International Journal of Antimicrobial Agents 53 (2019) 533–534

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Letter to the Editor

Emergence of NDM-7-producing multi-drug-resistant *Enterobacter hormaechei* sequence type ST-78 in Spain: a high-risk international clone

Sir,

Enterobacter cloacae complex (ECC) is an important nosocomial pathogen that is intrinsically resistant to many antibiotics, and harbours clonal lineages of increased epidemic potential that may be associated with the spread of resistance [1]. In recent years, a global emergence of carbapenem-resistant *E. cloacae* complex (CR-ECC) has been observed, mainly due to VIM-type carbapenemase, followed by NDM, KPC, OXA-48 and IMP [1]. In Spain, VIM is the most common carbapenemase in ECC, although KPC and OXA-48 have also been detected [2,3]. NDM has been described in *Klebsiella pneumoniae* and *Escherichia coli*, but never in ECC [3,4].

Here, we report three cases of NDM-producing multi-drug-resistant ECC infection in our tertiary hospital. The three patients were elderly, had many comorbidities, and had never travelled outside of Spain. Patient 1 was a 76-year-old woman who underwent kidney transplantation. During hospitalization, she was diagnosed with a urinary tract infection with CR-ECC (Table 1). Contact isolation and active surveillance protocols were initiated. During the following 7 months, she was admitted to the nephrology ward on several occasions, and four isolates of CR-ECC were obtained from urine and rectal swab cultures (10/2016, 01/2017, 02/2017 and 03/2017). All isolates showed the same antimicrobial resistance pattern, with the exception of the last two isolates which were resistant to trimethoprim-sulfamethoxazole. Patient 2, a 76-year-old man, was diagnosed with CR-ECC urinary tract infection at a primary care centre in January 2017. The patient had a history of admission to the hospital from September to December 2016. Patient 3, an 81-year-old woman, was admitted to the nephrology ward in February 2017. After 7 days, she was diagnosed with CR-ECC skin and soft tissue infection. The nine isolates obtained from the three patients were identified as *E. cloacae* using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. All were resistant to beta-lactams (including aztreonam, ceftolozane-tazobactam and ceftazidime-avibactam), carbapenems, aminoglycosides and fluoroquinolones (Table 1). The modified Hodge test and MBL-Etest yielded positive results which indicated that they were metallo-beta-lactamase (MBL) producers. Polymerase chain reaction amplification and nucleotide sequence analysis detected NDM-7-MBL in the isolates. Previously, $bla_{\text{VIM-1}}$ had been the most prevalent mechanism of CR-ECC in the study hospital [2]. Pulsed-field gel electrophoresis after chromosomal DNA digestion with *Xba*I was performed, and all CR-ECC isolates showed identical patterns of DNA macrorestriction.

In order to complete the molecular epidemiology study, whole-genome sequencing (WGS) was performed on the first isolate of CR-ECC obtained from each patient (see online supplementary material). The isolates were identified by pairwise average nucleotide identity as *Enterobacter hormaechei* (>99% identity). WGS analysis confirmed that the three isolates had an NDM-7 carbapenemase gene and other resistance genes such as $bla_{\text{CTXM-15}}$ (Table 1). Three plasmid replicons were identified that belonged to IncA/C2, IncFIB and IncX3 groups. The $bla_{\text{NDM-7}}$ gene was located on a 45 122-bp IncX3 plasmid, inserted into an *IS*26-*dsb*C-*trp*F-bla_{MBL}-$bla_{\text{NDM-7}}$-Δ*ISAba*125-*IS*5-Δ*ISAba*125 genetic element (Figs 1S and 2S, see online supplementary material). The plasmid structure, pEC-NDM-7, was identical in all three sequenced isolates, and showed 100% identity to pOM26-1 (KP776609). Interestingly, IncX3 plasmid was involved in the spread of NDM-7 among different species of Enterobacteriaceae [5]. Core-genome single-nucleotide-polymorphism-based phylogenetic analysis revealed that the three isolates were closely related (Fig. 3S, see online supplementary material), and belonged to sequence type ST-78. It is important to highlight that ST-78 represents an international lineage that has been identified in several countries in Europe (Poland, Spain, Italy, Greece, Turkey) and the Asian-Pacific region, carrying different resistant determinants, including OXA-48, VIM-1, KPC-2 and IMP-1/4/8 [1].

A recent study reported the first outbreak of NDM-7-producing *K. pneumoniae* in Spain and demonstrated the spread of this clone among patients from three hospitals in Madrid [6]. Although we do not have sufficient information to trace the micro-evolution of this clone, it is likely that strain EH_1 had evolved, and another isolate EH_1 resistant to trimethoprim-sulfamethoxazole had been transmitted to Patient 3. However, this cannot be confirmed as only the first EH_1 trimethoprim-sulfamethoxazole-susceptible isolate was sequenced. It is not known how Patient 2 could have been infected with this strain, because he was admitted to a different unit, and did not share a room, healthcare equipment or personnel with Patients 1 and 3. It is likely that there were patients colonized by *E. hormaechei* ST-78 NDM-7 in the hospital who were not detected, or NDM-7 may have been circulating in other enterobacterial species and could have been acquired by this ST-78 clone. We report here the emergence of NDM-7-producing *E. hormaechei* ST-78 in Spain. This high-risk clone has been identified in several European countries associated with different carbapenemase types, and now with NDM-7.

Nucleotide sequence accession numbers

The Whole Genome projects have been deposited at the DDBJ/EMBL/GenBank under the following accession numbers and BioProjects: EH_1 (QNVP00000000, PRJNA476574), EH_2 (QNVQ00000000, PRJNA476575) and EH_3 (QNVR00000000, PRJNA476576).

https://doi.org/10.1016/j.ijantimicag.2018.11.009

Table 1
Characteristics of NDM-7-producing *Enterobacter cloacae* complex isolates.

