Treatment of ESBL-producing *Klebsiella pneumoniae* bacteraemia with carbapenems or flomoxef: a retrospective study and laboratory analysis of the isolates

Chen-Hsiang Lee1,2, Lin-Hui Su3,4, Ya-Fen Tang2 and Jien-Wei Liu1,2*

1Division of Infectious Diseases, Department of Internal Medicine, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Chang Gung University College of Medicine, Taiwan; 2Infection Control Team, Chang Gung Memorial Hospital-Kaohsiung Medical Center, 123 Ta-Pei Road, Niao-Sung Hsiang, Kaohsiung Hsien 833, Taiwan; 3Department of Clinical Pathology, Chang Gung Memorial Hospital-Lin-Kou Medical Center, 5 Fu-Hsin Street, Kweishan, Taoyuan 333, Taiwan; 4Department of Medical Biotechnology and Laboratory Science, Chang Gung University College of Medicine, 259 Wenhua 1st Road, Kweishan, Taoyuan 333, Taiwan

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**Objectives:** To better understand the clinical outcomes of patients with extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* (ESBL-KP) bacteraemia treated with either flomoxef or a carbapenem, and to evaluate the *in vitro* activities of these antibiotics against ESBL-KP.

**Methods:** Retrospective analyses to identify risk factors for mortality in patients with flomoxef-susceptible ESBL-KP, especially addressing the therapeutic roles of flomoxef and carbapenem. *In vitro* activities of flomoxef and carbapenem against flomoxef-susceptible ESBL-KP isolates were evaluated by susceptibility testing and time–kill study.

**Results:** Twenty-seven patients (flomoxef group, *n* = 7; carbapenem group, *n* = 20) were included. Clinical severity reflected by high Pitt bacteraemia score (≥6) was an independent risk factor for mortality (OR 13.43; 95% CI, 1.08–166.73; *P* = 0.043), while use of flomoxef or a carbapenem was not. The MICs of flomoxef and carbapenem indicated that the tested ESBL-KP were susceptible to these antibiotics regardless of the inoculum size of 10⁵ or 10⁷ cfu/mL. Time–kill study showed that these antibiotics (flomoxef 8 mg/L and meropenem 4 mg/L) each acted actively against and inhibited the regrowth of the tested ESBL-KP for at least 24 h.

**Conclusions:** Flomoxef might be as clinically effective as a carbapenem in treating flomoxef-susceptible ESBL-KP bacteraemia.

Keywords: clinical outcome, *in vitro* activity, inoculum effect

**Introduction**

*Klebsiella pneumoniae* has been frequently found to produce extended-spectrum β-lactamases (ESBLs). Infections caused by ESBL-producing pathogens are problematic because when co-resistance to other antimicrobial class is present, limited antibiotic options are available. Currently, imipenem or meropenem is regarded as the drug of choice for infections caused by ESBL-producing pathogens. However, the selective pressure from increasing use of carbapenems will lead to development of carbapenem-resistant microbes. An alternative to carbapenems may relieve this selective pressure and offer an option to carbapenem-allergic patients, when necessary.

Cephamycins (i.e. cefmetazole, cefotetan and flomoxef), characterized by their 7-α-methoxy β-lactam, have been reported to be highly active *in vitro* against both low inocula (10⁵–10⁶ cfu/mL) and high inocula (10⁷–10⁸ cfu/mL) of TEM- or SHV-producing Enterobacteriaceae. Unfortunately, few clinical reports evaluating treatment of infections caused by ESBL producers with cephamycins have been published. Flomoxef is unique among cephamycins in having a difluoromethylthio-acetamido group at position 7 giving it better *in vitro* activity against ESBL-producing Enterobacteriaceae, and may therefore offer a treatment alternative to carbapenems. The objectives of this study were to better understand the outcomes of patients with various agents in the treatment of ESBL-producing *K. pneumoniae*.
Flomoxef and meropenem activities against ESBL-KP

(ESBL-KP) bacteraemia, and to evaluate the in vitro activities of flomoxef and meropenem against ESBL-KP.

Materials and methods

Hospital setting and study design

Adult patients with flomoxef-susceptible ESBL-KP bacteraemia that received either flomoxef or a carbapenem (meropenem or imipenem) during admissions between 1 March 2004 and 28 February 2005 at Chang Gung Memorial Hospital-Kaohsiung Medical Center were included in a retrospective study. For each included patient, the prescribed flomoxef or carbapenem was used for at least 2 days, which started within 5 days of the availability of the blood culture result indicating flomoxef-susceptible ESBL-KP bacteraemia. The use of either flomoxef or a carbapenem was at the discretion of each patient’s attending physician. The medical charts of the included patients were reviewed for collection of their demographic, clinical and laboratory data. Variables used for assessment of the illness severity of patients included Pitt bacteraemia score, 2 admission to intensive care unit (ICU) and length of prior hospital stay. The study endpoint was mortality resulting from bacteraemia within 14 days of culturing blood that subsequently grew a flomoxef-susceptible ESBL-KP.

