International Journal of Antimicrobial Agents 45 (2015) 485–490

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

In vitro activity of flomoxef and comparators against *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* producing extended-spectrum β-lactamases in China

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ARTICLE INFO

Article history:
Received 5 November 2014
Accepted 25 November 2014

Keywords:
Flomoxef
Extended-spectrum β-lactamases
Escherichia coli
Klebsiella pneumoniae
Proteus mirabilis

ABSTRACT

The objective of this study was to better understand the in vitro activity of flomoxef against clinical extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae. A total of 401 ESBL-producing isolates, including 196 *Escherichia coli*, 124 *Klebsiella pneumoniae* and 81 *Proteus mirabilis*, were collected consecutively from 21 hospitals in China in 2013. Minimum inhibitory concentrations (MICs) were determined by broth microdilution methods. Phenotypic identification of ESBL production was detected as recommended by the Clinical and Laboratory Standards Institute (CLSI). ESBL genes were detected by PCR and sequencing. Flomoxef, doripenem, meropenem, ertapenem, cefmetazole and piperacillin/tazobactam exhibited good activity against ESBL-producing isolates, with susceptibility rates >90%. Tigecycline showed good activity against *E. coli* and *K. pneumoniae* (100% and 97.6%, respectively). Cefotaxime and cefepime showed very low activities against ESBL-producing isolates, with susceptibility rates of 0–0.8% and 1.0–13.6%, respectively. $bla_{\text{CTX-M}}$ were the major ESBL genes, with occurrence in 99.5% of *E. coli*, 91.1% of *K. pneumoniae* and 97.5% of *P. mirabilis*. $bla_{\text{CTX-M-14}}$ was the predominant ESBL gene, detected in 46.9% (188/401) of the isolates, followed by $bla_{\text{CTX-M-15}}$ (21.4%), $bla_{\text{CTX-M-55}}$ (17.2%), $bla_{\text{CTX-M-65}}$ (12.7%) and $bla_{\text{CTX-M-3}}$ (6.7%). Flomoxef exhibited excellent activity against the different CTX-M-type ESBL-producing

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http://dx.doi.org/10.1016/j.ijantimicag.2014.11.012

isolates, with MIC_{50} and MIC_{90} values of 0.064–0.125 μg/mL and 0.25–0.5 μg/mL, respectively. Against the isolates solely producing CTX-M-14, -15, -55, -3 or -65, flomoxef showed susceptibility rates of 98.6%, 98.0%, 98.1%, 100.0% and 97.4%, respectively. In conclusion, flomoxef showed good activity against ESBL-producing Enterobacteriaceae and may be a choice to treat infections caused by these isolates in China.

1. Introduction

A number of surveillance programmes exist to monitor the susceptibility of clinically important pathogens at national and international levels [1–3]. The Enterobacteriaceae are a major group of pathogens causing community- and hospital-acquired infections. With the increase in the prevalence of extended-spectrum β-lactamases (ESBLs) in *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*, clinicians have a limited choice of antimicrobial agents to use, which in turn leads to an increase in the abuse of certain antimicrobial agents such as the carbapenems. ESBL rates in *E. coli* and *K. pneumoniae* in China have been reported as 60–70% and 30–40%, respectively [2,3], which has resulted in a challenge to Chinese clinicians in terms of selecting appropriate antimicrobial agents to treat infections caused by ESBL-producing isolates. Flomoxef is a unique β-lactam antibiotic with oxygen substituted for the sulphur, a 7-α-methoxy group in the cephalosporin core and a difluoromethylthio-acetamido group at position 7, which gives it better in vitro activity against ESBL-producing Enterobacteriaceae [4]. However, the activity of this drug in China has not been reported.

In view of this, in this study consecutive community- and hospital-associated ESBL-producing *E. coli*, *K. pneumoniae* and *P. mirabilis* isolates were collected and antimicrobial susceptibility testing was conducted on these strains to evaluate the in vitro activity of flomoxef and other comparators. Although there have been some reports describing the activity of flomoxef [1–6], this is the first systematic report to provide an overview specifically of the results in China.

