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Short Communication

Outbreak of NDM-1+CTX-M-15+DHA-1-producing *Klebsiella pneumoniae* high-risk clone in Spain owing to an undetectable colonised patient from Pakistan

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ABSTRACT

Here we describe an outbreak due to NDM-1+CTX-M-15+DHA-1-producing *Klebsiella pneumoniae* (NDM-1-Kp) in Spain related to a patient previously admitted to a healthcare centre in an endemic area (Pakistan). Nine colonised patients were detected in the Neurosurgery ward between September 2015 and February 2016 during the R-GNOSIS European Project. NDM-1-Kp isolates from clinical samples were also recovered in three of these patients. Surveillance culture at admission was negative in the index case, but NDM-1-Kp colonisation was detected 27 days later after receiving antibiotic treatment. Co-colonisation with a second NDM-1-Kp isolate was identified in this patient 61 days post-admission. Overall length of stay (LOS = 75 days) ($P < 0.01$) and LOS until carbapenemase detection (LOS-1 = 36 days) was longer in NDM-1-Kp carriers than in patients with other carbapenemase-producing Enterobacterales. Intervention strategies were implemented after the outbreak declaration and NDM-1-Kp transmission was contained. Among the NDM-1-Kp isolates, two clones [ST437 (index case and Patient 2) and ST101 (index case and Patients 3–9)] with different IncFIB NDM-1-containing plasmids were identified. Whole-genome sequencing revealed a high content of antimicrobial resistance genes in both isolates in addition to a large number of virulence factors. Colonisation with other epidemic (OXA-48-ST11-*K. pneumoniae* and VIM-1-ST54-*K. pneumoniae*) and non-epidemic (VIM-1-ST908-*K. pneumoniae* and VIM-ST431-*Escherichia coli*) clones was also detected in two NDM-1 carriers. Implementation of adequate infection control measures and uninterrupted active surveillance programmes for detecting patients with a low colonisation status are crucial to prevent the introduction and dissemination of NDM-type enzymes in our region.

1. Introduction

The emergence and uncontrolled dissemination of carbapenemase-producing Enterobacterales (CPE) has caused great concern to healthcare systems worldwide. In the last decades, a large variety of acquired carbapenemases have been identified in nosocomial pathogens, although the carbapenemases of greatest clinical relevance among Enterobacterales species are usually grouped in three classes: class A (KPC); class B or metallo-β-lactamases (MBLs) (VIM, IMP and NDM); and class D or oxacillinases (OXA-48) [1].

New Delhi metallo-β-lactamase 1 (NDM-1) corresponds to one of the latest and most important carbapenemases identified among Gram-negative bacilli. Both NDM-1 colonisation and infection were first detected in 2008 in a Swedish patient with a prior hospital stay in New Delhi, India [2]. Since then, worldwide dissemination of Enterobacterales harbouring NDM-1 has been mainly attributed to intercontinental travel to endemic areas, which include the Indian subcontinent, the Balkans and Middle East countries [3,4]. Remarkably, not all NDM-1-positive bacteria have been linked to nosocomial acquisition. Community circulation and environmental sources have also been described as reservoirs of these enzymes in endemic countries [5]. In Spain, sporadic hospital outbreaks have

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been reported and in most cases a link with endemic regions has been established [6,7].

The NDM-1 enzyme has been detected in a wide variety of Enterobacterales species, although *Klebsiella pneumoniae* and *Escherichia coli* are the most frequent. Moreover, successful spread of $bla_{\text{NDM-1}}$ is often mediated by a broad range of genetic structures such as plasmids and transposable elements [3]. NDM-1 exhibits higher carbapenemase activity compared with other carbapenemases. It is also commonly associated with other antimicrobial resistance mechanisms resulting in a multidrug-resistant (MDR) phenotype. As a consequence, therapeutic options are usually limited to very few antibiotics such as colistin, amikacin, tigecycline and fosfomycin [2,3,8].

Here we describe an outbreak of NDM-1-producing Enterobacterales isolates in a tertiary hospital in Spain during a cluster randomised study evaluating isolation strategies for carriers of extended-spectrum β-lactamase (ESBL)-producing Enterobacterales (R-GNOSIS European Project).

