Comparison of *in vitro* activity of carbapenems and tosufloxacin against clinically-isolated strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis*

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The activities of three carbapenems, imipenem, meropenem, and panipenam, as well as that of tosufloxacin, against 88 strains of *Pseudomonas aeruginosa*, 48 strains of *Staphylococcus aureus* and 37 strains of *Enterococcus faecalis* were examined. In *P. aeruginosa*, the activities of piperacillin, ceftazidime, amikacin, tobramycin, ofloxacin, and carumonam were also evaluated. To compare activities the ratios of the minimum inhibitory concentration (MIC) of imipenem to the respective MICs of meropenem, panipenem, and tosufloxacin were calculated. In *P. aeruginosa*, meropenem had the lowest MICs among the carbapenems. In *S. aureus* as well as *E. faecalis*, imipenem had the lowest MICs among the carbapenems. To evaluate the cross-antimicrobial activity between imipenem and the other antimicrobial agents, we introduced a parameter called the MIC ratios distribution index. In *P. aeruginosa*, MIC ratios distribution indexes between meropenem and imipenem, panipenem and imipenem, tosufloxacin and imipenem, piperacillin and imipenem, ceftazidime and imipenem, amikacin and imipenem, tobramycin and imipenem, ofloxacin and imipenem, and carumonam and imipenem were 1.01, 0.41, 1.86, 1.47, 1.26, 1.66, 1.82, 1.82, and 1.41, respectively, indicating cross-antimicrobial activity between imipenem and panipenem. In *S. aureus*, the MIC ratios distribution indexes between meropenem and imipenem, panipenem and imipenem, and tosufloxacin and imipenem were 0.35, 0.42, and 0.75, respectively, indecating cross-antimicrobial activity between carbapenems. In *E. faecalis*, the MIC ratios distribution indexes between meropenem and imipenem, panipenem and imipenem, and tosufloxacin and imipenem were 0.65, 0.19, and 2.57, respectively, indicating cross-antimicrobial activity between carbapenems, but no cross-antimicrobial activity between imipenem and tosufloxacin. These results seem helpful in providing useful guidelines for choosing an effective treatment against clinical isolates of *P. aeruginosa*, *S. aureus*, and *E. faecalis*.

Key words: *in vitro* activity, carbapenems, tosufloxacin, MIC ratios distribution index

Introduction

Pseudomonas aeruginosa, *Staphylococcus aureus* and *Enterococcus faecalis* are currently recognized as the most important pathogens in severe hospital infections. Carbapenem antibiotics and new quinolones have potent antimicrobial activities against *P. aeruginosa*, *S. aureus* and *E. faecalis*. However, few studies comparing the activities of carbapenems and new quinolones against clinically-isolated strains of *P. aeruginosa*, *S. aureus* and *E. faecalis* have been conducted. With this background, the *in vitro* activities of three carbapenems (imipenem, meropenem, and panipenem) and tosufloxacin against clinical isolates of *P. aeruginosa*, *S. aureus* and *E. faecalis* were examined. In addition, the activities of piperacillin, ceftazidime, amikacin, tobramycin, ofloxacin, and carumonam against *P. aeruginosa* were also evaluated.

Materials and Methods

Bacterial strains

Strains of *P. aeruginosa*, methicillin-sensitive *S. aureus* and *E. faecalis*, isolated clinically at Kagawa Medical School from June 1993 to October 1993, were used. Where multiple isolates of bacteria were received from the same patient, only the first isolate was used; hence, 88 strains of *P. aeruginosa* from 88 patients, 48 strains of *S. aureus* from 48 patients, and 37 strains of *E. faecalis* from 37 patients were evaluated in this study.

