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Piperacillin/tazobactam MIC and Disk Correlation Study for ESBL and Non-ESBL Producing Organisms Using NCCLS Published Guidelines versus Proposed Breakpoints

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Abstract

Background The NCCLS is considering the possibility of lowering the MIC breakpoints for selected antimicrobials against enterobacteriaceae to compensate for the increasing prevalence of extended spectrum beta-lactamase (ESBL) producing strains in this group of organisms. The first antimicrobial group to be examined will be the beta-lactam/beta-lactamase inhibitor compounds. This study investigates the effects that a one or two log_2 dilution change would have upon in vitro susceptibilities and the resultant Very Major Errors (VME), Major Errors (ME) for piperacillin/tazobactam. **Methods** This study analyzed 632 clinical strains (44% ESBL, 56% non-ESBL producers) of Enterobacteriaceae consisting of Escherichia coli, Klebsiella pneumoniae and Enterobacter species. All isolates were tested against piperacillin/tazobactam by broth microdilution and disk diffusion methodologies according to NCCLS guidelines. **Results** A decrease from current MIC breakpoints of one and two log2 dilutions resulted in increases of VME from 0% to 2.8% and 6.6%, respectively, for all strains. The percent susceptible rates of piperacillin/tazobactam against all strains of Enterobacteriaceae at current/-1 dilution/-2 dilution breakpoints were (%) 60.6 / 56.3 / 47.2. The percent resistant rates of piperacillin/tazobactam against all strains of Enterobacteriaceae at current/-1 dilution/-2 dilution breakpoints were (%) 25.3 / 31.6 / 39.4. The one and two log2 dilutional changes affect E. coli > K. pneumoniae > Enterobacter spp. **Conclusion** This study demonstrates significant, undesirable changes in VME, susceptibility and resistant rates for piperacillin/tazobactam against all Enterobacteriaceae, both ESBL positive and ESBL negative, strains when MIC breakpoints are lowered either one or two log_2 dilutions.

Introduction

Piperacillin-tazobactam is a broad-spectrum beta-lactam/beta-lactamase inhibitor compound first approved by the FDA in October 1993. The current breakpoints for piperacillin-tazobactam against Enterobacteriaceae were adopted by the National Committee for Clinical Laboratory Standards (NCCLS) in 1994 defined as (in terms of the piperacillin component in mcg/mL) susceptible ≤ 16, intermediate 32 – 64, and resistant ≥ 128. The NCCLS committee on antimicrobials is currently evaluating the breakpoints of this drug and other selected classes of antimicrobials that may result in a potential change of current values for some "bug-drug combinations." In particular, carbapenems, beta-lactams and beta-lactamase inhibitor combinations are the committee's immediate focus.

Clinicians currently use piperacillin-tazobactam for the treatment of many infections caused by pathogens in the Enterobacteriaceae group. Decision by the NCCLS to change these breakpoints may significantly impact the use of this compound by clinicians. Any changes made to piperacillin-tazobactam breakpoints should only be made with sufficient data to support those changes. This study is designed to determine the impact that the proposed changes may have on the calculation of Very Major Errors (VME) and percent susceptible determinants of piperacillin-tazobactam against selected extended spectrum beta-lactamase producing and non-beta-lactamase producing Enterobacteriaceae.

Materials and Methods

- All 632 strains were obtained prospectively from clinically documented nosocomial infections isolated from blood, respiratory tract, urine, skin, wound and body fluids.
- Study enrolled 32 individual collection sites in 18 countries: Africa: Egypt, South Africa. Europe: Austria, Belgium, Croatia, France, Germany, Greece, Italy, Portugal, Slovenia, Spain, Switzerland, The Netherlands, Turkey. Middle East: Lebanon, Saudi Arabia, Turkey.
- Only one isolate per patient was allowed.
- Pathogens in this study included four species: *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Enterobacter cloacae* (listed collectively as *Enterobacter* spp).
- Processing, handling and testing was performed by a single reference laboratory, Laboratories International for Microbiology Studies (LIMS, Schaumburg, IL, USA).

Antimicrobial Susceptibility Testing

- Antimicrobial agent tested: piperacillin-tazobactam microdilution PML panels (PML Microbiologicals, Inc., Wilsonville, OR) with concentrations of 0.12/4 - 128/4 mcg/mL. Piperacillin-tazobactam disks: 100/10 mcg (Becton Dickinson. Franklin Lakes, NJ).
- All testing was conducted using guidelines published by the National Committee for Clinical Laboratory Standards [1] [2]. MIC and Zone Diameter interpretive criteria followed published guidelines established by the NCCLS [3].
- *Enterobacteriaceae* broth microdilution panels and disk diffusion agar plates were incubated in ambient air at 35°C for 16-20 hrs.
- Quality Control of panels and disks was performed using *Escherichia coli*, ATCC 25922 and *Pseudomonas aeruginosa*, ATCC 27853.

