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 10^8 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 agents[1~11].

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

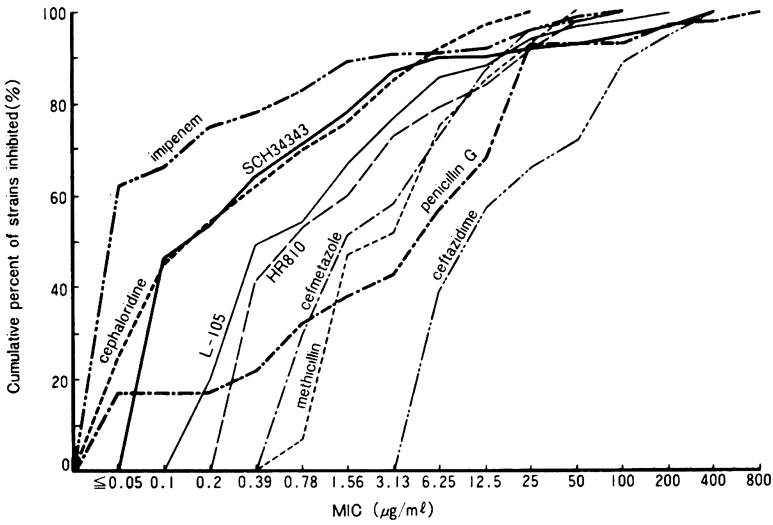
It is the purpose of this paper to compare the antibacterial activities of new β-lactam antibiotics against *S. aureus* from patients with septicemia and to know the relationship between methicillin resistance and mice pathogenicity.

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 following antibiotics were used; imipenem (Merck),

Fig. 1 Antibacterial activity of β-lactam antibiotics against 96 *S. aureus* strains isolated from septicemic patients



SCH 34343 (Schering), L-105 (Lederle Japan), HR 810 (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 (Nissui) 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 μl) 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[12], using penicillin G as a substrate. Methicillin was used for 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 10^6/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.

Table 1 Mouse mortality after intraperitoneal infection with 96 *S. aureus* strains isolated from septicemic patients

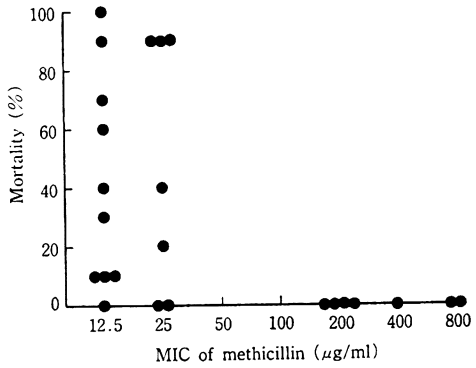
Strain	Mortality (%)	No. of strains
Clinical isolates	100	13
	90	15
	80	5
	70	3
	60	2
	50	5
	40	3
	30	7
	20	4
	10	11
	0	28
Total	—	96
Smith strain	100	1
Total	—	1

Ten mice were used for each strain.
Challenge dose, 10^8 cells/ml.

RESULTS

MICs. The antibacterial activities of 9 β-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

Fig. 2 Mouse mortality after intraperitoneal infection with 24 methicillin-resistant strains of *S. aureus*



order. There were no highly resistant (≧100 μg/ml) strains to both cephaloridine and penicillin G. The MIC_{90s} 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 10^8 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 (≧12.5 μg/ml) strains was 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 10^6 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 10^6 cells/ml (Table 2).

Table 2 Mouse mortality after intraperitoneal infection with 43 *S. aureus* strains*

Strain	No. of mice died	Mortality (%)
S. aureus Smith	9	90
43 *S. aureus* strains	0	0

* Forty-three strains were selected from 96 strains by 50 percent or more of mortality rate after intraperitoneal infection with 10^8 cells/ml. Ten mice were used for each strain. Challenge dose, 10^6 cells/ml.

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 plasmid[21]. 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 MRSA[22~24]. 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.

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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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東京都養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，とくに 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，これらの耐性菌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 株のような強病原性を示す株は認められなかった。とくに methicillin 高度耐性菌（MIC ≧200 μg/ml）はマウスに対し極めて弱い病原性を示したことで注目された。