IN VITRO SUSCEPTIBILITY OF 14 *BACILLUS* SPECIES FROM DIFFERENT SOURCES AGAINST 34 ANTIMICROBIAL AGENTS

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In vitro susceptibilities of a total of 86 strains of *Bacillus* from different sources against 34 antimicrobial agents were evaluated. Susceptibilities of 28 strains of *Bacillus pumilus* were compared with those of 20 strains of *B. subtilis* and also the other 15 strains of *Bacillus*. No significant differences in susceptibility was observed between *B. pumilus* and *B. subtilis* strains tested, except susceptibilities to lincomycin and cefotaxime. $MIC_{s_{90\%}}$ of *B. pumilus* strains to lincomycin and cefotaxime were 25 and 100 μg/ml respectively, whereas those of *B. subtilis* strains were 6.25 and 3.13 μg/ml respectively. All strains of both species were uniformally susceptible to penicillin G, ampicillin, carbenicillin, oxacillin, cephalothin, cefazolin, cefmetazole, cefotiam, cefoperazone, latamoxef, aminoglycosides, tetracyclines, erythromycin and vancomycin; $MIC_{s_{90\%}}$ of these agents were less than 0.78 μg/ml. The newer cephalosporins were less active than the older ones against *B. pumilus* strains. Susceptibilities of *B. anthracis*, *B. cereus* and *B. thuringiensis* strains were compared each other. *B. anthracis* strains were highly susceptible to groups of penicillins, aminoglycosides, tetracyclines and macrolides; $MIC_{s_{90\%}}$ of these agents were less than 0.39 μg/ml. Strains of *B. cereus* and *B. thuringiensis* were resistant to a wider variety of agents than those of *B. anthracis*. Beta-lactamase could be detected by all strains of *B. cereus* and *B. thuringiensis* tested, but not by *B. anthracis* strains tested. The difference in susceptibility against beta-lactam antibiotics among these three species seems to be paralleled by the difference of beta-lactamase production. No significant difference in susceptibility was found between the isolates from clinical specimens or soil samples and the reference strains from culture collections in each of the species tested.

INTRODUCTION

Bacillus species are usually regarded as non-pathogenic or harmless "contaminant" when isolated from clinical specimens. Numerous reports[1,2], however, have indicated a wide variety of clinical infections associated with *Bacillus* species other than *Bacillus anthracis*, a causative agent of anthrax. *Bacillus cereus* is widely distributed in nature and has been documented as being involved in food poisoning[3,4]. It is commonly found in cereals, milk, dried food ingredients and has also named as a causative agent of abscesses, endocarditis, osteomyelitis and bacteremia[5,6]. There were also reports of infections associated with the other *Bacillus* species including *B. alvei*, *B. licheniformis*, *B. pumilus*, *B. spharicus* and *B. thuringiensis*[7~11]. Recently, *B. pumilus* has been investigated since the elucidation of its causative role in antibiotic-associated diarrhea or colitis after administration of antimicrobial agents[11]. The elucidation of the susceptibility of the strains to antimicrobial agents is essential for the understanding of the pathogenesis of antibiotic-associated diarrhea or colitis, since the administration of agents ap-

pears to be essential for the development of the diseases. *B. pumilus* is closely related to *B. subtilis*, which is also widely distributed in nature, but is differentiated from *B. subtilis* by its ability of hippurate hydrolysis and the reduction of nitrate to nitrite[12]. In the present paper, antimicrobial susceptibilities of *B. pumilus* strains to 34 antimicrobial agents were determined and compared with those of *B. subtilis* strains and also the other *Bacillus* strains as the references. Furthermore, antimicrobial susceptibilities of *B. anthracis*, *B. cereus* and *B. thuringiensis*, which are closely related each other in biological characters[12], were also compared. Since a selective media is necessary to demonstrate the etiological and pathogenic role of *Bacillus* strains in infections, knowledge of antimicrobial susceptibilities of various species of *Bacillus* strains should be useful in formulating the selective media for these organisms and also in comparing the clinical isolates with those from different sources. Also knowledge of susceptibility is undoubtedly necessary for treating patients infected with *Bacillus* strains[13]. For these reasons, the *in vitro* susceptibilities to 34 antimicrobial agents of 86 strains representing 14 species of *Bacillus* were investigated.

