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## Iberian Lynx (*Lynx pardinus*) from the captive breeding program as reservoir of antimicrobial resistant enterococci and *Escherichia coli* isolates

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### ABSTRACT

A total of 98 faecal samples from captive specimens of Iberian lynx were collected and analysed for enterococci (96 isolates) and *Escherichia coli* (90 isolates) recovery. These 186 isolates were tested for antimicrobial resistance, molecular mechanisms of resistance, and detection of virulence genes. Among the enterococci, *Enterococcus hirae* was the most prevalent species (35 isolates), followed by *Enterococcus faecalis* (30 isolates), *Enterococcus faecium* (27 isolates), and *Enterococcus durans* (4 isolates). High rates of resistance to tetracycline, erythromycin and high-level-kanamycin were detected among enterococcal isolates (41%, 26%, and 19%, respectively). The *tet(M)* and/or *tet(L)*, *erm(B)*, *aac(6')-Ie-aph(2'')-Ia*, *ant(6)-Ia*, or *aph(3')-IIIa* genes were detected among resistant enterococci. Likewise, high rates of resistance were detected in *E. coli* isolates to tetracycline, streptomycin, sulfamethoxazole-trimethoprim (SXT), nalidixic acid, ampicillin, and ciprofloxacin (34%, 28%, 26%, 21%, 17%, and 16%, respectively). Furthermore, the *bla<sub>TEM</sub>* or *bla<sub>SHV</sub>*, *tet(A)* and/or *tet(B)*, *aadA* or *strA-strB*, *aac(3)-II* and/or *aac(3)-IV*, and different combinations of *sul* genes were detected among most resistant isolates. Fifteen isolates contained class 1 and/or class 2 integrons and 3 different gene cassette arrays were identified (*aadA1*, *dfrA1+aadA1*, and *estX+psp+aadA2*). The *E. coli* isolates were ascribed to phylo-groups A (12%); B1 (40%); B2 (37%), and D (11%), being *fimA* the most prevalent virulence gene (n=84), followed by *aer* (n=13), *cnf1* (n=13), *papC* (n=10) and *papG*-allele III (n=9). This study showed specimens of Iberian lynx acting as reservoirs of resistance genes, and in future (re)introductions they could spread resistant bacteria throughout the environment.

**Keywords:** Antimicrobial resistance; enterococci; *Escherichia coli*; Iberian lynx; virulence genes.

### 1. Introduction

The selection pressure exerted by the intensive use of antimicrobial agents, in human and veterinary medicine, has contributed to the selection and dissemination of resistant bacteria [1]. Moreover, resistant commensal bacteria constitute a reservoir of resistance genes that might be transferred to other commensal or pathogenic bacteria [2]. *Enterococcus* spp. and *Escherichia coli* inhabit the gastrointestinal tract of

human and animals and are occasionally associated with both community- and hospital-acquired infections [3]. The levels of antimicrobial resistance in these microorganisms can be used as indicators that might help to track the evolution of antimicrobial resistance in different ecosystems [4]. Recent studies in wild animals confirm that they might act as reservoirs of antimicrobial resistant genetic elements that

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could be spread across the environment [4].

The Iberian lynx is a critically endangered species native of the Iberian Peninsula [5]. This species typically hunts the European wild rabbit (*Oryctolagus cuniculus*). The decline of prey population along with habitat loss caused a restriction in the Iberian lynx geographical distribution. Total wild population for this species is estimated to a maximum of 150 adults, surviving in two breeding population on Southern Spain (Doñana and Sierra Morena) [6]. Studying captive animals of Iberian lynx is of capital importance since the susceptibility of this endangered species to bacterial infection may be affected by the presence of virulence and resistance genes.

Previous studies performed by our group analysed the carriage of *E. coli* producing extended-spectrum beta-lactamases or vancomycin resistant enterococci in faecal samples of Iberian lynx (free range and captive), using for that purpose antibiotic-supplemented plates for resistant-bacteria recovery [7, 8]. In addition, the faecal *E. coli* and enterococci population of free-range lynx (obtained in non-antibiotic-supplemented plates) has been recently evaluated for antimicrobial resistance and virulence [9], but no data do exist, so far, about this population in captive Iberian lynx.

