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β-Lactamases and β-Lactam Resistance in *Escherichia coli*

GEORGE A. JACOBY* AND LORRAINE SUTTON
Massachusetts General Hospital, Boston, Massachusetts 02114

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***Escherichia coli* strains determining 17 different plasmid-determined β-lactamases were tested for resistance to new broad-spectrum β-lactam antibiotics. Several β-lactamases demonstrated enhanced resistance to cefamandole but only low-level resistance to other agents. High production of cloned *E. coli* chromosomal β-lactamase, however, provided resistance to cefamandole, cefoxitin, cefotaxime, ceftazidime, and aztreonam but not to BMY-28142 or imipenem.**

In gram-negative bacteria, a variety of plasmid-determined β-lactamases can hydrolyze ampicillin, carbenicillin, cephalothin, and related drugs (19, 27). An ever-growing number of new β-lactam antibiotics have been developed to overcome this resistance through molecular modifications that diminish sensitivity to β-lactamase inactivation. However, resistance to such drugs has been observed with strains, particularly of *Enterobacter cloacae*, that overproduce chromosomal β-lactamase (24). It has been proposed that large amounts of β-lactamase in the periplasmic space either bind (28) or slowly hydrolyze (29) the antibiotic before it can reach its targets. With either mechanism the rate of antibiotic entry into the cell is also an important variable (3, 29). β-Lactams penetrate the outer membrane of gram-negative bacteria through specific porin channels (22, 31). Several of these channels exist in *Escherichia coli*, and mutations that prevent production of the outer membrane porin proteins OmpC and OmpF also provide low levels of β-lactam resistance (4, 11).

In the present study, the role of these variables in resistance to representative β-lactam antibiotics was investigated. A set of *E. coli* C600 (1) derivatives was constructed containing plasmids determining 18 different β-lactamase types (Table 1). Since the plasmids were all present in the same host, variations in antibiotic susceptibility were attributed to the β-lactamases and not to other genetic alterations. In addition, five of the enzymes were expressed in either low or high amounts. Plasmids R1 (7), RP4 (7), R1010 (18), R997 (18), RGN238 (7), R46 (7), pMG203 (20), pMG54 (20), pMG202 (20), pMG217 (21), pUZ8-R151 (6), Rms149 (5), and pUZ8-DAL (6) have low copy numbers. Strains producing high levels of certain β-lactamases were obtained by transposition of their genes to multicopy kanamycin resistance plasmids pMK20 and pCR1 (13) by selecting for enhanced β-lactam resistance (14) in a strain containing pMK20 or pCR1 and low-copy-number plasmids R1 (for Tn*3*), RP4 (for Tn*1*), RGN238 (for Tn*2603*), or pMG217 (for Tn*1401*). pNU81 and pNU104 are multicopy plasmids containing the cloned *E. coli ampC* gene (12) which determines the CEP-1 β-lactamase (16). In pNU104, an up-promoter mutation has increased the level of β-lactamase expression (12). The CEP-1 enzyme was of particular interest since its gene is homologous to chromosomal β-lactamases of other enterobacterial species (2) that develop resistance to β-lactamase-stable drugs by β-lactamase overproduction. To transfer β-lactamase genes found on *Pseudomonas* plasmids to *E. coli*, recombinant plasmids were formed (9) between low-copy-number plasmid pUZ8 (6) and the following plasmids: (i) pMG76 (26), determining LCR-1 (pUZ8:Tn*412*); (ii) pMG25 (8), determining OXA-3 (pUZ8::Tn*1411*); pMG39 (20), determining OXA-6 (pUZ8-pMG39); and the CARB-3 (15) *bla* gene of strain AP115 (pUZ8::Tn*1408*). Evidence that transposons were involved in recombinant plasmid formation will be presented elsewhere (manuscript in preparation).

β-lactamase activity in sonic extracts of these strains was assayed by using nitrocefin (23) in 0.05 M phosphate buffer, pH 7, at 30°C and was expressed as milliunits per milligram of protein (determined by the procedure of Lowry et al. [17], where 1 U represents 1 μmol of substrate hydrolyzed per min per ml of extract. Each strain was assayed at least twice. Strain C600 R^- produced a low level of chromosomal β-lactamase (Table 1). C600 derivatives containing low-copy-number plasmids varied in β-lactamase activity from little more than that of C600 R^- to 9,270 mU/mg for C600(RP4). Strains containing high-copy-number plasmids or pNU104 with an up-promoter mutation produced more β-lactamase, as much as 71,300 mU/mg for C600 (pMK20::Tn*1*).

