Article

# Sodium deoxycholate synergistically enhances the antimicrobial activity of β-lactam antibiotics against β-lactamase-producing *Staphylococcus aureus*

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**Background** β-lactamase-producing *Staphylococcus aureus* synthesizes a β-lactamase which decomposes the β-lactam ring, and consequently almost all β-lactam antibiotics are inactivated by the enzyme. The appearance of β-lactamase-producing bacterial strains has diminished the usefulness of β-lactam antibiotics. New therapeutic agents or new approaches are urgently needed for drug-resistant bacteria.

**Objective** We investigated the antimicrobial activity of sodium deoxycholate against β-lactamase-producing *S. aureus* strains, and the synergistic effects of sodium deoxycholate on the antimicrobial activity of β-lactam antibiotics against those strains. The possibility of this combination as a new therapeutic method against infectious diseases caused by β-lactamase-producing bacteria was examined.

**Methods** The synergistic effects of the combined use of sodium deoxycholate on the antimicrobial activities of β-lactam antibiotics against β-lactamase-producing *S. aureus* strains were tested by using an MIC (minimum inhibitory concentration) assay. The inhibitory effect of sodium deoxycholate on the β-lactamase activity was examined by nitrocefin assay.

**Results** The antimicrobial activities of β-lactam antibiotics, including benzylpenicillin, ampicillin and piperacillin, against β-lactamase-producing *S. aureus* strains were obviously enhanced by the combination with sodium deoxycholate. In addition, it was demonstrated that sodium deoxycholate remarkably eliminated the synthesis of β-lactamase in β-lactamase-producing *S. aureus*.

**Conclusion** The combined use of sodium deoxycholate with β-lactam antibiotic is expected to be a new therapeutic method and may contribute to an effective utilization of β-lactam antibiotics against infectious diseases caused by β-lactamase-producing bacteria.

**Key Words** β-lactamase-producing *Staphylococcus aureus*, sodium deoxycholate, β-lactam antibiotics, antimicrobial activity, *blaZ* gene.

デオキシコール酸ナトリウムとβ-ラクタム系抗菌薬との併用による
β-ラクタマーゼ産生黄色ブドウ球菌に対する抗菌活性相乗効果

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*Staphylococcus aureus* is a facultative anaerobic Gram-positive coccal bacterium, and one of the most important bacterial pathogens (Eykyn *et al.*; 1990, Schaberg *et al.*; 1991). It is frequently part of the skin flora found in the nose and on skin. It may be the causative organisms in pneumonia, meningitis, empyema, endocarditis, or sepsis with suppuration in any organ. Although β-lactam antibiotics such as benzylpenicillin (PCG) and ampicillin (ABPC) are effective against the *S. aureus* infection, penicillin-resistant *S. aureus* strains were found to produce a β-lactamase (penicillinase). The enzyme decomposes the β-lactam ring, and consequently β-lactam antibiotics are inactivated. The appearance of β-lactamase-producing bacterial strains have diminished the usefulness of β-lactam antibiotics (Medeiros; 1984).

The β-lactamase-producing bacterial strains have a *blaZ* gene, which encodes the β-lactamase (Okamoto *et al.*: 1996). Combination of β-lactam antibiotics with either sulbactam, tazobactam or clavulanic acid, all of which are β-lactamase inhibitors, is a useful therapeutic method for treating infections of β-lactamase-producing bacteria (Rizwi *et al.*; 1989, Maddux; 1991). However, it was reported that bacterial strains which acquired inhibitor resistance appeared (Blasquez *et al.*; 1993, Chaibi *et al.*; 1999). Drug resistance in pathogenic bacteria is a serious global problem. New therapeutic agents or new approaches are urgently needed for drug-resistant bacteria.

Deoxycholic acid is one of the secondary bile acids, which are metabolic byproducts of intestinal bacteria. The compound is used in the emulsification of fats for absorption in the intestine in humans. Deoxycholic acid and sodium deoxychol ate, the sodium salt of deoxycholic acid, contain a steroid ring component, and they are often used as biological detergents to lyse cells and solubilize cellular and membrane components. The detergent property also confers potent antimicrobial activity, primarily through the lysis of bacterial membranes (Begley *et al.*; 2005). Actually, it has been reported that deoxycholic acid has an antibacterial effect on *Helicobacter pylori* (Itoh *et al.*; 1999). The refore, a synergistic effect on the antimicrobial activities of antibiotics against bacteria (especially multiple drug-resistant microorganisms) was expected by a combination with deoxycholic acid or sodium deoxycholate.

