Outbreak Caused by an Ertapenem-Resistant, CTX-M-15-Producing
Klebsiella pneumoniae ST101 Clone Carrying an OmpK36 Porin Variant

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Running title: Outbreak due to ertapenem-resistant, CTX-M-15-producing Klebsiella
pneumoniae with OmpK36 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 are limited. We describe an outbreak caused by an ertapenem-resistant, CTX-M-15-producing *K. pneumoniae* clonal strain expressing an OmpK36 porin variant.

From May 2012-November 2012, 37 ertapenem-resistant, phenotypically-negative for carbapenemase production *K. pneumoniae* isolates 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 in all isolates the presence of *bla*CTX-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 *K. pneumoniae* environmental isolates belonged to the same clonal type and were assigned to MLST sequence type ST101. As all colonized/infected patients were hospitalized in overlapping periods, cross-infection was considered as 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 weekly basis was found valuable for early recognition and subsequent successful management of the outbreak.

**Keywords:** ESBL, OmpK36 porin variant, carbapenemase, *Klebsiella pneumoniae*, infection control
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 constituted the antibiotic treatment of choice in multidrug-resistant 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 (OXAs) 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 either the ompK35 or ompK36 gene. Less frequently, point mutations may cause protein structure changes or deletions which interfere with porin expression (8). Regardless however of the mechanism implicated, porin deficiency results in low ertapenem concentrations in the periplasmic space, which further facilitate even the activity of enzymes with weak carbapenemase proprieties (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 solely 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 \textit{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 report an outbreak caused by an ertapenem-resistant CTX-M-15-producing \textit{K. pneumoniae} porin deficient strain, in the intensive care unit (ICU) of a Greek hospital. Awareness is raised to the possibility that carbapenem resistance may derive and 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 \textit{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 plotted, aiming to identify other such isolates from both clinical and active surveillance samples, collected from patients hospitalized in the 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 of 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 from the hospital internal review board. Nosocomial infections were defined by standard Centers for Disease Control 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 substrates according to CLSI guidelines (24). The MBL Etest (bioMérieux, Marcy l’Étoile, 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, ESBLs including the SHV, TEM, CTX-M, IBC/GES enzymes and plasmid-mediated AmpCs in single PCR reactions 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 templates for sequencing 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 rectal swabs upon patient admission and on a weekly basis was implemented. 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, respectively. All Gram-negative colonies growing after 24 h of incubation at 37°C were identified and \textit{K. pneumoniae} isolates were phenotypically tested for carbapenemase, ESBL and plasmid-mediated AmpC production. Phenotypic testing results were further verified by molecular techniques. Additionally, at least one bronchial aspirate sample was obtained from each patient during his 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 \textit{K. pneumoniae} isolates, pulse-field electrophoresis (PFGE) of the \textit{XbaI}-digested genomic DNA was performed with a CHEF-DRIII system (Bio-Rad, Hemel Hempstead, UK), with 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 \textit{K. pneumoniae} isolates [www.pasteur.fr/recherche/genopole/PF8/mlst](http://www.pasteur.fr/recherche/genopole/PF8/mlst) with sequence types (STs) assigned using online database tools.

Conjugation experiments and plasmid analysis. The potential for conjugational transfer of carbapenem or third-generation cephalosporin resistance was examined in biparental matings using LB broth cultures of three representative isolates and \textit{Escherichia coli} strain 26R793 (lac\textsuperscript{-}, Rif\textsuperscript{R}) as the recipient strain. Donor and recipient cells were mixed in a ratio of 1:5 and transconjugant clones were screened on Mac
Conkey agar plates containing rifampicin (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* 39R861 as a control strain.

