Tetracycline Resistance Profile in Darwin Finches in the Galapagos Islands

María Inés Baquero, Marylin Cruz, Viviana Duque, Alberto Velez, Vanessa Lopez, Christian Vinueza and Gabriela Giacoboni

Department of Bacteriology, Universidad Central del Ecuador, Ecuador
Agencia de Regulación Para la Bioseguridad y Cuarentena Para Galapagos (ABG), Ecuador
Department of Surveillance and Quality, Agencia de Regulación Para la Bioseguridad y Cuarentena Para Galapagos (ABG), Ecuador
Department of Quality Control, Agencia de Regulación Para la Bioseguridad y Cuarentena Para Galapagos (ABG), Ecuador
Department of Bacteriology, Facultad de Medicina Veterinaria y Zootecnia, Universidad Central del Ecuador, Ecuador
Foodborne Disease and Antimicrobial Resistance Unit (UNIETAR), Facultad de Medicina Veterinaria y Zootecnia, Universidad Central del Ecuador, Ecuador
Department of Microbiology, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina

Abstract: Antimicrobial Resistance (AMR), which is the ability of microorganisms to withstand attack by antimicrobial drugs, has a worldwide impact. Thus, it is important to identify resistance mechanisms circulating in the environment. Among antimicrobials widely used in human and veterinary medicinal practices against Gram-positive and Gram-negative bacteria (Marosevic et al., 2017), has been categorized as highly critical (WHO, 2018). Since this microbial is considered to be a protected zone. Phenotypic antibiotic resistance was analyzed by the Kirby-Bauer disk diffusion method in Escherichia coli (n = 136) and Enterococcus spp. (n = 332) isolates. PCR was performed for the detection of the tetA gene in E. coli strains and of the tetM gene in Enterococcus isolates. Resistance for at least one of nine and one of eight antimicrobials was observed in 62.5% (85/136) and 28.6% (95/332) of E. coli and Enterococcus isolates, respectively. A high percentage of phenotypic resistance to tetracycline was identified for both bacteria. The tetA and tetM genes were identified in 83.7% (36/43) of E. coli strains and 75.4% (49/65) of Enterococcus spp. isolates, respectively. The highest percentages of AMR were observed in the agricultural zone. We also found the presence of multi-drug-resistant strains. These results show that Darwin finches might be proposed as sentinels of AMR in the Galapagos Islands.

Keywords: Antimicrobial Resistance, Tetracycline, Darwin Finches, Galapagos, Multi-Resistance

Introduction

Antimicrobial Resistance (AMR), which is the ability of microorganisms to withstand attack by antimicrobial drugs, is a global health problem related to several factors, including bacterial genetics and the use of antibiotics in human, veterinary and agricultural usage of antibiotics (Marston et al., 2016). Among important antimicrobials, tetracycline, which is a broad-spectrum antimicrobial that acts against Gram-positive and Gram-negative bacteria (Marosevic et al., 2017), has been categorized as highly critical (WHO, 2018). Since this microbial is considered to be ideal due to its safety (Grossman, 2016), it is widely used in human and veterinary medicinal practices (Thaker et al., 2010). However, some bacteria can develop resistance against tetracycline. The main mechanisms of tetracycline resistance are the activation of efflux pumps and ribosomal protection (Rossolini et al., 2017). Efflux...
pumps are codified by 23 different genes, among which tetA is the most common one, whereas ribosomal protection involves 11 genes, among which tetM is frequently detected (Sigirci et al., 2019).

Studies in wildlife have allowed the understanding of the role of mobile genetic elements in AMR dissemination (Alonso et al., 2021) and identifying those related to AMR. In particular, wild birds have been proposed as sentinels of AMR dissemination in diverse environments (Bonnedahl and Järhult, 2014). In this context, isolated environments such as the Galapagos Islands, which are located approximately 1000 Km from Ecuador’s continental coast (UNESCO, 2022), can be useful to evaluate the anthropogenic impact on the spread of AMR (Nieto-Claudín et al., 2021). Among the passerine endemic birds that inhabit the Galapagos Islands, Darwin finches have been widely studied as a model of adaptive radiation (Hau and Wikelski, 2001), which is a phenomenon related to the fact that these birds adjust the morphology of their beaks in association with their nutritional habits (Michel et al., 2018). Eighteen species of finches, most of which belong to the group of ground or tree finches (Geospiza spp.), have been described on the islands (Hau and Wikelski, 2001). Ground finches feed especially on seeds but can be opportunistic in terms of their diet (Knutie et al., 2019).

To identify AMR patterns circulating in the Galapagos Islands, this study aimed to analyze sentinel AMR strains of Escherichia coli and Enterococcus spp. isolated from Darwin finches in three zones of Santa Cruz Island with different anthropogenic impacts: An urban, an agricultural, and a protected zone.

