STAPHYLOCOCCUS AUREUS FROM SEPTICEMIC PATIENTS:  
ANTIMICROBIAL SUSCEPTIBILITY TO NEW β-LACTAM  
ANTIBIOTICS AND PATHOGENICITY IN MICE  

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Ninety-six Staphylococcus aureus strains were isolated from septicemic patients at the  
Tokyo Metropolitan Geriatric Hospital and their in vitro antimicrobial susceptibility to 9  
β-lactam antibiotics was examined. Twenty-five % of the isolates were not inhibited  
by methicillin at a concentration of 6.25 μg/ml. All of these methicillin-resistant strains  
possessed inducible penicillinase activity.

Most drugs showed strong antibacterial activity against 96 S. aureus strains. Especially,  
imipenem was found to be most active and was followed by cephaloridine, SCH 34343,  
L-105, and HR 810, in that order.

Pathogenicity of these strains toward mice was examined, indicating that there were  
no Smith-like strains possessing high pathogenic to mice among the isolates. Especially,  
it was found that the highly methicillin-resistant (≥200 μg/ml) strains didn’t show any  
pathogenicity toward mice, when infected with saline suspension (0.5 ml) of 10⁶ cells/ml  
of these strains.

INTRODUCTION  
Staphylococci are known to be one of the main  
pathogenic bacteria which cause various infectious  
diseases. Accordingly various antimicrobial agents  
have been used for staphylococcal infections, resulting  
in the occurrence of multiple resistance in these  
organisms. Recently the isolation of frequencies  
of S. aureus strains carrying resistance to methicillin,  
aminoglycosides, penicillins, cephalosporins,  
etc. have increased owing to the widespread of  
various chemotherapeutic agents1-11).

It is known that the newly developed cepham-  
antibiotics have increased their activity against  
gram-negative bacteria including β-lactamase-pro-
ducing strains but are not enough effective against  
gram-positive bacteria.

It is the purpose of this paper to compare the  
antibacterial activities of new β-lactam antibiotics  
against S. aureus from patients with septicemia and  
to know the relationship between methicillin re-
sistance and mice pathogenicity.

MATERIALS AND METHODS  
Bacterial strains. Ninety-six S. aureus strains  
were isolated from patients with septicemia at the  
Tokyo Metropolitan Geriatric Hospital from 1972  
to 1983. Smith strain was used as a control.

Antibiotics. Fresh stock solutions of the fol-
lowing antibiotics were used; imipenem (Merck),
Fig. 1 Antibacterial activity of \(\beta\)-lactam antibiotics against 96 *S. aureus* strains isolated from septicemic patients

**SCH 34343** (Schering), L-105 (Lederle Japan), HR 310 (Hoechst), ceftazidime (Glaxo), cefmetazole (Sankyo), cephaloridine (Shionogi), methicillin (Banyu), and penicillin G (Meiji).

**Determination of MICs.** Minimum inhibitory concentrations (MICs) were determined using the agar dilution method. Sensitivity Test agar (Nisui) was used. An overnight broth culture was adjusted to the density of a 0.5 McFarland standard (about 10^8 cells/ml). One loopful (about 5 \(\mu\)l) of 100-fold diluted culture was inoculated onto 10 ml agar layers containing serial two-fold dilutions of drug. The plates were incubated at 37°C for 18 hr. The MIC was defined as the lowest drug concentration which inhibited visible bacterial growth.

**Measurement of penicillinase activity.** Penicillinase activity was determined by the modification of NOVICK's micro-iodometric assay, using penicillin G as a substrate. Methicillin was used as inducer of penicillinase.

**Strain pathogenicity for mice.** The ddY strain mice were used in these experiments. They were 4-week old male mice weighing 19 to 22 g. All cultures were grown in Brain Heart Infusion broth (Difco). After incubation for 18 hr at 37°C, the cells were adjusted spectrophotometrically to 10^8/ml and 10^9/ml with sterile physiological saline solution. Saline suspension (0.5 ml) of each strain was injected intraperitoneally. The animals were observed for 4 days after injection.

