

Emergence of an extensive drug resistant ArmA- and KPC-2-producing
ST101 *Klebsiella pneumoniae* clone in Italy

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Sir,

KPC-producing *Klebsiella pneumoniae* (KPC-Kp) strains have become the most frequent class A carbapenemase-producing pathogens worldwide. Since the first KPC, described in 2001 in the USA, there are currently 10 KPC-type enzymes (KPC-2 to KPC-11) (<http://www.lahey.org/studies>); among them KPC-2 and KPC-3 variants are the most common in clinical specimens, accounting for most epidemic outbreaks in the USA and Europe. Kp-KPC dissemination is associated with a highly epidemic international clone of multidrug-resistant (MDR) *K.pneumoniae* sequence type (ST 258), with susceptibility observed only to colistin, tigecycline and gentamicin.¹ Furthermore, recent Italian studies described the dissemination and the predominance of a KPC-2 variant belonging to ST 101.^{2,3} This MDR clone has recently acquired a new resistance determinant, the 16S rRNA methylase ArmA, encoded by the *armA* (aminoglycoside resistance methyltransferase) gene, conferring the extensive drug-resistance (XDR) phenotype.

ArmA gene was found on the same plasmid of the KPC-2 strains previously isolated in Italy³ and China⁴ and on different plasmids in isolates from Poland.⁵

In the present study, we describe five *K. pneumoniae* isolates from 5 patients in two Italian hospitals (IRCCS Neurolesi, Messina and Careggi Hospital, Florence), harboring *bla*_{KPC-2} and *armA* genes in isolates of sequence type (ST101) belonging to a clonal complex different from those containing the habitual sequence clone ST258 isolated in Italy.^{6,7}

The identification and antimicrobial susceptibility testing of the 5 isolates were preliminarily performed by the Vitek 2 system (bioMérieux, Marcy l'Etoile, France). The identified species level was centrally reconfirmed by API 20E (Bio Mérieux (SA- Marcy l'Etoile, France) and the Minimum Inhibitory Concentrations (MICs) were determined by microdilution method interpreted according to EUCAST guidelines v.3.1, 2013. These isolates presented a profile of XDR; two of them were resistant all classes of antibiotics except of tigeciclina and colistin and three were resistant to colistin. All strains were also highly resistant to gentamicin, amikacin and kanamicin (MICs between 128 and ≥ 512 mg/l) in addition to carbapenems (Table 1).

Multilocus sequence type, determined according to the protocol described on the *K. pneumoniae* multilocus sequence typing (MLST) website (<http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html>)⁷, revealed that all isolates belonged to ST101, an ST already found in other Italian hospitals.^{2,3}

Our 5 ST101 strains also possessed an identical macrorestriction profile by PFGE, performed after *Xba*I digestion, demonstrating the strong epidemic character of this clone.⁷

In order to fully characterize the profile of resistance of these strains, amplification and sequencing for detection of carbapenemases (KPC, IMP, VIM, OXA), ESBLs (TEM, SHV, CTX-M)⁸, and aminoglycoside modifying enzymes (AAC, APH, AAD, 16S methylase), was performed using previously described primers.^{5,9}

