

Large Nosocomial Outbreak of Colistin-Resistant, Carbapenemase-Producing *Klebsiella pneumoniae* Traced to Clonal Expansion of an *mgrB* Deletion Mutant

Tommaso Giani,^a Fabio Arena,^a Guendalina Vaggelli,^b Viola Conte,^a Adriana Chiarelli,^a Lucia Henrici De Angelis,^a Rossella Fornaini,^c Maddalena Grazzini,^d Fabrizio Niccolini,^d Patrizia Pecile,^b Gian Maria Rossolini^{a,b,e,f}

Department of Medical Biotechnologies, University of Siena, Siena, Italy^a; Clinical Microbiology and Virology Unit,^b Hospital Pharmacy Unit,^c and Hospital Medical Direction,^d Florence Careggi University Hospital, Florence, Italy; Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy^e; Don Carlo Gnocchi Foundation, Florence, Italy^f

We describe a large hospital outbreak (93 bloodstream infections) of colistin-resistant *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* isolates which was mirrored by increased colistin consumption. The outbreak was mostly traced to the clonal expansion of an *mgrB* deletion mutant of an ST512 strain that produced KPC-3.

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP), especially its isolates that produce *K. pneumoniae* carbapenemase (KPC)-type enzymes and belong to clonal complex 258 (CC258), are challenging pathogens due to the limited treatment options, high mortality rates, and potential for rapid dissemination in health care settings (1–4).

Polymyxins are among the few agents that retain activity against CRKP, and they are a key component of anti-CRKP regimens (5, 6). As a likely consequence of increased polymyxin usage, infections caused by polymyxin-resistant (colistin-resistant [COL^r]) strains of CRKP have been increasingly reported (7–10). In Italy, where KPC-producing CRKP is endemic, a remarkable dissemination of COL^r CRKP has recently been reported at a countrywide level (11).

Acquired polymyxin resistance usually results from modification of the lipid A polymyxin target following mutational upregulation of the endogenous lipid A modification systems (12). Inactivation of the *mgrB* gene, which encodes a negative feedback regulator of the PhoQ/PhoP signal transduction system, was found to be one of the most common mutational mechanisms responsible for polymyxin resistance among clinical isolates of CRKP (12–14). Alterations of the PmrA/PmrB and other two-component signal transduction systems have also been identified as causes of polymyxin resistance in *K. pneumoniae* (15–19).

In this paper, we report on a large hospital outbreak of COL^r CRKP which mirrored colistin consumption in the hospital and was traced mostly to the clonal expansion of a COL^r *mgrB* deletion mutant of a CC258 strain of *K. pneumoniae* producing the KPC-3 carbapenemase.

Data on bacterial infections were retrospectively obtained from our hospital laboratory records. Only episodes of bloodstream infections that occurred in different patients were counted. Bacterial identification was routinely carried out by using the Vitek2 or Vitek-MS system (bioMérieux, Marcy l’Etoile, France), and susceptibility testing was routinely carried out with the Vitek2 system. Interpretation of results was performed according to the EUCAST breakpoints (www.eucast.org/clinical_breakpoints/).

The first case of CRKP in the hospital was reported in late 2008 (20). An increased number of cases were observed in 2009 and led to a large hospital outbreak during the following years. Considering bloodstream infections (BSI) caused by *K. pneumoniae*, the

proportion of cases caused by CRKP exhibited a progressive and remarkable increase from 2009 to 2012 and thereafter stabilized to approximately two-thirds of cases (Table 1). Interestingly, an absolute increase in the number of *K. pneumoniae* BSI was initially observed, and this mirrored the emergence of CRKP. This trend was apparently reversed in 2013, when the overall number of *K. pneumoniae* BSI decreased (Table 1).

The first case of *K. pneumoniae* BSI caused by a COL^r strain was observed in 2010. The proportion of these cases (i.e., those caused by COL^r strains) exhibited a remarkable increase in 2012 and remained high in 2013 (Table 1). Overall, a total of 93 cases of BSI caused by COL^r CRKP (49.7% of all CRKP BSI) were observed in the study period. Patients with BSI caused by COL^r CRKP were reported from 38 hospital wards, of which 29 also reported cases of BSI by COL^s CRKP and 9 reported only cases of BSI by COL^r CRKP. To our best knowledge, this is the largest outbreak of COL^r CRKP thus far reported. Colistin MICs ranged from 4 to >16 µg/ml (MIC₅₀, >16 µg/ml). Notably, colistin resistance was only observed among CRKP.

