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In vitro activity of CXA-101 plus tazobactam (CXA-201) against CTX-M-14– and CTX-M-15–producing *Escherichia coli* and *Klebsiella pneumoniae*

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Abstract

CXA-101, a novel cephalosporin with good antipseudomonal activity, was evaluated against a consecutive and polyclonal collection of extended-spectrum-β-lactamase (ESBL)–producing *Escherichia coli* (n = 149) and *Klebsiella pneumoniae* (n = 20), mainly CTX-M-15- (69%) or CTX-M-14 producing (22%). A total of 41% of the *E. coli* isolates belonged to the international clone O25b-ST131. Broth microdilution versus CXA-101, CXA-tazobactam 4 and 8 mg/L (CXA-201), ceftazidime–tazobactam (CAT), ceftazidime–clavulanate (CAC), piperacillin–tazobactam (TZP), amoxicillin–clavulanate (ACL), ampicillin–sulbactam (ASU), and other comparators was performed, using EUCAST methodology and breakpoints. Susceptibility to CXA-201 was 96% (tazobactam 8 mg/L, tentative breakpoint S ≤ 1 mg/L), CAT 93%, CAC 95%, ACL 24%, ASU 2%, TZP 58%, ciprofloxacin 25%, levofloxacin 30%, gentamicin 54%, tobramycin 34%, amikacin 90%, and tigecycline 98%. Ninety-four percent of the TZP-resistant and all ACL-resistant isolates were CXA-201 susceptible. CXA-201 has good in vitro activity against ESBL-producing Enterobacteriaceae and might be a future therapeutic option for infections caused by TZP- and ACL-resistant isolates.

Keywords: ESBLs; 025b-ST131; β-Lactamase inhibitors; Ceftazidime; Piperacillin; Clavulanate

1. Introduction

Extended-spectrum-β-lactamases (ESBL) are spreading rapidly among *Escherichia coli* and *Klebsiella* spp. worldwide, and their clinical impact is undisputable (Giske et al., 2008). Although the majority of strains are still susceptible to carbapenems, the resistance levels to all other therapeutic alternatives are on the rise (Pitout and Laupland, 2008; Pitout 2010). Furthermore, the increasing prevalence of carbapenemase-producing *Enterobacteriaceae* calls for a reduction in carbapenem consumption (Nordmann et al., 2009; Vatopoulos 2008). Although the level of susceptibility to β-lactam/β-lactamase inhibitors such as piperacillin-tazobactam is relatively high, resistance is still found, not the least in *Klebsiella pneumoniae* (Paterson and Bonomo, 2005).

CXA-101 is a novel cephalosporin with good activity against Gram-negative bacilli, including *Pseudomonas aeruginosa*. The compound is now being combined with tazobactam, CXA-201, which should also theoretically display good activity against *Enterobacteriaceae* producing classical ESBLs. In a recent study, it was shown that around half of the ESBL-producing clinical isolates of *P. aeruginosa* were inhibited by CXA-201 (Giske et al., 2009). Furthermore, a study of 59 ESBL-producing *Enterobacteriaceae* demonstrated high activity of CXA-201, particularly when

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using tazobactam at a fixed concentration of 8 mg/L (Livermore et al., 2010).

In order to evaluate the in vitro activity of CXA-201 against ESBL-producing *Enterobacteriaceae*, a consecutive collection of ESBL-producing clinical isolates of *E. coli* and *K. pneumoniae*, out of which the majority produced CTX-M-14 and -15, were subjected to MIC testing against CXA-201 and relevant comparators, including β-lactam/β-lactamase inhibitors. The strains were all derived from Stockholm County and were subjected to epidemiologic typing to investigate the clonal diversity in the strain collection. Furthermore, polymerase chain reaction (PCR) targeting the *pabB* gene was done to determine the fraction of isolates that belonged to *E. coli* O25b-ST131, the dominant international clone among ESBL-producing *Enterobacteriaceae* (Clermont et al., 2009).

2. Materials and methods

2.1. Selection of isolates

All ESBL-positive non-replicate clinical isolates of *E. coli* and *K. pneumoniae* detected at the clinical microbiology laboratory at Karolinska University Hospital (covering approximately 80% of the population in Stockholm County) during 2005 were collected (n = 169). Production of ESBL was detected with ESBL combination disks (Oxoid, Basingstoke, UK) or ESBL Etest (bioMérieux, Marcy l'Etoile, France) according to the manufacturers' instruction. Of the 169 isolates, 149 were *E. coli* and 20 *K. pneumoniae*. Isolates were derived from the urinary tract (n = 130), secretions (n = 28), and blood (n = 11). Isolates came from primary care (n = 49), long-term care facilities (n = 23), hospital outpatients (n = 48), and hospital inpatients (n = 49).

