IMP-Producing Carbapenem-Resistant *Klebsiella pneumoniae* in the United States

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The emergence and spread of carbapenem-resistant *Enterobacteriaceae* (CRE) producing acquired carbapenemases have 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. health care 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, an MBL carbapenemase not previously published as present among *Enterobacteriaceae* in the United States.

*Enterobacteriaceae* are an abundant part of the normal human gut flora and also a common cause of both health care- 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 2 decades ago as a result of porin loss in combination with an overexpressed AmpC enzyme (4). More recently, we have seen the emergence of CRE due to production of carbapenemase enzymes that efficiently hydrolyze carbapenems and other β-lactam antibiotics. *Klebsiella pneumoniae* carbapenemase (KPC), an Ambler class A enzyme (35), is the most common carbapenemase produced by *Enterobacteriaceae* in the United States (32). The *bla*KPC gene is carried on a mobile genetic element and confers resistance to all β-lactam agents (5). Since it was first reported in 2001 (45), this widespread enzyme has been documented in 37 territories and states of the United States in various *Enterobacteriaceae*, including *K. pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli*, *Serratia marcescens*, *Proteus mirabilis*, and *Citrobacter*, *Enterobacter* and *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 *Acinetobacter* and *Pseudomonas* spp. (2, 6, 33, 37, 41).

Another group of carbapenemases, the Ambler class B metallo-β-lactamases (MBLs), are of greater clinical significance worldwide (6, 34). MBLs can confer resistance to carbapenems and all β-lactam agents except aztreonam although many MBL-producing strains carry additional resistance mechanisms (e.g., extended-spectrum β-lactamases [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 *Pseudomonas 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 *Enterobacter cloacae*, *K. pneumoniae*, and *E. coli* isolates from Massachusetts, California, Illinois, and Virginia, and VIM-producing *K. pneumoniae* bacteria 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) (7, 9, 17, 24, 29).

Here, we describe three *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 the United States.

**MATERIALS AND METHODS**

**Patient demographic data, history, and infection control investigation.** Patient information was collected by review of medical records and discussion with the primary health care 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

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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 the CDC for reference antimicrobial susceptibility and characterization based on unusual susceptibility profiles: intermediate or resistant to third-generation cephalosporins and carbapenems and susceptible to aztreonam, fluorquinolones, 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 in 0.8% agarose (data not shown). Multiple plasmids were present in each isolate were prepared with QIAGEN Plasmid Midi kits (QIAGEN, Valencia, CA) for Southern analysis by capillary transfer (38) and hybridized against the digoxigenin-labeled 448-bp IMP-specific probe described above using a DIG Nucleic Acid Detection Kit as recommended by the manufacturer (Roche).

**RESULTS**

**Patient 1** is a premature male infant born at 27 weeks gestational age with a history of chronic lung disease and multiple gastrointestinal surgeries who was admitted at birth to the neonatal intensive care unit (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 workup grew 10^7 colonies/ml of a carbapenemase-producing *K. pneumoniae* that was negative for ESBL production and bla_{KPC} 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 6 months until he was discharged home. The isolate from patient 1 is designated 1000308.

**Patient 2** is a premature 5-month-old female infant born at a gestational age of 29 weeks. 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 postoperative 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 4-day NICU admission, all of which overlapped with patients A and B. At 5 months of age, patient C presented to the emergency department with a 2-day history of fever and drainage from his left ear. Urine collected as part of a rule-out sepsis workup grew 5,000 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 the CDC for reference broth microdilution antimicrobial susceptibility testing and further characterization. Each of the isolates was intermediate or resistant to the third-generation cephalosporins and to three or more carbapenems but susceptible to aztreomycin (Table 1), according to current CLSI breakpoints (13). Two isolates demonstrated a decrease of one doubling dilution in the 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 µg/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 (data not shown).
TABLE 1. Results of antimicrobial susceptibility testing in IMP-producing strains

<table>
<thead>
<tr>
<th>Antimicrobial class and agent or screening test</th>
<th>Susceptibility of the indicated IMP-producing K. pneumoniae parent isolate (date cultured [mo/yr])</th>
<th>Susceptibility of E. coli transformant of the indicated isolate*</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1000308 (11/09)</td>
<td>1002002 (5/10)</td>
</tr>
<tr>
<td></td>
<td>MIC (μg/ml)</td>
<td>Int</td>
</tr>
<tr>
<td>Antimicrobials</td>
<td></td>
<td></td>
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<tr>
<td>Aminoglycocides</td>
<td></td>
<td></td>
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<tr>
<td>Amikacin</td>
<td>2</td>
<td>S</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≥16</td>
<td>R</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>8</td>
<td>I</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>≤2</td>
<td>S</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td></td>
<td></td>
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<tr>
<td>Ceftazidime</td>
<td>16</td>
<td>≤4</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&gt;32</td>
<td>R</td>
</tr>
<tr>
<td>Carbapenems</td>
<td></td>
<td></td>
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<tr>
<td>Doripenem</td>
<td>4</td>
<td>R</td>
</tr>
<tr>
<td>меропenem</td>
<td>2</td>
<td>R</td>
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<tr>
<td>Imipenem</td>
<td>1</td>
<td>S</td>
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<tr>
<td>Meropenem</td>
<td>2</td>
<td>I</td>
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<tr>
<td>Fluoroquinolones</td>
<td></td>
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<tr>
<td>Ciprofloxacin</td>
<td>0.5</td>
<td>S</td>
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<tr>
<td>Levofloxacin</td>
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<td>S</td>
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<tr>
<td>Lipopeptides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymixin</td>
<td>0.5</td>
<td>NA</td>
</tr>
<tr>
<td>Colistin</td>
<td>0.5</td>
<td>NA</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minocycline</td>
<td>≤4</td>
<td>S</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>≤2</td>
<td>S</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>≤0.5</td>
<td>S</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>1/19</td>
<td>S</td>
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</tbody>
</table>

