Risk Factors and Molecular Epidemiology of Community-Onset Extended-Spectrum β-Lactamase-Producing Escherichia coli Bacteremia

Yoon Soo Park,1 Il Kwon Bae,2 Juwon Kim,3 Seok Hoon Jeong,2 Seung-sik Hwang,4 Yiel-Hea Seo,5 Yong Kyun Cho,1 Kyungwon Lee,2 and June Myung Kim6

1Department of Internal Medicine, Gachon University, Gil Medical Center, Incheon; 2Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, Seoul; 3Department of Laboratory Medicine, Yonsei University Wonju College of Medicine, Wonju; 4Department of Social and Preventive Medicine, Inha University School of Medicine, Incheon; 5Department of Laboratory Medicine, Gachon University, Gil Medical Center, Incheon; 6Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea.

Purpose: Inadequate empirical therapy for severe infections caused by extended-spectrum β-lactamase-producing Escherichia coli (ESBLEC) is associated with poor outcomes. This study was designed to investigate risk factors for community-onset ESBLEC bacteremia at admission to a tertiary care hospital. Materials and Methods: A case-control study was performed that included all episodes of ESBLEC bacteremia in the outpatient department or within 48 hours of admission from January 2005 to March 2009. Data on predisposing factors were collected. The molecular epidemiology of ESBLEC clinical isolates was also determined. Results: Among 25281 blood cultures, 60 episodes of ESBLEC bacteremia were studied, which accounted for 7% of all Escherichia coli bacteremia at admission. Healthcare-associated infection [odds ratio (OR), 8.3; 95% confidence interval (CI), 2.4-28.7; p=0.001], malignancy (OR, 4.6; 95% CI, 1.3-16.3; p=0.018), urinary tract infection (OR, 139.1; 95% CI, 24.6-788.2; p<0.001), hepatobiliary infection (OR, 79.1; 95% CI, 13.5-463.8; p<0.001), third generation cephalosporin usage during preceding 3 months (OR, 16.4; 95% CI, 2.0-131.8; p=0.008), and severe sepsis/septic shock (OR, 73.7; 95% CI, 12.4-438.5; p<0.001) were determined as independent risk factors for community-onset ESBLEC bacteremia. The most common extended-spectrum β-lactamase (ESBL) gene identified was blaCTX-M-15 (n=31) followed by blaCTX-M-14 (n=23). Conclusion: The most common types of ESBLs in E. coli causing community-onset bacteremia were CTX-M-15 and CTX-M-14 in Korea. By result of decision tree analysis, the empirical use of carbapenems is suggested only for patients with severe sepsis/septic shock, hepatobiliary infection, or healthcare-associated urinary tract infection.

Key Words: Risk factors, beta-lactamase, Escherichia coli, CTX-M

INTRODUCTION

Extended-spectrum β-lactamase-producing Escherichia coli (ESBLEC) is an emerg-
ing cause of nosocomial, healthcare-associated, and community-acquired infections worldwide. Inadequate empirical antibiotic therapy for infections caused by this microorganism is associated with poor outcomes, especially in severe infections. Considering the increased prevalence of ESBLEC, elucidation of risk factors for ESBLEC bacteremia is critical in terms of empirical treatment of the patients. Although there have been several studies for infections caused by extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, only a few studies have investigated the risk factors for ESBLEC bacteremia.

The CTX-M enzymes are spreading rapidly and are now the dominant type of ESBL in E. coli in many parts of the world. Among CTX-M enzymes, members of the CTX-M-1 and CTX-M-9 clusters have repeatedly been found worldwide including Korea.

This study was designed to investigate risk factors for community-onset ESBLEC bacteremia at the time of admission to a tertiary care hospital. Decision-tree analysis using the classification and regression tree (CART) algorithm was performed to predict which subgroup of patients who had a blood culture within 48 hours of admission was at increased risk of being infected by ESBLEC bacteremia. The molecular epidemiology of ESBLEC isolates obtained from patients with bacteremia was also determined.

**MATERIALS AND METHODS**

**Background, setting, and design**

This study was conducted at the Gachon University Gil Medical Center, a 1200 bed tertiary care facility located in Incheon, Republic of Korea. The ESBLEC strains were isolated from the blood cultures of patients from January 2005 through March 2009.

