**Carbapenemase-producing Klebsiella pneumoniae**
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### Abstract

The continuing emergence of infections due to multidrug resistant bacteria is a serious public health problem. *Klebsiella pneumoniae*, which commonly acquires resistance encoded on mobile genetic elements, including ones that encode carbapenemases, is a prime example. *K. pneumoniae* carrying such genetic material, including both *blaKPC* and genes encoding metallo-β-lactamases, have spread globally. Many carbapenemase-producing *K. pneumoniae* are resistant to multiple antibiotic classes beyond β-lactams, including tetracyclines, aminoglycosides, and fluoroquinolones. The optimal treatment, if any, for infections due to these organisms is unclear but, paradoxically, appears to often require the inclusion of an optimally administered carbapenem.

### Introduction

The following experience is a stark expression of the epidemiologic and therapeutic problems presented by carbapenem-resistant *K. pneumoniae* (CR-KP).

A 43-year-old woman with pulmonary alveolar proteinosis was discharged from the National Institutes of Health (NIH) Clinical Center on July 15, 2012 after 32 days of hospitalization [1]. Initially transferred from a facility in New York City and known to be colonized with a CR-KP, she was immediately placed into enhanced isolation. The organism was not detected again until three weeks after discharge of this index case when it was recovered from a tracheal aspirate specimen of a mechanically ventilated patient and was eventually recovered from a total of 17 patients. The index isolate was resistant to all antibiotics tested, with the exception of gentamicin, tigecycline, and colistin. As the outbreak progressed, however, further resistance emerged to those three antibiotics as well, so that there were no antibiotics with *in vitro* activity against the organism available for treatment of some patients. Of the 17 affected patients 10 died and the outbreak organism was responsible for death in 6 of the 10.

The experience at the NIH Clinical Center exemplifies the problem highlighted in a 2013 report on antibiotic resistance in which the US CDC identified carbapenem-resistant *Enterobacteriaceae* (CRE) among the top three “urgent (antibiotic resistance) threats” to US public health. In fact, of the 9300 healthcare facility-associated CRE infections and 600 deaths annually in the US, a preponderance are due to *Klebsiella pneumoniae* carbapenemases (KPCs) [2], while other carbapenemases are often more prevalent outside the US. The proportion of US acute care hospitals reporting at least one hospital-acquired infection due to CRE to the CDC’s National Healthcare Safety Network (NHSN) increased from 1.2% to 4.2% between 2001 and 2011, with the largest share of the increase occurring among *Klebsiella* species [3]. KPCs, named for the species from which these enzymes were first isolated in 1996, consist of at least a dozen subtypes (KPC2-13) with varying substrate specificities [4-6]. Despite the name, KPCs have now appeared in a variety of other *Enterobacteriaceae*, as well as in other Gram-negative bacilli, including *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [7]. Invasive infections due to organisms producing KPCs are associated with mortality rates approaching 50% [1,4,8-10]. The effect
of these carbapenemases may be difficult to detect in the laboratory, and what constitutes optimal antibiotic therapy remains uncertain. Isolates may phenotypically appear susceptible to carbapenems, delaying both time to administration of appropriate antibiotic therapy and implementation of infection control policies, leading to transmission within an institution.

**Laboratory identification**

The US CDC, for surveillance purposes, currently defines CRE as Enterobacteriaceae isolates that are nonsusceptible to doripenem, imipenem, and/or meropenem, together with resistance to ceftriaxone, cefotaxime, and ceftazidime [11]. Phenotypic detection of carbapenem resistance, and the presence of a carbapenemase by standard in vitro susceptibility testing, especially with some automated systems, can be problematic [7]. This results, at least in part, from variable carbapenemase expression, as well as the frequent presence of additional resistance mechanisms. This observation led both the European Committee on Antimicrobial Susceptibility (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) to lower minimum inhibitory concentration (MIC) breakpoints in order to improve their sensitivity in the detection of CRE (Table 1). The CDC currently recommends the use of these new interpretive criteria for screening for the possible presence of a carbapenemase. As always, however, improved sensitivity is accompanied by reduced specificity. Thus, while the presence of reduced susceptibility to ertapenem may be the most sensitive indicator of the presence of a carbapenemase, it is the least specific indicator since full resistance to this antibiotic may result from the simultaneous presence of other mechanisms such as altered porin proteins together with derepressed AmpC or an extended spectrum beta-lactamase (ESBL).

High level resistance to carbapenems in *K. pneumoniae* carrying KPCs is associated with increased *bla*KPC copy number and/or non-functional outer membrane protein (Omp) K35 or Omp K36 [12]. In addition to these issues, some automated susceptibility testing methods may have difficulty detecting isolates carrying KPC [7].

The CDC recommends that, in order to detect the presence of a carbapenemase, isolates with reduced susceptibility to one or more carbapenems undergo further testing with the modified Hodge test (MHT) [13]. The CLSI indicates that testing by MHT is not necessary when the isolate is found to be intermediate or resistant to all carbapenems tested, since use of the recently reduced breakpoints should preclude the possibility of misclassification of CR-KP as carbapenem susceptible [14]. They do, however, suggest its use for epidemiological investigations.

