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Presence of the Narrow-Spectrum OXA-1 Beta-Lactamase Enzyme is not Associated with Elevated Ceftolozane-Tazobactam MIC Values among ESBL-Producing *Escherichia coli* Clinical Isolates (CANWARD, 2011-2018)

A. Walkty[1,2], J. A. Karlowsky[1,2], P. R. S. Lagace-Wiens[1,2], A. R. Golden[3], M. R. Baxter[1], A. J. Denisuik[1], M. McCracken[3], M. R. Mulvey[3], H. J. Adam[1,2], G. G. Zhanel[1]

[1]Department of Medical Microbiology and Infectious Diseases, Max Rady College of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada; [2]Shared Health, Winnipeg, Manitoba, Canada; [3]National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada

Name: Andrew Walkty
Address: MS673B, Microbiology
Health Sciences Centre
Winnipeg, Manitoba, Canada, R3A1R9
Phone: (204) 787-1161
Email: Awalkty@sharedhealthmb.ca

Abstract

Introduction: Beta-lactam/beta-lactamase inhibitor combinations have been proposed as an alternative to carbapenems for the treatment of infections caused by extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* as an antimicrobial stewardship initiative. However, in a recent randomized trial evaluating patients with bacteremia, presence of the narrow spectrum OXA-1 beta-lactamase enzyme among ESBL-producing Enterobacterales was associated with higher piperacillin-tazobactam MICs, and in turn excess mortality among patients treated with piperacillin-tazobactam relative to meropenem. The purpose of this study was to determine whether the *in vitro* activity of ceftolozane-tazobactam versus ESBL-producing *E. coli* is similarly compromised by the presence of OXA-1.
Methods: *E. coli* clinical isolates were obtained from patients evaluated at hospitals across Canada (January 2011 to December 2018) as part of an ongoing national surveillance study (CANWARD). ESBL production was confirmed using the Clinical and Laboratory Standards Institute phenotypic method. Susceptibility testing was carried out using custom broth microdilution panels, and all isolates underwent whole genome sequencing for beta-lactamase gene detection.
Results: In total, 485 ESBL-producing *E. coli* identified as part of the CANWARD study were included. The majority of isolates (91.3%; 443/485) harbored a CTX-M ESBL enzyme. OXA-1 was present in 39.6% (192/485) of isolates. OXA-1 was detected in 62.5% (187/299) of isolates with a CTX-M-15 ESBL enzyme versus only 2.7% (5/186) of isolates with other ESBL enzyme types. Ceftolozane-tazobactam MIC_{50} and MIC_{90} values were identical (0.25 µg/mL and 1 µg/mL, respectively) for the isolate subsets with and without OXA-1. Overall, 97.4% and 96.9% of isolates with and without OXA-1 remained susceptible (≤2/4 µg/mL, CLSI breakpoint) to ceftolozane-tazobactam.
Conclusions: The presence of OXA-1 among ESBL-producing *E. coli* clinical isolates was not associated with an elevation in MIC values for ceftolozane-tazobactam. These data support further evaluation of ceftolozane-tazobactam as an alternative to carbapenems for the treatment of infections caused by ESBL-producing *E. coli*, as is being done with the MERINO-3 trial.

