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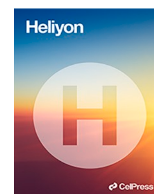

Research article

Vancomycin resistance and virulence genes evaluation in *Enterococci* isolated from pork and wild boar meat

Laura Andriani [a], Martina Rega [a,*], Paolo Bonilauri [b], Giovanni Pupillo [b], Giorgia De Lorenzi [b], Silvia Bonardi [a], Mauro Conter [a], Cristina Bacci [a]

[a] Food Hygiene and Inspection Unit, Veterinary Science Department, University of Parma, Strada del Taglio, 10, 43126, Parma, Italy
[b] Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna, via Pitagora, 2, 42124, Reggio Emilia, Italy

A R T I C L E I N F O

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A B S T R A C T

Enterococci are considered valuable sentinel Gram-positive bacteria for monitoring vancomycin antibiotic resistance due to their widespread presence and characteristics. The use of antimicrobials in farming animals has a role in the increasing of Antimicrobial Resistance (AMR) and the anthropogenic transformation of the landscape has forced wildlife into greater contact with humans and their livestock. The transmission of resistant bacteria by their meat products is a significant contributor to AMR development.

The present study aimed to assess the prevalence of vancomycin resistant Enterococci spp. In antimicrobial-treated farmed pigs meat and in antimicrobial-free wild boars meat.

A total of 341 Enterococci were isolated from 598 pork meat samples (57 %) and 173 Enterococci were isolated from 404 wild boar meat samples (42.8 %). Data found showed that low-resistance was detected more in wild boars meat *Enterococci* (52.6 %) than in pork meat once (48.4 %). However, the prevalence of resistance genes was at low level (33.9 % in pork meat Enterococci and 4.4 % in wild boar meat ones) and the only gene found was *vanC1/C2*, related to intrinsic AMR. Normally, *Enterococci* persist in the normal intestinal flora of animals including humans. However, the presence of resistance genes was frequently linked to the detection of pathogenic genes, mostly *gelE* in pork meat isolates and *asa1* in wild boars meat isolates. Pathogenic bacteria can cause severe infections in human that can become more risky if associated to the presence of AMR. Pathogenic bacteria were characterized and a high presence of *E. gallinarum* and *E. casseliflavus* was found.

Given the growing interest in wild game meat consumption the monitoring of AMR in these matrices is essential. Further surveillance studies are needed to fully evaluate the emergence and spread of vancomycin-resistant *Enterococci* (VRE) and pathogenic *Enterococci* from animal-derived food to humans, including the role of wildlife in this phenomenon. Giving the higher interest in wild animals meat consumption, it is important to better evaluate the spread of AMR phenomenon in the future and intensify hygienic control of wild animals derived food.

* Corresponding author.
E-mail addresses: laura.andriani@unipr.it (L. Andriani), martina.rega@unipr.it (M. Rega), paolo.bonilauri@izsler.it (P. Bonilauri), giovanni.pupillo@izsler.it (G. Pupillo), giorgia.delorenzi@izsler.it (G. De Lorenzi), silvia.bonardi@unipr.it (S. Bonardi), mauro.conter@unipr.it (M. Conter), cristina.bacci@unipr.it (C. Bacci).

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1. Introduction

Enterococci are Gram-positive lactic acid bacteria (LAB) belonging to the normal microbiota of animals and humans and that are able to survive in biotic and abiotic environments [1]. They are generally non-pathogenic, but some species of *Enterococcus* can cause nosocomial infections in immunocompromised patients [2–4].

