SOCIEDAD ESPAÑOLA DE MICROBIOLOGÍA

XIV REUNIÓN DEL GRUPO DE MICROBIOLOGÍA MOLECULAR DE LA SEM

Santander, 17-19 de junio de 2024

ibbtec

MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES

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Extended-spectrum beta-lactamase-producing *Escherichia coli* isolated from chicken meat in Spain involve rare and complex resistance plasmids and ST lineages.

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Introduction: Animal food products are important sources of zoonotic agents, increasing the risk of exposure to antibiotic-resistant bacteria (AMR) from farm to fork. Therefore, we aimed to detect ESBL-producing *E. coli* (Ec) from the poultry sector and to fully characterise them as well as the genetic determinants carrying resistance genes with a One Health approach.

Methods: From December 2021/to March 2022, 48 chicken meat samples (thigh/breast/minced meat) were collected from 16 establishments (nine local shops and seven hypermarkets) in La Rioja (Northern Spain). Both selective (MacConkey) and enriched selective media (cefotaxime, 2 µg/mL) were used. Identification was confirmed by MALDI-TOF. Antibiotic susceptibility testing was assessed by the disk-diffusion method (CLSI, 2023). Long-read (Oxford Nanopore) and short-read (Illumina) WGS were performed on all ESBL-Ec isolates.

Results: Fifty-seven Ec isolates (1-2 isolates per sample) were recovered from 33 out of 48 chicken meat samples tested (68.8%). In addition, six ESBL-Ec (10.5%) were obtained, which belonged to ST1140-E/*bla*$_{\text{CTX-M-32}}$ (n=1), ST752-A/*bla*$_{\text{TEM-52}}$ (n=1), ST117-B2/*bla*$_{\text{CTX-M-1}}$/*bla*$_{\text{SHV-12}}$ (n=2), ST10-A/*bla*$_{\text{SHV-12}}$ (n=1) and ST223-B1/*bla*$_{\text{SHV-12}}$ (n=1). Phylogenetic analysis revealed that ST117-Ec (from different farms) differed by 135 allelic differences (ADs). Three IncI1-plasmids (pST3-CC3) were found carrying the *bla*$_{\text{SHV-12}}$/*bla*$_{\text{CTX-M-1}}$/*bla*$_{\text{CTX-M-32}}$ genes in different genetic environments: i) IS*26*-*smc*-*glpR*-*bla*$_{\text{SHV-12}}$-IS*26* and ii) *wbuC*-*bla*$_{\text{CTX-M-32}}$/*bla*$_{\text{CTX-M-1}}$-*ISEcp1*. The bla$_{\text{TEM-52}}$ gene was in a P1-phage flanked by an IS*4*-mediated composite transposon. The IncHI2 plasmid harboured a *bla*$_{\text{SHV-12}}$ gene flanked by an IS*26*-mediated composite transposon inserted in a Tn*21*-derived. This transposon also harboured a non-classical integron (*sul3*-associated), indicating the sequential acquisition of several AMR genes (aminoglycosides-chloramphenicol-sulfonamides) with an embedded Tn*1721*. To analyse the cross-sectoral relatedness of our ESBL-Ec, we mapped our six genomes with those framed within the “EU AMR monitoring in livestock and meat project” establishing links between 53-136 ADs with caecal contents of dairy cows, beef calves or broiler chickens’ genomes.

Conclusion: This study demonstrates that ESBL genes are widely disseminated in chicken meat in Spain due to the diversity of clones and genetic backgrounds. Nevertheless, the predominance of ST117 and the IncI1-*bla*$_{\text{CTX-M-1-32}}$/*bla*$_{\text{SHV-12}}$ plasmids might indicate the presence of successful clones and plasmids adapted to the chicken host.