Variety of β-Lactamases Produced by Amoxicillin-Clavulanate-Resistant *Escherichia coli* Isolated in the Northeastern United States

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Received 5 September 2003/Returned for modification 20 November 2003/Accepted 27 January 2004

This study analyzed the enzymatic basis and molecular epidemiology of amoxicillin-clavulanate-resistant *Escherichia coli* isolated by the microbiology laboratory of a United States tertiary care hospital. From October 1998 to December 1999, all *E. coli* isolates were screened for ampicillin-sulbactam resistance. Of 283 isolates that tested resistant to ampicillin-sulbactam, 69 unique patient isolates were also resistant to amoxicillin-clavulanate by disk diffusion testing (zone diameter ≤ 13 mm). These amoxicillin-clavulanate-resistant *E. coli* isolates underwent agar dilution testing, pulsed-field gel electrophoresis, PCR analysis, and isoelectric focusing. The mean age of study patients was 52 years; 78% were female. Among the isolates, 12 were nosocomial (rate of amoxicillin-clavulanate resistance = 4.7%) and 57 were community acquired (rate of amoxicillin-clavulanate resistance = 2.8%). No predominant strain was identified. By agar dilution testing, 67 isolates were nonsusceptible (39 resistant and 28 intermediate) to amoxicillin-clavulanate and 37 were piperacillin-tazobactam resistant but only 8 were ceftazidime resistant (ceftazidime MIC ≥ 32 μg/ml). Two isolates were susceptible to amoxicillin-clavulanate acid by agar dilution, although they were resistant by disk diffusion testing. The distribution of β-lactamases was as follows: the TEM type alone was found in 52 isolates, the AmpC type was found in 4 isolates (2 identified as containing CMY-2), the TEM type and CMY-2 were found in 2 isolates, and the OXA type was found in 1 isolate. Also, there was one isolate with the TEM type and the SHV type and one with the TEM type and a second, unidentified enzyme. Among the isolates with TEM-type enzymes, two extended-spectrum β-lactamase-producing isolates were identified but two isolates with inhibitor-resistant TEM (IRT) enzymes (one with TEM-34 [IRT-6] and the other with a novel enzyme [tentatively assigned the designation TEM-122]) were more interesting.

Amoxicillin-clavulanate is a β-lactam–β-lactamase inhibitor combination that has antimicrobial activity against gram-positive, gram-negative, and anaerobic organisms (1, 10, 22, 26). *Escherichia coli*, one of the most common pathogens in community-acquired and nosocomial infections (5, 15), is usually susceptible to amoxicillin-clavulanate; with increasing use of this antimicrobial agent, however, resistance has begun to emerge (8, 12, 17, 29, 30). Resistance is most often conferred by plasmid-encoded TEM-type β-lactamase production (23, 28). Other mechanisms that have been described include acquired plasmid-encoded cephalosporinases (AmpC type), hyperproduction of the chromosomal AmpC intrinsic to *E. coli*, the alteration of porin channels, production of OXA β-lactamases, and inhibitor-resistant mutants of TEM and SHV enzymes (14, 19, 23, 28). The latter β-lactamases have been described only for isolates from Europe to date and have been designated Bush-Jacoby-Medeiros group 2br (2).

Discrepancies among the results produced by various antimicrobial susceptibility testing methods have been reported for several of the β-lactam–β-lactamase inhibitor combinations (6, 9, 25, 30). For example, Oliver et al. showed that various results were obtained when a fixed ratio of β-lactam to β-lactamase inhibitor (as opposed to a fixed concentration of inhibitor) was used to test the in vitro activity of β-lactam–β-lactamase inhibitor combinations, such as amoxicillin-clavulanate, against *E. coli* (25). Since disk diffusion and broth microdilution methods may give discrepant results, it is often difficult to compare the results for β-lactam–β-lactamase inhibitor combinations reported by various surveillance systems. Making comparisons even more complicated, the standards-setting bodies of various countries use differing susceptibility breakpoints for amoxicillin-clavulanate as well as differing susceptibility-testing methods (30).

