Outbreak Caused by an Ertapenem-Resistant, CTX-M-15-Producing
Klebsiella pneumoniae Sequence Type 101 Clone Carrying an OmpK36
Porin Variant

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Although numerous studies have documented outbreaks of carbapenem-resistant Klebsiella pneumoniae (CRKP) possessing various carbapenemases, reports on outbreaks due to CRKP possessing extended-spectrum β-lactamases (ESBLs) and/or AmpCs with porin lesions have been limited. Here, we describe an outbreak caused by an ertapenem-resistant, CTX-M-15-producing clonal K. pneumoniae strain expressing an OmpK36 porin variant. From May 2012 to November 2012, 37 ertapenem-resistant K. pneumoniae isolates phenotypically negative for carbapenemase production were recovered from 19 patients hospitalized in the intensive care unit of a Greek hospital. The isolates were either susceptible or intermediate to other carbapenems and resistant to all remaining β-lactams but cefotetan. Phenotypic and molecular analysis revealed the presence in all isolates of the blaCTX-M-15 gene on a conjugative 100-kb plasmid, disruption in the expression of the ompK35 gene, and the production of an Ompk36 porin variant. The index case was a patient admitted from another hospital. Active surveillance upon admission and on a weekly basis was immediately initiated; environmental samples were also periodically tested. Molecular typing showed that all clinical isolates as well as two ertapenem-resistant environmental K. pneumoniae isolates belonged to the same clonal type and were assigned to multilocus sequence typing (MLST) sequence type 101 (ST101). As all colonized/infected patients were hospitalized during overlapping periods, cross-infection was considered the main route for the dissemination of the outbreak strain. Despite reinforcement of infection control measures and active surveillance, the outbreak lasted approximately 7 months. Identification of hidden carriers upon admission and by screening on a weekly basis was found valuable for early recognition and subsequent successful management of the outbreak.

Klebsiella pneumoniae is currently one of the most challenging pathogens worldwide and a leading cause of infections, especially of the respiratory system in hospitalized patients. Carbapenems have constituted the antibiotic treatment of choice in multiresistant isolates; however, excess use over the past years has resulted in an increase in outbreaks due to carbapenem-resistant K. pneumoniae (CRKP) strains (1). This restricted susceptibility profile is commonly attributed to the production of carbapenem-hydrolyzing enzymes of class A (KPCs), class B (metallo-β-lactamases [MBLs]), and class D (OXA) β-lactamases, and several studies have documented large dissemination or outbreaks due to carbapenemase-producing Klebsiella pneumoniae strains (1–4). Nevertheless, outer membrane porin loss has also been highlighted as yet another likely cause of carbapenem resistance and has been associated with the simultaneous production of an extended-spectrum β-lactamase (ESBL) or plasmid-mediated AmpC (5, 6).

The outer membrane proteins serve as protein channels that regulate the exchange of small hydrophilic molecules, such as iron, nutrients, and antibiotics. They also have a significant structural role in maintaining cellular integrity (7). The main mechanism responsible for porin deficiency is the direct interruption by insertion sequences of the ompK35 or ompK36 gene. Less frequently, point mutations may cause protein structure changes or deletions which interfere with porin expression (8). Regardless of the mechanism implicated, however, porin deficiency results in low ertapenem concentrations in the periplasmic space, which further facilitate even the activity of enzymes with weak carbapenem properties (9). Pressure applied by treatment with carbapenems is considered to contribute to the selection of porin-deficient mutants among susceptible ESBL- or AmpC-producing populations (10–12). However, the loss of only one of the involved porins is not commonly linked to a significant increase in carbapenem MICs, in contrast to simultaneous depletion of both porins, which is associated with higher levels of resistance (13, 14).

