

Failure of Ceftazidime-Amikacin Therapy for Bacteremia and Meningitis Due to *Klebsiella pneumoniae* Producing an Extended-Spectrum β -Lactamase

CRAIG E. SMITH,¹ B. SUSAN TILLMAN,² ANNE W. HOWELL,³ ROBERT N. LONGFIELD,¹
AND JAMES H. JORGENSEN^{3*}

Infectious Disease Service, Brooke Army Medical Center, Fort Sam Houston, Texas 78234,¹ and Departments of Microbiology and Medicine² and Department of Pathology,³ University of Texas Health Science Center, San Antonio, Texas 78284

Received 11 July 1989/Accepted 18 March 1990

A multiple trauma patient failed treatment with ceftazidime and amikacin for bacteremia and meningitis due to a *Klebsiella pneumoniae* strain that produced a novel, plasmid-mediated β -lactamase. Both pre- and posttreatment isolates were resistant to ceftazidime (MIC, ≥ 64 $\mu\text{g/ml}$) and various penicillins but not to other expanded-spectrum cephalosporins. The β -lactamase had a pI of 5.25 and was encoded on a conjugal plasmid of approximately 150 kilobases. DNA hybridization studies indicated that the enzyme was a TEM derivative.

A number of expanded-spectrum cephalosporins have been developed and marketed in recent years. A principal feature of many of these compounds has been their relative resistance to hydrolysis by β -lactamases of aerobic gram-negative bacilli (17); however, strains of *Klebsiella* spp., *Escherichia coli*, *Citrobacter freundii*, and *Serratia marcescens* containing transferable β -lactamases conferring broad β -lactam resistance, including unique resistance to certain expanded-spectrum cephalosporins, have been reported (8-11, 22, 23). Strains containing β -lactamases especially active against ceftazidime have now been described in France (3, 4, 6, 18, 24), Germany (1), and the United Kingdom (27) but only rarely in the United States (7, 21). We describe herein clinical isolates of *Klebsiella pneumoniae* and *S. marcescens* that produce a broad-spectrum, transferable β -lactamase conferring high-level resistance to ceftazidime, which in the former case resulted in a therapeutic failure.

A 44-year-old man was admitted to Brooke Army Medical Center in August 1988, after involvement in a motor vehicle accident. The patient had multiple injuries, including bilateral mandibular and sinus wall fractures, which required surgical repair on hospital day 2. The patient did well postoperatively on penicillin and clindamycin therapy until hospital day 9, when he became obtunded and developed meningismus. Lumbar puncture revealed viscous, yellow cerebrospinal fluid with gram-negative rods seen on direct Gram stain. Blood cultures obtained on day 7 of hospitalization also grew a gram-negative rod. Penicillin and clindamycin therapy was replaced with amikacin (500 mg every 8 h) and ceftazidime (2 g every 8 h).

The gram-negative rod from blood and cerebrospinal fluid was identified as *K. pneumoniae* and was found to be resistant to ceftazidime but susceptible to cefotaxime and amikacin by the automated Vitek instrument (Vitek Systems, Inc., Hazelwood, Mo.). Because of apparent initial clinical improvement, ceftazidime and amikacin were continued, but repeat susceptibility tests were requested. However, the patient again deteriorated, and lumbar puncture yielded hazy, xanthochromic cerebrospinal fluid that was again positive for *K. pneumoniae*. Repeat susceptibility

tests, by both disk diffusion and the Vitek instrument, confirmed resistance of the organism to ceftazidime. The cephalosporin therapy of the patient was then changed to cefotaxime (2 g every 4 h), and amikacin was continued. The patient rapidly improved on cefotaxime and amikacin and recovered without further complications.

