ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1986, p. 918–920
0066-4804/86/050918-03$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 29, No. 5

NOTES

Transfer of β-Lactamase-Associated Cefoxitin Resistance in *Bacteroides fragilis*

GEORGE J. CUCHURAL, JR.,[1] FRANCIS P. TALLY,[2]* JAMES R. STOREY,[3]† AND MICHAEL H. MALAMY[3]

Infectious Disease Service, Department of Medicine,[1] and Clinical Microbiology Laboratory, Department of Pathology,[2] Tufts-New England Medical Center Hospital, and Department of Molecular Biology,[3] Tufts University School of Medicine, Boston, Massachusetts 02111

Received 21 January 1986/Accepted 3 February 1986

A cefoxitin-resistant *Bacteroides fragilis* isolate, TAL 4170, which inactivates cefoxitin, was able to transfer β-lactamase-mediated cefoxitin resistance to a susceptible *B. fragilis* recipient. Cefoxitin-resistant transconjugants acquired a new β-lactamase with a pI of 8.1 and were able to inactivate cefoxitin and retransfer cefoxitin resistance. No plasmids were detected in the donor or transconjugants.

Cefoxitin is one of the preferred drugs for the treatment of intra-abdominal infections that frequently harbor *Bacteroides fragilis*. The superior activity of cefoxitin against *B. fragilis* is due to its resistance to hydrolysis by the β-lactamases found in *Bacteroides* spp. (3, 6, 12). In this report, we characterize a new mechanism of cefoxitin resistance in a *B. fragilis* isolate and describe the conjugal transfer of cefoxitin inactivating activity.

The cefoxitin-resistant *B. fragilis* strain TAL 4170 was isolated at the University of Miami/Jackson Memorial Medical Center, Miami, Fla. TM4170.23 was made by transferring the Tet transfer element from TM230 to TAL 4170 by selecting for clindamycin-resistant transconjugants. The Tet transfer element, which transfers tetracycline and clindamycin resistance at a high frequency from TM230, has previously been used to mobilize determinants for ampicillin resistance in *B. fragilis* (T. Butler, F. P. Tally, S. L. Gorbach, and M. H. Malamy, Clin. Res. **28**:365A, 1980). The frequency of cotransfer of the Tet transfer element with clindamycin resistance in transconjugants from a TM230 mating is 100% (14). *B. fragilis* recipient strain JCF101 is susceptible to ampicillin (Amp^s) and cefoxitin (Cfx^s) and resistant to rifampin (Rif^r) and fusidic acid (Fus^r) and requires histidine and arginine to grow on minimal media (13, 14).

The isolates were transported, stored, and incubated as previously described (10, 13, 14). MICs were determined by broth dilution, using microtiter techniques as previously described (11). Resistance breakpoints were defined as follows: cefoxitin, >16 μg/ml; tetracycline, >4 μg/ml; clindamycin, >4 μg/ml; and ampicillin, >16 μg/ml (10). The inoculum effect was investigated by increasing the initial inoculum density from 10^6 to 10^8 CFU/ml. Induction of cefoxitin resistance was tested by growing the organisms to be tested for MICs in media with and without cefoxitin (5 μg/ml). Filter mating techniques and plasmid analysis were used as previously described (13, 14).

Cefoxitin concentrations in broth cultures were determined by high-pressure liquid chromatography or bioassay as previously described (2). Intact cells, cells treated with 1% toluene for 1 h at 22°C, or crude cell sonicates served as sources of β-lactamase. β-Lactamase activity and "crypticity" were determined as described below (see Table 2). β-Lactamase induction was investigated by incubating BHIS broth cultures with and without cefoxitin (5 μg/ml) or ampicillin (20 μg/ml).

