

# Carbapenemase-Producing *Enterobacteriaceae*

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## Abstract

Carbapenemase-producing *Enterobacteriaceae* (CPE) were almost nonexistent up to the 1990s, but are today encountered routinely in hospitals and other healthcare facilities in many countries including the United States. KPC-producing *Klebsiella pneumoniae* was the first to emerge and spread globally and is endemic in the United States, Israel, Greece, and Italy. Recently, NDM-producing *Enterobacteriaceae* and OXA-48-producing *K. pneumoniae* appear to be disseminating from South Asia and Northern Africa, respectively. They are almost always resistant to all  $\beta$ -lactams including carbapenems and many other classes. Mortality from invasive CPE infections reaches up to 40%. To obtain the maximal benefit from the limited options available, dosing of antimicrobial agents should be optimized based on pharmacokinetic data, especially for colistin and carbapenems. In addition, multiple observational studies have associated combination antimicrobial therapy with lower mortality compared with monotherapy for these infections. The outcomes appear to be especially favorable when patients are treated with a carbapenem and a second agent such as colistin, tigecycline, and gentamicin, but the best approach is yet to be defined.

## Keywords

- antimicrobial resistance
- carbapenemases
- *Klebsiella pneumoniae*
- *Enterobacteriaceae*

Carbapenem resistance in *Enterobacteriaceae* had been a negligible phenomenon before 2000. Back then, the rare occurrence of reduced susceptibility to carbapenems in *Enterobacteriaceae* was mostly attributed to a combination of production of extended-spectrum  $\beta$ -lactamase or AmpC  $\beta$ -lactamase and deficiency of porins in the outer membrane.<sup>1,2</sup> Reports on carbapenem resistance due to production of carbapenemases ( $\beta$ -lactamases capable of hydrolyzing carbapenems), such as IMP or VIM-type metallo- $\beta$ -lactamases, were beginning to emerge,<sup>1</sup> but the prevalence of carbapenemase-producing *Enterobacteriaceae* (CPE) was exceedingly low. This picture changed when *Klebsiella pneumoniae* producing KPC-type carbapenemase appeared in the late 1990s and spread worldwide in the 2000s,<sup>3</sup> recently followed by expansion of *Enterobacteriaceae* producing NDM-type carbapenemase and *K. pneumoniae* producing OXA-48-type carbapenemase. These organisms are almost always resistant to

carbapenems and many other classes of commonly used antimicrobial agents; thus, managing infections caused by them poses a substantial challenge in clinical practice. In this review, we will briefly examine the epidemiology and microbiology of these emerging CPEs, and review the current knowledge regarding their clinical management, including prevention and treatment.

## Epidemiology of Carbapenemase-Producing *Enterobacteriaceae*

### KPC-Producing *K. pneumoniae*

KPC stands for *Klebsiella pneumoniae* carbapenemase and is a class A  $\beta$ -lactamase which has the capacity to hydrolyze penicillins, cephalosporins, and carbapenems. KPC was initially reported from a *K. pneumoniae* strain isolated in North Carolina in 1996.<sup>3</sup> By 1997, KPC-producing *K. pneumoniae*

appeared in some hospitals in New York City,<sup>4</sup> and continued to spread.<sup>5</sup> In a city-wide surveillance study conducted in 2006, 38% of *K. pneumoniae* clinical isolates produced KPC, making this a truly concerning epidemic.<sup>5</sup> KPC-producing *K. pneumoniae* has since spread across the United States,<sup>6</sup> but not evenly, with eastern census regions showing higher prevalence than western and southern regions. KPC-producing *K. pneumoniae* appears to be especially endemic in the mid-Atlantic, Midwest regions, as well as in Florida and Puerto Rico. The overall prevalence of carbapenem resistance among *Klebsiella* spp. isolates causing hospital-acquired infections in U.S. hospitals was approximately 12% between 2009 and 2010 according to data from the National Healthcare Safety Network.<sup>7</sup>

KPC-producing *K. pneumoniae* has since spread worldwide. The first country besides the United States that experienced a nationwide outbreak was Israel.<sup>8</sup> Emergence and a sharp increase in the number of KPC-producing *K. pneumoniae* isolates was identified in Tel Aviv hospitals between 2005 and 2006, which were shown to be genetically related to the isolates circulating in U.S. hospitals.<sup>9</sup> It was apparent by early 2007 that this was a nationwide outbreak, which led the Ministry of Health to issue infection control guidelines mandating contact precaution of hospitalized carriers and the use of dedicated staffing which was enforced by a task force.<sup>10</sup> These measures were successful in controlling the nationwide incidence of KPC-producing *K. pneumoniae* acquisitions, and the downward trend has continued as of 2012.<sup>11</sup>

