

Exploring the Role of Autochthonous Portuguese Hens in Antimicrobial Resistance dissemination: A Study of *Escherichia coli* in Cloacal and Eggshell Samples

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Introduction and Aim

The use of antibiotics in intensive animal farming is a significant contributor to antimicrobial resistance (AMR), a global public health concern [1]. However, the role of autochthonous Portuguese laying hens as reservoirs of antibiotic-resistant *Escherichia coli* remains underexplored. These breeds, raised in extensive systems with minimal antibiotic use, provide a unique opportunity to study AMR under lower selective pressure [2].

This study investigated the role of autochthonous Portuguese laying hens (*Preta Lusitânica*, *Amarela*, *Branca*, and *Pedrês Portuguesa*) as carriers of antibiotic-resistant *E. coli*, using cloacal and eggshell swabs to assess their antimicrobial resistance profiles.



Methods

- A total of 64 cloacal and 64 eggshell swabs were collected from 31 selected flocks within extensive production systems, with two swabs of each sample type per flock (corresponding to 16 per breed) [2].
- From these, 67 *E. coli* isolates were obtained through microbiological culture – 33 from cloacal and 34 from eggshell samples (Figure 1). Confirmation of *E. coli* was performed using multiplex PCR targeting the *uidA* gene and flanking regions of *uspA*, as previously described, following DNA extraction by the boiling method [3].
- Antimicrobial susceptibility testing against 10 antibiotics was assessed according to EUCAST/CLSI guidelines [4].

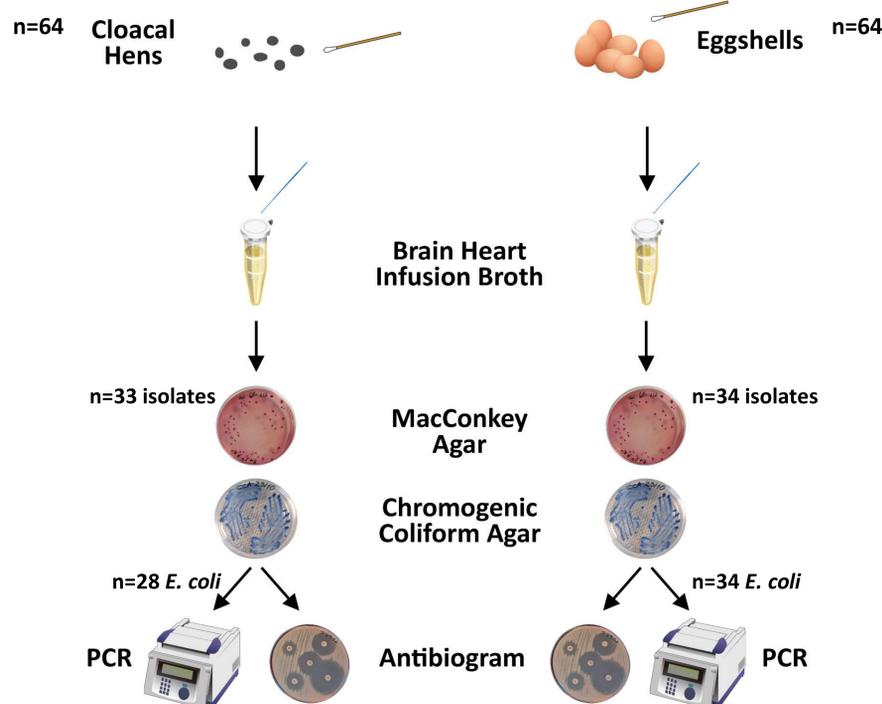
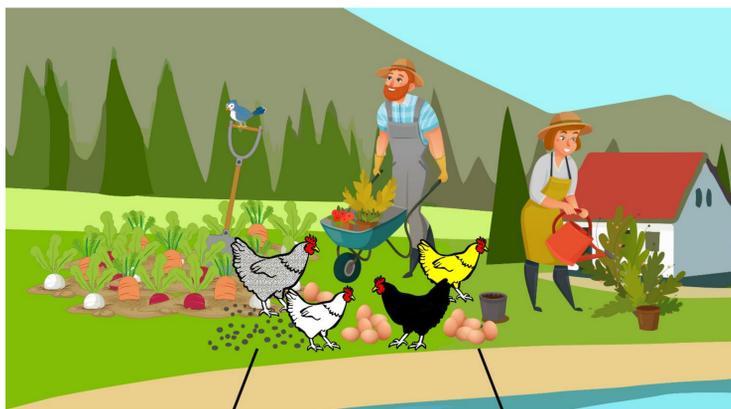


Figure 1: Schematic representation of the methodology used in this study (bacterial identification and phenotypic characterization).

Results

- All cloacal *E. coli* isolates (100%) were resistant to gentamicin, resulting in 100% resistance to at least one antibiotic (Figure 2a and b).
- Additional resistance among cloacal isolates was observed to tetracycline (29%, *Branca*, *Pedrês Portuguesa*, and *Amarela*), ampicillin (14%, *Branca*, *Preta Lusitânica*, and *Amarela*), trimethoprim+sulfamethoxazole (14%, *Branca*, *Pedrês Portuguesa*, and *Amarela*), and amikacin (7%, *Branca* and *Amarela*) (Figure 2a).
- Multidrug resistance was identified in 14% of cloacal isolates (*Branca* and *Amarela*), (Figure 2b).