In this study, we investigated the antimicrobial activities of sodium deoxycholate (because sodium deoxycholate dissolves in water more easily than deoxycholic acid) and the synergistic effects on the antimicrobial activities of β-lactam antibiotics against β-lactamase-producing *S. aureus* strains of the combined use with sodium deoxycholate, aiming at developing new therapeutic agents or new approaches against infectious diseases caused by β-lactamase-producing bacteria.

Five strains of penicillin-resistant *S. aureus* (SA-24, SA-69, SA-78, SA-85 and SA-91) were isolated from five healthy adult volunteers (all males, 22- to 50-year-old). The SA-12732 strain was used as the standard strain, which was obtained from the National Institute of Technology and Evaluation Biological Resource Center, Chiba, Japan. These strains were identified by PCR analysis for the presence of the *blaZ* gene employing the primer pair described in our previous report (Horie *et al.*; 2010). The DNA fragment of 325 bp of the *blaZ* gene was amplified in each of five penicillin-resistant strains, but not in the standard strain (Fig.1). Therefore, these penicillin-resistant strains should produce this β-lactamase and will be resistant to other β-lactam antibiotics. The MICs (minimum inhibitory concentrations) of β-lactam antibiotics against those *S. aureus* strains are shown in Table 1. The MIC was determined by a

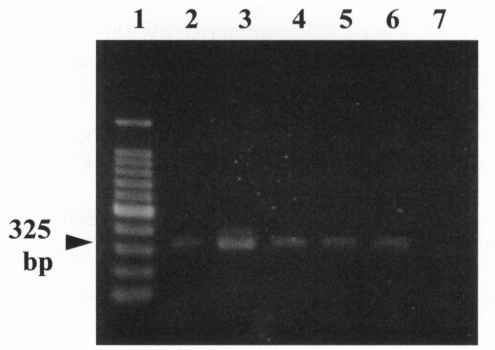


Fig. 1 PCR analysis of *blaZ* gene in *S. aureus*. Lane 1, 100-bp DNA ladder (molecular weight marker); lane 2, *S. aureus* SA-24; lane 3, SA-69; lane 4, SA-78; lane 5, SA-85; lane 6, SA-91; lane 7, SA-12732. Expected size of PCR products (325 bp) is shown by arrow.

**Table 1 Effect of sodium deoxycholate in sensitizing β-lactamase-producing *S. aureus* to β-lactam antibiotics.**

| | MIC of β-lactam antibiotics (U, μg/mL) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Without DOCNa | | | | With 100 g/mL of DOCNa | | |
| S. aureus | PCG | MPIPC | ABPC | PIPC | PCG | ABPC | PIPC |
| SA-24 | 32 | < 0.5 | 64 | 128 | 2 | 4 | 2 |
| SA-69 | 64 | < 0.5 | 32 | 64 | 2 | 2 | 2 |
| SA-78 | 32 | < 0.5 | 32 | 64 | 4 | 2 | 8 |
| SA-85 | 32 | < 0.5 | 32 | 128 | 2 | 4 | 4 |
| SA-91 | 32 | < 0.5 | 32 | 64 | 2 | 2 | 4 |
| SA-12732 | < 0.125 | 0.25 | 0.25 | 0.5 | ND | ND | ND |

PCG, benzylpenicillin (U/mL); ABPC, ampicillin (μg/mL); MPIPC, oxacillin (μg/mL); PIPC, piperacillin (μg/mL); DOCNa, sodium deoxycholate; ND, not done

liquid microdilution method in 96-well microtiter plates according to the procedure recommended by the National Committee for Clinical Laboratory Standards(National Committee for Clinical Laboratory Standards; 1997). Three kinds of β-lactam antibiotics, PCG, ABPC and piperacillin (PIPC), hardly showed any activity against such strains carrying the *blaZ* gene as described in Fig. 1. The MIC range of the three β-lactams was 32 to 128 μg/mL. On the other hand, the strains were highly susceptible to oxacillin (MPIPC), because MPIPC is not decomposed by β-lactamase. Thus, the results show that these strains indeed produce the β-lactamase. The standard strain showed high susceptibility against all antibiotics.

