Title: IMP-producing carbapenem resistant *Klebsiella pneumoniae* in the United States

Running title: U.S. IMP-producing *K. pneumoniae*

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**Article summary:** Carbapenem resistant Enterobacteriaceae carrying the IMP metallo-β-lactamase were recovered from three pediatric patients with no history of international travel.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.
Abstract

The emergence and spread of carbapenem resistant Enterobacteriaceae (CRE) producing acquired carbapenemases has created a global public health crisis. In the United States, CRE producing the *Klebsiella pneumoniae* carbapenemase (KPC) are increasingly common, and are endemic in some regions. Metallo-β-lactamase (MBL)-producing CRE have recently been reported in the United States among patients who received medical care in countries where such organisms are common. Here we describe three carbapenem resistant *K. pneumoniae* isolates recovered from pediatric patients at a single U.S. healthcare facility, none of whom had a history of international travel. The isolates were resistant to carbapenems but susceptible to aztreonam, trimethoprim-sulfamethoxazole and fluoroquinolones. The three isolates were closely related to each other by pulsed-field gel electrophoresis, and contained a common plasmid. PCR and sequence analysis confirmed that these isolates produce IMP-4, a MBL carbapenemase not previously published as present among Enterobacteriaceae in the United States.

Introduction

Enterobacteriaceae are an abundant part of the normal human gut flora, and also a common cause of both healthcare- and community-associated infections in humans. Unfortunately, resistance to antimicrobial agents has been steadily increasing among Enterobacteriaceae, such that resistance to multiple antibiotic classes is not uncommon (19). Broad-spectrum carbapenem agents are often considered the last option for effective therapy of infections with these resistant organisms, but the emergence of carbapenem resistant Enterobacteriaceae (CRE) over the past decade has left clinicians with few treatment options, and the spread of CRE has created a serious threat to public health.

CRE were first described in the United States more than two decades ago as a result of porin loss in combination with an over-expressed AmpC enzyme (7). More recently, we have seen the emergence of CRE due to production of carbapenemase enzymes that efficiently hydrolyze carbapenems and other β-lactam antibiotics. Production of the *Klebsiella pneumoniae* carbapenemase (KPC), an Ambler class A enzyme (35), is...
the most common carbapenemase among Enterobacteriaceae in the United States (32). The \textit{bla}_{KPC} gene is
carried on a mobile genetic element and confers resistance to all \textit{\beta}-lactam agents (8). Since it was first reported
in 2001 (45), this widespread enzyme has been documented in 37 U.S. states and territories among various
mirabilis} and \textit{Citrobacter}, \textit{Enterobacter} and \textit{Salmonella spp} (21, 22, 28, 32, 39). KPC-producing
Enterobacteriaceae are now being recognized in numerous countries in Europe, the Middle East and Asia, and
KPC has also been documented in \textit{Acinetobacter} and \textit{Pseudomonas spp} (5, 9, 33, 37, 41).

Another group of carbapenemases, the Ambler class B metallo-\textit{\beta}-lactamases (MBL), are of greater clinical
significance worldwide (9, 34). MBLs can confer resistance to carbapenems and all \textit{\beta}-lactam agents except
aztreonam, although many MBL-producing strains carry additional resistance mechanisms (e.g., ESBLs) that
render them resistant to aztreonam (42). Unlike KPC, MBLs have previously been unrecognized as mechanisms
of carbapenem resistance among Enterobacteriaceae in the United States, although they have been found in \textit{P.
aeruginosa} (18, 25). Recently, two different MBL enzymes, the newly-described New Delhi MBL (NDM) and
Verona Integron-encoded MBL (VIM), have been reported among Enterobacteriaceae in the United States.
NDM has been identified in \textit{E. cloacaes}, \textit{K. pneumoniae} and \textit{E. coli} isolates from Massachusetts, California,
Illinois, Virginia, and VIM-producing \textit{K. pneumoniae} have been isolated from patients in Washington and
California. In each instance, the patient carrying an MBL-producing strain had a history of recent medical care
in India or Pakistan (NDM), Greece or Italy (VIM) (1, 3, 17, 24, 29).

Here we describe three \textit{K. pneumoniae} isolates that are carbapenem resistant due to production of an IMP-type
MBL. Unlike the NDM- and VIM-producing isolates described above, the pediatric patients carrying these
IMP-producing organisms had no history of travel or receipt of medical care outside of the United States.

