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Short report

Intestinal persistence of a plasmid harbouring the OXA-48 carbapenemase gene after hospital discharge

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SUMMARY

To study intestinal colonization by OXA-48-producing *Klebsiella pneumoniae* (KpO48) after hospital discharge, stool samples from 22 previously colonized subjects were collected. Time from discharge was 33–611 days, without readmissions. Eight subjects (36%) were identified as *bla*OXA-48 gene carriers. In all of them the hospital-acquired strain of KpO48 had been lost, and the gene was harboured by other strains of *K. pneumoniae*, *Klebsiella oxytoca* and/or *Escherichia coli*. Our findings show intestinal persistence for several months of a plasmid harbouring the OXA-48 carbapenemase gene in a significant proportion of individuals in the absence of antibiotic treatment.

Introduction

In many healthcare facilities, carbapenemase-producing *Klebsiella pneumoniae* (CPKP) outbreaks are drifting towards an endemic situation in which infections are usually the tip of the iceberg and most affected patients are asymptomatic intestinal carriers. In most cases, the CPKP is cleared spontaneously in a few weeks or months after hospital discharge, but some of the patients become long-term carriers. This raises concerns about the possibilities of community-spread of CPKP and patient-to-patient transmission if a new hospitalization occurs. In fact, the number of community-onset CPKP infections seems to be increasing in our environment [1].

Long-term colonization studies have focused primarily on KPC-2-producing *K. pneumoniae* (KP_{KPC}) concluding that, in colonized patients, the carrier state may persist for up to 28 months [2–4]. However, in most cases the monoclonal nature of KP_{KPC} precludes the differentiation between long-term carriage and recolonization.

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In our hospital, KP_{KPC} isolations are rare; nonetheless there is a high prevalence of OXA-48-producing *K. pneumoniae* (KpO48). To date, no studies have been made regarding the behaviour of KpO48 in outpatients. During the course of a clinical trial we searched for previously colonized patients who had been discharged and who remained outside the hospital, and we investigated their colonization status as well as the colonizing micro-organisms.

Methods

Sample collection

Samples were obtained from 22 subjects who met the criteria to be included in the clinical trial "Pilot Study of the Effectiveness of Probiotics and Lactitol for the Decolonisation of OXA-48 (Carbapenemase) Producing *Klebsiella pneumoniae* Among Rectal Carriers (DESPROBIOXA), (NCT02307383)", to evaluate the effectiveness of lactitol and probiotics to achieve intestinal decolonization of KP_{OXA-48}. This study was finally stopped due to insufficient recruitment.

Study subjects were non-hospitalized individuals colonized by KpO48 during a previous admission (≥1 month before recruitment) who agreed to participate. Exclusion criteria included severe health conditions, diarrhea or antibiotic treatment at the moment of the study among others (most relevant inclusion and exclusion criteria are referred to in Box 1). The study was approved by the institutional review board.

Isolation and identification of OXA-48-producing strains

The subjects were screened with a rectal swab plated in OXA-48-producing Enterobacteriaceae (OXA-PE) selective medium chromID™ OXA-48 (bioMérieux, Marcy l'Etoile, France) and then introduced into tryptic soy broth (TSB) enrichment medium with an ertapenem disk (1 μg/mL) for 18 h at 37°C. After 18 h, TSB medium was plated on the same agar media and isolates were confirmed to carry the *bla*OXA-48 by specific quantitative polymerase chain reaction (qPCR) (Progenie Molecular®, Valencia, Spain). All subjects who tested positive for any OXA-PE in the initial screening were invited to participate in the study and asked to collect a stool sample before initiating treatment. Stool samples were plated using the same method as rectal swabs. All OXA-PE isolated by this method were subsequently analysed.

Enterobacterial isolates growing on chromogenic agar plates were identified using matrix-assisted laser desorption/ionization mass spectrometry (Biotyper®; Bruker Daltonik GmbH, Bremen, Germany). Isolates belonging to the same species but showing different morphologies at visual inspection were isolated and conserved at −80°C for further analysis. Antibiotic susceptibility of all isolates was determined by MicroScan® (MahWah, NJ, USA) panels and interpreted according to the European Committee on Antimicrobial Susceptibility Testing standards.

Box 1
Most relevant inclusion and exclusion criteria

Inclusion criteria

- Adults aged between 18 and 85 years.
- Have signed the informed consent to participate.
- Testing positive for the screening test of EPC-OXA-48.
- First positive screening for *K. pneumoniae* OXA-48 ≥6 months.

