SUSCEPTIBILITY TO CARBENICILLIN OF CLINICAL ISOLATES OF GRAM-NEGATIVE BACTERIA IN 1972 AND 1973

YUKIO YAMAZI, KAZUO NIKI*, MASAMI TAKAHASHI**,
YUKO TODOME, MASARU SUGANUMA,
EIJI WATARI and HIDEYO IWAGUCHI
Department of Microbiology and Immunology,
Nippon Medical School
* Microbiology Laboratory, Pfizer-Taito Co., Ltd.
** Department of Microbiology, St. Marianna University
School of Medicine

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Disodium α-carboxybenzylpenicillin (CB-PC), carbenicillin, is a semisynthetic penicillin, which has a wider range of activity against gram-negative bacteria than previously available penicillins including ampicillin, and in particular is active against *Pseudomona aeruginosa* and most *Proteus* species[1~14]. The low degree of toxicity of the drug[1,3,15] permits the use of dose large enough to achieve adequate levels for those bacterial strains which have relatively high MICs[1,16~23]. Thus, intravenous administration of up to 20 g per day of CB-PC is recommended for the treatment of patients with serious infection of *Ps. aeruginosa*.

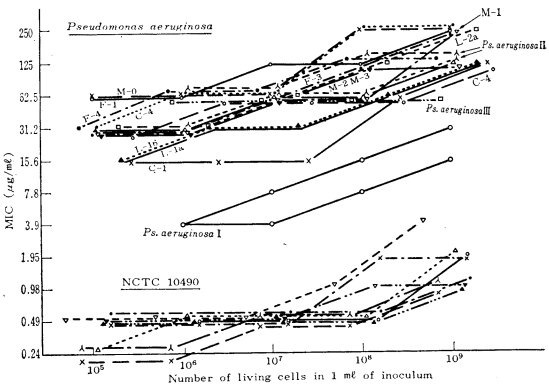
Administration of the massive dose of this compound was not officially adopted in this country until 1974. From 1971 to 1973, experiments on this problem was undertaken as a program of the Carbenicillin Research Conference (Chairman, Prof. J. ISHIGAMI, Dept. Urol., Kobe Univ.). This paper describes the susceptibility to CB-PC of 142 isolates of gram-negative bacteria, which were isolated from patients involved in the program in 1972 and 1973, and several findings from laboratory experiments.

Inoculum Size in Sensitivity Testing

To compare the susceptibility of many bacterial strains to antibacterial agents, reproducibility and sharp end-point of the minimum inhibitory concentration (MIC) are preferable. Serial 10-fold dilutions from 10^0 to 10^{-4} of overnight Tryptosoy Broth (Eiken) cultures of stocked strains and recent clinical isolates of gram-negative bacteria were inoculated on 2-fold serial dilutions of CB-PC in Heart Infusion Agar (Eiken) in Petri dishes using Typing Apparat (Muto), with which one loopful of 27 different bacterial suspensions can be inoculated onto a plate with one action[24]. The plate of CB-PC showing no growth after overnight incubation at 37°C was interpreted as the MIC.

The results with *Ps. aeruginosa* are illustrated in Fig. 1. A CB-PC-sensitive strain of *Ps. aeruginosa*, NCTC 10490 gave a same MIC of 0.49 μg/ml in 9 times repeated tests, when 10^{-2} dilution was used as the inoculum. Results with 18 strains of *Ps. aeruginosa* and a number of strains of another species suggested that great variation of MIC due to the slight difference of inoculum size could be avoided by using 10^{-2} dilution of the broth culture as the inoculum except one strain of *Enterobacter aerogenes*. In general, sharp end point of MIC was obtained with 10^{-2} dilution of the broth culture. As research with *Ps. aeruginosa* was a major project, 10^{-2} dilution was employed in this study except special notice.

Fig. 1. MIC of CB-PC against 18 strains of *Pseudomonas aeruginosa* and NCTC 10490 (9 different tests) with different inoculum size.



MIC of CB-PC against Clinical Isolates of Gram-Negative Bacteria

E. coli, *Klebsiella*, *Proteus vulgaris*, *Prot. mirabilis*, *Rettgerella*, *Morganella*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Serratia* and *Acinetobacter* were studied in this experiment. Numbers of test strains are listed in Figs. 2 and 3. They were isolated from 87 patients in 14 hospitals listed in Table 1. Patients were so severe that large amount dose of CB-PC was administered, and isolation of bacteria was attempted before and after the CB-PC-treatment.

