Multiclonal epidemic of *Klebsiella pneumoniae* isolates producing DHA-1 in a Spanish hospital

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Abstract

Between June 2007 and January 2008, 26 *Klebsiella pneumoniae* isolates carrying bla_{DHA-1} on an IncL/M plasmid were obtained from clinical samples at Granollers Hospital, Barcelona, Spain. Three of the isolates also carried a $bla_{CTX-M-15}$ gene. The 26 isolates showed 11 pulsed-field gel electrophoresis (PFGE) patterns. Multilocus sequence typing showed that PFGE patterns A, B and C belonged to sequence type (ST)17, D to ST13, E to ST427, F and G to ST416, H to ST37, I to ST440, J to ST326, and K to ST428. Results demonstrated the effectiveness of the infection control programme in place at the centre. This study reports the first characterization of STs for bla_{DHA-1}-producing *K. pneumoniae* isolates.

Keywords: AmpC, epidemiology, ESBL, multilocus sequence typing, resistance mechanisms

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These results were partially presented at the 2009 Annual Meeting of the Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica in Sevilla, Spain (Epidemiología de las cepas de *Klebsiella pneumoniae* productoras de cefamicinasa en un hospital comarcal), and at the 20th European Congress of Clinical Microbiology and Infectious Diseases, 2010, Vienna, Austria (Multilocus sequence typing of DHA-1-producing *Klebsiella pneumoniae* isolates in a Spanish hospital).

Gram-negative bacilli expressing chromosomal AmpC β-lactamases could acquire resistance to cephalosporins by hyperproduction of their β-lactamase [1]. Moreover, since 1989, these AmpC genes or genes derived from AmpC genes have been detected on plasmids and in species that do not naturally produce AmpC enzymes, such as *Klebsiella oxytoca*, *Salmonella* spp. and *Proteus mirabilis*, and especially *Klebsiella pneumoniae* isolates [1,2]. The plasmid-mediated AmpC β-lactamases described to date can be grouped in families: ACC, CMY, DHA, FOX, MIR, ACT or MOX.

The present study aimed to document nosocomial infections caused by different clusters of AmpC-producing *K. pneumoniae* isolates between June 2007 and January 2008 at Granollers Hospital (Barcelona, Spain). This is a medical referral centre with 297 acute-care beds, 22 intensive-care beds and an average of 17 161 admissions per year. In June 2007, the Microbiology and Infectious Diseases Department reported the detection of five nosocomial *K. pneumoniae* cefotaxime-resistant and cefoxitin-resistant isolates. The infection control team immediately began to investigate a possible outbreak.

Between June 2007 and January 2008, a total of 2885 enterobacterial isolates were obtained from 2459 patients consecutively admitted to Granollers Hospital. Of these, 26 *K. pneumoniae* isolates (corresponding to 26 patients) showed resistance, by the disk diffusion method, to amoxycillin–clavulanic acid, cefoxitin, cefotaxime, ceftazidime and aztreonam, and were susceptible to carbapenem according to CLSI breakpoints [3]. This resistance pattern suggested the production of an AmpC enzyme. Additionally, three of these isolates showed synergy between amoxycillin–clavulanic acid and cefotaxime on Mueller–Hinton agar, suggesting production of extended-spectrum β-lactamases (ESBLs).

The 26 isolates were obtained from 17 male and nine female patients with mean ages of 60 and 75 years, respectively. One of the patients was transferred from another hospital, three patients had community-acquired infection or colonization with no relation to our hospital, and the other 23 patients were inpatients or patients related to healthcare facilities. Urine was the most frequent source of *K. pneumoniae* isolation (17 isolates, 62.9%) (Table 1). Thirty-five per cent of patients had previously been treated with amoxycillin–clavulanic and 11.5% with cephalosporins. By the time the microbiological result was known, five patients had already started inadequate treatment with β-lactams. Nevertheless, four developed favourably without change in the treatment. The fifth patient died before the treatment was changed.