2. Materials and methods

2.1. Hospital setting and study design

Ramón y Cajal University Hospital is a 1118-bed tertiary healthcare facility located in the north of Madrid (Spain) that provides specialised assistance to 558373 citizens and is also the referral hospital of the International Madrid–Barajas Adolfo Suárez Airport. From March 2014 to April 2016, 15556 rectal swabs from 8209 patients were recovered as a part of the R-GNOSIS project (R-GNOSIS-FP7-HEALTH-F3-2011-282512) [9]. Following Spanish guidelines, contact isolation and standard precautions were immediately implemented following detection of each new case of carbapenemase colonisation/infection [10]. Between September 2015 and February 2016, nine patients were involved in an outbreak due to NDM-1-producing *K. pneumoniae* (NDM-1-Kp) in the Neurosurgery ward of Ramón y Cajal University Hospital. All clinical and epidemiological data were retrospectively reviewed, including patient age, sex, length of stay (LOS), LOS until the first (LOS-1) and second (LOS-2) positive culture, infection and colonisation sites, antibiotic therapy and underlying diseases. Epidemiological differences with respect to patients colonised with other CPE [OXA-48 ($n = 104$), VIM-1 ($n = 50$) and KPC-3 ($n = 6$)] during the R-GNOSIS study were also analysed. The study was approved by the Ethical Committee of Ramón y Cajal University Hospital.

2.2. Bacterial isolates and determination of carbapenemase production

Rectal swabs were seeded on chromID® CARB/OXA-48 selective chromogenic agar (bioMérieux, Marcy-l'Étoile, France) plates and were incubated at 37 °C for 48 h. A unique colony per colour and morphology was selected for microbiological analysis. Bacterial identification was performed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker Daltonik GmbH, Bremen, Germany). Carbapenemase production was phenotypically confirmed by the KPC/MBL/OXA-48 Confirm Kit (Rosco Diagnostica, Taastrup, Denmark) and the modified Hodge test.

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the broth microdilution method (MicroScan; Beckman Coulter, Brea, CA). Meropenem and fosfomycin minimum inhibitory concentrations (MICs) were determined using MIC gradient strips (MIC Test Strip; Liofilchem, Roseto degli Abruzzi, Italy). All results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2018 guidelines (http://www.eucast.org). Isolates categorised as intermediate were considered non-susceptible.

2.4. Identification and characterisation of antimicrobial resistance genes

Carbapenemase and CTX-M-group genes were preliminarily identified using the eazyplex® SuperBug CRE system [11]. Genes encoding carbapenemases (bla_{VIM}, bla_{OXA}, bla_{NDM} and bla_{KPC}), ESBLs (bla_{TEM}, bla_{SHV} and $bla_{\text{CTX-M}}$) and AmpC β-lactamases (bla_{DHA}) were then characterised by PCR and sequencing [9].

2.5. Population structure

Genetic relatedness was studied by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) [9].

2.6. Plasmid typing

Plasmids carrying carbapenemase genes were transferred into azide-resistant *E. coli* J53 cells by conjugation assay. In those isolates in which conjugation was not effective, transformation and electroporation experiments were performed. Plasmid size and β-lactamase gene location were studied by *S1* nuclease PFGE digestion and hybridisation following standard procedures. Plasmids encoding carbapenemases were also typed by PCR (updated PBRT scheme; *repA, traU* and *parA* gene amplification) as previously described [12].

2.7. Whole-genome sequencing

DNA was extracted using a Wizard® Genomic DNA Purification Kit (Promega, Madison, WI). The genomes of two NDM-1-Kp isolates were fully sequenced using a standard 2×100-bp protocol with a Genome Analyzer IIx HiSeq® 2500 platform (Illumina Inc., San Diego, CA). *De novo* assembly was performed using SPAdes genome assembler [13]. Antimicrobial resistance genes and virulence factors were screened using ResFinder, NCBI and VFDB tools with an identity threshold of 95%. Plasmid analysis was performed using PlasmidFinder, PLACNETw (Plasmid Constellation Network) and plasmidSPAdes tools [14,15]. Prokka v.1.13.3 was used to annotate contigs [16]. Easyfig v.2.2.2 was used for drawing the schemes and comparisons [17].

2.8. Statistical analysis

Differences in continuous variables were analysed using analysis of variance (ANOVA) and Tukey's HSD test (RStudio v.1.0.44; RStudio Team, Boston, MA). In all cases, a P-value of <0.05 was considered statistically significant.