### Materials and Methods

#### Sample Collection

A total of 384 Darwin ground finches (Geospiza spp.), were caught using mist nets from the urban (n = 128), agricultural (n = 128), and protected zones (n = 128), between March and August 2019. Cloacal swabs were collected from each bird. All cloacal swab samples were placed in 700 µL of brain heart infusion broth (BHI broth BBL™) at 37°C for 24 h in the laboratory of the Agency for the Regulation and Control of Biosafety and Quarantine for the Galapagos (ABG) at Santa Cruz Island. Samples were preserved in BHI with 20% of glycerol and transported to the laboratory of the School of Veterinary Medicine of the Universidad Central del Ecuador in Quito city, Ecuador, for further analysis.

#### Isolation and Identification of Enterococcus spp. and Escherichia Coli

For isolation of Enterococcus spp., samples were incubated in 10 mL BHI medium at 37°C for 24 h and one loopful was streaked in BBL CHRO Magar Orientation agar (Becton Dickinson, Heidelberg, Germany). For E. coli, one loopful was streaked in Levine eosine methylene blue agar (Becton Dickinson, Heidelberg, Germany). At least one colony phenotypically compatible with Enterococcus spp. was picked from the medium and isolated for further biochemical identification with bile esculin (Becton Dickinson, USA), pyrrolidinyl arylamidase (PYR-A-ENT, Britannia, CABA, Argentina), leucine aminopeptidase (Britannia, CABA Argentina) and sodium chloride 6.5% assays (Lopardo, 2016). At least one colony compatible with E. coli in Levine agar was identified using a biochemical battery (Triple Sugar, Iron Agar (BBL), Sulfide Indole Motility medium (BBL), Simmons Citrate Agar (BBL), Lysine Iron Agar (BBL) and urease broth (BBL)). Identification of Enterococcus and E. coli isolates was further confirmed by MALDI TOF MS analysis.

#### Antibiotic Resistance Analysis

Phenotypic antibiotic resistance was analyzed by the Kirby-Bauer disk diffusion method (Hudzicki, 2009). For Enterococcus spp., eight classes of antibiotics were included: Ampicillin (10 µg), gentamicin (120 µg), chloramphenicol (30 µg), tetracycline (30 µg), vancomycin (30 µg), streptomycin (300 µg), teicoplanin (30 µg) and ciprofloxacin (5 µg), whereas for E. coli the antibiotics tested were: Ampicillin (10 µg), ciprofloxacin (5 µg), ceftoxitine (30 µg), cefotaxime (30 µg), cefepime (30 µg), gentamicin (10 µg), chloramphenicol (30 µg), tetracycline (30 µg) and trimethoprim/sulfamethoxazole (1.25/23.75 µg).

The results were interpreted following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2019). Tetracycline phenotypic resistant isolates were further considered for tet gene analyses.

#### DNA Extraction and PCR for the Detection of the tetA and tetM Genes

DNA from the Enterococcus spp. and E. coli isolates was extracted by the boiling method (Millar et al., 2000). Both tetA and tetM PCR reactions included nuclease-free water and a final concentration of Buffer 1X, 0.2 mm dNTPs, 1.5 mm MgCl₂ and 0.025 U/µL Taq polymerase (GoTaq®DNA Polymerase, Promega). All primers were used at a concentration of 0.1 pM/µL.

Specific primers for tetA were tetA-F 5’-TTTCGGCGAGGATCGTTTACTG-3’ and tetA-R 5’-ATCCACCTGCCTGGACAACAT TGC -3’ (product size of 283 bp), whereas for the identification of the tetM gene, the primer sequences were TETM3 5’-GAACTCGAACAAGAGGAAAGC -3’ and 5’-ATGGGAAGCCCAAGAAGAT-3’ (amplicon length of 740 bp) (Cochetti et al., 2005). PCR conditions were the ones described elsewhere (Cochetti et al., 2005; Pantozzi, 2018). Amplicons were visualized by electrophoresis in 1.5% agarose gel and stained with SYBR safe (Invitrogen™).
Statistical Analysis

The association between AMR and the zone of sample collection was analyzed by the Chi-square test for differences among proportions, considering a p-value (<0.05) with a 95% confidence interval.

Results

Isolation and Identification of Enterococcus spp. and Escherichia Coli

A total of 136 E. coli and 332 Enterococcus spp. isolates were recovered from Darwin finches. Isolate distribution was associated with the sampling zone (p<0.0001). E. coli was isolated in a higher percentage in the agricultural zone at 47% (64/136), followed by the protected zone at 34% (46/136) and the urban zone at 19% (26/136). On the other hand, Enterococcus spp. was isolated more often in the urban zone with 41% (135/332), followed by the agricultural zone with 34% (114/332) and the protected zone with 25% (83/332).