Resistance to amoxicillin-clavulanate in *E. coli* is an emerging problem that has not been extensively studied in the United States. This study investigated the enzymatic basis and clinical epidemiology of amoxicillin-clavulanate-resistant *E. coli* isolates recovered from a tertiary care clinical microbiology laboratory in the Northeastern United States.

(This study was presented in part at the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy in Toronto, Canada, in 2000 and at the 39th Annual Meeting of the Infectious Diseases Society of America in San Francisco, Calif., in 2001.)
AMPC-Resistant Escherichia Coli

MATERIALS AND METHODS

Study design. The study was designed to assess the frequency and mechanisms of resistance to β-lactam-β-lactamase inhibitor combinations and to identify any E. coli that produced inhibitor-resistant TEM (IRT) β-lactamases. At the initiation of this study, the clinical microbiology laboratory was performing amoxicillin-clavulanate resistance is a more effective method for identification of IRT-producing E. coli than is resistance to ampicillin-sulbactam (4), so all ampicillin-sulbactam-resistant isolates were tested for resistance to amoxicillin-clavulanate.

Study setting. Beth Israel Deaconess Medical Center (BIDMC) is a tertiary care teaching hospital in Boston, Massachusetts, with 685 beds and approximately 39,000 patient discharges per year. During the study period, there were two adjoining campuses where both inpatients and outpatients received care. Healthcare workers often, though not uniformly, worked at both locations.

Collection of clinical isolates. Patient isolates were obtained from the clinical microbiology laboratory at the BIDMC. E. coli isolates were identified by using MicroScan panels (Dade MicroScan, West Sacramento, Calif.) and API strips (21 October 1998 to 31 May 1999) (bioMérieux, Hazelwood, Mo.) or VITEK (1 June 1999 to 27 November 1999) (bioMérieux). Amoxicillin-sulbactam-resistant isolates were identified using disk diffusion and Microscan conventional panels from 21 October 1998 to 31 May 1999 and VITEK from 1 June 1999 to 27 November 1999. A variety of identification and susceptibility panels were used during the study period due to the merger of the clinical microbiology laboratories of the former Beth Israel and Deaconess Hospitals. After the ampicillin-sulbactam-resistant isolates were identified in the clinical microbiology laboratories, disk diffusion testing for amoxicillin-sulbactam was performed in the research laboratory. There were no discrepancies between the disk diffusion results for amoxicillin-sulbactam in the research laboratory and the disk diffusion and MIC testing results in the clinical microbiology laboratories. All ampicillin-sulbactam-resistant isolates were then screened for amoxicillin-clavulanate susceptibility by disk diffusion. Only those isolates resistant to amoxicillin-clavulanate by disk diffusion were included in the analysis described below.

Media, chemicals, and enzymes. Mueller-Hinton agar (BD Biosciences, Sparks, Md.) was used for agar dilution susceptibility testing. Antimicrobial powder preparations of amoxicillin, ticarcillin, clavulanic acid, and ceftazidime (GlaxoSmithKline, Middlesex, United Kingdom), piperacillin and tazobactam (Wyeth, Pearl River, N.Y.) and imipenem (Merek, Whitehouse Station, N.J.) were gifts from their respective manufacturers. Cephalexin and cefotixin powders were purchased through Sigma Chemical Company (St. Louis, Mo.). Other chemical reagents were purchased from Sigma unless otherwise stated.

Antimicrobial susceptibility testing methods. Agar dilution susceptibility testing was performed for piperacillin-ampicillin-clavulanate (fixed ratio of amoxicillin-clavulanate of 2:1), piperacillin-tazobactam (fixed tazobactam concentration of 4 μg/ml), cephalexin, cefotixin, and imipenem. Agar dilution and disk diffusion susceptibility testing methods were performed in accordance with the National Committee for Clinical Laboratory Standards guidelines (20, 21). Isolates that were resistant to ceftazidime and that did not produce AmpC (as determined by isoelectric focusing [IEF]) were tested for extended-spectrum β-lactamase (ESBL) activity. Zones of inhibition were measured using disks (BD Biotics) with 30 μg of cefotaxime and 30 μg of ceftriaxone prepared with and without 10 μg of clavulanic acid. An increase of ≥5 mm in the zone diameter for either of these cephalosporins in the presence of clavulanic acid indicated the presence of an ESBL (20). The susceptibility of the isolates to ceftazidime was also determined, but these data did not increase the sensitivity of the ESBL screening test in this study.

