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Characterization and Prevalence of the Different Mechanisms of Resistance to Beta-Lactam Antibiotics in Clinical Isolates of *Escherichia coli*

ANTONE A. MEDEIROS, RALPH L. KENT, AND THOMAS F. O'BRIEN

Department of Medicine, Peter Bent Brigham Hospital, and Harvard Medical School, Boston, Massachusetts 02115

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A survey of clinical isolates from a hospital laboratory showed that *Escherichia coli* could be grouped into three classes of beta-lactam-antibiotic resistance by results of routine susceptibility testing to ampicillin, cephalothin, and carbenicillin. *E. coli* highly resistant to ampicillin and carbenicillin but not to cephalothin (class I) were found to have one of two levels of R factor-mediated, periplasmic-β-lactamase which resembled R_{TEM} and was located behind a permeability barrier to penicillins but not to cephalosporins. This permeability barrier appeared to act synergistically with the β-lactamase in producing high levels of resistance to penicillins. *E. coli* highly resistant to ampicillin and cephalothin but not carbenicillin (class II) were found to have a β-lactamase with predominantly cephalosporinase activity which was neither transferable nor releasable by osmotic shock. *E. coli* moderately resistant to one or to all three of these antibiotics (class III) were found to have low levels of different β-lactamases including a transferable β-lactamase which resembled R_{1818}. Thus, different mechanisms producing resistance to β-lactam antibiotics could be deduced from the patterns of resistance to ampicillin, cephalothin, and carbenicillin found on routine susceptibility testing. *E. coli* of class I were much more prevalent than the other classes and the proportion of *E. coli* that were class I increased with duration of patient hospitalization. The incidence of class I *E. coli* rose only slightly over the past 7 years and that of class II *E. coli* remained constant despite increased usage of both cephalothin and ampicillin. These observations emphasize that the properties of the apparently limited number of individual resistance mechanisms that exist in a bacterial flora, such as their genetic mobility and linkages and the spectrum of their antibiotic inactivating enzymes and permeability barriers, may govern the effect that usage of an antibiotic has upon the prevalence of resistance to it and to other antibiotics.

A gram-negative bacillus which is resistant to penicillin or cephalosporin antibiotics usually contains one of many different types of cell-bound β-lactamase (7, 12, 30, 34, 42). The levels of resistance of the bacillus to different β-lactam antibiotics, however, often do not correlate with the rates at which these antibiotics are hydrolyzed by the β-lactamase released from disrupted bacilli (17, 28, 30, 40). The β-lactamase appears to act synergistically with other resistance mechanisms, such as permeability barriers to the entry into the bacillus of some antibiotics, in producing resistance to them (28, 30, 40). Thus, the particular β-lactamase together with the other resistance mechanisms that an isolate may possess appear to determine the isolate's distinctive pattern of levels of resistance to different β-lactam antibiotics.

Conversely, from observation of such patterns of resistance it might be possible to deduce the mechanisms of β-lactam antibiotic resistance in an isolate, or survey the prevalence of particular mechanisms in large numbers of isolates. This would be an important type of surveillance, because the prevalence that particular mechanisms attain in gram-negative bacilli will be a major determinant of the relative usefulness of different members of the growing family of β-lactam antibiotics. Moreover, understanding of how various different mechanisms come to be prevalent could provide a rationale for policies regarding usage and restriction of antibiotics.

The following studies seek to identify biochemical and genetic characteristics of the different mechanisms of resistance to β-lactam antibiotics found in a prevalence sample of clinical isolates of *Escherichia coli*, the most commonly isolated of the gram-negative bacilli, and to survey prevalence of these different mechanisms in all *E. coli* isolates from one hospital over a 7-year period.

MATERIALS AND METHODS

The data base consisted of isolates of *E. coli* routinely tested for antibiotic susceptibility by the single-disk-diffusion method (16) in the Clinical Microbiology Laboratory of the Peter Bent Brigham Hospital over the 7-year period 1967–73. The format of the data file and the programs used for generating plots of frequency distribution of diameters of zones of inhibition (25) and plots of resistance prevalence by hospital day have been described. For the present studies repeat isolates from the same patient were excluded unless they differed in resistance to ampicillin. Frequency counts were made of all possible groupings of resistance to eight different antibiotics (tetracycline, chloramphenicol, streptomycin, kanamycin, sulfonamide, cephalothin, ampicillin, and carbenicillin). For carbenicillin, the standard for high-level resistance was used (zone <13 mm) and for all agents intermediate zones were considered not resistant. Routine testing with carbenicillin disks began in 1971. Analyses were performed utilizing an IBM 360/75 computer and plots were generated on a Cal Comp model 890 plotter.