2.2. MIC and susceptibility testing

MICs were determined with broth microdilution (ISO, International Organization for Standardization, 2006) (Trek Diagnostic Systems, Cleveland, OH, USA) versus CXA-101, CXA-101 plus tazobactam fixed concentrations of 4 and 8 mg/L (CXT/4 and CXT/8, respectively), ceftazidime–tazobactam, ceftazidime–clavulanate, piperacillin–tazobactam, amoxicillin–clavulanate, ampicillin–sulbactam, ceftazidime, cefotaxime (CTX), ceftriaxone, cefepime, piperacillin, imipenem, ertapenem, ciprofloxacin, levofloxacin, amikacin, gentamicin, tobramycin, and tigecycline. We used a fixed concentration of 4 mg/L for tazobactam and sulbactam, and 2 mg/L for clavulanic acid. EUCAST interpretive breakpoints were used (http://www.eucast.org/clinical_breakpoints/, accessed on 2010/11/15). For CXA-101, we tentatively used a susceptible breakpoint of S ≤ 2 mg/L, due to improved pharmacokinetic properties compared to other extended-spectrum cephalosporins (Ge et al., 2010), as well as S ≤ 1 mg/L, which is identical to the S breakpoint used for other extended-spectrum cephalosporins in the EUCAST system. We included *E. coli* ATCC 25922 for quality control (QC range for CXA-101 0.12–0.5 mg/L).

2.3. ESBL Characterization

Amplification of the $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} genes was made prior to DNA sequencing, using primers according to Sundsfjord et al., 2004 ($bla_{\text{CTX-M}}$ and bla_{TEM}) and Jouini et al., 2007 (bla_{SHV}) Sequencing primers for CTX-M were designed specifically for this study; $bla_{\text{CTX-M}}$ seq1, forward: 5-ATG GTT AAA AAA TCA CTG CG-3; $bla_{\text{CTX-M}}$ seq1, reverse: 5-TTA CAA ACC GTY GGT GAC GA-3; $bla_{\text{CTX-M}}$ seq2, forward: 5-ATG GTG ACA AAG AGA GTG CA-3; and $bla_{\text{CTX-M}}$ seq2, reverse: 5-TYA CAG CCC YTY GGC GAC GA-3. DNA extraction was performed by boiling. A PCR master mix (47 μL) was prepared according to the Perkin-Elmer standard protocol: 10× PCR buffer II, $MgCl_2$ 2.5 mmol/L, dNTP 200 μmol/L, forward and reverse primer 5 μmol/L, *Taq* Gold 1.2 U/reaction, and sterile H_2O. Three microliters of template DNA was added to the master mix, for a final volume of 50 μL in each PCR tube. All reagents except for dNTP (Amersham Pharmacia Biotech, Uppsala, Sweden) were purchased from Applied Biosystems (Foster City, CA, USA). Amplifications were performed on a Perkin-Elmer Applied Biosystems GeneAmp PCR System 9700, using the following temperature profile: 35 cycles of 45 s at 94 °C, followed by 45 s at variable annealing temperatures (Table 1) and 60 s at 72 °C. Analysis of PCR products and purified templates for sequencing was performed on 1% agarose gels (Agarose NA, Amersham Biosciences, Uppsala, Sweden) in TBE buffer at 100 V for 40 min.

DNA sequencing was performed with the dideoxy-chain termination method (Sanger et al., 1977). PCR products were purified by the JETquick Spin Column Technique (Genomed, Bad Oeynhausen, Germany). Cycle sequencing was done with the BigDye Terminator Ready Reaction Kit (Applied Biosystems) on the GeneAmp PCR System 9700 (Applied Biosystems). Extension products were purified by ethanol/sodium acetate precipitation, and sample electrophoresis was performed on the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Experimentally determined nucleotide sequences were compared against sequence databases with BLAST (http://blast.ncbi.nlm.nih.gov).

2.4. Pulsed-field gel electrophoresis

Epidemiologic typing of *E. coli* isolates using pulsed-field gel electrophoresis (PFGE) was performed with all isolates according to the PulseNet protocol for *E. coli* O157:H7 (http://www.cdc.gov/pulsenet/protocols.htm) using *Xba*I for restriction of DNA. Unique PFGE types were defined as similarity indices above 0.90 (Dice coefficient), corresponding to a difference of ≤3 bands (Tenover et al., 1995).