The risk factors for community-onset ESBLEC bacteremia were investigated using a case-control design. A case was defined as an adult (>18 years) with ESBLEC bacteremia that was present in the outpatient department or within 48 hrs of admission to the hospital. Patients with positive blood cultures for ESBLEC, which recovered after 48 hours of admission, were excluded from the study. The first blood isolate per case was studied. Controls were chosen among the patients who had a blood culture performed in the outpatient department or within 48 hrs of admission in the study period if their blood culture did not yield ESBLEC. For each case, three controls were randomly selected. Patients who were ≤18 years old or did not have a blood culture within 48 hrs of admission were excluded from the control group.

Variables analyzed as possible risk factors included age, sex, associated diseases, severity of comorbidity according to the Charlson score, healthcare-associated infection, source of bacteremia, invasive procedure such as urinary catheter or tracheostomy during the preceding three months, antimicrobial therapy during preceding three months, presence of severe sepsis or septic shock, and severity of illness as calculated by the Pitt bacteremia score.

The presence of the following associated diseases was documented: diabetes mellitus, heart failure, chronic pulmonary disease, chronic renal insufficiency, liver cirrhosis, and malignancy. Healthcare-associated infections were classified in accordance with the definition by Friedman, et al., with some modifications. Any of the following criteria were considered as healthcare-associated infections: intravenous therapy, wound care, or nursing care received at home 30 days before the bloodstream infection; attendance at a hospital or hemodialysis clinic or receipt of intravenous chemotherapy 30 days before the bloodstream infection; >48-hour hospital admission or performance of invasive procedures such as urinary catheter, endoscopy, and naso-gastric tube 90 days before the bloodstream infection; or residence at a nursing home or long-term care facility. Source of the infection was determined to be the urinary tract, hepatobiliary, gastrointestinal, respiratory, other soft-tissue infection, or primary bloodstream infection.

The study was approved by the Institutional Review Boards of the hospital (GIRBA 2212).

**Microbiologic studies**

Isolates were identified using a Vitek GNI card (bioMérieux, Marcy l’Etoile, France). Antimicrobial susceptibilities were tested by disk diffusion test on Mueller-Hinton agar (Difco, Cockeysville, MI, USA) and by the agar dilution method according to the interpretative criteria proposed by the Clinical and Laboratory Standards Institute. The phenotypic confirmatory test for ESBL and/or AmpC β-lactamase was performed. Detection of genes coding for plasmid-borne ESBLs and AmpC β-lactamas was performed by PCR amplification with primers as described previously. The templates for PCR amplification in clinical isolates were whole cell lysates, and the PCR products were subjected to direct sequencing. The agar mating method was used to test transferability of oxyimino-cephalosporin resistance determinants using azide-resistant E. coli J53 as a re-
cipient. Transconjugants were selected on Mueller-Hinton agar plates supplemented with 2 µg/mL cefotaxime and 100 µg/mL sodium azide.13

Pulsed-field gel electrophoresis (PFGE) was performed with XbaI restriction enzyme using a CHEF-DRII device (Bio-Rad, Hercules, CA, USA). Tiff format gel images were exported to Molecular Analyst Fingerprinting Software Ver. 3.2 (Bio-Rad) for analysis. Comparisons for E. coli isolates were made by using the band-based dice coefficient. Dendrograms were generated using the unweighted pair group method with arithmetic averages method with 1.0% position tolerance. Multilocus sequence typing (MLST) was performed on ESBL-producing isolates using seven conserved housekeeping genes (adk, fumC, gyrB, icd, mdh, purA, and recA) following protocols at http://mlst.ucc.ie/mlst/dbs/Ecoli.

Statistical analysis
Univariate analyses were performed separately for each of the variables. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for binomial variables. p values were calculated using Fisher’s exact test for categorical variables. Variables with a p value of <0.1 in the univariate analysis were candidates for multivariate analysis using a backward elimination method. The area under the receiver operator characteristic (ROC) curve was calculated to evaluate the performance of the models. To split patients into more homogeneous subgroups, a CART analysis was used to build a binary classification tree through recursive partitioning. All tests were 2-tailed, and a p value of <0.05 was considered significant in the multivariable model. STATA software package version 10.0 (StataCorp, College Station, TX, USA) was used to perform the multiple logistic regression analysis, and R 2.4.1 (The R foundation for statistical computing) was used to construct the CART algorithm.

