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ORIGINAL ARTICLE

The impact of revised CLSI cefazolin breakpoints on the clinical outcomes of *Escherichia coli* bacteremia

Kung-Ching Wang [a], **Meei-Fang Liu** [a], **Chin-Fu Lin** [c], **Zhi-Yuan Shi** [a,b,*]

[a] *Division of Infectious Diseases, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan*
[b] *School of Medicine, National Yang-Ming University, Taipei, Taiwan*
[c] *Microbiology Section of the Medical Laboratory, Taichung Veterans General Hospital, Taichung, Taiwan*

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Abstract *Background/Purpose:* The susceptibility breakpoints of cephalosporins for Enterobacteriaceae were revised by the Clinical and Laboratory Standards Institute (CLSI) in 2010 and 2011. The clinical outcome and susceptibility data were analyzed to evaluate the impact of revised CLSI cefazolin breakpoints on the treatment of *Escherichia coli* bacteremia.
Methods: Forty-three bacteremic *Escherichia coli* isolates from Taichung Veterans General Hospital, Taichung, Taiwan, during the period from January 2013 to December 2013, were selected to analyze the minimum inhibitory concentration (MIC) distributions of cefazolin and the correlated clinical responses to cefazolin therapy.
Results: The modal cefazolin MIC among the 43 isolates was 1 μg/mL and accounted for 18 (42%) isolates. The cumulative percentage for MICs $\leq$ 2 μg/mL was 79%. The conventional dosing regimens achieved clinical cure in 33 (97%) of 34 patients with bacteremia due to *E. coli* with a cefazolin MIC $\leq$ 2 μg/mL, in all of the six patients with a cefazolin MIC of 4 μg/mL, and all of the three patients with a cefazolin MIC of 8 μg/mL.
Conclusion: The microbiological data support the revised CLSI breakpoints of cefazolin. The conventional cefazolin dosing regimens can still achieve satisfactory clinical cure rates for bacteremia of *E. coli* with a cefazolin MIC $\leq$ 2 μg/mL in patients without severe septic shock.

* Corresponding author. Section of Infectious Diseases, Taichung Veterans General Hospital, Number 1650, Section 4, Taiwan Boulevard, Taichung 40705, Taiwan.
E-mail address: zyshi@vghtc.gov.tw (Z.-Y. Shi).

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Before the approval of the efficacy of cefazolin for the treatment of *E. coli* isolates with a cefazolin MIC of 4 μg/mL, it is prudent to use cefazolin only when a high drug level can be achieved in the infection site, such as the urinary tract.

Introduction

The breakpoints of cefazolin against Enterobacteriaceae had been in existence for >30 years, before new breakpoints were revised by the Clinical and Laboratory Standards Institute (CLSI) in January 2010.[1] The CLSI 2010 performance standards revised the interpretive criteria for cephalosporins and aztreonam.[1] The minimum inhibitory concentration (MIC) breakpoints for cefazolin were changed from susceptible, ≤ 8 μg/mL; intermediate, 16 μg/mL; and resistant, ≥ 32 μg/mL in CLSI 2009[2] to susceptible, ≤ 1 μg/mL; intermediate, 2 μg/mL; and resistant, ≥ 4 μg/mL in CLSI 2010.[1] The choice of cefazolin breakpoints (susceptible, ≤ 1 μg/mL) was based on the conventional dose of cefazolin (i.e., 1 g every 8 hours) according to Monte Carlo Simulation in two pharmacokinetic studies of cefazolin.[3,4] However, the target attainment rates for 50% T > MIC can achieve 94∼100% at the dose of 2 g every 8 hours suggested that a breakpoint of susceptibility of ≤ 2 μg/mL may be acceptable.[3,4] To prevent the impact of eliminating cefazolin as a useful agent for the treatment and prevention of infections caused by some common Enterobacteriaceae without a resistance mechanism, the breakpoints of cefazolin for Enterobacteriaceae were further revised by the CLSI in 2011 (i.e., susceptible, ≤ 2 μg/mL; intermediate, 4 μg/mL; and resistant, ≥ 8 μg/mL, based on a dosage regimen of 2 g every 8 hours).[5,6] However, there were only a few studies describing the cefazolin dosing regimens and the correlated clinical and/or bacteriological outcome.[6–8] They did not provide information for MIC data and the decision of breakpoints.[6]

Therefore, the aims of this study were analysis of MIC distribution data of *Escherichia coli* and the impact of revised CLSI cefazolin breakpoints on the clinical outcome of *E. coli* bacteremia.