Isoelectric focusing of β-lactamase-containing samples was performed on polyacrylamide gel plates, Ampholine (R) Pagplate (LKB Stockholm, Bromma, Sweden), pH gradient 3.5 to 9.5, at 4°C over 4 h. TEM-2 β-lactamase and the visible proteins myoglobin and cytochrome *c* were used as controls. β-Lactamase-containing bands were detected by overlaying the gel with a nitrocefin solution (256 μg of nitrocefin per ml of 0.1 M potassium phosphate buffer [pH 7]) and observing the development of red bands.

Both TAL 4170 and TM4170.23 were able to transfer cefoxitin and ampicillin resistance to strain JCF101 (Table 1). Strain TM4170.23 was able to transfer β-lactam drug resistance about 10 times more frequently than TAL 4170 was. Only one Tet^r transconjugant was found to be cefoxitin resistant. Pretreatment of the donor strains with tetracycline, cefoxitin, or clindamycin did not increase the frequency of cefoxitin resistance transfer. Induction with tetracycline increased tetracycline transfer frequency 10-fold for TM4170.23.

The transfer of cefoxitin resistance occurred by conjugation, since transfer required cell-to-cell contact on filters, was DNase insensitive, and did not occur with supernatants of chloroform-treated cells.

The MICs for the donors, recipient, and transconjugants to ampicillin and cefoxitin are listed in Table 2. β-Lactam-resistant transconjugants TM5334 and TM5335 were initially selected on plates containing 150 μg of ampicillin per ml; while TM5336 and TM5337 were selected on plates containing 50 μg of cefoxitin per ml. There was no induction to higher MICs when these transconjugants were pretreated with cefoxitin (5 μg/ml).

The β-lactam-resistant transconjugants were able to rapidly inactivate 100 μg of cefoxitin per ml in broth cultures; when incubated at a high inoculum (10^8 to 10^9 CFU/ml), less

* Corresponding author.
† Present address: Cambridge BioScience Corp., Hopkinton, MA 01748.

TABLE 1. Transfer of cefoxitin resistance from *B. fragilis* donor cells to *B. fragilis* recipient cells

Donor	Recipient	Selected markers[a]	Transfer frequency	Frequency of cotransfer (%)[b]		
				Cfx	Amp	Tet
TAL 4170	JCF101	Amp (150)[c]	10^{-7}	100	100	0
		Cfx (50)	10^{-7}	100[d]	100[d]	0
		Cfx (100)	10^{-8}	100	100	0
		Tet (10)	10^{-7}	0	0	100
TM4170.23	JCF101	Amp (150)	10^{-6}	100	100	2[e]
		Cfx (50)	10^{-6}	100	100	0
		Cfx (100)	10^{-6}	100	100	0
		Tet (10)	10^{-5}	0	0	100
		Cln (50)	10^{-6}	0	0	100

[a] In addition to rifampin (50 μg/ml).
[b] Number of transconjugants divided by the number of input donor cells.
[c] Drug concentrations in parentheses are the plate concentration micrograms per milliliter, used for the selection.
[d] Cefoxitin transfer frequencies were the same on selection plates containing 25 and 50 μg of cefoxitin per ml.
[e] One isolate.

than 1% remained at 24 h. Strain JCF101 and the drug alone had 71 and 90% remaining, respectively.

The β-lactam-resistant transconjugants had a substantial increase in β-lactamase specific activity compared with that of the recipient JCF101 (Table 2). The β-lactamase specific activity of the isolates selected on cefoxitin-containing plates, TM5336 and TM5337, was about fourfold higher than that of the transconjugants selected on ampicillin-containing plates, TM5334 and TM5335. No induction of β-lactamase was detected when either donors or transconjugants were exposed to either ampicillin (20 μg/ml) or cefoxitin (5 μg/ml) in broth cultures and sampled every 2 h for 8 h. Crude sonicates of TAL 4170 and TM4170.23 showed two bands of β-lactamase activity, with pI's of 8.1 and 4.2, while JCF101 displayed one weak band at 4.2. β-Lactamase samples from the β-lactam-resistant transconjugants displayed a band with pI of 8.1 as well as a band at 4.2. These data indicate that all β-lactam-resistant transconjugants acquired cefoxitin-inactivating enzyme, but transconjugants selected on ampicillin plates had lower β-lactamase specific activity, with a resultant lower cefoxitin MIC. Ampicillin and cefoxitin resistance were mediated by the β-lactamase with a pI of 8.1.

