Domestic pigs as reservoirs of resistant and virulent Gram-Negative bacteria

Eva Cunha
DVM, PhD

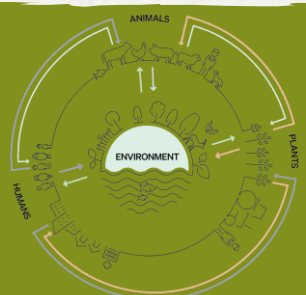
17as JORNADAS
INTERNACIONAIS
HOSPITAL VETERINÁRIO
MURALHA DE ÉVORA

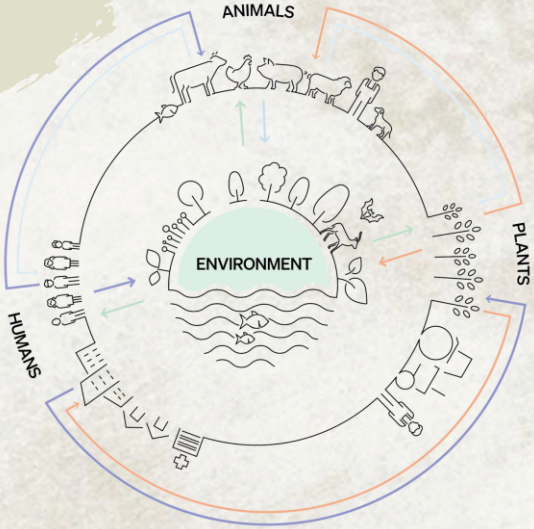
Antimicrobial resistance

Ability of microorganisms to persist or grow in the presence of drugs designed to inhibit or kill them.

- ✓ “one of the **top global public health”**
- ✓ “directly responsible for **1.27 million global deaths in 2019”**
- ✓ “Deaths due to AMR estimated to reach **10 million people by 2050**”

World Health Organization





ONE HEALTH

Domestic Pigs

- Important **reservoirs** of pathogens [Eiamsam-Ang et al., 2024; Huang et al. 2025]
- **Swine outdoor farming systems**
- Contact with other animals – domestic and wild animals
- **First research work - Wild Boars**

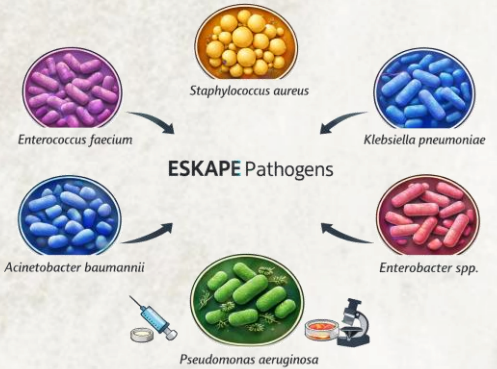
Figure 1. Bísaro pigs and sheeps (Original)

Domestic Pigs

Figure 2. Bisaro pigs (Original)

- ✓ **First research work – Wild Boars**

ESKAPE pathogens, ESBL producing bacteria, zoonotic pathogens



World Health Organization

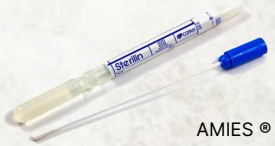
Goal

To evaluate **pig's role as reservoirs of pathogenic bacteria** by characterizing the AMR and virulence profiles of Gram-negative isolates from pigs bred in **outdoor farming systems**

Figure 3. Iberic pigs (Original)

Methodology

1. Sample collection

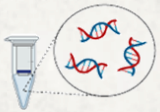


AMIES ®

2. Classical bacteriologic evaluation
[Microbiome analysis]

chromID® ESBL

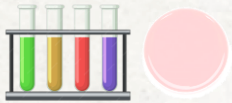
Vitek® Identification



3. Antimicrobial susceptibility testing

Kirby-Bauer method

4. Virulence phenotypic evaluation



Methodology

1 Sample collection

Nasal samples from domestic pigs

AMIES ®

Figure 4. Bisaro pig restraining (Original)

Figure 5. Alentejano pigs (Original)

Methodology

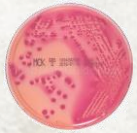
2 Classical bacteriologic evaluation

AMIES ®

BHI
[24h, 37ºC]

chromID® ESBL

Blood agar

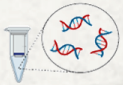


MCK agar

Gram staining
Oxidase test
Lactose fermentation

Vitek® Identification

Genomic approach



MGI Easy Nucleic Acid

16S rDNA gene amplification
(V1-V9 region)

