

# The clinical impact of multidrug-resistant gram-negative bacilli in the management of septic shock

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Multi-antibiotic drug-resistant (MDR) gram-negative bacilli are becoming a major threat to the standard care of septic patients. Empiric antimicrobial drug regimens to cover likely bacterial pathogens have to be altered in keeping with the spread of MDR pathogens in the health care setting and in the community. Reliable antibiotics for broad spectrum coverage for sepsis such as extended spectrum  $\beta$ -lactam antibiotics, carbapenems, and fluoroquinolones can no longer be counted upon to provide activity against a range of common, virulent pathogens that cause sepsis. In some regions of Asia, South America, and Eastern Europe in particular, MDR pathogens have become a major concern, necessitating the use of potentially toxic and costly antibiotic combinations as initial antibiotic therapy for septic shock. In this brief review, we will focus on the emergence of MDR gram-negative pathogens, resistance mechanisms, and suggest some management and prevention strategies against MDR pathogens.

## Introduction

Medical care is an increasingly complex environment. Patients undergo ever more sophisticated surgeries, transplants, implanted devices, immunotherapies and chemotherapies, and their compromised immune defenses make them ever more vulnerable to infection. Unfortunately, the global spread of multidrug antimicrobial resistance and the paucity of novel antimicrobials in development are limiting clinicians' ability to provide effective and safe treatments for these very susceptible patients. Resistance to antimicrobial agents in gram-negative bacilli is particularly challenging. Of the famous ESKAPE pathogens identified as the most important emerging threats in antimicrobial resistance (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter* species, *Pseudomonas aeruginosa*, and *Enterobacter* species), the majority are gram-negative bacilli.<sup>1</sup>

In this review we will address the epidemiology, mechanisms of resistance, impact on clinical outcomes, treatment and

prevention of the most important multidrug-resistant gram-negative pathogens encountered in clinical practice. Resistance mechanisms, phenotypes, and therapeutic options for the most common multidrug-resistant organisms encountered in clinical practice are summarized in Table 1.

## Epidemiology

The burden of multidrug antibiotic resistance has substantially increased worldwide,<sup>2</sup> with important regional differences in prevalence. For example, in the Study for Monitoring Antimicrobial Resistance Trends which collected isolates from 28 countries during 2004, extended spectrum  $\beta$ -lactamase (ESBL) producing bacteria were highest in Latin America, the Middle East, Africa, and Asia.<sup>3</sup> A recent survey of ICUs throughout the world found a similar distribution of ESBL producing gram negative bacilli and found that gram-negative bacilli now outnumber gram-positive pathogens in ICU infections,<sup>4</sup> reversing a 20 year trend where gram-positive pathogens were the previously dominant, causative pathogens of sepsis.<sup>5</sup> In the United States, the recent spread of carbapenem resistance is most troublesome. Data from CDC's National Healthcare Safety Network (NHSN) shows that nosocomial *Klebsiella pneumoniae* isolates resistant to carbapenem increased from 2% in 2001 to 10% in 2011.<sup>6</sup>

The importation of multidrug-resistant pathogens through travel to endemic areas is increasingly being recognized. For example, in a prospective study of 100 Swedish volunteers screened prior and post-travel, the abroad acquisition of ESBL-producing organisms was 24%. The average trip duration was 2 weeks, and India was the most common destination.<sup>7</sup> In the US and the UK, emergence of the NDM-1 producing bacteria was similarly associated with foreign travel and receipt of medical care in India and Pakistan.<sup>4,5</sup> In this regard, the frequency of contamination with *Enterobacteriaceae*, aeromonads, and nonfermenters carrying the NDM-1 gene found in a New Delhi environmental point prevalence study is particularly telling,<sup>8</sup> as is the 2011 report that 18% of the tap water samples in New Delhi showed evidence of fecal contamination.<sup>9</sup>

Multidrug-resistant pathogens make ideal nosocomial pathogens, as they can survive for a prolonged time in the hospital environment,<sup>10</sup> and can be transferred easily between patients

