# Clinical and molecular epidemiology of community-onset, extended-spectrum β-lactamase-producing *Escherichia coli* infections in Thailand: A case-case-control study

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***Background:*** Extended-spectrum β-lactamase (ESBL)-producing organisms, first identified in Germany in 1983, are now widely recognized as clinically relevant causes of infections in community.
***Methods:*** Our objective was to evaluate the clinical and molecular epidemiology of community-onset, extended-spectrum β-lactamase (CO-ESBL)-producing *Escherichia coli* infections. We used a case-case-control study undertaken in a 450-bed, tertiary care hospital. Patients included case group (CG) I, which had confirmed CO-ESBL-producing *E coli* infections (n = 46). Case group (CG) II (n = 46) included patients with CO-non-ESBL-producing *E coli* infections. Controls (n = 138) were patients without infections.
***Results:*** By multivariate analysis, diabetes (95% confidence interval [CI]: 1.9-13.2, $P < .001$), prior ESBL *E coli* colonization (<90 days) (95% CI: 1.2-67.8, $P < .001$), recent receipt of antibiotics (<90 days) (95% CI: 4.2-44.2, $P = .004$), and previous exposure to third-generation cephalosporins (95% CI: 2.2-16.4, $P = .001$) and fluoroquinolones (95% CI: 1.4-18.3; $P = .003$) were associated risks among CG I. Diabetes (95% CI: 1.6-15.4, $P = .005$), stroke (95% CI: 1.5-17.1, $P = .001$), and diarrhea (95% CI: 3.8-65.8, $P = .001$) were risks among CG II. Patients with CO-ESBL in CG I versus controls were more likely to die (30% vs 0%, respectively; $P < .001$), had prolonged hospital length of stay (8 vs 5 days, respectively; $P < .001$), and had higher hospitalization costs (median, US $528 vs $108, respectively; $P < .001$). The plasmid carrying the CTX-M-15 gene was identified in 13 of 25 (52%) available CO-ESBL-producing *E coli* isolates.
***Conclusion:*** CO-ESBL-producing *E coli* is an emerging multidrug-resistant microorganism in Thailand. Patients with prior ESBL colonization and recent antibiotic exposures, especially to third-generation cephalosporins and fluoroquinolones, were at risk for CO-ESBL-producing *E coli* infection. (Am J Infect Control 2007;35:606-12.)

Extended-spectrum β-lactamase (ESBL)-producing organisms, first identified in Germany in 1983,[1] are now widely recognized as clinically relevant causes of infections.[2-8] The majority of persons infected with these microorganisms have been exposed to hospital intensive care units, but such exposure is not necessary for infection.[9,10] Identified risks for acquisition include hospital length of stay (LOS), severity of illness, intensive care unit LOS, mechanical ventilation, urinary or arterial catheterization, and previous exposure to antibiotics.[9,10] More recent data have suggested that ESBL-producing enterobacteriaciae are prevalent in community-based settings.[11-30] However, most of these studies simply noted the incidence of ESBL-induced infection,[13-17,19,24] ESBL enteric carriage,[17,21-23,28,30] or described clinical cases and associated risks for ESBL acquisition.[12,29]

In Thailand, where antibiotic-management programs are uncommon and antibiotics can be purchased without a prescription, the rate of antibiotic resistance among gram-negative organisms, especially with ESBL, have increased significantly over the past decade.[31,32] We, therefore, conducted a case-case-control study to evaluate relevant clinical and molecular epidemiologic factors associated with community-onset (CO), ESBL-producing *Escherichia coli* infections among hospitalized adults. In addition, we characterized hospital resource utilization and estimated costs associated with the medical care of this population.

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## MATERIALS AND METHODS

### Setting and patients

This study was conducted at Thammasart University Hospital, a 450-bed academic tertiary care medical center in Pratumthani, Thailand. Study subjects were identified from source records of the hospital's clinical microbiological laboratory, which performs cultures for bacteria for all sterile-body fluid clinical specimens obtained at the institution. Records from the Division of Infection Control were also reviewed to ensure that all eligible subjects were identified. All adult inpatients with initial clinical cultures positive for ESBL-producing *E coli* and who met the definition of CO-ESBL-producing *E coli* infection during the period of July 1, 2003 through June 30, 2004, were eligible for the study.