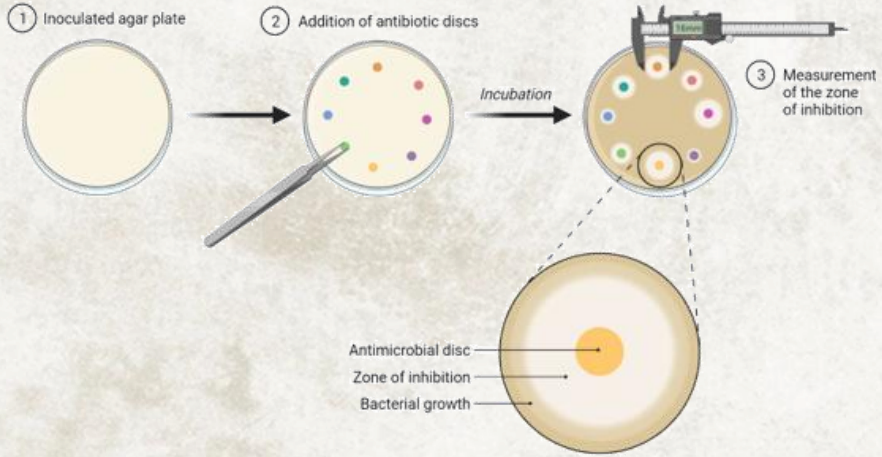
Long-read **nanopore** sequencing

ZymoBIOMICS Microbial Community

Methodology

3 Antimicrobial susceptibility testing – disc diffusion technique

✓ AST –**Disc Diffusion technique** [CLSI guidelines, Abreu et al., 2025]



1. Enterobacterales
2. *Pseudomonas*
3. *Acinetobacter*

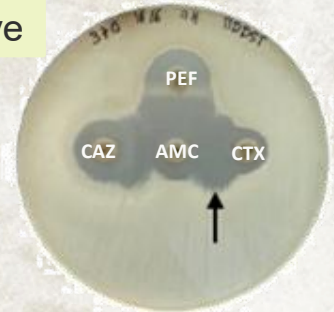
Beta-lactams
Tetracyclines
Aminoglycosides
Fluoroquinolones
Sulfonamides
Phenicols

Methodology

3 Antimicrobial susceptibility testing – disc diffusion technique

- ✓ AST –**Disc Diffusion technique** [CLSI guidelines, Abreu et al., 2025]
- ✓ ESBL detection – **Modified double disk synergy test** [Kaur et al., 2013]

Positive



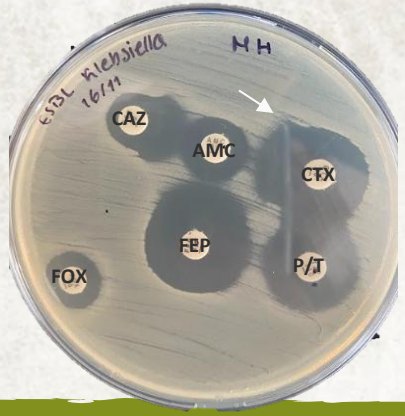


Figure 6. Modified double disk synergy tests (Original)

Methodology

3 Antimicrobial susceptibility testing – disc diffusion technique

- ✓ AST –**Disc Diffusion technique** [CLSI guidelines, Abreu et al., 2025]
- ✓ ESBL detection – **Modified double disk synergy test** [Kaur et al., 2013]
- ✓ Detection of **multidrug resistance (MDR)** isolates [Magiorakos et al., 2012]
- ✓ **Multiple antimicrobial resistance (MAR)** classification [Sing et al., 2017]

$$MAR = \frac{\text{Nº resistant antimicrobials}}{\text{Total nº of antimicrobials tested}}$$

Methodology

4 Virulence phenotypic evaluation

- ✓ Phenotypic production of 6 virulence factors using specific media: [Santos et al., 2025]