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**Table 1.** Resistance mechanisms, phenotypes, and therapeutic options for common multidrug-resistant organisms encountered in clinical practice

Pathogen	Resistance mechanisms	Resistance phenotype (antibiotics affected)	Therapy options
<i>Escherichia coli</i>	Extended-spectrum $\beta$ -lactamases (ESBL) <sup>a</sup>	All penicillins, narrow spectrum cephalosporins, oxymino- $\beta$ -lactams (cefotaxime, ceftazidime, cefepime), aztreonam	Carbapenems
<i>Klebsiella pneumoniae</i> <sup>a</sup>	Carbapenemases (i.e., KPC, NDM)	All penicillins, cephalosporins, carbapenems	Polymyxins, <sup>b</sup> tigecycline <sup>c</sup>
<i>Pseudomonas aeruginosa</i>	Active efflux, porin loss, carbapenemases	Quinolones, aminoglycosides, anti-pseudomonal penicillins, cephalosporins, carbapenems	Polymyxins <sup>d</sup>
<i>Acinetobacter baumannii</i>	Active efflux, porin loss, amp-C cephalosporinases, carbapenemases	Quinolones, penicillins, cephalosporins, carbapenems	Polymyxins <sup>e</sup> , tigecycline <sup>c</sup>

<sup>a</sup>Genes conferring resistance to fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole are often co-transmitted with mobile  $\beta$ -lactamases. <sup>b</sup>For serious infections, combination therapy (i.e., polymyxin with tigecycline or extended-infusion meropenem) is preferred over monotherapy.<sup>49</sup> <sup>c</sup>Tigecycline monotherapy not recommended for bacteremia or nosocomial pneumonia. <sup>d</sup>Polymyxins in combination therapy with other antipseudomonal agents or rifampin were reportedly active in vitro, but clinical data are rare. <sup>e</sup>Polymyxins in combination with rifampicin reported successful in small, retrospective series.<sup>52,56</sup>

through the hands of healthcare workers.<sup>11</sup> However, antimicrobial resistance is no longer a phenomenon restricted to the healthcare setting. Emergence of community-acquired urinary tract infections with *E. coli* organisms producing CTX-M extended spectrum  $\beta$ -lactamases and fluoroquinolones have been increasingly documented in the US, Europe, and Asia.<sup>12-14</sup>

### Mechanisms of Antibiotic Resistance

Gram-negative bacteria can employ a variety of mechanisms to achieve resistance to a single class or to multiple classes of antibiotics, as depicted in Figure 1. Mobile genetic elements such as plasmids can facilitate further spread of resistance genes between species.

