

## Outbreak of Colistin-Resistant, Carbapenem-Resistant *Klebsiella pneumoniae* in Metropolitan Detroit, Michigan<sup>∇</sup>

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**Carbapenem-resistant *Klebsiella pneumoniae* has spread worldwide and throughout the United States. Colistin is used extensively to treat infections with this organism. We describe a cluster of colistin-resistant, carbapenem-resistant *K. pneumoniae* infection cases involving three institutions in Detroit, MI. A cluster of five cases of colistin-resistant, carbapenem-resistant *K. pneumoniae* was identified at Detroit Medical Center (DMC) from 27 July to 22 August 2009. Epidemiologic data were collected, and transmission opportunities were analyzed. Isolates were genotyped by using pulsed-field gel electrophoresis and repetitive extragenic palindromic PCR. Data regarding the use of colistin were obtained from pharmacy records. The index case of colistin-resistant, carbapenem-resistant *K. pneumoniae* was followed 20 days later by four additional cases occurring in a 6-day interval. All of the patients, at some point, had stayed at one particular institution. The mean number of opportunities for transmission between patients was  $2.3 \pm 0.5$ , and each patient had at least one opportunity for transmission with one of the other patients. Compared to 60 colistin-susceptible, carbapenem-resistant *K. pneumoniae* controls isolated in the previous year at DMC, case patients were significantly older ( $P = 0.05$ ) and the carbapenem-resistant *K. pneumoniae* organisms isolated from them displayed much higher MICs to imipenem ( $P < 0.001$ ). Colistin use was not enhanced in the months preceding the outbreak. Genotyping revealed two closely related clones. This report of a colistin-resistant, carbapenem-resistant *K. pneumoniae* outbreak is strongly linked to patient-to-patient transmission. Controlling the spread and novel emergence of bacteria with this phenotype is of paramount importance.**

Carbapenem-resistant members of the family *Enterobacteriaceae* have spread across the world and within the United States (2, 5, 15). In the United States, carbapenem-resistant *Klebsiella pneumoniae* constitutes 92% of all carbapenem-resistant *Enterobacteriaceae* and carbapenemase production mediated by *bla*<sub>KPC</sub> is the most prevalent mechanism conferring resistance to carbapenems (5). Carbapenem-resistant *Enterobacteriaceae* are coresistant to almost all classes of antimicrobials (29), and infections are associated with extremely high rates of morbidity and mortality and high costs (30). Colistimethate sodium, i.e., colistin, is one of the few remaining therapeutic options available to treat infections due to carbapenem-resistant *Enterobacteriaceae*. Despite the toxicity of this relatively old agent, colistin is frequently used to treat infections due to carbapenem-resistant *Enterobacteriaceae* because (i) therapeutic alternatives are not yet available and (ii) colistin has a long history and track record in the treatment of invasive infections due to Gram-negative bacilli (28, 31, 35). In most

cases, colistin is the last viable effective option for the treatment of invasive bloodstream infections due to carbapenem-resistant *Enterobacteriaceae*.

In the past few years, there have been sporadic reports of colistin-resistant, carbapenem-resistant *Enterobacteriaceae* cases from various parts of the world, e.g., Greece (2, 37), Israel (28), South Korea (32), and Singapore (33). In Athens, Greece, an outbreak during the years 2004 and 2005 involving 13 patients in a single hospital unit was reported (2). Colistin-resistant, carbapenem-resistant *Enterobacteriaceae* cases have never been reported from the United States. There have been case reports of polymyxin B-resistant, carbapenem-resistant *Enterobacteriaceae* from the New York City area (usually, resistance to polymyxin B confers resistance to polymyxin E, i.e., colistin, and vice versa), but these events remain sporadic and rare, with no outbreaks reported thus far (4, 8, 17). The mechanism(s) of colistin resistance in carbapenem-resistant *Enterobacteriaceae* is not yet known. The genetic basis of colistin resistance in *Acinetobacter baumannii* is being investigated (1, 13, 18), but it remains unclear whether the same mechanism(s) is associated with colistin resistance in carbapenem-resistant *Enterobacteriaceae*. Additionally, the microbiological definitions of colistin resistance are also not well established, as formal Clinical and Laboratory Standards Institute (CLSI) cri-

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teria and breakpoints have not been established (7). Lastly, the clinical significance of colistin resistance remains to be defined.

