

Original Article

Title: Cefotaxime for the detection of extended-spectrum β -lactamase or plasmid-mediated AmpC β -lactamase and clinical characteristics of cefotaxime-non-susceptible *Escherichia coli* and *Klebsiella pneumoniae* bacteraemia

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Abstract

Purpose: We investigated the performance of cefotaxime for the detection of extended-spectrum β -lactamase (ESBL) or plasmid mediated-AmpC β -lactamase (pAmpC) and clinical characteristics of cefotaxime-non-susceptible *E. coli* or *K. pneumoniae* (CTXNS-EK) bacteraemia.

Methods: All of the consecutive bloodstream isolates between 2005 and 2010 in a Japanese university hospital were characterized using polymerase chain reaction. Risk factors and outcomes of CTXNS-EK were analysed by multivariate logistic regression analysis.

Results: We identified 58 CTXNS-EK (15.6%) from 249 *E. coli* and 122 *K. pneumoniae*. Cefotaxime with minimum inhibitory concentration of $>1 \mu\text{g/mL}$ had a sensitivity of 98.3% and a specificity of 99.7% for the detection of ESBL or pAmpC. CTXNS-EK had increased from 4.5% in 2005 to 23% in 2009. Risk factors for CTXNS-EK were previous isolation of multidrug-resistant bacteria, use of oxyimino-cephalosporins or fluoroquinolones, and high Sequential Organ Failure Assessment (SOFA) score. Patients with CTXNS-EK bacteraemia less frequently received appropriate empirical therapy than patients with cefotaxime-susceptible EK bacteraemia (81% vs. 97%, $P<0.001$) and died within 30 days (21% vs. 5%, $P=0.001$).

Conclusions: Using the current breakpoint of CLSI or EUCAST, cefotaxime alone can identify ESBL or pAmpC producers. CTXNS-EK is an important and increasingly prevalent bacteraemia pathogen.

1 **Keywords:** cefotaxime, bloodstream infection, risk factor, prognosis, ESBL, AmpC

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1 Introduction

2 Bacteraemia caused by *Enterobacteriaceae*, especially *Escherichia coli* or *Klebsiella*
3 *pneumoniae* (EK), is a common and significant problem in both community and healthcare-associated
4 settings [1, 2]. In recent years, extended-spectrum β -lactamase (ESBL)-producing EK have
5 dramatically increased worldwide [3, 4]. In addition, plasmid-mediated AmpC β -lactamase
6 (pAmpC)-producing EK that also confer resistance to broad-spectrum cephalosporins are also
7 increasing [5]. Clinical data show that prognosis of infections caused by ESBL or pAmpC-producing
8 EK is worse than that caused by non-producers [3, 6-8].

9 ESBL screening and confirmation tests described by the Clinical and Laboratory Standards
10 Institute (CLSI) are useful for identifying ESBL-producing organisms [9]. Although pAmpC
11 producers are positive for ESBL screening, standard guidelines for the detection of pAmpC are
12 lacking [10]. However, using the CLSI breakpoints revised in 2010 [11] or the European Committee
13 on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [12], ESBL screening and
14 confirmation tests are unnecessary for selecting antimicrobials for treatment. From the viewpoint of
15 patient therapy, breakpoints are considered to be more important than identification of resistance
16 mechanisms.

17 Third-generation cephalosporins, such as cefotaxime and ceftriaxone, are commonly used as
18 first-line empirical therapies for the treatment of EK-related infections. Although clinical features of
19 ESBL producing EK have been investigated well [13, 14], little data is available for the
20 microbiological and clinical features of bacteraemia due to cefotaxime-non-susceptible EK
21 (CTXNS-EK) with the clinical breakpoint. Herein, we confirm the performance of cefotaxime for the
22 detection of ESBL and pAmpC-producing EK in comparison with broad-spectrum cephalosporins and
23 CLSI ESBL screening test. We also evaluate the risk factors and outcomes of bacteraemia due to
24 CTXNS-EK using comparisons with bacteraemia caused by cefotaxime-susceptible EK (CTXS-EK).

26 Materials and Methods

27 Setting and study design

This study was conducted at Kyoto University Hospital, a tertiary care 1182-bed university hospital located in Japan. All episodes of bacteraemia in our hospital were notified and followed up by our infectious disease physicians. Changes in antimicrobial treatment and general management were advised if considered necessary. All patients with bacteraemia due to *Escherichia coli* or *Klebsiella pneumoniae* that occurred from April 2005 to March 2010 were enrolled in this study. Each patient was included in the study only once, at the time of the initial positive blood culture. A retrospective cohort study design was used. Patients who were <18 years of age were excluded from the clinical analysis. The Ethics Committee of Kyoto University Graduate School and Faculty of Medicine approved this study and waived the need for obtaining informed consent from each patient.

Variables and definitions

Cefotaxime-non-susceptible isolates with minimum inhibitory concentration (MIC) of >1 µg/mL were defined to be CTXNS-EK, and isolates with MIC ≤1 µg/mL to be CTXS-EK. Polymicrobial infection was identified when additional microorganisms were recovered from the blood cultures. Bacteraemia was categorized as nosocomial, health care-associated, or community-acquired in accordance with the criteria of Friedman et al. [15]. Neutropenia was defined as an absolute neutrophil count below 500/mm³. Multidrug-resistant (MDR) bacteria included ESBL, metallo-β-lactamase producers (detected using mercaptoacetic acid) [16], multidrug (imipenem, amikacin, and ciprofloxacin)-resistant *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus*, and vancomycin-resistant enterococci. Empirical therapy was defined as the initial therapy during the first 24 hours after the blood sample was obtained. Antimicrobial therapy was considered to be appropriate if an active antimicrobial agent determined by in vitro susceptibility testing was administered at the usual recommended dose. The susceptibilities of β-lactam/β-lactamase inhibitors and cefepime were categorised following the CLSI breakpoints revised in 2011 [11], irrespective of ESBL confirmation test.

