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Are Food-Producing Animals a Source of Multidrug-resistant *E. coli* and *Salmonella* spp.?

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BACKGROUND

- Zoonoses have been responsible for several human diseases, many associated with the consumption of contaminated food, where pathogenic and drug-resistant *Escherichia coli* and *Salmonella* spp. are among the principal bacterial agents.
- Food-producing animals can be healthy carriers of these zoonotic agents and meat contamination can occur in any stage of the production chain, including by cross-contamination in the slaughter line.
- In accordance with Directive 2003/99/EC, in the European Union, data on animals, food and feed must be reported on a mandatory basis for eight zoonotic agents, including *Salmonella spp.* and Shiga toxin-producing *E. coli* (STEC)[1], which were responsible for the 2nd and 4th most reported zoonoses in humans in 2021, respectively[1].

OBJECTIVES

- To assess the role of food-producing animals as potential transmission vehicles of *Salmonella* spp. and *E. coli* to Humans, in order to understand the epidemiology and population structure of these zoonotic agents in Portugal.

METHODS

- Between October 2022 and March 2023, fecal samples were collected in several Portuguese slaughterhouses.
- For *E. coli*, samples were pre-enriched and platted on selective and non-selective media, whereas isolation of *Salmonella* spp. was performed according to ISO 6579-1:2017[4] with slight modifications. Presumptive colonies were isolated on Tryptone Soy Agar, and confirmed on VITEK2 or by *16SrRNA* amplification (only for *E. coli*)[2]. *Salmonella* serotyping was achieved by the slide agglutination method for O and H antigens, using the Kauffmann-White-Le Minor scheme [5], while detection of *E. coli* Shiga toxins *stx1* and *stx2* was performed by PCR [3].
- For a sub-set of the isolates, antimicrobial susceptibility testing (AST) was performed by disk-diffusion method, according to EUCAST guidelines (http://www.eucast.org).

CONCLUSIONS

- **Overall, our preliminary findings revealed the presence of MDR *E. coli* isolates in fecal samples collected from pigs, chickens and turkeys slaughtered for human consumption, highlighting the role of these animal reservoirs as potential source of this zoonotic agent.**
- **This is an alarming scenario, as the resistance genes responsible for these phenotypes can be transmitted between bacteria, but also to other animals and to humans. Therefore, surveillance of these zoonotic agents as well monitoring of their antimicrobial resistance should be reinforced in food-producing animals.**

RESULTS

- A total of 254 fecal samples (124 from pigs, 83 from chickens and 47 from turkeys) were studied, mostly from Centro and Lisboa e Vale do Tejo regions (Figure 1).
- *Salmonella* spp. (*S. enterica enterica* serovar Cremieu) was only detected in one turkey sample, while *E. coli* was identified in all studied samples from the three animal species, with STEC (*stx2e* gene) detected in two pig samples.

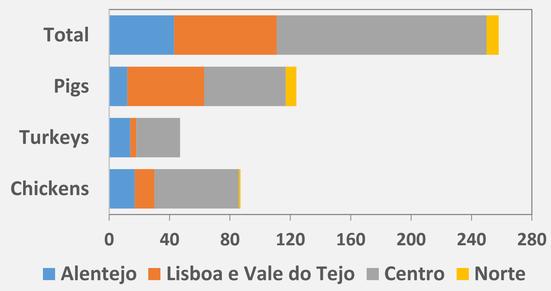

Figure 1 | Geographic location (NUTS II) of the collected fecal samples.

- Whereas *S. Cremieu* was susceptible to all the tested antimicrobials, AST performed on 100 pig, 18 chicken and 18 turkey *E. coli* isolates showed that 73.0%, 88.9% and 94.4% were resistant to at least one tested antibiotic (Figure 2), respectively, with AMP/TET occupying the top-rank on pigs and turkeys, and AMP/CIP on chickens.

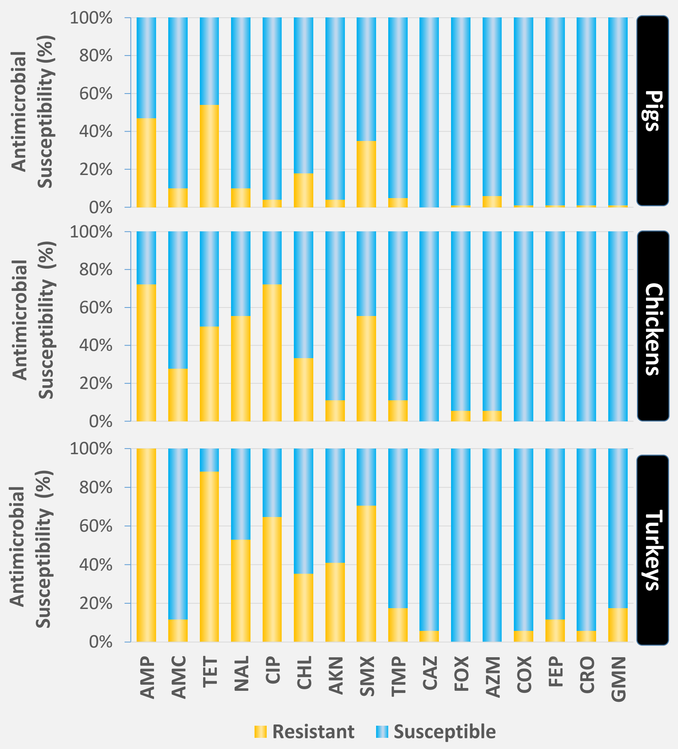

Figure 2 | Antimicrobial susceptibility of *E. coli* isolates against a panel of 16 antimicrobials: ampicillin (AMP), amoxicillin (AMC), tetracycline (TET), nalidixic acid (NAL), ciprofloxacin (CIP), chloramphenicol (CHL), amikacin (AKN), sulfamethoxazole (SMX), trimethoprim (TMP), ceftazidime (CAZ), cefoxitin (FOX), azithromycin (AZM), cefotaxime (COX), cefepime (FEP), ceftriaxone (CRO), gentamicin (GMN).

- Overall, 61.3% (65/106) of all resistant *E. coli* isolates displayed a multidrug-resistance (MDR) profile (Figure 3): 35.4% to three (n=23), 38.5% to four (n=25), and 26.1% (n=17) to five or more different classes of antimicrobials. One *E. coli* recovered from a turkey sample revealed resistance to 9 antibiotics: AMP, AMC, TET, NAL, CIP, CHL, AKN, TMP, and SMX.
- Interestingly, one MDR isolate (AMP, TET, CHL, SMX, AZM) was a STEC.
- Resistance to some critically important antimicrobials for humans, was observed in 35,8% of the isolates: 26.4% to ciprofloxacin (mainly in chickens), 6.6% to azithromycin (mainly in pigs) and 2.8% to cefepime (mainly in turkeys).

Figure 3 | MDR profile of *E. coli* isolates.

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