MIC of bacterial strains was determined with the agar plate method described above, and results are shown in Figs. 2 and 3. Among 55 strains of *Ps. aeruginosa*, MICs of 11% were 12.5～25 μg/ml ; 46%, 50 μg ; 13%, 100 μg ; 22%, 200 μg ; 9%, 400 μg or more. Fourteen out of 18 strains of *E. coli* were inhibited at 3.12～6.25 μg/ml ; one strain, 25 μg ; 3 strains were not inhibited at 400 μg. Sixteen out of 23 isolates of *Klebsiella* were not inhibited with CB-PC at the concentration of less than 400 μg/ml, and the other 7 gave MICs of 100～200 μg/ml. MICs of 7 strains of *Prot. mirabilis* were 0.78～1.56 μg/ml ; 2 out of 4 strains of *Prot. vulgaris* showed MICs of 50～100 μg/ml and the other 2, 6.25～12.5 μg/ml. One strain of *Rettgerella* was inhibited with 0.39 μg/ml ; and 3 strains of *Morganella* included one resistant with MIC of 200 μg/ml and 2 sensitive strains with 0.78 and 6.25 μg/ml, respectively. Two out of 3 strains of *Enterobacter cloacae* showed MICs of 200～400 μg/ml and the other strain, 25 μg. Two strains of *Enterobacter aerogenes* were inhibited at 6.25 and 25 μg/ml. Sixteen out of 22 strains of *Serratia* were not influenced with 400 μg/ml and the other 6 were inhibited at 6.25～12.5 or 50 μg/ml. Two strains of *Acinetobacter* gave MICs of 6.25 and 100 μg/ml.

Fig. 2. MIC of CB-PC against clinical isolates. Serial dilutions of CB-PC in nutrient agar were prepared in Petri dishes. One loopful of 1/100 dilution of each broth culture was inoculated onto the surface of the plate using Typing Apparatus. MIC was determined after overnight incubation at 37°C. Number of test strains were described in parentheesis.

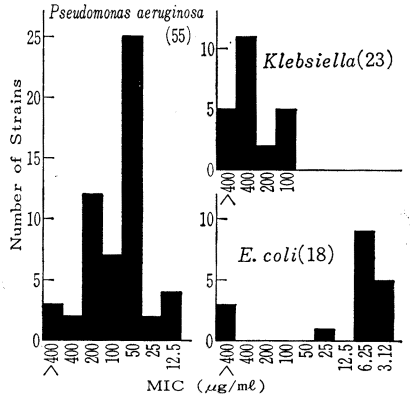


Fig. 3. Legend is same as Fig. 2.

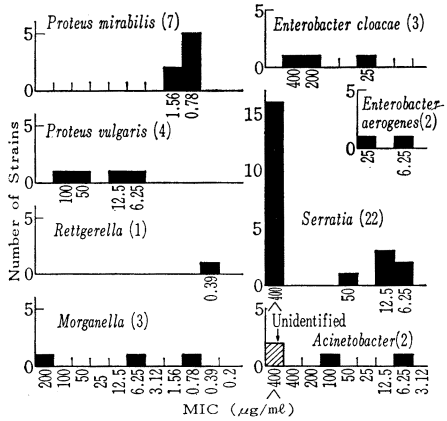


Table 1 Hospitals from which test strains were presented

Dept. Medicine, Hokkaido University, School of Medicine
Dept. Urology, Sapporo Medical School
Dept. Medicine, Jikei-Kai Medical School
Dept. Medicine, Tokyo Kyosai Hospital
Dept. Urology, Keio University, School of Medicine
Dept. Gynecology, Juntendo Medical School
Dept. Surgery, Nagoya City University, School of Medicine
Dept. Pediatrics, Osaka Medical School
Dept. Urology, Kobe University, School of Medicine
Dept. Dermatology, Kyushu University, School of Medicine
Dept. Gynecology, Kobe Central Hospital
Dept. Dermatology, Kurume Medical School
Dept. Gynecology, Himeji Red Cross Hospital
Dept. Urology, Kurume Medical School

Change of Susceptibility to CB-PC between Isolates before and after the Treatment with the Drug

From 13 patients, same species of bacteria were isolated before and after the treatment with CB-PC. MICs of these strains are shown in Table 2. With strains of *Ps. aeruginosa* from patient T.N. and M.T., increase of the resistance to CB-PC was observed after the adminstration of 10 g/day of the

drug. Strains of *E. coli* and *Klebsiella* were isolated from patient S.T. before and after the treatment with 20 g/day of CB-PC. *E. coli* isolates showed definite increase of the resistance to CB-PC after the treatment, though *Klebsiella* gave same MIC of 400 μg/ml before and after the treatment. Increase of the resistance after CB-PC-treatment was observed with *Klebsiella* strains isolated from patient O.M.