All strains harboured KPC allele 2, TEM-1, APHA1 and ArmA contributing to the complex phenotype of resistance of these strains. To better characterize the localization of KPC-2, which was found as part of the 10 kb Tn3-like element Tn4401, PCR assays, with specific primers for Tn4401, were performed.¹⁰ Amplicon sequencing revealed that the *bla*_{KPC-2} gene was in all cases embedded in a Tn4401-like transposon. Published papers reported that Tn4401 has been found on IncN and IncFII_k plasmids (pKpQIL-IT, S9, S12, S15, pKPN101-IT), therefore for the detection of these plasmids we used the following primers: S9-F (5'-GCATTGACCTTGGCATCTTC-3'), S9-R (5'-GTGATTTACACCACCACCTCATCA-3'); (S12-F (5'-CGGACGGTTGATCAGAATCGGATG-3'); S12-R (5'-ATTGCTGCTGTAGGGGCTGTCATTCT-3'); S15-F (5'-GGGGATCGGTTTTCGCCAGCA-3'); S15-R (5'-GCTTTACCGAGGGAGAATGGCTACTG-3'); pSLMT-F (5'-GCATTGACCTTGGCATCTTC-3'), pSLMT-R (5'-CTAATAAACTGGTGCTCGGACAGA-3'); pNYC-F (5'-GCATCAAACGGAAGCAAAAG-3'), pNYC-R (5'-CTTAGCAAATGTGGTGAACG-3'); pKpQIL-IT-F (5'-GGTTATTGGGTGAGGTAAGCATTAGGCG-3'); pKpQIL-IT-R (5'-GAGTGAGCGAGGAAGCACCAGGG-3') designed on the basis of published sequences and

specific for each plasmid (GenBank accession no: FJ223607.1, FJ223605.1, FJ223606.1, HQ589350.1, EU176011.1, GU595196.1 respectively.).¹⁰

In all strains amplicon sequence analysis (1071 bp) showed that plasmid sequences matched the pKpQIL-IT plasmid, circulating in Italy and already detected in a strain of *K.pneumoniae* ST258 background.⁶

Furthermore, as regards the coexistence of methylase *armA* in KPC producing *K.pneumoniae*, already found to be associated on pETKp90 and pETKp50 plasmids and on the same pKP048 plasmid^{5,4}, southern blot experiments on genomic and plasmid DNAs with the *bla*_{KPC}, *armA* and pKpQIL-IT probes obtained by PCR fragments were performed. A hybridization signal on the same fragment of 97.kb kb in all strains was found, suggesting that these genes are located on the same element. Further studies are in progress in our laboratory in order to identify the element carrying the *armA* gene.

In conclusion, our findings suggest that KPC-2 and ArmA producing *K.pneumoniae* strains are emerging in a ST101 background. These clones are extensively resistant, also due to lateral gene transfer, rendering all families of drugs useless and requiring only antibiotic combinations (Ceccarelli G, Falcone M, Giordano A, Mezzatesta ML, Caio C, Stefani S and Venditti M, unpublished results). Furthermore, the diffusion of these epidemic clones requires the activation of infection control procedures.

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Transparency Declaration

None to declare.

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Table 1. Clinical characteristics of patients and antibiotic susceptibility of KPC-2 and ArmA producing *K.pneumoniae*

Patients	Date	Place	Wards	Specimens	PFGE	ST	MIC (mg/L) *												
							IPM	MEM	ETP	TZP	CEF	CAZ	CTX	CT	TGC	LVX	GEN	AMK	KAN
1	23/11/2011	Messina	Medicine	Bronchial aspirate	A	101	4	64	64	512	>512	>512	>512	0,06	0,19	32	512	512	512
2	07/09/2011	Florence	ICU	Pharyngeal swab	A	101	128	32	512	256	64	>512	64	0,12	1	16	256	128	256
3	11/11/2011	Florence	ICU	Blood	A	101	128	256	>512	512	128	>512	>512	16	0,25	16	256	512	>512
4	10/12/2011	Florence	Infectious Diseases	Bronchial aspirate	A	101	128	256	>512	>512	128	>512	>512	64	0,25	32	512	>512	512
5	13/01/2012	Florence	Oncology	Blood	A	101	128	32	256	256	64	>512	64	4	2	16	256	128	256

Abbreviations : IPM, imipenem; MEM, meropenem; ETP, ertapenem; TZP, piperacillin/tazobactam, CEF, cefepime; CAZ, ceftazidime; CTX, cefotaxime; CT, colistin;

TGC, tigecycline; LVX, levofloxacin; GEN, gentamicin; AMK Amikacin; KAN, kanamicin; ICU intensive care units.

* MIC was performed by Broth Microdilution Method

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