According to the records of the hospital pharmacy, colistin consumption showed a remarkable increase from 2009 to 2011 and thereafter decreased and stabilized (Table 1). Overall, consumption data were consistent with those reported at the national level (ESAC-NET system) for hospital consumption of polymyxins (http://www.ecdc.europa.eu/en/activities/surveillance/ESAC-Net/about_ESAC-Net/Pages/about_network.aspx). Data on colis-

Received 21 April 2015 Returned for modification 14 May 2015

Accepted 14 July 2015

Accepted manuscript posted online 22 July 2015

Citation Giani T, Arena F, Vaggelli G, Conte V, Chiarelli A, Henrici De Angelis L, Fornaini R, Grazzini M, Niccolini F, Pecile P, Rossolini GM. 2015. Large nosocomial outbreak of colistin-resistant, carbapenemase-producing *Klebsiella pneumoniae* traced to clonal expansion of an *mgrB* deletion mutant. *J Clin Microbiol* 53:3341–3344. doi:10.1128/JCM.01017-15.

Editor: K. C. Carroll

Address correspondence to Gian Maria Rossolini, gianmaria.rossolini@unifi.it.

T.G. and F.A. contributed equally to this work.

Copyright © 2015, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JCM.01017-15

TABLE 1 Observed BSI caused by *K. pneumoniae* during the study period^a

Yr	No. of <i>K. pneumoniae</i> BSI	No. (%) of <i>K. pneumoniae</i> isolates that were:			
		Carbapenemase sensitive	Carbapenemase resistant ^b	COL ^r CRKP ^{b,c}	Colistin consumption ^d
2009	29	28 (97)	1 (3)	0 (0; 0)	0.004
2010	49	38 (78)	11 (22)*	1 (3; 9)	0.013
2011	76	44 (58)	32 (42)*	4 (5; 12)	0.018
2012	128	46 (36)	82 (64)*	53 (41; 65)*	0.014
2013	93	32 (34)	61 (66)	35 (38; 57)	0.015
Total	375	188 (50)	187 (50)	93 (25; 50)	

^a Numbers and proportions of BSI cases caused by carbapenem-susceptible, carbapenem-resistant, and carbapenem- and colistin-resistant (COL^r CRKP) strains. For patients with recurrent BSI episodes, only the first episode was considered.

^b An asterisk indicates that the difference in the proportion of resistant isolates was statistically significantly different ($P < 0.05$) from that for the previous year. For statistical analysis, the chi-squared test with Yates' correction or Fisher's exact test (as appropriate) was used.

^c Proportions are reported in relation to both *K. pneumoniae* BSI and CRKP BSI. (Values are shown in parentheses and separated by semicolons.) COL^r *K. pneumoniae* was only observed among CRKP cases.

^d Data on colistin consumption in the hospital during the study period, expressed as the defined daily dose per 1,000 inhabitants per day, are also reported.

tin use could be retrieved for 38 patients with a BSI caused by COL^r CRKP. These data revealed that in most cases (35 of 38; 92%), the patient had not received colistin prior to isolation of COL^r CRKP (since admission or, in case of prolonged or repeated admissions, for a period of up to 3 months), while in only 3 cases had the patient received colistin (range, 5 to 32 days). These findings are in agreement with previous observations from smaller outbreaks (21, 22).

The COL^r CRKP isolated from BSI during 2013 and a portion of those isolated in 2012 and 2011 were available for further investigation. These included a total of 59 nonreplicate isolates, corresponding to an equal number of BSI episodes (35 from 2013, 23 from 2012, and 1 from 2011). In the investigated isolates, the COL^r phenotype was confirmed by broth microdilution using Sensititre custom plates (TREK Diagnostic Systems, Cleveland, OH).

Genotyping, carried out by analysis of pulsed-field gel electrophoresis (PFGE) profiles of chromosomal DNA digested with XbaI and by multilocus sequence typing (MLST) (23), revealed an oligoclonal structure with a strong predominance of isolates with

a single PFGE profile (profile A) belonging to sequence type 512 (ST512; $n = 56/59$) and a small minority of isolates with different PFGE profiles and belonging to ST101 (Table 2). Characterization of carbapenemase genes (23) revealed the presence of *bla*_{KPC-3} or *bla*_{KPC-2} in the ST512 or ST101 isolates, respectively (Table 2). Interestingly, a KPC-3-producing ST512 strain with PFGE profile A was also the most prevalent (81%) among 31 colistin-susceptible (COL^s) CRKP isolates representative of the outbreak (data not shown).