Methods

This study was approved by the Institutional Review Board (IRB) of Taichung Veterans General Hospital, Taichung, Taiwan (IRB number CE14247).

Bacterial isolates

During the period from January 2013 to December 2013, 506 *E. coli* nonduplicate isolates from blood specimens were identified at the Microbiology Laboratory of Taichung Veterans General Hospital. The disk diffusion method was used for susceptibility tests of these isolates and read according to the CLSI 2009 recommendations.[2] There were 320 isolates susceptible to cefazolin and 186 isolates resistant to cefazolin (by the disk diffusion test, CLSI 2009).[2]

The medical records were reviewed for all of these 320 patients with bacteremia of *E. coli* susceptible to cefazolin (by the disk diffusion test, CLSI 2009). Patients with *E. coli* bacteremia due to cefazolin-susceptible isolates (by the disk diffusion test, CLSI 2009) were included with the conditions that cefazolin was the only beta-lactam antimicrobial agent administered throughout the course and used for at least 7 days; or the empiric antimicrobial agents other than cefazolin had not been used for longer than 2 days, and then cefazolin was used instead for at least 7 days. Patients with the following conditions were excluded: ≤ 18 years of age, malignancy, neutropenia, AIDS, negative culture for *E. coli* at the source of infection other than the bloodstream (Figure 1). Finally, only 43 isolates had met these criteria and were selected for MIC study.

Susceptibility test

The MICs of cefazolin (Sigma-Aldrich, St. Louis, MO, USA) were assessed using the broth microdilution method according to CLSI recommendations.[9] The antibiotics were serially diluted twofold in 4 mL of cation-adjusted Mueller-Hinton broth. The final range of antibiotic concentrations was 0.01–256 μg/mL. The bacterial suspension was prepared from actively growing bacteria in 2 mL of cation-adjusted Mueller-Hinton broth, and diluted to a bacterial cell density of 1×10^6 colony forming units/mL. To yield a final inoculum of approximately 5×10^4 colony forming units/mL, the 10 μL of bacterial suspension was then added to the wells containing 100 μL of serially diluted antimicrobial agents. The MICs were read after overnight incubation (16–20 hours) at 35°C. The MIC breakpoints were interpreted according to the guidelines established by the CLSI.[1,5] *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as the quality control strains.

Clinical and bacteriological assessments

The following variables identified from medical records were assessed: age, sex, shock status, source of infection, length of hospital stay, time of defervescence after antimicrobial treatment, clinical response, intensive care unit (ICU) stay, and death. Shock was defined as a systolic pressure < 90 mmHg that was unresponsive to fluid treatment.[10] The source of bacteremia was determined by clinical assessment and positive growth of *E. coli* at the source of infection.[11] Heart failure was defined as the clinical diagnosis of heart failure and ejection

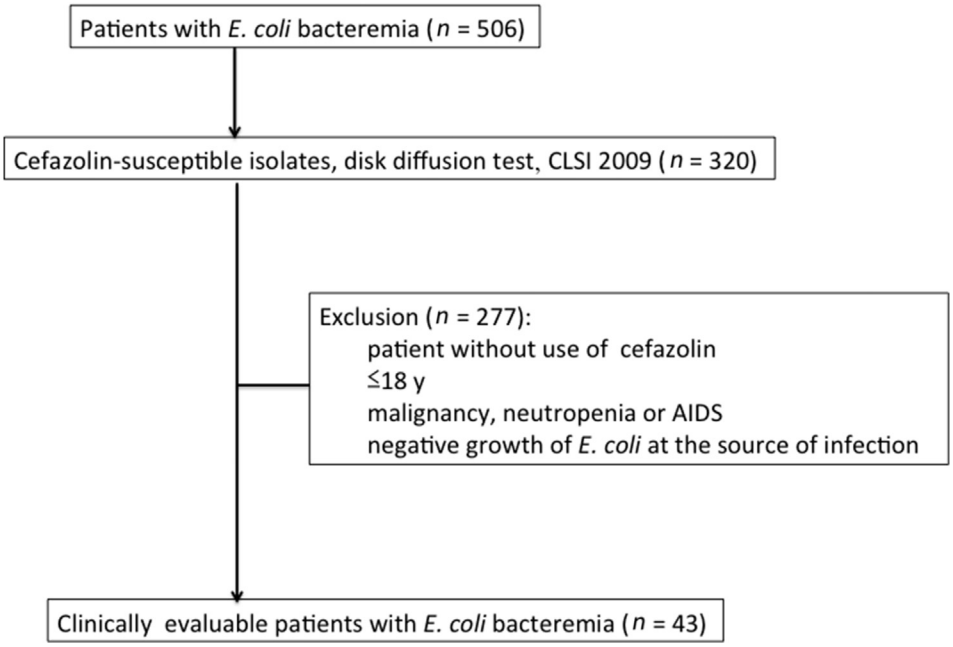

Figure 1. Flowchart of patient enrollment. *E. coli* = *Escherichia coli*.