RESULTS

During the study period, 25281 blood cultures were taken from adult patients in the outpatient department or within 48 hours of admission to the Gil Hospital. Among them, 3452 episodes of positive blood cultures (13.7%) were for any organism including 891 E. coli were found, among which 62 were ESBL producers by phenotypic analysis. Since the ESBL gene could not be detected in two isolates, risk factors and microbiological profiles were assessed in 60 patients with ESBL-producing isolates in community-onset bacteremic E. coli.

Risk factors for ESBL-producing isolates

Univariate analysis showed that age >60 years old; liver cirrhosis; malignancy; Charlson comorbidity index; healthcare-associated infection; urinary tract, hepatobiliary, or respiratory tract as the infection source; indwelling urinary catheter; naso-gastric tube; third generation cephalosporin and quinolone usage during preceding three months of admission; septic shock/severe sepsis; and severity of illness as calculated by the Pitt bacteremia score were associated with ESBL-producing isolates in community-onset bacteremic E. coli. Annual percentages of different ESBL genes were shown in Table 1. The most common type of ESBL gene identified was blaCTX-M-15 (n=31) followed by blaCTX-M-14 (n=23). Other types of ESBL gene, such as blaCTX-M-22 (n=2), blaCTX-M-24 (n=1), blaCTX-M-57 (n=1), and blaSHV-12 (n=2) were also identified. Annual percentages of ESBL-producing isolates in community-onset bacteremic E. coli were calculated using Fisher’s exact test for categorical variables. Values of <0.1 in the univariate analysis were candidates for multivariate analysis using a backward elimination method. The area under the receiver operator characteristic (ROC) curve was calculated to evaluate the performance of the models. To split patients into more homogeneous subgroups, a CART analysis was used to build a binary classification tree through recursive partitioning. All tests were 2-tailed, and a p value of <0.05 was considered significant in the multivariable model. STATA software package version 10.0 (StataCorp, College Station, TX, USA) was used to perform the multiple logistic regression analysis, and R 2.4.1 (The R foundation for statistical computing) was used to construct the CART algorithm.

Microbiologic results

All 60 isolates showed positive results in the phenotypic confirmatory test, indicating ESBL production. The most common type of ESBL gene identified was blaCTX-M-15 (n=31) followed by blaCTX-M-14 (n=23). Other types of ESBL gene, such as blaCTX-M-22 (n=2), blaCTX-M-24 (n=1), blaCTX-M-57 (n=1), and blaSHV-12 (n=2) were also identified. Annual percentages of ESBL-producing isolates in community-onset bacteremic E. coli.
was identified in three isolates carrying the \textit{bla}_{CTX-M-15} gene and one isolate carrying the \textit{bla}_{CTX-M-14} gene. Both isolates carrying the \textit{bla}_{SHV-12} gene also carried the \textit{bla}_{DHA-1} gene.

Despite repeated attempts, transconjugants were obtained from only 21 of 60 isolates.

After all of the 60 isolates were subjected to MLST analysis, 15% of isolates showed the CTX-M-1 group in 2005-2007 and the CTX-M-9 group in 2008-2009 (86% and 57%, respectively; \( p<0.001 \)).

Table 1. Univariate Analysis of Risk Factors for Community-Onset ESBL-Producing \textit{Escherichia coli} Bacteremia

| Risk factor                              | Cases (n=60) | Controls (n=180) | OR (95% CI) | \( p \) value |
|------------------------------------------|--------------|------------------|-------------|-------------|
| Age >60 yrs                               | 44 (73)      | 73 (41)          | 4.0 (2.1-7.7)| <0.001      |
| Female sex                                | 28 (47)      | 78 (43)          | 1.1 (0.6-2.1)| 0.66        |
| Associated disease                        |              |                  |             |             |
| Diabetes mellitus                         | 16 (27)      | 32 (18)          | 1.7 (0.8-3.3)| 0.14        |
| Heart failure                             | 7 (12)       | 12 (7)           | 1.8 (0.7-4.9)| 0.27        |
| Chronic pulmonary disease                 | 1 (2)        | 9 (5)            | 0.3 (0.04-2.6)| 0.46       |
| Chronic renal insufficiency               | 3 (5)        | 5 (3)            | 1.8 (0.4-8.0)| 0.42        |
| Liver cirrhosis                           | 11 (18)      | 4 (2)            | 9.9 (3.0-32.4)| <0.001     |
| Malignancy                                | 24 (40)      | 19 (11)          | 5.6 (2.8-11.4)| <0.001     |
| Charlson comorbidity index \( \geq 2 \)  | 40 (67)      | 49 (27)          | 5.3 (2.9-10.0)| <0.001     |
| Healthcare-associated infection           | 51 (85)      | 87 (48)          | 6.1 (2.8-13.0)| <0.001     |