The β-lactam resistance of these strains was determined by agar dilution as previously described (10). Antibiotics which were not commercially available were obtained from the following sources: aztreonam was from E. R. Squibb & Sons, Princeton, N.J.; BMY-28142 was from Bristol Laboratories, Syracuse, N.Y.; ceftazidime was from Glaxo Pharmaceuticals, Ltd., Greenford, United Kingdom; and imipenem was from Merck & Co., Rahway, N.J. All the β-lactamase-producing strains demonstrated enhanced resistance to ampicillin, and high β-lactamase production was correlated with high levels of ampicillin resistance (Table 1). More diversity was observed between lower levels of β-lactamase production, as measured by nitrocefin hydrolysis, and the level of ampicillin resistance. This was probably, at least in part, due to variations in the relative activity of each enzyme toward ampicillin and nitrocefin. If a fourfold or greater increase in MIC is considered significant, then resistance was demonstrated by nine β-lactamase types to cefamandole, by six types to BMY-28142, by five types to ceftazidime, by three types of aztreonam, by two types to cefotaxime, and by one type to cefoxitin or to imipenem. Relative to achievable serum antibiotic concentrations, however, the level of resistance produced toward aztreonam, BMY-28142, cefotaxime, cefoxitin, ceftazidime, or imipenem would not be clinically significant except with CEP-1 β-lactamase. Like other gram-negative strains that overproduce chromosomal β-lactamase (3, 24, 25, 29), C600, which produced high amounts of CEP-1, was resistant to

* Corresponding author.

TABLE 1. β-Lactamase levels and antimicrobial susceptibilities of *E. coli* C600 derivatives

β-Lactamase type	Plasmid	Plasmid *bla* expression	β-Lactamase activity (mU/mg)	MIC (μg/ml)							
				Ampicillin	Cefamandole	Cefoxitin	Cefotaxime	Ceftazidime	BMY-28142	Aztreonam	Imipenem
	None	None	8	8	1	16	0.125	0.25	0.063	0.125	1
TEM-1	R1	Low	368	1,000	4	16	0.125	0.25	0.063	0.125	0.5
TEM-1	pMK20::Tn*3*	High	2,420	4,000	8	16	0.125	0.25	0.125	0.125	0.5
TEM-2	RP4	Low	9,270	16,000	63	16	0.125	0.5	0.25	0.25	0.5
TEM-2	pMK20::Tn*1*	High	71,300	≥16,000	250	16	0.25	1	1	0.5	4
SHV-1	R1010	Low	2,230	8,000	16	16	0.125	2	0.5	0.25	1
HMS-1	R997	Low	66	8,000	32	16	0.125	0.5	0.125	0.125	0.5
LCR-1	pUZ8::Tn*1412*	Low	29	250	1	16	0.125	0.25	0.063	0.125	1
OXA-1	RGN238	Low	51	500	1	16	0.25	0.25	1	0.125	1
OXA-1	pMK20::Tn*2603*	High	1,680	4,000	2	16	2	0.25	8	0.125	2
OXA-2	R46	Low	85	250	8	16	0.125	8	0.125	0.125	0.5
OXA-3	pUZ8::Tn*1411*	Low	157	250	16	16	0.125	2	0.032	0.125	0.5
OXA-4	pMG203	Low	22	125	1	16	0.125	0.25	0.25	0.125	1
OXA-5	pMG54	Low	97	32	1	16	0.125	0.25	0.063	0.125	1
OXA-6	pUZ8-pMG39	Low	58	32	2	16	0.125	0.5	0.125	0.25	1
OXA-7	pMG202	Low	4,480	1,000	32	16	0.25	0.5	0.25	1.0	0.5
PSE-1	pMG217	Low	443	2,000	4	16	0.125	0.25	0.125	0.125	1
PSE-1	pCR1::Tn*1401*	High	4,334	16,000	8	16	0.125	0.25	0.5	0.125	1
PSE-2	pUZ8-R151	Low	91	63	2	16	0.125	0.25	0.125	0.25	1
PSE-3	Rms149	Low	13	1,000	2	16	0.125	0.25	0.125	0.125	1
PSE-4	pUZ8-DAL	Low	800	2,000	2	16	0.125	0.25	0.25	0.125	1
CARB-3	pUZ8::Tn*1408*	Low	3,750	4,000	2	16	0.125	0.25	0.125	0.125	1
CEP-1	pNU81	Low	105	63	4	32	0.5	2	0.125	2	1
CEP-1	pNU104	High	16,100	1,000	63	500	8	16	0.25	32	1

cefoxitin, cefotaxime, ceftazidime, and aztreonam, although it was unchanged in susceptibility to BMY-28142 and imipenem.