During a survey period June through August 1971, the Clinical Microbiology Laboratory isolated for further study *E. coli* resistant to ampicillin, carbenicillin, or cephalothin from 60 patients. Speciation was reconfirmed; all formed typical colonies on endo agar, were indole and nitrate positive, and were citrate, urease, hydrogen sulfide, gelatin, and oxidase negative.

Minimal inhibitory concentrations (MIC) were determined by making serial twofold dilutions of antibiotic in 2- to 5-ml volumes of glucose-phosphate broth (Albimi). Samples from each tube were then distributed in the corresponding cups of a microtiter plate (Cooke Engineering Co., Alexandria, Va.). An equal volume of diluted overnight culture was added to each cup to make final inocula of approximately 10^7 and 10^3 organisms per ml in order to measure the effect of inoculum size on levels of resistance. The plates were read after 24-h of incubation at 37 C. Determinations with the large and small inocula were made in duplicate.

Standard powders of known potency were a gift of the following: penicillin G (Upjohn Laboratories), ampicillin and Cloxacillin (Bristol Laboratories), carbenicillin (Beecham Laboratories), and cephalothin and cephaloridine (Lilly Laboratories).

Assay for β-lactamase activity was performed according to the iodometric method of Perret (29) as described previously (17). Michaelis constants were determined for penicillin G, ampicillin, and carbenicillin using the microiodometric method (24), and for cephalothin and cephaloridine using the spectrophotometric method (26) measuring decrease in absorption at 255 mμ.

Enzyme preparations used were: (i) whole cell suspensions prepared by diluting overnight broth culture 1:10 in fresh broth, incubating for 3 h, then washing and resuspending the cells in 0.2 M PO_4 buffer, pH 6.5, with 0.5% gelatin; (ii) sonic extracts prepared by sonicating (biosonik) a whole cell suspension for four 30-s periods while refrigerating in an ice bath. The suspension was then centrifuged at 15,000 × *g* for 20 min and the supernatant was used as enzyme; (iii) osmotic shock extracts prepared according to the method of Neu and Heppel (20) as modified by Nossal and Heppel (23).

Induction of β-lactamase was tested by growing bacteria for 3 h in broth containing 1 mg of penicillin G per ml before preparing sonic extracts for enzyme assay. Cloxacillin inhibition was tested by measuring hydrolysis rates of penicillin G by sonic extracts in the presence of 1 mg/ml of cloxacillin.

R factor transfer was accomplished by methods described previously (18). Recipients used were a *Klebsiella pneumoniae* (K15520) and a sodium azide-resistant mutant of *E. coli* K-12 F^- (K-12A_zR). R factor-infected recipients were selected either on plates containing citrate as sole carbon source (K15520) or on plates containing 250 μg of sodium azide per ml (K-12A_zR) and an antibiotic to which the R factor mediated resistance. To facilitate identification of R^+ recipients on selector plates, transfer of R factors from wild *E. coli* to K-12A_zR was done by first mating the wild isolate with K15520, then mating R^+ K15520 recipients with K-12A_zR.

RESULTS

Combined resistance to β-lactam antibiotics. In Fig. 1 the diameters of the zones of inhibition around the cephalothin and carbenicillin disks of 540 *E. coli* isolates resistant to ampicillin are compared with those of 3008 *E. coli* susceptible to ampicillin, all isolated during 1971 and 1972. Ampicillin-susceptible *E. coli* were virtually never resistant to carbenicillin and only infrequently to cephalothin. In contrast 73.3% of ampicillin-resistant *E. coli* were resistant to carbenicillin alone, and another 10.6% were resistant to cephalothin alone.

Overall, 11.8% of the 3,361 patients who had an *E. coli* isolate had one resistant to both ampicillin and carbenicillin but not cephalothin, defined in these studies as class I. A 1.7% had an *E. coli* isolate resistant to both ampicillin and cephalothin but not carbenicillin, defined as class II; and 4.7% had an *E. coli* isolate resistant to only one or to all three of these antibiotics, which were grouped into a miscellaneous class III.