Clinical information acquired from medical charts included age, sex, the duration of the hospital stay before the onset of bacteraemia, underlying diseases, the Charlson weighted index of

comorbidity [17], history of MDR bacteria isolation, surgery during the previous 30 days, receipt of corticosteroids or other immunosuppressive agents (immunosuppressive therapies) during the previous 30 days, any antimicrobial therapy during the previous 30 days, neutropenia, presence of an intravenous catheter, an indwelling urinary catheter, or any other artificial device, site of infection, Sequential Organ Failure Assessment (SOFA) score [18], and the antimicrobial regimen.

The main outcome measure was based on 30-day mortality rates. Intensive care unit (ICU) admission and time of response to treatment were also analysed. The response to treatment was assessed every 24 hours after the start of antimicrobial therapy and was classified as follows: complete response for patients with resolution of fever, leukocytosis and all signs of infection; failure for patients with no abatement or with deterioration of any of the clinical parameters; and death.

Microbiological analysis

The species were determined using the Vitek 2 system (bioMérieux). Antibiotic susceptibility was evaluated by microdilution using Dry Plate Eiken (Eiken, Tokyo, Japan) and interpreted according to the CLSI criteria [11]. ESBL screening was performed according to the CLSI microdilution methodology using cefotaxime, ceftazidime, cefpodoxime, and aztreonam [11]. ESBL confirmation test was done by the double disk synergy test, following the CLSI guidelines.

All isolates were subjected to PCR amplification of *bla*_{SHV}, *bla*_{TEM}, *bla*_{CTX-M} and six main groups of the pAmpC-type genes, as described previously [19-21]. Amplicons of the pAmpC-type genes were directly sequenced. The entire genes were amplified and sequenced for *bla*_{SHV}- [22] or *bla*_{TEM}- [23] positive isolates.

Clonal relatedness of CTXNS-EK was determined by random amplified polymorphic DNA (RAPD) fingerprinting using a DAF4 primer, as described previously [24]. Isolates with identical RAPD patterns were also studied using Pulsed-field gel electrophoresis (PFGE) and *SpeI* endonuclease. Digitalized gel images were subjected to analysis with GelCompar II, version 4.6 (Applied Maths). Cluster analysis was performed using the unweighted pair-group method based on Dice coefficients to quantify the similarities.

Statistical analysis

Categorical variables were compared using the Fisher exact test. Continuous variables were compared using the Mann-Whitney U test. To determine the association of independent variables with risk factors for cefotaxime-resistance and 30-day mortality, all variables with a P-value of less than 0.05 on univariate analyses were subjected to further selection by using a forward stepwise logistic procedure. We forced the inclusion of Charlson index, and SOFA score in the multivariate models, and CTXNS-EK bacteraemia was also included in mortality analysis. The goodness of fit of the last model was evaluated by the Hosmer and Lemeshow test. $P < 0.05$ was considered statistically significant. We conducted statistical analysis using Stata version 11.2 (StataCorp, College Station, TX, USA).

Results

Microbiological results

During the study period, a total of 371 patients with bacteraemia due to EK were identified, which consisted of 249 *E. coli* and 122 *K. pneumoniae* isolates (Table 1). Fifty-eight (15.6%) of the 371 isolates were CTXNS-EK. Yearly CTXNS-EK prevalence is shown in Figure 1. CTXNS-EK increased from 4.5% in 2005 to 23.0% in 2009. Isolates with pAmpC emerged in 2007. Fifty-seven of 58 (98.2%) CTXNS-EK isolates had ESBL or pAmpC, while only 1 of 313 (0.3%) CTXS-EK had ESBL. Table 1 shows that CTX-M, especially the CTX-M9 group, was the most prevalent type of ESBL, and CMY-2 was in pAmpC. All isolates were susceptible to imipenem ($\text{MIC} \leq 1 \mu\text{g/mL}$). Except for imipenem, the susceptibility rates of β -lactams, aminoglycosides, and levofloxacin were higher in CTXS-EK than in CTXNS-EK. The sensitivity and specificity for the screening of ESBL or pAmpC producers are shown in Table 2. Cefpodoxime (100.0%), cefotaxime (98.3%), and the CLSI ESBL screening test (98.3%) had higher sensitivity than aztreonam, cefepime, or ceftazidime. Of these 3 agents, cefotaxime had the highest specificity (99.7%).

Forty of 50 cefotaxime-non-susceptible *E. coli* were clonally unrelated by RAPD analysis. Three clusters (6, 2, and 2 isolates) underwent PFGE analysis (Figure not shown). Three and two

isolates of a larger cluster showed an identical pattern, all of which had CTX-M9. The others were unrelated. Eight cefotaxime-non-susceptible *K. pneumoniae* showed distinct RAPD patterns.

Risk factors and outcomes for cefotaxime-non-susceptible bacteraemia

Twenty patients (5 CTXNS-EK and 15 CTXS-EK) were <18 years of age. Four patients with CTXS-EK bacteraemia were lost to follow-up. Therefore, 53 patients with CTXNS-EK bacteraemia and 294 patients with CTXS-EK bacteraemia were included for clinical analysis.

Risk factors for the case patients are listed in Table 3. The factors significantly associated with CTXNS-EK bacteraemia in univariate analysis included nosocomial or healthcare-associated infections, previous isolation of MDR bacteria, previous antimicrobial use (any antibiotic, oxyimino-cephalosporins, fluoroquinolones, and trimethoprim/sulfamethoxazole), high Charlson index, transplantation, haemodialysis, liver disease, neutropenia, intravascular catheterisation, and high SOFA score. In multivariate analysis, previous isolation of MDR bacteria (odds ratio [OR] 3.2, 95% confidence interval [CI] 1.5-7.1), use of oxyimino-cephalosporins (OR 2.8, 95% CI 1.3-6.2), use of fluoroquinolones (OR 3.2, CI 1.3-7.8), and SOFA score (OR 1.2, CI 1.1-1.4) were independent factors for CTXNS-EK bacteraemia when controlled for Charlson index.

