CRITICAL CARE

REVIEW

New treatment options against gram-negative organisms

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Introduction

In recent years, infections caused by multi-drug resistant (MDR) pathogens have become a serious problem, especially in the nosocomial setting. The World Health Organization (WHO) has identified antimicrobial resistance as one of the three most important problems for human health. Some authors have summarized this phenomenon with the word 'ESKAPE', to include the most frequent MDR microorganisms: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp. [1]. Resistance to the current library of antibacterial drugs is a serious problem in all parts of the world including the Asia-Pacific region, Latin America, Europe, and North America.

Numerous classes of antimicrobials are currently available for physicians to use in the treatment of patient with infections; however, the pace of antibiotic drug development has slowed during the last decade (Fig. 1). In particular, the pharmaceutical pipeline of antibiotics active against MDR Gram-negative bacteria is very limited. New antibiotics that have been discovered and introduced into clinical practice in the last few years are active mostly against Gram-positive organisms, whereas when targeting resistant Gram-negative bacteria, clinicians are forced to rediscover old drugs, such as polymixins and fosfomycin. Among new antibacterials active against Gram-negative microorganisms that are already on the market, tigecycline, the first Food and Drug Administration (FDA)-approved representative of the glycylcyclines, and doripenem, a new carbapenem, seem the most promising.

Since 2001, different agencies and societies have tried to draw attention to the significant lack of new antibiotics for Gram-negative pathogens. In fact, in 2004 the Infectious Diseases Society of America (IDSA) issued their report, "Bad Bugs, No Drugs: As Antibiotic Discovery Stagnates, A Public Health Crisis Brews," which proposed incentives to reinvigorate pharmaceutical investment in antibiotic research and development [2]. In 2007, the IDSA and the FDA repeated their call for an increase in new antibacterial research to develop next-generation drugs [3]. Recently, the IDSA supported an initiative of developing 10 new systemic antibacterial drugs through the discovery of new drug classes, as well as exploring possible new molecules from existing classes of antibiotics (the "10 x '20" initiative, endorsed by the American Academy of Pediatrics, American Gastroenterological Association, Trust for America's Health, Society for Healthcare Epidemiology of America, Pediatric Infectious Disease Society, Michigan Antibiotic Resistance Reduction Coalition, National Foundation for Infectious Diseases, and European Society of Clinical Microbiology and Infectious Diseases) [4].

The profile of resistance to currently used antimicrobial agents and the development of new anti-Gram-negative agents, with a particular attention to cephalosporins, β-lactamase inhibitors and carbapenems will be discussed.

Mechanism of resistance to currently used antimicrobial agents in multi-drug resistant gram-negative bacteria

β-lactamase-mediated resistance is the most important and efficient method of β-lactam resistance for Gram-negative bacteria. The origin of β-lactamases is presumably ancient and their development evolved to combat natural β-lactams. However, resistance has been heavily influenced over the years by the widespread administration of these antibiotics in clinical practice. For example, the rapid increase in resistance to the widely-used ampicillin in the early 1960s turned out to be due to

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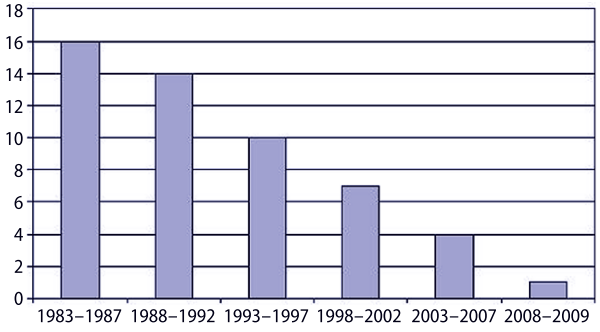

Figure1. New antibacterial agents approved in the United States, 1983–2009. From [3] with permission.

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 *Eschericihia coli* isolated from a patient named Temoniera, hence named TEM). The further selection of resistant mutants led to the appearance of extended-spectrum β-lactamases (ESBLs) that now compromise the use of even third-generation cephalosporins. In the 1990s, the pharmaceutical industry introduced carbapenems, which are extremely stable to degradation by β-lactamases. However, a variety of β-lactamases that are capable of hydrolyzing these antibiotics, including imipenemase (IMP), Verona integron-encoded MBL (VIM), *K. pneumoniae* carbapenemase (KPC) and oxacillinase (OXA) are being increasingly seen in Gram-negative bacterial isolates.

