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 polymyxins 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 glycyclines, 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...
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 *Escherichia coli* isolated from a patient named Temoniera, hence named TEM). The further selection of resistant mutants led to the appearance of extended-spectrum β-lactamas (ESBLs) that now compromise the use of even third-generation cephalosporins. In the 1990s, the pharmaceutical industry introduced carbapenams, which are extremely stable to degradation by β-lactamas. However, a variety of β-lactamas 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-lactamas have been proposed, but the Ambler classification is the most widely used and divides β-lactamas 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 sulphydryl reagent variable (SHV) subclasses. Some evolve class A β-lactamas 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-lactamas (MBL) with broad substrate specificity that includes not only penicillins and cephalosporins, but also carbapenams. Class C enzymes are primarily chromosomally encoded cephalosporinas and carbapenemas, including *K. pneumoniae* carbapenemase (KPC)-hydrolyzing β-lactamas [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 carbapenemas for treating infections due to ESBL-producing bacteria. Moreover, carbapenem-resistant *Enterobacteriaceae* are increasingly recognized

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 carbapenams), 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 cephalosporinas and carbapenemas, including *K. pneumoniae* carbapenemase (KPC)-hydrolyzing β-lactamas [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 carbapenemas for treating infections due to ESBL-producing bacteria. Moreover, carbapenem-resistant *Enterobacteriaceae* are increasingly recognized

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**Figure 1.** New antibacterial agents approved in the United States, 1983–2009. From [3] with permission.

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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 potentiates 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

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**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 cephemycins | *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 (ceftaxime, 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 ceftirome | 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 carbapenem, oxyimino-β-lactams, cephemycins | KPC-2, IMI-1, SME-1 |
| 3a                      | **B** (B1) Carbapenems    | Broad-spectrum hydrolysis including carbapenem, but not monobactams | IMP-1, VM-1, CcrA, IND-1 |
| 3b                      | **B** (B3) Carbapenems    | Preferential hydrolysis of carbapenem | CphA, Sfh-1 |
| Ni                      | **Unknown**               |                           |                           |

Adapted from [5].

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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 bicyclic or tricyclic substituents connected by a methyldiene 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].

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**Table 2. Old and new β-lactam 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
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 Enterobacteriaceae 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 derivatives

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. baumannii and K. pneumoniae [24].

New cephalosporins

New cephalosporins are very resistant to penicillinas and two of them have demonstrated anti-methicillin resistant S. aureus (MRSA) activity in animal models 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 prodrg 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 AmpCderepressed 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-I 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. faecium*, 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 *Enterobacteriaceae* resistant to other carbapenems because of outer membrane protein alterations, hyper-expression 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
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-(thiazol-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

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

| Drug          | FDA status       | Dose          | Administration | Half-life (h) | Active against                  |
|---------------|------------------|---------------|----------------|--------------|---------------------------------|
| 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            | –                               |
| 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
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
ESBL: extended-spectrum β-lactamase; IDSA: Infectious Diseases Society of America; IMP: imipenemase; KPC: K. Pneumoniae carbapenemase; MBL: metallo-β-lactamases; MDR: multi-drug resistant; MIC: minimum inhibitory concentrations; MRSA: methicillin-resistant S. aureus; NDM: New Delhi MBL; OXA: oxacillinase; SHV: sulfhydryl reagent variable; VAP: ventilator-associated pneumonia; VIM: Verona integron-encoded MBL; WHO: World Health Organisation.

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References
1. Rice LB: Federal funding for the study of antimicrobial resistance in nosocomial pathogens: No ESKAPE. J Infect Dis 2008, 197:1079–1081.
2. Infectious Diseases Society of America Bad Bugs, No Drugs: As Antibiotic Discovery Stagnates... A Public Health Crisis Brews. Clin Infect Dis 2008, 46:155–164.
3. Spellberg B, Guidos R, Gilbert D, et al.: The epidemic of antibiotic-resistant infections: A call to action for the medical community from the Infectious Diseases Society of America. Clin Infect Dis 2008, 46:155–164.
4. The 10 x 20 initiative: Pursuing a global commitment to develop 10 new antibacterial drugs by 2020. Clin Infect Dis 2010, 50:1081–1083.
5. Bush K, Jacoby GA, Medeiros AA: A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 1995, 39:1211–1233.
6. Ambler RP: The structure of beta-lactamases. Philosopical transactions of the Royal Society of London 1980, 289:321–331.
7. Kumarasamy KK, Toleman MA, Walsh TR, et al.: Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: A molecular, biological, and epidemiological study. Lancet Infect Dis 2010, 10:597–602.
8. Moore RA, Chan L, Hancock RE: Evidence for two distinct mechanisms of resistance to polymyxin b in pseudomonas aeruginosa. Antimicrob Agents Chemother 1984, 26:539–545.
9. Poulakou G, Kontopidou FV, Paramythiotou E, et al.: Tigecycline in the treatment of infections from multi-drug resistant gram-negative pathogens. J Infect 2009, 58:273–284.
10. Keeney D, Ruzin A, Bradford PA: Ram A, a transcriptional regulator, and acrAB, an rmd-type efflux pump, are associated with decreased susceptibility to tigecycline in Enterobacter cloacae. Microb Drug Resist 2007, 13:1–6.
11. Gilbert D: “The truth, if it exists, is in the details”. Crit Care Med 2008, 36:1368–1369.
12. Deshpande LM, Jones RN, Fritsche TR, Sader HS: Occurrence and characterization of carbapenemase-producing Enterobacteriaceae: Report from the sentry antimicrobial surveillance program (2000–2004). Microb Drug Resist 2006, 12:223–230.
13. Schwaber MJ, Carmeli Y: Mortality and delay in effective therapy associated with extended-spectrum beta-lactamase production in