MATERIALS AND METHODS

Strains tested. A total of 86 strains were used. The names and origins of the strains are listed in Table 1. Two clinical isolates of *B. pumilus* were kindly given by Dr. F. C. KNOOP (Creighton University, Omaha, USA). Eight strains (Soil-1~8) of *B. pumilus*, 5 strains (Soil-9~13) of *B. subtilis* and 7 strains (Soil-14~20) were isolated from soil in Japan. Four strains (Clinical-1~4) of *B. pumilus*, 5 strains (Clinical-7~11) of *B. subtilis* and 5 strains (Clinical-12~18) of *B. cereus* were isolated from clinical specimens in St. Marianna University Hospital. Three strains (Clinical-19~21) of *B. anthracis* were iso-

Table 1 *Bacillus* strains tested

Species	No. of strains tested	Name and source of strain*
B. pumilus	28	ATCC 4520, ATCC 6632, ATCC 7061, ATCC 12140, ATCC 14484, NCTC 7576, NCIB 8600, NCIB 8738, CCM 340, CCM 386, CCM 1697, CCM 1725, NRRL B-1489, NRRL B-1875, Clinical-1~6, Soil-1~8
B. subtilis	20	ATCC 6051, ATCC 6633, IFO 3009, IFO 3026, IFO 3034, IFO 3035, IFO 3037, IFO 3108, IFO 12210, NRRL B-558, Clinical-7~11, Soil-9~13
B. cereus	17	ATCC 10702, ATCC 11778, IFO 3131, Clinical-12~18, Soil-14~20
B. thuringiensis	3	ATCC 13366, strain T84 AL, strain R_6
B. anthracis	3	Clinical-19~21
B. alvei	1	ATCC 6344
B. brevis	1	ATCC 8246
B. circulans	2	ATCC 4513, ATCC 7049
B. firmus	1	ATCC 8247
B. licheniformis	1	ATCC 9800
B. macerans	1	NRRL B-388
B. megaterium	4	ATCC 12872, ATCC 14945, ATCC 33726, NRRL B-349
B. polymyxa	1	IFO 3020
B. spharicus	3	ATCC 4525, ATCC 7063, IFO 3341

* Details of names and sources of strains tested are shown in Materials and Methods section.

lated from pig in Japan. These isolates were basically identified according to the methods described by GORDON et al.[12] and also by using the API 50 CHB (API Laboratory Products, Basingstoke, Hants, UK) system[13]. Fourty-four strains of *Bacillus* were selected from the culture collections of the American Type Culture Collections (ATCC ; Rockville, Maryland, USA), Czechoslovak Collection of Microorganism (CCM ; Brno, Czechoslovakia), Institute for Fermentation (IFO ; Osaka, Japan), National Collection of Industrial Bacteria (NCIB ; Aberden, Scotland, UK), Northern Regional Research Center, U. S. Department of Agriculture (NRRL ; Peoria, Illinois, USA). *B. thuringiensis* T 84 AL and R_6 strains were kindly given by Dr. M. KONDO (Osaka University, Osaka, Japan) and by Dr. P. GERHARDT (Michigan State University, East Lansing, Michigan, USA) respectively.

Antimicrobial agents. A total of 34 antimicrobial agents were tested. They were kindly supplied by the following organizations : penicillin G, ampicillin, kanamycin, streptomycin, amikacin, dibekacin and fosfomycin (Meiji Seika, Tokyo, Japan) ; carbenicillin, cefoperazone and doxycycline (Pfizer Taito, Tokyo) ; latamoxef, gentamicin, erythromycin and metronidazole (Shionogi, Osaka, Japan) ; piperacillin, cefmetazole and chloramphenicol (Sankyo, Tokyo) ; cefazolin and ceftizoxime (Fujisawa, Osaka) ; cefotiam and cefsulodin (Takeda, Osaka) ; tetracycline and minocycline (Japan Lederle, Tokyo) ; lincomycin and clindamycin (Japan Upjohn, Tokyo) ; cephalothin (Torii, Tokyo) ; cefotaxime (Roussel Medica, Tokyo) ; sisomicin (Yamanouchi, Tokyo) ; nalidixic acid (Daiichi, Tokyo) ; pipemidic acid (Dainippon, Osaka) ; vancomycin (Eli Lilly, Indianapolis, Indiana, USA). Solutions of antimicrobial agents were freshly prepared for each test as reported previously[14].

