Clinical Features and Molecular Epidemiology of CMY-Type β-Lactamase–Producing Escherichia coli

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Background. Knowledge of the clinical features of infections caused by Escherichia coli strains that produce plasmid-mediated AmpC β-lactamase is limited. Of the several groups of plasmid-mediated AmpC β-lactamases, CMY-type β-lactamase is the most common in the United States.

Methods. We prospectively identified patients infected or colonized with E. coli strains that produce CMY-type β-lactamase, and we collected clinical data over a 7-month period. A retrospective cohort study was performed to identify features associated with these cases. Patients with extended-spectrum β-lactamase–producing E. coli were used as a control group. Pulsed-field gel electrophoresis, plasmid analysis, and phylogenetic typing were performed.

Results. Twenty-two patients with infection or colonization due to CMY-type β-lactamase–producing E. coli and 25 patients with infection or colonization due to extended-spectrum β-lactamase–producing E. coli were identified. The demographic characteristics of the patients were similar in both cohorts. Patients with CMY-type β-lactamase–producing E. coli were significantly more likely to have symptomatic infection than were patients with extended-spectrum β-lactamase–producing E. coli (P = .028). The CMY-type β-lactamase was identified as CMY-2 or its variants. Ninety-four percent of the CMY-type β-lactamase–producing isolates belonged to E. coli phylogenetic groups B2 and D, which are associated with virulence. Many of the isolates shared similar plasmid profiles, whereas the pulsed-field gel electrophoresis profiles were diverse. Co-resistance to non–β-lactam antimicrobials was common.

Conclusion. In Pittsburgh, Pennsylvania, CMY-type β-lactamase–producing E. coli strains are almost as common as extended-spectrum β-lactamase–producing E. coli strains, and they cause symptomatic infection in the majority of cases.
comparison with cases involving ESBL-producing *E. coli*, which share in common resistance to third-generation cephalosporins.

**PATIENTS, MATERIALS, AND METHODS**

**Cohort study.** The CMY cohort included all patients from whom CMY-producing *E. coli* was isolated from any clinical sample obtained from 1 September 2006 through 31 March 2007. The CMY cohort was compared with the ESBL cohort, which included all patients from whom ESBL-producing *E. coli* was isolated within the same period of time. The study was performed at the University of Pittsburgh Medical Center (Presbyterian-Shadyside Campuses), which is a 1300-bed tertiary teaching hospital with affiliated outpatient clinics, located in Pittsburgh, Pennsylvania.

**Laboratory surveillance of CMY-producing E. coli.** Prospective laboratory-based surveillance was conducted during the study period. Isolates that met the screening criteria for ESBL production (ceftazidime or aztreonam zone size ≤27 mm on disk-diffusion testing) but had negative phenotypic confirmatory test results were collected [7]. Of these isolates, those showing nonsusceptibility to ceftazidime on disk-diffusion testing were checked for the presence of CMY-type β-lactamase gene by PCR analysis. Primers used in this study were 5′-CCG GAC ACC TTT TTG CTT TT-3′ for CMY-F and 5′-TAT CCT GGG CCT CAT CGT CAG TTA-3′ for CMY-R. The amplification was conducted with an annealing temperature of 60°C for 30 cycles using a 9700 GeneAmp thermocycler (Applied Biosystems). The PCR products were sequenced on an ABI3100 instrument (Applied Biosystems). Susceptibility of each isolate to various β-lactam and non-β-lactam antimicrobial agents was tested using the disk-diffusion method [7]. Phenotypic detection of plasmid-mediated AmpC β-lactamase using a boronic acid compound was also performed as described elsewhere [8].

In brief, 300 μg of 3-aminophenyl boronic acid hydrochloride (3-APB) was applied to a ceftazidime disk placed on a Mueller-Hinton agar plate (Becton Dickinson) inoculated with the test isolate. An increase in zone diameter of ≥5 mm, compared with that on a ceftazidime disk without 3-APB, was interpreted as a positive test result [8].

For the ESBL cohort, *E. coli* isolates that had phenotypic confirmatory test results positive for ESBL production were also collected during the same period. PCR analyses for the detection of TEM-, SHV-, and CTX-M–type ESBL genes were conducted as described elsewhere [9], followed by sequencing of the PCR products. For those isolates with test results negative for any of these ESBL genes, we conducted PCR analysis for the CMY-type β-lactamase gene as described above.

