







Article

Culturomics Profiling of Nasal Cavities of European Wild Rabbits on the Iberian Peninsula: Antimicrobial Resistance and Detection of Microorganisms of Public Health Interest

Carmen González-Azcona ¹, Saúl Jiménez-Ruiz ² , Nuno Santos ³, Inés Del Campo-Fernández ¹, Katherine Rojas-Tigasi ¹, Tamara Álvarez-Gómez ¹, Irene Marañón-Clemente ¹ , Paula Eguizábal ¹, Idris Nasir Abdullahi ^{1,4} , Carla Andrea Alonso ⁵ , Carmen Torres ^{1,*}  and Carmen Lozano ^{1,*} 

- ¹ Area of Biochemistry and Molecular Biology, OneHealth-UR Research Group, University of La Rioja, 26006 Logroño, Spain; carmen.gonzalezaz@unirioja.es (C.G.-A.); ines.del-campo@alum.unirioja.es (I.D.C.-F.); katherine-andrea.rojas@alum.unirioja.es (K.R.-T.); tamara.alvarez@unirioja.es (T.Á.-G.); irene-olga.maranon@unirioja.es (I.M.-C.); paula.eguibalm@unirioja.es (P.E.); eedris888@yahoo.com (I.N.A.)
- ² Departamento de Sanidad Animal, Grupo de Investigación en Sanidad Animal y Zoonosis (GISAZ), UIC Zoonosis y Enfermedades Emergentes ENZOEM, Universidad de Córdoba, 14014 Córdoba, Spain; saul.jimenez.ruiz@gmail.com
- ³ Centro Investigação Biodiversidade e Recursos Genéticos (CIBIO), Laboratório Associado (InBIO), Universidade do Porto, 4485-661 Vairão, Portugal; pygargusv@sapo.pt
- ⁴ Department of Medical Laboratory Science, College of Medical Sciences, Ahmadu Bello University, Zaria 810107, Nigeria
- ⁵ Servicio de Análisis Clínicos, Laboratorio de Microbiología, Hospital San Pedro, 26006 Logroño, Spain; caalonso@riojasalud.es
- * Correspondence: carmen.torres@unirioja.es (C.T.); carmen.lozano@unirioja.es (C.L.); Tel.: +34-941299750 (C.T.); +34-941299752 (C.L.)



Academic Editor: Valentina Virginia Ebani

Received: 19 February 2025

Revised: 8 March 2025

Accepted: 13 March 2025

Published: 26 March 2025

Citation: González-Azcona, C.; Jiménez-Ruiz, S.; Santos, N.; Del Campo-Fernández, I.; Rojas-Tigasi, K.; Álvarez-Gómez, T.; Marañón-Clemente, I.; Eguizábal, P.; Abdullahi, I.N.; Alonso, C.A.; et al. Culturomics Profiling of Nasal Cavities of European Wild Rabbits on the Iberian Peninsula: Antimicrobial Resistance and Detection of Microorganisms of Public Health Interest. *Pathogens* **2025**, *14*, 317. <https://doi.org/10.3390/pathogens14040317>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Background: European wild rabbits (*Oryctolagus cuniculus*) are closely connected to the natural environment and might be a potential source of pathogenic bacteria and/or antimicrobial-resistant bacteria. The objective was to identify the bacterial community (species and genera) that colonizes the nasal cavities of European wild rabbits as well as to study the antimicrobial resistance (AMR) phenotypes of bacteria of public health interest. **Methods:** A total of 147 nasal swabs individually collected from wild rabbits in Spain and Portugal (between 2022 and 2024) were studied. Samples were inoculated in different culture media, and isolates were identified by MALDI-TOF. The AMR phenotypes of staphylococci, mammaliococci, enterococci and Enterobacterales were evaluated by the disk-diffusion method. **Results:** Overall, 557 non-repetitive isolates were obtained (1 isolate per species and AMR phenotype of each animal). A wide diversity of genera (n = 40) and species (n = 90) was found. *Staphylococcus* (21.2%), *Mammaliococcus* (11.7%), *Enterococcus* (23.3%), *Enterobacter* (9.2%), *Citrobacter* (4.5%) and *Escherichia* (5.9%) were the most detected genera. Most animals presented more than one genera (78.9%), and in 15.7% of them, at least five genera were identified. Susceptibility to all antimicrobials tested was found in 37.2%, 38.5% and 51.6% of staphylococci/mammaliococci, enterococci and *Escherichia coli* isolates; moreover, multidrug resistance was detected in 10.4%, 14.6% and 9.6% of these groups of bacteria. Moreover, important species of pathogenic bacteria were found, such as *Yersinia enterocolitica* (0.5%) and *Bordetella bronchiseptica* (0.2%), among others. **Conclusions:** A high bacterial diversity was detected in the nasal cavities of European wild rabbits from the Iberian Peninsula, including pathogenic species and/or resistant strains of public health interest.

Keywords: wild rabbits; nasal colonization; *Staphylococcus*; *Enterococcus*; *Enterobacter*; *Citrobacter*; wildlife; resistance; surveillance

1. Introduction

Wildlife has been recognized to be an important link in One Health ecosystems, as it can be a source of microorganisms that could cause emerging and re-emerging infectious diseases that could spill over to urban settlements and infect humans and domestic animals [1]. In this regard, bacterial colonization of wild animals could be an indicator of environmental contamination or anthropogenic activities in the wild [2,3]. The management of wildlife health is closely linked to human and veterinary public health under a One Health context. In this sense, it is highly important to monitor and counteract the spread of antimicrobial resistance (AMR) in the natural environment [4,5].

Wildlife is not directly exposed to clinical antimicrobial agents but can acquire antimicrobial-resistant bacteria through contact with humans, animals and the contaminated environment [6]. Water polluted with feces appears to be the most significant source of contamination for wild animals [1]. This condition promotes the emergence and persistence of AMR, and wildlife commensal bacterial strains may become reservoirs of AMR genes [3,7]. Moreover, other factors could favor the presence of antibiotic-resistant bacteria in wildlife, such as the presence of resistance genes in mobile genetic elements or the emergence of spontaneous mutations [8]. AMR might be selected not only by the presence of antimicrobial agents but also by other chemical substances such as heavy metals and/or biocides [9]. To provide a broader picture of pathogenic bacteria in wildlife, prevalence studies across different habitats and animal species are necessary.

The European wild rabbit is an herbivore mammal of small size, native to the Iberian Peninsula, where two subspecies diverged around 1.8 million years ago [10]: *Oryctolagus cuniculus algirus* and *Oryctolagus cuniculus cuniculus*. *Oryctolagus c. algirus* is smaller and occurs in the southeastern Iberian Peninsula, North Africa, the Mediterranean and the Atlantic islands [11]. *Oryctolagus c. cuniculus* is larger and occurs in the northeastern Iberian Peninsula, France, and many other areas around the world where they were introduced by humans [11]. *Oryctolagus c. cuniculus* is also an ancestor of all domestic rabbits [12].

The European wild rabbit is an important species for hunting activities and can enter the food chain [13,14]. Moreover, it plays a fundamental role in the ecology of Iberian ecosystems and is also economically relevant, particularly in Europe [15]. The European wild rabbit might be a potential source of bacteria carrying relevant AMR genes for other domestic and wild animals, even for humans, because interactions may occur across different ecosystems [5,6,15]. The role of wild rabbits in infectious disease epidemiology is still uncertain. However, it is known that wild animals can act as reservoirs and could be sources of zoonotic diseases during anthropogenic activities in wildlife [16,17].

It has previously been observed that wild rabbits can host a wide variety of bacteria with AMR phenotypes of relevance in public health [18–20]. However, few studies have addressed the nasal microbiota of these animals [15,21]. In the present study, the main objective was to isolate and identify the bacterial community (species and genera) that colonizes the nasal cavities of European wild rabbits from Spain and Portugal as well as to study the AMR phenotypes of genera of public health interest.

2. Material and Methods

2.1. Sample Collection, Transport and Preservation

Nasal samples of 147 European wild rabbits were obtained in different areas of the Iberian Peninsula from November to December 2022, February 2023, and August 2024 (Figure 1). Sampling was as follows: a) 87 samples of wild rabbits from different regions of Spain (La Rioja, Aragón, País Vasco, Castilla y León and Andalucía) and b) 60 samples of wild rabbits from different regions of Portugal (Santarém and Beja). All samples from Spain were obtained during the game season from legally hunted animals. Portuguese

rabbits were sampled both from hunting ($n = 26$; from the counties Ourique and Almeirim, in Satarém) and captures for a longitudinal capture–mark–recapture study ($n = 8$; at Parque de Natureza de Noudar, Beja) [22]. The study was approved by the ethical committee of the CIBIO's Animal Welfare and Ethics Review Board (ORBEA/2023_01).

