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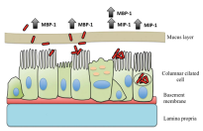

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LETTER TO THE EDITOR

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Infections caused by carbapenem-resistant *Klebsiella pneumoniae* with hypermucoviscous phenotype: A case report and literature review

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In the mid 1980s, a hypervirulent variant of *Klebsiella pneumoniae* (hvKP) causing serious community-acquired clinical syndromes with pyogenic liver abscesses, possibly associated with bacteraemic extrahepatic disseminations, was identified in Taiwan. Generally, patients presenting with these syndromes were young and without significant comorbidities, with the exception of diabetes that was found to be a major risk factor.[1,2] After those first reports from the Far East, similar cases have been subsequently reported worldwide.[3-5] The hvKP strains differ from classical *K. pneumoniae* strains for an increased virulence potential, that can be evaluated in animal models of infection (usually mouse or *Galleria mellonella*).[6] The increased virulence potential has been associated with the expression of several traits, present in variable combinations, including: i) iron-scavenging systems (e.g. the IucA aerobactin, the EntH enterobactin, the IroB salmochelin and the Irp2 yersiniabactin);[7] ii) the allantoin metabolism pathway;[8] iii) the Kpc fimbriae;[9] and iv) certain capsular types (e. g. K1 and K2) produced in increased abundance to form a so-called "hypercapsule."[10] Due to production of the hypercapsule, the colonies of these strains typically exhibit the hypermucoviscous (HM) phenotype, denoted by an abundant production of capsular material and a positive "string test."[4] The HM phenotype has been related with the acquisition of plasmid-borne *rmpA* and *rmpA2* genes, encoding transcriptional regulators that activate capsular biosynthesis,[11,12] or with mutations of the chromosomal *rcsA* and *rcsB* genes, encoding a signaling system involved in the regulation of capsular biosynthesis.[10,11] However, in some strains the mechanism(s) underlying the HM phenotype remain elusive.[13,14] The HM phenotype apparently contributes to the virulence of hvKP strains and is widely considered a surrogate marker of increased virulence.[15,16] However, the relationship between hvKP and the HM phenotype, i. e. whether all hvKP are HM and *vice versa*, remains unclear.[4] Of the hvKP strains thus far described, most belong to a single clonal group (CG), namely CG23, although hvKP strains of other lineages (e. g. of sequence type ST86) have occasionally been reported. Consistently with their community origin, the hvKP strains are usually susceptible to antibiotics.[4]

Unlike the hvKP strains, classical *K. pneumoniae* strains typically behave as opportunistic pathogens of lower virulence potential,[17-19] mostly causing infections in hospitalized patients with some degree of impairment of the host defenses.[20] On the other hand, these strains often carry multiple resistance determinants to antibiotics which make treatment more difficult.[20] Carbapenem-resistant *K. pneumoniae* (CRKP), in particular, have emerged as one of the ultimate challenges for public health because of their extended antibiotic resistance phenotypes and ability to rapidly disseminate in the hospital setting and eventually even outside.[21] The spread of CRKP is mostly linked to the expansion of successful high-risk clones producing carbapenemases of various

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types (e. g. KPC, NDM, OXA-48 or VIM), with a paradigmatic example represented by the CG258 clonal lineage harbouring bla_{KPC} carbapenemase genes.[22-24]

The current dichotomy between CRKP and hvKP populations in terms of resistance and virulence, however, could eventually be blurred, and the emergence of CRKP with an increased virulence potential is a worrisome perspective.[25] In fact, HM strains producing extended-spectrum β-lactamases (ESBL) and carbapenemases have recently been reported,[26-34] being a matter of considerable concern.

In this work we describe a case of liver abscess followed by fatal bacteremic infection in a liver transplant patient, caused by a CRKP strain that showed an HM phenotype (CRHMKP). We also reviewed the recent literature reporting cases of CRKP with an HM phenotype.

