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Concomitant and multiclonal dissemination of OXA-48-producing *Klebsiella pneumoniae* in a Spanish hospital

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Sir,

Carbapenemase-producing Enterobacteriaceae, particularly *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter* spp., are emerging worldwide as a major public health concern. According to the European survey on carbapenemase-producing Enterobacteriaceae, including 38 countries in 2013 (EuSCAPE) (http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-carbapenemase-producing-bacteria-europe.pdf), the epidemiological situation has significantly worsened over recent years and now represents a major threat to patient healthcare.[1] Here, we identified and characterized a series of carbapenem-resistant *K. pneumoniae* circulating in a single hospital in northern Spain during a 3 month period.

All *K. pneumoniae* isolates with reduced susceptibility to at least one carbapenem (imipenem and/or ertapenem) recovered at the University Central Hospital of Asturias, northern Spain, from September to December 2014, were selected for the study. A total of 68 isolates were recovered from 41 patients admitted to different wards, but only a single isolate per patient (the first obtained) was considered for further characterization. These isolates were involved in urinary tract infections (33.3%), respiratory infections (19%), sepsis (17%) and surgical wound infections (14.3%), while 9.7% of the patients were colonized.

Antimicrobial susceptibility testing was performed by disc diffusion assays (Becton Dickinson, Sparks, MD, USA), and the Microscan System (Beckman Coulter, Brea, CA, USA) was used for determination of MICs and bacterial identification. Susceptibility to colistin was determined by a broth culture microdilution method as recommended by the CLSI.[2] The results of susceptibility tests were interpreted according to CLSI breakpoints;[2] however, the MICs of tigecycline and colistin were determined and interpreted according to EUCAST guidelines.[3] The Carba NP test was applied for the detection of carbapenemase activity.[4] All 41 isolates were resistant to amoxicillin/clavulanate and piperacillin/tazobactam. In addition, all showed reduced susceptibility or resistance to ertapenem and imipenem, except 15 isolates (36.6%) that remained susceptible to imipenem. Interestingly, nine isolates (21.9%) were resistant to cefoxitin, and resistance to broad-spectrum cephalosporins was observed in all but one isolate (97.6%). Additionally, 93% of the isolates were resistant to ciprofloxacin, 78% to trimethoprim/sulfamethoxazole, 68% to gentamicin, 80% to tobramycin, 41% to amikacin, 20% to tigecycline, 7% to fosfomycin and 10% to colistin. The Carba NP test was positive for all 41 isolates.

Genes encoding resistance to carbapenems (bla_{OXA-48}, bla_{NDM}, bla_{VIM}, bla_{KPC}, bla_{IMP}) and to broad-spectrum cephalosporins (bla_{CTX-M}, bla_{SHV}) were screened by PCR amplification followed by sequencing.[5] PCRs were also performed to determine the genetic environment of the bla_{OXA-48} gene.[6] All isolates were positive for the bla_{OXA-48} carbapenemase gene that was located in transposon Tn*1999.2*.[6] In addition, 80% of the isolates resistant to broad-spectrum cephalosporins were positive for the $bla_{CTX-M-15}$ gene, while the remaining 20% were positive for the bla_{SHV-12} gene.

Conjugation assays were performed using each of the bla_{OXA-48}-positive isolates as donors and *E. coli* J53 resistant to sodium azide as the recipient strain. Transconjugants were selected on eosin methylene blue agar containing sodium azide (100 mg/L) plus temocillin (50 mg/L). Plasmid DNA was extracted using the Kado and Liu technique and the incompatibility group of the bla_{OXA-48}-carrying plasmids was established, as previously described.[5] The bla_{OXA-48} gene was carried by a conjugative plasmid of ~62 kb belonging to the IncL/M group, corresponding to the epidemic plasmid bearing the bla_{OXA-48} gene.[7] The remaining β-lactamase genes identified among the OXA-48 producers were always carried on distinct plasmids. To establish the genetic relationships between the isolates, 25 of them, selected as representatives of the different resistance phenotypes and hospital wards (Figure 1), were analysed by PFGE performed using endonuclease XbaI (www.pulsenetinternational.org). Overall, 19 different PFGE profiles were identified, with similarities evaluated by the Dice coefficient and cluster analysis performed using the software program MVSP (Multivariate Statistics Package for PCs, RockWare Inc.). Using a coefficient of similarity of 0.85, the 19 profiles could be distributed into six clusters termed CX1–CX6. One isolate per cluster was typed by MLST according to the Pasteur Institute scheme (www.pasteur.fr/mlst), giving rise to the subsequent clones ST326 (CX1), ST405 (CX2), ST147 (CX3), ST104 (CX4 and CX5) and ST15 (CX6) (Figure 1). Of note, all clones except ST104 had previously been associated with OXA-48 production in Europe and/or Spain.[1,8,9]

The concomitant occurrence of five different *K. pneumoniae* clones producing OXA-48 in a single hospital might be explained, at least in part, by the high transfer frequency of the IncL/M plasmid carrying the bla_{OXA-48} gene.[10] In view of our data, it is likely that the spread of OXA-48-producing *K. pneumoniae* in Spain will rapidly mirror the endemic situation observed in Italy and the USA with KPC-producing *K. pneumoniae* isolates. Once again,