In the past there have been mainly sporadic case reports of carbapenem-resistant K. pneumoniae isolates with porin defects and simultaneous production of an ESBL or AmpC type enzyme (9, 15–20). However, reports presenting outbreaks or large dissemination of such strains are limited (14, 15). In the present study, we investigated an outbreak caused by an ertapenem-resistant, CTX-M-15-producing K. pneumoniae porin-deficient strain in the intensive care unit (ICU) of a Greek hospital. This outbreak raises awareness of the possibility that carbapenem resistance may derive from and be spread by a mechanism other than carbapenemase production, which to date has been prevalent in this region (4, 21, 22).
MATERIALS AND METHODS

Bacterial isolates and patients. Following the initial detection in May 2012 of a K. pneumoniae isolate phenotypically negative for carbapenemase production but exhibiting resistance to third-generation cephalosporins (ceftazidime and cefotaxime) and ertapenem (MIC, 4 µg/ml), a prospective study was performed to identify other such isolates from both clinical and active surveillance samples collected from patients hospitalized in the intensive care unit (ICU) of the Serres General Hospital, Greece.

This institution serves as an acute care facility of approximately 410 beds, serving a population of more than 200,000 inhabitants. It has a combined medical and surgical open ICU, comprising 6 beds, and its structure allows the isolation of at least four ICU patients. Patient demographics along with patient medical records were retrieved and evaluated. This investigation was approved by the hospital internal review board. Nosocomial infections were defined by standard Centers for Disease Control and Prevention definitions (23).

**Bacterial identification and susceptibility testing.** Species identification and initial antibiotic susceptibility testing of the recovered isolates were performed using the MicroScan WalkAway system (Siemens Healthcare Diagnostics, West Sacramento, CA). In addition, MICs of selected antibiotics were evaluated by the agar dilution method and interpreted according to the updated CLSI criteria (24).

**Phenotypic testing.** Preliminary phenotypic testing for the production of a carbapenem-hydrolyzing enzyme was conducted with the modified Hodge test (MHT) using ertapenem and meropenem disks as the substrates according to CLSI guidelines (24). The MBL Etest (bioMérieux, Marcy l’Etoile, France) and the combined-disk tests using meropenem and ertapenem with and without phenylboronic acid (PBA), EDTA, or both were used to screen for class A and B carbapenemases (25). Possible coproduction of an ESBL was tested using a modified CLSI ESBL combined-disk test (26). The production of an AmpC β-lactamase was evaluated using a combined-disk test with cefotetan as a substrate with and without the addition of PBA as an inhibitor (27) as well as the AmpC Etest strips (bioMérieux).

**PCR assays and nucleotide sequencing.** Isolates were screened for β-lactamase genes by PCR amplification using a panel of primers for the detection of MBLs, KPCs, OXA-48, and ESBLs, including the SHV, TEM, CTX-M, and IBC/GES enzymes and plasmid-mediated AmpC genes in single PCRs for each gene (22, 28). The structural genes of ompK35 and ompK36 were also amplified specifically in order to define the DNA sequences of the omp genes (18, 29). PCR products which were subjected to direct sequencing were purified using ExoSAP-IT reagent (USB Corporation, Cleveland, OH) and both strands were used as the templates for sequencing using with an ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, CA). Strains used for the comparison of ompK35 and ompK36 porin gene sequences were K. pneumoniae AJ011501 and FJ577673, respectively.

**Bacteriological studies for infection control purposes.** Following the index case, surveillance was implemented in the form of rectal swabs obtained upon patient admission and on a weekly basis. The samples were collected using premoistened swabs and plated onto two MacConkey agar plates supplemented with 1 µg/ml meropenem or 4 µg/ml ceftazidime. All Gram-negative colonies growing after 24 h of incubation at 37°C were identified, and K. pneumoniae isolates were phenotypically tested for production of carbapenemase, ESBL, and plasmid-mediated AmpC. Phenotypic testing results were further verified by molecular techniques. Additionally, at least one bronchial aspirate sample was obtained from each patient during hospitalization.

Over the study period, environmental samples were also taken from inanimate surfaces, personalized medical devices, and wet surroundings. Staff carriage status was investigated via surveillance cultures from pharyngeal and hand samples as described previously (30).

**Molecular typing.** In order to determine the clonal relationships of the K. pneumoniae isolates, pulse-field gel electrophoresis (PFGE) of the XbaI-digested genomic DNA was performed with a CHEF-DR III system (Bio-Rad, Hemel Hempstead, United Kingdom), with a running time of 21 h and pulse times ranging from 3 to 40 s. The PFGE patterns were compared visually following previously described criteria (31). Multilocus sequence typing (MLST) was used to assess the relatedness of the K. pneumoniae isolates (www.pasteur.fr/recherche/genopole/PF8/mlst) with sequence types (STs) assigned using online database tools.