Source of infection                        |              |                  |             |             |
| Primary                                   | 6 (10)       | 22 (12)          | 0.8 (0.3-2.1)| 0.82        |
| Urinary                                   | 33 (55)      | 22 (12)          | 8.8 (4.5-17.3)| <0.001     |
| Hepatobiliary                             | 26 (43)      | 15 (8.3)         | 8.4 (4.0-17.5)| <0.001     |
| Gastrointestinal                          | 5 (8)        | 26 (14)          | 0.5 (0.2-1.5)| 0.27        |
| Respiratory                               | 5 (8)        | 48 (27)          | 0.3 (0.09-0.7)| 0.002       |
| Skin and soft tissue                      | 1 (2)        | 14 (8)           | 0.2 (0.03-1.6)| 0.12        |

Device                                     |              |                  |             |             |
| Urinary catheter                          | 15 (25)      | 10 (6)           | 5.7 (2.4-13.5)| <0.001     |
| Tracheostomy/intubation                    | 3 (5)        | 3 (2)            | 3.1 (0.6-15.8)| 0.17        |
| Naso-gastric tube                          | 6 (10)       | 5 (3)            | 3.9 (1.1-13.2)| 0.03        |

Previous antimicrobial usage                |              |                  |             |             |
| Cephalosporins                            |              |                  |             |             |
| First generation                           | 4 (7)        | 4 (2)            | 3.1 (0.8-13.0)| 0.11        |
| Second generation                          | 2 (3)        | 3 (2)            | 2.0 (0.3-12.5)| 0.60        |
| Third generation                           | 17 (28)      | 7 (4)            | 9.8 (3.8-25.0)| <0.001     |
| Penicillins                                | 2 (3)        | 1 (1)            | 6.2 (0.6-69.3)| 0.15        |
| Quinolones                                 | 10 (17)      | 5 (3)            | 7.0 (2.3-21.4)| 0.001       |
| Septic shock/severe sepsis                | 26 (43)      | 10 (6)           | 13.0 (5.7-29.4)| <0.001     |
| Pitt bacteremia score \( \geq 2 \)        | 24 (40)      | 25 (14)          | 4.1 (2.1-8.1)| <0.001      |

ESBL, extended-spectrum \( \beta \)-lactamase; OR, odds ratio; CI, confidence interval.

Data are presented as no. (%) of patients.

Table 2. Multivariate Analysis of Risk Factors for Community-Onset ESBL-Producing \textit{Escherichia coli} Bacteremia

| Risk factor                              | Adjusted OR (95% CI) | \( p \) value |
|------------------------------------------|----------------------|--------------|
| Healthcare-associated infection           | 8.3 (2.4-28.7)       | 0.001        |
| Malignancy                               | 4.6 (1.3-16.3)       | 0.018        |
| Urinary tract infection                   | 139.1 (24.6-788.2)   | <0.001       |
| Hepatobiliary infection                   | 79.1 (13.5-463.8)    | <0.001       |
| Previous third generation cephalosporin usage | 16.4 (2.0-131.8)    | 0.008        |
| Severe sepsis/septic shock                | 73.7 (12.4-438.5)    | <0.001       |

ESBL, extended-spectrum \( \beta \)-lactamase; OR, odds ratio; CI, confidence interval.

\textit{Escherichia coli} increased from 1% in 2005 to 21% in 2009 (\( p<0.001 \)). Among ESBL-EC isolates harboring \textit{bla}_{CTX-M} genes, the predominant CTX-M group was the CTX-M-1 group in 2005-2007 and the CTX-M-9 group in 2008-2009 (86% and 57%, respectively; \( p=0.002 \)) (Table 3). Six ESBL-producing isolates also carried an AmpC gene. The \textit{bla}_{CMY-2} gene was identified in three isolates carrying the \textit{bla}_{CTX-M-15} gene and one isolate carrying the \textit{bla}_{CTX-M-14} gene. Both isolates carrying the \textit{bla}_{SHV-12} gene also carried the \textit{bla}_{DHA-1} gene. Despite repeated attempts, transconjugants were obtained from only 21 of 60 isolates.

