Infect Dis Ther (2015) 4:513–518
DOI 10.1007/s40121-015-0094-6

BRIEF REPORT

Defining Extended Spectrum β-Lactamases: Implications of Minimum Inhibitory Concentration-Based Screening Versus Clavulanate Confirmation Testing

Christina A. Sutherland · Jared L. Crandon · David P. Nicolau

To view enhanced content go to www.infectiousdiseases-open.com
Received: September 21, 2015 / Published online: October 20, 2015

ABSTRACT

Introduction: While the Clinical and Laboratory Standards Institute (CLSI) recommends against routine screening for extended spectrum β-lactamases (ESBLs), knowledge of these data can provide valuable insights regarding epidemiology and drug therapy decisions. The purpose of this study was to compare the impact of minimum inhibitory concentration (MIC)-based screening versus phenotypic confirmatory testing of ESBLs on the susceptibility profile of selected antimicrobials.

Methods: Broth microdilution MICs were determined for various antimicrobial agents against a collection contemporary clinical *Escherichia coli* and *Klebsiella pneumoniae* isolates. Isolates identified as ESBL-positive by MIC screening were then subjected to confirmatory phenotypic testing. Percent susceptibility was based on CLSI or United States Food and Drug Administration breakpoints.

Results: Four-hundred and forty-two (18%) isolates screened positive for ESBL production. Of these, 274 (62%) were confirmed positive for ESBL production; 28 (10%) were also carbapenem non-susceptible. We found an under-prediction of activity for ceftolozane/tazobactam (C/T), ertapenem (ETP), meropenem (MEM), and piperacillin/tazobactam (TZP) when considering only the screen-positive testing.

Conclusion: For agents with potential activity against ESBLs such as C/T, TZP, ETP, and MEM, reduced susceptibility was noted when only considering the MIC screen-positive test. Although phenotypic screening selects for resistant organisms, inclusion of other genotypes besides ESBL (i.e., AmpC, carbapenemase) may falsely under-predict the potency against some ESBL producers and may limit applicability of surveillance data to geographic areas not plagued with carbapenemase producers.

Funding: Cubist Pharmaceuticals.

Electronic supplementary material The online version of this article (doi:10.1007/s40121-015-0094-6) contains supplementary material, which is available to authorized users.

C. A. Sutherland · J. L. Crandon · D. P. Nicolau (✉)
Center for Anti Infective Research and Development, Hartford Hospital, Hartford, CT, USA
e-mail: david.nicolau@hhchealth.org

Present Address:
J. L. Crandon
Actavis plc, Jersey City, NJ, USA

Keywords: Extended spectrum β-lactamase (ESBL); Minimum inhibitory concentration (MIC); Screening

INTRODUCTION

Extended spectrum β-lactamases (ESBL) are enzymes capable of hydrolyzing penicillins, broad-spectrum cephalosporins, and monobactams [1]. ESBL-based infections have been noted to result in increased morbidity and mortality. These infections increase cost of care if inappropriate therapy, defined as non-susceptibility of pathogen to therapy by laboratory criteria, is administered [2]. At present, the Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) do not recommend routine screening for ESBLs or subsequent phenotypic confirmation testing that had been advocated previously [3, 4]. While this approach has been supported in the clinical practice setting by the introduction of reduced breakpoints for many beta-lactams [5], it may be of value, particularly when evaluating novel therapies, to understand the potency of various agents against ESBL producers. However, if one utilizes ESBL classification with aztreonam (ATM), ceftriaxone (CRO), or ceftazidime (CAZ) minimum inhibitory concentrations (MIC) as a simple screen, without phenotypic confirmation via the clavulanate test, this approach may include other resistant genotypes (i.e., carbapenemase producers) that may not accurately predict the clinical utility of therapeutic agents under consideration [6].

Phenotypic screening tests to detect resistance mechanisms such as carbapenemase production or AmpC expression are available and can be used. While molecular testing would yield the most definite results, it is also cost prohibitive and unavailable to most clinical microbiological laboratories. In this study, we tested the recently US Food and Drug Administration (FDA)-approved compound, ceftolozane/tazobactam (C/T), which has demonstrated in vitro activity against some ESBL organisms [7]. The purpose of this study was to compare the impact of MIC-based screening versus confirmatory testing of ESBLs on the susceptibility profile of C/T versus selected antimicrobials.