\textbf{Methods}
Patient demographic data, history and infection control investigation. Patient information was collected by review of medical records and discussion with the primary healthcare providers for each patient. The identification of the novel resistance mechanisms in these three isolates took place after the inpatient clinical care of these patients had been completed and this limited the epidemiologic investigation. No travel was identified among the patients or their mothers, but travel information among other family members was not known. Microbiology laboratory records were reviewed retrospectively to identify previously unrecognized CRE that might have represented additional cases, and prospectively to identify isolates with similar antimicrobial susceptibility patterns.

Bacterial isolates: Three *K. pneumoniae* urine isolates collected between November 2009 and June 2010 from infants at a single pediatric intensive care unit were submitted to CDC for reference antimicrobial susceptibility and characterization based on unusual susceptibility profiles: intermediate or resistant to 3rd generation cephalosporins and carbapenems, susceptible to aztreonam, fluoroquinolones and trimethoprim-sulfamethoxazole. An NDM-1–producing *K. pneumoniae* and a *K. pneumoniae* isolate from the same institution that was determined to not contain an MBL or KPC carbapenemase by the methods described below, were included as comparators for pulsed-field gel electrophoresis only. *E. cloacae* strains JMI10526 and JMI03839 were included as positive and negative *bla*IMP controls, respectively.

Antimicrobial susceptibility: Initial clinical testing was performed with the Vitek$^\text{®}$2 system (bioMérieux Inc, Durham, NC) and disk diffusion (11). Reference broth microdilution (BMD) was performed at CDC using frozen in-house prepared panels (10, 12) for the following antimicrobial agents: amikacin, aztreonam, cefepime, cefotaxime, cefoxitin, ceftazidime, ciprofloxacin, colistin, doripenem, ertapenem, gentamicin, imipenem, levofloxacin, meropenem, minocycline, polymyxin B, tetracycline, tigecycline, tobramycin, and trimethoprim-sulfamethoxazole. MIC to colistin and polymyxin B were reported without interpretation, as CLSI does not have Enterobacteriaceae breakpoints for these agents. BMD panels included a series of investigational MBL screening wells containing imipenem (range 0.25–1024 µg/ ml) plus the chelating agents, EDTA [0.2 mM] and...
1,10-phenanthroline [0.02 mM], adapted from Migliavaca et al. (27). A positive MBL screen was interpreted as a ≥2 doubling dilution decrease in imipenem MIC in the presence of chelating agents as compared to standard imipenem MIC. Each isolate was evaluated for carbapenemase production by the modified Hodge test (12) with 10 µg/ml meropenem, ertapenem, and imipenem disks (BD, Franklin Lakes, NJ); K. pneumoniae ATCC® BAA-1705™ and BAA-1706™ were included as positive and negative controls, respectively. MBL production was evaluated with Etest® MBL strips (ABBiodisk, Solna, Sweden) and the direct MBL test (20) using 10 µg imipenem disk and 0.1mM Tris-EDTA disks (BD; K. pneumoniae ATCC® BAA-2146™ and P. aeruginosa ATCC® 27853™ were included as positive and negative controls, respectively. Etest® MBL strips were performed and interpreted according to the manufacturer’s instructions.

PCR amplification and sequence analysis. PCR for blaKPC and blaNDM were performed as described previously (24). For screening, a multiplex PCR for detection of IMP-type, VIM-type, GIM-1, SPM-1 and SIM-1 MBLs was performed as described (14). Isolates positive for IMP-type PCR amplicons were further characterized by PCR amplification using primers described by Yan et al. (44) for the separation of IMP-1 from IMP-2. Amplicons obtained with IMP-1-specific primers were then sequenced with the same primers using BigDye® terminator v3.1 (ABI, Carlsbad, CA) chemistry and an ABI 3130xl (ABI) sequence analyzer. A 448-bp blaIMP probe labeled with digoxigenin (PCR DIG Probe Synthesis Kit; Roche, Indianapolis, IN) was generated using forward primer 5'-CATGGTTTGGTGGTTCTTGT-3' and reverse primer 5'ATAATTTGGCGGACTTTTG-GC-3'(30).

