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FULL PAPER

In vitro Activity of Cefmetazole and Flomoxef among Extended-Spectrum Beta-Lactamase producing Enterobacterales

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SUMMARY

In this age of antimicrobial resistance (AMR), improving treatment using existing antibiotics is desirable. Extended-spectrum beta-lactamase (ESBL)-producing *Enterobacterales* (ESBL-E) are high priority AMR pathogens according to the World Health Organization. Cephamycin-class beta-lactams are tolerant to hydrolysis by ESBL activity and have bactericidal effects on ESBL-E. The aim of the present study was to compare the *in vitro* minimum inhibitory concentration (MIC) of cefmetazole (CMZ) and flomoxef (FMOX) among ESBL-E strains. This was a retrospective study using microbiology laboratory data from Okayama University Hospital (Japan) from January 2014 to June 2022. The MIC was determined by broth microdilution method and the ESBL phenotypes were determined by double-disk method. Antimicrobial use density (AUD) data for CMZ and FMOX were also gathered. Annual proportions of ESBL-producing organisms in *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* complex were 20.4-30.6%, 3.5-13.7%, and 0-3.1%, respectively. The ESBL-producing bacteria with MIC levels ≤1 μg/mL for CMZ and FMOX ranged from 57 to 84% and 97 to 100%, respectively, for *E. coli*, and from 50 to 92% and 80 to 100%, respectively, for *K. pneumoniae*. *E. cloacae* strains showed MIC levels ≥32 μg/mL for both agents. The AUD ratio for CMZ to FMOX ranged from 5.31 to 12.27, with no apparent upward or downward trend. Proportions of ESBL-producing *E. coli* and *K. pneumoniae* strains with MIC ≤1 μg/mL were greater in FMOX than in CMZ. To corroborate the clinical superiority of FMOX in treating ESBL-E infections, a randomized controlled study, as well as pharmacokinetic/pharmacodynamic analysis, is required.

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INTRODUCTION

Among the various genotypic and phenotypic antimicrobial resistance (AMR) pathogens emerging worldwide, third-generation cephalosporin-resistant *Enterobacterales,* or extended-spectrum beta-lactamase (ESBL)-producing *Enterobacterales* (ESBL-E), are high-priority organisms according to the World Health Organization (WHO) (World Health Organization, 2017). Carbapenems, such as meropenem and doripenem, are conventionally the drugs of choice for the treatment of ESBL-E infection, but the increasing use of these compounds can lead to microbial resistance (Doi Y., 2019). In 2017, the WHO promoted the development of new antimicrobials potentially active against ESBL-E (World Health Organization, 2015); however, an epoch-making drug has yet to be developed, probably because of the depletion of research seeds and lower interest by pharmaceutical manufacturers in comparison with other medical needs. In this context, the revival of existing antimicrobial agents could alleviate the global threat of AMR.

Cefmetazole (CMZ), a cephamycin-class beta-lactam agent, possesses a 7 α-methoxy side chain and is hence stable against ESBLs (Pitout *et al.*, 2008; Matsumura *et al.,* 2016); therefore, it is receiving increasing attention as a carbapenem-alternative therapy for ESBL-E infections. The *in vitro* potency and clinical effectiveness of CMZ against ESBL-E, even in bacteremia cases, has been well clarified, mainly by Japanese researchers (Doi A. *et al.*, 2013; Fukuchi *et al.*, 2016; Matsumura *et al.*, 2015). However, the utility of flomoxef (FMOX), an oxacephem group antibiotic

Key words:
Antibiotics, antimicrobial, Cefmetazole, *Enterobacterales*, extended-spectrum beta-lactamase, Flomoxef, minimum inhibitory concentration.

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that is also unique in possessing a difluoromethylthio-acetamido group at position 7 (Bauernfeind *et al.*, 1991), has yet to be thoroughly examined, and its efficacy should be investigated, considering the shortage of antimicrobials. This is mainly because FMOX has been marketed only in East Asian countries, such as Japan, China, South Korea, and Taiwan since manufacturing began in the 1980s. Currently, we acknowledge that the CMZ resistance rate in ESBL-producing *Escherichia coli* has been shown to be higher than that of FMOX (Komatsu *et al*., 2018); however, the number of reported strains was very small (n=30), and further evaluation is required with the use of additional data. Therefore, the aim of the present study was to compare the distribution of the minimum inhibitory concentration (MIC) of CMZ and FMOX among ESBL-E isolates at a hospital laboratory.