After all of the 60 isolates were subjected to MLST analy-
molecular epidemiology of community-onset ESBLEC bloodstream infection. Because ESBL-producing bacteria are often resistant to various antimicrobials including fluoroquinolones and oxyimino-cephalosporins, the presence of these enzymes complicates the selection of empirical antimicrobials while the results of cultures and antimicrobial susceptibility profiles are awaited. Several studies have addressed the impact of inadequate empirical antimicrobial treatment in patients with infections caused by ESBL producers. A delay or failure in initiating adequate antimicrobial therapy was associated with increased morbidity and mortality in severe infections. Moreover, in septic patients, the importance of appropriate empirical therapy has also emphasized. For effective empirical treatment, risk factor analysis for any bloodstream infection by ESBL producers has important clinical implications. Although there have been numerous reports of infections or colonization with ESBL-producing organisms in recent years, only five studies have been conducted to analyze the risk factors of ESBLEC.

Table 3. Percentage of ESBL Production and Distribution of CTX-M Enzyme in Community-Onset Escherichia coli Bacteremia

| Yr | No. of isolates | Blood culture | E. coli | ESBL producer (% of E. coli) | CTX-M | CTX-M-1 group | CTX-M-9 group | SHV-12 |
|----|----------------|---------------|--------|-----------------------------|-------|---------------|---------------|--------|
|    |                |               |        |                             |       | M-15          | M-22          | M-57   |
|    |                |               |        |                             |       | M-14          | M-24          |        |
| 2005 | 5598           | 191           | 2 (1)  | 2                           | 2     |               |               |        |
| 2006 | 5280           | 201           | 12 (6) | 10                          | 8     | 1             |               |        |
| 2007 | 5897           | 187           | 9 (5)  | 9                           | 6     | 1             |               |        |
| 2008 | 6768           | 245           | 23 (9) | 23                          | 9     |               |               | 14     |
| 2009*| 1738           | 67            | 14 (21)| 14                          | 6     | 1             |               | 6      |
| Total| 25281          | 891           | 60 (7) | 58                          | 31    | 2             | 1             | 23     |

ESBL, extended-spectrum β-lactamase.
*From January to March, 2009.

This study was designed to identify the best predictors and...
bloodstream infections. Although two studies have investigated the risk factors for community-onset bloodstream infections of ESBL EC, as risk factors may differ according to geographic variation and patient population, empirical antibiotic choices should be individualized.

In the present study, we selected control patients from all adult patients who had a blood culture in the outpatient department or within 48 hours of admission to Gil Hospital to investigate the risk factors of ESBL-EC bacteremia among patients who were septic; therefore blood cultures were taken at presentation. Although some of the documented risk factors in this study might be those of a susceptible organism (non-ESBL producing E. coli), we believe that our results certainly give clinical clues as to what subpopulation of the patients need empirical therapy for ESBL EC bacteremia. If patients infected with the antimicrobial susceptible organism were used as control patients, these ‘susceptible control patients’ (i.e., patients with non-ESBL producing E. coli bacteremia) would not be representative of the ‘source population’ for antimicrobial resistant organisms, and this may lead to an overestimation of the association between antimicrobial exposure and cases.

The CART analysis is a statistical method based on a recursive partitioning analysis. Unlike multivariate logistic regression, by which the odds ratio produced can be difficult to translate, CART is well suited clinical decisions on rules and produces decision trees that are simple to interpret. CART has been successfully used to assist in the diagnosis of various clinical conditions including infections and neurological, oncological, and cardiac disorders. According to our results, the empirical use of carbapenems is suggested for patients with severe sepsis/septic shock, hep-

