

**The frequency of Tolerance to Antimicrobial biocides in  
*Enterococcus faecalis* and *Enterococcus faecium* isolates collected  
from human and environmental samples in Ardabil-2019**

**Abstract**

**Introduction and Objective:** Enterococci are among the most common causes of nosocomial infections worldwide. Antimicrobial biocides are extensively used in hospitals to control the growth of microorganisms on different surfaces. The purpose of this study was to determine the tolerance to four common biocide agents, formaldehyde (FOR), benzalkonium chloride (BZC), triclosan (TRE), and chlorhexidine di-gluconate (CHDG), in *E. faecalis* and *E. faecium* isolates collected in Ardabil, Iran. Additionally, the frequency of biocide tolerance associated genes (BTA genes), *qacA/B*, *qacED1*, *emeA*, *sigV* and *gasp65*, and possible genetic relationship between isolates were investigated.

**Materials and Methods:** In this study, a total of 222 *E. faecalis* and 425 *E. faecium* isolates previously collected during the period between 2017 and 2019 from clinical and non-clinical sources were included. Minimum Inhibitory Concentration (MIC) of biocide agents was determined using an agar dilution method. Biocides Epidemiological cutoff values (ECOFFs) were determined using 95% rule. BTA genes were identified using the PCR testing. The genetic relationship between isolates was determined by ERIC-PCR method.

**Findings:** ECOFFs for CHDG, BZC, TRE and FOR were determined to be 8 µg/ml, 16 µg/ml, 32 µg/ml and 512 µg/ml for both species respectively. According to MIC<sub>90</sub> values the distribution of isolates with high-level tolerance rates to antimicrobial biocides were significantly different among isolates based on the sources isolated. The BTA genes, *qacA/B*, *qacED1*, *emeA*, *sigV* and *gasp65* were detected in 19.4% (43), 19.8% (44), 42.8% (95), 89.6% (199) and 70.2% (156) of *E. faecalis* and 10.3% (44), 17.2% (73), 27.8% (118), 42.2% (188) and 82.8% (352) of *E. faecium* isolates, respectively. Based on the distribution pattern of BTA genes 14 and 18 different profiles were identified in *E. faecalis* and *E. faecium* isolates. Generally, the isolates carrying at least a single BTA gene showed higher MIC<sub>90</sub> values against all biocides compared to isolates with no BTA genes. However there were no clear association between MIC<sub>90</sub> values and carrying particular BTA genes combination.

**Conclusions:** The results of this study can be used as part of a global study to determine the breakpoint of resistance to biocides.

**Keywords:** *E. faecalis*, *E. faecium*, Antimicrobial biocides, Biocide tolerance associated genes, Epidemiological cutoff