Enterococci are either intrinsically resistant or acquire resistance to many antimicrobials used to treat human infections and Antimicrobial resistance (AMR) is becoming a significant public health concern [5]. Resistance to vancomycin requires particular attention due to its use against Multi Drug Resistant Gram-positive bacteria [6] and the World Health Organization (WHO) has classified vancomycin as a “Highest Priority” Critical Important Antimicrobial for human health [7]. Vancomycin belongs to the glycopeptide class of antimicrobials, whose mechanism of action consists in the inhibition of bacterial cell wall peptidoglycan chain cross-linking by binding the NAM-pentapeptide final residues (*d*-Ala-d-Ala). Resistance occurs when this dipeptide is modified in *d*-Ala-d-Lac (high resistance level) or in *d*-Ala-d-Ser (low resistance level) and vancomycin affinity to target is reduced [8]. It is mediated by eight different acquired *van* operons (*vanA*, *vanB*, *vanD*, *vanE*, *vanG*, *vanL*, *vanM*, and *vanN*) and one intrinsic operon *vanC*, with its three variants *vanC1, vanC2 and vanC3* [9].

Enterococci are frequently found in animal- and plant-derived foods, posing a risk to consumers [10]. Indeed, *Enterococci* are considered as hygienic indicators in food due to possible fecal contamination of meat products during slaughter or from environmental contamination. Furthermore, these bacteria are used during food fermentation (e.g. cheese, fermented meat) and as probiotics to improve human health [11] and are present in food spoilage. *Enterococci* can survive pasteurization temperatures, can grow in 6.5 % NaCl and at pH 9.6 and therefore can be easily found in meat products and ready-to-eat foods [12]. The genus *Enterococcus* contains 61 species [13] and over 90 % of the species found in food are *Enterococcus faecalis* and *Enterococcus faecium*, which are frequently associated with nosocomial infections in humans. Other species, such as *E. hirae*, *E. avium*, *E. durans*, *E. raffinosus, E. gallinarum*, *E. casseliflavus*, and *E. flavescens* are considered opportunistic bacteria, but the last three species have been reported as intrinsically non-susceptible to vancomycin harbouring VanC operon [14].

These characteristics and the ubiquitous behavior make *Enterococci* good sentinel bacteria for antibiotic resistance surveillance [15]. The direct transmission of resistant bacteria from animals to humans contributes to AMR, particularly from food producing animals [16] and their derived meat [17–19]. Thus, surveillance needs to have a multi-sectorial approach, including the role of animal food [20].

The spread of AMR is leading by the persistence of Enterococci which is promoted by virulence determinants, the most common are *asa1* (aggregation substance), *cylA* (cytolysin), *esp* (enterococcal surface protein), *hyl* (hyaluronidase) and *gelE* (gelatinase) [21].

Pork meat is widely consumed around the world and the use of antimicrobials during pig farming favors the development of resistant bacteria [22]. Meat is considered sterile in healthy animals, but during carcass dressing and retail, products can be cross contaminated by different sources such as skin and gut microorganisms of slaughtered animals, by human workers hands that can be a vehicle of bacteria during working processing, and from the slaughter environment in contact with carcasses [23,24]. Furthermore, pig farms produce a vast amount of pig manure and farm wastewater, which are released into the environment as fertilizer or waste products and which can contain antimicrobial residues or antimicrobial resistant bacteria [25]. Finally, the increasing anthropogenic transformation of the landscape has forced wildlife into greater contact with humans and their livestock, increasing the AMR transmission risk to different populations [14]. Antimicrobial resistance has already been reported in commensal bacteria in wildlife animals [26] and their meat products [18].

In this context, the present study is aimed at evaluating the role of meat as a transmission route of antimicrobial resistant *Enterococci* from animals to consumers by comparing meat from *antimicrobial-treated farmed* pigs and *antimicrobial-free* wild boars.

2. Material and methods

2.1. Sample collection

Meat samples were collected by the Istituto Zooprofilattico Sperimentale della Lombardia e Emilia Romagna (IZSLER) located in Reggio Emilia, Italy (44°42′34″56 N, 10°37′13″80 E) between September 2021 and April 2022. The sample set was composed of 598 pork and 404 wild boar fresh meat samples collected from processing companies and slaughterhouses. Samples were given to IZSLER by official veterinarians for official analysis on food products and in the context, *Enterococci* isolation was performed.

2.2. Enterococci isolation

Enterococci isolation on the food samples reported previously was performed by the IZSLER following the procedures of the International Commission on Microbiological Specifications for Foods [27].