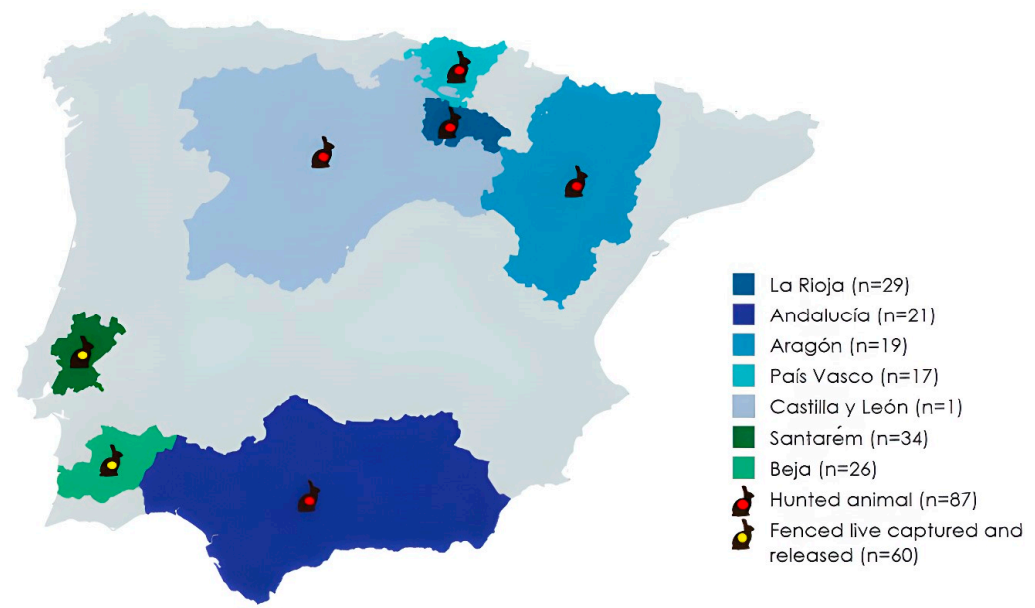


Figure 1. Geographical areas of the Iberian Peninsula in which the European wild rabbit samples were obtained and how animals were captured.

For nasal sampling, sterile cotton-tipped swabs were used and briefly inserted into the nostrils. They were immediately transferred to commercial tubes of Amies transport medium and stored at 4 °C until arrival at the laboratory for processing. In case they could not be processed in one or two days, they were frozen at −80 °C until laboratory analysis.

2.2. Bacterial Isolation and Identification

Nasal swab samples were placed in Brain Heart Infusion broth (BHI, Condalab, Madrid, Spain). Samples were inoculated in duplicate: one in BHI broth with 6.5% sodium chloride (NaCl) and the other one non-supplemented with NaCl. Both media were incubated for 24 h at 37 °C. After incubation, the broth samples were inoculated into five culture media. Broth samples supplemented with NaCl were inoculated on mannitol salt agar (MSA, Condalab, Madrid, Spain) and MRSA chromogenic media for the isolation of staphylococci (methicillin-susceptible and methicillin-resistant, respectively). The unsupplemented broth samples were inoculated on blood agar (BioMerieux, Madrid, Spain), MacConkey agar (OXOID, Madrid, Spain) and Slanetz Bartley agar (Scharlab, Sentmenat, Spain) for the isolation of Gram-positive and Gram-negative bacteria. Plates were incubated for 24 to 48 h at 37 °C for bacterial recovery. After overnight growth, up to 13 different colonies per sample (based on morphology, color and haemolysis) were randomly selected. Colonies were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF; Bruker Daltonics, Bremen, Germany) using the standard extraction protocol recommended by the manufacturer. For calibration of the spectrometer, the protein profile of the DH5 peptide from the *Escherichia coli* strain was used [23].

2.3. Antimicrobial Susceptibility Testing

The antimicrobial susceptibility phenotypes were analyzed in all staphylococci, enterococci, *Escherichia coli*, *Klebsiella* spp. and *Yersinia enterocolitica* isolates obtained. For

this purpose, the disk-diffusion method was used and the following antimicrobial agents were employed (charge of disk in mcg): (a) in staphylococci: penicillin (10), cefoxitin (30), erythromycin (15), clindamycin (2), gentamicin (10), tobramycin (10), tetracycline (30), ciprofloxacin (5), chloramphenicol (30), linezolid (30), trimethoprim–sulfamethoxazole (1.25 + 23.7) and mupirocin (200); (b) in enterococci: penicillin (10), vancomycin (30), teicoplanin (30), erythromycin (15), gentamicin (120), streptomycin (300), tetracycline (30), ciprofloxacin (5), chloramphenicol (30) and linezolid (30); and c) in Gram-negative bacteria: ampicillin (10), cefoxitin (30), amoxicillin/clavulanic acid (30), cefotaxime (30), ceftazidime (30), imipenem (10) gentamicin (10), tobramycin (10), tetracycline (30), ciprofloxacin (5), trimethoprim-sulfamethoxazole (25) and chloramphenicol (30). The staphylococcal disk diffusion results were interpreted according to the European Committee of Antimicrobial Susceptibility Testing and the enterococcal, *E. coli*, *Klebsiella* spp. and *Y. enterocolitica* susceptibility testing data according to the Clinical and Laboratory Standards Institute [24,25].

2.4. Statistical Analysis

A correlation matrix of bacteria species detected in European wild rabbits was formulated. To analyze the correlation between whole diversity, a graphic model was performed in R 4.4.1 (R Core Team, 2024) using the R packages ‘corrplot’ (0.9.5) and readxl (1.4.3).

3. Results

3.1. Isolates and Genera Detected in Nasal Samples

A total of 598 isolates were recovered. After AMR phenotyping, 557 non-repetitive isolates (1 isolate per species and antimicrobial resistance phenotype per animal) were selected for further analysis. The distribution of isolates from the 147 nasal samples from European wild rabbits was as follows: 291 isolates of 60 samples from Portugal and 266 isolates of 87 samples from Spain. A total of 40 genera and 90 species were identified. Overall, 344 isolates were Gram-positive, and there were 213 Gram-negative bacteria (Tables 1 and 2). Among the Gram-positive bacteria, the most common genera were the following ones (number of isolates in brackets): *Enterococcus* (130), *Staphylococcus* (118) and *Mammaliicoccus* (65). For the Gram-negative bacteria, the most common genera were (number of isolates in brackets): *Enterobacter* (48), *Escherichia* (33) and *Citrobacter* (25).

3.2. Diversity of Genera and Species Detected

In all 147 European wild rabbits, at least one bacterial isolate was obtained and identified. In general, a high diversity of genera and species was found. Most of the animals tested presented more than 1 genera (78.9%), and in 15.7% of them, ≥ 5 genera were identified. Seven different genera were obtained in four of the animals tested, and they were the following ones: *Achromobacter*, *Butiauxella*, *Citrobacter*, *Enterobacter*, *Enterococcus*, *Escherichia*, *Hafnia*, *Klebsiella*, *Kocuria*, *Lelliottia*, *Mammaliicoccus*, *Micrococcus*, *Raoultella*, *Stenotrophomonas*, *Staphylococcus* and/or *Streptococcus*. A single genus was recovered in only 31 of the rabbits (21.1%) (Figure 2).

Table 1. Distribution pattern of Gram-positive bacteria from nasal samples of European wild rabbits.

Genera	Species	Number of Isolates *	Number of Animals with: This Species	Number of Animals with: This Genus
<i>Staphylococcus</i>	<i>S. aureus</i>	28	20	65
	<i>S. pseudintermedius</i>	10	10	
	<i>S. xylosus</i>	20	18	
	<i>S. saprophyticus</i>	14	14	
	<i>S. epidermidis</i>	13	12	
	<i>S. hominis</i>	9	6	
	<i>S. succinus</i>	7	6	
	<i>S. equorum</i>	4	4	
	<i>S. borealis</i>	3	3	
	<i>S. capitis</i>	3	3	
	<i>S. haemolyticus</i>	2	2	
	<i>S. gallinarum</i>	1	1	
	<i>S. hyicus</i>	1	1	
	<i>S. pasteurii</i>	1	1	
	<i>S. simulans</i>	1	1	
	<i>S. warneri</i>	1	1	
<i>Mammaliicoccus</i>	<i>M. sciuri</i>	34	30	45
	<i>M. lentus</i>	30	22	
	<i>M. vitulinus</i>	1	1	
<i>Enterococcus</i>	<i>E. faecium</i>	41	36	92
	<i>E. casseliflavus</i>	26	24	
	<i>E. hirae</i>	23	20	
	<i>E. faecalis</i>	19	17	
	<i>E. mundtii</i>	13	13	
	<i>E. gallinarum</i>	4	4	
	<i>E. durans</i>	2	2	
	<i>E. gilvus</i>	1	1	
	<i>E. hermannienseis</i>	1	1	
<i>Aerococcus</i>	<i>A. viridans</i>	9	9	9
<i>Lactococcus</i>	<i>L. lactis</i>	6	6	6
<i>Cellulosimicrobium</i>	<i>C. cellulans</i>	2	2	2
<i>Micrococcus</i>	<i>M. luteus</i>	2	2	2
<i>Paenibacillus</i>	<i>P. glucanolyticus</i>	1	1	2
	<i>P. lautus</i>	1	1	
<i>Streptococcus</i>	<i>S. gallolyticus</i>	1	1	2
	<i>S. parauberis</i>	1	1	
<i>Aeromicrobium</i>	<i>A. massiliense</i>	1	1	1
<i>Carnobacterium</i>	<i>C. maltanomaticum</i>	1	1	1
<i>Corynebacterium</i>	<i>C. afermentans</i>	1	1	1
<i>Jeotgalicoccus</i>	<i>J. halotolerans</i>	1	1	1
<i>Kocuria</i>	<i>K. rhizophila</i>	1	1	1
<i>Oceanobacillus</i>	<i>O. picturae</i>	1	1	1
<i>Pseudoclavibacter</i>	<i>P. helvovus</i>	1	1	1
<i>Vagococcus</i>	<i>V. penai</i>	1	1	1

