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# Patterns and Mechanisms of β-Lactam Resistance among Isolates of *Escherichia coli* from Hospitals in the United States

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**To study the national distribution of β-lactam resistance patterns and mechanisms among *Escherichia coli* organisms isolated in U.S. hospitals, 652 ampicillin-resistant ($Am^r$) or ampicillin-intermediate ($Am^i$) isolates were submitted to the Centers for Disease Control from March 1983 through July 1984 by nine hospitals participating in the National Nosocomial Infections Study. Among the isolates (most of which caused urinary tract infections), 78% were $Am^r$ and 22% were $Am^i$ by the interpretative criteria established by the National Committee for Clinical Laboratory Standards. Resistance to carboxypenicillins ranged from 73 to 74%, and that to acylureidopenicillins ranged from 43 to 66%. A total of 26% of the isolates were resistant to cephalothin, and 4% were resistant to cefazolin. Resistance to cefoxitin was 1%, while resistances to cefuroxime and cefamandole were 2 and 7%, respectively. With the exception of cefsulodin (98% resistant) and cefoperazone (1% resistant), there was no resistance to newer cephalosporins or aztreonam. In general, only minor differences in the incidence of resistance to β-lactam antibiotics were noted in hospital-acquired versus non-hospital-acquired isolates as well as among isolates from various regions of the United States. TEM β-lactamases were produced by 87% of the 237 $Am^r$ isolates examined. By our methods, OXA and chromosomal (type I) β-lactamases were detected in 2 and 28 isolates, respectively, and plasmid-mediated extended-spectrum cephalosporinases were detected in none of the isolates. Disk substrate and clavulanic acid inhibition assays revealed that TEM β-lactamase conferred $Am^r$ and resistance to carboxypenicillins, acylureidopenicillins, cephalothin, cefamandole, cefsulodin, and cefoperazone. A total of 391 isolates were screened for plasmids, and 259 isolates were examined by DNA hybridization with a TEM probe. Among 462 plasmids probed, 129 plasmids, ranging from 4 to 140 megadaltons, harbored TEM sequences. Although β-lactam resistance in clinical isolates of *E. coli* is predominantly mediated by TEM β-lactamase, the diverse spectrum of resistance appears to be related to additional strain-dependent factors.**

*Escherichia coli* has remained the most frequently isolated nosocomial pathogen in U.S. hospitals, accounting for approximately 18.6% of hospital-acquired infections (5).This species is the most frequently reported pathogen from adult services, including medicine, gynecology, obstetrics, and surgery (5). From January 1985 through August 1988, *E. coli* organisms were associated with 26.7% of urinary tract infections, 6.4% of pneumonias, 6.8% of bloodstream infections, 7.3% of infections among patients in intensive care, and 9.8% of surgical wound infections (T. Horan, D. Culver, W. Jarvis, G. Emori, S. Banerjee, W. Martone, and C. Thornsberry, Antimicrob. Newsl. 5:65–68, 1988). Treatment of *E. coli* infections is usually empirical, often varying with the site of infection, and a variety of compounds, including β-lactam antibiotics, are used. Several genera of gram-negative bacilli, including *E. coli*, innately produce low basal amounts of a chromosomally encoded (type I) β-lactamase (1). Especially when they are hyperproduced (for example, through the effects of "up" promoter mutations or gene repetitions), these type I enzymes may confer resistance to clinically used compounds such as cefamandole or cefuroxime (1, 19). Most resistance to the β-lactam antibiotics that are used for the treatment of *E. coli* infections, however, is conferred by TEM-1, a plasmid-encoded constitutive broad-spectrum β-lactamase. Alterations in permeability of the outer membrane to some β-lactams as well as the level of enzyme production may affect this expression of resistance (7, 9, 10). A strain-specific increase in accumulation of TEM-1 in the periplasm, which is related to hyperproduction, may likewise diminish the activity of some β-lactam compounds (22). Just as important are changes (point mutations) in β-lactamase structural genes that have generated enzyme variants with extended substrate profiles, some of which include newer cephalosporins such as cefotaxime and ceftazidime (13, 24, 26). Although these variants may usually be differentiated from the parent enzymes (e.g., TEM-1, TEM-2, and SHV-1) on the basis of isoelectric point determinations, their genes share extensive nucleotide sequence homology with parent sequences, preventing their distinction with restriction fragment probes. Newer probes derived from synthetic oligonucleotides are proving useful, however, for distinguishing minor nucleotide differences among these β-lactamase genes (3; C. Mabilat, I. Guilvout, and P. Courvalin, Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 667, 1989).

We evaluated β-lactamase-mediated resistance among clinical isolates of *E. coli* obtained over a 2-year period (1983 through 1984) from nine geographically diverse U.S. hospitals participating in the National Nosocomial Infections Study. In addition to surveying for the presence of novel broad-spectrum β-lactamases among these isolates, our goal was to characterize β-lactamases and their encoding plas-

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mids and to relate these enzymes to β-lactam susceptibility patterns.

