

# ROLE OF THE COMMON HEDGEHOG AS A RESERVOIR FOR ANTIMICROBIAL RESISTANCE GENES

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## INTRODUCTION

The overuse of antimicrobials has provoked the adaptation of the microorganisms and the generation of antimicrobial resistances (AMR). The FAO, OIE and WHO work on a tripartite initiative to combat AMR with a “One Health” approach. The collaboration of different sectors is necessary to guarantee the human, animal and environmental health.

Prevalence of ARM is high, and the main mechanisms for antimicrobial resistance are beta-lactamase enzymes.

The study of wildlife as AMR bacteria carrier is taking importance worldwide. The hedgehog lives near residential areas where gets in contact with anthropogenic sources of AMR. That is why it is a good environment bioindicator.

## HYPOTHESSIS

The hedgehog has a high prevalence of multidrug resistant bacteria, and consequently is an antimicrobial resistant gene reservoir and environmental AMR sentinel. Moreover, the hospitalization in Wild Fauna Rehabilitation Centres (WFRC) has an enhancing effect on the selection of MDR bacteria.

## OBJECTIVES

- Study the prevalence of AMR in wild hedgehogs treated in WFRC in Catalonia.
- Determine the profiles of phenotypic (antibiogram) and genotypic resistance (PCR of AMR genes) to antimicrobials of relevance in veterinary and human medicine.
- Study the effect of hospitalization on WFRC on the selection of AMR bacteria in hedgehogs.

## METODOLOGY

- For microbiological analysis faecal samples of *Erinaceus europaeus* were obtained **before** giving treatment.
- Plates of MacConkey Agar, MC Agar supplemented with ceftriaxone and colistin and XLT4 were used for the culture and **selective** isolation of RAM bacteria (37°C for 24h).
- All grown colonies were isolated and identified by the oxidase test, biochemical tests, API 20NE and API 20E.

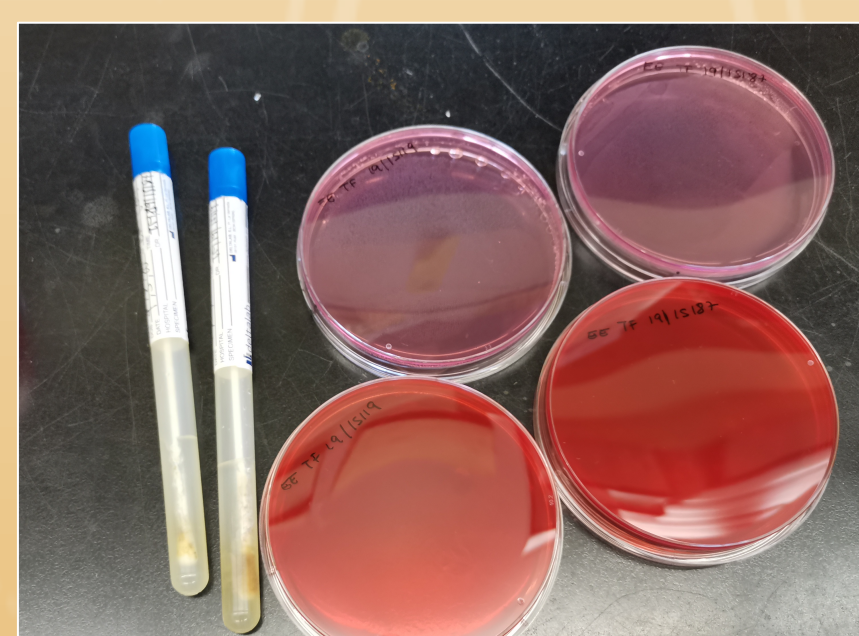


Image 1: rectal swabs, MC Agar and XLT4 plates.



Image 2: grown colonies after incubation.

