephalosporins.

■ Ceftobiprole

Ceftobiprole (formerly BAL-9141) is an active component of the prodrug named ceftobiprole medocaril (formerly BAL-5788), and in comparison to older compounds, expanded activity against Gram-positive bacteria was observed. A randomized, double-blind trial published in 2008, reported that ceftobiprole monotherapy was as effective as vancomycin combined with ceftazidime for treating adults with complicated skin and skin-structure infections (SSSIs) due to Gram-positive and Gram-negative bacteria [56]. Following these results, ceftobiprole has been approved for this indication in Canada and Switzerland, but the US and European approval procedures are ongoing. Ceftobiprole has a low potential for inducing chromosomal AmpC β-lactamases, but it is hydrolyzed by most ESBLs and MBLs [57].

■ Ceftaroline

Ceftaroline is a novel semisynthetic anti-MRSA cephalosporin with a broad-spectrum activity, approved by the FDA in 2010 for the treatment of acute bacterial SSSIs and community-acquired bacterial pneumonia (CAP) [58]. *In vitro*, ceftaroline was synergistic with tazobactam against MDR Gram-negative pathogens such as ESBL-producing *E. coli* and *K. pneumoniae* [59]. Nevertheless, in Phase III clinical trials, ceftaroline

was less active than currently used antimicrobial agents against Gram-negatives, as a combination of vancomycin plus aztreonam demonstrated higher favorable microbiological response rates (86.3 vs 93.6%) [60]. In particular, the efficacy of ceftaroline against non-ESBL-producing *E. coli* and *K. pneumoniae* was comparable to that of aztreonam, but was lower against *P. aeruginosa* and *Proteus mirabilis* infection [60].

CXA-101

CXA-101 (previously FR264205), is a novel cephalosporin of particular interest in the treatment of Gram-negative infections. It has a potent activity against *P. aeruginosa,* which is not diminished by AmpC overexpression, porin mutations or efflux pumps [61]. CXA-101 is under development as a single agent and in combination with tazobactam (Phase II) [62].

New carbapenems

Carbapenems are a class of broad-spectrum β-lactams identified in the late 1970s. The main advantage of this class of antibiotics is due to their stability to hydrolysis by many ESBLs. At present, meropenem and imipenem/cilastatin are widely used and recommended for treatment of severe infections. Imipenem is hydrolyzed by renal dehydropeptidase I (DHP-I) and this process produces a nephrotoxic compound; consequently cilastatin, the DHP-I inhibitor without antibacterial activity, is always coadministered with imipenem with a 1:1 ratio. Apart from panipenem, older and new carbapenems do not require DHP-1 inhibitors. Several mechanisms of resistance to carbapenems are known:

- Carbapenemases or other β-lactamases with weak hydrolyzing activity;
- Changes in membrane permeability through the loss of specific porins;
- Efflux pumps;
- Structural changes in protein-binding proteins; but the resistance phenotype is usually a result of an interplay involving more than one mechanism [63].

Following evaluation of pharmacokinetic–pharmacodynamic properties, clinical data and MIC distributions that include recently described carbapenemase-producing strains, the Clinical and Laboratory Standards Institute (CLSI) established revised interpretative criteria for carbapenems (Table 6) [201]. The method currently endorsed by the CLSI is the modified Hodge Test, but it may not be the ideal phenotypic confirmatory test for KPCs since false positive results have been reported [64].

Over ten novel compounds are reported in different phases of clinical development; two of them are currently marketed and available (ertapenem and doripenem), others are in Phase II clinical trials, while several are still being investigated in preclinical studies. Of note, tebipenem is a novel carbapenem that can be administered orally.

Ertapenem

Ertapenem was licensed in the USA in 2001 and in Europe in 2002, with indications including: intra-abdominal infections, complicated SSSIs, complicated UTIs, acute pelvic infections and CAP. The main limitation of ertapenem is its poor activity against nonfermenting Gram-negative bacteria, such as *P. aeruginosa, Acinetobacter* spp and *B. cepacia* [65]. The role of ertapenem in the treatment of ventilator-associated pneumonia was investigated and a pilot study found ertapenem useful for treating early-onset ventilator-associated pneumonia due to ESBL producers, with clinical success achieved in 80% of patients and microbiological success in 75% of cases [66].

Doripenem

Doripenem is a new broad-spectrum, recently marketed, parenteral carbapenem. It is as active against Gram-positive cocci such as methicillin-susceptible *S. aureus* (MSSA) and coagulase-negative staphylococci, but activity against MDR *Enterobacteriaceae* is similar to that of meropenem, and two- to three-fold superior to imipenem [67–69].