AMR Profiles in E. Coli and Enterococcus spp. Isolates

AMR in E. coli was tested for nine antimicrobials, whereas that in Enterococcus spp. isolates were tested for eight antimicrobials (WHO/AGISAR, 2017). Resistance to at least one antimicrobial was observed in 62.5% (85/136) of E. coli strains. Resistance to ampicillin was identified in 50% of the isolates, to tetracycline in 31.6%, trimethoprim/sulfamethoxazole in 18.4%, chloramphenicol in 14.7%, to cefoxitin in 11.1%, to ciprofloxacin in 6.6%, to cefotaxime in 0.7% and cefepime in 0.7%. No resistance was identified for gentamicin. A total of 51 (37.5%) E. coli strains were susceptible to all the antimicrobials tested. Results showed no significant association between ampicillin resistance and the sampling zone. Differently, resistance to tetracycline, which was the second antimicrobial with a higher percentage, was significantly observed in the agricultural zone of the island (p<0.0001). E. coli isolates showed 23 resistance patterns, presenting resistance from one to eight antimicrobials (Table 1). Resistance pattern 21 (30.6%) was the most frequent one, followed by patterns 22 (10.6%), 15 (9.4%), and 7 (7.1%). Also, 26 E. coli isolates showed a Multi-Resistant (MDR) pattern.

For Enterococcus spp., resistance to at least one antimicrobial was recorded in 28.6% (n=95) of the isolates. High resistance rates to tetracycline (19.6%) were found, followed by streptomycin (6.9%) and ciprofloxacin (5.7%). Low AMR resistance rates were recorded for vancomycin (2.4%), ampicillin, chloramphenicol (1.8%), gentamicin, and teicoplanin (1.2%). Tetracycline resistance was observed in a higher proportion in the agricultural zone (p<0.0001). Enterococcus spp. isolates showed 14 resistance patterns, presenting resistance from one to four antimicrobials (Table 2). Resistance pattern 12 was the most frequent one (43.2%), followed by patterns 14 (9.5%), 8 (10.5%), and 13 (9.5%). Moreover, seven Enterococcus spp. isolates presented MDR phenotypes.

Table 1: Antimicrobial resistance patterns in E. coli

| Pattern | Resistance Pattern | No of Antimicrobials | No of E. coli strains |
|---------|--------------------|----------------------|-----------------------|
| 1       | A+F+C+FE+T+SXT+CI+CL | 8                    | 1                     |
| 2       | A+F+T+SXT+CL        | 5                    | 1                     |
| 3       | A+F+T+SXT+CI        | 5                    | 1                     |
| 4       | A+T+SXT+CI+CL       | 5                    | 1                     |
| 5       | A+F+T+CL            | 4                    | 2                     |
| 6       | A+T+SXT+CIP         | 4                    | 3                     |
| 7       | A+T+SXT+CL          | 4                    | 6                     |
| 8       | A+T+CIP+CL          | 4                    | 1                     |
| 9       | T+SXT+CI+CL         | 4                    | 1                     |
| 10      | A+F+T               | 3                    | 2                     |
| 11      | A+T+SXT             | 3                    | 2                     |
| 12      | A+T+CL              | 3                    | 1                     |
| 13      | A+SXT+CI            | 3                    | 1                     |
| 14      | T+SXT+CL            | 3                    | 3                     |
| 15      | A+F                 | 2                    | 8                     |
| 16      | A+T                 | 2                    | 7                     |
| 17      | A+SXT               | 2                    | 3                     |
| 18      | A+CI                | 2                    | 1                     |
| 19      | A+CL                | 2                    | 1                     |
| 20      | T+SXT               | 2                    | 2                     |
| 21      | A                  | 1                    | 26                    |
| 22      | T                  | 1                    | 9                     |
| 23      | CI                 | 1                    | 2                     |

A: Ampicillin; F: Cefoxitin; C: Cefotaxime; Fe: Cefepime; T: Tetracycline; SXT: Trimethoprim/sulfamethoxazole; CI: Ciprofloxacin; CL: Chloramphenicol
Due to its good safety and tolerability, this antimicrobial has been used widely, a fact reflected in the resistance pattern observed in clinical and veterinary medicine (Grossman, 2016). In addition, the presence and dissemination of AMR genes in the environment have been associated with the use of manure as fertilizer in agriculture (Lima et al., 2020). Since tetracycline is excreted as an active compound, the presence of tet genes increases after manure is used as a fertilizer (Guo et al., 2018; Xiong et al., 2018).

In the present study, we found tetracycline resistance in 31.6% of E. coli strains. High tetracycline resistance profiles in E. coli isolated from wild birds have also been reported by Guenther et al. (2010) in Germany, where 46.6% (n = 7/15) of the strains were resistant to this antimicrobial. Also, Giacopello et al. (2016); Nowacek et al. (2021) have reported 48.2% (40/83) and 50% (n = 16/32) of tetracycline resistance respectively, in diverse wild birds from Europe. Similarly, Wheeler et al. (2012) reported resistance to tetracycline as the most frequent one in reptiles in the Galapagos Islands. In the present study, resistance to tetracycline was higher in the agricultural zone of the island, which may be related to its use in farming practices in the zone. It has to be considered that tetracycline is an antibiotic commonly used in livestock and poultry production (Michalova and Schlegelova, 2004).