## MATERIALS AND METHODS

**Bacterial isolates.** Isolates of *E. coli* (one from each patient) were received from March 1983 through July 1984 from nine U.S. hospitals participating in the National Nosocomial Infections Study from the following geographic areas of the United States: northeast, 101 isolates; south, 199; west, 168; and north central, 204. Patient status included 581 inpatients, 68 outpatients, 20 emergency room patients, and 3 patients of unreported status. Approximately 78% of the isolates were obtained from urine cultures, 334 of 529 (63%) of the reported isolates were diagnosed as agents of infection, and 27.6% were hospital-acquired isolates. We requested only ampicillin-resistant ($Am^r$) isolates, and upon receipt all isolates were evaluated for purity and reidentified by using Micro Scan Combo plates (American Micro Scan, Campbell, Calif.). In vitro testing of susceptibility to 29 antimicrobial agents, including 20 β-lactam antibiotics, was performed by disk agar diffusion and broth microdilution methods according to the standards of the National Committee for Clinical Laboratory Standards (17, 18). The compounds tested, along with intermediate disk zones of inhibition and resistance breakpoints for MICs, are given in Table 1. Qualitative testing for β-lactamase production was performed in microdilution plate wells by using a chromogenic cephalosporin (nitrocefin).

**β-Lactamase characterization.** β-Lactamase was extracted from cells that were grown overnight at 37°C with shaking in 50 ml of brain heart infusion broth. Duplicate cultures contained a β-lactamase inducer (0.12 μg of cefoxitin per ml or 50 to 100 μg of penicillin G per ml). Cells were harvested by centrifugation, washed in 0.1 M $PO_4$ (pH 7), suspended in 6 ml of deionized HOH, and sonicated for 2 min under conditions of optimum cooling. Cellular debris was removed by centrifugation, and supernatants were stored in 1.0-ml portions at −20°C. β-Lactamase activity was estimated by reacting 50 μl of serial dilutions of sonic extracts with an equal volume of nitrocefin (500 μg/ml of stock solution) in microdilution plate wells and recording the highest titer that was capable of strongly hydrolyzing the substrate after incubation for 15 min at 25°C. Additional treatments of sonic extracts included concentration (5 or 10 times) with concentrators (Minicon; Amicon Corp., Danvers, Mass.), removal of salt and low-molecular-weight proteins by using prepacked Sephadex G-25 columns (PD-10 column; Pharmacia LKB Biotechnology, Inc., Piscataway, N.J.), or both, according to the instructions of the manufacturers.

β-Lactamase pIs were determined by analytical isoelectric focusing of sonic extracts (30 μl) applied to filter paper strips (5 by 10 mm) that were placed on the surface of commercially prepared gels (PAGplates; Pharmacia LKB Biotechnology, Inc.) and electrophoresed by the conditions recommended by the manufacturer. Samples were initially screened on broad-range gels (pH ranges, 3.0 to 9.5) to detect multiple enzymes if they were present in the same sample. Enzymes that focused within the range for TEM β-lactamases were then retested on narrow-range gels (pH 4.0 to 6.5) to better differentiate between the TEM isoenzymes. The following four β-lactamase control sonic extracts were routinely included: TEM-1 (pI 5.4), TEM-2 (pI 5.6), OXA-1 (pI 7.4), and PSE-4 (pI 5.3) (4). β-Lactamases were detected in the gels by the agar-nitrocefin overlay method of Sanders et al. (23). All samples were tested in duplicate to confirm the pIs that were observed.

Semiquantitative enzyme substrate patterns were evaluated by using a modification of the disk method of Masuda et al. (14). A strain of *Sarcina lutea* (ATCC 9641), which is susceptible to β-lactam antibiotics, was inoculated into Mueller-Hinton agar to a final inoculum of $10^6$ CFU/ml and poured into tissue culture plates (25 by 25 cm). After the agar solidified, drug disks were placed on the agar surface, and after overnight incubation, zones of inhibition were recorded. The procedure was then repeated except that, after seeding and placing drug disks, 6-mm-diameter paper disks impregnated with 25 μl of sonic extract (endpoint titer) were placed near the drug disks at the edge of the expected radius of inhibition. The extent of drug inactivation was judged visually by truncation of the normal zone of inhibition as 1+ through 4+.

β-Lactamase inhibition was evaluated by disk agar diffusion as described above, except that 20 μl of clavulanic acid (1-mg/ml freshly prepared stock solution) was spotted onto the drug disks after they were placed on the agar. Competitive substrate inhibition was performed by preincubating sonic extracts (endpoint titer) with a battery of 11 β-lactam compounds (400-μg/ml stock solutions) for 10 min at 25°C in microdilution plate wells and then adding nitrocefin (500 μg/ml). The volume of each reagent added was 50 μl. After an additional incubation of 10 min, wells that contained β-lactams that inhibited hydrolysis of nitrocefin were recorded.

Genetic probes to detect nucleotide sequences homologous to the TEM β-lactamase structural gene included a 1-kilobase-pair (kb) *Eco*RI-*Hin*fI fragment of plasmid pBR322 and a 0.42-kb *Bgl*I-*Hin*cII fragment of the same plasmid prepared as described previously (4, 8). Representative isolates were examined with both probes to evaluate the probes for TEM specificity, especially considering that the former fragment contains promoter sequences external to the structural gene. Restriction fragment probes were labeled with $^{32}P$ by random primer synthesis or nick translation (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). Target nucleic acids were prepared by the method of Birnboim and Doly (2), boiled for 3 min, and applied to the surface of charged nylon membranes (Biotrace RP; Gelman Sciences, Inc., Ann Arbor, Mich.) by using a slot blot vacuum apparatus (Minifold II; Schleicher & Schuell, Inc., Keene, N.H.). The lysates were also subjected to electrophoresis in vertical agarose-Tris-borate gels and electroblotted as described previously (4). Blots were hybridized with denatured probes under conditions of high stringency as described previously (4). Slot blot hybridizations served to screen for the presence of probe-homologous sequences among the isolates, and gel blot hybridizations were performed to localize the sequences to specific genetic elements.