**Study sample and data collection.** The study was approved by the Institutional Review Boards of the University of Pittsburgh. Medical records of those patients from whom CMY-type β-lactamase–producing *E. coli* or ESBL-producing *E. coli* were isolated were collected to document patient demographic characteristics, contact with the health care system, underlying medical conditions, prior administration of antibiotics, the role of the organism (symptomatic infection vs. colonization), the type of infection, the antibiotic treatment received, and the clinical and microbiologic outcome. The role of the organisms was determined by detailed chart review and in accordance with the Centers for Disease Control and Prevention/National Nosocomial Infections Surveillance guidelines whenever applicable [10]. These data were supplied to the investigators without patient identification. The same patient was reenrolled only if a positive culture result was obtained ≥30 days after the initial enrollment.

Hospital-acquired infection was defined by a positive culture specimen obtained from a patient who had been hospitalized for ≥48 h. If a patient was transferred from another hospital, the duration of inpatient stay was calculated from the date of the first hospital admission.

Health care–associated infection was defined by a positive culture specimen obtained from a patient at the time of hospital admission or within 48 h after admission if the patient fulfilled any of the following criteria: (1) received intravenous therapy at home; received wound care or specialized nursing care through a health care agency, family, or friends; or had self-administered intravenous medical therapy within 30 days before onset of the infection (patients whose only home therapy was oxygen were excluded); (2) attended a hospital or hemodialysis clinic or received intravenous chemotherapy in the 30 days before the infection; (3) was hospitalized in an acute care hospital for ≥2 days within 90 days before the onset of infection; (4) resided in a nursing home or long-term care facility.

Community-associated infection was defined by a positive culture specimen obtained at the time of hospital admission or <48 h after admission from a patient who did not meet the criteria for a health care–associated infection.

**Phylogenetic and molecular typing.** The phylogenetic groups of the study isolates were determined by multiplex PCR analysis [11]. Among the 4 main phylogenetic groups (A, B1, B2, and D) in *E. coli*, virulent extraintestinal strains belong mainly to groups B2 and D [12, 13].

To determine the genetic relatedness of the study isolates, PFGE analysis was performed using *XbaI* as a restriction endonuclease and electrophoresing the genome in a CHEF DR III system (Bio-Rad) at 6 volts with pulse times of 2.2–54.2 s and linear ramping at a temperature of 14°C for 22 h. Digitalized gel images were saved and subjected to analysis with BioNumerics software, version 4.0 (Applied Maths). Cluster analysis was performed by using the unweighted pair-group method based on Dice coefficients to quantify the similarities.

**Plasmid analysis.** Plasmids encoding CMY-type β-lactama genes were first transferred to *E. coli* DH10B by electro-
poration. The presence of the CMY-type β-lactamase genes in the transformants was confirmed by PCR. The plasmids in the transformants were extracted by the standard alkaline lysis method [14], digested with restriction enzyme PstI (New England Biolabs) and subjected to electrophoresis in 0.8% agarose gel. The DNA ladders were then hybridized with a digoxigenin-labeled DNA probe specific for CMY-type β-lactamase gene using PCR DIG detection system (Roche Diagnostics). Cluster analysis for quantification of similarities was performed as for PFGE.

Statistical analysis. Categorical variables were compared using χ² test. Continuous variables were compared using the Mann-Whitney U test. P values <.05 were considered to be statistically significant.