The purpose of our study was to investigate the antimicrobial resistance, the molecular mechanisms of resistance, and the detection of virulence genes in faecal *Enterococcus* spp. and *E. coli* isolates from captive specimens of Iberian lynx.

## 2. Material and Methods

### 2.1. Faecal samples and bacterial isolates

Antimicrobial resistance in enterococci isolates and *E. coli* was studied in 98 fresh faecal samples recovered from captive specimens of Iberian lynx (*Lynx pardinus*), between 2008 and 2010. Each faecal sample collected belonged to a different animal. Sample collection from captive animals was obtained in the breeding facilities of the Iberian Lynx Captive Breeding Program, Iberian Peninsula, and took place during the daily handling or during clinical procedures.

For enterococci recovery, samples were seeded in Slanetz-Bartley agar (Oxoid Limited, Basingstoke, UK) plates. One colony with typical enterococcal morphology was identified to the genus and species level by Gram-staining, catalase test, bile-aesculin reaction, and by biochemical tests using the API ID20 Strep system (BioMérieux, La Balme, Les Grottes, France). Species identification was confirmed by polymerase chain reaction (PCR) using specific primers and conditions for *E. faecalis*, *E. faecium*, *E. casseliflavus* [10, 11], *E. gallinarum* [12], *E. hirae* and *E. durans* [13].

For *E. coli* isolation, samples were seeded in Levine agar (Oxoid Limited, Basingstoke, UK) plates. One colony per sample with typical *E. coli* morphology was selected and identified by classical biochemical methods (Gram staining, catalase, oxidase, indol, Methyl-Red-Voges-Proskauer, citrate, and urease) and by the API 20E system (BioMérieux, La

Balme Les Grottes, France).

### 2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility was performed by the disk diffusion method according to the criteria of the CLSI [14]. The enterococci susceptibility to 11 antimicrobial agents (vancomycin; teicoplanin; ampicillin; chloramphenicol; tetracycline; erythromycin; quinupristin-dalfopristin; ciprofloxacin; streptomycin; gentamicin; and kanamycin) was tested. *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923 strains were used for quality control.

Similarly, susceptibility of the *E. coli* isolates was performed to 16 antimicrobial agents [ampicillin, amoxicillin plus clavulanic acid, cefoxitin, cefotaxime, ceftazidime, aztreonam, imipenem, gentamicin, amikacin, tobramycin, streptomycin, nalidixic acid, ciprofloxacin, sulfamethoxazole-trimethoprim (SXT), tetracycline, and chloramphenicol] by the disk diffusion method [14]. *E. coli* ATCC 25922 was used as a quality-control strain. Additionally, ESBL-phenotypic detection was carried out by double-disk diffusion test [14].

### 2.3. Assay of gelatinase and beta-hemolytic activities in enterococci isolates

Gelatinase is an extracellular metalloendopeptidase that can hydrolyse gelatin, collagen, and other bioactive peptides which suggests that it may take part in inflammatory processes. Moreover, haemolysin has been demonstrated to contribute to the severity of enterococcal disease (Archimbaud *et al.*, 2002). Evaluation of gelatinase and hemolysin production in enterococci isolates was performed as previously reported [15].

### 2.4. Characterization of antimicrobial resistance mechanisms and detection of virulence genes

Among resistant-enterococci, the presence of genes encoding resistance to erythromycin [*erm*(A) and *erm*(B)], tetracycline [*tet*(M), *tet*(L), and *tet*(K)], kanamycin [*aph*(3')-IIIa], streptomycin [*ant*(6)-Ia], gentamicin [*aac*(6)-Ie-*aph*(2'')-Ia], chloramphenicol [*catA*], quinupristin-dalfopristin [*vatD* and *vatE*] and the presence of Tn916- and Tn5397-specific sequences were analysed by PCR using primers and conditions previously reported [16-19]. The presence of genes encoding different virulence factors (*gelE*, *agg*, *ace*, *cpd*, *fsr*, *esp*, *hyl* and *cylLLLSABM*) was also studied by PCR [19, 20].

Likewise, in the resistant *E. coli* isolates the presence of resistance genes to ampicillin [*bla*<sub>TEM</sub> and *bla*<sub>SHV</sub>], tetracycline [*tet*(A) and *tet*(B)], streptomycin [*aadA* and *strA-strB*], gentamicin [*aac*(3)-II, *aac*(3)-IV], SXT [*sul1*, *sul2* and *sul3*] and chloramphenicol [*cmlA* and *floR*] were studied by PCR [21]. Additionally, the incidence of the *int11* and *int12* genes, encoding classes 1 and 2 integrases, respectively, and their variable regions were also analysed by PCR and sequencing

[21].