* Considering non-repetitive isolates: one isolate per species and antimicrobial resistance phenotype per animal.

Table 2. Distribution pattern of Gram-negative bacteria from nasal samples of wild rabbits.

Genera	Species	Number of Isolates *	Number of Animals with: This Species	This Genus
<i>Enterobacter</i>	<i>E. cloacae</i>	22	22	38
	<i>E.r kobei</i>	9	9	
	<i>E. hormaechei</i>	5	5	
	<i>E. ludwigii</i>	5	5	
	<i>E. asburiae</i>	4	4	
	<i>E. bugandensis</i>	2	2	
	<i>E. cancerogenus</i>	1	1	
<i>Citrobacter</i>	<i>C. braakii</i>	11	11	22
	<i>C. gillenii</i>	9	9	
	<i>C. freundii</i>	5	5	
<i>Serratia</i>	<i>S. marcescens</i>	5	5	16
	<i>S. liquefaciens</i>	3	3	
	<i>S. ficaria</i>	2	2	
	<i>S. fonticola</i>	2	2	
	<i>S. rubidaea</i>	2	2	
	<i>S. odorifera</i>	1	1	
	<i>S. plymuthica</i>	1	1	
<i>Escherichia</i>	<i>E. coli</i>	31	25	26
	<i>E. hermannii</i>	2	2	
<i>Klebsiella</i>	<i>K. oxytoca</i>	13	12	14
	<i>K. pneumoniae</i>	2	2	
<i>Hafnia</i>	<i>H. alvei</i>	14	14	14
<i>Pantoea</i>	<i>P. agglomerans</i>	12	12	12
<i>Achromobacter</i>	<i>A. spanius</i>	5	5	6
	<i>A. piechaudii</i>	1	1	
	<i>A. xylosoxidans</i>	1	1	
<i>Lelliottia</i>	<i>L. amnigena</i>	7	7	7
<i>Raoultella</i>	<i>R. ornithinolytica</i>	4	4	7
	<i>R. terrigena</i>	2	2	
	<i>R. planticola</i>	1	1	
<i>Acinetobacter</i>	<i>A. radioresistens</i>	3	3	4
	<i>A. pittii</i>	1	1	
<i>Leclercia</i>	<i>L. adecarboxylata</i>	4	4	4
<i>Ranella</i>	<i>R. aquatilis</i>	3	3	3
<i>Buttiauxella</i>	<i>B. agrestis</i>	2	2	3
	<i>B. gaviniae</i>	1	1	
<i>Yersinia</i>	<i>Y. enterocolitica</i>	3	2	2
<i>Kluyvera</i>	<i>K. cryocrescens</i>	1	1	2
	<i>K. intermedia</i>	1	1	
<i>Kosakonia</i>	<i>K. cowanii</i>	2	2	2
<i>Ochrobactrum</i>	<i>O. grignonense</i>	2	2	2
<i>Pseudoescherichia</i>	<i>P. vulneris</i>	2	2	2
<i>Aeromonas</i>	<i>A. hydrophila</i>	1	1	1
<i>Advenella</i>	<i>A. incanta</i>	1	1	1
<i>Bordetella</i>	<i>B. bronchiseptica</i>	1	1	1
<i>Stenotrophomonas</i>	<i>S. maltophilia</i>	1	1	1

* Considering non-repetitive isolates: one isolate per species and antimicrobial resistance phenotype per animal.

Overall, 90 bacterial species (44 Gram-positive and 46 Gram-negative) were identified. Only 17 (11.6%) animals presented a single bacterial species. The remaining 130 European wild rabbits showed more than one, most of them containing from two to four species (63.3%). From 8 to 12 bacterial species were identified in six individuals (4.1%) (Figure 2). In the sample in which the highest diversity of species was detected (seven genera and 12 species), the following species were found: *Citrobacter freundii*, *Citrobacter gillenii*, *Enterococcus casseliflavus*, *Enterococcus faecium*, *Enterococcus hermanni*ensis, *Enterococcus hirae*, *Enterococcus mundtii*, *Escherichia coli*, *Hafnia alvei*, *Klebsiella oxytoca*, *Mammaliicoccus lentus* and *Streptococcus parauberis*.

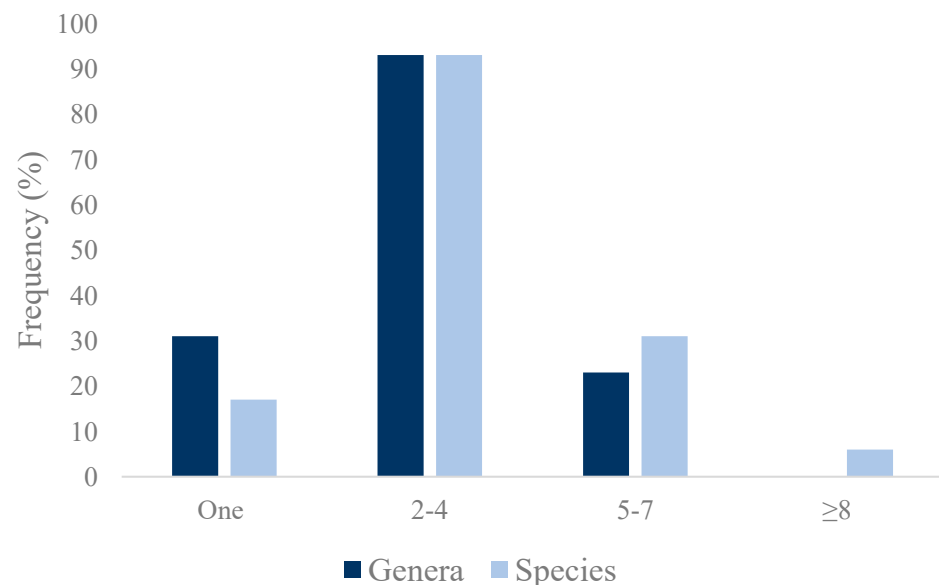


Figure 2. Frequency of bacterial genera and species identified in the 147 nasal samples from European wild rabbits.

3.3. Diversity of Gram-Positive Bacteria

Most of the animals were colonized by enterococcal (62.6% of the animals), staphylococcal (44.2%) and mammaliicoccal (30.6%) isolates, and a wide diversity of bacterial species (9 *Enterococcus* spp., 16 *Staphylococcus* spp. and 3 *Mammaliicoccus* spp.) was identified among these genera (Figure 3, Table 1). Two coagulase-positive staphylococci (CoPS) species were detected: *S. aureus* in 20 animals (13.6%) and *S. pseudintermedius* in 10 animals (6.8%) (Table 1). In addition, no staphylococci were recovered in 82 animals (55.8%) (Figure 3). In most of the nasal samples, only one staphylococcal species was identified (25.9%), and eight animals carried three staphylococcal species: *S. aureus* + *S. pseudintermedius* + *S. hyicus* or *S. xylosus*; *S. aureus* + *S. xylosus* + *S. saprophyticus* or *S. simulans*; *S. epidermidis* + *S. saprophyticus* + *S. gallinarum* or *S. xylosus*; *S. epidermidis* + *S. borealis* + *S. haemolyticus*; and *S. capitis* + *S. equorum* + *S. saprophyticus*. Two animals carried four staphylococcal species: *S. aureus* + *S. pseudintermedius* + *S. xylosus* + *S. hominis* and *S. aureus* + *S. pseudintermedius* + *S. pasteurii* + *S. warneri*.