IEF of β-lactamases. β-lactamase preparations were obtained by the freeze-thaw procedure (K. Bush and S. B. Singer, Letter, J. Antimicrob. Chemother. 24:82-84, 1989). β-lactamases were characterized by analytical IEF in the manner described by Matthew et al. (18). Crude lysate was placed onto polyacrylamide gel plates (Pharmacia LKB, Piscatway, N.J.) (pH 3.5 to 9.5); after electrophoresis, an overlay of filter paper saturated with 0.05% nitrocel lulose solution (BD Biotech) was used to develop the gel (27). The following β-lactamases were used as standards in IEF studies: TEM-1 (pl 5.4), SHV-5 (8.2), TEM-3 (6.3), and MIR-1 (8.6).

PCR assays. PCR assays for blaTEM and blablaSHV were performed on crude lysates of all study isolates. Primers were selected to screen for blaTEM and blablaSHV (Table 1). Primers TEMU2 and TEML2 were predicted to produce an amplification product of 374 bp. Primers SHVU2 and SHVL2 were predicted to produce a product of 477 bp. The primers were run in a multiplex PCR using the following PCR program: 95°C for 5 min and then 35 cycles of 94°C for 30 s, 62°C for 30 s, and 72°C for 30 s followed by 72°C for 10 min and storage at 4°C. The PCR products were separated using 1.5% agarose gels, stained with ethidium bromide, and imaged with UV illumination.

Select isolates with characteristics thought to be the most compatible with the presence of an IRT β-lactamase underwent additional PCR for the purpose of sequencing the blaTEM. These isolates were resistant to amoxicillin-clavulanate (amoxicillin-clavulanate MIC ≥ 32/16 μg/ml) but remained susceptible to cefazolin (cefasolin MIC ≤ 8 μg/ml), had IEF results consistent with the presence of a TEM-related enzyme, and gave positive test results for the presence of blaSHV by PCR as above. Only two isolates met these criteria. Crude lysates and preparations of plasmid DNA (the latter obtained using a standard alkaline lysis technique) served as the templates for these PCRs. Primers TEMU1 and TEML1 were used (Table 1). The PCR program was as follows: 95°C for 5 min and then 35 cycles of 94°C for 60 s, 58°C for 60 s, and 72°C for 60 s followed by 72°C for 10 min and storage at 4°C (27).

To detect genes encoding acquired AmpC-type enzymes, PCR was performed on crude lysates of amoxicillin-clavulanate-resistant E. coli isolates with IEF results consistent with the presence of AmpC enzymes (pl > 8.3). We used degenerate primers designed to amplify genes encoding plasmid-mediated AmpC enzymes but not the chromosomal β-lactamase of E. coli. The primers were CDEg1 and CDEg2 (Table 1). The PCR program was 95°C for 5 min and 30 cycles of 94°C for 30 s, 62°C for 30 s, and 72°C for 45 s followed by 72°C for 10 min and storage at 4°C. A subset of isolates was evaluated by PCR (using the methodology of Steward et al. (31)) for the presence of blaelaOXA. The following primers were used: OXA-1F, OXA-2F, OXA-2R, OXA-10F, and OXA-10R (Table 1). Cycling parameters for OXA-1F and OXA-1R and OXA-10F and OXA-10R included a 5-min denaturation cycle at 96°C followed by 35 cycles of denaturation at 98°C for 1 min, annealing at 61°C for 1 min, and extension at 72°C for 2 min and a final extension period of 10 min at 72°C. OXA-2F and OXA-2R, the primers were identical except that the annealing temperature was 65°C.

