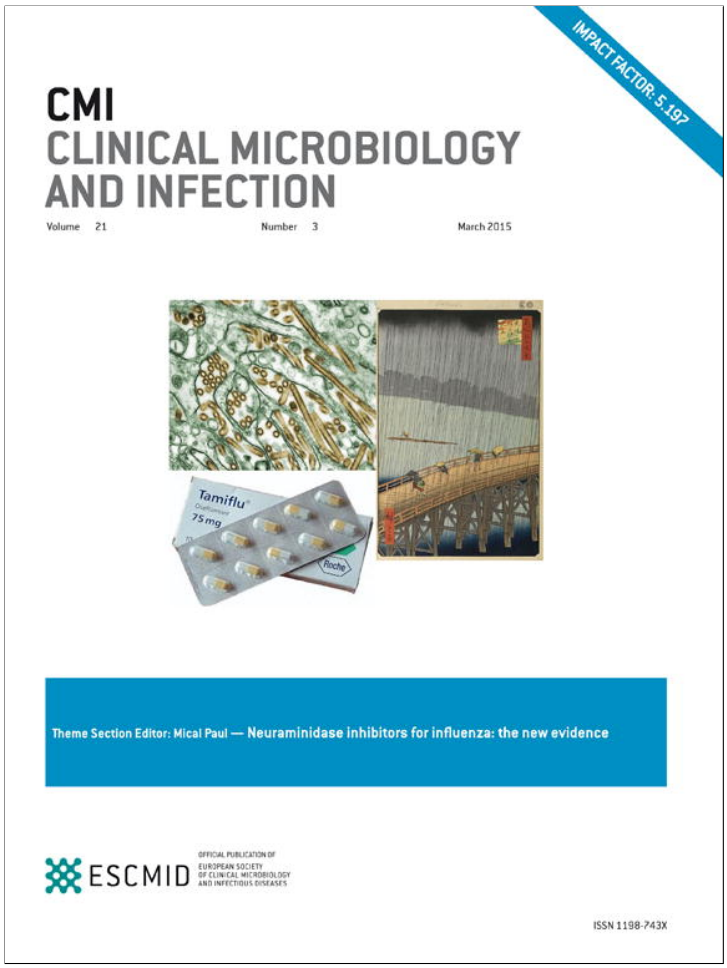

Is the monoclonal spread of the ST258, KPC-3-producing clone being replaced in southern Italy by the dissemination of multiple clones of carbapenem-nonsusceptible, KPC-3-producing *Klebsiella pneumoniae*?

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Spread of carbapenemase-producing *Klebsiella pneumoniae* has been identified as an issue of serious worldwide concern from clinical and public health perspectives [1]. Italy is a country endemic for and a reservoir of *K. pneumoniae* carbapenemases (KPC) [1,2].

In March 2014, during a period of active surveillance in five neonatal intensive care units (NICUs) of Palermo, Italy, three carbapenem-nonsusceptible isolates of *K. pneumoniae* (CR-Kp) were identified from rectal swabs of three infants admitted to two different NICUs. The first isolate was detected from an outborn infant admitted at the first NICU on March 3; the second isolate was identified on March 10 from an infant staying in the same NICU as a probable secondary colonization case. Of interest, a carbapenem-nonsusceptible *Escherichia coli* isolate was also detected in the rectal swab of the first infant. The third *K. pneumoniae* isolate was identified on March 27 from an outborn infant admitted to a second NICU. Isolation/cohorting of colonized patients and reinforcement of infection control measures were able to interrupt the transmission in both NICUs, and no further cases were identified in the following 3 months.

Molecular typing showed that the three *K. pneumoniae* isolates carried bla_{KPC-3} and belonged to two different sequence types (STs), ST307 and ST323 (Table 1). This finding, along with the report by Gona *et al.* [3] of a multiclonal cluster of cases of colonization and infection by KPC-producing *K. pneumoniae* (KPC-Kp) at the Mediterranean Institute for Transplantation and Advanced Specialized Therapies (ISMETT) in Palermo, Italy, prompted us to look for a possible spread of KPC-Kp isolates other than ST258 in the healthcare facilities of this city.

All the CR-Kp isolates identified in April and May 2014 by the microbiology laboratories of the two largest acute general hospitals of Palermo (ARNAS 'Civico, Di Cristina & Benfratelli' and Azienda Ospedaliera 'Villa Sofia-V. Cervello') were collected and characterized. Antibiotic susceptibility testing was performed by the VITEK-2 automated system (bio-Mérieux, Marcy l'Etoile, France) and the results interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (http://www.eucast.org/clinical_breakpoints/).