Regarding *Enterococcus*, at least one enterococcal species was found in 62.6% of the animals. The combination *E. faecium* + *E. casseliflavus* was found in three animals and *E. faecalis* + *E. gallinarum* in two animals. Moreover, three different species were detected in two animals (*E. faecium* + *E. hirae* + *E. mundtii*, and *E. faecalis* + *E. gallinarum* + *E. mundtii*) and five different enterococcal species (*E. faecium* + *E. casseliflavus* + *E. hermanni*ensis + *E. hirae* + *E. mundtii*) were detected in one of the nasal samples tested (Figure 3).

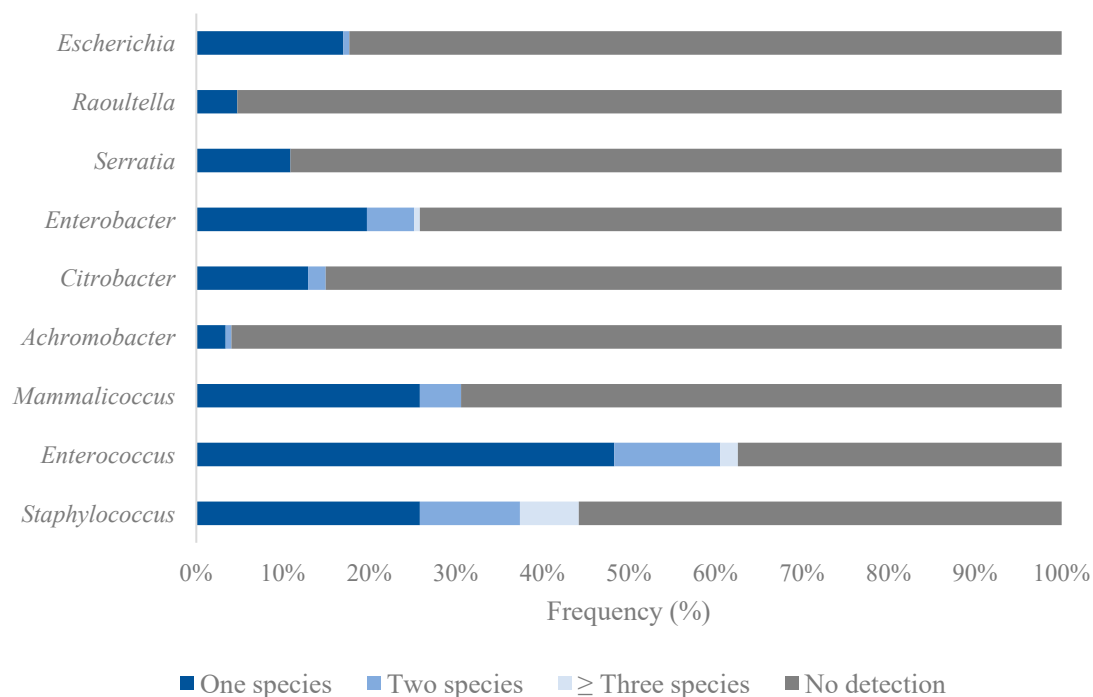


Figure 3. Frequency of bacterial species detected among the 147 European wild rabbits. Genera in which ≥ 3 different species were identified are represented.

3.4. Diversity of Gram-Negative Bacteria

Among the Gram-negative bacteria, 23 different genera were identified (Table 2). Two different *Enterobacter* species were detected in eight animals (*E. cloacae* + *E. kobei* in four animals, *E. cloacae* + *E. asburiae*, *E. bugandensi* or *E. hormaechei* in three animals, *E. asburiae* + *E. ludwigii* in one animal). Three different *Enterobacter* species were detected in one animal: *E. cloacae* + *E. hormaechei* + *E. ludwigii*. Moreover, two *Citrobacter* species (*C. gillenii* + *C. freundii* in two animals and *C. gillenii* + *C. braakii* in one) were recovered in three animals. In addition, two *Achromobacter* species (*A. spanius* + *A. xylosoxidans*) and two *Escherichia* species (*E. coli* + *E. hermannii*) were obtained in one animal each (Figure 3).

3.5. Co-Colonization of Bacterial Species in Nasal Samples of European Wild Rabbits

The correlation between the detection of the different bacterial species is shown in Figure 4. Cohabiting species with the highest positive correlation ($\rho = 1$) were (big green circles) one combination of two Gram-positive species (*Streptococcus parauberis* + *Enterococcus hermanni* and *Staphylococcus pasteuri* + *Staphylococcus warneri*) and different combinations of Gram-positive and Gram-negative species (*Buttiauxella gaviniae* + *Enterococcus gilvus*; *Aeromonas hydrophila* + *Vagococcus penai* and *Stenotrophomonas maltophilia* + *Kocuria rhizophila*). A relevant positive correlation was also found for some staphylococcal species: *S. haemolyticus* + *S. borealis* ($\rho = 0.40$) and *S. pseudintermedius* + *S. aureus*, *S. hyicus*, *S. pasteuri* or *S. warneri* ($\rho \approx 0.30$), Gram-negative species from different genera [*Raoultella terrigena* + *Kocuria rhizophila* or *S. maltophilia* ($\rho = 0.7/\rho = 1$), *Bordetella bronchiseptica* + *Escherichia hermannii* ($\rho = 0.70$), and *Serratia fonticola* + *Ochrobactrum grignonense* or *Pseudoescherichia vulneris* and *Bordetella bronchiseptica* + *Raoultella ornithinolytica* ($\rho = 0.49$)] and Gram-positive and Gram-negative species [*Staphylococcus equorum* + *Rahnella aquatilis* and *Lactococcus lactis* + *Serratia ficaria* ($\rho = 0.57$), and *Enterococcus durans* + *E. hermannii* and *Cellulosimicrobium cellulans* + *Serratia rubidarea* ($\rho = 0.49$)].

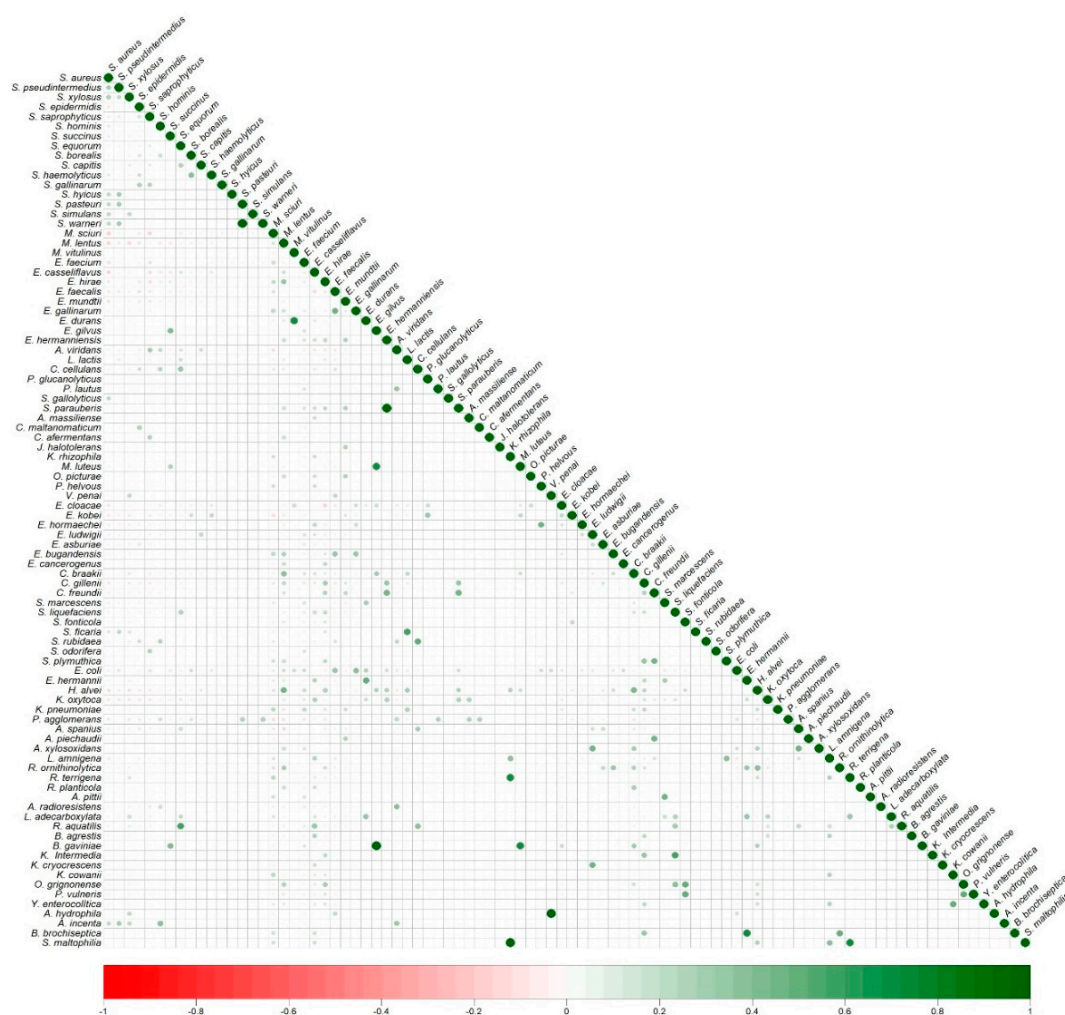


Figure 4. Correlation matrix of bacteria in the nasal cavities of European wild rabbits. The color and size of the circle correspond to the level of correlation: positive correlation (green) and negative correlation (red).