β-Lactamase genes were characterized by PCR and sequenced as previously described [4]. The results revealed

TABLE 1. Clinical, resistance gene, pulsed-field gel electrophoresis (PFGE) and associated resistance data of the patients with DHA-1-producing *Klebsiella pneumoniae* isolates

Date of isolation (month/year)	Age/sex	Type of sample	Service	β-Lactamase	PFGE type	Associated resistance	Main diagnosis	Previous medication	*Klebsiella* treatment	Clinical outcome
06/07	71/F	Urine	Convalescent care	DHA-1	A	NAL	Cerebral haemorrhage	AMC	CIP	Microbiological and clinical cure
06/07	67/M	Tracheal exudate	ICU	DHA-1	C2	AMK	Pneumonia	LVX, AMK	Unknown	Death not related
06/07	77/M	Bronquial aspirate	Surgery	DHA-1, CTX-M-15	J	GEN, TOB, KM, AMK, CIP, SXT, TET	Gastric adenocarcinoma	AMC	Unknown	Related death with no microbiological cure
06/07	52/M	Blood	Surgery	DHA-1	K	–	Sclerosing cholangitis	CTX, GEN, AMC	IPM + AMK	Microbiological and clinical cure
07/07	15 days/M	Urine	Community	DHA-1	A	–	Emesis episode	No	No	Colonization, no infection
07/07	44/M	Urine	Community	DHA-1, CTX-M-15	J	GEN, TOB, KM, AMK, CIP, SXT, TET	Acute prostatitis	No	AMC	Microbiological and clinical cure
07/07	67/M	Tracheal exudate	ICU	DHA-1, CTX-M-15	J	GEN, TOB, KM, AMK, CIP, SXT, TET	Pharyngeal cancer	TZP, GEN	IPM	Microbiological and clinical cure
08/07	72/M	Urine	Medicine	DHA-1	C	NAL	Prostate adenocarcinoma	No	No	Asymptomatic bacteriuria
08/07	72/M	Urine	Other hospital	DHA-1	C1	–	Obstructive pyelonephritis	No	Unknown	Microbiological and clinical cure
09/07	84/F	Urine	Palliative care	DHA-1	A	CIP	Gastric neoplasia	Unknown	GEN	Death attributable to underlying disease
09/07	11 months/M	Urine	Paediatric	DHA-1	A	–	Urinary tract infection	CTX, CFM	CFR	Microbiological and clinical cure
09/07	61/F	Urine	Gynaecology	DHA-1	A	–	Genital prolapse, hysterectomy	CXM	Unknown	Clinical cure
09/07	64/F	Abdominal exudate	Surgery	DHA-1	A	–	Diverticulitis	AMC	AMC	Microbiological and clinical cure
09/07	89/M	Urine	Healthcare centre	DHA-1	A1	–	Urinary tract infection	Unknown	LVX	Microbiological and clinical cure
09/07	58/M	Urine	Urology	DHA-1	F	SXT, NAL	Acute pyelonephritis	CIP	CIP + GEN	Microbiological and clinical cure
10/07	62/M	Urine	Medicine	DHA-1	B	NAL	Paraneoplasia encephalopathy	TZP, VAN	TZP, CIP	Death attributable to underlying disease
10/07	76/F	Urine	Healthcare centre	DHA-1	B	NAL	Urinary tract infection	AMC	CIP	Microbiological and clinical cure
11/07	77/F	Urine	Geriatric	DHA-1	E	NAL	Vascular dementia and Alzheimer's disease	AMC	CIP	Unknown
11/07	71/M	Catheter	Medicine	DHA-1	H	0	Cardiopulmonary arrest	AMC	No	Catheter colonization
11/07	63/F	Blood	Medicine	DHA-1	I	NAL	Cirrhosis	Unknown	Unknown	Death
12/07	88/F	Urine	Geriatric	DHA-1	B	0	Failure to thrive	Unknown	CIP	Microbiological cure
12/07	76/M	Urine	Convalescent care	DHA-1	B	NAL	Colon adenocarcinoma	AMC	CIP	Microbiological and clinical cure
12/07	64/M	Ascitic liquid	Medicine	DHA-1	D	0	Cirrhosis and spontaneous bacterial peritonitis Pulmonary tuberculosis	RIF + INH	CTX	Related death with no microbiological cure
12/07	88/F	Urine	Geriatric	DHA-1	E	NAL	Heart failure	AMC	CXM	Clinical cure
12/07	52/M	Anal exudate	Community	DHA-1	E	NAL	Perianal abscess	Unknown	Unknown	Unknown
01/08	90/M	Urine	Convalescent care	DHA-1	G	SXT	Dementia–Parkinson's disease	AMC	CIP	Microbiological and clinical cure

