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First report of OXA-producing Kluyvera ascorbata isolates recovered from patients admitted in a hospital in Madrid

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Background: Occurrence and dissemination of carbapenemase-producing Enterobacteriaceae (CPE) have been reported worldwide in last years, mainly in healthcare services. The main reservoir of OXA-

48 encoding-plasmids is *Klebsiella pneumoniae*, but other *Enterobacteriaceae* species also contribute to the endemicity of this enzyme. We study emergence of OXA-48+CTX-M-9-*like* producing *Kluyvera ascorbata* isolates recovered from colonized patients admitted in our Hospital in Madrid during an active surveillance-screening program for detecting extended-spectrum β-lactamase (ESBL)-carriers (R-GNOSIS project).

Material/methods: Rectal swabs were collected and seeded onto Chromo ID-ESBL and Chromo CARBA SMART agars (BioMérieux, France). Bacterial identification was performed by MALDI-TOF MS (Bruker-Daltonics, Germany). Carbapenemase and ESBL production was detected by Modified Hodge test and KPC/MBL Confirm Kit and ESBL Screen Kit tests (Rosco Diagnostica, Germany). Antibiotic susceptibility testing was determined by microdilution (MicroScan, Beckman, CA) and clonal relatedness was established by *Xba*I-PFGE. Plasmids carrying *bla*$_{\text{carbapenemase}}$ were transferred into DH5-α *E. coli* cells by heat shock, and transformants were confirmed by PCR. S1-PFGE and SB-Hybridization were performed, and OXA-48 encoding plasmids were typed by PCR and RFLP. Genomic DNA extraction was performed with Wizard® Genomic DNA purification Kit and whole genome sequencing (WGS) was performed using Illumina HISeq 2500 platform. Bioinformatics tools were used to annotate (Uniprot and ARG-ANNOT databases), analyze (Plasmid Constellation Network - PLACNET) and compare (Artemis comparison tool – ACT) genome sequences.

Results: Six unrelated patients (4 male; Median age=75, range 59-81) colonized with OXA-48-producing *K. ascorbata* were detected in three different wards in our hospital from April 2014 to July 2015. All isolates exhibited a very closed PFGE pattern (>95% similarity). In two patients, OXA-48-*K. ascorbata* coexisted with other OXA-48-producers (*Raoultella ornithinolytica* and *K. pneumoniae*). Successful transformants carrying *bla*$_{\text{OXA-48}}$ were confirmed and comparable restriction profiles among them were showed by plasmid DNA digestion. Hybridization and PCRs revealed that OXA-48 gene was harbored onto a ~75kb IncL plasmid with a backbone related to IncL/M-pOXA-48a. WGS analysis showed that plasmid pOXA-48 shared a 99% similarity with the *K. pneumoniae* plasmid pKPoxa-48N1 and carried a 15kb insertion including a *parA/B* system and a *repB* replication gene. Additionally, a chromosomally novel CTX-M variant that differed from CTX-M-13 by a single amino acid substitution (K56E) was identified in all wild types. Moreover, we also found a chromosomally located *pbp2* gene, a class 1 integron encoding *sul1* and *aadA2* genes and a *bla*$_{\text{FOX-8}}$ in an ~8kb IncQ plasmid.

Conclusions: We describe the first *bla*$_{\text{OXA-48}}$ plasmid harbored by *K. ascorbata* isolates, also harboring a new chromosomal CTX-M variant. We highlight the threat of further nosocomial OXA-48 dissemination through *K. ascorbata* either by clonal transmission or lateral transfer of a plasmid highly related to IncL/M-pOXA-48a, despite the sporadic isolation of Enterobacteriaceae species other than *E. coli* or *Klebsiella* spp. in hospital settings.