Comparison of MICs with Typing Apparat and Hand Streaking

In 1968 the Japan Society of Chemotherapy (JSC) recommended a standard for bacterial sensitivity testing, which indicated to inoculate one loopful of undiluted Casein Soy Mixed Pepton Broth (*e. g.* Trypto Soy Broth) culture of a test strain on to 2-fold serial dilutions of a chemotherapeutic agent in Heart Infusion Agar plates by hand streaking of 2 cm long. In our research, as described before, sensitivity of bacteria to CB-PC was measured with the Typing Apparat, which is used at many laboratories in Japan, inoculating one loopful of 10^{-2} dilution of Trypto Soy Broth culture of each isolate. MICs of 13 isolates were measured with JSC standard method and with our method, using same Trypto Soy Broth cultures as original bacterial preparations. Results are illustrated in Table 3.

Six out of 13 strains gave more than 4 times

Table 2 Sensitivity to CBPC of strains isolated before and after the treatment with CBPC

Patient	Bacterial species	Date of isolation	Strains	MIC of CBPC (μg/ml)
A.K.	*Pseudomonas aeruginosa*	1/29/73	M-1	50
		1/31/73	M-2	50
		2/3/73	M-3	50
K.M.	*Pseudomonas aeruginosa*	9/18/72	M-9a	200
		9/21/72	M-10a	200
			M-11	200
	Serratia	9/18/72	M-9b	>400
		9/21/72	M-10b	>400
S.K.	*Pseudomonas aeruginosa*	9/16/72	S-5a	50
		10/13/72	P-6	50
		10/12/72	P-7	50
T.N.	*Pseudomonas aeruginosa*	10/9/72	P-8	50
		10/20/72	P-9	100
		10/26/72	P-12	200
M.T.	*Pseudomonas aeruginosa*	1/19/73	Ni-6	100
		1/27/73	Ni-7	100
		1/27/73	Ni-8	400
I.T.	*Escherichia coli*	6/2/72	A-8b	6.25
		6/23/72	A-10	6.25
K.K.	*Escherichia coli*	1/10/72	D-2	6.25
		1/26/72	D-4	6.25
		2/4/72	D-5	6.25
	Klebsiella	1/25/72	D-3	400
		2/4/72	D-6	200
S.T.	*Escherichia coli*	4/17/72	D-7	3.12
		4/25/72	D-9	>400
	Klebsiella	4/17/72	D-8	400
		4/25/72	D-10	400
M.J.	*Klebsiella*	2/8/72	A-3	>400
		2/15/72	A-4	>400
O.M.	*Klebsiella*	4/6/72	P-3	100
		5/9/72	P-3-2	400
K.D.	*Klebsiella*	1/24/73	I-6	400
		1/30/73	I-7	400
		2/5/73	I-8	400
K.T.	*Serratia*	10/24/72	Ni-2	>400
		11/2/72	Ni-1	>400
H.G.	*Serratia*	12/13/72	M-14b	>400
		12/26/72	M-15b	>400

Table 3 MICs obtained by different procedures for inoculation

Organisms		Typing 10^{0}	Apparatus 10^{-2}	Hand 10^{0}	Streaking 10^{-2}
Pseudomonas aeruginosa	C-1	50	25	50	
	C-2	200	50	200	
	C-3a	50	12.5	100	
	C-3b	200	50	200	
	C-4	200	100	200	100
	B-1	400	200	200	
	B-2	>400	200	>400	
	NCTC10490	3.12	0.39	3.12	
Klebsiella	A-3	>400	>400	>400	
	A-4	>400	>400	>400	
Escherichia coli	A-5	6.25	3.12	6.25	
Enterobacter aerogenes	A-6	25	12.5	25	12.5
Morganella	C-5	6.25	0.78	6.25	
Pseudomonas aeruginosa	Q-1	400	100		100
	Q-2	100	50		100
	O-1	100	100		100
	F-4	100	50		50
	NCTC10490	1.56	0.78		0.78
Klebsiella	A714423	200	100		100
	A714464	200	100		100
	D-3	>400	400		400
	D-6	400	200		400
Escherichia coli	Q-3	6.25	6.25		6.25
	D-4	12.5	6.25		6.25
	D-5	12.5	6.25		6.25
Proteus mirabilis	M-1-2	1.56	0.78		0.78

10^{0} : Undiluted Trypto Soy broth culture was inoculated.
10^{-2} : 1/100 dilution of the culture was inoculated.
Numbers in the table mean MIC (μg/ml) of CB-PC.

higher MICs with JSC method than with our method (upper half of Table 3), as expected from the experiment for determining the inoculum size described before in this paper (Fig. 1). However, no meaningful difference of MICs was observed between spotting with the Typing Apparat and hand streaking, as far as same bacterial dilution was used as the inoculum.

