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The Carbapenemase-Producing *Klebsiella pneumoniae* Population Is Distinct and More Clonal than the Carbapenem-Susceptible Population

Andrés Esteban-Cantos,[a] Belén Aracil,[a,b] Verónica Bautista,[a] Adriana Ortega,[a,b] Noelia Lara,[a] David Saez,[a] Sara Fernández-Romero,[a] María Pérez-Vázquez,[a,b] Ferran Navarro,[b,c] Hajo Grundmann,[d] José Campos,[a,b] Jesús Oteo,[a,b] the EuSCAPE-Spain Network

Laboratorio de Referencia e Investigación en Resistencia a Antibióticos e Infecciones relacionadas con la Asistencia Sanitaria, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain[a]; Red Española de Investigación en Patología Infecciosa (REIPI), Seville, Spain[b]; Servei de Microbiología, Hospital de la Santa Creu i Sant Pau, Institut d'Investigació Biomèdica Sant Pau, Barcelona, Spain[c]; Department of Infection Prevention and Hospital Hygiene, Faculty of Medicine, University of Freiburg, Freiburg, Germany[d]

ABSTRACT We studied in parallel the population structure of 90 carbapenemase-producing and 88 carbapenemase-susceptible *Klebsiella pneumoniae* isolates collected in 20 Spanish hospitals, in the context of the EuSCAPE project. Fourteen and 50 multilocus sequence types (MLSTs) were detected among the carbapenemase-producing and carbapenem-susceptible isolates, respectively. ST11 and ST15 clones were more frequent in the carbapenemase-producing group than in the carbapenemase-susceptible group ($P < 0.0001$). Among the members of the carbapenem-suceptible group, the cefotaxime-resistant population showed population parameters that differed between the populations of the wild-type strains and the carbapenemase producers.

KEYWORDS carbapenem resistance, population structure, MLST, carbapenemases, *Klebsiella pneumoniae*

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Address correspondence to Jesús Oteo, jesus.oteo@isciii.es.

Carbapenemase-producing *Enterobacteriaceae* (CPE) have emerged in recent years as being among of the most important threats to public health. CPE have been detected in almost all European countries, although with a highly variable geographical distribution (1). Some high-risk clones of *Klebsiella pneumoniae*, mainly KPC-producing sequence type 258 (ST258) but also OXA-48-producing ST395 or ST15, have been implicated in the worldwide spread of carbapenemases (2–4).

Previous studies have shown that the population of *Escherichia coli* strains resistant to amoxicillin-clavulanic acid is less diverse than the susceptible population (5). Little is known about the population structure of carbapenemase-producing *K. pneumoniae* in comparison with the carbapenem-susceptible population. The aim of this study was to test the hypothesis that the carbapenemase-producing *K. pneumoniae* strains isolated in Spanish hospitals constitute a distinct and more homogeneous population than the carbapenemase-susceptible isolates.

The European Survey on Carbapenemase-Producing *Enterobacteriaceae* (EuSCAPE) is an initiative funded by the European Centre for Disease Prevention and Control (ECDC) and coordinated by the Department of Medical Microbiology of the University Medical Center Groningen (Netherlands) (6). EuSCAPE carried out a structured survey between November 2013 and April 2014 involving national networks of representative laboratories from 35 European countries (7). Each participant laboratory collected up to 10 carbapenem-nonsusceptible clinical isolates of *K. pneumoniae* or *E. coli* and 10 susceptible same-species comparator isolates. All clinical specimens were accepted, except for

TABLE 1 Comparisons of clinical and epidemiological data of *Klebsiella pneumoniae* isolates from carbapenemase-producing cases and carbapenem-susceptible controls[a]

Parameter	No. of cases ($n = 90$)	No. of controls ($n = 88$)	OR	95% CI	*P*
Age (yrs)					
<18	0	3	0.32	0.03–3.12	0.30
>65	65	44	2.6	1.49–4.85	**0.002**
Sex					
Female	40	45	0.76	0.42–1.38	0.37
Clinical relevance					
Infected	84	86	0.32	0.64–1.66	0.16
Hospital location					
ICU	27	11	3	1.38–6.52	**0.004**
Outpatient/emergency department	22	34	0.51	0.27–0.98	**0.04**
Previous hospital admission in the last 6 mo	56	33	2.7	1.50–5.03	**0.001**
Sample source					
Urine	42	47	0.76	0.42–1.38	0.37
Blood	16	22	0.65	0.31–1.34	0.24