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) [5,6]. Briefly, class A enzymes are plasmid-mediated penicillinases, constitutively expressed and susceptible to inhibition by β-lactamase inhibitors; representative enzymes include TEM and sulfhydryl reagent variable (SHV) subclasses. Some evolved class A β-lactamases accept extended-spectrum cephalosporins as substrates and are known as ESBLs, even if 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 β-lactamase inhibitors. Finally, class D β-lactamases have a substrate preference for oxacillin and are therefore called oxacillinases. This class diversity is a crucial aspect for antimicrobial therapy. Recently, a new plasmid MBL, the New Delhi MBL (NDM-1) was identified in *K. pneumoniae* and *E. coli* recovered from a Swedish patient who was admitted to hospital in New Delhi, India [7]. Of particular concern is that NDM enzymes are present in *E. coli*, the most common cause of community-associated urinary tract infections. The NDM-producing bacteria are resistant to many groups of antibiotics, including fluoroquinolones, aminoglycosides, and β-lactams (especially carbapenems), and are susceptible only to colistin and tigecycline [7]. Nevertheless, even these two agents might lose their activity.

The target of the antimicrobial action of colistin is the bacterial cell membrane and studies on colistin-resistant *P. aeruginosa* strains have reported alterations at the outer membrane of the cell, leading to resistance [8]. Thus, colistin might not be a long-standing treatment option for MDR Gram-negative bacteria. 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 minimum inhibitory concentrations (MICs) of the targeted pathogen exceed the Cmax of the drug, which is almost the rule for all targeted *A. baumannii* strains [9]. The genetic basis of development of resistance has been investigated with molecular studies and efflux pumps seem to be the most important mechanism of decreased susceptibility. Various efflux pumps have been reported in *E. coli, E. cloacae, K. pneumoniae* and *A. calcoaceticus-A. baumannii* [10].

Gram-negative resistant bacteria and drug development needs

Given the continuous increase in antibiotic resistance, the IDSA's Antimicrobial Availability Task Force identified development needs for the ESKAPE pathogens, including Gram-negatives such as *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *P. aeruginosa* and *Acinetobacter* spp. [1,11].

In *Enterobacteriaceae*, the main resistance problems stem from production of ESBL, inducible chromosomal cephalosporinases and carbapenemases, including *K. pneumoniae* carbapenemase (KPC)-hydrolyzing β-lactamases [12]. Infections due to ESBL-producing *E. coli* and *Klebsiella* spp. continue to increase in frequency and severity. In an interesting meta-analysis of 16 studies, bacteremias caused by ESBL-producing pathogens were significantly associated with delayed initiation of effective therapy and increased crude mortality [13]. Additionally, *Enterobacter* causes an increasing number of health care-associated infections and is increasingly resistant to multiple antibacterials [12]. *Enterobacter* infections, especially bloodstream infections, are associated with significant morbidity and mortality [14]. Unfortunately, drugs in late stage development, as well as the recently approved doripenem, offer little advantage over already existing carbapenems for treating infections due to ESBL-producing bacteria. Moreover, carbapenem-resistant *Enterobacteriaceae* are increasingly recognized

Table 1. Classification schemes for bacterial β-lactamases.

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

Adapted from [5].

as the cause of sporadic infections and outbreaks worldwide [15,16]. Thus, tigecycline and the polymyxins, including colistin, have been used with variable success rates and there are currently no antibacterials in advanced development for these highly resistant pathogens [17]. Aggressive infection-control practices are required to abort epidemic outbreaks.

Rates of infection by resistant *P. aeruginosa* continue to increase in the United States and globally, as does resistance to β-lactams, quinolones, aminoglycosides, and carbapenems [18]. Resistance of *P. aeruginosa* to polymyxins has also been reported. Patients at risk include those in the intensive care unit (ICU), particularly if they are ventilator dependent, and individuals with cystic fibrosis. To date, no drug in clinical development addresses the issue of MDR or offers a less toxic alternative to the polymyxins for treating *P. aeruginosa*.

Last but not least, the incidence of infections due to MDR *Acinetobacter* spp. continues to increase globally [19]. Unfortunately, no agents against *Acinetobacter* spp. are under development and infections caused by this pathogen are emblematic of the mismatch between unmet medical needs and the current antimicrobial research and development pipeline.

