A pilot survey of carbapenem-resistant
*Escherichia coli* and *Klebsiella pneumoniae*
mediated by *K pneumoniae* serine carbanpenemases in a regional referral hospital
in British Columbia

To the Editor:
The first Canadian report of a *Klebsiella pneumoniae*-producing Enterobacteriaceae in a *K pneumoniae* carbapenemase (*KPC*) was described in 2009 by Pillai et al (1). Hospitals in British Columbia (BC) have yet to document increases in carbapenem treatment failures or in vitro resistance; however, the true prevalence is unknown. To better understand the current situation in BC, we undertook a limited pilot study of patient isolates to evaluate the prevalence of carbapenem resistance in a hospital patient population of Kamloops and the surrounding areas.

A six-month pilot study of *Escherichia coli* and *Klebsiella* species was performed as a preliminary evaluation of the prevalence of *KPC*-encoded resistance in these commonly isolated bacteria. The study was conducted on patient isolates from Royal Inland Hospital (RIH) – a 250-bed regional referral hospital located in Kamloops, BC. A total of 154 nonduplicate *E coli* and *K pneumoniae* isolates (144 *E coli* and 10 *K pneumoniae* isolates) were collected over a period of six months (September 2009 to February 2010). Initial identification was performed at RIH using a Vitek 2 analyzer (bioMérieux Inc, USA) with the microdilution technology. Using the Clinical and Laboratory Standards Institute (CLSI) standardized disk-diffusion method (2), each study isolate was tested for phenotypic resistance to ertapenem, meropenem and noncarbapenem antibiotics: cefazolin, cefoperazone, ceftriaxone, ceftazidime, amoxicillin-clavulanic acid, gentamicin, trimethoprim-sulfamethoxazole and ciprofloxacin (Figure 1, Table 1). The isolates included in the study were resistant to at least one antibiotic; most were resistant to multiple antibiotics. Additionally, each of the isolates was investigated for *bla*-KPC genes using conventional molecular methods described by Cole et al (3). Universal primers were used to detect *bla*-KPC genes. We used *bla*-KPC-positive controls (*K pneumoniae* strains BAA ATCC 1705) that were generously provided by Dr JD Pitout of Calgary Laboratory Services (Calgary, Alberta), and Dr M Desjardines of The Ottawa Hospital (Ottawa, Ontario). As for *bla*-KPC-negative control bacteria, we used *K pneumoniae* BAA ATCC 1706 and *E coli* ATCC 25922, while the reagent control was reagent water plus master mix (primers, Taq polymerase and buffers).

Using the disk-diffusion susceptibility method, it was noticed that all patient isolates exhibited a susceptible phenotype for meropenem and ertapenem. The *KPC*-universal primer set, validated by Cole et al (3), was confirmed to amplify *K pneumoniae* *bla*-KPC-positive controls. Each positive control amplicon aligned with the 399 bp location on the ladder marker (Figures 2, 3 and 4). The Cole et al (3) universal primer set did not produce amplicon product for any *E coli* or *K pneumoniae* patient isolate (ie, molecular amplification testing for the most common genetic determinants of transmissible carbapenem resistance, *bla*-KPC, was negative for all isolates in our RIH study).

This sample size was too small to exclude the presence of *KPC*-mediated carbapenemase resistance, particularly in *K pneumoniae*. However, using molecular amplification methods, the fact that 144 resistant *E coli* isolates were found to be negative for *bla*-KPC genes was reassuring. Consequently, at RIH, a routine confirmatory test for *KPC*-mediated resistance is probably not justified at this time; however, based on this preliminary study, multiresistant Enterobacteriaceae isolates that demonstrate reduced phenotypic susceptibility to ertapenem and other carbapenems could be tested using the modified Hodge method as per CLSI recommendations, and/or through molecular methods that amplify *bla*-KPC genes.

Carbapenem resistance has emerged as a significant concern in an era of limited antibiotic options and increasing resistance; this antibiotic class is a therapeutic ‘last resort’ in the treatment of serious infections caused by multiresistant, Gram-negative bacteria (4-6). While a resistant phenotype is not always due to KPCs, it is these isoenzymes that have been associated with large community outbreaks. Initially described in New York City and other northeastern

![Figure 1](https://example.com/figure1.png)

**Figure 1** The number of resistance patterns observed in isolates of *Escherichia coli* and *Klebsiella pneumoniae* collected at Royal Inland Hospital in Kamloops, British Columbia (n=154). AMC Amoxicillin-clavulanic acid; CAZ Cefazidime; CFP Cefoperazone; CIP Ciprofloxacin; CTX Cefotaxime; CZ Cefazolin; ETP Ertapenem; GM Gentamicin; MEM Meropenem; SXT Trimethoprim-sulfamethoxazole

