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Original article

Identification and characterisation of antimicrobial resistance of *Enterococcus* spp. isolated from pork and poultry meat

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Summary There is a great concern on the presence of antimicrobial-resistant bacteria in meat, since they can cause adverse effects in the consumers' health. The purpose of this work was to identify and characterise the antimicrobial resistance of *Enterococcus* spp. isolated from meat. A total of 209 meat samples (forty pork, fifty chicken, fifty-one turkey, thirty-one duck, and thirty-seven quail) were collected at retail level. *E. faecalis* was the dominant enterococci isolated from all the types of meat evaluated, followed by *E. faecium, E. gallinarum*, and *E. hirae,* among others which were also isolated. Of the 317 strains evaluated for antimicrobial resistance 48 (15.14%) were multi-resistant. Most of the multi-resistant strains were *E. faecium* and *E. faecalis*, and in lesser extent *E. gallinarum* and *E. hirae*. Multi-resistant strains were isolated from all the types of meat studied, mainly from turkey (sixteen strains), chicken (fifteen strains), and quail (fourteen strains). Two multi-resistant strains showed resistance to vancomycin (*E. faecium* isolated from turkey meat, and *E. faecalis* isolated from quail meat). Resistance to tigecycline, teicoplanin, linezolid and quinolones was found among enterococci isolates. Special measures should be taken to avoid faecal contamination of carcasses in order to reduce enterococci prevalence in meat.

Keywords Antimicrobial resistance, chicken, duck, enterococci, food safety, meat, pork, quail, turkey.

Introduction

Chicken meat is the most consumed meat worldwide followed by pork (FAO, 2022, OECD/FAO, 2022). Other poultry meats such as turkey, duck and quail are less consumed, although in the last decades their consumption has increased (FAO, 2022). Nowadays, antimicrobial resistance is considered a major threat to human health. It should be noted that the excessive use of antimicrobials in animals contributes to the spread of resistance to antimicrobials (Fatoba *et al.*, 2022), where the meat that is consumed plays a very important role.

The problem with antimicrobial resistance is a complicated global problem that requires a multisectoral approach called One Health, which includes human, animal and environmental health (Thu *et al.*, 2019). This approach not only seeks the good health of humans, but also that of animals and the environment (Serrano *et al.*, 2022).

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Enterococci are ubiquitous microorganisms that can be often found in the environment and are part of the usual microbiota of the intestinal tract of animals and humans (Hammerum *et al.*, 2010; Souillard *et al.*, 2022). Enterococci are used as stool indicator microorganisms (Byappanahalli *et al.*, 2012). On the other hand, enterococci are very important opportunistic bacteria that cause a range of infections (Asgharzadeh Marghmalek *et al.*, 2021; Souillard *et al.*, 2022). As they have the ability to survive in adverse ambient conditions, these bacteria appear in nosocomial diseases with increasing frequency. *Enterococcus fecalis* and *Enterococcus faecium* cause common infections in humans mainly in hospitals (Asgharzadeh Marghmalek *et al.*, 2021). Most of the studies on enterococci in poultry meat have been carried out in chicken and turkey meat, while no information is available in duck and quail meat (Hayes *et al.*, 2003; McGowan *et al.*, 2006; Kročko *et al.*, 2011; Maasjost *et al.*, 2015; Tyson *et al.*, 2018; Manson *et al.*, 2019). The predominant enterococci in chicken and turkey meat are *E. faecalis* and *E. faecium* (Hayes *et al.*, 2003; McGowan *et al.*, 2006; Kročko *et al.*, 2011; Maasjost *et al.*, 2015; Tyson

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et al., 2018). *E. faecium* was the prevailing specie in pork (Hayes *et al.*, 2003; Kročko *et al.*, 2007; Tyson *et al.*, 2018). On the other hand, most of the studies only evaluate the prevalence of enterococci in meat (Hayes *et al.*, 2003; McGowan *et al.*, 2006; Kročko *et al.*, 2011; Maasjost *et al.*, 2015; Tyson *et al.*, 2018). Few works include data on enterococci counts in meat (Kročko *et al.*, 2007; Kim & Koo, 2020).

Enterecocci are intrinsically resistant to several antimicrobials. Moreover, these microorganisms have the capability to acquire resistance to antibiotics (Aslam *et al.*, 2012). This poses a problem for treatment of the infections they cause in humans. In addition, enterococci have been recognised as a reservoir of antimicrobial resistance genes, being able of transferring these genes to other bacteria (Sanlibaba *et al.*, 2018). In the 1940s, the introduction of antimicrobials for the treatment of clinical infectious changed medicine. However, their use and misuse has also conducted to the development and growth of antimicrobial-resistant bacteria. It should be pointed out that in the European Union above 25 000 people die every year from diseases originated by antimicrobial resistant bacteria (WHO, 2011).

