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 μg/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.

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

Introduction

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

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

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

Materials and Methods

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/mm^3. 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 *Spe*I 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 (MIC $\leq$1 μg/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 μg/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 μg/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

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

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

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Conflict of interest

The authors declare that they have no conflict of interest.

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

	Cefotaxime-non-susceptible (n=58)		Cefotaxime-susceptible (n=313)	
Bacteria				
Escherichia coli	50	(86.2%)	199	(63.6%)
Klebsiella pneumoniae	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.

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

	ESBL or pAmpC producers		ESBL producers		pAmpC producers	
Antimicrobial agent	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%

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

[a] ESBL screening was performed according to the CLSI microdilution methodology using cefotaxime, ceftazidime, cefpodoxime, and aztreonam.

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

Characteristics	Cefotaxime-non-susceptible (n=53)	Cefotaxime-susceptible (n=294)	Univariate analysis		Multivariate analysis	
			OR (95% CI)	*P*	OR (95% CI)	*P*
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 cephems	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

MDR, multidrug-resistant; OR, odds ratio; CI, confidence interval.

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 multivariate analysis. Stepwise logistic regression 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 fit of the last model was evaluated by Hosmer and Lemeshow test (P=0.56).

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

Characteristics	Cefotaxime-non-susceptible (n=53)	Cefotaxime-susceptible (n=294)	Univariate analysis 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

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

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

Characteristics	Non-survivors (n=26)	Survivors (n=321)	Univariate analysis OR (95% CI)	*P*	Multivariate analysis[a] 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		
E. coli 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 cephems	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

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 multivariate analysis. Cefotaxime-non-susceptible bacteraemia and severe sepsis or septic shock were forced into the models. Stepwise logistic regression 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 fit of the last model was evaluated by Hosmer and Lemeshow test (*P*=0.65).

[a] None of the specific antibiotic was significantly associated with mortality.

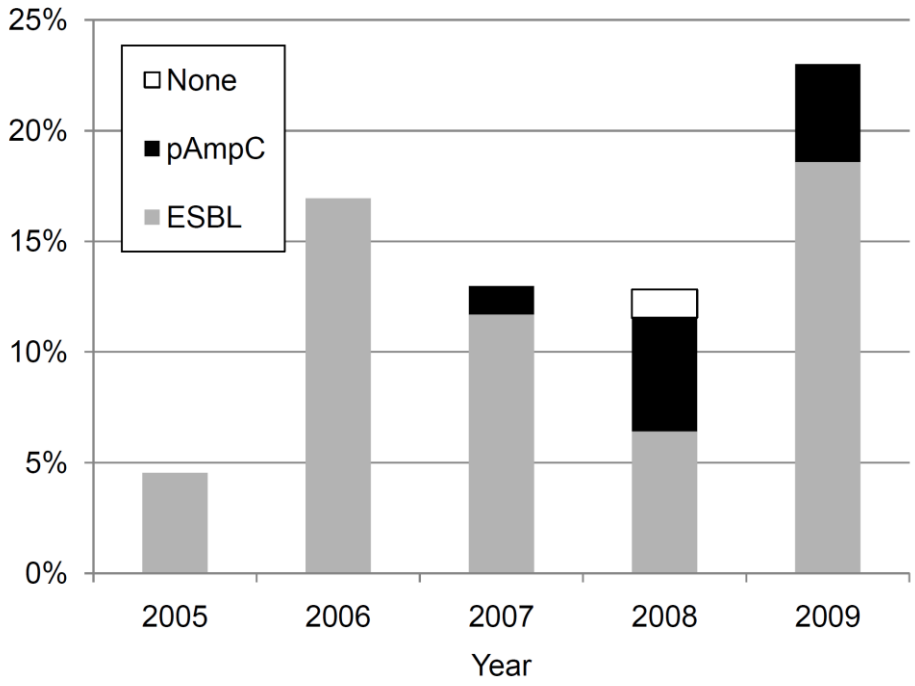

Fig. 1 Prevalence of bacteraemia due to cefotaxime-non-susceptible *E. coli* or *K. pneumoniae* stratified by extended-spectrum β-lactamase (ESBL) and plasmid mediated-AmpC β-lactamase (pAmpC) production. CTXNS-EK increased from 4.5% in 2005 to 23.0% in 2009. All cefotaxime-non-susceptible isolates had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