RESULTS

Clinical isolates and antimicrobial susceptibility. During the seven month study period in question (May-November 2012) a total of 37 ertapenem-resistant (MIC >1 μg/ml) phenotypically-negative for carbapenemase production *K. pneumoniae* isolates, were retrieved from clinical and rectal surveillance samples derived from 19 patients hospitalized in the ICU of Serres General Hospital. Twenty from the 37 isolates were recovered from rectal swabs, 8 from bronchial aspirates, 5 from blood cultures and 4 from intravenous catheters. It should be mentioned that 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 of 2-8 μg/ml). They were also susceptible or intermediate to meropenem (MICs ranging from 0.5-2 μg/ml) but all remained susceptible to imipenem (MICs ranging from 0.25-1 μg/ml). All isolates were highly resistant to cefotaxime (MICs 128->256 μg/ml), cefoxitin (MICs 64-256 μg/ml), ceftazidime (MICs 32-256 μg/ml), cefepime (MICs 64-256 μg/ml), piperacillin/tazobactam (MICs 128-256 μg/ml), amoxicillin/clavulanate (MICs 32-64 μg/ml), and aztreonam (MICs 64-256 μg/ml), while remained susceptible to cefotetan (4-16 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 69-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 of 8.9 days), of which 19 (31.7%) were colonized and/or infected by ertapenem-resistant phenotypically carbapenemase-negative isolates. Patients’ 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 in overlapping periods (Figure 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 iv (3 million units every 8 h). In three of these patients the 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 of the study. Also, subsequent MBL Etasting 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 in all K. pneumoniae of the
blaCTX-M-15 ESBL gene. 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. An insertion of 6 nucleotides (5′-GGCGAC-3′) encoding Gly and
Asp was 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 the Gly (134)-Asp (135)-Gly (136)-Asp (137), similarly to previous
observations (14). Additionally, a 9-nucleotide deletion was detected encoding Leu(184),
Ser(185) and Pro(186) in the wild strain (19). These deletions involved amino acids
located at L4, a loop facing the cell exterior (31).

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 blaCTX-M-15 gene, produced the OmpK 36
porin variant detected in the clinical isolates and showed a disruption in the expression of
the ompK35 gene.

During the study period, medical and non-medical staff was also evaluated for likely
pharyngeal and hand carriage of the implicated K. pneumoniae, yet 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, Ic) with 17, 3 and 1 isolates, respectively. The two environmental isolates belonged to the major subtype (Figure 3). MLST assigned representative isolates from the three subtypes to a single sequence type 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 plasmid approximately 100-kb in size and three smaller ones ranging from 1.8-3 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 0.125-0.25 µg/ml and harbored the *bla*$_{CTX-M-15}$ gene (data not shown).

**Infection control measures.** Following the detection of the initial carbapenemase-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. Medical staff was alerted regarding possible ertapenem-resistant isolates with the completion of antibiotic susceptibility testing and initial phenotypic characterization.

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 and after contact with the infected/colonized patient was routinely
adhered to. 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, with emphasis on the patient’s beds, using
sodium hypochloride solutions and sodium dichloroisocyanurate tablets was performed.
These measures allowed the containment of the outbreak after 7 months, in November
2012. Carbapenemase-negative ertapenem-resistant \textit{K. pneumoniae} isolates were not
detected thereafter from clinical or active surveillance specimens.

**DISCUSSION**

Loss or compromise of outer membrane porins has been associated in \textit{K. pneumoniae}
isolates 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 $\beta$-lactamase (6, 9, 16, 20). The present survey describes for the
first time the large dissemination of an ertapenem-resistant \textit{K. pneumoniae} outbreak strain
possessing \textit{bla}_{CTX-M-15} gene. Ertapenem resistance was associated with a disruption in the
expression of \textit{ompK35} gene as well as the presence of an OmpK36 porin variant.

Indeed, outbreaks involving ESBL or AmpC-producing \textit{K. pneumoniae} isolates with
OmpK35 and/or OmpK36 impairment remain limited. Thus, a CTX-M-1-producing \textit{K. pneumoniae}
clonal strain with OmpK35 porin deficiency has been associated with
slightly elevated ertapenem MICs in isolates recovered from several patients hospitalized
in an ICU in Spain during 2002-2005 (13). In two clonal isolates 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 \textit{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 also described previously among ESBL-producing strains (12, 14, 20). The OmpK36 variant of our clonal isolates had both a nucleotide insertion and nucleotide deletions in L3 and L4 loops, respectively. Loop 3 seems to play a major functional role as it is the most conserved loop of OmpK36 porin and extend 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 co-producing OXA-163 β-lactamase (33). In addition, *K. pneumoniae* isolates exhibiting similar modifications in 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 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 decrease significantly the virulence (34).

In our survey, in an attempt to provide helpful insight, epidemiological data were assessed and evaluated. Despite the fact 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 an 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 was able to give a comprehensive depiction of the underlying situation at hand, as the total number of patients colonized was estimated to be approximately double in comparison to those exhibiting infection as it was also previously described (35). Infected 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, it is of note the relatively high day average prior to colonization/infection with the outbreak strain as well as the fact that resistant isolates were selected following selective pressure due to previous antibiotic consumption (Table 1).

