Third-generation cephalosporin-resistant Enterobacterales in healthy cats: implications for One Health surveillance

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The detection of Enterobacterales resistant to third-generation cephalosporins is of particular concern, as these strains are classified by the World Health Organization (WHO) as critical priority pathogens. Within a One Health framework, clinically healthy companion animals may contribute to the silent circulation of antimicrobial-resistant Enterobacterales. In this context, this study aimed to characterize the antimicrobial resistance profiles and genomic features (resistome, virulome and mobilome) of third-generation cephalosporin-resistant Enterobacterales isolates recovered from healthy cats. A total of four Enterobacterales isolates (2 *Klebsiella pneumoniae,* 1 *Escherichia coli* and 1 *Enterobacter hormaechei)* obtained from 4 healthy cats were selected on MacConkey agar supplemented with cefotaxime (2 µg/mL). Isolates were identified by MALDI-TOF MS and the MALDI TypeR platform, and antimicrobial susceptibility was determined by disk diffusion according to CLSI guidelines. Whole-genome sequencing was performed using the Illumina MiSeq platform. Reads were assembled with SPAdes and genomes were annotated using PGAP and assessed with CheckM. Antimicrobial resistance genes (ARGs), virulence factors, sequence types, mobile genetic elements, and plasmid replicons were identified using CGE tools and ABRicate v1.2.0 against multiple AMR databases (NCBI, CARD, ResFinder, ARG-ANNOT, MEGARES), with MGEs (ISs, transposons, and integrons) further manually curated following TnCentral guidelines. *E. coli* and both *K. pneumoniae* isolates exhibited a multidrug resistance (MDR) phenotype, with resistance to beta-lactams, quinolones/fluoroquinolones, aminoglycosides, tetracycline and sulfonamides. *Enterobacter hormaechei*, although not classified as MDR, showed resistance to all tested beta-lactams, including ertapenem. Genomic analysis revealed that *E. coli* GAP95 (ST2, O8:H17) and *K. pneumoniae* GP129b (ST429) and GAP138a (ST40) harbored a broad resistome, including ESBL genes ($bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1}}$, bla_{SHV}), additional ARGs, mutations in topoisomerase genes, and efflux pump determinants. Virulence profiling identified genes associated with adhesion, biofilm formation, iron acquisition, stress response, and the type VI secretion system (T6SS). Multiple mobile genetic elements (e.g., IS families and Tn*3*-like transposons) and plasmid replicons (mainly IncF and Col types) were identified, frequently associated with $bla_{\text{CTX-M-15}}$ – IS*Ecp1* structures. *Enterobacter hormaechei* GAP298 (ST346) presented a narrower resistome, including $bla_{\text{ACT-15}}$ and other ARGs, but also displayed diverse virulence determinants and mobile genetic elements,

with plasmids assigned to IncFIB, IncFII and Col groups. These findings suggest that clinically healthy cats may act as reservoirs of Enterobacterales with multidrug resistance, clinically relevant ESBL genes, and diverse virulence and mobile genetic elements, highlighting their potential role as reservoirs for antimicrobial resistance dissemination within a One Health framework.

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