Enterococci were isolated on Kanamycin Aesculin Azide Agar (KAA Biolife Italiana, Milan, Italy) designed by Mossel et al., 1978 [28] to detect *Enterococci* in foodstuffs.

For all the sample types, from each plate, a single typical colony (presumptive *Enterococci*) was considered: round, white or grey colonies about 2 mm in diameter, surrounded by black zones of at least 1 cm diameter. Incubation is carried out aerobically at 37 °C ± 1 °C for 18–24 h. Confirmation of presumptive *Enterococci* was achieved by phenotypic and biochemicals test: Gram stain (Gram-positive cocci); catalase (−); growth (+) at 44 ± 1 °C detected in Brain Hearth Infusion Broth (BHI broth, BHI Biolife - Italia) after 48 ± 2 h; growth (+) in BHI containing 6.5 % NaCl detected after incubation at 37 ± 1 °C for 48 ± 2 h.

Strains were sent to the laboratory of Food Hygiene and Inspection of the Veterinary Science Department, University of Parma where biomolecular confirmation testing through end point PCR was performed following Foka et al., 2019 [29] with some modifications (see Table 1).

2.3. Antimicrobial resistance evaluation

Resistance to vancomycin was evaluated in all isolates through Minimum Inhibitory Concentration (MIC) detection determined by standard broth micro-dilution method following EUCAST, 2023 [30].

Concentration from 0.125 μg/mL to 256 μg/mL were tested. As defined by EUCAST, 2023 [30], the break point determining non-susceptibility of *Enterococci* to vancomycin is > 4 μg/mL (IC 95 %; 2–8 μg/mL following ECOFF).

De Moura et al., 2013 [31] and Schwaiger et al., 2012 [32] reported that the presence of different Van operon changes the resistance MIC range in *Enterococci*. In fact, the presence of VanA determine a MIC value of 64–256 μg/mL, VanB determine a MIC of 64–128 μg/mL and VanC1/2 of 2–32 μg/mL. These cut off values were considered to select resistant isolates for further analysis.

All resistant strains were analysed genotypically by multiplex PCR and *vanA*, *vanB* and *vanC1/2* genes presence was evaluated (Table 1).

The *Enterococci* harboring vancomycin-related resistance genes were further analysed to evaluate virulence profiles by PCR protocol reported in Table 1 (*asa1, gelE, cylA, esp, hyl*). Resistant pathogens were then classified in species using biochemical gallery RapidID 32 STREP (bioMérieux Italia Spa, Florence, Italy).

2.4. Statistical analysis

To evaluate the statistical difference between the variables considered, the *p* value was calculated (MedCalc Software Ltd.–free version, Ostend, Belgium). Particularly, the percentage of *Enterococci* isolation and the MIC values obtained from the analysis of pork and wild boar *Enterococcus* were compared using a Chi-Square test, and a p value < 0.05 was considered statistically significant. Using the same method, the statistical difference between the presence/absence of resistance genes in resistant strains was evaluated.

The data collected must respect the following relation to be statistically considered:

$$n > 30;\ np > 5, n(1 - p) > 5$$

n = the number of animals; p = the proportion of *Enterococci* strains with the characteristics that are being studied.

Table 1
PCR conditions for the detection of vancomycin resistance genes and virulence genes.