Polymerase chain reaction (PCR) screening for carbapenemase encoding genes of classes A (bla_{KPC}, bla_{GES}), B (bla_{VIM}, bla_{IMP}, bla_{NDM}) and D (bla_{OXA-48}) for extended-spectrum β-lactamases encoding genes bla_{TEM}, bla_{OXA}, bla_{SHV} and bla_{CTX-M} and for plasmid-mediated quinolone resistance (PMQR) genes *qnrA*, *qnrB* and *aac(6′)-Ib-cr* was performed [4]. Multiplex PCRs for the detection of plasmid-mediated 16S RNA methylase genes (*armA, rmtA, rmtB, rmtC, rmtD* and *npmA*) were also carried out as previously described [5]. The identity of resistance genes was confirmed by DNA sequence analysis. To assess clonality, the isolates were submitted to pulsed-field gel electrophoresis (PFGE) after *Xba*I DNA digestion. Moreover, multilocus sequence typing (MLST) was performed according to the protocol described online on the *K. pneumoniae* MLST website (http://www.pasteur.fr/recherche/genopole/ PF8/mlst/Kpneumoniae.html).

Nineteen isolates of CR-Kp from 16 different patients were identified in the two hospitals in the period under study. The main characteristics of the three NICU isolates and 16 further isolates from the two hospitals, each from an individual patient or site, are summarized in Table 1. Besides those detected in the NICUs, most isolates (12/16) were from three adult intensive care units (ICUs). However, additional isolates were identified from four different wards (Table 1). Seven of 16 isolates belonged to ST307, six to ST258 and three to ST273.

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TABLE 1. Main phenotypic and genetic characteristics of the isolates of carbapenem-nonsusceptible isolates of *Klebsiella pneumoniae* identified in Palermo, Italy, March to May 2014

Ward	Clinical sample	ST	Resistance genetic determinants				MICs (μg/mL)										
			KPC-3	CTX-M-15	*aac(6′)-Ib-cr*	*qnrB*	CTX	CAZ	FEP	ETP	IPM	MEM	AK	GN	CIP	TIG	FOS	CT	SXT
NICU	RS	307	+	+	+	+	≥64	≥64	≥64	≥8	≥16	≥16	≤2	≥16	≥4	2	128	≤0.5	≥320
NICU	RS	307	+	+	+	+	≥64	≥64	32	≥8	8	≥16	≤2	≥16	≥4	2	≤16	≤0.5	≥320
NICU	RS	323	+	+	−	+	≥64	≥64	≥64	≥8	≥16	≥16	≤2	≥16	−	4	≥256	2	≥320
ICU	Blood	307	+	+	+	+	≥64	≥64	≥64	≥8	≥16	≥16	8	≥16	≥4	2	32	≥16	40
ICU	BA	307	+	+	+	+	≥64	≥64	≥64	≥8	≥16	≥16	4	≥16	≥4	2	64	≥16	40
ICU	BA	307	+	+	+	+	≥64	≥64	≥64	≥8	≥16	≥16	≤2	≥16	≥4	≥8	32	2	40
Cancer surg	WS	258	+	−	−	−	≥64	≥64	2	≥8	≥16	8	≥64	2	≥4	4	≥256	≤0.5	≥320
ICU	BA	273	+	−	+	+	8	≥64	2	≥8	≥16	8	≤2	≥16	≥4	2	64	8	≤20
ICU	BA[a]	307	+	+	+	+	16	≥64	8	≥8	≥16	≥16	4	≥16	≥4	2	64	≥16	40
ICU	U[a]	307	+	+	+	+	16	≥64	8	≥8	≥16	≥16	≤2	≥16	≥4	−	≤16	≥16	≤20
ICU	U	258	+	−	+	−	16	≥64	8	≥8	≥16	≥16	≥64	−	≥4	−	128	≥0.5	≥320
ICU	WS	307	+	+	+	+	16	≥64	8	≥8	≥16	≥16	8	≥16	≥4	−	32	≥16	≤20
ICU	BA	273	+	−	−	−	8	≥64	2	≥8	≥16	≥16	≤2	≥16	≥4	2	64	≥16	80
ICU	Blood	273	+	−	−	−	8	≥64	2	4	8	≥16	≤2	≥16	≥4	−	64	≥16	80
LTCF	U	258	+	−	+	−	≥64	≥64	≥64	≥8	≥16	≥16	≥64	2	≥4	≥8	32	≥16	≥320
Neurosurg	U	307	+	+	+	+	16	≥64	8	≥8	≥16	≥16	8	≥16	≥4	−	32	≥16	≤20
ICU	U	258	+	−	+	−	16	≥64	≥64	≥8	≥16	≥16	≥64	−	≥4	2	128	≥16	≥320
Inf dis	SP	258	+	−	+	−	16	≥64	≥64	≥8	≥16	≥16	≥64	2	≥4	2	128	≥16	≥320
ICU	RS	258	+	−	+	−	16	≥64	≥64	≥8	≥16	≥16	≥64	−	≥4	2	128	≤0.5	≥320