The other countries heavily affected by this organism include Greece and Italy. In Greece, a hospital outbreak was detected in 2007,<sup>12</sup> and a nationwide epidemic ensued. Carbapenem resistance rate of 60.5% was recorded in 2012 for *K. pneumoniae* in Greece in the ERAS-Net surveillance (<http://www.ecdc.europa.eu>). While KPC-producing *K. pneumoniae* accounts for the majority of this, *K. pneumoniae* producing VIM-type metallo- $\beta$ -lactamase is also endemic in Greece,<sup>13</sup> further complicating the picture. Italy also now has a high rate of carbapenem resistance among *K. pneumoniae* (28.8% in the above surveillance report), following initial reports of hospital outbreak occurring around 2009.<sup>14</sup> Nearly 90% of them are producing KPC.<sup>15</sup> High burdens of KPC-producing *K. pneumoniae* have also reported from China, Brazil, and Colombia.<sup>16</sup>

The rapid global spread of KPC-producing *K. pneumoniae* is now understood as a largely clonal phenomenon.<sup>17</sup> A specific clone of KPC-producing *K. pneumoniae*, called ST258, is globally distributed. ST stands for sequence type, and is assigned by multilocus sequence typing, which is a nucleotide sequence-based bacterial typing method where seven genes on the chromosome are sequenced to decipher global relatedness among strains. ST258 predominates among KPC-producing *K. pneumoniae* in the United States.<sup>6</sup> ST258 as well as ST512, which is closely related to ST258, has been found commonly in Israel and Italy,<sup>18,19</sup> whereas ST11 and ST437 appear to predominate in China and Brazil, respectively.<sup>20,21</sup> These STs are all closely related to ST258 suggesting the presence of a common origin, most likely in the mid-Atlantic United States. On the other hand, plasmids carrying the KPC

gene are diverse in structure and often capable of self-transmission to other strains by conjugation.<sup>22</sup>

While carbapenem resistance mediated by KPC production is most conspicuous in *K. pneumoniae*, the KPC gene can be acquired by other species of *Enterobacteriaceae* including *Enterobacter* spp. and *Escherichia coli*,<sup>23,24</sup> and on rare occasions *Pseudomonas aeruginosa* and *Acinetobacter baumannii* as well.<sup>25</sup> Among these species, the potential of *E. coli* acquiring KPC is especially concerning because of its community-wide distribution as a commensal organism.

### NDM-Producing *Enterobacteriaceae*

NDM stands for New Delhi metallo- $\beta$ -lactamase. It is a class B  $\beta$ -lactamase and is capable of hydrolyzing penicillins, cephalosporins, and carbapenems, but unlike KPC, it does not hydrolyze aztreonam. NDM-producing *K. pneumoniae* and *E. coli* were first identified in an Indian patient residing in Sweden who had hospitalization for wound infections in New Delhi before returning to Sweden in early 2008.<sup>26</sup> It was soon reported that NDM-producing *Enterobacteriaceae* were present in Indian hospitals as early as 2006.<sup>27</sup> In a study where *Enterobacteriaceae* clinical isolates were collected from three Indian hospitals in 2010, the prevalence of the NDM production was 5.2%.<sup>28</sup> In Pakistan, prevalence of 18.5% has been reported for NDM-producing *Enterobacteriaceae* among stool samples collected at two hospitals in 2010.<sup>29</sup> In addition to the Indian Subcontinent as the primary reservoir, it has been speculated that secondary but significant reservoirs of NDM-producing organisms might exist in the Balkans and the Gulf region.<sup>30,31</sup> NDM-producing bacteria have now been reported from all corners of the world, including the United States, therefore having shown even more rapid spread compared with those producing KPC since the initial appearance. NDM is also distinct from KPC in that its spread is occurring both in healthcare settings and the community. NDM-producing organisms have been detected in tap water and seepage samples collected in New Delhi,<sup>32</sup> and in species that are considered community-acquired pathogens, such as *Salmonella enterica* and *Vibrio cholerae*.<sup>33,34</sup>

### OXA-48-Producing *K. pneumoniae*

OXA stands for oxacillinase and is a diverse group of  $\beta$ -lactamases classified to class D. Some of OXA  $\beta$ -lactamases additionally have the capability to hydrolyze carbapenems. In *Enterobacteriaceae*, OXA-48 and its related enzymes are the ones that require attention. OXA-48 was first found in a *K. pneumoniae* strain isolated in Turkey in 2001.<sup>35</sup> Its production mediates resistance to penicillins and carbapenems (especially imipenem), but not to cephalosporins. The OXA-48 gene is usually encoded on a plasmid and may spread to other species of *Enterobacteriaceae*, but most cases have been reported in *K. pneumoniae*. OXA-48-producing *K. pneumoniae* often also coproduces an extended-spectrum  $\beta$ -lactamase. These isolates are then resistant to all  $\beta$ -lactams including cephalosporins. *K. pneumoniae* producing OXA-48 and its related  $\beta$ -lactamases have been reported mostly from Turkey, North Africa, and recently the Gulf region and India.<sup>30,36</sup> Outbreaks have occurred in Europe as well. However, their

prevalence has not been estimated, mostly because of the difficulty in detecting OXA-48-producing isolates, which could present with variable levels of carbapenem resistance and may remain susceptible to cephalosporins.<sup>36</sup> They remain extremely rare in the United States, but imported cases have been reported.<sup>37</sup>