Several selective agars, such as CHROMagar KPC and ChromID CARBA may be of use in screening for carbapenem resistance [15,16]. The presence of a carbapenemase may also be detected by phenotypic methods, such as inhibition of its activity by ethylenediaminetetraacetic acid (EDTA) and phenylboronic acid [6,17,18]. Direct phenotypic methods of carbapenemase detection include the identification of hydrolytic products of imipenem by UV spectrophotometry [19], by the Carba NP test [16], or by matrix-assisted laser desorption time-of-flight mass spectrometry [20].

The most sensitive method for establishing the presence of known carbapenemases is the detection of genes encoding these enzymes, and the CDC has published a protocol for multiplex real-time PCR detection of KPC and New Delhi metallo-β-lactamase (NDM)-1 genes [13]. A variety of test systems are in use [15]. Among the newer ones is the Biofire FilmArray, which is a multiplex PCR system that detects the gene encoding KPC as well as two other resistance genes and 24 pathogens (19 bacteria and 5 yeasts) in positive blood cultures [21]. The Verigene Gram Negative Blood Culture Test is a microarray system that detects, in addition to nine genus/species target pathogens, the genes encoding KPC, NDM, oxacillinase class D β-lactamase (OXA), verona integrin-encoded metallo-β-lactamase (VIM) and imipenemase metallo-β-lactamase (IMP), as well as CTX-M (β-lactamase showing preferential hydrolytic activity for cefotaxime [CTX] first identified in Munich [M]) [22]. The GeneXpert multi-drug resistant organism (MDRO) test, under development, detects genes encoding KPC, NDM and VIM [23]. Identification of the gene responsible may be of importance in epidemiological investigations, but is not currently of value in designing therapeutic regimens.

| Carbapenem   | Previous Breakpoints (M100-S19) | Current Breakpoints (M100-S22) |
|--------------|--------------------------------|------------------------------|
|              | MIC (µg/ml) | Susceptible | Intermediate | Resistant | MIC (µg/ml) | Susceptible | Intermediate | Resistant |
| Doripenem    | -            | -           | -            |          |
| Ertapenem    | ≤2           | 4           | ≥8           |          |
| Imipenem     | ≤4           | 8           | ≥16          |          |
| Meropenem    | ≤4           | 8           | ≥16          |          |
However resistance is detected, it remains important that clinical laboratories test isolates with reduced susceptibility to carbapenems for susceptibility to tigecycline, fosfomycin, and colistin or polymyxin B, without requiring a specific request from a clinician, in order to reduce any delay in appropriate treatment.

**Epidemiology**

Initially identified in 1996, the first description of CRE was not published until 2001[4]. Early reports of CREs were concentrated in and around the New York metropolitan area and primarily involved KPC-producing Klebsiella species [24,25]. In the US, KPCs (which are Ambler class A serine proteases) are the dominant carbapenemases and have also become important in several other countries, such as Israel and Greece [26]. A single sequence type, ST258, which belongs to clonal complex 11, is the predominant strain of *K. pneumoniae* carrying KPC-2 in the US and Europe [27]. Since the early 2000s there have been several reported CRE outbreaks that have shaped our epidemiologic understanding, including a 2013 outbreak of CRE related to endoscopic retrograde cholangiopancreatography that was notably due to NDM-producing organisms, rather than KP-KPC organisms [28]. As of February 2014, CRE had been identified in all states in the US except Alaska, Maine, and Idaho, but its distribution is geographically heterogeneous with varying causes of resistance identified in different regions of the country [29,30]. In many countries outside the US, a number of metallo-β-lactamases, including NDM, VIM, and IMP are the dominant carbapenemases.

**Genetics**

While some carbapenemases are chromosomally encoded, most are present on mobile genetic elements that have allowed dissemination to a number of different Gram-negative bacteria [31]. As an example, the KPC-encoding gene, blaKPC, is present on a Tn3-based transposon, Tn4401, and carried on plasmids, many of which also carry QmrA and/or QmrB, which elevate fluoroquinolone MICs, and rmtB, which methylates the 16S rRNA target of many aminoglycosides [7,32]. Carbapenemases may coexist with other β-lactamases, such as ESBLs, and more than one carbapenemase may be simultaneously present, as in a *K. pneumoniae* isolate identified in China that carried both blaKPC-2 and the metallo-β-lactamase gene, IMP-4 [33].