New β-lactamase inhibitors

In β-lactam agent/β-lactamase inhibitor combinations, the latter agent 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 and various ESBLs, whereas activity against class C and class D enzymes is poor [20,21].

Several compounds are now under investigation as potential β-lactamase inhibitors, in different stages of pre-clinical and clinical studies. They can be classified as β-lactams and non-β-lactams according to their molecular structure. Their main advantage over the older β-lactamase inhibitors is conferred by their ability to

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	FDA Status
Inhibitors with β-lactam structure					
Clavulanic acid	++	–	+	+	Approved
Tazobactam	++	–	+	+	Approved
Sulbactam	++	–	+	+	Approved
BLI-489	++	UA	++	++	Pre-clinical
Ro 48–1220	+++	UA	++	UA	Pre-clinical
4-phenyl cyclic phosphate	+++	UA	++	UA	Pre-clinical
C3-methylene-modified group penicillin sulfone	UA	UA	++	UA	Pre-clinical
BAL 30376	UA	+	++	UA	Pre-clinical
LK-157	++	UA	UA	UA	Pre-clinical
Oxapenems	++	UA	++	++	Pre-clinical
Inhibitors without β-lactam structure					
NXL104	+++	+	++	++	Phase II
ME1071	UA	++	UA	UA	Pre-clinical

UA, unknown activity; FDA: Food and Drug Administration

inhibit class C and D enzymes. Thus, the MICs of various currently used β-lactams, such as piperacillin or ceftazidime, is decreased when administered together with a novel β-lactam inhibitor, and these antibiotics become active against ESBL-producing strains. Moreover, their combined use with carbapenems, makes the latter active against MBL-producing strains.

Although the results of studies on the clinical usefulness of new β-lactam inhibitors are not yet available, they seem particularly promising as therapeutic agents. Details of new β-lactam inhibitors are outlined in Table 2.

Inhibitors with a β-lactam structure

Imidazole-substituted 6-methylidene-penem molecules

The unique structure of these compounds (they contain byciclic or triciclic substituents connected by a methydilene linkage to the 6 position of the β-lactam ring) imparts potent activity against class A and C β-lactamases, such as the AmpC enzyme, which is not observed with the currently used inhibitors. Several novel compounds demonstrated excellent *in vitro* inhibition of the TEM-1 enzyme (class A β-lactamases) and AmpC enzyme with significantly higher activity compared with tazobactam [22]. *In vitro* tests showed synergistic activity of these compounds when combined with piperacillin with susceptibility of 90% of the tested organisms; animal models confirmed the synergistic effect with piperacillin [22,23]. Among these agents, BLI-489 is the compound with the most promising clinical data. It has shown activity against molecular class A, C and D enzymes, including ESBL as well as class C β-lactamases; some strains that were class C or ESBL producers, classified as non-susceptible to piperacillin/tazobactam, were found to be susceptible to piperacillin/BLI-489 [24].

2β-alkenyl penam sulfones

2β-alkenyl penam sulfones, another group of inhibitors with β-lactam structure, inhibit most of the common types of β-lactamases, with a level of activity depending strongly on the nature of the substituent in the 2β-alkenyl group. Richter et al. demonstrated that Ro 48-1220, the most active inhibitor from this class of compounds, enhanced the action of ceftriaxone against a broad selection of organism producing β-lactamases, including strains of cephalosporinase-producing *Enterobacteriaceae* [25]. In a different study, Ro 48-1220 was at least 15 times more effective than tazobactam against the class C enzymes and reduced the MIC values of ceftriaxone and ceftazidime against the class A plasmid-mediated β-lactamases; less potency was exerted towards SHV-type β-lactamases [26].

4-phenyl cyclic phosphate

4-phenyl cyclic phosphate is a monocyclic acyl phosphonate. It has an irreversible reaction with *E. Cloacae* P99 β-lactamase (Class C). This compound also bound TEM-2 and P99 β-lactamases non-covalently. Similar to other novel inhibitors, it is effective against class A and class C enzymes [27].

C3-modified penicillin sulfones

Buynak et al. reported that C3-methylene-group penicillin sulfones were 10-fold more active against class C β-lactamases compared to sulbactam [28].