**Conjugation experiments and plasmid analysis.** The potential for conjugal transfer of carbapenem or third-generation cephalosporin resistance was examined in biparental matings using LB broth cultures of three representative isolates and Escherichia coli strain 26R793 (Lac⁺, Rifr) as the recipient strain. Donor and recipient cells were mixed in a ratio of 1:5, and transconjugant clones were screened on MacConkey agar plates containing rifampin (100 µg/ml) and amoxicillin (100 µg/ml) or ertapenem (0.5 µg/ml). All β-lactamase genes were sought by PCR amplification. Plasmid extraction was performed by using an alkaline lysis protocol and Escherichia coli 39B861 as a control strain.

RESULTS

**Clinical isolates and antimicrobial susceptibility.** During the 7-month study period in question (May to November 2012), a total of 37 ertapenem-resistant (MIC, >1 µg/ml) K. pneumoniae isolates that were phenotypically negative for carbapenemase production were retrieved from clinical and rectal surveillance samples derived from 19 patients hospitalized in the ICU of Serres General Hospital. Twenty of the 37 isolates were recovered from rectal swabs, 8 from bronchial aspirates, 5 from blood cultures, and 4 from intravenous catheters. In addition, during the study period 5 KPC- and 8 KPC- and SHV-12-producing K. pneumoniae isolates were additionally recovered from clinical or rectal surveillance samples of 7 patients.

**Antibiotic susceptibility testing verified that all 37 isolates of the study exhibited resistance to ertapenem (MICs, 2 to 8 µg/ml). They were also susceptible or intermediate to meropenem (MICs ranging from 0.5 to 2 µg/ml), but all of them remained susceptible to imipenem (MICs ranging from 0.25 to 1 µg/ml). All isolates were highly resistant to ceftazidime (MICs, 128 to >256 µg/ml), cefoxitin (MICs, 64 to 256 µg/ml), ceftazidime (MICs, 32 to 256 µg/ml), cefepime (MICs, 64 to 256 µg/ml), piperacillin-tazobactam (MICs, 128 to 256 µg/ml), amoxicillin-clavulanate (MICs, 32 to 64 µg/ml), and aztreonam (MICs, 64 to 256 µg/ml), while remaining susceptible to cefotetan (MIC, 4 to 16 µg/ml). Furthermore, all isolates exhibited resistance to gentamicin, ciprofloxacin, and cotrimoxazole and susceptibility to amikacin, tigecycline, and colistin.

**Clinical and epidemiological characteristics.** The index ertapenem-resistant K. pneumoniae isolate was retrieved in May 2012 from a bronchial aspirate sample of a 39-year-old patient hospitalized due to a stroke and lower respiratory tract infection. The patient suffered from diabetes mellitus, pulmonary hypertension, and malignant obesity. The index K. pneumoniae was identified on day 4 of hospitalization following his transfer from another tertiary care facility, where the patient had been subjected to tracheostomy. During the ICU outbreak, 60 patients were admitted to the ICU (mean ICU stay, 8.9 days); of these patients, 19 (31.7%) were colonized and/or infected by ertapenem-resistant phenotypically carbapenemase-negative isolates. Patient age ranged from 18 to 87 years (mean age, 63.6 years); 12 were males (63.2%). It is of note that all colonized/infected patients were hospitalized during overlapping periods (Fig. 1). More specifically, 16 patients (84.2%) were colonized, while 7 (36.8%) exhibited infections attributable to the isolates under investigation. Four of the
latter patients were colonized prior to developing infection, while carriage was not verified in 3 patients who developed infection (Table 1). It is of note that none of the patients was determined to be colonized on admission. Rectal swabs during weekly surveillance revealed 15 colonized patients, while the resistant strain was recovered from the bronchial aspirate of 7 patients. Figure 2 shows the distribution of new cases of colonization and/or infection on a monthly basis during the study period. The mean time for colonization was 10.7 days, while the mean time until the development of infection was 15.1 days. All infected patients were treated with meropenem alone (2 g every 8 h) or in combination with colistin intravenously (3 million units every 8 h). In three of these patients, death was attributed to the infection caused by the ertapenem-resistant pathogen (Table 1).