3. Results

3.1. Outbreak emergence, evolution and infection control measures

The index case (patient 1) was a 36-year-old Pakistani man living in Valencia, a city located 360 km from Madrid on the Mediterranean coast. On 26 August 2015, on return from Pakistan, he reported cervical pain and was transferred from Madrid–Barajas Adolfo Suárez Airport to Ramón y Cajal Hospital and was admitted to the neurosurgery ward. The patient had received healthcare assistance in Pakistan following a traffic accident in November 2014. CPE colonisation status was investigated at admission by a rectal swab cultured on chromogenic media. As the results were negative, the patient was not placed under contact precaution

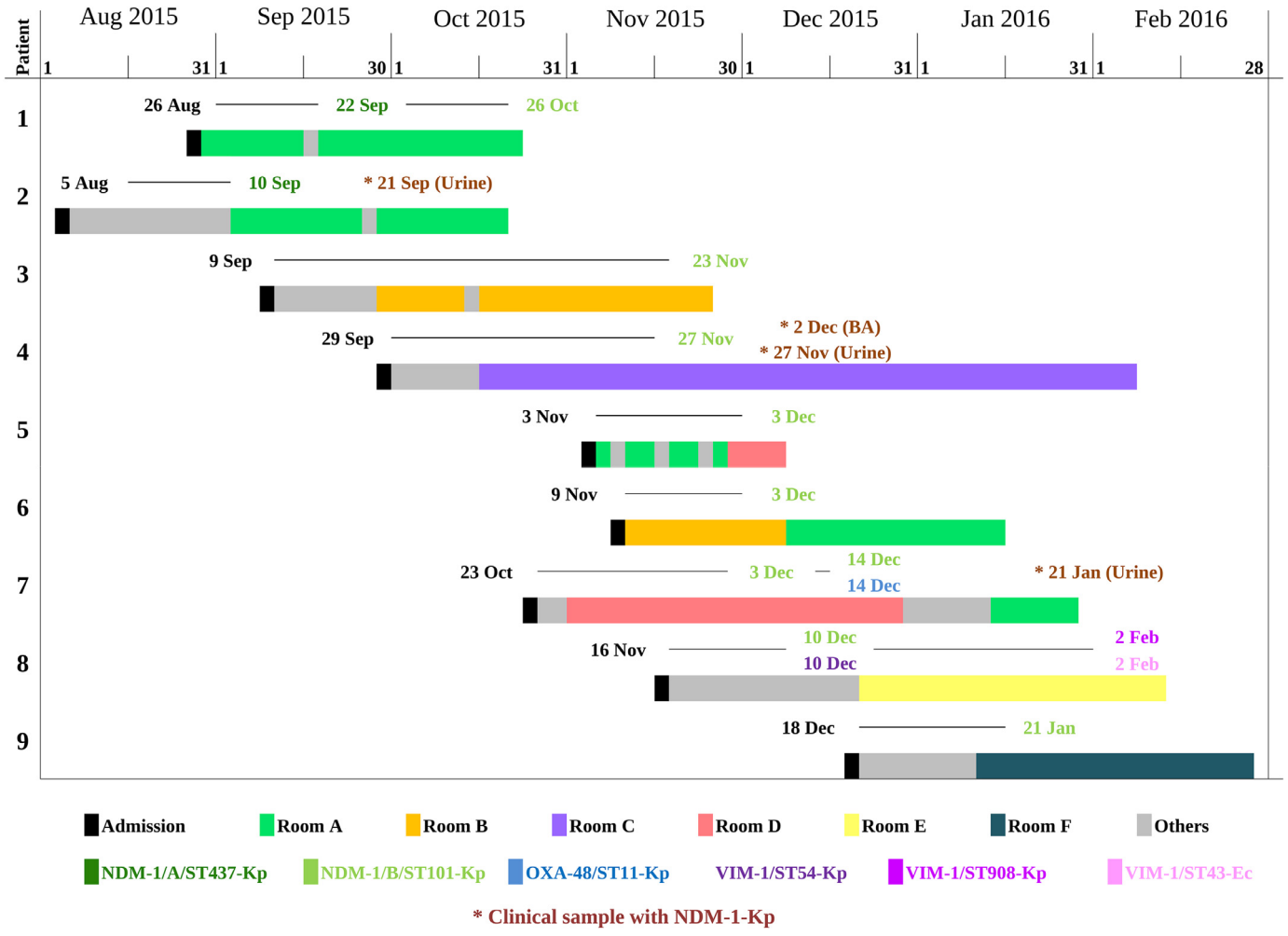

Fig. 1. Timeline of events during the outbreak of NDM-1-producing *Klebsiella pneumoniae* (Kp). Dates are given as day/month. BA, bronchial aspirate; Ec, *Escherichia coli*.

in a single room. On 15 September 2015 the patient underwent surgery by aseptic loosening of a thoracolumbar prosthesis, and on 22 September 2015 a *K. pneumoniae* with a MDR phenotype compatible with a MBL-type carbapenemase (isolate 1A) was recovered from a rectal surveillance culture. The eazyplex® SuperBug CRE system demonstrated the presence of bla_{NDM} and $bla_{CTX\text{-}M\text{-}1\text{-}group}$ genes.