## Detection of Carbapenemase-Producing *Enterobacteriaceae*

CPE may or may not be frankly “resistant” to carbapenems. This is because, while production of carbapenemase always elevates the minimum inhibitory concentrations (MICs) of carbapenems, they may not be high enough to be called resistant or intermediately resistant. To address this issue, the Clinical and Laboratory Standards Institute (CLSI) lowered the MIC breakpoints for carbapenems in 2010. For example, an MIC of 16 mg/L was required for meropenem resistance prior to 2010, whereas an MIC of 4 mg/L has been defined as resistant since this revision. However, the uptake of the revised breakpoints has been slow, mostly because of validation delays on commercially available, automated susceptibility testing instrument.<sup>38</sup> It has been well documented that a substantial portion of CPE is missed by the old breakpoints.<sup>39</sup> In general, resistance to ertapenem (under the current breakpoints) is considered to have the best sensitivity but less than ideal specificity in screening of carbapenemase-producing isolates.<sup>39</sup> Therefore, those that are resistant or intermediately resistant (i.e., MIC of  $\geq 1$  mg/L) should then undergo confirmatory testing for carbapenemase production.

In Europe, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) defines susceptibility to imipenem and meropenem as an MIC  $\leq 2$  mg/L, with resistance being defined as an MIC  $> 8$  mg/L. The EUCAST guidelines note that in many areas, carbapenemase detection and characterization is recommended or mandatory for infection control purposes.<sup>39</sup>

There are several approaches to confirmatory testing, using either molecular or nonmolecular methods. In clinical microbiology laboratories, nonmolecular methods are more feasible because they do not require expensive instrument and reagents. The most widely adopted and endorsed by the CLSI is the modified Hodge test.<sup>40</sup> It is a culture-based test to detect release of carbapenemase into agar media and can be performed without any special equipment or reagent, but the interpretation of the results may be subjective. The test performs reasonably well with KPC-producing *K. pneumoniae*, but false-positive results are frequent in *Enterobacter* spp. One caveat is that this test is designed to detect carbapenemase in general and not specifically KPC. In countries with high burden of KPC producers, a positive test is likely to result from KPC production, but could on rare occasions result from production of OXA-48, NDM, or other infrequent metallo- $\beta$ -lactamases. A positive test should therefore be reported out as “carbapenemase producer” and not “KPC producer.”

A rapid chromogenic test (“Carba NP test”) has also been developed, where hydrolysis of imipenem by crude carbapenemase extracted from the isolates is observed by color

changes in a microtiter well.<sup>41</sup> This test has equivalent sensitivity and superior specificity in comparison with the modified Hodge test,<sup>42</sup> and has the added advantage of not needing an extra day of culturing to read the results.

In countries or regions where KPC-producing organisms are endemic, inhibitor-based testing using a boronic acid compound is a viable alternative to the aforementioned methods. Boronic acid was initially studied as an inhibitor of AmpC  $\beta$ -lactamases, but was later found to also inhibit the activity of KPC. Therefore, by adding aminophenyl boronic acid (300 or 400  $\mu$ g) to a carbapenem disk (ertapenem or meropenem), enlargement of the inhibitory zone in comparison with a carbapenem disk alone can be used to infer KPC activity.<sup>43,44</sup> In this case, a positive test can be reasonably signed out as “KPC producer.” This test would not detect NDM or OXA-48-producing isolates.

Phenotypic detection of NDM, which is a metallo- $\beta$ -lactamases, depends on the use of a zinc chelator such as ethylenediaminetetraacetic acid (EDTA), sodium mercaptoacetic acid (SMA), and dipicolinic acid, where these compounds are used to inhibit the activity of NDM.<sup>45</sup> Etest MBL strips, which use EDTA as the inhibitor, are commercially available. Currently, no nonmolecular test for detection of OXA-48 is available.