RESULTS

E. coli clinical isolates producing CMY-type β-lactamase. During the study period, a total of 2583 E. coli isolates were identified in the microbiology laboratory. Of these isolates, a total of 29 unique E. coli isolates were phenotypically confirmed as ESBL producers. Of the 18 unique isolates that had positive ESBL screening results, negative confirmation test results, and were nonsusceptible to ceftazidime, all were found to possess the CMY-type β-lactamase gene. E. coli isolates that produced other types of plasmid-mediated AmpC β-lactamase were not found. The DNA sequences were consistent with CMY-2 for all of these isolates, except for 1 isolate that was consistent with CMY-18, which is a variant of CMY-2. Of 29 unique isolates phenotypically confirmed as ESBL producers, 25 possessed SHV, TEM, or CTX-M–type ESBL genes. The remaining 4 isolates were found to possess the CMY-type β-lactamase gene but not an ESBL gene, indicating false-positive ESBL confirmatory test results. They included CMY-2 and its variants CMY-32 and 33. These 4 cases were added to the CMY cohort for the clinical analysis. Overall, 22 unique CMY-producing and 25 unique ESBL-producing E. coli isolates were identified, and the patients from whom these isolates were obtained constituted the CMY cohort and ESBL cohort, respectively, for this study. All 22 CMY-producing isolates had phenotypic detection test results positive for AmpC β-lactamase production using 3-APB, whereas none of the 25 ESBL-producing isolates had positive results, indicating that this test had 100% sensitivity and specificity among the study isolates.

Clinical features of patients with CMY-producing and patients with ESBL-producing E. coli infection. Twenty-two patients in the CMY cohort and 25 patients in the ESBL cohort were included in the analysis (table 1). The patients were predominantly female in both cohorts. The mean ages of the patients were 62.0 and 62.4 years in the CMY cohort and the ESBL cohort, respectively. In the CMY cohort, 12 patients (55%) were admitted from home, whereas 11 patients (50%) had a history of hospitalization within the previous 3 months.

Table 1. Demographic and clinical characteristics of patients with infection or colonization due to CMY-type β-lactamase–producing (CMY cohort) or extended-spectrum β-lactamase (ESBL)–producing (ESBL cohort) Escherichia coli.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CMY cohort (n = 22)</th>
<th>ESBL cohort (n = 25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years ± SD</td>
<td>62.0 ± 21.0</td>
<td>62.4 ± 20.0</td>
<td>.97</td>
</tr>
<tr>
<td>Female sex</td>
<td>18/22 (82)</td>
<td>19/25 (76)</td>
<td>.63</td>
</tr>
<tr>
<td>White race</td>
<td>20/21 (95)</td>
<td>18/23 (78)</td>
<td>.10</td>
</tr>
<tr>
<td>Transfer from another hospital</td>
<td>3/22 (14)</td>
<td>0/25 (0)</td>
<td>.056</td>
</tr>
<tr>
<td>Hospitalization within previous 3 months</td>
<td>11/21 (52)</td>
<td>14/25 (56)</td>
<td>.81</td>
</tr>
<tr>
<td>Location within previous 30 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Home</td>
<td>12/20 (60)</td>
<td>13/24 (54)</td>
<td>.70</td>
</tr>
<tr>
<td>Nursing home/LTCF</td>
<td>5/20 (25)</td>
<td>7/24 (29)</td>
<td>.76</td>
</tr>
<tr>
<td>Hospital</td>
<td>2/20 (10)</td>
<td>4/24 (17)</td>
<td>.52</td>
</tr>
<tr>
<td>Site of infection acquisition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital acquired</td>
<td>11/21 (52)</td>
<td>7/25 (28)</td>
<td>.091</td>
</tr>
<tr>
<td>Health care associated</td>
<td>7/21 (33)</td>
<td>14/25 (56)</td>
<td>.12</td>
</tr>
<tr>
<td>Community associated</td>
<td>3/21 (14)</td>
<td>3/25 (12)</td>
<td>.82</td>
</tr>
<tr>
<td>Chronic underlying disease</td>
<td>19/21 (90)</td>
<td>23/25 (92)</td>
<td>.86</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>13/21 (62)</td>
<td>16/25 (64)</td>
<td>.88</td>
</tr>
<tr>
<td>Antibiotic use within previous 30 days</td>
<td>8/21 (38)</td>
<td>9/25 (36)</td>
<td>.88</td>
</tr>
<tr>
<td>Urinary tract infection or colonization</td>
<td>17/22 (77)</td>
<td>18/25 (72)</td>
<td>.68</td>
</tr>
<tr>
<td>Symptomatic infection</td>
<td>15/19 (79)</td>
<td>10/22 (45)</td>
<td>.028</td>
</tr>
</tbody>
</table>