METHODS

We retrospectively collected microbiology laboratory data from Okayama University Hospital (Japan) covering January 2014 to June 2022 (8.5 years). The data included *Enterobacterales* species and their MICs for CMZ and FMOX from routine clinical laboratory work. Data from both inpatients and outpatients were included, while duplicate isolates from the same patient in a year were excluded. Bacterial identification was performed using a MALDI Biotyper (Bruker Daltonics Inc., Billerica, MA, USA) and the MIC was determined by the broth microdilution method using Dry Plate Eiken (Eiken Chemical Co., Ltd, Tokyo, Japan) based on the Clinical and Laboratory Standards Institute (CLSI) M100 document (Clinical and Laboratory Standards Institute, 2021). The presence of ESBL was determined by phenotypic identification, as recommended by the CLSI. We calculated the number and percentage of MIC values for each year for the *E. coli, Klebsiella pneumoniae*, and *Enterobacter cloacae* complex. In addition, we determined the MIC_{50} and MIC_{90} for CMZ and FMOX for each ESBL-E organism. To compare the administration of CMZ and FMOX, we calculated the antimicrobial use density (AUD; defined daily doses divided by 100 patient-days) of these drugs at the hospital. The requirement for informed consent was waived because the data were fully anonymized and no patient-identifiable information was included.

RESULTS

During the study period, the strain numbers of *E. coli*, *K. pneumoniae*, and *E. cloacae* complex isolated in our laboratory were 2,615, 1,258, and 1,131, respectively. The total number (proportion) of ESBL-producing strains was 683 (26.1%), 96 (7.6%), and 17 (1.5%), respectively. The annual proportion of ESBL-E in each of these organisms is shown in *Figure 1*. *E. coli* had the highest annual proportions of between 20.4 and 30.6%, followed by *K. pneumonia* with 3.5 to 13.7%. In contrast, isolation of the ESBL-producing *E. cloacae* complex was rare, ranging from 0 to 3.1%.

The annual proportions of ESBL-E by MIC levels for CMZ and FMOX by year are shown in *Figure 2*. The proportion of ESBL-producing *E. coli* with MIC levels ≤1 μg/mL for CMZ ranged from 57 to 84%, with no increasing or decreasing trend. In contrast, the levels in FMOX remained high, between 97 and 100%, throughout the study period. The proportions