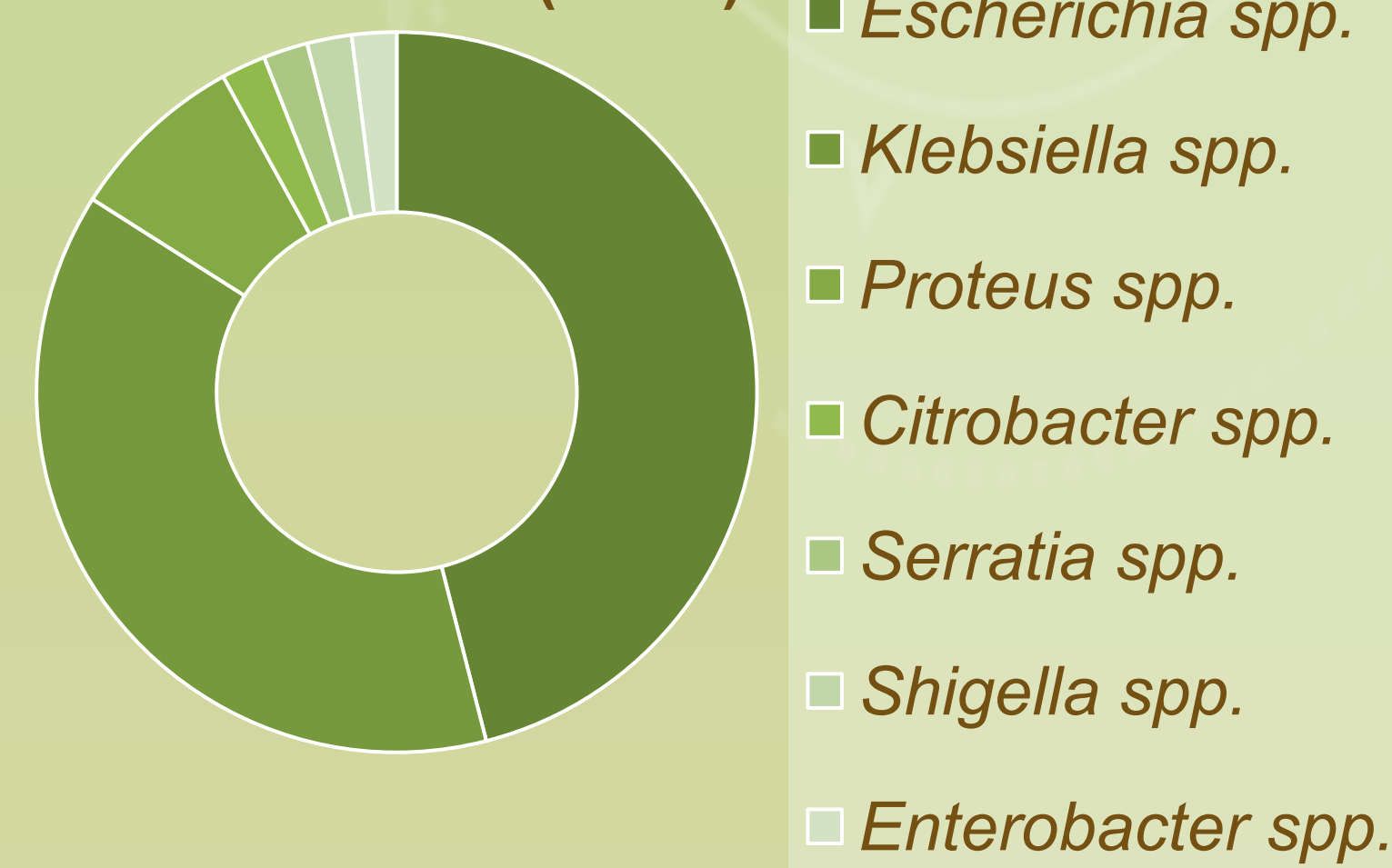
- Antimicrobial susceptibility testing was done in *Klebsiella spp.* using the disk diffusion method (Kirby-Bauer) for: AM, AMC, XML, CRO, CEQ, TE, GM, ENR, CIP, E, LS, SXT, C and CL.
- For the molecular characterization of AMR genes, DNA extraction and then the PCR for the beta-lactamase genes - bla<sub>SHV</sub>, -bla<sub>CTX</sub>, y -bla<sub>CMY-2</sub> was performed in *E. coli* and *Klebsiella spp.*