### Definitions and data collection

CO-ESBL infection was defined as an infection that (1) occurred <48 hours after admission to the hospital in an adult who had never been hospitalized or occurred <48 hours after hospitalization in a patient without a recent hospital admission (within the preceding 30 days), (2) did not fulfill the criteria for nosocomial infection as delineated by the Centers for Disease Control and Prevention (CDC),[33] and (3) occurred in a patient who was not transferred from an outside hospital or nursing home. Patients who did not meet the criteria of infection were considered to be colonized. Because all patients had positive microbiologic cultures, inadequate empiric antimicrobial therapy was defined as the use of nonefficacious antibiotics for more than 48 hours after specimens were taken.[34] Inadequate antimicrobial therapy included the absence of a prescribed antimicrobial agent directed against the specific class of recovered microorganisms and/or administration of antimicrobial agents to which the microorganism responsible for the infection was resistant.[34] Data collection was abstracted from the inpatient medical record and included age, sex, underlying diseases, hospital unit, hospital LOS, severity of illness calculated by admission APACHE-II score, previous ESBL *E coli* colonization, exposure to the health care system in the previous year, site of infection, ESBL production, *E coli* antimicrobial susceptibility profile, corticosteroid use, use of an immunosuppressive agent and antibiotic(s) (≥1 standard dose in >2 hours) in the previous year before admission, antimicrobial therapy, receipt of inadequate antimicrobial therapy (as previously defined),[34] crude mortality, and estimated costs of hospitalization. Cost data were obtained from the hospital cost accounting database. Hospital costs represented the sum of direct and indirect costs required to provide health care services and medications per the participants' evaluation and management plan. All costs in Thai baht currency were converted to US dollars at an exchange rate of 40 baht per 1 US dollar.

### Case-case-control study

The primary objectives of this study were to identify the clinical risks and molecular epidemiology (antimicrobial susceptibility patterns, transmission, and strain types) associated with CO-ESBL-producing *E coli* infections. In addition, we characterized associated hospital resource utilization and estimated costs of care for these hospitalized adults. A case-case-control study was performed. Case group (CG) I patients were infected with ESBL-producing *E coli* and met the definition of CO-ESBL. Case group (CG) II patients were infected with CO-non-ESBL-producing *E coli*. They were randomly selected from source records of the hospital's clinical microbiological laboratory of patients admitted within ±7 days that CG I was identified and had comparable site of infections to CG I. Control patients were without infections and were randomly selected from the same source record of patients admitted within ±7 days that CG I was identified. Risk factors were evaluated for an interval of 1 year prior to admission up until the occurrence of CO-ESBL-producing *E coli* for CG I and until the occurrence of CO-non-ESBL-producing *E coli* for CG II and until end of hospitalization for control patients.

### Microbiology methods

Identification of *E coli* isolates was performed by the VITEK System (bioMerieux, Hazelwood, MO). Susceptibilities to all antimicrobial agents were determined by the VITEK system and disk diffusion method and were interpreted according to the guidelines by the Clinical and Laboratory Standards Institute (CLSI).[35] ESBL production was confirmed by double disk synergy test according to CLSI guidelines, and minimum inhibitory concentration (MIC) values were determined by the E-test method (AB Biodisk, Solna, Sweden). We considered ESBL-producing *E coli* isolates to be multidrug resistant (MDR) if resistant to more than 2 classes of other antimicrobial agents (quinolones, trimethoprim/sulfamethoxazole, or aminoglycosides).

### Polymerase chain reaction detection, molecular characterization, and typing of ESBL genes

ESBL-producing *E coli* isolates were further characterized for genes encoding for β-lactamase enzymes. Specific polymerase chain reaction (PCR) amplifications of $bla_{TEM}$, $bla_{SHV}$, and $bla_{CTX\text{-}M}$ genes were performed to determine the presence of TEM, SHV, and CTX-M families, respectively, using primers described previously.[36,37] The PCR reaction used was 95°C for 5

minutes, 30 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, followed by 72°C for 5 minutes. The PCR products of the entire ESBL gene were subject to DNA sequencing to characterize genetically the type of ESBL in each family. The DNA sequences were analyzed by the 3100 Genetic Analyzer (Applied Biosystems, CA) according to the manufacturer's recommendation. Genomic DNA of 25 CO-ESBL-producing *E coli* isolates were compared by pulsed-field gel electrophoresis analysis for molecular typing.[38] Genomic DNA was prepared and cleaved by *Xba*I. The pulsed-field gel electrophoresis DNA patterns were analyzed by using a CHEF Mapper XA system (Bio-Rad, Hercules, CA).