METHODS

In this study, 44 US hospitals collected non-duplicate, non-urine *Escherichia coli* ($n = 1306$) and *Klebsiella pneumoniae* ($n = 1205$) over the period of June 2013 and October 2014. Participating sites identified the organisms using their established method and then transferred each isolate to trypticase soy agar slants for shipping. Isolates were transferred onto trypticase soy agar plates containing 5% sheep blood at the central processing laboratory (Center for Anti-Infective Research and Development, Hartford Hospital, Hartford Hospital, Hartford, CT, USA) for MIC determination using broth microdilution as described by CLSI [5]. MICs were determined for C/T, cefepime (FEP), CRO, CAZ, ciprofloxacin (CIP), ATM, ertapenem (ETP), piperacillin/tazobactam (TZP), meropenem (MEM), and tobramycin (TOB) against this collection of 2511 contemporary clinical Enterobacteriaceae isolates. MIC trays were prepared using the Biomek 3000 (Beckman Instruments, Inc., Fullerton, CA, USA) and frozen at −80 °C until use. C/T was provided by Cubist Pharmaceuticals, while all others antibiotics were purchased from Sigma-Aldrich

(St. Louis, MO, USA). *E. coli* 25922 and *Pseudomonas aeruginosa* 27853 were utilized as quality control strains as recommended by CLSI.

CLSI states that the initial MIC-based ESBL screening for *E. coli* and *K. pneumoniae* should be performed by evaluating the MIC profile of one of the following drugs: cefpodoxime 4 μg/mL, CAZ 1 μg/mL, ATM 1 μg/mL, or cefotaxime 1 μg/mL. It is noted that the use of more than one of these agents will improve detection of ESBLs. We defined a positive ESBL screen as having an MIC of ≥1 μg/mL to 2 of the following: ATM, CRO, or CAZ. Subsequent CLSI defined, phenotypic, ESBL confirmation studies were undertaken using CAZ and cefotaxime with and without clavulanate [8]. Probable carbapenemase-producing organisms were excluded based on ETP (≥1 μg/mL) or MEM (≥2 μg/mL) susceptibility profiles. While molecular-based confirmation of these enzymes would have been definitive, methodologies readily available to the clinical microbiology laboratory were utilized. Percent susceptibility (%S) was based on CLSI breakpoints [3]. Recently, C/T has been approved in the US and susceptibility interpretative criteria have now been provided by the FDA of ≤2 μg/mL for the Enterobacteriaceae [7].

This surveillance program was reviewed by the institutional review board (IRB) at the coordinating center, Hartford Hospital. Since all samples were collected as part of routine

Table 1 Percent susceptibility of all agents against MIC-based screen-positive (Screen +) and phenotypically confirmed (Confirmed +) ESBL *Escherichia coli* and *Klebsiella pneumoniae* isolates

	Percent susceptibility for antimicrobials									
	C/T	**FEP**	**CRO**	**CAZ**	**CIP**	**ATM**	**ETP**	**TZP**	**MEM**	**TOB**
All										
Screen + ($n = 442$)	64	21	10	18	23	16	74	53	79	45
Confirmed + ($n = 274$)	75	8	2	9	14	6	91	61	93	42
Confirmed +, carbapenem NS removed ($n = 246$)	82	9	2	9	13	7	100	67	100	42
E. coli										
Screen + ($n = 231$)	79	29	13	24	27	23	86	69	90	54
Confirmed + ($n = 146$)	88	9	3	10	10	10	96	75	98	47
Confirmed +, carbapenem NS removed ($n = 139$)	91	9	4	11	10	10	100	78	100	47
K. pneumoniae										
Screen + ($n = 211$)	47	12	5	11	18	9	62	36	65	34
Confirmed + ($n = 128$)	61	7	1	7	18	2	82	46	88	35
Confirmed +, carbapenem NS removed ($n = 107$)	71	7	1	7	16	2	100	52	100	36

ATM aztreonam, *CAZ* ceftazidime, *CIP* ciprofloxacin, *CRO* ceftriaxone, *C/T* ceftolozane/tazobactam, *ETP* ertapenem, ESBL extended spectrum β-lactamase, *FEP* cefepime, *MEM* meropenem, *MIC* minimum inhibitory concentration, *NS* non-susceptible, *TOB* tobramycin, *TZP* piperacillin/tazobactam

medical care and no interventions were undertaken as a result of this testing, informed consent was deemed unnecessary by the IRB.