Six blood cultures drawn on hospital days 7, 9, and 12 and the two cerebrospinal fluid cultures (days 9 and 12) grew *K. pneumoniae* that were identical by routine biochemical identification tests and that had identical antimicrobial susceptibility patterns. Disk diffusion and broth microdilution tests (15, 16) indicated susceptibility to imipenem, amikacin, and ciprofloxacin but resistance to ampicillin, amoxicillin-clavulate, ticarcillin, ticarcillin-clavulanate, gentamicin, tobramycin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole. Cephalosporin susceptibilities were unpredictable; the isolates were susceptible to cefotetan, cefoxitin, and cefuroxime and moderately susceptible to cefamandole and cefazolin but resistant to cephalothin. Of the expanded-spectrum cephalosporins, the isolates were susceptible to cefotaxime, ceftizoxime, and ceftriaxone, moderately susceptible to cefoperazone, and resistant to ceftazidime.

After the index patient was recognized, all gram-negative rod isolates recovered at Brooke Army Medical Center during the subsequent 2 months were screened carefully for possible ceftazidime resistance. One additional ceftazidime-resistant isolate, *S. marcescens* DU-1, was recovered from the sputum of a second patient with nosocomial pneumonia in a separate intensive care unit of the same hospital.

Since all of the isolates from the initial patient exhibited identical antimicrobial susceptibilities during repeated testing, one clinical isolate (designated LD-1) was further examined for β -lactamase production and plasmid content. Transconjugants were prepared from LD-1 in two *E. coli* recipient strains (HB101 and C600) by direct mating experiments. Resistance to gentamicin, tobramycin, chloramphenicol, and tetracycline was transferred (data not shown) along with β -lactam resistance. The HB101-derived transconjugant was designated LD-tr1 in further experiments, and the C600-derived transconjugant was designated LD-tr2. For comparative purposes, three previously described multiply resistant

* Corresponding author.

TABLE 1. MICs of selected β -lactam antibiotics alone and with clavulanic acid or sulbactam against *K. pneumoniae* LD-1, *S. marcescens* DU-1, and *E. coli* transconjugant LD-tr as well as CTX-1-, CAZ-1-, and RHH-1-producing strains

Antimicrobial agent ^a	MIC ($\mu\text{g/ml}$) ^b for:					
	LD-1	LD-tr	DU-1	CTX-1	CAZ-1	RHH-1
Cefotaxime	≤ 0.5 –1	≤ 0.5	8–16	16–32	8	4–8
Cefotaxime + clav	≤ 0.5	≤ 0.5	16	≤ 0.5 –4	≤ 0.5 –4	≤ 0.5 –2
Cefotaxime + sulb	≤ 0.5 –1	2–4	4–8	≤ 0.5	4	≤ 0.5 –2
Ceftazidime	≥ 64	>64	16–64	32–>64	64	>64
Ceftazidime + clav	1–2	≤ 0.5	≤ 0.5	1	1	2–4
Ceftazidime + sulb	1–2	4–8	1–2	≤ 0.5 –1	32–64	32–>64
Aztreonam	2–8	>64	2–4	16	8–16	≥ 64
Aztreonam + clav	≤ 0.5	≤ 0.5	1–2	≤ 0.5	≤ 0.5	≤ 0.5
Aztreonam + sulb	≤ 0.5	≥ 64	≤ 0.5 –1	≤ 0.5	8	2–>64

^a Clavulanic acid (clav) and sulbactam (sulb) were added at 2 $\mu\text{g/ml}$.

^b On repeat testing, some values resulted in a range due to difficulty in interpreting trailing endpoints.

strains known to harbor plasmid-mediated β -lactamases were examined in parallel with LD-1 and DU-1. These included *K. pneumoniae* strains CF504 (producing CAZ-1 [TEM-5] and SHV-1 [18, 24]) and CF104 (producing CTX-1 [TEM-3] and SHV-1 [22]) and a transconjugant strain of *E. coli* J53 R2633E (producing RHH-1 [TEM-9] [27]), all kindly provided by Antone A. Medeiros, Brown University, Providence, R.I.