***In vitro* susceptibility test.** The minimal inhibitory concentrations (MICs) were determined by the agar dilution method in modified Mueller-Hinton agar (Nissui, Tokyo)[15]. Overnight cultures of the test organisms were diluted to a density of approximately 10^6 colony-forming units/ml in Mueller-Hinton broth (Difco) and supplied by means of the multiple inocula replicator (Sakuma, Tokyo) to the surface of the agar plates containing two fold dilutions of antimicrobial agents. The MICs were determined as the lowest concentration of antimicrobial agent inhibiting growth after overnight incubation at 37°C. A few slow growing strains were incubated for 48 hr. These procedures were performed as directed by Japan Society of Chemotherapy[16]. Results were expressed as the range of MIC (μg/ml), in addition to $MIC_{50\%}$ and $MIC_{90\%}$ (the concentration of antimicrobial agent inhibiting 50% and 90% of the strains respectively).

Beta-lactamase detection. *B. anthracis*, *B. cereus* and *B. thuringiensis* strains were tested for beta-lactamase production by the use of the Cefinase disc (BBL Microbiology Systems, Cockeysville, Maryland, USA) method[17].

RESULTS

Susceptibilities of strains of *B. pumilus*, *B. subtilis* and other *Bacillus* to 34 antimicrobial agents, expressed as MIC range, $MIC_{50\%}$ and $MIC_{90\%}$, are shown in Table 2. The reproducibility of the MIC determinations against control organisms was excellent for all agents and varied only within one twofold dilution in separate measurements. In general, the penicillins, aminoglycosides and tetracyclines, as a group, were highly active against *B. pumilus* strains tested. All of the strains were uniformally susceptible to penicillin G, ampicillin, carbenicillin, oxacillin, cefazolin, cephalothin, cefmetazole, cefotiam, cefoperazone, latamoxef, aminoglycosides (kanamycin, streptomycin, gentamicin, dibekacin, sisomicin), tetracyclines (tetracycline, doxycycline, minocycline), erythromycin and vancomycin : $MICs_{90\%}$ of these agents were less than 0.78 μg/ml. All of *B. pumilus* strains tested were resistant to metronidazole. Susceptibilities to the other agents were variable. The newer (beta-lactamase resistant) cephalosporins were less active than the older (beta-lactamase sensitive) ones (Table 2). MICs of lincomycin to *B. pumilus* strains ranged from 3.13 to 25 μg/ml and the $MIC_{90\%}$ was 25 μg/ml, whereas MICs of clindamycin ranged from 0.025 to 1.56 μg/ml and the $MIC_{90\%}$ was 1.56 μg/ml. Although no significant difference in susceptibility to antimicrobial agents was observed between *B. pumilus* and *B. subtilis* strains tested, there was a distinct species-related difference in susceptibility to lincomycin and cefotaxime. $MIC_{90\%}$ of cefotaxime and

Table 2 MICs of 34 antimicrobial agents against *B. pumilus*, *B. subtilis* and the other *Bacillus* strains

