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# Immunological Properties of Beta-Lactamases That Hydrolyze Cefuroxime and Cefotaxime

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Antiserum against purified β-lactamase from *Proteus vulgaris* GN7919 cross-reacted with β-lactamases produced by strains of *Pseudomonas cepacia* in a neutralization test. Anti-*P. cepacia* β-lactamase serum, however, did not show any cross-reaction with *P. vulgaris* β-lactamases. Each of these enzymes can hydrolyze cefuroxime and cefotaxime.

We have previously studied the enzymatic and physicochemical properties of purified β-lactamases from many different species of gram-negative bacteria (3, 5, 7-9, 13, 15). We used substrate profiles with novel cephalosporins and inhibitory profiles with clavulanic acid, CP-45,899, and cefoxitin to classify β-lactamases of gram-negative bacteria into three main groups: (i) penicillinase; (ii) typical cephalosporinase; and (iii) cefuroxime-hydrolyzing β-lactamase (10). The cefuroxime-hydrolyzing β-lactamases were found to be produced by *Pseudomonas cepacia* GN11164 (3), *Proteus vulgaris* GN7919 (5), and *Bacteroides fragilis* GN11477 (13). These enzymes were able to hydrolyze at a high rate cefuroxime and cefotaxime, which are known to be resistant to hydrolysis by cephalosporinases and penicillinases (1, 10, 11). In addition, the activity of these enzymes was found to be inhibited by clavulanic acid and CP-45,899, compounds known to be penicillinase inhibitors (2, 6, 12).

In this paper, we compare the immunological properties of purified β-lactamases from *P. cepacia* GN11164, *P. vulgaris* GN7919, and *B. fragilis* GN11477 with typical cephalosporinases produced by *Escherichia coli* (8), *Enterobacter cloacae* (9), *Citrobacter freundii* (15), *Serratia marcescens, Pseudomonas aeruginosa, Proteus morganii,* and *Proteus rettgeri* (6).

β-Lactamase was extracted from cultured cells of each strain. Cells were harvested by centrifugation, suspended in 0.05 M phosphate buffer (pH 7.0), and disrupted by ultrasonic treatment. After centrifugation at 10,000 × *g* to remove cell debris, clear supernatants (crude enzyme) were prepared and purified by methods described previously (3, 5, 7-9, 13, 15). β-Lactamase activity was determined by a spectrophotometric method as described previously (3).

Antisera against purified *P. vulgaris* GN7919, *P. cepacia* GN11164, and *B. fragilis* GN11477 enzymes were prepared in two or three rabbits. The rabbits were given two injections at 1-week intervals. One milligram of enzyme protein was dissolved in 0.5 ml of saline, emulsified with 0.5 ml of Freund complete adjuvant (Difco), and used for one injection. An injection of 0.5 ml was given under the skin of the footpad, and the remaining dose was given intramuscularly in the thigh. Three weeks after the last injection, a booster injection containing 0.5 mg of enzyme protein in 0.5 ml of saline was administered intravenously. Antisera were collected 2 weeks after the last injection.

The immunological identification of the β-lactamase was examined by a neutralization test of the hydrolyzing activity by the method described previously (15). The enzyme solution (4 U) was mixed with the antiserum at 37°C for 1 h and then kept at 4°C for 18 h. The mixture was centrifuged for 15 min at 3,000 × *g*, and the remaining enzyme activity in the supernatant was assayed.

The β-lactamase activity of purified enzyme from *P. vulgaris* GN7919, *P. cepacia* GN11164, or *B. fragilis* GN11477 was inhibited by the respective antisera. Figure 1 shows the neutralization curves of *P. vulgaris, P. cepacia,* and *B. fragilis* enzyme activities by each homologous antiserum. The enzyme (4 U) activities were completely neutralized after 60 min of incubation at 37°C by 100 μl of homologous antiserum.

The neutralizing activities of antisera to *P. vulgaris* GN7919, *P. cepacia* GN11164, and *B. fragilis* GN11477 were examined by using various β-lactamases obtained from 17 strains of clinical isolates including the three strains named.