The use of antimicrobials in food-producing animals has relevant public health implications, as it encourages the increase of antimicrobial-resistant bacteria that can be transmitted to people. Since edible animals and animal-based foods are traded around the world, they contribute to antimicrobial resistance in areas far from where the problem was originated (WHO, 2011). Since vancomycin has been used for severe enterococci infections treatment, it is of great concern the emergence of vancomycin-resistant enterococci (Cercenado, 2011; Maasjost *et al.*, 2015). The emergence of vancomycin resistance has conducted to the use of other antimicrobials, such as linezolid, teicoplanin, daptomycin, fosfomycin and daptomycin (Riccardi *et al.*, 2021).

As there is a great concern on the role of meat on the spread of antimicrobial resistance, it is of interest to evaluate the antimicrobial resistance of bacteria present in those meat more consumed such as pork and chicken meat, besides other poultry meats such as turkey, duck and quail meat that are increasing their consumption (González-Fandos *et al.*, 2022). Several authors have studied the antimicrobial resistance of strains isolated from pork, chicken and turkey meat, while there is no information available from duck and quail meat (Hayes *et al.*, 2003; McGowan *et al.*, 2006; Kročko *et al.*, 2011; Maasjost *et al.*, 2015; Tyson *et al.*, 2018).

Thus, the purpose of this work was to enumerate and identify the enterococci present in pork, chicken, turkey, duck and quail meat. The antimicrobial resistance of *Enterococcus* spp. isolated from these types of meat was also evaluated.

Material and methods

Sampling and microbiological analysis

A total of 209 meat samples (forty pork, fifty chicken, fifty-one turkey, thirty-one duck and thirty-seven quail) were purchased in different retailers in La Rioja (Spain). The amount of samples of each animal species depended on consumption data, availability and diversity of commercial brands (MAPA, 2019). The samples were analysed as quickly as possible and always maintaining the cold chain.

For enumeration of enterococci ten grams of each meat were weighed and homogenised using 90 mL of 0.1% sterile peptone water (Oxoid, Basingstoke, Hampshire, UK) in a Masticator blender (IUL Instruments, Barcelona, Spain) for 2 min. Serial dilutions were prepared and plated on Kanamycin Esculin Azide agar (Scharlau, Barcelona, Spain), incubating for 48 h at 37 °C. In addition, the isolation of enterococci strains suspected of being resistant to vancomycin (VRE) was performed using a selective chromogenic medium CHROMID VRE® (BioMérieux, Marcy l'Etoile, France) as described by González-Fandos *et al.* (2022).

Isolation and identification of enterococci

From each culture media and sample between 3 and 5 suspected colonies were randomly selected and isolated. After, the purified isolates were maintained at −80 °C. Microbial identification was carried out using a Matrix-Assisted Laser Desorption/Ionisation-Time of Flight Mass-Spectrometry Biotyper (Bruker, Billerica, MA, USA).

Antimicrobial susceptibility of enterococci

Of the identified strains, a total of 317 enterococci isolates were tested against a panel of 16 antimicrobials. The method used was the disk diffusion method on Mueller–Hinton agar. The following antibiotic disks (Oxoid, Basingstoke, Hampshire, UK) were used: vancomycin (30 µg), ciprofloxacin (5 µg), enrofloxacin (5 µg), levofloxacin (5 µg), norfloxacin (10 µg), doxycycline (30 µg), tetracycline (30 µg), chloramphenicol (30 µg), tigecycline (15 µg), teicoplanin (30 µg), linezolid (30 µg), ampicillin (10 µg), gentamicin (120 µg), imipenem (5 µg), minocycline (30 µg), and nitrofurantoin (300 µg). After incubation at 37 °C for 24 h, inhibition areas were measured and scored as resistant, susceptible, or intermediate according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2020). The minimum inhibitory concentration (MIC) for vancomycin was evaluated by E-test strips (Biomerieux® Marcy l'Etoile, France) in those enterococci isolates that showed resistance to this antibiotic.

Statistical analysis

Analysis of variance was performed using SPSS version 26 software (IBM SPSS Statistics). Tukey's test for comparison of means was performed using the same program. The level of significance was determined at $P < 0.05$.

Results and discussion

Enumeration and prevalence of enterococci isolated from pork and poultry meat

In the present work *Enterococcus* spp. were isolated in 15 of 39 pork samples (38.46%), 33 of 50 chicken samples (66%), 11 of 31 of duck samples (35.48%), 31 of 37 of quail (83.78%), and 39 of 51 of those of turkey (76.47%). The other samples showed enterococci counts below 1 log CFU g^{-1}. Higher percentages of samples with presence of enterococci have been reported by Tyson *et a*l. (2018) in chicken (95%) and turkey meat (94.4%), while lower percentages were reported by Onaran *et al*. (2019) in chicken (30%). We observed a high percentage of quail meat samples with presence of enterococci, while a lower level was observed in duck meat. We observed that the percentage of samples with presence of enterococci was lower in pork meat samples than in chicken, turkey or quail meat. In contrast, Pesavento *et al*. (2014) reported higher prevalence of enterococci in pork meat (44.3%) than in poultry meat (28.6%).