**Infection control and public health**

Rapid detection of CRE producing organisms, followed by aggressive infection prevention tactics, forms the basis of treatment and control of these organisms. The CDC has recently asked infection preventionists to enhance their vigilant surveillance for CRE and they have published a “toolkit” for clinicians and infection preventionists to assist in the task [11]. According to the CDC, institutions should, at a minimum, be aware of whether or not they have circulating CREs, at least among *Klebsiella* and *Escherichia coli* species. Additionally, the toolkit reinforces core measures that all hospitals should follow to reduce the CRE threat. Bundled interventions to disrupt CRE transmission among susceptible patients were evaluated in a recent review [34]. Given the multi-faceted nature of infection prevention bundles, the individual contribution of each intervention has not clearly been established, but the authors conclude that traditional targeted interventions (such as contact isolation), and systems approaches (including hand hygiene compliance and feedback) are essential to CRE mitigation. The role of these infection control practices and their inclusion in several national guidelines is largely based on outbreak experience rather than clinical trial data and was recently reviewed by Kruse and colleagues [35]. Clinicians involved in the NIH Clinical Center CRE outbreak echoed the need for diligent compliance with hand hygiene and other infection prevention protocols in a recent editorial on the future of CRE management [36]. They also highlighted the need to incorporate molecular diagnostics, rational antimicrobial utilization, new drug development and administrative leadership on patient safety issues as essential factors in effective CRE control. Countries other than the US have directed attention toward infection prevention to stop the spread of CRE. In Israel, for example, expanded national oversight of strategic infection control interventions has been successfully employed to control the spread of nosocomial CREs since 2007 [37].

**Emerging therapies**

In 2009, we stated in this journal regarding KP-KPC that “The optimal therapy for infections due to these multidrug-resistant pathogens is not well defined and depends upon the susceptibilities of individual isolates, and the choices are often severely limited” [38]. This assessment, which applies to CRE in general, unfortunately, remains largely unchanged [7,39]. The antibiotics most likely to be active *in vitro* against CR-KP, including KP-KPC, are gentamicin, tigecycline, fosfomycin, colistin, and polymyxin B. Therapy with a single antibiotic to which the pathogen is susceptible *in vitro* appears to be inferior to combination therapy with two or three active antibiotics and, furthermore, monotherapy is associated with an increased likelihood of emergence of resistance [7,39–41]. Daikos and colleagues in Athens, Greece, provide details of a large and highly instructive experience with the problem of treatment of infections due to CRE [41]. Of 205 patients with CR-KP bloodstream infection seen at
their institution during 2009 and 2010. 163 (79.3%) were infected with KPC-KP, with 36 also producing VIM-1. Another 42 produced VIM-1 alone. Despite the fact that all isolates contained carbapenemases, the proportions of isolates resistant to imipenem, meropenem, and doripenem were only 53.7%, 52.7%, and 57.1%, respectively, with application of EUCAST resistance breakpoints then in use (>8 \( \mu \)g/ml for imipenem and meropenem, >4 \( \mu \)g/ml for doripenem). Resistance to gentamicin was seen in 31.2% and to amikacin in 68.3%, while 97.6% were resistant to ciprofloxacin. The mortality rate in patients who received monotherapy was 44.4%, while it was only 27.2% with combination therapy, with the lowest mortality in those who received a carbapenem-containing combination regimen. Among patients who received a carbapenem together with another agent with in vitro activity against the etiologic pathogen, the mortality varied depending on the carbapenem MIC (19.3% with an MIC \( \leq 8 \) \( \mu \)g/ml and 35.5% with MIC >8 \( \mu \)g/ml). None of the 11 patients who received a carbapenem together with tigecycline and either an aminoglycoside or colistin died. Combination therapy was an independent predictor of survival. In agreement with this experience, Tumbarello and colleagues found improved survival in patients with KPC-KP bloodstream infections who received two or more drugs with in vitro activity against the pathogen [42]. Once antimicrobial susceptibility data was available, the lowest mortality was documented in those receiving the combination of tigecycline, meropenem, and colistin.

This experience suggests that the optimal therapy for treatment of CR-KP involves combination therapy that, seemingly paradoxically, includes a carbapenem (imipenem, meropenem, or doripenem) together with tigecycline and either an aminoglycoside (usually gentamicin) or colistin, with at least two of the antibiotics being active against the pathogen in vitro. The doses and administration should be optimized, taking into account relevant pharmacokinetic and pharmacodynamic principles.

**Fosfomycin**

In a sample of 68 clinical KPC-KP isolates collected in the eastern US, 93% were susceptible to fosfomycin based on CLSI breakpoints for *E. coli* urinary tract infections, the only infection for which CLSI fosfomycin breakpoints currently exist [43]. Analysis of a subset of 23 isolates, each resistant to both tigecycline and colistin, found that 87% were susceptible to fosfomycin. In Germany, the MIC50 of 50 isolates of CR-KP (all but four produced one or more carbapenemase) was 16 \( \mu \)g/ml and the MIC90 was 256 \( \mu \)g/ml; 16 (32%) were resistant by EUCAST criteria (susceptible: \( \leq 32 \) \( \mu \)g/ml; resistant: >32 \( \mu \)g/ml) [44]. Fosfomycin is not recommended as a monotherapy because of the likelihood of rapid emergence of on-treatment resistance to this agent; it should always be used as part of a combination regimen. Unfortunately, this strategy is not always successful and resistance may nonetheless emerge during combination therapy [45].