A. **Protease** - Skim Milk Agar 48h 37°C

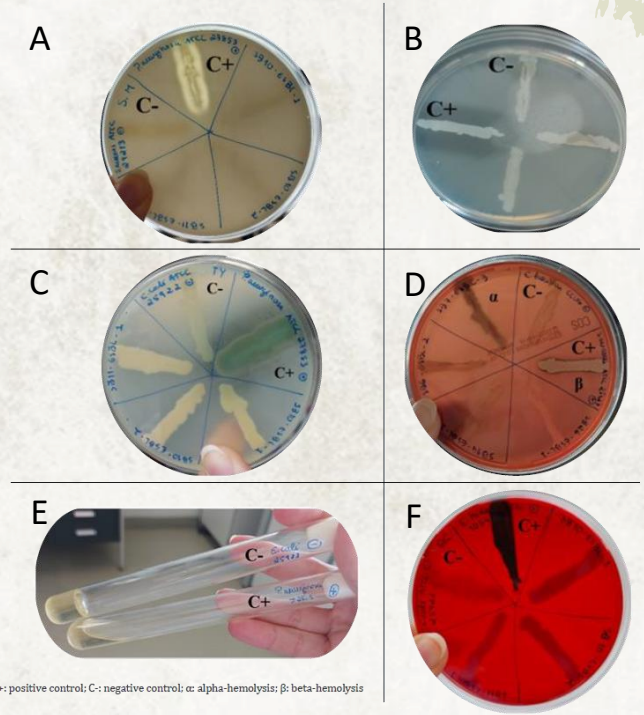
B. **Dnase** - DNase Test Agar 48h 37°C

C. **Lecitinase** - Tryptic Soy Agar + 10% egg yolk 48h 37°C

D. **Hemolysis** – Columbia Agar + 5% Sheep Blood 24h 37°C

E. **Gelatinase** – Nutrient Gelatine 48h 37°C

F. **Biofilm** production - Congo Red Agar 72h 37°C



C+: positive control; C-: negative control; α: alpha-hemolysis; β: beta-hemolysis

- ✓ **Virulence index** was determined to characterize the pathogenic potential of the bacterial collection [Sing et al., 2017]

Figure 7. Culture mediums for virulence phenotypic expression determination (Original)

Results

- **30 Bisaro pigs – 3 farms**
- **6 Alentejano pigs**
- **4 Iberian Pigs**
- **2 Vietnamese pigs**

73 Gram-negative isolates

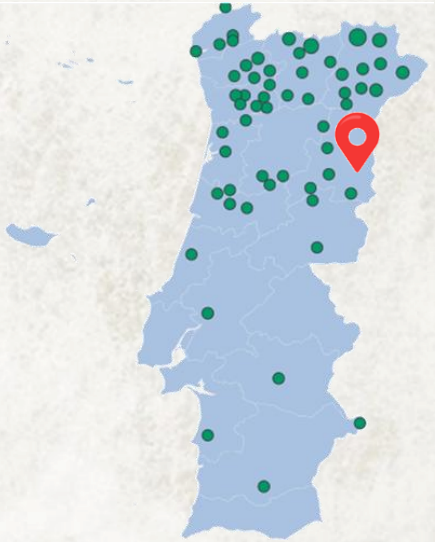


Figure 8. Map identification of the sampling location
(Source: ANCSUB)