Pulsed-field Gel Electrophoresis (PFGE). Relatedness of the three K. pneumoniae isolates was assessed with Xba I pulsed-field gel electrophoresis as previously described (21), using Bionumerics 5.0 (Applied Maths, Austin, TX) software with the Dice coefficient and UPGMA, standardized to the Salmonella ser. Braenderup H9812 standard. Isolates were also compared to the CDC database of more than 400 carbapenem resistant K. pneumoniae strains, and to contemporary carbapenemase-negative K. pneumoniae from the same institution.
Plasmid analysis, transfer, and Southern blot. Plasmid preparations from each isolate were prepared with Qiagen Plasmid Midi kits (Qiagen, Valencia, CA), digested with HindIII (NEB, Beverly, MA), and separated by gel electrophoresis in 0.8% agarose (not shown). Multiple plasmids were present in each strain, therefore these plasmid preparations were used to transform E. coli DH10B™ cells (Invitrogen, Carlsbad, CA) by electroporation. Transformants were selected on LB agar containing 8 µg/ml ceftazidime. Transformants from each IMP-positive parent strain were screened for the presence of blaIMP by PCR with the IMP-1 family primers as described above. IMP-positive transformants were characterized by broth microdilution as described above.

Uncut parent and transformant plasmid DNA was compared by agarose gel electrophoresis to ensure that only one plasmid was present in each of the transformants, and that this plasmid corresponded in size to a plasmid band in the parent. Plasmid DNA from each parent strain and one transformant was digested with Xmn I and separated by electrophoresis in 0.75% agarose. DNA digests were transferred to Zeta-Probe nylon membranes (Bio-Rad, Hercules, CA) for Southern analysis by capillary transfer (38) and hybridized against the digoxigenin-labeled 448-bp IMP-specific probe described above using the DIG Nucleic Acid Detection Kit as recommended by the manufacturer (Roche).

Results

Patient 1 is a premature male infant born at 25 weeks gestational age with a history of chronic lung disease and multiple gastrointestinal surgeries who was admitted at birth to the NICU at Hospital A. He developed difficulty breathing during the 17th week of his NICU admission. Urine collected from an indwelling catheter as part of a rule-out sepsis work-up grew $10^4$ colonies/ml of a carbapenemase producing K. pneumoniae that was negative for ESBL production and blaKPC by PCR. Due to the low numbers of organisms, no treatment specific to this organism was initiated. He remained in the NICU for more than six months until he was discharged home. The isolate from Patient 1 is designated 1000308.
Patient 2 is a premature five month-old female infant born at 29 weeks gestational age. She was admitted to the NICU at Hospital A at birth and remained there for 60 days, the first 46 of which overlapped with Patient 1. Three months after she was discharged from the NICU, she returned to the hospital for a scheduled surgical repair of an atrioventricular canal defect. On post-operative day 4 a urine culture from the patient grew *Enterococcus faecalis* and carbapenem resistant *K. pneumoniae*. The patient completed a 10-day course of ampicillin and trimethoprim-sulfamethoxazole and was discharged home. The isolate from Patient 2 is designated 1002002.

Patient 3 is a male infant born at 37 weeks gestational age by emergency C-section. At birth, he required continuous positive airway pressure therapy in the delivery room and was admitted to the NICU at Hospital A for continued respiratory support and monitoring. He was discharged home after a four day NICU admission, all four of which overlapped with Patients A and B. At five months of age, Patient C presented to the emergency department with a two-day history of fever and drainage from his left ear. Urine collected as part of a rule-out sepsis work-up grew 5000 colonies/ml of carbapenemase-producing *K. pneumoniae*. The patient was prescribed amoxicillin and Tylenol/Motrin for his ear infection, and discharged home. The isolate from Patient 3 is designated 1002003.

Due to their unusual susceptibility patterns, *K. pneumoniae* isolates from each of the case patients above were submitted to CDC for reference broth microdilution antimicrobial susceptibility testing and further characterization. Each of the isolates was intermediate or resistant to the 3rd generation cephalosporins and to three or more carbapenems, but susceptible to aztreonam (Table 1) according to current CLSI breakpoints (13). Two isolates demonstrated a decrease of one doubling dilution in imipenem MIC in the presence of the metal chelating agents with the MBL broth screen, and one demonstrated a decrease of two dilutions (Table 1). The isolate from Patient 1 consistently yielded an imipenem MIC of 1 ug/ml and was negative for MBL production with the broth MBL screen. The modified Hodge test was positive for all three isolates with meropenem (Fig. 1A), ertapenem and imipenem disks (not shown).
The Etest® MBL was interpreted as a positive for MBL production for each isolate. The ellipse for each of the 159
*bla*<sub>IMP</sub>-positive *K. pneumoniae* isolates would have intersected the strip below the lowest numeric value
(imipenem <4 µg/ml, imipenem+EDTA <1 µg/ml) and demonstrated a deformed zone of inhibition (Fig. 1B)
(www.abbiodisk.com/pdf/pi/75001546.PDF). Likewise, the Direct MBL test demonstrated an extension of the
zone of inhibition surrounding the imipenem disk toward the TE disk, suggesting that the isolates were
producing a MBL (Fig. 1B).