Published clinical experience with fosfomycin therapy of infections due to CR-KP is limited. In 11 Greek intensive care units (ICUs), 68 patients with infections due to multidrug resistant Gram-negative bacilli, 41 (60.3%) of which were due to CR-KP, received fosfomycin intravenously in a median total daily dose of 24 g for a median duration of 10 days, usually in combination with tigecycline or colistin [46]. In the entire evaluable cohort, bacterial eradication was achieved in 56.3% of cases and the 28 day crude mortality was 37.5%. The most frequently encountered adverse event was hypokalemia; resistance emerged in three cases. In the US, fosfomycin is only available in an oral formulation, making the achievement of systemic antibiotic exposure comparable to that seen with a 24 gram/day intravenous dose highly problematic, if not impossible.

**Rifampin**

*In vitro* time-kill experiments with a small number of NDM-producing *K. pneumoniae* evaluated several two and three drug combinations and found that the most active was the combination of rifampin with meropenem and fosfomycin [47]. The combination of rifampin, doripenem and colistin had previously been found to be bactericidal against KPC-KP isolates [48], and dual combinations of rifampin with colistin have been reported to exhibit synergy against this organism [49,50], as has rifampin with polymyxin B [51]. Clinical data are, however, lacking.

**Double-carbapenems**

The combination of doripenem and ertapenem was more active than either drug alone in both an in vitro chemostat model and a murine thigh infection model [35].

A limited number of case reports describe the use of double-carbapenem combination therapy. Giamarellou et al. successfully treated three patients with pan-resistant KPC-KP infections (two with bacteremia, one with urinary tract infection) with the combination of doripenem or meropenem (with prolonged infusion) plus ertapenem [52]. In another report, three patients with KPC-KP infections were successfully treated with a combination of meropenem and ertapenem and *in vitro* time-kill assay demonstrated bactericidal synergy against one of the isolates [53]. One proposed mechanism of action is as follows: the least potent carbapenem against
carbapenemase-producing *Enterobacteriaceae*, ertapenem, binds the carbapenemase with greater affinity, thereby protecting the more potent carbapenem from hydrolysis. This effect, if it exists at all, may not be universal across KPC subtypes, however, as doripenem has been found to have approximately threefold greater affinity than ertapenem for KPC-6 [54].

**Azhetreonam**

Aztreonam, combined with meropenem and colistin, demonstrated *in vitro* synergistic or bactericidal activity against VIM and NDM-1 producing *K. pneumoniae*, despite the presence of high level resistance to the monobactam and non-susceptibility to meropenem [47]. It is speculated that this may result from the fact that aztreonam is a competitive inhibitor of metallo-β-lactamases, while meropenem is an inhibitor of ESBLs and AmpC enzymes, which may be co-produced by some organisms.

**Glycopeptides**

Potent synergy, with reduction of the MIC of vancomycin from 256 μg/ml to 1 μg/ml with each of six isolates (four *E. coli*, two *K. pneumoniae*) carrying NDM in combination with one or more ESBL, was observed when this glycopeptide was combined with colistin *in vitro* [55]. A retrospective review examining therapy with this combination in critically ill patients with Gram-negative bacillary infections included 24 patients with CR-KP infection [56]. Of these, 15 received colistin alone, 5 received it with a glycopeptide, 5 with another antibiotic targeting Gram-negative bacilli, and one received the combination with another anti-Gram-negative antibiotic. The number of patients was too small to identify significant differences in outcome. Furthermore, while similar synergy of vancomycin with colistin has been demonstrated *in vitro* against isolates of *Acinetobacter baumanii*, a retrospective study of patients with ventilator-associated pneumonia due to this organism failed to identify evidence of benefit from the addition of this glycopeptide to colistin [57–59].

**Avibactam**

Avibactam is an investigational non-β-lactam β-lactamase inhibitor being studied in combination with several β-lactam antibiotics [60]. It inhibits enzymes of Ambler classes A (including KPC) and C, as well as some in class D. It has, however, been reported to be slowly hydrolyzed by KPC [61]. In a US national survey, the combination of avibactam with ceftazidime inhibited all ESBL and KPC producing *Enterobacteriaceae* at a ceftazidime MIC <4 μg/ml [62]. Of 112 meropenem non-susceptible (MIC ≥2 μg/ml) *K. pneumoniae*, 29 (85.2%) had an MIC to the combination of ≤1 μg/ml.

**BAL30072**

BAL30072 is an investigational siderophore monosulfactam that, like aztreonam, is stable to metallo-β-lactamases of Ambler class B and is not hydrolysed by VIM or IMP. It is believed to be resistant to KPC, but its activity against KPC-KP is often poor because of the frequent simultaneous presence of SHV and/or derepressed AmpC [63,64].

**Other novel agents**

Several novel antibiotics have been reported to have *in vitro* activity against some CRE. These include the fluorocycline, eravacycline [65-67] and the neoglycoside sisomicin derivative, plazomicin (ACHN-490) [68].