Bacterial isolates and β-lactamase identification

All K. pneumoniae isolates were identified by standard methods, and the presence of ESBLs was evaluated using the CLSI criteria for ESBL screening and disc confirmation test. 1 Specific β-lactamases were identified for each of the included flomoxef-susceptible ESBL-KP isolates. Briefly, three primer sets previously described for detection of blaTEM, blaSHV and blaCTX-M genes were used in the amplification procedure; the purified and sequenced nucleotides were compiled and analysed by matching homologous sequences searched from the GenBank database. 5

Antimicrobial agents and susceptibility testing

Standard powders of meropenem (Sumitomo Ltd, Japan) and flomoxef (Shionogi Ltd, Japan) were used in susceptibility testing with microdilution methods. MICs were determined with different inocula (10^5 and 10^7 cfu/mL) for each KP-ESBL isolate. 6 Escherichia coli ATCC 25922 and K. pneumoniae ATCC 700603 were used as control strains. MICs considered susceptible were ≤4 mg/L for meropenem and ≤8 mg/L for flomoxef, and MICs considered intermediate were 4-8 mg/L for meropenem and 8–16 mg/L for flomoxef. 6,7

Time–kill study

Four ESBL-KP isolates were randomly chosen and used throughout the time–kill study, in which specific concentration of each flomoxef-susceptible (8 mg/L) or meropenem (4 mg/L) was adjusted; these antibiotic concentrations were the mean steady-state antibiotic levels in sera when normal doses of flomoxef or meropenem were used in healthy volunteers. 8,9 The concentrations of flomoxef and meropenem were fixed regardless of the inoculum sizes of the tested bacteria (10^5 or 10^7 cfu/mL) in each experiment. ESBL-KP of the same strain with similar inoculum size simultaneously inoculated in antibiotic-free broth was used as control. Bacterial colony counts were measured at 0, 2, 4, 6, 8, 12 and 24 h. The lower limit of viable counts was set at 10^5 cfu/mL. All tests were performed twice to assure their reproducibility.

Statistical analysis

Patients with ESBL-KP bacteraemia grouped by therapy with flomoxef or with carbapenem were compared to clarify whether there were demographic and clinical differences between them. All study patients were additionally divided into deceased and survived groups for analyses to identify risk factor(s) for mortality. Variables from different groups were compared with each other; Mann–Whitney U-test was used to assess the differences in variables, while χ² test or Fisher’s exact test was used to assess the differences in dichromatic variables. To eliminate confounding factors in predicting risks for mortality, variables with P values ≤0.2 in univariate analyses between patients of deceased and survived groups were entered in a logistic regression model for further assessment. A 2-tailed P ≤ 0.05 was considered statistically significant.

Results

Analyses of demographic and clinical data

Thirty-five patients with ESBL-KP bacteraemia were identified during the study period. We excluded one patient with recurrent ESBL-KP bacteraemia, five (14.3% of the overall ESBL-KP bacteraemic patients) with flomoxef non-susceptible ESBL-KP bacteraemia and two who died of sepsis on the admission day whose blood cultures subsequently grew ESBL-KP. As a result, 27 eligible patients were included, of which 7 were treated with flomoxef and 20 were treated with carbapenems (14 with meropenem and 6 with imipenem). Seven (25.9%) study patients died within 14 days after sampling blood for culture that ultimately grew flomoxef-susceptible ESBL-KP. Demographics, underlying diseases (mainly neutropenia and renal failure), source of infection, illness severity (28.6% versus 60% admitted to ICU; P = 0.16) and mortality between patients treated with flomoxef and those with a carbapenem were not significantly different. Among the overall included individuals, patients in the deceased group had a significantly higher proportion of admission to ICU (85.7% versus 35.0%; P = 0.02) and higher Pitt bacteraemia score (mean, 8.1 versus 4.3; P = 0.002; Pitt bacteraemia score ≥6 points, 100% versus 50%; P = 0.02) than those in the survived group (Table 1). Of note, no significant difference in proportion of flomoxef use was found between the deceased and survived groups (P = 0.86). Logistic regression disclosed that Pitt bacteraemia score ≥6 (OR 13.43 with 95% CI, 1.08–166.73; P = 0.043) was an independent risk factor for mortality in patients with flomoxef-susceptible ESBL-KP bacteraemia.

Identified ESBL

Among the 27 ESBL-KP isolates, four had two ESBL genes, one had three ESBL genes and the remaining isolates each carried one ESBL gene. Of the 33 identified ESBL genes, CTX-Ms were detected in 21 (77.8%) ESBL-KP isolates (CTX-M3 in 13, CTX-M14 in 7, and combined CTX-M3 and CTX-M14 in 1), and SHVs were detected in 11 (40.7%) ESBL-KP isolates (SHV-12 in 6, SHV-28 in 2, SHV-5 in 2 and SHV-2 in 1). MICs for the K. pneumoniae isolates

When the inoculum size was 10^5 cfu/mL, MICs of meropenem and flomoxef were ≤4–8 mg/L for meropenem and ≤8–16 mg/L for flomoxef, and MICs considered susceptible were ≤4 mg/L for meropenem and ≤8 mg/L for flomoxef. 6,7

