POSSIBLE β-LACTAMASE ACTIVITIES DETECTABLE IN INFECTIVE CLINICAL SPECIMENS

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Biological β-lactam antibiotic-inactivating activities were detected in bacteriuria and suppurating pleural fluids. Clinical specimens were sterilized with membrane filters and the amounts of ampicillin and/or cephalothin which were being inactivated by 1 ml of each filtrate were determined. In general, filtrates which originally yielded *Klebsiella* sp. tended to show activity against ampicillin; whereas those yielding *Enterobacter* sp. and *Pseudomonas aeruginosa* showed activity against cephalothin.

A number of clinical bacterial isolates have been reported to produce β-lactamases which inactivate penicillins and cephalosporins. In the present paper, the authors have attempted to study β-lactam antibiotic-inactivating activities (possible β-lactamase activities) detectable in infected fluid specimens; the activities considered to have been produced by the microorganisms isolated from each clinical material.

Materials and Methods

Antibiotics

Laboratory standards of ampicillin (Fujisawa Pharmaceutical Co., Ltd., Osaka) and cephalothin (Shionogi Pharmaceutical Co., Ltd., Osaka) were employed.

A phosphate buffer solution (pH adjusted to 7.2) was used for the dilution of antibiotics and specimens throughout the experiments.

Media

Heart infusion broth (Difco) and heart infusion agar (Difco) were used.

Assay of possible β-lactamase activities in filtrates of clinical specimens

Urine specimens which contained more than 10^5 viable cells per ml and suppurating pleural fluids were sterilized with membrane filters (GA-metricel filter GA-6, pore size 0.45 μ, Gelman Instrument Co., Ann Arbor, Michigan). Each of the filtrates was diluted in 1: 5, 1: 50 and 1: 500 with phosphate buffer solution. Each of these solutions was mixed thoroughly with required amounts of solutions containing 20, 200 and 2,000 μg per ml respectively of ampicillin and/or cephalothin. The antibiotic concentration in each of these mixtures was 10, 100 or 1,000 μg per ml, and the final dilution of the filtrates was 1: 10, 1: 100 or 1: 1,000 at the time of preparation. These mixtures were allowed to stand in an incubator at 37°C for up to 3 hours. The antibiotic activities remaining in each of these filtrates were then assayed microbiologically by the cylinder-plate method, and the amounts of antibiotic which had been inactivated by 1 ml of each original undiluted filtrate were calculated. These amounts were taken to represent β-lactam antibiotic-inactivating activities and used as a measure of "possible β-lactamase activities" in this paper.

Possible β-lactamase activities detectable in filtrates of bacterial culture fluids

Clinical isolates of *Staphylococcus aureus* (30 strains), *Pseudomonas aeruginosa* (20 strains), *Escherichia coli* (28 strains), *Klebsiella* sp. (23 strains), *Enterobacter* sp. (29 strains) and *Serratia marcescens* (20 strains) were obtained at Keio University Hospital, Tokyo. The identification was

performed by the method described by BUCHANAN *et al.*[1]. These strains were inoculated with a heat-sterilized loop from agar slants into heart infusion broth which was allowed to stand in an incubator at 37°C without shaking. These cultures were harvested at 48 hours and then membrane-sterilized. The filtrates were stored at −20°C until used. The possible β-lactamase activities were determined microbiologically by the method as described above with the clinical specimens.

Standard strains of *S. aureus* FDA 209P and *Bacillus subtilis* ATCC 6633 were also employed for assaying ampicillin and cephalothin, respectively.

Results

Possible β-Lactamase Activities Detectable in Filtrates of Clinical Specimens

Table 1 shows that the filtrates of some bacteriuric specimens were capable of inactivating ampicillin and/or cephalothin. It was apparent that the activities were not so high in bacteriuria; one ml of these specimens inactivated at most 100 μg of either ampicillin and/or cephalothin. Urine specimens with activities against ampicillin generally yielded *Klebsiella* sp. Three specimens with slight activities against cephalothin yielded *P. aeruginosa*.

More potent activities were detected in some of the filtrates of suppurating pleural fluids as shown in Table 2; one ml of one of these specimens inactivated up to 5,000 μg of both ampicillin and cephalothin.

A control study with filtrates of urine and pleural fluids which originally detected no micro-organisms showed no activities of possible β-lactamase (not shown).

