

Dissemination of *Enterococcus* spp. across One Health Settings: Insights from fingerprinting analysis

Joana Monteiro Marques^{1,2*}, Nicolau Fialho^{1,2*}, Maria do Mar Duarte^{1,2*}, Maria Teresa Barreto-Crespo^{3,4}, Teresa Semedo-Lemsaddek^{1,2,5}

*Equal contributors to the study

¹ Center for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal.

² Associate Laboratory for Animal and Veterinary Sciences (AL4Animals), Portugal.

³ Institute of Experimental Biology and Technology (IBET), Oeiras, Portugal.

⁴ Institute of Chemical and Biological Technology António Xavier (ITQB), Nova University of Lisbon, Oeiras, Portugal.

⁵ Biosystems & Integrative Sciences Institute (BioISI), Faculty of Sciences, University of Lisbon, Lisbon, Portugal.

INTRODUCTION & AIM

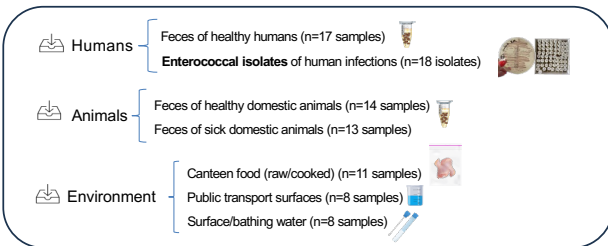
Enterococcus spp. are ubiquitous Gram-positive bacteria present in the gastrointestinal tract of humans and animals, as well as a widely distributed in the environmental sector, being present in the soil, plants, food, water and sewage through faecal contamination.

Over recent decades, enterococci have emerged as significant opportunistic pathogens in healthcare-associated infections, due to their rapid adaptation to their host and high tendency to acquire both virulence and antibiotic resistance genes. Among the species of this genus, the vancomycin-resistant *E. faecium* were identified as a critical health threat. In this context, the One Health concept underscores the interconnection of human, animal and environmental health, highlighting the importance of intervention fields such as antimicrobial resistance and food safety.

This study aims to isolate and analyse the genetic diversity of *Enterococcus* spp. recovered from a diverse range of samples across One Health sectors, using RAPD-PCR.

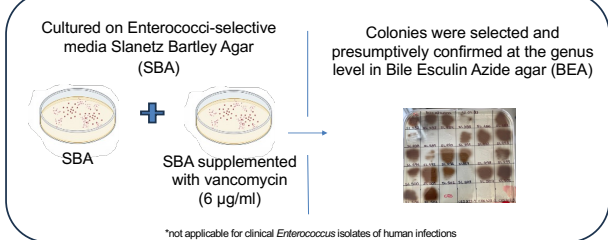
METHOD

Sampling
n = 89



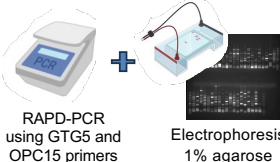
Isolation

If needed:
> Samples were homogenized in Maximum Recovery Broth
> Overnight enrichment at 37 °C

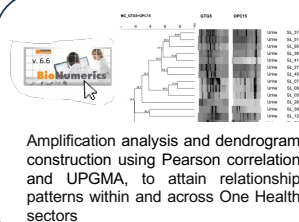


DNA Extraction and RAPD-PCR

DNA extraction of ≈20% colonies (boiling method)



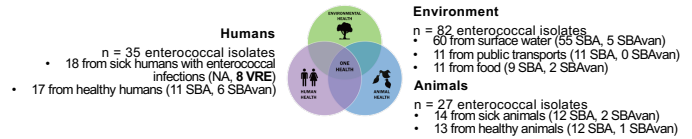
Fingerprinting analysis Genetic diversity



RESULTS & DISCUSSION

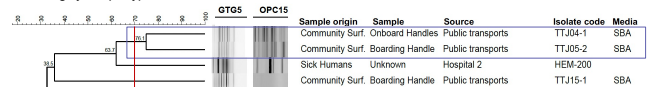
Collection of enterococcal isolates

148 *Enterococcus* isolates (presumptively confirmed in BEA) → 16 *Enterococcus* from vancomycin supplemented media SBAvan (12%) → 8 VRE from human infections (44% of infection isolates)

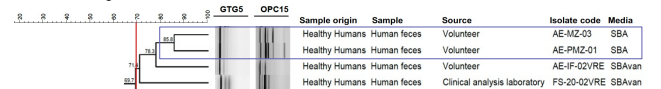


RAPD-PCR Genetic diversity analysis

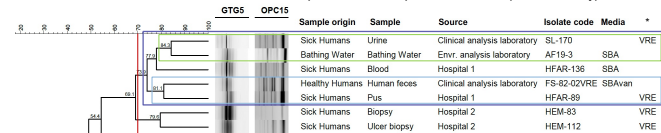
• Clustering by sample type – isolates from different samples at the same setting share similar profiles (>76% similarity).



• Cluster containing isolates from healthy humans. Isolates highlighted belong to related individuals (>85% similarity).



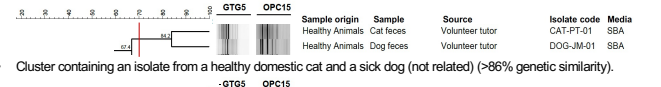
• Cluster of isolates from enterococcal infections (clinical), healthy humans and surface water (purple) (>85% similarity).
• Cluster composed of on VRE clinical isolate and surface water (green) (>84% similarity).
• Cluster containing isolates from a healthy and a sick human (blue) (>81% similarity).
• Cluster of two clinical VRE isolates from the same hospital, but different patients and dates (>79% similarity).



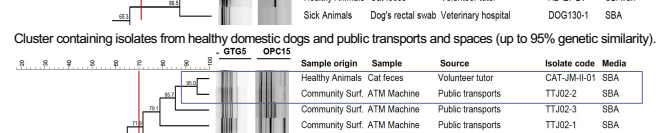
• Cluster formed of isolates from surface water and a clinical VRE isolate from a blood infection (70% similarity).



• Cluster containing isolates from healthy domestic animals from different households (>84% genetic similarity).



• Cluster containing isolates from healthy domestic dogs and public transports and spaces (up to 95% genetic similarity).



* Column only applicable for clinical isolates of enterococcal infections. Reproducibility level was set at 70% (red line).

CONCLUSION

- This study used RAPD-PCR to analyze the genetic relatedness of *Enterococcus* isolates across settings and explore their dissemination and foresee potential reservoirs of vancomycin resistance in clinical isolates.
- Overall, no significant clusters by sample type or One Health sector were highlighted in the dendrogram, suggesting the identification of genetically diverse enterococci across settings.
- However, RAPD analysis suggested that similar *Enterococcus* were found in surface water and clinical isolates, isolates from domestic animals and public transports and spaces, and healthy and sick animals.
- This study confirmed the widespread dissemination of similar enterococci across specific One Health sectors, including presumptive vancomycin-resistant enterococci.

FUTURE WORK / REFERENCES

- Characterize the pathogenic potential of these isolates:
 - Antibiotic susceptibility testing and resistance gene confirmation by Multiplex PCR
 - Presence of virulence factors
- Selection of isolates for WGS based on pathogenicity and genetic relationship between One Health sectors

References:
Monteiro Marques, J., Coelho, M., Santana, A. R., Pinto, D. & Semedo-Lemsaddek, T. Dissemination of Enterococcal Genetic Lineages: A One Health Perspective. *Antibiotics* 12, 1140 (2023).
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