Broth microdilution MICs of cefotaxime, ceftazidime, and aztreonam with LD-1, LD-tr1, and other strains tested are shown in Table 1. The LD strains exhibited high-level resistance to ceftazidime, as did the CTX-1-, CAZ-1-, and RHH-1-producing strains. The susceptibilities to cefotaxime and aztreonam varied among the strains; the LD strains were in general more susceptible. The LD transconjugant ap-

peared more resistant to aztreonam than the parent strain LD-1; however, LD-tr1 produced very faint growth and trailing endpoints in the microdilution test wells, making precise MIC determinations difficult. The addition of 2 μg of clavulanate or sulbactam per ml markedly lowered the MICs of ceftazidime and aztreonam for LD-1. The *S. marcescens* isolate, DU-1, exhibited resistance to cefoxitin and cefuroxime in addition to ceftazidime. Both clavulanate and sulbactam lowered the ceftazidime and aztreonam MICs for DU-1, but the inhibitors had little effect on the susceptibility of that strain to cefotaxime (Table 1).

Crude β -lactamase extracts prepared from LD-1, LD-tr1, DU-1, and strains containing previously characterized β -lactamases were focused (13, 14) on 7% polyacrylamide gels containing pH 3.5 to 9.5 ampholines at 4°C with 10-W power for 3 h. Gels were then stained with a 0.05% nitrocefin solution. LD-1, LD-tr1, and DU-1 revealed identical bands with a pI of 5.25 (Fig. 1) in multiple experiments. LD-1 also contained a band at pI 7.7, consistent with SHV-1. DU-1 contained a second band at pI 8.2, consistent with a chromosomally mediated β -lactamase.

Plasmid-enriched DNA from strains LD-1, LD-tr2, DU-1, and CF504 was isolated by the method of Portnoy et al. (20). The DNA was subjected to electrophoresis on 0.7% agarose gel with TBE buffer at 10 V/cm for 3 h. Several supercoiled plasmids of known size (kindly provided by Fred C. Tenover, Veterans Administration Medical Center, Seattle, Wash.) were used as reference standards to determine the sizes of the isolated plasmids of interest. These standards

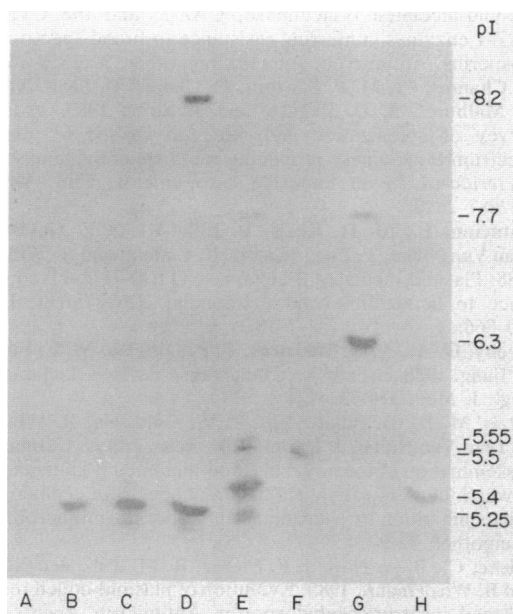


FIG. 1. Isoelectric focusing of β -lactamases on polyacrylamide gel with nitrocefin for detection. Lanes: A, *E. coli* HB101; B, *E. coli* LD-tr (pI 5.25); C, *K. pneumoniae* LD-1 (pI 5.25 and 7.7); D, *S. marcescens* DU-1 (pI 5.25 and 8.2); E, *K. pneumoniae* CF504 (CAZ-1; pI 5.4, 5.55, and 7.7); F, *E. coli* J53 R2633E (RHH-1; pI 5.5); G, *K. pneumoniae* CF104 (CTX-1; pI 6.3 and 7.7); H, *E. coli* K12 J53 (TEM-1; pI 5.4).