Definitive identification of carbapenemases in *Enterobacteriaceae* still relies on nucleic acid-based tests, including PCR. PCR-based commercial test kits are now available (e.g., Check-Points products).<sup>46</sup>

## Active Screening of CPE Carriage

While not routinely performed in most hospitals, active screening of CPE carriage may need to be considered under circumstances where there is a high incidence rate of CPE infection and ongoing transmission within the hospital. Early detection of CPE carriage allows for implementation of appropriate infection control measures in a timely manner before transmission occurs. Patients who are considered to be at high risk for acquiring CPE include contacts of newly discovered carriers, those transferred from another hospital with high incidence of CPE infection, and those hospitalized in ICUs or floors (wards) with high incidence of CPE infection. Rectal swab or stool is the preferred specimen for surveillance because *Enterobacteriaceae* constitute the intestinal microbiota. They are typically plated on selective medium that preferentially grows CPE. Examples include direct inoculation of a MacConkey plate with an ertapenem disk on it,<sup>47</sup> direct inoculation of a MacConkey plate containing 1 mg/L of imipenem,<sup>48</sup> and overnight enrichment in broth containing an imipenem disk followed by plating on a MacConkey plate.<sup>49</sup> Commercially manufactured selective media include CHROMagar KPC (CHROMagar, Paris, France) and Spectra CRE (Oxoid, Basingstoke, Hampshire, UK), where these specimens can be directly cultured on these plates. While these chromogenic media allow for distinction between *K. pneumoniae*, *E. coli*, and lactose-non-fermenters, they do not identify species or type of carbapenemase. PCR-based detection of the KPC and NDM genes has also been proposed and may be

more sensitive than culture-based methods,<sup>50</sup> but the higher costs would suggest that they may be appropriate in settings with high prevalence of CPE.

## Antimicrobial Agents Used for Treatment of CPE Infections

### General Considerations

Carbapenems are often reserved for treatment of infections caused by otherwise drug-resistant organisms as efficacious and safe agents of last resort. Carbapenem resistance due to production of carbapenemase thus leaves clinicians and patients with very few treatment options. With few exceptions, these carbapenemases render penicillins (including  $\beta$ -lactamase combinations such as piperacillin-tazobactam) and cephalosporins (including cefepime) inactive, effectively wiping out the entire  $\beta$ -lactams from therapeutic consideration, at least in the context of standalone therapy. In terms of in vitro susceptibility, two agents that maintain activity relatively well across CPEs are polymyxins (colistin and polymyxin B) and tigecycline. Fosfomycin and doxycycline (or minocycline) are active against some CPE strains and their use could be considered especially in the case of urinary tract infection. In addition, some, but not all, KPC-producing *K. pneumoniae* strains remain exquisitely susceptible to gentamicin. However, this is not the case for NDM-producing *Enterobacteriaceae* strains, many of which produce 16S ribosomal RNA methyltransferase that render them highly resistant to all aminoglycosides including gentamicin. Finally, although OXA-48 itself does not hydrolyze cephalosporins well, OXA-48-producing *K. pneumoniae* strains appear to coproduce ESBL in most instances, and as a result are resistant to cephalosporins as well as carbapenems.

### Polymyxins (Colistin and Polymyxin B)

Polymyxins are cationic cyclic polypeptides linked to a fatty acid chain. Colistin and polymyxin B differ only by one amino acid and share similar biological activity, and exert their bactericidal activity by binding to lipid A of lipopolysaccharide followed by uptake across the outer membrane.<sup>51</sup> They were discovered over 50 years ago and were used for treatment of gram-negative bacterial infection. The advent of safer alternatives (e.g., cephalosporins) since led to their disuse, but they have been brought back to widespread use since the turn of the century for treatment of infection caused by carbapenem-resistant bacteria including CPE as well as *P. aeruginosa* and *A. baumannii*. Colistin is active against most gram-negative bacterial species, except for *Proteus* spp., *Providencia* spp., and *Serratia* spp., which are intrinsically resistant. Both colistin and polymyxin B are available in clinical use in some countries in the Americas including the United States, but only colistin is available in many countries worldwide. Therefore, more data are available on colistin compared with polymyxin B. Major adverse effects are nephrotoxicity and neurotoxicity. Nephrotoxicity occurs in as many as 50% of patients and more among critically ill patients,<sup>52,53</sup> but these rates may not be higher than controls overall.<sup>54</sup> It is usually reversible upon discontinuation of the agents. Neurotoxicity such as neuromuscular

blockade may also occur with polymyxin use, but they appear to be rare events in recent clinical series.<sup>54</sup>

Polymyxin B is administered to patients as the active drug, but colistin is administered as its prodrug colistin methanesulfonate (CMS), which sequentially undergoes hydrolysis to form a mixture of partially sulfomethylated as well as active colistin. The complex pharmacokinetics of CMS and colistin began to be elucidated only recently and remain a subject of intensive investigation. The half-life of CMS and colistin is approximately 2.2 and 18.5 hours, respectively.<sup>55</sup> CMS undergoes net tubular secretion with extensive renal clearance, whereas colistin is subjected to tubular reabsorption and its clearance is largely nonrenal. Only approximately 7% of the administered CMS is converted to colistin systemically.<sup>56</sup> As a result, the maximum concentration of colistin in plasma after the first dose given at 90 mg colistin base activity (CBA) is only 0.6 mg/L, well below the MICs of many organisms that require therapy with this agent, and colistin is undetectable in bronchoalveolar lavage 2 hours after administration of CMS.<sup>57,58</sup> When CMS is given at 90 mg CBA every 8 hours, the steady state concentration of approximately 2.3 mg/L is achieved only after 2 or 3 days of therapy. If the first dose is given at 180 mg CBA instead of 90 mg as a loading dose, then the maximum colistin concentration of approximately 1.3 mg/L is achieved in 8 hours.<sup>55</sup> It therefore appears that a loading dose is warranted to obtain a therapeutic plasma concentration of colistin in a timely manner. Another consideration is that a combination with another active agent is likely to be beneficial in improving patient outcome and preventing development of resistance, unless colistin MIC is very low. In addition, colistin does not achieve therapeutic levels in the cerebrospinal fluid via intravenous administration. Treatment of meningitis thus requires either intrathecal or intraventricular routes of administration.