Patients with CTXNS-EK bacteraemia received less frequently the appropriate empirical therapy than patients with CTXS-EK (81% vs. 97%; $P=0.001$; Table 4). In addition, patients with CTXNS-EK had worse outcomes than patients with CTXS-EK in terms of complete response within 7 days (70% vs. 85%), ICU admission (19% vs. 8%), and 30-day mortality (21% vs. 5%). However, durations between appropriate therapy and complete response were similar (median 3 days in each group).

Predictors of mortality

Factors significantly associated with 30-day mortality are listed in Table 5. Bacterial species was not associated with mortality. After stepwise logistic regression analysis, Charlson index (OR 1.6, CI 1.2-2.1) and SOFA score (OR 1.4, CI 1.2-1.6) were the independent predictors, while CTXNS-EK bacteraemia was not (OR 1.6, CI 0.5-4.5).

Discussion

In this study, we evaluated 371 EK bacteraemias including 58 CTXNS-EK cases. At concentrations $>1 \mu\text{g/mL}$, cefotaxime detected ESBL and pAmpC producers with excellent sensitivity and specificity. CTXNS-EK bacteraemia has been increasing and results in worse outcomes than CTXS-EK. The risk factors for CTXNS-EK were also investigated.

Increased prevalence of CTXNS-EK correlated with an increase of ESBL or pAmpC producers. However, RAPD and PFGE analyses indicated that the increase of CTXNS-EK was not due to clonal spread of a unique isolate. A high prevalence of CTXNS-EK was observed in 2009 (23.0%), and cefotaxime non-susceptibility was more common in *E. coli* than in *K. pneumoniae*. Although a few nationwide surveillance of the prevalence of CTXNS-EK or ESBL-producers have been conducted in Japan, in 2003, inpatient urine isolated in 37 hospitals in Japan was studied, and the prevalence of ESBL-producing *E. coli* was 14% [25]. A study from Fukuoka, Japan in 2009 showed that 17.1% of *E. coli* and 10.5% of *K. pneumoniae* isolates were ESBL-producers [26]. In Europe, although geographic differences have been observed, *K. pneumoniae* has been reported to display an ESBL phenotype more frequently than *E. coli* [27]. SENTRY surveillance in the Asia-Pacific region in 2009 showed that the cefotaxime non-susceptibility rates were 55% in *E. coli* and 65% in *K. pneumoniae* [28]. Our data are consistent with these data.

Within ESBL, CTX-M, especially the CTX-M9 group, was the dominant type. CTX-M is now spreading worldwide [4], and CTX-M has been prevalent in Japan since the emergence of ESBL [22]. Among CTX-M, the CTX-M9 group is now the most prevalent [29]. The prevalence of CTX-M may have contributed to the ability of cefotaxime to efficiently detect ESBL because CTX-M has better hydrolyzing activity against cefotaxime than TEM or SHV [4]. CMY-2 is the most common pAmpC worldwide [5]. Surveillance in the Kinki region of Japan, where the study site was located, showed that CMY-2 was most prevalent, but the prevalence rate from our data (2.7%) seems to be substantially higher (0.1%) [30]. One possible explanation for this difference is that our study used pAmpC isolated between 2007 and 2009, whereas the surveillance was conducted between 2002 and

2008.

We did not assess the overproduction of chromosomal AmpC in *E. coli*, which represents another mechanism known to underlie broad-spectrum cephalosporin resistance [5]. However, among 49 of 50 cefotaxime-non-susceptible *E. coli* isolates, the resistance mechanism could be explained by ESBL or pAmpC. The other isolate was susceptible to cefmetazole and produced a positive result in an ESBL confirmation test, suggesting that rather than overproducing chromosomal AmpC, the isolate produced ESBL of a type other than CTX-M, TEM, or SHV.

Association with long-term care facility or hospitalization, exposure to antibiotics, indwelling devices, and severe underlying disease are all reported to be risk factors for ESBL bacteraemia [13, 14]. Courpon-Claudinon et al. conducted the only study of which the design can be compared with ours [31]. They investigated bacteraemia due to third-generation cephalosporin-resistant *E. coli*, including ESBL and AmpC hyperproducers, and found underlying chronic disease and prior use of antibiotics as risk factors. Our data also showed prior antibiotic use, particularly oxyimino-cephalosporins and fluoroquinolones as independent risk factors for CTXNS-EK bacteraemia. This is not surprising, because CTXNS-EK isolates were more resistant to oxyimino-cephalosporins and fluoroquinolones than CTXS-RE isolates. In addition, the use of cephalosporins and fluoroquinolones were reported as risk factors for pAmpC- or ESBL-producing EK bacteraemia [32-35].

In the study by Courpon-Claudinon et al., the mortality rate of resistant bacteraemia was significantly higher (31% vs. 12%) [31]. Our patients with CTXNS-EK bacteraemia also had a higher mortality rate. The difference in outcomes between CTXNS-EK and CTXS-EK might be associated with severity of illness and appropriate empirical therapy. Patients with CTXNS-EK bacteraemia experienced a more severe illness, even after controlling confounders by multivariate analysis. Appropriate empirical therapy has been considered to be an important predictor of mortality [34, 36] and our patients with CTXNS-EK bacteraemia less frequently received appropriate empirical therapy. Cefotaxime resistance was associated with mortality in univariate analysis but was not associated in multivariate analysis. One possible explanation is that the presence of cefotaxime resistance is a strong confounding factor of severity of illness (SOFA score).