DHP: dehydopeptidase; ESBL: extended-spectrum β-lactamase; IDSA: Infectious Diseases Society of America; IMP: imipenemase; KPC: K. Pneumoniae carbapenemase; MBL: metallo-β-lactamases; MDR: multi-drug resistant; MIC: minimum inhibitory concentrations; MRSA: methicillin-resistant S. aureus; NDM: New Delhi MBL; OXA: oxacillinase; SHV: sulfhydryl reagent variable; VAP: ventilator-associated pneumonia; VIM: Verona integron-encoded MBL; WHO: World Health Organisation.
Enterobacteriaceae bacteraemia: A systematic review and meta-analysis. 
J Antimicrob Chemother 2007, 60:913–920.

14. Lin YC, Chen TL, Ju HL, Chen HS, Wang FD, Yu KW, Liu CY: Clinical characteristics and risk factors for attributable mortality in Enterobacter cloacae bacteraemia. J Microbial Immunol Infect 2006, 39:67–72.

15. Poleg NY, Franklin C, Bell JM, Spelman DW: Dissemination of the metallo-beta-lactamase gene blaimp-4 among gram-negative pathogens in a clinical setting in Australia. Clin Infect Dis 2005, 41:1549–1556.

16. Bratu S, Landman D, Haag R, et al: Rapid spread of carbapenem-resistant Klebsiella pneumoniae in New York City: A new threat to our antibiotic armamentarium. Arch Int Med 2005, 165:1430–1435.

17. Pintado V, San Miguel LG, Grill F, et al: Intravenous colistin sulphonmethate sodium for therapy of infections due to multidrug-resistant gram-negative bacteria. J Infect 2008, 56:185–190.

18. EARS (2008) European Antimicrobial Resistance Surveillance System: Annual Report. Available at: http://www.ecdc.europa.eu/en/activities/surveillance/EARS-Net/Documents/2008_EARS_Annual_Report.pdf Accessed Nov 2010

19. Munoz-Price LS, Weinstein RA: Penicillanic acid sulfone metabolism and pharmacokinetics. J Biol Chem 2000, 275:26674–26682.

20. Bassetti M, Righi E, Viscoli C: Novel beta-lactam antibiotics and inhibitor combinations. Expert Opin Investig Drugs 2006, 15:285–296.

21. Wiss JW, Petersen PJ, Murphy TM, et al: In vitro and in vivo activities of novel 6-methylidene penems as beta-lactamase inhibitors. Antimicrob Agents Chemother 2004, 48:4589–4596.

22. Venkatesan AM, Agarwala A, Abe T, et al: Novel imidazole substituted 6-methylidene-penems as broad-spectrum beta-lactamase inhibitors. Bioorg Med Chem 2004, 12:5807–5817.

23. Shahid M, Sobia F, Singh A, et al: Beta-lactams and beta-lactamase-inhibitors in current- or potential-clinical practice: A comprehensive update. Crit Rev Microbiol 2009, 35:81–108.

24. Richter HG, Angenoh P, Huibschwerlen C, et al: Design, synthesis, and evaluation of 2-beta-alkenyl penam sulfonic acids as inhibitors of beta-lactamases. J Med Chem 1996, 39:3712–3722.

25. Tzouvelekis LS, Gazouli M, Prinarakis EE, Telepi E, Legakis NJ: Comparative evaluation of the inhibitory activities of the novel penicillanic acid sulfone 48–1220 against beta-lactamases that belong to groups 1, 2b, and 2be. Antimicrob Agents Chemother 1997, 41:475–477.

26. Kaur K, Adediran SA, Lan MJ, Pratt RF: Inhibition of beta-lactamases by monocyclic acyl phosph(on)ates. Biochemistry 2003, 42:1529–1536.

27. Buyck JD, Aadishadchanda VR, Vogeli L, Zhang J, Chen H: Synthesis and evaluation of 3-(carbomethoxy)- and 3-(carboxymethyl) penicillines as inhibitors of beta-lactamase. J Org Chem 2005, 70:4510–4513.

28. Page M, Desabre E, Geer C, Holier B: Activity of BAL30376 against gram-negative bacteria, 47th Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society of Microbiology. Abstract F1–229.

29. Cherry PC, Newall CE, Watson NS: Preparation of the 7-oxo-oxa-1-azaacyclo(3.2.0)hept-2-ene system and the reversible cleavage of its oxazoline ring. J Chem Soc Chem Commun 1978, 11:469–470.