NOTE: Data are no. or proportion of patients (%), unless otherwise indicated. LTCF, long-term care facility.
The source of CMY-producing E. coli was the urinary tract in 17 patients (77%). Three patients (14%) had community-associated cases, all of which originated in the urinary tract. The first patient had cystitis and was treated with piperacillin-tazobactam. The second patient had pyelonephritis and was treated with imipenem. Both experienced clinical cure. The third patient had E. coli colonization. Seven (32%) of the patients had health care–associated cases, and 11 (50%) of the patients had hospital-acquired cases. The site of acquisition could not be determined for 1 patient. Thirteen patients (59%) had at least 1 immunosuppressive condition, including diabetes, receipt of immunosuppressive therapy, malignancy, and receipt of transplant. Whether the patient had symptomatic infection or colonization could be determined in 19 of 22 cases in the CMY cohort. Fifteen patients (86%) had symptomatic infection, which was statistically significantly higher than the incidence in the ESBL cohort (10 patients; 45%) \( (P = .028) \). Three patients in the CMY cohort, including 1 from the community, had pyelonephritis, whereas none of the patients in the ESBL cohort had pyelonephritis.

**CMY-producing E. coli clinical isolates.** All CMY-producing clinical isolates were resistant to cefoxitin and cefpodoxime, had intermediate resistance or resistance to cefotaxime, and were variably resistant to cefotaxime. Co-resistance to non-β-lactam antimicrobials used for the treatment of E. coli infection was common. Only 7 (32%) of 22 isolates obtained from the CMY cohort and 5 (20%) of 25 isolates obtained from the ESBL cohort were susceptible to ciprofloxacin. Fourteen (64%) of the isolates obtained from the CMY cohort and 8 (32%) of the isolates obtained from the ESBL cohort were susceptible to gentamicin, and 11 (50%) obtained from the CMY cohort and 8 (32%) obtained from the ESBL cohort were susceptible to trimethoprim-sulfamethoxazole. PFGE analysis revealed that they constituted a relatively diverse population, with 13 distinct PFGE types, as shown in figure 1. In the CMY cohort, 20 (91%) of 22 isolates belonged to the 2 phylogenetic groups associated with virulence (2 isolates belonged to B2, and 18 isolates belonged to D), whereas 21 (88%) of 25 isolates from the ESBL cohort belonged to these 2 phylogenetic groups (12 isolates to B2 and 9 isolates to D) \( (P \not= \text{not significant}) \).

**Plasmid analysis.** The results of restriction analysis of the isolates obtained from the CMY cohort are shown in figure 2. Plasmids from the isolates belonging to phylogenetic group D, the most commonly observed group in the CMY cohort, shared restriction patterns with >70% similarity, suggestive of a common origin. The plasmid from 1 of the 2 isolates in phylogenetic group B2 also belonged to this cluster.

**DISCUSSION**

Despite the broad spectrum β-lactam resistance that they confer, there have been many unanswered questions about plasmid-mediated AmpC β-lactamase in *E. coli*. First, there is the question of prevalence. A surveillance study conducted in the United
States reported detection of plasmid-mediated AmpC \(\beta\)-lactamase in 4\% and ESBL in 40\% of \textit{E. coli} isolates with reduced susceptibility to broad-spectrum cephalosporins collected from 1992 through 2000 [15]. In the present study, a total of 2583 \textit{E. coli} isolates were identified. The identification of 22 unique cases due to CMY-producing \textit{E. coli} implies that \(\sim0.9\%\) of all \textit{E. coli} produce CMY-type \(\beta\)-lactamase, although the actual rate is likely to be slightly higher because of the exclusion of additional isolates obtained from patients in our study. Meanwhile, at least 1.0\% of \textit{E. coli} isolates were found to produce ESBL during the same period. Although the number of cases is much smaller in the present study, our data suggest that the incidence of cases due to CMY-producing \textit{E. coli} may be almost as high as that of cases due to ESBL-producing \textit{E. coli} in certain epidemiologic contexts; these results are consistent with recent findings in Nebraska [6]. Of note, the majority of isolates obtained from the CMY cohort (18 [82\%] of 22) belonged to phylogenetic group D and shared common restriction profiles of the plasmids encoding the CMY genes. This was in contrast with findings from a small study conducted in France, in which all CMY-producing \textit{E. coli} isolates belonged to phylogenetic group B1 [16]. Our findings suggest that certain groups of extraintestinal \textit{E. coli} strains may have affinity with CMY-type \(\beta\)-lactamase–bearing plasmids and warrant further investigation.