**Conclusion**

The emergence of multidrug resistant *K. pneumoniae* is a perfect storm of antibiotic resistant threats — pervasive, transmissible and deadly. With the emergence of resistance to what many consider antibiotics of last resort — carbapenems — the problem is reaching critical proportions. Mortality associated with infections due to these organisms remains high and the optimal therapy uncertain. In some instances, no antibiotics with *in vitro* activity against the pathogen are available. Effective infection control and aggressive antimicrobial stewardship are imperative to prevent infections by these organisms. In the absence of the development of new antibiotics effective against these pathogens, however, the future may prove to be bleak. At present, the keys to dealing with the problem are prevention through antimicrobial stewardship and infection control, swift laboratory detection, and combination therapy with an existing carbapenem (imipenem, meropenem or doripenem) and at least one other antibiotic with *in vitro* activity against the pathogen.

It goes without saying that these drugs need to be administered in the optimal dose and, in the case of β-lactams, optimal duration of infusion.

**Abbreviations**

CLSI, Clinical and Laboratory Standards Institute; CR-KP, carbapenem-resistant *Klebsiella pneumoniae*; CRE, carbapenem-resistant *Enterobacteriaceae*; ESBL, extended spectrum β-lactamase; EUCAST, European Committee on Antimicrobial Susceptibility Testing; NIH, National Institutes of Health; IMP, imipenemase metallo-β-lactamase; KPC, Klebsiella pneumoniae carbapenemase; MHT, modified Hodge test; MIC, minimum inhibitory concentration; NDM, New Delhi metallo-β-lactamase; Omp, outer membrane protein; OXA, oxacillinase class D β-lactamase; VIM, Verona integrin-encoded metallo-β-lactamase.

**Disclosures**

The authors declare that they have no disclosures.
References

1. Snitkin ES, Zelazny AM, Thomas PJ, Stock F, Group NCSP, Henderson DK, Palmore TN, Segre JA: Tracking a hospital outbreak of carbapenem-resistant Klebsiella pneumoniae with whole-genome sequencing. Sci Transl Med 2012, 4:148ra116.

2. Centers for Disease Control and Prevention: Antibiotic Resistance Threats in the United States, 2013. U.S. Department of Health and Human Sciences; 2013. [http://www.cdc.gov/drugresistance/threat-report-2013/pdf/afar-threats-2013-508.pdf]

3. Centers for Disease Control and Prevention: Vital signs: carbapenem-resistant Enterobacteriaceae. MMWR Morb Mortal Wkly Rep 2013, 62:165-70.

4. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, Alberti S, Bush K, Tenover FC: Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. Antimicrob Agents Chemother 2001, 45:151-61.

5. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, Alberti S, Bush K, Tenover FC: Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. Antimicrob Agents Chemother 2008, 52:809.

6. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL: Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. Clin Microbiol Rev 2012, 25:682-707.

7. Chen LF, Anderson DJ, Paterson DL: Overview of the epidemiology and the threat of Klebsiella pneumoniae carbapenemases (KPC resistance). Infect Drug Resist 2012, 5:133-41.

8. Hussein K, Raz-Pasteur A, Finkelstein R, Neuberger A, Shachor-Snovitz M, Assaf J, Ben-Dov I, Ofek O, Kassal I: Impact of carbapenem resistance on the outcome of patients' hospital-acquired bacteremia caused by Klebsiella pneumoniae. J Hosp Infect 2013, 83:307-13.

9. Schwaber MJ, Klarfeld-Lidji S, Navon-Venezia S, Schwartz D, Leavitt A, Loebenberg G, Solomon M, Schaffner W: Antimicrobial resistance in Klebsiella pneumoniae isolates in the clinical laboratory. J Clin Microbiol 2009, 47:362-7.

10. Hussein K, Raz Pasteur A, Finkelstein R, Neuberger A, Shachor-Snovitz M, Assaf J, Ben-Dov I, Ofek O, Kassal I: Impact of carbapenem resistance on the outcome of patients' hospital-acquired bacteremia caused by Klebsiella pneumoniae. J Clin Microbiol 2013, 51:4130-6.

11. Centers for Disease Control and Prevention: 2012 CRE Toolkit - Guidance for Control of Carbapenem-resistant Enterobacteriaceae (CRE). U.S. Department of Health and Human Sciences; 2012. [http://www.cdc.gov/healthyliving/cre-guidance-508.pdf]

12. Kitchel B, Rasheed JK, Patel JB: Genetic factors associated with elevated carbapenem resistance in KPC-producing Klebsiella pneumoniae. Antimicrob Agents Chemother 2010, 54:4201-7.

13. CDC: Multiplex Real-Time PCR Detection of Klebsiella pneumoniae Carbapenemase (KPC) and New Delhi metallo-

14. Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement (January 2014). 2014. [http://shopping.mdsite.com/123739/site/Sample.pdf?M100524_sample.pdf]

15. Gazin M, Paszty C, F. Goossens H, Malhota-Kumar S, Moser WP, Teams SWS: Current trends in culture-based and molecular detection of extended-spectrum-beta-lactamase-harbouring and carbapenem-resistant Enterobacteriaceae. J Clin Microbiol 2012, 50:1140-6.