We describe here the first cluster, as far as we know, of colistin-resistant, carbapenem-resistant *K. pneumoniae* in the United States, which involved two hospitals and a long-term acute-care (LTAC) facility, in the summer of 2009. The epidemiologic and molecular outbreak investigations that followed are detailed.

## MATERIALS AND METHODS

**Study design and setting.** This outbreak investigation and case-cohort study were conducted in the Detroit Medical Center (DMC) health care system, which consists of eight hospitals and more than 2,000 inpatient beds. The outbreak involved two of these hospitals; both are university hospitals located in Detroit. Hospital A is a 360-bed hospital, and hospital B is a 570-bed hospital. An LTAC facility is housed within one of the study hospitals (hospital A) but is not part of the DMC network. This LTAC facility has 35 beds and is located inside the main inpatient building. Although the LTAC facility is administratively separate from the inpatient hospital, patients from this LTAC facility use, on a routine basis, multiple services of this particular DMC facility, such as radiology, operating rooms, the emergency room, dialysis, respiratory therapy, and laboratory services.

The first part of the outbreak investigation was conducted prospectively during an infection control investigation. Molecular analyses were conducted retrospectively. This study was approved by the Institutional Review Boards at DMC and Wayne State University.

**Patients and variables.** Patients were included in the outbreak investigations if they had one or more clinical isolates of *K. pneumoniae* from 30 July to 22 August 2009 that were resistant to both imipenem and colistin. Imipenem resistance was defined as an MIC of  $\geq 8$   $\mu\text{g/ml}$ , and colistin resistance was defined as an MIC of  $\geq 4$   $\mu\text{g/ml}$  (7, 11).

Patients were included in the case-cohort study if they had one or more clinical isolates of carbapenem-resistant *K. pneumoniae* either colistin susceptible or colistin resistant at DMC from 1 September 2008 to 22 August 2009 (from the time testing for carbapenem-resistant *Enterobacteriaceae* was initiated at DMC to the last day of the outbreak). A cumulative case-cohort analysis was conducted comparing the colistin-resistant, carbapenem-resistant *K. pneumoniae* case patients (from the outbreak investigation) to the colistin-susceptible, carbapenem-resistant *K. pneumoniae* controls.

The epidemiologic data collected included demographics, admission source, comorbid conditions, severity-of-illness indices, hospital exposures and procedures, and in-hospital mortality. In addition, opportunities for transmission between patients were analyzed in order to quantify the number of encounters and investigate the possible role of patient-to-patient transmission in the outbreak (19). An opportunity for transmission was defined as two case patients residing at the same hospital ward/unit on the same day before the isolation of colistin-resistant, carbapenem-resistant *Enterobacteriaceae* from one or both of the patients. Transmission opportunities for each patient were analyzed for the 3 months prior to the initial isolation of colistin-resistant, carbapenem-resistant *K. pneumoniae* to 2 weeks after the final positive culture.

Cocolonization with non-glucose-fermenting Gram-negative bacilli (such as *A. baumannii* and *Pseudomonas aeruginosa*) was assessed in order to examine the possible role of gene transfer from nonfermenters to *K. pneumoniae* conferring high levels of carbapenem resistance and colistin resistance. Cocolonization was defined as isolation of *A. baumannii* or *P. aeruginosa* from a clinical culture on the same day as isolation of the colistin-resistant, carbapenem-resistant *K. pneumoniae* strain or during the period from 1 week before to 1 week after colistin-resistant, carbapenem-resistant *K. pneumoniae* isolation.

Data regarding the use of colistin during the outbreak and in the preceding 18 months were obtained from pharmacy records. Colistin defined daily doses (DDDs) were based on the WHO-recommended daily dose of 3 million U/day (i.e., 90 mg colistin base activity) (3, 20, 27, 34).