Although the negative correlations showed less intensity, they were detected for the enterococcal species *Enterococcus faecalis* + *Enterococcus casseliflavus* ($\rho = -0.16$), and among mammaliococcal, staphylococcal or enterococcal species [*Mammaliococcus sciuri* + *S. aureus* ($\rho = -0.19$), *S. aureus* + *Enterococcus casseliflavus* ($\rho = -0.18$), *M. sciuri* + *Staphylococcus saprophyticus* and *E. faecalis* + *E. casseliflavus* ($\rho = -0.16$), *E. casseliflavus* + *S. saprophyticus* ($\rho = -0.14$), and even among Gram-negative and Gram-positive species *E. coli* + *S. saprophyticus* ($\rho = -0.14$)].

3.6. Phenotype of Antimicrobial Resistance

The AMR rates detected in staphylococci/mammaliococci, enterococci, *E. coli*, *Klebsiella* spp. and *Y. enterocolitica* isolates are shown in Figure 5. Sixty-eight of the total staphylococci/mammaliococci isolates (37.2%) were susceptible to all antimicrobials tested, and the remaining showed resistance to one or more antimicrobial agents: penicillin (40.5%), cefoxitin (15.3%), erythromycin (17.0%), clindamycin (11.5%), tetracycline (19.7%), gentamicin (3.8%), tobramycin (3.3%), ciprofloxacin (6.6%), chloramphenicol (1.7%), and mupirocin (3.8%). In the case of *M. sciuri*, all isolates had intrinsic resistance to clindamycin (and this resistance phenotype was not considered among AMR rates). Nineteen staphylococci of the total staphylococci/mammaliococci (10.4%) showed a multidrug resistance phenotype (MDR) (resistance to three or more antimicrobial families) and linezolid resistant isolates

were not identified. Twenty-two of the 38 CoPS isolates (57.9%) were methicillin-resistant, all of them *S. aureus* (MRSA), and they were obtained of 18/147 animals (12.2%).

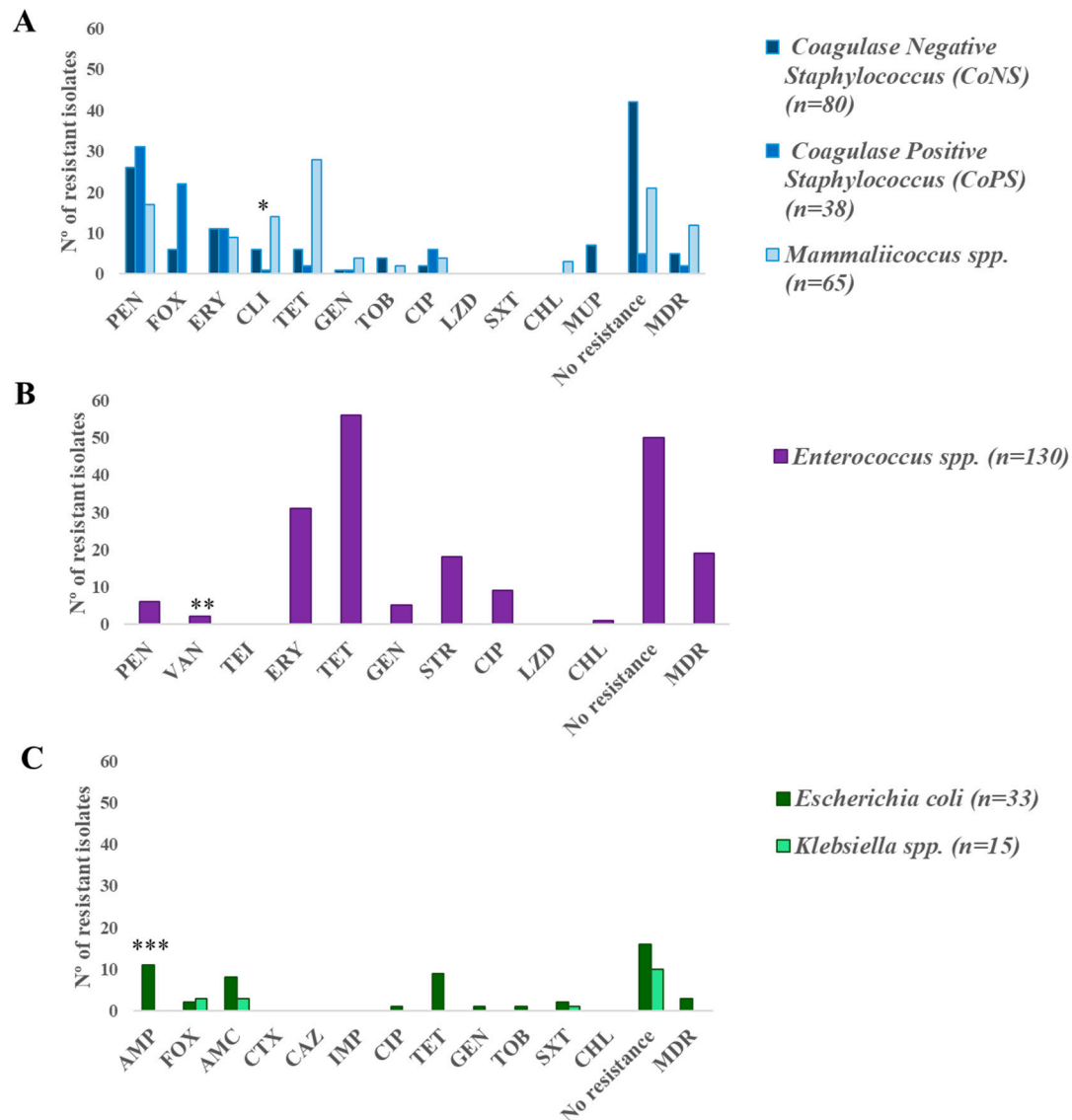


Figure 5. (A) Coagulase-negative *Staphylococcus* (CoNS), coagulase-positive *Staphylococcus* (CoPS) and *Mammaliicoccus* spp. (B) *Enterococcus* spp., (C) *Escherichia coli* and *Klebsiella* spp. PEN: penicillin; FOX: cefoxitine; AMP: ampicillin; AMC: amoxicillin–clavulanic; CTX: cefotaxime; CAZ: ceftazidime; IMP: imipenem; ERI: erythromycin; CLI: clindamycin; TET: tetracycline; CN: gentamicin, STR: streptomycin; TOB: tobramycin; CIP: ciprofloxacin, SXT: trimethoprim-sulphamethoxazole; LZD: linezolid, CHL: chloramphenicol; MUP: mupirocin; VAN: vancomycin; TEI: teicoplanin. * Intrinsic clindamycin resistance of *M. sciuri* was not considered. ** Intrinsic vancomycin resistance of *E. casseliflavus* and *E. gallinarum* was not represented. *** Intrinsic ampicillin resistance of *Klebsiella* spp. was not represented.

Among enterococci isolates, intrinsic vancomycin resistance was detected in 15 isolates (13 *E. casseliflavus* and 2 *Enterococcus gallinarum*). Two *E. faecalis* isolates showed decreased susceptibility to vancomycin, but teicoplanin resistance was not detected. High tetracycline resistance rates (43.1%) were identified, followed by erythromycin (23.9%) and streptomycin (13.9%) resistance. Fifty isolates (38.5%) were susceptible to all antimicrobial agents, nineteen (6.9%) were MDR, and no linezolid resistant enterococci isolates were identified.

Regarding *E. coli*, almost half of the isolates (51.6%) were susceptible to all tested antimicrobial agents. In the remaining *E. coli* isolates, the following resistance rates were detected: ampicillin (35.5%), cefoxitin (6.5%), amoxicillin–clavulanic acid (25.8%), ciprofloxacin, gentamicin, tobramycin (3.3%, each one), tetracycline (29.0%) and trimethoprim–sulphamethoxazole (6.5%). In *Klebsiella* spp., in addition to ampicillin intrinsic resistance, resistance to other beta-lactams such as cefoxitin and amoxicillin–clavulanic were detected in three *K. oxytoca* isolates (23.1%). Moreover, one *K. oxytoca* isolate was trimethoprim–sulphamethoxazole-resistant. None of the *E. coli* or *Klebsiella* isolates produced extended-spectrum β -lactamases (ESBL).

