

Containment of an Outbreak of KPC-3-Producing *Klebsiella pneumoniae* in Italy[∇]

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Received 22 June 2011/Returned for modification 2 August 2011/Accepted 29 August 2011

From March 2009 to May 2009, 24 carbapenem-resistant *Klebsiella pneumoniae* isolates were recovered from 16 patients hospitalized in an Italian intensive care unit (ICU). All isolates contained KPC-3 carbapenemase and belonged to a single pulsed-field gel electrophoresis (PFGE) clone of multilocus sequence type 258 (designated as ST258). A multimodal infection control program was put into effect, and the spread of the KPC-3-producing *K. pneumoniae* clone was ultimately controlled without closing the ICU to new admissions. Reinforced infection control measures and strict monitoring of the staff adherence were necessary for the control of the outbreak.

The class A *Klebsiella pneumoniae* carbapenemases (KPCs), which are predominantly harbored by *Enterobacteriaceae*, have over the past few years exhibited an international dissemination (15). There are currently 10 recognized KPC types (KP-2 to KPC-11) (<http://www.lahey.org/studies>), of which KPC-2 and KPC-3 are the most commonly detected variants (7, 15). KPC-2 appears to be more predominant worldwide, with outbreaks arising from the United States but also from Europe and China (7, 17, 18). KPC-3 is mainly detected in the United States, Latin America, and Israel (3, 7, 14, 15), while reports from Europe still remain rare, mainly involving patients with a history of previous hospitalization in these regions of endemicity (2, 9, 23). In Italy, KPC-2- and KPC-3-producing *K. pneumoniae* strains have already been introduced into hospital units (8, 9, 13). We report the emergence of a KPC-3-producing *K. pneumoniae* clone in an Italian intensive care unit (ICU) and the containment of this outbreak.

The study was conducted in the 12-bed ICU at the Cannizzaro Hospital, Catania, Italy, a 500-bed acute care hospital. In the period from March 2009 to May 2009, clinical and screening specimens (oropharyngeal, rectal, nasal, and axillary swabs) were collected to detect *K. pneumoniae* isolates exhibiting resistance to at least one of the carbapenems (imipenem, meropenem, or ertapenem) (4). Screening cultures were collected from each patient on the day of admission using cysteine lactose electrolyte-deficient (CLED) agar plates. Standard definitions of carriage, colonization, and infections were used (1). Particularly, carriers were considered patients with positive screening cultures in the absence of, or before isolation of, positive clinical specimens. Patients with positive clinical specimens were considered to be colonized in cases where there was a lack of clinical data confirming infection.

In total, 24 carbapenem-resistant *K. pneumoniae* isolates were recovered from clinical and screening specimens of 16 patients hospitalized in the ICU. Table 1 presents the characteristics of the patients and their *K. pneumoniae* isolates. Most of the patients (13 of 16; 81.3%) had evidence of clinical infection due to KPC-producing *K. pneumoniae*. The crude mortality rate was 37.5%. The index carbapenem-resistant *K. pneumoniae* was recovered from blood culture of a patient who was transferred to the unit from the neurosurgery department 23 days before the isolation. It was this initial detection of a carbapenem-resistant *K. pneumoniae* isolate in the ICU that alerted us to the possibility of a carbapenem-resistant clone being introduced into our hospital. It is of note that upon ICU admission, screening cultures were taken from this patient and were negative for carbapenemase-producing isolates.

Agar dilution susceptibility testing (4) was performed on the 24 isolates. MICs for imipenem ranged from 2 to >32 μg/ml and those for meropenem from 2 to >32 μg/ml, while all isolates were highly resistant to ertapenem, with MICs of >32 μg/ml. Furthermore, they were resistant to several β-lactams including aztreonam, amikacin (MICs of 64 to 128 μg/ml), trimethoprim-sulfamethoxazole (MIC >32 μg/ml), and ciprofloxacin (MIC >32 μg/ml). Antimicrobials that demonstrated *in vitro* activity were gentamicin, tigecycline, and colistin, with MICs of ≤4 μg/ml, ≤2 μg/ml, and ≤2 μg/ml, respectively, with the exception of index isolate K1, which was resistant to colistin (MIC of 32 μg/ml).

Phenotypic testing using the boronic acid potentiation disk test with meropenem (20) was indicative of the production of a KPC carbapenemase in all isolates, while the combined-disk test with imipenem and EDTA was negative for metallo-β-lactamase (MBL) production. Also, the modified extended-spectrum β-lactamase (ESBL) CLSI confirmatory test (21) was negative for ESBL production in all cases.