Discussion

AMR is a multifactorial problem affecting both human and animal health (White and Hughes, 2019). In this context, environmental studies of AMR are important to understand AMR mechanisms circulating in diverse ecological niches. Wild species are good sentinels of AMR in the environment (Ramey and Ahlstrom, 2020) and, particularly, wild birds are important reservoirs and spreaders of AMR genes (Santos et al., 2013; Bonnedahl and Järhult, 2014; Foti et al., 2017).

In the present study, we identified tetracycline as an antimicrobial with a very high percentage of resistance in both E. coli and Enterococcus spp. isolates. Previous studies have shown that AMR could be associated with the use of antimicrobials in agricultural practices (Blanco et al., 2007). The use of antibiotics is known to exert selection pressure, favoring AMR in these microbial communities (Wall et al., 2016). Tetracycline is a broad-spectrum antibiotic used in both humans and animals (Thaker et al., 2010). Due to its good safety and tolerability, this antimicrobial has been used widely, a fact reflected in the resistance pattern observed in clinical and veterinary medicine (Grossman, 2016).

Fig. 1: Identification of the tetA gene in E. coli and the tetM gene in Enterococcus spp. in isolates with phenotypic tetracycline resistance, collected from Santa Cruz Island.

Detection of Tetracycline Resistance Genes

The tetA gene was identified in 83.7% (n = 36) of the E. coli isolates phenotypically resistant to tetracycline. The proportion of tetA gene-positive isolates was higher in the agricultural zone (p<0.0001) than in the urban and protected zones (Fig. 1).

The tetM gene was detected in 75.4% (n = 49) of the Enterococcus spp. strains phenotypically resistant to tetracycline. The proportion of tetM gene-positive isolates was higher in the agricultural zone (p<0.05) than in the urban and protected zones (Fig. 1).

Table 2: Antimicrobial resistance patterns in Enterococcus spp.

| Pattern | Resistance pattern | N° of Antimicrobials | N° of Enterococcus spp. strains |
|---------|--------------------|----------------------|---------------------------------|
| 1       | A+T+VA+TC          | 4                    | 3                              |
| 2       | T+S+CI             | 3                    | 2                              |
| 3       | CL+T+CI            | 3                    | 1                              |
| 4       | CL+T+S             | 3                    | 1                              |
| 5       | CL+T               | 2                    | 4                              |
| 6       | S+CI               | 2                    | 1                              |
| 7       | T+CI               | 2                    | 3                              |
| 8       | T+S                | 2                    | 10                             |
| 9       | VA+TC              | 2                    | 1                              |
| 10      | A                  | 1                    | 3                              |
| 11      | CN                 | 1                    | 4                              |
| 12      | T                  | 1                    | 41                             |
| 13      | S                  | 1                    | 9                              |
| 14      | CI                 | 1                    | 12                             |

A: Ampicillin; T: Tetracycline; VA: Vancomycin; TC: Teicoplanin; S: Streptomycin; CI: Ciprofloxacin; CL: Chloramphenicol
Antimicrobials are usually absorbed in low amounts in the intestinal tracts of animals (Sarmah et al., 2006) and are consequently excreted in the environment without being degraded (Liu et al., 2020). Therefore, manure use in agriculture has been associated with the presence and dissemination of AMR genes in the ecosystem (Lima et al., 2020). Livestock fecal matter may act as a deposit of AMR genes in the soil, promoting resistance to clinically important antimicrobials (Lee et al., 2017). As tetracycline is excreted as an active compound, the presence of tet genes increases after manure is used as a fertilizer (Guo et al., 2018; Xiong et al., 2018).

Another important result of the present study was that Enterococcus spp. isolates were mostly non-susceptible (71.4%) to any of the antimicrobials tested in this investigation. Remarkably, in those resistant strains, high resistance rates were observed for tetracycline (19.6%). This result is similar to the one reported by Radimersky et al. (2010), who identified 20% of resistance for this antimicrobial in Enterococcus spp. strains isolated from feral pigeons in the Czech Republic. Also, Klibi et al. (2015) reported 19.2% of resistance to tetracycline in Enterococcus spp. isolated from wild birds in Tunisia.

In the present study, 19.1% of E. coli isolates presented an MDR profile. This result is in agreement with those of a study on seagulls in Alaska, where 22% of E. coli isolates were MDR (Atterby et al., 2016). Also, in Italy, Gambino et al. (2021) reported that 23% of E. coli strains from wild birds were MDR.

As in this investigation, other studies have reported Enterococcus spp. MDR strains were isolated from wild birds (Silva et al., 2018; Stepien-Pyshna et al., 2019). Although the percentage was low (2.1%), these observations show that wild birds may be reservoirs of MDR bacterial strains, a fact that might be related to an anthropogenic impact mediated by contact with human or agricultural wastes (Skarzyńska et al., 2021).