Exclusion criteria

- Hospitalization due to an acute process at the moment of inclusion.
- Use of systemic antibiotics (oral, intramuscular, or intravenous) at the moment of inclusion.
- Diarrhoea in the two weeks before inclusion.
- Hydroelectrolytic disorders that require supplements.
- Suffer from diseases that affect intestinal absorption or alter intestinal transit (e.g. Cröhn's disease, ulcerative colitis).
- Taking antisecretory inhibitors, proton pump, or anti-H2.

Clonality study

Clonal relatedness between KpO48 isolated during hospitalization and those isolated from stool samples was studied. If participants had been previously colonized by KP_{OXA-48} belonging to sequence types (ST) 405 or 11, a clone-specific qPCR assay was performed [5]. In participants previously colonized by KpO48 with other sequence types, relatedness between hospital-acquired and long-term colonization strain was determined by repetitive element palindromic (REP) PCR using the DiversiLab® system (bioMérieux). Random amplified polymorphic DNA (RAPD) was used to study clonality in OXA-48-producing *Escherichia coli* isolates, including those obtained from the same patient that had different morphologies and/or antibiogram.

Results

To evaluate the effectiveness of the use of lactitol and probiotics for intestinal decolonization of KP_{OXA-48} we carried out the clinical trial "Pilot Study of the Effectiveness of Probiotics and Lactitol for the Decolonisation of OXA-48 (Carbapenemase) Producing *Klebsiella pneumoniae* Among Rectal Carriers (DESPROBIOXA), (NCT02307383)". In all, 918 patient histories were reviewed and 22 subjects met the inclusion criteria (Box 1) and agreed to participate. We report the colonization status of these 22 study subjects before starting the use of lactitol plus probiotics.

Fourteen (64%) of the 22 tested negative and in eight (36%) of them at least one OXA-48-producing enterobacterial isolate was obtained. The median number of days from the last hospital discharge to screening was significantly larger in the negative subjects (mean: 309; range: 218–611) than in the positive ones (mean: 173; range: 33–313) (Table I). Other epidemiological data are shown in Table I. No differences between the two groups were observed in epidemiological and clinical variables (age, gender, weight, smoking, hypertension, diabetes, dislipidaemia, hyperuricaemia). Functional status indices were similar between the two groups (Barthel index: >90; Katz index: 0–1), as was the Charlson comorbidity score (3–5). None of the subjects had antibiotic therapy during the previous month.

Table I
Epidemiological data of studied patients

	Long-term *bla*OXA-48 carriers ($N = 8$)	Negative as outpatients ($N = 14$)
Sex		
Female	3	8
Male	5	6
Age (years)[a]	66 (36–79)	60 (36–71)
Days from hospital discharge[a,b]	173 (33–313)	319 (218–611)
Days of hospitalization during last hospital admission[a]	35 (0–371)	14 (7–95)

[a] Median (range).
[b] $P = 0.0027$, unpaired *t*-test.

Two of the participants carried KpO48 as the unique OXA-48-producing enterobacterial species. In four of them the *bla*OXA-48 gene was found in *K. pneumoniae* and *E. coli*, in another it was detected in *K. oxytoca* and *E. coli*, and in one subject it was found only in *E. coli*. Some of the subjects carried several different *E. coli* OXA-48-producing strains.

Overall, 18 different OXA-48-producing enterobacterial isolates were obtained from the eight participants, including six *K. pneumoniae*, 11 E. *coli* and one *K. oxytoca*.

Whereas all the KpO48 isolated from the eight study subjects during their hospitalization were identified as extended-spectrum β-lactamase producers, three of the six KpO48 isolated in this study were not. These isolates presented the phenotypic pattern associated with the expression of the *bla*OXA-48 gene (resistance to penicillins, cephalothin, and ertapenem) plus resistance to other antibiotics such as tobramycin plus quinolones or fosfomycin. One KpO48 isolate had the resistance phenotype associated with the expression of the OXA-48 carbapenemase alone and was susceptible to every other antibiotic tested.

Regarding the 11 OXA-48-producing *E. coli* isolates, six had the resistance profiles associated with expression of OXA-48 carbapenemase, and five were also resistant to quinolones. The *K. oxytoca* isolate presented the resistance phenotype of OXA-48 expression.

Clone-specific qPCR of the three KpO48 isolates obtained from individuals previously colonized by ST11 or ST405 tested negative, indicating that the original strains had been replaced by new ones. In the three participants previously colonized by other clones, REP-PCR showed also that the hospital clones had been replaced by new ones. RAPD of the 11 E. *coli* isolates showed that they were not clonally related.