**Microbiology and molecular analyses.** Identification of bacteria and determination of susceptibility to carbapenems (imipenem and meropenem) were initially done by using an automated broth microdilution system (MicroScan, Renton, WA). A modified Hodge test was conducted to assess carbapenemase production according to CLSI criteria (7). Etests (AB Biodisk, Solna, Sweden) were used to determine susceptibility to colistin and determine the MIC to imipenem (the automated panel tests concentrations of 4 and 8  $\mu\text{g/ml}$  only). Etests were conducted according to packet insert instructions, using Mueller-

Hinton agar with an inoculum determined by a turbidity of 0.5 in a 0.85% NaCl suspension. The MIC endpoints were defined at the zone of complete inhibition of growth. Quality control tests were performed according to FDA package insert for imipenem Etest strips and included the following organisms and MIC ranges for imipenem: *Staphylococcus aureus* ATCC 29213, 0.016 to 0.064  $\mu\text{g/ml}$ ; *Enterococcus faecalis* ATCC 29212, 0.5 to 2.0  $\mu\text{g/ml}$ ; *Escherichia coli* ATCC 25922, 0.064 to 0.25  $\mu\text{g/ml}$ ; *P. aeruginosa* ATCC 27853, 1.0 to 4.0  $\mu\text{g/ml}$ . Resistance to colistin was defined as an MIC of  $\geq 4$   $\mu\text{g/ml}$  (11).

The presence of a *bla*<sub>KPC</sub> gene was determined by PCR as follows. Bacterial DNA was obtained by suspending two to three colonies of each isolate grown on MacConkey agar plates in 500  $\mu\text{l}$  of nuclease-free water (UBS Corporation, Cleveland, OH) and heating them at 90°C for 10 min. Samples were spun at 10,000 rpm for 10 min, and the resulting supernatant was diluted 1:10 with nuclease-free water. Amplification reactions were performed using high-fidelity rTth DNA Polymerase, XL (GeneAmp XL PCR kit; Applied Biosystems, Foster City, CA). The temperature cycling conditions included an initial denaturation at 94°C for 1 min, followed by 30 cycles of 94°C for 30 s, annealing for 30 s at 59°C, and extension at 72°C for 30 s. Cycling was followed by a final extension at 72°C for 10 min. Samples were incubated in the PTC-225 Peltier thermal cycler (MJ Research, Waltham, MA). Each reaction mixture (5  $\mu\text{l}$ ) containing the PCR product was mixed with 2  $\mu\text{l}$  of UltraClean gel dye (Mo Bio Laboratories Inc., Carlsbad, CA) and analyzed by electrophoresis in a 1% ultrapure molecular biology grade agarose gel (USB Corporation). For these experiments, previously characterized KPC-producing *K. pneumoniae* isolates were used as controls (9, 10).

The expression level of *K. pneumoniae* carbapenemase (KPC)  $\beta$ -lactamase was determined by immunoblotting according to the following procedure. Rabbit polyclonal antibodies were raised against the KPC-2  $\beta$ -lactamase in order to measure *bla*<sub>KPC</sub> expression. The *bla*<sub>KPC-2</sub> gene was expressed from pBR322-*cat*-*bla*<sub>KPC-2</sub> in *E. coli* DH10B. The KPC-2  $\beta$ -lactamase was isolated and purified (24). Anti-KPC-2 polyclonal antibodies were raised by Sigma-Genosys (The Woodlands, TX) and isolated from serum using protein G column purification (Sigma-Genosys) (24). KPC-producing *K. pneumoniae* isolates were grown in Luria-Bertani broth to an optical density at 600 nm of 0.8. Thirty microliters of each culture was mixed with sodium dodecyl sulfate-polyacrylamide gel electrophoresis sample buffer, boiled, subjected to electrophoresis, and transferred to polyvinylidene difluoride membranes (Invitrogen, Carlsbad, CA) (14). After overnight incubation in blocking buffer (5% bovine serum albumin, 20 mM Tris HCl-buffered 150 mM saline, pH 7.5 [Sigma, St. Louis, MO]), the presence of KPC  $\beta$ -lactamase was detected by addition of 0.5  $\mu\text{g/ml}$  anti-KPC antibody and incubation for 3 h at room temperature. Membranes were then washed four times, 10 min each, with Tris-buffered saline (pH 7.5) containing 0.05% Tween and further incubated with a 1:10,000 dilution of horseradish peroxidase-conjugated protein G (Bio-Rad, Hercules, CA) in blocking buffer. After an additional four washes, the membranes were processed for film exposure using the Amersham ECL Plus Western blotting detection system (GE Healthcare, Buckinghamshire, United Kingdom) according to the protocol provided. For these experiments, 200 ng of purified KPC was used as a positive control.

