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Molecular Characterization of Carbapenem-Resistant *Escherichia coli* From Hospital Effluent

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Structured Abstract

Background: Carbapenem-resistant *Escherichia coli* (CREc) is an emerging public health concern due to its resistance to multiple antibiotics, limiting treatment options and increasing the risk of healthcare-associated infections. Hospital effluents serve as potential reservoirs for CREc, contributing to environmental contamination and resistance dissemination. This study aims to isolate *E. coli* from hospital wastewater, determine their antibiotic resistance profiles, evaluate biofilm formation, and confirm their identity through molecular characterization.

Methods: Samples were collected from hospital wastewater and cultured on MacConkey agar and Eosin Methylene Blue (EMB) agar for bacterial isolation. Identification was based on colony morphology and Gram staining. Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method for imipenem, meropenem, ampicillin, gentamicin, and tetracycline. Biofilm formation was assessed using a 96-well microtiter plate assay. Molecular identification of selected isolates was conducted using 16S rRNA gene sequencing, followed by BLAST analysis for genetic confirmation.

Results: This study shows that *E. coli* isolates were successfully identified from hospital effluents. Antibiotic profiling revealed that all isolates were resistant to meropenem and tetracycline, with some exhibiting multidrug resistance (MDR) due to additional resistance to ampicillin. However, all isolates remained susceptible to imipenem and gentamicin. Biofilm assays demonstrated weak biofilm formation in certain isolates, while others exhibited no biofilm formation. Molecular analysis confirmed the identity of selected isolates as *E. coli* with 100% sequence similarity through BLAST identification.

Conclusion: This study successfully isolated carbapenem-resistant *E. coli* from hospital effluents, with some classified as MDR strains. While biofilm formation was weak or absent, indicating potentially lower virulence, the presence of antibiotic resistance highlights the potential risk of environmental dissemination. These findings emphasize the need for continuous monitoring of hospital effluents to control the spread of resistant bacteria and support antimicrobial stewardship programs.

Keywords: Carbapenem-resistant *Escherichia coli*, hospital effluent, antibiotic resistance, biofilm formation, molecular characterization.

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