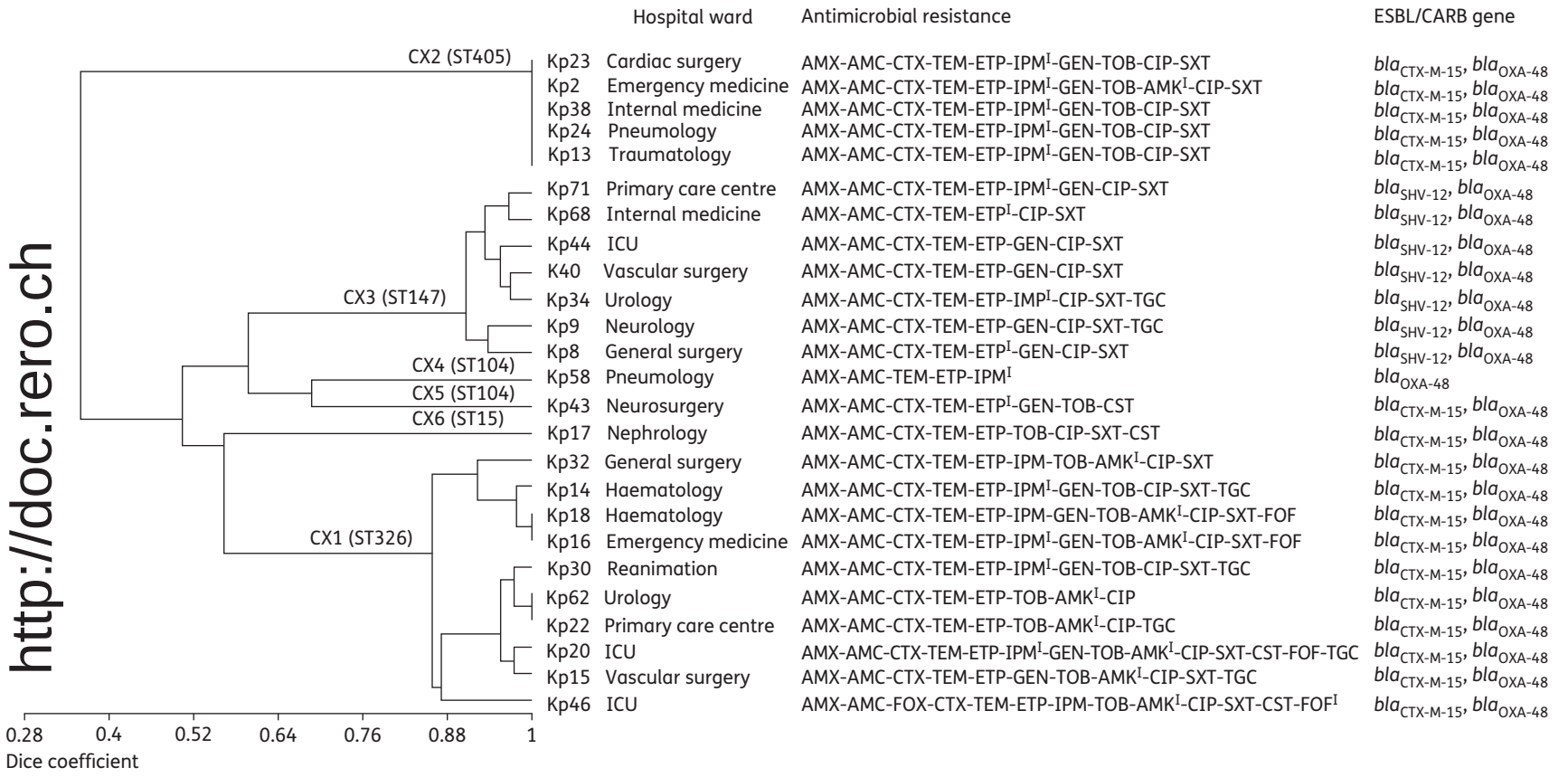

Figure 1. Dendrogram showing the relationships between 25 OXA-48-producing isolates of *K. pneumoniae* (Kp), selected as representative of different antimicrobial resistance phenotypes and hospital wards. In all isolates, the bla_{OXA-48} gene was carried by an ~62 kb conjugative IncL/M plasmid, and associated with transposon Tn*1999.2*. I, intermediate resistance; AMX, amoxicillin; AMC, amoxicillin/clavulanate; CTX, cefotaxime; TEM, temocillin; ETP, ertapenem; IPM, imipenem; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; AMK, amikacin; TGC, tigecycline; FOF, fosfomycin; FOX, cefoxitin; CST, colistin; CARB, carbapenemase.

this bacterial species is playing a pivotal role in the emergence and dispersion of resistance traits in hospital settings. Urgent hygiene measures have to be taken to prevent further consolidation of a difficult-to-control situation.

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Transparency declarations

None to declare.

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