

Clonal dissemination of OXA-48-ST11 and VIM-ST54 *Klebsiella pneumoniae* within a carbapenemase-producing Enterobacteriaceae polyclonal situation at a University Hospital in Madrid (Spain)

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Objectives: The prevalence of the carbapenemase-producing *Enterobacteriaceae* (CPE) faecal carriers is increasing worldwide and has become a matter of great concern. We describe prevalence and the microbiological features of CPE in patients admitted in a Hospital in Madrid (Spain) during an active surveillance screening program for detecting extended spectrum β -lactamase (ESBL)-carriers (R-GNOSIS project).

Material and Methods: Rectal swabs from 2,908 patients at two medical and two surgical wards were collected (March-August, 2014). Rectal swabs were obtained at admission and weekly for patients staying longer >7 days until discharge. They were seeded on ChromoID-ESBL and -CARBA and -OXA-48 agars (BioMérieux, France). Growing colonies were identified by MALDI-TOF MS (Bruker Daltonics, Germany). Carbapenemase production was detected by the modified Hodge and KPC/MBL Confirm Kit tests (Rosco Diagnostica, Germany). Antibiotic susceptibility testing was performed by microdilution (MicroScan, Siemens, CA). Presence of the *bla*_{VIM}, *bla*_{KPC}, *bla*_{NDM}, *bla*_{OXA-48} and *bla*_{ESBL} genes were characterized (PCR, sequencing). Population structure was established by PFGE and MLST. Clinical chart were also reviewed.

Results: A total of 41 CPE isolates (16 *Klebsiella pneumoniae*, 10 *E. coli*, 3 *Citrobacter* spp., 8 *Enterobacter* spp., 3 *Kluyvera ascorbata*, 1 *Raoultella ornithinolytica*) were identified among 38/2,908 patients (1.3%). Three patients were co-colonized by two different CPE (*K. ascorbata*-*R. ornithinolytica* and *K. pneumoniae*-*E. coli*). CPE isolates were detected in 13 patients at admission and 25 patients during hospitalization. All patients with a positive CPE culture at admission were hospitalized during previous 6-months. OXA-48 was the most frequent carbapenemase (24 isolates: 12 *K. pneumoniae*, 7 *E. coli*, 3 *K. ascorbata*, 1 *C. koseri* and 1 *R. ornithinolytica*) followed by VIM (15 isolates: 6 *E. cloacae*, 4 *K. pneumoniae*, 2 *E. coli*, 2 *C. freundii* and 1 *E. asburiae*) and KPC (2 isolates: 1 *E. coli* and 1 *E. cloacae*) enzymes. NDM-carbapenemases were not detected. CTX-M-15 and CTX-M-9 production was also characterized in 12/41 (29.2%) and 4/41 (9.7%) isolates, respectively. CTX-M-15 was associated with OXA-48-producing *K. pneumoniae* (n=12) and CTX-M-9 with OXA-48-producing *E. coli* (n=1) and *K. ascorbata* (n=3). *K. pneumoniae* isolates were clustered into 2 major PFGE-types which correspond to OXA-48-ST11 (n=10) and VIM-ST54 (n=3) clones disperse in different wards. Among the other CPE isolates, a high genetic diversity was found: *E. coli* (9 pulsotypes and 9 STs), *E. cloacae* (7 pulsotypes and 7 STs), *K. ascorbata* (1 pulsotypes) and *C. freundii* (2 pulsotypes and 2 STs).

Conclusions: We describe a complex CPE hospital epidemiology with the co-existence of an epidemic dissemination of two high-risk clones (OXA-48-ST11 and VIM-ST54 *K. pneumoniae*), and the presence of OXA-48 and VIM-1 unrelated clones from different bacterial species.