TABLE 1. PCR primers used in this work

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5’-3’)</th>
<th>Predicted product size (bp)</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEMU2</td>
<td>ACT GCC GCC AAC TTA CTT CTG</td>
<td>374</td>
<td>This work</td>
</tr>
<tr>
<td>TEML2</td>
<td>CGG GAG GGC TTA CCA TCT G</td>
<td>374</td>
<td>This work</td>
</tr>
<tr>
<td>SHVU2</td>
<td>CGG CCG CTT GAG CAA A</td>
<td>477</td>
<td>This work</td>
</tr>
<tr>
<td>SHVL2</td>
<td>GCT GCC GGG GGT AGT GGT GTC</td>
<td>477</td>
<td>This work</td>
</tr>
<tr>
<td>TEMU1</td>
<td>ATG AGT ATT CAA CAT TTC CG</td>
<td>867</td>
<td>28</td>
</tr>
<tr>
<td>TEML1</td>
<td>CTG ACA GTT ACC AAT GCT TA</td>
<td>867</td>
<td>28</td>
</tr>
<tr>
<td>CDce1</td>
<td>CAR ACS CTT TTY GAG MTR GG</td>
<td>800</td>
<td>This work</td>
</tr>
<tr>
<td>CDce2</td>
<td>GCC AAA ICC RYG RGT SGA GCG</td>
<td>800</td>
<td>This work</td>
</tr>
<tr>
<td>OXA-1F</td>
<td>ACA CAA TAC ATA TCA TCT C</td>
<td>813</td>
<td>31</td>
</tr>
<tr>
<td>OXA-1R</td>
<td>AGT GTG TTT AGA ATG GTG ATC</td>
<td>813</td>
<td>31</td>
</tr>
<tr>
<td>OXA-2F</td>
<td>TTC AGA CCA AAG GCA CGA TAG</td>
<td>702</td>
<td>31</td>
</tr>
<tr>
<td>OXA-2R</td>
<td>TCC GAG TTG ACT GGC GGG TTG</td>
<td>702</td>
<td>31</td>
</tr>
<tr>
<td>OXA-10F</td>
<td>CTT GGT TTA AAG TAG CAG</td>
<td>651</td>
<td>31</td>
</tr>
<tr>
<td>OXA-10R</td>
<td>CAT GAT TTT GGG GGG AAT GG</td>
<td>651</td>
<td>31</td>
</tr>
</tbody>
</table>

* I, inosine; M, A or C; R, G or A; S, G or C; Y, C or T.
DNA sequencing. The nucleotide sequence of \textit{bla}\textsubscript{TEM} was determined (using the PCR primers described above) from two isolates. Sequences were obtained from cloned PCR products inserted into plasmid PCR 2.1 and transformed into One Shot competent \textit{E. coli} using the protocol provided by the manufacturer of a TA cloning kit (Invitrogen, Carlsbad, Calif.). To confirm these sequences, a PCR assay was performed and \textit{bla}\textsubscript{TEM} sequences were obtained by direct sequencing of the PCR product after gel purification and extraction (Qiagen, Valencia, Calif.). The reported sequence is the consensus of sequencing of both strands of at least two colonies containing the cloned PCR product and both strands of the PCR product of a second PCR obtained by direct sequencing. PCR products obtained by using the degenerate 

\textit{bla}\textsubscript{SHV}-primers were cloned using a TA cloning kit and sequenced. Sequencing was performed at the Molecular Biology Core Facility at the Dana-Farber Cancer Center, Boston, Mass.

Clinical epidemiology. Data abstracted from medical records included patient's age, sex, the anatomical site from which the \textit{E. coli} isolate was obtained, and whether the isolate was nosocomial or community acquired. An isolate was considered nosocomial in origin only if the patient had been hospitalized for \geq 48 h at the time of specimen collection.

Statistical analysis. Continuous variables were analyzed with the Wilcoxon rank sum and Spearman correlation tests, and ordinal variables were analyzed with the Spearman correlation test. Dichotomous variables were analyzed with the Fisher's exact test. Analyses were performed using an SAS software system for Windows, version 6.12 (SAS Institute, Cary, N.C.).

Nucleotide sequence accession numbers. The nucleotide sequence of TEM-122 identified in this study were deposited in the GenBank repository (http://www.ncbi.nlm.nih.gov) under accession number AY307100.