Surveillance cultures for carbapenem-resistant *Enterobacteriaceae* were obtained during and following the outbreak. Rectal cultures were obtained and processed according to the CDC laboratory protocol published in 2009 (6).

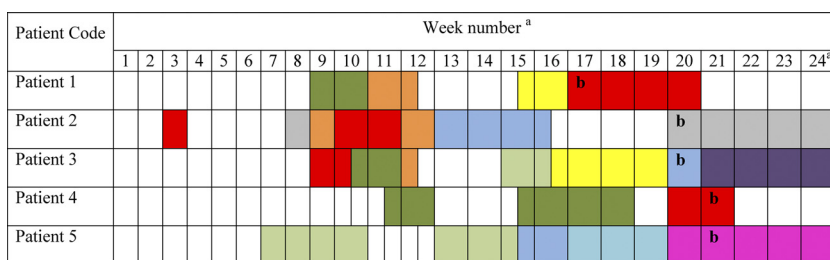
In order to assess clonality, pulsed-field gel electrophoresis (PFGE) was conducted. Bacterial DNA was prepared and cleaved with 20 U SpeI endonuclease (New England Biolabs, Boston, MA). Electrophoresis was performed in a 1% agarose gel (BMA Products, Rockland, ME) prepared and run in 0.5 $\times$  Tris-borate-EDTA buffer on a CHEF-DR III apparatus (Bio-Rad Laboratories, Richmond, CA). The initial switch time was 10 s, the final switch time was 45 s, and the run time was 23 h at 6 V/cm. Gels were then stained in ethidium bromide, destained in distilled water, and then photographed (GelDoc 2000; Bio-Rad) (16). GelCompar software was used to construct a dendrogram.

As an additional method to determine genetic relatedness among strains, repetitive extragenic palindromic PCR (Rep-PCR) using a semiautomated system (DiversiLab; bioMérieux, Marcy l'Etoile, France) was applied. Genomic DNA was extracted using the UltraClean microbial DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA). PCR amplification of highly conserved, repetitive DNA sequences occupying distinct positions in the bacterial genome was performed using the DiversiLab *Klebsiella* kit. Rep-PCR products were separated by electrophoresis using a microfluidics chip. Analysis with the Agilent 2100 Bio-analyzer (Agilent Technologies, Santa Clara, CA) yielded a profile of bands for each strain. Band patterns were compared with the modified Kullback-Leibler method in order to generate a dendrogram, using the DiversiLab software. As in previous studies, isolates with band patterns that were  $\geq 95\%$  similar were con-

TABLE 1. Clinical characteristics of patients with colistin-resistant, carbapenem-resistant *K. pneumoniae*, Detroit, MI, 27 July to 22 August 2009

Patient code	Age (yr)	Gender	Comorbid conditions	Admission source	Functional status at the time of admission	Days in hospital prior to isolation of CRE <sup>m</sup>	Anatomic site of isolation	Primary clinical infectious syndrome	Associated bacteremia	MIC (µg/ml)		LOS <sup>n</sup>	Discharge disposition
										Imipenem	Colistin		
1	86	Female	Colon cancer, HTN <sup>a</sup> , CRF, <sup>b</sup> dementia, stage IV pressure ulcers, recurrent UTI <sup>c</sup>	Nursing home	Bedridden	9	Urine	Colonization	No	>32	16	18	Alive
2	77	Female	Parkinson's disease, HTN, DM, <sup>d</sup> dementia, dyslipidemia, stage IV pressure ulcers	LTAC	Partially dependent	1	Wound	Colonization	No	>32	8	39	Dead
3	76	Male	CHF, <sup>e</sup> HTN, DM, COPD, <sup>f</sup> dementia, gastrostomy, stage IV pressure ulcers, CRF, pulmonary HTN	LTAC	Bedridden	1	Urine	UTI	No	>32	64	21	Dead
4	68	Female	ESRF, <sup>g</sup> DM, AKA, <sup>h</sup> HTN, IHD, <sup>i</sup> CHF, DVT, <sup>j</sup> CVA, <sup>k</sup> hemiplegia, eye enucleation	Nursing home	Partially dependent	11	Tissue of legs <sup>l</sup> infected wound	Deep soft-tissue infection	Yes	>32	8	14	Alive
5	76	Female	HTN, CHF, dyslipidemia, DM, neuropathy, morbid obesity, stage IV pressure ulcers, OA, <sup>m</sup> COPD, gastrostomy	Nursing home	Bedridden	33	Blood	Deep soft-tissue infection, UTI	Yes	>32	8	71	Alive

