

# Multidrug-resistant *Escherichia coli* and *Salmonella* spp. in pigs slaughtered for human consumption, a potential source for humans?

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## INTRODUCTION

- Foodborne diseases are a serious public health problem worldwide. Food-producing animals are considered a major source of these diseases through contamination of food-products, 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), which were responsible for the 2<sup>nd</sup> and 4<sup>th</sup> most reported zoonoses in humans in 2021, respectively [1].

## OBJECTIVES

- To assess the role of pig reservoir as potential transmission vehicle 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 plated on selective and non-selective media, whereas isolation of *Salmonella* spp. was performed according to ISO 6579-1:2017 [2] with slight modifications. Presumptive colonies were isolated on Tryptone Soy Agar, and confirmed on VITEK2 or by 16S rRNA amplification (only for *E. coli*) [3]. *Salmonella* serotyping was achieved by the slide agglutination method for O and H antigens, using the Kauffmann-White-Le Minor scheme [4], while detection of *E. coli* Shiga toxins *stx1* and *stx2* was performed by PCR [5].
- Antimicrobial susceptibility testing (AST) was performed by disk-diffusion method, according to EUCAST guidelines (<http://www.eucast.org>).

## REFERENCES

- [1] EFSA & ECDC. The European Union One Health 2021 Zoonoses Report. EFSA J. 2022, 20:e07666.
- [2] ISO 6579-1:2017; Microbiology of the Food Chain-Horizontal Method for the Detection, Enumeration and Serotyping of Salmonella— Part 1: Detection of Salmonella spp. ISO: Geneva, Switzerland, 2017.
- [3] Sabat et al. Appl. Environ. Microbiol. 2000, 66:844-849.
- [4] Grimont & Weill. Antigenic formulae of the Salmonella serovars. In WHO Collaborating Centre for Reference and Research on Salmonella, 9th ed. Institute Pasteur: Paris, France, 2007; pp. 1–166.
- [5] Scheutz et al. J. Clin. Microbiol. 2012, 50:2951-2963.

## RESULTS

- Overall, 124 stool samples were studied, mostly from Centro and Lisboa e Vale do Tejo Regions (Figura 1).

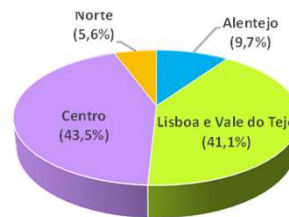


Figure 1 | Geographic location (NUTS II) of the collected fecal samples.

- While no *Salmonella* spp. was detected, *E. coli* was identified in all pig samples, with STEC (*stx2e* gene) detected in two pig samples.

- AST performed on 100 pig *E. coli* isolates showed that 73.0% of them were resistant to at least one of the 16 tested antibiotics (Figure 2), with AMP/TET occupying the top-rank.

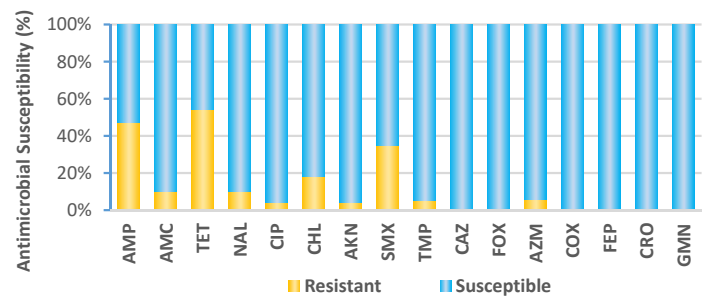


Figure 2 | Antimicrobial susceptibility of *E. coli* isolates against a panel of 16 antimicrobials: ampicillin (AMP), amoxicillin (AMC), tetracycline (TET), nalidixic acid (NAL), ciprofloxacin (CIP), chloramphenicol (CHL), amikacin (AKN), sulfamethoxazole (SMX), trimethoprim (TMP), ceftazidime (CAZ),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

- Overall, 50.7% (38/75) of all resistant *E. coli* isolates displayed a multidrug-resistance (MDR) profile (Figure 3): 42.1% to three (n=16), 44.7% to four (n=17), and 13.2% (n=5) to five or more different classes of antimicrobials. One of these MDR isolates was a STEC.

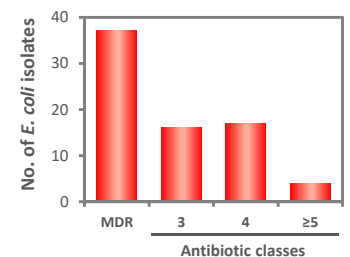


Figure 3 | MDR profile of *E. coli* isolates.

## CONCLUSIONS

- Although this study is still ongoing, our preliminary findings revealed the presence of MDR *E. coli* isolates in fecal samples of pigs slaughtered for human consumption, highlighting the role of this animal reservoir as potential source of these bacteria.

## ACKNOWLEDGMENTS

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