fraction $\leq$ 40% or evidence of abnormal left ventricular diastolic dysfunction that can be determined by Doppler echocardiography or cardiac catheterization.[12] Renal insufficiency was defined as glomerular filtration rate $<$ 60 mL/min/1.73 m^2 which presented $>$ 3 months.[13] Diabetes mellitus was defined as fasting plasma glucose $\geq$ 126 mg/dL or the 2 hour plasma glucose value after a 75 g oral glucose tolerance test $\geq$ 200 mg/dL.[14] Immune compromised status included chronic steroid use (daily equivalent of 20 mg prednisone for $\geq$ 1 mo/y) and/or solid organ transplantation.[15]

The efficacy was assessed by the clinical and bacteriological response.[16,17] The clinical response was evaluated at the end of antimicrobial treatment and defined as cure, failure, or indeterminate (a clinical assessment was not possible for any reason). The bacteriological response was evaluated at the 7th day after the discontinuation of antimicrobial treatment and defined as eradication, presumed eradication (absence of evaluable culture in a patient with clinical cure), persistence, presumed persistence (absence of evaluable culture in a patient with clinical failure of treatment), or indeterminate (if bacteriological response was not evaluable for any reason). Bacteriological success was defined if eradication or presumed eradication were present. Bacteriological failure was rated as persistence or presumed persistence. The 14-day, 28-day and in-hospital mortality rates were analyzed.

Statistical analysis

Categorical variables were analyzed by the Pearson χ^2 test; continuous variables were analyzed by the Student *t* test and analysis of variance test. A $p < 0.05$ was considered statistically significant. All statistical analyses were performed with SPSS version 22.0.0 for Windows (IBM corp., Armonk, NY, USA).

Results

A total of 43 *E. coli* isolates and their related infected patients were enrolled in this study. There were 2 (5%), 4 (9%), 18 (42%), 10 (23%), 6 (14%), and 3 (7%) isolates with the cefazolin MICs of 0.25 μg/mL, 0.5 μg/mL, 1 μg/mL, 2 μg/mL, 4 μg/mL, and 8 μg/mL, respectively. The modal cefazolin MIC was 1 μg/mL and accounted for 18 (42%) isolates. The cumulative percentage for cefazolin MICs $\leq$ 2 μg/mL was 79%.

The clinical characteristics and outcomes of 43 patients with *E. coli* bacteremia were classified into three groups, i.e., the cefazolin MICs $\leq$ 2 μg/mL, 4 μg/mL, and 8 μg/mL (Table 1). There were no statistically significant differences in the demographic characteristics, comorbidity, length of stay, time to defervescence, duration of therapy, and mortality rates among the three groups. *E. coli* was isolated from urinary tracts in 41 (95%) patients, and from biliary tracts in two (5%) patients. The length of stay in hospital in the three groups of cefazolin MICs $\leq$ 2 μg/mL, 4 μg/mL, and 8 μg/mL were 15.7 $\pm$ 12.4 days, 14.3 $\pm$ 4.8 days, and 18.0 $\pm$ 16.6 days, respectively ($p = 0.91$). The periods to defervescence in the three groups of cefazolin MICs $\leq$ 2 μg/mL, 4 μg/mL, and 8 μg/mL were 3.47 $\pm$ 1.91 days, 3.33 $\pm$ 2.4 days, and 3.67 $\pm$ 2.0 days, respectively ($p = 0.97$). The duration of treatment in the three groups of cefazolin MICs $\leq$ 2 μg/mL, 4 μg/mL, and 8 μg/mL were 13.4 $\pm$ 4.4 days, 13.8 $\pm$ 4.0 days, and 11.0 $\pm$ 6.0 days, respectively ($p = 0.63$). The clinical cure rates in the three groups of patients with cefazolin MICs $\leq$ 2 μg/mL, 4 μg/mL, and 8 μg/mL were 97%, 100%, and 100%, respectively. The total bacteriological success rates (eradication plus

Table 1 The demography and clinical outcomes of 43 patients with *Escherichia coli* bacteremia, stratified by cefazolin minimum inhibitory concentrations (MICs).