Minimum Bactericidal Concentration (MBC) of CB-PC

MBCs were measured on 96 isolates with the method described by SILVERBLATT and TURCK[5]. One loopful of each isolate was transferred from stock medium to 5 ml of Trypto Soy Broth and incubated overnight at 37°C. The broth culture were then diluted further in Nutrient Broth to final dilution of 10^{-4}; at this concentration, 1 ml of broth contained approximately 10^{5} organisms. A 0.5 ml aliquot of the bacterial suspension was then added to 0.5 ml of CB-PC, giving final concentration ranging from 0.39 to 400 μg/ml in broth. The tube of antibiotic macroscopically showing complete

Fig. 4. Comparison of MIC and MBC in the broth dilution test. Trypto Soy Broth culture of each isolate was diluted in Nutrient Broth to 1/10000. A 0.5 ml of the bacterial suspension was added to 0.5 ml of CB-PC, giving final concentration of 0.39 to 400 μg/ml in broth. The tube of antibiotic macroscopically showing complete inhibition of growth after overnight incubation at 37°C was interpreted as the MIC. The lowest concentration of antibiotic in which no visible colonies were recovered after subculture on antibiotic-free agar was designated the MBC.

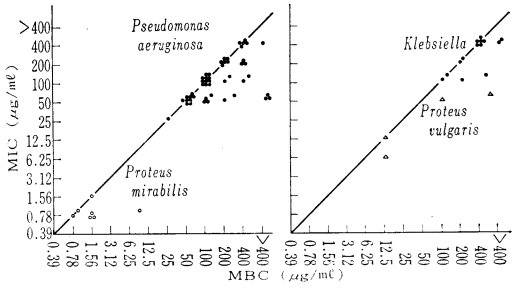
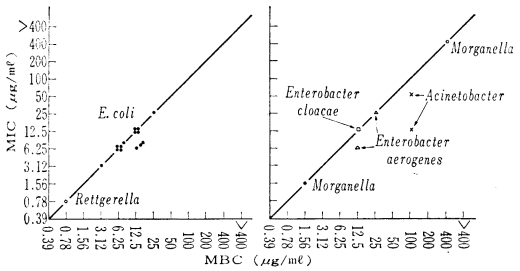


Fig. 5. Legend is same as Fig. 4.



inhibition of growth after overnight incubation at 37°C was interpreted as the bacteriostatic end point (MIC). The lowest concentration of antibiotic in which no visible colonies were recovered when one loopful of broth from each clear tube was subcultured on antibiotic-free agar was designated as the bactericidal end point (MBC).

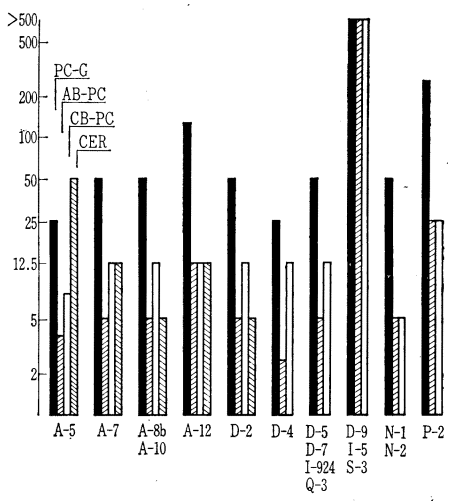
Results are shown in Figs. 4 and 5. Seven out of 49 isolates of *Ps. aeruginosa*, 1 out of 4 isolates of *Prot. vulgaris*, 1 out of 14 isolates of *Klebsiella*, 1 out of 7 isolates of *Prot. mirabilis*, and none of 14 isolates of *E. coli* showed the MBC which was higher than the MIC by times or more. Therefore the action of CB-PC against most strains is considered to be bactericidal. MIC of each strain by this broth dilution method was substantially identical with that with agar dilution method using 10^{-2} dilution as the inoculum described before in this paper.

Comparison of the Antibacterial Effect of Benzylpenicillin (PC-G), Aminobenzylpenicillin (AB-PC), Carbenicillin (CB-PC) and Cephaloridine (CER)

Effect of 3 penicillins and CER against 14 isolates of *E. coli* was compared with a broth dilution method. One drop of 37°C overnight culture of each isolate in Nutrient Broth was inoculated in 2-fold serial dilutions of antibiotic in Nutrient Broth. The lowest dilution of each antibiotic showing macroscopically complete inhibition of growth after overnight incubation at 37°C was designated as the MIC.

Results are shown in Fig. 6. In general, AB-PC gave the lowest MIC, MIBs of CB-PC were identical with those of AB-PC or higher than AB-PC by 2 to 4 times, and PC-G gave highcst MICs. In 6 strains tested, antibacterial effect of CER was identical with AB-PC or CB-PC except one strain, A-5 which gave rather high MIC of CER.