### Statistical analysis

Data analysis was performed using SPSS version 10.0 (SPSS, Chicago, IL). Categorical variables were compared using $\chi^2$ or Fisher exact test, as appropriate. Continuous variables were compared using the Wilcoxon rank sum test. All *P* values were 2-tailed; $P < .05$ was considered statistically significant. Adjusted odds ratios (aOR) and 95% confidence intervals (CI) were computed for the significant factors. Variables that were present in >10% of CO-ESBL or CO-non-ESBL patients with $P < .20$ or that had a priori clinical significance were entered into backward stepwise logistic regression models.

Building of the model began with inclusion of certain key variables based on a priori hypotheses (ie, previous antibiotic exposure and type of antibiotic exposure) as well as variables (eg, age, sex, and underlying disease) considered likely to influence the association between the key variables and the outcome of interest. Significant variables that were thought to covary were grouped, and only 1 variable from each group was chosen for entry into the model. The final model was chosen on the basis of biologic plausibility and by selecting the logistic regression model with the lowest −2-log likelihood function. In evaluating the association between CO-ESBL-producing *E coli* or CO-non-ESBL-producing *E coli* infection and various outcomes (crude mortality, hospital LOS, cost of hospitalization), we controlled for certain variables (ie, APACHE II score), which, on the basis of a priori hypotheses, were believed likely to influence the association between CO-ESBL-producing *E coli* or CO-non-ESBL-producing *E coli* infections and outcomes of interest.

## RESULTS

### Patient characteristics

During the study period, 74 (9%) of 788 *E coli* culture specimens from 46 patients were confirmed as CO-ESBL *E coli*. Each patient had a single episode of CO-ESBL infection. Among the 46 patients, the median age was 64 years (range, 16-89 years), 33 (72%) were female, 19 (41%) had diabetes, 12 (26%) had stroke, 18 (39%) had received antibiotics within the prior 3 months, and 12 (26%) had prior (<3 months) documented ESBL *E coli* colonization. Twenty-eight patients (61%) had never been hospitalization, and 18 (39%) had been hospitalized >3 months prior to the index admission. Sixty-seven percent (31/46) of patients with CO-ESBL infections had urinary tract infection, 20% (9/46) had bloodstream infection, and 8.7% (4/46) had pneumonia.

### Risk factors and outcomes

Demographic and baseline clinical comorbidities for case and control patients were summarized in Table 1. In multivariate analyses, CG I were more likely than control patients to have diabetes (aOR, 4.41; 95% CI: 1.9-13.2; $P < .001$), prior (<90 days) ESBL-producing *E coli* colonization (aOR, 11.4; 95% CI: 1.2-67.8; $P < .001$), and recent receipt of antibiotics (<90 days) (aOR, 15.1; 95% CI: 4.2-44.2; $P = .004$), especially with third-generation cephalosporins (aOR, 5.69; 95% CI: 2.2-16.4; $P = .001$) and fluoroquinolones (aOR, 3.63; 95% CI: 1.4-18.3; $P = .003$). By multivariate analyses, CG II were more likely than control patients to have diabetes (aOR, 4.2; 95% CI: 1.6-15.4; $P = .005$), stroke (aOR, 3.46; 95% CI: 1.5-17.1; $P = .001$), and diarrhea (aOR, 16; 95% CI: 3.8-65.8; $P = .001$). Antibiotic exposures and multivariate analysis of risk factors for CO-ESBL-producing *E coli* and CO-non-ESBL-producing *E coli* infections in the study population are summarized in Tables 2 and 3, respectively.

The crude mortality among patients with CO-ESBL infection was 30% (14/46). Seventeen patients (37%) received inadequate antimicrobial therapy, and the median time of delay (with the interval onset at time of specimen's procurement) to receive appropriate antibiotic regimens was 2 days (range, 1-6 days). CG I patients had prolonged hospital LOS (median, 8 vs 5 days, respectively; $P < .001$), higher mortality (30% vs 0%; $P < .001$, respectively), and excess total hospital costs (median, US $528 vs $108, respectively; $P < .001$), and there was no difference in the outcomes between CG II patients compared with control patients (Table 1). Notably, no risk factors associated with MDR CO-ESBL-producing *E coli* infections were predictors of mortality in both CGs.