16. Nordmann P, Poirel L, Dortet L: Rapid detection of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 2012, 18:1503-7.

17. Yan Y, Sun K, Pan L, Fan H, Yang H, Lu Y, Shi Y: A screening strategy for phenotypic detection of carbapenemase in the clinical laboratory. Can J Microbiol 2014, 60:211-5.

18. Tsakris A, Kristo I, Poulou A, Themeli-Digalaki I, Ikonomidou A, Petropoulou D, Pourmaras S, Sofianou D: Evaluation of boric acid disk tests for differentiating KPC-possessing Klebsiella pneumoniae isolates in the clinical laboratory. J Clin Microbiol 2009, 47:362-7.

19. Bernabei S, Poirel L, Nordmann P: Spectrophotometry-based detection of carbapenemase producers among Enterobacteriaceae. Diagn Microbiol Infect Dis 2012, 74:88-90.

20. Burckhardt I, Zimmermann S: Using matrix-assisted laser desorption ionization-time of flight mass spectrometry to detect carbapenem resistance within 1 to 2.5 hours. J Clin Microbiol 2011, 49:3321-4.

21. Altun O, Almuhsaywi M, Ullberg M, Ozenci V: Clinical evaluation of the FilmArray blood culture identification panel in identification of bacteria and yeasts from positive blood culture bottles. J Clin Microbiol 2013, 51:4130-6.

22. Sullivan KV, Deburger B, Roundtree SS, Ventrola CA, Blecker-Shelly DL, Mortensen JE: Rapid Detection of Inpatient Gram-Negative Bacteremia; Extended-Spectrum Beta-Lactamases and Carbapenemase Resistance Determinants with the Verigene BC-GN Test: A Multi-Center Evaluation. J Clin Microbiol 2014, 52:2416-21.

23. Tenover FC, Canton R, Kop J, Chan R, Ryan J, Weir F, Ruiz-Garbajosa P, LaBombard V, Persing DH: Detection of colonization by carbapenemase-producing Carbapenemase-producing and carbapenem-resistant Enterobacteriaceae. Lancet Infect Dis 2013, 13:785-96.

25. Bratu S, Mooty M, Nichani S, Landman D, Gullans C, Pettinato B, Karumudi U, Tolaney P, Quale J: Emergence of KPC-possessing Klebsiella pneumoniae in Brooklyn, New York: epidemiology and recommendations for detection. Antimicrob Agents Chemother 2005, 49:3018-20.

26. Munoz-Price LS, Poirel L, Bonomo RA, Schwaner MJ, Daikos GL, Cormican M, Cornaglia G, Garau J, Gniasdowski M, Hayden MK, et al.: Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. Lancet Infect Dis 2013, 13:785-96.

27. Kitchel B, Rasheed JK, Patel JB, Srivivasan A, Navon-Venezia S, Carmeli Y, Brolund A, Giske CG: Molecular epidemiology of KPC-producing Klebsiella pneumoniae isolates in the United
States: clonal expansion of multilocus sequence type 258. Antimicrob Agents Chemother 2009, 53:3365-70.

28. Centers for Disease C, Prevention: Notes from the Field: New Delhi Metallo-β-Lactamase—Producing Escherichia coli Associated with Endoscopic Retrograde Cholangiopancreatography — Illinois, 2013. MMWR Morb Mortal Wkly Rep 2014, 62:1105.

29. Healthcare-associated Infections (HAI): Tracking CRE. [http://www.cdc.gov/hai/organisms/cre/TrackingCRE.html]

30. Guh AY, Limbago BM, Kallen AJ: Epidemiology and prevention of carbapenem-resistant Enterobacteriaceae in the United States. Expert Rev Anti Infect Ther 2014, 12:565-80.

31. Mathers AJ, Cox HL, Kitchel B, Bonatti H, Brassinga AK, Carroll J, Scheld WM, Hazen KC, Sifri CD: Molecular dissection of an outbreak of carbapenem-resistant enterobacteriaceae reveals Intergenus KPC carbapenemase transmission through a promiscuous plasmid. Mbio 2011, 2:e00204-11.

32. Naas T, Cuzon G, Villegas MV, Laritge MF, Quinn JP, Nordmann P: Genetic structures at the origin of acquisition of the beta-lactamase bla KPC gene. Antimicrob Agents Chemother 2008, 52:1257-63.

33. Wang Y, Cao W, Zhu X, Chen Z, Li Z, Zhang B, Wang B, Tian L, Wang F, Liu C, et al.: Characterization of a novel Klebsiella pneumoniae sequence type 476 carrying both bla KPC-2 and bla IMP-4. Eur J Clin Microbiol Infect Dis 2012, 31:1867-72.

34. Munoz-Price LS, Quinn JP: Deconstructing the infection control bundles for the containment of carbapenem-resistant Enterobacteriaceae. Curr Opin Infect Dis 2013, 26:378-87.

35. Kruse EB, Aurbach U, Wisplinghoff H: Carbapenem-Resistant Enterobacteriaceae: Laboratory Detection and Infection Control Practices. Curr Infect Dis Rep 2013. [Epub ahead of print].