Fig. 6. MICs of Benzylpenicillin (PC-G), Aminobenzylpenicillin (AB-PC), Carboxybenzylpenicillin (CB-PC), and Cephaloridine (CER) to clincal isolates of *E. coli* in broth dilution test. One drop of 37°C, overnight Nutrient Broth cultures of 17 isolates was inoculated into 5 ml of antibiotic dilutions in Nutrient Broth, and incubated at 37°C overnight. Minimum dilution showing complete inhibition of the growth of bacteria was designated as MIC.



Discussion

Inoculum size is an important factor to obtain the reproducible results, which are sufficient for the comparison of the drug-susceptibility between different bacterial strains. It was suggested that a number of strains of *Ps. aeruginosa*, *E. coli*, *Klebsiella*, *Prot. vulgaris*, *Prot. mirabilis*, *Providencia*, *Alcaligenes faecalis*, *Morganella* and *Rettgerella* gave stable figure of MIC, if 10^{-2} dilution of Trypto Soy Broth culture was used as the inoculum in agar dilution technique. A marked inoculum effect has been noted with penicillins including CB-PC by several authors[1,3,5,6,9,12,13], though WILLIAMS *et al.*[7] found that in a broth dilution method variation in inoculum size from 10^4 to 10^6 organisms produced no consistent differences with a few exceptions.

MITSUHASHI *et al.*[40,41], who studied drug-resistance in *Shigella* strains, found that decrease of inoculum size could eliminate the error caused by development of resistant mutant, and they recommended to use one loop of 10^{-2} dilution of overnight broth culture for inoculum size on assay plate.

After this preliminary experiment, 142 clinical

isolates of gram-negative bacteria were tested for the susceptibility to CB-PC, and it was confirmed that, *in vitro*, CB-PC was relatively effective in inhibiting the growth of a number of gram-negative bacterial species. Among 55 strains of *Ps. aeruginosa*, 70% showed MICs of 100 μg/ml or less, and the concentration could be available in blood after massive dose of CB-PC[16~23]. Twenty-two per cent was inhibited at the concentration of 200 μg/ml, and 9% showed MICs of 400 μg/ml or mose. These findings are substantially similar to those by some researchers[2,5~7,10,12,14,17,26~30,39]. Another some workers[1,4,8] reported rather low MICs for *Ps. aeruginosa*, but BODEY *et al.*[30] and ISHIGAMI *et al.*[31] reported high MIC. The explanation for these divergent results is not completely clear. Inoculum size does not always seem to be a major factor[30], and difference in media, in technique, and criterion of MIC-reading[1] may be answers. The activity of CB-PC against *Ps. aeruginosa* was considerably lower than that of polymyxin B or gentamicin[9,24,28], but the low toxicity[1,8,15] will favor the clinical usefulness of the compound.

Among 18 strains of *E. coli* 78% gave MICs of 6.25 μg/ml or less and 17% were greatly resistant being not inhibited at 400 μg/ml. Thus *E. coli* strains were sharply divided into the sensitive and the resistant strains, These data are similar to those observed by others[2,4,5,7,17,30].

Recently, nonpigmented *Serratia marcescens* has been isolated with increasing frequency from patients with a variety of clinical illness[32~34]. Also in this research, 22 clinical isolates of nonpigmented *Serratia* were presented from several hospitals. Sixteen of these strains were not inhibited the growth with CB-PC at the concentration of 400 μg/ml, and MICs of other strains were 6.25~12.5 or 50 μg. Thus *Serratia* strains are divided into major members of the resistant and a few sensitive ones. THORNTON *et al.*[35], who examined the sensitivity of 52 isolates of nonpigmented *Serratia*, found that MICs of CP-PC ranged from 6.25 to 1,000 μg/ml or more, *i.e.*, a few strains were observed to be moderately sensitive. Resistance of this species to CB-PC was observed also by WILLIAMS *et al.*[7], the MEYERS *et al.*[11] observed somewhat lower resistance with light inoculum. Nevertheless, our data suggest that CB-PC might be effective therapy for infections caused by the occurrence of nonpigmented *Serratia* that are susceptible *in vitro* especially for infections of the urinnary tract, since very high concentration of CB-PC can be achieved in the urine[1~3,6,8,17].