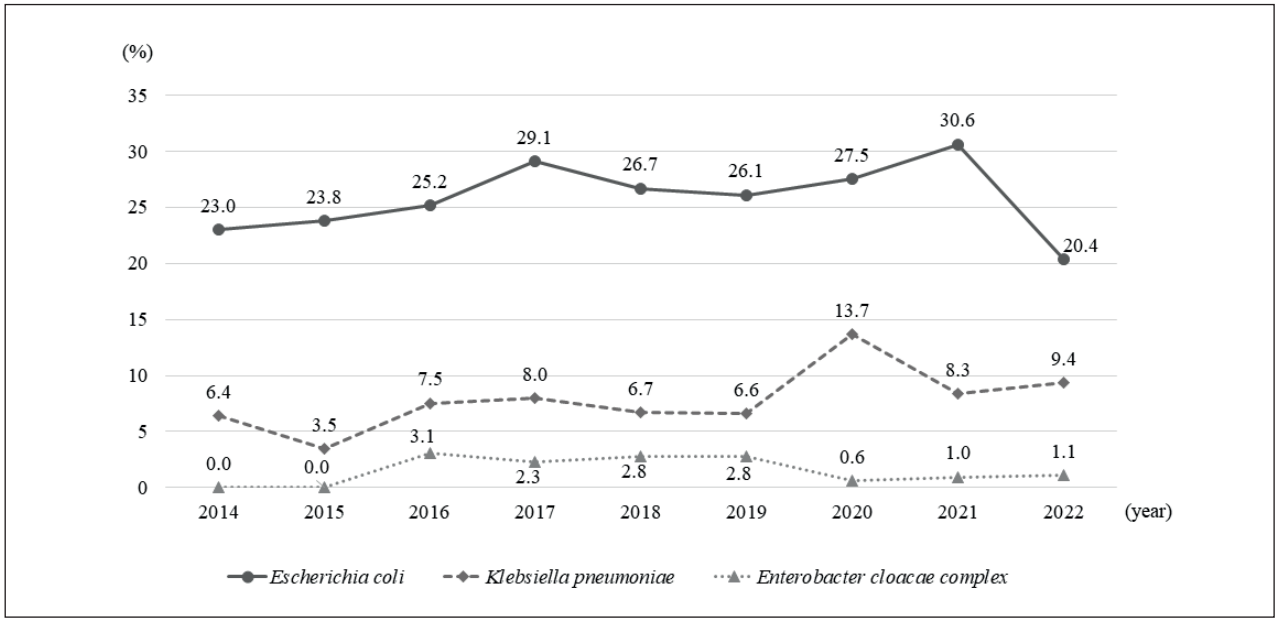

Figure 1 - *Trends in annual proportions of extended-spectrum beta-lactamase (ESBL) producing Enterobacterales.*

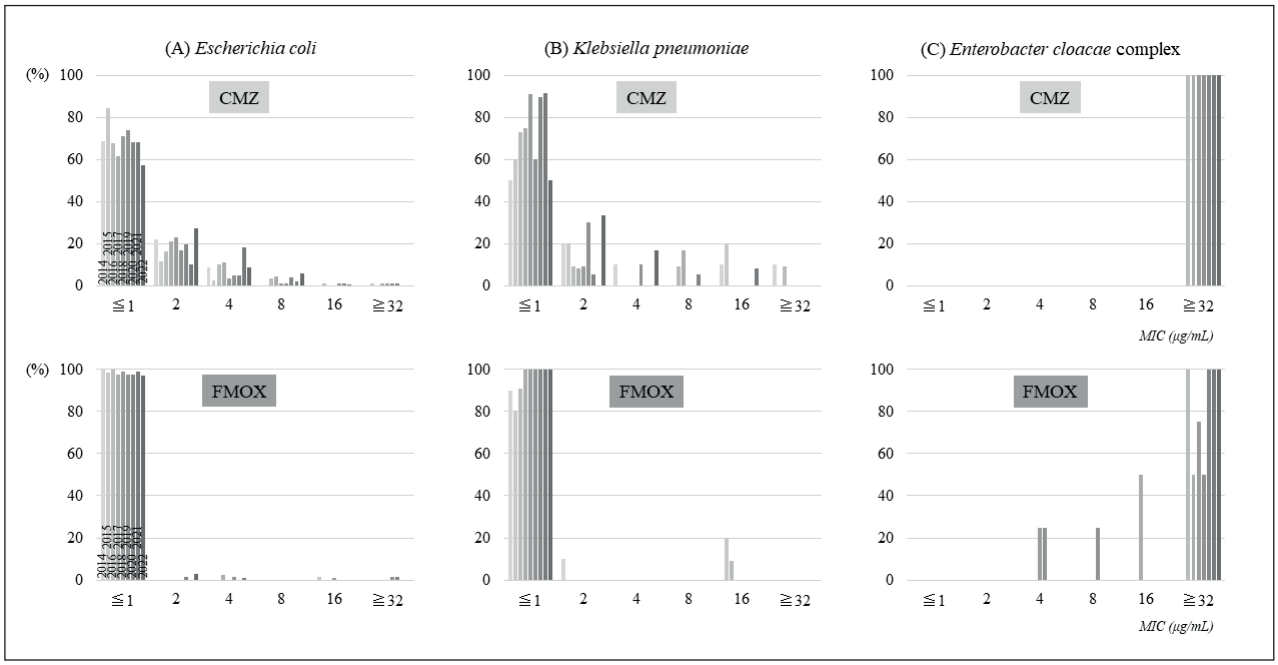

Figure 2 - *Annual isolation proportions of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli, Klebsiella pneumonia, and Enterobacter cloacae complex by minimum inhibitory concentration (MIC) levels of cefmetazole (CMZ) and flomoxef (FMOX), 2014 to 2022. Bars (left to right) indicate proportions in each year from 2014 to 2020.*