[a]Boldface indicates statistically significant differences. OR, odds ratio; 95% CI, 95% confidence interval; ICU, intensive care unit.

stool and surveillance screening samples; isolates of colonizers obtained from clinical specimens were also included (7). In Spain, 20 hospital laboratories from 16 provinces distributed across the country were enrolled according to EuSCAPE criteria. A total of 116 carbapenem-nonsusceptible *K. pneumoniae* isolates were collected, 102 (87.9%) of which produced carbapenemases (81 produced OXA-48, 12 VIM, and 9 KPC). A total of 14 carbapenem-nonsusceptible *E. coli* strains were also detected, 4 (28.5%) of which produced OXA-48 carbapenemases (7).

Our main purpose was to study two groups of representative isolates that were as comparable as possible according to the EUSCAPE project guidelines. Accordingly, a subset of 90 nonduplicated isolates of carbapenemase-producing *K. pneumoniae* strains was analyzed and compared in detail with 88 carbapenem-susceptible isolates; all of them were selected according to the following criteria: (i) clinical impact (isolates producing infections were prioritized) and (ii) geographic representation (isolates from all 20 participating hospitals were included). An isolate was considered carbapenem susceptible and carbapenemase negative when it was susceptible to all carbapenems according to EUCAST guidelines (8) and when both the Hodge modified test (meropenem disk with 600 μg cloxacillin) and the Carba NP test were negative (9). Carbapenem-susceptible and carbapenemase-producing isolates were matched by hospital and temporal origin. The presence of genes encoding carbapenemases or extended-spectrum β-lactamase (ESBLs) was determined using PCR and DNA sequencing assays (10).

All isolates were subjected to multilocus sequence type (MLST) analysis according to the Institut Pasteur scheme (http://bigsdb.web.pasteur.fr/klebsiella/) (11) A simple-diversity index (SDI) (12) was calculated. The phylogenetic relationships among the different sequence types (STs) were established according to the eBURST program (version 3) (http://eburst.mlst.net).

The statistical analysis was performed using GraphPad Prism software (version 3.02) (GraphPad Software, Inc., San Diego, CA, USA). Population differences were assessed using Fisher's exact test.

Comparisons of clinical and epidemiological data from carbapenemase-producing and carbapenem-susceptible isolates are detailed in Table 1.

Of the 90 carbapenemase-producing *K. pneumoniae* isolates, 70 (77.8%) produced OXA-48, 12 (13.3%) VIM, and 8 (8.9%) KPC. Eighty-four (93.3%) produced clinical infections (mainly urinary tract infections [46.7%], wound infections [20.2%], and bacteremia [17.8%]), and 6 were colonizers. Among the 90 isolates, 14 MLSTs were detected (SDI = 15.6) (Table 2). The mean number of isolates per ST was 6.4 (range, 1 to 31); the most prevalent STs were ST15 (34.4%), ST11 (26.7%), ST405 (13.3%), ST147 (10%), and ST258 (3.3%) (Table 2). The three STs with more than 10 isolates each came from eight hospitals located in six (ST15) and five (ST11 and ST405) provinces.