## RESULTS

**Antimicrobial susceptibilities.** Among the 672 nosocomial isolates of *E. coli* submitted, 20 isolates (3%) were determined to be susceptible to ampicillin and were discarded (Table 1). Among the remaining 652 isolates, 511 (78%) were $Am^r$, and 141 (22%) were intermediate ($Am^i$). Resistances to carbenicillin and ticarcillin were 73 and 74%, respectively.

Resistance to acylureidopenicillins was 43% for the isolates, with the exception of azlocillin (66% of the isolates were resistant). Resistances to cefazolin and cephalothin were 4 and 26%, respectively. Susceptibility to cefamandole was 86%, that to cefuroxime was 94%, and that to cefoxitin

TABLE 1. Antimicrobial susceptibility patterns of ampicillin-resistant *E. coli* isolates from nine U.S. hospitals

| Drug | Breakpoint[a] | | No. of isolates tested | Percent[b]: | | | % Resistance of isolates from the following sources[c]: | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Zone (mm) | MIC (μg/ml) | | r | i | s | In | Out | HA | CA |
| Ampicillin | 12–13 | ≥32 | 652 | 78 | 22 | 0 | 75 | 87 | 76 (54) | 72 (79) |
| Carbenicillin | 18–22 | ≥32 | 652 | 73 | 2 | 25 | 70 | 84 | 71 (46) | 69 (74) |
| Ticarcillin | 2–14 | ≥128 | 652 | 74 | 1 | 25 | 71 | 84 | 71 (50) | 69 (79) |
| Piperacillin | 15–17 | ≥128 | 652 | 43 | 21 | 36 | 43 | 35 | 39 (29) | 40 (52) |
| Mezlocillin | 13–15 | ≥256 | 652 | 43 | 21 | 36 | 41 | 41 | 37 (21) | 38 (48) |
| Azlocillin | 15–17 | ≥256 | 652 | 66 | 12 | 22 | 63 | 75 | 65 (46) | 58 (67) |
| Cefazolin | 15–17 | ≥32 | 517 | 4 | 4 | 92 | 4 | 0 | 2 (0) | 1 (0) |
| Cephalothin | 15–17 | ≥32 | 652 | 26 | 27 | 47 | 26 | 16 | 32 (33) | 19 (21) |
| Cefoxitin | 15–17 | ≥32 | 652 | 1 | 3 | 96 | 1 | 0 | 2 (0) | 0 (0) |
| Cefuroxime | 15–17 | ≥32 | 652 | 2 | 4 | 94 | 2 | 1 | 5 (0) | 1 (0) |
| Cefamandole | 15–17 | ≥32 | 652 | 7 | 7 | 86 | 7 | 3 | 5 (8) | 5 (7) |
| Cefmenoxime | ?[d] | ? | 652 | 0 | 0 | 100 | 0 | 0 | 0 (0) | 0 (0) |
| Cefotaxime | 15–22 | ≥64 | 652 | 0 | 0 | 100 | 0 | 0 | 0 (0) | 0 (0) |
| Ceftizoxime | 15–19 | ≥32 | 652 | 0 | 0 | 100 | 0 | 0 | 0 (0) | 0 (0) |
| Ceftriaxone | 14–20 | ≥64 | 519 | 0 | 0 | 100 | 0 | 0 | 0 (0) | 0 (0) |
| Ceftazidime | 15–17 | ≥32 | 652 | 0 | 0 | 100 | 0 | 0 | 0 (0) | 0 (0) |
| Moxalactam | 15–22 | ≥64 | 652 | 0 | 0 | 100 | 0 | 0 | 0 (0) | 0 (0) |
| Cefoperazone | 16–20 | ≥64 | 652 | 1 | 2 | 96 | 1 | 0 | 0 (4) | 0 (0) |
| Cefsulodin | ? | ≥32 | 518 | 98 | 1 | 0 | 98 | 100 | 99 (95) | 97 (100) |
| Aztreonam | 16–21 | ≥32 | 123 | 0 | 0 | 100 | 0 | 0 | 0 (0) | 0 (0) |
| Kanamycin | 14–17 | ≥25 | 141 | 23 | 23[e] | 77 | 22 | 23 | 50 (0) | 16 (25) |
| Gentamicin | 13–14 | ≥8 | 652 | 0 | 3 | 97 | 1 | 0 | 1 (0) | 0 (0) |
| Tobramycin | 13–14 | ≥8 | 652 | 0 | 4 | 96 | 1 | 0 | 1 (0) | 0 (0) |
| Amikacin | 15–16 | ≥32 | 652 | 0 | 0 | 100 | 0 | 0 | 0 (0) | 0 (0) |
| Netilmicin | 13–14 | ≥32 | 652 | 0 | 0 | 100 | 0 | 0 | 0 (0) | 0 (0) |
| Chloramphenicol | 13–17 | ≥25 | 652 | 21 | 21[e] | 78 | 21 | 18 | 21 (29) | 18 (26) |
| Sulfamethoxazole-trimethoprim | 11–15 | ≥8/152 | 652 | 8 | 1 | 92 | 8 | 4 | 8 (13) | 5 (17) |
| Tetracycline | 15–18 | ≥16 | 652 | 0 | 40 | 60 | 0 | 0 | 0 | 0 |

[a] National Committee for Clinical Laboratory Standards standard zone diameters for intermediate or moderately susceptible isolates; MIC is a correlate for the zone sizes of resistant isolates.