Susceptibility testing

The activity of each antimicrobial agent was determined by measuring the minimum inhibitory concentration (MIC) of each agent with the MIC 2000 Plus System (Dynatech Laboratories, U.S.A.). To compare the activity of imipenem with those of the other antimicrobial agents, the ratios of the minimum inhibitory concentration (MIC) of imipenem to the respective MICs of meropenem, panipenem, and tosufloxacin were calculated. In *P. aeruginosa*, the ratios of the MIC of imipenem to the MICs of piperacillin, ceftazidime, amikacin, tobramycin, ofloxacin, and carumonam were also calculated.

Results

Table 1 shows the activities of imipenem, meropenem, panipenam, and tosufloxacin against 88 strains of *P. aeruginosa*, 48 strains of *S. aureus* and 37 strains of *E. faecalis*. The range of MICs, MIC 50% and MIC 90% for each bacterial species are listed. For *P. aeruginosa*, meropenem had the lowest MICs among the carbapenems. For *S. aureus* as well as *E. faecalis*, imipenem had the lowest MICs among the carbapenems.

Fig. 1 shows the distribution patterns of the

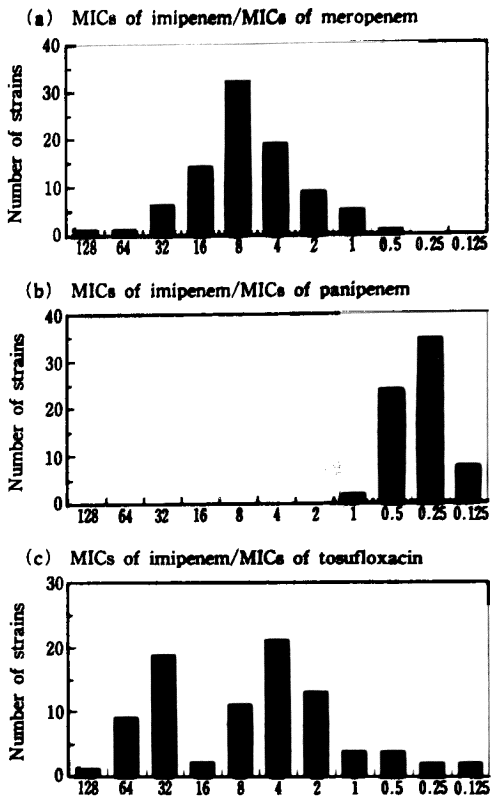

Fig. 1. Distribution patterns of the ratios of MICs of imipenem to MICs of meropenem (a), MICs of imipenem to MICs of panipenem (b), and MICs of imipenem to MICs of tosufloxacin (c) in *Pseudomonas aeruginosa*.

Table 1. Comparative *in vitro* activity of imipenem, meropenem, panipenem, and tosufloxacin against clinically-isolated *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*

Organism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)		
		range	50%	90%
Pseudomonas aeruginosa (88)	imipenem	2–64	8	32
	meropenem	0.03–32	1	16
	panipenem	4–128	16	64
	tosufloxacin	0.25–32	1	4
Staphylococcus aureus	imipenem	0.015–0.06	0.03	0.03
Methicillin-susceptible strains (48)	meropenem	0.125–0.5	0.25	0.25
	panipenem	0.03–0.06	0.06	0.06
	tosufloxacin	0.06–1	0.06	0.25
Enterococcus faecalis (37)	imipenem	0.125–8	1	4
	meropenem	0.06–32	8	32
	panipenem	0.125–8	1	4
	tosufloxacin	0.25–32	1	32

MIC ratios of imipenem to meropenem (a), imipenem to panipenem (b), and imipenem to tosufloxacin (c) for *P. aeruginosa*. Figs. 2 and 3 show the distribution patterns of the MIC ratios of imipenem to meropenem (a), imipenem to panipenem (b), and imipenem to tosufloxacin (c) for *S. aureus* and *E. faecalis*, respectively. These figures clearly demonstrate the comparative activity of the 4 antimicrobial agents.

Fig. 4 shows the distribution patterns of the MIC ratios of imipenem to piperacillin (a), imipenem to ceftazidime (b), imipenem to amikacin (c), imipenem to tobramycin (d), imipenem to ofloxacin (e), and imipenem to carumonam (f) for *P. aeruginosa*.