E. coli strains with a phenotypic tetracycline resistance pattern were analyzed for the tetA gene. In general, tetracycline resistance in the family Enterobacteriaceae is given by the acquisition of efflux pump-coding genes, particularly tetA, which has been reported in other studies (Sigirci et al., 2019; Handrova and Kmet, 2019). In the present study, we identified this gene in 83.7% of E. coli strains isolated from Darwin finches. In agreement with this, Santos et al. (2013) identified the tetA gene in 60% (3/5) of tetracycline phenotypic resistant E. coli isolates in wild birds from the Azores archipelago. Also, Radhouani et al. (2012) reported the tetA gene in 59.3% (n = 16/27) of common buzzards in Portugal. The high proportion of this gene could be influenced by the transference of genetic resistance determinants through mobile elements such as conjugative transposons (Schell, 2019).

In the present study, we also determined the tetM gene in 75.4% of Enterococcus spp. strains from finches. The tetM gene is associated with ribosomal protection and it is usually identified in these bacteria (Frazzon et al., 2010). A high percentage of this gene has been reported in Enterococcus spp. strains isolated from wild birds (Radimersky et al., 2010; Santos et al., 2013; Yahia et al., 2018; Stepien-Pyshna et al., 2019). In agreement with our results, in fecal samples from giant tortoises (Chelonoidis spp.) from Santa Cruz Island, determined 97.2% of tet genes, 35.5% of which corresponded to tetM genes. Nevertheless, these researchers did not associate the gene with the bacterial genus.

AMR in Galapagos finches may be related to an anthropogenic impact especially related to agricultural practices on the island. Wild birds act as reservoirs and disseminators of AMR. This research shows their importance to better understanding resistance mechanisms circulating in this specific niche, as well as to gain insights into the impact of human practices in the Galapagos Islands.

**Conclusion**

To our knowledge, this is the first AMR study carried out on finches from the Galapagos Islands. Finches, like other wild birds, might be proposed as sentinels of AMR in the archipelago. In this study, we observed a high AMR percentage towards tetracycline in E. coli and Enterococcus isolates, especially in the agricultural zone of Santa Cruz Island. It is known that the antimicrobials usually used as therapeutic and prophylactic agents in production animals are absorbed only in low amounts by the gut of animals. Moreover, tetracycline is mostly evacuated as an active compound within excreta. In this way, livestock manure, which is widely used as fertilizer in agriculture, may act as an important reservoir of AMR genes in the soil of the agricultural zone, due to anthropogenic practices exerted on the island. Also, AMR bacteria have been found in high levels in manure from animals with no history of antibiotic use due to the bacteria intrinsically resistant to antimicrobials harbored in their intestinal tracts. In our study, we were able to determine circulating MDR bacteria as well as AMR profiles in E. coli and Enterococcus spp. isolates. However, further studies should be carried out to clarify the AMR patterns circulating in the Galapagos islands, as well as to better understand the role of anthropogenic variables in the development of AMR.

**Acknowledgment**

The present study was supported by the Galapagos National Park Direction (DPNG) and the Agency for the Regulation and Control of Biosafety and Quarantine for the Galapagos (ABG). We are grateful to María Belén Cevallos, Carlos Gómez, José Luis Medina, and Sofía de Janon for their contribution to this study. We also thank Clara López for her help in the statistical data analysis.
Funding Information

This study was funded by the Universidad Central del Ecuador, Ecuador. Sampling logistics at Galapagos were provided by the ABG.

Author’s Contributions

María Inés Baquero: Design of the study, field and laboratory work, data analysis, and manuscript writing.

Marylin Cruz: Head of logistics in the Galapagos Islands.

Viviana Duque: Sample logistics in Santa Cruz Island.

Alberto Velez: Sample and permission logistics in Santa Cruz Island.

Vanessa Lopez: Contributed to field and laboratory analyses.

Christian Vinueza: Conceptualization, methodology, supervision, and manuscript writing.

Gabriela Giacoboni: Conceptualization, methodology, supervision, contribution to laboratory work, and manuscript writing.

Ethics

This study was approved by the Institutional Committee for the Care and Use of Laboratory Animals of the Universidad Nacional de La Plata, Buenos Aires, Argentina (88-2-18T). All samples were collected under the Contra to Marco MAE-DNB-CM-2028-0094 and the DPNG authorization No PC 85-18.