Results

73 Gram-negative isolates

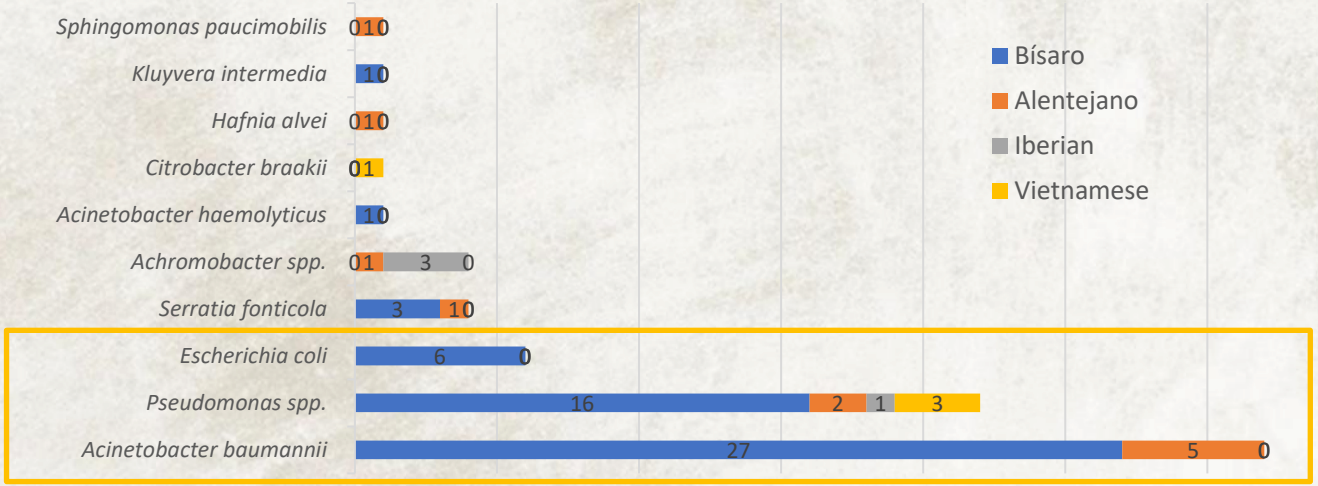
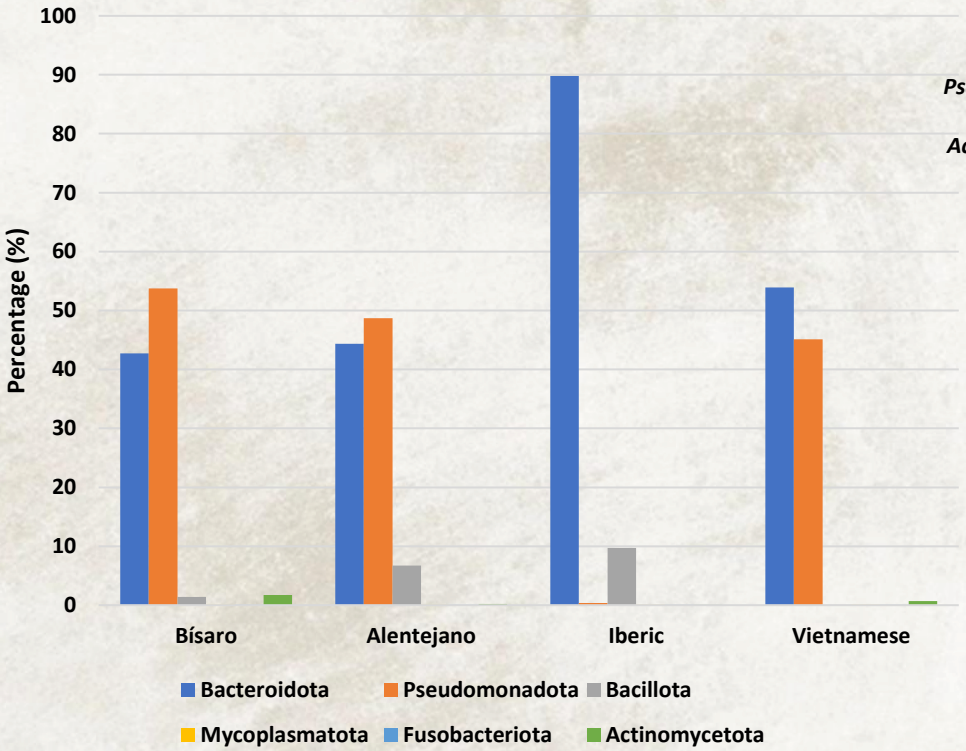


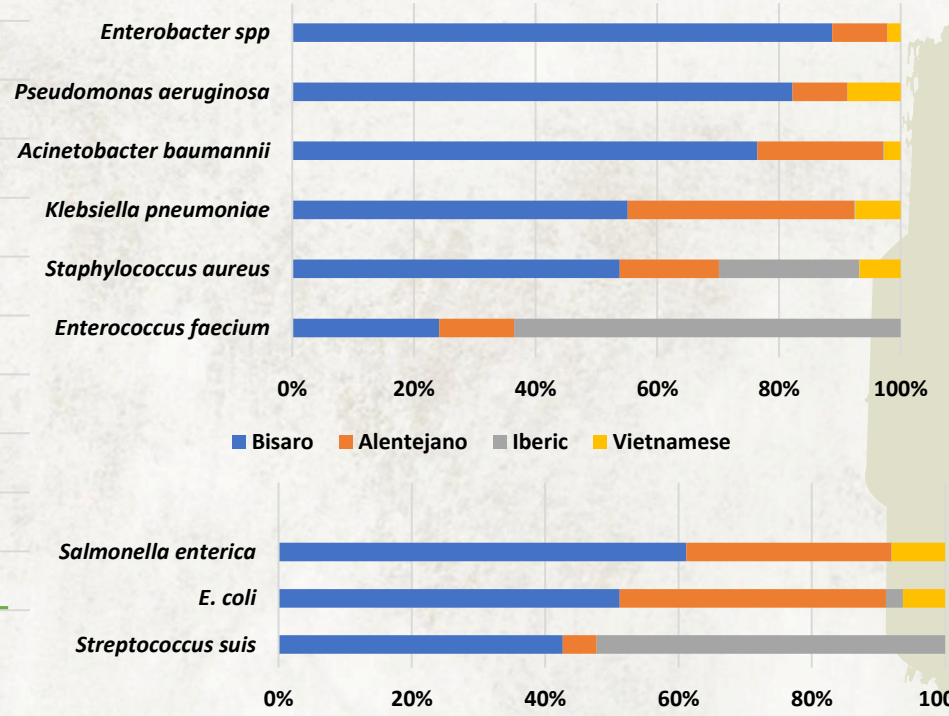
Figure 9. ESBL medium
(Original)