[b] s, Susceptible; i, intermediate; r, resistant by disk diffusion.

[c] Data were not available for all study isolates. Abbreviations: In, inpatient; out, outpatient; HA, hospital acquired; CA, community acquired. Values in parentheses are percent resistant isolates among colonized patients.

[d] ?, Unknown.

[e] Resistant and intermediate values are reported as single percentages.

was 96%. With the exceptions of cefsulodin (98% resistant and 2% intermediate) and cefoperazone (1% resistant and 2% intermediate), the isolates were susceptible to the other cephalosporins tested and to aztreonam. All 652 isolates were susceptible to amikacin and netilmicin, 96% were susceptible to tobramycin, and 97% were susceptible to gentamicin; and 77% of 141 isolates tested were susceptible to kanamycin. From a previously tested sample (423 isolates) of these isolates, aminoglycoside-modifying enzymes were detected in 19 of 21 isolates that were found to be resistant or intermediate to at least one aminoglycoside (6). A total of 78% of the isolates were susceptible to chloramphenicol, 92% were susceptible to sulfamethoxazole-trimethoprim, and 60% were susceptible to tetracycline (40% were intermediate).

Resistance to penicillin-type compounds tended to be more common overall among outpatients than among inpatients and among hospital-acquired isolates compared with non-hospital-acquired isolates. The greatest difference was observed for resistance of the isolates to carboxypenicillins (84% resistance for isolates from outpatients versus 71 to 72% resistance for isolates from inpatients). The exceptions were piperacillin and mezlocillin, for which differences by these comparisons were only minor. Colonized, compared with infected, patients, however, more often yielded isolates that were resistant to penicillins when the isolates were non-hospital acquired. Geographic differences for the penicillins were also minor (data not shown). Inpatient and hospital-acquired isolates were generally more often resistant to the older cephalosporins, and although isolates from western and northeastern U.S. hospitals more often tended to be resistant to older cephalosporins, the overall differences appeared to be minor.

**β-Lactamase characterization.** β-Lactamase was detected in all isolates that were $Am^r$ or $Am^i$, and enzymes were characterized in 237 isolates (Table 2). Isolates were chosen at random, except that the representation of hospital-acquired and non-hospital-acquired isolates was approximately equal and that representatives of those isolates with unusual resistance to cephalosporins were included. Although at least 42 patterns of susceptibilities to the β-lactam antibiotics were observed, the most common patterns were represented among the 237 isolates that were characterized further. TEM was the only β-lactamase detected in 207 isolates (87.3%) representing 20 patterns. By disk substrate testing, sonic extracts containing TEM characteristically demonstrated strong inactivation (3+ to 4+) of ampicillin, carbenicillin, ticarcillin, piperacillin, mezlocillin, azlocillin, cephalothin, cefazolin, and cefamandole and weak inactivation (1+ to 2+) of cefoperazone, cefoxitin, and cefuroxime. No activity against other, newer cephalosporins was detected. By competitive substrate inhibition testing, nitrocefin hydrolysis by

TABLE 2. β-Lactam resistance patterns and β-lactamase types among 237 randomly selected study Am$^r$ *E. coli* isolates

