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Detection of Plasmid-Mediated KPC-Producing *Klebsiella pneumoniae* in Ottawa, Canada: Evidence of Intrahospital Transmission[∇]

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***Klebsiella pneumoniae* isolates from three patients admitted to the Ottawa Hospital, a 1,040-bed teaching hospital, were found to contain the plasmid-borne *K. pneumoniae* carbapenemase (KPC)-producing *bla* gene (bla_{KPC}). There was evidence of person-to-person transmission for two patients. Screening of 186 clinical isolates revealed no additional bla_{KPC}-containing isolates.**

The first *Klebsiella pneumoniae* carbapenemase (KPC) was reported for an isolate collected from a North Carolina hospital through the Intensive Care Antimicrobial Resistance Epidemiology (ICARE) project (17). Since then, KPC-producing isolates have been reported across the United States, especially in New York City, as well as among other *Enterobacteriaceae* (2, 3, 6, 9, 10). These enzymes are particularly concerning because they confer resistance to all beta-lactam antibiotics and are carried on plasmids. We report the first three cases of KPC-producing *K. pneumoniae* in Canada and a retrospective surveillance for KPC-producing *K. pneumoniae*.

Case 1. An 87-year-old woman admitted to our hospital in June 2008 with a stroke had a urine culture obtained in August 2008 due to increasing confusion. The culture grew $>100 \times 10^6$ colonies/liter of a *K. pneumoniae* strain which by Vitek 2 (bioMérieux, Canada) was susceptible to trimethoprim-sulfamethoxazole (TMP-SMX), intermediate to amikacin, and resistant to other aminoglycosides, all beta-lactams (including meropenem) and beta-lactam–beta-lactamase inhibitor combinations, ciprofloxacin, and nitrofurantoin. Prior to hospital admission, she lived at home, and her only recent travel had been a brief trip to New York City 3 months previously, although there was no known contact with the health care system there. Shortly after admission to our hospital she was treated with ciprofloxacin for an *Escherichia coli* urinary tract infection (UTI) and then metronidazole for *Clostridium difficile*-associated diarrhea. She did not have an indwelling urinary catheter and was not admitted to the intensive care unit. For the *K. pneumoniae* UTI, she was placed on contact precautions and treated with oral TMP-SMX. Repeat urine culture following treatment showed no growth.

Case 2. An 87-year-old man was admitted to our hospital with advanced Alzheimer's disease in October 2007. Due to benign prostatic hypertrophy, he had a chronic indwelling urinary catheter which was changed monthly. He also received multiple courses of antibiotics (including ciprofloxacin, TMP-SMX, amoxicillin, and cephalexin) for recurrent UTIs, although he had not previously grown *K. pneumoniae*. In October 2008, a urine culture obtained during catheter change grew $>100 \times 10^6$ colonies/liter each of *K. pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa.* By Vitek 2, his *K. pneumoniae* isolate was susceptible to amikacin and resistant to other aminoglycosides, all beta-lactams (including meropenem) and beta-lactam–beta-lactamase inhibitor combinations, ciprofloxacin, nitrofurantoin, and TMP-SMX. He was in a room adjacent to case 1 for several days prior to identification of that patient's KPC producer. He was treated with oral ciprofloxacin, and a repeat catheter urine culture continued to grow *K. pneumoniae* (with the same susceptibility profile) and *S. aureus*.

Case 3. In December 2008, a 67-year-old woman with a history of primary sclerosing cholangitis was admitted to a hospital in Florida while on vacation with increasing right-upper-quadrant pain and obstructive jaundice requiring placement of a biliary stent. Upon transfer to our institution, she developed fever (38.5°C), tachycardia, and worsening of her right upper-quadrant pain and was treated with ciprofloxacin and metronidazole intravenously. Blood cultures grew *K. pneumoniae* (isolate 3) which by Vitek 2 was intermediate to gentamicin, susceptible to meropenem, and resistant to other aminoglycosides, all other beta-lactams and beta-lactam–beta-lactamase inhibitor combinations, ciprofloxacin, and TMP-SMX. Repeat blood cultures again grew *K. pneumoniae*, and antibiotics were changed to meropenem. The patient defervesced, her tachycardia resolved, and repeat blood cultures after 2 days of meropenem were negative. The biliary stent was replaced with a percutaneous drain; she received an additional month of meropenem and tigecycline and was discharged home without antibiotics. However, she was readmitted 3 days later with a fever and relapse of her bacteremia. The blood culture isolate of *K. pneumoniae* was now resistant to meropenem and tigecycline but susceptible to colistin (MIC = 1 mg/liter). She was treated with colistimethate, and subsequent blood cultures became negative. Since then, the KPC isolate has been persistently recovered from the biliary drainage fluid and has become resistant to all antibiotics, including colistin.