Genes	Primers	Sequences (5′-3′)	Size (bp)	PCR conditions		Reference
16S RRNA	16S rRNA	F: TGCATTAGCTAGTTGGTG R: TTAAGAAACCGCCTGCGC	356	Denaturation 95 °C for 4 min, 30 cycles at 95 °C 30s, 54 °C 60s, 72 °C 60s, and 72 °C 10 min	Final volume 25 μL: 1x Green GoTaq Flexi Buffer, 2 mM of $MgCl_2$, 0.2 mM of each dNTPs, 1.5 U of GoTaq G2 Flexi DNA Polymerase, primers at 1 μM,1 μL of DNA sample, Nuclease Free Water to final volume	Foka and Ateba, 2019
Vancomycin resistance genes	*VanA*	F:GGGAAAACGACAATTGC R:GTACAATGCGGCCGTTA	732	Denaturation 94 °C 3 min, 30 cycles at 94 °C 30 s, 54 °C 60 s, 72 °C 60 s, and 72 °C 10 min	Final volume 25 μL: 1x Green GoTaq Flexi Buffer, 2 mM of $MgCl_2$, 0.2 mM of each dNTPs, 1.5 U of GoTaq G2 Flexi DNA Polymerase, primers at 1 μM,1 μL of DNA sample, Nuclease Free Water to final volume	Foka and Ateba, 2019
	VanB	F:ACGGAATGGGAAGCCGAR: TGCACCCGATTTCGTTC	647			
	$VanC_{1/2}$	F:ATGGATTGGTAYTKGTATR: TAGCGGGAGTGMCYMGTAA	815/827			
Virulence genes	*asa1*	F:GCACGCTATTACGAACTATGA R: TAAGAAAGAACATCACCACGA	375	Denaturation 95 °C 3 min, 30 cycles at 95 °C 30 s, 55 °C 30 s, 72 °C 60 s, and 72 °C 10 min	Final volume 25 μL: 1x Green GoTaq Flexi Buffer, 2 mM of $MgCl_2$, 0.2 mM of each dNTPs, 1.5 U of GoTaq G2 Flexi DNA Polymerase, primers at 0.2 μM (asa1, gelE), 0.4 μM (cylA, esp, hyl),5 μL of DNA sample, Nuclease Free Water to final volume	Foka and Ateba, 2019
	gelE	F:TATGACAATGCTTTTTGGGATR: AGATGCACCCGAAATAATATA	213			
	cylA	F:ACTCGGGGATTGATAGGCR: GCTGCTAAAGCTGCGCTT	688			
	esp	F:AGATTTCATCTTTGATTCTTGG R:AATTGATTCTTTAGCATCTGG	510			
	hyl	F:ACAGAAGAGCTGCAGGAAATG R:GACTGACGTCCAAGTTTCCAA	278			

3. Results

3.1. Enterococci isolation from pork and wild boar meat

The protocol described above resulted in the isolation of 341/598 (57 %) *Enterococci* from pork and 173/404 (42.8 %) from wild boar meat. The difference between the two isolation rates and the animal species was statistically significant ($P < 0.001$).

3.2. Antimicrobial resistance evaluation

In the present study, all the isolates showed MIC values at lower antimicrobial concentration than break point resistance values (MIC >4 μg/mL). Isolates from pork and wild boar meat showed MIC = 4 μg/mL in 76/341 (22.3 %) and 83/173 (48 %), respectively. In addition, 89/341 (26.1 %) and 8/173 (4.6 %) isolates from pork and wild boar samples respectively had MIC = 2 μg/mL. Statistically significant differences were found between pork and wild boar enterococci for both MIC values ($P < 0.001$) with a prevalence of wild boar enterococci with MIC = 4 μg/mL and a prevalence of pork enterococci with MIC = 2 μg/mL. No statistical differences was found between pork and wild boar enterococci carry low vancomycin resistance.

As mentioned above, the strains showing MIC = 4 μg/mL and MIC = 2 μg/mL were considered for further analysis. The isolates that showed MIC values < 2 μg/mL were considered completely susceptible to vancomycin (Fig. 1).

The total *Enterococci* considered for detection of AMR-related genes were 165/341 (48.4 %) isolated from pork and 91/173 (52.6 %) isolated from wild boar meat.

VanC1/2 was the only gene detected and was harboured by 56/165 (33.9 %) pork meat isolates; 38/56 (67.8 %) strains had MIC = 4 μg/mL and 18/56 (32.1 %) had MIC = 2 μg/mL (Table 2). A statistically significant difference was found ($P = 0.001$) between the presence of *vanC1/2* gene and the MIC values.

Among bacteria isolated from wild boar meat, 4/91 (4.4 %) harboured *vanC1/2* gene and all the strains had MIC = 4 μg/mL (Table 2); no statistical difference was found.