of ESBL-producing *E. coli* with MIC levels of 2 μg/mL or more were higher in CMZ than in FMOX, and the MIC_{50}/MIC_{90} of CMZ and FMOX were ≤1/4 μg/mL and ≤1/≤1 μg/mL, respectively. Similarly, the proportion of ESBL-producing *K. pneumoniae* with MIC levels ≤1 μg/mL for CMZ ranged from 50 to 92%, which was lower than those in FMOX, showing 80 to 100%. As observed with *E. coli*, the proportion of ESBL-producing *K. pneumoniae* with MIC levels of 2 μg/mL or more was also higher in CMZ than in FMOX. The MIC_{50}/MIC_{90} of CMZ and FMOX was ≤1/4 μg/mL and ≤1/≤1 μg/mL, respectively. As for the *E. cloacae* complex, all ESBL-producing strains showed MIC levels of CMZ ≥32 μg/mL. However, some isolates had relatively low MIC levels for FMOX. The MIC_{50}/MIC_{90} of CMZ and FMOX were ≥32/≥32 μg/mL

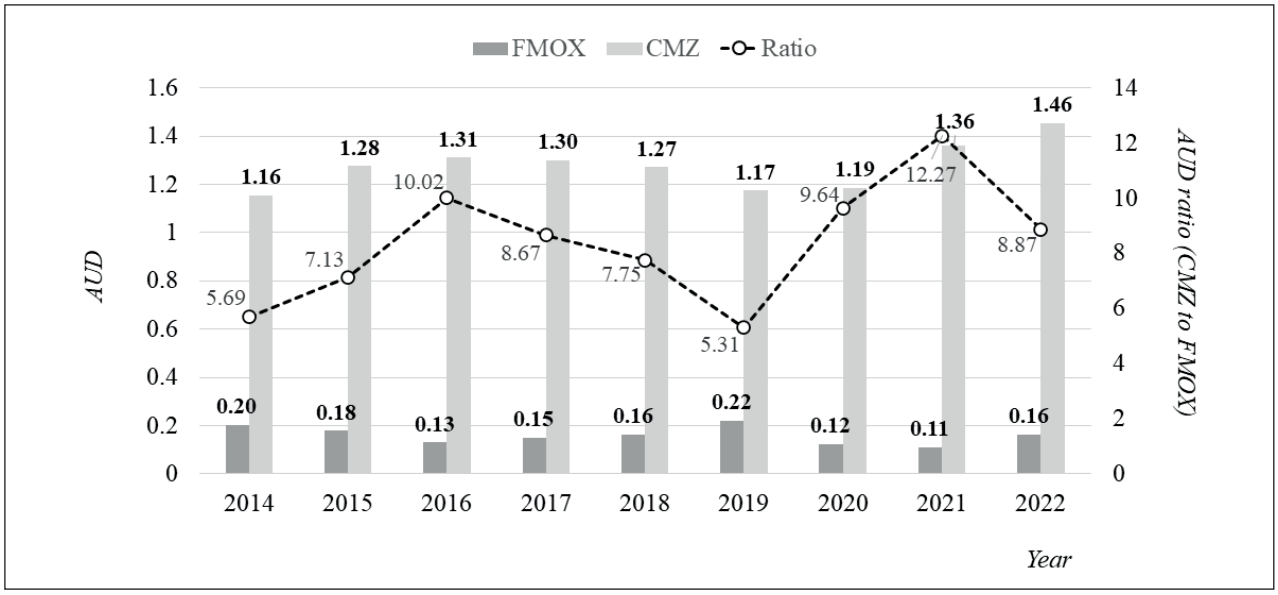

Figure 3 - *Antimicrobial use density (AUD) of cefmetazole (CMZ) and flomoxef (FMOX).*

and ≥32/≥32 μg/mL, respectively, and no *E. cloacae* complex strain presented a MIC ≤1 μg/mL.

