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NOTE

In vitro Activity of Sodium Benzoate Against Clinically Relevant *Enterococcus faecalis* and *Enterococcus faecium* Isolates

Oguz Karabay[1], Esra Kocoglu[2,*], Nevin Ince[1], Tufan Sahan[3] and Davut Ozdemir[4]

[1]*Department of Infectious Diseases and Clinical Microbiology, Izzet Baysal Medical Faculty, Abant Izzet Baysal University, Bolu, Turkey*
[2]*Department of Microbiology and Clinical Microbiology, Izzet Baysal Medical Faculty, Abant Izzet Baysal University, Bolu, Turkey*
[3]*Microbiology laboratory of Izzet Baysal Medical Faculty, Abant Izzet Baysal University, Bolu, Turkey*
[4]*Department of Infectious Diseases and Clinical Microbiology, Duzce Medical Faculty, Abant Izzet Baysal University, Bolu, Turkey*

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The antimicrobial effects of sodium benzoate against *Enterococcus faecalis* and *Enterococcus faecium* were investigated. The MIC_{90} of sodium benzoate were 64 mg/L for *E. faecalis* and 32 mg/L for *E. faecium*, while the MBC_{90} were 128 mg/L and 64mg/L, respectively. Although further studies are required for clinical evidence, sodium benzoate seems to be effective against *Enterococcus* spp.

Keywords: sodium benzoate, *Enterococcus faecalis*, *Enterococcus faecium*

Enterococci are normal inhabitants of the alimentary tract and cause urinary tract infections, bacteremia, and endocarditis. *E. faecalis* causes the majority of all enterococcal infections. *E. faecium* causes a substantial proportion of nosocomial enterococcal infections (Murray, 1990; Facklom.& Sahm, 1995; Gold, 2001).

Data collected by the National Nosocomial Infection Surveillance System on infections in intensive care units from 1989 through 1998 showed that enterecocci were the third most common isolate from surgical site infections and the fourth most common isolate from all sites (Gold, 2001). The most striking attribute of enterococci is the relative and absolute resistance of these organisms to a variety of antimicrobial agents commonly used to treat infections of gram-positive organisms (Fridkin and Gynes, 1999; Gold, 2001).

Today, vancomycin-resistant enterococci (VRE) are important pathogens in the hospital settings. VRE was first reported in 1988.The addition of high-level aminoglycoside resistance, β-lactamase, to enterococcal infections indicates that enterococci will cause therapeutic problems for years to come. Thus, current drugs against enterococci are limited and new drugs are needed for the treatment of enterococcal infections. Sodium benzoate (SB), or "benzoate of soda," is the sodium salt of benzoic acid, an FDA-approved polyunsaturated fat used by food manufacturers to inhibit microbial growth for over 80 years. It can prevent growth of almost all microorganisms (bacteria, yeast and moulds), and has been used for the treatment of congenital metabolic diseases such as urea cycle disorders. Recently, we showed effective *in vitro* activity of sodium benzoate against methicillin-resistant *S. aureus* (MRSA), and we hypothesized that SB might be effective against enterococcal isolates also (Karabay and Sahin, 2005). To our knowledge, there is no study focusing on the activity of SB against enterococci, so here we investigate the *in vitro* effects of SB on enterococcus isolates.

A total of 90 clinical strains of *Enterococcus faecalis* and 8 of *Enterococcus faecium* were obtained from the Microbiology Laboratories of Izzet Baysal Medical Faculty and Duzce Medical Faculty of Abant Izzet Baysal University, Turkey, between March 2003 and January 2005. *Enterococcus* spp. were identified by classical tests (Gold, 2001). All enterococci were identified by a commercial kit (API 20 Strep, bioMerieux, France) on the species level.

Susceptibility testing for SB was performed with a commercial kit (Onur Kimya-Istanbul), and antimicrobial susceptibility tests were performed in accordance with

* To whom correspondence should be addressed.
(Tel) 90-374-2534656
(E-mail) kocogluesra@yahoo.com

procedures recommended by National Committee for Clinical Laboratory Standards (NCCLS, 2003). The minimal inhibitory concentration (MIC) was the lowest antibiotic concentration at which there were no colonies on cation-adjusted Mueller-Hinton (MH) agar, or no visible growth in the wells of MH broth. Minimal bactericidal concentrations (MBCs) were determined by removal of 0.1 ml from each well of the microplate in which no growth was evident after subculture on MH II agar. The plates were then incubated at 37°C for 24 h. MBCs were read as the lowest concentrations of antibiotic that resulted in 0.1% survival in the subculture.

