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A single-day point-prevalence study of faecal carriers in long-term care hospitals in Madrid (Spain) depicts a complex clonal and polyclonal dissemination of carbapenemase-producing Enterobacteriaceae

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Objectives: The objective of this study was to describe the prevalence and microbiological characteristics of carbapenemase-producing Enterobacteriaceae (CPE) colonizing patients in long-term care hospitals (LTCHs) in Madrid, Spain.

Methods: Three LTCHs were included in a single-day point-prevalence survey (September 2013). Rectal swabs, collected from all hospitalized patients (137 in LTCH-A, 121 in LTCH-B and 83 in LTCH-C), were plated onto chromogenic media. Population structure (PFGE and MLST), genes encoding carbapenemases and ESBLs and plasmids carrying carbapenemase genes were characterized.

Results: The prevalence of CPE carriers was 4.1% (14/341) [2.9% (4/137), LTCH-A; 4.1% (5/121), LTCH-B; and 6.0% (5/83), LTCH-C]. OXA-48 was the most prevalent carbapenemase (nine *Klebsiella pneumoniae*, two *Escherichia coli*, one *Enterobacter cloacae* and one *Citrobacter braakii*) followed by VIM-1 (one *K. pneumoniae* and one *Raoultella ornithinolytica*). One patient (LTCH-C) was co-colonized with OXA-48-producing *K. pneumoniae* and *E. coli*. *K. pneumoniae* and *E. coli* isolates also coproduced CTX-M-15 ($n=11$) or CTX-M-9 ($n=1$) enzymes. *K. pneumoniae* clustered into six PFGE types corresponding to ST11 ($n=1$), ST15 ($n=6$), ST307 ($n=1$) and ST405 ($n=2$). *E. coli* from LTCH-A and LTCH-C exhibited two different PFGE types associated with ST68. OXA-48 and VIM-1 enzymes were found in different clones in LTCH-A and LTCH-C. However, OXA-48 was the only carbapenemase detected in LTCH-B, mainly associated with *K. pneumoniae* ST15. KPC, IMP and NDM enzymes were not detected. $bla_{\text{OXA-48}}$ was located on an ~60 kb plasmid with a pOXA-48a-IncL/M backbone.

Conclusions: We describe the first point-prevalence study of CPE faecal carriers in LTCHs in Spain. OXA-48, the most prevalent carbapenemase, showed a complex dissemination pattern with clonal and polyclonal bacterial populations.

Introduction

The worldwide dissemination of carbapenemase-producing Enterobacteriaceae (CPE) constitutes an important challenge for the healthcare system.[1] Initially, CPE appeared to cause hospital-acquired infections, but more recently they have spread into different healthcare settings, including long-term care hospitals (LTCHs), and also into the community.[2–4]

LTCHs constitute important reservoirs for MDR bacteria, such as ESBL-producing Enterobacteriaceae.[5] Previous admittance into these institutions has been identified as a risk factor for intestinal colonization of ESBL producers and more recently for CPE.[6,7] Moreover, it has been demonstrated that continuous bidirectional movement of patients between these institutions and acute care hospitals facilitates the spread and maintenance of MDR bacteria.[8] As a result, control interventions to curtail this spread have been successfully implemented in countries with a high prevalence of CPE in healthcare institutions, including LTCHs.[9] Despite these facts, the prevalence of CPE faecal carriers in LTCHs remains unknown in most geographical areas. Our objective was to describe the prevalence and microbiological characteristics of CPE colonizing patients hospitalized in three different LTCHs in Madrid, Spain.

Materials and methods

Facilities and patient recruitment

Three LTCHs in the Madrid area (LTCH-A, LTCH-B and LTCH-C) were recruited for a single-day (September 2013) point-prevalence survey. LTCH-A (198 beds) was located in the southern area of the Madrid community, while LTCH-B (192 beds) and LTCH-C (144 beds) were in the northern area. In each LTCH, specimen collection was undertaken by local hospital staff. Screening of patients for carriage of CPE is part of a regional plan to control and prevent infections caused by CPE, promoted by the public health authorities of our region.[10]