PCR was performed for genes encoding the most common MBLs, including NDM, VIM, IMP, SIM, GIM and
SPM, as well as for KPC, the most common carbapenemase in the United States. All three of the isolates
yielded a PCR amplicon with IMP-family primers (14), but no products were produced with primers to any of
the other gene targets. Additional PCR with primers specific to IMP-1 or IMP-2 sub-groups of the *bla*<sub>IMP</sub> gene
(44) demonstrated that all three isolates were carrying an allele belonging to the IMP-1 family, and sequence
analysis demonstrated that it was indistinguishable from *bla*<sub>IMP-4</sub> (http://www.lahey.org/Studies/).

All three IMP-producing *K. pneumoniae* were very closely related by PFGE, but were unrelated to another
IMP-negative carbapenemase-negative *K. pneumoniae* from the same institution (Fig. 2) or to other
carbapenemase-producing *K. pneumoniae* isolates in the CDC database (data not shown). Multiple plasmids
were apparent in each of the IMP-producing isolates, making it difficult to determine whether a single plasmid
was common to all three isolates (Fig 3A). Therefore, plasmid DNA from each parent was electroporated into
naïve *E. coli*. Ceftazidime resistant transformants from each of the IMP-positive parent strains were shown by
PCR to contain *bla*<sub>IMP</sub>. Analysis of uncut parent and transformant plasmid DNA demonstrated the presence of a
single plasmid approximately 100 kb in size in each transformant, corresponding to a plasmid band in each
parent (not shown). *Xmn I* plasmid DNA digests (Fig 3A) and Southern blot analysis demonstrated a single
*Xmn I* fragment of approximately 3.5 kb containing *bla*<sub>IMP</sub> in plasmid preparations from both parent and
transformant strains (Fig 3B).
Antimicrobial susceptibility testing was performed on the transformants and compared to that of the naïve E. coli recipient strain. Each of the transformant strains demonstrated increased MICs to cephalosporins and carbapenems but not aztreonam, consistent with IMP production. All three transformants were positive for carbapenemase production by the modified Hodge test (not shown) and demonstrated a decrease of > 2 doubling dilutions in imipenem MIC in the presence of chelating agents compared to imipenem alone (Table 1). Each of the transformant strains also demonstrated increased MICs to tobramycin and gentamicin when compared to the naïve recipient strain, suggesting that a mechanism for aminoglycoside resistance was co-resident on the IMP-plasmid.

Although the IMP-producing isolate from patient one was recognized as unusual, it would not have been classified as carbapenem resistant according to the interpretive criteria in use at the time of its isolation (11). Isolates from patients two and three, however, were recovered within weeks of the release of the newer interpretive criteria, which lowered the clinical breakpoints for all of the carbapenems. According to the revised breakpoints, all three isolates demonstrated resistance to multiple carbapenem agents (12). No additional clinical isolates with similar antimicrobial susceptibility profiles were identified by review of microbiology reports from one month prior to the arrival of patient one through the present. By the time that carbapenem resistant K. pneumoniae was recognized among these patients, all three had been discharged from the hospital and were residing at home, and thus posed limited risk for further transmission in the healthcare setting.

Discussion

The continued emergence and spread of CRE worldwide is an important concern among clinicians, infection prevention experts and public health agencies. Internationally, MBL-type carbapenemases in particular have been of concern for some time, while KPC-producing isolates have been a far greater problem in the United States to date. This report describes three cases of probable domestically-acquired IMP-producing Enterobacteriaceae and adds to reports of likely internationally-acquired MBLs that have already been recognized in the United States (1, 3). Although the ultimate contribution of MBL-producing isolates to the
prevalence of CRE remains to be determined, this potential increase in mechanisms highlights the need for vigorous infection prevention efforts aimed at preventing transmission.

