ADAPTING THE MODIFIED CARBAPENEM INACTIVATION METHOD TO ASSESS FOR POSSIBLE BETA-LACTAMASE MEDIATED RESISTANCE IN PIPERACILLIN-TAZOBACTAM RESISTANT/ CEFTRIAXONE SUSCEPTIBLE *ESCHERICHIA COLI*

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Background

Piperacillin-Tazobactam (PTZ) is a commonly prescribed empiric antibiotic for patients with sepsis in many centres. Recently, there was an increase in the isolation of ceftriaxone (CTX) susceptible, Piperacillin-Tazobactam resistant *Enterobacterales* from blood at our centre (Figure 1). The resistance mechanism behind this phenotype was not clear.

Objectives

- To identify the potential for a beta-lactamase to confer the ceftriaxone susceptible, piperacillin-tazobactam resistant phenotype
- To determine the potential for variations on the modified carbapenem inactivation method to be applied to probe for other beta-lactamase production

Methods

We identified all *Enterobacterales* isolates from blood cultures at our institution between 2017 and 2019 that had the CTX-S, PTZ-R phenotype.
The resistance phenotype was confirmed using routine automated antimicrobial susceptibility testing with the Vitek2. AST system and N208 cards.

In order to assess for a beta-lactamase mediated resistance, we adapted the modified carbapenem inactivation method.

- The isolates were grown overnight on blood agar plates and then 1 µL loopfuls were inoculated into Tryptic Soy Broth broth
- Ceftriaxone (30mcg, Oxoid Canada) or piperacillin-tazobactam (100/10mcg, Oxoid Canada) discs were then added to the inoculum and allowed to incubate at 35ºC for 4 hours
- The discs were then placed onto Mueller Hinton agars that had been inoculated with a pan susceptible E. coli strain (ATCC 25922) and allowed to grow overnight in ambient air at 35C.
- The zones of growth around the discs were then measured.
- As controls, the discs were also incubated in TSB alone, or in broths inoculated with susceptible *E. coli*, KPC producing *K.pmeumoniae* (ATCC 1705) , or ampicillin resistant *Enterococcus faecium* (ATCC 51559).

Results

We identified a notable increase in the rates of PTZ-R/CTX-S Enterobacterales being isolated from blood in the first half of 2019 as shown in Figure 1 below.

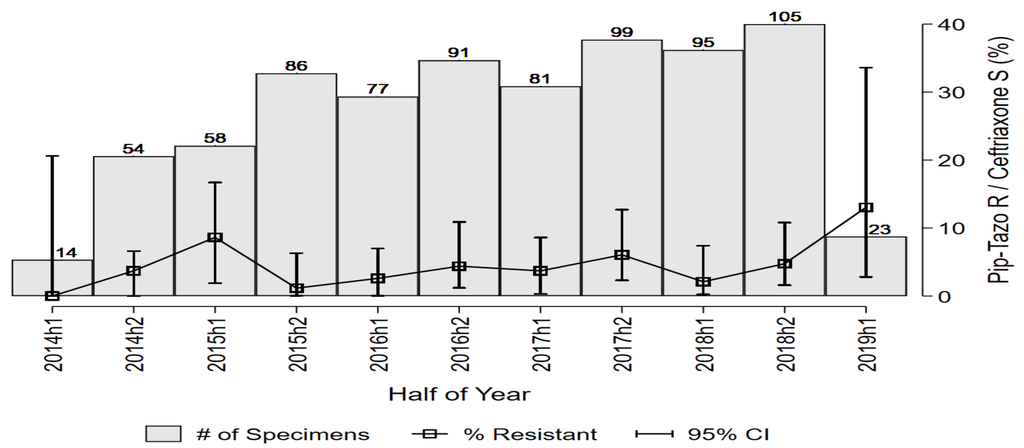

Figure 1. Frequency of isolation of PTZ R/CRTX S E. coli from 2014-2019

The adapted mCIM test results are shown in Table 1. 7/9 PTZ-R, CTX-S isolates rendered the PTZ disc inert while sparing the ceftriaxone disc. The other 2/7 reduced the zone size. ESBL isolates rendered the CTX disc inert while sparing the PTZ disc. KPC producing *K. pneumoniae* rendered both discs inert while the ampicillin resistant *E. faecium* didn't affect either disc.

			MIC according to Vitek2 N208 AST (mg/L; Interpretation*)			Zone size (mm) of adapted mCIM	
Isolate ID	Species	Phenotype	Pip-Tazo	Ceftriaxone	Cefazolin**	Pip-Tazo	Ceftriaxone
1	E. coli	PTZ R/ CTX S	64; I	≤1; S	≤4; S/I	6	27
2	E. coli	PTZ R/ CTX S	64; I	≤1; S	≤4; S/I	6	24
3	E. coli	PTZ R/ CTX S	64; I	≤1; S	≤4; S/I	6	28
4	E. coli	PTZ R/ CTX S	64; I	≤1; S	≤4; S/I	6	27
5	E. coli	PTZ R/ CTX S	64; I	≤1; S	≤4; S/I	6	28
6	E. coli	PTZ R/ CTX S	≥128; R	≤1; S	16; R	10	28
7	E. coli	PTZ R/ CTX S	≥128; R	≤1; S	32; R	13	30
8	E. coli	PTZ R/ CTX S	≥128; R	≤1; S	≤4; S/I	6	27
9	E. coli	PTZ R/ CTX S	≥128; R	≤1; S	≤4; S/I	6	26
10	E. coli	ESBL	≤4; S	≥64; R	≥8; R	18	6
11	E. coli	ESBL	≤4; S	≥64; R	>8; R	14	6
12	E. coli	ESBL	≤4; S	≥64; R	>8; R	14	22
ATCC 25922	E. coli	Pan S	≤4; S	≤1; S	≤4; S/I	16	27
ATCC 1705	K. pneumoniae	CPE	≥128; R	≥64; R	≥8; R	6	6
ATCC 51559	E. faecium	Ampicillin R	≥128; R	***	***	16	26
TSB alone						18	26

*Interpretation based on 2018 CLSI M100S.
***Intrinsic resistance.

Table 1. Results of the adapted mCIM assay

Discussion

We postulate that piperacillin-tazobactam resistance for these isolates is mediated by a diffusible beta-lactamase because the assay behaves similarly to carbapenemase producing *Enterobacteriaceae* in the mCIM. While the mCIM is well validated for the detection of enzymatic destruction of carbapenems, our results suggest that the theory behind the assay can also be applied to detect a range of beta-lactamase enzymes even when the genetics remain unknown. We noted that the assay correctly identified the ESBL phenotype, as well as the KPC producing control. It also showed that the piperacillin-tazobactam resistance seen in the *E. faecium* isolate was not mediated by a beta-lactamase.

A number of enzymes exist that could explain this phenotype, notably hyperproduction of TEM-1 or inhibitor resistant TEM-1. Other possibilities include certain members of the OXA family. As this phenotype increases in prevalence, the implications for clinical reliance upon piperacillin-tazobactam will need careful consideration.

Limitations

- This study was performed on a limited number of clinical isolates.
- The assay can only determine the mechanism of resistance (enzymatic destruction) and is not likely to be specific to a type of beta-lactamase. Further genetic work is required to determine the identity of the genes responsible.

Conclusion

The mCIM can be adapted to quickly ascertain if a novel resistance pattern is due to beta-lactamase production. The emerging phenotype, Piperacillin-tazobactam resistance with ceftriaxone susceptibility being seen in *Enterobacterales,* is due to a beta-lactamase.

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