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ECM 2023 – Abstract

2 Isolation and characterization of *Enterococcus*

<u>spp. from animal settings: surveillance for</u> antimicrobial resistance

5 Daniel Pinto^{1,2}, Joana Monteiro Marques^{*1,2}, Maria Teresa Barreto-Crespo^{3,4} and Teresa Semedo-Lemsaddek^{*1,2}

- 6 1: Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon.
 7 Av. Universidade Técnica, 1300-477 Lisboa
- 8 e-mail: {daniel.fs.pinto@tecnico.ulisboa.pt; jmarques@fmv.ulisboa.pt; tcrespo@ibet.pt
- 9 tlemsaddek@fmv.ulisboa.pt}, web: https://ciisa.fmv.ulisboa.pt/en/.
- 10 2: Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Portugal.
- 11 3: iBET Institute of Experimental Biology and Technology, PO box 12, 2781-901 Oeiras, Portugal.
- 12 4: ITQB NOVA Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Avenida
- 13 da República, 2780-157 Oeiras, Portugal. e-mail: <u>tcrespo@ibet.pt</u>
- 14 * Equal contributors to the work.
- 15

Enterococcus spp. are ubiquitous bacteria that inhabit the normal intestinal microbiota of most animals and are widespread in the environment. Enterococci have emerged as opportunistic pathogens, due to rapid adaptation features and ability to acquire virulence and antibiotic resistance genes. Considering the low numbers of investigations on the characterization of enterococcal isolates from animals and animal-related samples, the goal of the present study was to isolate enterococci from those settings and evaluate the associated antibiotic susceptibility.

Briefly, enterococci were isolated from faecal samples of stabled animals (horses, cows, and goats), healthy domestic cats and dogs, and from surface swabs and dogs' anal exudates at a veterinary teaching hospital. Each sample was spread-plated in enterococcal selective media, Slanetz and Bartley agar, with/without vancomycin supplementation. Subsequently, 20% of the characteristic colonies were selected, submitted to DNA extraction and genetic comparison using RAPD-PCR. RAPD-pattern analysis and dendrogram construction allowed the selection of 110 genomically distinct representative isolates, further characterized for antibiotic susceptibility.

Regarding the results, around 3% were found to be vancomycin-resistant (two from goat and one from a domestic cat), while 10% were considered multidrug-resistant (isolates from one goat, one dogs' scale, 1 tickets' machine and 8 dogs' anal exudates). Most enterococci recovered from stabled animals, and some from domestic dogs/cats, were classified as susceptible to all tested antibiotics.

33 Overall, relevant antibiotic resistances were detected, and resistance phenotypes were found to be34 disseminated among distinct sample sources.

35 Keywords: *Enterococcus*, animals, environment, antibiotic resistance, genetic lineages

This study was supported by FCT – Fundação para a Ciência e Tecnologia IP Portugal, through projects,
UIDB/00276/2020 (CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary
Medicine, University of Lisbon) and LA/P/0059/2020-AL4ANIMALS (AL4AnimalS). Teresa SemedoLemsaddek is financially supported by national funds through FCT under the Transitional Standard –
DL57/2016/CP1438/CT0004, Daniel Florindo dos Santos Pinto holds a master fellowship – CIISA/2023/2 –
supported by national funds Joana Monteiro Marques holds a doctoral fellowship – BIPM CIISA 2/2022 –
supported by national funds