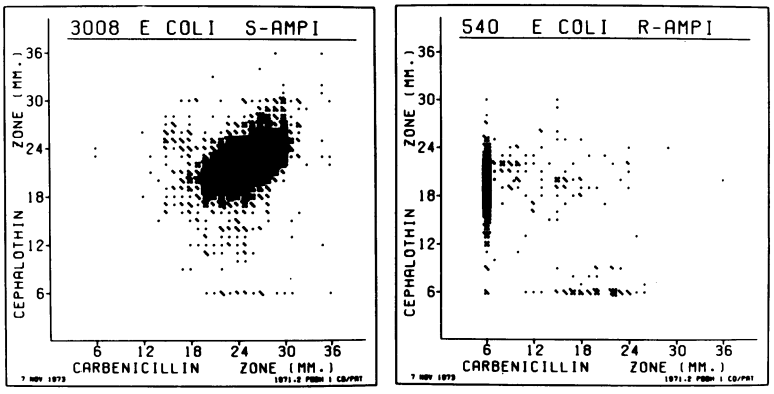

FIG. 1. *Computer generated plots of diameters of inhibition zones around the cephalothin and carbenicillin disks for 3,008 unrepeated E. coli isolates susceptible to ampicillin and 540 resistant to ampicillin, all isolated from 1971 through 1972.*

Seven-year comparison. Table 1 shows tnat the percentage of *E. coli* isolates with the class I resistance pattern remained relatively constant from 1971 through 1973, although a slight increase is suggested. Carbenicillin testing was not done before 1971. However the resistance pattern *ampi*^{R} *kef*^{S}, which is composed mainly of class I isolates, showed a slight increase over the 7-year period, 1967 through 1973. There was no increase in the percentage of class II *E. coli* isolates. The prevalence of class I isolates among *E. coli* isolated from patients late in their hospital course was twice that of isolates from recently admitted patients (Fig. 2).

Associated resistance to non-β lactam antibiotics. Resistance to β-lactam antibiotics was associated frequently with grouped resistance to sulfonamide, streptomycin, tetracycline, kanamycin, and chloramphenicol (Table 2). Eighty percent of β-lactam-resistant *E. coli* were resistant to one or more, and 42% to three or more of these antibiotics. In contrast, only 23% of β-lactam-susceptible *E. coli* were resistant to one or more, and only 5% to three or more of these agents. Grouped resistance to sulfonamide, streptomycin, and tetracycline was particularly common among β-lactam-resistant *E. coli*.

TABLE 1. *Yearly incidence of class I and class II patterns of resistance of Escherichia coli isolates*

Year	Sample no. of patients with an *E. coli* isolate	Patients (%) with an *E. coli* isolate who have one that is:			
		ampi^R *kef*^{S}	*ampi*^{R} *kef*^{S} *carb*^{R} (class I)	*ampi*^{R} *kef*^{R}	*ampi*^{R} *kef*^{R} *carb*^{S} (class II)
1973	1865	14.4	12.8	3.5	2.0
1972	1785	13.4	12.3	2.3	1.2
1971	1575	13.1	11.1	3.0	2.2
1970	1331	12.8		3.9	
1969	584	15.4		3.3	
1968	759	11.5		2.6	
1967	768	8.5		3.4	

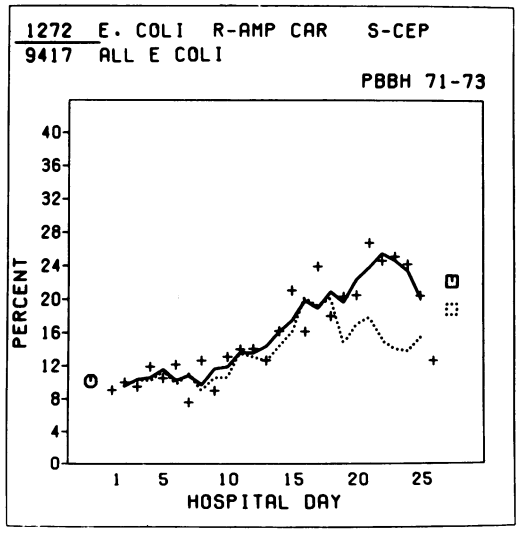

FIG. 2. *Computer generated plot of percent of E. coli isolates testing resistant to ampicillin and carbenicillin but susceptible to cephalothin (class I) plotted according to the patient hospital day on which the specimen was obtained. The circle at the left represents isolates from outpatients, and the squares at the right those from patients hospitalized for more than 25 days. Individual values for all isolates are plotted as + and a 3-day running average of these values is shown as a solid line. The dotted line is a similar plot of first isolates only from any patient. Separation between the lines represents repeated isolations from the same patient.*

TABLE 2. *Comparison of resistance to five non-β-lactam antibiotics of Escherichia coli resistant and susceptible to β-lactam antibiotics, isolated 1971 through 1973*[a]

