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Determination of Genes Encoding Antibiotic and Copper Resistance in Enterococcus Originated from Different Ecosystems

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Introduction

- Antibiotic resistance is a global problem for human and animal health, and food can serve as a vector for the transmission of antimicrobial-resistant bacteria. Therefore, it is important to determine the presence of genes encoding for resistance for such heavy metals e.g., copper and linked antibiotics.
- Bacteria of the genus Enterococcus are an integral part of the gastrointestinal (GI) tract of the animal microbiome and can be found in fermented meat and dairy products, mostly due to poor food handling and their ability to grow under extreme conditions. Bacterial infections are treated with antibiotics, and the rise of enterococci, which are resistant to a variety of antibiotics and have the ability to transfer genetic material, is a notable problem in modern medicine. The uptake of heavy metals in animal feed and resistance to them has been associated with resistance to glycopeptides and macrolides for many years.
- The enterococcal isolates were obtained from the faeces of wild boar (*Sus scofa*) (n = 30), spontaneously fermented sausages produced from the meat of wild boar and domestic pigs (*Sus domesticus*) (n = 10), and traditionally produced Istrian cheese (n = 5).

Aim

- This study aimed to investigate the genetic basis of acquired resistance to two antibiotics and heavy metal copper, in enterococcal isolates from diverse ecological sources.
- Specifically, we employed the multiplex PCR method to detect and confirm the presence of resistance genes in enterococci isolated from wild boar feces and animal-derived food products, including sausages and cheese.
- Antibiotic-resistant genes for **erythromycin** and **tetracycline** and those for heavy metal **copper** were evaluated using multiplex PCR methods.

Methodology

- The genomic DNA used in this study was selected based on the results of our previous research. Isolates were cultured on selective media, and their identification was determined through sequencing (Table 1.).
- To identify genes encoding resistance to erythromycin, tetracycline and copper (Table 2.), a reaction mixture with a total volume of 25 µL was prepared using two pairs of oligonucleotide primers as suggested by Mazaheri Nezhad Fard *et al.*, 2011.
- The amplification was checked by horizontal gel electrophoresis in a 1% agarose gel.

Table 1. Species of Enterococcus isolates for the detection of antibiotic and copper resistance genes collected from wild boar feces, sausage and cheese.

FECES		SAUSAGE	CHEESE
1. *E. durans*	16. *E. hirae*	1. *E. faecalis*	1. *E. faecalis*
2. *E. durans*	17. *E. faecalis*	2. *E. faecalis*	2. *E. faecium*
3. *E. durans*	18. *E. hirae*	3. *E. faecium*	3. *E. faecium*
4. *E. durans*	19. *E. durans*	4. *E. faecium*	4. *E. durans*
5. *E. durans*	20. *E. faecalis*	5. *E. faecium*	5. *E. casseliflavus*
6. *E. durans*	21. *E. gallinarum*	6. *E. faecium*	
7. *E. durans*	22. *E. gallinarum*	7. *E. durans*	
8. *E. durans*	23. *E. faecalis*	8. *E. casseliflavus*	
9. *E. durans*	24. *E. durans*	9. *E. casseliflavus*	
10. *E. durans*	25. *E. faecium*	10. *E. casseliflavus*	
11. *E. faecalis*	26. *E. faecalis*		
12. *E. faecalis*	27. *E. hirae*		
13. *E. faecalis*	28. *E. faecium*		
14. *E. faecalis*	29. *E. faecalis*		
15. *E. faecalis*	30. *E. faecalis*		

Table 2. Oligonucleotide primers used in the PCR reaction for the detection of genes encoding erythromycin, tetracycline, and copper.

Antibiotic / Heavy metal	Primer for the gene	Sequences (5'→3')	Fragment size	Reference
Erythromycin	*ermB*	F 5′-CATTTAACGACGAAACTGGC-3′ R 5′-GGAACATCTGTGGTATGGCG-3′	738 bp	Jensen et al., (1999)
Tetracycline	*tet*(M)	F 5′-GTTAAATAGTGTTCTTGGAG-3′ R 5′-CTAAGATATGGCTCTAACAA-3′	657 bp	Aarestrup et al., (2000)
Copper	*tcrB*	F 5′-CATCACGGTAGCTTTAAGGAGATTTTC-3′ R 5′-ATAGAGGACTCCGCCACCATTG-3′	663 bp	Hasman et al., (2006)