Among CTXNS-EK, imipenem and amikacin resulted in susceptibilities of more than 90%. These agents were also active for CTXS-EK isolates. Carbapenem-resistant EK are extremely rare in Japan, and were not identified in our cohort of patients. Thus, when CTXNS-EK bacteraemia is suspected, antibiotic regimens including carbapenems or amikacin would be the preferred choice. As the emergence of carbapenem resistance is a major concern [37], recommendations of using carbapenems as the empirical therapy must be made with caution. However, for severely ill patients with neutropenia or multiple organ failure, physicians may choose a broad-spectrum antibiotic to increase the probability of susceptibility in the clinical practice. It has been suggested that patients with severe infections receive carbapenem monotherapy or a combination therapy including aminoglycosides [38]. Among patients in this study with a previous history of MDR bacterial isolation, oxyimino-cephalosporin use, or fluoroquinolone use, 46% (33/71) had CTXNS-EK bacteraemia. Therefore, those patients might also be considered for the antibiotic regimens including carbapenems or amikacin.

In the present study, we defined an isolate with an MIC of cefotaxime $>1 \mu\text{g/mL}$ as a resistant organism, because the value is identical to the clinical breakpoint of the CLSI [11] and the EUCAST. Cefotaxime had a reasonable performance of detecting both the ESBL and pAmpC producers, as did the ESBL screening test and cefpodoxime. However, ESBL screening test usually require multiple antibiotics [11] and cefpodoxime is much less frequently used in clinical setting than cefotaxime. Although the detection of ESBL or pAmpC producers requires further testing for phenotypes or for resistant genes, the exact distinction between ESBL and pAmpC is difficult. Furthermore, the mortality rate from pAmpC bacteraemia is worse than from non-resistant bacteraemia [33], and is similar to ESBL bacteraemia [6]. Considering the clinical importance of CTXNS-EK described in this study, the approach of identifying cefotaxime resistance seems to be feasible in the clinical practice.

Limitations in the present study were that the populations examined were from a large university hospital, and most of the bacteraemias occurred in the health care-associated setting. Despite these limitations, the data in this study can be used as a guide for making clinical decisions in

1 situations when EK are suspected to be the cause of sepsis. We believe that our results may be
2 applicable, especially in the absence of carbapenem-resistant isolates or clonal outbreaks.

3 In conclusion, cefotaxime resistance can identify ESBL or pAmpC producers without
4 another confirmatory test. CTXNS-EK bacteraemia is increasing, and is associated with a delay in
5 appropriate therapy and with severe outcomes. Independent predictors for CTXNS-EK bacteraemia
6 were previous isolation of MDR bacteria, use of oxyimino-cephalosporins or fluoroquinolones, and
7 high SOFA score.

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15 16 **Conflict of interest**

17 The authors declare that they have no conflict of interest.

References

- [1] Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB (2004) Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis 39 (3):309-317
- [2] Biedenbach DJ, Moet GJ, Jones RN (2004) Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from the SENTRY Antimicrobial Surveillance Program (1997-2002). Diagn Microbiol Infect Dis 50 (1):59-69
- [3] Ramphal R, Ambrose PG (2006) Extended-spectrum beta-lactamases and clinical outcomes: current data. Clin Infect Dis 42 Suppl 4:S164-172
- [4] Rossolini GM, D'Andrea MM, Mugnaioli C (2008) The spread of CTX-M-type extended-spectrum beta-lactamases. Clin Microbiol Infect 14 Suppl 1:33-41
- [5] Jacoby GA (2009) AmpC beta-lactamases. Clin Microbiol Rev 22 (1):161-182
- [6] Pai H, Kang CI, Byeon JH, Lee KD, Park WB, Kim HB, Kim EC, Oh MD, Choe KW (2004) Epidemiology and clinical features of bloodstream infections caused by AmpC-type-beta-lactamase-producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother 48 (10):3720-3728
- [7] Park YS, Yoo S, Seo MR, Kim JY, Cho YK, Pai H (2009) Risk factors and clinical features of infections caused by plasmid-mediated AmpC beta-lactamase-producing Enterobacteriaceae. Int J Antimicrob Agents 34 (1):38-43
- [8] Sidjabat HE, Paterson DL, Qureshi ZA, Adams-Haduch JM, O'Keefe A, Pascual A, Rodríguez-Baño J, Doi Y (2009) Clinical features and molecular epidemiology of CMY-type beta-lactamase-producing *Escherichia coli*. Clin Infect Dis 48 (6):739-744
- [9] Falagas ME, Karageorgopoulos DE (2009) Extended-spectrum beta-lactamase-producing organisms. J Hosp Infect 73 (4):345-354
- [10] Doi Y, Paterson DL (2007) Detection of plasmid-mediated class C beta-lactamases. Int J Infect Dis 11 (3):191-197
- [11] Clinical Laboratory Standards Institute (CLSI) (2011) Performance standards for

antimicrobial susceptibility testing; 21st informational supplement. CLSI document M100-S21. CLSI, Wayne, PA

[12] European Committee on Antimicrobial Susceptibility Testing. (2011) Breakpoint tables for interpretation of MICs and zone diameters, Version 1.3, http://www.eucast.org/clinical_breakpoints/. Last date accessed 31 October 2011

[13] Pfaller MA, Segreti J (2006) Overview of the epidemiological profile and laboratory detection of extended-spectrum beta-lactamases. *Clin Infect Dis* 42 Suppl 4:S153-163

[14] Stürenburg E, Mack D (2003) Extended-spectrum beta-lactamases: implications for the clinical microbiology laboratory, therapy, and infection control. *J Infect* 47 (4):273-295

