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Letter to the Editor

Emergence of carbapenem-resistant NDM-1 -producing *Klebsiella pneumoniae* high-risk sequence type 147 in a tertiary care hospital in Tenerife, Spain

Sir,

Since its first identification in 2009, New Delhi metallo-β-lactamase 1 (NDM-1) has been found in different species and clones around the world. In Spain, few sporadic cases and outbreaks due to NDM-1-producing isolates have been described [1]. The bla_{NDM-1} gene in *Klebsiella pneumoniae* has been acquired by plasmids, which often carry multiple antimicrobial resistance determinants. Significantly, NDM-1-producing bacteria frequently display extensively drug-resistant (XDR) or pandrug-resistant phenotypes and frequently cause difficult-to-treat infections [2,3]. Here we report the emergence and spread of XDR NDM-1-producing *K. pneumoniae* for the first time at our tertiary care hospital in Spain.

A collection of 2782 Enterobacteriaceae was prospectively recovered from clinical specimens of hospitalised patients between January and August 2018. Species identification was performed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (VITEK® MS; bioMérieux; Marcy-l'Étoile, France). *Escherichia coli* (48%), *K. pneumoniae* (26%) and *Enterobacter cloacae* complex (5.5%) were the most prevalent species isolated. Preliminary antimicrobial susceptibility testing was performed using a VITEK®2 system with AST-N243 and AST-N245 cards (bioMérieux). A total of 161 isolates (160 *K. pneumoniae* and 1 *E. cloacae*) showed an antibiogram compatible with the presence of a carbapenemase. The isolates were subsequently subjected to phenotypic screening, confirming carbapenem resistance by Etest (bioMérieux) and using disk inhibition methods (Rosco Diagnostica A/S, Taastrup, Denmark) for detection of the possible carbapenemase type. Colistin and tigecycline susceptibility was determined by broth microdilution according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2018 guidelines. Inhibition of carbapenemase activity by dipicolinic acid demonstrated that 10 carbapenem-resistant *K. pneumoniae* (CPRK) isolates produced a metallo-β-lactamase (MBL) and these isolates were further investigated.

The 10 CRKP isolates were classified as XDR and were obtained from single patients (Table 1). Interestingly, very high variability in terms of carbapenem minimum inhibitory concentrations were observed. Furthermore, three isolates were resistant to fosfomycin and one isolate was resistant to colistin, but no isolates showed resistance to both antibiotics simultaneously.

Multiplex real-time PCR (Real Cycler Universal INOCVK-U INOCVK-G v.2; Progenie Molecular SL, Valencia, Spain) for detection of the bla_{VIM}, bla_{IMP}, $bla_{NDM-like}$, $bla_{OXA48-lilke}$ and bla_{KPC} carbapenemase genes and bla_{CTX-M} extended-spectrum β-lactamase (ESBL) genes was performed on each confirmed carbapenemase-producing isolate. $bla_{OXA48-like}$ was detected in 150 *K. pneumoniae* isolates, whilst bla_{VIM} was detected in the single *E. cloacae* isolate. The 10 MBL-producing *K. pneumoniae* isolates carried bla_{NDM} and bla_{CTX-M}. DNA sequence analysis confirmed the presence of bla_{NDM-1} and $bla_{CTX-M-15}$. Clonality was assessed by pulsed-field gel electrophoresis (PFGE) with restriction enzyme *Xba*I and the isolates were grouped into the same PFGE type. Multilocus sequence typing (MLST) was carried out according to the Institut Pasteur scheme (https://bigsdb.pasteur.fr/klebsiella/klebsiella.html) and all of the isolates belonged to ST147.

In Europe, the main circulating carbapenemases are those of types OXA-48, KPC and VIM, and to a lesser extent NDM-1 [3]. In accordance with data in Spain [4], our results show that the vast majority of CRKP carried OXA-48 (150/160; 93.8%). In Spain, the first NDM-1-producing *K. pneumoniae* isolate was reported in 2012. Since then, limited sporadic cases and outbreaks of infection due to Enterobacteriaceae carrying NDM-1 have been detected [2]. Remarkably, NDM-1-producing CRKP account for 6.2% of the total carbapenemase-producing Enterobacteriaceae and their emergence in our hospital is highly worrisome. The cases had no proven connection with endemic regions. To the best of our knowledge, this is the first study reporting carbapenem-resistant NDM-1-producing isolates in the Canary Islands.

