J Antimicrob Chemother 2016; **71**: 913–915
doi:10.1093/jac/dkv419 Advance Access publication 13 December 2015

Journal of Antimicrobial Chemotherapy

1,3-β-D-Glucan contamination of common antimicrobials

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Received 5 October 2015; returned 23 October 2015; revised 2 November 2015; accepted 5 November 2015

Background: 1,3-β-D-Glucan (BDG) is a fungal cell wall constituent used in the diagnosis of invasive fungal infections. BDG testing, although endorsed by the European Organization for Research and Treatment of Cancer, suffers from limited specificity. False-positive results have been linked to haemodialysis membranes, blood products, antineoplastic agents and antimicrobial use.

Objectives: The aim of this study was to determine whether false-positive BDG results in the context of antimicrobial use are caused by BDG present in infusion solutions.

Methods: We obtained 35 antimicrobial drugs (30 antibiotics and 5 antifungals) and analysed their BDG content using two different assays.

Results: Twenty-five antimicrobials (20 antibiotics and all the tested antifungals) contained enough BDG to trigger a positive test. Depending on the substance, BDG varied between 9 and 2818 pg/mL.

Conclusions: A majority of the available antimicrobial substances contained BDG, potentially limiting the utility of BDG testing in the context of prior exposure to these drugs. As the cumulative effects of repeated BDG exposure are unknown, efforts to reduce contamination should be considered.

Introduction

1,3-β-D-Glucan (BDG), like galactomannan, is a fungal cell well constituent used to establish the diagnosis of invasive fungal infections (IFIs) in accordance with official guideline criteria.[1,2] In analogy to galactomannan,[3] it has a known potential for false-positive results correlated to e.g. haemodialysis, blood product transfusion and wound gauze.[4–6]

The correlation of false-positive BDG results with antibiotic exposure in haematology/oncology patients[7] is especially alarming, as high-risk patients are very likely to have received antibiotic therapy prior to attempts to diagnose an IFI. Indeed, persistent fever despite broad-spectrum antibiotic treatment is a major indicator of IFI.[8]

To examine an underlying causality for this observed correlation, we measured BDG in 30 routinely used antibiotics, as well as 5 antifungals.

Materials and methods

All antimicrobial substances were obtained through the pharmacy of the University Clinic of Cologne. The drugs were freely sold, unspoiled and fit for intravenous administration. Samples were either ready-to-use infusion solutions or lyophilized powders. The powders were reconstituted according to the manufacturer's instructions using glucan-free (<1 pg/mL BDG) instruments and reagents. Investigational products and topical or oral drugs were excluded from the analysis.

The antimicrobial substances were tested for their respective BDG content using two different BDG tests [Fungitell assay (Associates of Cape Cod, MA, USA) and BioAssay (United States Biological, Salem, MA, USA)] according to the instructions of the respective manufacturer. Briefly, for the Fungitell assay, 5 μL of the drug was pretreated with 20 μL of alkaline reagent, incubated with 100 μL of Fungitell reagent at 37°C and monitored at 405 nm kinetically for 40 min. For the BioAssay, 100 μL of the drug was treated with 10 μL of balance solution and incubated with 50 μL of enzyme conjugate for 1 h at 37°C followed by incubation with 50 μL of substrates A+B for 15 min at room temperature. Absorbance was measured at 450 nm after the addition of 50 μL of stop solution. All tests were run in triplicate using glucan-free (<1 pg/mL BDG) pipette tips and reagents.

Results and discussion

Of the tested antimicrobials, 25 contained sufficient amounts of BDG to cause a positive assay result (Table 1). The strength of this study lies in analysing a broad cross-section of commonly