Biapenem

Biapenem was approved in Japan in 2002 and its prominent feature is related to its high concentration in respiratory tissue and other body fluids. It has a broad spectrum of activity including Gram-positive bacteria such as *S. pneumoniae* (also penicillin-resistant strains), MSSA and Gram-negatives including *A. baumannii*, and ESBL-producing *Enterobacteriaceae*, while, moderate activity (median MIC 8 mg/l) was found against *P. aeruginosa* [70,71].

Tomopenem

Tomopenem (CS-023) seems to have a very low rate of spontaneous emergence of resistance and *in vitro* activity against β-lactam-susceptible and -resistant strains, including MRSA, ceftazidime-resistant *P. aeruginosa* and ESBL-producing *Enterobacteriaceae* [72]. Its activity against Gram-negatives was found to be similar or better than meropenem [68]. It seems more effective than imipenem and meropenem against MRSA (with a MIC of 4 mg/l) and is characterized by a low protein binding ratio, a feature that can be useful since the plasmatic active fraction achieves rapid equilibrium with intracellular fluid [44].

Razupenem

Razupenem (previously know as SMP-601) is a novel compound in Phase II of evaluation. *In vitro*, razupenem was found active against ESBL-producers, but its activity was significantly reduced by AmpC enzymes and carbapenemases [73].

Panipenem/betamipron

The combination of panipenem with betamipron, similar to imipenem/cilastatin, is necessary because betamipron inhibits the renal uptake of panipenem. It is approved in Asia for the treatment of lower respiratory tract infections, UTIs, obstetrical/gynecological and surgical infections. The clinical efficacy of panipenem/betamipron was demonstrated in three large, randomized, Phase III clinical trials comparing this drug with imipenem/cilastatin in adults with respiratory and UTIs [74]. Its spectrum of activity includes *Enterobacteriaceae* and common respiratory tract pathogens, although meropenem remains the most active carbapenem against *Haemophilus influenzae* [75]. Panipenem is not active against *E. faecium,* and *Stenotrophomonas maltophilia* and *P. aeruginosa* seem to be resistant, showing MIC_{90} values of 12.5–25 mg/l [75].

Tebipenem

Tebipenem pivoxil is a prodrug of an oral carbapenem with a high degree of stability to DHP-I. While tebipenem is inactive against MBL-producing pathogens and MRSA, good activity against *K. pneumoniae* and *E. coli* has been reported [67]. It might become a specific antibiotic for the treatment of persistent otitis media, UTI and bacterial pneumonia in pediatric patients, given its favorable pharmacokinetic profile and oral administration [76,77].

ME1036

ME1036, previously named CP5609, is a novel parenteral carbapenem, active against *Enterobacteriaceae* and Gram-positives [78]. Recently, the activity of ME1036 and comparators was evaluated against clinical blood culture isolates from patients with bacteraemic CAP requiring hospitalization, and ME1036 had an excellent activity against all isolates, including MRSA [79].

The latter three carbapenems, similarly to ertapenem, are all inactive against *P. aeruginosa*. Particular advantages of tebipenem include good oral bioavailability and activity against *Acinetobacter* spp.

Novel monobactams

BAL30072 is a monosulfactam with siderophore side chain and penicillin-binding protein affinities. Good activity against MDR nonfermenting rods has been reported [80], and activity against MDR *Acinetobacter* spp. is further enhanced by iron limitation, the natural condition in the human body. Such enhanced activity results from induction of the siderophore receptors in *Acinetobacter* [81,82].

Table 6. New carbapenem breakpoints for *Enterobacteriaceae*.

	Susceptible	Intermediate	Resistant
New carbapenem breakpoints, MIC (μg/ml)			
Doripenem	≤1	2	≥4
Ertapenem	≤0.25	0.5	≥1
Imipenem	≤1	2	≥4
Meropenem	≤1	2	≥4
Old carbapenem breakpoints, MIC (μg/ml)			
Ertapenem	≤2	4	≥8
Imipenem	≤4	8	≥16
Meropenem	≤4	8	≥16

MIC: Minimum inhibitory concentration.
Adapted from [201].