RESULTS

Collection of isolates and patient characteristics. Between 26 October 1998 and 27 November 1999, there were 2,263 \textit{E. coli} isolates recovered from specimens submitted to the BIDMC microbiology laboratory. Of these isolates, 283 were resistant to ampicillin-sulbactam by disk diffusion and MIC testing. Among these strains, 69 unique isolates of amoxicillin-clavulanate-resistant \textit{E. coli} (zone diameter \leq 13 mm) were identified from 69 individuals. The rate of resistance to amoxicillin-clavulanate was higher among nosocomial isolates (12/254 [4.7%]) than among community isolates (57/2,009 [2.8%]) \textit{(P = 0.08)}. The combined rate of resistance to amoxicillin-clavulanate resistance was 3.0%. The majority of isolates \textit{(n = 55)} were recovered from urine. Other sites of isolation included pulmonary secretions \textit{(n = 4)}, blood \textit{(n = 3)}, cutaneous ulcers \textit{(n = 3)}, abdominal fluid \textit{(n = 2)}, cervix \textit{(n = 1)}, and an unspecified fluid source \textit{(n = 1)}. The cohort included 54 women \textit{(78\%)} and the mean cohort age was 52 years.

Molecular epidemiology. A total of 57 unique PFGE patterns were identified among the 69 clinical isolates. No more than five isolates of any one PFGE type were identified, and these five were not clustered with respect to time (data not shown).

In vitro susceptibility test results. The results of the agar dilution susceptibility tests for the 69 \textit{E. coli} isolates that were determined to be amoxicillin-clavulanate resistant by disk diffusion are shown in Table 2. Most isolates were resistant to cefalothin, piperacillin, and ticarcillin but were susceptible to cefoxitin; only eight (11.6\%) isolates were resistant to ceftazidime. None of the isolates were resistant to imipenem. A total of 37 (53.6\%) of the isolates were resistant to piperacillin-tazobactam. Although all isolates were resistant to amoxicillin-clavulanate by disk diffusion testing, 2 isolates were susceptible and 28 were intermediate to amoxicillin-clavulanate by agar dilution testing. All 30 (100\%) isolates that were susceptible or intermediate to amoxicillin-clavulanate by agar dilution testing were TEM phenotype, as opposed to the 22 of 39 (56\%) isolates that tested resistant by agar dilution testing \textit{(P < 0.001)}.

Characterization of \textit{β}-lactamases. The classification of \textit{β}-lactamases produced (as determined by PCR and IEF) by the 69 isolates in our cohort is shown in Table 3. PCR detected \textit{bla}\textsubscript{TEM} in 56 isolates, but only 1 isolate contained \textit{bla}\textsubscript{SHV}. The latter isolate also contained \textit{bla}\textsubscript{TEM}. Two isolates (EK143 and EK518) that had phenotypes thought to be consistent with the presence of inhibitor-resistant TEM (IRT) enzymes (Bush-Jacoby-Medeiros group 2r) underwent sequencing of \textit{bla}\textsubscript{TEM}; these isolates were resistant to amoxicillin-clavulanate (amoxicillin-clavulanate MICs were 32/16 and 64/32 \text{µg/ml}, respectively) but remained susceptible to cefazolin (cefazolin MICs were 8 and 4 \text{µg/ml}, respectively). Both had \textit{bla}\textsubscript{TEM} mutations that produced changes in amino acids known to be important for resistance to \textit{β}-lactamase inhibitors—specifically, Met-69 and Arg-275. The \textit{bla}\textsubscript{TEM} of EK518 had the Met\textsubscript{69}Val that has previously been described for TEM-34 (IRT-6) \textit{(32)}. The \textit{bla}\textsubscript{TEM} of EK143 had an Arg\textsubscript{275}Gln which has not been reported in the absence of other amino acid substitutions in an IRT enzyme and has been given the \textit{β}-lactamase designation TEM-122 (http://www.lahey.org/studies/temtable.htm). IEF revealed that this enzyme had a pI of 5.25.
plasmid-mediated AmpC-type β-lactamases were performed on 15 amoxicillin-clavulanate-resistant *E. coli* isolates that demonstrated β-lactamases with pI values of ≥8. Four of these yielded an 810-bp product with a nucleotide sequence that was identical to an intragenic fragment of *bla*<sub>CMY-2</sub>, an AmpC-type β-lactamase. Two of the four isolates with *bla*<sub>CMY-2</sub> also demonstrated the presence of *bla*<sub>TEM</sub> by PCR and contained β-lactamases with pIs of 5.4 (consistent with the presence of a TEM enzyme).