The recent heightened attention to CRE is largely due to the emergence of the New Delhi Metallo-β-lactamase, an MBL that has been strongly associated with travel and receipt of medical care on the Indian subcontinent. This has been the case among U.S. NDM-producing isolates as well; five of six patients from whom NDM-producing CRE were identified by U.S. public health agencies had a history of recent receipt of medical care in India or Pakistan (1, 17), A. Kallen, unpub. data). Similarly, the two VIM-producing CRE identified in the United States were isolated from patients who had been recently hospitalized in Greece or Italy, countries in which these organisms have been reported (26, 35, 40). In contrast, the IMP-producing K. pneumoniae described here were from pediatric patients who had no travel history and, in the case of Patient 1, had never been outside of the hospital prior to CRE isolation.

IMP was originally identified in a Japanese P. aeruginosa isolate collected in 1988 that was resistant to imipenem, and was named for this phenotype (active on imipenem) (43). blaIMP was located on a plasmid that readily transferred among P. aeruginosa strains, and within five years of that first report, blaIMP was found among Enterobacteriaceae in other Japanese hospitals in association with Class 3 integrons (4, 30, 42). IMP variants have now been identified among multiple species of gram-negative bacteria around the world (42), but have been reported infrequently in the United States, and only among Pseudomonas species (18). This is the first report of this enzyme from U.S. Enterobacteriaceae; however, outbreaks caused by IMP-producing Pseudomonas have been reported from both Canada and Mexico in association with Class 1 integrons (15, 16, 23, 36).

PFGE analysis demonstrated that all three IMP-producing K. pneumoniae isolates were related to each other, but not to a contemporary carbapenemase-negative isolate from the same institution or to other carbapenemase-producing isolates in the CDC database. All three IMP-producing isolates harbored a common plasmid that carried blaIMP on an XmnI fragment of approximately 3.5 kb. These results and the fact that IMP-producing
Enterobacteriaceae have not been previously recognized in the United States suggest a common source for these organisms. As the common link between these patients was their overlapping stays in the NICU, it is possible that transmission occurred there, and that at least 2 of the patients remained colonized for months afterward. In the case of Patient 2, IMP-producing *K. pneumoniae* was detected in a clinical culture collected three months later, and Patient 3’s isolate was detected as an incidental finding five months after his discharge. The original source and potential route of transmission of this IMP-producing *K. pneumoniae* isolate for these three patients are not known. It is possible that these strains are circulating widely in the community, and that all three patients acquired CRE from independent sources. At least two, and possibly all three, of these cases represent asymptomatic colonization with CRE rather than true infection. It is also possible that they represent cross transmission (e.g., by healthcare personnel hands) or a common environmental source. Since surveillance cultures were not obtained to identify additional cases of CRE carriage, the true extent of colonization with IMP-producing *K. pneumoniae* in this NICU cannot be known.

Each of the CRE described here was recognized by routine antimicrobial susceptibility testing performed in the hospital laboratory as resistant to the 3rd generation cephalosporins and carbapenems according to the revised (lowered) CLSI guidance for carbapenems (12), but unusual in being susceptible to aztreonam. This antimicrobial susceptibility pattern is characteristic of MBL-producing organisms, although it is not frequently encountered since most MBL-producing organisms also carry multiple other resistance mechanisms. The isolates described here are also unusual because although they carry an MBL, they remain susceptible to fluoroquinolones, trimethoprim-sulfamethoxazole and tetracyclines. Although one isolate demonstrated intermediate susceptibility to minocycline, we believe this was likely due to variability in the test rather than acquired resistance. One of these three isolates consistently yielded an imipenem MIC within the susceptible range, although it was non-susceptible to doripenem, ertapenem and meropenem.

The IMP-producing *K. pneumoniae* yielded variable results with the phenotypic tests used to detect the presence of MBLs. All three were clearly positive for carbapenemase production with the MHT, but the direct MBL test,
the Etest® MBL and the broth microdilution MBL screen yielded equivocal results. Two isolates demonstrated a decrease of only one doubling dilution with the broth microdilution MBL screen, which is within the acceptable variation for broth microdilution testing and would not be a reliable indicator of MBL production. Interestingly, the IMP-containing *E. coli* transformants were readily detected with the broth MBL screen, suggesting that some factor present in the parent strain interfered with this assay. The Etest MBL demonstrated only a subtle deformation of the ellipse in both the *K. pneumoniae* parent and *E. coli* transformant strains. Variability in the performance of phenotypic tests for detection of MBLs and difficulty with their interpretation has been described previously (29), and our results demonstrate the need to include multiple methods to ensure reliable detection of MBLs. In this case, the pattern of non-susceptibility to all β-lactam agents except aztreonam was an important indicator of MBL presence.