Extended-Spectrum Beta-Lactamase(ESBL) Determinations

- *Escherichia coli* and *Klebsiella pneumoniae* were screened and confirmed for ESBL activity according to NCCLS guidelines [3].
- Preliminary ESBL activity was determined by screening cefotaxime, ceftazidime and ceftriaxone with MICs >1 mcg/mL using broth microdilution panels.
- Confirmation of ESBL production was made if there was an increase of > 5 mm in the inhibition zone of the combination disc (Oxoid, Inc. Ogdensburg, New York) when compared to that of the cephalosporin disc alone: cefotaxime/clavulanic acid – cefotaxime > 5 mm or ceftazidime/clavulanic acid – ceftazidime > 5 mm.
- ESBL activity in the *Enterobacter* species was confirmed using isoelectric focusing (IEF), PCR (PCR Master Kit (Roche, Basel, Switzerland) and DNA sequencing (Applied Biosystems automated DNA sequencing system 3700 (Foster City, CA, USA).

Results

The results are included in the following tables and graphs:

Table 1. Study Organisms with number and percentage of ESBL and non-ESBL producers.

Organism/Phenotype	ESBL Negative n (% Total N)	ESBL Positive n (% Total N)	Total N (% of all strains)
Escherichia coli	103 (51.8)	96 (48.2)	199 (31.5)
Klebsiella pneumoniae	124 (44.8)	153 (55.2)	277 (43.8)
Enterobacter spp	129 (82.7)	27 (17.3)	156 (24.7)
Total *Enterobacteriaceae*	356 (56.3)	276 (43.7)	632 (100)

Figure 1. Scattergram Analysis of Piperacillin-tazobactam vs. Enterobacteriaceae at Current NCCLS Breakpoints

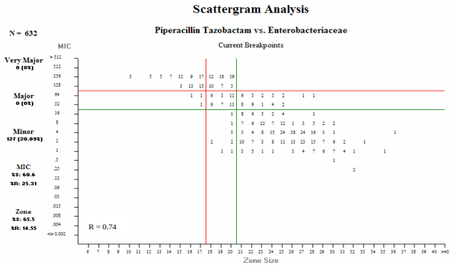

Figure 2. Scattergram Analysis of Piperacillin-tazobactam vs. Enterobacteriaceae at One Log_2 Dilution Below Current NCCLS Breakpoints

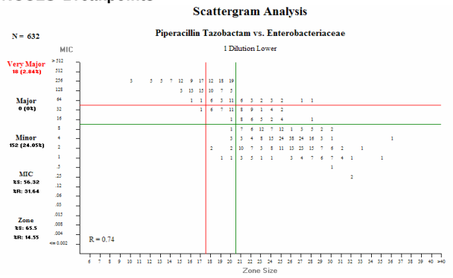

Figure 3. Scattergram Analysis of Piperacillin-tazobactam vs. Enterobacteriaceae at Two Log_2 Dilutions Below Current NCCLS Breakpoints

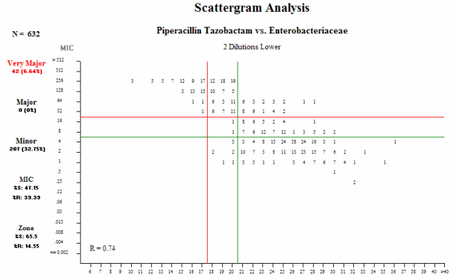

Table 2. Changes in Very Major Errors resulting from a 1 or 2 log_2 decrease in MIC breakpoints compared to current NCCLS breakpoints for piperacillin-tazobactam

Organism/Phenotype	No. of Strains	Very Major Errors n (%) Current BPs	- 1 log_2 Dilution	- 2 log_2 Dilution
Enterobacteriaceae, All	632	0 (0)	18 (2.8)	42 (6.6)
Enterobacteriaceae, ESBL_Pos	276	0 (0)	12 (4.4)	28 (10.1)
Enterobacteriaceae, ESBL_Neg	356	0 (0)	6 (1.7)	14 (3.4)
E. coli, All	199	0 (0)	7 (3.5)	10 (9.0)
E. coli, ESBL_Pos	96	0 (0)	4 (4.5)	14 (14.6)
E. coli, ESBL_Neg	103	0 (0)	3 (2.9)	4 (8.9)
K. pneumoniae, All	277	0 (0)	9 (3.2)	18 (6.5)
K. pneumoniae, ESBL_Pos	153	0 (0)	7 (4.6)	13 (8.5)
K. pneumoniae, ESBL_Neg	124	0 (0)	2 (1.6)	5 (4.0)
Enterobacter spp, All	156	0 (0)	2 (1.3)	6 (3.8)
Enterobacter spp, ESBL Pos	27	0 (0)	1 (3.7)	1 (3.7)
Enterobacter spp, ESBL Neg	129	0 (0)	1 (0.8)	5 (3.9)

Red denotes any value above the acceptable NCCLS value of 1.5% for VME's.