AMC, amoxycillin–clavulanic acid; AMK, amikacin; CIP, ciprofloxacin; CFM, cefixime; CFR, cefadroxil; CTX, cefotaxime; CXM, cefuroxime; GEN, gentamicin; ICU, intensive-care unit; INH, isoniazid; IPM, imipenem; KM, kanamycin; LVX, levofloxacin; NAL, nalidixic acid; RIF, rifampicin; SXT, trimethoprim–sulphamethoxazole; TET, tetracycline; TOB, tobramycin; TZP, piperacillin–tazobactam; VAN, vancomycin.

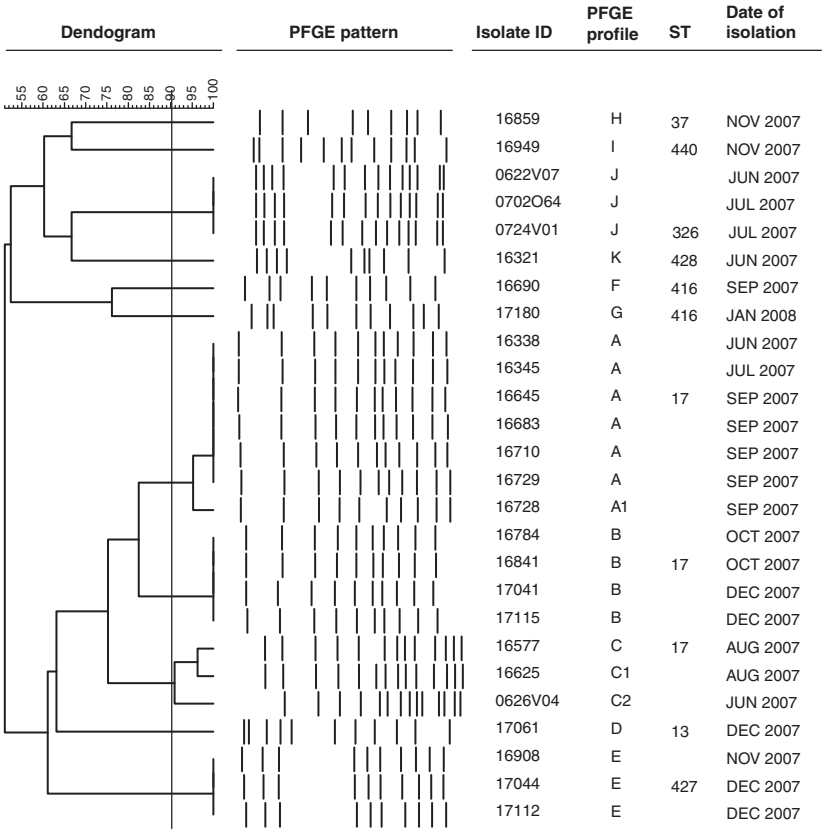

FIG. 1. Dendogram, pulsed-field gel electrophoresis (PFGE) patterns and their correspondence with multilocus sequence typing profiles of the 26 *Klebsiella pneumoniae* strains producing DHA-1. Similarity was calculated by using Dice coefficients with a position tolerance of 1% (Fingerprinting II Software; Bio-Rad). The cut-off level for identity was set at 90%. ST, sequence type.