References

Alonso, C. A., de Toro, M., de la Cruz, F., & Torres, C. (2021). Genomic insights into drug resistance and virulence platforms, CRISPR-Cas systems and phylogeny of commensal E. Coli from wildlife. Microorganisms, 9(5), 999. https://doi.org/10.3390/microorganisms9050999

Atterby, C., Ramey, A. M., Hall, G. G., Jährult, J., Börjesson, S., & Bonnedahl, J. (2016). The increased prevalence of antibiotic-resistant E. coli in gulls sampled in Southcentral Alaska is associated with urban environments. Infection Ecology & Epidemiology, 6(1), 32334. https://doi.org/10.3402/iee.v6.32334

Blanco, G., Lemus, J. A., Grande, J., Gangoso, L., Grande, J. M., Donázar, J. A., ... & Hiraldo, F. (2007). Retracted Geographical variation in cloacal microflora and bacterial antibiotic resistance in a threatened avian scavenger concerning diet and livestock farming practices. Environmental Microbiology, 9(7), 1738-1749. https://doi.org/10.1111/j.1462-2920.2007.01291.x

Bonnedahl, J., & Jährult, J. D. (2014). Antibiotic resistance in wild birds. Upsala Journal of Medical Sciences, 119(2), 113-116. https://doi.org/10.3109/03009734.2014.905663

CLSI. (2019). Performance Standards for Antimicrobial Susceptibility Testing - 29th Edition (Clinical and Laboratory Standards Institute [CLSI] ed.); 29th ed.). CLSI.

Cochetti, I., Vecchi, M., Mingoia, M., Tili, E., Catania, M. R., Manzin, A., ... & Montanari, M. P. (2005). Molecular characterization of pneumococci with efflux-mediated erythromycin resistance and identification of a novel mef gene subclass, mef (I). Antimicrobial Agents and Chemotherapy, 49(12), 4999-5006. https://doi.org/10.1128/AAC.49.12.4999-5006.2005

Foti, M., Mascetti, A., Fischella, V., Fulco, E., Orlandella, B. M., & Lo Piccolo, F. (2017). Antibiotic resistance assessment in bacteria isolated in migratory Passeriformes transiting through the Metaponto territory (Basilicata, Italy). Avian Research, 8(1), 1-11. https://doi.org/10.1186/s40657-017-0085-2

Frazzon, A. P. G., Gama, B. A., Hernes, V., Bierhals, C. G., Pereira, R. I., Guedes, A. G., ... & Frazzon, J. (2010). Prevalence of antimicrobial resistance and molecular characterization of tetracycline resistance mediated by tet (M) and tet (L) genes in Enterococcus spp. isolated from food in Southern Brazil. World Journal of Microbiology and Biotechnology, 26(2), 365-370. https://doi.org/10.1007/s11274-009-0160-x

Gambino, D., Vicari, D., Vitale, M., Schirò, G., Mira, F., Giglia, M. L., ... & Gargano, V. (2021). Study on Bacteria Isolates and Antimicrobial Resistance in Wildlife in Sicily, Southern Italy. Microorganisms, 9(1), 203. https://doi.org/10.3390/microorganisms9010203

Giacopello, C., Foti, M., Mascetti, A., Grosso, F., Ricciardi, D., Fischella, V., & Lo Piccolo, F. (2016). Antibiotic resistenza in ceppi di Enterobacteriaceae isolati da avifauna europea ricoverata presso un centro di recupero per la fauna selvatica. Vet. Ital, 52, 139-144. https://doi.org/10.12834/VetIt.327.1374.2

Grossman, T. H. (2016). Tetracycline antibiotics and resistance. Cold Spring Harbor Perspectives in Medicine, 6(4), a025387. https://doi.org/10.1101/cshperspect.a025387

Guenther, S., Grobbel, M., Lüke-Becker, A., Goedecke, A., Friedrich, N. D., Wieler, L. H., & Ewers, C. (2010). Antimicrobial resistance profiles of Escherichia coli from common European wild bird species. Veterinary Microbiology, 144(1-2), 219-225. https://doi.org/10.1016/j.vetmic.2009.12.016
Guo, T., Lou, C., Zhai, W., Tang, X., Hashmi, M. Z., Murtaza, R., ... & Xu, J. (2018). Increased occurrence of heavy metals, antibiotics, and resistance genes in surface soil after long-term application of manure. *Science of the Total Environment, 635*, 995-1003. https://doi.org/10.1016/j.scitotenv.2018.04.194

Handrova, L., & Kmet, V. (2019). Antibiotic resistance and virulence factors of Escherichia coli from eagles and goshawks. *Journal of Environmental Science and Health, Part B, 54*(7), 605-614. https://doi.org/10.1080/0360361234.2019.1608103

Hau, M., & Wikelski, M. (2001). Darwin's Finches. *Life Sciences, January*, 1–8. https://doi.org/10.1038/npg.els.0001791

Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. *American Society for Microbiology, 15*, 55-63.

