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21877 | A dive into diversity and antimicrobial resistance of *Enterococcus* from rivers used for drinking water production

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Background & Aim: Antimicrobials affect microbial diversity and the spread of antimicrobial-resistance (AMR) in surface waters. Here we studied the occurrence and diversity of antimicrobial-resistant *Enterococcus*, a water quality indicator, in rivers used for drinking water production. **Methods:** Water (n=42) and sediments (n=34) samples were collected (n=6 rivers-A-F; Porto region; 10 months/2022-2023) and *Enterococcus* recovered by standard water quality protocols. Species were studied by PCR, antimicrobial (antibiotics/biocides) susceptibility by disk diffusion/broth-microdilution (EUCAST/CLSI, 2023)**,** linezolid resistant (*optrA*/*poxtA*) or metal tolerance (copper-*tcrB*/*cueO*; arsenic-*arsA*; mercury-*merA*) genes by PCR and metals by ICP-MS. **Results:** *Enterococcus* were in 89% of the samples (2-6000CFU/100ml), below the maximum advised values for surface waters supporting chemical+physical+disinfection treatment. Isolates (n=208) belong to 9 species. The clinical-relevant *E.faecium (Efm)* and *E.faecalis* were less detected than other species (2-5%, 17-36% or 83-100% of samples, respectively). Resistance to chloramphenicol, linezolid, gentamicin, ciprofloxacin (0-17%, each), quinuspristin-dafopristin (0-33%), streptomycin (7-25%), erythromycin (21-50%) or tetracycline (42-50%) was similar (p>0,05; Fisher) among rivers. Linezolid-resistance (MIC=8-16mg/L) was found in *Efm* (*optrA*+*poxtA*), *E. gallinarum* or *E.durans* (*optrA*) from two rivers. Benzalkonium-chloride MIC/MBC values were of wild-type strains (0.5-4mg/L; n=62). Few samples carried *tcrB*±*cueO* (8-33%/river; including the linezolid-resistant-*Efm*), *arsA_I* (17%/river-C) or *merA*_IV (0-17%/river), supporting low concentrations of metals found in water (copper:0.27-1.94µg/L; arsenic: 0.78-15.6 µg/L; mercury: <0.048µg/L). **Conclusions:** The year-round presence of MDR-*Enterococcus*

emphasizes the role of surface waters in spreading AMR. Of concern are MDR+linezolid-resistant+copper-tolerant isolates occurring in samples with an *Enterococcus* load below the advised legal limits if treatment barriers prove ineffective.

Keywords: Water Quality Indicators, *Enterococcus*, Antimicrobial Resistance, Surface Waters, Environment, Public Health.

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