KP04C62 was isolated in August 2011 from the blood cultures of a 52 years-old caucasic patient with septic shock. The strain exhibited an HM phenotype and was resistant to carbapenems (meropenem MIC, >64 μg/ml), extended-spectrum cephalosporins, conventional β-lactamase inhibitor combinations (amoxicillin-clavulanate, piperacillin-tazobactam), amikacin, fluoroquinolones, trimethoprim-sulfamethoxazole, and colistin (MIC, 32 μg/ml), while retaining susceptibility to gentamicin (MIC, 1 μg/ml) and tigecycline (MIC, 1 μg/ml). The patient was diabetic and had been subjected to liver transplantation for end-stage liver disease 6 months before. Immunosuppression had been with cyclosporine, methylprednisolone and mycophenolate mofetil. Four months after the transplantation a voluminous abscess in the VI and VII liver segments was diagnosed, and a CRHMKP with the same resistance profile as KP04C62 was isolated from a drainage. Despite drainage, hyperbaric oxygen treatment and combination antibiotic therapy with meropenem, gentamicin, and colistin, the abscess persisted. The patient was then subjected to surgical resection of the V, VI, VII and VIII liver segments, and the antimicrobial therapy was modified (substitution of meropenem with tigecycline). However, the clinical condition of patient worsened and he died of peritonitis and septic shock few days after surgery.

A PubMed search (accessed on December 27th 2016), using as search terms "*Klebsiella,*" "hypermucoviscous," and "resistance" revealed a total of 8 reports describing cases of human infections or colonizations caused by CRHMKP strains. These reports, summarized in Table 1, are briefly reviewed below.

Zhang *et al.* (2015), in a multi-center retrospective study which analyzed the clinical and laboratory features of 28 cases of CRKP infections from 9 cities in China, observed between 2012 and 2013, detected 5 HM strains (17.8%) of which only 3 were positive for *rpmA*/*rpmA2* genes. The CRHMKP strains caused 2 cases of pneumonia and 3 of bloodstream infection. All patients for whom information were available (4 out of 5) survived the infection. The infected patients, aged from one day to 84 years, suffered of multiple underlying diseases and were all hospitalized. Three out of the 5 strains belonged to ST11 and were non-typeable by conventional serotyping methods. Another non-typeable strain was assigned to ST1700, while the remaining strain was an ST65 of serotype K2. Interestingly, the latter strain, isolated from a one-day-old infant who developed septicaemia during treatment of bronchopulmonary dysplasia and survived the infection, showed a remarkable *in vitro* resistance to serum killing and a high virulence in a mouse peritonitis infection model. By contrast, the other 4 strains were avirulent in the same murine model. The $ST65^{K2}$ strain carried the aerobactin and the enterobactin siderophores, and was resistant to carbapenems due to decreased expression of OmpK35 and OmpK36 associated with SHV-11 and TEM-53 β-lactamases production.[30]

Andrade *et al.* (2014) reported on a CRHMKP strain obtained from the blood cultures of a 36 years-old patient, during a hospital outbreak of ST11 *K. pneumoniae* producing KPC-2 occurred in 2013 in a tertiary-care university hospital in Ribeirão Preto (Brazil).[27] The patient, admitted for acute myeloid leukemia, died for septic shock after a few days. The strain showed a multidrug resistant phenotype including colistin resistance. Capsular serotyping was not performed. The *rmpA*/*rmpA2* genes were not detected and the mechanism underlying the HM phenotype remained unknown.

Wei *et al.* (2015) reported on a CRHMKP strain obtained in January 2014 from a 47 years-old patient with multiple traumatic injuries due to a traffic accident admitted in the Intensive Care Unit of a university teaching hospital in NanChang (China). The strain was isolated 20 d after hospital admission from blood, a chronic wound, and a decubitus ulcer, and the patient eventually died of infection. The strain belonged to ST11, carried the bla_{KPC-2} gene, expressed a K1 capsular serotype, and was positive for the *rmpA*/*rmpA2* genes.[32]

Yao *et al.* (2015) performed a retrospective surveillance study aimed to identify HM strains in a collection of CRKP (selection criteria were positive string test and imipenem and/or meropenem minimum inhibitory concentration $\geq$ 4mg/L) from a large Chinese hospital in the period January 2010-August 2014. Among the 60 CRKP isolated during the study period from 33 patients, 7 (isolated since February 2013 from 4 patients) were positive for the HM phenotype. These CRHMKP strains were responsible for 2 cases of pneumonia, one bloodstream infection secondary to urinary tract infection, and one gut colonization. All cases were hospital-acquired and

Table 1. Summary of articles available at PubMed database, accessed on December 27th 2016, describing cases of infections or colonization caused by CRHMKP. Legend: pattern 1, strains with K1, positive for *rmpA*/*rmpA2* genes, mostly of ST23 but also of other sequence types; pattern 2, strains with non-typeable serotype, mostly negative for *rmpA*/*rmpA2*); *, deduced from *wzi* gene sequence; -, data not available.