## RESULTS

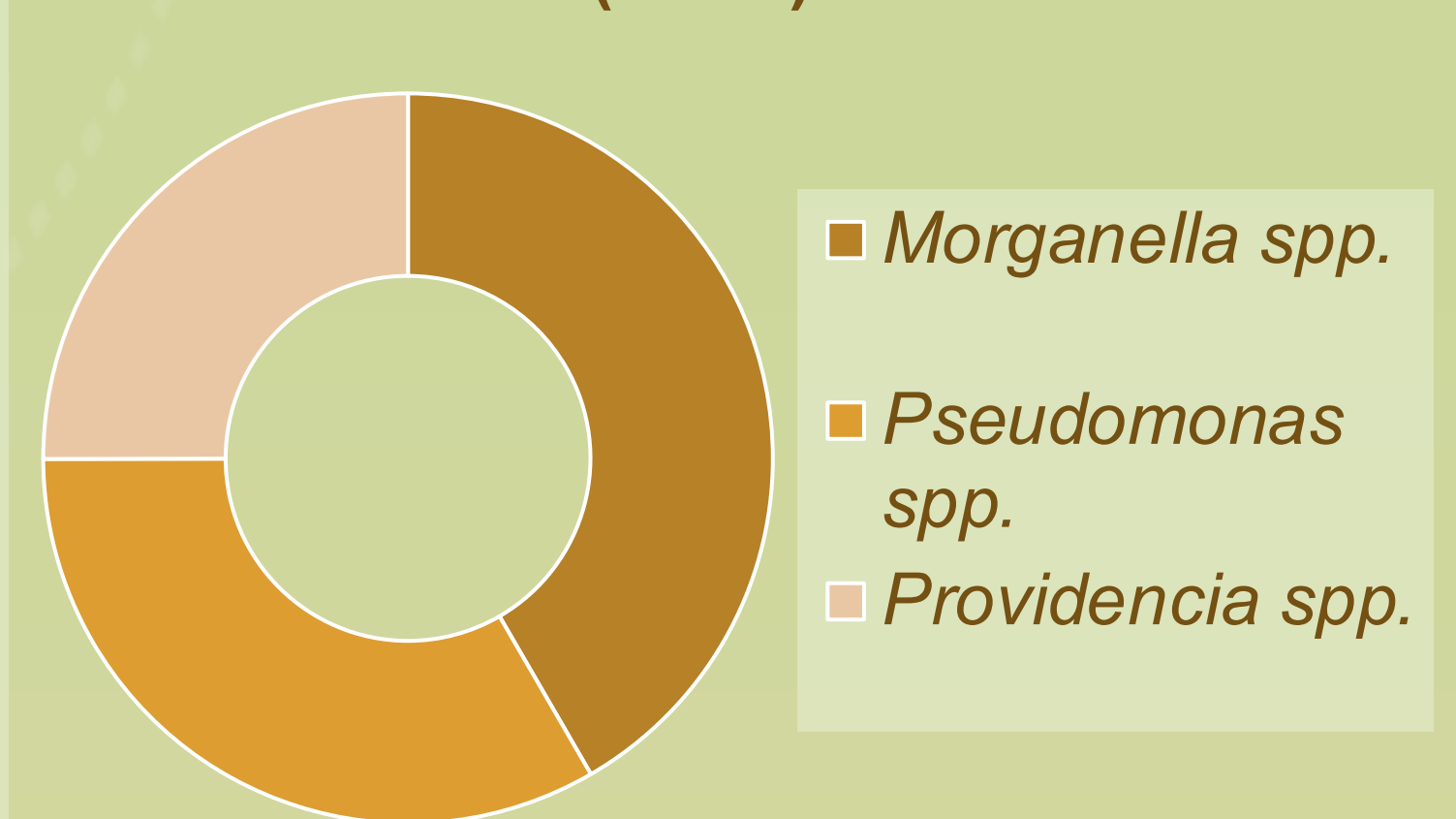
Study population → 70 hedgehogs analysed in the entrance and a follow up of 36 (106 samples in total).