<sup>a</sup> HTN, hypertension.  
<sup>b</sup> CRF, chronic renal failure.  
<sup>c</sup> UTI, urinary tract infection.  
<sup>d</sup> DM, diabetes mellitus.  
<sup>e</sup> CHF, congestive heart failure.  
<sup>f</sup> COPD, chronic obstructive pulmonary disease.  
<sup>g</sup> ESRF, end-stage renal failure.  
<sup>h</sup> AKA, above-knee amputation.  
<sup>i</sup> IHD, ischemic heart disease.  
<sup>j</sup> DVT, deep-vein thrombosis.  
<sup>k</sup> CVA, cerebrovascular attack.  
<sup>l</sup> OA, osteoarthritis.  
<sup>m</sup> CRE, carbapenem-resistant *Enterobacteriaceae*.  
<sup>n</sup> LOS, length of hospital stay.



Legend to figure colors:

Medicine 1
Medicine 2
Medicine 3
Medicine 4
LTAC
Medicine 5
Medicine 6
Medicine 7
Surgery 1
Medicine 8

FIG. 1. Time line and transmission opportunities among patients during the outbreak. Each row represents a patient, and each column represents a week. The colors represent the different units/wards as presented in the legend at the bottom. A transmission opportunity was deemed to have occurred if two patients were in the same ward during the same time period. For example, patients 1 and 2 had a transmission opportunity during week 12 in the Medicine 2 ward. Superscript a: week 1, 29 March to 4 April 2009; week 24, 5 to 11 September 2009. Superscript b: date of clinical culture.

sidered genetically related (10). Results of Rep-PCR typing were compared with those of previously characterized KPC-producing *K. pneumoniae* isolates (9, 10).

**Statistical analysis.** All analyses were performed using SPSS software (SPSS, Chicago, IL, 2009). Fisher's exact test was used for the analysis of categorical variables, and the independent-sample *t* test or the Wilcoxon rank-sum test was used for the analysis of continuous variables. *P* values of  $\leq 0.05$  were considered significant. All *P* values were two sided.

## RESULTS

The first culture of colistin-resistant, carbapenem-resistant *K. pneumoniae* was obtained on 27 July 2009, followed by four additional cases occurring between 16 and 22 August 2009. All isolations were from clinical cultures; none were from surveillance cultures. Table 1 depicts the patients' characteristics and outcomes. In general, patients were elderly, frail individuals with multiple complicated comorbid conditions who had numerous exposures to environments and settings where nosocomial infections can occur. Three of the patients reside permanently in nursing homes (different ones), and two reside in the LTAC facility attached to hospital A. In addition to the antimicrobials displayed in Table 1, all of the colistin-resistant, carbapenem-resistant *K. pneumoniae* strains were resistant to meropenem, ceftazidime, amikacin, and ciprofloxacin, while two out of five were susceptible to tigecycline (MIC = 2  $\mu$ g/ml for both) and one was susceptible to tobramycin.

Isolates were from hospitals A and B and from one LTAC facility (Fig. 1). The index patient (patient 1, Fig. 1) was identified in hospital A on outbreak day 0 (week 17 in Fig. 1). Twenty days later (on outbreak day 20), patient 2 was identified at hospital B and patient 3 was identified at the LTAC facility. On day 25 and day 26, patients 4 and 5 were identified at hospital A. All of the patients, at some point, had stayed at hospital A, and patients 2, 3, and 5 had also stayed in the LTAC facility attached to hospital A. The mean number of opportunities for transmission between patients was  $2.3 \pm 0.5$ , and each patient had at least one opportunity for transmission

involving one of the other patients. Figure 1 represents the time line and transmission opportunities for the patients. The order in which patients are presented does not necessarily reflect the true chronological sequence in which patients actually acquired the organism but is based on temporal isolation of the organism from a clinical culture.