Isolate no.	Date (dd/mm/yyyy)	Clinical department	Antimicrobial resistance pattern[a]	PFGE// MLST	Inc replicon type	Antibiotic resistance genes						
						β-lactams	Aminoglycosides	Fluoroquinolones	Phenicol	Fosfomicyn	MLS	Sulphonamide
EH_1	03/08/2016	Nephrology	IPM; MEM; ETP; GEN; TOB; NAL; CIP; FOF	A //ST-78	IncA/C2, IncFIB, IncX3	bla_{NDM-7}, bla_{ACT-5}, $bla_{CTXM-15}$, bla_{TEM-1B}, bla_{OXA-1}	*aac(6′)Ib-cr*, *aac(3)*-IIa	*Qnr*S-1, *aac(6′)Ib-cr*	*flo*R, *cat*B4	*flo*R, *cat*B4	*mph(A)*	*sul*-2
EH_2	17/01/2017	PCC	IPM; MEM; ETP; GEN; TOB; NAL; CIP; FOF									
EH_3	21/02/2017	Nephrology	IPM; MEM; ETP; GEN; TOB; NAL; CIP; SXT; FOF									

CIP, ciprofloxacin; ETP, ertapenem; FOF, fosfomycin; GEN, gentamicin; IPM, imipenem; MEM, meropenem; NAL, naldixic acid; SXT, trimethopim-sulfamethoxazol; TOB, tobramycin; MLS, macrolide, lincosamide, streptogramin; MLST, multi-locus sequence type; PFGE, pulsed-field gel electrophoresis; PCC, primary care centre.
[a] Antimicrobial resistance pattern [minimum inhibitory concentration (mg/L)]: IPM (>32); MEM (>32); ETP (>32); GEN (>8); TOB (>8); NAL (>16); CIP (>2); FOF (64); SXT (>4/78, only in EH_3).All isolates were susceptible to amikacin, SXT (EH_1 and EH_2), tigeciclin and colistin.

Funding

This work was supported by Plan Nacional de I+D+i 2008-2011 and Instituto de Salud Carlos III, Subdirección General de Redes y Centros de Investigación Cooperativa, Ministerio de Economía y Competitividad (PI13/00045 and Spanish Network for Research in Infectious Diseases REIPI RD16/0016), co-financed by European Development Regional Fund, 'A way to achieve Europe'.

Competing interests

None declared.

Ethical approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2018.11.009.

References

[1] Peirano G, Matsumura Y, Adams MD, Bradford P, Motyl M, Cheng L, et al. Genomic epidemiology of global carbapenemase-producing *Enterobacter* spp., 2008–2014. Emerg Infect Dis 2018;24:2008–14.
[2] Villa J, Viedma E, Brañas P, Orellan MA, Otero JR, Chaves F. Multiclonal spread of VIM-1-producing *Enterobacter cloacae* isolates associated with In624 and In488 integrons located in an IncHI2 plasmid. Int J Antimicrob Agents 2014;43:451–5.
[3] Oteo J, Saez D, Bautista V, Fernández-Romero S, Hernández-Molina JM, Pérez-Vázquez M, et al. Carbapenemase-producing Enterobacteriaceae in Spain in 2012. Antimicrob Agents Chemother 2013;57:6344–7.
[4] Solé M, Pitart C, Roca I, Fàbrega A, Salvador P, Muñoz L, et al. First description of an *Escherichia coli* strain producing NDM-1 carbapenemase in Spain. Antimicrob Agents Chemother 2011;55:4402–4.
[5] Chen L, Peirano G, Lynch T, Chavda KD, Gregson DB, Church DL, et al. Molecular characterization by using next-generation sequencing of plasmids containing bla NDM-7 in Enterobacteriaceae from Calgary, Canada. Antimicrob Agents Chemother 2016;60:1258–63.
[6] Seara N, Oteo J, Carrillo R, Pérez-Blanco V, Mingorance J, Gómez-Gil R, et al. Interhospital spread of NDM-7-producing *Klebsiella pneumoniae* belonging to ST437 in Spain. Int J Antimicrob Agents 2015;46:169–73.

Jennifer Villa
Department of Microbiology, Universitary Hospital 12 de Octubre, Madrid, Spain
Research Institute Hospital 12 de Octubre (i+12), Universitary Hospital 12 de Octubre, Madrid, Spain

Octavio Carretero
Department of Microbiology, Universitary Hospital 12 de Octubre, Madrid, Spain

Esther Viedma*
Department of Microbiology, Universitary Hospital 12 de Octubre, Madrid, Spain
Research Institute Hospital 12 de Octubre (i+12), Universitary Hospital 12 de Octubre, Madrid, Spain

Jaime Lora-Tamayo
Research Institute Hospital 12 de Octubre (i+12), Universitary Hospital 12 de Octubre, Madrid, Spain
Department of Internal Medicine, Universitary Hospital 12 de Octubre, Madrid, Spain

Jesús Mingorance
University Hospital la Paz, IdiPAZ, Madrid, Spain

Fernando Chaves
Department of Microbiology, Universitary Hospital 12 de Octubre, Madrid, Spain
Research Institute Hospital 12 de Octubre (i+12), Universitary Hospital 12 de Octubre, Madrid, Spain

*Corresponding author. Address: Department of Microbiology, Universitary Hospital 12 de Octubre, Avenida de Córdoba sn, Madrid 28041, Spain. Tel.: +34 917792414.
E-mail address: ester.viedma@salud.madrid.org (E. Viedma)

Received 25 September 2018
Accepted 17 November 2018