Microbiological study → 62 positive growths in the first sampling in the selective mediums and 36 in the second.

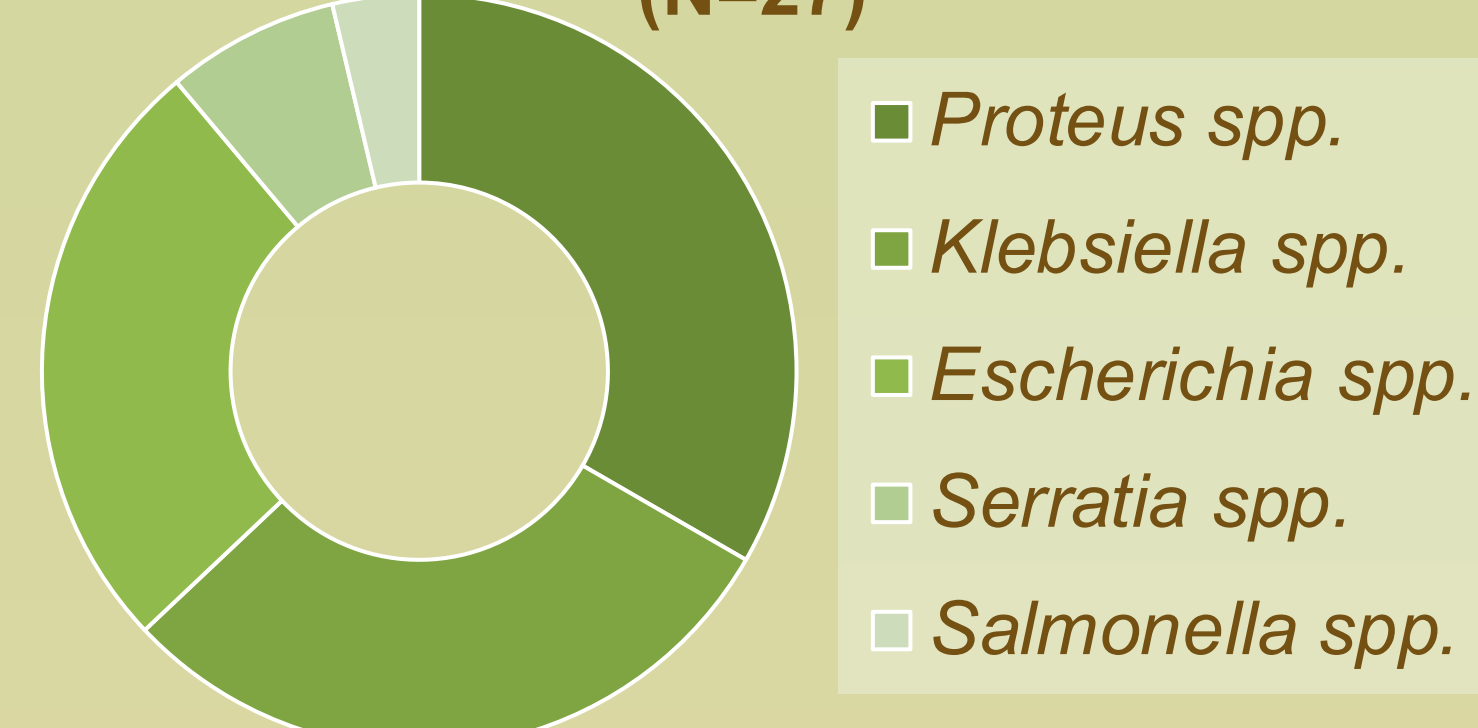
ENTEROBACTERIA 1<sup>st</sup> sampling (N=50)



NO ENTEROBACTERIA 1<sup>st</sup> sampling (N=12)