The PFGE and Rep-PCR analysis results are displayed in Fig. 2A and B, respectively. Both methods reveal that there are two clones (I and II). Four of the five colistin-resistant, carbapenem-resistant *K. pneumoniae* isolates belonged to a single clone designated clone II (Fig. 2A). This clone consisted of isolates 1, 3, 4, and 5 from Table 1 and Fig. 1. There was a second clone of carbapenem-resistant *K. pneumoniae* at DMC, designated clone I (Fig. 2A), which included the fifth colistin-resistant case (case 2 in Table 1 and Fig. 1), and "representative" colistin-susceptible, carbapenem-resistant *K. pneumoniae* from DMC (isolates 29 and 52, Fig. 2A). Immunoblotting revealed that there is increased expression of KPC  $\beta$ -lactamase in clone II compared to that in clone I (Fig. 3).

Once the cluster was recognized, infection control practices were enforced at all three study institutions. Patients were subjected to strict contact precautions, in single-occupancy rooms with dedicated staff, and infection control preventionists educated health care workers regarding the importance of hand hygiene and strict adherence to precautions. Infection control interventions were implemented, including active surveillance culturing (rectal) of all patients who were exposed to case patients and/or were in the same patient care unit as case patients. Overall, 112 surveillance rectal cultures were obtained and none revealed colistin-resistant, carbapenem-resistant *K. pneumoniae*. All patients admitted from LTAC facilities to a DMC facility were empirically subjected to contact precautions, and surveillance cultures for carbapenem-resistant *Enterobacteriaceae* were obtained. At the LTAC facility, infection control interventions included active surveillance of all