At the time the first isolate was recovered (November 2009), it would have been characterized as susceptible to ertapenem, imipenem and meropenem, and non-susceptible only to doripenem (11), (http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/022106s002s005lbl.pdf). Since all three patients had been discharged to their homes by the time that CRE colonization was recognized, infection prevention staff at the facility elected to perform laboratory-based surveillance for organisms with the same antimicrobial susceptibility pattern rather than active surveillance of the NICU in which these patients resided. However, consideration should be given to performing point prevalence surveys and/or active surveillance testing when novel cases of CRE infection or colonization are recognized in a healthcare facility (2). The emergence of this new mechanism of carbapenem resistance among Enterobacteriaceae in the United States has the potential to add to the growing burden of CRE. CRE present treatment dilemmas and are associated with high rates of mortality (31). This is particularly concerning in light of a 2009 IDSA report highlighting the fact that there were no antimicrobials in advanced development with activity against these organisms (6). In order to slow the emergence of these strains, aggressive infection prevention practices aimed at preventing infections with— and transmission of— these organisms should be employed. Current prevention recommendations for acute-care facilities are available from CDC (2). Foremost among these recommendations is recognizing when CRE are
present in facilities so infected or colonized patients can be put in Contact Precautions and patient contacts can be screened for evidence of transmission.

In summary, this is the first published report of IMP-producing Enterobacteriaceae in the United States. The isolates appear to have been acquired domestically although the mechanism of transmission to these three patients is not known. The emergence of this and other metallo-β-lactamases (i.e., VIM and NDM) among Enterobacteriaceae in the United States have the potential to add to the already substantial burden of CRE due to the emergence and spread of KPC-producing strains. CRE are an important public health problem; in order to limit their impact, implementation of recommended interventions to prevent transmission of these organisms should be a priority for institutions where these organisms are identified.

Acknowledgements: We thank JMI laboratories for providing control strains JMI10526 and JMI03839.
Table 1. Results of antimicrobial susceptibility testing in IMP-producing strains

| Strain ID | IMP-producing Klebsiella pneumoniae parent isolates | Escherichia coli transformants |
|-----------|----------------------------------------------------|--------------------------------|---|
|           | From 1000308 | From 1002002 | From 1002003 | Naïve Recipient |
| Date cultured | Nov. 2009 | May 2010 | June 2010 | |
| Antimicrobial agents | MIC µg/ml | Int. | MIC µg/ml | Int. | MIC µg/ml | Int. | MIC µg/ml | Int. | MIC µg/ml | Int. | |
| Aminoglycocides | | | | | | | | | | | |
| Amikacin | 2 S | ≤ 1 S | ≤ 1 S | 4 S | 2 S | 2 S | 4 S | | | | |
| Gentamicin | > 16 R | 16 R | 8 I | > 16 R | > 16 R | > 16 R | | | | 0.5 S | |
| Tobramycin | 8 I | 4 S | > 16 R | 8 I | 8 I | 8 I | 1 S | | | | |
| Aztreonam | ≤ 2 S | ≤ 2 S | ≤ 2 S | ≤ 2 S | ≤ 2 S | ≤ 2 S | | | | | |
| Cepheims | | | | | | | | | | | |
| Cefepime | 16 I | 4 S | 16 I | 4 S | 4 S | 2 S | ≤ 1 S | | | | |
| Cefotaxime | 32 R | > 64 R | > 64 R | 128 R | 32 R | 64 R | | | | | |
| Ceftazidime | > 64 R | 64 R | 64 R | 128 R | 64 R | > 128 R | | | | | |
| Cefoxitin | > 32 R | > 32 R | > 32 R | > 16 R | > 16 R | > 16 R | | | | 8 S | |
| Carapenems | | | | | | | | | | | |
| Doripenem | 4 R | 8 R | 2 I | 2 I | 1 S | 1 S | ≤ 0.25 S | | | | |
| Ertapenem | 2 R | 4 R | 1 R | 1 R | 1 R | 1 R | ≤ 0.12 S | | | | |
| Imipenem | 1 S | 2 I | 2 I | 2 I | 1 I | 1 S | | | | ≤ 0.5 S | |
| Meropenem | 2 I | 4 R | 2 I | 2 I | 1 I | 1 S | ≤ 0.25 S | | | | |
| Fluoroquinolones | | | | | | | | | | | |
| Ciprofloxacin | 0.5 S | 0.5 S | 0.5 S | ≤ 0.25 S | ≤ 0.25 S | ≤ 0.25 S | | | ≤ 0.25 S | | |
| Levofoxacin | 0.5 S | 0.5 S | 1 S | ≤ 0.25 S | ≤ 0.25 S | ≤ 0.25 S | | | ≤ 0.25 S | | |
| Lipopeptides | | | | | | | | | | | |
| Polymixin | 0.5 n/a | ≤ 0.25 n/a | 0.5 n/a | 1 n/a | 1 n/a | 1 n/a | | | 1 n/a | n/a | |
| Colistin | 0.5 n/a | ≤ 0.25 n/a | 0.5 n/a | ≤ 0.5 n/a | ≤ 0.5 n/a | ≤ 0.5 n/a | | | ≤ 0.5 n/a | |
| Tetracyclines | | | | | | | | | | | |
| Minocycline | ≤ 4 S | ≤ 4 S | 8 I | ≤ 4 S | ≤ 4 S | ≤ 4 S | | | ≤ 4 S | | |
| Tetracycline | ≤ 2 S | ≤ 2 S | ≤ 2 S | ≤ 2 S | ≤ 2 S | ≤ 2 S | | | ≤ 2 S | | |
| Tigecycline | ≤ 0.5 S | ≤ 0.5 S | ≤ 0.5 S | ≤ 0.5 S | ≤ 0.5 S | ≤ 0.5 S | | | ≤ 0.5 S | | |
| Trimethoprim-sulfmethoxazole | 1/19 S | 0.5/9.5 S | ≤ 0.2 S/5/4.25 S | ≤ 0.2 S/5/4.25 S | ≤ 0.2 S/5/4.25 S | | | ≤ 0.2 S/5/4.25 S | | |
| Screening tests | MIC | Int. | MIC | Int. | MIC | Int. | MIC | Int. | MIC | Int. | MIC | |