It should be mentioned, that reevaluation of laboratory data archives failed to identify other similar isolates exhibiting ertapenem resistance due to porin modifications, which 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 no doubt facilitated their prompt identification (24).

ST101 to which these *K. pneumoniae* isolates were assigned is detected in the Greek region for the first time. Previous reports implicating ST101 in the European and wider Mediterranean region have derived from Italy involving KPC-2 producers, Spain and
Tunisia with OXA-48 and CTX-M-15 harboring isolates and France and Czech Republic with ESBL-producing strains (36, 37).

Reinforcement of infection control measures can hinder intra-hospital dissemination of resistant isolates (38). In the current epidemic diligent disinfection of inanimate surfaces, adherence to an intensified cleaning routine and patient cohorting seem to have contributed to effectively control the outbreak after 7 months. However, the inability to provide dedicated staff care is likely to have compromised effectiveness of these measures and contributed to the long duration of the clonal strain dissemination.

The use of simple and reliable phenotypic methods for the evaluation of antibiotic resistance patterns enabled the early detection of these isolates, which resulted not only in the reinforcement of infection control measures but also in the administration of appropriate antibiotic treatment. The necessity for timely detection of emerging resistant isolates such as these CTX-M bearing, porin compromised \textit{K. pneumoniae} which are currently a novelty in the Greek region, cannot be once again overemphasized given the already high prevalence of carbapenemase-producing clones (4, 22, 28, 39).

Finally, active surveillance upon admission and on a weekly basis thereafter, was highlighted as a valuable tool in the early detection, monitoring and containment of this outbreak. Screening for carriage status should not be limited to isolates resistant to 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.
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during an outbreak involving multiple carbapenem-resistant *Enterobacteriaceae* species

and of reduced amounts of OmpK36 porin in *Klebsiella pneumoniae* results in increased


### TABLE 1. Characteristics of patients infected and/or colonized with the ertapenem-resistant CTXM-15-producing and porin deficient K. pneumoniae clonal isolates

