Detection of ESBL-producing *Escherichia coli* and Vancomycin Resistant Enterococci (VRE) from farm slurry in Portugal

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Keywords: Cattle slurry; extended spectrum beta-lactamase resistance; Vancomycin Resistant Enterococci.

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Use of organic manures such as cow slurry is the most economic, practical, environmentally beneficial and useful option for improving soil quality and fertility. The most common organisms that are generally found in these wastes are bacteria and fungi. These microorganisms use the components of the waste as the substrate for their growth. The different aspects of environmental and public health concerns are caused due to the abundance of antibiotics resistance (AR), and the effects of released leachate on the various environmental reservoirs and human health by this waste (Soares *et al.,* 2019 Anand *et al.,* 2021).

In this study, slurry samples on a herd level were evaluated instead of individual faeces samples from all cattle, to classify herds with regard to the presence of ESBL producing *Escherichia coli* or Vancomycin Resistant Enterococci (VRE). Herds that tested positive for ESBL producing *E. coli* in the slurry sample was defined as positive herds to ESBL and herds that tested positive for VRE in the slurry sample were defined as positive herds to ESBL. Each sample was seeded in Levine agar (Oxoid, Basingstoke, UK) plates supplemented with cefotaxime (2 mg/mL) and incubated during 24 h at 37 ºC. *E. coli* isolates were selected and identified by classical biochemical methods (Gram staining, catalase, oxidase, indol, Methyl-Red-Voges-Proskauer and citrate) and by the API 20E system (BioMérieux, La Balme Les Grottes, France). Susceptibility to 14 antimicrobial agents [ampicillin (10 µg), amoxicillin plus clavulanic acid (20+10 µg), cefotaxime (30 µg), ceftazidime (30 µg), aztreonam (30 µg), imipenem (10 µg), gentamicin (10 µg), tobramycin (10 µg), streptomycin (10 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), sulfamethoxazole-trimethoprim (25 µg), tetracycline (30 µg) and chloramphenicol (30 µg)] was determined by the disc diffusion method. ESBL-phenotypic detection was carried out by double-disc diffusion test [EUCAST, 2017]. One isolate per slurry sample were selected for further studies. To avoid isolation of intrinsically resistant enterococcal species, the samples were spread onto Slanetz Bartley agar (Oxoid) plates supplemented with 4 mg/L of vancomycin and incubated at 37 ºC for 48 hr. Colonies with typical enterococcal morphology were identified by Gram-staining, cultural characteristics, bileaesculin reaction, biochemical tests using the API ID20 Strep system, and catalase test (BioMérieux, La Balme Les Grottes, France). Antimicrobial susceptibility was tested by the disk diffusion method according to the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2017) for enterococci. Five antibiotics were tested: ampicillin (10 mg), ciprofloxacin (5 mg), tetracycline (30 mg), teicoplanin (30 mg), vancomycin (30 mg). Minimal inhibitory concentrations (MICs) of teicoplanin, vancomycin, and ampicillin were determined by the agar dilution method and by using the susceptibility breakpoints recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2017).

From the slurry samples of 7 of the 32 dairy herds, ESBL producing *E. coli* were cultured in 21.9%. From the slurry samples of 6 of the 32 dairy herds, VRE were cultured in 18.8%. Three farmers (3/32) (2, 29 and 30) simultaneously presented ESBL and VRE. Ten farms were positive at least for one microorganism under study. Amongst the strains tested, the highest percentage of resistance was to ampicillin (56.3%), followed by oxytetracycline, streptomycin and sulphonamide (41.1, 39.6 and 37.3%, respectively).

With the quantity of slurry applied on the soils as fertiliser every year, there is a need of studies to measure the leaching of pathogenic agents, antibiotics residues normally present in slurry, and their fate in the environment using for that a One Health approach. The use of high doses of antibiotics in animal feed increases the number of antibiotic-resistant bacteria that may spread their genes into the environment after slurry disposal on fields (Gonçalves *et al.*, 2010; Poeta *et al.*, 2005; Soares *et al.*, 2021).

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Soares, A.S., Miranda, C., Trindade, H. and Coelho, A.C. (2021). Microorganisms Control in Animal Effluents-A One Health Approach. *E-BOOK ISBN: 978-81-952994-2-3*