that bla_{DHA-1} was present in all 26 isolates, and three of them also had $bla_{CTX-M-15}$. Resistance to other antibiotic agents is shown in Table 1. DHA-1-producing isolates were, in some cases, resistant only to nalidixic acid, whereas CTX-M-15-producing isolates were resistant to aminoglycosides, co-trimoxazole, tetracycline and quinolones. The presence of plasmid-mediated quinolone resistance (*qnrA*, *qnrB*, *qnrS* and *aac(6′)-Ib-cr*) was investigated by PCR [5]. Twenty-five of the isolates contained *qnrB*, and the three ESBL-producing isolates also carried *aac(6′)-Ib-cr*. Associations between bla_{DHA-1} and *qnrB*, between *aac(6′)-Ib-cr* and $bla_{CTX-M-15}$ and between bla_{DHA-1} and $bla_{CTX-M-15}$ have been reported previously [5–7].

The clonal relationships between *K. pneumoniae* clinical isolates were analysed by comparing pulsed-field gel electrophoresis (PFGE) profiles of genomic DNA, as previously described [8]. A total of 11 major patterns were obtained among the 26 *K. pneumoniae* isolates (Fig. 1); five of them were clusters with seven isolates (A), four isolates (B) or three isolates (C, E and J). The three DHA-1-producing and CTX-M-15-producing isolates belonged to the J pattern.

Multilocus sequence typing was applied according to the Institute Pasteur scheme for *K. pneumoniae* [9]. Only one isolate for each PFGE pattern was selected for multilocus sequence typing. The 11 PFGE patterns corresponded to different *K. pneumoniae* sequence types (STs): PFGE A, B and C belonged to ST17, D to ST13, E to ST427, F and G to ST416, H to ST37, I to ST440, J to ST326, and K to ST428. ST13 has been previously found in Seoul and Madrid, associated with CTX-M-14 and CTX-M-15, respectively [9–11] (Valverde *et al.*, 19th ECCMID, 2009, P1195), ST17 in Cadiz, associated with CTX-M-15, in Freiburg and in Seoul (ESBL association not shown) [9,10,12], ST37 in Madrid, associated with SHV-12, in Rome, in Germany and in Seoul (ESBL association not shown) [9–11,13] (Valverde *et al.*, 19th ECCMID, 2009, P1195), and ST326 in two hospitals in Barcelona, associated with CTX-M-15 [13]. By eBURST (http://eburst.mlst.net), all STs except ST17 and ST37 were considered to be singletons. ST17 belongs to the ST17 complex, which contains four single-locus variants and six double-locus variants, and ST37 to the ST37 complex, with only two single-locus variants. ST416, ST427, ST428 and ST440 are described here for the first time.

We selected one isolate from each clone (A, B, C, E and J) to obtain transconjugants and characterize plasmids. Transconjugants were obtained by filter matting experiments, as previously described [4]. The incompatibility group of plasmids carrying *bla* was determined by PCR, PFGE with S_1

nuclease and Southern blot hybridization methods, as described previously [14,15]. The bla_{DHA-1} gene was located in a plasmid of 180 kb belonging to the L/M incompatibility group in all isolates except for one with a plasmid of 120 kb. The $bla_{CTX-M-15}$ gene was detected in the same L/M plasmid that contained bla_{DHA-1}. Moreover, this plasmid also contained an FII replicon. The nucleotide sequence corresponding to the FII replicon obtained by PCR showed more than 98% identity with the IncFII replicon of plasmids pKPN3, pKPN4 and pKpQIL from *K. pneumoniae* (GenBank accession numbers: CP000648, CP000649 and GU595196).