Among the 88 carbapenem-susceptible isolates, 50 STs were detected (SDI = 56.8%) (Table 2). The mean number of isolates determined by STs was 1.8 (range, 1 to 6);

TABLE 2 Distribution of different population markers indicating genetic variation between carbapenemase-producing and carbapenem-susceptible *Klebsiella pneumoniae* isolates[a]

Population marker	Value(s)				*P* value		
	Phenotype						
		CBP-S[b]					
	CBP-R	Total	WT	CTX-R	CBP-R vs CBP-S	CBP-R vs WT	CBP-R vs CTX-R/CBP-S
No. of isolates	90	88	66	22			
No. of STs	14	50	44	10			
Mean no. of isolates per ST (range)	6.4 (1–31)	1.8 (1–6)	1.5 (1–5)	2.2 (1–5)			
No. (%) of single isolates per ST	7 (7.8)	36 (40.9)	33 (50)	5 (22.7)	**<0.0001**	**<0.0001**	0.13
SDI	15.6	56.8	66.7	45.5			
No. (%) of ST15 isolates	31 (34.4)	6 (6.8)	1 (1.5)	5 (22.7)	**<0.0001**	**<0.0001**	0.22
No. (%) of ST11 isolates	24 (26.7)	5 (5.7)	3 (4.5)	2 (9.1)	**0.0002**	**0.0001**	0.18
No. (%) of ST405 isolates	12 (13.3)	5 (5.7)	2 (3)	3 (13.6)	0.12	**0.01**	0.75
No. (%) of ST35 isolates	0	5 (5.7)	5 (7.6)	0	**0.03**	**0.01**	
No. (%) of ST147 isolates	9 (10)	2 (2.3)	2 (3)	0	0.06	0.13	0.20
No. (%) of ST307 isolates	0	5 (5.7)	1 (1.5)	4 (18.2)	0.06	0.41	**0.002**

[a]CBP-S, carbapenem susceptible isolates; WT, wild-type fully susceptible isolates; CTX-R, cefotaxime-resistant ESBL-producing isolates; CBP-R, carbapenem-resistant carbapenemase-producing isolates; SDI, single-diversity index [(number of STs/total number of isolates) × 100].
[b]CBP-S include WT and CTX-R. Boldface indicates statistically significant differences.

the most prevalent STs were ST15 (6.8%), ST11 (5.7%), ST35 (5.7%), ST307 (5.7%), and ST405 (5.7%). eBURST analysis provided a representation of the different population structures of carbapenemase-producing and carbapenem-susceptible *K. pneumoniae* isolates (Fig. 1).

Only seven STs (ST11, ST15, ST16, ST45, ST104, ST147, and ST405) were identified in both groups, but their prevalences strongly differed between the groups, as they comprised 80 isolates in the carbapenemase-producing group (88.9%) and 25 isolates in the carbapenem-susceptible group (28.4%) ($P < 0.0001$). The ST11 and ST15 clones were much more predominant among the carbapenemase-producing isolates (55/90, 61.1%) than among the members of the carbapenemase-susceptible group (11/88, 12.5%) ($P < 0.0001$).

An additional finding of interest was that the 88 carbapenem-susceptible isolates in fact consisted of two subpopulations: a wild-type population fully susceptible to all tested β-lactam antibiotics except ampicillin and ticarcillin ($n = 66$, 75%) and a cefotaxime-resistant population also producing ESBLs ($n = 22$, 25%): 14 producing CTX-M-15, 4 SHV-2a, 2 SHV-12, 1 SHV-36, and 1 CTX-M-1. The most prevalent STs among the ESBL-producing isolates were ST15 (3 producing SHV-2a, 1 SHV-12, 1 CTX-M-15), ST405 (3 producing CTX-M-15, 1 SHV-12), ST307 (4 producing CTX-M-15), and ST11 (1 isolate each producing CTX-M-15, CTX-M-1, and SHV-2a). These two subpopulations also showed other important differences (Table 2); for instance, in the fully susceptible subpopulation, the SDI and the prevalence of ST15 were 69.7 and 1.5%, respectively, while the corresponding values were 50 and 22.7%, respectively, in the cefotaxime-resistant subpopulation. Among ESBL-producing *K. pneumoniae* isolates, the prevalence of successful clones such as ST15 and ST11 has been well documented (13–15).