**Phenotypic and molecular testing.** Phenotypic testing using the MHT with meropenem and ertapenem disks was negative for carbapenemase production in all 37 isolates in the study. Also, subsequent MBL Etesting and combined-disk tests gave negative results for the production of both class A and class B carbapenemases as well as the production of plasmid-mediated AmpC β-lactamases. All isolates tested positive with the modified CLSI ESBL combined-disk test.

PCR amplification and sequencing verified the presence of the *bla*<sub>CTX-M-15</sub> ESBL gene in all *K. pneumoniae* isolates. Other expanded-spectrum β-lactamase (carbapenemase, ESBL, or AmpC) genes were not detected in any isolate. Sequencing analysis of the *ompK35* gene revealed a deletion of a G at codon 61 leading to a premature stop codon (TGA) at amino acid position 63. This nonsense mutation resulted in disruption of the porin-coding sequence (13).

Sequencing of the *ompK36* gene indicated an OmpK36 protein variant. Two major lesions were identified. Insertions of 6 nucleotides (5'-GGCGAC-3') encoding Gly and Asp were detected at amino acid positions 136 and 137, respectively. This modification was apparently due to a duplication of the adjacent region in the L3 loop, resulting in the generation of Gly (134)-Asp (135)-Gly (136)-Asp (137), similarly to previous observations (15). Additionally, a 9-nucleotide deletion was detected encoding Leu (184), Ser (185), and Pro (186) in the wild strain (20). These deletions involved amino acids located at L4, a loop facing the cell exterior (32).

**Environmental sampling.** During the evolution of the outbreak, a total of 119 environmental samples from inanimate surfaces, personalized medical devices, and wet surroundings (sinks and baths) were collected. Ertapenem-resistant, phenotypically carbapenemase-negative *K. pneumoniae* isolates were recovered from two beds on different checks. These isolates harbored the *bla*<sub>CTX-M-15</sub> gene, produced the OmpK36-porin variant detected in the clinical isolates, and showed a disruption in the expression of the *ompK35* gene.

During the study period, medical and nonmedical staff members were also evaluated for likely pharyngeal and hand carriage of the implicated *K. pneumoniae*, but none tested positive.

**PFGE and MLST typing, plasmid analysis, and conjugation experiments.** PFGE analysis was performed in 19 patient single isolates and the two environmental ertapenem-resistant isolates. PFGE clustered the *K. pneumoniae* isolates from both clinical and environmental samples into a single clonal type, which contained three subtypes (Ia, Ib, and Ic) with 17, 3, and 1 isolates, respectively. The two environmental isolates belonged to the major subtype (Fig. 3). MLST assigned representative isolates from the three subtypes to a single sequence type, sequence type 101 (ST101). It should be noted that the outbreak clonal type differed from the concurrent circulating clonal types of KPC- and KPC/SHV-12-producing *K. pneumoniae* isolates (data not shown).

Plasmid profiling revealed that all isolates harbored one large
TABLE 1  Characteristics of patients infected and/or colonized with the ertapenem-resistant, CTX-M-15-producing, and porin-deficient clonal K. pneumoniae isolates