In 2000, FOX-4 was the first plasmid-mediated AmpC β-lactamase to be detected in Spain (in the Canary Islands) [16]. Since then, the prevalence of AmpC-producing enterobacteria has increased [4,16–19]. However, detection of these enzymes is difficult, and they could be undetected or misidentified as ESBLs [1,20].

Few outbreaks of DHA-1-carrying *K. pneumoniae* have been described in the literature [1,21,22]. One of these included the presence of isolates co-producing AmpC and ESBLs [22]. In the present article, we describe the association of bla_{DHA-1} and $bla_{CTX-M-15}$ in the same plasmid of the L/M-FII incompatibility group. The isolate belonged to ST326. An ST326 isolate carrying only a CTX-M-15 ESBL had previously been found in two hospitals in Barcelona[13]. Moreover, the most predominant plasmid-mediated AmpC β-lactamase observed since 2005 in *K. pneumoniae* in Barcelona is DHA-1 [17].

The distinctive characteristic of the present study is the multiclonal aspect of the supposed outbreak, which subsided in a short period of time. Our hypothesis is that community clones entered the hospital and spread by cross-infection, because three patients with community-acquired infections with no previous relation to our hospital were clonally related to isolates of three different nosocomial clusters.

The small number of patients per cluster was probably attributable to the infection control programme in place in the centre. This programme includes contact precautions for all *K. pneumoniae* ESBL-positive patients and their environments, and an internal combined hand hygiene and antibiotic policy. The fact that all 26 patients were isolated in single rooms may also have played a role.

From this study, we can conclude that an increase in the incidence of a resistance mechanism does not always imply an outbreak. This appears to be true even when a low-prevalence resistance phenotype is observed. Accordingly, it is important to stress the relevance of the community as a reservoir of antimicrobial-resistant *K. pneumoniae* to explain this non-clonally related diffusion.

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Transparency Declaration

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A novel complex class 1 integron found in a *Klebsiella pneumoniae* isolate from Portugal

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Abstract

Klebsiella pneumoniae Kp1 carrying a novel complex class 1 integron was isolated from an inanimate surface of a female ward sanitary facility in the Hospital Infante D. Pedro, Aveiro, central Portugal. The integron consists of two variable regions (VRs); VR1 was previously described in *Escherichia coli* and *Vibrio cholerae*, and VR2 contains an In*37*-like structure and is located downstream of an IS*CR1* element. The integron was found on a plasmid of 225 kb. The *qnrB10* gene, although present, is not associated with the complex class 1 integron.

Keywords: Complex class 1 integron, inanimate surface, IS*CR1*, *Klebsiella pneumoniae*, multidrug resistance

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Klebsiellae are widespread in the environment and in the intestinal flora of humans and other mammals. *Klebsiella pneumoniae* is an important opportunistic pathogen causing urinary tract and intra-abdominal infections, neonatal meningitis and pneumonia in immunocompromised patients [1].

The intensive use of broad-spectrum antibiotics in hospitalized patients led to the development of multidrug-resistant (MDR) strains. In *K. pneumoniae*, resistance is associated with the production of β-lactamase enzymes, mainly extended-spectrum β-lactamases [2]. However, more recently, metallo-β-lactamases have also been described [3].

K. pneumoniae strains carrying complex class 1 integrons are becoming more common. Usually, within the second variable region (VR2), only one or two genes conferring resistance to β-lactamases, fluoroquinolones, aminoglycosides, chloramphenicol or trimethoprim are found [4–7]. Thus, the use of antibiotics that are commonly used to treat infections caused by *Klebsiella* MDR strains, such as cephalosporins, β-lactamase-stable penicillins and aminoglycosides, can be compromised [8].

Herein, we report and characterize a novel complex class 1 integron found in a *K. pneumoniae* isolate collected from an inanimate surface within the Hospital Infante D. Pedro, Aveiro, Central Portugal.