Analysis of the *mgrB* locus of the 59 COL^r CRKP, carried out by PCR and sequencing using primers targeting amplification of the *mgrB* coding sequence and promoter region (14), revealed that the majority of them (50 of 59; 85%), all belonging to the same clonal lineage (PFGE profile A; ST512), carried a deletion of 11 bp in the *mgrB* coding sequence, while inactivation of *mgrB* by an insertion sequence was detected in a single isolate of the same clonal lineage (Table 2). Both alterations were previously associated with colistin resistance in CRKP (24). The 50 patients infected by the COL^r ST512 clone carrying the *mgrB*_{Δ109/119} deletion were from 22 different wards.

TABLE 2 Characterization of the 59 COL^r CRKP isolates investigated in this work

No. of isolates	PFGE profile ^a	ST	KPC variant	Status of <i>mgrB</i> locus ^b	Status of PmrA and PmrB ^c	Yr of isolation (n)
50	A	512	KPC-3	Δnt109/119 (frameshift and premature termination)	NT	2011 (1) 2012 (19) 2013 (30)
1	A	512	KPC-3	Insertional inactivation by ISKpn26 at nt 75 (FW)	PmrA WT PmrB WT	2013
5	A	512	KPC-3	WT	PmrA WT PmrB WT	2012 (2) 2013 (3)
2	B	101	KPC-2	WT	PmrA ^{C650T} PmrB WT	2012 (1) 2013 (1)
1	C	101	KPC-2	WT	PmrA ^{C650T} PmrB WT	2012 (1)

^a Different PFGE profiles were defined as differences of more than 4 bands.

^b The nucleotide (nt) numbers indicate the positions of deletions (Δ) or of the insertion site of the insertion sequence ISKpn26; numbering is in reference to the coding sequence of the *mgrB* gene (accession no. AVFC01000053, region 155460 to 155655), considering number 1 as the first base of the GTG start codon. FW, the transposase gene is in the same orientation as the *mgrB* gene; WT, wild-type sequence.

^c NT, not tested; PmrB WT, wild-type deduced PmrB protein sequence, identical to that of KPb-1 (15) (accession no. NZ_AYOV00000000); PmrA WT, wild-type deduced PmrA protein sequence, identical to that of KPb-1 (accession no. NZ_AYOV00000000).

Data on prior colistin use, available for 19 of these patients, revealed that 18 (95%) of them had not received colistin.

Analysis of the eight COL^r CRKP isolates with a wild-type *mgrB* locus for the presence of alterations in PmrA and PmrB, by using PCR and sequencing (16), revealed no alterations in the ST512 isolates and a single amino acid substitution (A271V) in PmrA of the ST101 isolates (Table 2). However, an identical substitution was also detected in COL^s isolates of ST101 (data not shown), suggesting that it represents a protein polymorphism not relevant to polymyxin resistance.

Altogether, our present results (i) suggest that the large outbreak of COL^r CRKP was primarily attributable to the clonal expansion of a single *mgrB* deletion mutant that originated from the dominant ST512 KPC-3-producing CRKP clone that was spreading in the hospital; (ii) confirm the relevance of the *mgrB* gene alterations as a mechanism of acquired polymyxin resistance among CRKP; and (iii) underscore the potential for clonal expansion of similar mutants. Indeed, the presence of an identical deletion of the *mgrB* gene in isolates with the same PFGE profile was strongly suggestive of clonal expansion, although the lack of a whole-genome sequencing analysis of these isolates, which could have provided a definitive confirmation of this phenomenon, was a limitation of this study.

The outbreak we have described here was initially associated with increased colistin consumption, pointing to an important role of the selective pressure generated by antibiotic usage in the selection of resistant mutants. On the other hand, the mostly clonal nature of the outbreak and the lack of prior colistin exposure in several cases of BSI caused by COL^r CRKP also revealed that, once selected, COL^r *mgrB* mutants are able to persist and rapidly disseminate in the hospital setting.

ACKNOWLEDGMENT

This work was partially supported by European Community FP7 HEALTH-F3-2011-282004 (EvoTAR) to G.M.R.