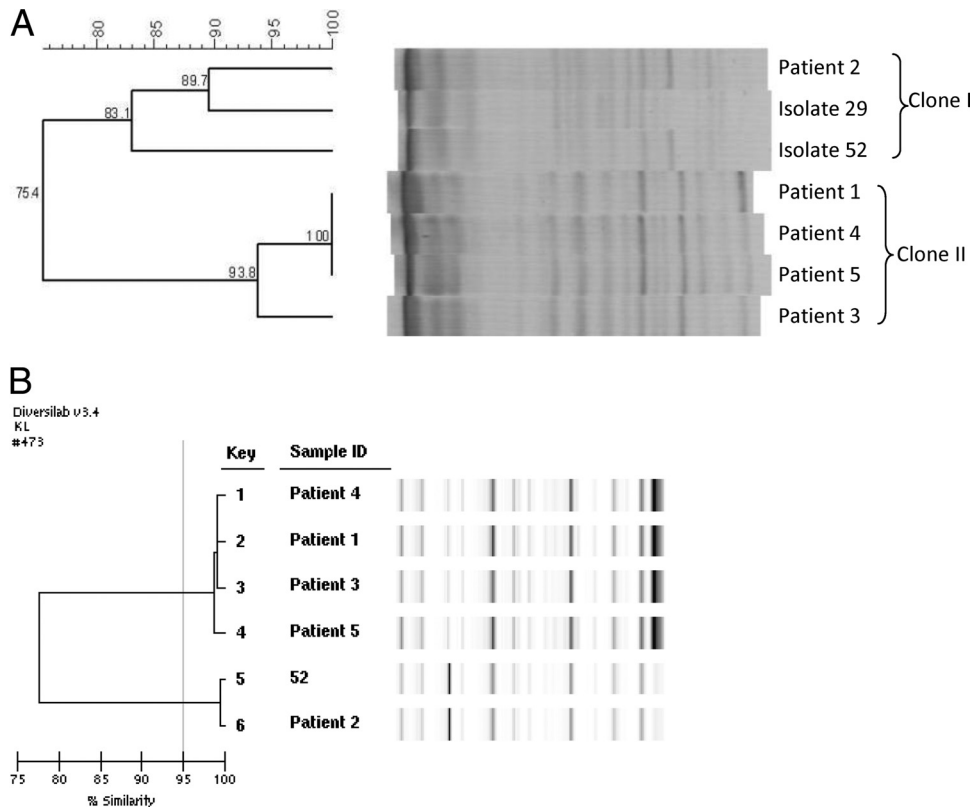


FIG. 2. Dendrograms of carbapenem-resistant *Klebsiella* species strains, DMC, 2009. (A) Dendrogram showing the relatedness of colistin-resistant KPC-producing *K. pneumoniae* based on PFGE patterns after digestion with XbaI. Isolates showing 80% similarity are considered related. (B) Dendrogram showing the relatedness of colistin-resistant isolates based on Rep-PCR. Isolates 95% similar are considered related. According to both methods, isolates from patients 1, 3, 4, and 5 are related and represent a single clone. Isolates 29 and 52 are representative colistin-susceptible, KPC-producing *K. pneumoniae* strains isolated at DMC during the outbreak period.

new admissions to the LTAC facility, cohorting of patients who were culture positive for carbapenem-resistant *Enterobacteriaceae*, and initiation of daily baths with chlorhexidine (2%) for all LTAC facility patients. Additional colistin-resistant, carbapenem-resistant *Enterobacteriaceae* cases were not reported from DMC hospitals in the subsequent months.

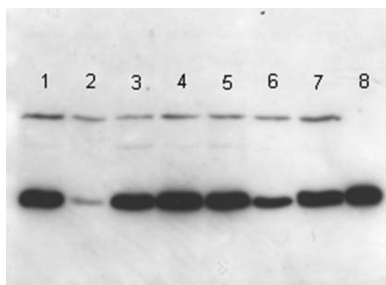


FIG. 3. Immunoblot analysis of KPC production by colistin- and carbapenem-resistant *K. pneumoniae* isolates. Lanes 1 to 5 are the colistin-resistant isolates (MIC, >32 µg/ml for patients 1 through 5). Lanes 6 and 7 are two representative strains of colistin-susceptible, carbapenem-resistant (MIC = 2 µg/ml for both) *K. pneumoniae* (isolates 29 and 52) isolated at DMC during the outbreak period. Lane 8 represents the activity of 200 ng of purified KPC beta-lactamase. Comparison of the blots gives an indication of the relative levels of KPC production.

When the 5 colistin-resistant, carbapenem-resistant *K. pneumoniae* case patients were compared to 60 patients with colistin-susceptible, carbapenem-resistant *K. pneumoniae*, the MIC to imipenem was significantly higher for case patients (a median of 64 µg/ml versus a median of 2 µg/ml;  $P < 0.001$  between groups). The mean age of case patients was greater than the mean age of patients with colistin-susceptible, carbapenem-resistant *K. pneumoniae* ( $77 \pm 6$  years and  $62 \pm 8$  years, respectively,  $P = 0.05$ ). The mortality rate and length of hospital stay were higher among case patients than among controls, though these differences did not reach statistical significance (mortality rates were 40% and 26% for case patients and controls, respectively, and the mean lengths of hospital stay were  $33 \pm 23$  days and  $30 \pm 23$  days, respectively;  $P > 0.05$  for both comparisons). All five case patients had cocolonization with a nonfermenter; three were cocolonized with *P. aeruginosa*, and two were cocolonized with *A. baumannii*. In contrast, only 23 (38%) of 60 controls were cocolonized with a nonfermenter (odds ratio, 2.44; 95% confidence interval, 1.8 to 3.3;  $P = 0.01$ ). The two cocolonizing strains of *A. baumannii* were not resistant to colistin, and the three *P. aeruginosa* isolates were not tested for colistin susceptibility. All five nonfermenters had MICs of >32 µg/ml to imipenem.

Colistin use during the 3-month period prior to the outbreak (1 April to 30 June 2009) did not differ from that in the

previous quarter (1 January to 31 March 2009) (mean DDD,  $20.7 \pm 3.4/1,000$  patient days for the entire 18 months studied). None of the five patients received colistin in the 3 months preceding their colistin-resistant, carbapenem-resistant *K. pneumoniae* isolation. Polymyxin B had rarely been used at DMC in the past 4 years.