Antimicrobial agent	*B. pumilus*			*B. subtilis*			Other *Bacillus* strains*		
	MIC range	$MIC_{50\%}$	$MIC_{90\%}$	MIC range	$MIC_{50\%}$	$MIC_{90\%}$	MIC range	$MIC_{50\%}$	$MIC_{90\%}$
Penicillin G	<0.025-0.05	0.025	0.05	<0.025-0.39	0.05	0.2	<0.025-0.78	0.2	0.78
Ampicillin	0.05-0.2	0.1	0.2	<0.025-0.78	0.2	0.78	<0.025-3.13	0.78	3.13
Carbenicillin	0.025-0.2	0.05	0.2	<0.025-0.78	0.39	0.78	<0.025-1.56	0.78	1.56
Oxacillin	<0.025-1.56	0.39	0.78	0.025-1.56	0.1	0.39	<0.025-12.5	0.39	6.25
Methicillin	0.2-12.5	6.25	6.25	0.39-6.25	3.13	6.25	0.78-100	3.13	6.25
Piperacillin	0.05-3.13	0.78	1.56	0.05-12.5	1.56	3.13	<0.025-12.5	1.56	12.5
Cefazolin	<0.025-0.2	0.05	0.1	<0.025-0.2	0.025	0.05	<0.025-1.56	0.2	1.56
Cephalothin	<0.025-0.05	<0.025	0.05	<0.025	<0.025	<0.025	<0.025-0.78	0.05	0.78
Cefmetazole	0.05-0.78	0.2	0.78	0.05-0.39	0.1	0.2	0.05-100	0.39	1.56
Cefotiam	0.05-0.78	0.1	0.2	0.025-0.1	0.025	0.1	<0.025-1.56	0.2	1.56
Cefoxitin	0.39-6.25	3.13	6.25	0.39-1.56	0.78	1.56	0.39-25	0.78	3.13
Cefoperazone	0.05-1.56	0.78	0.78	0.05-3.13	0.39	0.78	0.025-3.13	0.39	3.13
Latamoxef	0.1-1.56	0.39	0.78	0.1-0.78	0.1	0.78	0.025-12.5	0.39	6.25
Cefotaxime	0.78-100	100	100	0.2-6.25	0.78	6.25	0.025-6.25	0.78	6.25
Ceftizoxime	3.13-100	100	100	0.025-100	3.13	50	<0.025-50	0.39	6.25
Cefsulodin	1.56-100	100	100	0.2-100	12.5	100	0.2-100	12.5	50
Kanamycin	0.1-0.39	0.2	0.39	0.1-0.78	0.2	0.78	0.1->100	0.78	12.5
Streptomycin	0.1-0.78	0.39	0.78	0.39-25	1.56	12.5	0.05-3.13	0.78	3.13
Gentamicin	0.025-0.39	0.2	0.2	0.1-0.39	0.2	0.2	0.05-0.78	0.1	0.39
Amicacin	0.05-0.2	0.1	0.2	0.1-0.78	0.2	0.39	0.05-6.25	0.1	1.56
Dibekacin	0.05-0.39	0.1	0.2	0.05-0.39	0.1	0.2	0.05-100	0.1	3.13
Sisomicin	0.025-0.2	0.2	0.2	0.1-0.2	0.2	0.2	0.05-3.13	0.2	1.56
Tetracycline	0.025-0.39	0.2	0.2	0.1-3.13	1.56	3.13	0.05-12.5	0.2	3.13
Doxycycline	0.025-0.2	0.1	0.1	<0.025-0.78	0.1	0.39	<0.025-0.2	0.05	0.2
Minocycline	0.025-0.2	0.1	0.2	0.05-0.39	0.1	0.2	<0.025-1.56	0.1	1.56
Erythromycin	0.025-0.39	0.1	0.2	0.05-0.1	0.05	0.1	<0.025-0.39	0.05	0.39
Lincomycin	3.13-25	12.5	25	0.39-12.5	3.13	3.13	1.56-12.5	6.25	12.5
Clindamycin	0.025-1.56	0.78	1.56	0.05-0.39	0.1	0.2	0.05-6.25	0.2	6.25
Chloramphenicol	0.78-6.25	3.13	3.13	0.39-6.25	0.78	1.56	0.2-3.13	0.78	3.13
Fosfomycin	1.56->100	100	>100	12.5->100	50	>100	6.25->100	50	>100
Nalidixic acid	3.13-50	25	25	1.56-6.25	3.13	6.25	0.2->100	1.56	>100
Pipemidic acid	3.13-25	12.5	25	1.56-12.5	3.13	6.25	1.56-100	6.25	25
Metronidazole	25-100	50	100	25-100	50	100	25-100	50	100
Vancomycin	0.05-0.39	0.2	0.39	0.05-0.78	0.2	0.39	0.05-1.56	0.2	1.56

* *B. alvei*, *B. brevis*, *B. firmus*, *B. licheniformis*, *B. macerans*, *B. megaterium*, *B. polymyxa* and *B. spharicus*. Details are shown in Table 1.

** MIC (μg/ml) at which 50% or 90% of the strains were inhibited, respectively.

lincomycin to *B. pumilus* strains were 100 and 25 μg/ml respectively, whereas those to *B. subtilis* strains were 6.25 and 3.13 μg/ml respectively. In the other group of *Bacillus* strains which included *B. alvei*, *B. brevis*, *B. firmus*, *B. licheniformis*, *B. macerans*, *B. megaterium*, *B. polymyxa* and *B. spharicus*, all of the strains tested were uniformally susceptible to penicillin G, cephalothin, gentamicin, doxycycline and erythromycin : $MIC_{90\%}$ of these agents were less than 0.78 μg/ml (Table 2).