Lastly, the phylogenetic groups and virulence determinants often found in pathogenic *E. coli* (*stx1-stx2*, *fimA*, *papG* allele III, *cnf1*, *papC* and *aer*) were investigated [3, 22].

Positive and negative controls from the collection of strains of the University of Trás-os-Montes and Alto Douro (Portugal) were included in all PCR assays.

### 3. Results

Enterococci were obtained from 96 (98%) of the 98 samples analysed. *E. hirae* was the most prevalent detected species (35 isolates), followed by *E. faecalis* (30 isolates), *E. faecium* (27 isolates) and *E. durans* (4 isolates). *E. coli* isolates were detected in 90 (92%) of the 98 Iberian lynx faecal samples.

Fifty-five of the 96 enterococci isolates (57%) showed susceptibility to all the antimicrobial agents tested (24 *E. hirae*, 19 *E. faecium*, 8 *E. faecalis*, and 4 *E. durans*). Most of *E. faecalis* isolates showed a multiresistant phenotype.

No ampicillin-, vancomycin- or teicoplanin-resistant enterococcal isolates were found in this study and quinupristin-dalfopristin resistance, as expected, was found in *E. faecalis* isolates. On the other hand, higher levels of resistance to tetracycline (41%), erythromycin (26%), and kanamycin (19%) were detected among our isolates. Moderate level of resistance was observed to quinupristin-dalfopristin (10%), ciprofloxacin (9%), and streptomycin (9%). Additionally, low level of resistance was detected to gentamicin (high-level, 6%) and chloramphenicol (3%).

Table 1 presents the antimicrobial resistance genes detected among resistant-enterococci. Different associations of *tet* (M) and *tet*(L) genes with the genetic elements *Tn916* and *Tn5397* were found in tetracycline resistant isolates. All erythromycin-resistant *E. hirae* and *E. faecium* isolates harbored the *erm*(B) gene. On the other hand, only 12 of the 20 *E. faecalis* isolates that showed resistance to erythromycin presented the *erm*(B) gene. The *aac*(6')-Ie-*aph*(2'')-Ia gene was observed in 4 of the 6 high-level gentamicin-resistant *E. faecalis* isolates and the *ant*(6)-Ia gene in 1 of the 9 high-level streptomycin-resistant isolates. The *aph*(3')-IIIa gene, encoding an aminoglycoside-modifying enzyme, was detected in all kanamycin resistant isolates (Table 1).

No virulence genes were detected among enterococci isolates and none of these microorganisms showed beta-hemolytic or gelatinase activities.

Fifty of the 90 *E. coli* isolates (56%) showed susceptibility to all tested antimicrobial agents. No resistances were detected to amoxicillin plus clavulanic acid, ceftazidime, ceftazidime, aztreonam, and imipenem. High percentages of resistance were observed to tetracycline (34%), streptomycin (28%), sulfamethoxazole-trimethoprim (26%), nalidixic acid (21%), ampicillin (17%), and ciprofloxacin (16%), and lower levels to gentamicin (13%), chloramphenicol (10%), tobramycin (2%), and amikacin (1%). Additionally, it is noteworthy that 27 isolates (30%) showed a multiresistant pheno-

type.

Table 1 presents the antimicrobial resistance genes detected among resistant *E. coli* isolates. From the 15 ampicillin-resistant *E. coli* isolates, 6 harbored the *bla*<sub>TEM</sub> gene and 1 harbored the *bla*<sub>SHV</sub> gene. The *tet*(A) and *tet*(B) genes were detected in 18 and 7 tetracycline-resistant *E. coli* isolates, respectively. The presence of the *aadA* and the *strA-strB* genes were shown in 16 and 6 streptomycin-resistant *E. coli* isolates, respectively. Among the gentamicin-resistant *E. coli* isolates, 9 harbored the *aac*(3)-II gene and 1 isolate the *aac*(3)-II plus *aac*(3)-IV genes. Different combinations of *sul1*, *sul2*, and *sul3* genes were identified in 22 of the 23 sulfamethoxazole-trimethoprim-resistant *E. coli* isolates. In addition, the *cmlA* gene was identified in 7 of the 11 chloramphenicol-resistant isolates (Table 1).