[15] Friedman N, Kaye K, Stout J, McGarry S, Trivette S, Briggs J, Lamm W, Clark C, MacFarquhar J, Walton A, Reller L, Sexton D (2002) Health care-associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. *Ann Intern Med* 137 (10):791-797

[16] Arakawa Y, Shibata N, Shibayama K, Kurokawa H, Yagi T, Fujiwara H, Goto M (2000) Convenient test for screening metallo-beta-lactamase-producing gram-negative bacteria by using thiol compounds. *J Clin Microbiol* 38 (1):40-43

[17] Charlson ME, Pompei P, Ales KL, MacKenzie CR (1987) A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 40 (5):373-383

[18] Vincent J, de Mendonça A, Cantraine F, Moreno R, Takala J, Suter P, Sprung C, Colardyn F, Blecher S (1998) Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. *Crit Care Med* 26 (11):1793-1800

[19] Dallenne C, Da Costa A, Decré D, Favier C, Arlet G (2010) Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in

Enterobacteriaceae. J Antimicrob Chemother 65 (3):490-495

[20] Xu L, Ensor V, Gossain S, Nye K, Hawkey P (2005) Rapid and simple detection of blaCTX-M genes by multiplex PCR assay. J Med Microbiol 54 (Pt 12):1183-1187

[21] Pérez-Pérez F, Hanson N (2002) Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 40 (6):2153-2162

[22] Yagi T, Kurokawa H, Shibata N, Shibayama K, Arakawa Y (2000) A preliminary survey of extended-spectrum beta-lactamases (ESBLs) in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* in Japan. FEMS Microbiol Lett 184 (1):53-56

[23] Briñas L, Zarazaga M, Sáenz Y, Ruiz-Larrea F, Torres C (2002) Beta-lactamases in ampicillin-resistant *Escherichia coli* isolates from foods, humans, and healthy animals. Antimicrob Agents Chemother 46 (10):3156-3163

[24] Wiedmann-al-Ahmad M, Tichy H, Schön G (1994) Characterization of *Acinetobacter* type strains and isolates obtained from wastewater treatment plants by PCR fingerprinting. Appl Environ Microbiol 60 (11):4066-4071

[25] Muratani T, Matsumoto T (2006) Urinary tract infection caused by fluoroquinolone- and cephem-resistant Enterobacteriaceae. Int J Antimicrob Agents 28 Suppl 1:S10-13

[26] Chong Y, Yakushiji H, Ito Y, Kamimura T (2011) Clinical and molecular epidemiology of extended-spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in a long-term study from Japan. Eur J Clin Microbiol Infect Dis 30 (1):83-87

[27] Coque TM, Baquero F, Canton R (2008) Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Euro Surveill 13 (47)

[28] Chen YH, Hsueh PR, Badal RE, Hawser SP, Hoban DJ, Bouchillon SK, Ni Y, Paterson DL (2011) Antimicrobial susceptibility profiles of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections in the Asia-Pacific region according to currently established susceptibility interpretive criteria. J

Infect 62 (4):280-291

[29] Shibata N, Kurokawa H, Doi Y, Yagi T, Yamane K, Wachino J, Suzuki S, Kimura K, Ishikawa S, Kato H, Ozawa Y, Shibayama K, Kai K, Konda T, Arakawa Y (2006) PCR classification of CTX-M-type beta-lactamase genes identified in clinically isolated gram-negative bacilli in Japan. *Antimicrob Agents Chemother* 50 (2):791-795

[30] Yamasaki K, Komatsu M, Abe N, Fukuda S, Miyamoto Y, Higuchi T, Ono T, Nishio H, Sueyoshi N, Kida K, Satoh K, Toyokawa M, Nishi I, Sakamoto M, Akagi M, Nakai I, Kofuku T, Orita T, Wada Y, Jikimoto T, Kinoshita S, Miyamoto K, Hirai I, Yamamoto Y (2010) Laboratory surveillance for prospective plasmid-mediated AmpC beta-lactamases in the Kinki region of Japan. *J Clin Microbiol* 48 (9):3267-3273

[31] Courpon-Claudinon A, Lefort A, Panhard X, Clermont O, Dornic Q, Fantin B, Mentré F, Wolff M, Denamur E, Branger C, group obotC (2011) Bacteraemia caused by third-generation cephalosporin-resistant *Escherichia coli* in France: prevalence, molecular epidemiology and clinical features. *Clin Microbiol Infect* 17 (4):557-65

[32] Lee CH, Su LH, Li CC, Chien CC, Tang YF, Liu JW (2010) Microbiologic and clinical implications of bacteremia due to extended-spectrum-beta-lactamase-producing *Klebsiella pneumoniae* with or without plasmid-mediated AmpC beta-lactamase DHA-1. *Antimicrob Agents Chemother* 54 (12):5395-5398

[33] Yan JJ, Ko WC, Wu JJ, Tsai SH, Chuang CL (2004) Epidemiological investigation of bloodstream infections by extended spectrum cephalosporin-resistant *Escherichia coli* in a Taiwanese teaching hospital. *J Clin Microbiol* 42 (7):3329-3332

[34] Du B, Long Y, Liu H, Chen D, Liu D, Xu Y, Xie X (2002) Extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* bloodstream infection: risk factors and clinical outcome. *Intensive Care Med* 28 (12):1718-1723

[35] Lautenbach E, Patel JB, Bilker WB, Edelstein PH, Fishman NO (2001) Extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for infection and impact of resistance on outcomes. *Clin Infect Dis* 32

(8):1162-1171

[36] Rodríguez-Baño J, Picón E, Gijón P, Hernández JR, Ruíz M, Peña C, Almela M, Almirante B, Grill F, Colomina J, Giménez M, Oliver A, Horcajada JP, Navarro G, Coloma A, Pascual A, for the Spanish Network for Research in Infectious Diseases (REIPI) (2010) Community-onset bacteremia due to extended-spectrum beta-lactamase-producing *Escherichia coli*: risk factors and prognosis. Clin Infect Dis 50 (1):40-48

