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Emergence of an MDR *Klebsiella pneumoniae* ST231 producing OXA-232 and RmtF in Switzerland

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Sir,

The increasing incidence of carbapenem-resistant *Klebsiella pneumoniae* is a major challenge to public health. Despite the fact that the prevalence of carbapenemases among carbapenem-resistant *K. pneumoniae* varies geographically, the incidence of OXA-48-like enzymes has soared in recent years and is particularly high in some European countries, such as Spain and France (74% and 78% among carbapenemase-producing *K. pneumoniae*, respectively).[1] A significant number of OXA-48 variants have been reported in the last decade. This includes OXA-232, a carbapenemase firstly identified in France in 2011[2] and thereafter found in several countries.[3,4] Recently, an MDR *K. pneumoniae* ST231 co-producing OXA-232, the ESBL CTX-M-15 and the 16S rRNA methyltransferase RmtF conferring broad-spectrum resistance to aminoglycosides has emerged as a successful epidemic clone in South-East Asia, with related outbreaks being reported in Singapore and in Brunei Darussalam between 2013 and 2015.[5,6] Here, we report on a nosocomial spread of this emerging resistant strain in Switzerland.

Six *K. pneumoniae* clinical isolates with reduced susceptibility or resistant to carbapenems were recovered from February to April 2017 from five different patients, namely three hospitalized in the medicine ward of a regional hospital in Western Switzerland, and two non-hospitalized at the private Ear, Nose and Throat (ENT) centre located near to the hospital (Table 1) and were sent to the Swiss National Reference Center for Emerging Antibiotic Resistance for further characterization. All the patients reported no recent travel abroad. Clinical and epidemiological analyses failed to detect any obvious route of transmission for those isolates.

Antimicrobial susceptibility testing was performed by disc diffusion assay (Sanofi-diagnostic Pasteur, France) and MICs were determined using Etest (bioMérieux, France) and broth microdilution techniques, with susceptibility defined according to CLSI breakpoints (https://clsi.org/standards/products/microbiology/documents/m100/). Four isolates were resistant to penicillins, broad-spectrum cephalosporins, meropenem and ertapenem and showed intermediate resistance to imipenem. The remaining two isolates displayed a typical ESBL phenotype, with resistance towards all penicillins, to expanded-spectrum cephalosporins (antagonized by β-lactamase inhibitors) and to ertapenem, and susceptibility to imipenem and meropenem. Interestingly, one isolate presenting carbapenemase activity and one exhibiting an ESBL phenotype had been recovered from a single patient (Table 1). All six *K. pneumoniae* isolates also exhibited broad-spectrum resistance to aminoglycosides and were additionally resistant to sulphonamides, fluoroquinolones, trimethoprim/sulfamethoxazole, chloramphenicol, tetracycline and to the recently developed ceftolozane/tazobactam combination. Notably, all six clinical isolates were susceptible to colistin and to the ceftazidime/avibactam combination.

Multiplex PCRs performed to detect Ambler class A, B and D carbapenemases and 16S rRNA aminoglycoside resistance genes[7,8] followed by sequencing revealed that all six isolates possessed the *rmtF* 16S rRNA methyltransferase gene, and that the four carbapenem-resistant isolates possessed the $bla_{\text{OXA-232}}$ carbapenemase gene. In addition, all the isolates possessed the ESBL $bla_{\text{CTX-M-15}}$ and the $bla_{\text{TEM-1}}$ gene (Table 1). Transferability of the $bla_{\text{OXA-232}}$, $bla_{\text{CTX-M-15}}$ and *rmtF* genes was attempted by mating-out assays using the azide-resistant *Escherichia coli* J53 as a recipient strain. Transconjugants were obtained on azide (100 mg/L) and either amikacin/gentamicin (50 mg/L each) or ceftazidime (1 mg/L), but not imipenem (1 mg/L), indicating the transferability of the plasmids harbouring the *rmtF* and $bla_{\text{CTX-M-15}}$, but not that one carrying the $bla_{\text{OXA-232}}$ gene. Analysis of the plasmid content by using the Kieser technique revealed the presence of several plasmids in all isolates. The $bla_{\text{OXA-232}}$ was carried on a 6141 bp plasmid identical to that identified by Potron *et al.*,[2] as further supported by sequence analysis performed as previously reported.[6] Noticeably, the *rmtF* and $bla_{\text{CTX-M-15}}$ genes were located on the same 160 kb plasmid. PFGE of the SpeI-digested genomic DNA obtained from the six *K. pneumoniae* isolates revealed that they were clonally related. MLST showed that they belonged to ST231 (https://cge.cbs.dtu.dk/services/MLST/).