Because of biologically close similarities among *B. anthracis*, *B. cereus* and *B. thuringiensis* strains each other, susceptibilities of these species to 34 agents were compared (Table 3). Strains of *B. anthracis* tested were resistant to cefoxitin, ceftizoxime, cefsulodin, fosfomycin and metronidazole, whereas they were highly susceptible to penicillins, aminoglycosides, tetracyclines and macrolides as a group : $MIC_{90\%}$ of these agents were less than 0.39 μg/ml. Significant difference in susceptibility was observed in *B. cereus* strains to carbenicillin, oxacillin, cefazolin, cephalothin, cefotiam,

Table 3 MICs of 34 antimicrobial agents against *B. anthracis*, *B. cereus* and *B. thuringiensis* strains

Antimicrobial agent	MIC (μg/ml) range of		
	B. anthracis	*B. cereus*	*B. thuringiensis*
Penicillin G	<0.025	1.56-3.13	50-100
Ampicillin	<0.025	6.25-12.5	25
Carbenicillin	<0.025	25-50	50-100
Oxacillin	0.025	25-50	100
Methicillin	0.039-1.56	100	100
Piperacillin	0.2-0.39	3.13-6.25	6.25
Cefazolin	<0.025	12.5-25	50
Cephalothin	<0.025	12.5-25	50
Cefmetazole	0.1-0.2	3.13-6.25	6.25
Cefotiam	0.025	25-50	50
Cefoxitin	6.25	6.25	50
Cefoperazone	0.1-0.2	1.56-3.13	6.25-12.5
Latamoxef	0.05	12.5-25	50-100
Cefotaxime	1.56-3.13	25-50	100
Ceftizoxime	100	100	100
Cefsulodin	25	100	100
Kanamycin	0.1	1.56-3.13	1.56-3.13
Streptomycin	0.1	0.78-3.13	1.56
Gentamicin	0.05-0.1	0.78-3.13	0.78-3.13
Amikacin	0.05	0.78-1.56	0.78-1.56
Dibekacin	0.05-0.1	1.56-3.13	0.78
Sisomicin	0.05	1.56-3.13	0.78
Tetracycline	0.025	0.39-0.78	0.39-0.78
Doxycycline	<0.025	0.1-0.2	0.2-0.39
Minocycline	0.025	0.2-0.78	0.2-0.39
Erythromycin	0.05	0.2-0.39	0.2-0.39
Lincomycin	0.05	1.56	1.56
Clindamycin	<0.025	0.2-0.39	0.2-0.39
Chloramphenicol	0.2-0.39	1.56-6.25	0.78-3.13
Fosfomycin	>100	3.13-12.5	6.25-12.5
Nalidixic acid	1.56	12.5-25	3.13
Pipemidic acid	1.56	25-50	6.25
Metronidazole	25	25-50	25-50
Vancomycin	0.05	0.1-0.2	0.1-0.2

latamoxef, cefotaxime, pipemidic acid and nalidixic acid, compared with those of *B. anthracis* strains. Strains of *B. cereus* were more resistant to these agents than those of *B. anthracis*. The MICs of fosfomycin to *B. anthracis* strains were over 100 μg/ml, whereas those of *B. cereus* strains ranged from 3.13 to 12.5 μg/ml. Furthermore, three strains of *B. thuringiensis* were more resistant to many agents than *B. anthracis* and *B. cereus* strains tested. Especially significant difference in susceptibility was observed in penicillins and cephalosporins, as a group. MICs of these agents to *B. thuringiensis*, except piperacillin, cefmetazole and cefoperazone, were more than 25 μg/ml. Beta-lactamase could be demonstrated by all strains of *B. cereus* and *B. thuringiensis* tested, but not by *B. anthracis* strains tested. The difference of susceptibility among these three species against beta-lactam antibiotics was paralleled by the difference of beta-lactamase production. Both *B. cereus* and *B. thuringiensis* were uniformally susceptible to aminoglycosides, tetracyclines, erythromycin, lincomycin and clindamycin. No significant difference in susceptibility was observed between the isolates from clinical specimens or soil samples and the reference strains from the culture collections in each of the species tested.

DISCUSSION

The present study revealed antimicrobial susceptibilities of a wide variety of *Bacillus* strains to 34 agents. Little difference in susceptibility was observed between *B. pumilus* and *B. subtilis* strains tested, with the exception of susceptibilities to clindamycin and cefotaxime. *B. cereus* strains were found to be resistant to more antimicrobial agents than *B. anthracis* strains were. Furthermore, *B. thuringiensis* strains were more resistant than the two species described above. Since beta-lactamase could be demonstrated by all strains of *B. thuringiensis* and *B. cereus* tested, but not by *B. anthracis* strains tested, these results seem to reflect the difference of beta-lactamase production among these three species. Since *Bacillus* strains were found to be foundamentally susceptible to at least some of the antimicrobial agents, a strain of *Bacillus* becomes noticed in clinical infections only when the strain is resistant to the initial antimicrobial therapy. This might be a major reason why only *B. cereus* strains, which are normally beta-lactamase producer and therefore more resistant to beta-lactam antibiotics than the other *Bacillus* species, have frequently been found to be pathogenic. In many cases of clinical infections, specific identification of *Bacillus* strains has not been carried out precisely and the terms of "*B. cereus*" or "*B. subtilis*" are easily used in this generic sense, because of complex taxonomic relationship and identification procedures[5].