Second, in contrast with ESBLs, plasmid-mediated AmpC \(\beta\)-lactamase, including CMY-type \(\beta\)-lactamase, does not have detection methods that have been standardized by the Clinical and Laboratory Standards Institute or any other authority, which is a major barrier in defining its epidemiology. At present, the isolates producing this group of \(\beta\)-lactamas are typically identified as being negative for ESBL and do not undergo additional testing. Recently, however, there has been a growing interest in the use of boronic acid compounds as specific AmpC inhibitors for the detection of plasmid-mediated AmpC \(\beta\)-lactamase production in \textit{E. coli} and \textit{Klebsiella} species [8, 17, 18]. In our study, the use of the disk-based method (adding 3-APB to a ceftazidime disk) gave a sensitivity and specificity of 100\% for the detection of CMY-type \(\beta\)-lactamase production. Routine use of the boronic acid–based method for isolates that have positive results of the initial screen test for ESBL production (i.e., reduced susceptibility to broad-spectrum cephalosporins) would greatly enhance detection of \textit{E. coli} strains that produce CMY or other types of plasmid-mediated AmpC-type \(\beta\)-lactamases. Four isolates producing CMY-type \(\beta\)-lactamase (1 CMY-2 isolate, 2 CMY-32 isolates, and 1 CMY-33 isolate) were misidentified as ESBL producers by the phenotypic confirmatory test in the clinical microbiology laboratory (results that were reproducible in the research laboratory, as well). These isolates all also had positive results according to the boronic acid–based method, but they were definitely CMY-type \(\beta\)-lactamase producers and were negative for ESBLs according to genotypic tests. For the 2 isolates that produced CMY-32, enhanced susceptibility of CMY-32 to clavulanic acid may account for this phenomenon [19]. We are currently investigating the mechanism underlying the false-positive ESBL test results for the other 2 isolates.

Third, largely as the consequence of the lack of a standardized detection method, the clinical significance of CMY-producing \textit{E. coli} has not been known. In the present study, approximately one-half of the cases were acquired outside of the hospital, with the urinary tract being the most common site of infection or colonization. Of note, CMY-producing \textit{E. coli} were significantly more likely to cause symptomatic infection (as opposed to colonization) than were ESBL-producing \textit{E. coli}. This was a
surprising finding that needs to be confirmed in a study involving a larger number of cases. What is the basis for the virulence of CMY-producing \textit{E. coli}? There was no difference with respect to membership in a virulence-associated phylogenetic group (group B2 or D) between the 2 cohorts. Therefore, it will be worth investigating the specific virulence gene contents of these isolates. In particular, it has been suggested that some of the plasmids carrying the CMY-type $\beta$-lactamase genes also carry a cluster of genes encoding the type IV pili, which contributes to adhesion and invasion [20]. The clinical implications of the potentially enhanced virulence of CMY-producing \textit{E. coli}, if confirmed, are paramount. We also noted high rates of co-resistance to non-$\beta$-lactam antimicrobials commonly used to treat \textit{E. coli} infection, which were much higher than those in a previous report [5] and were likely attributable to the difference in patient populations. Although our study was too small to systematically assess clinical outcome, multidrug resistance in CMY-producing \textit{E. coli} is a concern in terms of empirical management of these infections.

Our study has several limitations. The study was performed at a single center in the United States and involved a relatively small number of cases in both cohorts. Because of the retrospective nature of the clinical study, some of the clinical data were not available, leaving room for potential bias, although the number of cases with missing data was small.

In conclusion, we have shown that, in Pittsburgh, Pennsylvania, clinical cases due to \textit{E. coli} that produce CMY-type broad-spectrum $\beta$-lactamase are almost as common as cases due to \textit{E. coli} that produce ESBLs. Routine screening for CMY-producing \textit{E. coli} using a simple phenotypic method will help to identify these clinical cases in the clinical microbiology laboratory. A larger, multicenter clinical study is warranted to assess the clinical significance of these \textit{E. coli} isolates.

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