

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

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INTRODUCTION

Antimicrobial resistance (AMR) is a public health concern involving food-producing animals. Animals act as reservoirs/source of antibiotic resistant *Escherichia coli* that can be spread to humans through the food chain or the environment [1-3]. In Portugal, there are fifteen bovine autochthonous breeds with a relevant historical-cultural, social and economic impact. However, AMR available data about these breeds are scarce.

➤ This study aimed to characterize the AMR profiles of *E. coli* from fecal samples of three autochthonous Portuguese breeds – Barrosã, Cachena and Minhota, with Protected Designation of Origin (PDO) native of the Northern region of Portugal.



MATERIAL and METHODS

From thirty extensive producing farms (10 per breed) in Northern Portugal, 480 collected fecal samples (May-June 2023) were pooled, based on age group (8 calves and 8 cows per farm). Samples were isolated on MacConkey Agar supplemented with or without antibiotics (4 µg/ml cefotaxime or 3 µg/ml colistin). *Escherichia coli* ATCC 25922 was used as control.

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 (Figure 1).

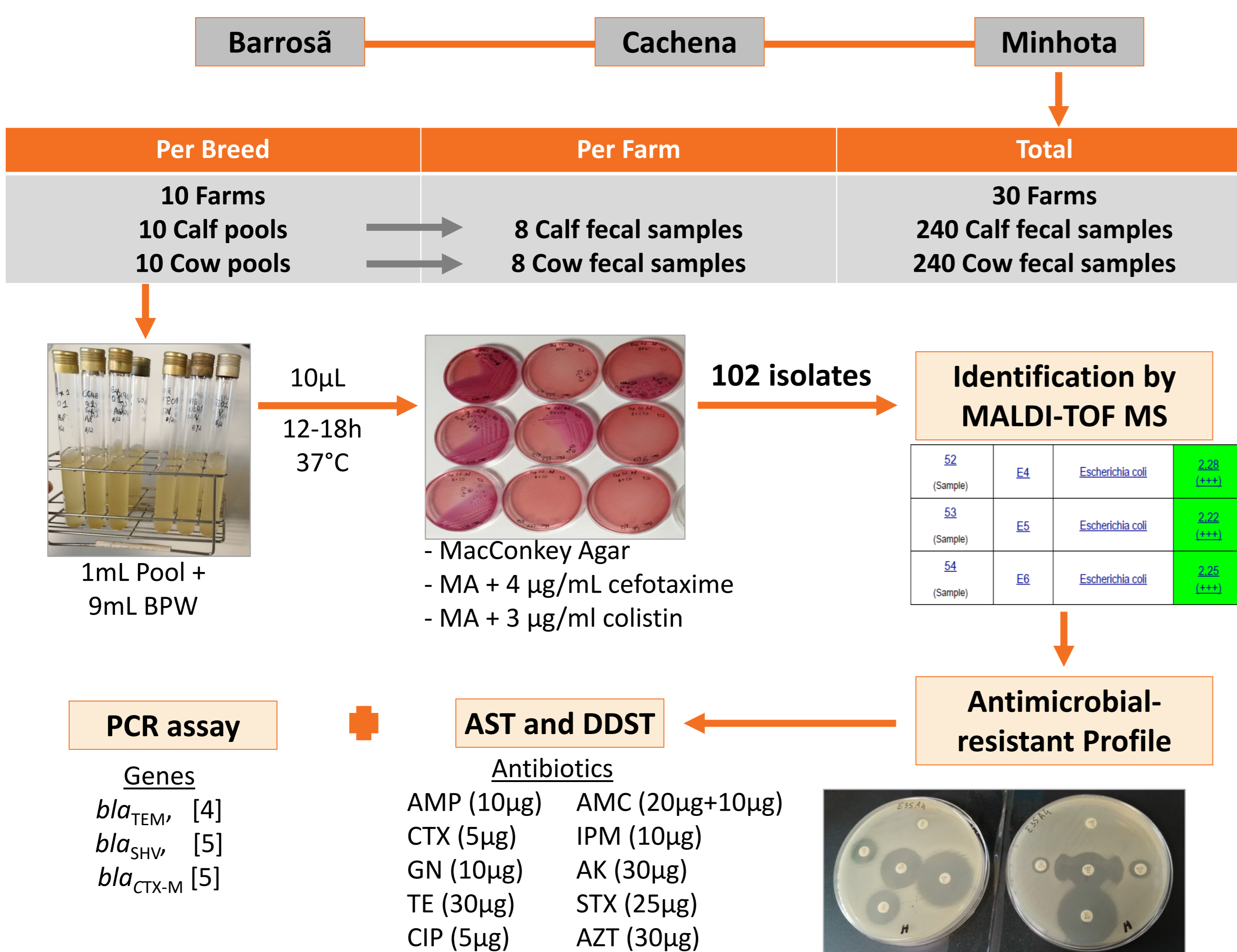


Figure 1. Methodology of the processing of fecal samples from autochthonous Portuguese bovine, including the microbiological analyses and phenotypic and genotypic characterization of the antibiotic-resistant *E. coli*. BPW- Buffered peptone water. MA- MacConkey agar. AST-Antimicrobial susceptibility testing. DDST- ESBL phenotype. AMP- ampicillin; AMC-amoxicillin/clavulanic acid; CTX-cefotaxime; IPM-imipenem; GN-gentamicin; AK-amikacin; TE-tetracycline; SXT-trimethoprim/sulfamethoxazole; CIP-ciprofloxacin; AZT-aztreonam.