TABLE 2. Susceptibilities, β-lactamase activity, and crypticity

Strain	MIC (μg/ml)		β-Lactamase	
	Cfx	Amp	Sp act[a]	Crypticity[b]
Recipient				
JCF101	8	16	0.013	1
Transconjugants				
TM5334	32	128	0.7	15
TM5335	32	128	0.8	15
TM5336	128	>256	1.8	20
TM5337	128	>256	3.8	20
Donors				
TAL 4170	128	>256	2.0	30
TM4170.23	64	>256	2.6	30

[a] β-Lactamase specific activity of crude bacterial sonicates, expressed in units per milligram of protein. β-Lactamase activity was determined spectrophotometrically (Coleman model 124; Perkin-Elmer Corp., Maywood, Ill., using the chromogenic substrate nitrocefin at 482 nm at a temperature of 30°C in 0.1 M phosphate buffer at pH 7.0 (7). Protein concentration was determined by a modified Lowry method (5). One unit of β-lactam activity was defined as that amount of enzyme resulting in the destruction of 1 μM/min.
[b] Crypticity was defined as the ratio of β-lactamase activity of disrupted cells to whole cells (4).

The crypticity of β-lactamase activity in TAL 4170 and TM4170.23 was 30, while that of JCF101 was approximately 1. The cefoxitin-resistant transconjugants had crypticities in the range of 15 to 20 (Table 2). No plasmid bands were seen after agarose gel electrophoresis of DNA harvested from TAL 4170, TM4170.23, and β-lactam-resistant transconjugants.

β-Lactamases are the most important mechanism of β-lactam antibiotic resistance in aerobic and anaerobic bacteria. β-Lactamase-mediated cefoxitin resistance in *B. fragilis*, although rare, has been well documented (2, 15). Our findings further demonstrate that β-lactamase-mediated cefoxitin resistance is transferable in *B. fragilis*. β-Lactamase expression is constitutive, and transconjugant strains with higher levels of β-lactamase production can be found associated with higher cefoxitin MICs. Other investigators have demonstrated β-lactamase transfer in *B. fragilis* (9; Butler et al., Clin. Res., 1980). Cefoxitin resistance transfer was reported for a strain of *Bacteroides thetaiotaomicron* by Rashtchian et al. (8); however, the mechanism of the resistance in that isolate was not determined.

Limited drug permeability and periplasmic localization of β-lactamase are considered to be synergistic in maintaining low periplasmic β-lactam drug concentrations (16). β-Lactam crypticity values are indirect assays for assessing outer membrane β-lactam drug permeability. The range of values we obtained for the cefoxitin-resistant donors and transconjugants indicates some degree of inhibition of drug permeation, which probably contributes to the effectiveness of the β-lactamase in maintaining low periplasmic drug concentrations.

Cefoxitin resistance in *B. fragilis* is important because it is associated with high frequency of cross-resistance to other β-lactam agents (1), and outbreaks of cefoxitin resistance seen at different centers (1, 10) may yield isolates resistant to virtually all currently available β-lactam agents. The spread of these resistances will undoubtedly depend on selection pressure of antibiotic usage and other epidemiologic factors.