36. Palmore TN, Henderson DK: Carbapenem-resistant enterobacteriaceae: a call for cultural change. Ann Intern Med 2014, 160:567-9.

37. Schwaber MJ, Lev B, Israeli A, Salter E, Smollan G, Rubinovich B, Shalit I, Carmeli Y: Israel Carbapenem-Resistant Enterobacteriaceae Working G: Containment of a country-wide outbreak of carbapenem-resistant Klebsiella pneumoniae in Israeli hospitals via a nationally implemented intervention. Clin Infect Dis 2011, 52:648-55.

38. Dereiinski SC, Schirmer P: Management of infections due to KPC-producing Klebsiella pneumoniae. F1000Med Rep 2009, 1:79.

39. Petrozillo N, Giannella M, Lewis R, Viale P: Treatment of carbapenem-resistant Klebsiella pneumoniae: the state of the art. Expert Rev Anti Infect Ther 2013, 11:159-77.

40. Lee GC, Burgess DS: Treatment of Klebsiella pneumoniae carbapenemase (KPC) infections: a review of published case series and case reports. Ann Clin Microbiol Antimicrob 2012, 11:32.

41. Daikos GL, Tsoussi S, Tzouvelekis LS, Anyfantis I, Psichogiou M, Argyropoulou A, Stefanou I, Syssa V, Miragou V, Nefkis M, Georgiadou S, Markogiannakis A, Goulios D, Skoulidis A: Carbapenemase-Producing Klebsiella pneumoniae Bloodstream Infections: Lowering Mortality by Antibiotic Combination Schemes and the Role of Carbapenems. Antimicrob Agents Chemother 2014, 58:2322-8.

42. Tumarello M, Viale P, Viscoli C, Trecarichi EM, Tumietto F, Marchese A, Spanu T, Ambretti S, Ginocchio F, Cristini F, Losito AR, Tesedici S, Cauda R, Bassetti M: Predictors of mortality in bloodstream infections caused by Klebsiella pneumoniae carbapenemase-producing K. pneumoniae: importance of combination therapy. Clin Infect Dis 2012, 55:943-50.

43. Endimiani A, Patel G, Hujer KM, Swaminathan M, Perez F, Rice LB, Jacobs MR, Bonomo RA: In vitro activity of fosfomycin against blaKPC-containing Klebsiella pneumoniae isolates, including those nonsusceptible to ß-lactam and/or colistin. Antimicrob Agents Chemother 2010, 54:526-9.

44. Kaase M, Szabados F, Anders A, Gatermann S: Fosfomycin Susceptibility in Carbapenem-Resistant Enterobacteriaceae from Germany. J Clin Microbiol 2014, 52:1893-7.

45. Karageorgopoulos DE, Miriagou V, Tzouvelekis LS, Syридopoulou K, Daikos GL: Emergence of resistance to fosfomycin used as adjunct therapy in KPC Klebsiella pneumoniae bacteraemia: report of three cases. J Antimicrob Chemother 2012, 67:2777-9.

46. Pontikis K, Kariakos I, Bastani S, Dimopoulos G, Kalogirou M, Katsiaris M, Okonomou A, Poulakou G, Roilides E, Giamarellou H: Outcomes of critically ill intensive care unit patients treated with fosfomycin for infections due to pandrug-resistant and extensively drug-resistant carbapenemase-producing Gram-negative bacteria. Int J Antimicrob Agents 2014, 43:52-9.

47. Tenn T, Hickman RA, Forsberg P, Lagerback P, Giske CG, Cars O: Evaluation of Double- and Triple-Antibiotic Combinations for VIM- and NDM-Producing Klebsiella pneumoniae by In Vitro Time-Kill Experiments. Antimicrob Agents Chemother 2014, 58:1757-62.

48. Urban C, Mariano N, Rahal JJ: In vitro double and triple bacterial activities of doripenem, polymyxin B, and rifampin against multidrug-resistant Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Escherichia coli. Antimicrob Agents Chemother 2010, 54:2732-4.

49. Elemam A, Rahimian J, Doymaz M: Synergistic activity of colistin plus rifampin against colistin-resistant KPC-producing Klebsiella pneumoniae. J Antimicrob Chemother 2010, 65:3558-62.

50. Cascini C, Tagliaferri E, Giani T, Leonildi A, Flaminini S, Casini B, Lewis R, Ferranti S, Rossolini GM, Menichetti F: Synergistic activity of colistin plus rifampin against colistin-resistant KPC-producing Klebsiella pneumoniae. Antimicrob Agents Chemother 2013, 57:3990-3.

51. Pankey GA, Ashcraft DS: Detection of synergy using the combination of polymyxin B with either meropenem or rifampin against carbapenemase-producing Klebsiella pneumoniae. Diagn Microbiol Infect Dis 2011, 70:561-4.