REFERENCES

- Nordmann P, Naas T, Poirel L. 2011. Global spread of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis* 17:1791–1798. <http://dx.doi.org/10.3201/eid1710.110655>.
- Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, Cornaglia G, Garau J, Gniadkowski M, Hayden MK, Kumarasamy K, Livermore DM, Maya JJ, Nordmann P, Patel JB, Paterson DL, Pitout J, Villegas MV, Wang H, Woodford N, Quinn JP. 2013. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. *Lancet Infect Dis* 13:785–796. [http://dx.doi.org/10.1016/S1473-3099\(13\)70190-7](http://dx.doi.org/10.1016/S1473-3099(13)70190-7).
- Tangden T, Giske K. 2015. Global dissemination of extensively drug-resistant carbapenemase-producing Enterobacteriaceae: clinical perspectives on detection, treatment and infection control. *J Intern Med* 277:501–512. <http://dx.doi.org/10.1111/joim.12342>.
- Tzouvelekis LS, Markogiannakis A, Psychogiou M, Tassios PT, Daikos GL. 2012. Carbapenemases in *Klebsiella pneumoniae* and other Enterobacteriaceae: an evolving crisis of global dimensions. *Clin Microbiol Rev* 25: 682–670. <http://dx.doi.org/10.1128/CMR.05035-11>.
- Petrosillo N, Giannella M, Lewis R, Viale P. 2013. Treatment of carbapenem-resistant *Klebsiella pneumoniae*: the state of the art. *Expert Rev Anti Infect Ther* 11:159–177. <http://dx.doi.org/10.1586/eri.12.162>.
- Daikos GL, Tsaousi S, Tzouvelekis LS, Anyfantis I, Psychogiou M, Argyropoulou A. 2014. Carbapenemase-producing *Klebsiella pneumoniae* bloodstream infections: lowering mortality by antibiotic combination schemes and the role of carbapenems. *Antimicrob Agents Chemother* 58:2322–2328. <http://dx.doi.org/10.1128/AAC.02166-13>.
- Ah YM, Kim AJ, Lee JY. 2014. Colistin resistance in *Klebsiella pneumoniae*. *Int J Antimicrob Agents* 44:8–15. <http://dx.doi.org/10.1016/j.ijantimicag.2014.02.016>.
- Zagorianou A, Sianou E, Iosifidis E, Dimou V, Protonotariou E, Miyakis S, Roulides E. 2012. Microbiological and molecular characteristics of carbapenemase-producing *Klebsiella pneumoniae* endemic in a tertiary Greek hospital during 2004–2010. *Euro Surveill* 17:pil=20088. <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20088>.
- Pena I, Picazo JJ, Rodríguez-Avial C, Rodríguez-Avial I. 2014. Carbapenemase-producing Enterobacteriaceae in a tertiary hospital in Madrid, Spain: high percentage of colistin resistance among VIM-1-producing *Klebsiella pneumoniae* ST11 isolates. *Int J Antimicrob Agents* 43:460–464. <http://dx.doi.org/10.1016/j.ijantimicag.2014.01.021>.
- Papadimitriou-Olivgeris M, Christofidou M, Fligou F, Bartzavali C, Vrettos T, Filos KS, Marangos M, Anastassiou ED. 2014. The role of colonization pressure in the dissemination of colistin or tigecycline resistant KPC-producing *Klebsiella pneumoniae* in critically ill patients. *Infection* 42:883–890. <http://dx.doi.org/10.1007/s15010-014-0653-x>.
- Monaco M, Giani T, Raffone M, Arena F, Garcia-Fernandez A, Pollini S, Network EuSCAPE-Italy, Grundmann H, Pantosti A, Rossolini GM. 2014. Colistin resistance superimposed to endemic carbapenem-resistant *Klebsiella pneumoniae*: a rapidly evolving problem in Italy, November 2013 to April 2014. *Euro Surveill* 19(42):pii=20939. <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20939>.
- Olaitan AO, Morand S, Rolain JM. 2014. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. *Front Microbiol* 5:643. <http://dx.doi.org/10.3389/fmicb.2014.00643>.