Klibi, N., Ben Amor, I., Rahmouni, M., Dziri, R., Douja, G., Ben Said, L., ... & Torres, C. (2015). Diversity of species and antibiotic resistance among fecal enterococci from wild birds in Tunisia. Detection of vanA-containing Enterococcus faecium isolates. *European Journal of Wildlife Research*, 61(2), 319-323. https://doi.org/10.1007/s10344-014-0884-2

Knutie, S. A., Chaves, J. A., & Gotanda, K. M. (2019). Human activity can influence the gut microbiota of Darwin's finches in the Galapagos Islands. *Molecular Ecology, 28*(9), 2441-2450. https://doi.org/10.1111/mec.15088

Lee, J., Shin, S. G., Jang, H. M., Kim, Y. B., Lee, J., & Kim, Y. M. (2017). Characterization of antibiotic resistance genes in representative organic solid wastes: Food waste-recycling wastewater, manure, and sewage sludge. *Science of the Total Environment, 579*, 1692-1698. https://doi.org/10.1016/j.scitotenv.2016.11.187

Lima, T., Domingues, S., & Da Silva, G. J. (2020). Manure as a potential hotspot for antibiotic resistance dissemination by horizontal gene transfer events. *Veterinary Sciences, 7*(3), 110. https://doi.org/10.3390/vetsci7030110

Liu, C., Chen, Y., Li, X., Zhang, Y., Ye, J., Huang, H., & Zhu, C. (2020). Temporal effects of repeated application of biogas slurry on soil antibiotic resistance genes and their potential bacterial hosts. *Environmental Pollution*, 258, 113652. https://doi.org/10.1016/j.envpol.2019.113652

Lopardo, H. Á. (2016). Introducción a la microbiología clínica. *Libros de Catedra*. http://sedici.unlp.edu.ar/handle/10915/52389

Marosevic, D., Kaevska, M., & Jaglic, Z. (2017). Resistance to the tetracyclines and macrolide-lincosamide-streptogramin group of antibiotics and its genetic linkage—A review. *Annals of Agricultural and Environmental Medicine, 24*(2), 338. https://doi.org/10.26444/aaem/74718

Marston, H. D., Dixon, D. M., Knisely, J. M., Palmore, T. N., & Fauci, A. S. (2016). Antimicrobial resistance. *Jama, 316*(11), 1193-1204. https://doi.org/10.1001/jama.2016.11764

Michalova, E., & Schlegelova, J. (2004). Tetracyclines in veterinary medicine and bacterial resistance to them. *Veterinarni Medicina, 49*(3), 79. https://doi.org/10.17221/5681-VETMED

Michel, A. J., Ward, L. M., Goffredí, S. K., Dawson, K. S., Baldassarre, D. T., Brenner, A., ... & Chaves, J. A. (2018). The gut of the finch: uniqueness of the gut microbiome of the Galápagos vampire finch. *Microbiome, 6*(1), 1-14. https://doi.org/10.1186/s40168-018-0555-8

Millar, B. C., Jiru, X. U., Moore, J. E., & Earle, J. A. (2000). A simple and sensitive method to extract bacterial, yeast and fungal DNA from blood culture material. *Journal of Microbiological Methods, 42*(2), 139-147. https://doi.org/10.1016/S0167-7012(00)00174-3

Nieto-Claudín, A., Deem, S. L., Rodríguez, C., Cano, S., Moity, N., Cabrera, F., & Esperón, F. (2021). Antimicrobial resistance in Galapagos tortoises as an indicator of the growing human footprint. *Environmental Pollution, 284*, 117453. https://doi.org/10.1016/j.envpol.2021.117453

Nowaczez, A., Dec, M., Stepienn-Pyśniak, D., Urban-Chmiel, R., Marek, A., & Rózański, P. (2021). Antibiotic Resistance and Virulence Profiles of Escherichia coli Strains Isolated from Wild Birds in Poland. *Pathogens, 10*(8), 1059. https://doi.org/10.3390/pathogens10081059

Pantozzi, F. L. (2018). *Evaluación fenotípica y genotípica de la resistencia a tetraciclina en cepas de Escherichia coli de origen animal* (Doctoral dissertation, Universidad Nacional de La Plata). http://sedici.unlp.edu.ar/handle/10915/70535

Radhouani, H., Poeta, P., Goncalves, A., Pacheco, R., Sargo, R., & Igrejas, G. (2012). Wild birds as biological indicators of environmental pollution: Antimicrobial resistance patterns of Escherichia coli and enterococci isolated from common buzzards (Buteo buteo). *Journal of Medical Microbiology, 61*(6), 837-843. https://doi.org/10.1099/jmm.0.038364-0

Radimersky, T., Frolikova, P., Janoszowská, D., Dolejská, M., Svec, P., Roubalová, E., ... & Literák, I. (2010). Antibiotic resistance in feral pigeons. *Journal of Applied Microbiology, 109*(5), 1687-1695. https://doi.org/10.1111/j.1365-2672.2010.04797.x