Among the 56 *vanC Enterococci* isolated from pork, 38/56 (67.8 %) harboured virulence genes: 34/38 (89.5 %) *gelE*, 1/38 (2.6 %) *gelE* + *asa1*, 1/38 (2.6 %) *asa1*, 1/38 (2.6 %) *hyl* and 1/38 (2.6 %) *esp*.

Three out of four (75 %) *vanC Enterococci* isolated from wild boar meat harboured virulence genes, in particular 1/3 (33.3 %) *esp* and 2/3 (66.7 %) *asa1*.

Virulent strains isolated from pork were mostly *E. gallinarum* (30/38–78.9 %), 5/38 (13.1 %) strains were *E. casseliflavus*, 2/38 (5.3 %) *E. faecalis* and 1/38 (2.6 %) *E. faecium*.

Pathogens isolated from wild boar meat were classified as *E. casseliflavus* (2/3–66.7 %) and *E. faecalis* (1/3–33.3 %).

4. Discussion

According to the United Nations Food and Agriculture Organization, pork is the most widely consumed meat in the world (36 %) [22]. The production of pork in Europe is approximately 23.7 million tons/year and in Italy the consumption is 27.9 kg/person/year [33]. The emergence of vancomycin resistant *Enterococci* (VRE) in food products across Europe was first reported in the 1990s, with the *vanA* genotype. Different studies associated the phenomenon to the inappropriate use of the avoparcin glycopeptide as growth promoter, which lead to selection of VRE in pigs and poultry [34–36]. These bacteria were consequently found in high rates in animal feces and in their derived food products [14]. The use of avoparcin was banned in 1997. However, VRE persisted in meat products, even if at lower rates [37]. The monitoring of VRE is currently a low priority; despite this, it is recommended because *Enterococci* are indicator microorganisms that can be used as sentinels of Gram-positive resistance incidence [38]. Our study evaluated the presence of VRE in pork and wild boar meat products to monitor the differences between food derived from farmed animals and wildlife.

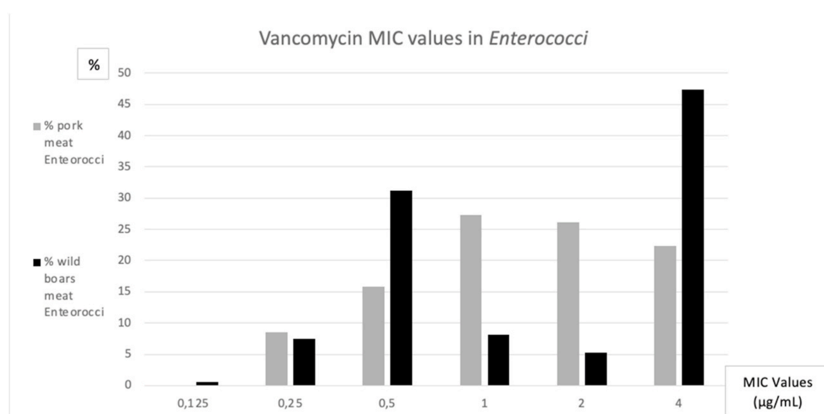

Fig. 1. Vancomycin MIC values in *Enterococci* isolated from pork and wild boar meat.

Table 2
Minimum Inhibitory Concentration (MIC) values, genotypic characteristic, and species of *Enterococci* with low resistance profile. (–) = not tested.

SAMPLES	N° *ENTEROCOCCI*	MIC (MG/ML)	Resistance genes	Virulence genes	Species
Pork meat	25	4	*vanC1/2*	*gelE*	*E. gallinarum*
	1	4	*vanC1/2*	*gelE*	*E. faecium*
	1	4	*vanC1/2*	*gelE- asa1*	*E. faecalis*
	1	4	*vanC1/2*	*hyl*	*E. casseliflavus*
	10	4	*vanC1/2*	*ABSENT*	-
	38	4	*ABSENT*	–	-
	5	2	*vanC1/2*	*gelE*	*E. gallinarum*
	3	2	*vanC1/2*	*gelE*	*E. casseliflavus*
	1	2	*vanC1/2*	*esp*	*E. faecalis*
	1	2	*vanC1/2*	*asa1*	*E. casseliflavus*
	8	2	*vanC1/2*	*ABSENT*	–
	71	2	*ABSENT*	–	–
Total	**165**				
Wild boar meat	1	4	*vanC1/2*	*asa1*	*E. casseliflavus*
	1	4	*vanC1/2*	*asa1*	*E. faecalis*
	1	4	*vanC1/2*	*esp*	*E. casseliflavus*
	1	4	*vanC1/2*	*ABSENT*	-
	79	4	*ABSENT*	-	-
	8	2	*ABSENT*	–	-
Total	**91**				