Antibiotics	β-lactam-resistant *E. coli* (n = 968) (%)	β-lactam-susceptible *E. coli* (n = 4564) (%)
Not resistant to:[a]		
sulfa, strep, tetra, kana, or chlor	19.8	76.9
Resistant to:		
Sulfa	7.6	5.2
Strep	4.0	1.1
Tetra	3.2	4.4
Kana	0.3	0.6
Chlor	0.2	0
Sulfa, tetra	8.1	3.1
Strep, tetra	6.7	1.2
Sulfa, strep	5.1	2.2
Sulfa, chlor	1.5	<0.1
Sulfa, strep, tetra	16.9	2.5
Sulfa, tetra, chlor	2.8	0.2
Sulfa, tetra, kana	2.1	0.6
Strep, tetra, kana	1.7	0.2
Sulfa, strep, tetra, kana	9.8	1.0
Sulfa, strep, tetra, chlor	3.9	0.2
Sulfa, strep, tetra, kana, chlor	1.5	0.1
Miscellaneous groupings	4.7	0.5

[a] Sulfa, sulfonamide; strep, streptomycin; tetra, tetracycline; kana, kanamycin; chlor, chloramphenicol.

Ninety percent of class I, but only 64% of class II and III *E. coli*, were resistant to one or more of these non-β-lactam antibiotics. Chloramphenicol resistance was more frequent among class I *E. coli* (16%) than among class II and III (5%) or β-lactam-susceptible *E. coli* (1%) and was always associated with resistance to some other antibiotic.

Resistance levels in 62 consecutive resistant isolates. The isolates of β-lactam-resistant *E. coli* which came from the 1971 survey were assigned by disk testing as described above to class I (40 isolates), class II (12 isolates), and class III (10 isolates). Serial dilution susceptibility determinations using small inocula (about 10^2 colonies/ml) of these isolates (Fig. 3) showed MICs of ampicillin to be high for all three classes. Carbenicillin MICs were 500-fold higher than cephalothin MICs in class I, but 16-fold lower in class II. Cephalothin MICs were generally similar to cephaloridine MICs except in class II where they were eightfold higher. *E. coli* isolates resistant to any one β-lactam antibiotic generally had higher MICs to all the other β-lactam antibiotics than did those isolates not resistant to any (control *E. coli*). When large inocula were used, MICs for class I *E. coli* increased most and the MICs of cephaloridine had the greatest increment (Table 3).

β-lactamase activity of sonic extracts. Class I isolates had much higher levels of β-lactamase activity than those of class II or III (Table 4). Some of the class I isolates, subclassified IB, had distinctly greater activity than the remainder, subclassified IA. Attempts to induce additional activity by preincubation with penicillin G were unsuccessful in four isolates each from class I and II, and one from class III. Cloxacillin partially inhibited the β-lactamase activity of the class I *E. coli* isolates tested and completely inhibited the activity of class II isolates. In contrast, class III isolates were not inhibited by cloxacillin. Heating of sonic extracts for 5 min at 60 C caused no loss of activity for the class I isolates tested, and nearly complete loss of activity for class II and III isolates.

The substrate profiles of β-lactamases from class I isolates differed substantially from those of class II and III isolates (Table 4). The β-lactamases of class I *E. coli* hydrolyzed cephaloridine and ampicillin at rates similar to penicillin G, but were much less active against carbenicillin, cloxacillin, and cephalothin. The β-lactamase from class II isolates had primarily cephalosporinase activity. Substrate profiles of the β-lactamases of class III isolates varied, but those from three of them had distinctively high levels of hydrolytic activity against cloxacillin.

Determinations of K_m values of the β-lactamases for a variety of substrates are shown in Table 5. These results show that β-lactamase from the three class I and two class II isolates tested had much greater affinity for the penicillins than for the cephalosporins.

β-lactamase activity of intact cells. Intact cell suspensions of class I isolates hydrolyzed penicillin, ampicillin, or carbenicillin at rates only one-tenth or less of those of their sonically treated materials (Table 6). However, intact cell suspensions hydrolyzed cephalothin and cephaloridine at rates nearly equal to those of their sonic extracts. In contrast, intact cell suspensions of class II hydrolyzed the cephalosporin antibiotics at rates several-fold greater than their sonic extracts, an effect observed also in some class III isolates.

