

Iconic autochthonous breeds of cattle in Northern Portugal are reservoirs of multidrug-resistant ESBL-carrying *Escherichia coli*

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Antimicrobial resistance (AMR) is a public health concern involving food-producing animals. Animals act as reservoirs/source of antibiotic resistant *Escherichia coli* that can spread to humans through the food chain or the environment.

This study aimed to characterize the AMR profiles of *E. coli* from fecal samples of three native Portuguese breeds (Barrosã, Cachena and Minhota) since this information is non-existent.

From thirty extensive producing farms (10 per breed) in Northern Portugal, 480 collected samples (May-June 2023) were pooled, based on age group (8 calves and 8 cows per farm) and isolated on MacConkey Agar supplemented with or without antibiotics (4 µg/ml cefotaxime; 3 µg/ml colistin). A total of 102 characteristic *E. coli* colonies representing the 3 different breeds were selected for confirmation by MALDI-TOF MS, antimicrobial susceptibility testing (AST) for 10 antibiotics (EUCAST/CLSI guidelines), ESBL phenotype (DDST) screening and detection of *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M} genes by PCR.

From both calves and cows, 77% and 20% of the *E. coli* exhibited resistance to ≥1 antibiotic and multidrug resistance (MDR, resistance to ≥3 antimicrobial classes), respectively, from all breeds. Isolates were mostly resistant to gentamycin (72%), tetracycline (27%) and ampicillin (21%). ESBL activity was observed in 10% of *E. coli* isolates (from Cachena and Minhota breeds). Ongoing assays have already shown the presence of *bla*_{TEM} and *bla*_{CTX-M} genes for one isolate. This pioneering study revealed the concerning presence of MDR *E. coli* in iconic native Portuguese cattle breeds raised in production regimes where antibiotic use is theoretically low.

Keywords: Antimicrobial resistance; *Escherichia coli*; Livestock; One Health; Native breeds