The antimicrobial phenotype of the three *Y. enterocolitica* isolates obtained was also analyzed, with two of them being susceptible to all antimicrobials tested and one isolate being resistant to ampicillin.

4. Discussion

There is very little information about nasal microbial diversity in European wild rabbits [15]. Most studies are focused on pet rabbits and their intestinal microbiota. Moreover, in these studies, the microbiome has been mostly analyzed by genomic sequencing technologies (culture-independent approach). In these cases, *Firmicutes*, *Proteobacteria*, *Euryarchaeota*, *Tenericutes* or *Bacteroidota* phyla are usually the most predominant ones [19,26,27]. Although the majority of studies have been focused on the gut microbiome, important differences have been observed in some of them among mouth or oral parts in comparison with intestinal sites [19].

In this study, a high diversity of Gram-positive and Gram-negative genera was found. In the oral cavity of healthy pet rabbits in France, the most frequently cultivable isolated bacteria were *Streptococcus* spp. (19.8%), *Rothia* spp. (17.9%), *Enterobacter* spp. (7.0%), *Staphylococcus* spp. (6.6%) and *Actinomyces* spp. (5.7%) [28]. Although the genera detected were identified at different frequencies, in comparison with our present study, a predominance of Gram-positive genera was detected.

The predominance of *Staphylococcus* in nasal samples of European wild rabbits was not unexpected. As was corroborated by previous findings by our research group, this genus has been frequently detected in other wild animals such as small mammals, mouflon, wild boar, red deer, or several bird species [17,18,29,30]. Among staphylococci, the most common species identified was *S. aureus* (23.7% of total staphylococci), detected in 13.6% of animals analyzed (12.2% of animals carried MRSA). *Staphylococcus aureus* is an important opportunistic pathogen associated with important infectious diseases in humans and animals [31,32]. This species has also been associated with the acquisition and spread of clinically relevant AMR genes. In other studies, in which nasal and/or fecal samples of wild mammals (including rabbits) were analyzed, a similar prevalence of MRSA was detected [18,33,34]. In the present study, 15.3% of staphylococci and mammaliococci were methicillin-resistant. For these, future studies will be carried out to determine the mechanism of AMR. Interestingly, 10.4% of staphylococci/mammaliococci showed an MDR phenotype, and most of them were coagulase-negative *Staphylococcus* (*S. saprophyticus*, *S. epidermidis*, *S. xylosus*, *S. hominis*) or *Mammaliococcus* species (*M. sciuri*, *M. lentus*). Coagulase-negative *Staphylococcus* and *Mammaliococcus* appear to be important reservoirs of AMR genes that are often located on mobile genetic elements and, therefore, might be transferred to more pathogenic bacterial species [35,36].

Enterococcus spp. were also very prevalent, mainly the species *E. faecium*. Enterococci are among the most widely distributed genera in animal gut microbiota [37]. In one study in which rectal samples of 103 different wild mammals were analyzed, the prevalence of enterococci ranged from 0.8% to 26.4%. Curiously, the highest rate (26.4%) was found in

European wild rabbits [38]. However, there is less information on its prevalence at the nasal/oral level. In nasotracheal samples of storks, the prevalence of enterococci among cultivable microbiota was 20.5% [17]. The detection of high-level contamination in nasal samples could be indicative of nasal contamination from an environment contaminated with fecal material, where the rabbits reside and feed [39]. High tetracycline resistance rates were identified among enterococci isolates, and 19 isolates were MDR. Linezolid- and vancomycin-acquired resistance has been identified previously in other wild animals such as boars, small mammals and different bird species [17,40–42]. In our study, two *E. faecalis* species showed decreased susceptibility to vancomycin, and none were linezolid-resistant. The absence of linezolid and vancomycin in this category of animals could be associated with less antibiotic pressure, as boars are largely and evolutionarily related to pigs (important livestock species with high AMR bacteria).

Few Gram-negative bacteria were obtained from the nasal cavities of European wild rabbits. The combination of a predominantly Gram-positive microbiota, natural defense mechanisms and a less favorable environment for Gram-negative bacteria explains their lower presence in the nasal cavities of rabbits [43,44]. Moreover, the isolates presented fewer AMR and MDR phenotypes. Worryingly, in other works, higher rates of MDR *E. coli* have been detected in wild animals [45–47].

Isolates producing extended-spectrum β -lactamases were not detected among our samples. However, two important pathogenic species, *Y. enterocolitica* and *B. bronchiseptica*, were identified in three animals and one animal, respectively. *Yersinia enterocolitica* is known to cause human enteric infections, but some biotypes have been related to septicemia [48]. This relevant pathogenic species has been detected previously in pets [49], but its presence in wildlife should be monitored. Regarding *B. bronchiseptica*, it has been mainly associated with respiratory diseases in humans and animals, being one of the main causes of snuffles in rabbits [50]. Although *B. bronchiseptica* infections are rare in humans, patients with compromised immunity are at increased risk of opportunistic infections with this species, and infections in healthy adults and children have also been reported [51]. It has been suggested that wild animals are involved in the epidemiology of most zoonoses [52]. According to our results, wild rabbits can be an important reservoir for the transmission of zoonotic agents to domestic animals and humans.

In this culturomics study, the nasal microbial diversity of wild rabbits was analyzed. It must be considered that a culture-dependent methodology was used. Therefore, only a fraction of the microorganisms that can grow in artificial culture media were identified, and a large part of the microbial diversity could not be detected. Future metagenomic studies could help to provide more data on the microbiota of these animals in the regions analyzed.

5. Conclusions

In conclusion, a high bacterial diversity was detected in nasal samples from European wild rabbits on the Iberian Peninsula, including pathogenic and/or resistant species of public health interest. A predominance of Gram-positive genera was detected, and some microbial genera or groups could act as reservoirs of important antimicrobial resistance mechanisms (coagulase-negative *Staphylococcus*, *Mammaliococcus* or *Enterococcus*). Important pathogenic species such as *Y. enterocolitica* and *B. bronchiseptica* were detected, indicating that wild rabbits can be involved in the epidemiology of relevant zoonoses. This finding warrants close monitoring and surveillance due to the related implications for public health.

Author Contributions: Conceptualization: C.G.-A., C.L. and C.T.; methodology: C.G.-A., S.J.-R., N.S., C.L. and C.T.; laboratory experiments: C.G.-A., S.J.-R., N.S., I.D.C.-F., K.R.-T., T.Á.-G., I.M.-C., P.E. and C.A.A.; software analysis: C.G.-A.; validation: C.G.-A., C.T. and C.L.; formal analysis: C.G.-A., C.L. and C.T.; writing—original draft preparation, C.G.-A., I.N.A., C.L. and C.T.; writing—review

and editing: C.G.-A., I.N.A., S.J.-R., N.S., C.L. and C.T.; supervision: C.T. and C.L.; project administration: C.T.; funding acquisition: C.T. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by MICIU/AEI/10.13039/501100011033/ and FEDER, EU (Projects PID2022-139591OB-I00 and PID2023-151954NB-I00), and by Fundação para a Ciência e Tecnologia (FCT) [grant SFRH/BPD/116596/2016 to N.S.]. C.G.-A. and P.E. have a FPI predoctoral fellowship from the University La Rioja (Spain). T.Á.-G. has a contract in the project PID2022-139591OB-I00. S.J.R was supported by the European Union-NextGeneration EU/PRTR (JDC2022-048850-I). This study was co-funded by the project NORTE-01-0246-FEDER-000063, supported by Norte Portugal Regional Operational Programme (NORTE2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

Institutional Review Board Statement: The protocols and methodology used in this study have been reviewed and approved by the ethical committee of the CIBIO's Animal Welfare and Ethics Review Body (ORBEA), number: ORBEA/2023_01, date 16 October 2023.

Informed Consent Statement: Not applicable.

Data Availability Statement: All the data derived from this study are comprehensively presented in this article. However, additional information may be requested from the corresponding author.