Novel aminoglycosides

In this old class of antibacterials, ACHN-490 is a novel compound that evades plasmid-mediated aminoglycoside-modifying enzymes and is currently undergoing a Phase II evaluation in complicated UTI [32,83]. Compared to currently used aminoglycosides, it has shown better activity against 82 carbapenem-resistant *Enterobacteriaceae*. However, 16 of 17 isolates with NDM-1 enzyme were resistant to ACHN-490 (with MICs ≥64 mg/l) and all the other currently used aminoglycosides [83]. Recently, antimicrobial activity of ACHN-490 has been tested against clinical isolates of *A. baumannii, P. aeruginosa, E. coli* and *K. pneumoniae* [84,85]. The MICs of ACHN-490 for *A. baumannii* were lower than those of traditional aminoglycosides, whereas against *P. aeruginosa* the activity was similar to that of amikacin [84]. ACHN-490 was active against most isolates of *E. coli* and *K. pneumoniae*, including MDR strains, and MIC_{90} values for ACHN-490 were four-times lower than for amikacin [85].

Novel tetracyclines

TP-434 is a broad spectrum fluorocycline with *in vitro* antimicrobial activity against all major pathogens (except for *P. aeruginosa*) and efficacy in animal models against MDR Gram-negatives [86]. TP-434 and comparator agents were tested against 398 clinical strains of Gram-negatives [87]. An intravenous formulation of TP-434 is currently undergoing Phase I trial, while an oral formulation is in preclinical development [8].

Novel polymyxin analogs

CB-182804 is a novel polymyxin B analog currently in Phase I of clinical development, which is active against MDR *P. aeruginosa*, *A. baumannii*, *K. pneumoniae* and *E. coli* [88,89]. Similarly to polymyxin B, CB-182804 has been shown to bind to lipopolysaccharide leading to an increase in cell membrane permeability, leakage of cell contents and cell death [90]. Resistance to CB-182804 in *A. baumannii* may result from alterations in membrane structure and permeability, since these mutants also show increased susceptibilities to other antibiotics [91]. Recently, CB-182804 has been tested against colistin-susceptible and -resistant isolates, and was very active against colistin-susceptible strains that were resistant to all other currently available antimicrobials, but cross-resistance with colistin was observed in the colistin-resistant bacteria [92]. Moreover, the addition of rifampicin was reported to lower MICs of CB-182804 more than MICs of polymyxin B and to restore activity against resistant isolates [93]. In monkeys, CB-182804 demonstrated less nephrotoxicity when compared with polymyxin B [94].

Bis-indole antibiotics

The *bis*-indole antibiotics enter bacterial cells and interact with DNA, resulting in inhibition of DNA and RNA synthesis, ssDNA breaks and induction of the SOS response. Therefore, it is likely that the DNA binding activity of these compounds is directly related to their mechanism of antibacterial action [95]. Eight *bis*-indole agents have shown potent *in vitro* activity against *K. pneumoniae,* including MDR and carbapenem-resistant strains, but further studies are warranted to determine their potential for clinical use [96].

Boron-containing antibacterial protein synthesis inhibitors

The novel boron-containing antibacterial, AN3365, inhibits protein synthesis by inhibiting tRNA-synthetase [97]. An excellent *in vitro* activity against various Gram-negative bacteria, including ciprofloxacin-resistant isolates, was reported [98,99], as well as a favorable pharmacokinetic profile in mice [100].

Novel outer membrane synthesis inhibitors

LpxC is a deacetylase involved in the biosynthesis of lipopolysaccharide in cell of Gram-negatives and it is a validated target for developing novel antimicrobial agents such as CHIR-090, a potent inhibitor of LpxC [101,102]. These agents have a novel mechanism of action and are active against MDR *P. aeruginosa* and MDR *E. coli.* (MIC <1 mg/ml) [8]. Moreover, they have the potential to be administered orally as well as intravenously [8].

Antibiotics targeting novel sites of the 50S ribosomal subunit

Following a novel approach to antibiotic research and development, the Rχ-04 program has identified 1400 crystal structures of known and new antibiotics bound to the 50S ribosome, thereby defining an available antibiotic design space in a large ribosomal binding site [8]. Among 100 new analogs, several showed activity against *E. coli* and *P. aeruginosa,* including MDR strains [103].

Novel antimicrobial peptides

Antimicrobial peptides are a group of molecules produced by all types of living organisms, considered to be part of the host innate immunity. The ability of these natural molecules to kill MDR microorganisms has gained them considerable attention and clinical interest [104]. Among them, a group of peptides was investigated as antibacterial agents against *A. baumannii,* and one of these compounds, mastoparan, showed good activity against both colistin-susceptible and -resistant strains, suggesting that the mechanism of action of this antimicrobial peptide may be different than that used by colistin [105]. For more details on this interesting new class of antimicrobial, see a review recently published [104].

