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Outbreak of KPC-3-producing, and colistin-resistant, *Klebsiella pneumoniae* infections in two Sicilian hospitals

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Abstract

We report the first outbreak caused by colistin-resistant *Klebsiella pneumoniae* producing KPC-3 carbapenamase in two Italian hospitals. This spread occurred in 1 month, and was caused by eight colistin-resistant and carbapenem-resistant *Klebsiella pneumoniae* isolates from eight patients. A further three isolates were obtained from the intestinal tract and pharyngeal colonization. All isolates were multidrug-resistant (MDR), including being resistant to colistin, but they were susceptible to gentamicin and tigecycline. PCR detection showed that all isolates harboured the bla_{KPC-3} gene associated with bla_{SHV-11}, bla_{TEM-1} and bla_{OXA-9}. All *K. pneumoniae* isolates, genotyped by pulsed-field gel electrophoresis and multilocus sequence typing, belonged to the same sequence type (ST)258 clone. From our data and a review of the international literature, *K. pneumoniae* ST258 seems to be the most widespread genetic background for KPC dissemination in Europe.

Keywords: Colistin resistance, colonization, *Klebsiella pneumoniae*, KPC-3, ST258

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Among class A carbapenemases, KPC enzymes have eclipsed all other major groups i.e. GES, SME, IMI and NMC-A, to become the dominant enzymes, with endemic behaviour and/or outbreaks in 27 states in the USA, as well as in China, South America and Israel [1–5]. Since 2005, KPC-positive strains have been isolated in Europe: in France, Germany, the UK, Greece and, more recently, Italy [6–10].

Until very recently, KPC enzymes had only been found in *Enterobacteriaceae*; however, they have now been characterized from *Pseudomonas aeruginosa* [11,12] and from several *Acinetobacter* spp. strains [13].

In 2009–2010 in Italy, a few reports outlined the isolation of two KPC variants, i.e. multidrug-resistant (MDR) KPC-2 and KPC-3, belonging to ST258, and susceptible only to colistin, tigecycline and gentamicin [14,15].

We report a hospital outbreak, occurring between 19 August and 27 October 2010, caused by eight colistin-resistant and carbapenem-resistant *Klebsiella pneumoniae* isolates from eight patients in two different hospitals in Catania (University and Vittorio Emanuele hospitals). A further three isolates were obtained from the intestinal tract and pharyngeal colonization of three patients: these strains were included in the study for their characterization.

The first KPC-producing *K. pneumoniae* isolate was obtained from abdominal drainage fluid of a patient aged 84 years admitted to the intensive-care unit (ICU) of the University Hospital and coming from another Sicilian hospital. It was not possible to establish whether patient 1 could be considered the index case, because an active surveillance culture system was not in place at the time of admission. In the month after the first isolate had been obtained, there was a rapid intrahospital outbreak of these strains isolated from seven patients in different wards (ICU, surgery, internal medicine, transplant unit and paediatric haematology). Subsequently, after 20 days, one case of bloodstream infection in the nephrology ward of Vittorio Emanuele Hospital occurred, in one patient coming from the ICU of the University Hospital, where three other cases were previously confirmed.

The rapid spread of *K. pneumoniae* with this MDR resistance pattern in such a short period caused concern, and activated an investigation to determine the possible source of this outbreak, to establish the increasing risk of transmission, and to start the infection containment procedure.

In fact, infection control measures, including undertaking contact precautions, segregating infected/colonized patients, and using dedicated staff and equipment as much as possible, were implemented at that time [16].

Therefore, all patients included in our study, after infection, were screened weekly for colonization, by the use of pharyngeal and rectal swabs, with a previously published method in which the culture was grown in the presence of a 10-μg disk of imipenem and subsequently plated on MacConkey agar [17]: only three of eight patients were colonized, but it was not possible to establish whether they were colonized at the time of admission. The patients remained colonized until discharge. In all cases, targeted combination treatment, including gentamicin plus tigecycline and/or carbapenems and/or an anti-Gram-positive drug, was used. With regard to the outcomes, seven patients are still alive, and one died from causes not related to the infection.