[37] Nordmann P, Cuzon G, Naas T (2009) The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. Lancet Infect Dis 9 (4):228-236

[38] Rodríguez-Baño J, Navarro MD, Romero L, Muniain MA, de Cueto M, Ríos MJ, Hernández JR, Pascual A (2006) Bacteremia due to extended-spectrum beta-lactamase-producing *Escherichia coli* in the CTX-M era: a new clinical challenge. Clin Infect Dis 43 (11):1407-1414

1 **Table 1. Microbiological data of 371 bloodstream *Escherichia coli* or *Klebsiella***
2 ***pneumoniae* isolates.**

	Cefotaxime-non-susceptible (n=58)	Cefotaxime-susceptible (n=313)
Bacteria		
<i>Escherichia coli</i>	50 (86.2%)	199 (63.6%)
<i>Klebsiella pneumoniae</i>	8 (13.8%)	114 (36.4%)
In vitro susceptibility		
ESBL-screening test	58 (100.0%)	6 (1.9%)
Aztreonam	27 (46.6%)	313 (100.0%)
Cefpodoxime	0 (0%)	303 (96.8%)
Ceftazidime	34 (58.6%)	313 (100.0%)
Cefmetazole	48 (82.8%)	309 (98.7%)
Cefepime	35 (60.3%)	313 (100.0%)
Piperacillin-tazobactam	51 (87.9%)	310 (99.0%)
Imipenem	58 (100.0%)	313 (100.0%)
Amikacin	55 (94.8%)	312 (99.7%)
Gentamicin	44 (75.9%)	302 (96.5%)
Levofloxacin	25 (43.1%)	275 (87.9%)
Type of β -lactamase		
CTX-M	46 (79.3%)	0 (0%)
CTX-M1 group	9 (15.5%)	0 (0%)
CTX-M2 group	6 (10.3%)	0 (0%)
CTX-M9 group	31 (53.4%)	0 (0%)
TEM (ESBL type)	3 (5.2%)	1 (0.3%)

SHV (ESBL type)	3 ^a (5.2%)	0 (0%)
CMY-2	10 ^b (17.2%)	0 (0%)

Data are presented as no. (%) of isolates. One cefotaxime-non-susceptible *E. coli* isolate was negative for both ESBL and pAmpC. The isolate produced a positive result in an ESBL confirmation test and was susceptible to cefotaxime, cefmetazole, cefepime, and piperacillin-tazobactam, but non-susceptible to aztreonam, cefpodoxime, and ceftazidime. One cefotaxime-susceptible isolate produced TEM-20-type ESBL. The isolate was susceptible to cefotaxime, aztreonam, ceftazidime, cefmetazole, cefepime, and piperacillin-tazobactam, but non-susceptible to cefpodoxime.

^a Of the three SHV-positive isolates, one isolate was also positive for CTX-M9 group.

^b Of the 10 CMY-2-positive isolates, three isolates were also positive for CTX-M9 group and one isolate was also positive for CTX-M1 group.

1 **Table 2. Antimicrobial performance for the detection of ESBL- or pAmpC-producing *E.***
2 ***coli* or *K. pneumoniae*.**

Antimicrobial agent	ESBL or pAmpC producers		ESBL producers		pAmpC producers	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Aztreonam	51.7%	99.7%	55.8%	99.4%	30.0%	92.2%
Cefepime	39.7%	100.0%	44.2%	100.0%	10.0%	93.9%
Cefotaxime	98.3%	99.7%	98.1%	97.8%	100.0%	86.7%
Cefpodoxime	100.0%	96.8%	100.0%	95.0%	100.0%	83.9%
Ceftazidime	39.7%	99.7%	34.6%	98.1%	90.0%	95.8%
CLSI ESBL screening ^a	98.3%	97.8%	98.1%	95.9%	100.0%	85.0%

3 Among 371 bloodstream *E. coli* and *K. pneumoniae* isolates, 58 ESBL or pAmpC producers,
4 52 ESBL producers, and 10 pAmpC producers were included.

5 ^a ESBL screening was performed according to the CLSI microdilution methodology using
6 cefotaxime, ceftazidime, cefpodoxime, and aztreonam.

1 **Table 3. Characteristics of patients with *E. coli* or *K. pneumoniae* bacteraemia.**

Characteristics	Cefotaxime-non-su	Cefotaxime-suscepti	Univariate analysis		Multivariate analysis	
	sceptible (n=53)	ble (n=294)	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
Age (years)	64 (58-74)	67 (57-76)		0.41		
Male sex	29 (55%)	154 (52%)	1.1 (0.6-2.0)	0.77		
Nosocomial or healthcare-associated bacteraemia	43 (81%)	171 (58%)	3.1 (1.5-6.4)	0.001		
Previous isolation of MDR bacteria	18 (34%)	29 (10%)	4.7 (2.4-9.3)	<0.001	3.2 (1.5-7.1)	0.003
Previous antimicrobial use						
Any antibiotic	34 (64%)	130 (44%)	2.3 (1.2-4.1)	0.01		
Penicillins	2 (4%)	4 (1%)	2.8 (0.5-15.9)	0.23		
Oxyimino-cephalosporins	14 (26%)	32 (11%)	2.9 (1.4-6.0)	0.007	2.8 (1.3-6.2)	0.01
Other cephe	5 (9%)	28 (10%)	1.0 (0.4-2.7)	1		
β-lactam/β-lactamase inhibitors	8 (15%)	35 (12%)	1.3 (0.6-3.0)	0.50		
Carbapenems	8 (15%)	23 (8%)	2.1 (0.9-5.0)	0.11		
Fluoroquinolones	13 (25%)	22 (7%)	4.0 (1.9-8.6)	0.001	3.2 (1.3-7.8)	0.009
Aminoglycosides	5 (9%)	9 (3%)	3.3 (1.1-10.3)	0.046		