Monobactam-based structure compounds

BAL 30376 is a β-lactamase inhibitor and is a combination of BAL 0019764 (a siderophore monobactam), BAL 0029880 (a bridged monobactam which is a class C inhibitor), and clavulanic acid [24]. Page et al. [29] demonstrated the *in vitro* activity of BAL 30376 against various Gram-negative bacteria. MICs were observed in a range of ≤ 0.06–4 mg/l, including most carbapenem-resistant strains. Higher MICs were observed for a few strains of *Acinetobacter* spp., *Enterobacter* spp. and *P. aeruginosa*.

Tricyclic carbapenem inhibitors

LK-157 is a tricyclic carbapenem inhibitor of serine β-lactamases [24]. LK-157 decreased the MICs of aztreonam, ceftazidime, and cefuroxime for *B. fragilis* and a wide range of β-lactamases-producing *Enterobacteriaceae* members. However, LK-157 did not affect the MICs of aztreonam, ceftazidime or cefuroxime against CTX-M producing members of *Enterobacteriaceae* [24].

Oxapenems

Four β-lactamase inhibitors, members of the oxapenems, are being developed (AM-112 – AM-115) and express activity against class A, C, and D enzymes [30]. AM-114 and AM-115 displayed the most potent activity against class A enzymes, comparable to that of clavulanic acid. Activity against class C and class D enzymes was similar to that of AM-112 and AM-113 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*. Enhanced activity of oxapenems in combination with ceftazidime was also noted against *Pseudomonas* strains and MRSA [31].

Inhibitors with no β-lactam structure

NXL104

NXL104 is a non-β-lactam compound which inhibits β-lactamases through the formation of a stable covalent carbamoyl linkage. In combination with ceftazidime and cefotaxime against *Enetrobacteriaceae* producing CTX-M ESBLs, it showed a 4 to 8000-fold potentiation of the cephalosporins, with MIC values ≤ 1 for all organisms irrespective of CTX-M type [24]. Against P99, NXL104 showed a stronger inhibition than tazobactam, whereas clavulanic acid was inactive. Another study showed that combination with NXL104 restored the activity of ceftazidime and cefotaxime against isolates producing class A carbapenemases [24]. NXL104/ceftazidime combination is currently undergoing Phase II clinical trials in patients admitted for complicated intra-abdominal and complicated urinary tract infections [32].

Maleic acid derivates

ME1071, previously known as CP3242, is a metallo β-lactamase inhibitor that competitively inhibits IMP-1 and VIM-2. It significantly lowered the MICs of biapenem in a concentration-dependent manner against MBL-producing *P. aeruginosa*. MIC lowering by ME1071 was also shown for IMP- or VIM-producing *E. coli*, *S. marcescens*, *A. baumanii* and *K. pneumoniae* [24].

New cephalosporins

New cephalosporins are very resistant to penicillinases and two of them have demonstrated anti-methicillin resistant *S. aureus* (MRSA) activity in animal mod els of infections. Some of these compounds also showed potent anti-Gram-negative activity. However, there is no evidence of better activity against MDR Gram-negative bacteria compared to older cephalosporins.

Ceftobiprole

Ceftobiprole (formerly BAL-9141) is the active component of the prodrug ceftobiprole medocaril (formerly BAL-5788), and represents a novel cephalosporin with expanded activity against Gram-positive bacteria. It has been engineered to bind highly to penicillin binding protein 2a (PBP2a). Ceftobiprole is stable against some enzymes (non-ESBL class A), but is hydrolyzed by ESBLs and carbapenemases [33]. A study published in 2008 reported that ceftobiprole monotherapy was as effective as vancomycin combined with ceftazidime for treating patients with a broad range of complicated skin and skin-structure infections and infections due to Gram-positive and Gram-negative bacteria [32]. Ceftobiprole is an effective anti-MRSA agent that also has activity against important Gram-negative bacteria, but there is no evidence that ceftobiprole has better activity against class A and class C β-lactamase-producing Gram-negative bacteria compared to ceftazidime.