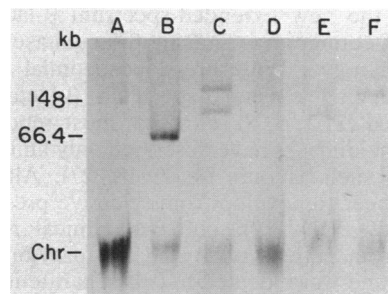


FIG. 2. Agarose gel electrophoresis of isolated plasmid DNA. Lanes: A and B, supercoiled plasmids of known size (R40-a [148 kb] and R446b [66.4 kb], respectively); C, plasmids of CF 504 (CAZ-1); D, E, and F, plasmids isolated from strains LD-tr, LD-1, and DU-1, respectively. Chr, Chromosomal DNA.

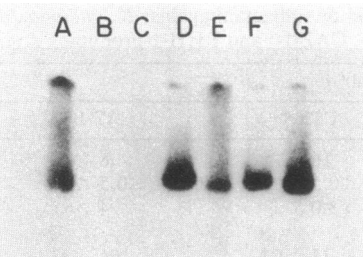


FIG. 3. Southern blot analysis. Lanes: A and D, DNA of plasmid R40a and the plasmid DNA encoding for CAZ-1 hybridized with a ^{32}P -labeled fragment of the TEM-1 gene under high-stringency conditions; E, F, and G, probe hybridized with plasmid DNA from LD-tr, LD-1, and DU-1, respectively; B and C, DNA of plasmids R16 and R446b, respectively, as controls that do not contain TEM genes.

included R40a (148 kilobases [kb]), R16 (104 kb), R446b (66.4 kb) and pSa (34.7 kb) (28). Both LD strains and DU-1 contained a plasmid of approximately 150 kilobases (Fig. 2), similar to the larger (150-kb) plasmid from CF-504 (18).

A 298-base-pair *Pst*I-*Hinc*II fragment (internal fragment of the TEM-1 gene of pBR322) labeled with ^{32}P was used as a probe (18) for Southern blot analysis (26) of plasmid DNA prepared from LD-1, LD-tr2, DU-1, and CF-504 by using standard techniques and high-stringency conditions (12). Homology was demonstrated in that all of the above plasmids contained TEM-type β -lactamase sequences (Fig. 3) and are thus likely TEM derived.

The antimicrobial susceptibility pattern conferred by the extended-spectrum β -lactamase produced by the *K. pneumoniae* and *S. marcescens* isolates encountered in our two patients was similar to those conferred by other recently described enzymes, e.g., CAZ-1 (18, 19, 21), CAZ-2 (4), and RHH-1 (27). Analysis of the extended-spectrum β -lactamases has allowed categorization of the new enzymes as either TEM or SHV derivatives. Analytical isoelectric focusing has revealed that the TEM derivatives possess isoelectric points in the 5 to 6 range, whereas the SHV derivatives have been in the pI 7 to 8 range (19). Currently, at least 10 TEM-derived β -lactamases produced by clinical isolates have been described (19, 21). The β -lactamase produced by the LD-1 and DU-1 isolates in our study had a unique pI of 5.25, which is similar to that of other TEM β -lactamases, especially CAZ-3 (pI 5.2) (19). Plasmid isolation and hybridization studies with a TEM-1 probe confirmed that the β -lactamase produced by our isolates was encoded by a TEM-related gene located on a plasmid of approximately 150 kilobases.

Although the new extended-spectrum β -lactamases are mutations of commonly occurring β -lactamases, their prevalence is unknown. Widespread nosocomial outbreaks of strains producing extended-spectrum β -lactamases have been reported (2, 3, 5, 8), although most reports of TEM-derived ceftazidimases have described only limited, sporadic occurrences such as ours (4, 7, 18, 27). Although these enzymes appear to develop from selective pressure of antibiotic use (2, 5, 6, 19), our two clinical isolates were recovered from patients who had not previously received ceftazidime and were located in two separate intensive care units with no apparent relationship. Moreover, no subsequent ceftazidime-resistant isolates of either genus have been recognized in the hospital since the inception of this study. Spencer et al. (27) retrospectively tested 14,000 clinical isolates without identifying an additional RHH-

1-producing isolate. Thus, the ultimate epidemiologic importance of these strains remains to be determined.