ENTEROBACTERIA 2<sup>nd</sup> sampling (N=27)



NO ENTEROBACTERIA 2<sup>nd</sup> sampling (N=9)

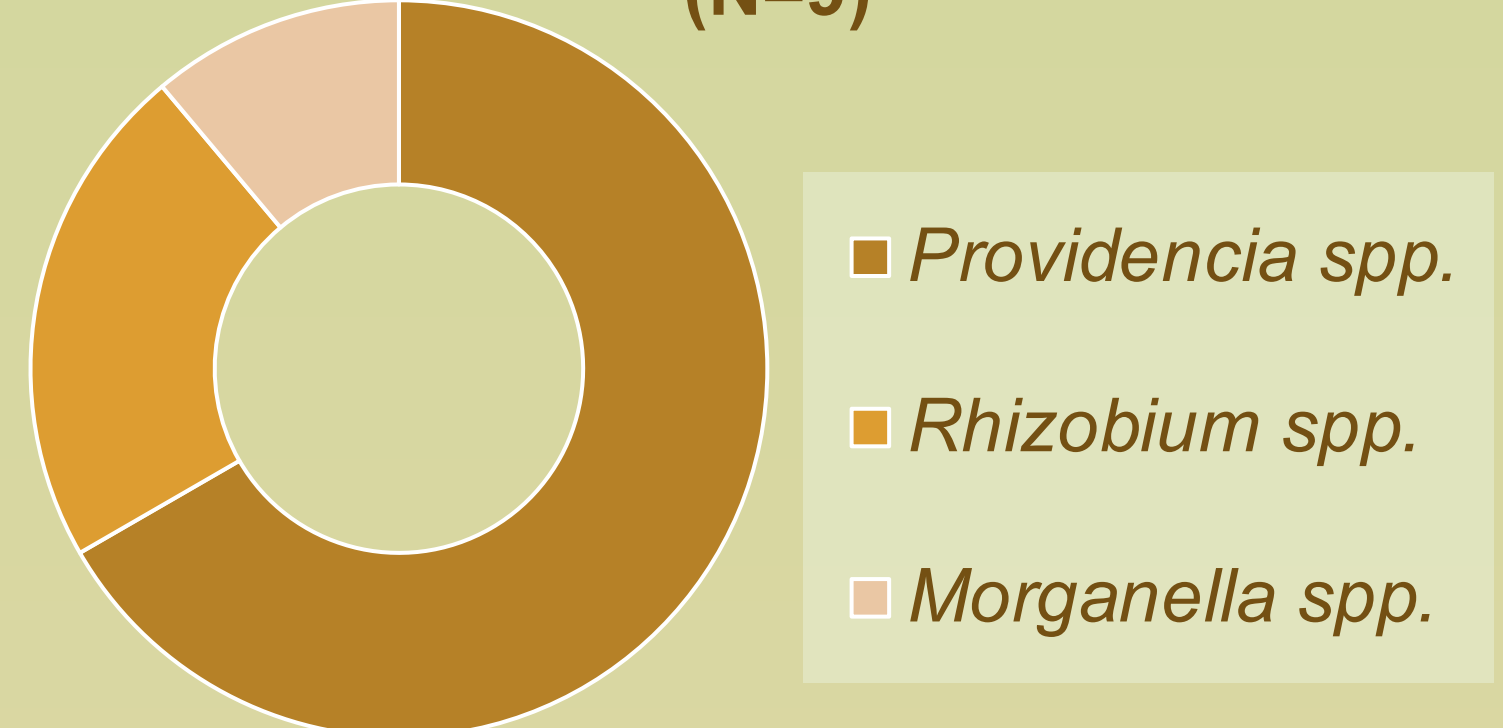


Figure 1: Proportion of identified bacterial genera from the study samples.