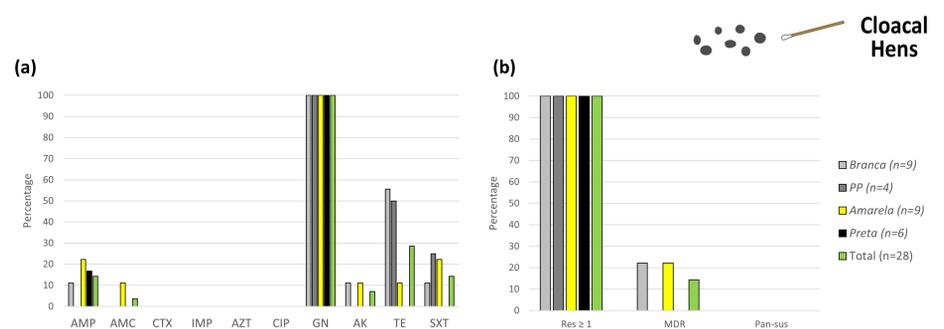


Figure 2: Phenotypic characterization of antimicrobial resistance in *E. coli* from cloacal swabs (n=28): (a) antimicrobial resistance profiles; and (b) antimicrobial resistance of *E. coli* isolates.

AMP - ampicillin; AMC - amoxicillin/clavulanic acid; CTX - cefotaxime; IMP - imipenem; AZT - aztreonam; CIP - ciprofloxacin; GN - gentamicin; AK - amikacin; TE - tetracycline; SXT - trimethoprim /sulfamethoxazole. Pan-sus - pan-susceptibility; Res ≥ 1 - resistance to ≥ 1 antibiotic; MDR - multidrug-resistant phenotype; PP - *Pedrês Portuguesa*

- Eggshell *E. coli* isolates exhibited 94% resistance to gentamicin (Figure 3a).
- Resistance was also observed to tetracycline (38%, all breeds), ampicillin (15%, *Pedrês Portuguesa* and *Amarela*), amikacin (6%, *Preta Lusitânica*), and trimethoprim+sulfamethoxazole (3%, *Branca*) (Figure 3a).
- Overall, 97% were resistant to at least one antibiotic (Figure 3b).
- Multidrug resistance was observed in 18% of eggshell isolates (*Preta Lusitânica*, *Amarela*, and *Pedrês Portuguesa*) (Figure 3b).

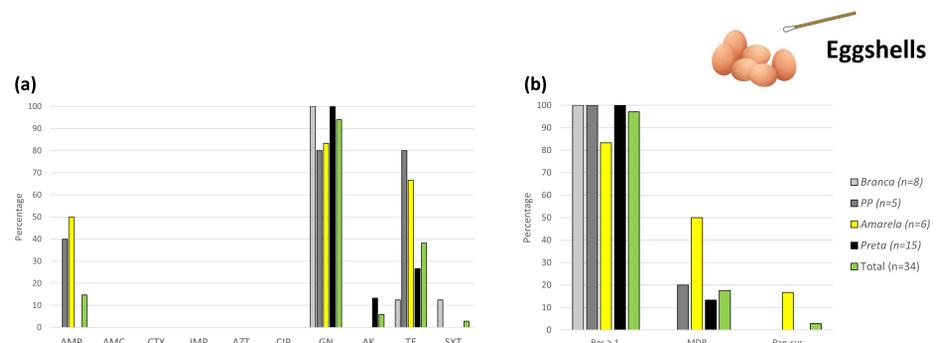


Figure 3: Phenotypic characterization of antimicrobial resistance in *E. coli* from eggshell swabs (n=34): (a) antimicrobial resistance profiles; and (b) antimicrobial resistance of *E. coli* isolates.

AMP - ampicillin; AMC - amoxicillin/clavulanic acid; CTX - cefotaxime; IMP - imipenem; AZT - aztreonam; CIP - ciprofloxacin; GN - gentamicin; AK - amikacin; TE - tetracycline; SXT - trimethoprim /sulfamethoxazole. Pan-sus - pan-susceptibility; Res ≥ 1 - resistance to ≥ 1 antibiotic; MDR - multidrug-resistant phenotype; PP - *Pedrês Portuguesa*

Conclusion

- ✓ This study provides novel insights into AMR in autochthonous hens, highlighting the role of environmental factors (soil, water, feed, and human contact) in resistance transmission to cloacal and eggshell samples.
- ✓ High resistance levels, particularly to gentamicin (a Critically Important Antimicrobial) and the presence of multidrug-resistant strains were confirmed.
- ✓ As sustainable poultry farming gains importance, this study provides valuable insights into the role of autochthonous hens and their by-products, particularly eggs, in the transmission of AMR, underscoring implications for food safety and the potential spread of resistant *E. coli* through the food chain.

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