The synergistic effects of the combined use of 100 μg/mL sodium deoxycholate on the antimicrobial activity of the β-lactam antibiotics against the β-lactamase-producing *S. aureus* strains are shown in Table 1. The MIC of sodium deoxycholate against the strains was 500 μg/mL (data not shown). Sodium deoxycholate was used at a concentration at which the proliferation of *S. aureus* was not inhibited. The antimicrobial activities of three β-lactams (PCG, ABPC and PIPC) against those strains were obviously enhanced in combination with 100 μg/mL of sodium deoxycholate (Table 1). The MIC range of the three β-lactams was 2 to 8 μg/mL. The synergistic effect was hardly observed with 50 μg/mL of sodium deoxycholate (data not shown).

In addition, to elucidate the mechanism of the synergistic effects of sodium deoxycholate on the antimicrobial activity of the β-lactam antibiotics, the inhibitory effect of sodium deoxycholate against β-lactamase activity was examined. Each *S. aureus* (SA-24, SA-69, SA-78, SA-85 or SA-91) was inoculated in 100 μL of sensitivity test broth (ST-broth, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) with or without 100 μg/mL of sodium deoxycholate and cultured at 37° C for 24 h. Also, the cultivation medium to which 100 μg/mL of sodium deoxycholate was added after the cultivation period was prepared as a control. Each cultivation fluid was incubated with nitrocefin (Kanto Chemical Co., Inc., Tokyo, Japan) as a substrate of β-lactamase at 37° C for 30 to 60 min. Absorbance at 492 nm was measured with a spectrophotometer. The test was performed three times independently and the data were analyzed using Student's t-test. A value of $P<0.05$ was considered as statistically significant. The cultivation fluid without sodium deoxycholate was observed to have high β-lactamase activity (Fig. 2, white bar), because the activity of β-lactamase was proportional to the absorbance (Zhao *et al.*; 2002). However, the activity decreased significantly ($p<0.01$) when the medium containing 100 μg/mL of sodium deoxycholate was used for cultivation (Fig. 2, black bar). On the other hand, in the medium to which 100 μg/mL of sodium deoxycholate was added after the cultivation period, a significant decrease in activity was not observed (Fig. 2, gray bar).

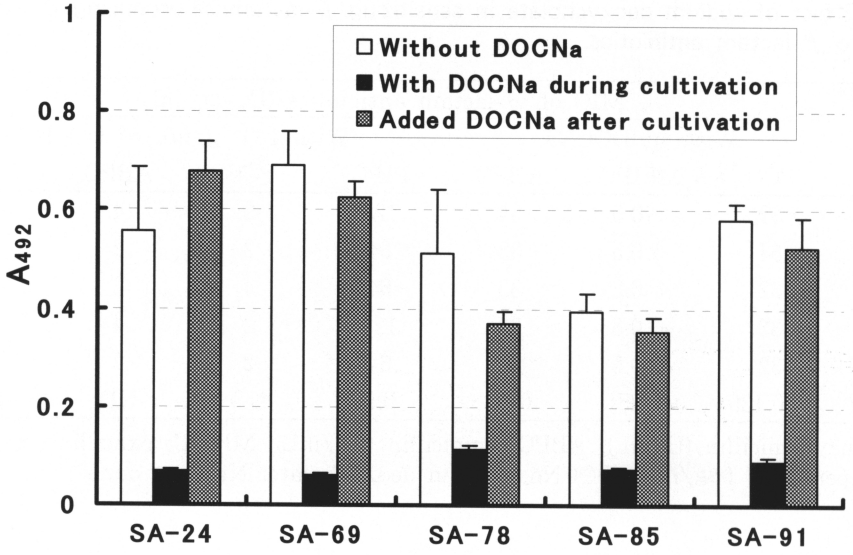


Fig. 2 Inhibition effect of sodium deoxycholate against β-lactamase activity. Each *S. aureus* (SA-24, SA-69, SA-78, SA-85 or SA-91) was inoculated in 100 μL of ST-broth with (black bar) or without (white bar) 100 μg/mL of sodium deoxycholate and cultured at 37° C for 24 h. The cultivation medium to which 100 μg/mL of sodium deoxycholate was added after the cultivation period was prepared (gray bar) as a control. Each cultivation fluid was incubated with nitrocefin as a substrate of β-lactamase at 37° C for 30 to 60 min. Absorbance at 492 nm was measured with a spectrophotometer. The test was performed three times independently. The mean and S.D. values are described.