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Table 1. Comparisons of demographic and clinical data between the deceased and survived groups of patients with ESBL-producing *Klebsiella pneumoniae* bacteraemia

<table>
<thead>
<tr>
<th>Variable</th>
<th>Deceased group n = 7 (%)</th>
<th>Survived group n = 20 (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flomoxef treatment</td>
<td>2 (28.6)</td>
<td>5 (25.0)</td>
<td>0.86</td>
</tr>
<tr>
<td>Male gender</td>
<td>3 (42.8)</td>
<td>10 (50.0)</td>
<td>0.77</td>
</tr>
<tr>
<td>Age (median range)</td>
<td>80 (69–89)</td>
<td>70 (12–91)</td>
<td>0.16</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>2 (28.6)</td>
<td>1 (5.0)</td>
<td>0.09</td>
</tr>
<tr>
<td>Renal failure</td>
<td>3 (42.8)</td>
<td>5 (25.0)</td>
<td>0.39</td>
</tr>
<tr>
<td>Other underlying disease</td>
<td>7 (100)</td>
<td>17 (85.0)</td>
<td>0.29</td>
</tr>
<tr>
<td>Source of infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>6 (85.6)</td>
<td>9 (45.0)</td>
<td>0.07</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>0</td>
<td>5 (25.0)</td>
<td>0.15</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>0</td>
<td>3 (15.0)</td>
<td>0.30</td>
</tr>
<tr>
<td>Soft tissue</td>
<td>0</td>
<td>1 (5.0%)</td>
<td>0.56</td>
</tr>
<tr>
<td>Other</td>
<td>1 (14.4)</td>
<td>2 (10.0)</td>
<td>0.77</td>
</tr>
<tr>
<td>Admission to ICU</td>
<td>6 (85.7)</td>
<td>7 (35.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>Pitt bacteraemia score</td>
<td>8.1 (6–11)</td>
<td>4.3 (0–7)</td>
<td>0.002</td>
</tr>
<tr>
<td>Pitt bacteraemia score ≥6</td>
<td>7 (100)</td>
<td>5 (50.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>Previous LOS (median days)</td>
<td>47.4</td>
<td>28.5</td>
<td>0.08</td>
</tr>
</tbody>
</table>

ICU, intensive care unit; LOS, length of stay.

*For univariate analyses of data between the deceased and survived groups, with the exception of using Mann–Whitney *U*-test in comparing difference in age, $\chi^2$ test or Fisher’s exact test was used to assess the differences in other variables.*

MIC$_{50}$ = 0.064 mg/L and MICs of flomoxef ranged from 0.032 to 2 mg/L (MIC$_{50}$ = 0.125 mg/L and MIC$_{90}$ = 1 mg/L). When the inoculum size was 10$^7$ cfu/mL, MICs of meropenem ranged from 1 to 4 mg/L (MIC$_{50}$ = 2 mg/L and MIC$_{90}$ = 4 mg/L) and MICs of flomoxef ranged from 1 to 8 mg/L (MIC$_{50}$ = 4 mg/L and MIC$_{90}$ = 8 mg/L); meropenem and flomoxef remained active against the tested ESBL-KP.

**Time–kill study**

Time–kill study on four randomly chosen ESBL-KP isolates (CTX-M3, CTX-M14 and SHV-28 enzymes were found in one isolate; while CTX-M3 and SHV-12 enzymes were found in another isolate). Solid lines, inoculum 10$^5$ cfu/mL; broken lines, inoculum 10$^7$ cfu/mL; filled diamonds, flomoxef; filled squares, meropenem; filled triangles, control.

In general, the larger the inoculum effect, the more vulnerable the tested antibiotic to hydrolysis of the organism’s $\beta$-lactamase(s). When incubating ESBL-KP with a third- or fourth-generation cephalosporin, the inoculum effect is pronounced. However, little information about the inoculum effect of the ESBL-producing pathogens tested with cephamycins has been published. In this report, the effectiveness of flomoxef against ESBL-KP indicated by susceptibility testing was further supported by time–kill study.

One concern in treatment with a cephamycin in infections caused by Enterobacteriaceae is the potential *in vivo* selection of porin-deficient mutations, which was previously reported in cases involving therapy with cefoxitin. Further study is needed to clarify whether flomoxef can overcome the *in vivo* selection of porin-deficiency mutations in Enterobacteriaceae because of the markedly lower MIC of flomoxef than that of cefoxitin. Our study provides intriguing insights into therapy with flomoxef for ESBL-KP bacteraemia and suggests that flomoxef is a potential alternative for such infections. Of note, 60% of patients in the carbapenem arm of the study were admitted to ICU (as compared with 28.6% in the flomoxef arm); although not statistically significant, this suggests that patients in the carbapenem group might be more severely ill. Given the limitations of small size and being a retrospective study, our report may lack the power to discriminate real difference in outcome. Further study is warranted to establish the therapeutic roles of cephemycins in the treatment of infections caused by ESBL-producing Enterobacteriaceae.

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**Transparency declarations**

None to declare.
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References