NICU, neonatal intensive care unit; ICU, intensive care unit; Cancer surg, cancer surgery unit; LTCF, long-term care facility; Neurosurg, neurosurgery unit; Inf dis, infectious disease unit; RS, rectal swab; BA, bronchial aspirate; WS, wound swab; U, urine; SP, sputum; MIC, minimum inhibitory concentration; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; ETP, ertapenem; IPM, imipenem; MEM, meropenem; AK, amikacin; GN, gentamicin; CIP, ciprofloxacin; TIG, tigecycline; FOS, fosfomycin; CT, colistin; SXT, sulfamethoxazole-trimethoprim.
[a]Isolates from the same patient.

All isolates were KPC-3 producers. Moreover, the ST307 isolates as well as the ST323 NICU isolate harboured the $bla_{\text{CTX-M-15}}$ gene. The array of the PMQR genes differed by ST; indeed, *K. pneumoniae* ST307 tested positive for *aac(6′)-Ib-cr* and *qnrB*, ST258 for *aac(6′)-Ib-cr* only, ST323 for *qnrB* only, whereas ST273 tested negative for the PMQR genes. None of the *K. pneumoniae* isolates carried any of the plasmid-mediated 16S RNA methylase genes tested in this study. The carbapenem-nonsusceptible *E. coli* strain from the NICU index case proved to carry $bla_{\text{KPC-3}}$.

PFGE of KPC-Kp isolates yielded electrophoretic patterns that coincided with attribution to STs.

All KPC-Kp isolates, except for the ST323 isolate, were resistant to ciprofloxacin. The resistance pattern to aminoglycosides appeared to be associated to ST; indeed, the isolates belonging to STs other than ST258 were susceptible to amikacin and resistant to gentamicin, whereas the ST258 isolates were susceptible to gentamicin but resistant to amikacin (Table 1). Conversely, nonsusceptibility to tigecycline and colistin was not related to ST and involved 5 and 12 isolates, respectively (Table 1). The KPC *E. coli* strain was susceptible to cefepime and all non-β-lactams and, except for meropenem, exhibited lower MIC values for carbapenems compared with those observed in KPC-Kp isolates (ertapenem, 1 μg/mL; imipenem, 4 μg/mL).

Some troublesome considerations arise from our preliminary investigation about the evolving epidemiology of KPC-Kp in an area that has been previously reported as highly endemic [6]. First, the monoclonal spread of the successful pandemic ST258 clone is apparently being replaced by a simultaneous dissemination of multiple clones of KPC-Kp, a probable consequence of the horizontal transfer of mobile genetic elements carrying the bla_{KPC} gene. This is confirmed by the isolation of a KPC-producing *E. coli* isolate from a NICU infant colonized with KPC-Kp ST307. Our finding confirms a previous report by Gona *et al.* [3] about co-carriage of multiple clones of KPC-Kp including ST307 and KPC–*E. coli* by patients admitted to ISMETT, a highly specialized organ transplant center in Palermo. ST307, in particular, has proved to have entered different hospitals and wards, including NICUs. Moreover, it is noteworthy to highlight that *K. pneumoniae* ST273 had been identified in 2011 in two ICUs of Palermo as a colistin-resistant but carbapenem-susceptible clone [5].

Second, colistin resistance was shown to be highly prevalent within KPC-Kp isolates. Treatment with colistin has been proven to be a major risk factor for the emergence of resistance and the increasing need to use colistin for the treatment of carbapenem-resistant gram-negative organisms, such as Enterobacteriaceae and *Acinetobacter baumannii,* is likely in turn

driving healthcare-associated emergence and spread of colistin resistance.

A prospective collection of isolates is ongoing to answer questions about cross-transmission of multiple clones of KPC-Kp and consequent clonal expansion, lateral transmission of mobile genetic resistance determinants or both. Our preliminary data emphasize the increasing difficulties in controlling the spread of KPC-Kp [1].

Transparency declaration

All authors report no conflicts of interest relevant to this article.

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