| Code | Resistance pattern[a] | | | | | | | | | | | | | β-Lactamase | | Other β-lactamases obtained (no.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Am | Cb | Tic | Pip | Mz | Az | Cfs | Cf | Ma | Cz | Cfp | Fox | Cxm | No. tested | No. TEM-1 | |
| 1 | + | + | + | + | + | + | + | | | | | | | 24 | 22 | TEM-2 (2) |
| 2 | + | + | + | + | i | + | + | | | | | | | 7 | 7 | |
| 3 | + | + | + | i | i | + | + | | | | | | | 22 | 21 | OXA-1 (1) |
| 4 | + | + | + | + | i | i | + | | | | | | | 2 | 2 | |
| 5 | + | + | + | i | i | i | + | | | | | | | 2 | 2 | |
| 6 | + | + | i | + | + | i | + | | | | | | | 3 | 3 | |
| 7 | + | + | + | | + | + | + | | | | | | | 11 | 11 | |
| 8 | + | + | + | i | | + | + | | | | | | | 6 | 6 | |
| 9 | + | + | + | | i | + | + | | | | | | | 3 | 3 | |
| 10 | + | + | + | | | i | | | | | | | | 14 | 14 | |
| 11 | + | + | + | | | + | | | | | | | | 4 | 4 | |
| 12 | + | + | + | + | + | + | + | i | | | | | | 35 | 35 | |
| 13 | + | + | + | + | i | + | + | i | | | | | | 9 | 9 | |
| 14 | + | + | + | i | i | + | + | i | | | | | | 10 | 9 | OXA-2 (1) |
| 15 | + | + | + | + | + | + | + | + | | | | | | 9 | 9 | |
| 16 | + | + | + | i | i | + | + | + | | | | | | 4 | 4 | |
| 17 | + | | | | | i | + | + | | | | | | 3 | 0 | Type I (3) |
| 18 | + | + | + | + | + | + | + | + | + | | | | | 7 | 7 | |
| 19 | + | + | + | + | + | + | + | + | i | | | | | 16 | 16 | |
| 20 | + | + | + | + | + | + | + | i | i | | | | | 5 | 5 | |
| 21 | + | + | + | + | + | + | + | + | + | i | | | | 7 | 7 | |
| 22 | + | + | + | + | + | + | + | + | + | + | | | | 2 | 2 | |
| 23 | + | + | + | + | + | + | + | + | + | + | + | | | 7 | 7 | |
| 24 | + | + | + | + | + | + | + | + | | + | | + | + | 2 | 0 | Type I (2) |
| 25 | + | + | + | i | i | + | + | + | + | + | | + | + | 6 | 0 | Type I (6) |
| 26 | + | | i | | | + | + | | | | | + | + | 2 | 0 | Type I (2) |
| 27 | + | | | | | | + | | | | | | | 15 | 0 | Type I (15) |
| Total | | | | | | | | | | | | | | 237 | 205 (86.5%) | 32 (13.5%) |

[a] +, Resistant; i, intermediate; determined by the breakpoints shown in Table 1. Abbreviations: Am, ampicillin; Cb, carbenicillin, Tic, ticarcillin; Pip, piperacillin; Mz, mezlocillin; Az, azlocillin; Cfs, cefsulodin; Cf, cephalothin; Ma, cefamandole; Cz, cefazolin; Cfp, cefoperazone; Fox, cefoxitin; Cxm, cefuroxime.

the TEM β-lactamase was competitively inhibited by clavulanic acid and methicillin; weakly inhibited by cloxacillin, oxacillin, and carbenicillin; and uninhibited by cefamandole, cefazolin, cefoperazone, cefotaxime, moxalactam, cefmenoxime, or ceftizoxime. TEM-mediated resistance was further confirmed for 41 isolates (representing 20 β-lactam resistance patterns) by testing the ability of clavulanic acid to reverse disk agar diffusion resistance patterns. By incorporating 20 μg of clavulanic acid into β-lactam disks, zones of resistance and intermediate resistance were consistently enlarged to susceptible zones for ampicillin, carbenicillin, ticarcillin, piperacillin, mezlocillin, azlocillin, cefsulodin, cephalothin, cefamandole, cefazolin, and cefoperazone.

β-Lactam susceptibility patterns for 205 TEM-producing isolates are shown in Table 3. All of these isolates were resistant to ampicillin, carbenicillin, and cefsulodin, and none of the isolates was resistant or intermediate to cefoxitin and cefuroxime. Although none of the isolates was susceptible to azlocillin, 10% were intermediate. Susceptibilities of the isolates to the remaining β-lactam compounds tested ranged from 15.6% to piperacillin to 96.6% to cefoperazone.

By isoelectric focusing of TEM-containing sonic extracts, 2 β-lactamases were determined to be TEM-2 and 205 were determined to be TEM-1 on the basis of a pI of 5.6 for TEM-2 and a pI of 5.4 for TEM-1. Other β-lactamases detected among the 237 isolates were OXA-1 and OXA-2, each of which was produced by a single isolate with a resistance pattern that was identical to the TEM patterns (Table 2). The pIs of these enzymes were 7.4 (OXA-1) and 7.7 (OXA-2). The remaining 28 isolates in which only a type I β-lactamase was detected represented five resistance patterns that were distinct from the TEM patterns. These enzymes were detectable (by nitrocefin hydrolysis) only when cells were grown in the presence of penicillin G followed by concentration of the lysates. When the enzymes were detectable, their pIs varied between 8.4 and 9.0. Their hydrolytic activities against nitrocefin were competitively inhibited by cloxacillin, cefotaxime, moxalactam, cefmenoxime, methicillin, oxacillin, carbenicillin, and usually cefamandole and ceftizoxime, but not by cefazolin or clavulanic acid.

Slot blots containing the 207 isolates that produced only

TABLE 3. β-Lactam susceptibility patterns of 205 *E. coli* producing TEM-1[a]

| Susceptibility category | No. of isolates | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Am | Cb | Tic | Pip | Mz | Az | Cfs | Cf | Ma | Cz | Cfp | Fox | Cxm |
| Resistant | 205 | 205 | 202 | 131 | 126 | 184 | 205 | 52 | 23 | 9 | 7 | 0 | 0 |
| Intermediate | 0 | 0 | 3 | 42 | 55 | 21 | 0 | 58 | 21 | 7 | 0 | 0 | 0 |
| Susceptible | 0 | 0 | 0 | 32 | 24 | 0 | 0 | 95 | 161 | 189 | 198 | 205 | 205 |

[a] Drug abbreviations are as given in footnote *a* of Table 2, and breakpoints are as given in Table 1.

