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

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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 (MIC_{50}, >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-

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

Yr	No. of *K. pneumoniae* BSI	No. (%) of *K. pneumoniae* 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 $mgrB_{\Delta 109/119}$ 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 *mgrB* 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 IS*Kpn26* 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 IS*Kpn26*; 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.

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