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Reactivity of Platelia *Aspergillus* Galactomannan Antigen with Piperacillin-Tazobactam: Clinical Implications Based on Achievable Concentrations in Serum

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The possible reactivities of commonly used antibiotics of fungal, nonfungal, and nonmicrobial or synthetic sources with the Platelia *Aspergillus* galactomannan assay were assessed. For drugs that tested positive, the minimal concentration of the antibiotic in serum that yielded a positive test (index, >0.5) was determined. At undiluted concentrations, piperacillin and multiple lots of piperacillin-tazobactam tested positive, whereas amoxicillin, ampicillin-sulbactam, nafcillin, cefazolin, ceftazidime, erythromycin, gentamicin, and levofloxacin tested negative. All three lots of piperacillin-tazobactam and all bags within each lot tested positive, with a mean index value of 5.168. At achievable concentrations in serum, however, only one of three lots of piperacillin-tazobactam yielded a positive test. Concentrations of 75, 150, and 300 μg/ml of serum tested positive with the Platelia *Aspergillus* enzyme immunoassay, whereas lower concentrations, mimicking the trough levels, tested negative. Thus, while achievable serum piperacillin-tazobactam concentrations may potentially result in a positive test for galactomannan, the timing of the collection of serum samples from patients may influence the test results, with reactivity being less likely in samples collected at trough levels or prior to the administration of a dose of the antibiotic.

Galactomannan is a polysaccharide component of the cell wall of *Aspergillus* spp. that is released into the circulation in varying amounts during invasive aspergillosis (3, 7, 15). Galactomannan detection by the Platelia *Aspergillus* enzyme immunoassay (EIA) has proven to be a potentially promising tool for the early diagnosis of invasive aspergillosis. False-positive test results, however, have been reported for ~6 to 8% of neutropenic and hematopoietic stem cell transplant recipients, for 13% of liver transplant recipients, and for 20% of lung transplant recipients (4, 5, 9, 10, 12). Cytotoxic chemotherapeutic agents, autoreactive antibodies or paraproteins, or yet-unidentified serum components may account for the false-positive tests. The high rate of false EIA reactivity in neonates may result from cross-reactivity with the lipoteichoic acid of *Bifidobacterium bifidum* in the gut (P. E. Verweij, R. R. Klont, A. Warris, H. J. M. Op Den Camp, and M. A. S. Menninkk-Kersten, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1027, 2003). Reactivity with the galactomannan of *Paecilomyces* and *Penicillium* spp. has been noted previously (13).

In 1997, Ansorg et al. first reported that drugs of fungal origin, such as antibiotics and uricase, might be associated with false-positive test results (1a). Galactomannan was detected in a batch of ampicillin-sulbactam and in two batches of piperacillin (1a). Of our liver transplant recipients with false-positive test results, 55% had received these antibiotics (5). Recent reports from Europe have documented false-positive tests related to the use of piperacillin-tazobactam in patients with hematologic malignancy or those who had undergone bone marrow transplantation (1, 11, 16).

The goals of this study were to systematically assess whether commonly used antibiotics (of fungal, nonfungal, and nonmicrobial sources) would test positive in the Platelia *Aspergillus* EIA. For the drugs that tested positive as undiluted samples, we sought to determine whether achievable concentrations of these antibiotics in serum, based on a normal dosing regimen, could potentially result in reactivity with the galactomannan assay.

MATERIALS AND METHODS

A total of 10 antibiotics were tested for galactomannan by using the Platelia *Aspergillus* EIA, Bio-Rad Laboratories, Redmond, Wash. These included intravenous formulations of piperacillin, ampicillin-sulbactam, nafcillin, piperacillin-tazobactam, cefazolin, ceftazidime, gentamicin, erythromycin, and levofloxacin, in 0.9% saline or 5% dextrose (Table 1). Three separate lots of piperacillin-tazobactam (one bag from lot A, three bags from lot B, and five bags from lot C) were tested. Since amoxicillin powder is insoluble in water, it was tested as a solution reconstituted in a phosphate buffer (pH 6.0) to yield the same concentration of the drug that is present in oral suspension (50 mg/ml).