- Cannatelli A, D'Andrea MM, Giani T, Di Pilato V, Arena F, Ambretti S, Gaibani P, Rossolini GM. 2013. *In vivo* emergence of colistin resistance in *Klebsiella pneumoniae* producing KPC-type carbapenemases mediated by insertional inactivation of the PhoQ/PhoP *mgrB* regulator. *Antimicrob Agents Chemother* 57:5521–5526. <http://dx.doi.org/10.1128/AAC.01480-13>.
- Poirel L, Jayol A, Bontron S, Villegas MV, Ozdamar M, Türkoglu S, Nordmann P. 2015. The *mgrB* gene as a key target for acquired resistance to colistin in *Klebsiella pneumoniae*. *J Antimicrob Chemother* 70:75–80. <http://dx.doi.org/10.1093/jac/dku323>.
- Cannatelli A, Di Pilato V, Giani T, Arena F, Ambretti S, Gaibani P, D'Andrea MM, Rossolini GM. 2014. *In vivo* evolution to colistin resistance by PmrB sensor kinase mutation in KPC-producing *Klebsiella pneumoniae* is associated with low-dosage colistin treatment. *Antimicrob Agents Chemother* 58:4399–4403. <http://dx.doi.org/10.1128/AAC.02555-14>.
- Jayol A, Poirel L, Brink A, Villegas M-V, Yilmaz M, Nordmann P. 2014. Resistance to colistin associated with a single amino acid change in protein PmrB among *Klebsiella pneumoniae* isolates of worldwide origin. *Antimicrob Agents Chemother* 58:4762–4766. <http://dx.doi.org/10.1128/AAC.00084-14>.
- Jayol A, Nordmann P, Brink A, Poirel L. 2015. Heteroresistance to colistin in *Klebsiella pneumoniae* associated with alterations in the PhoPQ regulatory system. *Antimicrob Agents Chemother* 59:2780–2784. <http://dx.doi.org/10.1128/AAC.05055-14>.
- Wright MS, Suzuki Y, Jones MB, Marshall SH, Rudin SD, van Duin D, Kaye K, Jacobs MR, Bonomo RA, Adams MD. 2015. Genomic and transcriptomic analyses of colistin-resistant clinical isolates of *Klebsiella pneumoniae* reveal multiple pathways of resistance. *Antimicrob Agents Chemother* 59:536–543. <http://dx.doi.org/10.1128/AAC.04037-14>.
- Cheng YH, Lin TL, Pan YJ, Wang YP, Lin YT, Wang JT. 2015. Colistin resistance mechanisms in *Klebsiella pneumoniae* strains from Taiwan. *Antimicrob Agents Chemother* 59:2909–2913. <http://dx.doi.org/10.1128/AAC.04763-14>.
- Giani T, D'Andrea MM, Pecile P, Borgianni L, Nicoletti P, Tonelli F, Bartoloni A, Rossolini GM. 2009. Emergence in Italy of *Klebsiella pneumoniae* sequence type 258 producing KPC-3 carbapenemase. *J Clin Microbiol* 47:3793–3794. <http://dx.doi.org/10.1128/JCM.01773-09>.
- Bogdanovich T, Adams-Haduch JM, Tian G-B, Nguyen MH, Kwak EJ, Muto CA, Doi Y. 2011. Colistin-resistant, *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* belonging to the international epidemic clone ST258. *Clin Infect Dis* 53:373–376. <http://dx.doi.org/10.1093/cid/cir401>.
- Zarkotou O, Pournaras S, Voulgari E, Chrysos G, Prekates A, Voutsinas

- D, Themeli-Digalaki K, Tsakris A. 2010. Risk factors and outcomes associated with acquisition of colistin-resistant KPC-producing *Klebsiella pneumoniae*: a matched case-control study. *J Clin Microbiol* 48:2271–2274. <http://dx.doi.org/10.1128/JCM.02301-09>.
23. Giani T, Pini B, Arena F, Conte V, Bracco S, Migliavacca R, AMCLI-CRE Survey Participants, Pantosti A, Pagani L, Luzzaro F, Rossolini GM. 2013. Epidemic diffusion of KPC carbapenemase-producing *Klebsiella pneumoniae* in Italy: results of the first countrywide survey, 15 May to 30 June 2011. *Euro Surveill* 18(22):pii=20489. <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20489>.
24. Cannatelli A, Giani T, D'Andrea MM, Di Pilato V, Arena F, Conte V, Tryfinopoulou K, the COLGRIT Study Group, Vatopoulos A, Rossolini GM. 2014. MgrB inactivation is a common mechanism of colistin resistance in KPC-producing *Klebsiella pneumoniae* of clinical origin. *Antimicrob Agents Chemother* 58:5696–5703. <http://dx.doi.org/10.1128/AAC.03110-14>.