Clinical characteristics	Cefazolin MIC (μg/mL)			*p*
	≤ 2 (*n* = 34)	4 (*n* = 6)	8 (*n* = 3)	
Age (y)	68.1 ± 14.2	68.2 ± 16.0	55.0 ± 26.3	0.37
Gender				0.59
Female	28 (82)	4 (67)	2 (67)	
Male	6 (18)	2 (33)	1 (33)	
Source of infection				
Urinary tract	33 (97)	5 (83)	3 (100)	0.31
Biliary tract	1 (3)	1 (17)	0 (0)	
Comorbidity				
Renal insufficiency	12 (35)	3 (50)	1 (33)	0.99
Heart failure	11 (32)	2 (33)	1 (33)	0.99
Diabetes mellitus	17 (50)	3 (50)	1 (33)	0.65
Immunosuppressive	4 (12)	0 (0)	0 (0)	0.56
Length of stay in hospital (d)	15.7 ± 12.4	14.3 ± 4.8	18.0 ± 16.6	0.91
Time to defervescence (d)	3.47 ± 1.91	3.33 ± 2.4	3.67 ± 2.0	0.97
Duration of therapy (d)	13.4 ± 4.4	13.8 ± 4.0	11.0 ± 6.0	0.63
Shock	2 (6)	1 (17)	1 (33)	0.22
ICU stay	2 (6)	1 (17)	1 (33)	0.22
Clinical response				
Cure	33 (97)	6 (100)	3 (100)	0.87
Failure	0 (0)	0 (0)	0 (0)	
Indeterminate	1 (3)	0 (0)	0 (0)	
Bacteriological response				
Eradication	22 (64)	3 (50)	3 (100)	0.33
Presumed eradication	12 (36)	3 (50)	0 (0)	
14-Day mortality	1 (3)	0 (0)	0 (0)	0.87
28-Day mortality	1 (3)	0 (0)	0 (0)	0.87
In hospital mortality	2 (6)	0 (0)	0 (0)	0.76

Data are presented as *n* (%) or mean ± standard deviation.
ICU = intensive care unit.

presumed eradication) were 100% for the 43 patients, evaluated at the 7th day after the discontinuation of antimicrobial treatment.

The in-hospital mortality rate was 4.6% (2/43). Two patients in the group of cefazolin MIC ≤ 2 μg/mL expired during the hospitalization. Although one patient had achieved clinical cure, she expired due to congestive heart failure 7 days after the end of cefazolin therapy. Another patient died from aspiration pneumonia at the 10th day of cefazolin therapy, although fever had subsided and the symptoms of bacteremia had improved. Therefore, her clinical response was regarded as indeterminate.

The conventional dosing regimens (i.e., 1 g every 8 hours, 1 g every 6 hours, and the reduced dose regimens for impaired renal function) achieved clinical cure in 33 (97%) of 34 patients with bacteremia due to *E. coli* with a cefazolin MIC ≤ 2 μg/mL, in all of the six patients with a cefazolin MIC of 4 μg/mL, and in all of the three patients with a cefazolin MIC of 8 μg/mL (Table 2). Six patients were treated with cefazolin 1 g every 12 hours as a result of renal insufficiency and three patients were treated with cefazolin 1 g every day due to end stage renal disease.

Table 2 Cefazolin dosing regimens and the clinical cure rates in 43 patients with *Escherichia coli* bacteremia.

Dosage	Number of patients with clinical cure (%)			
	≤ 1 μg/mL	2 μg/mL	4 μg/mL	8 μg/mL
	n = 24	*n* = 10	*n* = 6	*n* = 3
1 g every 6 h	13/13 (100)	6/6 (100)	3/3 (100)	2/2 (100)
1 g every 8 h	6/6 (100)	3/3 (100)	1/1 (100)	—
1 g every 12 h[a]	2/3 (67)	1/1 (100)	2/2 (100)	—
1 g every 24 h[a]	2/2 (100)	—	—	1/1 (100)

[a] 1 g every 12 hours and 1 g every 24 hours were used for patients with impaired renal function.