Possible β-Lactamase Activities Detectable in Filtrates of Bacterial Culture Fluids

The possible β-lactamase activities in 48 hours' culture filtrates of *S. aureus, P. aeruginosa, E. coli, Klebsiella* sp., *Enterobacter* sp. and *S. marcescens* were compared with ampicillin and cephalothin as the substrates (Fig. 1). Among the *S. aureus* used, 17 strains showed no activities against the two β-lactam antibiotics. Thirteen strains showed some activities against ampicillin; one ml of some of these filtrates inactivated up to 1,000 μg of ampicillin. There was no strain of this bacterial species which inactivated cephalothin.

P. aeruginosa failed to inactivate ampicillin. It was shown that more than half of the *P. aeruginosa* strains showed a low activity in inactivating cephalothin.

In contrast to these bacteria, many strains of *E. coli, Klebsiella* sp., *Enterobacter* sp. and *S. marcescens* showed markedly high activities for inactivating ampicillin and/or cephalothin. One ml of the filtrates of some of these strains inactivated up to 300,000 μg of these drugs. It was shown that the activities were especially potent among the strains of *Enterobacter* sp. It was also shown that, among these enterobacteria, most strains of *Klebsiella* sp. inactivated ampicillin, whereas *E. coli, Enterobacter* sp. and *S. marcescens* inactivated cephalothin.

Discussion

Antibiotic concentration at infected sites is one of the major determinants of clinical effectiveness. A number of bacterial isolates obtained from clinical specimens are reported recently to be β-lactamase-producers and these strains sometimes show resistance to β-lactam antibiotics[2~7]. The bacterial enzyme inactivates β-lactam antibiotics which penetrate into the sites of infection. In some special clinical situations, this enzyme could also produce "indirect pathogenicity" which prevents elimination of causal bacteria by inactivating the β-lactam antibiotics even when the causative organisms were susceptible to the drugs[8].

From the clinicians' viewpoint, it would sometimes be more convenient to determine the biological activities for drug inactivation than to estimate "chemical" β-lactamase activities, as described in this tentative study. As it is widely accepted that β-lactamase plays a considerable role in the inactivation of β-lactam antibiotics[6,9,10], the authors consider the "possible β-lactamase activities" to be due

Table 1. Microorganisms isolated from bacteriuria and possible β-lactamase activities detectable in each specimen

Specimen	Organisms isolated	Activities against*	
		Ampicillin	Cephalothin
1	*E. coli*	—	—
2	*E. coli*	—	—
3	*E. coli*	—	—
4	*E. coli*	—	—
5	*E. coli*	—	—
6	*E. coli*	—	—
7	*E. coli*	—	—
8	*E. coli*	—	—
9	*E. coli*	—	—
10	*E. coli*	—	—
11	*E. coli*	—	—
12	*Klebsiella* sp.	—	—
13	*Klebsiella* sp.	—	—
14	*P. aeruginosa*	—	—
15	Micrococcus	—	—
16	Enterococcus	—	—
17	*Candida* sp.	—	—
18	*E. coli*	30	—
19	*E. coli*	30	—
20	*Klebsiella* sp.	30	—
21	*Klebsiella* sp.	30	—
22	*Klebsiella* sp.	50	—
23	*Klebsiella* sp.	70	—
24	*Klebsiella* sp.	100	—
25	*Klebsiella* sp., *E. coli*, *Proteus* sp.	30	—
26	*Klebsiella* sp., Enterococcus, *P. aeruginosa*	20	—
27	*Klebsiella* sp., *E. coli*, *P. aeruginosa*	70	—
28	*P. aeruginosa*	—	20
29	*P. aeruginosa*	—	30
30	*E. coli*, *Klebsiella* sp., *P. aeruginosa* Enterococcus	20	100

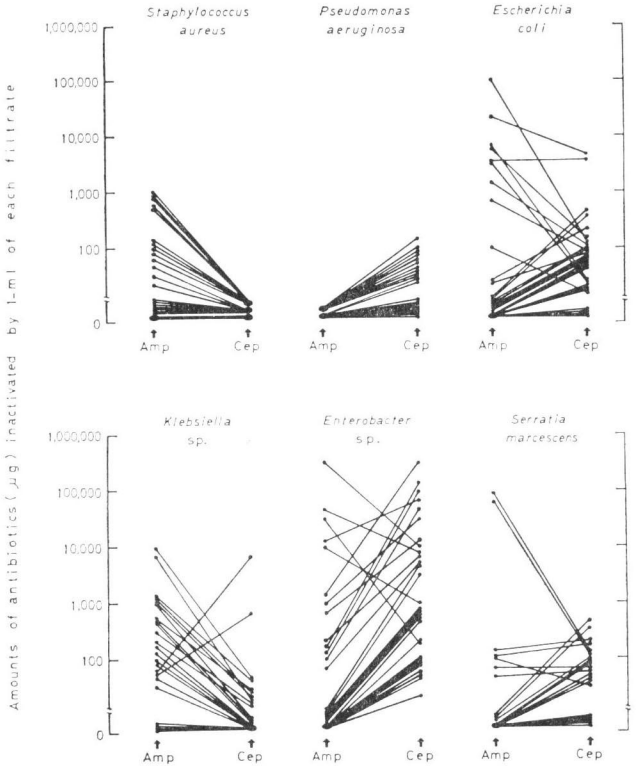
* The — signs indicate that no inactivation of the antibiotic was detected. The numbers indicate the amounts of antibiotics which had been inactivated by 1 ml of the filtrates of each specimen.