Based on the data of the distribution patterns in Figs. 1~4, it was speculated that when the distribution pattern was broad, cross-antimicrobial activity (cross-resistance or cross-sensitivity) did not exist, and when the distribution pattern was narrow, cross-antimicrobial activity existed. To evaluate the degree of cross-antimicrobial activity between several antimicrobial agents and imipenem, we introduced a parameter called the MIC ratios distribution index. The calculation of this index is demonstrated by taking the example of Fig. 1 (a). In Fig. 1 (a), 32 strains had a MIC ratio of imipenem to meropenem of 8. We considered this category cross-antimicrobial activity grade 0. Fourteen strains had a MIC ratio of 16, and 19 strains had a ratio of 4. We considered this category cross-antimicrobial activity grade 1, and $(14+19)\times1=33$ points (No. of points=total number of strains×grade) were assigned. Six strains had a MIC ratio of 32, and 9 strains had a ratio of 2. We considered this category cross-antimicrobial activity grade 2, and $(6+9)\times2=30$ points were assigned. One strain had a MIC ratio of 64, and 5 strains had a ratio of 1. We considered this category cross-antimicrobial activity grade 3, and $(1+5)\times3=18$ points were assigned. One strain had a MIC ratio of 128, and 1 strain had a ratio of

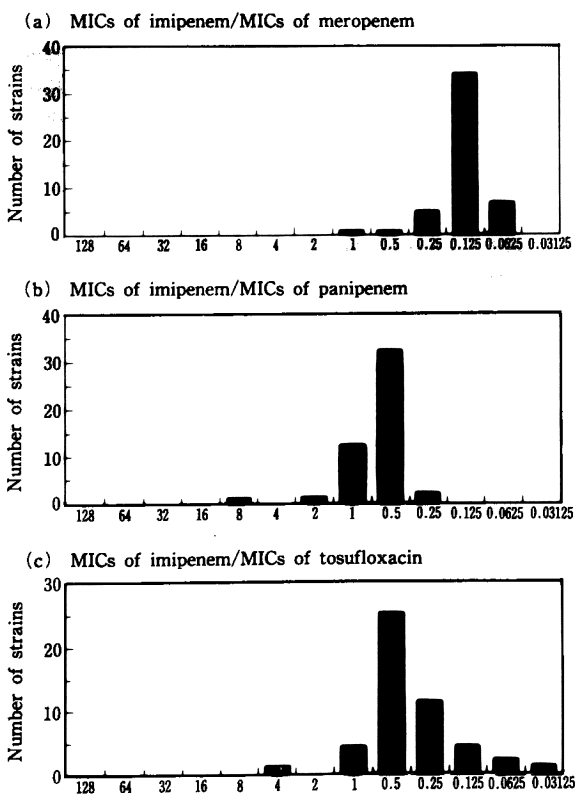

Fig. 2. Distribution patterns of the ratios of MICs of imipenem to MICs of meropenem (a), MICs of imipenem to MICs of panipenem (b), and MICs of imipenem to MICs of tosufloxacin (c) in *Staphylococcus aureus*.