#### $\beta$ -lactam resistance

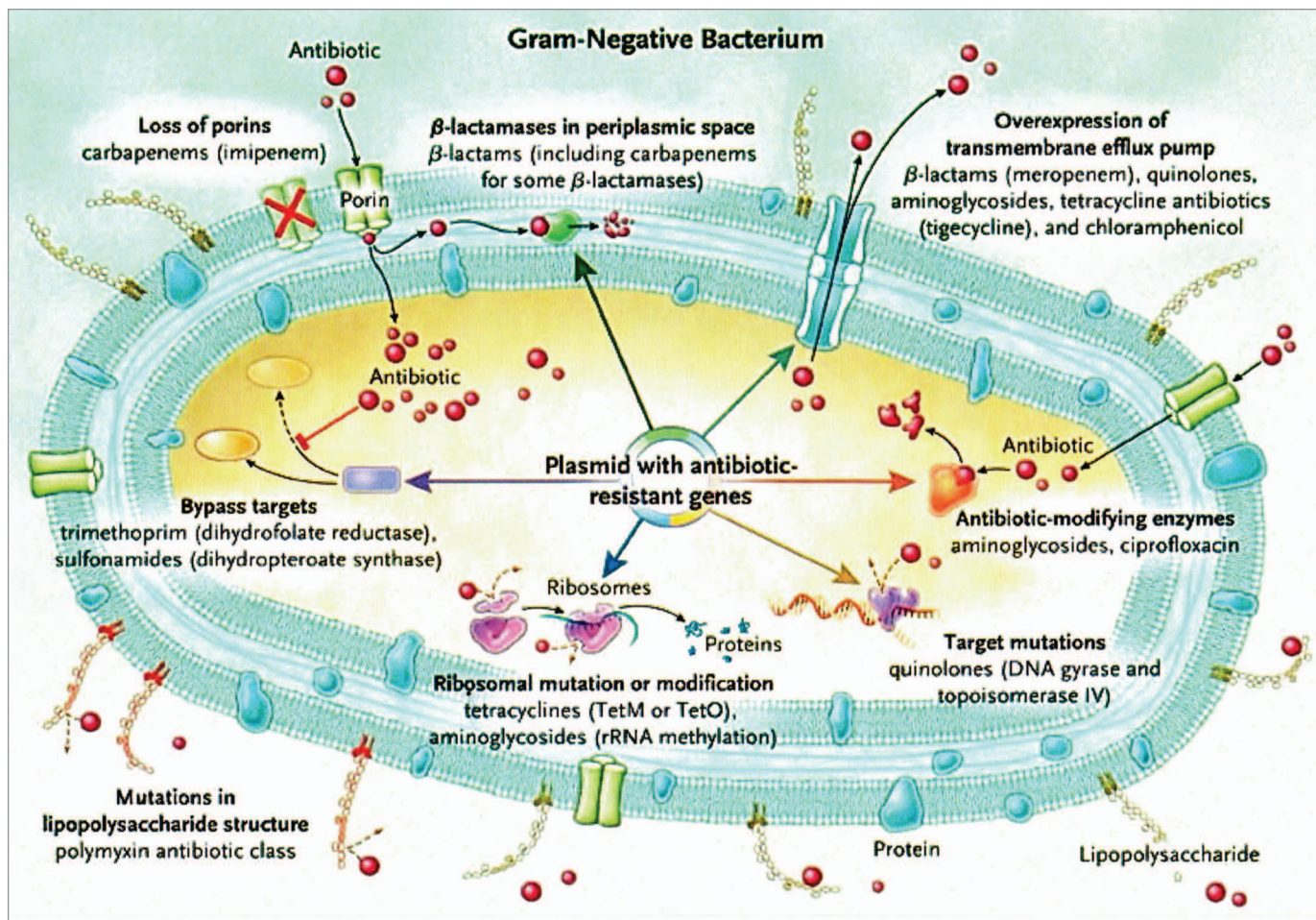
##### *Penicillin, cephalosporin, and aztreonam*

Penicillin, cephalosporin, and aztreonam resistance occurs through production of extended- $\beta$ -lactamases (ESBL), which inactivate the antibiotics by splitting the amide bond of the  $\beta$ -lactam ring. Based on their molecular structure and amino-acid homology, ESBL are classified into Ambler classes A, C, and D (have serine at their active site), and class B (have zinc at their active site, and are thus called metallo- $\beta$ -lactamases).<sup>15</sup> Major ESBL families include TEM, SHV, CTX-M, (class A) found in *Enterobacteriaceae*, and OXA (class D), found in *Pseudomonas* isolates. They all hydrolyze oxyimino- $\beta$ -lactam substrates, and are susceptible to  $\beta$ -lactamase inhibitors such as clavulanic acid and tazobactam. The CTX-M enzymes preferentially inactivate cefotaxime and ceftazidime, and are the most common ESBL worldwide.<sup>16</sup> These plasmid-borne enzymes in *Klebsiella* and *E. coli* isolates have been recovered from both nosocomial as well as community-acquired isolates.<sup>17</sup> In the United States and Europe, the increasing prevalence in CTX-M enzymes have been linked to the ST131 (O:25:H24) *E. coli* clone that accounts for most of the spread of isolates resistant to fluoroquinolone and broad-spectrum  $\beta$ -lactam antibiotics.<sup>18,19</sup> Among class C ESBL, the most important clinically are the AmpC enzymes. They confer resistance to cephalothin, ceftazolin, cefoxitin, most penicillins, and  $\beta$ -lactamase inhibitor- $\beta$ -lactam combinations, and their production is induced in the