The presence of integrons was studied by PCR and sequencing, and the presence of class 1 integrons were detected in 14 isolates (all contained the *intI1*, but two isolates lacked the *qacEΔ1/sulI* 3'-conserved region) containing the following gene cassette arrays: *aadA1* (6 isolates), *dfrA1+aadA1* (6 isolates), and *estX+psp+aadA2* (2 isolates, both lacking the *qacEΔ1/sulI* 3'-conserved region). The presence of class 2 integrons was confirmed in 3 isolates (2 of them also harboring a class 1 integron), containing the gene cassette array *dfrA1+aadA1*.

Among the *E. coli* isolates, 84 of them contained at least one virulence gene. The *fimA* virulence gene was the most prevalent, detected in the 84 isolates. The *aer* and/or *cnf1* genes were found in 13 *E. coli* isolates, respectively. Moreover, the *papC* and *papG*-allele III virulence genes were shown in 10 and 9 isolates, respectively. The distribution of the phylogenetic groups was: 11 isolates ascribed to the phylogenetic group A, 36 isolates to B1, 33 isolates to B2 and 10 isolates into the phylogroup D (Table 2).

### 4. Discussion

*E. faecium* and *E. faecalis* are usually the most prevalent enterococcal species among isolates recovered from wild animals [4, 19]. In our study *E. hirae* was the predominant species followed by *E. faecalis* and *E. faecium*. Similar results were obtained in wild boars and Iberian wolf [23, 24].

No vancomycin resistant enterococci (VRE) were detected in this study in faecal samples of captive lynxes, and similar results were also demonstrated in free range lynxes [9]. Even when vancomycin-supplemented plates were used for VRE recovery, negative results were also demonstrated both in captive and free range lynxes, with the exception of enterococci with an intrinsic-mechanism of vancomycin-resistance [8].

The *tet*(M) and *tet*(L) genes, coding for tetracycline resistance, are frequently reported in tetracycline-resistant enterococcal isolates [4, 25]. In our study, most of the tetracycline-resistant isolates harbored simultaneously these two genes. The *erm*(B) gene, responsible for acquired erythromycin resistance, is frequently linked with the *tet*(M) gene on

**Table 1** Resistance genes detected among antimicrobial resistant enterococci and *E. coli* isolates obtained from faecal samples of Iberian lynx.

Bacteria	Antimicrobial agent	No. of resistant isolates	Genes detected by PCR	
			Resistance genes and genetic elements	No. of isolates
Enterococci (n= 96)				
<i>E. hirae</i> (n= 35)	Chloramphenicol (30 µg)	1	<i>catA</i>	1
		11	<i>tet(M) + tet(L)</i>	3
	Tetracycline (30 µg)		<i>tet(M) + tn916</i>	2
			<i>tet(M) + tet(L) + tn916</i>	5
			<i>erm(B)</i>	2
Kanamycin (120 µg)	1	<i>aph(3')-IIIa</i>	1	
<i>E. faecalis</i> (n= 30)	Chloramphenicol (30 µg)	2	<i>catA</i>	0
		22	<i>tet(L)</i>	8
	Tetracycline (30 µg)		<i>tet(M) + tet(L)</i>	9
			<i>tet(M) + tet(L) + tn916</i>	3
			<i>tet(M) + tet(L) + tn5397</i>	2
	Erythromycin (15 µg)	20	<i>erm(B)</i>	12
	Quinupristin-Dalfopristin (15 µg)	10	<i>vatD</i> or <i>vatE</i>	0
	Ciprofloxacin (5 µg)	8	-	-
	Streptomycin (300 µg)	9	<i>ant(6)-Ia</i>	1
	Gentamicin (120 µg)	6	<i>aac(6')-Ie-aph(2'')-Ia</i>	4
Kanamycin (120 µg)	16	<i>aph(3')-IIIa</i>	16	
<i>E. faecium</i> (n= 27)	Tetracycline (30 µg)	6	<i>tet(L)</i>	2
			<i>tet(M) + tet(L)</i>	4
	Erythromycin (15 µg)	3	<i>erm(B)</i>	3
	Ciprofloxacin (5 µg)	1	-	-
	Kanamycin (120 µg)	1	<i>aph(3')-IIIa</i>	1
<i>E. coli</i> (n= 90)	Ampicilin (10 µg)	15	<i>bla<sub>TEM</sub></i>	6
			<i>bla<sub>SHV</sub></i>	1
	Tetracycline (30 µg)	31	<i>tet(A)</i>	18
			<i>tet(B)</i>	7
	Gentamicin (10 µg)	12	<i>aac(3)-II</i>	9
			<i>aac(3)-II + aac(3)-IV</i>	1
	Amikacin (30 µg)	1	-	-
	Tobramycin (10 µg)	2	-	-
	Streptomycin (10 µg)	25	<i>aadA</i>	16
			<i>strA-strB</i>	6
	Nalidixic acid (30 µg)	19	-	-
	Ciprofloxacin (5 µg)	14	-	-
	Sulfamethoxazol.Trimethopim (25 µg)	23	<i>sul1</i>	9
			<i>sul2</i>	3
			<i>sul3</i>	3
		<i>sul1 + sul2</i>	4	
		<i>sul1 + sul3</i>	1	
		<i>sul2 + sul3</i>	2	
		<i>cmIA</i>	7	