FINEGOLD et al.[18-20] demonstrated that between 47 and 92% of normal healthy people contain in their feces members of the genus *Bacillus* with counts of 10^3 to 10^6 colony-forming units/g of feces. The normal intestinal flora is thought to protect against colonization by virulent or pathogenic organisms. Disturbance of the normal flora following administration of antimicrobial agents may be responsible for the overgrowth of enteropathogens such as *B. pumilus*[11] or *Clostridium difficile*[21,22], as well as toxin elaboration by the organisms. The extended-spectrum β-lactam antibiotics are frequently used as the initial empirical therapy for a wide variety of clinical situations in which a *Bacillus* strain may be a potential pathogen. It is important to note that *Bacillus* species as well as *Clostridium* species form spores in their life-cycle and the spores formed are extremely resistant to almost all antimicrobial agents. Although the growth of the vegetative cells of *Bacillus* strains could be inhibited easily by the effective antimicrobial agents at the moderate concentrations, the spore-form of the strains could survive in the interior of human body. The spores could easily germinate and multiply abundantly after the disappearance of the antimicrobial agents. From this reason, it might not be easy to control infectious diseases associated with invasive sporeformers because of their pecularities. Furthermore, there might be the other numerous causative sporeformers associated with antibiotic-associated diarrhea or colitis[23,26], in addition to anaerobic sporeformer *C. difficile*, the role of which has already been established[22,27,28].

Although vancomycin has not usually been tested against *Bacillus* strains, it appears to be one of the most effective drugs of choice when specific therapy of antibiotic-associated diarrhea or colitis is indicated[21,27,29,30]. In the present study, all the *Bacillus* strains tested were susceptible to van-

comycin and, therefore, it should prove to be equally effective in the treatment of *B. pumilus*-associated diarrhea or colitis[11]. Because of the variable susceptibility of some strains of *Bacillus* to β-lactam antibiotics reported in this paper, susceptibility testing should always be performed before the agents are used. The present results should be useful in selection of appropriate antimicrobial agents for treatment of infections and also in formulating the selective media for isolation of *Bacillus* strains.

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Bacillus 属 14 菌種の 34 薬剤に対する感受性

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Bacillus 属 14 種 86 株の，34 薬剤に対する *in vitro* 感受性を検討した。近縁菌種である *B. pumilus* (28 株) と *B. subtilis* (20 株) および他の *Bacillus* (9 種 15 株) の感受性を比較した結果，顕著な差は認められなかった。しかし LCM および CTX について，*B. subtilis* の $MIC_{90\%}$ はそれぞれ 6.25 および 3.13μg/ml であったのに対し，*B. pumilus* の $MIC_{90\%}$ はそれぞれ 25 および 100μg/ml であった。上記各菌種に対し，PCG, ABPC, CBPC, MPIPC, CET, CEZ, CMZ, CTM, CPZ, LMOX, KM, SM, GM, AMK, DKB, TC, DOXY, MINO, EM, VCM の $MIC_{90\%}$ は 0.78μg/ml 以下であった。*B. pumilus* に対し，第 3 世代セフェム剤は第 1，第 2 世代薬剤に比べ顕著に劣った抗菌力を示した。近縁菌種である *B. anthracis*, *B. cereus* および *B. thuringiensis* の場合，*B. anthracis* に対し，ペニシリン系，アミノグリコシド系，テトラサイクリン系，マクロライド系薬剤の $MIC_{90\%}$ は 0.39μg/ml 以下であった。しかし *B. cereus* および特に *B. thuringiensis* は多くの薬剤に対しより高度の耐性を示した。これら 3 菌種においては，β-ラクタマーゼ産生能と β-ラクタム系薬剤に対する感受性の間に相関性が認められた。検討した各菌種において，臨床材料分離株と，土壌由来株あるいは教室保存標準株の間に感受性の差は認められなかった。