A total of 186 recent (2008) clinical isolates from urine (125

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TABLE 1. Clinical and microbiological characteristics of the KPC-producing *K. pneumoniae*

Patient isolate	Age (yr)	Underlying illness	Source of isolate	Possible origin of the isolate	bla_{KPC} type	Susceptibility[b] (MIC, mg/liter) to: Meropenem	Ertapenem	Tigecycline	Colistin
1	87	Stroke	Urine	NYC[a]	3	>128	>128	0.5	0.125
2	87	Alzheimer's disease	Urine	Patient 1	3	>128	>128	0.5	0.25
3	67	Primary sclerosing cholangitis	Blood	Florida	3	2	>128	2	0.5

[a] Although the patient did travel to New York City (NYC), there was no known contact with a health care facility.
[b] MICs of isolates were determined by the Etest.

isolates), wounds (14 isolates), blood (9 isolates), and other sources (38 isolates) were tested for production of KPC. Isolates were tested for susceptibility to meropenem and ertapenem using Etest (AB Biodisk, Piscataway, NJ) according to the manufacturer's recommendations and incubated for 18 h at 35°C in ambient air. MICs were interpreted according to Clinical Laboratory Standards Institute (CLSI) guidelines (5). Isolates were also tested using the modified Hodge test and for the KPC-producing *bla* (bla_{KPC}) gene, as previously described (8, 16). An amplified product of the expected size was confirmed as KPC by sequencing. For typing of the bla_{KPC} gene, overlapping PCRs were performed using the following primer pairs: for amino acids 89 to 399, the 5′-CGGAACCATTCGC TAAACTC-3′ (forward) and 3′-GGCGGCGTTATCACTGT ATT-5′ (reverse) primers, and for amino acids 372 to 874, the 5′-CGCCGTGCAATACAGTGATA-3′ (forward) and 3′-CG TTGACGCCCAATCC-5′ (reverse) primers. Amplification products were purified using the QIAquick gel extraction kit (Qiagen, Valencia, CA), and sequencing was performed by the Ottawa Genome Center DNA Sequencing Institute (Ottawa, Canada). Sequences were aligned and compared using the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/). The relatedness of the two clinical isolates from patients 1 and 2 was determined by pulsed-field gel electrophoresis after digestion of genomic DNA with XbaI as previously described (14, 15).

Both isolates 1 and 2 were multidrug resistant, including to meropenem (MIC $\geq$ 128 mg/liter), and carried the type 3 bla_{KPC} gene (Table 1). Comparison of both isolates by pulsed-field gel electrophoresis revealed identical profiles, suggesting person-to-person transmission. Isolate 3 was found to be multidrug resistant but initially susceptible to meropenem. Screening with ertapenem indicated the possibility of a KPC, which was confirmed by the modified Hodge test and amplification of the bla_{KPC} gene, which typed as KPC-3 (Table 1). Although the patient with isolate 3 improved clinically with meropenem, she soon relapsed with a fully resistant isolate, suggesting that meropenem may not be relied upon in this setting.

Retrospective surveillance of recent clinical *K. pneumoniae* isolates failed to identify KPC producers other than the three reported here. The meropenem and ertapenem MICs for all isolates tested were <0.032 mg/liter, and none were positive by the modified Hodge test or found to carry the bla_{KPC} gene (data not shown).

Several surveillance studies have demonstrated the difficulties of identifying KPC-producing strains (2, 3, 4, 9). As with detection of extended-spectrum beta-lactamases and other broad-spectrum serine beta-lactamases, detection of carbapenemase-producing organisms can be complicated by false susceptibility to the carbapenems on routine testing (11). Two of our isolates were found to have elevated MICs to meropenem and therefore were not missed on routine susceptibility testing. Recently, ertapenem nonsusceptibility was found to have a low positive predictive value for identification of the bla_{KPC} phenotype in a study of *Enterobacteriaceae* clinical isolates (10). Although we did not test other *Enterobacteriaceae*, we did not find non-ertapenem-susceptible isolates other than those confirmed as KPC producers. However, isolate 3 was susceptible to meropenem (MIC = 2 mg/liter) but resistant to ertapenem, supporting the need to include ertapenem as a screening marker for KPC production (1, 4, 9). Based on these results, our laboratory now screens *Enterobacteriaceae* for KPC production by determining reduced susceptibility to ertapenem and by confirmatory testing using the modified Hodge test (5).

Previous national surveillance data from intensive care units across Canada revealed no in vitro resistance to meropenem among *K. pneumoniae* isolates, with the highest identified MIC being 0.25 mg/liter (18). To our knowledge, these are the first published cases of KPC-producing *K. pneumoniae* infections in Canada, including evidence of intrahospital transmission. Receiving medical care in the United States was a risk factor for case 3. Although the evidence that case 1 acquired her resistant organism while she visited the United States is uncertain, she did have several other risk factors for acquisition, including prior receipt of a fluoroquinolone and poor functional status, as did case 2 (7, 13). The dissemination of KPC-producing organisms is no longer restricted to the United States and has emerged as a global concern, aided by the recently described mobile genetic element Tn*4401*, which carries the KPC genes (12). Regardless of geographic location, microbiology laboratories in Canada as well as those outside the United States need to ensure that they have methods in place for the accurate detection of KPC-producing organisms and be aware of the potential for their spread.

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