Table 3. Changes in Percents Susceptible and Resistant resulting from a 1 or 2 log_2 decrease in MIC breakpoints compared to current NCCLS breakpoints for piperacillin-tazobactam

Organism/Phenotype	No. of Strains	% Sus by Disk ** Current BPs	% Susceptible by MIC (p Value)* Current BPs	- 1 Dilution	- 2 Dilution	% Resistant by MIC (p Value) Current BPs	- 1 Dilution	- 2 Dilution
Enterobacteriaceae, All	632	65.5	60.6	56.3 (<0.001)	47.2 (<0.001)	25.3	31.6 (<0.001)	39.4 (<0.001)
Enterobacteriaceae, ESBL_Pos	276	48.9	40.2	36.2 (0.01)	27.5 (<0.001)	43.8	51.4 (<0.001)	59.8 (<0.001)
Enterobacteriaceae, ESBL_Neg	356	78.4	76.4	71.9 (0.005)	62.4 (<0.001)	1.7	16.3 (<0.001)	23.6 (<0.001)
E. coli, All	199	89.9	80.9	77.4 (0.07)	68.3 (<0.001)	6.5	12.1 (0.005)	19.1 (<0.001)
E. coli, ESBL_Pos	96	82.3	67.7	61.5 (0.07)	47.9 (<0.001)	11.5	18.8 (0.03)	32.3 (<0.001)
E. coli, ESBL_Neg	103	97.1	93.2	92.2 (0.5)	89.3 (0.1)	1.9	5.8 (0.18)	6.8 (0.09)
K. pneumoniae, All	277	59.2	53.4	51.6 (0.16)	43.3 (<0.001)	37.9	43 (0.01)	46.6 (0.002)
K. pneumoniae, ESBL_Pos	153	26.8	19.6	18.3 (0.34)	14.4 (0.03)	68.6	75.8 (0.03)	80.4 (0.002)
K. pneumoniae, ESBL_Neg	124	99.2	95.2	92.7 (0.32)	79.0 (<0.001)	0	2.4 (0)	4.8 (1)
Enterobacter spp, All	156	45.5	47.4	37.8 (0.002)	25.6 (<0.001)	26.9	36.5 (0.02)	52.6 (<0.001)
Enterobacter spp, ESBL Pos	27	55.6	59.3	48.1 (0.006)	29.6 (<0.001)	18.5	29.6 (0.24)	40.7 (0.06)
Enterobacter spp, ESBL Neg	129	43.4	45	35.7 (0.19)	24.8 (0.04)	28.7	37.9 (<0.001)	55 (<0.001)

*Current NCCLS breakpoints (mcg/mL) for piperacillin-tazobactam by broth microdilution are: susceptible ≤16; intermediate = 32 to 64; and resistant ≥ 128.
**Current NCCLS breakpoints (mm) for piperacillin-tazobactam by disk diffusion are: resistant ≤ 17; intermediate = 18 to 20; and susceptible ≥ 21.

Conclusions

- A decrease in the MIC breakpoint of piperacillin-tazobactam of one and two log_2 dilutions below the current NCCLS breakpoint resulted in a significant rise in the VME of 2.8% and 6.6%, respectively, for all strains.
- A decrease in the MIC breakpoint of piperacillin-tazobactam of one and two log_2 dilutions below the current NCCLS breakpoint resulted in significant changes in percent susceptibility of all *Enterobacteriaceae* from 60.6% to 56.3% (p <0.001) and 47.2 (p=0.001), respectively.
- A decrease in the MIC breakpoint of piperacillin-tazobactam of one log_2 dilution effects ESBL negative strains (p=0.01) more so than ESBL producing strains (p=0.005).
- This study demonstrates significant, undesirable changes in VME, susceptible and resistant rates for piperacillin-tazobactam against all *Enterobacteriaceae* including both ESBL positive and ESBL negative strains when breakpoints are lowered either one or two log_2 dilutions.
- The lowering of breakpoints for piperacillin-tazobactam against *Enterobacteriaceae* strains in this study is not supported by these data.

References

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3. NCCLS, *Performance Standards for Antimicrobial Susceptibility Testing; Thirteenth Informational Supplement*, in *NCCLS document M100-S13*. 2003: NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

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