| Isolate | ESBL gene | Conjugability | ST | adk | fumC | gyrB | icd | mdh | purA | recA |
|---------|-----------|---------------|----|-----|------|------|-----|------|------|------|
| E09258  | blaCTX-M-15| No            | 10 | 10  | 11   | 4    | 8   | 8    | 8    | 2    |
| E07483  | blaCTX-M-15| No            | 617| 10  | 11   | 4    | 8   | 8    | 13   | 74   |
| E09600  | blaCTX-M-15| No            | 648| 92  | 4    | 87   | 96  | 70   | 58   | 2    |
| E07354  | blaCTX-M-15| No            | 648| 92  | 4    | 87   | 96  | 70   | 58   | 2    |
| E08141  | blaCTX-M-15| Yes           | 95 | 37  | 38   | 19   | 37  | 17   | 11   | 26   |
| E08342  | blaCTX-M-15| Yes           | 96 | 8   | 7    | 1    | 8   | 8    | 6    |
| E09156  | blaCTX-M-15| No            | 405| 35  | 37   | 29   | 25  | 4    | 5    | 73   |
| E09971  | blaCTX-M-15| No            | 131| 53  | 40   | 47   | 13  | 36   | 28   | 29   |
| E09115  | blaCTX-M-15| No            | 648| 92  | 4    | 87   | 96  | 70   | 58   | 2    |
| E06238  | blaCTX-M-15| No            | 131| 53  | 40   | 47   | 13  | 36   | 28   | 29   |
| E061324 | blaCTX-M-15| No            | 95 | 37  | 38   | 19   | 37  | 17   | 11   | 26   |
| E06269  | blaCTX-M-15| Yes           | 648| 92  | 4    | 87   | 96  | 70   | 58   | 2    |
| B070151 | blaCTX-M-22| Yes           | 69 | 21  | 35   | 27   | 6   | 5    | 5    | 4    |
| E08581  | blaCTX-M-15| No            | 939| 80  | 4    | 33   | 16  | 7    | 8    | 6    |
| E06070  | blaCTX-M-22| Yes           | 410| 6   | 4    | 12   | 1   | 20   | 18   | 7    |
| E07348  | blaCTX-M-15| No            | 131| 53  | 40   | 47   | 13  | 36   | 28   | 29   |
| E06027  | blaCTX-M-15| No            | 648| 92  | 4    | 87   | 96  | 70   | 58   | 2    |
| E09626  | blaCTX-M-15, blaCTX-M-2 | No | 1177| 4  | 26   | 2   | 211 | 5   | 5    | 19   |
| E09086  | blaCTX-M-15| Yes           | 410| 6   | 4    | 12   | 1   | 20   | 18   | 7    |
| B061647 | blaCTX-M-15| Yes           | 1011| 6  | 4    | 159  | 44  | 112  | 1    | 17   |
| E09601  | blaCTX-M-15| Yes           | 69 | 21  | 35   | 27   | 6   | 5    | 5    | 4    |
| E08013  | blaCTX-M-15| No            | 44 | 10  | 11   | 4    | 8   | 8    | 8    | 7    |
| E09630  | blaCTX-M-15, blaCTX-M-2 | No | 410| 6   | 4    | 12   | 1   | 20   | 18   | 7    |
| E09637  | blaCTX-M-15| Yes           | 410| 6   | 4    | 12   | 1   | 20   | 18   | 7    |
| B061803 | blaCTX-M-15| No            | 648| 92  | 4    | 87   | 96  | 70   | 58   | 2    |
| E08396  | blaCTX-M-15, blaCTX-M-2 | Yes | 410| 6   | 4    | 12   | 1   | 20   | 18   | 7    |
| E05051  | blaCTX-M-15| No            | 410| 6   | 4    | 12   | 1   | 20   | 18   | 7    |
| E07042  | blaCTX-M-15| No            | 648| 92  | 4    | 87   | 96  | 70   | 58   | 2    |
| E07294  | blaCTX-M-15| No            | 131| 53  | 40   | 47   | 13  | 36   | 28   | 29   |
| B061459 | blaCTX-M-15| No            | 44 | 10  | 11   | 4    | 8   | 8    | 8    | 7    |
| E06476  | blaCTX-M-15| No            | 410| 6   | 4    | 12   | 1   | 20   | 18   | 7    |
| E06190  | blaCTX-M-15| No            | 410| 6   | 4    | 12   | 1   | 20   | 18   | 7    |
| E08284  | blaCTX-M-15| Yes           | 112| 13  | 44   | 9    | 22  | 16   | 30   | 34   |
| E08437  | blaCTX-M-15| No            | 964| 35  | 183  | 29   | 25  | 4    | 5    | 73   |