RESULTS

Four-hundred and forty-two (18%) isolates [*E. coli* ($n = 231$), *K. pneumoniae* ($n = 211$)] screened positive for ESBL production based on MIC-based criteria. Of these, 274 (62%) isolates [*E. coli* ($n = 146$), *K. pneumoniae* ($n = 128$)] were phenotypically confirmed positive for ESBL production. With further exclusion of the 28 (10%) isolates [*E. coli* ($n = 13$), *K. pneumoniae* ($n = 14$)] that demonstrated carbapenem non-susceptible, a left shift in the MIC distribution (i.e., enhanced potency) against phenotypically confirmed ESBL was noted for C/T, ETP, TZP, and MEM (Table 1). Figure 1 highlights these screening- and confirmation-based observations for C/T and TZP. Improvements in %S between 8% and 17% were noted for confirmed versus screened-positive ESBL isolates for C/T, ETP, TZP, and MEM. (Table 1) Overall, %S of the other β-lactams, TOB and CIP, was poor against phenotypically confirmed ESBLs. Comparing the MIC_{90} values for all ESBL MIC-based screen-positive versus phenotypically confirmed positive, we observed a reduction of twofold for C/T, sixfold for ETP, and eightfold for MEM (Table 2).

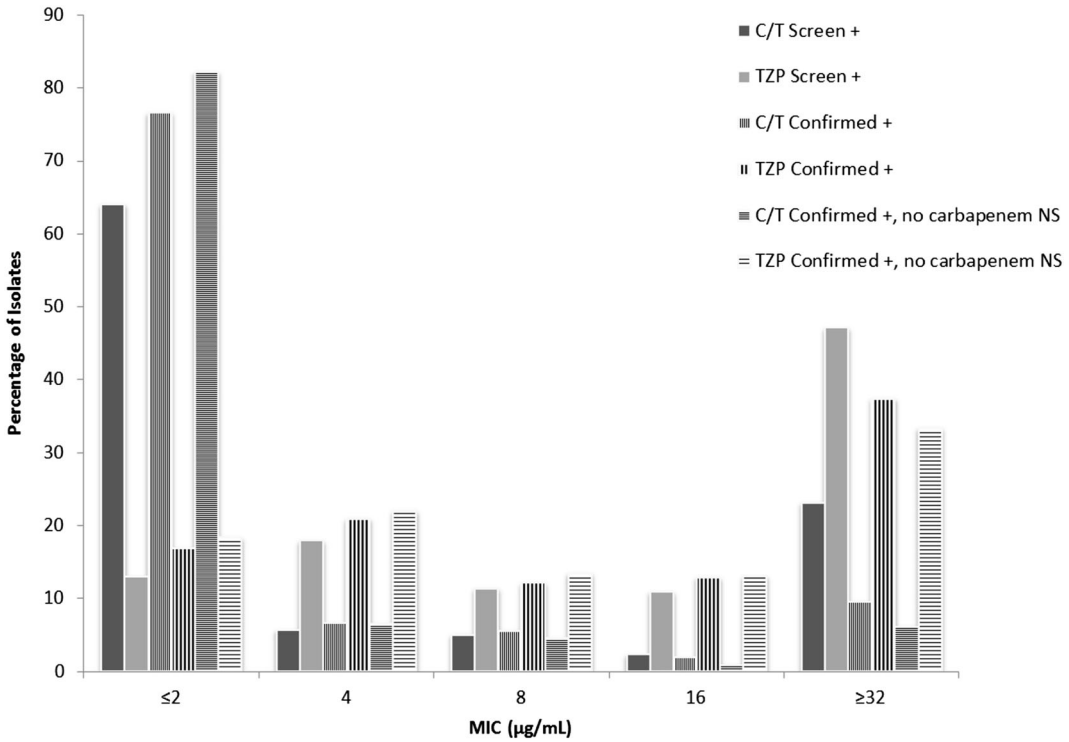

Fig. 1 The MIC distribution of C/T and TZP for the MIC-based screen-positive isolates (Screen +), phenotypically confirmed positive (Confirmed +) and Confirmed + with carbapenem NS isolates removed (Confirmed +, no carbapenem NS) of all Enterobacteriaceae. *C/T* ceftolozane/tazobactam, *MIC* minimum inhibitory concentration, *NS* non-susceptible, *TZP* piperacillin/tazobactam

Table 2 MIC_{90} (μg/mL) of all agents against MIC-based screen-positive (Screen +) and phenotypically confirmed (Confirmed +) *Escherichia coli* and *Klebsiella pneumoniae* isolates