As shown in Table 1, antisera to *P. cepacia*

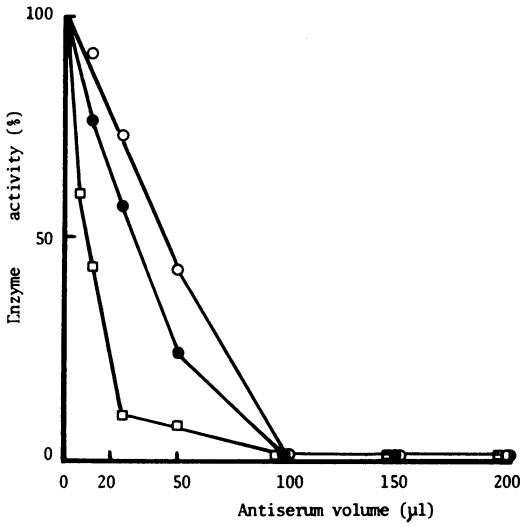


FIG. 1. *Neutralization of the activities of β-lactamase from P. vulgaris GN7919 (●), P. cepacia GN11164 (○), or B. fragilis GN11477 (□) by each homologous antiserum. β-Lactamase activity was assayed by spectrophotometry. Cephaloridine (100 μM) was used as a substrate.*

TABLE 1. *neutralization of β-lactamase activities with anti-β-lactamase sera*

| β-Lactamase prepared from | Neutralization (%) by antisera against the enzyme of[a]: | | |
|---|---|---|---|
| | *P. vulgaris* GN7919 | *P. cepacia* GN11164 | *B. fragilis* GN11477 |
| *P. vulgaris* GN7919 | 100 | 0 | 0 |
| *P. vulgaris* GN76 | 96 | 0 | 0 |
| *P. vulgaris* GN4413 | 98 | 0 | 0 |
| *P. cepacia* GN11164 | 87 | 100 | 0 |
| *P. cepacia* GN11127 | 89 | 100 | 0 |
| *P. cepacia* GN11152 | 90 | 94 | 0 |
| *P. cepacia* GN11155 | 86 | 92 | 0 |
| *B. fragilis* GN11477 | 0 | 0 | 100 |
| *B. fragilis* GN11480 | 0 | 0 | 85 |
| *B. fragilis* GN11482 | 0 | 0 | 81 |
| *E. coli* GN5482 | 0 | 0 | 0 |
| *E. cloacae* GN7471 | 0 | 0 | 0 |
| *C. freundii* GN7391 | 0 | 0 | 0 |
| *S. marcescens* GN10857 | 0 | 0 | 0 |
| *P. aeruginosa* GN10362 | 0 | 0 | 0 |
| *P. rettgeri* GN4430 | 0 | 0 | 0 |
| *P. morganii* GN5407 | 0 | 0 | 0 |

[a] Neutralization of β-lactamase activity was expressed by the following equation: $(a - b)/a$ (%), where $a$ is enzyme activity without antiserum, and $b$ is enzyme activity with antiserum.

GN11164 and *B. fragilis* GN11477 β-lactamases cross-reacted with enzymes from other strains of *P. cepacia* and *B. fragilis*, respectively, but not those from other species. This result suggests that antiserum to these chromosomal β-lactamases might be species specific as reported by Sykes and Matthew (14).

Besides cross-reacting with enzymes from other strains of *P. vulgaris*, antiserum to *P. vulgaris* GN7919 β-lactamase cross-reacted with the β-lactamases produced by *P. cepacia* strains, although the β-lactamases produced by *P. vulgaris* GN7919 and *P. cepacia* GN11164 were different in their molecular weights (*P. cepacia* GN11164, 22,000; *P. vulgaris* GN7919, 30,000) and in their isoelectric points (*P. cepacia* GN11164, 9.3; *P. vulgaris* GN7919, 8.8). The two enzymes were similar, however, in their activity against cefuroxime and cefotaxime and their inhibition by clavulanic acid and CP-45,899.