We obtained enterococci counts between 1.30 log CFU g^{-1} and 4.05 log CFU g^{-1}, 1.00 log CFU g^{-1} and 3.27 log CFU g^{-1}, 1.30 log CFU g^{-1} and 3.85 log CFU g^{-1}, 1.30 log CFU g^{-1} and 3.79 log CFU and 1.0 log CFU g^{-1} and 3.13 log CFU g^{-1} in pork, chicken, duck, quail and turkey meat, respectively. No significant differences ($P > 0.05$) in enterococci counts were observed among the different types of meat. Other authors have reported counts between 0.60 and 6.47 CFU cm^{-2} in pork meat (Kročko *et al*., 2007; Kim & Koo, 2020). Kročko *et al*. (2011) have reported higher enterococci counts in chicken meat between 1.48 and 5.79 CFU cm^{-2}. When comparing these results, it should be considered if enterococci counts are reported by cm^2 or g.

In the current study only two samples were positive in chromID VRE, one from quail meat and another from turkey meat. The isolates from VRE were identified as *E. faecium* in the sample from quail and *E. faecalis* in the sample from turkey.

Table 1 shows the *Enterococcus* spp. distribution by type of meat. The largest number of enterococci strains were obtained from turkey (37.86%), followed by quail (24.29%), chicken (23.34%), pork (7.57%) and duck (6.94%). *E. faecalis* was the dominant enterococci (46.37%), followed by *E. faecium* (22.71%). As in the present study, other investigations have shown that the dominant enterococci found in pork, chicken and turkey meat was *E. faecalis*, followed by *E. faecium* (Aslam *et al*., 2012; Maasjost *et al*., 2015; Onaran *et al*., 2019; Kim & Koo, 2020). However, other authors have pointed out that the dominant enterococci in pork and chicken meat was *E. faecium* (Kročko *et al*., 2007). In addition, we isolated *E. devriesei*, *E. gilvus* and *E. hirae* from pork meat samples. However, other researchers have isolated other species such as *E. durans*, *E. gallinarum, E. raffinosus,*

Table 1 Species of *Enterococcus* isolated from pork, chicken, duck, quail and turkey meat

Species	Type of meat					
	PORK	CHICKEN	DUCK	QUAIL	TURKEY	TOTAL
Enterococcus casseliflavus	0[†]	2	0	0	3	5
Enterococcus cecorum	0	9	0	1	8	18
Enterococcus devriesei	1	0	0	0	0	1
Enterococcus durans	0	1	4	0	1	6
Enterococcus faecalis	15	34	11	30	57	147
Enterococcus faecium	2	18	5	15	32	72
Enterococcus gallinarum	0	5	1	15	9	30
Enterococcus gilvus	3	3	0	2	4	12
Enterococcus hirae	2	1	1	11	5	20
Enterococcus mundtii	0	0	0	3	0	3
Enterococcus pasteuri	0	0	0	0	1	1
Enterococcus phoeniculicola	0	1	0	0	0	1
Enterococcus thailandicus	1	0	0	0	0	1
Total	24	74	22	77	120	317

[†]Number of strains isolated.

E. avium, and *E. mundtii* (Kročko *et al*., 2007; Pesavento *et al*., 2014; Kim & Koo, 2020). As in the present work Aslam *et al*. (2012) found *E. hirae* in pork meat. Onaran *et al*. (2019) also isolated *E. casseliflavus, E. durans*, and *E. gallinarum* from chicken meat, but not other *Enterococcus* spp. that were isolated in the present work such as *E. cecorum, E. gilvus* or *E. hirae*. *E. cecorum* represented 12.16% and 6.67% of the enterococci isolated from chicken and turkey meat, respectively. However, Aslam *et al*. (2012) also isolated *E. hirae* from chicken meat. Furthermore, we isolated *E. casseliflavus* from turkey meat, *E. gilvus* from quail and turkey meat and *E. hirae* from duck, quail and turkey meat. We observed a higher prevalence of *E. hirae* in quail meat (14.28%) than in chicken (4.05%), duck (4.55%) or turkey meat (4.16%). Manson *et al*. (2019) also isolated *E. cecorum* (3.3%), *E. hirae* (3.3%), *E. durans* and *E. gallinarum* (0.3%) from chicken meat.

It should be noted that *E. faecalis* and *E. faecium* cause nosocomial infections in humans (Asgharzadeh Marghmalek *et al*., 2021; Souillard *et al*., 2022). Moreover, other enterococci such as *E. casseliflavus* can also be pathogenic to humans (Yoshino, 2023). These species have been isolated from meat in the present work. Meat may become contaminated with enterococci, mainly *E. faecalis*, *E. faecium* at slaughter. As *enterococci* are commensals in the gut of animals, contamination of carcasses by faecal bacteria can occur if the hygienic standards are low (González-Fandos *et al*., 2022).