All drugs were initially tested at full strength or undiluted for the galactomannan assay. Drug diluents (0.9% saline, 5% dextrose, or a phosphate buffer [pH 6.0]) were used as controls, and all tests were conducted in duplicate wells. Briefly, 50 μl of the undiluted antibiotic sample was added to the wells containing 50 μl of the conjugate, and the plates were incubated at 37°C for 90 min. The plates were then washed with an automated washer, and 200 μl of the chromogen substrate solution was added. After incubation at 18 to 25°C for 30 min in the dark, stop solution was added. The plates were read at a wavelength of 450 nm by using a reference filter of 630 nm. The index for each sample was calculated by dividing its optical density (OD) by the cutoff value (mean OD) of the threshold control. Indices of >0.5 were considered positive per the cutoff values for serum samples noted in the manufacturer's package insert.

Drugs that tested positive for galactomannan as undiluted samples were further tested at achievable concentrations in serum. We also sought to determine the minimal concentration of the antibiotic in serum that yielded a positive test. For this experiment, the drug was diluted in serum which had been pretested and

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TABLE 1. Antibiotics tested with the galactomannan assay

Antibiotic (manufacturer)	Lot no.	Diluent
Amoxicillin powder (Sigma)	112K0481	Phosphate buffer, pH 6.0
Nafcillin (Apothecon)	PS137083	5% dextrose
Piperacillin (Lederle)	800468	5% dextrose
Ampicillin-sulbactam (Pfizer)	PS138115	0.9% saline
Piperacillin-tazobactam (Wyeth), lot:		
A	LNO39263	5% dextrose
B1	LNO36947	5% dextrose
B2	LNO36947	5% dextrose
B3	LNO36947	5% dextrose
C1	LNO37143	5% dextrose
C2	LNO37143	5% dextrose
C3	LNO37143	5% dextrose
C4	LNO37143	5% dextrose
C5	LNO37143	5% dextrose
Cefazolin (SmithKline Beecham)	LDO86751	5% dextrose
Ceftazidime (Glaxo Wellcome)	LDO86728	5% dextrose
Gentamicin (American Pharmaceutical Partners)	PS137083	5% dextrose
Erythromycin (Abbott)	PS138305	0.9% saline
Levofloxacin (Ortho-McNeil)	07-003-JT	5% dextrose

shown to be negative for galactomannan (index of <0.20). Antibiotic dilutions tested were based on the achievable peak concentration in serum as the target level for each drug, with 1 twofold dilution above and 2 serial dilutions below the target level. The serum sample (300 μl) was pretreated to remove immune complexes and proteins per the package insert instructions, boiled for 3 min, and centrifuged at 10,000 × *g* for 10 min. Fifty microliters of the supernatant was then added to the duplicate wells, and the experiment for each dilution was completed by following the steps outlined for assaying the undiluted samples. Sterile water and pretested negative serum were used as controls.

RESULTS

When tested full strength or undiluted, piperacillin-tazobactam and piperacillin tested positive in the galactomannan assay, whereas amoxicillin, nafcillin, ampicillin-sulbactam, cefazolin, ceftazidime, gentamicin, erythromycin, and levofloxacin tested negative. The index (the mean of two values when the drug was tested in duplicate wells) for piperacillin was 3.681 (Table 2). All lots of piperacillin-tazobactam and all bags within each lot yielded a positive result, with index values calculated at >5.168 (Table 2). The OD of the piperacillin-tazobactam wells was out of the absorbance range of the plate reader; i.e., it was >3.000. The index was therefore calculated using an OD value of >3.000.

Since piperacillin and piperacillin-tazobactam tested positive as undiluted samples, dilutions of these antibiotics above and below the achievable peak concentrations in serum were tested for galactomannan assay positivity (Table 3). Given that the peak concentration of piperacillin in the serum following an intravenous dose of 4.5 g of piperacillin-tazobactam or 4 g of piperacillin is 298 μg/ml (or ~300 μg/ml), the dilutions tested were 600 μg/ml (1 twofold dilution higher than the peak serum drug concentration), 300 μg/ml (achievable peak serum drug concentration), 150 μg/ml (1 twofold dilution lower than the peak serum drug concentration), and 75 μg/ml (2 twofold dilutions lower than the peak serum drug concentration). Piperacillin yielded a negative result at all concentrations tested, i.e., 75, 150, 300, and 600 μg/ml. For piperacillin-tazobactam, one bag from each lot was tested. Piperacillin-tazobactam from lot A tested negative at concentrations of 75, 150, 300, and 600 μg/ml (Table 3). Piperacillin-tazobactam from lot B tested negative at concentrations of 75, 150, and 300 μg/ml but positive at 600 μg/ml (Table 3).