To investigate the influence of altered porin channels on β-lactam resistance, we transferred plasmids producing low and high levels of TEM-2, OXA-1, and CEP-1 β-lactamases to *E. coli* JF568, JF703, and JF694, which produce different outer membrane porins (22). Strains containing the same plasmid produced comparable amounts of β-lactamase and had rather similar levels of β-lactam resistance (Table 2). Compared with strain JF568 with intact porins, some plasmids in JF694 *ompC ompF* demonstrated four or eight times

TABLE 2. Antimicrobial susceptibility of *E. coli ompC* and *ompF* strains

Strain	OmpC	OmpF	PhoE	β-Lactamase type	Plasmid *bla* expression	β-Lactamase activity (mU/mg)	MIC (μg/ml)							
							Ampicillin	Cefamandole	Cefoxitin	Cefotaxime	Ceftazidime	BMY-28142	Aztreonam	Imipenem
JF568	+	+	−	−	None	14	4	1	8	0.063	0.25	0.032	0.063	0.5
				TEM-2	Low	10,500	16,000	63	8	0.063	0.25	0.125	0.125	0.5
				TEM-2	High	90,500	≥16,000	125	8	0.125	0.5	0.5	0.25	1
				OXA-1	Low	111	250	0.5	8	0.125	0.125	0.125	0.063	0.5
				OXA-1	High	1,300	2,000	1	8	1	0.25	2	0.125	0.5
				CEP-1	Low	213	63	2	32	0.5	0.5	0.032	1	0.5
				CEP-1	High	12,300	1,000	63	500	16	16	0.125	16	1
JF703	+	−	−	−	None	9	8	2	8	0.125	0.25	0.032	0.125	0.5
				TEM-2	Low	8,300	16,000	125	16	0.125	0.5	0.25	0.125	0.5
				TEM-2	High	79,700	≥16,000	500	8	0.125	1	0.5	0.5	1
				OXA-1	Low	180	250	1	8	0.25	0.25	0.5	0.125	0.5
				OXA-1	High	1,470	2,000	2	8	1	0.25	4	0.125	0.5
				CEP-1	Low	373	63	1	32	0.25	0.5	0.032	1	0.5
				CEP-1	High	10,600	1,000	63	500	8	32	0.25	32	1
JF694	−	−	+	−	None	11	8	0.25	8	0.063	0.25	0.063	0.032	0.5
				TEM-2	Low	10,900	16,000	16	8	0.25	0.5	0.5	0.125	0.5
				TEM-2	High	89,600	≥16,000	16	8	0.125	2	0.25	0.125	0.5
				OXA-1	Low	100	500	0.25	8	0.25	0.25	1	0.063	0.5
				OXA-1	High	1,370	8,000	0.5	8	1	0.5	8	0.125	0.5
				CEP-1	Low	366	63	1	32	0.5	4	0.125	1	0.5
				CEP-1	High	19,900	1,000	16	500	8	32	0.25	8	1

greater resistance to BMY-28142, cefotaxime, or ceftazidime, but in all these cases the strains remained susceptible to 8 μg of antibiotic per ml or less. In *E. coli*, which is much more permeable to β-lactams than *E. cloacae* is (29), variations in outer membrane porins had relatively little effect on the level of β-lactamase-mediated resistance.

These studies document the considerable variation in enzyme activity associated with different types of plasmid-determined β-lactamases. Some of this diversity may be due to differences in the relative activity of individual enzymes toward nitrocefin, but variation in efficiency of the *bla* gene promoters may also play a role (30). Recently described β-lactamases such as LCR-1, OXA-4, OXA-5, OXA-6, OXA-7, and CARB-3 were no more efficient in providing resistance to new broad-spectrum β-lactams than were previously studied enzymes. While a number of β-lactamases showed an increment in resistance to cefamandole, even very high levels of TEM β-lactamase failed to give clinically significant resistance to aztreonam, BMY-28142, cefotaxime, ceftazidime, or imipenem. However, resistance to aztreonam, cefamandole, cefotaxime, cefoxitin, and ceftazidime was observed in strains constructed to overproduce the *E. coli* chromosomal β-lactamase, so that such strains serve as models for chromosomal β-lactamase overproducers in other genera. Since the set of strain C600 derivatives is representative of the types of β-lactamase that a new drug might confront in *E. coli* or other gram-negative pathogens, it should be useful in evaluating new β-lactams or β-lactamase inhibitors for this mechanism of resistance.

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