Supernatant fluid of four class I isolates subjected to osmotic shock had approximately three-fourths as much enzymatic activity as their sonic extracts, whereas osmotic shock

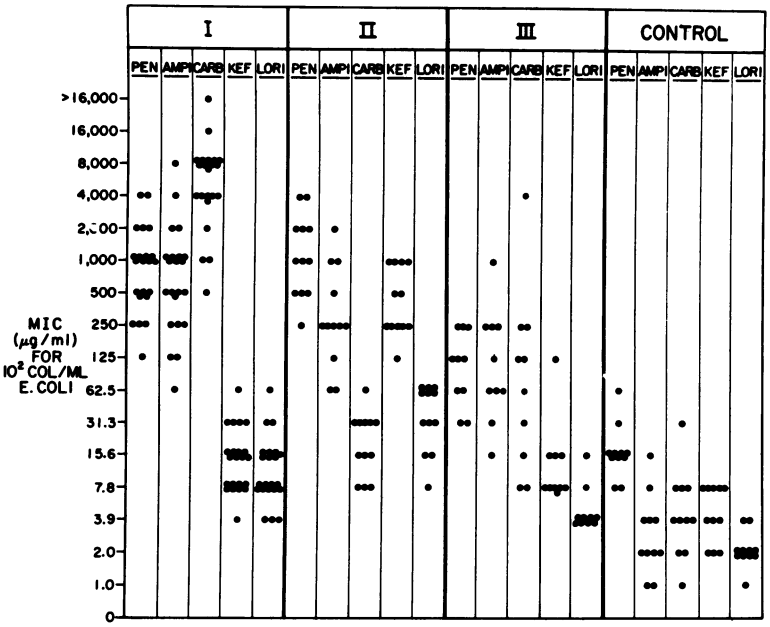

FIG. 3. *MIC of penicillin (PEN), ampicillin (AMPI), carbenicillin (CARB), cephalothin (KEF), and cephaloridine (LORI) for small inocula (approximately 10^2 colonies/ml) of isolates of each of the three classes (I, II, III) of β-lactam-resistant E. coli and for non-β-lactam-resistant E. coli (control).*

released little or no activity from two class II and one class III isolate tested.

Resistance transfer. Nearly all of the class I isolates that were mated with *Klebsiella* 15520 transferred resistance to both non-β lactam and β-lactam antibiotics (Table 7). Most of the class III isolates also transferred resistance to non-β-lactam antibiotics and half transferred resistance to β-lactam antibiotics as well. In contrast, half of the class II isolates transferred resistance to non-β-lactam antibiotics but none transferred their β-lactam antibiotic resistance.

In further mating experiments all but 3 of 18 R^+ *Klebsiella* 15520 recipients tested then transferred to *E. coli* K-12 both their β-lactam antibiotic resistance and β-lactamase activity. The level of β-lactamase activity of each of these R^+ K-12 recipients was characteristic of the class IA, IB, or III clinical isolate that had been the original host of its R factor. One of these R^+ K-12 recipients, however, did have appreciable activity even though its R factor had mediated only barely detectable activity in its class III donor (25967).

DISCUSSION

Bacteria may resist the action of an antibiotic by a variety of known mechanisms which include enzymatic inactivation of the antibiotic, barriers to its entry into the bacterial cell, and alterations in the site of action of the antibiotic (3, 6, 15, 22, 30, 31, 33, 37). Each of these mechanisms may produce a different level of resistance to the antibiotic. Multiple mechanisms may also be present in a single bacterial isolate (30, 31). The usefulness of an antibiotic is probably limited by the number of existing mechanisms of resistance to it and by the prevalence these mechanisms attain in pathogenic bacteria.

A resistance mechanism or a combination of resistance mechanisms may mediate different levels of resistance to different congeners of an antibiotic (40). If the patterns of such levels of resistance were sufficiently characteristic for certain mechanisms, it should be possible to infer prevalence of such mechanisms in large scale data from routine susceptibility testing. In the results presented here, routine susceptibil-

TABLE 3. *Effect of increase in inoculum from approximately 10^2 to 10^7 colonies per ml on MIC*

Escherichia coli class	No. tested	Mean tube increase in MIC				
		Pen	Ampi	Carb	Kef	Lori
I	22	4.0	4.8	2.2	4.6	6.4
II	12	1.9	1.3	1.6	2.7	4.1
III	10	2.9	2.6	2.6	3.6	2.8
Control	11	1.6	0.9	1.5	3.0	2.0

TABLE 4. *β-lactamase activity of isolates from three classes of Escherichia coli resistant to β-lactam antibiotics*