At the achievable peak concentrations in serum (300 μg/ml) and at 1 dilution higher (600 μg/ml), piperacillin-tazobactam from lot C yielded a positive test (Table 3). The index values for duplicate wells at a concentration of 300 μg/ml were 1.45 and 1.42, and at 600 μg/ml, they were 2.50 and 2.36. The test

TABLE 2. Galactomannan assay reactivity with antibiotics tested at full strength or undiluted

Sample	Index[a]	Interpretation of the test
Negative control	0.167	NA[b]
Threshold control	NA	NA
Positive control	3.228	NA
0.9% saline	0.081	Negative
	0.086	Negative
5% dextrose	0.067	Negative
	0.071	Negative
Amoxicillin powder in phosphate buffer	0.125	Negative
	0.110	Negative
Piperacillin	4.901	Positive
	4.912	Positive
Piperacillin-tazobactam, lot:		
A	>5.168[c]	Positive
B1	>5.168	Positive
B2	>5.168	Positive
B3	>5.168	Positive
C1	>5.168	Positive
C2	>5.168	Positive
C3	>5.168	Positive
C4	>5.168	Positive
C5	>5.168	Positive
Nafcillin	0.067	Negative
	0.069	Negative
Ampicillin-sulbactam	0.127	Negative
	0.127	Negative
Cefazolin	0.047	Negative
	0.048	Negative
Ceftazidime	0.048	Negative
	0.047	Negative
Gentamicin	0.062	Negative
	0.062	Negative
Erythromycin	0.072	Negative
	0.067	Negative
Levofloxacin	0.069	Negative
	0.078	Negative

[a] Index values represent the test results for duplicate wells.
[b] NA, not applicable.
[c] The index for piperacillin-tazobactam for each bag in lots A, B, and C was >5.168 for duplicate wells; the value is therefore presented only once per lot. The OD of the piperacillin-tazobactam wells was out of the absorbance range of the plate reader. The index was calculated with an OD value of >3.000.

TABLE 3. Galactomannan assay reactivity with antibiotic dilutions at achievable peak concentrations in serum and at a two-fold dilution above and at 2 dilutions below the peak

Concn (μg/ml) of the antibiotic in serum[b]	Index[a]	Interpretation of the test
Piperacillin		
600	0.241	Negative
	0.241	Negative
300[b]	0.213	Negative
	0.209	Negative
150	0.216	Negative
	0.193	Negative
75	0.216	Negative
	0.135	Negative
Piperacillin-tazobactam, lot A		
600	0.378	Negative
	0.344	Negative
300[b]	0.309	Negative
	0.275	Negative
150	0.177	Negative
	0.218	Negative
75	0.240	Negative
	0.230	Negative
Piperacillin-tazobactam, lot B2		
600	0.686	Positive
	0.698	Positive
300[b]	0.391	Negative
	0.405	Negative
150	0.252	Negative
	0.249	Negative
75	0.210	Negative
	0.208	Negative
Piperacillin-tazobactam, lot C3		
600	2.505	Positive
	2.362	Positive
300[b]	1.450	Positive
	1.423	Positive
150	0.840	Positive
	0.788	Positive
75	0.509	Positive
	0.452	Negative
10	0.170	Negative
	0.170	Negative
5	0.212	Negative
	0.205	Negative

[a] Index values for each dilution are the test results for duplicate wells.
[b] The values at 300 μg/ml are the achievable peak levels in serum for the antibiotic indicated.

was also positive at concentrations of 150 μg/ml (indices of 0.84 and 0.78), approached negativity at 75 μg/ml (indices of 0.45 and 0.50), and was negative at concentrations of 10 μg/ml (index of 0.17 for both wells) and 5 μg/ml (indices of 0.21 and 0.20) (Table 3). Saline, dextrose, the phosphate buffer, and serum controls tested negative in all experiments.

DISCUSSION

Patients at risk for or being evaluated for invasive aspergillosis are likely to have been on broad-spectrum antibiotics. A false-positive galactomannan test in this setting may lead to unnecessary diagnostic procedures or employment of antifungal therapy (11). On the other hand, a positive test may be attributed to false EIA reactivity and may therefore delay the appropriate investigations for aspergillosis. Thus, reactivity of the Platelia *Aspergillus* EIA with antibiotics is of potentially significant clinical relevance.