On 22 September 2015, another NDM-1-Kp isolate was confirmed in a rectal sample from a second patient (Patient 2), a 68-year-old male who shared a room with the index case. After these events, all CPE isolates collected in the neurosurgery ward in the weeks leading up to 22 September 2015 were retrospectively characterised. From Patient 2, another positive sample on 10 September 2015 with the same resistance phenotype was identified. Thereafter, a second NDM-1-Kp isolate with a different MDR profile (isolate 1B) was also recovered from the index patient at discharge. The following seven cases of NDM-1-Kp colonisation occurred between November 2015 and February 2016 (Fig. 1). NDM-1-Kp were also recovered from clinical samples in three patients (3/9; 33%), affecting the urinary ($n = 3$) and respiratory ($n = 1$) tracts (Table 1).

In Patients 7 and 8, colonisation with other CPE was also detected: isolate 7B (OXA-48-Kp); isolates 8B and 8C (VIM-1-Kp); and isolate Ec-8D (VIM-1-*E. coli*). It should be noted that isolates 7B and 8B were detected colonising simultaneously with the NDM-1-producing isolates (7A and 8A, respectively), whilst isolates 8C and Ec-8D were collected 54 days after NDM-1-Kp detection (isolate 8) (Fig. 1).

All patients involved in the outbreak, including the index case, had a negative sample for CPE colonisation at admission. However, colonisation with ESBL-producing *K. pneumoniae* was demonstrated in Patients 1, 2, and 4.

Prior to this outbreak, no cases of colonisation and/or infection with NDM-producing Enterobacterales were identified in the hospital. All patients were placed under contact precaution in single rooms on the same day that CPE colonisation/infection was detected. Isolation protocols included washing patients' skin with an antiseptic gel of 4% soapy chlorhexidine, emphasising hand hygiene of healthcare workers and visitors with an alcohol-based hand rub solution, decontamination of the patients' environment twice daily with a combined detergent–chlorine solution, and cleaning and disinfection of medical equipment. Moreover, following the R-GNOSIS Project protocol, active surveillance cultures were performed in all patients at admission in the unit and weekly until discharge.

3.2. Patient characteristics

The median age of patients colonised with NDM-1-Kp was 80 years and 89% of them were male (8/9). The median LOS was 75 days (range 58–132 days) and until the detection of the first NDM-1-producing isolate (LOS-1) was 34 days (range 27–75 days). In Patient 1, the LOS until the detection of co-colonisation with a second NDM-1-Kp isolate (LOS-2) was 61 days. During the R-GNOSIS study, patients colonised with NDM-1-producers had a significant

Table 1
Epidemiological and clinical data of patients colonised with NDM-1-producing *Klebsiella pneumoniae* (NDM-1-Kp).

Patient	Sex	Age (years)	Admission diagnosis	LOS (days)	LOS-1 (days)	LOS-2 (days)	NDM-1 isolate	Clinical sample (+) NDM-1	Previous antibiotic treatment (day start: day end)
1	M	36	Cervical apophyseal joint pain	58	27	61	1A/1B	–	AMC (+14:+43), CZO (+21:+22), CIP (+55:+58)
2	M	68	Parkinson's	76	36	–	2	Urine	CZO (+13), LVX (+29:+33), CAZ+LVX+VAN (+34:+36)
3	M	84	Brain tumour	75	75	–	3	–	CZO (+6:+8, +20:+21, +35:+37), AMC (+45:+61), CIP (+64:+69)
4	F	80	Cranioencephalic trauma	132	59	–	4	Urine and bronchial aspirate	AMC (+1:+4), VAN+MEM (+6:+15, +37:+45), MEM (+16:+20), TZP (+24:+35), VAN+MEM+CIP (+46:+52)
5	M	29	Intracranial injury	34	30	–	5	–	CTX+VAN+CAZ (+1:+9), VAN+CAZ (+10:+30)
6	M	87	Epileptic crisis	65	24	–	6	–	CAZ+VAN+MTR (+1:+8), AMC (+9:+24)
7	M	92	Cranioencephalic trauma	92	41		7A	Urine	CIP (+28:+36)
8	M	49	Subarachnoid haemorrhage	88	29	–	8A	–	VAN+MEM (+12:+13), MEM+LNZ (+14:+29)
9	M	89	Chronic subdural haematoma	69	34	–	9	–	CIP (+2:+14)