Screening tests:
- MIC
- Int.
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Interpretations based on CLSI M100-S21(13)

Interpretation per decrease of ≥2 doubling dilutions (Broth MBL screen) or package insert (Etest® MBL)
Figure legends

Figure 1. Phenotypic tests demonstrating metallo-β-lactamase production by a bla_IMP-positive *K. pneumoniae* isolate 1002003 (A & B) and bla_IMP-negative *K. pneumoniae* isolate 1002001 (C). (A) Modified Hodge test with meropenem demonstrating carbapenemase production by the test isolate and positive control, but not the negative control, (B) Direct MBL test demonstrating subtle distortion of the zone of inhibition around the imipenem disk toward the EDTA disk and MBL Etest® demonstrating deformation of the IP and IPI ellipses (arrow), (C) Example of negative results for the direct MBL test (round, undistorted zone of inhibition) and MBL Etest®.

Figure 2. Dendrogram showing strain relatedness of bla_IMP-positive *K. pneumoniae* isolates compared to two contemporary bla_IMP-negative carbapenem-resistant *K. pneumoniae* isolates from California based on pulsed-field gel electrophoresis patterns. * Isolate did not produce an MBL.

Figure 3. *XmnI* digest and Southern blot analysis of plasmid DNA demonstrating presence of multiple plasmids in bla_IMP-positive *K. pneumoniae* isolates and a single plasmid in bla_IMP-positive *E. coli* transformants. Lanes: 1, *E. cloacae* bla_IMP positive control JMI10526; 2, *E. cloacae* bla_IMP negative control JMI03839; 3, *K. pneumoniae* 1000308; 4, *E. coli* transformant of 1000308; 5, *K. pneumoniae* 1002002; 6, *E. coli* transformant of 1002002; 7, *K. pneumoniae* 1002003; 8, *E. coli* transformant of 1002003; 9, *HindIII* digest of phage lambda DNA size standard. A) Gel-electrophoresis of plasmid DNA. B) Southern blot analysis of DNA from (A) with bla_IMP-specific probe demonstrating presence of a single hybridizing band in both the parent and transformant strains.
References


12. **Clinical_and_Laboratory_Standards_Institute.** 2010. Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement. M100-S20-U. Clinical and Laboratory Standards Institute, Wayne, PA