### Antimicrobial susceptibility and MIC 90%

The 74 CO-ESBL-producing *E coli* isolates demonstrated variable resistance to other antibiotics and antibiotic classes (Fig 1). Although 29 CO-ESBL-producing

**Table 1.** Demographic and clinical characteristics and outcomes among patients with community-onset extended-spectrum β-lactamase-producing *Escherichia coli*, CG I, and patients with CO-non-ESBL-producing *Escherichia coli*, CG II, compared with control patients

| Variable | CG I (n = 46) | CG II (n = 46) | Control (n = 138) |
|---|---|---|---|
| Age, median yr (range) | 64 (16-89) | 63 (18-82) | 62 (15-88) |
| Male sex | 13 (29) | 13 (28) | 45 (33) |
| Underlying diseases | | | |
| Diabetes | 19 (41)* | 18 (39)* | 19 (14) |
| Stroke | 12 (26) | 17 (37)* | 20 (15) |
| Chronic kidney disease | 7 (15) | 6 (14) | 17 (12) |
| Malignancy | 6 (13) | 5 (11) | 17 (12) |
| Others† | 3 (6) | 3 (6) | 11 (8) |
| Admission APACHE-II score, median (range) | 10 (1-25) | 8 (2-20) | 7 (1-19) |
| Diarrhea‡ | 5 (11) | 9 (20)§ | 8 (6) |
| Previous receipt of corticosteroid or immunosuppressant therapy[c] | 3 (6) | 3 (6) | 6 (4) |
| Previous hospitalization‖ | 19 (41)* | 0 (0)§ | 21 (15) |
| Previous colonization with ESBL-producing *E coli*‖ | 12 (26)* | 0 (0) | 0 (0) |
| Previous receipt of antibiotic‖ | 18 (39)* | 6 (4) | 6(4) |
| Receipt of inadequate antimicrobial therapy | 17 (37) | 6 (4) | NA |
| Outcomes | | | |
| Crude mortality | 14 (30)* | 3 (6) | 0 (0) |
| Length of hospitalization, median (range) | 8 (1-43)* | 6 (3-14) | 5 (3-13) |
| Cost (US $), median (range) | 528 (43-3173)* | 120 (29-850) | 108 (23-790) |

Data are number (%), unless otherwise indicated.
*CG*, case group; *NA*, nonapplicable; *APACHE-II* score, Acute Physiology and Chronic Health Evaluation-II score.
*$P < .001$.
†Included human immunodeficiency virus and chronic liver diseases.
‡Seventy-two hours prior to isolation of CO-ESBL for CG I and CO-non-ESBL *E coli* for CG II versus for the entire duration of hospitalization for control patients.
§$P < .05$.
‖Defined as positive clinical cultures in patients without evidence of infection and not treated with antibiotic within previous 3 months.

*E coli* (39%) isolates fulfilled the MDR criteria, none were resistant to imipenem, meropenem, or ertapenem. The MIC 90% ($MIC_{90}$) for ceftazidime, cefotaxime, ceftriaxone, imipenem, meropenem, and ertapenem were 96 mg/L (range, 0.5-256 mg/L), 256 mg/L (range, 4-256 mg/L), 256 mg/L (range, 2-256 mg/L), 0.5 mg/L (range, 0.09-1.5 mg/L), 0.09 mg/L (range, 0.01-4 mg/L), and 0.38 mg/L (0.01-1.5 mg/L), respectively. Notably, there was a high prevalence of resistance to piperacillin-tazobactam (60%) and cefpirome (70%), with 81% concordance for susceptibility of these 2 agents. The proportion of patients with isolates resistant to other agents was similar for isolates with resistance to cefepime and piperacillin-tazobactam.

**Table 2.** Cumulative exposures to antimicrobial agents within 90 days of hospitalization among 230 study participants in a case-case-control study of community-onset *Escherichia coli* infections.

| Antibiotic class | CG I (n = 46) | CG II (n = 46) | Control (n = 138) |
|---|---|---|---|
| First-generation cephalosporin | 2 (5) | 2 (5) | 17 (12) |
| Second-generation cephalosporin | 3 (7) | 2 (5) | 11 (8) |
| Third-generation cephalosporin | 15 (33)* | 5 (11) | 11 (8) |
| Macrolides | 2 (5) | 3 (7) | 7 (5) |
| Penicillin | 3 (7) | 2 (5) | 7 (5) |
| Quinolones | 13 (28)† | 8 (17) | 20 (15) |
| Trimethoprim-sulfamethoxazole | 2 (5) | 4 (9) | 10 (7) |
| Aminoglycosides | 8 (18) | 6 (13) | 17 (12) |
| Others‡ | 1 (3) | 1 (3) | 7 (5) |

Data are number (%), unless otherwise indicated. Case group (CG) I: Patients with CO-ESBL-producing *E coli* infections. Case group (CG) II: Patients with CO-non-ESBL-producing *E coli* infections.
*$P < .001$.
†$P < .05$.
‡Including doxycycline, chloramphenicol, glycopeptides and carbapenems.