Surveillance cultures and CPE detection

Rectal swabs were collected and then processed at Ramón y Cajal University Hospital. Samples were plated onto ChromoID-ESBL, CARBA and OXA-48 agars (bioMérieux, Marcy-l'Étoile, France) and incubated at 37°C for 48 h. A unique colony per colour and morphology growing on each selective chromogenic agar-media was selected for microbiological studies. Bacterial identification was confirmed by the MALDI-TOF MS method (Bruker Daltonics, Germany). Screening for carbapenemase production included both the Carba-NP and modified Hodge tests and assessment of the inhibition-based profile using the ROSCO KPC/MBL and OXA-48 Confirm Kit (ROSCO Diagnostica, Taastrup, Denmark).[11,12] Production of ESBLs was also screened for by the double-disc synergy test.[4] The presence of genes encoding carbapenemases (bla_{VIM}, bla_{OXA-48}, bla_{KPC}, bla_{IMP} and bla_{NDM}) and ESBLs (bla_{TEM}, bla_{SHV} and bla_{CTX-M}) as well as the genetic environment of bla_{OXA-48} were investigated by PCR and sequencing.[3,4,13] Antibiotic susceptibility testing was performed by microdilution (MicroScan; Beckman Coulter, CA, USA) and Etest (bioMérieux) according to EUCAST guidelines (http://www.eucast.org). Isolates classified as intermediate and resistant were considered as non-susceptible.

Clonal relatedness

Isolates were typed by PFGE using XbaI-digested DNA.[4] *Klebsiella pneumoniae* and *Escherichia coli* isolates were also characterized by MLST (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html and http://mlst.ucc.ie/mlst/dbs/Ecoli).

Conjugation assay and plasmid characterization

Conjugation was attempted with all carbapenemase-producing isolates per duplicate using *E. coli* K-12 BM21 as recipient. Determination of the size of plasmids harbouring bla_{OXA-48} and RFLP was performed in all *E. coli* transconjugants.[4] In WT isolates in which no transconjugants were obtained, the bla_{OXA-48} hybridization assay was performed. The plasmid incompatibility group was determined by the PCR-based replicon typing (PBRT) scheme.[14] The *repA*, *traU* and *parA* genes were detected by PCR to relate the OXA-48-encoding plasmids to the pOXA-48a-IncL/M plasmid.[15]

Statistical analysis

Statistical significance for comparison of proportions was calculated by Fisher's exact test ($P<0.05$ was considered statistically significant). Exact binomial methods were used to calculate 95% CIs for prevalence rates.

Results

Rate of CPE and ESBL-producing Enterobacteriaceae colonization

A total of 341 patients were screened across all three LTCH sites ($n=137$, LTCH-A; $n=121$, LTCH-B; and $n=83$, LTCH-C). The total prevalence of CPE faecal carriers was 4.1% (14/341; 95% CI 2%–6.2%). Among the different LTCHs, colonization rates were as follows: 2.9% (4/137; 95% CI 0.1%–5.7%), LTCH-A; 4.1% (5/121; 95% CI 0.6%–7.6%), LTCH-B; and 6.0% (5/83; 95% CI 0.9%–11.4%), LTCH-C ($P>0.05$) (Figure 1). The mean age of CPE-colonized patients was 81 years (range=30–88 years) and 7/14 were female. Moreover, 28.6% (4/14) of CPE carriers were also co-colonized by ESBL-producing Enterobacteriaceae (3 *K. pneumoniae* and 1 *E. coli*). Overall, 30.2% (103/341; 95% CI 25.3%–35.0%) of patients were colonized with ESBL-producing Enterobacteriaceae. The corresponding frequency in different centres is also shown in Figure 1.

Bacterial isolates and carbapenemase characterization

Fifteen CPE isolates were identified from 14 patients with positive CPE faecal carriage. *K. pneumoniae* ($n=10$) was the most prevalent species, followed by *E. coli* ($n=2$), *Enterobacter cloacae* ($n=1$), *Citrobacter braakii* ($n=1$) and *Raoultella ornithinolytica* ($n=1$) (Table 1). The majority of the isolates (13/15) were OXA-48 producers (Table 1).

All *K. pneumoniae* and *E. coli* isolates coproduced CTX-M enzymes (CTX-M-15 and CTX-M-9) (Table 1). The highest bacterial