Table 1. Tested substances and BDG assay results

Antimicrobial substance	Manufacturer	Batch	BDG (pg/mL)			
			modified limulus amoebocyte lysate assay	SD	immunological assay	SD
Amikacin	B. Braun	16FM0040	41	4.2	36	3.3
Amoxicillin/clavulanic acid	Hikma	J001	**429**	31.1	**420**	28.2
Ampicillin	ratioPharm	N49967	**120**	8.5	**114**	7.8
Ampicillin/sulbactam	Teva	2859	**347**	24.4	**340**	22.8
Cefazolin	Hikma	147087.2	**1569**	71.6	**1672**	61.5
	Fresenius Kabi	147074,1	**982**	67.8	**982**	65.5
Cefepime	Bristol-Myers Squibb	4D02695	**1134**	77.1	**1150**	77.7
Cefotaxime	Fresenius Kabi	B14002	**445**	32.0	**445**	30.4
Ceftazidime	GlaxoSmithKline		**871**	60.8	**864**	58.1
Ceftriaxone	Stragen Nordic	18F3760	**313**	23.0	**305**	20.9
Cefuroxime	Fresenius Kabi	136177,1	**877**	59.9	**872**	58.3
Ciprofloxacin	Fresenius Kabi	137245.2	17	2.3	16	1.4
Clarithromycin	Martindale Pharma	J4	21	2.5	17	1.5
Clindamycin	Fresenius Kabi	141067,2	31	3.0	29	2.3
Colistin	Sobi	7916	**1006**	69.4	**987**	65.9
Trimethoprim/sulfamethoxazole	ratioPharm	N37410	43	3.0	36	2.9
Daptomycin	Novartis	CDF092H	**553**	37.5	**546**	37.2
Doxycycline	ratioPharm	D13761	**2808**	88.9	**2818**	78.7
Erythromycin	Stragen Nordic	318020	**498**	33.7	**482**	33.1
Flucloxacillin	Stragen Nordic	4P66HH	**211**	16.9	**195**	13.9
Gentamicin	B. Braun	147154	19	1.7	9	0.7
Imipenem	Fresenius Kabi	IDEA1294	**432**	31.2	**424**	28.7
Linezolid	Pfizer	14K23U84	**526**	37.6	**521**	35.4
Meropenem	Hospira	600E006A	**213**	15.4	**208**	14.6
Metronidazole	B. Braun	B8953	16	3.3	10	1.4
Moxifloxacin	Fresenius Kabi	42806	23	1.7	14	1.8
Penicillin G	INFECTOPHARM	B011401,1	33	4.3	23	2.3
Piperacillin/tazobactam	IBIGEN	1FL5001DE	**254**	17.8	**226**	15.2
Rifampicin	RIEMSER	4124	**89**	7.3	**83**	6.4
Tobramycin	B. Braun	1965140	41	2.8	40	3.3
Vancomycin	Fresenius Kabi	234/14	32	3.3	23	2.5
Amphotericin B deoxycholate	Bristol-Myers Squibb	46204TB23	**1315**	88.6	**1308**	87.3
Liposomal amphotericin B	Gilead	042471AD	**712**	50.4	**710**	47.4
Caspofungin	MSD	216260	**119**	8.1	**109**	7.5
Fluconazole	B. Braun	13284403	**168**	13.0	**139**	10.1
Voriconazole	Pfizer	Z316902	**229**	15.6	**224**	15.6

Values shown in bold are above the positivity cut-off.

used antimicrobials. However, the resulting data need to be interpreted cautiously as only samples from a single batch were analysed for most drugs and BDG concentrations may fluctuate between batches, as well as manufacturers, as seen with cefazolin (Table 1). This variation in BDG contamination between manufacturers and batches may explain the discrepancy between our findings and prior reports of minimal BDG amounts in antimicrobials.[9]

Among other factors, the contamination of most antimicrobial substances with BDG may contribute to the limited usefulness of BDG testing in patients with prior exposure to antibiotics or antifungals. This finding may also partially explain the variability of previously suggested positivity cut-offs, as patients exposed to varying amounts of BDG through infusion solutions may exhibit varying BDG levels even in the absence of a fungal infection. A burdensome approach would be to establish an individual positivity cut-off for each patient based on the amount of BDG infused and then adjust for the plasma kinetics of BDG.[10]

In the face of possible immunomodulatory properties of BDG,[11] an intervention to reduce contamination on the manufacturing side should be considered. The feasibility of such an intervention has been recently demonstrated for 'galactomannan-containing' piperacillin/tazobactam.[12] If adjusting the manufacturing process to reduce contamination, e.g. through eliminating cellulose membranes, is not immediately possible, the BDG content could be declared in the package leaflet. At least in the EU, regulatory statutes may actually require such a declaration (e.g. under Title V, Article 54, EU Directive 2001/83/EC).

Acknowledgements

The data contained in this paper were presented as 'Potential interaction of the β-D-glucan test with antimicrobial agents' in the slide session 'Clinical Mycology' (session number: 047) at the Interscience Conference of Antimicrobial Agents and Chemotherapy/International Congress of Chemotherapy and Infection (ICAAC/ICC) 2015 in San Diego, CA, USA.

Funding

This study was supported by internal funding.

Transparency declarations

O. A. C. is supported by the German Federal Ministry of Research and Education, has received research grants from 3M, Actelion, Astellas, AstraZeneca, Basilea, Bayer, Celgene, Cubist/Optimer, Genzyme, Gilead, GSK, Merck/MSD, Miltenyi, Pfizer, Quintiles, Shionogi and Viropharma, is a consultant to Astellas, Basilea, Cidara, Da Volterra, Daiichi Sankyo, F2G, Genentech, Gilead, Merck/MSD, Merck Serono, Pfizer, Sanofi Pasteur, Summit, Vical and Vifor, and received lecture honoraria from Astellas, Basilea, Gilead, Merck/MSD and Pfizer, all outside the submitted work. D. H. reports personal fees from Alexion and personal fees from Beckman Coulter, outside the submitted work. H. W. has received research grants from, is an advisor to or received lecture honoraria from Beckmann, bioMérieux, Bruker Daltonics, Cepheid, Hologic, r-biopharm and Siemens. B. L. and V. D.: none to declare.

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