presence of antibiotics. Many strains of *Enterobacter* have small populations of mutants that permanently overexpress AmpC enzymes.<sup>20</sup> Consequently, exposure to third generation cephalosporins can select out these resistant sub-populations and lead to antibiotic resistance during therapy, as described in reports of treatment failure in patients with *Enterobacter* bacteremia treated with ceftriaxone.<sup>21</sup>

##### *Carbapenem resistance*

Carbapenem resistance is mainly achieved through the production of carbapenemases—ESBL capable of hydrolyzing a broad-spectrum of  $\beta$ -lactam antibiotics, including all penicillins, cephalosporins, and carbapenems. Among class A carbapenemases, *Klebsiella pneumoniae* carbapenemases (KPC) are clinically the most important. They are plasmid-based, confer resistance to all  $\beta$ -lactams, and can be transferred to other gram-negative species, such as *E. coli*,<sup>22</sup> *Enterobacter*, *Pseudomonas*,<sup>23</sup> and *Salmonella*,<sup>24</sup> which have all been recovered from clinical isolates of hospitalized patients. Among the metallo- $\beta$ -lactamases (class B), most clinically relevant enzymes are the IMP-type, VIM-type, SPM-type, and the NDM-type.<sup>15</sup> The New Delhi metallo- $\beta$ -lactamase (NDM-1) has received the most attention recently. Originally described in a Swedish patient hospitalized with *K. pneumoniae* infection in India in 2008, NDM-1 enzymes have since been reported in the US and UK primarily in connection with travel to India or Pakistan.<sup>4,25</sup> These enzymes confer resistance to all  $\beta$ -lactams except aztreonam. However, most metallo- $\beta$ -lactamases reside on mobile gene cassettes inserted into integrons which harbor additional antibiotic resistance genes to other antimicrobial classes. This multidrug resistance can be transferred to other species via transposons and plasmids, severely limiting therapeutic options in serious infections.<sup>15,24</sup> Class D carbapenemases belong mostly to the OXA-type family, and are found primarily in *P. aeruginosa* and *Acinetobacter* species. Carbapenem resistance can also occur through other mechanisms, such as impermeability and efflux, especially in *Pseudomonas* isolates.<sup>26</sup> Production of cephalosporinases such as AmpC enzymes combined with reduction in antimicrobial diffusion across bacterial membranes through alterations in the genes regulating porin channels can also confer carbapenem resistance in gram-negative bacteria.<sup>25</sup>



**Figure 1.** Mechanisms of antimicrobial resistance in gram-negative bacteria. Re-printed with permission from reference 43.

### Fluoroquinolone resistance

Fluoroquinolone resistance is mediated primarily through alteration of drug targets (DNA gyrase and/or topoisomerase IV). In *E. coli*, the main mutations occur at specific hotspots associated with the quinolone resistance determining region within the genes *gyrA* and *parC* encoding the drug target DNA gyrase.<sup>27</sup> Efflux pumps may also contribute to fluoroquinolone resistance in *E. coli* (*acrAB*–*tolC* system) and *Pseudomonas aeruginosa* (*mex*-encoded operons).<sup>27</sup>

### Aminoglycoside resistance

Aminoglycoside resistance occurs through inactivating enzymes which modify the antibiotics through phosphorylation, adenylation, or acetylation. Alternatively, methylation of the 16S rRNA drug target confers high-level resistance to the entire aminoglycoside class, including novel aminoglycosides in development.<sup>17,28</sup> These plasmid-mediated methylases are often disseminated in association with carbapenemases, contributing to the spread of multidrug resistance among different species of gram-negative bacteria.<sup>29</sup> In patients with cystic fibrosis harboring *Pseudomonas aeruginosa* isolates, resistance through decreased permeability by active efflux has also been described.<sup>30</sup>