**Table 2** Virulence genes detected among the *E. coli* isolates collected from faecal samples of Iberian lynx.

Bacteria	Virulence genes profiles detected by PCR	No. of isolates	Phylogenetic group			
			A	B1	B2	D
<i>E. coli</i> (n= 90)	<i>fimA</i>	56	5	24	19	8
	<i>aer; fimA</i>	11	1	9	1	0
	<i>cnf1; fimA</i>	6	0	0	5	1
	<i>papC; fimA</i>	1	0	0	1	0
	<i>papGIII; fimA</i>	1	1	0	0	0
	<i>aer; papC; fimA</i>	1	0	1	0	0
	<i>papC; papGIII; fimA</i>	1	0	0	1	0
	<i>papC; cnf1; papGIII; fimA</i>	6	1	0	5	0
	<i>aer; papC; cnf1; papGIII; fimA</i>	1	0	0	1	0
Virulence genes not detected	6	3	2	0	1	

the highly mobile conjugative transposon Tn1545, which predominates in clinically important Gram-positive bacteria [18]. In our study, it was shown that 50% of the *erm*(B) and *tet*(M)-positive isolates harbored the Tn916/Tn1545-like element. Nonetheless, the association of *tet*(M) and *erm*(B) genes with this transposon was not analysed. The *aac*(6')-*aph* (2''), *ant*(6)-Ia and *aph*(3')-IIIa genes detected in our HLR-aminoglycoside enterococci, has been frequently detected in previous reports from wild animals [4, 19, 23, 25].

None of our *E. coli* isolates produced extended-spectrum  $\beta$ -lactamases (ESBL). Nevertheless, in a previous study, ESBL-producing *E. coli* were recovered from 9 faecal samples of captive specimens of Iberian lynx when Levine-supplemented agar plates were used [7]. Thus, ESBL-producing *E. coli* could be present in the faecal microbiota of captive animals in lower rates when compared with ESBL-nonproducing *E. coli* isolates. In our study, 44% of *E. coli* isolates showed resistance to at least one antimicrobial agent tested and high levels of resistance to tetracycline, streptomycin, SXT, nalidixic acid, ampicillin, and ciprofloxacin were found. Similar results were detected in other studies performed with faecal *E. coli* isolates from wild animals [26, 27], and food-producing animals [28]. Still, higher levels of resistance have been previously detected in *E. coli* isolates from wild birds in Portugal [25].

The classical TEM enzymes are the predominant plasmid-mediated  $\beta$ -lactamases of gram-negative bacteria and have been previously found in ampicillin-resistant *E. coli* isolates from different origins [29]. Similarly, in our study 6 of the 10 ampicillin-resistant *E. coli* isolates harboured the *bla*<sub>TEM</sub> gene and 1 isolate harboured the *bla*<sub>SHV</sub> gene. Likewise, the *tet*(A) and *tet*(B) genes, detected among our isolates, are the most frequent ones reported among resistant isolates from different origins [21, 27]. The CmlA family is the largest family of