Fig. 2. Dendrogram based on XbaI-macrorestriction patterns of E. coli isolates producing CTX-M-1-type ESBLs. The dashed line indicates 80% similarity. E. coli isolates exhibiting similarities of <80% were considered unrelated. * XbaI-macrorestriction analysis yielded no DNA banding patterns due to the degeneration of the genomic DNA during preparation of the agarose plugs. ESBL extended-spectrum β-lactamase.
atobiliary infection, or healthcare-associated urinary tract infection. Before the present risk factors might be applied to clinical decisions for empirical therapy, the limitations for our study must be considered. This study was performed with a relatively small number of patients in a single center. In addition, documented risk factors in our study were based on data that is several years old. Clinicians should take into account that the epidemiology of Enterobacteriaceae changes rapidly, which was underlined by the major change in ESBL prevalence in this study population. Moreover, increasing carbapenem resistance, mediated by porin loss or BLEC prevalence in this study population.

The predominance of CTX-M enzymes and clonally unrelated isolates are consistent with the fact that ESBLEC is a true community pathogen. Although healthcare-associated infection was associated with an increased risk of ESBLEC bacteremia in our study, it could be a surrogate marker because ESBLEC colonization is mainly a problem in the community. The most prevalent CTX-M-9 group enzymes are the most prevalent ESBLs in Spain, China, and Taiwan, currently, the most prevalent CTX-M enzyme worldwide is CTX-M-15, which has been reported in Europe, Asia, Africa, North America, South America, and Australia. The predominance of CTX-M-15 and CTX-M-14 is in agreement with recent reports in Korea. Moreover, the presence of the CTX-M-9 group is increasing in Korea. In the present study, the most predominant CTX-M group was the CTX-M-1 group in 2005-2007 and the CTX-M-9 group in 2008-2009 among all ESBLEC isolates having blactx-m genes (86% and 57%, respectively, p=0.002). The rate of ESBL production among E. coli from the blood of patients with community-onset bacteremia was 7% and increased 21 times from 2005 to 2009. This change may be attributable to the dissemination of CTX-M enzymes since the blactx-m genes were detected in 58 (97%) of 60 ESBLEC isolates. Further studies are required to obtain more information on the prevalence of ESBL producers and changes to the molecular epidemiology of CTX-M enzymes in the Republic of Korea.

The E. coli O25:H4-ST131 has been recognized as an emerging intercontinental clonal group expressing CTX-M-type ESBL. Most previous studies from Europe and North America, South America, and Australia.