Discussion

We have studied the KpO48 intestinal colonization status of a series of individuals after leaving hospital and the microorganisms harbouring the *bla*OXA-48 gene in those subjects who remained positive. The number of days from discharge was higher in the negative subjects, showing that colonization status cleared with time, but while the original, hospital-acquired KpO48 strains had been cleared as soon as one month after discharge, the *bla*OXA-48 gene persisted, circulating among different enterobacterial strains for up to ten months. In almost all the subjects studied, the plasmid harbouring the *bla*OXA-48 gene was found in different isolates of non-multidrug-resistant Enterobacteriaceae, mostly *E. coli*, but also *K. pneumoniae* and *K. oxytoca*, suggesting that the plasmid had been transmitted *in vivo* to some strains from the endogenous microbiota. Similar observations have recently been reported during a KpO48 outbreak and in long-term carriers of KP_{KPC} in which the plasmid dynamics over time included several rearrangements and in-vivo transmission to other strains and species [6,7]. Plasmids carrying resistance genes are thought to be a burden for the host cells in the absence of antibiotic pressure, and therefore should be outcompeted by commensal microbiota. Nonetheless, high transconjugation frequencies might facilitate the diffusion and persistence of the plasmid in the endogenous microbiota, while compensatory evolution might promote persistence of particular clones [8,9].

Despite the small sample size, the frequency of long-term persistence is high enough to suggest that it is significant and therefore should be regarded as a major epidemiological problem. The intestinal microbiota of these subjects may be a transient reservoir of OXA-48 carbapenemase genes in the community, allowing transmission to close contacts, incrementing the prevalence of community-onset carbapenemase-producing enterobacterial infections and allowing patient-to-patient transmission in new readmission episodes.

We have found no factors contributing to the long-term persistence of the plasmids harbouring the *bla*OXA-48 gene, and there is scarce information about it, but our data show that these events are far from negligible and might be important contributors to the epidemiology of the OXA-48 carbapenemase.

Conflicts of interest statement
None declared.

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References

[1] Paño-Pardo JR, López Quintana B, Lázaro Perona F, Ruiz Carrascoso G, Romero-Gómez Mp, Loeches Yagüe B, et al. Community-onset bloodstream and other infections, caused by carbapenemase-producing enterobacteriaceae: epidemiological, microbiological, and clinical features. Open Forum Infect Dis 2016;3:ofw136.

[2] Feldman N, Adler A, Molshatzki N, Navon-Venezia S, Khabra E, Cohen D, et al. Gastrointestinal colonisation by KPC-producing *Klebsiella pneumoniae* following hospital discharge: duration of carriage and risk factors for persistent carriage. Clin Microbiol Infect 2013;19:E190–6.

[3] Haverkate MR, Weiner S, Lolans K, Moore NM, Weinstein RA, Bonten MJM, et al. Duration of colonisation with *Klebsiella pneumoniae* carbapenemase-producing bacteria at long-term acute

care hospitals in Chicago, Illinois. Open Forum Infect Dis 2016;3:1—4.
[4] Lübbert C, Lippmann N, Busch T, Kaisers UX, Ducomble T, Eckmanns T, et al. Long-term carriage of *Klebsiella pneumoniae* carbapenemase-2-producing K pneumoniae after a large single-center outbreak in Germany. Am J Infect Control 2014;42:376—80.
[5] López-Camacho E, Rentero Z, Ruiz-Carrascoso G, Wesselink J-J, Pérez-Vázquez M, Lusa-Bernal S, et al. Design of clone-specific probes from genome sequences for rapid PCR-typing of outbreak pathogens. Clin Microbiol Infect 2014;20:O891—3.
[6] Göttig S, Gruber TM, Stecher B, Wichelhaus TA, Kempf VAJ. In vivo horizontal gene transfer of the carbapenemase OXA-48 during a nosocomial outbreak. Clin Infect Dis 2015;60:1808—15.
[7] Conlan S, Park M, Deming C, Thomas PJ, Young AC, Coleman H, et al. Plasmid dynamics in KPC-positive *Klebsiella pneumoniae* during long-term patient colonisation. MBio 2016;7:e00742—16.
[8] Hall JPJ, Brockhurst MA, Dytham C, Harrison E. The evolution of plasmid stability: are infectious transmission and compensatory evolution competing evolutionary trajectories? Plasmid 2017;91:90—5.
[9] Harrison E, Dytham C, Hall JPJ, Guymer D, Spiers AJ, Paterson S, et al. Rapid compensatory evolution promotes the survival of conjugative plasmids. Mob Genet Elements 2016;6:e1179074.