Our data suggest that carbapenemase-producing *K. pneumoniae* isolates have a population structure that is different from and less diverse than the population structure of carbapenem-susceptible *K. pneumoniae* isolates; the latter group in fact consisted of a mixed population of isolates in which ESBL production was relatively frequent (25%). The population structure of carbapenem-susceptible/ESBL-positive isolates showed population parameters that differed between populations of wild-type and carbapenemase producers (Table 2); in fact, some significant differences that obtained between wild-type isolates and carbapenemase-producing isolates were not observed between carbapenem-susceptible/ESBL-positive isolates and carbapenemase producers (Table 2). However, these data should be considered with caution due to the low number of carbapenem-susceptible/ESBL-positive isolates included in the analysis.

The concept of the impact of the consumption of antibiotics on the selection of successful clones is supported by results of a recent study suggesting that different

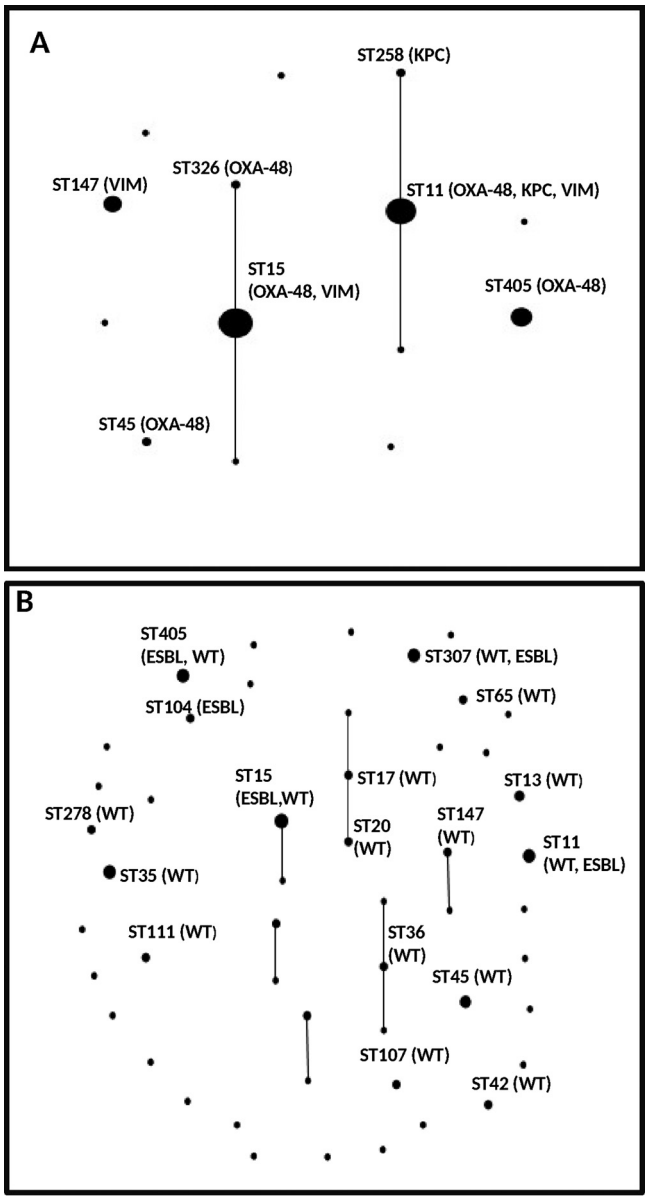

FIG 1 Population snapshot of *Klebsiella pneumoniae* sequence types (STs) of carbapenemase-producing ($n = 90$) (A) and carbapenem-susceptible ($n = 88$) (B) strains. The most frequent STs found in this study are emphasized. In panel A, carbapenemase types are depicted in the most relevant STs. In panel B, ESBL production is depicted in the most relevant STs; also, STs comprising isolates of the ESBL-producing and wild-type (WT) groups of isolates are emphasized.

clones of *E. coli* differ in their responses to antibiotics despite their comparable drug MICs (16). Our results suggest that carbapenem-resistant *K. pneumoniae* strains have undergone a population shift leading to a marked loss of genetic diversity as a consequence of strong selection pressure; production of ESBLs may be the first step in this process. Therefore, the ecologic and clinical impact of the consumption of antibiotics in the population structure of such pathogen bacteria as *K. pneumoniae* is of concern beyond the obvious selection of resistant isolates.

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