TABLE 4. Hybridization of TEM β-lactamase probe with plasmids and chromosomes of 259 isolates selected from 391 Am$^r$ *E. coli* isolates examined for plasmids

| Plasmid size (MDa) | No. (%) of: | | |
|---|---|---|---|
| | Isolates with plasmid[a] | Plasmids probed[b] | Plasmids hybridizing with TEM[c] |
| <4 | 383 (97.9) | 130 | 36 (27.6) |
| 5–8 | 67 (17.1) | 46 | 12 (26.1) |
| 9–12 | 25 (6.4) | 19 | 4 (21) |
| 13–16 | 25 (6.4) | 17 | 3 (17.6) |
| 17–20 | 24 (6.1) | 13 | 3 (23.1) |
| 21–24 | 24 (6.1) | 17 | 9 (53.9) |
| 25–28 | 35 (8.9) | 14 | 7 (50) |
| 29–32 | 23 (5.9) | 12 | 4 (33.3) |
| 33–36 | 33 (8.4) | 29 | 14 (48.3) |
| 37–40 | 21 (5.4) | 13 | 6 (46.1) |
| 41–45 | 22 (5.6) | 12 | 2 (16.7) |
| 46–50 | 20 (5.1) | 14 | 4 (28.6) |
| 51–60 | 58 (14.8) | 36 | 12 (33.3) |
| 61–70 | 60 (15.3) | 42 | 13 (30.9) |
| 71–80 | 30 (7.7) | 15 | 5 (33.3) |
| 81–90 | 21 (5.4) | 11 | 1 (9) |
| 91–100 | 17 (4.3) | 8 | 1 (13) |
| 101–120 | 27 (6.9) | 8 | 2 (25) |
| 121–140 | 10 (2.5) | 5 | 1 (20) |
| >140 | 3 (0.8) | 1 | 0 (0) |
| Total (<4 to >140) | 928 | 462 | 129 (27.9) |
| Chromosome | 391 | 259 | 35 (13.5) |

[a] Number of isolates with greater than or equal to one plasmid in the indicated size range; alternate forms of a common plasmid (e.g., open circles or multimers) are not discerned.

[b] Greater than or equal to one plasmid per isolate may be included.

[c] The TEM β-lactamase probe was a 998-base-pair *Eco*RI-*Hin*fI fragment of plasmid pBR322 labeled with $^{32}$P by nick translation and hybridized with the plasmids of Birnboim and Doly (2) that were electroblotted from agarose to charged nylon.

TEM and 2 of 28 type I β-lactamase producers (resistance patterns 26 and 27; Table 2) hybridized with the $^{32}$P-labeled, 1-kb TEM probe. None of the remaining 28 isolates, including the OXA-1- or OXA-2-producing isolates, hybridized with this probe. Confirmation of the presence of TEM sequences in the type I organisms was obtained by repeating the hybridization with the 0.42-kb probe bearing only sequences internal to the TEM structural gene.

A total of 391 randomly selected isolates were examined for plasmids (Table 4). Plasmid gel electroblots of 259 of these isolates, including 147 isolates representing the resistance patterns shown in Table 2, were hybridized with the 1-kb TEM probe. By comparison with the plasmid size standards that were included in the gels, plasmid band sizes ranging from <4 to >140 megadaltons (MDa) were observed. All isolates contained at least one plasmid band. The most frequently occurring plasmids were in the size ranges of <4 MDa (97.9% of isolates) and 51 to 70 MDa. Chromosomal and plasmid bands from 259 isolates were electroblotted, and 129 of 462 plasmid bands (27.9%) hybridized with the 1-kb TEM probe. Although TEM$^+$ plasmids were diverse in size, the highest frequencies of hybridization were for those between 20 and 40 MDa. Hybridization of the probe to chromosomes only occurred in 14 isolates (5.4%) (all harboring greater than or equal to one plasmid), including both TEM$^+$ type I β-lactamase producers. Although TEM$^+$ plasmids of any specific sizes did not appear to be pervasive among isolates from any one hospital, eight pairs of isolates (each pair having consecutive identification numbers, but each isolate in the pair being from a different patient) from four hospitals were observed to harbor TEM plasmids of a common size, but the sizes were different for each pair (Table 5). Isolates from five of these pairs were from specimens cultured on the same day, and five pairs each had matching β-lactam resistance patterns; and although information regarding patient location within each hospital was unknown, isolates from two pairs differed in that one isolate from each pair was hospital acquired.