Ramey, A. M., & Ahlstrom, C. A. (2020). Antibiotic-resistant bacteria in wildlife: Perspectives on trends, acquisition and dissemination, data gaps and future directions. *Journal of Wildlife Diseases, 56*(1), 1-15. https://doi.org/10.7589/2019-04-099
Rossolini, G. M., Arena, F., & Giani, T. (2017). Mechanisms of Antibacterial Resistance. In Infectious Diseases (Fourth Edi). Elsevier Ltd. https://doi.org/10.1016/b978-0-7200-6285-8.00138-6

Santos, T., Silva, N., Igrejas, G., Rodrigues, P., Micael, J., Rodrigues, T., ... & Poeta, P. (2013). Dissemination of antibiotic-resistant Enterococcus spp. and Escherichia coli from wild birds of Azores Archipelago. Anaerobe, 24, 25-31. https://doi.org/10.1016/j.anaerobe.2013.09.004

Sarmah, A. K., Meyer, M. T., & Boxall, A. B. (2006). A global perspective on the use, sales, exposure pathways, occurrence, fate, and effects of veterinary antibiotics (VAs) in the environment. Chemosphere, 65(5), 725-759. https://doi.org/10.1016/j.chemosphere.2006.03.026

Schell, C. M. B. (2019). Caracterización fenotípica y genotípica de cepas de Enterococcus spp. aisladas de líquidos obtenidos por punción provenientes de infecciones invasivas humanas (Doctoral dissertation, Universidad Nacional de La Plata). http://sedici.unlp.edu.ar/handle/10915/73675

Sigirici, B. D., Celik, B., Kahraman, B. B., Bagcigil, A. F., & Ak, S. (2019). Tetracycline resistance of enterobacteriaceae isolated from feces of synanthropic birds. Journal of Exotic Pet Medicine, 28, 13-18. https://doi.org/10.1053/j.jepm.2017.12.003

Silva, V., Igrejas, G., Carvalho, I., Peixoto, F., Cardoso, L., Pereira, J. E., ... & Poeta, P. (2018). Genetic Characterization of van A-Enterococcus faecium Isolates from Wild Red-Legged Partridges in Portugal. Microbial Drug Resistance, 24(1), 89-94. https://doi.org/10.1089/mdr.2017.0040

Skarżyńska, M., Zajac, M., Bomba, A., Bocian, Ł., Kozdruń, W., Polak, M., ... & Wasyl, D. (2021). Antimicrobial Resistance Glides in the Sky-Free-Living Birds as a Reservoir of Resistant Escherichia coli With Zoonotic Potential. Frontiers in Microbiology, 12, 656223. https://doi.org/10.3389/fmicb.2021.656223

Stepień-Pyśniak, D., Hauschild, T., Dec, M., Marek, A., & Urban-Chmiel, R. (2019). Clonal structure and antibiotic resistance of Enterococcus spp. from wild birds in Poland. Microbial Drug Resistance, 25(8), 1227-1237. https://doi.org/10.1089/mdr.2018.0461

Thaker, M., Spanogiannopoulos, P., & Wright, G. D. (2010). The tetracycline resistome. Cellular and Molecular Life Sciences, 67(3), 419-431. https://doi.org/10.1007/s00018-009-0172-6

UNESCO. (2022). Galápagos Islands. https://whc.unesco.org/en/list/1/

Wall, B. A., Mateus, A. L. P., Marshall, L., Pfeiffer, D. U., Lubroth, J., Ormel, H. J., ... & Patriarchi, A. (2016). Drivers, dynamics, and epidemiology of antimicrobial resistance in animal production. Food and Agriculture Organization of the United Nations.

Wheeler, E., Hong, P. Y., Bedon, L. C., & Mackie, R. I. (2012). Carriage of antibiotic-resistant enteric bacteria varies among sites in Galapagos reptiles. Journal of Wildlife Diseases, 48(1), 56-67. https://doi.org/10.1089/mdr.2018.0461

White, A., & Hughes, J. M. (2019). The critical importance of a one health approach to antimicrobial resistance. Eco Health, 16(3), 404-409. https://doi.org/10.1007/s10393-019-01415-5

WHO. (2018). WHO/WHO list of Critically Important Antimicrobials (CIA).

WHO/AGISAR. (2017). Integrated Surveillance of Antimicrobial Resistance in Foodborne Bacteria Application of a One Health Approach.

Xiong, W., Wang, M., Dai, J., Sun, Y., & Zeng, Z. (2018). The application of manure containing tetracyclines slowed down the dissipation of tet-resistance genes and caused changes in the composition of soil bacteria. Ecotoxicology and Environmental Safety, 147, 455-460. https://doi.org/10.1016/j.ecoenv.2017.08.061

Yahia, H. B., Chairat, S., Hamdi, N., Gharsa, H., Sallem, R. B., Ceballos, S., ... & Slama, K. B. (2018). Antimicrobial resistance and genetic lineages of fecal enterococci of wild birds: Emergence of vanA and vanB2 harboring Enterococcus faecalis. International Journal of Antimicrobial Agents, 52(6), 936-941. https://doi.org/10.1016/j.ijantimicag.2018.05.005