LOS, length of stay; LOS-1, length of stay until detection of first NDM-Kp; LOS-2, length of stay until detection of second NDM-Kp; AMC, amoxicillin/clavulanic acid; CZO, cefazolin; CIP, ciprofloxacin; LVX, levofloxacin; CAZ, ceftazidime; VAN, vancomycin; MEM, meropenem; TZP, piperacillin/tazobactam; CTX, cefotaxime; MTR, metronidazole; LNZ, linezolid.

longer LOS than patients with other CPE ($LOS_{OXA-48} = 14$ days vs. $LOS_{VIM-1} = 20.5$ days vs. $LOS_{KPC-3} = 12$ days; $P \leq 0.01$). Moreover, patients carrying NDM-1-Kp showed a higher LOS-1 than patients colonised with other CPE ($LOS\text{-}1_{OXA-48} = 16$ days vs. $LOS\text{-}1_{VIM-1} = 18$ days vs. $LOS\text{-}1_{KPC-3} = 12$ days; $P > 0.05$). Antibiotic therapy prior to the detection of the NDM-1-Kp in each patient is shown in Table 1. Overall, fluoroquinolones (67%) and cephalosporins (56%) were the antimicrobial groups most frequently used during the hospital stay until NDM-1-Kp detection. Moreover, Patient 1 had received antibiotic therapy with ciprofloxacin prior to the detection of co-colonisation with the second NDM-1-Kp strain.

3.3. Antimicrobial resistance gene characterisation and clonal relatedness

PCR and sequencing confirmed that all *K. pneumoniae* isolates involved in the outbreak co-produced NDM-1, CTX-M-15 and DHA-1. All of the isolates were grouped into two restriction profiles corresponding to two different sequence types (STs): clone A/ST437 (isolates 1A and 2); and clone B/ST101 (isolates 1B, 3, 4, 5, 6, 7A, 8A and 9). Isolate 7B was identified as the *K. pneumoniae* high-risk clone ST11-OXA-48+CTX-M-15 (clone C), whilst among the VIM-1-producing isolates the following PFGE patterns and STs were detected: clone D/ST54-VIM-1+SHV-12 (isolate 8B); clone E/ST908-VIM-1 (isolate 8C); and clone F/ST43-VIM-1 (isolate Ec-8D) (Supplementary Table S1).

The ESBL-producing *K. pneumoniae* isolates recovered in Patients 1, 2 and 4 harboured the $bla_{CTX-M-15}$ gene. They were not included in the subsequent analysis as different restriction profiles were observed among them with respect to NDM-producers.

3.4. Antimicrobial susceptibility

Overall, elevated carbapenem MICs [imipenem (4 mg/L to >8 mg/L), meropenem (3 mg/L to >32 mg/L) and ertapenem (>4 mg/L)] were observed in all NDM-1+CTX-M-15+DHA-1-Kp isolates. Moreover, all of these isolates exhibited high co-resistance rates to other antimicrobial groups, although differences were observed between clones. Isolates belonging to clone A/ST437 were susceptible to amikacin and resistant to trimethoprim/sulfamethoxazole (SXT), whilst all isolates from clone B/ST101 showed resistance to amikacin and susceptibility to SXT. Furthermore, except for one (isolate 2), all NDM-1+CTX-M-15+DHA-1-Kp isolates were susceptible to tigecycline, but only three (isolates 3, 8A and 9) were susceptible to fosfomycin (Supplementary Table S1).