Trimethoprim/sulfamethoxazole	16 (30%)	49 (17%)	2.2 (1.1-4.2)	0.03		
Glycopeptides	4 (8%)	20 (7%)	1.1 (0.4-3.4)	0.77		
Charlson index	3 (2-5)	2 (1-3)		0.002	1.1 (0.9-1.3)	0.31
Use of immunosuppressive drugs	17 (32%)	85 (29%)	1.2 (0.6-2.2)	0.63		
Haematological malignancy	13 (25%)	49 (17%)	1.6 (0.8-3.3)	0.18		
Solid malignancy	16 (30%)	106 (36%)	0.8 (0.4-1.4)	0.44		
Transplantation	12 (23%)	34 (12%)	2.2 (1.1-4.7)	0.045		
Haemodialysis	5 (9%)	9 (3%)	3.3 (1.1-10.3)	0.046		
Diabetes	8 (15%)	69 (23%)	0.6 (0.3-1.3)	0.21		
Liver disease	23 (43%)	63 (21%)	2.8 (1.5-5.2)	0.002		
Surgery	5 (9%)	27 (9%)	1.0 (0.4-2.8)	1		
Neutropenia	14 (26%)	40 (14%)	2.3 (1.1-4.6)	0.02		
Intravascular catheterisation	32 (60%)	123 (42%)	2.1 (1.2-3.8)	0.02		
Artificial devices other than intravascular catheter	19 (36%)	66 (22%)	1.9 (1.0-3.6)	0.06		
Site of infection						
Urinary tract	15 (28%)	118 (40%)	0.6 (0.3-1.1)	0.16		
Intra-abdominal infection	18 (34%)	97 (33%)	1.0 (0.6-1.9)	0.88		

Primary	17 (32%)	63 (21%)	1.7 (0.9-3.3)	0.11		
Others	2 (4%)	11 (4%)	1.0 (0.2-4.7)	1		
Polymicrobial bacteraemia	17 (32%)	63 (21%)	1.7 (0.9-3.3)	0.11		
SOFA score	5 (2-6)	2 (0-4)		<0.001	1.2 (1.1-1.4)	<0.001

-
- 1 MDR, multidrug-resistant; OR, odds ratio; CI, confidence interval.
- 2 Data are presented as the No. (%) or median (interquartile range). All variables with a P-value of less than 0.05 on univariate analyses were included in the
- 3 multivariate analysis. Stepwise logistic regression analysis was performed using forward selection and likelihood ratio. Only the variables in the last model
- 4 were presented as the final result. The goodness of fit of the last model was evaluated by Hosmer and Lemeshow test ($P=0.56$).

1 **Table 4. Treatment and outcomes of patients with *E. coli* or *K. pneumoniae* bacteraemia.**

	Cefotaxime-non-susceptible	Cefotaxime-susceptible	Univariate analysis	
Characteristics	(n=53)	(n=294)	OR (95% CI)	P
Empirical therapy				
Carbapenem	20 (38%)	47 (16%)	3.2 (1.7-6.0)	0.001
Oxyimino-cephalosporin	18 (34%)	123 (42%)	0.7 (0.4-1.3)	0.36
Other cephems	4 (8%)	39 (13%)	0.5 (0.2-1.6)	0.36
β -lactam/ β -lactamase inhibitor	11 (21%)	68 (23%)	0.9 (0.4-1.8)	0.86
Others	0 (0%)	17 (6%)	0.1 (0.0-2.5)	0.09
Appropriate empirical therapy	43 (81%)	285 (97%)	0.1 (0.0-0.4)	<0.001
Outcomes				
Complete response within 72 hours	20 (37%)	153 (52%)	0.6 (0.3-1.0)	0.07
Complete response within 7 days	38 (70%)	251 (85%)	0.4 (0.2-0.9)	0.03
Durations between appropriate therapy and complete response	3 (2-7)	3 (2-6)		0.72
ICU admission	10 (19%)	27 (8%)	2.6 (1.2-5.7)	0.02
30-day mortality	11 (21%)	15 (5%)	4.9 (2.1-11.3)	<0.001

2 Data are presented as the No. (%) or median (interquartile range).

1 **Table 5. Factors associated with 30-day mortality in patients with *E. coli* or *K. pneumoniae* bacteraemia.**

Characteristics	Non-survivors		Survivors		Univariate analysis		Multivariate analysis ^a	
	(n=26)		(n=321)		OR (95% CI)	P	OR (95% CI)	P
Age (years)	64	(59-70)	67	(57-76)		0.41		
Male sex	13	(50%)	170	(53%)	0.9 (0.4-2.0)	0.84		
<i>E. coli</i> bacteraemia	17	(65%)	218	(68%)	0.9 (0.4-2.1)	0.83		
Cefotaxime-non-susceptible bacteraemia	11	(42%)	42	(13%)	4.9 (2.1-11.3)	<0.001	1.6 (0.5-4.5)	0.41
ESBL bacteraemia	11	(42%)	36	(11%)	5.8 (2.5-13.6)	<0.001		
pAmpC bacteraemia	0	(0%)	10	(3%)	0.6 (0.03-9.8)	0.59		
Polymicrobial bacteraemia	3	(12%)	15	(5%)	2.7 (0.7-9.9)	0.14		
Nosocomial or healthcare-associated bacteraemia	19	(73%)	195	(61%)	1.8 (0.7-4.3)	0.29		
Previous isolation of MDR bacteria	8	(31%)	39	(12%)	3.2 (1.3-7.9)	0.01		
Previous antimicrobial use ^a	15	(58%)	149	(46%)	1.6 (0.7-3.5)	0.31		
Charlson index	4.5	(3-6)	2	(1-3)		<0.001	1.6 (1.2-2.1)	<0.001
Use of immunosuppressive drugs	6	(23%)	96	(30%)	0.7 (0.3-1.8)	0.66		
Haematological malignancy	8	(31%)	54	(17%)	2.2 (0.9-5.3)	0.11		
Solid malignancy	14	(54%)	108	(34%)	2.3 (1.0-5.1)	0.05		