Isolate identifcative	Country	Year	ST	K type	Carbapenems resistance mechanism	*rmpA* and/or *rmpA2*	Pattern	Polymixins susceptibility	Tigecycline susceptibility	animal model	clinical sample	disease	age (years)	sex	comorbidities	therapy	outcome	Reference n.
Strain 1	China	2013	11	non-typable	OmpK35/36 decreased expression associated with β-lactamases production	negative	2	–	susceptible	avirulent in murine peritonitis	sputum	pneumonia	84	famale	Coronary heart disease, Diabetes mellitus, Hypertension, Cerebral infarction	Moxifloxacin, Meropenem,	survived	30
Strain 2	China	2013	1700	non-typable	KPC-2, IMP-4	negative	2	–	resistant	avirulent in murine peritonitis	abdominal fluid	abdominal infection, septic shock	14	famale	Acute myocarditis, Acute renal insufficiency	Piperacillin/ tazobactam, Meropenem, Imipenem	unknown	30
Strain 3	China	2013	65	K2	OmpK35/36 decreased expression associated with β-lactamases production	positive	–	–	susceptible	highly virulent in murine peritonitis	blood	septicemia	1 day	male	Premature, Bronchopulmonary dysplasia, Hyaline membrane disease, Severe asphyxia, Brain damage, Hypoglycemia	Ceftazidime, Piperacillin/ tazobactam, Imipenem, Ceftazidime/ tazobactam, Meropenem	survived	30
Strain 4	China	2012	11	non-typable	KPC-2	positive	2	–	susceptible	avirulent in murine peritonitis	abdominal fluid	pneumonia	71	male	Cholecystectomy, Bile duct obstruction, Coronary heart disease, cancer, acute respiratory failure	Levofloxacin, Cefoperazone/ sulbactam, Piperacillin/ tazobactam	survived	30
Strain 5	China	2012	11	non-typable	KPC-2	positive	2	–	susceptible	avirulent in murine peritonitis	bile	biliary tract infection, pulmonary infection, sepsis	76	male	Cholecystectomy, common bile duct stent, Chronic bronchitis, Calculus of intrahepatic duct, Respiratory failure	Meropenem, Imipenem, Cefepime, Fosfomycin, Amoxicillin/ clavulanic acid, Tigecycline	survived	30
RP59	Brazil	2013	11	–	KPC-2	negative	2	resistant	susceptible	–	blood	bacteraemia	36	male	Acute myeloid leukemia	unknown	died	27
KP70–2	China	2013	23	K1	KPC-2	positive	1	susceptible	susceptible	–	sputum and blood samples	septic shock	50	male	brain injury	cefoperazone-sulbactam	died	31
KP1088–2	China	2013	23	K1	KPC-2	positive	1	susceptible	susceptible	–	sputum and blood samples	septic shock	67	male	multiple injury	unknown	died	31
KP86	China	2013	1797	K1	KPC-2	positive	1	susceptible	susceptible	–	sputum and blood samples	septic shock	88	male	Abdominal infection	unknown	died	31
KP91	China	2013	1797	K1	KPC-2	positive	1	susceptible	susceptible	–	sputum and blood samples	septic shock	73	famale	Septic arthritis	unknown	died	31
KP96	China	2013	1797	K1	KPC-2	positive	1	susceptible	susceptible	–	sputum and blood samples	septic shock	32	male	none	unknown	died	31
3089	Argentina	2013	23	K1	KPC-2	positive	1	susceptible	susceptible	–	tracheal secretion	suspected pneumonia	85	male	acute myeloid leukemia	unknown	died	28
Kp1500	China	2014	11	K1	KPC-2	positive	1	–	susceptible	highly virulent in murine peritonitis	blood, wound and decubitus ulcer	bacteraemia	47	famale	multiple traumatic injuries	unknown	died	32

(*Continued on next page*)

Table 1. (*Continued*)