Antimicrobial resistance of enterococci isolated from pork and poultry meat

The percentages of enterococci strains resistant to the antimicrobials tested by type of meat are shown in Fig. 1. We observed high resistance rates against tetracyclines, with values between 54.17% in pork meat and 70.27% in chicken meat. These results are in agreement with those found in the literature (Aslam *et al*., 2012; Noh *et al*., 2020; Rebelo *et al*., 2023). The resistance to tetracyclines found could be explained since these antimicrobials are often used in farm animal treatments. We did not detect any resistant strain in pork meat to doxycycline or in duck meat to minocycline. For doxycycline, we observed the highest resistance rates in quail meat (12.99%), followed by turkey meat (10.83%). The resistance rate to minocycline in pork meat isolates was 8.33%.

Table 2 shows the antimicrobial resistance of the different species of *Enterococcus* by type of meat. In general, the highest resistant rates were observed among *E. faecium* and *E. faecalis* strains isolated from all the types of meat studied. High resistance rates to fluoroquinolones and tetracyclines were observed. We also found resistance against tigecycline, teicoplanin, vancomycin, ampicillin, chloramphenicol, gentamicin, imipenem, and nitrofurantoin among *E. faecium* and *E. faecalis* strains, while resistance to linezolid was only observed in *E. faecalis* strains isolated from chicken and turkey meat.

We observed resistance rates above 50% against tetracyclines in *E. faecalis* strains isolated from all the types of meat and for *E. faecium* in strains isolated from poultry. Moreover, all the *E. casseliflavus* strains isolated from chicken and turkey meat showed resistance to tetracycline. Also, *E. cecorum* and *E. gallinarum* strains isolated from chicken, quail and turkey, *E. gilvus* strains isolated from pork, quail and turkey meat, *E. hirae* strains isolated from duck, chicken, quail and turkey, and *E. durans* isolated from chicken and duck meat showed resistance to tetracycline (Table 2).

We found resistance to fluoroquinolones, mainly against enrofloxacin with resistance rates of 22.97% in

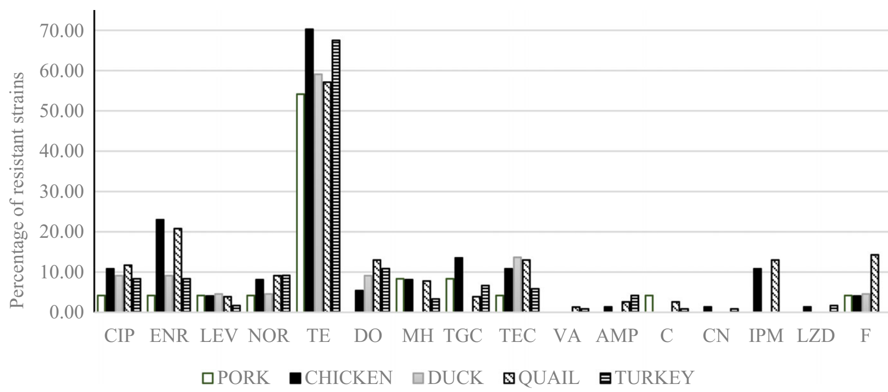

Figure 1 Percentage of antimicrobial-resistant enterococci strains isolated from pork, chicken, duck, quail and turkey meat. AMP, ampicillin; C, chloramphenicol; CIP, ciprofloxacin; CN, gentamicin; DO, doxycycline; ENR, enrofloxacin; F, nitrofurantoin; IMP, imipenem; LEV, levofloxacin; LZD, linezolid; MH, minocycline; NOR, norfloxacin; TE, tetracycline; TEC, teicoplanin; TGC, tigecycline; VA, vancomycin.

Table 2 Antimicrobial resistance of enterococci isolated from pork, chicken, duck, quail and turkey meat

