



MICROBIOLOGY

Assessing metal tolerance genes in enterococci isolated from animals in South Brazil

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Abstract: Heavy metal pollution is a global environmental problem. Enterococci have been recognized as sentinels of environmental pollution. This study investigates metal tolerance genes (*arsA*, *merA*, and *tcrB*) among enterococci isolated from fecal samples of domestic and wild animals from Atlantic Forest, Pampa biomes, and coastal regions of southern Brazil. Among the 238 enterococci analyzed, 38% harbored *arsA_I/arsA_II* and 10% contained *tcrB*, while none tested positive for *merA* genes. These results suggest that the selective pressure exerted by anthropogenic activities in the animals' habitats may contribute to the occurrence of heavy metal tolerance genes in host-associated enterococci.

Key words: bacteria, metal tolerance genes, environmental contamination, anthropogenic impacts, animals.

INTRODUCTION

The Brazilian ecosystems have faced significant challenges in recent decades. Among these challenges, heavy metal (HM) pollution has emerged as a significant problem in the Amazon, Atlantic Forest, and Pampa biomes, as well as in coastal regions and large cities. In southern Brazil, multiple anthropogenic activities, such as pesticide use, mining, industrial activities, and urbanization, are the major sources of HM pollution. As a result, some human and animal populations have shown high levels of HM (Ellwanger & Chies 2023).

Monitoring environmental pollution is an important issue addressed by One Health. Recently, Rebelo et al. (2021) proposed that enterococci, Gram-positive bacteria found in the animal gut microbiota, could serve as bioindicators of HM pollution influenced by anthropogenic activities. In this perspective, we sought to investigate the distribution of

metal tolerance genes (*arsA* for arsenic, *merA* for mercury, and *tcrB* for copper) among *Enterococcus* strains isolated from domestic and wild animals from the Atlantic Forest and Pampa biomes, as well as coastal regions in southern Brazil. Additionally, we aimed to explore the potential connections between these metal tolerance genes and anthropogenic activities in animal habitats.

To assess the distribution of *arsA*, *merA* and *tcrB* genes among enterococci, we selected 238 *Enterococcus* spp. isolates, including 133 *E. faecalis*, 44 *E. hirae*, 22 *E. faecium*, 14 *E. casseliflavus*, five *E. durans*, two *E. mundtii*, one *E. raffinosus*, one *E. gallinarum*, one *E. avium*, and 15 *Enterococcus* spp. These strains were isolated from fecal samples of domestic (broilers, n = 59) and wild animals (Capuchin monkeys, n = 72; fur seals, n = 34; sea turtles, n = 24; Brazilian free-tailed bats, n = 17; Geoffroy's cats, n = 8; Pampas foxes, n = 12; and Magellanic penguins, n = 12) between 2011 and 2020 from different geographic

areas of the southern Brazil (Figure 1). These strains were isolated, tested for antimicrobial susceptibility profiles and for the presence of antibiotic resistance genes (*tetM*, *tetL*, *ermB* and *msrC*) in our previous works (Cassenego et al. 2011, Santestevan et al. 2015, Prichula et al. 2016, Grassotti et al. 2018, Costa et al. 2019, Oliveira et al. 2020). All previous studies were conducted in accordance with the regulations of the Ethics Committees at the Federal University of Rio Grande do Sul.

The presence of the *arsA_I*, *arsA_II*, *tcrB*, *merA_III*, *merA_V*, and *merA_VI* genes was determined by PCR, as described by Rebelo et al. (2021). Fisher's exact and chi-square tests were performed to evaluate the associations between the occurrence of heavy metal tolerance genes and antimicrobial resistance. Differences were considered significant at $p < 0.05$.