Much less is known about the pharmacokinetics of polymyxin B, which is administered as the active drug upfront. It has a half-life of approximately 13.6 hours and is cleared mostly via nonrenal routes.<sup>59</sup>

Most CPE isolates remain susceptible to polymyxins, but colistin-resistant KPC-producing *K. pneumoniae* strains are increasingly reported.<sup>60–62</sup> Colistin-resistant *K. pneumoniae* producing NDM or OXA-48 appear to be rarer but have been reported as well.<sup>63,64</sup> These cases are mostly reported in those who have been exposed to colistin. Therefore, it would be prudent to test colistin susceptibility if the patient has been treated with colistin or polymyxin B before.

### Tigecycline

Tigecycline is a derivative of minocycline designed to circumvent efflux-mediated resistance mechanisms. It has a broad spectrum of activity against gram-positive and gram-negative bacteria, including CPE. However, nonsusceptibility to tigecycline is increasingly common in KPC-producing *K. pneumoniae*,<sup>65</sup> occurring in patients who have been treated with this agent.<sup>66</sup>

Tigecycline has a large volume of distribution resulting in low concentrations in blood, epithelial lining fluid of the lungs, and urinary tract.<sup>67</sup> The peak plasma concentration of



tigecycline is in the range of 0.6 to 0.9 mg/L.<sup>67</sup> Therefore, it is generally not considered a good option for treatment of patients with bacteremia, severe pneumonia, and urinary tract infection. It did not attain clinical approval for hospital-acquired pneumonia because of a phase 3 study which showed an inferior cure rate compared with imipenem.<sup>68</sup> This inferiority was primarily driven by those with ventilator-associated pneumonia, which is a relevant clinical presentation for CRE. A higher dose of tigecycline (100 mg every 12 hours) has resulted in a nominally better clinical cure rate compared with imipenem for hospital-acquired pneumonia in a phase 2 study.<sup>69</sup> This is not an approved dose, and further studies would be required before this approach can be recommended. Overall, despite the in vitro activity of tigecycline against CPE, its use in treatment is usually in the context of combination therapy given the above-mentioned reservations.

### Carbapenems

CPEs exhibit elevated MICs to carbapenems, but the level of resistance is highly variable, ranging from as low as 0.12 mg/L to >256 mg/L. For strains with low MICs up to 4 mg/L, prolonged infusion of high-dose carbapenem may achieve sufficient free time above MIC (i.e., >40%) required for bactericidal effect. Limited clinical experience suggests that monotherapy with carbapenem may indeed result in clinical cure when carbapenem MICs of the CPE isolates are low.<sup>70</sup> However, the majority of CPEs have carbapenem MICs which exceed this range, and there is also a concern for carbapenem therapy leading to elevation of MICs through mutations in porin genes.<sup>71</sup> Therefore, treatment of CPE infection with carbapenem alone is generally discouraged, perhaps with the exception of rare cases where carbapenem MICs are exceedingly low and the source of infection is well controlled.

A unique approach to treatment of KPC-producing *K. pneumoniae* infection using two carbapenems has been proposed. The rationale is that ertapenem, which has high affinity to the KPC enzyme, would serve as a decoy allowing for the second carbapenem (meropenem or doripenem) to be protected from KPC and bind to the target penicillin binding proteins better.<sup>72</sup> Anecdotal success of this approach in patients with KPC-producing *K. pneumoniae* infection has been reported.<sup>73</sup> However, controlled clinical data would be needed to determine if this is a unique effect from the combination or due to the higher net amount of carbapenems that are administered.

Despite these reservations regarding carbapenem-only regimens, carbapenems appear to constitute an essential element of combination therapy in treating CPE infections, which will be described in more detail later.

