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Short Communication

Extended-spectrum cephalosporins and the inoculum effect in tests with CTX-M-type extended-spectrum β-lactamase-producing *Escherichia coli*: Potential clinical implications of the revised CLSI interpretive criteria

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ABSTRACT

Based on the new recommendations of the Clinical and Laboratory Standards Institute (CLSI), the revised cephalosporin breakpoints may result in many CTX-M-producing *Escherichia coli* being reported as susceptible to ceftazidime. We determined the activity of ceftazidime and other parenteral β-lactam agents in standard- and high-inoculum minimum inhibitory concentration (MIC) tests against CTX-M-producing *E. coli* isolates. Antimicrobial susceptibility was determined using a broth microdilution MIC method with inocula that differed 100-fold in density. An inoculum effect was defined as an eight-fold or greater increase in MIC on testing with the higher inoculum. When the revised CLSI ceftazidime breakpoint of 4 μg/mL was applied, 34 (34.3%) of the 99 CTX-M-producers tested were susceptible. More specifically, for 42 CTX-M-14-producing *E. coli* isolates, 32 (76.2%) were susceptible at 4 μg/mL. Cefotaxime, ceftazidime, cefepime and piperacillin/tazobactam were found to be associated with inoculum effects in 100% of the evaluable tests for extended-spectrum β-lactamase-producing *E. coli* isolates. The MIC_{50} (MIC required to inhibit 50% of isolates) of ceftazidime was 16 μg/mL in the standard-inoculum tests and >512 μg/mL in the high-inoculum tests. In the high-inoculum tests including isolates encoding CTX-M-14, ceftazidime was dramatically affected, with susceptibility decreasing from 82.1% of isolates inhibited at 4 μg/mL in the standard-inoculum tests to 0% at high inoculum. Although further studies may demonstrate that ceftazidime has a role in the treatment of infections caused by these organisms, we suggest that until more data become available, clinicians should be cautious about treating serious CTX-M-producing *E. coli* infections with ceftazidime or cefepime.

1. Introduction

The international dissemination of $bla_{CTX\text{-}M}$ extended-spectrum β-lactamase (ESBL) genes over the last decade has been described as a pandemic [1,2]. Breakpoints for Enterobacteriaceae against extended-spectrum cephalosporins have recently been revised by the Clinical and Laboratory Standards Institute (CLSI), and it is now recommended that susceptibility results for cephalosporins be reported according to the minimum inhibitory concentration (MIC), regardless of whether the isolate produces an ESBL [3]. Based on these new recommendations, the revised cephalosporin breakpoints may result in many CTX-M-producing *Escherichia coli* being reported as susceptible to ceftazidime [4,5]. Previous studies have shown that when ceftazidime is used to treat infections due to TEM- and SHV-type ESBL-producing Enterobacteriaceae, clinical outcomes are generally poor [6,7]. Whether the same finding can be expected with CTX-M-producing Enterobacteriaceae is unknown, and this uncertainty is concerning because the latest CLSI

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Table 1
Minimum inhibitory concentrations (MICs) for 99 extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates according to inoculum size.

Antimicrobial agent	MIC (μg/mL) of antimicrobial agent at standard and high inocula							
	Standard inoculum (10^5 CFU/mL)				High inoculum (10^7 CFU/mL)			
	Range	MIC_{50}	MIC_{90}	Non-susceptible [*n* (%)]	Range	MIC_{50}	MIC_{90}	Non-susceptible [*n* (%)]
Cefotaxime	32 to >512	>512	>512	99(100)	256 to >512	>512	>512	99(100)
Ceftazidime	0.25–512	16	512	65(65.7)	16 to >512	>512	>512	99(100)
Cefepime	1 to >512	128	>512	80(80.8)	128 to >512	>512	>512	99(100)
TZP	2/4 to >1024/4	64/4	1024/4	65(65.7)	32/4 to >1024/4	>1024/4	>1024/4	99(100)
Ciprofloxacin	≤0.12–256	64	128	82(82.8)	0.5 to >512	512	>512	96(97.0)
Imipenem	0.12–0.5	0.12	0.12	0	0.03–4	1	2	16(16.2)
Meropenem	≤0.03–0.12	≤0.03	0.06	0	0.03–8	1	2	24(24.2)
Ertapenem	≤0.03–1	≤0.03	0.25	4(4.0)	0.03–16	0.5	2	58(58.6)

$MIC_{50/90}$, MICs for 50% and 90% of the organisms, respectively; TZP, piperacillin/tazobactam.

reporting recommendations effectively endorse ceftazidime for the treatment of serious infections caused by CTX-M-producing *E. coli*. A bacterial isolate may appear susceptible to a drug when tested in vitro using a standard inoculum; however, that same drug may be ineffective in vivo owing to a high inoculum. A high-inoculum effect was recently reported for cephalosporins on ESBL-producing Enterobacteriaceae, and it has been proposed that the presence of a major inoculum effect for a particular antimicrobial agent precludes its use [8,9]. Therefore, this study was designed to investigate the activity of ceftazidime against CTX-M-producing clinical isolates by determining the activities of this agent and other parenteral β-lactam agents in standard- and high-inoculum MIC tests.

