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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

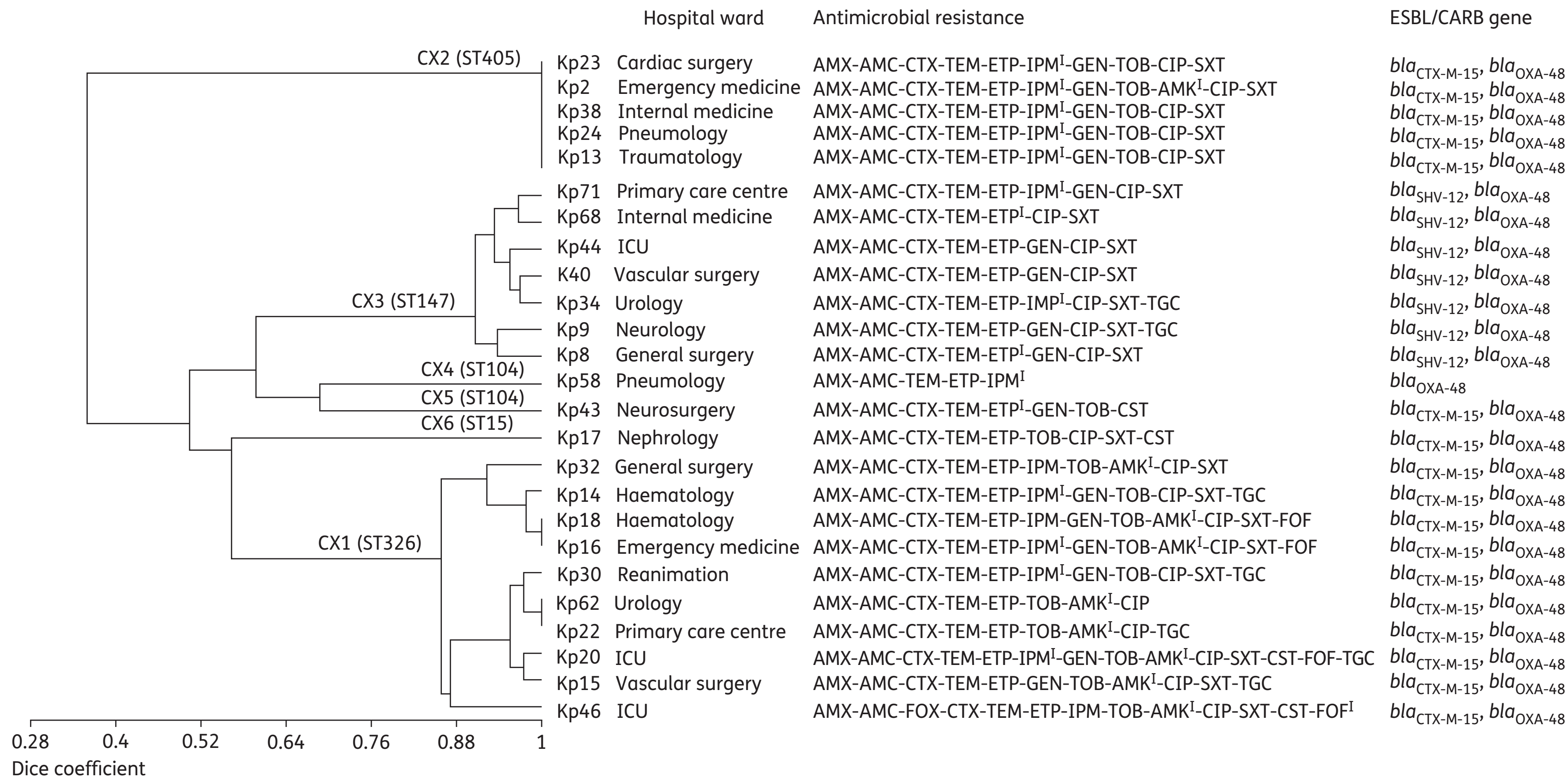

Figure 1. Dendrogram showing the relationships between 25 OXA-48-producing isolates of *K. pneumoniae* (Kp), selected as representative of different antimicrobial resistance phenotypes and hospital wards. In all isolates, the $bla_{\text{OXA-48}}$ gene was carried by an ~62 kb conjugative IncL/M plasmid, 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.

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, 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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In vivo emergence of ceftaroline resistance during therapy for MRSA vertebral osteomyelitis

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Sir,

Ceftaroline, the active metabolite of ceftaroline fosamil, was approved by the US FDA in 2010 and by the European Commission in 2012 for the treatment of acute bacterial skin and skin structure infections and community-acquired bacterial pneumonia.[1] This medication has also been reported to be used for the sporadic treatment of other severe MRSA infections, including osteomyelitis[2] and epidural abscesses.[3]

A middle-aged man with diabetes mellitus was admitted for incision and drainage of a septic left wrist. Source control was achieved. Tissue cultures grew MRSA (strain 89, Table 1), with ceftaroline MIC of 0.75 mg/L by broth microdilution (incremental dilution steps).[4] Vancomycin was initiated, but was switched to ceftaroline fosamil due to rising serum creatinine and impending cardiac catheterization for a myocardial infarction. He improved and was discharged on renally adjusted ceftaroline fosamil 600 mg every 12 h. During his hospitalization, the patient complained of chronic sciatica back pain. Physical examination was notable for no spinal tenderness. However, back pain progressed post-discharge, and MRI showed vertebral osteomyelitis and collections in bilateral psoas muscles. He was readmitted for CT-guided aspiration of the right psoas fluid collection, which grew MRSA (strain 91) with ceftaroline MIC 4–6 mg/L (Table 1) and antibiotics were changed to vancomycin. Peripheral blood cultures revealed two MRSA isolate morphologies (strains 86 and 88). He underwent laminectomy and facetectomy for epidural abscess drainage with lumbar debridement, fusion and fixation. Cultures from the epidural abscess again grew MRSA. Trans-thoracic echocardiogram was negative for vegetations. The patient was discharged and completed 12 weeks of intravenous antibiotics followed by 12 months of oral doxycycline after inflammatory markers normalized. More than 1 year after surgery the patient was ambulating independently with a cane and living at home, and inflammatory markers had remained normal for >4 months after completing antibiotics. The patient gave verbal and written informed consent for the publication of this case report.

The baseline MRSA (strain 89) and subsequent ceftaroline-resistant isolates (strains 86, 88 and 91) were subjected to WGS. Genome sequence data were utilized to extract epidemiological information related to isolate ST, *spa* and SCC*mec* determination, as previously described.[5] The DNA genes and/or