Four strains of *Prot. vulgaris* and 7 of *Prot. mirabilis* were tested, the former showing MICs from 6.25 to 100 μg/ml, the latter 0.78 to 1.56 μg/ml. One strain of *Rettgerella* was sensitive, and MICs of 3 strains of *Morganella* were variable. The susceptibility of indole negative *Proteus* (*Prot. mirabilis*) strains has been observed by some workers[7,13,30], but others[2,4~6,12,17,27] have indicated the susceptibility of indole positive strains, too. STANDIFORD *et al.*[27] indicated that *Prot. vulgaris* strains tested were variable in the susceptibilty. Rather high inoculum size effect was observed with a *Prot. vulgaris* strain in our data.

Isolates of *Klebsiella* showed high resistance to CB-PC. This has been found by nearly all obserrvers[1,2,4,7,12,30]. NEU *et al.*[17] observed overgrowth of *Klebsiella* in a few patients with pulmonary infections after treatment with CB-PC.

Two out of 3 isolates of *Enterobacter cloacae* were quite resis tant to CB-PC, but both of two isolates of *Enterobacter aerogenes* were inhibited at 25 μg/ml or less. MEYERS *et al.*[11] indicated that most *Klebsiella-Enterobacter* organisms were quite resistant to CB-PC, and SMITH *et al.*[6] reported a wide range of sensitivity of *Enterobater* to CB-PC. Species within *Enterobacter* genus, however, should be considered separately when the susceptibility to CB-PC is analyzed.

Increase in resistance to CB-PC of *Ps. aeruginosa* isolates following the treatment with the compound has been suggested by several authors[5,26,29,36]. In our data, increase of the resistance to the drug during CB-PC treatment was observed in 2/5 of *Ps. aeruginosa*, 1/3 of *E. coli*, and 1/5 of *Klebsiella* infections from which bacterial cultures were obtained before and after the treatment. Pyocin or phage typing was not done so that it is not passible to be certain that the strains before and after the treatment were identical. The mechanism of incidence of the resistant strain, *e.g.*, chromosomal mutation, resistance transfer, or succession by a new, resistant strain, should be studied further. LINDBERG *et al.*[37], who typed bacteriophages of *Ps. aeruginosa* isolated from burn wound in a hospital ward, found that in most instances the emergence of CB-PC resistant strains in each patient was actually colonization by a new, resistant type ; and

individual phage types did not always correlate to the resistance to the drug.

Like other penicillins, action of CB-PC has been evaluated to be bactericidal[3,4,7,9,11,26]. Great difference between MIC and MBC was observed only infrequently in this experiment, and 65% of *Ps. aeruginosa* strains, 78% of *E. coli*, 76% of *Klebsiella*, 43% of *Prot. mirabilis*, and 25% of *Prot. vulgaris* lost the viability after 24 hours' incubation with MIC of CB-PC. BODEY *et al.*[30] reported that CB-PC was bactericidal for only 39 of 143 strains of *Ps. aeruginosa*. It may be mentioned that difference between MIC and MBC will reflect the vague end point of MIC in the tube dilution technique, and some *Pseudomonas* strains were observed not to have a consistently sharp end point[30].

Carbenicillin was somewhat less active against some strains of *E. coli* than either AB-PC or CER. This is in agreement with reports by others[2,9,10,38]. However, one strain of *E. coli* resistant to CER was sensitive to CB-PC. ACRED *et al.*[1] and NEU *et al.*[17] pointed out that AB-PC and CB-PC were equally active to *E. coli* strains, if the inoculum was small.

Conclusion

Carbenicillin (CB-PC), a semisynthetic derivative of penicillin was tested against 142 gram-negative isolates by use of an agar dilution method. It was found to be active *in vitro* against many gram-negative bacterial species, including most *Pseudomonas*, *Escherichia coli*, *Proteus*, *Rettgerella*, *Morganella* and *Enterobacter aerogenes* strains. Most *Klebsiella* and *Serratia* strains and some strains of *Pseudomonas*, *E. coli* and *Morganella* were quite resistant, and were not suppressed by concentration of 200 μg/ml or higher. When several gram-negative bacterial species were used as test organisms, a marker effect of inoculum size on the MIC of the drug was apparent. Increased inoculum size required greater concentrations for inhibition. Emergence of resistance occurred during therapy in 2 of 5 patients of *Pseudomonas aeruginosa* infections, and 1 of 3 of *E. coli*. In general, there was very little difference between the MIC and MBC of the drug for most of the sensitive organisms.