| Isolate | ESBL gene | Conjugability | ST | adk | fumC | gyrB | icd | mdh | purA | recA |
|---------|-----------|---------------|----|-----|------|------|-----|-----|------|------|
| E02390 | blaCTX-M-9 | No | 131 | 53  | 40   | 47   | 13  | 36  | 28   | 29   |
| B090280 | blaCTX-M-9 | Yes | 95  | 37  | 38   | 19   | 37  | 17  | 11   | 26   |
| B080048 | blaCTX-M-9 | No | 648 | 92  | 4    | 87   | 96  | 70  | 58   | 2    |
| B084396 | blaCTX-M-9 | No | 405 | 35  | 37   | 29   | 25  | 4   | 5    | 73   |
| B085588 | blaCTX-M-9 | No | 405 | 35  | 37   | 29   | 25  | 4   | 5    | 73   |
| E083888 | blaCTX-M-9, blaCTX-M-2 | Yes | 131 | 53  | 40   | 47   | 13  | 36  | 28   | 29   |
| E083444 | blaCTX-M-9 | No | 131 | 53  | 40   | 47   | 13  | 36  | 28   | 29   |
| E090699 | blaCTX-M-9 | No | 131 | 53  | 40   | 47   | 13  | 36  | 28   | 29   |
| E08248 | blaCTX-M-9 | Yes | 773 | 6   | 165  | 4    | 17  | 7   | 8    | 6    |
| E08374 | blaCTX-M-9 | Yes | 69  | 21  | 35   | 27   | 6   | 5   | 4    | 4    |
| B08420 | blaCTX-M-9 | No | 131 | 53  | 40   | 47   | 13  | 36  | 28   | 29   |
| E09038 | blaCTX-M-9 | No | 38  | 4   | 26   | 2    | 25  | 5   | 5    | 19   |
| E07495 | blaCTX-M-9 | No | 405 | 35  | 37   | 29   | 25  | 4   | 5    | 73   |
| E08132 | blaCTX-M-9 | Yes | 393 | 18  | 106  | 17   | 6   | 5   | 4    | 4    |
| E08022 | blaCTX-M-9 | Yes | 131 | 53  | 40   | 47   | 13  | 36  | 28   | 29   |
| E08331 | blaCTX-M-9 | Yes | 457 | 101 | 88   | 97   | 108 | 26  | 79   | 2    |
| E09116 | blaCTX-M-9 | No | 38  | 4   | 26   | 2    | 25  | 5   | 5    | 19   |
| B090371 | blaCTX-M-9 | Yes | 10  | 10  | 11   | 4    | 8   | 8   | 2    | 2    |
| B081373 | blaCTX-M-9 | No | 38  | 4   | 26   | 2    | 25  | 5   | 5    | 19   |
| E09180 | blaCTX-M-9, blaCTX-M-2 | Yes | 131 | 53  | 40   | 47   | 13  | 36  | 28   | 29   |
| E07409 | blaCTX-M-9 | No | 254 | 85  | 88   | 78   | 29  | 59  | 58   | 62   |
| E080398 | blaCTX-M-9 | Yes | 2037| 58  | 88   | 78   | 37  | 59  | 58   | 62   |
| E06380 | blaCTX-M-9, blaCTX-M-1 | Yes | 95  | 37  | 38   | 19   | 37  | 17  | 11   | 26   |
| E063079 | blaCTX-M-9 | Yes | 405 | 35  | 37   | 29   | 25  | 4   | 5    | 73   |
| E06283 | blaCTX-M-9 | Yes | 393 | 18  | 106  | 17   | 6   | 5   | 4    | 4    |
| E09047 | blaCTX-M-9 | No | 38  | 4   | 26   | 2    | 25  | 5   | 5    | 19   |

Fig. 3. Dendrogram based on XbaI-macrorestriction patterns of E. coli isolates producing CTX-M-9-type and SHV ESBLs. The dashed line indicates 80% similarity. E. coli isolates exhibiting similarities of <80% were considered unrelated. *XbaI-macrorestriction analysis yielded no DNA banding patterns due to the degeneration of the genomic DNA during preparation of the agarose plugs. ESBL, extended-spectrum β-lactamase.

Community-Onset ESBL E. coli Bacteremia
America reported *E. coli* O25:H4-ST131 strains were CTX-M-15 producers.\(^{40-42}\) Our analysis of 60 community-onset ESBLEC determined that *E. coli* ST131 was the most prevalent (18%) clonal group. The *E. coli* ST131 isolates carried not only the *bla*\(_{CTX-M-15}\) but also *bla*\(_{CTX-M-14}\) or *bla*\(_{SHV-12}\). Although *E. coli* ST131 was the most common cause of community-onset ESBLEC infection, clonal expansion of strains carrying *bla*\(_{CTX-M-15}\) was not the main reason for the rapid spread in the community. This hypothesis is supported by the fact that these isolates show low level of similarity in PFGE analysis. Despite that many different types of STs among ESBLEC and the isolates showed a low-level similarity by PFGE, certain ESBL genes, such as *bla*\(_{CTX-M-14}\) or *bla*\(_{CTX-M-15}\), were predominantly found among the isolates. This may suggest that the plasmid or conjugative transposons harboring these antibiotic resistance genes were transferred horizontally among *E. coli* strains. The multi-clonal-ity of ESBLEC, shown in our study, comes not only from the type of ESBL genes but also from the whole genome including virulence and housekeeping genes, so a multi-clonal outbreak of ESBLEC does not always correlate with the diversity of ESBL genes. This means that the predominance of certain type of ESBL genes may not result in the mono-clonal spreading of ESBLEC.

Our results showed that physicians who care for patients with these risk factors should consider ESBLEC as the causative organism of community-onset bacteremia. The most common types of ESBLs in *E. coli* causing community-onset bacteremia were CTX-M-15 and CTX-M-14 in the Republic of Korea, which might have transferred horizontally.

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