

Research Article

Exposure to Biocides Leads to Ampicillin Resistance in Clinical *Enterococcus faecalis*: A Concise Summary

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Abstract

The emergence of antimicrobial resistance is a major global health concern, and increasing evidence suggests that exposure to sub-inhibitory concentrations of disinfectants (biocides) may contribute to this problem. This study investigated the effect of commonly used biocides on antibiotic susceptibility in clinically isolated *Enterococcus faecalis*. Clinical isolates were exposed to sub-lethal concentrations of ethanol, hydrogen peroxide, sodium hypochlorite, and chlorhexidine. Changes in antibiotic susceptibility were assessed using standard disk diffusion and minimum inhibitory concentration (MIC) assays. Molecular analyses, including PCR and gene sequencing, were performed to detect resistance-associated genes, while electron microscopy was used to observe potential morphological changes.

Keywords: General microbiology, Biocides, Antimicrobial susceptibility patterns, *Enterococcus faecalis*, pbp4 gene; Ampicillin resistant

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Introduction

Antimicrobial resistance is a growing global health concern. Besides the misuse of antibiotics, exposure to sublethal concentrations of disinfectants (biocides) may contribute to the development of antibiotic resistance. *Enterococcus faecalis*, an opportunistic pathogen associated with hospital-acquired infections, has increasingly exhibited resistance to commonly used antibiotics, including ampicillin. This study investigated whether exposure to commonly used biocides could alter antibiotic susceptibility in clinical isolates of *E. faecalis*.

Methods

Two clinical isolates of *E. faecalis* were exposed to sub-inhibitory concentrations of four commonly used biocides: ethanol, hydrogen peroxide, sodium hypochlorite, and chlorhexidine. Antibiotic susceptibility was evaluated using disk diffusion and broth microdilution methods. Polymerase chain reaction (PCR) and gene sequencing were performed to identify resistance-associated genes, while scanning and transmission electron microscopy were used to assess bacterial morphology and ultrastructure.

Results

Exposure to biocides reduced antibiotic susceptibility in approximately 27% of the tested cultures. Hydrogen peroxide produced the most notable effect, significantly increasing the minimum inhibitory concentration (MIC) of ampicillin in one clinical isolate. Following exposure, this isolate developed resistance to ampicillin. Molecular analysis revealed the presence of the *pbp4* gene, which is associated with β -lactam resistance. However, no mutations were detected in the gene after biocide exposure, suggesting that resistance may have arisen through mechanisms other than gene mutation. Microscopic examination showed no significant changes in bacterial morphology or cell ultrastructure following biocide treatment.

Discussion

The findings suggest that repeated exposure to low concentrations of disinfectants may promote reduced susceptibility to antibiotics in clinically important bacteria. Although the exact mechanism remains unclear, biocide-induced stress may facilitate adaptive responses that enhance bacterial survival under antimicrobial pressure. The absence of structural changes and genetic mutations in the *pbp4* gene indicates that alternative regulatory or physiological mechanisms may contribute to resistance development.

Conclusion

This preliminary study demonstrates that exposure to sub-inhibitory concentrations of biocides, particularly hydrogen peroxide, can decrease antibiotic susceptibility and induce ampicillin resistance in clinical isolates of *Enterococcus faecalis*. These findings highlight the importance of proper biocide usage and emphasize the need for further research into the relationship between disinfectant exposure and antimicrobial resistance in healthcare settings.

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