Discussion

The small number of cases in this study is due to several causes. First, to evaluate the bacteriological efficacy of cefazolin for the treatment of sources of infection other than the bloodstream, patients with negative culture of *E. coli* from the primary source of infection were excluded. Second, using potent antimicrobial agents as empiric treatment for ≥ 3 days will affect the assessment of cefazolin efficacy. Therefore, these patients were excluded

from this study. Third, patients with severe bacteremia could be complicated by a systemic inflammatory response, pulmonary edema, mechanic ventilation, and ventilator-associated pneumonia. Under these conditions, other potent antimicrobial agents would be used by clinicians instead of cefazolin. Therefore they were also excluded. The patient selection bias in this retrospective study reflects the real-world practice. Therefore, the evaluation of cefazolin efficacy focuses on the patient population of *E. coli* bacteremia without severe septic shock in this study.

From the viewpoint of microbiological data, the modal cefazolin MIC among the 43 isolates was 1 μg/mL and accounted for 18 (42%) isolates. The cumulative percentage for MIC ≤ 2 μg/mL was 79%. If the susceptibility breakpoint of cefazolin was 1 μg/mL according to the CLSI 2010, 10 (23%) isolates with MIC of 2 μg/mL would be interpreted as intermediate. This would not be compatible with the high clinical cure rate in the group of patients with a cefazolin MIC of 2 μg/mL. Setting the susceptibility breakpoints of cefazolin as 1 μg/mL would have an impact on the choice of cefazolin as an effective drug for the treatment and prophylaxis of infections cause by Enterobacteriaceae. The MIC distributions are similar to the results of the following two studies. The MIC distributions of wild-type microorganisms are available on the EUCAST website.[18] Among 274 wild-type *E. coli* isolates with cefazolin MIC ≤ 8 μg/mL, there were 37 (13.5%), 131 (47.8%), 67 (24.5%), 27 (9.8%), and 12 (4.4%) isolates with cefazolin MICs of 0.5 μg/mL, 1 μg/mL, 2 μg/mL, 4 μg/mL, and 8 μg/mL, respectively. The cumulative percentage for MIC ≤ 2 μg/mL was 85.8%. In another study, the SENTRY Antimicrobial Surveillance Program from 1997 to 2001 worldwide, 48,440 Enterobacteriaceae were analyzed for susceptibility.[19] The cumulative percentage for cefazolin MICs ≤ 2 μg/mL was 72.7% among the isolates with cefazolin MICs ≤ 8 μg/mL.[19] The cumulative percentages of the isolates with MIC ≤ 2 μg/mL ranged from 72.7% to 85.8%, and accounted for the majority of isolates without a resistance mechanism in these two previous reports and our study. These microbiological data are all in agreement with the susceptible breakpoint (≤ 2 μg/mL) of cefazolin MIC by CLSI 2011, rather than that (≤ 1 μg/mL) by CLSI 2010.

Automated systems for antimicrobial susceptibility testing have become more common in the clinical microbiology laboratories. However, there are differences between CLSI and U.S. Food and Drug Administration (FDA) breakpoints; commercial manufacturers must use the FDA breakpoints. For example, the current cefazolin concentration range on the AST-GN69 card of Vitek 2 (bioMérieux, Inc., Durham, NC, USA) is 4—64 μg/mL, which precludes an evaluation of the current CLSI susceptible breakpoints (≤ 2 μg/mL) for Enterobacteriaceae.[20] The impact of differences between CLSI and FDA breakpoints on automated antimicrobial susceptibility testing systems needs to be solved by further work.

From the viewpoint of PK-PD data, the cefazolin breakpoints by CLSI were revised in 2010 and 2011[1,5] based on the Monte Carlo simulation analysis in two studies.[3,4] The target attainment rates for 50% T > MIC could achieve 94～100% for the isolates with a cefazolin MIC of 1 μg/mL and 64～83% for those of 2 μg/mL at the conventional dose regimen of 1 g every 8 hours. The target attainment rates could achieve 94～100% for the isolates with a cefazolin MIC of 2 μg/mL at the higher dose of 2 g every 8 hours.[3,4] Therefore, the interpretive criteria for cefazolin in the CLSI 2011 were revised (susceptible, ≤ 2 μg/mL; intermediate, 4 μg/mL; and resistant, ≥ 8 μg/mL) based on a higher dosing regimen (2 g every 8 hours).[5] It is a less optimal condition that the target attainment rates for 50% T > MIC for isolates with a cefazolin MIC of 4 μg/mL can achieve only 42～51% at the dose of 1 g every 6 hours and 65～84% at the dose of 2 g every 8 hours. The bacteremia of *E. coli* with a cefazolin MIC of 4 μg/mL was eradicated with cefazolin of 1 g every 6 hours or 1 g every 8 hours in this study. It is prudent to use cefazolin for the treatment of *E. coli* isolates with a cefazolin MIC of 4 μg/mL when a high drug level can be achieved in the infection site. Cefazolin can reach a high drug concentration in the urinary tract and nonobstructive biliary tract.[21,22] Studies have shown that following intravenous administration of cefazolin to normal volunteers, mean serum concentrations peaked at ～188 μg/mL and were ～4 μg/mL at 8 hours for a 1 g dose.[21,22] Bile levels in patients without obstructive biliary disease can reach or exceed serum levels by up to five times. However, bile levels of cefazolin are considerably lower than serum levels in patients with obstructive biliary disease.