E. coli		β-lactamase activity [U/mg (dry weight) of cells (Pen G)]	Activity (%) after:				Relative rates of hydrolysis (Pen G = 100)				
Class	Isolate no.		Induction by Pen G	Inhibition by Clox	Inactivation at 60 C (min)						
					5	60	Ampi	Carb	Clox	Kef	Lori
IA	21707	47									107
	22341	23		47			185	11	3	12	132
	22408	55	119	59	126		135	10	2	10	89
	22727	43				73	139				108
	22791	47	93			59	130	11	2	9	105
	22966	15					155	9	5	13	125
	23359	59									106
	24769	45					135				100
	25011	62									103
	25800	31									
	26982	60					139				116
	27972	18					146				108
IB	16448	179		41			161	13	1	10	108
	22017	139	111	48		71	175	10	1	10	112
	22181	131		56	95	73	147	10	2	10	104
	22972	124					143				100
	23280	500					136	9	2	10	102
	24893	237									
	25988	110	102				179	11	2	11	126
II	19850	3.9		0	0	0	2	0	0	285	139
	24993	3.1	116	0		0	0	0	0	226	109
	26562	2.0	59				32	0	0	348	142
	26860	0.3									169
	27237	2.0	111	0			4	4	2	262	111
	27609	0.4									
	27790	1.0		0							
	28004	1.4					0	0	0	299	193
	30875	0.5									
	31837	3.4		0			2	0	0	282	210
	31974	1.1					0	0	0	204	522
III	21660	<0.2									
	24388	2.4	108	91	0	0	72	48	261	40	131
	24666	1.1					56	41	170	0	0
	25560	<0.2									
	25967	<0.2									
	26530	2.8					56				22
	26918	<0.2									
	29822	0.2					0		0	241	1000
	30736	0.7		105			69	36	148	28	25

ity testing with disks of three β-lactam antibiotic congeners distinguished three classes of resistant *E. coli*. Broth dilution susceptibility testing confirmed that class I *E. coli* were highly resistant to ampicillin and carbenicillin but not to cephalothin, class II were highly resistant to ampicillin and cephalothin but not to carbenicillin, and class III were heterogeneous with mostly intermediate levels of resistance. Each of these classes was found to have characteristic resistance mechanisms.

TABLE 5. *K_m of β-lactamases from class I and II Escherichia coli*

E. coli		K_m in μM				
Class	Isolate no.	Pen G	Ampi	Carb	Kef	Lori
I	22791	57	58	20	425	1160
	22181	37	78	23	338	1050
	22017	47	78		544	1250
II	24993	26				970
	27237	39			328	1000

Class I *E. coli* had an R factor-mediated β-lactamase which was cell bound and released by osmotic shock. It was constitutive, cloxacillin inhibited, and relatively heat stable. It hydrolyzed penicillin, ampicillin, and cephaloridine at similar rates, and had a 10-fold greater affinity for penicillin G than for cephaloridine. As such it resembled the R_{TEM}-type enzyme first described by Datta and Kontomichalou (4) and was designated type IIIa by Richmond and Sykes (30) and type I by Sawai et al. (34, 35). The two levels of TEM-type β-lactamase found in *E. coli* subclasses Ia and Ib are not due to host controlled gene expression (1, 32) since each plasmid mediated its characteristic level when transferred to the same *E. coli* K-12 recipient. Hedges et al. (11) found similarly that different R factors each conferred one of two levels of TEM-type β-lactamase activity. The two levels of activity may be due to structurally different enzymes as described in clinical isolates by Sawai, et al. (35) or due to repeated sequences of the *amp* gene on the R factor genome as produced in vitro by Odakura et al. (27). It is unlikely that class Ib *E. coli* have an increased number of copies of the R factor genome per bacterium (2, 41) since their MICs for chloramphenicol and streptomycin were no higher than those of class Ia *E. coli* (27).

The release by osmotic shock of the TEM-type β-lactamase of class I *E. coli* indicates that it is located between the plasma membrane and the outer cell membrane (20, 21), in the periplasmic space as reported also by other investigators (2, 41). Within this space the β-lactamase appeared located behind a permeability barrier to penicillin G, ampicillin, and carbenicillin, because intact class I cells hydrolyzed penicillin G, ampicillin, and carbenicillin at rates that were only one-tenth those of their sonically treated extracts. This barrier appeared permeable, however, to cephalothin and cephaloridine which were hydrolyzed at similar rates by both intact cells and their sonic extracts. Chemical (9) or mutational (19) alterations in the lipopolysaccharide content of cells have been associated with changes in permeability to penicillins suggesting that the permeability barrier is probably the outer membrane.

These differences in permeability to penicillins and cephalosporins as well as differences in enzyme K_m values for them probably explain why a class I isolate is resistant to ampicillin and suceptible to cephaloridine, for example, even though its β-lactamase hydrolyzes both antibiotics at similar maximal rates. Enzymatic hydrolysis may be more effective in keeping an antibiotic below a critical lethal concentration within a cell if, as with ampicillin, the antibiotic's rate of diffusion into the cell is restricted (28, 10). High affinity of the β-lactamase for ampicillin would further facilitate inactivation of low concentrations of ampicillin in the enzyme compartment. Cephaloridine may diffuse in so rapidly that the enzyme, which also has a lower affinity for cephaloridine, cannot keep the cephaloridine level below its lethal concentration within the cell.