Aspergillus fumigatus galactomannan is a polysaccharide comprised of a linear mannan core with α-(1-2)-linked mannotetraose units attached with α-(1-6) linkage (6). The side chains, consisting of an average of four to five β-(1-5)-galactofuranose units, are linked to C-6 and C-3 positions of α-(1-2)-linked mannose units of the mannan core (6). Galactomannan is widely distributed among *Aspergillus* and *Penicillium* species (6, 14). Although subtle chemical differences exist, the galactomannan of *Aspergillus* is strikingly similar in structure to that of *Penicillium*.

We tested several antibiotics commonly used in clinical practice, including those of fungal and nonfungal origins and those from nonmicrobial and synthetic sources. Penicillins and cephalosporins, with the exception of cephamycins (which are produced by actinomycetes rather than fungi), are of fungal origin (8). Penicillins are derived from *Penicillium* spp.; the drug originally isolated by Alexander Fleming in 1929 was from a strain of *Penicillium notatum* (2). The piperacillin component of piperacillin-tazobactam is a semisynthetic acylaminopenicillin, and tazobactam is a synthetic penicillinate sulfone. Gentamicin and erythromycin are naturally occurring compounds of nonfungal origin; gentamicin is derived from *Micromonospora purpurea*, and erythromycin is derived from *Streptomyces erythraeus*, formerly known as *Saccharopolyspora erythraea*. Finally, levofloxacin, a chiral fluorinated carboxyquinolone, is synthetically produced.

Undiluted samples of piperacillin-tazobactam and piperacillin in our study tested positive for galactomannan, whereas other antibiotics, i.e., amoxicillin, nafcillin, ampicillin-sulbactam, cephalosporins, gentamicin, levofloxacin, and erythromycin, tested negative. All batches of piperacillin-tazobactam and all bags within each batch tested strongly positive (index, 5.168). However, at achievable concentrations in serum, piperacillin-tazobactam, but not piperacillin, resulted in a positive test. Given that an index of 1.0 equals approximately 1 ng of galactomannan/ml (10), piperacillin-tazobactam at its achievable peak concentration in serum (300 μg/ml) had a level of reactivity that indicated the presence of ~1.4 ng of galactomannan per ml.

The precise reasons for the reactivity of piperacillin-tazobactam in the galactomannan assay are not known. It remains to be determined if the basis of reactivity is galactomannan or galactofuranose from either *Aspergillus* or a non-*Aspergillus* source such as *Penicillium*, a chemical reaction with the drug itself, or another compound found in piperacillin-tazobactam. Whether the reactivity is amenable to elimination in the manufacturing process or is part of the antibiotic molecule that cannot be modified is also unknown.

Following a 4.5-g dose, the peak levels (at 30 min) of piperacillin-tazobactam range from 155 to 298 μg/ml (Zosyn [piperacillin-tazobactam] product information, Wyeth Pharmaceuticals, Inc., Philadelphia, Pa.). Levels at 1, 2, 3, and 4 h are 141, 46.6, 16.4, and 6.9 μg/ml, respectively, and decline to <1.4 μg/ml 6 h after the dose or at a trough (Zosyn product information; Wyeth Pharmaceuticals, Inc.). Whereas piperacillin-tazobactam at concentrations of 75, 150, 300, and 600 μg/ml yielded false-positive reactivity in the galactomannan assay in

our study, lower concentrations (10 and 5 μg/ml) tested negative for galactomannan. These data therefore suggest that while achievable levels of piperacillin-tazobactam in the serum at 30 min and 1 h following a 4.5-g dose may potentially yield a false-positive galactomannan test result, concentrations in serum that approximate the trough level are unlikely to result in a false-positive test.

Clinicians evaluating the results of the galactomannan test should be aware that achievable concentrations of piperacillin-tazobactam in serum can result in a positive galactomannan test in patients receiving this antibiotic. Alternatively, since the dilutions of piperacillin-tazobactam in serum that mimicked the levels achievable at trough concentrations did not yield a positive test, the samples for the galactomannan test in patients receiving piperacillin-tazobactam could be timed so as to be collected prior to the administration of a dose or to coincide with a trough level of piperacillin-tazobactam. These findings warrant validation in future investigations with tests conducted on sera from patients receiving piperacillin-tazobactam.

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