# *In Vitro* Activity of Sodium-Benzoate against Isolates of Methicillin-resistant *Staphylococcus aureus*

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## *ABSTRACT*

***Background:*** *Worldwide, methicillin-resistant* Staphylococcus aureus *(MRSA) rates have increased dramatically during the last decades. Sodium benzoate (SB) is a chemical substance that is used for preparing food and drinks and in the treatment of some metabolic (urea cycle disorders and hepatic coma) diseases. No studies were found which focussed on the effects of SB in MRSA infections. The aim was to determine* in vitro *activity of sodium benzoate against MRSA clinical isolates.*

***Methods:*** *In this study, MIC for SB in 36 MRSA and 29 methicillin-sensitive* Staphylococcus aureus *(MSSA) isolates were determined by a broth microdilution method recommended by the National Committee for Clinical Laboratory Standards.*

***Results:*** *The MIC at which all of the MRSA and MSSA strains were inhibited was at 32 μg/ml and higher concentrations.*

***Conclusion:*** *Sodium benzoate showed good* in vitro *activity against clinically relevant MRSA and MSSA isolates. It is suggested in this study that this cheap substance, which has been used for systemic and local treatment of infection in humans, may be used alternatively for the treatment of MRSA infections. However, it is clear that more comprehensive and* in vivo *studies are needed to further elucidate the activity of SB against MRSA infections.*


# Actividad *In Vitro* del Benzoato de Sodio contra los Aislados de *Staphylococcus aureus* Resistentes a la Meticilina

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## *RESUMEN*

***Antecedentes:*** *A nivel mundial, los índices de* Staphylococcus aureus *resistente a la meticilina (SARM) han aumentado dramáticamente durante las últimas décadas. El benzoato de sodio (BS) es una sustancia química que se usa ampliamente en la preparación de comidas y bebidas, y en el tratamiento de algunas enfermedades metabólicas (trastornos del ciclo de la urea y coma hepático). No encontramos ningún estudio dedicado específicamente a los efectos del BS en las infecciones por SARM. Nos trazamos como objetivo determinar la actividad* in vitro *del benzoato de sodio contra los aislados clínicos de SARM.*

***Métodos:*** *En este estudio, se determinaron la concentración mínima inhibitoria (CMI) para el BS en 36 SARM y 29 aislados de* Staphylococcus aureus *sensible a la meticilina (SASM), mediante un método de microdilución en caldo, recomendado por el Comité Nacional para las Normas de Laboratorios Clínicos.*

***Resultados:*** *La CMI a la que se inhibieron todas las cepas de SARM y SASM fue de 32 μg/ml y concentraciones más altas.*

***Conclusión:*** *El benzoato de sodio demostró ser bueno en la actividad* in vitro *contra los aislados de SARM y SASM clínicamente relevantes. Sugerimos que esta sustancia económica, que se ha usado para el tratamiento sistémico y local de infecciones en los seres humanos, puede usarse alternativamente para el tratamiento de infecciones por SARM. Sin embargo, está claro que se requieren estudios* in vivo *y más exhaustivos, que contribuyan a un entendimiento aún más claro de la actividad del BS contra las infecciones por SARM.*


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## INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become increasingly prevalent worldwide. In the United States of America (USA) and some European countries, MRSA accounts for 10 to 40% of all *S aureus* isolates (1, 2). Recent studies suggest that the epidemiology of MRSA may be changing, as the isolation of MRSA is no longer limited to patients hospitalized or previously hospitalized (3, 4). Methicillin resistance and associated multiple drug resistance is a significant problem in nosocomial *S aureus* infection. Thus drugs that may be used against MRSA are limited and for this reason, new drugs are needed for the treatment of MRSA infections.

Also known as "benzoate of soda," sodium benzoate (SB) is the sodium salt of "benzoic acid," an FDA-approved, polyunsaturated fat that has been used by food manufacturers for over 80 years to inhibit microbial growth (2). Sodium benzoate has been used in the food industry for preserving food (ketchup, fruit juice) against almost all micro-organisms. It can prevent growth of almost all micro-organisms (bacteria, yeast and moulds) (6) and has been used for the treatment of congenital metabolic diseases such as urea cycle disorders (7, 8). We found no studies focussing on the effects of SB in MRSA infections. For this reason, in this study, the author aimed to determine the effect of SB in the MRSA isolates *in vitro*.

## MATERIALS AND METHODS

### *MRSA isolates*

Methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) isolates were obtained from hospitalized patients. All isolates were stored frozen in skimmed milk at -20°C and were sub-cultured twice onto trypticase soy agar with 5% sheep blood prior to testing. A total of 36 well-characterized clinical isolates of MRSA and 29 MSSA were selected for *in vitro* activity testing. Quality control for broth microdilution was performed with MRSA ATCC 33592 and MSSA ATCC 29213. The values were within the acceptable range of inhibition for these strains.