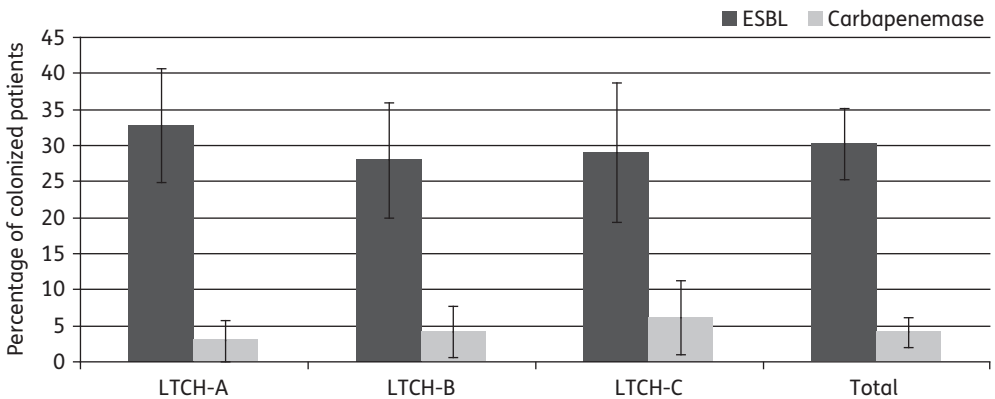

Figure 1. Colonization prevalence of ESBL-producing Enterobacteriaceae and CPE among patients admitted into LTCHs. Error bars represent 95% CIs.

Table 1. Microbiological characteristics of CPE isolates

LTCH (no. of isolates)	Bacterial species (no. of isolates)	Resistance genes		Molecular typing[a] (no. of isolates)		Coresistance profile	Plasmids in transconjugants[c], size (kb)
		$bla_{carbapenemase}$	bla_{ESBL}	PFGE type	ST		
LTCH-A (4)	*Klebsiella pneumoniae* (1)	VIM-1	CTX-M-15	Kpn-1	15	GEN, TOB, CIP, SXT	~60
	Escherichia coli (1)	OXA-48	CTX-M-9	Eco-1	68	GEN, TOB, AMK, CIP	~60
	Citrobacter braakii (1)	OXA-48	—	Cb-1	—	—	NT
	Enterobacter cloacae (1)	OXA-48	—	Eclo-1	—	TGC	NT
LTCH-B (5)	*Klebsiella pneumoniae* (5)	OXA-48	CTX-M-15	Kpn-2 (4)	15	GEN, TOB, CIP, SXT	~60
				Kpn-3 (1)	405	GEN, TOB, CIP, SXT	NT
LTCH-C (6)	*Klebsiella pneumoniae* (4)	OXA-48	CTX-M-15	Kpn-2[b]	15	GEN, TOB, CIP, SXT, TGC	~60
				Kpn-4	11	CIP, SXT	~60
				Kpn-5	307	GEN, TOB, CIP, SXT	~60
				Kpn-6	405	GEN, TOB, CIP, SXT, TGC	NT
	Escherichia coli (1)	OXA-48	CTX-M-15	Eco-2[b]	68	CIP	~60
	Raoultella ornithinolytica (1)	VIM-1	—	Ror-1	—	TOB, AMK, CIP, SXT	NT

AMK, amikacin; CIP, ciprofloxacin; GEN, gentamicin; TGC, tigecycline; TOB, tobramycin; SXT, trimethoprim/sulfamethoxazole; NT, no transconjugants were obtained.
[a]Underlined PFGE types and STs correspond to CPE clones detected in different LTCHs.
[b]Patient co-colonized by *K. pneumoniae* ST15 and *E. coli* ST68, both producing OXA-48.
[c]All plasmids had a pOXA-48a-IncL/M backbone and showed a highly related restriction profile.

diversity was detected in LTCH-A since all the carbapenemase-producing isolates ($n=4$) belonged to different bacterial species (*K. pneumoniae* VIM-1, *E. coli* OXA-48, *E. cloacae* OXA-48 and *C. braakii* OXA-48) (Table 1).

Antimicrobial susceptibility

All isolates were non-susceptible to ampicillin, extended-spectrum cephalosporins and β-lactam/β-lactamase inhibitor combinations. MICs of imipenem, meropenem and ertapenem ranged from 0.75 to 6 mg/L, 0.25 to 4 mg/L and 0.38 to 8 mg/L, respectively.

Non-susceptibility to ciprofloxacin, trimethoprim/sulfamethoxazole, tobramycin and gentamicin was 86.6%, 73.3%, 73.3% and 66.6%, respectively. In two *K. pneumoniae* and one *E. cloacae*, tigecycline MICs were ≥2 mg/L. Amikacin was one of the most active antibiotics (13.3% non-susceptible isolates). All isolates were susceptible to colistin.

K. pneumoniae and E. coli clonal relatedness

K. pneumoniae isolates were clustered into six PFGE types corresponding to ST11 ($n=1$), ST15 ($n=6$), ST307 ($n=1$) and ST405 ($n=2$). *K. pneumoniae* ST15 (PFGE types Kpn-1 and Kpn-2) was the most prevalent *K. pneumoniae* OXA-48 clone (Table 1). Interestingly, ST15 was associated with a VIM-1-producing isolate (PFGE type Kpn-1) in LTCH-A. Although LTCH-C was located in the northern area of Madrid as was LTCH-B, the OXA-48 dissemination was associated with a polyclonal *K. pneumoniae* population (ST11, ST15, ST307 and ST405) (Table 1). Finally, *E. coli* isolates that were isolated in two different LTCHs (A and C) exhibited two different PFGE types and both grouped into ST68.