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yr)/gender</th>
<th>Admission date</th>
<th>Days in the unit prior to colonization</th>
<th>Days in the unit prior to infection</th>
<th>Source of carriage</th>
<th>Type of infection</th>
<th>Reason for ICU admission</th>
<th>Underlying disease(s)</th>
<th>Antibiotic regimen prior to infection</th>
<th>Antibiotic regimen for infection</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>69/M</td>
<td>4/27/12</td>
<td>27</td>
<td></td>
<td>Bacteremia</td>
<td>Respiratory failure</td>
<td>Diabetes mellitus/tetraplegia</td>
<td></td>
<td>FEP MEM, CST</td>
<td>Death</td>
<td>11/1/12</td>
</tr>
<tr>
<td>2</td>
<td>80/F</td>
<td>5/6/12</td>
<td>23</td>
<td></td>
<td>Fecal</td>
<td>Stroke</td>
<td>TZP</td>
<td></td>
<td>TZP, FEP</td>
<td>Death</td>
<td>11/1/12</td>
</tr>
<tr>
<td>3</td>
<td>75/F</td>
<td>5/15/12</td>
<td>14</td>
<td></td>
<td>Fecal, bronchial</td>
<td>Stroke/respiratory failure</td>
<td></td>
<td></td>
<td>TZP</td>
<td>Death</td>
<td>11/1/12</td>
</tr>
<tr>
<td>4</td>
<td>75/F</td>
<td>5/16/12</td>
<td>13</td>
<td></td>
<td>Fecal</td>
<td>Stroke/septic shock</td>
<td>Hypothyroidism</td>
<td></td>
<td>VAN</td>
<td>Death</td>
<td>11/1/12</td>
</tr>
<tr>
<td>5</td>
<td>39/M</td>
<td>5/18/12</td>
<td>4</td>
<td></td>
<td>Pneumonia</td>
<td>Stroke/pneumonia</td>
<td>Pulmonary fibrosis/diabetes mellitus/morbid obesity</td>
<td></td>
<td>TZP, MEM, CST</td>
<td>Discharged</td>
<td>11/1/12</td>
</tr>
<tr>
<td>6</td>
<td>77/M</td>
<td>6/1/12</td>
<td>10</td>
<td></td>
<td>Fecal</td>
<td>Polytrauma</td>
<td>Chronic lymphocytic leukemia</td>
<td></td>
<td>TZP, FEP</td>
<td>Discharged</td>
<td>11/1/12</td>
</tr>
<tr>
<td>7</td>
<td>57/M</td>
<td>6/6/12</td>
<td>6</td>
<td>19</td>
<td>Fecal</td>
<td>Bacteremia</td>
<td>Stroke/Diabetes mellitus/alcoholic cirrhosis</td>
<td></td>
<td>TZP, MEM</td>
<td>Discharged</td>
<td>11/1/12</td>
</tr>
<tr>
<td>8</td>
<td>76/M</td>
<td>6/7/12</td>
<td>5</td>
<td></td>
<td>Fecal</td>
<td>Stroke</td>
<td>FEP</td>
<td></td>
<td></td>
<td>Discharged</td>
<td>11/1/12</td>
</tr>
<tr>
<td>9</td>
<td>60/M</td>
<td>6/17/12</td>
<td>15</td>
<td></td>
<td>Fecal</td>
<td>Respiratory failure</td>
<td>Guillain-Barré syndrome</td>
<td></td>
<td>TZP</td>
<td>Death</td>
<td>11/1/12</td>
</tr>
<tr>
<td>10</td>
<td>37/F</td>
<td>6/27/12</td>
<td>5</td>
<td></td>
<td>Fecal</td>
<td>Heart failure</td>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
<td>Discharged</td>
<td>11/1/12</td>
</tr>
<tr>
<td>11</td>
<td>50/M</td>
<td>7/13/12</td>
<td>14</td>
<td>23</td>
<td>Fecal</td>
<td>Pneumonia</td>
<td>Polytrauma/Alcoholic cirrhosis</td>
<td></td>
<td>TZP, MEM, CST</td>
<td>Discharged</td>
<td>11/1/12</td>
</tr>
<tr>
<td>12</td>
<td>56/F</td>
<td>7/20/12</td>
<td>12</td>
<td>14</td>
<td>Fecal</td>
<td>Bronchial</td>
<td>Stroke/methicillin diarrhea</td>
<td></td>
<td>TZP, MEM, CST</td>
<td>Discharged</td>
<td>11/1/12</td>
</tr>
<tr>
<td>13</td>
<td>79/M</td>
<td>7/23/12</td>
<td>14</td>
<td></td>
<td>Fecal, bronchial</td>
<td>Cerebral hemorrhage</td>
<td>FEP</td>
<td></td>
<td>TZP</td>
<td>Death</td>
<td>11/1/12</td>
</tr>
<tr>
<td>14</td>
<td>87/F</td>
<td>8/2/12</td>
<td>4</td>
<td></td>
<td>Fecal</td>
<td>Stroke/renal failure</td>
<td></td>
<td></td>
<td>TZP</td>
<td>Death</td>
<td>11/1/12</td>
</tr>
<tr>
<td>15</td>
<td>61/M</td>
<td>8/23/12</td>
<td>3</td>
<td></td>
<td>Fecal</td>
<td>Heart failure</td>
<td>SAM</td>
<td></td>
<td></td>
<td>Discharged</td>
<td>11/1/12</td>
</tr>
<tr>
<td>16</td>
<td>72/M</td>
<td>9/16/12</td>
<td>101</td>
<td>12</td>
<td>Fecal</td>
<td>Pneumonia</td>
<td>Stroke</td>
<td></td>
<td>FEP, MEM</td>
<td>Discharged</td>
<td>11/1/12</td>
</tr>
<tr>
<td>17</td>
<td>62/M</td>
<td>9/16/2</td>
<td>12</td>
<td></td>
<td>Bronchial</td>
<td>Stroke</td>
<td>Diabetes mellitus</td>
<td>AZM, FEP, MEM, CST</td>
<td>Discharged</td>
<td>11/1/12</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>79/F</td>
<td>9/25/12</td>
<td>7</td>
<td></td>
<td>Bacteremia</td>
<td>Abdominal surgery</td>
<td>TIM, AMK, MTZ, MEM, CST</td>
<td></td>
<td></td>
<td>Death</td>
<td>11/1/12</td>
</tr>
<tr>
<td>19</td>
<td>18/M</td>
<td>10/26/12</td>
<td>11</td>
<td></td>
<td>Fecal</td>
<td>Polytrauma</td>
<td>Hypothyroidism</td>
<td>CAZ</td>
<td>Discharged</td>
<td>11/1/12</td>
<td></td>
</tr>
</tbody>
</table>