Introduction

Escherichia coli is a common cause of urinary tract infections, intra-abdominal infections, and bacteremia. Of concern, *E. coli* isolates that produce an extended-spectrum beta-lactamase (ESBL) enzyme are being recovered with increasing frequency from patients in Canada and elsewhere in the world.[1-3] ESBL-producing *E. coli* often demonstrate a multidrug-resistant phenotype, leaving clinicians with a limited range of therapeutic options from which to choose.[4] Carbapenems are typically regarded as the antimicrobials of choice for the treatment of serious infections caused by ESBL-producing *E. coli*, but overuse of these agents has the potential to drive carbapenem resistance.[5,6]
Beta-lactam/beta-lactamase inhibitor combinations have been proposed as an alternative to carbapenems for the treatment of infections caused by ESBL-producing *E. coli* as an antimicrobial stewardship initiative.[5] However, in a recent randomized trial evaluating patients with bacteremia, presence of the narrow spectrum OXA-1 beta-lactamase enzyme among ESBL-producing Enterobacterales was associated with higher piperacillin-tazobactam MICs, and in turn excess mortality among those treated with piperacillin-tazobactam relative to meropenem.[6,7]
Ceftolozane-tazobactam is a novel beta-lactam/beta-lactamase inhibitor combination currently indicated for the treatment of complicated urinary tract infections, complicated intra-abdominal infections, and nosocomial pneumonia.[8,9] It is active *in vitro* versus a diverse range of Gram-negative bacterial pathogens, including many *E. coli* isolates that harbor an ESBL enzyme.[8] In a pooled analysis of randomized trial data, ceftolozane-tazobactam demonstrated high clinical cure rates among patients with a urinary tract infection or intra-abdominal infection caused by ESBL-producing Enterobacterales.[10] The purpose of this study was to determine whether the *in vitro* activity of ceftolozane-tazobactam versus ESBL-producing *E. coli* is compromised by the presence of OXA-1, similar to piperacillin-tazobactam.

Materials and Methods

Bacterial isolates: *E. coli* clinical isolates were obtained from patients evaluated at hospitals across Canada (January 2011 to December 2018) as part of an ongoing national surveillance study (CANWARD).[11] CANWARD is a Public Health Agency of Canada-PHAC/Canadian Antimicrobial Resistance Alliance (CARA) partnered surveillance study evaluating *in vitro* activities of antimicrobial agents against bacterial pathogens isolated by clinical laboratories from patients attending tertiary care hospitals across Canada. On an annual basis, each center was asked to submit clinical isolates (consecutive, one per patient/infection site) from blood, respiratory, urine, and wound infections. The medical centers submitted clinically significant isolates, as defined by their local site criteria. Isolate identification was performed by the submitting site and confirmed at the reference site as required (i.e., when morphological characteristics and antimicrobial susceptibility patterns did not fit the reported identification). Isolates were shipped on Amies semi-solid transport media to the coordinating laboratory (Health Sciences Centre, Winnipeg, Canada), subcultured onto appropriate media, and stocked in skim milk at -80˚ C until MIC testing was carried out.
Antimicrobial susceptibility testing: Following 2 subcultures from frozen stock, the *in vitro* activity of ceftolozane-tazobactam was determined by broth microdilution in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines.[12] In-house-prepared 96-well broth microdilution panels were used for antimicrobial susceptibility testing. MICs were interpreted according to current CLSI breakpoints.[13] Putative ESBL-producing *E. coli* were identified as isolates with a ceftriaxone and/or ceftazidime MIC of ≥1 µg/mL. ESBL-production was verified using the CLSI phenotypic confirmatory disk test.[13]
Whole genome sequencing: All phenotypically confirmed ESBL-producing isolates were sequenced using the Illumina MiSeq platform. Quality control was performed using the FastQC tool (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and contigs were assembled using SPAdes software.[14] Beta-lactamase genes were identified using ResFinder 4.0 at an identity threshold of 90%.[15] MLST alleles and sequence types were identified by scanning assembled contigs against available PubMLST databases (https://github.com/tseemann/mlst).