3.5. Plasmid characterisation

A total of 12 transconjugants with bla_{NDM-1}, bla_{OXA-48} or bla_{VIM-1} genes were obtained from the Kp-B/ST101, Kp-C/ST11, Kp-D/ST54, Kp-E/ST908 and Ec-F/ST43 clones (Table 2). Transconjugants or transformants could not be obtained from the two isolates (1A and 2) belonging to clone A/ST437. In both, bla_{NDM-1} was located on an ca. 100-kb plasmid along with the $bla_{CTX-M-15}$ and bla_{DHA-1} genes. Nevertheless, in all isolates from clone B/ST101 and their respective transconjugants, the bla_{NDM-1} gene was detected in an ca. 120-kb plasmid, whilst $bla_{CTX-M-15}$ and bla_{DHA-1} genes were only found in the wild-type in a larger plasmid (ca. 150 kb). The NDM-1-encoding plasmid could not be classified by PCR according to their incompatibility (Inc) group. On the other hand, bla_{OXA-48}, $bla_{CTX-M-15}$ and the bla_{VIM-1} genes were mostly detected on an ca. 60 kb IncL plasmid.

3.6. Genomic sequencing and bioinformatics tools

Whole-genome sequencing analysis was performed on one representative NDM-1-Kp isolate of each ST (isolate 1A-clone A/ST437 and isolate 3-clone B/ST101).

In the ST437 clone, a next-generation sequencing (NGS) approach confirmed the presence of multiple genes encoding resistance to β-lactams (bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{OXA-1}, bla_{OXA-10}, $bla_{SHV-155}$ and bla_{TEM-1}), aminoglycosides [*aac(3)-IIa, aadA1, aadA16, aadA2, aph(3'')-Ib, aph(3')-Ia* and *aph(6)-Id*], sulfonamides (*sul1* and *sul2*), trimethoprim (*dfrA12* and *dfrA27*), tetracycline [*tet*(A)], fosfomycin (*fosA6*), quinolones (*qnrB1*), rifampicin (*arr-3*), macrolides [*mph(A)*] and chloramphenicol (*catA1*). In the ST101 strain, resistance mechanisms were also detected against β-lactams (bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{DHA-1}, bla_{OXA-1} and $bla_{SHV-106}$), aminoglycosides [*aac(3)-IIa, aph(3'')-Ib* and *rmtF1*], sulfonamides (*sul1* and *sul2*), fosfomycin (*fosA* and *fosA6*), quinolones (*qnrB4*), rifampicin (*arr-3*) and chloramphenicol (*catB* and *catB3*). Furthermore, additional genes for antiseptic resistance (*qacEΔ1*), bleomycin resistance (ble_{MBL})

Table 2
Characterisation of plasmids containing bla_{NDM-1}, bla_{OXA-48} and bla_{VIM-1} genes.

WT isolates	Patient	ST (WT)	Resistance genes (WT)	Plasmids WT (kb)	Tc	Resistance genes (Tc)	Plasmids TC (kb)	Inc group	MIC (mg/L) (Tc)						Co-resistance profile (Tc)
									IMP	ETP	MEM	CAZ	CTX	FEP	
1A	1	437	NDM-1 CTX-M-15 DHA-1	~100 ~100 ~100	–	–	–	–	–	–	–	–	–	–	–
1B	1	101	NDM-1 CTX-M-15 DHA-1	~120 ~150 ~150	Tc-1B	NDM–1	~120	NT	8	2	16	>16	>16	>16	GEN, TOB, AMK
2	2	437	NDM-1 CTX-M-15 DHA-1	~100 ~100 ~100	–	–	–	NT	–	–	–	–	–	–	–
3	3	101	NDM-1 CTX-M-15 DHA-1	~120 ~150 ~150	Tc-3	NDM–1	~120	NT	>8	>1	>8	>16	>16	>16	GEN, TOB, AMK
4	4	101	NDM-1 CTX-M-15 DHA-1	~ 120 ~150 ~150	Tc-4	NDM–1	~120	NT	2	>1	>8	>16	>16	>16	GEN, TOB, AMK
5	5	101	NDM-1 CTX-M-15 DHA-1	~ 120 ~150 ~150	Tc-5	NDM–1	~120	NT	>8	>1	8	>16	>16	>16	GEN, TOB, AMK
6	6	101	NDM-1 CTX-M-15 DHA-1	~120 ~150 ~150	Tc-6	NDM–1	~120	NT	>8	>1	>8	>16	>16	16	GEN, TOB, AMK
7A	7	101	NDM-1 CTX-M-15 DHA-1	~ 120 ~150 ~150	Tc-7A	NDM–1	~120	NT	>8	>1	4	>16	>16	>16	GEN, TOB, AMK
7B	7	11	OXA-48 CTX-M-15	~60 ~60	Tc-7B	OXA–48 CTX–M–15	~60 ~60	IncL	8	>1	2	>16	>16	>16	–
8A	8	101	NDM-1 CTX-M-15 DHA-1	~ 120 ~150 ~150	Tc-8A	NDM–1	~120	NT	>8	>1	8	>16	>16	>16	GEN, TOB, AMK
8B	8	54	VIM-1 SHV-12	~90 ~90	Tc-8B	VIM–1 SHV–12	~90 ~90	NT	8	>1	2	>16	>16	>16	–
8C	8	908	VIM-1	~60	Tc-8C	VIM–1	~60	IncL	8	1	>8	>16	>16	>16	–
Ec-8D	8	43	VIM-1	~60	Tc-Ec-8D	VIM–1	~60	IncL	4	1	4	>16	>16	>16	–
9	9	101	NDM-1 CTX-M-15 DHA-1	~120 ~150 ~150	Tc-9	NDM–1	~120	NT	>8	>1	>8	>16	>16	>16	GEN