### Multidrug resistance

Multidrug resistance occurs through simultaneous activation of several resistance mechanisms such as production of chromosomally-encoded ESBL, and decreased permeability through loss of porin channels and activation of multidrug efflux pumps in response to antibiotic exposures. In addition, acquisition of plasmids and mobile elements carrying multiple resistance genes also contributes to the development of multidrug-resistant phenotypes. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the two clinical pathogens most likely to become multidrug-resistant in this fashion.<sup>26</sup>

### Clinical Impact of Antibiotic Resistance

Serious infections with gram-negative pathogens are associated with significant mortality, especially in immunocompromised hosts. For example, in studies of *Pseudomonas aeruginosa* bloodstream infections, mortality ranged from 39% to 42% in patients with stem-cell or solid organ transplants.<sup>31,32</sup> Studies of patients with ICU infections with MDR *Pseudomonas* or *Acinetobacter* species have a significantly worse outcome than

ICU patients with other types of infection.<sup>4,5</sup> Multidrug resistance further increases the risk of death in gram-negative sepsis. For example, in severe nosocomial infections with *Pseudomonas aeruginosa* isolates producing metallo- $\beta$ -lactamases, mortality ranged from 70% to 95%.<sup>32,33</sup> In patients infected with carbapenem-resistant organisms from the KPC-producing family, mortality ranged from 24% to 65%.<sup>34-36</sup> Receipt of antimicrobials that still retain activity against the multidrug-resistant isolates is crucial for survival. The impact of inadequate empiric antimicrobial therapy on mortality was illustrated in a study of 186 patients with bloodstream infections caused by ESBL producing organisms. The 3 week mortality associated with receipt of inadequate vs. adequate initial therapy was 60% vs. 19%, respectively.<sup>36</sup>

## Prevention

The cornerstone of reducing infections with multidrug-resistant organisms relies on practicing high-quality antimicrobial stewardship and infection control. Avoiding unnecessary antimicrobial exposure, distinguishing colonization from true infection, dosing antimicrobials adequately, and stopping antimicrobial treatment as soon as possible after symptom resolution are all essential to preventing development of antimicrobial resistance.<sup>37</sup> Active surveillance should strongly be considered in healthcare facilities caring for patients harboring multidrug-resistant organisms, since isolates recovered in clinical cultures often represent only a small fraction of the local antibiotic resistance burden. This is particularly true for carbapenemase-producing organisms, as illustrated in the study by Wiener-Well et al., where less than one-third of the patients identified as carriers through active surveillance had positive clinical cultures.<sup>38</sup> Strict adherence to infection control strategies such as compliance with hand-hygiene and contact precautions in all healthcare settings is essential and cannot be over-emphasized.<sup>25</sup>

## Treatment

Prompt initiation of antimicrobial therapy is critical for improving the clinical outcomes in severe sepsis, since the likelihood of survival in septic shock decreases by 8% for each hour of delay in antimicrobial administration.<sup>39,40</sup> Ensuring that the empirical antimicrobial regimen prescribed is actually effective in the treatment of sepsis with a multidrug-resistant pathogen remains challenging. Access to local institution-wide antimicrobial biograms, and knowledge of local prevalence of multidrug resistance as well as prior antimicrobial receipt could help guide initial antimicrobial choices.<sup>41</sup> Combination antimicrobial therapy initially can increase the chances that at least one drug retains activity against the suspected offending pathogen, and appears to improve survival in patients with severe sepsis.<sup>42-44</sup> Obtaining appropriate cultures prior to instituting antimicrobial therapy will allow de-escalation once susceptibility results are known. The following antibiotics constitute possible options in the treatment of severe infections with multidrug-resistant pathogens, depending on isolate antibiogram results:

### Carbapenems

Carbapenems remain the treatment of choice for severe infections with ESBL-producing organisms that do not have carbapenemase activity. This was illustrated in a prospective study of patients with ESBL-producing *Klebsiella pneumoniae* bacteremia: the 2 week mortality for patients treated with carbapenem monotherapy, other  $\beta$ -lactam therapy, and antibiotics without any activity against the offending organism was 3.7%, 44%, and 54%, respectively.<sup>45</sup> Although some strains with ESBL from the TEM or SHV-type families may appear sensitive in vitro to cefepime and piperacillin-tazobactam, they are no longer susceptible when the inoculum is high,<sup>46</sup> and therefore are not indicated in the treatment of sepsis or severe infections with a high bacterial burden (e.g., extensive pneumonia, peritonitis, or large abscesses).

### Colistin

Colistin (Polymyxin E) is an old antibiotic with cationic detergent properties which binds to the lipid A component of bacterial lipopolysaccharide and disrupts the outer cell membrane of gram-negative bacteria, causing leakage of cytoplasmic contents.<sup>47</sup> Some multidrug-resistant strains of *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas*, and some *Enterobacteriaceae* (*E. coli*, *Klebsiella*, and *Enterobacter*) remain susceptible to colistin. The experience with colistin therapy is limited to mostly non-comparative, retrospective studies where colistin was administered in varying doses and durations, and was often given in combination with other antimicrobials.<sup>48</sup> Prospective, randomized trials are needed before definitive recommendations regarding dosing and therapy duration can be made.

The literature to date suggests that colistin administered in combination with other antimicrobials appears to improve survival compared with monotherapy, at least for carbapenem-resistant *Enterobacteriaceae*.<sup>49</sup> One of the most common combination therapies studied includes colistin-polymyxin B with carbapenem, which had a mortality rate of 12.5% (1/6) in patients with KPC-producing *Klebsiella pneumoniae* bacteremia. The mortality for colistin or carbapenem monotherapy in this study was 66.7% (8/12)<sup>50</sup> Colistin combined with doripenem was also found to be bactericidal and synergistic in vitro for carbapenemase producing *Klebsiella pneumoniae* isolates.<sup>51</sup> The combination of tigecycline, colistin, and extended-infusion meropenem (2 g IV infused over 3 h every 8 h) was associated with the best outcomes in a multicenter retrospective study of 125 patients with sepsis from KPC-producing *K. pneumoniae* bacteremia.<sup>49</sup> Intravenous colistin has also been used with some success in the treatment of nosocomial infections with multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates, especially when used in combination with rifampicin.<sup>52-56</sup> Nephrotoxicity can develop in almost half of the patients receiving full doses of intravenous colistin, although it appears to be reversible for most patients.<sup>57</sup>

### Tigecycline

Tigecycline has in vitro activity against ESBL-producing organisms, including some of the carbapenemase-producing *Enterobacteriaceae*, *Acinetobacter*, and *Stenotrophomonas* species.

It is not active against *Pseudomonas* species, and *Proteus* isolates are intrinsically resistant. Tigecycline has been used with varying degrees of success in the treatment of infections with multidrug-resistant gram-negative organisms. For example, a retrospective analysis of 45 patients, primarily in the ICU with infections caused by multidrug-resistant *Acinetobacter* and *Klebsiella* species reported an 80% clinical success rate with tigecycline monotherapy.<sup>58</sup> On the other hand, tigecycline is not recommended in the treatment of bacteremia. The serum concentration of this bacteriostatic, lipophilic drug is rather low with a large volume of distribution, and does not adequately exceed the MIC for most resistant gram-negative organisms.<sup>59</sup> Development of resistance during therapy with tigecycline for initially susceptible isolates (carbapenemase and ESBL-producing *Enterobacteriaceae* and non-fermenters) raises further caution in the use of tigecycline as monotherapy for severe infections.<sup>60</sup>