Transplantation	3 (12%)	46 (14%)	0.8 (0.2-2.7)	1		
Haemodialysis	1 (4%)	13 (4%)	0.9 (0.1-7.5)	1		
Diabetes	6 (23%)	71 (22%)	1.1 (0.4-2.7)	1		
Liver disease	13 (50%)	73 (23%)	3.4 (1.5-7.7)	0.004		
Surgery	1 (4%)	31 (10%)	0.4 (0.0-2.9)	0.49		
Neutropenia	9 (35%)	45 (14%)	3.2 (1.4-7.7)	0.01	2.7 (0.8-8.4)	0.09
Intravascular catheterisation	19 (73%)	136 (42%)	3.7 (1.5-9.0)	0.003		
Artificial devices other than intravascular catheter	7 (27%)	78 (24%)	1.1 (0.5-2.8)	0.81		
Site of infection						
Urinary tract	5 (19%)	128 (40%)	0.4 (0.1-1.0)	0.04		
Intra-abdominal infection	10 (38%)	105 (33%)	1.3 (0.6-2.9)	0.53		
Primary	6 (23%)	74 (23%)	1.0 (0.4-2.6)	1		
Others	5 (19%)	14 (4%)	5.2 (1.7-15.9)	0.009		
SOFA score	2 (2-4)	1 (0-2)		<0.001	1.4 (1.2-1.6)	<0.001
Inappropriate empirical therapy	4 (15%)	15 (5%)	3.7 (1.1-12.1)	0.04		
Empirical therapy						
Carbapenem	5 (19%)	62 (19%)	1.0 (0.4-2.7)	1		

Oxyimino-cephalosporin	11 (42%)	130 (40%)	1.1 (0.5-2.4)	0.84
Other cepheids	2 (8%)	31 (13%)	0.6 (0.1-2.5)	0.76
β -lactam/ β -lactamase inhibitor	7 (27%)	72 (22%)	1.3 (0.5-3.2)	0.63
Others	1 (4%)	16 (5%)	0.8 (0.1-6.0)	1

1 Data are presented as the No. (%) or median (interquartile range). All variables with a *P*-value of less than 0.05 on univariate analyses were included in the
2 multivariate analysis. Cefotaxime-non-susceptible bacteraemia and severe sepsis or septic shock were forced into the models. Stepwise logistic regression
3 analysis was performed using forward selection and likelihood ratio. Only the variables in the last model were presented as the final result. The goodness of
4 fit of the last model was evaluated by Hosmer and Lemeshow test (*P*=0.65).

5 ^a None of the specific antibiotic was significantly associated with mortality.

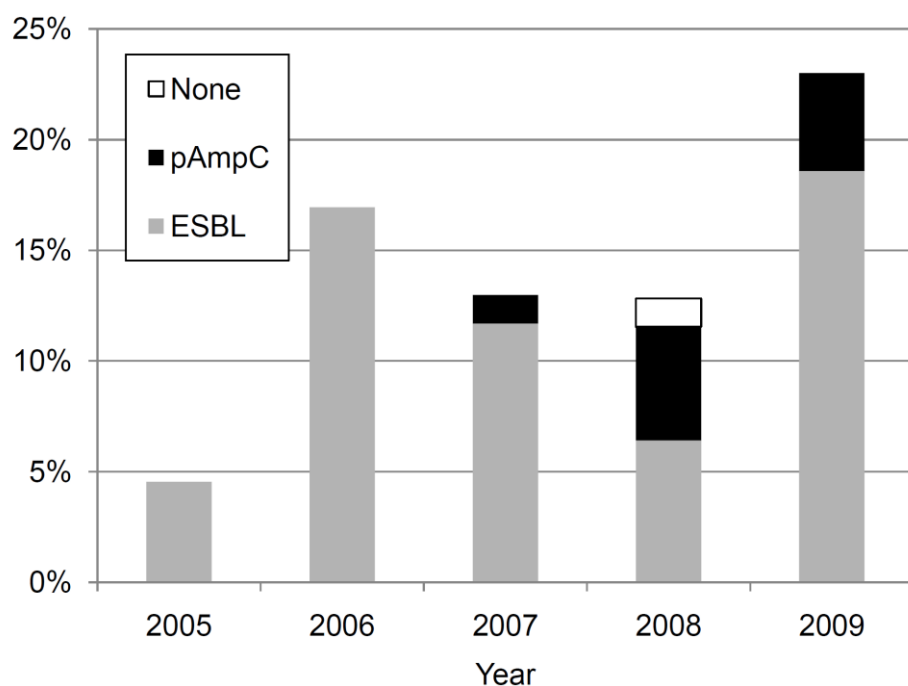


Fig. 1 Prevalence of bacteraemia due to cefotaxime-non-susceptible *E. coli* or *K. pneumoniae* stratified by extended-spectrum β -lactamase (ESBL) and plasmid mediated-AmpC β -lactamase (pAmpC) production. CTXNS-EK increased from 4.5% in 2005 to 23.0% in 2009. All cefotaxime-non-susceptible isolates had ESBL or pAmpC, except for one isolate in 2008. Only one cefotaxime-susceptible isolate in 2009 had ESBL.