Classical bacteriologic evaluation

Phylum distribution by breed



ESKAPE pathogens distribution



Results

- ✓ A total of **16.4%** (n=12/73) isolates presented a **MDR**
- ✓ **5 *E. coli* ESBL producers**
- ✓ Highest levels of resistance:
  - Cefotaxime (100% in *A. baumannii*)
  - Piperacillin (85% in *A. baumannii,* P+T 51%)
  - Aztreonam (78% in *Pseudomonas* spp.)
  - Ceftiofur (50% in Enterobacterales)
  - Tetracycline, S+T, Cloranfenicol, CTX e CEP (38% enterobacterales)
- ✓ **MAR index** ranged from 0 to 0.9, with 5 isolates (**9.6%)** presenting a value higher than 0.3

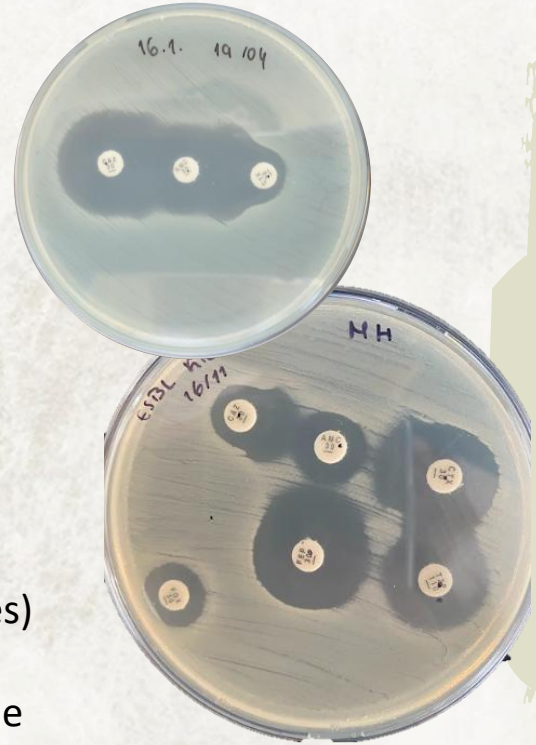


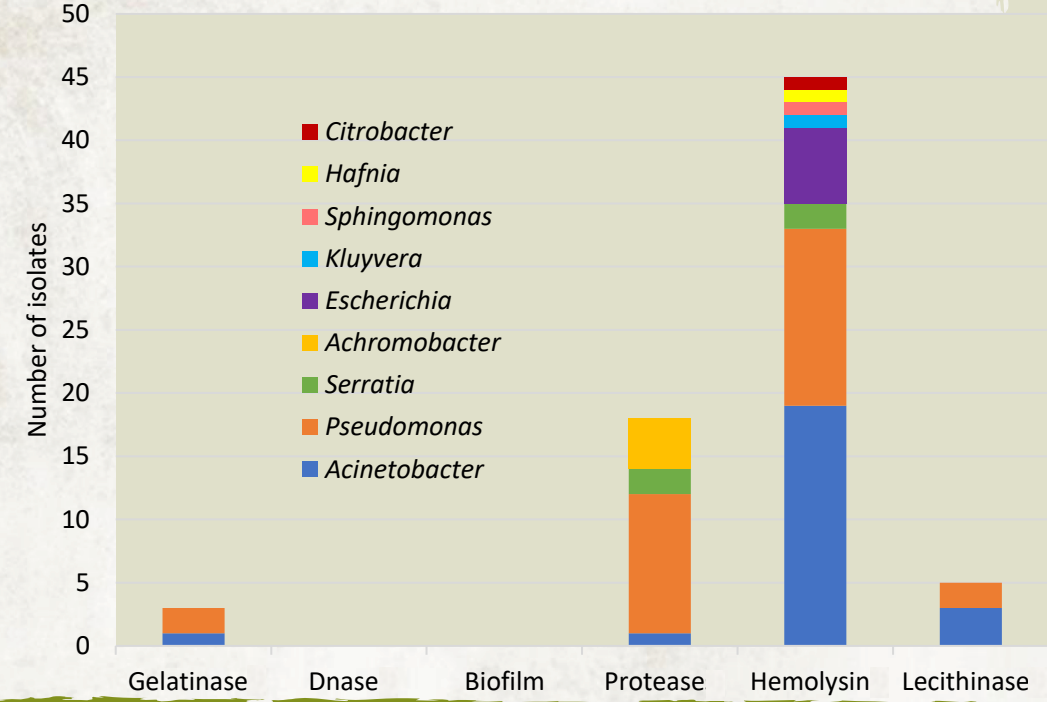
Figure 10. Modified double disk synergy tests (Original)