Screening tests*

| Broth MBL screen (IP/IP-EDTA-PA*) | 1/0.5 Neg | 2/1 Neg | 2/0.5 Pos | 2/≤0.25 Pos | 1/≤0.25 Pos | 1/≤0.25 Pos | ≤0.5/0.25 Neg |
| Etst MBL (IP/IP)                  | ≤<4/≤1 IND | ≤<4/≤1 IND | ≤<4/≤1 IND | ≤<4/≤1 IND | ≤<4/≤1 IND | ≤<4/≤1 IND | ≤<4/≤1 IND |

* Int, interpretation. For antimicrobial agents, interpretations are based on CLSI M100-S21 (13): S, susceptible; R, resistant; I, intermediate. For screening tests, a decrease of ≥2 doubling dilutions (broth MBL screen) or ≥3 doubling dilutions (Etest MBL) is considered positive (Pos). NA, not applicable; IND, indeterminate.

a In the Etest, deformation of the IP and IPI ellipses (positive result) was found for all isolates and transformants; results were negative for the naïve recipient.

b PA, 1,10-phenanthroline.

The Etest MBL was interpreted as a positive for MBL production for each isolate. The ellipse for each of the bla\textsubscript{IMP}\textsuperscript{-} positive K. pneumoniae isolates would have intersected the strip below the lowest numeric value (imipenem [IP], <4 μg/ml; imipenem plus EDTA [IPI], <1 μg/ml) and demonstrated a deformed zone of inhibition (Fig. 1B) (Etest MBL package insert; AB Biodisk, Solna, Sweden). 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 an 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 PCRs with primers specific to IMP-1 or IMP-2 subgroups of the bla\textsubscript{IMP}\textsuperscript{-} 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\textsubscript{IMP}\textsuperscript{-} (http://www.lahey.org/Studies/).

All three IMP-producing K. pneumoniae isolates 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. Cefazidime-resistant transformants from each of the IMP-positive parent strains were shown by PCR to contain \textit{bla\textsubscript{IMP}}. 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 (data not shown). XmnI plasmid DNA digests (Fig. 3A) and Southern blot analysis demonstrated a single XmnI fragment of approximately 3.5 kb containing \textit{bla\textsubscript{IMP}} in plasmid preparations from both parent and transformant strains (Fig. 3B).

Antimicrobial susceptibility testing was performed on the transformants, and the result was 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 (data not shown). XmnI plasmid DNA digests (Fig. 3A) and Southern blot analysis demonstrated a single XmnI fragment of approximately 3.5 kb containing \textit{bla\textsubscript{IMP}} in plasmid preparations from both parent and transformant strains (Fig. 3B).

DISCUSSION

The continued emergence and spread of CRE worldwide are important concerns 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 (7, 9). 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.
was named for this phenotype (active on imipenem) (43).

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ing presence of a single hybridizing band in both the parent and

VOL. 49, 2011 U.S. IMP-PRODUCING

E. cloacae bla

Pseudomonas Enterobacteriaceae enzyme from U.S. have been reported infrequently in the United States and only among Gram-negative bacteria around the world (42) but have been

variants have now been identified among multiple species of hospitals in association with class 3 integrons (1, 30, 42). IMP

16, 23, 36).

canada and Mexico in association with class 1 integrons (15, 30). 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 \( bla_{IMP} \) 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 two 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 3 months later, and patient 3’s isolate was detected as an incidental finding 5 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 health care 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 third-generation cephalosporins and carbapenems according to the revised (lowered) CLSI guidance for carbapenems (12), but the isolates were 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 nonsusceptible to doripenem, ertapenem, and meropenem.

The IMP-producing \( K. pneumoniae \) isolates 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

The recent heightened attention to CRE is largely due to the emergence of the New Delhi metallo-\( \beta \)-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 (7, 17; also A. Kallen, unpublished 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 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). \( bla_{IMP} \) was located on a plasmid that readily transferred among \( P. aeruginosa \) strains, and within 5 years of that first report, \( bla_{IMP} \) was found among Enterobacteriaceae in other Japanese hospitals in association with class 3 integrons (1, 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

FIG. 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. Lane 1, \( E. cloacae \) \( bla_{IMP} \) positive control JMI10526; lane 2, \( E. cloacae \) \( bla_{IMP} \) negative control JMI10539; lane 3, \( K. pneumoniae \) 1000308; lane 4, \( E. coli \) transformant of 1000308; lane 5, \( K. pneumoniae \) 1002002; lane 6, \( E. coli \) transformant of 1002002; lane 7, \( K. pneumoniae \) 1002003; lane 8, \( E. coli \) transformant of 1002003; lane 9, HindIII digest of phage lambda DNA size standard. (A) Gel electrophoresis of plasmid DNA. (B) Southern blot analysis of DNA from the experiment shown in panel A with a \( bla_{IMP} \)-specific probe demonstrating presence of a single hybridizing band in both the parent and transformant strains.
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 nonsusceptibility 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 nonsusceptible only to doripenem (11); http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/022106s002005bl.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 health care facility (8). 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 Infectious Diseases Society of America (IDSA) report highlighting the fact that there were no antimicrobials in advanced development with activity against these organisms (3). 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 (8). Foremost among these recommendations is recognizing when CRE are present in facilities so that 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-lactamasas (i.e., VIM and NDM) among Enterobacteriaceae in the United States has 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.

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REFERENCES