The isolates were screened for β-lactamase genes by PCR and sequencing using primers for the detection of MBL (10, 11), KPC (21), ESBL (12, 22), and plasmidic AmpC genes (16).

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[∇] Published ahead of print on 7 September 2011.

TABLE 1. Characteristics of patients and KPC-3-producing *K. pneumoniae* isolates in the ICU of the Cannizzaro hospital

Patient and isolate(s) ^a	Type of admission or isolation source	Date sample obtained (mo/day)	Type of sample	Pattern of acquisition	Discharge status from ICU
Patient 1 K1	Unscheduled Surgical	3/11	Blood from CVC ^b	CRI ^c	Dead
Patient 2 K2 K3	Medical	3/16 3/16	Blood Sputum	Infection Infection	Alive
Patient 3 K4	Unscheduled Surgical	3/23	Tonsillar swab	Carriage	Alive
Patient 4 K5 K6 K7 K8 K9	Medical	3/24 3/27 3/29 3/29 3/30	Arterial catheter tip Urine Blood Sputum Blood from CVC	— ^d Colonization CRI Colonization —	Dead
Patient 5 K10	Medical	4/4	Urine	colonization	Dead
Patient 6 K11	Medical	4/14	Blood	Infection	Alive
Patient 7 K12	Scheduled Surgical	4/14	Peritoneal fluid	Infection	Dead
Patient 8 K13 K14	Medical	4/27 4/27	Tonsillar swab Sputum	Carriage Infection	Dead
Patient 9 K15	Medical	4/27	Tonsillar swab	Carriage	Alive
Patient 10 K16	Medical	4/28	Sputum	Infection	Dead
Patient 11 K17	Medical	5/9	Urine	Infection	Alive
Patient 12 K18	Medical	5/11	Blood	Infection	Alive
Patient 13 K19	Scheduled Surgical	5/12	Sputum	Infection	Alive
Patient 14 K20	Medical	5/12	Sputum	Infection	Alive
Patient 15 K21	Medical	5/16	Arterial catheter tip	Infection	Alive
Patient 16 K22	Medical	5/18	Urine	Infection	Alive
Patient 16 K23 K24	Medical	5/24 5/25	Urine Sputum	Infection Infection	Alive

^a Isolate designations are arbitrary.

^b CVC, central venous catheter.

^c CRI, central venous catheter-related infection.

^d Dashes designate multiple isolates already associated with the pattern of acquisition.

In accordance with phenotypic results, molecular testing confirmed the presence of *bla*_{KPC-3} gene in all cases. Furthermore, all isolates contained the *bla*_{TEM-1} gene, and isolates K2 and K3, both deriving from a single patient's specimens, were positive for the *bla*_{OXA-1} gene. Conjugation experiments along with plasmid analysis failed to demonstrate the transfer of carbapenem resistance to all isolates.

Pulsed-field gel electrophoresis (PFGE) of the XbaI-digested genomic DNA was performed, and banding patterns were compared visually (19). PFGE analysis of the 24 *K. pneumoniae* isolates indicated the dissemination of clonally related KPC-3-positive isolates which belonged to a single clone designated as type E. It should be noted that this clonal type was distinctly different from the epidemic clone type A, preexisting in our unit, which did not exhibit resistance to carbapenems. Multilocus sequence typing (MLST) was performed on representative KPC-3-producing isolates according to the protocol described on the website <http://www.pasteur.fr/recherche>

[/genopole/PF8/mlst/Kpneumoniae.html](http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html), and results were compared to the international *K. pneumoniae* database (6). MLST showed that isolates of clonal type E shared the same MLST sequence type, designated as ST258.

Two epidemic curves were plotted to give a visual representation of the trend of *K. pneumoniae* colonization and infection events in the ICU, due to the carbapenem-resistant clone and to the previously circulated carbapenem-susceptible clone, respectively. Epidemic curves were used to provide information on the outbreak's pattern of spread, magnitude, and time trend.

Infection control measures were implemented following the detection of the index carbapenem-resistant *K. pneumoniae* isolate (Fig. 1). The ICU environment was cleaned with a neutral detergent, respiratory equipment was disinfected, and instructions regarding hand hygiene were given. Regardless of these initial measures, the outbreak continued to evolve with more isolates being detected. The epidemic curves (Fig. 1) and results of molecular typing suggested the occurrence of a prop-