WT, wild-type; ST, sequence type; Ec, *Escherichia coli*; Tc, transconjugant; NT, not typeable; MIC, minimum inhibitory concentration; IMP, imipenem; ETP, ertapenem; MEM, meropenem; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; GEN, gentamicin; TOB, tobramycin; AMK, amikacin.

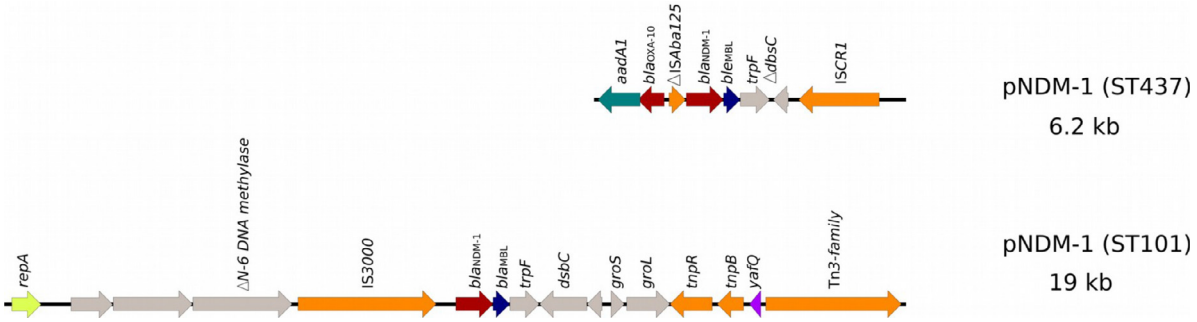

Fig. 2. Schematic structure of the bla_{NDM-1} genetic context of IncFIB plasmids from ST437 and ST101 NDM-1-producing *Klebsiella pneumoniae* clones. Genes and their corresponding transcription orientations are indicated by horizontal arrows. Antimicrobial resistance genes are indicated by coloured arrows as follows: red, β-lactam resistance genes (bla_{NDM-1} and bla_{OXA-10}); green, aminoglycoside resistance gene (*aadA1*); blue, bleomycin resistance gene (ble_{MBL}). Transposon-related genes and insertion sequences (ISs) are indicated by orange arrows. Replicons are indicated by yellow arrows. The toxin–antitoxin gene (*yafQ*) is indicated by a purple arrow. Δ, indicates genes that are truncated.

and efflux pump genes (*oqx* genes) were also identified in both strains. In addition, a high number of virulence genes were detected both in ST437 and ST101 NDM-1-Kp isolates (Supplementary Fig. S1).

The bla_{NDM-1} gene was encoded on an IncFIB plasmid both in ST437 and ST101 clones, although bla_{NDM-1} was located in the ST437 isolate in a class 1 integron flanked by the insertion sequences IS*CR1* and IS*Aba125* and as part of the composite transposon Tn*3* (IS*3000*) in the ST101 clone (Fig. 2).