TABLE 5. TEM β-lactamase plasmids of common sizes in consecutive nosocomial isolates of *E. coli* from different patients

| Hospital | Date of culture (mo/day/yr) | Source | Plasmid size (MDa) | Hospital-acquired | Infection | β-Lactam pattern |
|---|---|---|---|---|---|---|
| A | 1/8/83 | Urine | 74 | + | + | 21 |
| A | 1/8/83 | Urine | 74 | − | + | 19 |
| A | 3/28/83 | Stool | 55 | − | − | 13 |
| A | 3/28/83 | Stool | 55 | − | − | 13 |
| B | 3/31/83 | Urine | 101 | − | + | 11 |
| B | 4/2/83 | Blood | 101 | − | + | 9 |
| C | 3/16/83 | Urine | 4 | − | + | 1 |
| C | 3/16/83 | Blood | 4 | − | + | 1 |
| C | 4/9/83 | Urine | 62 | − | + | 19 |
| C | 4/11/83 | Urine | 62 | + | + | 19 |
| D | 3/15/83 | Urine | 5.6 | ?[a] | ? | 12 |
| D | 3/15/83 | Urine | 5.6 | ? | ? | 12 |
| D | 3/29/83 | Urine | 140 | ? | ? | 3 |
| D | 3/29/83 | Urine | 140 | ? | ? | 3 |
| D | 3/23/83 | Urine | 46 | ? | ? | 3 |
| D | 3/29/83 | Urine | 46 | ? | ? | 12 |

[a] ?, Unknown.

## DISCUSSION

Previous reports have established that the most frequently encountered plasmid-mediated β-lactamase in clinical isolates of *E. coli* is TEM-1 (15, 16, 21). In 1979, Matthew (15) reported this frequency to be 71.4% followed by 11.9% for OXA-1 among 84 isolates from 10 countries. In 1986, Roy et al. (21) found that TEM-1 accounted for 188 of 192 (98%) *E. coli* organisms that produced only one plasmid-mediated β-lactamase. Findings of several investigators from 1980 through 1987 were reviewed in 1989 by Medeiros (16) and revealed that, among 847 Am$^r$ *E. coli* organisms from five continents (at least 12 countries, including the United States), 77% produced TEM-1 as the only plasmid-mediated β-lactamase, followed by 6% for OXA-1 and 4% for SHV-1. A total of 9% of the isolates produced only chromosomal β-lactamase, and no more than 1% of the isolates produced other plasmid-mediated or multiple β-lactamases (16). Our data indicate that in 1983 and 1984, TEM-1 was the predominant β-lactamase produced by *E. coli* isolated within nine geographically distinct hospitals in the United States, accounting for 86.5% of detectable enzymes, and all were detected in Am$^r$ isolates. Multiple enzymes, which were previously reported in this species (15, 16, 25), were not detected in any of our isolates. The second most common enzyme detected among these isolates was type I cephalo-

sporinase in 28 (13.6%) isolates, 15 of which were $Am^i$. Considering that the *ampC* gene, which encodes type I β-lactamase, is ubiquitous in the *E. coli* chromosome, it is unclear why no type I enzymes could be detected among isolates that produced TEM or OXA enzymes. Only two each of OXA and TEM-2 enzymes and no plasmid-mediated extended-spectrum cephalosporinases were produced by the isolates. Considering the extensive distribution of TEM among U.S. hospitals and that several of the novel plasmid-mediated cephalosporinases are derived from TEM (26), the emergence of these novel enzymes among isolates in the United States may be predicted. Clusters of isolates of the family *Enterobacteriaceae* harboring these apparently novel enzymes have, in fact, been reported in several countries, including the United States (12, 20, 24; L. B. Rice, S. H. Willey, A. A. Medeiros, G. M. Eliopoulos, R. C. Moellering, Jr., and G. A. Jacoby. Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, A-64, p. 11; A. A. Medeiros, A. Bauernfeind, G. Papanicolaou, R. S. Hare, E. Papa, and G. Miller, 29th ICAAC, abstr. no. 670, 1989).

Despite the predominance of TEM-1 among our isolates, diverse β-lactam susceptibility patterns were observed. Although all TEM-1-producing isolates were resistant to ampicillin, carbenicillin, and cefsulodin and were susceptible to cefoxitin and cefuroxime, isolates in all three interpretative categories were found for the other β-lactams tested (except for cefoperazone, for which none of the isolates were intermediate). There were no differences in substrate patterns, however, when crude extracts containing β-lactamases with approximately equal activities against nitrocefin were tested. In conjunction, these observations support the interplay of multiple mechanisms that affect in vitro susceptibilities to some β-lactams. The highly variable patterns may be due to the amount of TEM-1 produced, strain-specific permeability, or both. Further studies of these factors as they relate to diverse phenotypic susceptibility patterns are warranted.

Also, in support of previous findings, the 1-kb probe for TEM β-lactamase has proven to be specific and sensitive for the detection of TEM-producing organisms (8, 11). This probe was also useful in demonstrating the diversity of TEM plasmids among the *E. coli* isolates. TEM plasmid bands varied in size, as did the number of TEM-positive bands per organism, among the isolates. Since the TEM gene is often associated with a transposon, the diversity in size of TEM plasmids and the frequency of chromosomal hybridization with the probe may not be surprising.

It was further apparent that there were no clusters of isolates from any hospital that shared TEM plasmids of a common size range. Exceptions may be the eight pairs of isolates (from patients having consecutive cultures numbers) shown in Table 5, each of which harbored TEM plasmids that were approximately equal in size. It is not known whether these plasmids were conjugally shared between isolates in each pair or whether one or more of these instances represent clonal acquisition of a common strain. Alternatively, the plasmids may have been unrelated except for their size and TEM homology. The observation that only one isolate from each of two pairs was hospital acquired may nonetheless emphasize the potential for community-acquired isolates to introduce resistance plasmids into hospital environments.