Species		Antibiotics[†]															
		CIP	ENR	LEV	NOR	TE	DO	MH	TGC	TEC	AMP	*C*	CN	IPM	LZD	*F*	VA
E. casseliflavus	Chicken (*n* = 2)	1[‡] (50.0)[§]	1 (50.0)	0	1 (50.0)	2 (100)	0	2 (100)	0	0	0	0	1 (50.0)	0	0	0	0
	Turkey (*n* = 3)	1 (33.3)	3 (100)	0	1 (33,3)	3 (100)	0	3 (100)	0	0	0	0	0	0	0	0	0
E. cecorum	Chicken (*n* = 9)	4 (44.4)	5 (55.6)	1 (11.1)	3 (33.3)	8 (88.9)	3 (33.3)	2 (22.2)	0	0	0	0	0	0	0	1 (11.1)	0
	Quail (*n* = 1)	1 (100)	1 (100)	0	0	1 (100)	0	0	0	0	0	0	0	0	0	0	0
	Turkey (*n* = 8)	2 (25.0)	3 (37.5)	1 (12.5)	2 (25.0)	6 (75.0)	3 (37.5)	3 (37.5)	0	0	0	0	0	0	0	0	0
E. durans	Chicken (*n* = 1)	0	0	0	0	1 (100)	0	1 (100)	0	0	0	0	0	0	0	0	0
	Duck (*n* = 4)	0	0	0	0	2 (50.0)	0	2 (50.0)	0	0	0	0	0	0	0	1 (25.0)	0
	Turkey (*n* = 1)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. faecalis	Pork (*n* = 15)	4 (26.7)	4 (26.7)	1 (6.7)	1 (6.7)	11 (73.3)	4 (26.7)	12 (80.0)	2 (13.3)	1 (6.7)	0	1 (6.7)	0	0	0	0	0
	Chicken (*n* = 34)	8 (23.5)	23 (67.6)	2 (5.9)	2 (5.9)	25 (73.5)	18 (52.9)	24 (70.6)	4 (11.8)	6 (17.6)	0	1 (2.9)	0	1 (2.9)	1 (2.9)	0	0
	Duck (*n* = 11)	3 (27.3)	4 (36.4)	0	0	7 (63.6)	2 (18.2)	7 (63.6)	0	1 (9.1)	0	0	0	0	0	0	0
	Quail (*n* = 30)	6 (20.0)	13 (43.3)	3 (10.0)	5 (16.7)	20 (66.7)	13 (43.3)	16 (53.3)	1 (3.3)	5 (16.7)	0	1 (3.3)	0	1 (3.3)	0	1 (3.3)	0
	Turkey (*n* = 57)	9 (15.8)	25 (43.9)	4 (7.0)	9 (15.8)	50 (87.7)	41 (71.9)	48 (84.2)	3 (5.3)	6 (10.5)	3 (5.3)	5 (8.8)	1 (1.8)	0	1 (1.8)	0	1 (1.8)
E. faecium	Pork (*n* = 2)	2 (100)	2 (100)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Chicken (*n* = 18)	9 (50.0)	14 (77.8)	1 (5.6)	2 (11.1)	16 (88.9)	7 (38.9)	16 (88.9)	1 (5.6)	0	1 (5.6)	0	0	7 (38.9)	0	4 (22.2)	0
	Duck (*n* = 5)	1 (20.0)	3 (60.0)	0	0	4 (80.0)	3 (60.0)	4 (80.0)	0	2 (40.0)	0	0	0	0	0	1 (20.0)	0
	Quail (*n* = 15)	12 (80.0)	15 (100)	1 (6.7)	7 (46.7)	11 (73.3)	9 (60.0)	11 (73.3)	1 (6.7)	5 (33.3)	2 (13.3)	1 (6.7)	0	4 (26.7)	0	7 (46.7)	1 (6.7)
	Turkey (*n* = 32)	12 (37.5)	31 (96.9)	0	1 (3.1)	16 (50.0)	8 (25.0)	12 (37.5)	5 (15.6)	1 (3.1)	1 (3.1)	0	1 (3.1)	0	0	3 (9.4)	0
E. gallinarum	Chicken (*n* = 5)	2 (40.0)	4 (80.0)	3 (60.0)	1 (20.0)	4 (80.0)	2 (40.0)	4 (80.0)	1 (20.0)	2 (40.0)	0	0	0	0	1 (20.0)	0	0
	Duck (*n* = 1)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Quail (*n* = 15)	8 (53.3)	12 (80.0)	3 (20.0)	8 (53.3)	15 (100)	5 (33.3)	14 (93.3)	0	0	0	1 (6.7)	0	1 (6.7)	0	0	0
	Turkey (*n* = 9)	5 (55.6)	8 (88.9)	2 (22.2)	5 (55.6)	8 (88.9)	2 (22.2)	7 (77.8)	0	0	0	0	0	0	1 (11.1)	0	0
E. gilvus	Pork (*n* = 3)	0	0	0	0	2 (66.7)	0	0	0	0	0	0	0	0	0	0	0
	Chicken (*n* = 3)	0	0	0	0	1 (33.3)	0	0	0	0	0	0	0	0	0	0	0
	Quail (*n* = 2)	0	0	0	0	2 (100)	0	0	0	0	0	0	0	0	0	0	0
	Turkey (*n* = 4)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. hirae	Pork (*n* = 2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Chicken (*n* = 1)	0	0	0	0	1 (100)	0	0	0	0	0	0	0	0	0	0	0
	Duck (*n* = 1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0	0	0	0	0	0	0	0	0
	Quail (*n* = 11)	1 (9.1)	3 (27.3)	1 (9.1)	1 (9.1)	4 (36.4)	0	2 (18.2)	0	0	0	0	0	4 (36.4)	0	7 (63.6)	0
	Turkey (*n* = 5)	0	1 (20.0)	0	0	3 (60.0)	2 (40.0)	3 (60.0)	0	0	0	0	0	0	1 (20.0)	1 (20.0)	0
E. mundtii	Quail (*n* = 3)	0	1 (33.3)	0	0	2 (66.7)	0	2 (66.7)	0	0	0	0	0	0	0	0	0
E. pasteuri	Turkey (*n* = 1)	0	0	0	0	0	0	0	0	0	1 (100)	0	0	0	0	0	0
E. phoeniculicola	Chicken (*n* = 1)	0	0	0	0	1 (100)	0	0	0	0	0	0	0	0	0	0	0
E. devriesei	Pork (*n* = 1)	0	0	0	0	1 (100)	0	1 (100)	0	0	0	0	0	0	0	0	0
E. thailandicus	Pork (*n* = 1)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (100)	0