As far as we know, cross-reactions between chromosomal β-lactamase antisera and chromosomal β-lactamases produced by different species of bacteria are rare. Exceptions include the antiserum to *E. coli* D31 β-lactamase, which showed cross-reaction with chromosomal β-lactamases from *Alcalescens dispar* and *Shigella sonnei* (14); antiserum to *Enterobacter aerogenes* MULB250 β-lactamase, which cross-reacted with *E. cloacae* cephalosporinase (4); and antiserum to *C. freundii* GN7391, which showed cross-reaction with cephalosporinases produced by strains of *E. cloacae* (15). This immunological cross-reactivity suggests that the active site or structure of β-lactamase from *P. vulgaris* GN7919 is similar to that of the enzyme from *P. cepacia* strains.

The high specificity of the immuno-neutralization method will be a useful tool for study of the structure and active site of β-lactamase and of the epidemiology of β-lactamase among gram-negative bacteria.

## LITERATURE CITED

1. **Fu, K. P., and H. C. Neu.** 1978. Beta-lactamase stability of a novel cephalosporin, compared to that of cefuroxime and cefoxitin. Antimicrob. Agents Chemother. **14:** 322–326.
2. **Fu, K. P., and H. C. Neu.** 1979. Comparative inhibition of β-lactamases by novel β-lactam compounds. Antimicrob. Agents Chemother. **15:**171–176.
3. **Hirai, K., S. Iyobe, M. Inoue, and S. Mitsuhashi.** 1980. Purification and properties of a new β-lactamase from *Pseudomonas cepacia*. Antimicrob. Agents Chemother. **17:**355–358.
4. **Letarte, R., M. Devaud-Felix, J. C. Pechere, and D. Allard-Leprohon.** 1977. Enzymatic and immunological characterization of a new cephalosporinase from *Enterobacter aerogenes*. Antimicrob. Agents Chemother. **12:**201–205.
5. **Matsubara, M., M. Inoue, and S. Mitsuhashi.** 1981. Purification and some properties of a cephalosporinase from *Proteus vulgaris*. Antimicrob. Agents Chemother. **19:**185–187.
6. **Matsuura, M., H. Nakazawa, T. Hashimoto, and S. Mitsuhashi.** 1980. Combined antibacterial activity of

amoxicillin with clavulanic acid against ampicillin-resistant strains. Antimicrob. Agents Chemother. **17**:908–911.

7. **Matsuura, M., H. Nakazawa, M. Inoue, and S. Mitsuhashi.** 1980. Purification and biochemical properties of β-lactamase produced by *Proteus rettgeri*. Antimicrob. Agents Chemother. **18**:687–690.
8. **Minami, S., M. Inoue, and S. Mitsuhashi.** 1980. Purification and properties of cephalosporinase in *Escherichia coli*. Antimicrob. Agents Chemother. **18**:77–80.
9. **Minami, S., M. Inoue, and S. M. Mitsuhashi.** 1980. Purification and properties of cephalosporinase from *Enterobacter cloacae*. Antimicrob. Agents Chemother. **18**:853–857.
10. **Mitsuhashi, S., M. Inoue, and S. Masuyoshi.** 1980. Antibacterial activity of cefotaxime. J. Antimicrob. Chemother. **6**(Suppl. A):37–46.
11. **Neu, H. C., and K. P. Fu.** 1978. Cefuroxime, a new beta-lactamase-resistant cephalosporin with a broad spectrum of gram-positive and -negative activity. Antimicrob. Agents Chemother. **13**:657–664.
12. **Neu, H. C., and K. P. Fu.** 1978. Clavulanic acid, a novel inhibitor of β-lactamase. Antimicrob. Agents Chemother. **14**:650–655.
13. **Sato, K., M. Inoue, and S. Mitsuhashi.** 1980. Activity of β-lactamase produced by *Bacteroides fragilis* against newly introduced cephalosporins. Antimicrob. Agents Chemother. **17**:736–737.
14. **Sykes, R. B., and M. Matthew.** 1976. The β-lactamases of gram-negative bacteria and their role in resistance to β-lactam antibiotics. J. Antimicrob. Chemother. **2**:115–157.
15. **Tajima, M., Y. Takeuchi, S. Sugawara, M. Inoue, and S. Mitsuhashi.** 1980. Purification and properties of β-lactamase from *Citrobacter freundii* GN7391. J. Gen. Microb. **121**(Part 2):449–456.