RESULTS

▪ A total of 102 representative *E. coli* isolates were identified by MALDI-TOF, of which 37 (20 calves and 17 cows) were obtained from Barrosã, 34 (20 calves and 14 cows) from Cachena and 31 (17 calves and 14 cows) from Minhota breeds. Isolates were mostly resistant to gentamycin (72%), and in a lesser extent to tetracycline (27%) and ampicillin (21%) (Table 1).

Table 1. Antimicrobial resistance phenotypes of the 102 tested *E. coli* isolates (%).

Antibiotic	Breed						Total % (Nr.)
	Barrosã		Cachena		Minhota		
	Calves % (Nr.)	Cows % (Nr.)	Calves % (Nr.)	Cows % (Nr.)	Calves % (Nr.)	Cows % (Nr.)	
AMP	15 (3)	12 (2)	5 (1)	0 (0)	59 (10)	36 (5)	21 (21)
AMC	0 (0)	6 (1)	10 (2)	0 (0)	12 (2)	0 (0)	5 (5)
CTX	0 (0)	0 (0)	5 (1)	0 (0)	29 (5)	29 (4)	10 (10)
IMP	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
AZT	0 (0)	0 (0)	0 (0)	0 (0)	23 (4)	29 (4)	8 (8)
CIP	5 (1)	6 (1)	0 (0)	0 (0)	0 (0)	21 (3)	5 (5)
GN	50 (10)	53 (9)	55 (11)	93 (13)	94 (16)	100 (14)	72 (73)
AK	5 (1)	6 (1)	0 (0)	0 (0)	12 (2)	29 (4)	8 (8)
TE	20 (4)	12 (2)	40 (8)	0 (0)	53 (9)	36 (5)	27 (28)
SXT	10 (2)	12 (2)	5 (1)	0 (0)	29 (5)	21 (3)	13 (13)

AMP-ampicillin; AMC-amoxicillin/clavulanic acid; CTX-cefotaxime; IPM-imipenem; GN-gentamicin; AK-amikacin; TE-tetracycline; SXT-trimethoprim/sulfamethoxazole; CIP-ciprofloxacin; AZT-aztreonam.

- 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, in all breeds (Figure 2A).
- In all breeds, the analysis carried out by the animal age group demonstrated a higher tendency of resistance in calves, perhaps resulting from the administration of colostrum originating from cows from the same farm.
- ESBL activity was observed in 10% of *E. coli* isolates, all obtained from Cachena (one calf) and Minhota (five calves and four cows) breeds (Figure 2A and 2B).