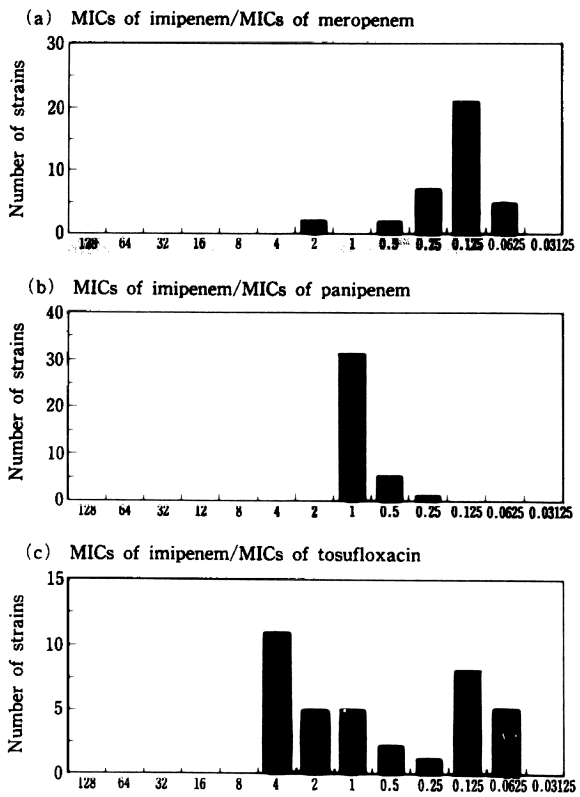

Fig. 3. Distribution patterns of the ratios of MICs of imipenem to MICs of meropenem (a), MICs of imipenem to MICs of panipenem (b), and MICs of imipenem to MICs of tosufloxacin (c) in *Enterococcus faecalis*.

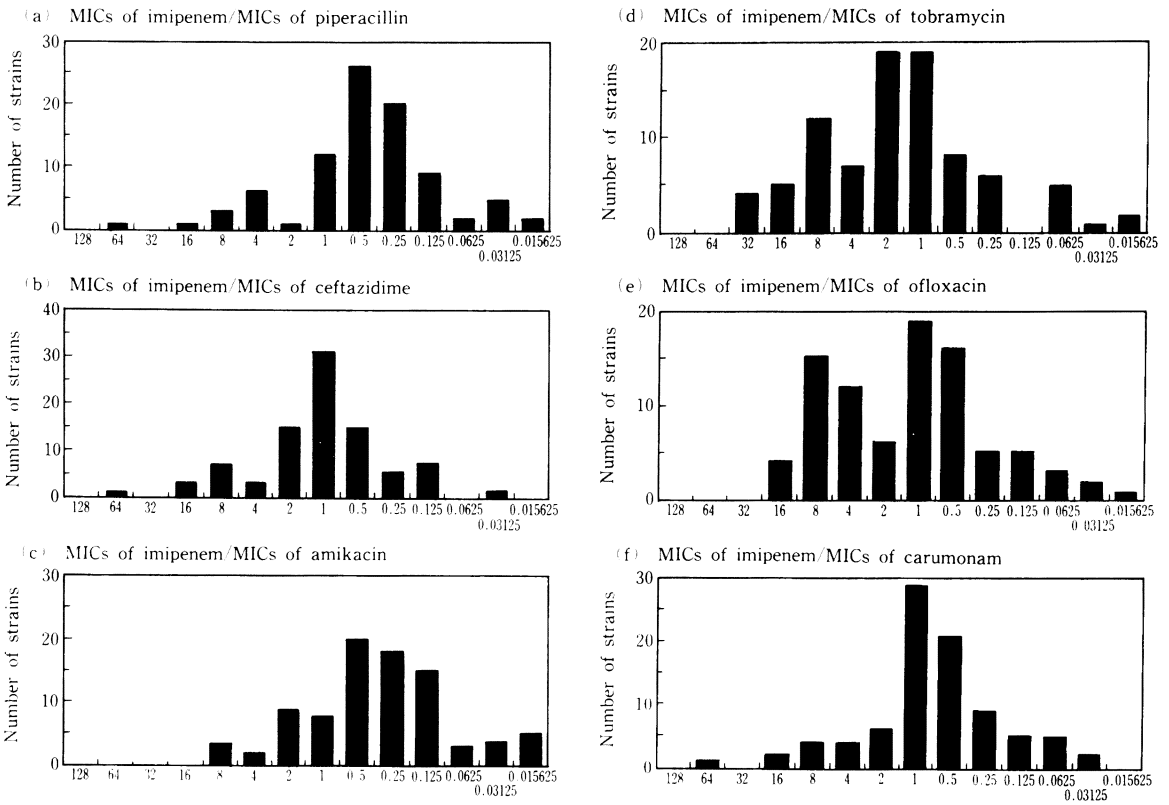

Fig. 4. Distribution pattern of the ratios of MICs of imipenem to MICs of piperacillin (a), MICs of imipenem to MICs of ceftazidime (b), MICs of imipenem to MICs of amikacin (c), MICs of imipenem to MICs of tobramycin (d), MICs of imipenem to MICs of ofloxacin (e), and MICs of imipenem to MICs of carumonam (f) in *Pseudomonas aeruginosa*.