Therefore, it is demonstrated that the sodium deoxycholate remarkably reduces the synthesis of the β-lactamase in β-lactamase-producing *S. aure us*, but does not suppress the β-lactamase activity.

Sodium deoxycholate is not a medical supply; therefore, its safety for the human body remains uncertain. Although further analysis is necessary, investigation of the synergistic effects of sodium deoxycholate on the antimicrobial activity is expected to be a new therapeutic method and may contribute to an effective utilization of β-lactam antibiotics against infectious diseases caused by β-lactamase-producing bacteria.

## Conclusion

The antimicrobial activities of three β-lactams (PCG, ABPC and PIPC) against β-lactamase-producing *S. aureus* were obviously enhanced by a combined use with sodium deoxycholate. In addition, it is demonstrated that sodium deoxycholate remarkably reduces the synthesis of the β-lactamase in β-lactamase-producing *S. aureus*, but does not suppress the β-lactamase activity. The combined use of sodium deoxycholate with β-lactam antibiotic is expected to be a new therapeutic method and may contribute to an effective utilization of β-lactam antibiotics against infectious diseases caused by β-lactamase-producing bacteria.

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# (要　旨)

**背景**　β-ラクタマーゼ産生黄色ブドウ球菌などが産生するβ-ラクタマーゼは、β-ラクタム系抗菌薬が有するβ-ラクタム環を開裂させることで、その抗菌活性を失活させる。そのため、β-ラクタマーゼ産生菌が原因となる感染症に対して、限られた薬剤しか使用できないのが現状である。β-ラクタマーゼ産生菌などの多剤耐性菌による感染症に対し、新しい治療薬や治療方法の開発が急務となっている。

**目的**　界面活性作用のあるデオキシコール酸ナトリウムは、抗菌活性も有していることが知られている。本研究では、この化合物のβ-ラクタマーゼ産生黄色ブドウ球菌に対する抗菌活性、並びに同化合物とβ-ラクタム系抗菌薬とを併用した場合の、同細菌に対する抗菌活性相乗効果について解析を行い、本化合物がβ-ラクタマーゼ産生細菌による感染症に対し、新しい治療薬・治療方法の開発に結び付く可能性について検討を行った。

**方法**　β-ラクタマーゼ産生黄色ブドウ球菌に対するデオキシコール酸ナトリウムの抗菌活性、および同化合物とβ-ラクタム系（ペニシリン系）抗菌薬とを併用した場合の抗菌活性相乗効果について、MIC（minimum inhibitory concentration）法で解析した。また、デオキシコール酸ナトリウムによるβ-ラクタマーゼの酵素活性阻害作用、および同酵素の生合成阻害作用に関して、ニトロセフィンを用いた吸光度測定法で解析した。

**結果**　β-ラクタマーゼ産生黄色ブドウ球菌に対して、ほとんど抗菌活性を示さなかったペニシリン系抗菌薬が、デオキシコール酸ナトリウムと併用することで顕著な抗菌活性を示した。この抗菌活性の増強効果は、デオキシコール酸ナトリウムによる同細菌に対するβ-ラクタマーゼの生合成阻害作用によるものであることが強く示唆された。

**結論**　デオキシコール酸ナトリウムとβ-ラクタム系（ペニシリン系）抗菌薬との併用による抗菌活性相乗効果に関する研究は、β-ラクタマーゼ産生黄色ブドウ球菌などの多剤耐性菌が原因となる感染症に対し、全く新しい治療方法の開発や既存抗菌薬の有効利用等に結びつく可能性が期待される。

**キーワード**　β-ラクタマーゼ産生黄色ブドウ球菌、デオキシコール酸ナトリウム、β-ラクタム系抗菌薬、抗菌活性、*blaZ*遺伝子