gene coding for a 244-amino-acid rRNA methylase was detected, which presented 93.9% identity with the Erm(T) protein of the *Streptococcus pyogenes* plasmid pGA2000 (accession number JF308631). Further analysis of the nucleotide sequence of the *erm*(T) gene indicated 83.7%–95.5% identity to the *erm*(T) genes described in lactobacilli,[5] streptococci[3] and staphylococci.[6] A translational attenuator is normally found upstream of the *erm*(T) gene. However, there is a 75 bp deletion in pFS39, which completely eliminated the regulatory peptide (Figure 1b). Moreover, the deletion removed inverted repeat (IR) 1 in the leader sequence of *erm*(T), but IR2, IR3 and IR4 remained intact. The deletion of the regulatory peptide and IR1 is expected to disrupt the translational attenuation mechanisms, leading to constitutive expression of *erm*(T) in pFS39. A similar finding has been reported with *erm*(T) in pKKS25, which was identified in methicillin-resistant *Staphylococcus aureus* ST398.[6]

The resistance gene region of plasmid pFS39 was related to that of plasmid pB1002, except that there was an insertion of *erm*(T) between the bla_{ROB-1} and IS*Apl1* in pFS39 and that the transposase gene of IS*Apl1* in pFS39 had an internal deletion of 659 bp, while the 3′ and 5′ ends were intact. The truncated IS*Apl1* linked with bla_{ROB-1} had been described on *Actinobacillus porcitonsillarum* plasmids[10] and another *H. parasuis* plasmid (accession number HQ015159). It is likely that several recombination events took place that finally resulted in the truncation of IS*Apl1*, but also in the integration of the resistance genes into a pFS39 precursor plasmid.

In conclusion, this is to the best of our knowledge the first report of the *erm*(T) gene in a Gram-negative bacterium and also the first description of the *erm*(T) gene located on a small plasmid in *H. parasuis*. This finding provides further evidence for gene transfer between Gram-positive and Gram-negative bacteria, which has been observed with other resistance genes. For example, the *tet*(L) gene in *Pasteurella multocida* and *Mannheimia haemolytica*[11] and the *cfr* gene in *Proteus vulgaris* and *Escherichia coli*[12,13] all likely originated from Gram-positive bacteria. These observations suggested that antibiotic resistance gene transfer between Gram-positive and Gram-negative bacteria is more common than previously thought. Furthermore, the co-location of the genes *erm*(T) and bla_{ROB-1} on the same plasmid could facilitate the spread of these resistance genes by co-selection. Further studies are warranted to show how widespread the *erm*(T) gene is in *H. parasuis* and other Gram-negative bacteria.

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Emergence of an extensively 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 Biomedical Sciences, Section of Microbiology, University of Catania, Catania, Italy;* [2]*Careggi Hospital, Florence, Italy;* [3]*IRCCS Neurolesi, Messina, Italy*

*Corresponding author. Tel: +39-095-2504733; Fax: +39-095-2504733; E-mail: mezzate@unict.it

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Sir,

KPC-producing *Klebsiella pneumoniae* 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. Dissemination of KPC-producing *K. pneumoniae* is associated with a highly epidemic international clone of multidrug-resistant (MDR) *K. pneumoniae* ST258 (where ST stands for sequence type), with susceptibility observed only to colistin, tigecycline and gentamicin.[1] Furthermore, recent Italian studies have described the dissemination and the predominance of a KPC-2 variant belonging to ST101.[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 extensively drug-resistant (XDR) phenotype.

The *armA* 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 five patients in two Italian hospitals (IRCCS Neurolesi, Messina, and Careggi Hospital, Florence) harbouring bla_{KPC-2} and *armA* genes in isolates of 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 five isolates were preliminarily performed by the Vitek 2 system (bioMérieux, Marcy l'Étoile, France). The identified species level was centrally reconfirmed by API 20E (bioMérieux) and the MICs were determined using the broth microdilution method, interpreted according to EUCAST guidelines (v.3.1, 2013). These isolates presented a profile of XDR; two of them were resistant to all classes of antibiotics except tigecycline and colistin and three were resistant to colistin. All strains were also highly resistant to gentamicin, amikacin and kanamycin (MICs between 128 and ≥512 mg/L) in addition to carbapenems (Table 1).

Multilocus STs, determined according to the protocol described on the *K. pneumoniae* multilocus sequence typing (MLST) web site (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 five ST101 strains also possessed an identical macrorestriction profile by PFGE, performed after XbaI 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 and OXA), extended spectrum β-lactamases (ESBLs; TEM, SHV and CTX-M)[8] and aminoglycoside-modifying enzymes (AAC, APH, AAD and 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

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

Patient	Date	Place	Ward	Specimen	PFGE	ST	MIC (mg/L)[a]												
							IPM	MEM	ETP	TZP	FEP	CAZ	CTX	CST	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	intensive care unit	pharyngeal swab	A	101	128	32	512	256	64	>512	64	0,12	1	16	256	128	256
3	11/11/2011	Florence	intensive care unit	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

IPM, imipenem; MEM, meropenem; ETP, ertapenem; TZP, piperacillin/tazobactam; FEP, cefepime; CAZ, ceftazidime; CTX, cefotaxime; CST, colistin; TGC, tigecycline; LVX, levofloxacin; GEN, gentamicin; AMK, amikacin; KAN, kanamycin.

[a]MICs were determined using the broth microdilution method.

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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 have 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′; and pKpQIL-IT-R, 5′-GAGTGAGCGAGGAAGCACCAGGG-3′—designed on the basis of published sequences and specific for each plasmid (GenBank accession numbers FJ223607.1, FJ223605.1, FJ223606.1, HQ589350.1, EU176011.1 and 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,[4,5] 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 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 an ST101 background. These clones are extensively resistant, also due to lateral gene transfer, rendering all families of drugs useless and requiring only antibiotic combinations (G. Ceccarelli, M. Falcone, A. Giordano, M. L. Mezzatesta, C. Caio, S. Stefani and M. Venditti, unpublished results). Furthermore, the diffusion of these epidemic clones requires the activation of infection control procedures.

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Isolation of carbapenem-resistant NDM-1-positive *Providencia rettgeri* in Mexico

Humberto Barrios[1]†, Ulises Garza-Ramos[1]†, Fernando Reyna-Flores[1], Alejandro Sanchez-Perez[1], Teresa Rojas-Moreno[1], Elvira Garza-Gonzalez[2], Jorge Martín Llaca-Diaz[3], Adrian Camacho-Ortiz[4], Santos Guzmán-López[5] and Jesus Silva-Sanchez[1]*

[1]Instituto Nacional de Salud Pública (INSP), CISEI, Cuernavaca, Morelos, México; [2]Servicio de Gastroenterología, Hospital Universitario Dr. José Eleuterio González, Universidad Autónoma de Nuevo León, Nuevo León, México; [3]Departamento de Patología Clínica, Hospital Universitario Dr. José Eleuterio González, Universidad Autónoma de Nuevo León, Nuevo León, México;

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