0.5. We considered this category cross-antimicrobial activity grade 4, and $(1+1)\times4=8$ points were assigned. The sum of these points (in this example, 89) divided by the number of strains (88) was calculated, and this value (1.01) was considered to be the MIC ratios distribution index. If this value was below 1.0, we considered it to represent cross-antimicrobial activity.

In *P. aeruginosa*, the MIC ratios distribution indexes between meropenem and imipenem, panipenem and imipenem, tosufloxacin and imipenem, piperacillin and imipenem, ceftazidime and imipenem, amikacin and imipenem, tobramycin and imipenem, ofloxacin and imipenem, and carumonam and imipenem were 1.01, 0.41, 1.86, 1.47, 1.26, 1.66, 1.82, 1.82, and 1.41, respectively, indicating cross-antimicrobial activity between imipenem and panipenem.

In *S. aureus*, the MIC ratios distribution indexes between meropenem and imipenem, panipenem and imipenem, and tosufloxacin and imipenem were 0.35, 0.42, and 0.75, respectively, indicating cross-antimicrobial activity between the cabapenems.

In *E. faecalis*, the MIC ratios distribution indexes between meropenem and imipenem, panipenem and imipenem, and tosufloxacin and imipenem were 0.65, 0.19, and 2.57, respectively, indicating cross-antimicrobial activity between the cabapenems, but no cross-antimicrobial activity between imipenem and tosufloxacin.

Discussion

During the past several years, a number of extended-spectrum antimicrobial agents have been developed, including the carbapenems and new quinolones. These agents offer a broad range of activity against both gram-positive and gram-negative aerobic, facultative, and anaerobic organisms. These agents also offer alternatives for many gram-negative isolates that are resistant to

other beta-lactam antimicrobial agents.

This study was designed to evaluate the efficacy of three carbapenems (imipenem, meropenem, and panipenem) and tosufloxacin against clinically-isolated strains of *P. aeruginosa, S. aureus* and *E. faecalis*. For *P. aeruginosa*, meropenem was 8-fold more active than imipenem, and 32-fold more active than panipenem. In contrast, for *S. aureus* and *E. faecalis*, imipenem and panipenem were 8-fold more active than meropenem.

In the previous study, we also evaluated the activity of six antimicrobial agents, piperacillin, ceftazidime, amikacin, ofloxacin, imipenem, and aztreonam against *P. aeruginosa*, and demonstrated cross-antimicrobial activity among piperacillin, ceftazidime, and aztreonam; however, amikacin and imipenem were effective antimicrobial agents, especially as salvage therapy, against *P. aeruginosa* resistant to one agent[1] In this study, we also evaluated the degree of cross-antimicrobial activity using a newly defined parameter called the MIC ratios distribution index. The data of the MIC ratios distribution indexes suggest that there was no cross-antimicrobial activity between imipenem and meropenem, imipenem and tosufloxacin, imipenem and piperacillin, imipenem and ceftazidime, imipenem and amikacin, imipenem and tobramycin, imipenem and ofloxacin and imipenem and carumonam in *P. aeruginosa.*

This parameter seemed to be useful to represent the degree of cross-antimicrobial activity between two drugs. In some instances, differences in cross-antimicrobial activity to several antimicrobial agents seem to be based on differences in the major mechanisms of resistance[2]. The major mechanism of resistance to imipenem in *P. aeruginosa* is related to loss of the porin channel, Opr D 2[3], and rarely due to β-lactamase. This difference in the major mechanism of resistance seems to explain why no cross-antimicrobial activity was demonstrated between imipenem and the other drugs in this study.