The identification and antimicrobial susceptibility testing of 11 isolates was preliminarily performed with the Vitek 2 system (bioMerieux, Marcy l'Etoile, France). The identified species level was centrally reconfirmed with the API 20E system (Bio Merieux), and the MICs were determined with a microdilution method, interpreted according to EUCAST guidelines [18]. These isolates were resistant to almost all antibiotics, including colistin (MIC 64 mg/L)

TABLE 1. Clinical characteristics of patients and antibiotic susceptibility of KPC-3-producing *Klebsiella pneumoniae*

					MIC (mg/L)										
Patients	Date	Hospital	Ward	Specimens	IPM	MEM	DOR	ETP	CEF	CAZ	CTX	TZP	TG	CT	CIP	GM
1	19 August 2010	University	ICU	Abdominal drainage	32	64	64	>128	128	>64	>64	>512	1	16	128	2
2	31 August 2010	University	Surgery	CVC	64	64	64	>128	>128	>64	>64	>512	1	32	128	2
3	10 September 2010	University	ICU	Bloodstream	64	128	64	128	>128	>64	>64	>512	1	16	128	2
4	19 September 2010	University	ICU	Bronchial aspirate	64	64	32	>128	>128	>64	>64	>512	1	32	128	2
5	20 September 2010	University	Internal Medicine	Urine	64	64	64	>128	>128	>64	>64	>512	1	8	256	2
6	23 September 2010	University	Transplant	Sputum	32	64	128	128	128	>64	>64	>512	1	64	128	2
7	26 September 2010	University	Paediatric Haematology	Bloodstream	64	64	256	>128	128	>64	>64	>512	1	32	128	2
8	19 October 2010	VE	Nephrology	Bloodstream	128	512	64	512	512	>512	256	>512	1	8	256	2
3[a]	20 October 2010	University	ICU	Pharyngeal swab	32	64	64	128	128	>64	>64	>512	1	32	128	2
7[a]	26 October 2010	University	Paediatric Haematology	Rectal swab	>512	>512	64	>512	256	>512	256	>512	1	8	128	2
4[a]	27 October 2010	University	ICU	Rectal swab	64	512	64	512	512	>512	256	>512	1	16	128	2

IPM, imipenem; MEM, meropenem; DOR, doripenem; ETP, ertapenem; CEF, cefepime; CAZ, ceftazidime; CTX, cefotaxime; TZP, piperacillin–tazobactam; TG, tigecycline; CT, colistin; CIP, ciprofloxacin; GM, gentamicin; VE, Vittorio Emanuele; CVC, central venous catheter; ICU, intensive-care unit.
[a]Colonization.

and carbapenems. All isolates were susceptible only to tigecycline (MIC 1 mg/l) and gentamicin (MIC 2 mg/L); see Table 1.

Analysis of β-lactamase genes by PCR and sequencing revealed the presence of bla_{KPC-3}, bla_{SHV-11}, bla_{TEM-1} and bla_{OXA-9} in all isolates, whereas genes encoding other enzymes (CTX type and VIM type) were not detected.

All *K. pneumoniae* isolates, genotyped after *Xba*I digestion by pulsed-field gel electrophoresis, belonged to the same clone, as they were indistinguishable from each other (100% identity) according to the criteria described previously by Tenover *et al.* [19], and with the multilocus sequence typing (MLST) scheme, according to the protocol described on the *K. pneumoniae* MLST website (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html) [20], all isolates were attributed to sequence type (ST)258.

This study reports, for the first time, an outbreak of KPC-3 colistin-resistant *K. pneumoniae* infection in Italy. However, in our country, there are only a few reports on KPC isolation (all colistin-susceptible). The first case detected was from an inpatient with a complicated intra-abdominal infection in 2009 [10]; subsequently, KPC-3 *K. pneumoniae* ST258 was found in Palermo [14] in 13 patients. Another isolate of KPC *K. pneumoniae* was identified in Rome, but the strain was not characterized [15], and a KPC-2 *K. pneumoniae* isolate was characterized in Switzerland from a Sicilian patient [21]. In all but one of these cases, the strains were ST258, and were susceptible to gentamicin, tigecycline and colistin.

Our isolates were colistin-resistant KPC-3 ST258 *K. pneumoniae*; colistin resistance is still uncommon among KPC producers (an outbreak of KPC-2 ST258 was recently described in Greece), and has been attributed mainly to the modification of lipid A of the outer membrane and the presence of an efflux pump [22,23]. In our study, the exact mechanism of colistin resistance was not investigated.

Even though, in our cases, all patients were cured, we observed that three of them tended to remain colonized until their discharge. It was not possible to continue the surveillance of these patients after their discharge from hospital. The other five patients were negative for all of the surveillance time.

All of these observations, together with the awareness of the limited therapeutic options for treatment these infections, lead to the alarming conclusion that we need urgent action to slow down and control the worldwide and epidemic spread of carbapenamase-producing *Enterobacteriaceae* resistant to colistin, which is one of the few drugs active against MDR Gram-negative bacteria.

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Transparency Declaration

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