Oxacillin resistance was confirmed by broth microdilution method in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) (3). Oxacillin MICs were read at 24 hours. Quality control for broth microdilution was performed with *S aureus* ATCC 29213.

### *Susceptibly testing*

Sodium benzoate was purchased commercially (Onur Kimya-Istanbul). MICs were determined by microdilution technique with an inoculum of 5 x $10^5$ CFU/ml by National Committee for Clinical Laboratory standards guidelines (3). Sodium benzoate was diluted at 0.1, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512 $\mu$g/ml concentrations in cation-supplemented Mueller-Hinton broth according to NCCLS guidelines.

## RESULTS

It was observed that 4/36 MRSA and 4/29 MSSA isolates were susceptible to SB at 16 µg/ml concentrations. All of the MRSA and MSSA isolates were susceptible to SB at >32 µg/ml concentrations. MRSA ATCC 33592 and MSSA ATCC 29213 isolates were found susceptible to SB at 16 µg/ml concentrations. The results are shown in the Table.

Table: *In vitro* activity of MRSA* and MSSA ** isolates to sodium benzoate

| | MRSA (n = 36) | | MSSA (n = 29) | |
|---|---|---|---|---|
| **SB concentrations** | **Resistance** | **Susceptible** | **Resistance** | **Susceptible** |
| | (n) | (n) | (n) | (n) |
| 0.1–1 µg/ml | 36 | 0 | 29 | 0 |
| 2 µg/ml | 36 | 0 | 29 | 0 |
| 4 µg/ml | 36 | 0 | 29 | 0 |
| 8 µg/ml | 36 | 0 | 29 | 0 |
| 16 µg/ml | 32 | 4 | 25 | 4 |
| 32 µg/ml | 0 | 36 | 0 | 29 |
| 64–256 µg/ml | 0 | 36 | 0 | 29 |
| 512 µg/ml | 0 | 36 | 0 | 29 |

*MRSA: Methicillin-resistant *Staphylococcus aureus*
** MSSA*:* Methicillin-sensitive *Staphylococcus aureus*

## DISCUSSION

Methicillin and multiple drug resistance is a significant problem in nosocomial as well as some community-acquired strains of *S aureus* (3, 10). The drugs that have been used in the treatment of MRSA infections are restricted because of resistance and side effects. Most strains of *Staphylococcus aureus* are now resistant to penicillin and many MRSA strains are resistant to erythromycin, clindamycin, aminoglycoside and quinolones (11, 12). Because a number of the drugs which have been used for the treatment of MRSA are restricted due to resistance, new drugs have been needed for the treatment of MRSA infections (13, 14). Further new drugs are needed for the prevention and treatment of MRSA infection.

In the present study, the aim was to study *in vitro* activity of SB on clinically relevant MRSA isolates. In this study SB, had a good *in vitro* activity against all of the MRSA isolates at 32 µg/ml and higher concentrations. When the medical usage of these doses were investigated against various diseases in humans in the literature we found no study that focussed on the effects of SB on clinically relevant MRSA isolates. This chemical substance has been used in the food industry for preserving food (*eg* ketchup and fruit juice) against almost all micro-organisms. It has been used in long-term treatment in ornithine transcarbamylase-deficient paediatric patients and this group had previously been treated with sodium benzoate at median dose 248 mg/kg/day (15). Takeda *et al* (16) 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 effect of sodium benzoate in clinical practice. These studies suggest that SB may be used *in vivo* at 32 µg/ml and higher concentrations.

However, Johansen and Berger (17) investigated *in vitro* activity of sodium benzoate on the function of polymorphonuclear leukocytes (PMN) stimulated by *S aureus* or the chemical agent phorbol myristate acetate (PMA), using assays of chemiluminescence, total bactericidal activity, intracellular recovery of bacteria, as well as release of lactate dehydrogenase, lysozyme and superoxide anion, *in vitro*. Sodium benzoate decreased chemiluminescence, superoxide anion and lysozyme release by PMN stimulated by *S aureus* but did not similarly affect these responses of PMN to the chemical agent PMA. The ability of PMN to kill *S aureus* was also impaired by sodium benzoate and associated with a reduced number of intracellular bacteria recovered after 90 minutes incubation, suggesting decreased uptake of *S aureus*.

This is the first study investigating *in vitro* activities of SB on clinically relevant MRSA and MSSA isolates. Sodium benzoate seems to be a good alternative drug for the treatment of MRSA infections. We thought that, good *in vitro* activities of sodium benzoate suggest that, in the future, it may represent an alternative choice in the treatment of infections with antibiotic-resistant *S aureus* infections. Animal models might be used to evaluate SB activity in *S aureus* infections, as well as synergy studies to determine SB activity in combination with other antibiotics, particularly against MRSA, and studies to monitor for SB resistance development during monotherapy. It is clear that more comprehensive and *in vivo* studies are needed to further elucidate the activity of SB against MRSA infections.

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