2. Materials and methods

From September 2010 to May 2011, samples were collected from Samsung Medical Center, a 1950-bed tertiary care university hospital in Seoul (South Korea), and Samsung Changwon Hospital, a 700-bed community-based university-affiliated hospital in Changwon (South Korea). Of the stored bacterial isolates in the deep freezers, a total of 99 CTX-M-producing *E. coli* isolates were collected and included in this study. One isolate per patient was included and there was no suspicious nosocomial outbreak. ESBL status was confirmed phenotypically using the double-disc diffusion test with ceftazidime ± clavulanic acid and cefotaxime ± clavulanic acid (BD Diagnostics, Sparks, MD). ESBL-related genes, including TEM, SHV, CTX-M and OXA, were amplified by PCR from clinical isolates as described previously [10,11]. PCR and sequencing of PCR products were performed to identify the bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ genes responsible for ESBL activity in the ESBL-producing strains. Antimicrobial susceptibility testing and ESBL confirmatory testing were performed using the broth microdilution method according to CLSI recommendations. Antimicrobial susceptibility was determined using a broth microdilution MIC method with inocula that differed 100-fold in density. The inocula comprised 10^5 CFU/mL (standard inoculum) and 10^7 CFU/mL (high inoculum) suspended in Mueller–Hinton broth (BD Diagnostics). An inoculum effect was defined as an eight-fold or greater increase in MIC on testing with the higher inoculum [8,9].

3. Results and discussion

The various strains were isolated from urine (n = 52), blood (n = 40), ascites (n = 3), pus (n = 3) and bile (n = 1). All isolates were confirmed to encode $bla_{CTX\text{-}M}$, and the predominant CTX-M types were CTX-M-14 (n = 39) and CTX-M-15 (n = 38), followed by CTX-M-27 (n = 10), CTX-M-57 (n = 6), CTX-M-24 (n = 2) and CTX-M-3 (n = 1); 3 isolates had both CTX-M-14 and CTX-M-15. All CTX-M-producing isolates were resistant to cefotaxime at a breakpoint of

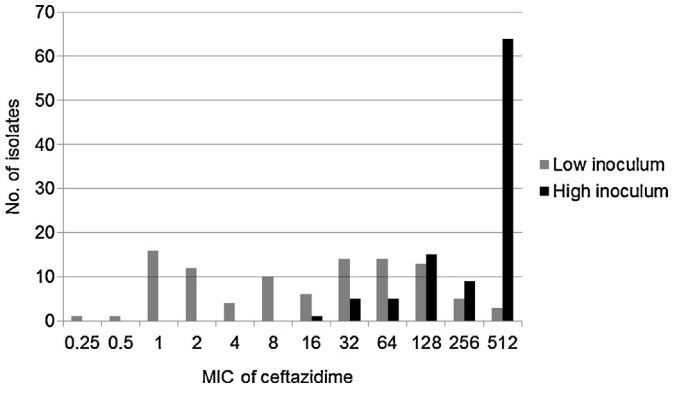

Fig. 1. Distribution of minimum inhibitory concentrations (MICs) (in μg/mL) of ceftazidime according to low inoculum and high inoculum.

1 μg/mL. When the revised CLSI ceftazidime breakpoint of 4 μg/mL was applied, 34 (34.3%) of the 99 CTX-M-producers tested were susceptible. More specifically, for 42 CTX-M-14-producing *E. coli* isolates, 32 (76.2%) were susceptible at 4 μg/mL. Cefotaxime, ceftazidime, cefepime and piperacillin/tazobactam (TZP) were found to be associated with inoculum effects in 100% of the evaluable tests for ESBL-producing *E. coli* isolates (excluding those that could not be evaluated because of off-scale MICs) (Table 1). The MIC_{50} (MIC required to inhibit 50% of the isolates) of ceftazidime was 16 μg/mL in the standard-inoculum tests and >512 μg/mL in the high-inoculum tests. Fig. 1 shows distribution of ceftazidime MICs according to low inoculum and high inoculum. Imipenem and meropenem inhibited all isolates at a concentration of 0.5 μg/mL in the standard-inoculum tests and also inhibited all isolates at 8 μg/mL in the high-inoculum tests (Table 1). The MICs of antimicrobial agents as determined by the standard- and high-inoculum tests for CTX-M-14 and CTX-M-15 ESBL-producing *E. coli* isolates are shown in Table 2. Inoculum effects in testing with cefotaxime, ceftazidime, cefepime and TZP were consistently detected regardless of CTX-M type. In standard-inoculum tests, all isolates encoding CTX-M-14 were susceptible to imipenem and meropenem, and ceftazidime was the next most active agent (82.1% susceptible). In the high-inoculum tests, ceftazidime was dramatically affected, with susceptibility decreasing from 82.1% of isolates inhibited at 4 μg/mL in the standard-inoculum tests to 0% with the high inoculum.

In the current study, we demonstrated that the inoculum effect was most pronounced in tests with ceftazidime and cefepime, even though CTX-M-producing *E. coli* isolates might be susceptible to ceftazidime or cefepime in vitro. The inoculum effect was smallest in tests with imipenem and meropenem. Studies determining