The major resistance mechanisms to quinolones in *P. aeruginosa* are both an altered target (DNA gyrase) and reduced antimicrobial agent uptake. Some mutants resistant to quinolones have altered DNA gyrase[4~6] In other mutants, the amount of the outer membrane porin protein is diminished and the accumulation of quinolones is decreased[6] Mutations in the DNA gyrase confer resistance only to quinolones, but alterations in the outer-membrane proteins result in cross-resistance to chemically unrelated antimicrobial agents[6]. In the present study, the non-cross-antimicrobial activity demonstrated between tosufloxacin and imipenem in *P. aeruginosa* and *E. faecalis* might indicate the existence of different major mechanisms of resistance to imipenem and tosufloxacin in the clinically-isolated strains in our institute.

Interestingly, partial non-cross-antimicrobial activity between imipenem and meropenem in *P. aeruginosa* has been suggested. Meropenem-resistant *P. aeruginosa* strains isolated from clinical sources have been reported to show cross-antimicrobial activity to cephems and quinolones, but not to imipenem and panipenem, and this cross-antimicrobial activity was associated with overproduction of an outer menbrane protein with an apparent molecular weight of 49,000[7] In addition, resistance to imipenem in *P. aeruginosa* has been recently demonstrated to be associated with decreased permeability and high-level production of chromosomal cephalosporinase, which was revealed by the use of cephalosporinase inhibitor BRL 42715[8] This could be explained by the apparently better stability of meropenem in the presence of class I chromosomal β-lactamase[9]

In conclusion, our results seem helpful in providing useful guidelines for choosing an effective treatment against clinical isolates of *P. aeruginosa*, *S. aureus*, and *E. faecalis*.

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緑膿菌，黄色ブドウ球菌，および腸球菌臨床分離株に対するcarbapenem系抗菌薬およびtosufloxacinの抗菌力の比較検討

藤田 次郎[1)]・根ヶ山 清[2)]・高原 二郎[1)]
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3種類のcarbapenem系抗菌薬 (imipenem, meropenem, およびpanipenem) とtosufloxacinの抗菌力の比較検討を臨床分離株である緑膿菌88株，黄色ブドウ球菌48株，および腸球菌37株を用いて行った。なお緑膿菌に関してはpiperacillin, ceftazidime, amikacin, tobramycin, ofloxacin, およびcarumonamについての抗菌力をも検討した。抗菌力を比較するためにそれぞれのMICをimipenemのMICで割った値を算出した。Carbapenem系抗菌薬の抗菌力の比較では緑膿菌に対してはmeropenem＞imipenem＞panipenemの順に抗菌力が強かった。一方黄色ブドウ球菌および腸球菌に対しては，imipenem＞panipenem＞meropenemの順に抗菌力が強かった。抗菌力を比較するために，MIC ratios distribution indexという指標を導入した。緑膿菌においてはimipenemと各種抗菌薬 (meropenem, panipenem, tosufloxacin, piperacillin, ceftazidime, amikacin, tobramycin, ofloxacin, およびcarumonam) とのMIC ratios distribution indexはそれぞれ1.01, 0.41, 1.86, 1.47, 1.26, 1.66, 1.82, および1.41であり，imipenemとpanipenemとの間にcross-antimicrobial activityの存在が示唆された。黄色ブドウ球菌においてはimipenemとmeropenem, panipenem, およびtosufloxacin間では，MIC ratios distribution indexはそれぞれ0.35, 0.42, 0.75，腸球菌においては0.65, 0.19, 2.57でcarbapenem間でのcross-antimicrobial activityの存在が示唆された。これらの結果は緑膿菌，黄色ブドウ球菌，および腸球菌に対する治療の選択に有用な情報を与えると考えられた。

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