### Gentamicin

Aminoglycosides, especially gentamicin, may have a role in the treatment of KPC-producing *K. pneumoniae* infection. In particular, the global epidemic clone ST258 is more likely to maintain susceptibility to gentamicin compared with other STs.<sup>74</sup> Gentamicin at a concentration of 10 mg/L has been shown to exert substantial bactericidal activity against gen-

tamicin-susceptible, KPC-producing *K. pneumoniae* ST258 strains within minutes of exposure.<sup>75</sup> In a murine thigh infection model, monotherapy with gentamicin was efficacious in the majority of KPC-producing *K. pneumoniae* strains tested.<sup>76</sup> In clinical practice, gentamicin is almost always used in the context of combination therapy, often in combination with colistin, a carbapenem, or tigecycline.<sup>77</sup>

In contrast, aminoglycosides are not considered as an option for NDM-producing *Enterobacteriaceae* because the majority of the isolates produce 16S ribosomal RNA methyltransferase, which renders them completely resistant to aminoglycosides.<sup>78</sup>

### Fosfomycin

Fosfomycin is a peptidoglycan synthesis inhibitor that has a wide spectrum of activity ranging from gram-positive to gram-negative bacteria. In the United States, it is available only for oral administration (fosfomycin tromethamine), but an intravenous formulation (fosfomycin disodium) is also available in some European countries. It is a small molecule with negligible protein binding, and is cleared well by glomerular filtration, achieving very high levels in the urine.<sup>79</sup> The oral formulation is used almost exclusively for treatment of urinary tract infection. The intravenous formulation, on the other hand, has been used for therapy of various types of infections where available.<sup>80</sup> CPE isolates including KPC-producing *K. pneumoniae* mostly remain susceptible to fosfomycin and likely could be used for treatment of urinary tract infection.<sup>81,82</sup> For systemic infections, intravenous fosfomycin is used in combination with another agent (e.g., colistin, tigecycline), making assessment of the efficacy difficult, but it appears to be well tolerated.<sup>83</sup>

### Rifampin

Rifampin is an inhibitor of RNA polymerase and has a very broad spectrum of activity. Its use is limited by rapid emergence of resistance due to amino acid substitutions in the target polymerase; thus, rifampin is always used in combination with other agents. Some studies have reported in vitro or in vivo synergy in the killing of CPE between rifampin and another agent (e.g., tigecycline, colistin).<sup>76,84</sup> Clinical data are scarce, however, and the significant interactions between rifampin and medications often used in the patient populations prone to CPE infections (e.g., tacrolimus, voriconazole) also limit its potential for use in the treatment of CPE infections.

### Doxycycline

Doxycycline is partially eliminated through glomerular filtration (20–30%), and achieves adequate concentration in the urine.<sup>85</sup> Doxycycline and, to a lesser extent, minocycline retain activity against KPC-producing *K. pneumoniae* and have been used successfully in the treatment of urinary tract infection.<sup>86,87</sup> Limited in vitro data suggest that isolates with a doxycycline MICs close to the susceptibility breakpoint may not be inhibited well by this agent,<sup>88</sup> and that doxycycline may have some synergy with gentamicin for KPC-producing *K. pneumoniae*, but synergy with other commonly used drugs

(e.g., colistin) is not robust. Therefore, its potential use is likely limited to therapy of urinary tract infection in patients who are not systemically ill.

### Eravacycline

Eravacycline is a fluorocycline, with a tetracycline core. Obviously, since it is not a  $\beta$ -lactam antibiotic, it will not be inhibited by  $\beta$ -lactamases. In vitro, it has activity against most KPC producers.<sup>89</sup> It remains to be tested clinically against substantial numbers of patients with infections with CPE.

### Ceftazidime-Avibactam

Avibactam is a new  $\beta$ -lactamase inhibitor which has activity against many  $\beta$ -lactamase types, except for those of class B (i.e., metallo- $\beta$ -lactamases). Thus, it has useful activity against class A carbapenemases such as the KPC-type. The in vitro effectiveness of the ceftazidime-avibactam combination has been well documented against contemporary isolates, with MIC<sub>50</sub> and MIC<sub>90</sub> values of 0.5 and 2 mg/L, respectively.<sup>90</sup> Unfortunately, given the lack of activity of avibactam against metallo- $\beta$ -lactamases, ceftazidime-avibactam does not have useful in vitro activity against NDM producers.<sup>90</sup> At present, there are no published data on the clinical use of ceftazidime-avibactam against CPE infections.

### Aztreonam-Avibactam

The combination of aztreonam and avibactam offers the potential for activity against metallo- $\beta$ -lactamase-producing organisms, such as NDM producers. The rationale for this is that aztreonam is stable to the effects of metallo- $\beta$ -lactamases. The addition of avibactam will inhibit other produced  $\beta$ -lactamases allowing aztreonam to act unhindered. The in vitro utility of this approach has been demonstrated against NDM,<sup>91</sup> IMP,<sup>91</sup> and VIM producers.<sup>92</sup> At present, there are no published data on the clinical use of aztreonam-avibactam against CPE infections.