Results

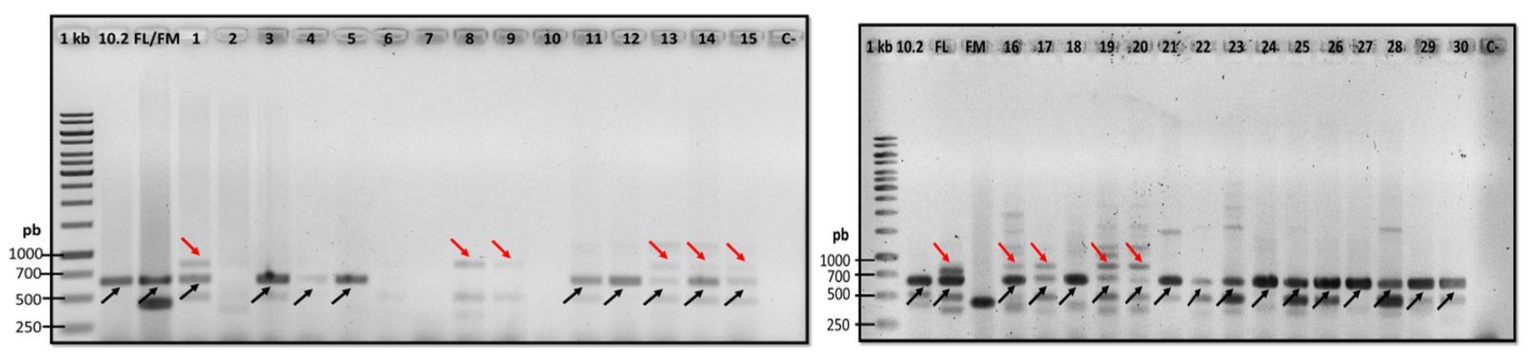

Figure 1. The PCR products of 738 bp *(ermB*) and 657 bp *tet*(M) confirm erythromycin and tetracycline gene presence in isolates (1-30) collected from wild boar feces. Similar bands were appreciated in other samples (*data not shown*). For positive control (10.2. and FL) we used strains of enterococci identified by sequencing as carriers of resistance genes for at least one mentioned antibiotic.

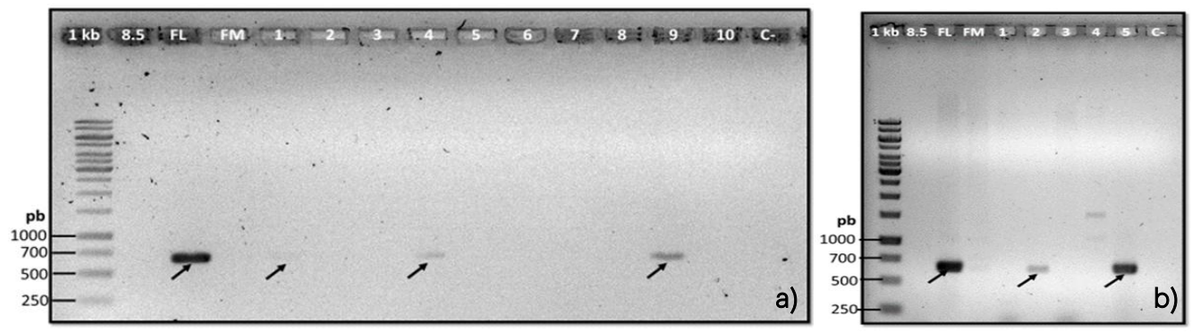

Figure 2. The PCR products of 663 bp (*tcrB*) confirm copper resistance in isolates a) (1-10) collected from sausage and b) isolates (1-5) collected from cheese samples. Similar bands were appreciated in other samples (*data not shown*). For positive control (FL) we used strain of enterococci identified by sequencing as carriers of resistance genes for the indicated heavy metal copper.

Conclusion

- This approach provides a comprehensive assessment of the distribution of resistance determinants across different environments, highlighting potential reservoirs of antimicrobial resistance within the food chain.
- The gene coding for **erythromycin** was found in 33.33% of fecal isolates, in 20% of cheese isolates, and in 10% of sausage isolates, and the gene coding for **tetracycline** was found in 80% of fecal isolates, in 20% of sausage isolates, and in none of the cheese isolates. A gene coding for **copper** was detected in 56.66% of the fecal isolates, then in 40% of the cheese isolates, and 30% of the sausage isolates.
- Our results suggest that genes encoding for acquired resistance to antibiotics and the heavy metal copper are present in the collected enterococcal isolates, and that this should be taken into consideration as a significant public health concern, given the potential for cross-resistance and horizontal gene transfer.

Reference

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