CIP, ciprofloxacin; ENR, enrofloxacin; LEV, levofloxacin; NOR, norfloxacin; TE, tetracycline; DO, doxycycline; MH, minocycline; TGC, tigecycline; TEC, teicoplanin; AMP, ampicillin; C, chloramphenicol; CN, gentamicin; IMP, imipenem; LZD, linezolid; F, nitrofurantoin; VA, vancomycin.

[†]Antibiotics tested.

[‡]Number of resistant strains.

[§]Percentage of resistant strains.

chicken, and 20.78% in quail. We observed lower resistance rates to fluoroquinolones in pork meat (4.17%), although all the strains from pork meat show the same resistance rate to all the fluoroquinolones tested (ciprofloxacin, enrofloxacin, levofloxacin and norfloxacin). It should be noted that in the present work the resistance rates against norfloxacin were especially high among strains isolated from quail meat (46.7%). Among fluoroquinolones, we noted the lowest resistance rates against levofloxacin, ranged between 1.67% in strains isolated from turkey meat, and 4.55% in those isolated from duck meat (Fig. 1). We found resistance rates to enrofloxacin specially high among *E. faecium* strains ranging between 60% in those isolated from duck meat, and 100% in those isolated from pork and quail meat, and above 50% in *E. casseliflavus* strains isolated from chicken and turkey meat. However, neither *E. durans* nor *E. gilvus* strains showed resistance to any of the four antimicrobials tested (Table 2). As other authors, we observed higher resistance to ciprofloxacin for *E. faecium* than for *E. faecalis*, except in duck meat (Rebelo *et al.*, 2023). In contrast, Aslam *et al.* (2012) did not find resistance to ciprofloxacin in *E. faecium* or *E. faecalis* strains isolated from pork, chicken, and turkey meat. As Rebelo *et al.* (2023) we observed that some *E. gallinarum* strains showed resistance to ciprofloxacin. In addition, we also found resistance to enrofloxacin, levofloxacin, and norfloxacin in some *E. gallinarum* strains isolated from chicken, quail and turkey meat. The high resistance rates to fluoroquinolones found are of special concern since these antimicrobials are categorised in “Category B: antimicrobials to restrict” in animals (EMA, 2019).

We did not detect any enterococci-resistant strain in ducks to tigecycline, while the resistance rate to tigecycline in pork isolates was 8.33% (Fig. 1). Resistance to tigecycline for *E. faecalis* isolated from pork, chicken and turkey meat has also been reported by other authors (Aslam *et al.*, 2012). Moreover, we also found tigecycline-resistant strains for *E. faecium* isolated from chicken, quail and turkey meat, as well as for *E. gallinarum* isolated from chicken meat (Table 2). This fact is relevant since tigecycline is categorised in “Category A: antimicrobial to avoid” in animals (EMA, 2019). Although other authors have not found resistance to teicoplanin in enterococci strains isolated from poultry and pork (Fracalanzza *et al.*, 2007; Kim & Koo, 2020), we observed resistance to this antimicrobial among *E. faecalis* isolated from all the types of meat studied, *E. faecium* strains isolated from duck, quail and turkey, and *E. gallinarum* isolated from turkey. Moreover, we detected resistance to linezolid in *E. faecalis* and *E. gallinarum* strains isolated from chicken and turkey meat, and in *E. hirae* isolated from turkey meat (Table 2). Other authors have not found resistance to linezolid in *E. faecalis, E. gallinarum* or *E. hirae* strains isolated from chicken or turkey (Aslam *et al.*, 2012; Rebelo *et al.*, 2023). The resistance to linezolid and teicoplanin is of special concern, since these antimicrobials are used for severe enterococci infections treatment, as an option to vancomycin-resistant enterococci (Riccardi *et al.*, 2021).