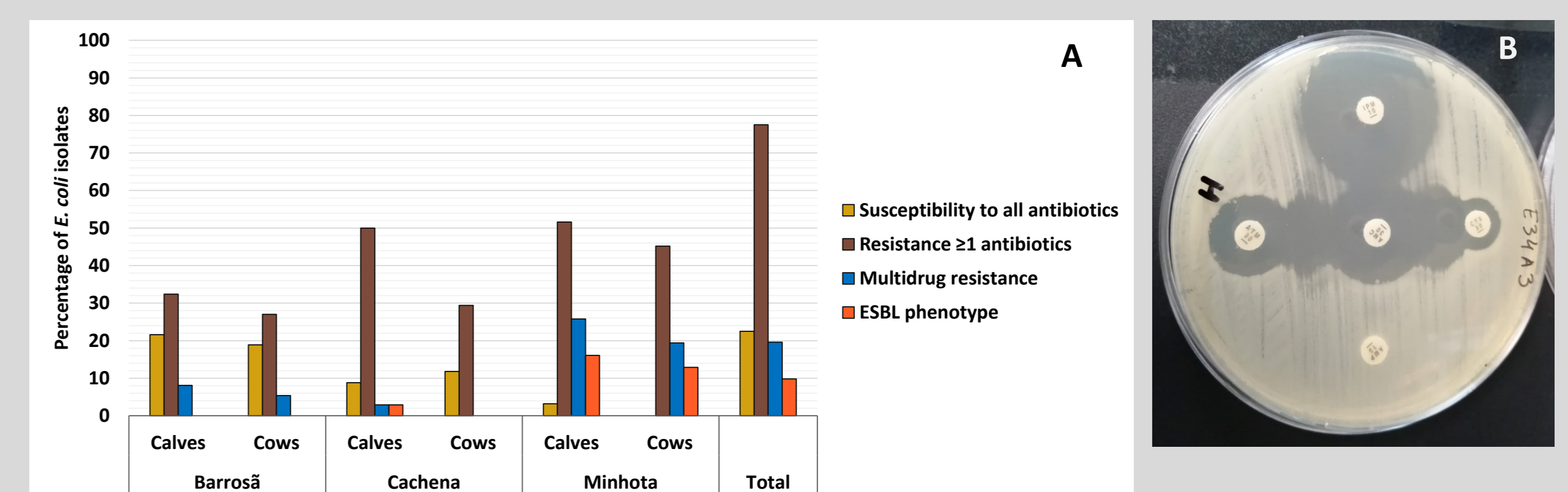


Figure 2. Percentage of the *E. coli* isolates that showed susceptibility to all tested antibiotics, resistance to ≥1 antimicrobial classes, multidrug resistance phenotype, and positive ESBL synergy test (A); an example of the positive ESBL synergy test (B).

- Ongoing assays have already shown the presence of *bla*_{TEM} and *bla*_{CTX-M} genes for one isolate obtained from a calf of the Cachena breed (Table 2).

Table 2. Identification of β-lactamase resistance genes isolated from *E. coli*.

<i>bla</i> _{TEM}	<i>bla</i> _{SHV}	<i>bla</i> _{CTX-M}
+	-	+

CONCLUSIONS

- ✓ This pioneering study revealed the concerning presence of multidrug-resistant *E. coli*, including resistance to antibiotics considered critical, in iconic autochthonous Portuguese cattle breeds raised in production regimes where antibiotic use is theoretically low. More studies are needed to explore the impact of these results in the public health.

Acknowledgements

This work was funded by CESPU, under the CORECattleGI2-CESPU-2022 project. Work supported by National Funds through FCT - Fundação para a Ciência e a Tecnologia in the scope of the project UIDP/50027/2020, as well as UIDB/50006/2020 and UIDP/50006/2020.

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