## Antimicrobial Susceptibility testing →

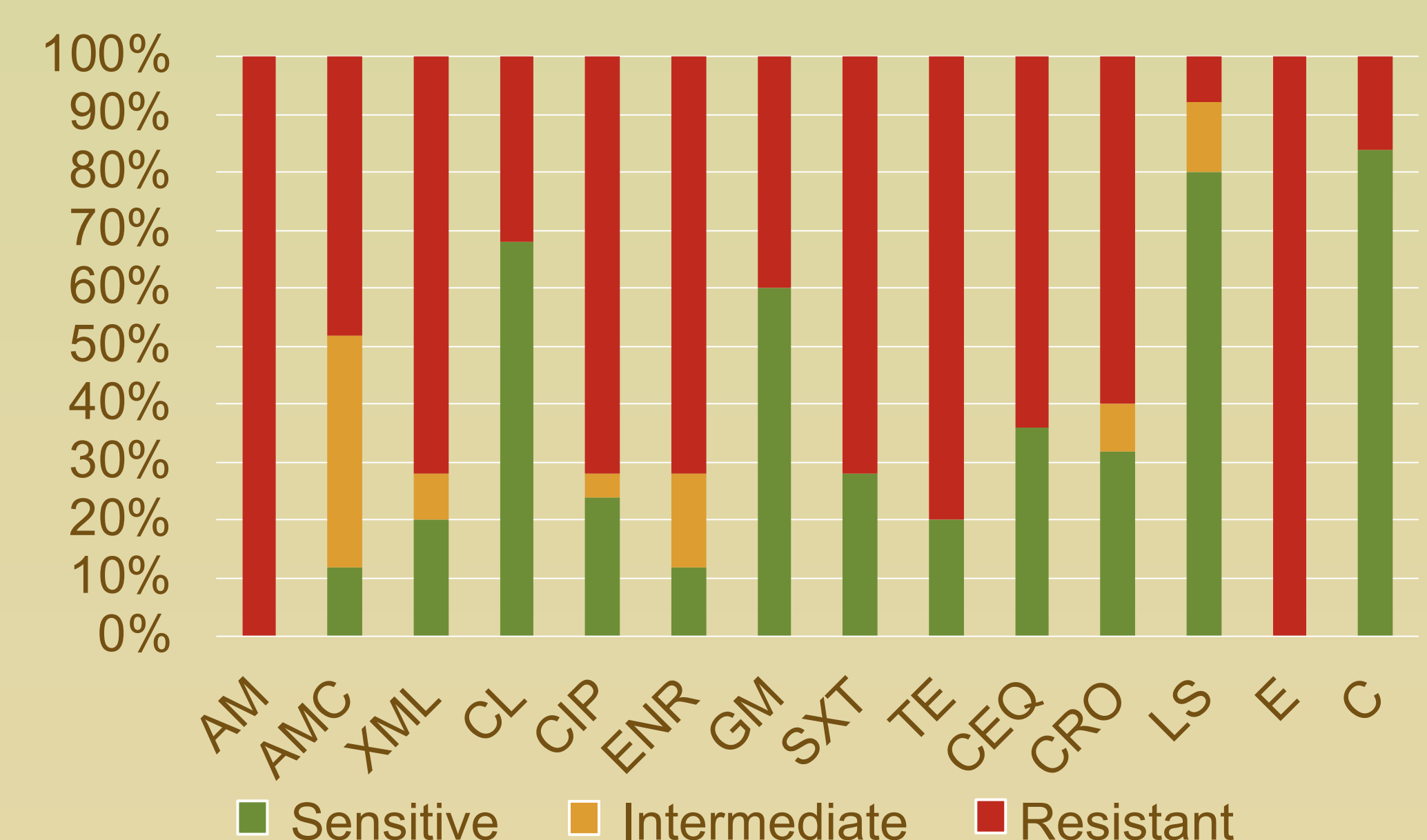


Figure 2: Antibiogram results in *Klebsiella spp.* AM, ampicillin; AMC, amoxicillin + clavulanic acid; XML, ceftiofur; CL, colistin; CIP, ciprofloxacin; ENR, enrofloxacin; GM, gentamicin; SXT, trimethoprim + sulfamide; TE, tetracycline; CEQ, cefquinoma; CRO, ceftriaxone; LS, lincospectin; E, erythromycin; C, chloramphenicol.