Results

In total, 485 ESBL-producing *E. coli* were identified as part of the CANWARD study. The majority of isolates (91.3%; 443/485) harbored a CTX-M ESBL enzyme. CTX-M-15 (61.6%; 299/485), CTX-M-27 (17.5%; 85/485), and CTX-M-14 (9.7%; 47/485) were the most common variants identified. The narrow spectrum OXA-1 beta-lactamase enzyme was present in 39.6% (192/485) of isolates. OXA-1 was detected in 62.5% (187/299) of isolates with a CTX-M-15 ESBL enzyme versus only 2.7% (5/186) of isolates with other ESBL enzyme types. The ceftolozane-tazobactam MIC_{50} and MIC_{90} values were identical (0.25 µg/mL and 1 µg/mL, respectively) for the isolate subsets with and without OXA-1 (Table 1). Overall, 97.4% and 96.9% of isolates with and without OXA-1 remained susceptible (≤2/4 µg/mL, CLSI breakpoint) to ceftolozane-tazobactam. In contrast, the piperacillin-tazobactam MIC_{50} and MIC_{90} values were 8 µg/mL and 32 µg/mL for isolates that possessed the OXA-1 enzyme versus 2 µg/mL and 8 µg/mL for those that did not (Table 2). The ceftolozane-tazobactam MIC distribution for isolates with the CTX-M-15 enzyme stratified by OXA-1 status (present or absent) is presented in Table 3. The presence or absence of TEM-1 also did not appear to influence the MIC distribution for ceftolozane-tazobactam (Table 4).

Table 1. Ceftolozane-tazobactam MIC distribution for ESBL-producing *E. coli*, stratified by the absence or presence of OXA-1

	Number of Isolates (Cumulative Percentage of Isolates) at MIC (µg/mL)											
Organism	**≤0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**≥128**	**Total**
ESBL-producing *E. coli*												485
OXA-1 Absent (n = 293)	43 (14.7)	114 (53.6)	85 (82.6)	31 (93.2)	11 (96.9)	2 (97.6)	2 (98.3)	1 (98.6)	2 (99.3)	0 (99.3)	2 (100.0)	293
OXA-1 Present (n = 192)	17 (8.9)	80 (50.5)	61 (82.3)	25 (95.3)	4 (97.4)	3 (99.0)	1 (99.5)	1 (100.0)				192

Table 2. Piperacillin-tazobactam MIC distribution for ESBL-producing *E. coli*, stratified by the absence or presence of OXA-1

	Number of Isolates (Cumulative Percentage of Isolates) at MIC (µg/mL)										
Organism	**≤1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**≥512**	**Total**
ESBL-producing *E. coli*											485
OXA-1 Absent (n = 293)	83 (28.3)	115 (67.6)	63 (89.1)	13 (93.5)	8 (96.2)	2 (96.9)	2 (97.6)	0 (97.6)	5 (99.3)	2 (100.0)	293
OXA-1 Present (n = 192)	10 (5.2)	23 (17.2)	39 (37.5)	62 (69.8)	37 (89.1)	3 (90.6)	8 (94.9)	5 (97.4)	2 (98.4)	3 (100.0)	192

Table 3. Ceftolozane-tazobactam MIC distribution for ESBL-producing *E. coli* (CTX-M-15 subset)

	Number of Isolates (Cumulative Percentage of Isolates) at MIC (µg/mL)											
Organism	**≤0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**≥128**	**Total**
***E. coli* (CTX-M-15 Positive)**												299
OXA-1 Absent (n = 112)	7 (6.3)	36 (38.4)	41 (75.0)	15 (88.4)	8 (95.5)	1 (96.4)	2 (98.2)	0 (98.2)	0 (98.2)	0 (98.2)	2 (100.0)	112
OXA-1 Present (n = 187)	15 (8.0)	78 (49.7)	60 (81.8)	25 (95.2)	4 (97.3)	3 (98.9)	1 (99.5)	1 (100.0)				187

Table 4. Ceftolozane-tazobactam MIC distribution for ESBL-producing *E. coli*, stratified by the absence or presence of TEM-1

	Number of Isolates (Cumulative Percentage of Isolates) at MIC (µg/mL)											
Organism	**≤0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**≥128**	**Total**
ESBL-producing *E. coli*												485
TEM-1 Absent (n = 368)	47 (12.8)	147 (52.7)	110 (82.6)	43 (94.3)	10 (97.0)	3 (97.8)	3 (98.6)	1 (98.9)	2 (99.5)	0 (99.5)	2 (100.0)	368
TEM-1 Present (n = 117)	13 (11.1)	47 (51.3)	36 (82.1)	13 (93.2)	5 (97.4)	2 (99.1)	0 (99.1)	1 (100.0)				117