We found two strains resistant to vancomycin, one *E. faecalis* from turkey and one *E. faecium* from quail (Table 2). In contrast, other authors have not found resistance to vancomycin in enterococci isolated from animals or meat (Fracalanzza *et al.*, 2007; Aslam *et al.*, 2012; Jahan *et al.*, 2013; Thu *et al.*, 2019; Noh *et al.*, 2020; Rebelo *et al.*, 2023). As Kim & Koo (2020) we did not isolate any resistant enterococci to vancomycin from pork meat. The occurrence of vancomycin-resistant enterococci in food of animal origin has been related to the use of avoparcin for animal growth promotion, because avoparcin is a vancomycin analogue that gives cross-resistance to vancomycin (Bager *et al.*, 1997). Despite avoparcin was banned in the European Union in 1997 (EC, 1997), the presence of vancomycin-resistant enterococci in meat is still detected (Onaran *et al.*, 2019). High resistance to vancomycin (MIC > 256 μg mL^{-1}) has also been reported by other authors (Onaran *et al.*, 2019). Although we only found 0.63% of the enterococci resistant to vancomycin, it is of special concern for public health. On the other hand, it has been suggested that enterococci have an amazing ability to acquire and transfer antibiotic resistance genes (González-Fandos *et al.*, 2022). Therefore, the risk that these strains can pose to human health must be taken into account, also knowing their multi-resistance to other antimicrobials.

As Rebelo *et al.* (2023), we found resistance to ampicillin in *E. faecium* isolated from chicken (5.6%). Moreover, we detected resistance to ampicillin in *E. faecium* isolated from turkey and quail meat (3.1% in turkey and 13.3% in quail). We also found resistance to ampicillin in 5.3% of *E. faecalis* and 100% of *E. pasteuri* strains isolated from turkey (Table 2). We observed resistance to chloramphenicol in pork (4.17%), quail (2.60%), and turkey (0.83%) meat, while resistance against gentamicin was only observed in strains isolated from turkey and chicken meat (Table 2). Previous works have also found resistance to chloramphenicol, and gentamicin among strains isolated from poultry meat (Pesavento *et al.*, 2014). Fracalanzza *et al.* (2007) also found resistance to imipenem among *E. faecium* strains isolated from poultry. In addition, we found resistance to imipenem among *E. faecalis*, *E. gallinarum* and *E. hirae* isolated from quail, and *E. faecalis* isolated from chicken meat (Table 2). We detected the highest resistance rates to nitrofurantoin in isolates from quail meat (14.29%) followed by pork, duck and chicken meat with resistant rates around 4% (Fig. 1). In contrast, Rebelo *et al*. (2023) did not find any resistance against nitrofurantoin in enterococci strains.

We found that only 2.84% of enterococci were susceptible to all the antimicrobials tested (8.33%, 1.36%, 4.55%, 0% and 4.17% in pork, chicken, duck, quail and turkey meat, respectively), none of them were identified as *E. faecalis* or *E. faecium*. Other authors have reported that only 3.4% of enterococci isolated from pork, chicken, and turkey meat were susceptible to all the antimicrobials tested (Aslam *et al*., 2012).

In the present study, of the 317 strains studied, a total of 48 (15.14%) were multi-resistant (resistance to three or more antibiotic classes). The antimicrobial resistance phenotype of multi-resistant *Entercoccus* spp. is shown in Table 3. Most of the multi-resistant strains were *E. faecium* (twenty-one strains) and *E. faecalis* (twenty-one strains), and in lesser extent *E. gallinarum* (four strains) and *E. hirae* (two strains). Multi-resistant strains were mainly isolated from turkey (16), chicken (15), and quail (14) meat, while only two strains were isolated from duck meat and one from pork meat. Of the forty-eight multi-resistant strains, only two *E. faecalis* strains were not resistant to any of the fluoroquinolones studied. All the multi-resistant strains showed resistance to tetracycline, except three *E. faecium* strains. Two multi-resistant strains showed resistance to vancomycin (*E. faecium*, and *E. faecalis*). These strains were isolated from CHROMID VRE®. Both strains showed a minimum inhibition concentration (MIC) to vancomycin above 256 μg mL^{-1}. We detected *E. faecalis* multi-resistant strains in pork (6.67%), chicken (14.71%), duck (9.09%), quail (13.33%), and turkey meat (17.54%). Other authors have also reported lower multi-resistant rates in *E. faecalis* isolated from pork meat than in those isolated from chicken, and turkey meat (Aslam *et al*., 2012). Multi-resistant *E. faecium* was found in chicken (44.44%), duck (20%), quail (60%), and turkey meat (9.38%), but not in pork meat. Moreover, 40% of the *E. gallinarum* isolated from chicken and 22% of those isolated from turkey were also multi-resistant, while multi-resistance was also observed in 9.09% of the *E. hirae* isolated from quail and 20% of those isolated from turkey. Also, Rebelo *et al*. (2023) pointed out a high multi-resistance rate for *E. gallinarum* isolated from chicken. Rebelo *et al*. (2023) indicated that multi-resistant rates were higher among *E. faecium* than in *E. faecalis* strains isolated from chicken. This finding is in agreement with our results, except in turkey meat where multi-resistance was higher among *E. faecalis* than in *E. faecium*.

The decline in antibiotic use at the farm level in Europe over the past decade has not been sufficient to reduce antibiotic resistance rates in meat (Rebelo *et al*., 2023). The consumption of meat containing antibiotic-resistant strains of enterococci is considered a likely route of transport of this agent from animals to humans (Thu *et al*., 2019).