This case report demonstrates the clinical relevance of accurately recognizing isolates that elaborate extended-spectrum β -lactamases. Our patient failed treatment with ceftazidime and amikacin but responded favorably to substitution of a cephalosporin to which his strain was susceptible in vitro, i.e., cefotaxime. This occurrence underscores the importance of performing specific susceptibility tests on clinical isolates for each antimicrobial agent that will be used for therapy, rather than relying on testing of only a spectrum class representative of the expanded-spectrum cephalosporins.

We thank Robert A. Radnik, Manjeri A. Venkatachalam, Alan G. Barbour, Carol Carter, and Fred C. Tenover for their advice, technical assistance, and support and Olga German for preparation of the manuscript. We gratefully acknowledge Antone A. Medeiros for providing us with the well-characterized β -lactamase-producing strains and Fred C. Tenover for providing us with the strains containing plasmids of known sizes.

LITERATURE CITED

- Bauernfeind, A., and G. Hörl. 1987. Novel R-factor borne β -lactamase of *Escherichia coli* conferring resistance to cephalosporins. *Infection* 15:257-259.
- Brun-Buisson, C., P. Legrand, A. Philippon, F. Montravers, M. Ansquer, and J. Duval. 1987. Transferable enzymatic resistance to third-generation cephalosporins during nosocomial outbreak of multiresistant *Klebsiella pneumoniae*. *Lancet* ii:302-306.
- Buré, A., P. Legrand, G. Arlet, V. Jarlier, G. Paul, and A. Philippon. 1988. Dissemination in five French hospitals of *Klebsiella pneumoniae* serotype K25 harbouring a new transferable enzymatic resistance to third generation cephalosporins and aztreonam. *Eur. J. Clin. Microbiol. Infect. Dis.* 7:780-782.
- Chanal, C. M., D. L. Sirot, R. Labia, A. Petit, A. Morand, J. L. Sirot, and R. A. Cluzel. 1988. Comparative study of a novel plasmid-mediated β -lactamase, CAZ-2, and the CTX-1 and CAZ-1 enzymes conferring resistance to broad-spectrum cephalosporins. *Antimicrob. Agents Chemother.* 32:1660-1665.
- De Champs, C., M. P. Sauviant, C. Chanal, D. Sirot, N. Gauzy, R. Malhuret, J. C. Baguet, and J. Sirot. 1989. Prospective survey of colonization and infection caused by expanded-spectrum- β -lactamase-producing members of the family *Enterobacteriaceae* in an intensive care unit. *J. Clin. Microbiol.* 27:2887-2890.
- Gutmann, L., M. D. Kitzis, D. Billot-Klein, F. Goldstein, G. Tran Van Nhieu, T. Lu, J. Carlet, B. Collatz, and R. Williamson. 1988. Plasmid-mediated β -lactamase (TEM-7) involved in resistance to ceftazidime and aztreonam. *Rev. Infect. Dis.* 10:860-866.
- Jacoby, G. A., A. A. Medeiros, T. F. O'Brien, M. E. Pinto, and H. Jiang. 1988. Broad spectrum, transmissible β -lactamases. *N. Engl. J. Med.* 319:723-724.
- Kitzis, M. D., D. Billot-Klein, F. W. Goldstein, R. Williamson, G. Tran Van Nhieu, J. Carlet, J. F. Acar, and L. Gutman. 1988. Dissemination of the novel plasmid-mediated β -lactamase CTX-1, which confers resistance to broad spectrum cephalosporins, and its inhibition by β -lactamase inhibitors. *Antimicrob. Agents Chemother.* 32:9-14.
- Kliebe, C., B. A. Nies, J. F. Meyer, R. M. Tolxdorff-Neutzling, and B. Wiedemann. 1985. Evolution of plasmid-coded resistance to broad-spectrum cephalosporins. *Antimicrob. Agents Chemother.* 28:302-307.
- Knothe, H., P. Shah, V. Kremery, M. Antal, and S. Mitsuhashi. 1983. Transferrable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. *Infection* 11:315-317.
- Labia, R., A. Morand, K. Tiwari, J. S. Pitton, D. Sirot, and J. Sirot. 1988. Kinetic properties of two plasmid-mediated β -