Ceftaroline

Ceftaroline is a novel semisynthetic anti-MRSA cephalosporin with broad-spectrum activity, which is currently undergoing Phase III clinical trials [35]. Ceftaroline maintains good activity against Gram-negative pathogens: MIC values were 0.06–0.5 for *E. coli*, *Klebsiella* spp., *M. morganii* and *Proteus*, and 0.12–1 mg/l for *Enterobacter*, *Serratia* and *Citrobacter* spp. MIC value rose to 1–2 mg/l for many *Enterobacteriaceae* with classical TEM β-lactamases and were much higher for those with ESBL, hyperproduced AmpC or K1 enzymes. Ceftaroline selected AmpC-derepressed *Enterobacter* mutants. Similar to cefotaxime in single-step experiments, in multistep procedures it selected ESBL variants of TEM [36]. Another study showed that ceftaroline was synergistic with the β-lactamase inhibitor, tazobactam,

(up to 500-fold) against MDR Gram-negative pathogens such as ESBL-producing *E. coli* and *K. pneumoniae* [37].

Despite being active against resistant Gram-positive bacteria, ceftaroline was less active than currently used antimicrobial agents against Gram-negatives. A combination of vancomycin plus aztreonam demonstrated higher favorable microbiological response rates than did ceftaroline monotherapy against Gram-negative infections. The efficacy of ceftaroline against non-ESBL-producing *E. coli* and *K. pneumoniae* was comparable to that of aztreonam; however, the efficacy of aztreonam against *P. aeruginosa* and *Proteus mirabilis* infection was better than that of ceftaroline [38].

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 their stability to hydrolysis by many ESBLs. At present, meropenem and imipenem/cilastatin are widely used and are recommended for treatment of several nosocomial infections such as pneumonia (if MRSA is excluded), complicated urinary tract infections, complicated intra-abdominal infections, febrile neutropenia, septicemia, complicated skin and skin-structure infections and meningitis. 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 co-administered with imipenem in a 1:1 ratio. Other carbapenems do not require DHP-1 inhibitors.

Three mechanisms of acquired resistance to carbapenems are known: 1) structural changes in PBPs; 2) carbapenemases; and 3) changes in membrane permeability through the loss of specific porins [39].

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 pre-clinical studies (Table 3). Of note, two of the novel carbapenems are developed to be administered orally.

Ertapenem

Ertapenem was licensed in the US in 2001 and in Europe in 2002. Its main indications include: Intra-abdominal infections, complicated skin and skin-structure infections, complicated urinary tract infections, acute pelvic infections and community acquired pneumonia. The most important pharmacokinetic feature of this drug is due to its net negative charge that increases its binding to plasma proteins (95%), which results in a long half-life permitting once-daily administration [40]. The main limitation of ertapenem is its limited activity against non-fermenting Gram-negative bacteria, such as *P. aeruginosa, Acinetobacter* spp. and *B. cepacia* [40]. Even though its activity against Gram-negative ESBL-producers seems to be lower than other carbapenems, ertapenem is approved for the treatment of infections caused by these bacteria. All three above-mentioned mechanisms of acquired resistance to carbapenems have been reported for ertapenem [40]. The role of ertapenem in the treatment of ventilator-associated pneumonia (VAP) was investigated in a pilot study, which reported that ertapenem was useful for treating early-onset VAP due to ESBL-producers, with clinical success achieved in 80% of patients and microbiological success in 75% of cases [41].

Doripenem

Doripenem is a new broad-spectrum, parenteral carbapenem with a chemical structure that confers β-lactamase stability and resistance to inactivation by renal DHP-I. It is as active as imipenem or ertapenem against Gram-positive cocci (methicillin-susceptible *S. aureus* [MSSA] and coagulase negative staphylococci), but anti-Gram-negative activity is similar to that of meropenem, and two to three fold superior to imipenem [42]. However, doripenem has no activity against MRSA, *E. fecium*, some strains of *Burkholderia* spp. and *Stenotrophomonas maltophilia* [42]. In an extensive study, in which the activity of 24 antibiotics was tested against 394 strains, doripenem was fully active against AmpC and other ESBL-producing *Enterobacteriaceae* [43]. Additionally, doripenem was found to be more active against *Acinetobacter* spp. and *P. aeruginosa* when the same susceptible and intermediate concentrations were used for imipenem and meropenem. Other strains that remained inhibited by doripenem concentrations ≤ 4 microg/ml were penicillin-resistant streptococci, *H. influenzae* with all resistance patterns tested, and many *Enterobactericeae* resistant to other carbapenems because of outer membrane protein alterations, hyperexpression of AmpC or acquisition of a Bush group 2f carbapenemase [43]. At a dose of 500 mg every 8 h, doripenem is effective against strains with a MIC < 2 mg/l and dose adjustment is required only when creatinine clearance is < 30 ml/min. *In vivo* animal studies demonstrated that the incidence of seizures with doripenem was lower than with other carbapenems and at the recommended dosage the most frequent adverse events are nausea (3.7%) and diarrhea (2.5%).

