

Galactomannan Detection from Piperacillin-Tazobactam Brands Available in the Brazilian Market

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Piperacillin-tazobactam is a broad spectrum antimicrobial agent that can cause false-positive results in the commercial Platelia *Aspergillus* EIA test. So far, no study has been performed in Latin America to evaluate the clinical implication of this finding. Here we studied the potential for galactomannan detection in piperacillin-tazobactam batches commercialized in the Brazilian market. Five batches from distinct laboratories were tested in duplicate in the Platelia *Aspergillus* EIA according to the manufacturer's instructions. Only one drug showed cross-reaction at a cut-off of 0.5. Human serum was spiked with this particular drug aiming to mimic achievable piperacillin-tazobactam concentrations in the serum. Results were all negative for galactomannan detection, even at high drug concentrations. Results from this pilot study suggest that piperacillin-tazobactam might not be a clinically significant cause of false-positive results in the Platelia *Aspergillus* EIA test in Brazil.

Key-Words: Piperacillin-tazobactam, aspergillosis, Platelia *Aspergillus*, galactomannan.

Galactomannan (GM) detection has gained importance in the diagnosis of invasive aspergillosis (IA) [1,2]. Several monoclonal antibodies have been developed to detect *Aspergillus* GM molecules, including EB-A2, the actual basis for a sandwich enzyme immunoassay commercial kit (Platelia™ *Aspergillus* EIA – Bio-Rad, USA). Although this test has been available in Europe for roughly a decade, it was not before 2003 that the assay was cleared by the Food and Drug Administration (FDA) for use in the United States [3]. In Brazil, approval was obtained by the national regulatory agency (ANVISA, *Associação Nacional de Vigilância Sanitária*) in late 2007 [4]. Therefore, experience with GM testing in South America is still quite limited.

The ability of GM testing to contribute to the diagnosis of IA is complicated by several factors. In addition to varied sensitivity [2], test performance is hampered by the occurrence of false-positive results, particularly occurring in patients taking antibiotics of fungal origin [5,6]. In many cases, a positive GM result can be obtained by testing directly the antibiotic batch. Piperacillin-tazobactam and amoxicillin-clavulanate are usually implicated as the main contributors to false-positive reactions [6-8]. Since IA mostly affects patients with prolonged neutropenia (and piperacillin-tazobactam is one the first-line options for empirical treatment of febrile neutropenia) cross-reactions might be of clinical significance. However, there is a controversy about the frequency and importance of piperacillin-tazobactam contamination with GM [9-12], with some authors arguing that we are now in use of a more pure drug [13]. Since no such a data is available for

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South America, here we investigate the relevance of piperacillin-tazobactam contamination with GM by testing piperacillin-tazobactam batches available in the Brazilian market.

Material and Methods

A list of companies manufacturing piperacillin-tazobactam in Brazil was initially obtained at the ANVISA website. We were able to get a vial from 5 out of the 7 branches available in the Brazilian market. These were named drugs A, B, C, D, and E.

Lyophilized drugs were diluted as for clinical use in sterile NaCl 0.9% to a final concentration of 45 mg/mL. Platelia *Aspergillus* test was performed according to manufacturer's instructions. In summary, 300 µL of the reconstituted drug was pre-treated as for serum samples. GM testing was performed in duplicate and in two laboratories by distinct scientists who were blinded to the other laboratory results. Sodium chloride was used as a negative control, in addition to the negative control provided in the Platelia *Aspergillus* kit. GM results were expressed as optical densities (OD) – samples were considered positive when the ratio between the OD observed for the sample and the mean cut-off OD was ≥ 0.5 .

In order to evaluate the potential for false-positive GM results in clinical samples, human serum was spiked with piperacillin-tazobactam using drug samples that showed positive results in the GM testing. Serum used for this experiment was known to be negative for GM (OD of 0.28). Different concentrations of piperacillin-tazobactam were tested, mimicking achievable peak and trough levels after a standard 4.5 g dose: 600 µg/mL, 300 µg/mL, 150 µg/mL and 75 µg/mL [10]. In this experiment, 300 µL of serum was also used as a negative control.

Results

Results of this study are summarized in Table 1. Apart from drug B, all other drugs tested negative in GM assay. Results were consistent even when tested by different

Table 1. Optical density indexes of galactomannan as determined by the commercial *Platelia Aspergillus* EIA in five brands of piperacillin-tazobactam commercialized in Brazil.