Acknowledgments: Authors acknowledge the collaboration of wildlife managers, hunters, technicians and students who assisted with the field or laboratory work. We acknowledge the contribution of the Laboratory Service of the University of La Rioja for its assistance in bacteria identification by Malditof. Also, we are grateful to the landowners of the different sampling points across Spain and Portugal. We acknowledge the contribution of Maria González-Azcona in the preparation of the graphical plot.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Zanardi, G.; Iemmi, T.; Spadini, C.; Taddei, S.; Cavirani, S.; Cabassi, C.S. Wild Micromammals as Bioindicators of Antibiotic Resistance in Ecopathology in Northern Italy. *Animals* **2020**, *10*, 1184. [CrossRef] [PubMed]
2. Larsson, D.G.J.; Flach, C.F. Antibiotic resistance in the environment. *Nat. Rev. Microbiol.* **2022**, *20*, 257–269. [CrossRef] [PubMed]
3. Abdullahi, I.N.; Fernández-Fernández, R.; Juárez-Fernández, G.; Martínez-Álvarez, S.; Eguizábal, P.; Zarazaga, M.; Lozano, C.; Torres, C. Wild Animals Are Reservoirs and Sentinels of *Staphylococcus aureus* and MRSA Clones: A Problem with “One Health” Concern. *Antibiotics* **2021**, *10*, 1556. [CrossRef]
4. OIE. World Organisation for Animal Health. 2018. Available online: <http://www.oie.int/> (accessed on 15 May 2020).
5. Benavides, J.A.; Salgado-Caxito, M.; Torres, C.; Godreuil, S. Public Health Implications of Antimicrobial Resistance in Wildlife at the One Health Interface. *Med. Sci. Forum* **2024**, *25*, 1. [CrossRef]
6. Radhouani, H.; Silva, N.; Poeta, P.; Torres, C.; Correia, S.; Igrejas, G. Potential impact of antimicrobial resistance in wild life, environment, and human health. *Front. Microbiol.* **2014**, *5*, 23. [CrossRef]
7. Mercato, A.; Cortimiglia, C.; Abualsha’ar, A.; Piazza, A.; Marchesini, F.; Milani, G.; Bonardi, S.; Cocconcelli, P.S.; Migliavacca, R. Wild Boars as an Indicator of Environmental Spread of ESβL-Producing *Escherichia coli*. *Front. Microbiol.* **2022**, *13*, 838383. [CrossRef]
8. Rodríguez-Beltrán, J.; DelaFuente, J.; León-Sampedro, R.; MacLean, R.C.; San Millán, Á. Beyond horizontal gene transfer: The role of plasmids in bacterial evolution. *Nat. Rev. Microbiol.* **2021**, *19*, 347–359. [CrossRef]
9. Ekhlas, D.; Sanjuán, J.M.O.; Manzanilla, E.G.; Leonard, F.C.; Argüello, H.; Burgess, C.M. Comparison of antimicrobial resistant *Escherichia coli* isolated from Irish commercial pig farms with and without zinc oxide and antimicrobial usage. *Gut Pathog.* **2023**, *15*, 8. [CrossRef]
10. Carneiro, M.; Ferrand, N.; Nachman, M.W. Recombination and speciation: Loci near centromeres are more differentiated than loci near telomeres between subspecies of the European rabbit (*Oryctolagus cuniculus*). *Genetics* **2009**, *181*, 593–606. [CrossRef]
11. Branco, M.; Ferrand, N.; Monnerot, M. Phylogeography of the European rabbit (*Oryctolagus cuniculus*) in the Iberian Peninsula inferred from RFLP analysis of the cytochrome b gene. *Heredity* **2000**, *85*, 307–317. [CrossRef]
12. Ferrand, N.; Branco, M. *The European Rabbit: The History and Biology of a Successful Colonizer*; Springer: Berlin/Heidelberg, Germany, 2007; pp. 207–235.

13. Delibes-Mateos, M.; Delibes, M.; Ferreras, P.; Villafuerte, R. Key role of the European rabbits in the conservation of the western Mediterranean basin hotspot. *Biol. Conserv.* **2008**, *22*, 301–309. [\[CrossRef\]](#)
14. Sevillano Morales, J.; Moreno-Ortega, A.; Amaro-López, M.A.; Arenas-Casas, A.; Cámara-Martos, F.; Moreno-Rojas, R. Game meat consumption by hunters and their relatives: A probabilistic approach. *Food Addit. Contam. Part A* **2018**, *35*, 1739–1748. [\[CrossRef\]](#)
15. Sousa, M.; Silva, V.; Silva, A.; Silva, N.; Ribeiro, J.; Tejedor-Junco, M.T.; Capita, R.; Chenouf, N.S.; Alonso-Calleja, C.; Rodrigues, T.M.; et al. Staphylococci among Wild European Rabbits from the Azores: A Potential Zoonotic Issue? *J. Food Prot.* **2020**, *83*, 1110–1114. [\[CrossRef\]](#)
16. Martin, C.; Pastoret, P.P.; Brochier, B.; Humblet, M.F.; Saegeman, C. A survey of the transmission of infectious diseases/infections between wild and domestic ungulates in Europe. *Vet. Res.* **2011**, *42*, 70. [\[CrossRef\]](#)
17. Abdullahi, I.N.; Juárez-Fernández, G.; Höfle, Ú.; Cardona-Cabrera, T.; Mínguez, D.; Pineda-Pampliega, J.; Lozano, C.; Zarazaga, M.; Torres, C. Nasotracheal Microbiota of Nestlings of Parent White storks with Different Foraging Habits in Spain. *EcoHealth* **2023**, *20*, 105–121. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Ruiz-Ripa, L.; Alcalá, L.; Simón, C.; Gómez, P.; Mama, O.M.; Rezusta, A.; Zarazaga, M.; Torres, C. Diversity of *Staphylococcus aureus* clones in wild mammals in Aragon, Spain, with detection of MRSA ST130-*mecC* in wild rabbits. *J. Appl. Microbiol.* **2019**, *127*, 284–291. [\[CrossRef\]](#)
19. Silva, V.; Almeida, F.; Carvalho, J.A.; Castro, A.P.; Ferreira, E.; Manageiro, V.; Tejedor-Junco, M.T.; Caniça, M.; Igrejas, G.; Poeta, P. Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* EMRSA-15 clone as the predominant cause of diabetic foot ulcer infections in Portugal. *Eur. J. Clin. Microbiol. Infect. Dis.* **2019**, *39*, 179–186. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Silva, N.; Igrejas, G.; Figueiredo, N.; Gonçalves, A.; Radhouani, H.; Rodrigues, J.; Poeta, P. Molecular characterization of antimicrobial resistance in enterococci and *Escherichia coli* isolates from European wild rabbit (*Oryctolagus cuniculus*). *Sci. Total Environ.* **2010**, *408*, 4871–4876. [\[CrossRef\]](#)
21. Hu, X.; Wang, F.; Yang, S.; Yuan, X.; Yang, T.; Zhou, Y.; Li, Y. Rabbit microbiota across the whole body revealed by 16S rRNA gene amplicon sequencing. *BMC Microbiol.* **2020**, *21*, 312. [\[CrossRef\]](#)
22. Jiménez-Ruiz, S.; Rafael, M.; Coelho, J.; Pacheco, H.; Fernandes, M.; Alves, P.C.; Santos, N. High Mortality of Wild European Rabbits during a Natural Outbreak of Rabbit Haemorrhagic Disease GI. 2 Revealed by a Capture-Mark-Recapture Study. *Transbound. Emerg. Dis.* **2023**, *9*, 3451338. [\[CrossRef\]](#)
23. Abdullahi, I.N.; Lozano, C.; Zarazaga, M.; Saidenberg, A.B.S.; Stegger, M.; Torres, C. Clonal relatedness of coagulase-positive staphylococci among healthy dogs and dog-owners in Spain. Detection of multidrug-resistant-MSSA-CC398 and novel linezolid-resistant-MRSA-CC5. *Front. Microbiol.* **2023**, *14*, 1121564. [\[CrossRef\]](#) [\[PubMed\]](#)
24. EUCAST, The European Committee on Antimicrobial Susceptibility Testing, Breakpoint Tables for Interpretation of MICs and Zone Diameters. 2024. Available online: <http://www.eucast.org/> (accessed on 15 December 2024).
25. CLSI, Clinical Laboratory Standards Institute. *Performance Antimicrobial Susceptibility*, 32nd ed.; CLSI: Wayne, PA, USA, 2024; Available online: <https://clsi.org/> (accessed on 15 December 2024).
26. Cotozzolo, E.; Cremonesi, P.; Curone, G.; Menchetti, L.; Riva, F.; Biscarini, F.; Marongiu, M.L.; Castrica, M.; Castiglioni, B.; Miraglia, D.; et al. Characterization of Bacterial Microbiota Composition along the Gastrointestinal Tract in Rabbits. *Animals* **2020**, *11*, 31. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Velasco-Galilea, M.; Piles, M.; Viñas, M.; Rafel, O.; González-Rodríguez, O.; Guivernau, M.; Sánchez, J.P. Rabbit Microbiota Changes Throughout the Intestinal Tract. *Front. Microbiol.* **2018**, *9*, 2144. [\[CrossRef\]](#)
28. Flenghi, L.; Mazouffre, M.; Le Loc’h, A.; Le Loc’h, G.; Bulliot, C. Normal bacterial flora of the oral cavity in healthy pet rabbits (*Oryctolagus cuniculus*). *Vet. Med. Sci.* **2023**, *9*, 1621–1626. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Gómez, P.; González-Barrio, D.; Benito, D.; García, J.T.; Viñuela, J.; Zarazaga, M.; Ruiz-Fons, F.; Torres, C. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) carrying the *mecC* gene in wild small mammals in Spain. *J. Antimicrob. Chemother.* **2014**, *69*, 2061–2064. [\[CrossRef\]](#)
30. Larsen, J.; Raisen, C.L.; Ba, X.; Sadgrove, N.J.; Padilla-González, G.F.; Simmonds, M.S.J.; Loncaric, I.; Kerschner, H.; Apfalter, P.; Hartl, R.; et al. Emergence of methicillin resistance predates the clinical use of antibiotics. *Nature* **2022**, *602*, 135–141. [\[CrossRef\]](#)
31. Mama, O.M.; Morales, L.; Ruiz-Ripa, L.; Zarazaga, M.; Torres, C. High prevalence of multidrug resistant *S. aureus*-CC398 and frequent detection of enterotoxin genes among non-CC398 *S. aureus* from pig-derived food in Spain. *Int. J. Food Microbiol.* **2020**, *320*, 108510. [\[CrossRef\]](#)
32. Quero, S.; Serras-Pujol, M.; Párraga-Niño, N.; Torres, C.; Navarro, M.; Vilamala, A.; Puigoriol, E.; de Los Ríos, J.D.; Arqué, E.; Serra-Pladevall, J.; et al. Methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* in pork industry workers, Catalonia, Spain. *One Health* **2023**, *16*, 100538. [\[CrossRef\]](#)
33. Haenni, M.; Murri, S.; Lefrère, C.; Larsen, J.; Drapeau, A.; Botman, J.; François, P.; Gourlay, P.; Meurens, F.; Madec, J.Y. Methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in French hedgehogs admitted to a wildlife health center. *One Health* **2024**, *19*, 100938. [\[CrossRef\]](#)