**Table 1**

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<th>Organisms used as positive and negative controls in the study</th>
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<td><strong>K pneumoniae</strong></td>
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<td>BAA 1705 (positive control for KPC)</td>
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<td><strong>Anti-microbial agents</strong></td>
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*Interpretation according to the Clinical and Laboratory Standards Institute (CLSI) criteria published in January 2009 (volume 29, Issue 3). AMC Amoxicillin-clavulanic acid; CAZ Cefazidime; CFP Cefoperazone; CIP Ciprofloxacin; CTX Cefotaxime; CZ Cefazolin; E coli Escherichia coli; ETP Ertapenem; GM Gentamicin; MEM Meropenem; SXT Trimethoprim-sulfamethoxazole*

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American jurisdictions (7,8), clonal spread has been subsequently reported in studies from Israel, Europe, the United Kingdom and South America (9-14). KPCs are currently the most common cause of carbapenem resistance worldwide. Initially believed to be a problem limited to *K* pneumoniae, KPCs are known to occur in other enteric bacteria including common clinical isolates such as *E coli*, *Enterobacter cloacae* and *Proteus mirabilis*. Dissemination has thus followed the familiar pattern of extended-spectrum beta-lactamases (ESBLs). Both ESBLs and KPCs are usually encoded on mobile conjugative plasmids, and associated with resistance to other antibiotics, notably quinolones, trimethoprim-sulfamethoxazole and, occasionally, aminoglycosides (15). Encoding plasmids are known to be sequestered in the uncharacterized reservoir of commensal bacteria, thereby limiting effective containment through traditional infection control isolation practices (7). While penicillins, cephalosporins and monobactams, to varying degrees, are all susceptible to ESBL isoenzyme hydrolysis, KPCs also hydrolyze carbapenems, although full resistance typically requires a coexisting porin defect and/or impaired membrane permeability (16-18).

The present study was undertaken as a hospital epidemiology investigation. The objective was to analyze a collection of *E coli* and *K pneumoniae* patient isolates to evaluate the prevalence of carbapenem resistance in rural BC. Although these bacteria represent a limited selection of resistant species recovered from RIH patients, none of the 154 isolates investigated were found to have phenotypic resistance to ertapenem or meropenem, and none harboured *bla*KPC genes. This, to a certain degree, can be interpreted to indicate that the Vitek 2 and CLSI's susceptibility testing can

**Figure 2** Gel electrophoresis polymerase chain reaction products. Lane 1: Polymerase chain reaction reactants plus water, no DNA present; lane 2: 15 colonies of sample organisms; lane 3: One colony of *Klebsiella pneumoniae* ATCC BAA 1705 (K pneumoniae carbapenemase [KPC]-1-positive control [currently KPC-2, according to Yigit et al (19)]; lane 4: 15 colonies of known negative *K pneumoniae* ATCC BAA 1705 and one KPC-positive colony; lane 5: 1 Kb marker. Bands in lanes 3 and 4 represent amplified 399 bp fragments of *bla*KPC-1 from the KPC-1-positive control organism ATCC BAA 1705.

**Figure 3** Gel electrophoresis polymerase chain reaction products. Lane 1: 1 Kb marker; lane 2: One colony of *Klebsiella pneumoniae* ATCC BAA 1705 (K pneumoniae carbapenemase [KPC]-1-positive control [currently KPC-2, according to Yigit et al (19)]; lane 3: One colony of KPC-3-positive control organisms; lane 4: Negative control lane, polymerase chain reaction reactants plus water, no DNA present; lane 5: 1 Kb marker. Bands in lanes 2 and 3 represent amplified 399 bp fragments of KPC genes.

**Figure 4** Mass screening of 154 patient isolates. Lane 1: *K pneumoniae* ATCC BAA 1705 (K pneumoniae carbapenemase [KPC]-1-positive control [currently KPC-2, according to Yigit et al (19)]; lane 2: K pneumoniae KPC-2 positive; lane 3 to 14: Polymerase chain reaction products of mass preliminary screening (15 different patient isolates per lane); lane 15: Negative control. Bands observed in lanes 1 and 2 are expected 399 bp fragments from the *bla*KPC-1-positive and *bla*KPC-2-positive organisms.
still be used to screen for KPC phenotypic resistance in a small regional hospital setting. CLSI recommendations should continue to be followed: all Enterobacteriaceae isolates demonstrating reduced susceptibility to ertapenem and other carbapenems should be further evaluated using the modified Hodge test. A negative result confirms that an isolate is probably bla-KPC negative. Positive isolates should not be reported as carbapenem resistant unless confirmed by a validated molecular method that amplifies bla-KPC genes [blaKPC-1/2 and blaKPC-3 genes].

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REFERENCES
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