According to our results, of the 238 enterococci strains tested for the presence of heavy metal tolerance genes, 79 strains (33%) were positive for *arsA_I* gene, 1 (0.4%) for the *arsA_II* gene, 11 (5%) for the *arsA_I* + *arsA_II* genes, and 23 (10%) for the *tcrB* gene. None of the strains were positive for *merA_III*, *merA_V*, or *merA_VI* genes. This finding is consistent with Rebelo et al. (2021), who showed the presence of these genes in enterococci collected from diverse epidemiological backgrounds, such as humans, food animal production setting and foodstuffs of animal/vegetable origin, and aquatic environment in Portugal and linked the occurrence of *arsA* and *tcrB* genes in the strains with the selective pressure due to arsenic and copper exposure, respectively. Moreover, we recently detected *ars* and *tcrB* genes in *Enterococcus* spp. isolated from seabird cloacal

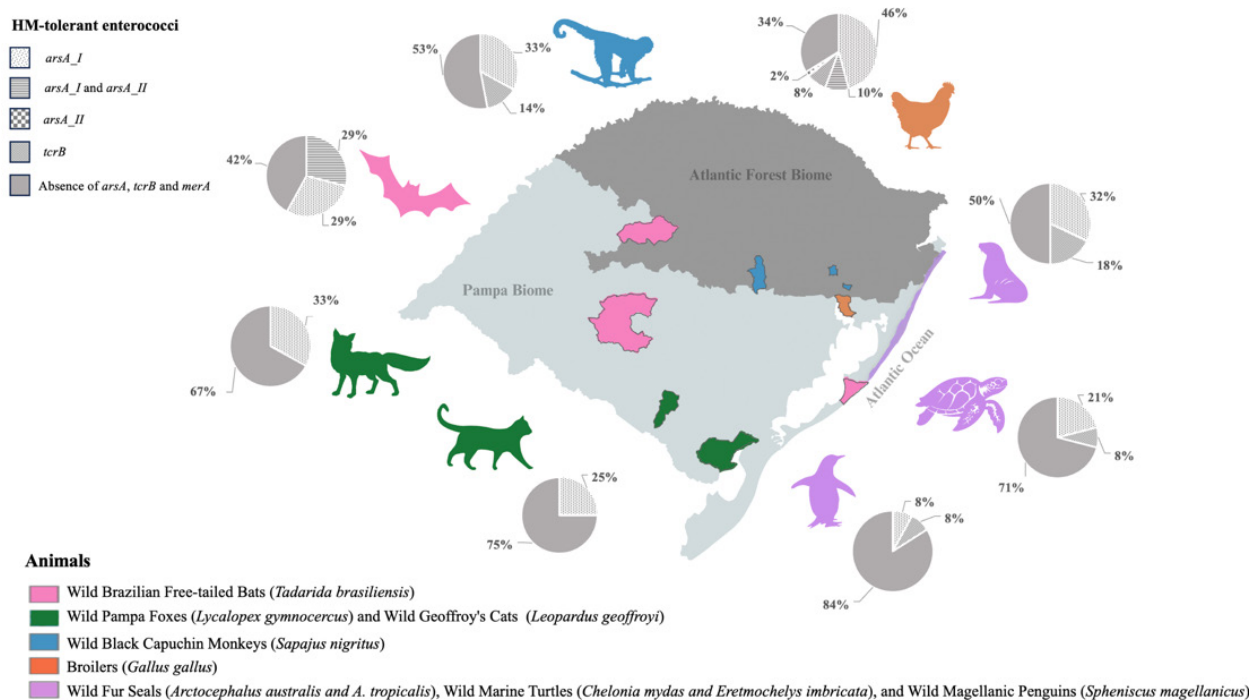


Figure 1. Distribution of heavy metal tolerance genes, *arsA* (arsenic), *merA* (mercury), and *tcrB* (copper), in *Enterococcus* isolated from fecal samples of domestic animals (broilers) and wild animals (Capuchin monkeys, fur seals, sea turtles, Brazilian free tailed bats, Geoffroy's cats, Pampas foxes, and Magellanic penguins) collected across three regions in southern Brazil: the Atlantic Forest biome (Iron), the Pampa biome (Gray), and coastal areas (Purple).

samples, where individuals showed heavy metal accumulation in blood and feathers, further supporting the potential of these bacteria as bioindicators of environmental pollution (Mocellin et al. 2024).