	MIC_{90} (μg/mL) for antimicrobials									
	C/T	FEP	CRO	CAZ	CIP	ATM	ETP	TZP	MEM	TOB
All										
Screen + ($n = 442$)	128	128	128	128	32	128	32	512	32	64
Confirmed + ($n = 274$)	32	128	128	128	32	128	0.5	512	0.125	64
Confirmed +, carbapenem NS removed ($n = 246$)	2	128	128	128	32	64	0.125	64	0.064	64

ATM aztreonam, *CAZ* ceftazidime, *CIP* ciprofloxacin, *CRO* ceftriaxone, *C/T* ceftolozane/tazobactam, *ETP* ertapenem, ESBL extended spectrum β-lactamase, *FEP* cefepime, *MEM* meropenem, *MIC* minimum inhibitory concentration, *NS* non-susceptible, *TOB* tobramycin, *TZP* piperacillin/tazobactam

CONCLUSION

After confirmatory ESBL testing using the clavulanate test, 38% of strains identified as potential ESBL producers using a simple MIC screen were excluded. For agents with potential activity against ESBLs such as C/T, TZP, ETP, and MEM, reduced susceptibility was noted when only considering MIC screen positivity. The reason for this discordance is that carbapenemase producers, AmpC, and other types of resistance would not be differentiated by MIC-based screen testing alone. As such, although MIC screening selects for resistant organisms, inclusion of these other genotypes may falsely under-predict the potency of several antibiotics against ESBL producers and if such a method is used during published surveillance studies, it may limit applicability of these data to geographic areas not plagued with carbapenemase producers. For this reason, in scenarios, such as the clinical laboratory, when molecular testing is not available, clavulanate-based phenotypic confirmatory testing is recommended to better evaluate empiric therapy options against ESBL.

ACKNOWLEDGMENTS

This study was supported by a grant from Cubist Pharmaceuticals, Lexington, MA, USA. The article processing charges for this publication were funded by The Center for Anti-Infective Research and Development at Hartford Hospital. The sponsor of the study was not involved in the design, collection, analysis or interpretation of the data or in decision to submit the manuscript for publication. We would like to thank Kimelyn Greenwood, Jennifer Hull, Lucinda Lamb, Sara Robinson, and Debra Santini for their collective efforts with MIC determination. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this manuscript, take responsibility for the integrity of the work as a whole, and have given final approval to the version to be published.

Disclosures. David P. Nicolau is on the speaker bureau for Cubist and has received grants from Cubist. Christina A. Sutherland and Jared L. Crandon have nothing to disclose. Jared Crandon was employed at Hartford Hospital during the study period.

Compliance with ethical guidelines. This surveillance program was reviewed by the institutional review board (IRB) at the coordinating center, Hartford Hospital. Since all samples were collected as part of routine medical care and no interventions were undertaken as a result of this testing, informed consent was deemed unnecessary by the IRB.

REFERENCES

1. Bush K, Jacoby GA. Updated functional classification of beta-lactamases. Antimicrob Agents Chemother. 2010;54(3):969–76.

2. Thabit AK, Crandon JL, Nicolau DP. Antimicrobial resistance: impact on clinical and economic outcomes and the need for new antimicrobials. Expert Opin Pharmacother. 2015;16(2):159–77.

3. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement. CLSI document M100-S24 U. Wayne: Clinical and Laboratory Standards Institute; 2014.

4. European Committee on Antimicrobial Susceptibility Testing. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 1; 2013.

5. Dudley MN, Ambrose PG, Bhavnani SM, Craig WA, Ferraro MJ, Jones RN, Antimicrobial Susceptibility Testing Subcommittee of the Clinical and Laboratory Standards Institute. Background and rationale for revised clinical and laboratory standards institute interpretive criteria (Breakpoints) for Enterobacteriaceae and *Pseudomonas aeruginosa*: I. Cephalosporins and Aztreonam. Clin Infect Dis. 2013;56(9):1301–9.

6. Thomas Kenneth. Extended-spectrum-β-lactamase, AmpC, and carbapenemase issues. J Clin Microbiol. 2010;48(4):1019–25.

7. Cubist Pharmaceuticals. Zebraxa-US prescribing information. Lexington: Cubist Pharmaceuticals; 2015.

8. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. CLSI document M07-A9. Wayne: Clinical and Laboratory Standards Institute; 2012.