Isolate identifcative	Country	Year	ST	K type	Carbapenems resistance mechanism	*rmpA* and/or *rmpA2*	Pattern	Polymixins susceptibility	Tigecycline susceptibility	animal model	clinical sample	disease	age (years)	sex	comorbidities	therapy	outcome	Reference n.
cr-hvKP1, cr-hvKP4	China	2013	65	K2	KPC-2	positive	–	–	susceptible	–	urine, tracheal secretion	colonization	78	male	Respiratory failure, Parkinson disease, cerebrovascular accident, duodenal ulcer, hypertension, chronic hepatitis, paroxysmal atrial fibrillation	none	survived	29
cr-hvKP2, cr-hvKP3 and cr-hvKP5	China	2014	65	K2	KPC-2	positive	–	–	susceptible	–	tracheal secretion, urine, blood	secondary bacteraemia after urinary tract infection	85	male	Respiratory failure, chronic obstructive pulmonary disease, hypertension, secondary epilepsy, cerebrovascular accident	imipenem; isepamicin	survived	29
cr-hvKP6	China	2014	25	K2	–	positive	–	–	susceptible	–	tracheal secretion	pneumonia	68	male	respiratory failure, cerebrovascular accident, hypertension, secondary epilepsy	cefoperazone–sulbactam, isepamicin	survived	29
cr-hvKP7	China	2014	11	non-typable	KPC-2	negative	2	–	resistant	–	tracheal secretion	pneumonia	91	famale	respiratory failure, chronic obstructive pulmonary disease, colon carcinoma, pleural effusion, coronary artery disease	ertapenem	died	29
Klebsiella pneumoniae U25	India	2009–10	14	K2*	OmpK36 mutation associated with β-lactamases production	negative	–	–	–	–	urine	–	–	–	–	–	–	33
B20143	India	2014	11	K24*	NDM-1	positive	–	–	–	–	blood	–	–	–	–	–	–	34
B1647	India	2015	231	non-typable*	OXA-232	negative	2	–	–	–	blood	–	–	–	–	–	–	34
B20038	India	2015	43	K30*	OXA-181	positive	–	–	–	–	blood	–	–	–	–	–	–	34

occurred in patients admitted for long periods, with several co-morbidities. In 3 cases the *K. pneumoniae* strains belonged to serotype K2 and carried the *rmpA/rmpA2* genes (2 of ST65 and one of ST25). The remaining was an ST11 strain of a non-typeable serotype, lacking the *rmpA/rmpA2* genes. All strains but one produced KPC-2. One of the infected patients, a 91 years-old patient affected by pneumonia caused by the ST11 strain, died for heart failure while the other patients survived the infection.[29]

Zhang *et al.* (2015) reported on 5 cases of infection by CRHMKP with K1 capsular type, occurred in hospitalized patients in the Zhejiang Province of China in 2013. All cases had a fatal outcome regardless of their original health status. Genotyping results revealed that 2 strains belonged to ST23 and the other 3 to a new genetically related ST (ST1797, a double locus variant of ST23), and that all strains carried the *rmpA/rmpA2* genes and a plasmid-borne bla_{KPC-2} gene. In 2 cases the acquisition of bla_{KPC-2} by a previously susceptible stain had occurred after or during imipenem therapy.[31] These are the first described cases of acquisition of carbapenem-resistance by clinical strains of a well known hypervirulent lineage.

Cejas *et al.* (2014) reported on a CRHMKP of ST23 and serotype K1, carrying the *rmpA*/*rmpA2* genes and producing the KPC-2 carbapenemase. The strain was isolated in 2013 from the tracheal aspirate of an 85 year-old man with a recent history of acute myeloid leukemia, admitted to an intensive care unit in Buenos Aires, Argentina. The patient, who was undergoing chemotherapy with methotrexate and prednisone, died 3 weeks after the isolation of the *K. pneumoniae* strain due to causes that were not specified.[28]

Most recently, 2 articles announcing the genome sequencing projects of 4 CRHMKP isolated in India, from 3 bloodstream infections and one urinary tract infection, have been published.[33,34] The strains were of different sequence types and capsular types, and carried different carbapenem-resistance mechanisms. Only 2 of them were positive for *rmpA*/*rmpA2*.