2. Materials and methods

2.1. Clinical isolates

A total of 401 ESBL-producing isolates, including 196 *E. coli*, 124 *K. pneumoniae* and 81 *P. mirabilis*, were collected consecutively from 21 hospitals in China in 2013. Specimens included 147 urine, 101 abdominal fluid, 37 pus, 32 blood, 31 sputum, 25 bile, 17 liver tissue, 6 pancreas, 2 appendix and 3 other specimens. Bacteria were identified by standard methods used in each site and were re-identified in Peking Union Medical College Hospital (Beijing, China) by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS). All organisms were deemed clinically significant according to local criteria. For the purpose of comparison of the activity of different antimicrobial agents against ESBL-producers, 244 non-ESBL-producing isolates were collected from the same hospitals during the same period, including 63 *E. coli*, 103 *K. pneumoniae* and 78 *P. mirabilis* isolates from 67 blood, 61 urine, 46 sputum, 29 gallbladder, 13 abdominal fluids, 9 liver tissue, 8 pus, 4 bile and 7 other specimens.

2.2. Antimicrobial susceptibility test method

Minimum inhibitory concentrations (MICs) were determined using a broth microdilution method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [7].Fifteen antimicrobial agents were tested, including flomoxef, ceftazidime, ceftazidime/clavulanic acid (fixed clavulanic acid concentration of 4 μg/mL), cefotaxime, cefotaxime/clavulanic acid (fixed clavulanic acid concentration of 4 μg/mL), cefepime, cefmetazole, piperacillin/tazobactam (TZP) (fixed tazobactam concentration of 4 μg/mL), cefoperazone/sulbactam (fixed ratio of 2:1), ertapenem, meropenem, doripenem, levofloxacin, tigecycline and amikacin. MIC_{50} and MIC_{90} values (MICs at which 50% and 90% of isolates were inhibited, respectively) were calculated using WHONET software v.5.6 (http://whonet.software.informer.com/5.6/). Susceptibility interpretations were based on CLSI clinical breakpoints [8]. The CLSI breakpoints for latamoxef [susceptible (S) ≤8 μg/mL, resistant (R) ≥64 μg/mL for Enterobacteriaceae] were used for flomoxef. US Food and Drug Administration (FDA)-approved breakpoints for tigecycline were used (S ≤2 μg/mL, R ≥8 μg/mL for Enterobacteriaceae). The cefoperazone CLSI breakpoints were used for cefoperazone/sulbactam. The reference strains *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *K. pneumoniae* ATCC 700603 (positive ESBL control) were used as quality control strains for each batch of MIC testing. Results were included in the analysis only when corresponding quality control isolates tested within the acceptable range according to CLSI guidelines.

2.3. Extended-spectrum β-lactamase detection

Phenotypic identification of ESBL production among *E. coli*, *K. pneumoniae* and *P. mirabilis* was detected according to the methods recommended by the CLSI [8]. If the cefotaxime or ceftazidime MIC was ≥2 μg/mL, then the MIC of cefotaxime or ceftazidime was compared with the MIC of cefotaxime/clavulanic acid or ceftazidime/clavulanic acid. A positive test for ESBL production was defined as a ≥8-fold (i.e. three-fold doubling dilution) decrease in the MIC for cefotaxime or ceftazidime when tested in combination with clavulanic acid versus their MIC when either drug was tested alone.

2.4. PCR amplification and DNA sequence analysis of extended-spectrum β-lactamase genes

Template DNA of the ESBL-producing *E. coli*, *K. pneumoniae* and *P. mirabilis* isolates was obtained by placing several small colonies of each strain in 200 μL of double-distilled water and boiling the sample for 10 min. After cooling on ice, 1–2 μL of each lysate was used in the PCR. The primers used in this study have been described previously [9]. The reaction was conducted in a PTC-200 PCR system (MJ Research Inc., Watertown, MA). The amplified genes included bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M\text{-}1\ group}$, $bla_{CTX\text{-}M\text{-}2\ group}$, $bla_{CTX\text{-}M\text{-}8\ group}$, $bla_{CTX\text{-}M\text{-}9\ group}$ and $bla_{CTX\text{-}M\text{-}25\ group}$. A positive and negative control was followed in each batch of PCR.

PCR products were purified with a QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) and were sequenced on an ABI PRISM 3730XL Sequencer Analyzer (Applied Biosystems, Foster City, CA). DNA sequencing data were analysed with the GenBank BLASTN tool (http://www.ncbi.nlm.nih.gov/blast).