From the viewpoint of clinical data, the conventional dosing regimens (i.e., 1 g every 8 hours, 1 g every 6 hours, and the reduced dose regimens for impaired renal function) achieved clinical cure rates in 42 (98%) of 43 patients with cefazolin MICs ≤ 8 μg/mL in this study. The previous study of *Klebsiella pneumoniae* bacteremia also showed similar results, i.e., the conventional dosing regimens of cefazolin (1 g every 6 hours or 8 hours) achieved a clinical cure in 70 (97.2%) of 72 patients with a cefazolin MIC ≤ 1 μg/mL, in 14 (87.5%) of 16 patients with a cefazolin MIC of 2 μg/mL, and in three (100%) of three patients with the cefazolin MICs of 4～8 μg/mL.[16] Cefazolin has been used according to breakpoints of 8 μg/mL for > 30 years.[6] It is widely believed that the conventional dosing schedule of 1 g every 8 hours has been used with satisfactory results for decades. This belief may be well founded for the treatment of noninvasive infections, especially those involving the urinary tract, where the drug can reach a high concentration.[6] It seems effective to use cefazolin for the treatment of bacteremia caused by Enterobacteriaceae with cefazolin MICs of 4～8 μg/mL in these two studies. This is discordant with the intermediate and resistant breakpoints by CLSI 2011. However, the numbers of patients in the group with the cefazolin MICs of 4～8 μg/mL are too small to make a conclusion in these two studies.

There were some limitations in this study. First, the conventional dosing regimen appears effective for the treatment of *E. coli* bacteremia with a cefazolin MIC ≤ 2 μg/mL in patients who had no severe septic shock. This is discordant with the higher dosing regimen recommended by CLSI 2011. Determination of the conventional or higher dosing regimen warrants further large scale clinical studies. Second, the relatively small number in the group of cefazolin MICs of 4～8 μg/mL was not sufficient to demonstrate a significant association between cefazolin MIC and clinical outcome, although the patients were successfully treated with cefazolin. Third, most critically ill patients had been

treated with extended-spectrum cephalosporins or carbapenems, and they were excluded from the analysis. Therefore, the evaluation of cefazolin efficacy in this study focuses on the patient population of *E. coli* bacteremia without severe septic shock. Fourth, Acute Physiology and Chronic Health Evaluation (APACHE) scores were calculated only when patients were admitted to the ICU. Most of the patients in our study were not admitted to the ICU, so APACHE scores were not listed in this study. Further prospective comparative trials of cefazolin versus extended-spectrum cephalosporins are also difficult to conduct due to ethical dilemmas. Fifth, patients with malignancies and neutropenia were excluded. Patients with malignancies could have persistent symptoms and signs in the organs of malignancies. Patients with immunocompromised conditions could have other unexpected concurrent infections. Patients with neutropenia could have a febrile condition without bacteremia. All of these conditions would affect the assessment of clinical response to antimicrobial treatment. Therefore, they were excluded in many clinical studies.[23–25] Sixth, the primary sources of *E. coli* bacteremia included only urinary tract and biliary tract in this study. This is because the most common sources of *E. coli* bacteremia are urinary tract and intraabdominal infections; the other sources of *E. coli* bacteremia are few.[26,27]

In conclusion, the microbiological data support the revised CLSI breakpoints of cefazolin. The conventional cefazolin dosing regimens can still achieve satisfactory clinical cure rates for bacteremia of *E. coli* with a cefazolin MIC $\leq$ 2 μg/mL in patients without severe septic shock. Before the approval of the efficacy of cefazolin for the treatment of *E. coli* isolates with a cefazolin MIC of 4 μg/mL, it is prudent to use cefazolin only when a high drug level can be achieved in the infection site, such as the urinary tract.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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