## Genetic characterization and molecular typing

Isolates from 25 cases were available for molecular characterization focused on the major ESBL types (TEM, SHV, and CTX-M). CTX-M type ESBL was detected in 16 patients' isolates: 13 had CTX-M-15, whereas 3 had CTX-M-55. Seven of the isolates also carried the TEM-1 gene. One patient had SHV-1. Among the other 9 patients, none of the CTX-M, TEM, or SHV types of ESBL were found. Eight of these isolates also carried TEM-1.

## DISCUSSION

Previous studies suggested that most ESBL-producing bacteria show cross-resistance to multiple antibiotics, and the prevalence of CO-ESBL-producing *E coli* infections varies widely by geographic region.[11-29] Data on risk factors of CO-ESBL-producing *E coli* infection are limited to 2 studies: one study in nonhospitalized patients[12] and another focused on urinary tract infections.[29] In nonhospitalized adults, recent hospitalization; advanced age; diabetes; male sex; *Klebsiella pneumoniae* infections; and prior use of second- and third-generation cephalosporins, quinolones, and penicillin were associated with CO-ESBL-producing *E coli* infections.[12] Among patients with urinary tract infections because of CO-ESBL-producing *E coli*, the only significant risk was exposure to second-generation cephalosporins.[29] Notably in these 2 studies, patients with non-ESBL-producing *E coli* infection were selected for the comparator group.

From the perspective of study design and analyses, studies such as these that evaluate risk factors in

**Table 3.** Multivariate analysis of risk factors among 2 case groups of patients infected with *Escherichia coli* versus uninfected control patients

| Variable | Adjusted odds ratio | 95% Confidence interval | *P* value |
|---|---|---|---|
| Case group I | | | |
| Diabetes | 4.41 | 1.9-13.2 | <.001 |
| Previous *E coli* ESBL colonization* | 11.4 | 1.2-67.8 | <.001 |
| Recent receipt of antibiotic(s)* | 15.1 | 4.2-44.2 | .004 |
| Recent exposure to third-generation cephalosporins* | 5.69 | 2.2-16.4 | .001 |
| Recent exposure to fluoroquinolone | 3.63 | 1.4-18.3 | .003 |
| Case group II | | | |
| Diabetes | 4.2 | 1.6-15.4 | .005 |
| Stroke | 3.46 | 1.5-17.1 | .001 |
| Diarrhea† | 16 | 3.8-65.8 | .001 |

Case group (CG) I: Patients with CO-ESBL-producing *E coli* infections. Case group (CG) II: Patients with CO-non-ESBL-producing *E coli* infections.

*Defined as positive clinical cultures in patients without evidence of infection and not treated with antibiotic within previous 3 months.

†Seventy-two hours prior to isolation of CO-ESBL for CG I and CO-non-ESBL-producing *E coli* for CG II versus for the entire duration of hospitalization for control patients.

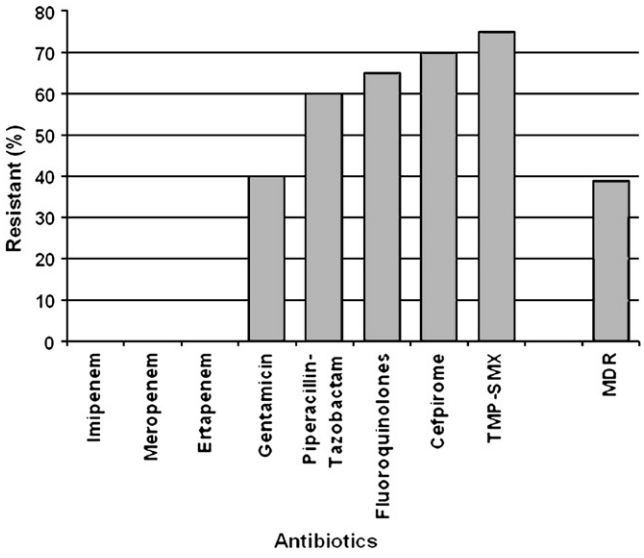


**Fig 1.** Antimicrobial susceptibilities of extended-spectrum β-lactamase-producing *E coli* for July 1, 2003, through June 30, 2004 (n = 74). MDR, multidrug.