Biapenem

Biapenem is a new parenteral agent that was approved in Japan in 2002 and it is currently undergoing phase II clinical studies in the USA. The prominent feature of this new carbapenem is related to its high concentration in respiratory tissue and other body fluids. Biapenem has a broad spectrum of activity including against Gram-positive

Table 3. FDA status and pharmacokinetic characteristics of new carbapenems.

					Active against			
Drug	**FDA status**	**Dose**	**Administration**	**Half-life (h)**	***P. aeruginosa***	**MRSA**	**VRE**	**PRP**
Ertapenem	Approved	1 g qd	i.v.	4	–	–	–	–
Doripenem	Approved	500 mg tid	i.v.	1	+	–	–	+
Biapenem	Phase II	300 mg bid	i.v.	1.03	+	–	–	+
Panipenem	Approved in Japan, China and Korea	0,5/0,5 g bid	i.v.	1.10–0.7	–	–	–	+
Tebipenem	Phase II	4 or 6 mg/kg bid	oral	U	–	–	U	+
Tomopenem	Phase II	700 mg	i.v.	1.7	+	+		
Razupenem	Phase II	U	i.v.	U	+	+	+	
Trinems		U	U	U	–	U	+/–	–

i.v.: intravenous; MRSA: methicillin-resistant *S. aureus*; PRP: penicillin-resistant pneumococci; U: unknown; VRE: vancomycin-resistant enterococci; +: active; –: non active; +/–: data only on small number of strains

bacteria such as *S. pneumoniae* (also penicillin-resistant strains), MSSA and Gram-negatives including *A. baumannii*, ESBL-producing *Enterobacteriaceae*, *E. cloacae*, *S. marcescens* and *Citrobacter freundii*. Moderate activity with median MIC of 8 mg/l was found against *P. aeruginosa* [44]. Biapenem has a mean plasma half-life of one hour and it is recommended at a dosage of 300 mg twice daily. It requires an adjustment in case of reduced glomerular filtration rate. Biapenem is generally well tolerated and clinical trials reported the incidence of adverse events ranging from 1.9% to 3.4% with nausea, skin eruption, vomiting and diarrhea as the most common side effects [45].

Panipenem/betamipron

The combination of panipenem with betamipron, like imipenem/cilastatin, is necessary because betamipron inhibits the renal uptake of panipenem. This combination is approved in Japan, China and Korea for the treatment of lower respiratory tract infections, urinary tract infections, obstetric/gynecological infections, and surgical infections at a dosage of 0.5/0.5 g twice daily as an intravenous infusion over 30–60 mins. 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 urinary tract infections [46–48]. Panipenem's spectrum of activity includes *Enterobacteriaceae* and common respiratory tract pathogens, although meropenem remains the most active carbapenem against *H. influenzae* [49]. Panipenem is not active against *E. faecium* and *S. maltophilia*, and *P. aeruginosa* seems to be resistant, showing MIC90 values of 12.5–25 mg/l [49].

Tebipenem

Tebipenem pivoxil is a prodrug of an oral carbapenem with a high degree of stability to DHP-I and absorption of the active metabolite into the blood from the intestine. While tebipenem is inactive against MBL-producing pathogens and MRSA, good activity against penicillin-susceptible and penicillin-resistant *S. pneumoniae, S. pyogenes, H. influenzae, K. pneumoniae, M. catarrhalis* and *E. coli* has been reported. It is likely to become a specific antibiotic for the treatment of persistent otitis media, upper respiratory infection and bacterial pneumonia in pediatric patients [50]. Phase II clinical studies are being conducted in Japan.

Tomopenem

Tomopenem is a novel 1-methyl carbapenem which inhibits the activity of PBP and disrupts bacterial cell wall peptidoglycan biosynthesis. Tomopenem seems to have a very low rate of spontaneous emergence of resistance. *In vitro* activity against β-lactam susceptible and resistant strains, including MRSA, ceftazidime-resistant *P. aeruginosa* and ESBL-producing *Enterobacteriaceae* has been demonstrated [51].

Other new carbapenems

Several novel compounds, still in pre-clinical phases of evaluation, are mentioned below, highlighting the results of *in vitro* studies aimed to define the activity spectrum of these new molecules.