Table 3 Antimicrobial resistance phenotype of multi-resistant enterococci isolated from pork, chicken, duck, quail and turkey meat

Type of meat	Species (Number of isolates)	Antibiotic resistance phenotype (Number of isolates)
Pork	*E. faecalis* (1)	CIP-ENR-TE-DO-MH-TEC (1)
Chicken	*E. faecalis* (5)	ENR-TE-DO-MH-TEC (1)
		ENR-TE-TEC (1)
		TE-DO-MH-C-LZD (1)
		CIP-ENR-TE-MH-TEC (1)
		CIP-ENR-TE-DO-MH-IPM (1)
	E. faecium (8)	CIP-ENR-LEV-NOR-TE-MH-AMP-IPM (1)
		CIP-ENR-TE-MH-IPM (2)
		CIP-ENR-TE-DO-MH-F (1)
		CIP-ENR-TE-DO-MH-IPM-F (2)
		CIP-ENR-NOR-TE-MH-IPM (1)
		CIP-ENR-TE-DO-MH-TGC-IPM-F (1)
	E. gallinarum (2)	ENR-LEV-TE-MH-TGC-TEC-LZD (1)
		CIP-ENR-LEV-TE-DO-MH-TEC (1)
Duck	*E. faecalis* (1)	CIP-ENR-TE-DO-MH-TEC (1)
	E. faecium (1)	ENR-TE-DO-MH-F (1)
Quail	*E. faecalis* (4)	CIP-ENR-NOR-TE-DO-MH-TEC (1)
		ENR-TE-MH-TEC (1)
		CIP-ENR-LEV-NOR-TE-DO-MH-IPM-F (1)
		ENR-TE-DO-MH-C (1)
	E. faecium (9)	CIP-ENR-TE-DO-MH-IPM-F (1)
		CIP-ENR-LEV-NOR-TE-DO-MH-F (1)
		CIP-ENR-TE-MH-TEC (1)
		ENR-TE-DO-MH-TEC-IPM (1)
		CIP-ENR-TE-DO-MH-AMP-IPM-F (1)
		ENR-AMP-IPM-F (1)
		CIP-ENR-TE-DO-MH-C-F (1)
		CIP-ENR-NOR-TE-MH-TEC-F (1)
		CIP-ENR-NOR-TGC-TEC-VA-F (1)†
	E. hirae (1)	CIP-ENR-LEV-NOR-TE-IPM-F (1)
Turkey	*E. faecalis* (10)	CIP-ENR-NOR-TE-DO-MH-AMP (3)
		CIP-ENR-LEV-NOR-TE-DO-MH-C (1)
		TE-DO-MH-TEC-C (1)
		ENR-TE-DO-MH-TEC (1)
		ENR-TE-DO-MH-TEC (1)
		CIP-ENR-LEV-NOR-TE-DO-MH-C (1)
		ENR-TE-DO-MH-CN (1)
		ENR-TE-DO-TEC-VA (1)†
	E. faecium (3)	ENR-TE-MH-CN (1)
		CIP-ENR-TE-MH-F (1)
		CIP-ENR-TEC-F (1)
	E. hirae (1)	ENR-TE-DO-MH-LZD-F (1)
	E. gallinarum (2)	CIP-ENR-TE-DO-MH (1)
		ENR-TE-MH-LZD (1)

AMP, ampicillin; C, chloramphenicol; CIP, ciprofloxacin; CN, gentamicin; DO, doxycycline; ENR, enrofloxacin; F, ni-trofurantoin; IMP, imipenem; LEV, levofloxacin; LZD, linezolid; MH, minocycline; NOR, norfloxacin; TE, tetracycline; TEC, teicoplanin; TGC, tigecycline; VA, vancomycin.
†Strain isolated from CHROMID VRE®.

Conclusions

E. faecalis was the predominant *Enterococcus* spp. found in pork, chicken, duck, quail, and turkey meat. The highest multi-resistant rates were found among *E. faecium*, and *E. faecalis* strains isolated from chicken, quail, and turkey meat.

Resistance to vancomycin and other critical antimicrobials such as tigecycline, linezolid, and quinolones was found among enterococci isolates, being of special concern for consumer's health. In consequence, special measures should be taken to avoid faecal contamination of carcasses during slaughter in order to reduce enterococci contamination of meat.

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Author contributions

Alba Martinez-Laorden: Formal analysis (equal); investigation (equal); writing – original draft (equal); writing – review and editing (supporting). **Celia Arraiz-Fernandez:** Data curation (equal); investigation (equal). **Elena Gonzalez-Fandos:** Conceptualization (lead); data curation (equal); formal analysis (lead); funding acquisition (lead); investigation (equal); methodology (lead); project administration (lead); resources (lead); supervision (lead); writing – original draft (lead); writing – review and editing (lead).

Conflict of interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval

Ethics approval was not required for this research.

Data availability statement

Research data are not shared.

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