The lack of a permeability barrier to cephaloridine in class I *E. coli* may also explain why MICs of cephaloridine which were relatively low with small inocula (Fig. 2) rose greatly when tested with large inocula (Table 3). Both increase of number of cells and increase of antibiotic access to the β-lactamase bound within the cells promote exhaustion of antibiotic in the tube before all the cells are killed and thus allow survivors to regrow. Increase of the amount of

TABLE 6. *Comparison of β-lactamase activity of sonic extracts and intact cells for penicillins and cephalosporins*

Escherichia coli		β-lactamase activity intact cells/ sonic extract × 100					
Class	Isolate number	Pen	Ampi	Carb	Clox	Kef	Lori
IA	21707	4					95
	22341	6	5	8	23	88	77
	22408	10	6	5	42	86	95
	22727	6	2				98
	22791	4	4	4	44	112	89
	22966	14	8	3	53	88	80
	23359	6					76
	25011	3					61
	26982	7	3	3	13	33	53
	27972	11	6				93
IB	16448	11	11	13	49	57	87
	22017	5	4	4	21	81	78
	22181	2	2	2	13	69	93
	23280	3	2	2	18	33	67
	25988	4	2	2	19	60	51
II	19850	30	—[a]	—	—	77	176
	23545	53					525
	24993	59	162	—	—	173	291
	26562	107	108	—	—	148	763
	27237	118	—	—	—	218	765
	28004	65	—	—	—	214	757
	31837	31	—	—	—	109	338
III	24388	36	50	—	7	83	62
	24666	311	479	—	—	—	109
	25967	—	—	—	—	612	482
	30736	106	135	—	54	274	435

[a] (—) indicates activity insignificant.

TABLE 7. *Transfer of antibiotic resistance from wild Escherichia coli to Klebsiella 15520 and from Klebsiella 15520 R+ to Escherichia coli K-12*

Wild *E. coli*		Resistance to non-β-lactam antibiotics	Resistance transferred to *Klebsiella* 15520		Comparison of beta-lactamase activity and resistance levels of wild *E. coli* and recipient *E. coli* K-12					
					β-lactamase activity of sonic extracts [U/mg (dry weight) cells (PenG)]		MIC (μg/ml) using 10^7 col/ml			
							Ampicillin		Cephaloridine	
Class	Isolate No.		Non-β-lactam	β-lactam	Wild *E. coli*	Recipient *E. coli* K-12	Wild *E. coli*	Recipient *E. coli* K-12	Wild *E. coli*	Recipient *E. coli* K-12
IA	21707	Su--SK	+	+	47	51	250	500	250	125
	22341	SuT---	+	+	23	42			63	63
	22408	SuTCS-	+	+	55	63	16,000	8,000	500	125
	22727	SuT-S-	+	+	43	41	8,000	8,000	500	125
	24769	-T-S-	+	+	–	–				
	22791	SuTCS-	+	+	47	37	8,000	8,000	250	125
	23359	---S-	+	+	59	50			1,000	125
	25011	Su--S-	+	+	62	66			250	125
	25800	SuT-S-	+	+	31	19			125	63
	26982	SuT-S-	–	–						
	27236	-T-S-	+	+						
	27972	Su-C--	–	–						
	30455	-T-SK	+	+						
IB	16488	Su-CSK	+	+	179	139	32,000	16,000	4,000	2,000
	22017	SuT-S-	+	+	139	160	32,000	16,000	4,000	4,000
	22181	SuTCS-	+	+	131	80	16,000	16,000	2,000	250
	22972	SuT-SK	+	+						
	23280	Su--S-	+	–						
	24893	SuT-S-	–	–						
	25988	-T-S-	+	+	110	188	>32,000	16,000	4,000	2,000
II	19850	-T---	–	–						
	23545	-T---	–	–						
	24993	-T---	–	–						
	26860	SuT-S-	–	–						
	27237	SuT-SK	+	–						
	27790	SuT-S-	+	–						
	28004	SuT-SK	+	–						
	31837	SuT-SK	+	–						
III	21660	SuT---	+	+	–	–				
	24388	SuT---	+	+	2.4	2.0	4,000	1,000	250	125
	24666	SuT-S-	+	+	–	–				
	25560	SuT-SK	+	–						
	25967	SuT-S-	+	+	<0.2	1.3	125	4,000	63	250
	26530	SuT-SK	+	–						
	26918	SuT-SK	–	–						
	29822	SuT-S-	+	–						
	30736	SuT-S-	+	+	0.7	0.6	4,000	500	250	125

β-lactamase within the cell would also promote this process and may explain why the MICs of cephaloridine were greater for class Ib *E. coli* than for class Ia *E. coli* when tested with large inocula.