## LITERATURE CITED


1. **Bergstrom, S., and S. Normark.** 1979. β-Lactam resistance in clinical isolates of *Escherichia coli* caused by elevated production of the *ampC*-mediated chromosomal β-lactamase. Antimicrob. Agents Chemother. **16**:427–433.
2. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
3. **Boissinot, M., J. Mercier, and R. C. Levesque.** 1987. Development of natural and synthetic DNA probes for OXA-2 and TEM-1 β-lactamases. Antimicrob. Agents Chemother. **31**:728–734.
4. **Cooksey, R. C., N. C. Clark, and C. Thornsberry.** 1985. A gene probe for TEM type β-lactamases. Antimicrob. Agents Chemother. **28**:154–156.
5. **Crowley, J. R., B. H. Edwards, and J. C. Mellinger.** 1986. Microorganisms in hospital acquired infections. J. Med. Technol. **3**:214–217.
6. **Gaynes, R. P., R. Cooksey, C. Thornsberry, J. M. Swenson, and J. M. Hughes.** 1987. Mechanisms of aminoglycoside resistance among β-lactam-resistant *Escherichia coli* in the United States. Diagn. Microbiol. Infect. Dis. **7**:45–50.
7. **Hiraoka, M., R. Okamoto, M. Inoue, and S. Mitsuhashi.** 1989. Effects of β-lactamases and *omp* mutation on susceptibility to β-lactam antibiotics in *Escherichia coli*. Antimicrob. Agents Chemother. **33**:382–386.
8. **Huovinen, S., P. Huovinen, and G. A. Jacoby.** 1988. Detection of plasmid-mediated β-lactamases with DNA probes. Antimicrob. Agents Chemother. **32**:175–179.
9. **Huovinen, S., P. Huovinen, K. Torniainen, and G. A. Jacoby.** 1988. Evaluation of plasmid-encoded beta-lactamase resistance in *Escherichia coli* blood culture isolates. Eur. J. Clin. Microbiol. Infect. Dis. **7**:651–655.
10. **Jacoby, G. A., and L. Sutton.** 1985. β-Lactamases and β-lactam resistance in *Escherichia coli*. Antimicrob. Agents Chemother. **28**:703–705.
11. **Jouvenot, M., M. L. Deschaseaux, M. Royez, C. Mougin, R. C. Cooksey, Y. Michel-Briand, and G. L. Adessi.** 1987. Molecular hybridization versus isoelectric focusing to determine TEM-type β-lactamases in gram-negative bacteria. Antimicrob. Agents Chemother. **31**:300–305.
12. **Kitzis, M. D., D. Billot-Klein, F. W. Goldstein, R. Williamson, G. Tran Van Nhieu, J. Carlet, J. F. Acar, and L. Gutmann.** 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.
13. **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.
14. **Masuda, G., S. Tomioka, and M. Hasegawa.** 1976. Detection of β-lactamase production by gram-negative bacteria. J. Antibiot. **29**:662–664.
15. **Matthew, M.** 1979. Plasmid-mediated β-lactamases of gram-negative bacteria: properties and distribution. J. Antimicrob. Chemother. **5**:349–358.
16. **Medeiros, A. A.** 1989. Plasmid-determined beta-lactamases, p. 101–127. *In* L. E. Bryan (ed.), Microbial resistance to drugs. Handbook of experimental pharmacology, vol. 91. Springer-Verlag KG, Berlin.
17. **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.
18. **National Committee for Clinical Laboratory Standards.** 1985. Dilution procedures for susceptibility testing of aerobic bacteria. Approved standard M7-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
19. **Normark, S., T. Edlund, T. Grundstrom, S. Bergstrom, and H. Wolf-Watz.** 1977. *Escherichia coli* K-12 mutants hyperproducing chromosomal beta-lactamase by gene repetitions. J. Bacteriol. **132**:912–922.
20. **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.

21. **Roy, C., C. Segura, M. Tirado, R. Reig, M. Hermida, D. Teruel, and A. Foz.** 1986. Frequency of plasmid-determined β-lactamases in 680 consecutively isolated strains of *Enterobacteriaceae*. Eur. J. Clin. Microbiol. **4**:146–147.
22. **Sanders, C. C., J. P. Iaconis, G. P. Bodey, and G. Samonis.** 1988. Resistance to ticarcillin-potassium clavulanate among clinical isolates of the family *Enterobacteriaceae*: role of PSE-1 β-lactamase and high levels of TEM-1 and SHV-1 and problems with false susceptibility in disk diffusion tests. Antimicrob. Agents Chemother. **32**:1365–1369.
23. **Sanders, C. C., W. E. Sanders, Jr., and E. S. Moland.** 1986. Characterization of β-lactamases in situ on polyacrylamide gels. Antimicrob. Agents Chemother. **30**:951–952.
24. **Sirot, D., C. Chanal, R. Labia, and J. Sirot.** 1989. Susceptibility of new β-lactams to the expanded-spectrum β-lactamase CTX-1. Infection **17**:28–30.
25. **Sirot, D., J. Sirot, P. Saulnier, B. Joly, M. Chanal, M. Cluzel, and R. Cluzel.** 1986. Resistance to beta-lactams in Enterobacteriaceae: distribution of phenotypes related to beta-lactamase production. J. Int. Med. Res. **14**:193–199.
26. **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.