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

Maria Lina MEZZATESTA[1*], Floriana GONA[1], Carla CAIO[1], Chiara ADEMBRI[2], Pia DELL'UTRI[3], Maria SANTAGATI[1] and Stefania STEFANI[1].

1) Department of Bio-Medical Sciences, section of Microbiology, University of Catania, Italy; 2) Careggi Hospital, Florence, Italy 3) IRCCS Neurolesi, Messina, Italy

* Corresponding Author:

Prof. Maria Lina MEZZATESTA - Department of Bio-Medical Sciences (Section Microbiology) – University of Catania[1], Via Androne 81, 95124 Catania Italy. Phone +39 095 2504733. Fax +39 095 2504733 Email: mezzate@unict.it

Keywords: Multidrug-resistance, 16S rRNA methylase, carbapenemase, Tn*4401*.

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 *arm*A (aminoglycoside resistance methyltransferase) gene, conferring the extensive drug-resistance (XDR) phenotype.

*Arm*A gene was found on the same plasmid of the KPC-2 strains previously isolated in Italy[3] and China[4] 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 *arm*A 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 (bioMerieux, Marcy l'Etoile, France). The identified species level was centrally reconfirmed by API 20E (Bio Merieux (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 Tn*4401*, PCR assays, with specific primers for Tn*4401*, were performed. [10] Amplicon sequencing revealed that the bla_{KPC-2} gene was in all cases embedded in a Tn*4401*-like transposon. Published papers reported that Tn*4401* 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 *arm*A 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*$_{KPC}$, *arm*A 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 *arm*A 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.

Acknowledgments

The authors express their gratitude to Antony Bridgewood for language revision.

Funding

This work was supported by the Italian Minister of Universities funding for SS and MM.

Transparency Declaration

None to declare.

References

1. Chen LF, Anderson DJ, Paterson DL. Overview of the epidemiology and the threat of Klebsiella pneumoniae carbapenemases (KPC) resistance. *Infect Drug Resist* 2012; **5**: 133-41
2. Mammina C, Bonura C, Aleo A *et al*. Sequence type 101 (ST101) as the predominant carbapenem-non-susceptible *Klebsiella pneumoniae* clone in an acute general hospital in Italy. *Int J Antimicrob Agents* 2012; **39**: 543-5.
3. Frasson I, Lavezzo E, Franchin E *et al*. Antimicrobial treatment and containment measures for an extremely drug-resistant *Klebsiella pneumoniae* ST101 isolate carrying pKPN101-IT, a novel fully sequenced *bla*(KPC-2) plasmid. *J Clin Microbiol* 2012; **50**: 3768-72
4. Jiang Y, Yu D, Wei Z *et al*. Complete nucleotide sequence of *Klebsiella pneumoniae* multidrug resistance plasmid pKP048, carrying *bla* $_{KPC-2}$, *bla*$_{DHA-1}$, *qnr*B4, and *arm*A. *Antimicrob Agents Chemother* 2010; **54**: 3967–3969.
5. Zacharczuk K, Piekarska K, Szych J *et al*. Emergence of *Klebsiella pneumoniae* coproducing KPC-2 and 16S rRNA methylase ArmA in Poland. *Antimicrob Agents Chemother* 2011; **55**: 443-6.
6. García-Fernández A, Villa L, Carta C *et al*. *Klebsiella pneumoniae* ST258 producing KPC-3 identified in Italy carries novel plasmids and OmpK36/OmpK35 porin variants. *Antimicrob Agents Chemother* 2012; **56**: 2143-5.
7. Mezzatesta ML, Gona F, Caio C *et al*. Outbreak of KPC-3-producing, and colistin resistant, *Klebsiella pneumoniae* infections in two Sicilian hospitals. *Clin Microbiol Infect* 2011; **17**: 1444-7.
8. Gona F, Mezzatesta ML, Corona D *et al*. *Klebsiella pneumoniae* ESBL producers responsible for severe UTIs in a renal transplant unit. *Infection* 2011; **39**: 83–85.

9. Perilli M, Mezzatesta ML, Falcone M *et al*. Class I integron-borne *bla*$_{\text{VIM-1}}$ carbapenemase in a strain of *Enterobacter cloacae* responsible for a case of fatal pneumonia. *Microb Drug Resist* 2008; **14**: 45-7.

10. Gootz TD, Lescoe MK, Dib-Hajj F *et al*. Genetic organization of transposase regions surrounding *bla*KPC carbapenemase genes on plasmids from Klebsiella strains isolated in a New York City hospital. *Antimicrob Agents Chemother* 2009; **53**: 1998-2004.

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