chloramphenicol specific exporters and the *cmlA* gene was identified in 7 of our chloramphenicol-resistant isolates. The *cmlA* determinant is typically associated with mobile genetic elements that carry additional resistance genes. This fact explains the persistence of chloramphenicol resistance determinants despite the longstanding prohibition of chloramphenicol use in food-producing animals or wild animals [30]. The *aadA* gene and the linked *strA-strB* gene pair found in our study encode for streptomycin resistance and are commonly found among streptomycin-resistant *E. coli* isolates from animals, food and humans [31]. Additionally, the AAC(3)-II and AAC(3)-IV gentamicin acetyltransferases have been recurrently reported within *E. coli* isolates of human, food, animal and environmental origin [21]. In our study, the genes encoding these enzymes, *aac*(3)-II and/or *aac*(3)-IV, were also detected in 10 gentamicin-resistant *E. coli* isolates. The acquisition and prevalence of alternative dihydropteroate synthase (DHPS) genes (*sul* genes) by our SXT-resistant isolates are in accordance with the findings of previously studies performed within *E. coli* isolates from wild animals in Portugal [4, 25].

The presence of integrons among our commensal *E. coli* isolates is a cause for concern, as this genetic structure might enhance the dissemination of resistance genes to other bacteria, by mobile elements such as plasmids and transposons. Gene cassettes that confer resistance to streptomycin/spectinomycin (*aadA1* and *aadA2*), and to trimethoprim (*dfrA1* and *dfrA12*) were present within the detected integrons. Among the variable region of these structures, 4 different gene cassette arrays were identified (*aadA1*, *dfrA1+aadA1*, *dfrA12+aadA2*, and *estX+psp+aadA2*). Similar structures have been reported in *E. coli* isolates from different sources [27, 28, 32].

A high number of virulence genes were detected among

our *E. coli* isolates. As showed, 95% of the isolates harbored at least one virulence gene. The predominance of *fimA* (type 1 fimbriae) is in accordance with previous works [25]. Still, this high value is unusual for wild animals and is higher than those previously found within *E. coli* isolates from wild animals and meat products [25, 33].

*E. coli* isolates can be ascribed into four main phylogenetic groups (A, B1, B2, and D) and whereas most commensal strains belong to groups A and B1, virulent extra-intestinal strains belong mainly to groups B2 and D [22]. The presence of virulence-encoding genes in our isolates is a cause of great concern especially since a vast number of them were ascribed to phylogenetic groups B2 and D. A similar ratio has been previously reported among intestinal *E. coli* clones from wild boars in Germany [34]. Nonetheless, this phylogenetic distribution was unexpected, as former studies performed within faecal *E. coli* isolates from wild animals detected a higher prevalence of strains belonging to groups A and B1 [25, 27].

There are several examples of resistant bacteria transfer between animals, and from animals to man via the food chain [2]. Therefore, Iberian lynx might be contaminated through the food chain, as the presence of resistant *E. coli* strains has been previously detected in their predominant prey [4]. Captive animals of Iberian lynx are fed with captive rabbits produced for human consumption. Previous works showed food-producing animals as reservoirs of antimicrobial resistant bacteria, which could explain the results obtained in this study [2, 35]. The use of antimicrobial agents in captive Iberian lynx is very restrict and infrequent. Still, their use during clinical procedures (e.g. clinical cases and surgical procedures) might create a selective pressure that could lead to a small increase in the levels of antimicrobial resistance.

The results obtained in this study with captive specimens are similar to those previously found in wild specimens of Iberian lynx [9]. The values of phenotypic resistance to the antimicrobial agents tested, the phylogenetic distribution, and the presence of virulence genes are closely related with the wild specimens of this species. Consequently, there is a possibility that the founder animals of the breeding program (caught in the wild) might have carried these resistant strains and resistance genes into the program.

## 5. Concluding Remarks

This study showed captive specimens of Iberian lynx acting as reservoirs of resistance genes. The same genes found in bacteria from environment and human origins were found in this study, indicating the possible circulation of bacteria and resistance genes between animal, environment and human ecosystems. Additionally, the high percentage of virulence determinants in *E. coli* isolates is a cause of concern since an increased risk of infection and therapeutic failure due to virulence/resistance genes can consequently represent a health problem for these animals.

Further research should be carried out in the future in order to better understand the dissemination and frequency of resistance genes and virulence determinants. This may be useful to verify the possible contamination of these animals through the food chain.

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