Altogether, according to our search, 21 CRHMKP strains have been described in the literature. In most cases the CRHMKP strains were from infections (only in one case from colonization). The cases were mostly from China (71.4%), but also from South America and India, and occurred in patients previously hospitalized for other causes. The most frequent site of isolation was bloodstream (12 cases), followed by the respiratory tract (often in concomitance with other sites; 11 cases). One third of these strains belonged to ST23 or to genetically related STs (ST25 and ST1797), one third to ST11, and the remaining ones to several unrelated sequence types (ST14, ST43, ST65, ST231 and ST1700). K1 and K2 were the most common capsular types (12 of 21), with K1 being almost exclusively associated with ST23 or genetically related STs, while K2 with ST65, ST25 and ST14. Several strains, mostly of ST11, were reported as non-typable with conventional serotyping methods. Overall, 6 of 21 strains (28.6%) were negative for the presence of *rmpA/rmpA2* genes, revealing the existence of alternative mechanisms underlying the HM phenotype. In most CRHMKP strains resistance to carbapenems was imputable to the production of the KPC-2 carbapenemase (in one case co-produced with IMP-4), but other carbapenemases (NDM-1 and OXA-48-like) were sporadically reported. In 3 strains carbapenem resistance was due to alterations in the major *K. pneumoniae* porins, OmpK35 and/or OmpK36, coupled with ESBL production. Regarding the phenotype toward other antimicrobial agents, CRHMKP strains retained, with few exceptions, susceptibility to colistin and tigecycline (Table 1).

The 17 cases for which clinical data are available occurred in patients of various ages (from 1 day to 91 years), with a predominance of males (70.5%) and a cumulative in-hospital mortality rate for infected patients of 56.2% (Table 1).

In summary, most CRHMKP strains described in the literature could be gathered into 2 groups with distinctive features: pattern 1, consisting of strains with K1 serotype, positive for the *rmpA*/*rmpA2* genes, mostly of ST23 or genetically related STs; and pattern 2, consisting of strains with a non-typable serotype, mostly of ST11 and mostly negative for *rmpA*/*rmpA2*.

Interestingly, the mortality rate for patients infected by strains with pattern 1 was significantly higher than that observed for patients infected by strains with pattern 2 (100% *vs.* 40%; p value calculated by the Two tailed Fisher's exact test = 0.045). Furthermore, the 2 fatal cases of infection by strains with pattern 2 were reported in patients with underlying conditions that could have significantly influenced the final outcome (age >90 y in one case, and severe hematologic malignancy in the other).

To investigate the genetic features of the KP04C62 strain, the genome was sequenced using the MiSeq platform (Illumina Inc., San Diego, CA) and a 2 × 300 bp paired-end approach. In total 946,759 reads were obtained, yielding an estimated average coverage of 103×, considering a genome size of 5.5 Mbp. Reads were assembled, using the SPAdes software,[35] into 147 contigs (N50 contig size, 268,882 bp). Scaffolds, annotated using the RAST software,[36] contained 5,598 coding sequences. The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession MIFX00000000. The version described in this article is version MIFX01000000. The genomic analysis showed that KP04C62 belonged to

Table 2. Acquired antimicrobial resistance genes detected in KP04C62 with the associated resistance phenotype.

Resistance genes	
Gene	**Associated phenotype**
*bla*TEM-1, *Δbla*OXA-9, *bla*SHV-11	β-lactams excluding carbapenems
*bla*KPC-3	β-lactams including carbapenems
aac(6′)Ib-cr	Aminoglycosides and quinolones
dfrA12, sul1	Trimethoprim, sulphonamides

ST512 (a single-locus variant of ST258), and that it was closely related to another ST512 strain isolated in another Italian hospital in the same period (KPB-1, accession number AYOV00000000), which did not exhibit an HM phenotype.[37] The 2 strains shared a common conserved genome of approximately 5.4 Mbp using the Panseq software,[38] with only 50 SNPs (CSI phylogeny, https://cge.cbs.dtu.dk/services/CSIPhylogeny/) in the core genome.

The content of acquired resistance genes of KP04C62 was consistent with the antibiotic resistance profile (Table 2). As described previously, colistin resistance in KP04C62 was attributed to the insertional inactivation of the *mgrB* gene by an IS5-like element at nt 126.[39]

The KP04C62 virulence genes content was investigated with a database of known *K. pneumoniae* virulence factors created *ad hoc*, expanding the already existing database available at http://bigsdb.pasteur.fr/klebsiella/klebsiella.html (Table S1). Interestingly, KP04C62 harboured none of the 76 putatively acquired virulence genes present in the database. Concerning the housekeeping virulence genes, we found a nonsense mutation in the regulatory *fimK* gene, resulting in a truncated FimK protein at position 440. The loss of FimK function in the *K. pneumoniae* TOP52 strain was previously reported to cause a higher expression of type 1 pili, with enhanced ability to form type 1-dependent biofilm and augmented virulence in a murine urinary tract infection model.[40] An analogous profile of virulence genes, including the nonsense mutation in the *fimK* gene, was found in the closely related KPB-1 strain, which did not exhibit an HM phenotype.