at-risk hospitalized patients are methodologically complex. Harris et al have suggested that using control patients infected with susceptible pathogens bias studies from the null hypothesis.[39] Furthermore, prior treatment with antimicrobial agents to which an organism is susceptible may prevent infection with that organism, and exposure to those antimicrobial agents are likely to be less frequent among the population from which cases arise.[40] Hence, we used case-case-control study to assess best the identifiable risk factors associated with CO-ESBL-producing *E coli*. Kaye et al first described the advantage of using this study design, which permits meaningful comparison of the 2 resulting risk models (ie, resistant phenotype vs control and susceptible phenotype vs control) because both CGs were compared with the same control patients.[41] After 2 separate risk models were compared and contrasted, we identified previous CO-ESBL colonization and recent exposure to antibiotics, especially to third-generation cephalosporins and fluoroquinolones, as risk factors associated with CO-ESBL-producing *E coli* and patient-level risk factor (stroke) and diarrhea as risk factors associated with CO-non-ESBL-producing *E coli*, whereas diabetes was a common risk factor among both CGs. The distinguishable pulsed-field gel electrophoresis patterns among all the ESBL isolates, together with the epidemiologic data, highlight the need for physicians to have differential clinical suspicion for CO-ESBL-producing *E coli* infections in at-risk adults, as well as the need for prudent antibiotic use in community-based settings to prevent and control the spread of such infections.

Previous reports suggested that infection with health care-associated ESBL-producing *E coli* or *K pneumoniae* were independent predictors of longer LOS, higher mortality, and excess hospital charges.[42,43] To our knowledge, we are the first to demonstrate an association between CO-ESBL-producing *E coli* infection and increased mortality, longer hospital LOS, and excess cost of hospitalization. No such associations were identified in patients with CO-non-ESBL-producing *E coli* infection. Because the major type of ESBL identified in our study belonged to the CTX-M-1 family (CTX-M-15 and CTX-M-55),[44] our result on outcomes may not be applicable to other ESBL types. As the number of reports increases for CO-ESBL-producing *E coli* CTX-M and associated disease,[28,29] it becomes clear that the epidemiology of CTX-M enzyme is distinct from that of TEM- and SHV-derived ESBLs.

There are several recognized limitations to our study. Although selection bias exists in all observational studies, we attempted to reduce such bias by inclusion of all consecutive cases through our hospital's clinical microbiological laboratory. To minimize misclassification bias, both CGs and control participants were identified from available antimicrobial susceptibility data. Differential misclassification was unlikely because microbiologic studies were conducted without knowledge of patients' exposures and outcomes.

Limitations related to case-case-control study design may also include the fact that the control group may not represent the exact source of the populations for each CG, and certain case variables were not used for matching when control patients are selected because there were 2 different case groups.[41] Among the available isolates, we examined for TEM-, SHV-, and CTX-M-derived types and, hence, may have under detected other ESBL strains. It is likely that other uncommon types of ESBL (eg, VEB, PER, GES) may also contribute to the resistance phenotypes of these strains. Furthermore, enteric carriage of CO-ESBL-producing *E coli* was not assessed, and only 48% of patients had specimens available for molecular investigations. The small sample size also limited our capacity to detect other possible risk factors and outcomes of CO-ESBL-producing *E coli* infections. Last, we only included patients who required hospitalization. Thus, risk factors and outcomes of patients with CO-ESBL-producing *E coli* infections who were treated as outpatients were not assessed.

In conclusion, CO-ESBL-producing *E coli* strains are emerging MDR organisms in Thailand. Patients with CO-ESBL-producing *E coli* infections also had prolonged hospital LOS, increased mortality, and excess estimated costs of hospitalization. Physicians should be aware of the occurrence of CO-ESBL-producing *E coli* among high-risk patients and target initial appropriate empiric antimicrobial therapy to reduce mortality in such patients. When treatment protocols are designed, the prevalence of CO-ESBL isolates must be taken into consideration, and, on this basis, a rational choice of empirical antibiotic therapy can then be recommended. Our data describe the first occurrence of CO-ESBL-producing *E coli* in Thailand and highlight the need for additional clinical and molecular epidemiologic studies, along with intervention trials, to help minimize the emergence of endogenous and exogenous CO-ESBL microorganisms.

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