LITERATURE CITED

1. **Cuchural, G. J., Jr., F. P. Tally, N. V. Jacobus, S. L. Gorbach, K. Aldridge, T. Cleary, S. M. Finegold, G. Hill, P. Iannini, J. P. O'Keefe, and C. Pierson.** 1984. Antimicrobial susceptibilities of

1,292 isolates of the *Bacteroides fragilis* group in the United States: comparison of 1981 with 1982. Antimicrob. Agents Chemother. **26**:145–148.
2. **Cuchural, G. J., Jr., F. P. Tally, N. V. Jacobus, P. K. Marsh, and J. W. Mayhew.** 1983. Cefoxitin inactivation by *Bacteroides fragilis*. Antimicrob. Agents Chemother. **24**:936–940.
3. **Darland, G., and J. Birnbaum.** 1977. Cefoxitin resistance to beta-lactamase: a major factor for susceptibility of *Bacteroides fragilis* to the antibiotic. Antimicrob. Agents Chemother. **11**:725–734.
4. **Dornbusch, K., B. Olsson-Liljequist, and C. E. Nord.** 1980. Antibacterial activity of new β-lactam antibiotics on cefoxitin-resistant strains of *Bacteroides fragilis*. J. Antimicrob. Chemother. **6**:207–216.
5. **Hanson, R. S., and J. A. Phillips.** 1981. Chemical composition, p. 328–364. *In* P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
6. **Neu, H.** 1983. β-Lactamase stability of cefoxitin in comparison with other β-lactam compounds. Diagn. Microbiol. Infect. Dis. **1**:313–316.
7. **O'Callaghan, C. H., A. Morris, S. M. Kirby, and A. H. Shingler.** 1972. Novel method for detection of β-lactamases by using a chromogenic cephalosporin substrate. Antimicrob. Agents Chemother. **1**:283–288.
8. **Rashtchian, A., G. R. Dubes, and S. J. Booth.** 1982. Transferable resistance to cefoxitin in *Bacteroides thetaiotaomicron*. Antimicrob. Agents Chemother. **22**:701–703.
9. **Sato, K., Y. Matsuura, M. Inoue, and S. Mitsuhashi.** 1982. Properties of a new penicillinase type produced by *Bacteroides fragilis*. Antimicrob. Agents Chemother. **22**:579–584.
10. **Tally, F. P., G. J. Cuchural, N. V. Jacobus, S. L. Gorbach, K. E. Aldridge, T. J. Cleary, S. M. Finegold, G. B. Hill, P. B. Iannini, R. V. McCloskey, J. P. O'Keefe, and C. L. Pierson.** 1983. Susceptibility of the *Bacteroides fragilis* group in the United States in 1981. Antimicrob. Agents Chemother. **23**:536–540.
11. **Tally, F. P., N. V. Jacobus, J. G. Bartlett, and S. L. Gorbach.** 1975. Susceptibility of anaerobes to cefoxitin and other cephalosporins. Antimicrob. Agents Chemother. **7**:128–132.
12. **Tally, F. P., J. P. O'Keefe, N. M. Sullivan, and S. L. Gorbach.** 1979. Inactivation of cephalosporins by *Bacteroides*. Antimicrob. Agents Chemother. **16**:565–571.
13. **Tally, F. P., D. R. Snydman, S. L. Gorbach, and M. H. Malamy.** 1979. Plasmid mediated, transferable resistance to clindamycin and erythromycin in *Bacteroides fragilis*. J. Infect. Dis. **139**: 83–88.
14. **Tally, F. P., D. R. Snydman, M. J. Shimell, and M. H. Malamy.** 1982. Characterization of pBFTM10, a clindamycin-erythromycin resistance transfer factor from *Bacteroides fragilis*. J. Bacteriol. **151**:686–691.
15. **Yotsuji, A., S. Minami, M. Inoue, and S. Mitsuhashi.** 1983. Properties of a novel β-lactamase produced by *Bacteroides fragilis*. Antimicrob. Agents Chemother. **24**:925–929.
16. **Zimmerman, W., and A. Rosselet.** 1977. Function of the outer membrane of *Escherichia coli* as a permeability barrier to beta-lactam antibiotics. Antimicrob. Agents Chemother. **12**: 368–372.