a  Index case.  

b  Due to infection.  

c  AMK, amikacin; AZM, azithromycin; CAZ, ceftazidime; CST, colistin; FEP, cefepime; MEM, meropenem; MTZ, metronidazole; SAM, ampicillin-sulbactam; TZP, piperacillin-tazobactam; TIM, ticarcillin-clavulanic acid; VAN, vancomycin.
plasmid approximately 100 kb in size and three smaller ones ranging in size from 1.8 to 3 kb. Conjugation experiments in representative isolates were successful in transferring cephalosporin resistance to the recipient strain. All transconjugants contained the large plasmid of approximately 100 kb, exhibited ertapenem MICs of 0.125 to 0.25 µg/ml, and harbored the blaCTX-M-15 gene (data not shown).

**Infection control measures.** Following the detection of the initial carbapenem-negative ertapenem-resistant isolates, infection control measures in the ICU were reinforced. Patient carrier status was evaluated both upon admission and on a weekly basis thereafter. Bronchial aspirate samples were also collected from all patients. At the completion of antibiotic susceptibility testing and initial phenotypic characterization, medical staff was alerted regarding possible ertapenem-resistant isolates.

The structure of the ICU allowed for the isolation of four out of a total of six hospitalized patients. Interventions concerning hand hygiene were intensified and training sessions were organized for the medical and nursing staff in order to strengthen awareness regarding infection control practices. Contact precautions were taken, with the use of gloves and disposable gowns for the duration of the hospitalization of the infected/colonized patients. Hand washing with antiseptic soap and alcohol-based hand rub solutions prior to and after contact with the infected/colonized patient was adhered to routinely. Nursing staff cohorting was applicable only in the morning shift due to the limited number of staff available. Sanitation of inanimate surfaces, equipment, and caring devices was vigorously implemented. Finally, following discharge, terminal cleaning and decontamination of the inanimate surfaces using sodium hypochloride solutions and sodium dichloroisocyanurate tablets was performed, with emphasis on the patients’ beds. These measures allowed the containment of the outbreak after 7 months, in November 2012. Carbapenemase-negative ertapenem-resistant *K. pneumoniae* isolates were not detected thereafter from clinical or active surveillance specimens.

**DISCUSSION**

Loss or compromise of outer membrane porins in *K. pneumoniae* isolates has been associated with a carbapenem-resistant profile, especially as far as ertapenem is concerned (6). Such isolates are sporadically reported and are usually accompanied by the presence of an ESBL or AmpC type β-lactamase (6, 9, 16, 20). We report for the first time the large dissemination of an ertapenem-resistant *K. pneumoniae* outbreak strain possessing the blaCTX-M-15 gene. Ertapenem resistance was associated with a disruption in the expression of the *ompK35* gene as well as the presence of an OmpK36 porin variant.

