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ARTICLE

High rates of susceptibility to ceftazidime among globally prevalent CTX-M-producing *Escherichia coli*: potential clinical implications of the revised CLSI interpretive criteria

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Abstract The CTX-M family of extended-spectrum β-lactamases (ESBLs) is a significant global public health threat. The prevalence of specific $bla_{\text{CTX-M}}$ genes varies geographically, but $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-14}}$ dominate in most countries. We applied the latest Clinical Laboratory Standards Institute (CLSI) interpretive criteria (M100-S20) to a diverse collection of ESBL-producing *Escherichia coli* strains obtained from clinical specimens in our laboratory. Whereas under previous CLSI recommendations *all* isolates in this strain collection would have been reported as ceftazidime-*resistant*, under the new recommendations, approximately 11% of CTX-M-15-producing *E. coli* and 93% of CTX-M-14-producing *E. coli* respectively tested as ceftazidime-*susceptible*. We also found that, whilst many CTX-M-14-producers had minimum inhibitory concentrations (MICs) less than the breakpoint of 4 mg/L, the MIC distribution for these strains was higher than that of wild-type *E. coli*, with one CTX-M-14-producing isolate having an MIC of >64 mg/L. Although the new CLSI recommendations imply that ceftazidime can be safely used to treat serious infections due to CTX-M-producing *E. coli*, clinical outcome data are lacking. Consequently, the widespread use of ceftazidime in this setting could have profound clinical implications.

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Introduction

The CTX-M family of extended-spectrum β-lactamases (ESBLs) is a significant global health threat [1]. The prevalence of specific $bla_{\text{CTX-M}}$ genes varies geographically, but $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-14}}$ dominate in most countries [2]. In the USA, TEM- and SHV-ESBLs still predominate, although this situation is rapidly changing, with CTX-M-14 and CTX-M-15 being reported in a number of states [3, 4].

Breakpoints for *Enterobacteriaceae* against third-generation cephalosporins have recently been revised by the Clinical Laboratory Standards Institute (CLSI) in the M100-S20 document [5]. Confirmatory disc testing for ESBL production is no longer recommended. Instead, it is recommended that susceptibility results for cephalosporins be reported according to the minimum inhibitory concentration (MIC), regardless of whether or not the isolate produces an ESBL. Of note, the new breakpoint MIC defining susceptibility to ceftriaxone and cefotaxime (1 mg/L) is lower than the corresponding new breakpoint for ceftazidime (4 mg/L). Importantly, although CTX-M ESBLs have activity against all cephalosporins, they have greater hydrolytic activity against cefotaxime and ceftriaxone than ceftazidime [1]. Based on these new recommendations, we hypothesized that the revised cephalosporin breakpoints may result in many CTX-M-producing *Escherichia coli* being reported as *susceptible* to ceftazidime.

Over the last five years at Auckland City Hospital, New Zealand, the incidence of bloodstream infections due to CTX-M-producing *Enterobacteriaceae* has increased significantly. Against the background of this concerning trend, we sought to determine the rate of ceftazidime susceptibility using the new CLSI M100-S20 breakpoint among a collection of CTX-M-producing *E. coli* isolates.

Methods

Between January 2006 and December 2007, we collected consecutive, non-duplicate isolates of ESBL-producing *E. coli* that tested positive by the CLSI combination disc test, as recommended in the CLSI M100-S20 document [5]. Both clinical isolates and isolates obtained by active surveillance (rectal swabs) were included. Resistance due to plasmid-mediated AmpC β-lactamase production was excluded by the use of the boronic acid disc synergy test [6]. Isolates testing positive for ESBL production underwent polymerase chain reaction (PCR) and sequencing of $bla_{\text{CTX-M}}$ genes and pulsed-field gel electrophoresis (PFGE) using previously described methods [7].

In order to compare (i) MIC distributions and (ii) ceftazidime and ceftriaxone susceptibility between ESBL-producing and "wild-type" *E. coli* isolates, consecutive, non-duplicate, non-ESBL-producing *E. coli* isolates were also collected between 15th September 2010 and 1st October 2010. Isolates were assumed not to produce an ESBL if there was either (i) no growth on Mueller–Hinton plates containing ceftriaxone 1 mg/L or ceftazidime 1 mg/L or (ii) if, following growth on these plates, the CLSI combination disc test and boronic acid synergy test were negative. Agar dilution susceptibility testing against ceftazidime was performed on all isolates according to current CLSI recommendations [5].