30. Jamieson CE, Lambert PA, Simpson IN: In vitro activities of novel oxapenem, alone and in combination with ceftazidime, against gram-positive and gram-negative organisms. Antimicrob Agents Chemother 2003, 47:2615–2618.

31. Lee JH, Bae IK, Hee Lee S: New definitions of extended-spectrum beta-lactamase conferring worldwide emerging antibiotic resistance. Med Res Rev, in press.

32. Queennan AM, Shang W, Kania M, Page MG, Bush K: Interactions of cefotibopirle with beta-lactamases from molecular classes A to D. Antimicrob Agents Chemother 2007, 51:3089–3095.

33. Noel GI, Bush K, Bachp I, Janus J, Strauss RS: A randomized, double-blind trial comparing cefotibopirle medocaril with vancomycin plus ceftazidime for the treatment of patients with complicated skin and skinstructure infections. Clin Infect Dis 2008, 46:6647–6655.

34. Sader HS, Fritsche TR, Kania K, Ge Y, Jones RN: Antimicrobial activity and spectrum of ppp-0903m (t-91825), a novel cephalosporin, tested against a worldwide collection of clinical strains. Antimicrob Agents Chemother 2005, 49:3501–3512.

35. Mushtaq S, Warner M, Ge Y, Kania K, Livermore DM: In vitro activity of ceftaroline (ppp-0903m, t-91825) against bacteria with defined resistance mechanisms and phenotypes. J Antimicrob Chemother 2007, 60:300–311.

36. Vidaillac C, Leonard SN, Sader HS, Jones RN, Rybak MJ: In vitro activity of ceftaroline alone and in combination against clinical isolates of resistant gram-negative pathogens, including beta-lactamase-producing enterobacteriaceae and pseudomonas aeruginosa. Antimicrob Agents Chemother 2009, 53:2360–2366.

37. Corey GR, Wilcox M, Talbott GH, et al: Integrated analysis of CANVAS 1 and 2: phase 3, multicenter, randomized, double-blind studies to evaluate the safety and efficacy of ceftaroline versus vancomycin plus aztreonam in complicated skin and skin-structure infection. Clin Infect Dis 2010, 51:641–650.

38. Bassetti M, Nicolini L, Esposto S, Righi E, Viscoli C: Current status of new carbapenems. Curr Med Chem 2009, 16:564–575.

39. Keating GD, Perry CM: Ertapenem: A review of its use in the treatment of bacterial infections. Drugs 2005, 65:2151–2178.

40. Bassetti M, Righi E, Fasce R, et al: Efficacy of ertapenem in the treatment of early ventilator-associated pneumonia caused by extended-spectrum beta-lactamase-producing organisms in an intensive care unit. J Antimicrob Chemother 2007, 60:435–435.

41. Anderson DL: Doripenem. Drugs Today (Barc) 2006, 42:399–404.

42. Jones RN, Huyhn HK, Biedenbach DJ: Activities of doripenem (t-9661) against drug-resistant clinical pathogens. Antimicrob Agents Chemother 2004, 48:3136–3140.

43. Chen HY, Livermore DM: Comparative in-vitro activity of biapenem against enterobacteria with beta-lactamase-mediated antibiotic resistance. J Antimicrob Chemother 1994, 33:543–561.

44. Perry CM, Ibbotson T: Biapenem. Drugs 2002, 62:2221–2234.

45. Kumaawa J, Matsumoto T, Kumamoto Y: Phase III comparative clinical trial of panipenem/betapenim (pam/bp) with imipenem/clastatin sodium (ipm/cis) in complicated urinary tract infections. Nishinom J Urol 1992, 54:254–271.

46. Hara K, Hiraga Y, Omichi M: A comparative study of panipenem/betapenim and imipenem/clastatin in bacterial pneumonia. Chemotherapy 1992, 40:509–531.

47. Hara K, Kousho SKH, Takebe K: A comparative study of panipenem/betapenim and imipenem/clastatin in respiratory tract infections. Chemotherapy 1992, 40:613–673.

48. Watanabe A, Tokue Y, Takahashi H, et al: Comparative in-vitro activity of carbapenem antibiotics against respiratory pathogens isolated between 1999 and 2000. J Infect Chemother 2001, 7:267–277.

49. Sato N, Kijima K, Koresawa T, et al: Intravenous colistin sulphomethate sodium for therapy of infections due to multidrug-resistant gram-negative bacteria. J Antimicrob Chemother 2009, 64:61–650.

50. Ueda Y, Sunagawa M: In vitro and in vivo activities of novel 2-(thiazol-2-ylthio)-1beta-methylcarbapenems with potent activities against multiresistant gram-positive bacteria. Antimicrob Agents Chemother 2008, 52:3849–3854.

51. Babin GS, Yuan M, Livermore DM: Interactions of beta-lactamases with sanfetrinem (gv 104326) compared to those with imipenem and with oral beta-lactams. Antimicrob Agents Chemother 1998, 42:1168–1175.

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