Indeed, outbreaks involving ESBL- or AmpC-producing *K. pneumoniae* isolates with OmpK35 and/or OmpK36 impairment remain limited. A CTX-M-1-producing clonal *K. pneumoniae* strain with an OmpK35 porin deficiency was associated with slightly elevated ertapenem MICs in isolates recovered from several patients hospitalized in an ICU in Spain during 2002 to 2005 (13). In two clonal isolates obtained during this outbreak, the lack of expression of both OmpK35 and OmpK36 porins was associated with high-level resistance to carbapenems (13). Also, CTX-M-15-producing *K. pneumoniae* clones with modified OmpK36 porins were found to affect a few hospitalized patients in different hospital settings (14, 15).

In the present study, regarding the OmpK35 porin, the TGA nonsense mutation at codon 63 resulted in early termination of translation and thus depletion of the porin. Similar porin deficiencies have been described previously among ESBL-producing strains (13, 14, 20). The OmpK36 variant of our clonal isolates had both a nucleotide insertion and nucleotide deletions in the L3 and L4 loops, respectively. Loop 3 seems to play a major functional role as it is the most conserved loop of the OmpK36 porin and extends inside the barrel defining the size of the transmembrane pore (32). It is of note that these specific lesions in OmpK36 porin were also identified in a *K. pneumoniae* strain coproducing OXA-163 β-lactamase (33). In addition, *K. pneumoniae* isolates exhibiting similar modifications in the L3 loop have been detected in limited cases reported from small outbreaks in Italy and Portugal (14, 15). In the current outbreak, the presence of the OmpK36 variant along with the OmpK35 porin deficiency did not obviously affect the potential for epidemic spread, though deletion of both porins was found to significantly decrease the virulence (34).

In our survey, in an attempt to provide helpful insight, we assessed and evaluated epidemiological data. Despite the fact that surveillance swabs were not obtained upon admission from our index patient, given his medical history, we assume that this isolate was introduced to our hospital setting from a different tertiary care facility. However, since the isolate was not detected until day 4 of hospitalization, it is also likely that the index patient acquired...
the isolate from an unknown source after hospital admission. Following this event, indirect transmission, probably through contaminated inanimate surfaces, led to further dissemination among newly admitted ICU patients. This is supported by the fact that clinical and environmental samples yielded isolates belonging to the same clonal type. Gastrointestinal colonization may have acted as a latent reservoir, facilitating the continuous cross-transmission of this clone among patients hospitalized in overlapping periods of time. In our study, pharyngeal and hand sampling of staff was negative and the indirect transmission was presumed because of the large number of patients involved, the extended span of the outbreak, and the absence of another source of contamination. Furthermore, such extensive clonal dissemination was unprecedented in our hospital, and in the period prior to this outbreak, other endemic KPC-producing clones were detected (28).

The implementation of active surveillance cultures not only upon admission but also on a weekly basis gave a comprehensive depiction of the underlying situation at hand, as the total number of patients colonized was estimated to be approximately double the number exhibiting infection, as previously described (35). Infection in patients manifested in the form of respiratory tract infections or septicemia, and death was the outcome in 42.8% of the cases, despite the administration of a targeted antibiotic regimen. Although a statistical analysis was not attempted, the relatively high average number of days prior to colonization/infection with the outbreak strain is notable, as well as the fact that resistant isolates appeared following selective pressure due to previous antibiotic consumption (Table 1).

Reevaluation of laboratory data archives failed to identify other similar isolates that exhibited ertapenem resistance due to porin modifications and may have gone undetected in the past. Given that these isolates did not exhibit high-level ertapenem resistance, the implementation of the novel CLSI breakpoints for carbapenems but should be expanded to involve third-generation cephalosporins, since via this wider screening ESBL-bearing strains with reduced carbapenem susceptibility, which have a documented potential for dissemination following selective pressure in the ICU environment, can be promptly identified.

REFERENCES