Piperacillin-tazobactam		Laboratory 1			Laboratory 2			Final results
Drug	Batch	Test 1	Test 2	Mean	Test 1	Test 2	Mean	Mean
A	133881E	0.50	0.31	0.40	0.22	0.18	0.20	0.30
B	006373	0.88	0.86	0.87	0.74	0.74	0.74	0.80
C	C09341	0.26	0.19	0.22	0.25	0.22	0.23	0.23
D	7100839	0.37	0.40	0.38	0.36	0.30	0.33	0.35
E	0760133	0.20	0.26	0.23	0.16	0.20	0.18	0.20

laboratories. Drug B was positive to GM detection in all tested wells, with GM OD indexes ranging from 0.74 to 0.88 (mean values of 0.80). Sodium chloride used to dilute drugs tested negative.

Spiking human serum with different concentrations of drug B resulted in negative GM results (OD of <0.5). Mean GM OD indexes for 600 µg/mL, 300 µg/mL, 150 µg/mL and 75 µg/mL of piperacillin-tazobactam were 0.38, 0.37, 0.37 and 0.21, respectively.

Discussion

To the best of our knowledge this is the first study evaluating cross-reaction in the *Platelia Aspergillus* EIA test using piperacillin-tazobactam batches commercialized in Brazil. Positive results in the GM test occurred for only one out of the 5 brands tested.

Several studies conducted in other countries have already evaluated the amount of GM contamination in piperacillin-tazobactam vials and the clinical implications for these findings. Results were somehow contradictory, suggesting that variation may occur within brands and even within different batches [6,9,10,12-17]. For instance, Singh et al. [10] tested 3 different batches of piperacillin-tazobactam from a single manufacturer in Pittsburgh and found very high positive indexes in all samples evaluated (OD of >5.2). Similarly, Aubry et al. [6] detected positive GM indexes in 7 out of 10 batches of piperacillin-tazobactam in France. Median GM index in their study was 2.5, ranging from 0.7-7.8. Also in France, Bart-Debasse et al. [5] described positivity in all piperacillin-tazobactam batches tested (n=10), with OD index ranging from 0.9-4.3. Similarly, an Italian study [9] found positive GM index in 26/30 (86%) piperacillin-tazobactam batches evaluated. Some other authors however have questioned these findings, stating that false-positive results are of limited clinical relevance [12, 15]. In addition, one study tested several piperacillin-tazobactam batches and found that their GM content was reduced over the time of study. The authors speculated about the possibility of a modification in the manufacturing process to have occurred over time, resulting in more purity and less GM contamination [13].

A study on the kinetics of GM decrease after cessation of β-lactam antibiotics showed that median time for a

negative *Platelia Aspergillus* EIA test result was 5.5 days. Quite impressively, results may remain falsely positive for up to 30 days [6]. Due to the possibility of cross-reaction with piperacillin-tazobactam in the *Platelia Aspergillus* EIA test some authors have suggested that testing of suspected antibiotic batches remains the only indicator of possible false EIA positivity [5]. Others have hypothesized that EIA false-positivity might be reduced by sampling serum at trough piperacillin-tazobactam levels or prior to the administration of the next dose [10,11,13]. In order to minimize false-positive results in the *Platelia Aspergillus* GM test in patients on piperacillin-tazobactam, separate tubes of the central venous line should be used for blood sampling and for administration of β-lactam antibiotics [13].

'Drug B' was the only piperacillin-tazobactam that resulted in a positive reaction in the *Platelia Aspergillus* EIA in this study. Conversely, at achievable concentrations of piperacillin-tazobactam in serum we did not observe any cross-reaction with this antibiotic. Drug concentrations ranging from 75-600 µg/mL resulted all in GM OD of <0.5, which was similar to negative controls.

Some limitations of this study deserve to be mentioned. First of all, only a single batch of each brand of piperacillin-tazobactam was tested – as already stated, marked variation in GM contamination might occur amongst batches. Secondly, it has been already shown that the kinetics of GM may vary according to the duration of antimicrobial treatment, with GM accumulating after time [5, 8]. Also, the amount of GM in the antibiotic batches does not always correlate with GM levels *in vivo* [13].

In conclusion, this pilot study suggests that treatment with piperacillin-tazobactam might not be an important cause of false-positive results in the *Platelia* EIA test in Brazil. More data is clearly needed on this field. Ideally, the amount of GM should be checked in each hospital, using different batches of piperacillin-tazobactam.

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