Results

- ✓ **80.8%** of the isolates (n=59/73) produced virulence factors:
  - Hemolysin (n=45/73; 61.6%)
  - Protease (n=18/73; 24.7%)
  - Lecithinase (n=5/73; 6.9%)
  - Gelatinase (n=3/73; 4.1%)

- ✓ *Pseudomonas* spp. presented high number of virulence factors

- ✓ **VIR index** ranged from 0 to 0.50, with 3 isolates **(4.1%)** presenting a value of 0.5



Virulence results

Conclusions

- ✓ Several bacteria were detected in domestic pigs, ***Acinetobacter baumanni*** was the most prevalent bacterial species found in Bísaro samples;
- ✓ Resistant and virulent bacteria can inhabit the commensal swine nasal microbiota
- ✓ Interspecies contact potential in outdoor production systems – **the case of wild boars**
- ✓ **Surveillance of ESKAPE and ESBL producing bacteria**

Why?

Promoting **biovigilance** and **biosecurity** measures is crucial for safeguarding animal health and **preventing disease spread**.

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Thank you !

Eva Cunha

evacunha@fmv.ulisboa.pt

Domestic pigs as reservoirs of resistant and virulent Gram-Negative bacteria

Manuela Oliveira[1,2,3], Ana Carolina Santos[1,2,] Mariana Cunha[1,2], Roberto Melo[4], Raquel Abreu[1,2], Hélder Pinto[5], Zita Martins Ruano[2,6,7,8], Madalena Vieira-Pinto[2,6,7], Luís Tavares[1,2], **Eva Cunha[1,2]**

[1]CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal
[2]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal
[3]cE3c - Centre for Ecology, Evolution and Environmental Changes & CHANGE - Global Change and Sustainability Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal
[4] Município da Guarda, Guarda, Portugal
[5] Unidade de Saúde Pública de Amadora/Sintra, Unidade Local de Saúde de Amadora/Sintra, Lisboa, Portugal
[6]CECAV – Animal and Veterinary Research Centre, University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal
[7]Departamento de Ciências Veterinárias, Escola de Ciências Agrárias e Veterinárias, Universidade de Trás-os-Montes e Alto (UTAD), Vila Real, Portugal
[8]CISAS - Center for Research and Development in Agrifood Systems and Sustainability, Instituto Politécnico de Viana do Castelo, Viana do Castelo, Portugal

Methodology

- ✓ **Antimicrobials selected:**

A. Enterobacteriaceae - Beta-lactams: Amoxicillin/Clavulanate, Ceftiofur, Ceftazidime, Cefotaxime, Cefepime, Cefoxitin, Piperacillin/tazobactam, Meropenem, Aztreonam; Tetracyclines: Tetracycline; Aminoglycosides: Gentamicin; Fluoroquinolones: Enrofloxacin; Sulfonamides: Sulfamethoxazole/trimethoprim; Phenicols: Chloramphenicol

B. *Pseudomonas* sp. - Beta-lactams:Aztreonam, Piperacillin, Meropenem, Ceftazidime, Cefepime, Piperacillin/tazobactam; Aminoglycosides: Gentamicin; Fluoroquinolones: Enrofloxacin,

C. *Acinetobacter* sp. - Beta-lactams: Ceftazidime, Cefotaxime, Cefepime, Piperacillin, Piperacillin-tazobactam, Meropenem; Tetracyclines: Tetracycline; Aminoglycosides: Gentamicin; Fluoroquinolones: Ciprofloxacin,Sulfonamides: Sulfamethoxazole/trimethoprim;