4. Discussion

The emergence and intercontinental dissemination of the NDM-1 enzyme is having an unprecedented impact on health systems worldwide [1]. According to the literature and unlike other carbapenemases such as OXA-48 or KPC, the spread of NDM-producers in Spain has been limited to sporadic outbreaks [7,18,19]. Currently, contact with endemic countries, either by tourism or health care, is considered the main risk factor for NDM-1 acquisition [3,4]. Coincidentally, in the outbreak described here, the index patient had received healthcare assistance in an endemic country (Pakistan) during the previous year. It was remarkable that the rectal swab culture performed at admission with chromogenic media was negative and that colonisation with NDM-1-Kp was detected 27 days later, after receiving antibiotic therapy. Several studies have demonstrated that prior fluoroquinolone exposure promotes the acquisition of carbapenem-resistant *K. pneumoniae* [20,21]. Nevertheless, in our index case, antibiotic use could have played a major role in the loss of intestinal microbiota diversity and the selection of different MDR strains, making NDM-1-Kp detection possible in subsequent cultures. Furthermore, culture of rectal swabs has demonstrated reduced sensitivity compared with molecular methods, particularly when the colonisation level is low [22]. In addition, co-colonisation with a second NDM-1-Kp strain was also identified in this patient 61 days post-admission. In this respect, a different intestinal density of ST437 and ST101 NDM-1-Kp clones could explain the low sensitivity of surveillance cultures in the detection of this second clone. However, although for ST437 clone no transconjugants were obtained with the in vitro conditions, we cannot rule out the in vivo transference of bla_{NDM-1} gene from ST437 to ST101 clone in the intestinal microbiome of the index patient under antibiotic pressure, and after nosocomial acquisition of a circulating ST101 clone during the hospital stay. Fortunately, despite the increasing CPE prevalence recently described in our hospital [9], cross-species transfer of bla_{NDM-1} from *K. pneumoniae* to other Enterobacterales species, particularly *E. coli*, was not detected during the outbreak.

Overall, bla_{NDM-1} has been reported to be harboured by a diverse range of conjugative plasmids (IncA/C, IncF, IncL/M, IncN or even untypeable) frequently associated with further resistance mechanisms, including not only other β-lactamases but also genes conferring resistance to other antimicrobial groups [2,3,8,23]. During this outbreak, a high co-resistance was also found among both NDM-1-Kp clones, leaving only two or three therapeutics options. Concurring with other studies [6], co-production of CTX-M-15 and DHA-1 was identified among all NDM-1-Kp isolates. Both $bla_{CTX-M-15}$ and bla_{DHA-1} genes were located along with bla_{NDM-1} in the same plasmid in the isolates belonging to the clone ST437, but were located in different plasmids in those identified as clone ST101. Whole-genome sequencing analysis demonstrated that both NDM-1-encoding plasmids belonged to the IncFIB group, although a distinct bla_{NDM-1} genetic context was observed between ST437 and ST101 clones. Interestingly, NGS revealed the presence in the ST437 clone of a conserved structure carrying bla_{NDM-1} similar to that found in other plasmids associated with bla_{NDM} genes [23,24]. The high diversity and plasticity of the genetic structures involved in the dissemination of NDM-1 β-lactamase along with the high resistance and virulence gene content of these clones results in the emergence of pandrug-resistant micro-organisms with a high capacity for adaptability and persistence both in hospital and community settings.

On the other hand, except in one case, bla_{OXA-48} and bla_{VIM-1} were located on a ca. 60-kb IncL plasmid related to the worldwide disseminated IncL/M-pOXA-48a [25]. During the R-GNOSIS Project, cross-species transfer of this IncL plasmid has been widely described among different OXA-48- and VIM-1-producing Enterobacterales isolates [12]. Although ST437 and ST101 *K. pneumoniae* were only related to NDM-1 production, both clones have been previously associated with other carbapenemases, such as OXA-48, KPC-2 or NDM-7 [18,26]. Furthermore, co-production of NDM-7 with OXA-48 by the *K. pneumoniae* ST437 high-risk clone has been recently reported in Madrid [27]. In the present outbreak, coexistence of NDM-1/ST101 along with other *K. pneumoniae* epidemic clones, such as VIM-1+SHV-12/ST54 and OXA-48+CTX-M-15/ST11, could have facilitated the acquisition of bla_{NDM-1} by these well-adapted hospital clones, favouring the introduction and successful establishment of NDM enzymes in our hospital, as happened with OXA-48 and VIM-1 [9] although, until now, this event has not been observed.

Although further dissemination of NDM-1-Kp isolates was controlled following reinforcement of infection control measures in the unit, the current results highlight the rapid dissemination of NDM-1 carbapenemase through epidemic clones with a high capacity for dispersion and persistence in the hospital environment. Admission of undetectable or asymptomatic colonised

patients from endemic countries should alert for the risk of introduction and establishment of these potential MDR strains in our local epidemiology.

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Competing interests

None declared.

Ethical approval

This study was approved by the Ethical Committee of Ramón y Cajal University Hospital (Madrid, Spain) [ref. 251/13].

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.05.021.

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