<table>
<thead>
<tr>
<th>Age (years)/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>Infection</th>
<th>Reason for ICU admission</th>
<th>Underlying disease(s)</th>
<th>Antibiotic regimen prior to infection</th>
<th>Antibiotic regimen</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 69/M</td>
<td>4/27/12</td>
<td>27</td>
<td>0</td>
<td>Fecal, bronchial</td>
<td>Bacteraemia</td>
<td>Respiratory failure</td>
<td>Diabetes mellitus/Tetraplegic</td>
<td>FEP, MEM, COL</td>
<td>deathb</td>
<td></td>
</tr>
<tr>
<td>2 80/F</td>
<td>5/06/12</td>
<td>25</td>
<td>0</td>
<td>Focal</td>
<td>Stroke</td>
<td></td>
<td>Pulmonary fibrosis</td>
<td>FEP, FEP</td>
<td>death</td>
<td></td>
</tr>
<tr>
<td>3 75/F</td>
<td>5/10/12</td>
<td>14</td>
<td>0</td>
<td>Focal, bronchial</td>
<td>Stroke/Respiratory failure</td>
<td>-</td>
<td>TIZP</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 75/F</td>
<td>5/16/12</td>
<td>13</td>
<td>0</td>
<td>Focal</td>
<td>Stroke/Septic shock</td>
<td>Hyperpyrexia</td>
<td>Pulmonary fibrosis/diabetes mellitus/obesity</td>
<td>TIZP, MEM, COL</td>
<td>discharged</td>
<td></td>
</tr>
<tr>
<td>5 50/M</td>
<td>5/18/12</td>
<td>4</td>
<td>0</td>
<td>Fecal</td>
<td>Pneumonia</td>
<td>Stroke/pneumonia</td>
<td>Chronic lymphocytic leukemia</td>
<td>TIZP, MEM, COL</td>
<td>discharged</td>
<td></td>
</tr>
<tr>
<td>6 77/M</td>
<td>6/01/12</td>
<td>10</td>
<td>0</td>
<td>Focal</td>
<td>Poly-trauma</td>
<td>Stroke/Septic shock</td>
<td>Pulmonary fibrosis/diabetes mellitus/obesity</td>
<td>FEP, AZM</td>
<td>discharged</td>
<td></td>
</tr>
<tr>
<td>7 76/F</td>
<td>6/06/12</td>
<td>6</td>
<td>0</td>
<td>Fecal</td>
<td>Bacteraemia</td>
<td>Stroke</td>
<td>Diabetes mellitus/Alcoholic cirrhosis</td>
<td>TIZP, MEM, COL</td>
<td>discharged</td>
<td></td>
</tr>
<tr>
<td>8 76/M</td>
<td>6/07/12</td>
<td>5</td>
<td>0</td>
<td>Focal</td>
<td>Stroke</td>
<td></td>
<td>-</td>
<td>FEP</td>
<td>discharged</td>
<td></td>
</tr>
<tr>
<td>9 40/M</td>
<td>6/17/12</td>
<td>15</td>
<td>0</td>
<td>Focal</td>
<td>Respiratory failure</td>
<td>Guillain Barre syndrome</td>
<td>-</td>
<td>TIZP</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10 37/F</td>
<td>6/27/12</td>
<td>5</td>
<td>0</td>
<td>Focal</td>
<td>Heart failure</td>
<td>Diabetes mellitus</td>
<td>-</td>
<td>-</td>
<td>discharged</td>
<td></td>
</tr>
<tr>
<td>11 50/M</td>
<td>7/13/12</td>
<td>14</td>
<td>23</td>
<td>Focal</td>
<td>Pneumonia</td>
<td>Poly-trauma</td>
<td>Diabetes mellitus/Alcoholic cirrhosis</td>
<td>FEP, MEM, COL</td>
<td>discharged</td>
<td></td>
</tr>
<tr>
<td>12 60/F</td>
<td>7/20/12</td>
<td>12</td>
<td>14</td>
<td>Focal, bronchial</td>
<td>Bacteraemia</td>
<td>Subacute leptomeningitis</td>
<td>Diabetes mellitus/Alcoholic cirrhosis/Hydrocephalus</td>
<td>COL, MEM</td>
<td>deathb</td>
<td></td>
</tr>
<tr>
<td>13 70/M</td>
<td>7/23/12</td>
<td>14</td>
<td>0</td>
<td>Focal, bronchial</td>
<td>-</td>
<td>Central haemorrhage</td>
<td>-</td>
<td>FEP</td>
<td>death</td>
<td></td>
</tr>
<tr>
<td>14 87/F</td>
<td>8/02/12</td>
<td>4</td>
<td>0</td>
<td>Focal</td>
<td>Stroke/Septic shock</td>
<td>-</td>
<td>TIZP</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 61/M</td>
<td>8/23/12</td>
<td>3</td>
<td>0</td>
<td>Focal</td>
<td>Heart failure</td>
<td>-</td>
<td>SAM</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 72/M</td>
<td>9/16/12</td>
<td>10</td>
<td>12</td>
<td>Focal</td>
<td>Pneumonia</td>
<td>Stroke</td>
<td>-</td>
<td>FEP, TIZP, MEM</td>
<td>discharged</td>
<td></td>
</tr>
<tr>
<td>17 62/M</td>
<td>9/16/2</td>
<td>12</td>
<td>0</td>
<td>Bronchial</td>
<td>Stroke</td>
<td>Diabetes mellitus</td>
<td>Azithromycin</td>
<td>-</td>
<td>discharged</td>
<td></td>
</tr>
<tr>
<td>18 79/F</td>
<td>9/25/12</td>
<td>7</td>
<td>0</td>
<td>Bronchial</td>
<td>Bacteraemia</td>
<td>Abdominal surgery</td>
<td>Ticarcillin-clavulanic acid</td>
<td>TIZP, AZM, MEM, COL</td>
<td>discharged</td>
<td></td>
</tr>
<tr>
<td>19 18/M</td>
<td>10/26/12</td>
<td>11</td>
<td>0</td>
<td>Focal</td>
<td>Poly-trauma</td>
<td>Hyperpyrexia</td>
<td>CAZ</td>
<td>-</td>
<td>discharged</td>
<td></td>
</tr>
</tbody>
</table>

*Index case

*death due to infection

AMK, amikacin; AZM, azithromycin; CAZ, ceftazidime; COL, colistin; FEP, cefepime; MEM, meropenem, MTZ metronidazole; TIZP, piperacillin-tazobactam; TIC, ticarcillin-clavulanic acid, VAN, Vancomycin
FIG 1. Hospitalization period of colonized/infected patients with ESBL-producing, porin deficient K. pneumoniae clonal isolates during the outbreak.
FIG 2. Total number of admissions per month and distribution of patients infected or only colonized with the CTX-M-15-producing, porin deficient K. pneumoniae clonal isolates during the study period (May 2012-November 2012).