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References

1) ACRED, P. ; BROWN, D. M, KNUDSEN, E. T., ROLINSON, G. N. & SUTHERLAND, R. : New semisynthetic penicillin active against *Pseudomonas pyocyanea*. Nature 215 : 25～30, 1967.
2) BRUMFITT, W., PERCIVAL, A. & LEIGH, D. A.: Clincal and laboratory studies with carbenicillin. Lancet 1 : 1289～1293, 1967.
3) KNUDSEN, E. T., ROLINSON, G. N. & SUTHERLAND, R. : Carbenicillin : a new semisynthetic penicillin active against *Pseudomonas aeruginosa*. Brit. Med. J. 8 : 75～78, 1967.
4) ROLINSON, G. N. & SUTHERLAND, R. ; Carbenicillin, a new semisynthetic penicillin active against *Pseudomonas aeruginosa*. Antibact. Agents & Chemoth. : 609～613, 1967.
5) SILVERBLATT, F. & TURCK, M. : Laboratory and clinical evaluation of carbenicillin (Carboxybenzyl penicillin). Antimicr. Agents & Chemoth. : 279～285, 1968.
6) SMITH, C. B. & FINLAND, M. : Carbenicillin. Activity *in vitro* and absorption and excretion in normal young men. Appl. Mcrobiol. 16 : 1753～1760, 1968.
7) WILLIAMS, T. W., Jr. & GLASGOW, N. M. : *In vitro* activity of carbenicillin against gram-negative bacilli. Antibact. Agents & Chemoth. : 388～391, 1968.
8) FUJII, R., KONNO, M., UNO, S., TAKESHITA, N., HACHIMORI, K. & OKADA, K. : Clinical and laboratory studies on carbenicillin in pediatric field. Chemotherapy 19 : 1184～1192, 1969.
9) NISHIDA, M., MATUBARA, T., MINE, Y., OKUI, M., YOKOTA, Y., GOTO, S. & KUWAHARA, S. : Fundamental studies on carbenicillin. Chemotherapy 17 : 1095～1105, 1969.
10) UEDA, Y., MATSUMOTO, F., NAKAMURA, N., SAITO, A., NODA, K., OMORI, M., FURUYA, C., NAKAMURA, Y. & UTSUNOMIYA, M. : Fundamental and clinical studies on carbenicillin. Chemotherapy 17 : 1140～1146, 1969.
11) MEYERS, B. R., SABBAJ, J. & WEINSTEIN, L. : Bacteriological pharmacological and clinical studies of carbenicillin. Arch. Intern. Med. 125 : 282～286, 1970.
12) TURK, M., SILVERBLATT, F., CLARK, H. & HOLMES, K. : The role of carbenicillin in treatment of infections of the urinary tract. J. Inf. Dis. 122 : S 29～S 33, 1970.
13) ADLER, J. L., BURKE, J. P., MARTIN, D. F. & FINLAND, M. : *Proteus* infections in a general hospital. I. Biochemical characeristics and antibiotic susceptibility of the organisms, with special reference to proticine typing and the Dienes phenomenon. Ann. Intern. Med. 75 : 517～530, 1971.
14) KAWARAJO, K., WATANABE, T., ISHII, T. & YAMAKI, S. : Studies on *Pseudomonas aeruginosa*. II. Serological typing, virulence for

mice and susceptibiltity to antibiotics of *P. aeruginosa* from experimental animals and nature. Japan. J. Bacteriol. 28:449～505, 1973.

15) ROBINSON, J. P. N. : Human pharmacology of carbenicillin, a semisynthetic penicillin active against *Pseudomonas aeruginosa*. Antimicr. Agents & Chemoth. : 614～619, 1967.

16) BODEY, G. P., RODRIGUEZ, V. & STEWART, D. : Clinical phamacological studies of carbenicillin. Amer. J. Med. Sci. 257 : 185～190, 1969.

17) NEU, H. D. & SWARZ, H. : Carbenicillin : Clinical and laboratoy experience with a parenterally administered penicillin for treatment of *Pseudomonas* infections. Ann. Intern. Med. 71 : 903～911, 1969.

18) KONDO, K., AMANO, M. & NIIJIMA, T. : Treatment of urinary tract infections with massive dose of carbenicillin. Jap. J. Antibiotics 25 : 175～180, 1972.

19) STANDIFORD, H. C., JORDAN, M. C. & KIRBY, W. M. M. : Clinical pharmacology of carbenicillin compound and other penicillins. J. Inf. Dis. 12 : S 9～S 13, 1970.

20) NAKATA, G., KAMEDA, H. & TANIMOTO, S. : Antibiotic therapy of respiratory infection with *Ps. aeruginosa*, Chemotherapy 21 : 1926～1927, 1973.

21) SHINAGAWA, T., SHIBATA, K., ITO, T., FUJII, S., MURAMATSU, Y. & SUZUKI, Y. : Administration of large dosis of carbenicillin in surgery. Chemotherapy 21 : 1899～1900, 1973.

22) OHKOSHI, M., NAIDE, Y., SUZUKI, K., KIMURA, S. & OKUBO, I. : Investigation on administration of large dosis of antibiotic. I. Adsorbtion and excretion of carbenicillin after administration of large dosis of the drug. Chemotherapy 22 : 139～140, 1974.

