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DIVERSITY AND ANTIBIOTIC RESISTANCE PATTERNS OF GRAM-NEGATIVE BACTERIA ISOLATED FROM WASTEWATER IN THE PRESENCE OF COPPER OR ZINC SALTS

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Some metals are nowadays worrying environmental pollutants. Although some, like copper and zinc, are essential for microorganisms, their presence in high concentrations can be toxic or exert a selective pressure in bacterial communities. Some studies propose that this selective pressure could also select for antibiotic resistant bacteria, and thus may play an important role in the maintenance and proliferation of antibiotic resistance. This is of particular concern considering that anthropogenic levels of heavy metals in the environment are currently several orders of magnitude above those of antibiotics. This study aims to investigate the effect of two metals (Cu^{2+} or Zn^{2+}) on the survival and antibiotic resistance profiles of bacteria isolated from raw wastewater (urban and hospital).

Enterobacteria were isolated on mFC agar or on this culture medium supplemented with 1 mM Cu^{2+} ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) or Zn^{2+} (ZnCl_2). Heterotrophic bacteria tolerant to the same metals were also enriched (3–5 successive transfers) from hospital effluent. Bacterial isolates were typed by RAPD-PCR, identified by 16S rRNA gene sequence analysis and characterized for antibiotic resistance. For *Escherichia coli*, the phylogenetic groups (A, B1, B2 and D) were also determined.

Enterobacterial counts were similar in urban and hospital wastewater. In both types of wastewater, the presence of metals in the culture medium inhibited the growth of enterobacteria, by up to 66% for Cu^{2+} and 98 % for Zn^{2+} . As expected, most of the isolates were *E. coli*, and the phylogenetic groups A and B2 were predominant. Resistance to ciprofloxacin, cephalothin and streptomycin was more prevalent among the isolates from Zn^{2+} supplemented culture medium than from metal-free medium.

Other heterotrophic bacteria isolated from metal enrichment cultures were identified as *Cupriavidus pauculus*, *Burkholderia anthina*, *Ralstonia picketti* and *Elisabethkingia anophelis*. Most (95%) of these isolates were resistant to three or more classes of antibiotics, contrary to what was observed for the enterobacteria in which only ~ 20% presented that phenotype.

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