Conclusions

- The presence of OXA-1 among ESBL-producing *E. coli* clinical isolates was not associated with an elevation in MIC values for ceftolozane-tazobactam..
- These data support further evaluation of ceftolozane-tazobactam as an alternative to carbapenems for the treatment of infections caused by ESBL-producing *E. coli*, as is being done with the MERINO-3 trial.[16]

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References

1. Denisuik AJ, Karlowsky JA, Adam HJ *et al.* Dramatic rise in the proportion of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* among clinical isolates identified in Canadian hospital laboratories from 2007 to 2016. *J Antimicrob Chemother* 2019;74:Suppl 4:iv64-71.
2. Peirano G, Pitout JDD. Extended-spectrum β-lactamase-producing Enterobacteriaceae: Update on molecular epidemiology and treatment options. *Drugs* 2019;79:1529-41.
3. McDanel J, Schweizer M, Crabb V *et al.* Incidence of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella infections* in the United States: A systematic literature review. *Infect Control Hosp Epidemiol* 2017;38:1209-15.
4. Karlowsky JA, Walkty A, Golden AR et al. ESBL-positive *Escherichia coli* and *Klebsiella pneumoniae* isolates from across Canada: CANWARD surveillance study, 2007-18. *J Antimicrob Chemother* 2021;76:2815-24.
5. Castanheira M, Simner PJ, Bradford PA. Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. JAC Antimicrob Resist 2021;3:dlab092. doi: 10.1093/jacamr/dlab092. eCollection 2021 Sep.
6. Harris PNA, Tambyah PA, Lye DC *et al.* Effect of piperacillin-tazobactam vs meropenem on 30-day mortality for patients with *E. coli* or *Klebsiella pneumoniae* bloodstream infection and ceftriaxone resistance: A randomized clinical trial. *JAMA* 2018;320:984-94.
7. Henderson A, Paterson DL, Chatfield MD *et al.* Association between minimum inhibitory concentration, beta-lactamase genes and mortality for patients treated with piperacillin-tazobactam or meropenem from the MERINO study. *Clin Infect Dis* 2021:73:e3842-50.
8. Zhanel GG, Chung P, Adam H *et al.* Ceftolozane/tazobactam: a novel cephalosporin/β-lactamase inhibitor combination with activity against multidrug-resistant gram-negative bacilli. *Drugs* 2014;74:31-51.
9. *Zerbaxa® Product Monograph.* Merck Canada Inc., Kirkland, QC, Canada, 2020.
10. Popejoy MW, Paterson DL, Cloutier D *et al.* Efficacy of ceftolozane/tazobactam against urinary tract and intra-abdominal infections caused by ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*: a pooled analysis of phase 3 clinical trials. *J Antimicrob Chemother* 2017;72:268-72.
11. Zhanel GG, Adam HJ, Baxter MR *et al.* Antimicrobial susceptibility of 42936 pathogens from Canadian hospitals: 10 years of results (2007-2016) from the CANWARD surveillance Study. *J Antimicrob Chemother* 2019;74 Suppl 4:iv5-21.
12. Clinical and Laboratory Standards Institute. *M07 Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, 11th Edition*. CLSI, Wayne, PA, USA, 2018.
13. Clinical and Laboratory Standards Institute. *M100 Performance Standards for Antimicrobial Susceptibility Testing, 32nd Edition*. CLSI, Wayne, PA, USA, 2022.
14. Bankevich A, Nurk S, Antipov D *et al.* SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455-77.
15. Bortolaia V, Kaas RS, Ruppe E *et al.* ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother* 2020;75:3491–500.
16. Stewart AG, Harris PNA, Chatfield MD *et al.* Ceftolozane-tazobactam versus meropenem for definitive treatment of bloodstream infection due to extended-spectrum beta-lactamase (ESBL) and AmpC-producing Enterobacterales ("MERINO-3"): study protocol for a multicentre, open-label randomised non-inferiority trial. *Trials* 2021;22:301.