23) UEDA, Y., KOBAYASHI, C., MATSUMOTO, H., SAITO, A., NODA, K., SHIMADA, J., OMORI, M., SHIBA, T. & YAMAJI, T. : Studies on the large dosis-treatment in chemotherapy. Chemotherapy 22 : 138～139, 1974.

24) YAMAZI, Y., TAKAHASHI, M., MIYANAGA, Y., TODOME, Y. & MOTOYAMA, M. : Antibiotic sensitivity of Gram-negative bacilli isolated from lesions since 1965. J. Antibiotics, Ser. B 21 : 25～33, 1968.

25) ISHIYAMA, T., UEDA, Y., KUWAHARA, S., KOSAKAI, N., KOYA, G., KONNO, M. & FUJII, Y. : On the standardization of the method for measuring the minimum inhibitory concentration. Chemotherapy 16 : 98～99, 1968.

26) JONE, R. J. & LOWBURY, E. J. L. : Prophylaxis and therapy for *Pseudomonas aeruginosa* infection with carbenicillin and with gentamicin. Brit. Med. J. 3 : 79～82, 1967.

27) STANDIFORD, H. C., KIND, A. C. & KIRBY, W. M. M. : Laboratory and clinical studies of carbenicillin against gram-negative bacilli. Antimicr. Agents & Chemoth. : 286～291, 1968.

28) HOMMA, J. Y., KUMAZAWA, Y. & YOKOTE, N. : Activity of carbenicillin on *Pseudomonas aeruginosa*. Chemotherapy 17:1106～1110, 1969.

29) PHAIR, J. P., TAN, J. S., WATANAKUNAKORN, C., SCHWAB, L. & SANDERS, L. W. : Carbenicillin treatment of *Pseudomonas* pulmonary infection. Amer. J. Dis. Child. 120:22～25, 1970.

30) BODEY, G.P. & TERREL, L.M. : *In vitro* activity of carbenicillin against gram-negative bacilli. J. Bact. 95 : 1587～1590, 1968.

31) ISHIGAMI, J., HARA, S., FUKUDA, Y. & HAYAMI, H. : Use of carbenicillin in urinary infections. Chemotherapy 17 : 1238～1243, 1969.

32) WILFERT, J. N., BARRETT, F. F. & KASS, E. H. : Bacteremia due to *Serratia marcescens*. New Eng. J. Med. 297 : 286～289, 1968.

33) DODSON, W.H. : *Serratia marcescens* septicemia. Arch. Intern. Med. 121 : 145～150, 1968.

34) CLAYTON, E. & VON GRAEVENITZ, A. : Nonpigmented *Serratia marcescens*. J. A. M. A. 197 : 1059～1064, 1966.

35) THORNTON, G. F. & ANDRIOLE, V. T. : Antibiotic sensitivities of nonpigmented *Serratia marcescens* to gentamicin and carbenicillin. J. Infect. Dis. 119 : 393～394, 1969.

36) LOWBURY, E. J. L., KIDSON, A., LILLY, H. A., AYLIFFE, G. A. J. & JONES, R, J. : Sensitivity of *Pseudomonas aeruginosa* of antibiotics : Enzyme of strains highly resistant to carbenicillin. Lancet 2 : 448～452, 1969.

37) LINDBERG, R. B., CURRERI, P. W. & PRUIT, B. A., Jr. : Microbiology of burns treated with carbenicillin : Experimental and clinical observations. J. Inf. Dis. 122 : S 34～S 39, 1970.

38) STEIGHIGEL, N. H., MCCALL, C. E., REED, C. W. & FINLAND, M. : Antibacterial action of "broad-spectrum" penicillins, cephalosporins, and other antibiotics against gram-negative bacilli isolated from bacteremic patients. Ann. N. Y. Acad. Sci. 145 : 224～236, 1967.

39) PHAIR, J. B., WATANAKUNAKORN, C. & BANNISTER, T. : *In vitro* susceptibility of *Pseudomonas aeruginosa* to carbenicllin and the combination of carbenicillin and gentamicin. Appl. Microbiology 18 : 303～306, 1969.

40) MITSUHASHI, S., NAGAI, Y., TANAKA, T. & HASHIMOTO, H. : Determination of drug-resistance in *Shigella* strains. Chemotherapy 17 : 1956～1963, 1963.

41) MITSUHASHI, S., NAGAI, Y., TANAKA, T. & HASHIMOTO, H. : Effect of innoculum size on the determination of drug sensitivity. Prog. Antimicr. & Anticanc. Chemotherapy 931～932, 1970.