### Ceftolozane-Tazobactam

While ceftolozane-tazobactam has good activity against ESBL-producing strains, it does not appear to have useful activity against CPE.<sup>93</sup>

### Plazomicin

Plazomicin is a new aminoglycoside which is not affected by most aminoglycoside-modifying enzymes.<sup>94</sup> Therefore, it has activity against most KPC-producing strains.<sup>94</sup> Unfortunately, many metallo- $\beta$ -lactamase producers also produce 16S rRNA methyltransferases rendering plazomicin inactive.<sup>95</sup> Results of clinical trials on the use of intravenous plazomicin are awaited.

### Inhaled Amikacin

Amikacin has long been available to clinicians in an intravenous formulation. A novel pulmonary drug delivery system is being developed to deliver high concentrations of amikacin to the lung, thereby facilitating treatment of pneumonia caused by multiresistant gram-negative organisms. Results of clinical trials of the use of inhaled amikacin are awaited.

## Therapy of CPE Infection: Review of Clinical Evidence

While data from randomized control studies would best address questions regarding appropriate therapeutic approaches for CPE infections, no such studies have been completed and reported till date. Therefore, information regarding the antimicrobial regimen and clinical outcome of CPE infection are mostly derived from observational studies. Most data have been published for bacteremia caused by KPC-producing *K. pneumoniae*, which is a frequently encountered condition with high mortality in countries where this organism is endemic (United States, Italy, and Greece). ► **Table 1** summarizes the findings of 4 such studies that included more than 20 patients. These studies sought to correlate the definitive therapy (or culture-directed therapy; antimicrobial treatment that is given after the culture data including susceptibility testing results become available) started 72 to 96 hours after cultures were collected and the mortality of the patients, either in hospital or at 28 or 30 days. While mortality at 14 days may better reflect the impact from sepsis, patients are usually still on definitive therapy at that point, which is why most studies use 28- or 30-day mortality as the primary outcome variable. The overall mortality is approximately 40% across these studies.

The available data overall support the superiority of combination therapy (two or more agents active in vitro against the infecting strain) over monotherapy (one agent active in vitro) in terms of patient survival for invasive CPE infections. Here, active agents usually include colistin, tigecycline, and sometimes carbapenems and gentamicin depending on the causative strains. The report by Zarkotou et al was the first to demonstrate this association.<sup>96</sup> The study was conducted at two hospitals in Greece and included 53 patients with KPC-producing *K. pneumoniae* bacteremia. Mortality was similar for those who received appropriate or inappropriate empiric therapy. Among 35 of them who survived to receive appropriate definitive therapy (i.e., at least one agent active in vitro), the infection-related mortality was 0% for 20 patients who received combination therapy and 46.7% for 15 patients who received monotherapy ( $p = 0.001$ ). The most common combination was colistin and tigecycline (9 patients; 0% infection-related mortality), and the most common monotherapy was colistin (7 patients; 66.7% infection-related mortality). Qureshi et al then examined the outcome of 41 patients at two hospitals in the United States.<sup>97</sup> Of the 34 patients who received definitive therapy, the 28-day mortality was 13.3% for 15 patients who received combination therapy and 57.8% for 19 patients who receive monotherapy ( $p = 0.01$ ). The combinations used were variable, whereas monotherapy largely consisted of colistin (or polymyxin B), tigecycline, or carbapenem. These findings corroborated the results by Zarkotou et al.<sup>96</sup>

Recently, two larger studies on the same topic were presented from Italy and Greece. These studies differed from the aforementioned ones in that colistin and meropenem were generally given at higher doses (270 mg a day and 6 g a day, respectively). A multicenter study conducted in Italy

**Table 1** Observational studies associating therapy and clinical outcome of bacteremia caused by KPC-producing *Klebsiella pneumoniae*

Study	Year	No. of patients	Treatment given (no. of patients)	Mortality	Comments	Reference
Zarkotou et al (Greece)	2008–2010	35	Combination therapy (20) Monotherapy (15)	0% in-hospital <sup>b</sup> 46.7% in-hospital	9 of the combination group received tigecycline and colistin	<sup>96</sup>
Qureshi et al (United States)	2005–2009	34	Combination therapy (15) Monotherapy (19)	13.3% at 28 d 57.8% at 28 d	9 patients received carbapenem-containing combinations; all were resistant to carbapenems	<sup>97</sup>
Tumbarello et al (Italy)	2010–2011	125	Combination therapy (79) Tigecycline–colistin (23) Tigecycline–gentamicin (12) Colistin–gentamicin (7) Tigecycline–colistin–meropenem (16) Tigecycline–gentamicin–meropenem (6) Monotherapy (46)	34.1% at 30 d 30.4% 50.0% 57.1% 12.5% 16.6% 54.3% at 30 d	36 patients received carbapenem-containing combinations; mortality was correlated with meropenem MICs	<sup>98</sup>
Daikos et al (Greece)	2009–2010	205 <sup>a</sup>	Combination therapy (103) Carbapenem-containing (31) Carbapenem-sparing (72) Monotherapy (72)	27.2% at 28 d 19.3% 30.6% 44.4% at 28 d	For carbapenem-containing combinations, mortality was higher for MIC of >8 mg/L (35.5%) than MIC of ≤8 mg/L (19.3%)	<sup>99</sup>

Abbreviations: KPC, *Klebsiella pneumoniae* carbapenemase; MICs, minimum inhibitory concentrations.