The prevalence of *Enterococci* in pork has been reported to be as high as 70 % [1,39], while in the present study it was lower (57 %). The *Enterococci* isolation rate in wild boar meat has been reported at 100 % [40], while in the present study the prevalence was 42.8 %. *Enterococci* can cause food intoxications because of biogenic amines production and can cause opportunistic infections associated worried by AMR characteristics [41]. The difference in prevalence between pork and wild boar can be due to their distinct lifestyle including environment, feed and hygienic condition during slaughtering [42].

Several authors have reported that resistance to vancomycin has a high variability, ranging from 5.6 % to 25 % in pork *Enterococci* [1,8,10,39]. In the present study, no resistant strains were found, but 48.4 % of pork isolates showed a low resistant profile (2 μg/mL ≤ MIC≤4 μg/mL).

Studies of the potential role of wild boar meat in the transmission of VREs to consumers are fewer. Guerrero-Ramos et al. (2016) [40] reported a rate of 48 % of resistant bacteria but in the present study, 52.6 % of strains showed a low resistance profile.

Low vancomycin resistance levels are usually reported in *Enterococci,* and they can be principally associated with the presence of intrinsic resistance (*vanC* gene) that confers a MIC value from 2 μg/mL [43]. The results from the present study would confirm this, with low resistance related to *vanC* presence in 33.9 % and 4.4 % of pork and wild boar meat isolates, respectively. The difference in *vanC* presence in pork and wild boar meat *Enterococci* was statistically significant. This may be due to environmental factors: wild boars live in an environment in which bacteria are subjected to less selective pressure from antibiotics. Indeed, was demonstrated that enterococci isolated from intensive farm carry more AMR than enterococci isolated from organic-extensive farms, and this highlight the production system impact on AMR dissemination in food chain [44]. *VanA* and *vanB* genes are usually located on plasmids, and different studies have reported the transmission risk of mobile genetic elements between bacteria both in pork and wild boar meat isolates [40]. Comfortingly, in accordance to the low MIC level found, no *vanA* and *vanB* genes were detected and the majority of low VRE did not harbour any of the resistance genes tested in this study.

The 66.1 % pork isolates and 95.7 % wild boar isolates did not harbour any resistance gene suggesting a different selective pressure that leads to a more aspecific or rare resistance mechanism in wildlife when compared to pork isolates [18].

Previous studies have reported that the most common VRE isolates from meat belong to *E. faecalis* and *E. faecium* and that they show high resistance levels associated with genes *vanA* and *vanB* [45]. Data found in this study highlight different prevalences: only one low VRE isolated from pork belonged to *E. faecium* and two to *E. faecalis*; only one *E. faecalis* was detect in low VRE isolated from wild boar meat. It is known that low resistance rates to vancomycin is usually not related to *E. faecium* and *E. faecalis* species [46].

On the other hand, *E. gallinarum* and *E. casseliflavus* isolates frequently have vancomycin MICs ranging from 2 to 16 μg/mL and 4–16 μg/mL, respectively [47]. In this study, high presence of *E. gallinarum* and *E. casseliflavus* was found in pork and wild boar meat isolates with a MIC of 4 μg/mL. Low MICs levels are frequently related to the presence of intrinsic VanC genes [47], as reported in this study.

Infection caused by these enterococcal species as rare and commonly related to immunocompromised patients however the intrinsic low vancomycin resistance can be a therapeutic challenge. Indeed vancomycin monotherapy to treat low resistance vancomycin enterococci infection was associated with clinical failure [43].