34. Moreno-Grúa, E.; Pérez-Fuentes, S.; Muñoz-Silvestres, A.; Viana, D.; Fernández-Ros, A.B.; Sanz-Tejero, C.; Corpa, J.M.; Selva, L. Characterization of Livestock-Associated methicillin-resistant *Staphylococcus aureus* isolates obtained from commercial rabbitries located in the Iberian Peninsula. *Front. Microbiol.* **2018**, *9*, 1812. [CrossRef]
35. Ruiz-Ripa, L.; Gómez, P.; Alonso, C.A.; Camacho, M.C.; Ramiro, Y.; de la Puente, J.; Fernández-Fernández, R.; Quevedo, M.Á.; Blanco, J.M.; Báguena, G.; et al. Frequency and characterization of antimicrobial resistance and virulence genes of coagulase-negative staphylococci from wild birds in Spain. Detection of *tst*-carrying *S. sciuri* isolates. *Microorganisms* **2020**, *8*, 1317. [CrossRef]
36. Otto, M. Coagulase-negative staphylococci as reservoirs of genes facilitating MRSA infection: Staphylococcal commensal species such as *Staphylococcus epidermidis* are being recognized as important sources of genes promoting MRSA colonization and virulence. *Bioessays* **2013**, *35*, 4–11. [CrossRef]
37. Schwartzman, J.A.; Lebreton, F.; Salamzade, R.; Shea, T.; Martin, M.J.; Schaufli, K.; Urhan, A.; Abeel, T.; Camargo, I.L.B.C.; Sgardoli, B.F.; et al. Global diversity of enterococci and description of 18 previously unknown species. *Proc. Natl. Acad. Sci. USA* **2024**, *121*, e2310852121. [CrossRef] [PubMed]
38. García, L.A.; Torres, C.; López, A.R.; Rodríguez, C.O.; Valencia, C.S. Antimicrobial Resistance of *Enterococcus* Species Isolated from Wild Mammals in Aragón, Spain. *J. Vet. Res.* **2022**, *66*, 151–159. [CrossRef] [PubMed]
39. Grünberg, W. *Salmonellosis in Animals*; Merck Sharp & Dohme Corp: Rahway, NJ, USA, 2024; Available online: <https://www.msddvetmanual.com/digestive-system/salmonellosis/salmonellosis-in-animals> (accessed on 9 February 2025).
40. Mallon, D.J.; Corkill, J.E.; Hazel, S.M.; Wilson, J.S.; French, N.P.; Bennett, M.; Hart, C.A. Excretion of vancomycin-resistant enterococci by wild mammals. *Emerg. Infect. Dis.* **2002**, *8*, 636–638. [CrossRef]
41. Lozano, C.; Gonzalez-Barrio, D.; Camacho, M.C.; Lima-Barbero, J.F.; de la Puente, J.; Höfle, U.; Torres, C. Characterization of fecal vancomycin-resistant enterococci with acquired and intrinsic resistance mechanisms in wild animals, Spain. *Microb. Ecol.* **2016**, *72*, 813–820. [CrossRef] [PubMed]
42. Smoglica, C.; Vergara, A.; Angelucci, S.; Festino, A.R.; Antonucci, A.; Marsilio, F.; Di Francesco, C.E. Evidence of Linezolid Resistance and Virulence Factors in *Enterococcus* spp. Isolates from Wild and Domestic Ruminants, Italy. *Antibiotics* **2022**, *11*, 223. [CrossRef]
43. McClure, D. *Disorders and Diseases of Rabbits*; Merck Sharp & Dohme Corp: Rahway, NJ, USA, 2020; Available online: <https://www.msddvetmanual.com/all-other-pets/rabbits/disorders-and-diseases-of-rabbits> (accessed on 9 February 2025).
44. Mayer, J. *Bacterial and Fungal Diseases of Rabbits*; Merck Sharp & Dohme Corp: Rahway, NJ, USA, 2021. Available online: <https://www.msddvetmanual.com/exotic-and-laboratory-animals/rabbits/bacterial-and-mycotic-diseases-of-rabbits> (accessed on 9 February 2025).
45. Sabença, C.; Romero-Rivera, M.; Barbero-Herranz, R.; Sargo, R.; Sousa, L.; Silva, F.; Lopes, F.; Abrantes, A.C.; Vieira-Pinto, M.; Torres, C.; et al. Molecular Characterization of Multidrug-Resistant *Escherichia coli* from Fecal Samples of Wild Animals. *Vet. Sci.* **2024**, *11*, 469. [CrossRef]
46. Martínez-Álvarez, S.; Châtre, P.; Cardona-Cabrera, T.; François, P.; Sánchez-Cano, A.; Höfle, U.; Zarazaga, M.; Madec, J.Y.; Haeni, M.; Torres, C. Detection and genetic characterization of blaESBL-carrying plasmids of cloacal *Escherichia coli* isolates from white stork nestlings (*Ciconia ciconia*) in Spain. *J. Glob. Antimicrob. Resist.* **2023**, *34*, 186–194. [CrossRef]
47. Chenouf, N.S.; Carvelho, I.; Messaï, C.R.; Ruiz-Ripa, L.; Mama, O.M.; Titouche, Y.; Zitouni, A.; Hakem, A.; Torres, C. Extended Spectrum β -Lactamase Producing *Escherichia coli* and *Klebsiella pneumoniae* from Broiler Liver in the Center of Algeria, with Detection of CTX-M-55 and B2/ST131-CTX-M-15 in *Escherichia coli*. *Microb. Drug Resist.* **2021**, *27*, 268–276. [CrossRef]
48. Ikeuchi, S.; Bui, H.T.; Sassa-O'Brien, Y.; Niwa, T.; Okumura, M.; Hara-Kudo, Y.; Taniguchi, T.; Hayashidani, H. Development of detection methods by multiplex real-time PCR for highly pathogenic *Yersinia enterocolitica*, low pathogenic *Yersinia enterocolitica*, and *Yersinia pseudotuberculosis* based on SYBR Green and TaqMan probes. *J. Microbiol. Methods* **2023**, *211*, 106779. [CrossRef] [PubMed]
49. Kameyama, M.; Yabata, J.; Obane, N.; Otsuka, H.; Nomura, Y. Detection of pathogenic *Yersinia enterocolitica* in pet Djungarian hamsters in Japan. *J. Vet. Med. Sci.* **2016**, *78*, 1639–1641. [CrossRef] [PubMed]
50. Wang, J.; Sun, S.; Chen, Y.; Chen, D.; Sang, L.; Xie, X. Characterisation of *Bordetella bronchiseptica* isolated from rabbits in Fujian, China. *Epidemiol. Infect.* **2020**, *148*, e237. [CrossRef] [PubMed]
51. Ahuja, U.; Liu, M.; Tomida, S.; Park, J.; Souda, P.; Whitelegge, J.; Li, H.; Harvill, E.T.; Parkhill, J.; Miller, J.F. Phenotypic and genomic analysis of hypervirulent human-associated *Bordetella bronchiseptica*. *BMC Microbiol.* **2012**, *12*, 167. [CrossRef]
52. González-Barrio, D. Zoonoses and Wildlife: One Health Approach. *Animals* **2022**, *12*, 480. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.