Antibody fragments

Using antibodies as antimicrobial agents is another novel field to be exploited in the research for solutions against MDR bacteria. KB001 is a high-affinity antibody fragment in the development of a treatment for *P. aeruginosa* infections [106]. The type-III secretion system is responsible for the delivery of exotoxins to target mammalian cells. KB001 binds to the PcrV protein of this secretion system, inhibits its activity and thereby reduces the pathogenicity of *P. aeruginosa* and its toxicity to host immune and epithelial cells [107].

Optimizing the use of already existing agents & limiting the spread of MDR strains

Pending the release of new drugs active against these MDR bacteria, the Probability of target attainment of Antibacterial agents Studied for Susceptibility and Pharmacodynamic Optimization in Regional Trials (PASSPORT) study demonstrated that, especially for nonfermenting Gram-negative bacilli such as *A. baumannii* and *P. aeruginosa*, high-dose prolonged infusions of cefepime, ceftazidime, doripenem and meropenem have higher probabilities of achieving bactericidal exposure compared with standard 30-min infusion regimens, reducing the risk of treatment failure [108]. For example, prolonged infusion of doripenem has been reported to increase its efficacy, particularly against strains with higher MIC values: doripenem 500-mg dose infused over 1 h every 8 h would be expected to be effective

for bacilli with MICs to doripenem of 1 µg/ml or less, while a 4-h infusion time improved target attainment for pathogens with a MIC as high as 4 µg/ml [109]. Thus, prolonged infusion seems to partially restore the efficacy of β-lactams against strains with decreased susceptibility.

Given the increase in MDR strains and limited therapeutic options available, containing the spread of resistant pathogens is crucial. Infection control policies should be revised and implemented to avoid transmission of MDR Gram-negatives [110].

Future perspective

Infections due to MDR Gram-negative bacteria, such as ESBL or carbapenemase-producing *Enterobacteriaceae* and *A. baumannii* or *P. aeruginosa* are a serious and emerging problem in healthcare and community settings, respectively. Although some promising novel molecules are in the late-stage of development, few new antibiotics have been advanced into clinical practice for the treatment of most of the ESKAPE pathogens. Moreover, among them, only few are active against MDR organisms.

Novel anti-Gram-negative agents include β-lactamase inhibitors, cephalosporins, carbapenems and other compounds belonging to old and new classes of antimicrobials. Fifth-generation cephalosporins acquired activity against MRSA, but except for CXA-101, they offer no advantage against MDR Gram-negatives. While some novel carbapenems are active against resistant Gram positives, when difficult Gram-negatives are involved their activity is similar to that of meropenem. Thus, β-lactamase inhibitors seem the most promising group of new compounds, as they might restore the activity of already known β-lactams against β-lactamase-producing strains. Although some of them seem to inhibit carbapenemases as well, their real clinical utility will be known only after results of clinical trials are available.

Innovative strategies against difficult Gram-negatives, such as *P. aeruginosa* have been proposed: antibody fragments can inhibit virulence factors reducing pathogenicity and are currently undergoing Phase II studies. Other novel antimicrobials include *bis*-indoles, boron-containing antibacterial protein synthesis inhibitors, outer membrane synthesis inhibitors and antimicrobial peptides. However, they are still undergoing preclinical or Phase I studies, so truly new therapeutic options against MDR strains are still far from clinical practice.

Finally, appropriate surveillance of infections due to MDR pathogens and antimicrobial stewardship programmers in human medicine, agriculture and animal husbandry are mandatory. This goal is achievable with the coordination between healthcare professionals, to ensure thorough surveillance and to optimize prescribing the most suitable antibiotic therapy.

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In the past 5 years, Matteo Bassetti has been an advisor/consultant for Gilead Scienses, Merck Sharp and Dohme, Novartis, Pfizer and Schering Plough. He has been paid for talks on behalf of Angelini, Astellas, Astra Zeneca, Aventis, Bayer, Cephalon, Glaxo SmithKline, Gilead Scienses, Jansen Cilag, Merck Sharp and Dohme, Novartis and Pfizer. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Executive summary

- A worldwide increase in antimicrobial resistance of Gram-negative bacteria has been noted, probably owing to the widespread use of antibiotics.
- β-lactamase inhibitors seem promising agents in restoring activity of already existing β-lactams, although few of them are active against carbapenemases.
- New cephalosporins and carbapenems offer little advantage against multidrug-resistant Gram-negative strains.
- New groups of antimicrobials include *bis*-indoles, boron-containing antibacterial protein synthesis inhibitors, outer membrane synthesis inhibitors and antimicrobial peptides.
- Very few agents will be shortly available for clinical practice.
- Hopefully, based on the growing knowledge of resistance mechanisms, other agents with a novel mechanism of action, designed specifically against resistant strains, will be developed.

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