

# RESISTANCE OF ENTEROCOCCUS FAECIUM BACTERIA TO COMMONLY USED DISINFECTANTS IN CLINICAL SETTING

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*Enterococcus faecium* bacteria are facultative anaerobic gram-positive opportunistic pathogens responsible for healthcare-associated infections due to their exceptional ability to colonize abiotic surfaces and tolerate antimicrobial stress, promoted by commonly used disinfectants [2] [3]. Recent studies indicate that biofilm formation plays a critical role in the survival of pathogens under biocidal exposure and contributes to elevated resistance and environmental contamination [1] [4]. Hence, this study aimed to identify essential genes associated with resistance to biocidal agents and to assess the impact of disinfectants on biofilm formation.

In this study, 59 clinical *E. faecium* isolates were collected from two Lithuanian hospitals between January and November 2024. The multiplex PCR genetic characterization method was used to detect genes associated with tolerance mechanisms to disinfectants and antiseptics (*qacE*, *qacEΔ1*, *qacG*, *qacH*, *qacA*, *cepA*). Biofilm formation by enterococci was determined using the microtiter plate method. 42 biofilm-positive isolates were assessed after exposure to 50% ethanol, 50% isopropanol, and 5% sodium hypochlorite using the crystal violet microtiter plate assay. The effects of alcohol-based antiseptics and sodium hypochlorite on biofilms were evaluated after 5- and 10-min. exposure, and biofilm optical density (OD) was measured spectrophotometrically at 595 nm wavelength. Isolates were classified as non-biofilm-forming, weak, moderate and strong biofilm producers. Statistical analysis of disinfectant effect was performed using Fisher's and McNemar's Chi-squared tests.

Multiplex PCR assessment revealed 4 distinct biocidal resistance genes: *qacE*, *qacEΔ1*, *qacH*, *qacA*. Among these, the *qacH* gene was the most prevalent, identified in 19 isolates (67.8%). Exposure to an alcohol-based antiseptic resulted in a statistically significant reduction in biofilm producers after 5 min ( $p < 0.001$ ), with a further reduction observed after prolonged exposure. In contrast, sodium hypochlorite resulted in less notable reduction of biofilm-forming isolates ( $p < 0.001$ ). The detection of resistance genes suggests a potential contribution of genetic traits and tolerance to antiseptics. This research demonstrates the potential threat posed by *E. faecium* biofilms to tolerance of commonly used antiseptics in healthcare institutions and may be linked to underlying genetic determinants and healthcare-associated infections. These findings emphasize the need to design new and effective infection control strategies in clinical environments.

**Keywords:** gram-positive bacteria, disinfectants, resistance, tolerance, Multiplex PCR, biofilm formation, statistical analysis.

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