Notably, the *rmpA* and *rmpA2* genes were not detected in KP04C62, while the *rcsABCD* genes of this strain were identical to those found in the non-HM KPB-1 strain. Compared to the latter strain, which has a capsular gene cluster typical of clade II strains of the CG258 lineage (cps_{BO-4} type with *wzi154* allele),[41] the sequence of the capsular gene cluster of KP04C62 exhibited 2 original differences: a missense T→C mutation at position 221 of the *wzc* gene, resulting in a Leu→Pro substitution at position 74 of the Wzc protein, and a T→C missense mutation at position 332 of a putative glycosyltransferase-encoding gene (Region 11007–11768 of AYOV01000044), resulting in a Cys→Ser substitution at position 110 of the corresponding protein. Wzc is a BY-kinase involved in the biosynthesis and transport of exopolysaccharides, which interacts with Wza (a transmembrane protein) for the translocation of the capsular polysaccharide from the periplasm across the outer membrane.[42] The amino acid substitution identified in the Wzc of KP04C62 is situated in the N-terminal periplasmic domain which carries the site of interaction with Wza. The role of these original mutations in expression of the HM phenotype of KP04C62 will be the subject of future investigation.

To investigate the virulence potential of KP04C62 in comparison with a known highly virulent, hvKP, strain (NTUH-K2044, a typical hvKP ST23 strain with the K1 capsular serotype),[43] we used a *Galleria mellonella* animal model, according to a described previously protocol.[6,17] In this model, KP04C62 showed a virulence potential that was significantly lower than that of NTUH-K2044 (LD_{50} at 72 hours, 6.1 ± 0.05 *vs.* 4.9 ± 0.24, P value <0.01; 3 independent replicates). This behavior was overall similar to that previously reported for another KPC-producing CG258 strain with a cps_{BO-4} capsular type (KKBO-1),[17] and revealed that KP04C62 did not behave as a typical hvKP strain, at least in this model. To assess whether the difference in the LD_{50} values could be, at least in part, attributed to a different growth pattern of the studied strains, we analyzed the growth of KP04C62, KKBO-1 and NTUH-K2044 at different pH values (pH 7, 6.5 and 6, in LB broth buffered with 1M HCl), considering that a lower pH (around 6.5) is encountered in the animal model [44] Growth was performed at 37°C for 24 h, in a volume of 5 ml, and was followed by monitoring A_{600} and CFU counts. Results of these experiments, performed in triplicate, did not reveal significant differences of growth patterns among the studies strains (data not shown).

Overall, the KP04C62 strain described in this work shared several characteristics with CRHMKP of pattern 2 (capsular locus organization typical of clade II of CG258 strains, i.e. cps_{BO-4}, non-typeable with conventional serotyping methods; negative for *rmpA/rmpA2*). This strain caused a fatal systemic infection, originating from a liver abscess, similarly to classic ST23 hvKP strains. However, in this case the infection occurred in a severely immunocompromised patient.

In conclusion, we described the clinical, epidemiological and genetic features of the first CRHMKP strain of ST512, producing the KPC-3 carbapenemase. The strain was isolated in 2011, i. e. before most other similar strains described in the literature, and shared several characteristics with other described previously CRHMKP of pattern 2, which have a lower virulence potential compared with other HM strains that we

identified as pattern 1 (more often belonging to ST23 or genetically related STs, with a K1 capsular serotype and an extensive set of virulence factors, including the *rmpA/rmpA2* genes). Indeed, the analysis of the existing literature suggested that pattern 2 strains are likely able to cause serious fatal infections (including liver abscesses) only in immunocompromised patients. This hypothesis was corroborated by the fact that, in the *Galleria mellonella* infection model, KP04C62 had a virulence potential inferior to a known hvKP strain and similar to that of another CG258 KPC-producing, $cps_{BO\text{-}4}$ strain, previously associated with low virulence.[17]

In our opinion, therefore, a laboratory positivity for the string test in a CRKP isolate should be interpreted as an alert for the possibility of an hvKP behavior, but confirmation requires further investigation of the genetic content of virulence determinants and possibly testing of virulence behavior in a suitable animal model. Further analysis will be necessary to characterize the mechanism underlying the HM phenotype in pattern 2 strains that generally lack *rmpA/rmpA2* determinants.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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