We also reviewed the clinical notes of patients who had a CTX-M-producing *E. coli* isolated in order to determine (i) the proportion of isolates associated with clinical infections and (ii) the nature of these infections. A complicated urinary tract infection was defined as one which occurred in the presence of a urinary catheter or in a patient with a functional or anatomic urinary tract abnormality.

Results and discussion

One hundred and sixteen ESBL-producing *E. coli* isolates were collected. Of these, 103 isolates with confirmed $bla_{\text{CTX-M}}$ were available for susceptibility testing. Sixty-six patients were identified as having clinical infections: 36/66 patients (54.5%) had uncomplicated cystitis; 19 (28.7%) had complicated urinary tract infection (including six bloodstream infections); 5 (7.5%) had deep abdominal infections; 3 (4.5%) had bloodstream infections associated with febrile neutropenia; 2 (3.0%) had surgical site infections; and 1 (1.5%) had a ventilator-associated pneumonia.

The predominant CTX-M type was CTX-M-15 (n=56; 54.3%), followed by CTX-M-14 (n=29; 28.1%) and CTX-M-9 (n=11; 10.6%). Seven isolates had other CTX-M types; CTX-M-3 (3; 2.9%), CTX-M-27 (1), CTX-M-57 (1), and CTX-M-65 (2). PFGE revealed diverse strains with 60–100% similarity. Seven CTX-M-9-producing isolates and two CTX-M-14-producing isolates had identical PFGE profiles.

All CTX-M-producing isolates were resistant to ceftriaxone at a breakpoint of 1 mg/L. When the revised CLSI ceftazidime breakpoint of 4 mg/L was applied, 48 (46.6%) of the 103 CTX-M-producers tested susceptible and 30 (29.1%) tested susceptible at 1 mg/L. More specifically, for CTX-M-15-producing *E. coli*, 6/56 (10.7%) and 1/56 (1.8%) were susceptible at the 4 and 1 mg/L concentrations respectively, whereas for CTX-M-14-producers, 27/29 (93.1%) were susceptible at 4 mg/L and 16/29 (55.2%) were susceptible at 1 mg/L.

The ceftazidime MICs for wild-type, CTX-M-15- and CTX-M-14-producing *E. coli* showed distinct distributions and peaks (Fig. 1). The highest MICs were seen with CTX-M-15-producers, followed by CTX-M-14, followed by wild-

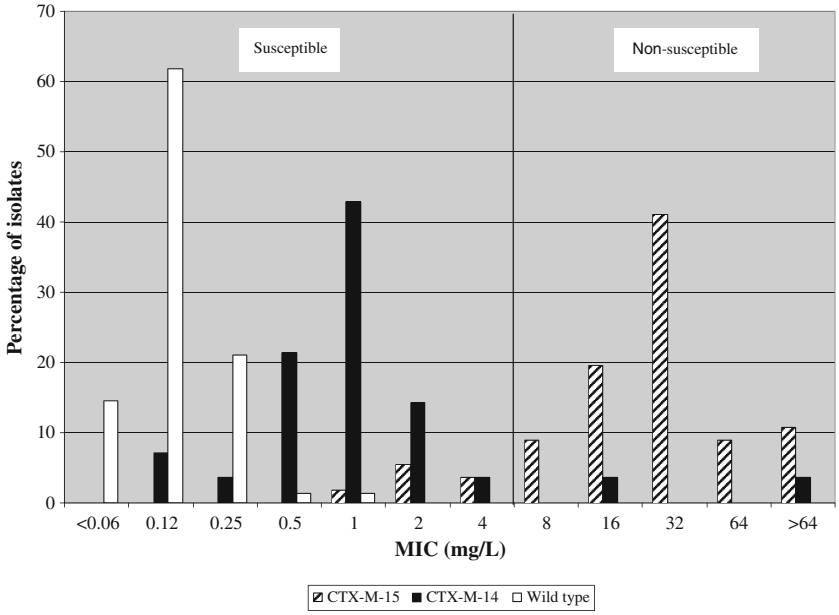

Fig. 1 Minimum inhibitory concentration (MIC) distributions for ceftazidime against CTX-M-15-producing, CTX-M-14-producing and "wild-type" *Escherichia coli*

type *E. coli*. Seventy-six "wild-type" isolates were collected, all of which remained susceptible to both ceftazidime and ceftriaxone (Fig. 1). Two of 29 CTX-M-14-producing strains had MICs greater than 4 mg/L; one with an MIC of 16 mg/L and one with an MIC of >64 mg/L.