Enterococci were resistant to concentrations below 2 mg/L of SB. Two *E. faecalis* isolates were sensitive to 4 mg/L. All *E. faecalis* isolates were resistant to 4 mg/L. At 8 mg/L, 26 (28.8%) of *E. faecalis*, and two *E. faecium* isolates were sensitive to SB. At 16 mg/L, 60 (66.7%) of *E. faecalis* isolates and all *E. faecium* species were sensitive to SB. All enterococci were sensitive to SB above 64 mg/L. In agar dilution tests, the median MIC at which 90% of bacteria were inhibited by SB (MIC_{90}) was 64 mg/L for *E. faecalis,* and 32 mg/L for *E. faecium*. The MIC_{50} of both *enterococci* were 16 mg/L. The bactericidal activity of SB was two to four times higher than the MIC level. The MBC_{90} of *E. faecalis* and *E. faecium* were 128 mg/L and 64 mg/L, respectively, and the MBC_{50} of both *enterococci* were 64 mg/L. No isolates were resistant to vancomycin. MIC and MBC concentrations of enterococcal isolates are presented in Table 1.

Enterococci are able to grow under extreme conditions, such as 6.5% NaCl at pH 9.6, and at temperatures ranging from 10°C to 45°C (Robert and Moellering, 2000). The most striking attribute of enterococci is the relative and absolute resistance to a variety of antimicrobial agents, and intrinsic resistance to cephalosporins (Robert and Moellering, 2000). Sensitivity to penicillins and other antibiotics varies widely, and clinical isolates must be tested for their susceptibility. Vancomycin resistance has been observed in enterococci and is a problem in high-dependency areas of some hospitals (Kilian, 2002). This high resistance of enterococci to antibiotics requires the development of new agents. Here we determined the *in vitro* activity of SB on clinically relevant enterococcus isolates at 64 mg/L and higher concentrations. We found no clinical studies on the effects of SB on clinically relevant enterococcus isolates in the literature.

Table 1. *In vitro* activity of sodium benzoate against enterococcal isolates

	E. faecalis	*E. faecium*
Range of MIC (mg/L)	4-64	8-32
MIC_{50}	16	16
MIC_{90}	64	32
Range of MBC (mg/L)	4-128	8-128
MBC_{50}	64	64
MBC_{90}	128	64

SB is used in the food industry to preserve food (e.g., ketchup and fruit juice) against almost all microorganisms. SB is reported to inhibit the growth of *Aspergilus flavus* in potato dextrose agar (Lopez-Malo, *et al.*, 2005). SB is used in the long-term treatment of ornithine transcarbamylase-deficient pediatric patients, and these patients had previously been treated with sodium benzoate at a mean dose of 248 mg/kg/day. Takeda and coworkers (Takeda, *et al.*, 1983) treated an eight-year old girl with partial ornithine-carbamyl transferase deficiency with sodium benzoate (200 mg/kg/day) for 13 months. These investigators have not observed any adverse effects of SB in clinical practice, suggesting that SB may be used *in vivo* at 64 mg/L and higher. In our study, 16 mg/L SB inhibited 50% of enterococci (MIC_{50}), and 64 mg/L killed 50% of them (MBC_{50}). The MIC_{90} level of SB was 32 mg/L for *E. faecium* and 64 mg/L for *E. faecalis*. The MBC_{90} was about two-fold higher than the MIC_{90}. Our findings suggest that sodium benzoate may be effective against enterococci in the doses between 32 mg/L and 128 mg/L.

This is the first study investigating *in vitro* activity of SB clinically relevant enterococcal isolates. Sodium benzoate seems to be a good alternative for the treatment of VRE infections. Animal models should be used to evaluate SB activity in enterococcal infections, and synergy studies are needed to determine SB activity in combination with other antibiotics.

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