<sup>a</sup>127 had KPC-producing *K. pneumoniae*, 36 had KPC/VIM-producing *K. pneumoniae*, and 42 had VIM-producing *K. pneumoniae*.

<sup>b</sup>Mortality attributable to KPC-producing *K. pneumoniae* bacteremia.

and reported by Tumbarello et al studied 125 patients with bacteremia caused by KPC-producing *K. pneumoniae*.<sup>98</sup> The 30-day mortality rates were 34.1% for 79 patients who received combination therapy and 54.3% for 46 patients who received monotherapy ( $p = 0.02$ ). Notably, the lowest mortality rates within the combination group were observed for those who received three active agents (12.5% for 16 patients treated with tigecycline, colistin, and meropenem and 16.6% for 6 patients who received tigecycline, gentamicin, and meropenem), and lower meropenem MICs were associated with lower mortality in the combination therapy group. Finally, Daikos et al examined the outcome of 205 patients with bacteremia caused by carbapenemase-producing *K. pneumoniae*, 163 of which produced KPC, at two hospitals in Greece.<sup>99</sup> The rate of resistance to colistin was high at 25.4%. Of the 175 patients who received active definitive therapy, 103 received combination therapy and 72 received monotherapy. The 28-day mortality rates were 27.2 and 44.4%, respectively ( $p = 0.003$ ), and the beneficial effect of combination therapy was maximized among those with rapidly fatal underlying diseases or septic shock. In addition, the mortality rate was seen among patients given carbape-

nem-containing combinations (19.3%), and lower carbapenem MICs were associated with lower mortality as was observed in the study by Tumbarello et al.

A recent systematic review by Tzouveleakis et al has compiled reports on the clinical outcome of CPE infections (mostly KPC producers with some VIM and OXA-48 producers) in 889 patients, which included those in the aforementioned studies.<sup>100</sup> Among them, 441 received combination therapy, 346 received monotherapy, and 102 received inappropriate therapy (i.e., no active agent in vitro). The mortality rates of monotherapy were 40.1% for carbapenem, 41.1% for tigecycline, and 42.8% for colistin, whereas the mortality for inappropriate therapy was 46.1%. In contrast, the mortality rates for combination therapy were 30.7% for carbapenem-sparing combinations and 18.8% for carbapenem-containing combinations. Again, these data are all derived from observational, uncontrolled studies. However, the two arms (combination therapy and monotherapy) are comparable in terms of demographics, underlying diseases, and severity of illness across these studies. In addition, the bias, if any, would be in the direction of adopting combination therapy for more ill-appearing patients for whom higher

mortality would be anticipated. Therefore, we recommend for the time being that invasive infections caused by CPE be treated with two in vitro active agents that include a carbapenem, with the second agent selected from colistin, tigecycline, or gentamicin depending on susceptibility. It remains an open question whether the addition of a third agent is warranted when the carbapenem MIC is high, as the benefits of combination therapy should be weighed against potential harms in terms of costs, adverse events, and interference with other therapy through the intravenous access, among others. Randomized clinical trials aimed at comparing colistin alone and colistin plus meropenem for CPE infections are ongoing in the United States and European Union (NCT01597973 and NCT01732250). The findings from these studies, when available, are expected to provide valuable insights on the efficacy of these approaches when pharmacokinetically optimized doses are used for these agents.

Currently, there are no substantial data correlating treatment and clinical outcome for infections caused by NDM-producing *Enterobacteriaceae*.

## Summary

CPE is one of the biggest infectious disease threats that have emerged in hospitals worldwide in the last decade. CPE infections are difficult to manage because of limited treatment options and are associated with high mortality. KPC-producing *K. pneumoniae* is the most prevalent CPE and the best studied as well. Treatment of invasive infections such as bacteremia usually consists of two active agents depending on the susceptibility patterns of the infecting strain (e.g., colistin and tigecycline, colistin and meropenem, meropenem and tigecycline, and meropenem and gentamicin), as this approach has been associated with lower patient mortality compared with treatment with a single active agent in multiple observational studies. At the same time, dosing for each agent should be optimized by using high doses, and in the case of colistin and tigecycline, with a loading dose. On the other hand, uncomplicated urinary tract infections caused by CPE can be safely managed with a variety of single agents with good clinical outcome. Of concern is the increasing number of reports documenting CPE resistance to colistin and tigecycline as we use more of these “salvage” agents for therapy. How these pandrug-resistant cases can be best managed remains an open question.

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