Title: IDENTIFICATION OF *Enterococcus* spp. FROM WASTEWATERS IN PARANÁ HEALTH CENTERS

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Abstract:

A great amount of environmental and clinical downsides in Brazil is derived from wastewater originated in health centers since most of the sewage produced by these entities are discharged directly in water bodies or in common sewage system without any previous treatment. This kind of wastewater presents a huge variety of microorganisms as well as antimicrobials agents, therefore providing a highly selective environment. Some Enterococcus species such as Enterococcus faecium and Enterococcus faecalis are frequently related to nosocomial infections. These microorganisms are extremely adaptable to a variety of environment conditions which make them able to survive in water bodies as well as in humans and other animals. The aim of this study was to isolate and identify *Enterococcus* spp. from wastewater of health centers of three different cities of Paraná, Brazil. We collected 30 mL of sewage of each health center and rapidly transported it to laboratory. The samples were concentrated by filtration in 0.22 µm ester cellulose membrane. These membranes were incubated in 10 mL of peptone water for 2 hours at 37 °C and following this suspension was serially diluted (1:10) until 10⁻⁵ fold. The suspensions were spread on plates containing Luria Bertani Agar. After growth, isolated colonies were submitted to presumptive identification for *Enterococcus* spp, including Gram staining, biochemical test of catalase and growth in Bile Esculin Agar - a selective and differential growth medium for Enterococcus spp. The identification was confirmed by PCR using primers for tuf gene, which are specific for genus Enterococcus spp. A total of 570 microorganisms was isolated from wastewater and 254 (44.6%) were identified as Grampositive. Catalase test revealed that 167 (29.3%) of the Gram-positive microorganisms are catalase-negative. Finally, Bile Esculin Ágar identified 75 isolates (13,2%) as Enterococcus. These data are very worrying since these microorganisms have become increasingly important as opportunistic pathogens and are often resistant to antimicrobials commonly used in clinical practice.

Keywords: *Enterococcus* spp., identification, wastewaters

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