Table 2. Microorganisms isolated from suppurating pleural fluids and possible β-lactamase activities detectable in each specimen

Specimen	Organisms isolated	Activities against*	
		Ampicillin	Cephalothin
1	*S. aureus*	—	—
2	α-hemolytic *Streptococcus*	—	—
3	*Salmonella* C2-group	—	—
4	*S. marcescens*	—	—
5	*Klebsiella* sp., *Bacteroides* sp., *P. aeruginosa* Enterococcus	—	—
6	*Aspergillus* sp.	—	—
7	*Peptococcus*, *Klebsiella* sp., *P. aeruginosa*	—	40
8	Enterococcus, *Klebsiella* sp., *P. aeruginosa*	—	50
9	Enterococcus, *Klebsiella* sp., *P. aeruginosa*	—	50
10	*Citrobacter freundii*, *Klebsiella* sp., *P. aeruginosa*	—	70
11	Enterococcus, *Klebsiella* sp., *Enterobacter* sp., *P. aeruginosa*	5,000	5,000
12	*Klebsiella* sp., *Enterobacter* sp., *P. aeruginosa*	40	600
13	*Klebsiella* sp., *Enterobacter* sp., *P. aeruginosa*	30	1,000
14	Enterococcus, *Citrobacter freundii*, *Klebsiella* sp., *P. aeruginosa*	50	1,000
15	Enterococcus, *Citrobacter freudii*, *Klebsiella* sp., *Pseudomonas maltophilia*, *P. aeruginosa*	30	100

* The — signs indicate that no inactivation of the antibiotic was detected. The numbers indicate the amounts of antibiotics which had been inactivated by 1 ml of the filtrates of each specimen.

Fig. 1. Possible β-lactamase activities in filtrates of bacterial culture fluids. The amounts of the ampicillin (Amp) and cephalothin (Cep) which had been inactivated by 1 ml of each membrane-sterilized 48-hour broth culture are comparatively shown. Symbols: • one strain; ● ten strains.



mostly to β-lactamase produced by the microorganisms. A previous paper published by our laboratory demonstrated that the substrate profiles of these activities against selected β-lactam antibiotics were similar to those of β-lactamase reported from other institutions[11]. However, factors such as amidase, protein-binding capacity, and pH of medium could also contribute to the activities.

The activities detected in our bacteriuric specimens were not high and would be almost negligible in β-lactam antibiotic chemotherapy. Some pleural fluid specimens revealed more potent activities. The specimen with the highest activities in the present series was taken from a patient with pyothorax which contained an estimated several liters of pleural fluid. In such a case, administration of hydrolysable β-lactam antibiotics would have been in vain, since the drugs could have been inactivated by the possible β-lactamase activities already present in the fluid even when they were given in large amounts. Fig. 1 would suggest that such high activities could be expected in clinical specimens which yield certain kinds of microorganisms, in particular, enterobacteria.

Some clinical materials used in our present study were taken from patients receiving no antibiotics for at least several days prior to specimen collection. A number of the patients, however, were receiving varying kinds of antimicrobials, systemically or topically prior to or even on the day when the specimens were being taken. Hence, the possible β-lactamase activities detected in these specimens, in some instances, would have been more potent if no antibiotics had been applied. However, the possibility should also be borne in mind that in other instances the activities could have been induced in the presence of the antibiotics used, as demonstrated *in vitro*[8,12,13,14].

The possible β-lactamase activities detected in some filtrates of our infected clinical specimens and bacterial culture fluids were more potent than expected. Taking also the crypticity problem and inducibility of bacterial β-lactamase into consideration[8,7,12,13,14], such activities should be considered

in the treatment of infections with β-lactam antibiotics.

Further studies should be carried out in this area.

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