PCR assays using multiple primers for *bla*<sub>OXA</sub> were performed on two isolates that were negative by PCR for *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>CMY-2</sub>. Both isolates produced IEF bands of pI 7.15 or 7.3. One of the isolates was positive by PCR (using the primers OXA-1F and OXA-1R) for *bla*<sub>OXA</sub>. Four isolates yielded an 810-bp product with a nucleotide sequence that was identical to an intragenic fragment of * bla*<sub>SHV</sub>. Isolates were not analyzed for the presence of SHV gene.

**DISCUSSION**

*E. coli* resistance to the β-lactam–β-lactamase inhibitor combinations is an emerging problem (8, 28, 29, 32). In one French study, 40% of *E. coli* isolates collected from hospitals and 18% of isolates from private laboratories were no longer susceptible to amoxicillin-clavulanate (8). A more recent 3-year French study revealed that a fairly stable proportion (5%) of *E. coli* isolates (predominantly from hospitalized patients) were resistant to amoxicillin-clavulanate (12). In 1998, 41% of *E. coli* isolates isolated in U.S. hospitals reporting to the Centers for Disease Control and Prevention were resistant to ampicillin-sulbactam; this rate of resistance had increased from 24% in 1990 (1,657 isolates tested in 1998 and 512 tested in 1990) (S. Fridkin, personal communication). Almost 15% of 708 North American urinary tract isolates of *E. coli* collected in 1997 to 1998 from hospitalized patients were nonsusceptible to amoxicillin-clavulanate (17).

<table>
<thead>
<tr>
<th>No. (%) of isolates</th>
<th>IEF result(s) (pI)</th>
<th>PCR result</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52* (75.4)</td>
<td>5.3–5.4</td>
<td><em>bla</em>&lt;sub&gt;TEM&lt;/sub&gt;</td>
<td>TEM producer</td>
</tr>
<tr>
<td>2 (2.9)</td>
<td>5.4, 5.4</td>
<td><em>bla</em>&lt;sub&gt;TEM&lt;/sub&gt; + * bla*&lt;sub&gt;CMY-2&lt;/sub&gt;</td>
<td>TEM + AmpC</td>
</tr>
<tr>
<td>2 (2.9)</td>
<td>8.4</td>
<td>* bla*&lt;sub&gt;CMY-2&lt;/sub&gt;</td>
<td>AmpC</td>
</tr>
<tr>
<td>1 (1.4)</td>
<td>5.4</td>
<td>No <em>bla</em> genes detected</td>
<td>Other AmpC</td>
</tr>
<tr>
<td>1 (1.4)</td>
<td>7.2</td>
<td><em>bla</em>&lt;sub&gt;TEM&lt;/sub&gt; + * bla*&lt;sub&gt;SHV&lt;/sub&gt;</td>
<td>TEM producer: ESBL</td>
</tr>
<tr>
<td>3 (4.3)</td>
<td>5.4, 5.7, 7.0</td>
<td><em>bla</em>&lt;sub&gt;OXA&lt;/sub&gt;</td>
<td>OXA producer</td>
</tr>
<tr>
<td>5 (7.2)</td>
<td>8.4, 8.5</td>
<td>No <em>bla</em> genes detected</td>
<td>TEM producer: ESBL</td>
</tr>
</tbody>
</table>

* Includes two inhibitor-resistant TEM (IRT) enzymes.
produced TEM-type $\beta$-lactamases, although one isolate also was positive for blaTEM by PCR (the second $\beta$-lactamase was not detected by IEF). Whereas most ESBL-producing isolates remain susceptible to piperacillin-tazobactam in vitro (14), both of these isolates were resistant. Extracts of both isolates showed rapid hydrolysis of nitrocefin, suggesting that hyperproduction of $\beta$-lactamases played a role in mediating resistance to the $\beta$-lactamase inhibitor combinations.