The international dissemination of $bla_{\text{CTX-M}}$ ESBL genes over the last decade has been described as a pandemic [2]. CTX-M-producing *E. coli* has emerged as a significant cause of both community-onset and hospital-acquired infections. Notably, the urovirulent CTX-M-15-producing 025:H4 ST131 clone is now widespread globally [2]. It seems probable, therefore, that the incidence of serious infection due to CTX-M-producing *E. coli* will continue to increase. In the face of this likely scenario, it is important that the optimal treatment of infections caused by these organisms is underpinned by clinically valid susceptibility testing interpretive criteria.

In New Zealand, as in most countries, ceftazidime has not been recommended to treat infections due to CTX-M-producing *Enterobacteriaceae*. However, when we applied the new CLSI ceftazidime breakpoint to a collection of CTX-M-producing *E. coli*, we found that 47% of all isolates (and for CTX-M-14-producing *E. coli*, 93% of all isolates) tested as ceftazidime-susceptible. Thus, application of the new CLSI reporting guidelines effectively "reclaimed" ceftazidime as a potential therapeutic agent against approximately half of all ESBL-producing *E. coli* in our locale.

Our data demonstrate that, although the ceftazidime MICs for many CTX-M-producing *E. coli* fall within the new susceptible range, their MIC distribution is higher than that of wild-type *E. coli* (Fig. 1). These findings are consistent with data published by the European Committee for Antimicrobial Susceptibility Testing (EUCAST) [8]. CTX-M-15-producing *E. coli* has enhanced ceftazidimase activity compared to its parent enzyme (CTX-M-3), due to the presence of the amino acid substitution Asp240Gly [1]. Of note, CTX-M-14 can also acquire increased activity against ceftazidime due to the Asp240Gly substitution through a single-point mutation [1].

Indeed, the evolutionary tendency for CTX-M enzymes to acquire more efficient hydrolytic activity against ceftazidime has recently been described [9]. This potential for ceftazidime resistance to develop in vivo may be underestimated by the murine thigh infection model that is used to determine pharmacodynamic (PD) targets because, in that model, shorter durations of antibiotic and lower inocula of bacteria are used than in most human infections [10, 11]. Furthermore, although PD targets have been determined for a wide variety of ESBL-producing isolates, no data (to our knowledge) have been published specifically for either CTX-M-15 or CTX-M-14 [10]. Finally, the Monte Carlo methods used to derive ceftazidime breakpoints are based on pharmacokinetic data obtained from healthy populations and should be applied with caution when treating critically ill patients with altered pharmacokinetics [12].

Most importantly, however, there are (to our knowledge) no published data specifically describing clinical outcomes when infections due to CTX-M-producing *E. coli* are treated with ceftazidime. Of the 66 patients we identified as having clinical infections, 30 patients (45%) had serious infections, including nine episodes of bacteremia. It is notable that, in some studies, the use of inappropriate empirical antibiotics to treat bacteremia caused by ESBL-producing *Enterobacteriaceae* has been associated with increased mortality [13]. For this reason, widespread application of these new reporting recommendations could, potentially, have substantial clinical impact. Previous studies have shown that, when ceftazidime is used to treat infections due to TEM- and SHV-type ESBL-producing *Enterobacteriaceae*, the clinical outcomes are generally poor, even when the MICs lie within the susceptible range [14]. Whether or not the same findings can be expected with CTX-M-producing *Enterobacteriaceae* is unknown. This uncertainty is concerning because the latest CLSI reporting recommendations effectively endorse ceftazidime for the treatment of serious infections caused by CTX-M-producing *E. coli*.

Two types of error are possible when devising clinical breakpoints. For the purposes of quality control, it is generally accepted that the clinical consequences of over-calling resistance ("major error") are less than the clinical consequences of over-calling susceptibility ("*very* major error"). Using the same logic, we suggest, therefore, that, in the absence of clinical outcome data, the risks associated with overcalling resistance to ceftazidime in *E. coli* are likely to be lower than the clinical risks associated with overcalling susceptibility.

In summary, the application of CLSI M100-S20 recommendations will lead to many CTX-M-producing *E. coli* being reported as susceptible to ceftazidime. Although future 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, laboratories should be cautious about reporting ceftriaxone-resistant *E. coli* as susceptible to ceftazidime.

Conflict of interest All authors report no conflict of interests.

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