To date, a few antimicrobial-resistant *K. pneumoniae* clones have become globally distributed. Specifically, ST147 is an emerging high-risk international clone whose prevalence is rapidly increasing. Carbapenem resistance in ST147 was detected for the first time in association with VIM. Later on, ST147 was associated with other carbapenemases (i.e. KPC, OXA, NDM, VIM and IMP), including NDM-1 [2]. It should be noted that until now, the emergence of NDM-1-producing *K. pneumoniae* belonging to ST147 has not been previously described in Spain. As in this study, co-production of NDM-1 and the ESBL CTX-M-15 in ST147 has been described previously [2]. Although we were unable to perform a detailed epidemiological investigation, the recovery of 10 clonally related NDM-1-producing *K. pneumoniae* isolates in the 6-month period (March–August 2018) suggest the occurrence of an infection outbreak in the hospital.

The detection for the first time in our hospital of NDM-1 and its association with ST147 is of great concern. In addition to being an increasingly prevalent clone related to humans, carbapenem-resistant ST147 has been recovered from wastewater and has also been identified in companion animals [2]. Remarkably, in vivo transfer of OXA-48 from ST147 to *Escherichia coli* clones in the human gut microbiota has been demonstrated [5]. Thus, it is important to highlight the recognised capacity of *K. pneumoniae*, and specifically of the ST147 lineage, to acquire and disseminate antimicrobial resistance in humans, animals and the environment [2].

Finally, the potential risk of ST147 dissemination in association with resistance genes to last-resort antibiotics such as

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Table 1
Clinical and microbiological data for patients infected or colonised with NDM-1-producing *Klebsiella pneumoniae* isolates.

Patient	Age (years)	Sex	Clinical characteristics[a]	Clinical outcome	Status	Date of sample isolation (dd/mm/yy)	Ward	Site of isolation	Antimicrobial susceptibility (MIC in μg/mL)[b]												
									TZP	CAZ	FEP	ETP	IPM	MEM	GEN	TOB	CIP	COL[c]	FOS	SXT	TGC
1	62	F	Intestinal perforation	Survived	Infected	19/03/2018	General surgery	Wound	>128	>64	>64	6	8	6	4	>16	>4	0.5	32	>320	2
2	35	M	Previous septic shock	Survived	Colonised	15/03/2018	Nephrology	Urine	>128	>64	16	1.5	0.5	1	4	>16	>4	0.5	≤16	>320	0.5
3	48	M	Exacerbated chronic kidney disease; alcoholic cirrhosis	Deceased (15/04/2018)	Infected	03/04/2018	Nephrology	Urine	>128	>64	32	>32	>32	4	>16	>16	>4	0.25	NT	>320	0.5
4	56	M	Subarachnoid haemorrhage	Deceased (17/04/2018)	Colonised	10/04/2018	ICU	Urine	>128	>64	>64	12	12	12	4	>16	>4	0.5	32	>320	2
5	55	M	Traffic accident; femur fracture	Survived	Infected	18/04/2018	Traumatology	Fistula	>128	>64	>64	8	3	3	8	>16	>4	0.5	32	>320	0.5
6	83	M	Renal neoplasia	Survived	Infected	21/04/2018	Emergency	Urine	>128	>64	>64	12	3	4	4	>16	>4	0.5	>256	>320	2
7	52	F	Pancreatic and hepatic neoplasia	Deceased (29/05/2018)	Infected	25/05/2018	Oncology	Ascitic fluid	>128	>64	>64	12	>32	4	>16	>16	>4	0.25	>256	>320	0.5
8	53	M	Chronic lymphocytic leukaemia	Survived	Infected	10/05/2018	ICU	Tracheal aspirate	>128	>64	32	12	6	8	>16	>16	>4	0.25	NT	>320	0.5
9	57	F	Chronic adrenal insufficiency	Survived	Colonised	09/07/2018	ICU	Rectal	>128	>64	>64	>32	>32	>32	>16	>16	>4	0.5	>256	>320	1
10	40	F	Previous breast neoplasia; acute promyelocytic leukaemia	Survived	Infected	30/08/2018	Haematology	Blood culture	>128	>64	>64	>32	>32	>32	>16	>16	>4	16	32	>320	0.5

MIC, minimum inhibitory concentration; TPZ, piperacillin/tazobactam; CAZ, ceftazidime; FEP, cefepime; ETP, ertapenem; IPM, imipenem; MEM, meropenem; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; COL, colistin; FOS, fosfomycin; SXT, trimethoprim/sulfamethoxazole; TGC, tigecycline; NT, not tested; ICU, intensive care unit.

[a] The main clinical characteristics of the patients are described.

[b] Except for carbapenems, colistin and tigecycline, MICs were determined by VITEK® 2. Carbapenem MICs were determined by Etest. Colistin and tigecycline MICs were determined by broth microdilution following European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2018 guidelines.

[c] The colistin-resistant isolate from patient No. 10 was negative for the *mcr-1*, *mcr-2* and *mcr-3* genes by PCR (data not shown).

carbapenems poses an unpredictable epidemiological situation. Consequently, thorough monitoring of ST147 is mandatory.

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Competing interests

None declared.

Ethical approval

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