52. Giamarello H, Galani L, Baziaka F, Kariakos I: Effectiveness of a double-carbapenem regimen for infections in humans due to carbapenemase-producing pandrug-resistant Klebsiella pneumoniae. Antimicrob Agents Chemother 2013, 57:2388-90.

53. Oliwa A, D’Abramo A, D’Agostino C, Iannetta M, Mascellini MT, Gallinelli C, Mastroianni CM, Vullo V: Synergistic activity and effectiveness of a double-carbapenem regimen in
pandrug-resistant Klebsiella pneumoniae bloodstream infections. J Antimicrob Chemother 2014, 69:1718-20.

54. Lamoureaux TL, Frase H, Antunes NT, Yakulenko SB: Antibiotic resistance and substrate profiles of the class A carbapenemase KPC-6. Antimicrob Agents Chemother 2012, 56:6006-8.

55. Wareham D, Phee L, Hornsey M: In-vitro activity of vancomycin combined with colistin versus multi-drug resistant E. coli and K. pneumoniae isolates producing NDM metallo-carbapenemases [abstract]. Presented at the 49th Annual Meeting of the Infectious Diseases Society of America: 20-23 October 2011; Boston, MA.

56. Petrosillo N, Giannella M, Antonelli M, Antonini M, Barsic B, Belancic L, Inlaka AC, De Pascale G, Grilli E, Tumbarello M, et al.: Clinical experience of colistin-glycopeptide combination in critically ill patients infected with Gram-negative bacteria. Antimicrob Agents Chemother 2014, 58:851-8.

57. Gordon NC, Png K, Wareham DW: Potent synergy and sustained bactericidal activity of a vancomycin-colistin combination versus multidrug-resistant strains of Acinetobacter baumannii. Antimicrob Agents Chemother 2010, 54:5316-22.

58. O’Hara JA, Ambe LA, Casella LG, Townsend BM, Pelletier MR, Ernst RK, Shanks RM, Doli Y: Activities of vancomycin-containing regimens against colistin-resistant Acinetobacter baumannii clinical strains. Antimicrob Agents Chemother 2013, 57:2103-8.

59. Garnacho-Montero J, Amaya-Villar R, Gutierrez-Pizarro A, Espejo-Gutierrez de Tena E, Artero-Gonzalez ML, Corcia-Palomo Y, Bautista-Paloma J: Clinical efficacy and safety of the combination of colistin plus vancomycin for the treatment of severe infections caused by carbapenem-resistant Acinetobacter baumannii. Chemotherapy 2013, 59:225-31.

60. Drawz SM, Papp-Wallace KM, Bonomo RA: New beta-Lactamase Inhibitors: a Therapeutic Renaissance in an MDR World. Antimicrob Agents Chemother 2014, 58:1835-46.

61. Ehmann DE, Jahic H, Ross PL, Gu RF, Hu J, Durand-Reville TF, Lahiri S, Thresher J, Livchak S, Gao N, et al.: Kinetics of avibactam inhibition against Class A, C, and D beta-lactamases. J Biol Chem 2013, 288:27960-71.

62. Sader HS, Castanheira M, Frazier KF, Farrell DJ, Jones RN: Antimicrobial Activity of Ceftazidime-Avibactam against Gram-Negative Organisms Collected from U.S. Medical Centers in 2012. Antimicrob Agents Chemother 2014, 58:1684-92.

63. Mushtaq S, Woodford N, Hope R, Adkin R, Livermore DM: Activity of BAL30072 alone or combined with beta-lactamase inhibitors or with meropenem against carbapenem-resistant Enterobacteriaceae and non-fermenters. J Antimicrob Chemother 2013, 68:1601-8.

64. Page MG, Dantier C, Desbarre E: In vitro properties of BAL30072, a novel siderophore sulfaftam with activity against multiresistant gram-negative bacilli. Antimicrob Agents Chemother 2010, 54:2291-302.

65. Solomkin JS, Ramesh MK, Cesnautkas G, Novikovs N, Stefanova P, Sutcliffe JA, Walpole SM, Horn PT: Phase 2, randomized, double-blind study of the efficacy and safety of two dose regimens of eravacycline versus ertapenem for adult community-acquired complicated intra-abdominal infections. Antimicrob Agents Chemother 2014, 58:1847-54.

66. Sutcliffe JA, O’Brien W, Fyfe C, Grossman TH: Antibacterial activity of eravacycline (TP-434), a novel fluorocycline, against hospital and community pathogens. Antimicrob Agents Chemother 2013, 57:5548-58.

67. Connors KP, Houman ST, Pope JS, Russomanno J, Salerno E, Shore E, Redican, S, Nicolau DP: Phase I, open-label, safety and pharmacokinetic study to assess bronchopulmonary disposition of intravenous eravacycline in healthy men and women. Antimicrob Agents Chemother 2014, 58:21113-8.

68. Livermore DM, Mushtaq S, Warner M, Zhang JC, Maharjan S, Doumith M, Woodford N: Activity of aminoglycosides, including ACHN-490, against carbapenem-resistant Enterobacteriaceae isolates. J Antimicrob Chemother 2011, 66:48-53.