- lactamases from *Klebsiella pneumoniae* with strong activity against third generation cephalosporins. *J. Antimicrob. Chemother.* **21**:301-307.
12. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 13. Marre, R., A. A. Medeiros, and A. W. Pasculle. 1982. Characterization of the β -lactamases of six species of *Legionella*. *J. Bacteriol.* **151**:216-221.
 14. Matthew, M., A. M. Harris, M. J. Marshall, and G. W. Ross. 1975. The use of analytical isoelectric focusing for detection and identification of β -lactamases. *J. Gen. Microbiol.* **88**:169-178.
 15. National Committee for Clinical Laboratory Standards. 1984. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
 16. National Committee for Clinical Laboratory Standards. 1985. Methods for dilution antimicrobial susceptibility tests for bacterial that grow aerobically. Approved standard M7-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
 17. Neu, H. C. 1982. The new beta-lactamase-stable cephalosporins. *Ann. Intern. Med.* **97**:408-419.
 18. Petit, A., D. L. Sirot, C. M. Chanal, J. L. Sirot, R. Labia, G. Gerbaud, and R. A. Cluzel. 1988. Novel plasmid-mediated β -lactamase in clinical isolates of *Klebsiella pneumoniae* more resistant to ceftazidime than to other broad-spectrum cephalosporins. *Antimicrob. Agents Chemother.* **32**:626-630.
 19. Philippon, A., R. Labia, and G. Jacoby. 1989. Extended-spectrum β -lactamases. *Antimicrob. Agents Chemother.* **33**:1131-1136.
 20. Portnoy, D. A., S. L. Moseley, and S. Falkow. 1981. Characterization of plasmids and plasmid-associated determinants of *Yersinia enterocolitica* pathogenesis. *Infect. Immun.* **31**:775-782.
 21. Quinn, J. P., D. Miyashiro, D. Sahm, R. Flamm, and K. Bush. 1989. Novel plasmid-mediated β -lactamase (TEM-10) conferring selective resistance to ceftazidime and aztreonam in clinical isolates of *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* **33**:1451-1456.
 22. Sirot, D., J. Sirot, R. Labia, A. Morand, P. Courvalin, A. Darfeuille-Michaud, R. Perroux, and R. Cluzel. 1987. Transferable resistance to third-generation cephalosporins in clinical isolates of *Klebsiella pneumoniae*: identification of CTX-1, a novel β -lactamase. *J. Antimicrob. Chemother.* **20**:323-334.
 23. Sirot, J., C. Chanal, A. Petit, D. Sirot, R. Labia, and G. Gerbaud. 1988. *Klebsiella pneumoniae* and other *Enterobacteriaceae* producing novel plasmid-mediated β -lactamases markedly active against third-generation cephalosporins: epidemiologic studies. *Rev. Infect. Dis.* **10**:850-859.
 24. Sirot, J., R. Labia, and T. Thabaut. 1987. *Klebsiella pneumoniae* strains more resistant to ceftazidime than to other third-generation cephalosporins. *J. Antimicrob. Chemother.* **20**:611-612.
 25. Sougakoff, W., S. Goussard, G. Gerbaud, and P. Courvalin. 1988. Plasmid-mediated resistance to third-generation cephalosporins caused by point mutations in TEM-type penicillinase genes. *Rev. Infect. Dis.* **10**:879-884.
 26. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**:503-517.
 27. Spencer, R. C., P. F. Wheat, T. G. Winstanley, D. M. Cox, and S. J. Plested. 1987. Novel β -lactamase in a clinical isolate of *Klebsiella pneumoniae* conferring unusual resistance to β -lactam antibiotics. *J. Antimicrob. Chemother.* **20**:919-921.
 28. Tenover, F. C. 1985. Plasmid fingerprinting—a tool for bacterial strain identification and surveillance of nosocomial and community-acquired infections. *Clin. Lab. Med.* **5**:413-436.