## Molecular characterization of antimicrobial resistance genes →

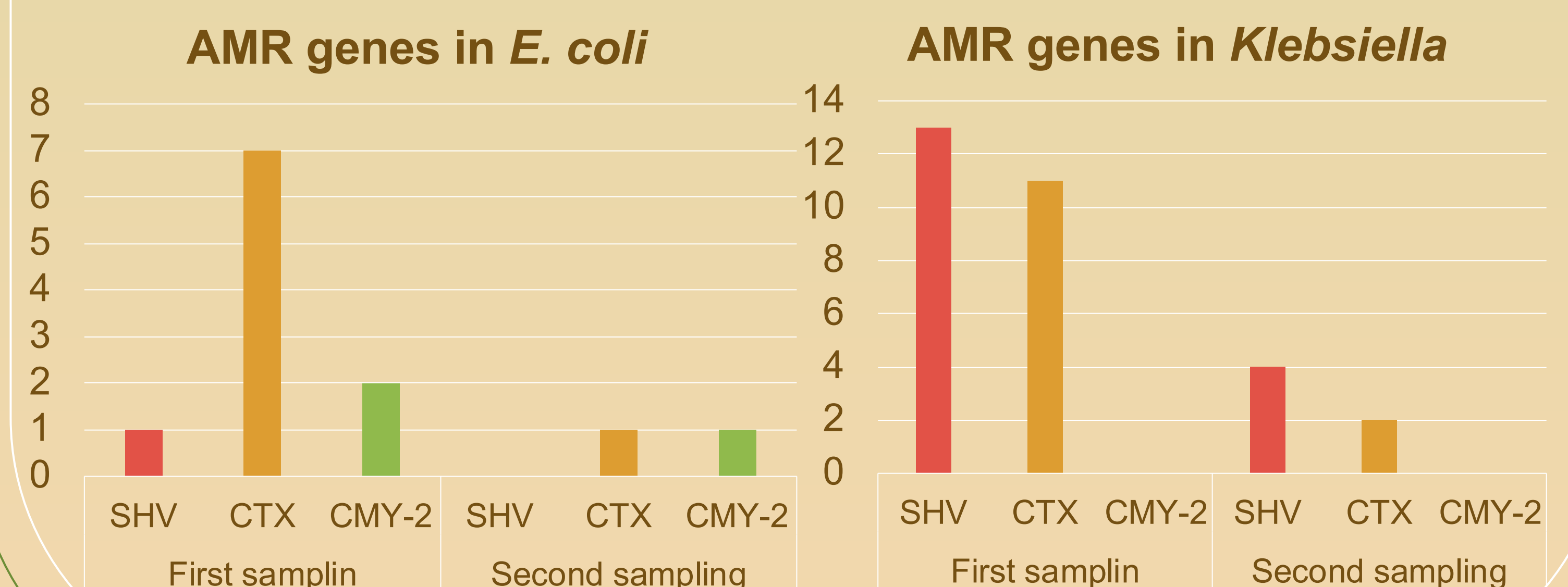


Figure 3: prevalence of AMR genes isolated in *E. coli* and *Klebsiella* strains in the study.

## CONCLUSIONS

- There is a high prevalence of bacteria with an antimicrobial multi-resistance profile in the hedgehog population of Catalonia.
- The most frequent bacterial genera were *Escherichia* and *Klebsiella* and the most detected genes were bla<sub>CTX</sub> and bla<sub>SHV</sub>.
- The great diversity of antimicrobial resistance obtained in *Klebsiella spp.* shows that the hedgehog is a good bioindicator of environmental trends of anthropogenic sources of AMR.
- We must adopt the One Health approach in order to reduce this prevalence of bacteria and AMR genes in the environment and thus reduce the risk to human and animal health.
- Hospitalization of hedgehogs in the WFRC does not seem to favour the selection of bacteria or generate AMR, especially in animals that have not received any antimicrobial treatment.