## DISCUSSION

This report of a colistin-resistant, carbapenem-resistant *K. pneumoniae* outbreak is notable for four reasons. First, as far as we know, it is the first report from the United States that describes an outbreak involving colistin-resistant, carbapenem-resistant *K. pneumoniae*. There have been previous case reports of infections of carbapenem-resistant *K. pneumoniae* resistant to polymyxin B in New York (4, 8, 17), but these cases occurred sporadically and not as part of an outbreak. Second, three different institutions are involved. Previous reports of outbreaks from other parts of the world involved only single institutions (2, 28, 37). Third, patient-to-patient transmission seems like the major mechanism instead of emergence of resistance. Finally, the MICs to imipenem were particularly high among these isolates (all were  $>32 \mu\text{g/ml}$ ). Most carbapenem-resistant *K. pneumoniae* strains within our health system and elsewhere usually display an MIC of 1 to 4  $\mu\text{g/ml}$  to imipenem according to automated systems. Although it is unclear whether the high level of resistance present in this outbreak is associated with increased resistance to other categories of antimicrobials, this issue is plausible and troubling. Based on pharmacokinetic synergy studies, infections caused by isolates that are nonsusceptible to carbapenems (MICs ranging between 4 and 8  $\mu\text{g/ml}$ ) can be treated with higher doses of carbapenem (21, 25), but a high level of resistance might not, therefore, be a major therapeutic issue.

Colistin is the mainstay of therapy for carbapenem-resistant *Enterobacteriaceae* and is often the last available active antimicrobial agent (12). Although we were unable to demonstrate an association between colistin use and colistin resistance, other investigators have demonstrated an association between the use of colistin and the emergence of resistance in carbapenem-resistant *K. pneumoniae*, though results are conflicting (2, 17, 37). The pharmacologic characteristics of colistin are still poorly understood. CLSI has not published susceptibility breakpoints for this agent against *Enterobacteriaceae*, and pharmacokinetic characteristics are controversial and not clearly defined (13, 26). The emergence of resistance to colistin is extremely troubling, particularly since colistin resistance might be associated with poor outcomes (37). Since optimal dosing regimens have not been determined for colistin, there is a great risk that inappropriate dosing might lead to continued spread of colistin resistance among carbapenem-resistant *K. pneumoniae* strains and other pathogens (36).

Patient-to-patient transmission appeared to play an important role in this outbreak. Hospital A, where all of the patients resided during the study period, was the location where the most transmission opportunities occurred. The outbreak terminated when strict infection control practices were implemented at all three health care institutions, including active surveillance and chlorhexidine bathing of residents at the LTAC facility. LTAC facilities care for patients with severe

illnesses, admit patients from a variety of different hospitals, and are an important reservoir of resistant organisms (9, 22, 23). LTAC facilities have been implicated in outbreaks of multidrug-resistant (MDR) Gram-negative bacilli (9). Interventions to limit the emergence and spread of carbapenem-resistant *Enterobacteriaceae* in hospitals should include referring LTAC facilities.

Another intriguing characteristic of this outbreak was that all five case patients were cocolonized with *A. baumannii* or *P. aeruginosa*, both Gram-negative bacilli which are non-lactose fermenters. High-level resistance to carbapenems and colistin is more common in these pathogens than in *Enterobacteriaceae*. The mechanisms of colistin resistance are not well described in nonfermenters or *Enterobacteriaceae*. While it is possible that genetic resistance determinants might have passed from nonfermenters to the outbreak strains, *in vitro* evidence to date does not support such a mechanism of transfer (1). A stronger hypothesis is that the association between cocolonization with nonfermenters and colistin-resistant, carbapenem-resistant *K. pneumoniae* is that the patient populations colonized or infected by nonfermenters share the same risk factors as patients colonized by or infected with MDR *Enterobacteriaceae*. Unfortunately, the nonfermenter strains were unavailable for molecular analysis. It seems advisable to separate patients colonized by or infected with carbapenem-resistant *Enterobacteriaceae* from patients with Gram-negative non-lactose fermenters. Further analysis of the impact of infection control interventions on the spread of genetic resistance determinants is warranted.

Ongoing studies further elucidating the mechanisms involved in colistin resistance and high-level carbapenem resistance among carbapenem-resistant *K. pneumoniae* strains are in progress. Infection control and antimicrobial stewardship interventions geared toward preventing the dissemination of these essentially untreatable pathogens and their associated resistance determinants are needed.

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