High occurrence of carbapenemase- and extended-spectrum-β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* from migratory birds (*Ciconia ciconia*) with detection of high-risk clones

Sandra MARTÍNEZ-ÁLVAREZ[1] Pierre CHÂTRE[2], Úrsula HÖFLE[3], Carla Andrea ALONSO[4], Pauline FRANÇOIS[2], Teresa CARDONA-CABRERA[2], Myriam ZARAZAGA[1], Jean-Yves MADEC[2], Marisa HAENNI[2], Carmen TORRES[1]

[1]*Area of Biochemistry and Molecular Biology, OneHealth-UR Research Group, University of La Rioja - Logroño (Spain)*
[2]*ANSES – Université de Lyon, Unité Antibiorésitance et Virulence Bactériennes - Lyon (France)*
[3]*Health and Biotechnology (SaBio) Research Group, Institute for Game and Wildlife Research IREC (CSIC-UCLM) - Ciudad Real (Spain)*
[4]*Hospital San Pedro, Logroño (Spain)*

Background: The presence of Enterobacterales producing carbapenemases (CP) and extended-spectrum β-lactamases (ESBL) is closely monitored in humans and animals, but the potential of migratory birds as carriers of resistance genes remains poorly understood. The aim of the study was to detect and characterize CP- and ESBL-producing *E. coli* (EC) and *K. pneumoniae* (KP) obtained from storks feeding on two landfills in Spain.

Methods: ESBL and CP-producing EC/KP were isolated from 211 stork faecal samples using chromogenic culture media, and collected isolates were sequenced using NovaSeq6000 (Illumina) and MinION (Oxford Nanopore) technologies. Resistome, virulome, sequence types (ST) and replicon profiles were determined using bioinformatic tools. Localization of ESBL/CP genes was performed using Southern blots on S1-PFGE gels.

Results: ESBL-EC/KP were detected in 71 samples (33.6%; 28.4%-EC and 5.2%-KP), while 28 samples (13.3%) contained CP-producing EC/KP (11.8%-EC and 1.4%-KP). Different sequence types (ST) (EC, n=33; KP, n=3) were identified, including high-risk clones associated with humans (EC: ST131, ST58 or ST69; KP: ST307), potential high-risk clones (EC: ST10 and ST48) and more ubiquitous clones (EC: ST46, ST155, ST117, ST617). A wide range of ESBL/pAmpC-conferring genes (*bla*$_{\text{CTX-M-1}}$/*bla*$_{\text{CTX-M-14}}$/*bla*$_{\text{CTX-M-15}}$/*bla*$_{\text{CTX-M-27}}$/*bla*$_{\text{CTX-M-32}}$/*bla*$_{\text{CTX-M-55}}$/*bla*$_{\text{CTX-M-65}}$/*bla*$_{\text{SHV-12}}$/*bla*$_{\text{CMY-2}}$/*bla*$_{\text{DHA-1}}$) was identified, as well as a large variety of CP-genes (*bla*$_{\text{KPC-2}}$, *bla*$_{\text{KPC-3}}$, *bla*$_{\text{NDM-1}}$, *bla*$_{\text{NDM-7}}$, *bla*$_{\text{OXA-48}}$, *bla*$_{\text{VIM-1}}$ and *bla*$_{\text{GES-7}}$), including in some cases a combination of up to three types of CP (*bla*$_{\text{KPC-2}}$, *bla*$_{\text{NDM-7}}$ and *bla*$_{\text{VIM-1}}$) and/or with co-detection of ESBL/pAmpC genes. Likewise, a variety of ESBL/pAmpC- and CP-carrying plasmids were identified, such as IncY (n=30), IncF (n=60), IncX3 (n=25) IncN (n=19) or IncL (n=8). The genetic characterization of these plasmids is ongoing.

Conclusions: This study demonstrates the role of storks as important reservoirs and potential vectors of antimicrobial resistance genes of very high clinical impact. The wide variety of STs, as well as resistance genes, suggests that these birds can acquire and accumulate resistant bacteria, including human high-risk clones, along their migratory routes, that should be monitored.