The β-lactamase of the class II *E. coli* isolates most resembled the type Ib enzyme of Jack and Richmond (12) in its substrate specificity and inhibition by cloxacillin. The partial inactivation of this β-lactamase by sonication, its retention by osmotically shocked cells, and its failure to transfer during mating suggest that it is a membrane-associated enzyme not located in the periplasmic space nor plasmid mediated.

Because sonication does partially inactivate this β-lactamase, the ratio of β-lactamase activity of intact cells to the activity of sonic extracts of these cells underestimates cell permeability barriers. Existence of such barriers in class II *E. coli* which could explain, in part, their resistance is suggested by the additional observation (unpublished data) that the rate of hydrolysis of penicillin by intact class II cells but not by their sonic extracts continued to increase as the penicillin concentration was increased above 2.5 mg/ml.

Isolates grouped under class III had several different patterns of resistance to ampicillin, carbenicillin, and cephalothin. They also were found to have several different β-lactamases. Three isolates (24388, 24666, 30736) had a transferable β-lactamase which hydrolyzed cloxacillin more rapidly than penicillin G. As such it resembled R_{1818}-type β-lactamase (4) (Mitsuhashi type II [7, 42] Richmond class V^{39}). Two isolates (25967, 21660) were unusual in having transferable β-lactam antibiotic resistance with barely detectable β-lactamase activity. The mechanism of this resistance is being investigated currently.

The prevalence of resistance to ampicillin and cephalothin in *E. coli* did not correlate with clinical usage of these antibiotics over the 7-year period surveyed here. Usage increased during this period as it had in an earlier 6-year period surveyed by Slocombe and Sutherland (38), but in neither period did resistance increase substantially. Possibly, β-lactam antibiotics are relatively poor selectors of resistant strains. Datta and her colleagues found in this regard that ampicillin had less effect than tetracycline in selecting fecal flora resistant to the relevant antibiotic (5, 36). It could also be that increasing β-lactam antibiotic usage has only compensated for declining usage of non-β-lactam antibiotics (e.g., chloramphenicol which was administered to 9% of patients in this hospital in 1962 and to 1% in 1970, unpublished data) in maintaining selection of these strains of *E. coli* which commonly are resistant to both β-lactam and non-β-lactam antibiotics (Table 2).

Alternatively, use of penicillin G may have preselected resistance to the newer β-lactam antibiotics before their introduction as suggested by Slocombe and Sutherland (39). The proportion of resistant strains in hospitals may have reached a level at that time that has been difficult to exceed subsequently because of the continuing large influx of susceptible *E. coli* in the flora of newly admitted patients.

The biochemical and genetic characteristics of the individual resistance mechanisms may also be determinants of their prevalence. Throughout the 7 years surveyed class I isolates of *E. coli* remained four times as prevalent as class II isolates, which showed no tendency to rise. Yet the mechanisms of class I mediate resistance to only one of the two most commonly used of these antibiotics, ampicillin, and not to the other, cephalothin, whereas those of class II mediate resistance to both antibiotics. This resistance of class II *E. coli* isolates to both ampicillin and cephalothin might have been expected to give them an advantage in surviving (8) in an environment where both antibiotics are used widely. Yet the relatively low prevalence of these class II *E. coli* suggests that some other factor, perhaps the inability of their resistance mechanisms to be transferred, has offset this apparent survival advantage. Transferable mechanisms, such as those of class I *E. coli* could have become widely distributed generally in different strains of *E. coli* (14) and could also become established in particular *E. coli* serotypes as they become predominant in hospital flora (13). Widespread resistance of *E. coli* to cephalosporin antibiotics may have to await the appearance of mechanisms of resistance to them that have greater genetic mobility.

The survival advantage of transferability, however, is not necessarily absolute. The R_{1818} type β-lactamase found in some class III isolates was R factor mediated and transferable but not prevalent. It also has the following two disadvantages previously discussed by Hedges et al. (11). They found the gene for this enzyme to be on R factors of fewer different compatibility groups than the gene for the R_{TEM}-type enzymes of class I isolates, which would tend to restrict its distribution. They also found the enzyme to be less efficient than that of R_{TEM}, hydrolyzing β-lactam antibiotics at substantially lower turnover numbers. This lower efficiency is reflected in the present study by the low rates of hydrolysis observed with this enzyme and also by the low levels of resistance it mediates, which may provide only a marginal survival advantage.

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