**RESULTS**

**MICs.** The antibacterial activities of 9 \(\beta\)-lactam antibiotics are shown in Fig.1. Imipenem exhibited the highest antibacterial activity against 96 *S. aureus* strains and was followed by cephaloridine, SCH 34343, L-105, and HR 810, in that
There were no highly resistant (≥100 µg/ml) strains to both cephaloridine and penicillin G. The MIC₉₀ of cephaloridine and penicillin G were 6.25 and 25 µg/ml, respectively.

**Penicillinase activity.** Penicillinase-negative strain was found in 16 out of 96 strains. However, all of the methicillin-resistant *S. aureus* (MRSA) strains (≥12.5 µg/ml) produced penicillinase.

**Pathogenicity to mice.** When a challenge dose (0.5 ml) of 10⁸ cells/ml was given, 50 to 100% mortality rate in 43 out of 96 strains (44.8%) was observed (Table 1). On the other hand, mice mortality was 100% when infected with Smith strain. Mouse pathogenicity among 24 methicillin-resistant *S. aureus* (MRSA) strains (≥12.5 µg/ml) were examined. The strains with MICs between 12.5 and 25 µg/ml showed various mouse pathogenicity. However, it was found that the highly resistant (≥200 µg/ml) strains didn't kill any of mice (Fig. 2). When mice were injected intraperitoneally with a challenge dose (0.5 ml) of 10⁶ cells/ml of 43 strains, any of mice didn't die within 4 days of observation. However, the Smith strain exhibited mouse pathogenicity (90% mortality), even when injected with a challenge dose of 10⁶ cells/ml (Table 2).

**DISCUSSION**

Antibacterial activities of 9 β-lactam antibiotics were examined against *S. aureus* strains isolated recently from patients with septicemia. It was found that imipenem, SCH 34343, HR 810, L-105, and cephaloridine were effective against almost all strains tested. These results indicate that resistance of these strains are due to the production of penicillinase and are owing to the stability of these antibiotics toward penicillinase.

The isolation frequency of MRSA has increased in the United States and Europe. The MRSA strains have not been, however, a serious problem in Japan, probably due to the wide use of newly developed cephalosporins instead of penicillins.

According to the wide and frequent use of β-lactam antibiotics for the treatment of aged-inpatients, we collected *S. aureus* strains from septicemic patients to know β-lactam resistance in these strains.

Twenty-five % of the strains was found to be resistant to methicillin (≥12.5 µg/ml). It is known that the mechanism of methicillin resistance is due to an alternation of penicillin-binding proteins (PBPs)¹⁷-²⁰, and the induction of PBP-2' occurs only in the presence of penicillinase plasmid²¹. All of the MRSA (≥12.5 µg/ml) strains used in this study produced inducible penicillinase.

Therefore, the methicillin resistance in these strains will be due to the formation of inducible PBP-2'.

There are some reports on pathogenicity in MRSA²²-²⁴. The comparison of the pathogenicity in MRSA showed that all of the highly resistant (≥200 µg/ml) strains were nonpathogenic toward mice, while the strains carrying the MIC values of 12.5 to 25 µg/ml showed various mouse pathogenicity. The ideal model of experimental infection of mice with *S. aureus* strains has not been well-established yet, and it is uncertain whether experiment animal (mice) and challenge route used in this study are adequate. But MRSA strains are not highly pathogenic to mice and the prevalence of MRSA strains should be paid attention in the future based on the epidemiological standpoints.
References


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敗血症患者から分離された黄色ブドウ球菌
β-ラクタム剤に対する感受性およびマウスに対する病原性について

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東京都療育院付属病院の敗血症患者より分離した黄色ブドウ球菌96株について、β-ラクタム剤9薬剤の感受性およびマウスに対する病原性を検討した。
imipenem, cephaloridine, SCH 34343, L-105, HR 810が優れた抗菌活性を示し、ときにimipenemが最も強い抗菌力を有していた。methicillinには供試菌株の25%がMIC 12.5 µg/ml以上の耐性を示し、これらの耐性株のすべてが誘導的にpenicillinaseを産生した。
methicillin耐性菌株のマウスに対する病原性を検討した結果、供試菌株の中にはSmith株のような強毒性を示す株は認められなかった。ときにmethicillin高度耐性菌（MIC ≥200 µg/ml）はマウスに対し極めて強い病原性を示したことで注目された。