Inhibitor-resistant TEM (IRT) enzymes (Bush-Jacoby-Medeiros group 2br) were found in two isolates that were resistant to amoxicillin-clavulanate and susceptible to cefazolin, had enzymes with pI's between 5.2 and 5.5, and had TEM-type enzymes detected by screening PCR. The predicted amino acid sequence of these enzymes contained amino acids substitutions known to be important to resistance to $\beta$-lactamase inhibitors.

One isolate had a TEM-type enzyme with the Met69Val that has previously been described for TEM-34 (IRT-6) (32). The other isolate had the Arg275Gln substitution, which has previously been described only for IRT enzymes with additional mutations, such as TEM-45 (IRT-14), in which Met69Leu is also present (3). It is possible that the elucidation of additional blaTEM sequences would have revealed more IRT-containing isolates in this collection. These are, to our knowledge, the first IRT enzymes reported in United States isolates. $\beta$-lactam-$\beta$-lactamase inhibitor combinations are widely used in the United States, so it is somewhat surprising that IRT enzymes had not been described in isolates from the United States in the past; however, this may only reflect a dearth of studies that included DNA sequencing to search for these enzymes.

After the TEM type, the second-most-common $\beta$-lactamase produced by our isolates was CMY-2, an AmpC-type $\beta$-lactamase, found in four isolates, two of which also produced TEM-type enzymes. Plasmid-mediated AmpC production has been recently identified as an emerging mechanism of resistance (7, 19, 24).

Two isolates displayed an AmpC phenotype (i.e., resistance to cefoxitin and extended-spectrum cephalosporins) and contained $\beta$-lactamases with pI's of 6.3 on IEF, but PCR was negative for genes encoding acquired AmpC enzymes, suggesting that these isolates were hyperproducers of the chromosomal AmpC. No SHV-type enzymes were identified among our isolates, although one isolate did have an OXA-type enzyme. Several organisms had detectable $\beta$-lactamases by IEF, but corresponding resistance determinants were not identified by PCR. These may be other OXA-type determinants not detected by our three primer sets or may be other $\beta$-lactamase genes.

Agar dilution susceptibility testing and disk diffusion testing results for amoxicillin-clavulanate were discrepant for 30 of 69 E. coli isolates, although of the 69 isolates that were resistant by disk testing, 67 were nonsusceptible by agar dilution testing. Two isolates were susceptible and 28 were intermediate by agar dilution testing. The significance of these discrepant results is not certain, but it would be of interest to determine which method provides the more clinically relevant results.

Piperacillin-tazobactam was the most active of the $\beta$-lactam-$\beta$-lactamase inhibitor combinations. The increased activity of this drug against E. coli compared to that of the other inhibitor combination agents has been previously demonstrated and is likely related to the strong inhibitory effect of tazobactam against TEM-type $\beta$-lactamases and the increased activity of piperacillin versus that of amoxicillin (6, 14, 17).

Our analysis of a set of amoxicillin-clavulanate-resistant E. coli isolates revealed that the majority were urinary tract isolates and were recovered from outpatients. They were genetically unrelated and carried a variety of enzymes, although most expressed standard TEM enzymes. The magnitude of expression of TEM determined the degree of resistance to $\beta$-lactam-$\beta$-lactamase inhibitor combinations. Acquired AmpC enzymes (particularly CMY-2) were found in several isolates. Additional study will be required to expand on the novel finding of IRT enzymes in United States isolates.

ACKNOWLEDGMENTS

We thank George M. Eliopoulos and Robert C. Moellering, Jr., for helpful discussions and expert advice.

This study was supported in part by the Centers for Disease Control and Prevention Postdoctoral Fellowship Training Program in Infectious Diseases (grant T01/CCT111438).

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