In an attempt to unravel the genetic factors contributing to the emergence and spread of this multiresistant pathogen, WGS of genomic DNA from isolate Kp1 (KP06–2017) was performed using a MiniSeq system (Illumina, USA), generating a total of 13 078 950 reads with an average length of 145.8 bp. Reads were *de novo* assembled using CLC Genomics Workbench version 7.5.1 (Qiagen, France). The draft genome revealed a size of 6 015 778 bp, with an average GC content of approximately 57%. The antimicrobial resistome was identified using ResFinder[9] and comprises genes

Table 1. Characteristics of the *Klebsiella pneumoniae* isolates under study

							MIC (mg/L)					
							carbapenems				novel cephalosporin/β-lactamase inhibitor combinations	
Isolate	Ward	Specimen	Date of isolation	ST	β-Lactamases identified by PCR	Approximate sizes of plasmids (kb)	IPM	MEM	ETP	colistin	ceftolozane/ tazobactam	ceftazidime/ avibactam
Kp1	ENT	sinus swab	28/02/17	231	$bla_{OXA-232}$, $bla_{CTX-M-15}$, bla_{TEM-1b}	3.6, 6.1, 9, 70, 160	2	8	>256	2	>256	2
Kp2	ENT	sinus swab	09/03/17	231	$bla_{OXA-232}$, $bla_{CTX-M-15}$, bla_{TEM-1b}	3.6, 6.1, 9, 70, 160	2	8	>256	2	>256	2
Kp3	Medicine	urine	21/03/17	231	$bla_{CTX-M-15}$, bla_{TEM-1b}	3.6, 4.8, 9, 70, 160	0.38	0.25	2	2	>256	2
Kp4	Medicine	rectal swab	23/03/17	231	$bla_{OXA-232}$, $bla_{CTX-M-15}$, bla_{TEM-1b}	3.6, 6.1, 9, 70, 160	2	8	>256	2	>256	2
Kp5	Medicine	rectal swab	29/03/17	231	$bla_{CTX-M-15}$, bla_{TEM-1b}	3.6, 4.8, 9, 70, 160	0.38	0.25	2	2	>256	2
Kp6	Medicine	rectal swab	10/04/17	231	$bla_{OXA-232}$, $bla_{CTX-M-15}$, bla_{TEM-1b}	3.6, 6.1, 9, 70, 160	2	8	>256	2	>256	2

IPM, imipenem; MEM, meropenem; ETP, ertapenem.

conferring resistance to aminoglycosides (*rmtF*, *aadB*, *aadA2* and *aacA4*), β-lactams ($bla_{OXA-232}$, $bla_{CTX-M-15}$, bla_{TEM-1b}), fluoroquinolones [*aac(6′)Ib-cr*], macrolides, lincosamides and streptogramin B (MLS) [*erm*(B) and *mph*(A)], phenicols (*catA1* and *catB4*), sulphonamides (*sul1*), trimethoprim (*dfrA12*) and rifampicin (*arr-2*) (Table 1). Plasmid finder[10] revealed the presence of IncFIB(pQil), IncFII(K), IncFII(pRSB107), IncFIA and ColKP3 replicons; the latter two present in the plasmids carrying the *rmtF*/$bla_{CTX-M-15}$ and $bla_{OXA-232}$ genes, respectively.

Overall, to our knowledge, we report here the first occurrence in Europe of an MDR *K. pneumoniae* ST231 clone, so far geographically confined in South-East Asia. This represents an important and worrying step toward the rise of another epidemic clone as a global public threat.

Accession number

The draft genome sequence of the *K. pneumoniae* KP06–2017 has been deposited in GenBank under accession number NTFP00000000.

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Transparency declarations

None to declare.

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