The calculated annual AUDs of CMZ and FMOX are shown in *Figure 3*. The AUD for CMZ ranged from 1.16 to 1.46, whereas that for FMOX was between 0.11 and 0.22. Therefore, the AUD ratio for CMZ to FMOX ranged from 5.31 to 12.27, without any apparent upward or downward trends during the study period.

DISCUSSION

In the present study, we compared the MIC levels of CMZ and FMOX in ESBL-E isolates from our institute. The clinically susceptible breakpoint of CMZ for *Enterobacterales* is set at ≤16 μg/mL by the CLSI (Clinical and Laboratory Standards Institute, 2021), and CMZ is considered effective against more than 99% of ESBL-producing *E. coli* and *K. pneumoniae* strains. Although the breakpoint of FMOX is not defined, the proportions of these ESBL-producing organisms showing an MIC ≤1 μg/mL were significantly higher in FMOX than in CMZ, suggesting the clinical superiority of FMOX. The generalizability in other clinical settings, the long-term trend of the discrepancy, and the clinical significance of this *in vitro* difference should be further investigated. In addition, since ESBL-E is listed as the most critical pathogen among various AMR organisms, our data may lead to a better treatment strategy for ESBL-E infections.

Recently, several studies have corroborated the *in vitro* superiority of FMOX over CMZ against ESBL-E (Miyazaki *et al.*, 2019). A single-center study in Malaysia revealed that 78% of *E. coli* and 56% of *K. pneumoniae* isolated from urinary samples showed MIC levels ≤1 μg/mL for FMOX (Ngoi *et al.*, 2021). The $MIC_{50/90}$ of FMOX was reportedly lower than that of CMZ in both *E. coli* (0.5/32 μg/mL vs. 4/64 μg/mL) and *K. pneumonia* (1/8 μg/mL vs. 2/16 μg/mL). A study from Korea evaluating the antimicrobial susceptibility of *E. coli* (n=93) and *K. pneumoniae* (n=83) also demonstrated the superiority of FMOX compared with CMZ, reporting an MIC_{50}/MIC_{90} for FMOX of 0.5/8 μg/mL (Jung *et al.*, 2019). Similarly, a Chinese study mentioned excellent activity of FMOX against various CTX-M-type ESBL-Es, with an MIC_{50}/MIC_{90} for FMOX of 0.064/0.125 μg/mL and that for CMZ of 0.25/0.5 μg/mL (Yang *et al.*, 2015). In addition, the MIC_{50}/MIC_{90} ratios of 35 ESBL-producing *E. coli* isolated from surgical site infections in Japan were <0.063/1 μg/mL for FMOX and 1/8 μg/mL for CMZ (Takesue *et al.*, 2017). Since the MIC data of the present study were derived from routine microbiological testing, they were examined at a range of 1 to 32 μg/mL, and a more detailed comparison of MIC values was unavailable. However, the *in vitro* superiority of FMOX (higher proportions of *E. coli* and *K. pneumonia* showing MIC ≤1 μg/mL) was apparent.

The clinical effectiveness of FMOX against ESBL-E infections has increasingly been reported in the literature. A multicenter retrospective study using a propensity score matching approach revealed that the administration of CMZ and FMOX was not inferior to carbapenem therapy against ESBL-producing *E. coli* bacteremia in terms of 30-day mortality rates and clinical success (Matsumura *et al.,* 2015), although the effectiveness of FMOX alone was not investigated in that study. Japanese single-facility studies indicated a good clinical response to FMOX in pediatric cases of ESBL-producing *E. coli* associated urinary tract infections (Horie *et al.*, 2019; Abe *et al.,* 2017). In contrast, when applying FMOX for ESBL-E treatment, previous studies have suggested that the MIC values of FMOX should be noted. Another propensity score matching study suggested that FMOX treatment was as effective as carbapenems against ESBL-producing *K. pneumoniae* bacteremia when the MIC for FMOX was ≤1 μg/mL (Lee *et al.,* 2015). Those authors found that ESBL-E isolates with FMOX MIC levels of 2-8 μg/mL were significantly associated with increased 30-day mortality. However, as shown in *Figure 2*, 97 to 100% of ESBL-producing *E. coli* and 80 to 100% of ESBL-producing *K. pneumoniae* revealed MIC levels of FMOX ≤1 μg/mL, indicating that FMOX can be used as an empirical treatment for ESBL-E infections in our current medical situation.