### Fosfomycin

Fosfomycin is another old broad-spectrum antibiotic that inhibits bacterial cell wall synthesis, and has in vitro activity against ESBL-producing organisms.<sup>61</sup> Fosfomycin may also inhibit carbapenemase-producing organisms, as evidenced by a recent study where 60% of the 81 carbapenem-resistant *Enterobacteriaceae* strains tested were found to be susceptible to this antimicrobial.<sup>62</sup> The experience with its use in severe infections is limited, as intravenous fosfomycin is not available in many countries, including the US. In a small prospective study of 11 critically ill patients with carbapenem-resistant *Klebsiella pneumoniae* infections, intravenous fosfomycin used in combination with other antimicrobials was reported to be associated with good clinical and bacteriological outcomes, and no significant adverse events.<sup>63</sup>

The additive benefit of intravenous fosfomycin in combination with colistin vs. colistin alone is currently being tested in patients with MDR *Acinetobacter baumannii* infections (clinicaltrials.gov NCT11297894). Fosfomycin aerosol, in combination with tobramycin aerosol administration, is also being studied in cystic fibrosis patients with difficult to treat lung infections with *Pseudomonas aeruginosa*. (NCT00794586)

### Antimicrobials in the pipeline

There are currently several parenteral antimicrobial therapies under investigation for the treatment of multidrug-resistant gram-negative infections. Ceftolozane (CXA-101) in combination with tazobactam is active against ESBL-producing bacteria (CTX-M-15  $\beta$ -lactamases specifically). It is currently undergoing phase 3 trials of complicated urinary tract and intra-abdominal infections. Ceftazidime in combination with avibactam (a new  $\beta$ -lactamase inhibitor) is active against *P. aeruginosa* and

ESBL-producers including KPC type  $\beta$ -lactamases, but not against metallo- $\beta$ -lactamases such as NDM and VIM. It is also in phase 3 studies for complicated urinary tract infection and intra-abdominal infections. Ceftaroline/avibactam is active in vitro against ESBL and KPC-producing organisms. The activity against resistant *Acinetobacter* species is limited for all these cephalosporin/ $\beta$ -lactamase inhibitor combinations. Imipenem in combination with another novel  $\beta$ -lactamase inhibitor, MK-7655, appears active in vitro against serine carbapenemases and against *P. aeruginosa*, but not against metallo-carbapenemases or *A. baumannii*. Plazomicin is a new generation aminoglycoside active against metallo- $\beta$ -lactamases, carbapenemases, fluoroquinolone-resistant, and aminoglycoside-resistant gram-negative bacteria, but has limited activity against *P. aeruginosa* or *A. baumannii*. Eravacycline is a novel tetracycline that is not susceptible to the efflux or protection of the ribosomal target that render older tetracyclines ineffective. It has in vitro activity against KPC-producing bacteria, but not against *Pseudomonas* or *Acinetobacter* species. Carbavance (a  $\beta$ -lactam/ $\beta$ -lactamase inhibitor combination of biapenem and RPX7009), currently in phase I development, has activity against ESBL and KPC-producing organisms, and is also active against resistant *Pseudomonas* and *Acinetobacter* strains, but not against metallo- $\beta$ -lactamases.<sup>64</sup>

## Conclusion

None of the antimicrobials currently in development have activity against the entire spectrum of multidrug-resistant gram-negative bacteria, with the therapeutic options for infections with *Acinetobacter*, *Pseudomonas*, and other metallo- $\beta$ -lactamase producing organisms particularly absent from both the current as well as the future drug armamentarium. Furthermore, none of the current drugs under investigation are in trials for the more severe sepsis syndromes like bloodstream infections, endocarditis, or invasive pneumonia that critical care clinicians are now increasingly being faced with. Therefore, death from sepsis caused by pathogens without therapeutic options is now an imminent threat. The best hope for the future is the development of a greater understanding of how antimicrobial resistance spreads globally, and whether the alarming trend in community-acquired multidrug resistance can be reversed. A concerted global commitment to intelligent use of antimicrobials, development of improved bacterial vaccines, better antibiotic stewardship, and the implementation of effective infection control strategies are urgently needed.<sup>65</sup>

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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