Among strains isolated from wild animals, the *arsA_I/arsA_II* genes were detected in high frequency in Brazilian free-tailed bats (58%; n=10/17), followed by Pampas foxes (33%; n=4/12), Capuchin monkeys (33%; n=24/72), fur seals (32%; n=11/34), Geoffroy's cats (25%; n=2/8), sea turtles (21%; n=5/24), and Magellanic penguins (8%; n=1/12). The *tcrB* gene was found in fur seals (18%, n=6/34), Capuchin monkeys (14%; 10/72), Magellanic penguins (8%; n=1/12), and sea turtles (4%; n=1/24) (Figure 1). Consistent with the literature, the presence of heavy metal tolerance genes in strains collected from Capuchin monkeys in the Atlantic Forest biome, as well as Brazilian free-tailed bats, Pampas foxes, and Geoffroy's cats in the Pampa biome, indicates potential anthropogenic pollution in their habitats. These animals inhabit highly productive livestock and agricultural regions of Rio Grande do Sul, where extensive use of pesticide and antibiotic might contribute to environmental contamination (Portella et al. 2023, Ziliotto et al. 2023). About marine animals, strains carrying the *arsA* and *tcrB* genes may be associated with contamination in Brazilian coastal waters (Ellwanger & Chies 2023, Mocellin et al. 2024). Furthermore, our previous studies indicate that anthropogenic activities, such as livestock farming, agricultural practices, urban effluent discharge, and direct or indirect contact with humans, contribute to the occurrence of antibiotic-resistant enterococci in the gut microbial communities of these animals (Cassenego et al. 2011, Santestevan et al. 2015, Prichula et al. 2016, Grassotti et al. 2018, Costa et al. 2019, Oliveira et al. 2020).

Regarding the enterococci isolated from domestic animals, 38 strains (64%) from cloacal samples of broilers were positive for heavy metal tolerance genes: 27 (46%) for *arsA_I*, 1 (2%) for *arsA_II*, 6 (10%) for *arsA_I + arsA_II* and 5 (8%) for *tcrB* (Figure 1). The occurrence of heavy metal tolerance genes in strains collected from broilers may be associated with their diets. The high frequency of arsenic-tolerance genes in these strains may be related to the selective pressure caused by the use of arsenic-derived compounds, commonly used as feed additives, in the poultry industry. Although the use of organoarsenic compounds, such as roxarsone, is banned in some countries due to their roles in promoting animal growth, controlling coccidiosis, improving meat pigmentation and serving as antiparasitics, these compounds are still used in Brazil (Baloš et al. 2019).

The absence of *merA_III*, *merA_V*, or *merA_VI* genes in the strains is consistent with Rebelo et al. (2021), who also reported a low frequency of MerA III, V and VI proteins in phylogenetic study of heavy metal tolerance proteins in *Enterococcus* spp. genomes isolated from animals. Furthermore, mercury contamination is relatively lower in southern Brazil than in other regions, such as the Amazon (Ellwanger & Chies 2023).

A significant association was found between the antibiotic resistance genes (*tetM*; $p=0.008$ and *msrC*; $p=0.028$) and the arsenic tolerance gene (*arsA_I*). Such associations suggest that these determinants may be co-located on plasmids or other mobile genetic elements, as previously reported (Hammerum et al. 2010, Rebelo et al. 2021). This facilitates co-selection and the simultaneous dissemination of resistance to both antibiotics and toxic metals, ultimately promoting bacterial persistence in contaminated environments.

In conclusion, our study identified heavy metal tolerance genes in *Enterococcus* spp. isolated from fecal samples of both wild and domestic animals in southern Brazil. The occurrence of these genes suggests that enterococci are facing selective pressures due to anthropogenic activities present in the environments inhabited by these animals.

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Data availability

Data will be made available upon reasonable request.

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Author contributions

C.C.S. led conceptualization, methodology, formal analysis, and prepared the original draft. R.R.M. and A.L.T. contributed to methodology. J.P. contributed to formal analysis, visualization, and manuscript revision. A.P.G.F. contributed to conceptualization, supervision, funding acquisition, and critical review of the manuscript.

