

Exploring the Antibiotics Resistance Profile of *Enterococcus faecium* and *Enterococcus faecalis* isolated from Municipal Wastewater

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Structured Abstract

Background: Enterococcus species, especially *Enterococcus faecium* and *Enterococcus faecalis*, are opportunistic pathogens recognised for their ability to thrive in various environments. They are also important for their involvement in hospital acquired infections. Enterococci demonstrate well known resistance to antibiotics, caused by mechanisms such as horizontal gene transfer, enzymatic modifications and biofilm development. Municipal wastewater acts as a significant source for antibiotic-resistant bacteria (ARB), playing a role in the dissemination of the resistance genes. This research explores the antibiotic resistance profiles and biofilm formation abilities of *E. faecium* and *E. faecalis* isolates obtained from municipal wastewater.

Methods: A total of 30 enterococci isolates originated from municipal wastewater were revived on Tryptic Soy Agar and characterised using Gram staining, catalase testing, followed by molecular identification via species-specific PCR targeting the *ddl* gene marker. These isolates were subjected to Antibiotic susceptibility testing (AST) against erythromycin (15 µg), penicillin (10 µg), and tetracycline (30 µg). Biofilm formation activity was also evaluated on selected isolates using the crystal violet assay.

Results: Based on the species-specific PCR result, among the 30 isolates, 70% were successfully identified as *E. faecium* and 30% as *E. faecalis*. The AST data indicates that antibiotic resistance was highest against tetracycline (53.1%), followed by erythromycin (37.5%) and penicillin (15.6%). Among 30 isolates, only four isolates exhibited multi-drug resistance. Biofilm assays evaluated for 18 resistance isolates, revealed that *E. faecalis* formed stronger biofilms than *E. faecium*, with one isolate classified as a moderate biofilm producer and six as weak producers.

Conclusion: In conclusion, this research highlights the widespread occurrence of antibiotic-resistant *E. faecium* and *E. faecalis* in municipal wastewater, with *E. faecalis* exhibiting more robust biofilm-forming abilities. The results highlight the significance of wastewater as a source of antibiotic-resistant bacteria and emphasize the importance of ongoing monitoring and control strategies to reduce the spread of resistance. Further research should expand into the molecular mechanisms underlying resistance and biofilm formation, while also broadening the focus to include additional environmental sources.

Keywords: *Enterococcus*, *E. faecium*, *E. faecalis*, antibiotic resistance, biofilm

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