Conjugation assay, plasmid characterization and bla_{OXA-48} genetic environment

Nine OXA-48-producing *E. coli* transconjugants were obtained (Table 1). In all transconjugants, PCR amplified for bla_{OXA-48}, which was located on an ~60 kb plasmid non-typable by the PBRT method. However, the presence of *repA*, *traU* and *parA* genes was detected, suggesting that those plasmids had a pOXA-48a-IncL/M backbone. All plasmids showed highly related restriction profiles and bla_{OXA-48} was part of the Tn*1999.2* composite transposon. No transconjugants were obtained from *C. braakii*, *E. cloacae* and *K. pneumoniae* ST405. For VIM-1-producing isolates, transconjugants were only obtained from *K. pneumoniae* ST15 (Table 1). The VIM-1-encoding plasmid had a restriction profile related to the OXA-48-encoding plasmids and also amplified for *repA*, *traU* and *parA* genes.

Discussion

We outline the first point-prevalence study of CPE carriers in LTCHs in Spain. This study was conducted due to the increasing prevalence of these isolates in acute care hospitals in our geographical area,[16] with the objective of identifying hidden CPE faecal carriers contributing to explain this prevalence and to support further control interventions.[1,10]

In some countries such as the USA or Israel, where carbapenemase epidemiology is characterized by a wide dissemination of KPC-producing *K. pneumoniae*, several studies have been conducted describing a prevalence of colonized LTCH patients, which ranged from 8% to 30%,[7,17,18] but currently scarce data about its prevalence are available in Europe. One study conducted in Italy reported a colonization rate with metallo-β-lactamase

producers of 6.3%,[8] while in another study in Belgian nursing homes, CPE colonization was not detected among residents.[19] We found that 4.1% of LTCH residents in the Madrid area were colonized by CPE. These data, in parallel with those from acute care hospitals in the same geographical area and national surveillance studies in Spain, demonstrate a successful penetration of CPE into healthcare facilities.[16] Moreover, the higher prevalence of ESBL carriers found in the studied LTCHs when compared with the most recent data from patients admitted into acute care hospitals in our country also depicts these centres as reservoirs for MDR isolates.[20]

In Spain, as in other European countries, CPE rates have increased dramatically during recent years. Currently, OXA-48 is the most common enzyme circulating in acute care hospitals, associated with *K. pneumoniae* epidemic clones such as ST11, ST405 and ST15.[16] In our study, we detected all these clones circulating in LTCHs, probably as a consequence of movement of patients between these sites and acute care hospitals. One of these clones, ST405, was detected for >1 year in a hospital-wide outbreak in Madrid[16] and ST11 is the main clone in our institution (D. Gijón, P. Ruiz-Garbajosa and R. Cantón, Ramón y Cajal University Hospital, unpublished data), denoting different local amplification in different institutions.

In LTCHs, cross-transmission events can occur resulting in the dissemination of not only one epidemic clone, but also the dissemination of carbapenemase genes in multiple clones and bacterial species, as we described particularly for bla_{OXA-48}. Currently, the spread of bla_{OXA-48} has been associated with the dissemination of a particular IncL/M plasmid backbone.[15] In this work, bla_{OXA-48} was also located in plasmids related to pOXA-48a, which were disseminated in different *K. pneumoniae* high-risk clones and even in other enterobacterial species. Moreover, these OXA-48-encoding plasmids showed the same restriction profile as plasmids found in the ST11 epidemic strain found in our institution (data not shown). This reflects a successful combination of high-risk epidemic clones, especially from *K. pneumoniae*, and plasmids prone to acquire antibiotic resistance genes.

In summary, this survey demonstrates an important reservoir of CPE and ESBL-producing Enterobacteriaceae among patients admitted into LTCHs in Madrid. LTCH patients should be considered as a high-risk group for CPE colonization that can act as hidden disseminators. As a consequence, admission surveillance cultures or molecular detection should be implemented in this high-risk group of patients for early detection of CPE carriage. The dissemination of CPE across healthcare institutions points to the need to establish coordinated infection control policies at regional and national levels involving both acute care hospitals and LTCHs.

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Transparency declarations

None to declare.

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