The underlying mechanism for the difference in MIC levels between CMZ and FMOX should be discussed. First, the administration of CMZ in our facility was approximately 5- to 12-fold higher than that of FMOX. This large discrepancy in antibiotic exposure might have resulted in the higher MIC of CMZ. However, our microbiology data included community strains, and the difference in antibiotic exposure did not necessarily influence the result, since CMZ is available only in an intravenous form. In addition, if the higher exposure to CMZ was mainly responsible for the higher MIC levels, MIC creeping phenomenon, as reported in the MIC level of vancomycin among *Staphylococcus aureus* (Fujimori *et al.,* 2022), could occur. However, such a trend was not observed in our data. Stratification of the data by its origin (community or nosocomial) would help to further evaluate the influence of drug administration on the MIC difference between CMZ and FMOX. Second, differences in the catalytic efficiency of ESBLs against CMZ and FMOX may explain the discrepancy in MIC levels. Generally, k_{cat}/K_m values are used to evaluate the enzymatic function of β-lactamases. To the best of our knowledge, no data are available in the literature comparing the kinetic parameters of these two drugs.

A recent pharmacokinetic/pharmacodynamic study reported that bactericidal activity is expected when

the time above the MIC is over 70% for both CMZ and FMOX (Hamada *et al.,* 2022). A Monte Carlo simulation (MCS) suggested that the optimal dosage of CMZ (in each creatinine clearance) was 1 g q12 h (<30 mL/min), 1 g q8 h (31-59 mL/min), and 1 g q6 h (60 mL/min). Similarly, those of FMOX were 1 g q24 h (<10 mL/min), 1 g q8 h or 12 h (10-50 mL/min), and 1 g q6 h (>50 mL/min) (Hamada *et al.,* 2022). More specifically, for the treatment of invasive ESBL-E urinary tract infections, 1 g every 8 h of CMZ would be adequate when the target strain shows an MIC of ≤4 mg/L (Hamada *et al.,* 2021). Considering that the MIC_{90} of CMZ in ESBL-producing *E. coli* and *K. pneumoniae* was 4 μg/mL in our study, the majority of the cases could be safely treated with CMZ. However, FMOX treatment may be more promising, considering the lower MIC levels.

The limitations of this study are as follows: first, due to the single nature of the study, the results may not be generalizable to other facilities, and a larger, multi-centered study is warranted. Second, we did not stratify the data by origin or infectious source because of the unavailability of the data in a retrospective manner. A comparison of nosocomial and community strains would be of interest because antimicrobial susceptibility is influenced, especially in nosocomial strains. Third, a certain percentage of ESBL-producing organisms might have been overlooked based on the CLSI criteria. Fourth, our data lacked a genetic investigation; thus, differences in ESBL genotypes were not considered. In addition, the presence of other potentially associated factors, such as AmpC β-lactamase, was not evaluated. Despite these limitations, we believe that the present data are worth sharing among researchers and clinicians considering the better use of existing drugs for AMR pathogens. In future studies, MIC comparisons with moxalactam (latamoxef), another compound classified as oxacepham, would be of interest.

In summary, we observed that the FMOX MIC tended to be lower than that of CMZ among ESBL-producing *E. coli* and *K. pneumoniae*. This finding suggests that FMOX may be a better choice for patients with ESBL-E infections. To investigate this assumption, a randomized controlled study investigating the clinical effectiveness of CMZ and FMOX is warranted for clinical application.

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Transparency declarations

None to declare.

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