1. The group of 2-(thyazol-2-ylthio)-1β-methyl carbapenems includes SM-197436, SM-232721 and SM-232724. These molecules are characterized by a unique 4-substituted thiazol-2-ylthio moiety at the side chain. They exhibit potent anti-MRSA activity but they have insufficient activity against *E. faecium*. As far as Gram-negative bacteria are concerned, these three carbapenems are highly active against *H. influenzae* (including ampicillin-resistant strains), *M. catarrhalis*, and *B. fragilis*, and show antibacterial activity equivalent to that of imipenem for *E. coli*, *K. pneumoniae* and

Proteus spp. [52]. Similar to other new carbapenems, these agents may be indicated for nosocomial bacterial infections due to Gram-positive and Gram-negative bacteria, especially multiresistant Gram-positive cocci, including MRSA and vancomycin-resistant enterococci (VRE) [52].

2. Another new compound is CS-023 (RO 4908463). It is more stable to hydrolysis by human DHP-I than meropenem or imipenem and has a broad spectrum of activity against Gram-positive and Gram-negative organisms. CS-023 seems more effective than imipenem and meropenem against MRSA, with an MIC of 4 mg/l. CS-023 is characterized by a low protein binding ratio, a feature which can be useful because the plasma active fraction achieves rapid equilibrium with intracellular fluid [24].
3. ME 1036, previously named CP5609, is a novel parenteral carbapenem. In a recent study, the activity of ME1036 and comparators was evaluated against clinical blood culture isolates from patients with bacteremic community-acquired pneumonia (CAP) requiring hospitalization. The results showed that ME1036 had excellent activity against CAP isolates causing serious invasive infections, including MRSA [53].
4. Razupenem (SMP-601) is a novel compound in phase II of evaluation. In a recent *in vitro* study, razupenem was found to be active against ESBL-producers, but its activity was significantly reduced by AmpC enzymes and carbapenemases [54]. Razupenem's activity can be improved by combining it with other antimicrobial agents: *In vitro* studies have shown a synergistic activity with amikacin or ciprofloxacin against *B. cepacia* and *S. marcescens* [24].
5. Trinems, previously called tribactams, have a carbapenem-related structure with a cyclohexane ring attached across carbon 1 and 2. One of these, sanfetrinem, is administered orally as a hexatil ester. Activity of sanfetrinem against *P. vulgaris* and *K. oxytoca*, which produce a potent class A β-lactamase, was reported in a study from 1998, but no recent studies of trinems have been published [55].

Conclusion

Infections due to MDR Gram-negative bacteria, such as ESBL or carbapenemase-producing Enterobacteriaceae and *A. baumannii* or *P. aeruginosa* remain a serious problem in the hospital setting. Although some promising novel molecules are in the late stages of development, few new antibiotics have been advanced for the treatment of most of the ESKAPE pathogens. Among agents potentially active against Gram-negatives are novel cephalosporins, carbapenems and β-lactamase inhibitors.

Fifth generation cephalosporins have acquired activity against MRSA, but they offer no advantage against Gram-negatives. They are inactive against MDR bacteria, and efficacy of ceftaroline was less than that of aztreonam against *P. aeruginosa*. Some of the novel carbapenems are active against resistant Gram-positives, but when difficult Gram-negatives are involved, their activity is similar to that of meropenem. Finally, β-lactamase inhibitors seem the most promising as they might restore the activity of already known β-lactams against β-lactamase-producing strains. However, their real clinical utility will be known only after results of large clinical trials are available.

Treating patients with infections due to resistant Gram-negative bacteria remains a serious challenge.

Competing interests

The authors declare that they have no competing interests.

List of abbreviations used

DHP: dehydopeptidase; ESBL: extended-spectrum β-lactamase; IDSA: Infectious Diseases Society of America; IMP: imipenemase; KPC: *K. Pneumonia* carbapenemase; MBL: metallo-β-lactamases; MDR: multi-drug resistant; MIC: minimum inhibitory concentrations; MRSA: methicillin-resistant *S. aureus;* MSSA: methicillin-susceptinle *S. aureus;* NDM: New Delhi MBL; OXA: oxacillinase; SHV: sulfhydryl reagent variable; VAP: ventilator-associated pneumonia; VIM: Verona integron-endcoded MBL; WHO: World Health Organisation.

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