The co-presence of antimicrobial resistance and bacterial pathogenicity represents a public health risk [48]. Different studies suggest that there is a relation between resistance and virulence determinants in clinical and in food producing animal isolates [41]. This can be due to antimicrobial treatments that favor the co-selection of both characteristics [5,49]. Moreover, this suggests the possibility of co-transfer of these genetic elements to other bacteria present in the same environment [50].

In our study, the pathogenicity was evaluated only in bacteria with evaluated resistant profile (all the strains evaluated were low

VRE as reported before). In particular, pathogenicity related genes *asa1*, *gelE, hyl, esp* were found in pork low VRE and *asa1, esp* in wild boar low VRE, in accordance to previous studies [5,51]. Frequently, the pathogenicity is related to *E. faecium* and *E. faecalis* [50], instead in the present study virulence genes are found also in *E. gallinarum* and *E. casseliflavus* strains. Although uncommon, *E. casseliflavus* infection can be seriously invasive and bacteremia is the most common form of infection [52]. *asa1* gene encode for the aggregation substance which facilitate the conjugative transfer of plasmid, *esp* gene encoded for the enterococcal surface protein which increased colonization and biofilm formation capability. Extracellular hydrolyzing protein are encoded by *gelE* and *hyl* genes [53].

The presence of these virulence genes in pork and wild boar meat *Enterococci* reduces the food safety. Moreover the presence of the same virulence factors in *Enterococci* isolated form food and from human clinical samples was demonstrated [54]. *Enterococci* are common residents of gastrointestinal tract of animals including humans but pathogenicity of those strains can cause severe infections worsend by antimicrobial resistance phenomenon. Moreover infections are usually related to antibiotic-treated hospitalized patients with perturbed intestinal microbiota giving a higher risk for human health [55]. To date, only a few studies have investigated the distribution and antibiotic susceptibility of *Enterococcus* species in wildlife, especially in wild boar meat products. This study shows that the presence of low resistance profile is common but rarely associated with the presence of resistance genes. Nevertheless, the presence of the latter is frequently related to pathogenic genes detection, increasing bacterial persistence and AMR dissemination as reported in previous studies [56]. Monitoring of this phenomenon is necessary, considering the current lack of data, the evidence of the increasing interest in consumption of wild game meat [57] and the frequent contact between domestic and wild animal species [58]. The limit of the present study is related to the possibility of testing all samples for virulence genes and *Enterococci* species and not only in resistant ones. It has been suggested that there is a relation between the AMR prevalence in bacteria isolated from wild animals and the level of their contact with human populations and farm animals [59].

Moreover, to reduce pork and wild boar meat bacterial contamination good manufacturing practices, good hygiene practices and sanitation procedure are required by food law in Europe [60].

Additionally, to improve the food safety has to be done a collective and integrative effort between food business operator, national competent authority, international agencies and consumers. The latter have responsibility in domestic environment and food preparation and they need to be aware of food risks [61].

5. Conclusion

As evidenced, *Enterococci* isolates in farmed pigs and wildlife animal meat can be a reservoir of virulence and AMR genes. The possible transmission of genetic elements can be a risk to humans and the direct transmission of bacteria cell through meat consumption is a risk to consumers. For this reason, wild animal-derived meat can be an opportunity to monitor the impact of the antibiotics usage upon the environment and can be a sentinel to monitor the phenomenon of AMR. The results reported here are comforting, highlighting the presence of low resistance levels and low prevalence of resistance genes. Despite this, further surveillance studies are needed to better understand the emergence and spread of VRE and pathogenic *Enterococci* directly from food of animal origin to humans and the role of wildlife in this phenomenon.

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CRediT authorship contribution statement

Laura Andriani: Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Martina Rega:** Writing – original draft, Investigation, Formal analysis, Conceptualization. **Paolo Bonilauri:** Resources, Project administration, Conceptualization. **Giovanni Pupillo:** Resources. **Giorgia De Lorenzi:** Resources. **Silvia Bonardi:** Visualization, Supervision. **Mauro Conter:** Writing – review & editing. **Cristina Bacci:** Visualization, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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