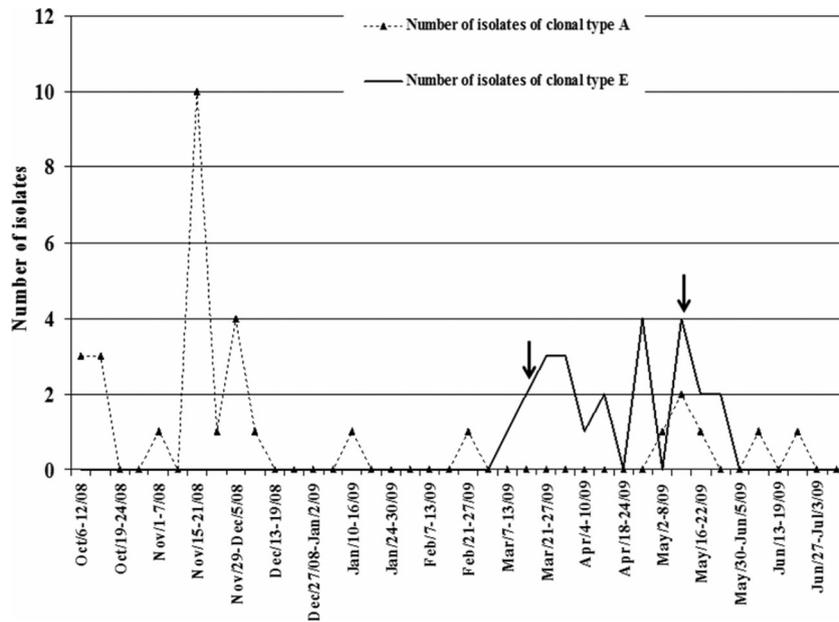


FIG. 1. Epidemic curves of the carbapenem-resistant clone (PFGE type E) and of the carbapenem-susceptible clone (PFGE type A) in the ICU of the Cannizzaro hospital. Isolates of both clonal types are represented according to their weekly distribution over a 9-month period of time. Arrows designate when the first and second rounds of infection control interventions were begun.

agated or progressive source mode of epidemic transmission, reflective of transmission within the health care facility, likely caused by person-to-person contact, although environmental or device exposure may have played a role. Infection control measures were therefore reinforced (Fig. 1). Colonized or infected patients were spatially segregated, they received care from a single nurse, and the use of disposable gloves and aprons was strictly implemented for all staff members. Compliance with hand hygiene was monitored, as were contact isolation measures and strict adherence of the personnel to environmental cleaning. Meetings between ICU staff and infection control teams were deemed necessary and were held on a weekly basis. The aim of these meetings was not only to provide timely and precise input on the number of new cases detected but also to monitor the implementation of the measures taken and optimize their efficacy. The outbreak was ultimately controlled within a 4-month period of time, with no novel carbapenem-resistant isolates being detected thereafter. ICU closure to new admissions was thus averted.

In European regions, the KPC-3 variant has been detected mostly from imported cases (2, 9, 23). In this study, the emergence of a KPC-3-producing *K. pneumoniae* clone in a European hospital is described. All isolates were found to belong to a single pulsotype, which seemingly replaced the previously established carbapenem-susceptible epidemic pulsotype of the hospital ICU rather than rapidly diffused the *bla*_{KPC-3} gene within the preexisting clone.

The index case isolate was retrieved from a patient with no history of a recent journey to countries where KPC-3 producers are widely spread, and the screening cultures during ICU admission were negative for KPC production. A distinct epidemiological link as to how this isolate was introduced into the unit was not identified in the current survey. This is a possible indication of the circulation of such KPC-producing isolates

within the hospital environment before they cause clinical infections (5). KPC-type enzymes are known for their potential for rapid dissemination among different bacterial species, which has been attributed to the fact that these genes are usually located on transferable broad-host-range plasmids which are associated with transposons and insertion sequence (IS) elements (15). Given the potential of KPC producers to rapidly disseminate and the difficulties involved in the containment of an outbreak due to such isolates, a multimodal infection control program was put into effect promptly after the identification of the first carbapenem-resistant isolate, in order to control the spread of the *K. pneumoniae* clone without closing the ICU to new admissions. Despite active surveillance and initial contact precautions, the outbreak continued to escalate. Reinforced infection control measures and strict monitoring of the staff adherence were necessary for the effective control of the outbreak within a 4-month period of time. Thus, we were able to prevent the closure of the ICU to new admissions.

The systematic description of the above measures and active surveillance is of importance due to the significant increase of KPC-producing pathogens in several regions. Such epidemics are also worrisome because of the potential for further dissemination of these KPC-3-possessing clonal isolates within the Italian and consequently the European region. This dissemination has been already described among sporadic cases of KPC-producing *K. pneumoniae* introduced into Switzerland from Italy (2). The need for accurate and rapid phenotypic methods is mandatory in order to detect such KPC-positive pathogens and timely implement the appropriate infection control measures.

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