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Potential Impact of a Microarray-Based Nucleic Acid Assay for Rapid Detection of Gram-Negative Bacteria and Resistance Markers in Positive Blood Cultures

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We evaluated the Verigene Gram-negative blood culture (BC-GN) test, a microarray that detects Gram-negative bacteria and several resistance genes. A total of 102 positive blood cultures were tested, and the BC-GN test correctly identified 97.9% of the isolates within its panel. Resistance genes (CTX-M, KPC, VIM, and OXA genes) were detected in 29.8% of the isolates, with positive predictive values of 95.8% (95% confidence interval [CI], 87.7% to 98.9%) in *Enterobacteriaceae* and 100% (95% CI, 75.9% to 100%) in *Pseudomonas aeruginosa* and negative predictive values of 100% (95% CI, 93.9% to 100%) and 78.6% (95% CI, 51.0% to 93.6%), respectively.

In cases of sepsis, timely microbiological diagnosis, including data on antimicrobial susceptibility, is crucial for prompt initiation of targeted drug therapy (1). This is not possible with currently used methods, thus causing a significant delay in specific treatment and the empirical use of broad-spectrum antimicrobials (2–4). Nucleic acid-based assays are considered to be a potential adjuvant tool for improving the microbiological diagnosis of sepsis (5–7). These assays may be classified into one of two groups (5–7): (i) those using positive blood cultures, which are potentially useful but burdened by the usual culture-associated drawbacks (i.e., interfering effect of ongoing antibiotics, long time to positivity, and the presence of fastidious pathogens), and (ii) those using blood samples, which are promising but still not developed for the sensitive detection of resistance markers (5–7).

In this pilot study, we evaluated the Verigene Gram-negative blood culture (BC-GN) test (Nanosphere, Northbrook, IL, USA), a microarray-based, almost fully automated, and random-access system allowing for bacterial identification (Table 1) and detection of several resistance genes (Table 2) from positive blood cultures. The turnaround time is 2 h, with a hands-on time of <10 min. The BC-GN test has been approved for clinical use in Europe and is currently under submission for use in the United States.

Several papers have already evaluated the Verigene panel dedicated to Gram-positive bacteria (8–12), but this is the first one on the BC-GN test. To investigate its potential clinical usefulness, we evaluated the following parameters: (i) the concordance of identification and of antibiotic susceptibility data with those obtained with the traditional blood culture flowchart, (ii) the time to definitive results, and (iii) the impact of the BC-GN test results on ongoing empirical therapy, evidencing the rate of potential BC-GN-induced antibiotic changes. In this analysis, the following phases of the standard management of blood cultures were considered: time from blood sampling to the loading of bottles into the bioMérieux BacT/Alert system, time to positivity, and time from positivity to Gram stain and subculturing on solid medium (positive bottles are downloaded every 2 h, from 8:00 a.m. to 6:00 p.m. Monday to Friday, 8:00 a.m. to 2:00 p.m. on Saturday, and 9:00 a.m. to 1:00 p.m. on Sunday).

Our study prospectively included all blood cultures positive for Gram-negative pathogens submitted to our center from June to September 2013, but only one positive bottle was considered per patient. Antibiotic susceptibility was phenotypically evaluated by disk diffusion from positive blood culture broth (preliminary antibiotic susceptibility testing [pAST]) and by automated microdilution using the Vitek 2 AST-GN202 card (definitive antibiotic susceptibility testing[dAST]). The resistance mechanisms were confirmed by phenotypic assays with *Enterobacteriaceae* (double-disk synergy test [DDST] for extended-spectrum β-lactamases [ESBLs], synergy with phenylboronic acid [PBA] for *Klebsiella pneumoniae* carbapenemases (KPC), and synergy with EDTA for metallo-β-lactamases) (13) and by PCR amplification and sequencing of resistance markers using already described primers (14, 15).

A total of 102 positive blood cultures (from 102 patients) yielding 104 Gram-negative organisms were tested using standard techniques and the BC-GN assay. Among the 96 (92.3%) isolates detectable by the BC-GN test panel, 94 (97.9%) were correctly identified by the BC-GN assay (Table 1); one positive blood culture for *K. pneumoniae* yielded inconclusive results even when repeating the BC-GN assay, whereas the unidentified *Pseudomonas aeruginosa* isolate was part of a polymicrobial blood culture (Table 1). Eight positive (7.7%) blood cultures included Gram-negative isolates belonging to genera not featured by the BC-GN panel, and importantly, none of them was misidentified, which would have yielded an incorrect genus or species (Table 1). Overall, the BC-GN assay correctly identified 94/104 of all the Gram-negative pathogens in this study, yielding a general sensitivity of 90.4% (95% confidence interval [CI], 90.4% ± 5.7%), which increased to 97.9% (94/96 [95% CI, 97.9% ± 2.1%]) if only the genera and species included in the panel were considered. In both

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TABLE 1 Gram-negative isolates detected in this study and concordance with the BC-GN assay identifications

Organism	No. (%) of isolates: Total	Correctly identified	Not detected	Misidentified
Klebsiella pneumoniae	20 (19.2)	19 (95)[a]	1 (5)	
Escherichia coli	45 (43.2)	45 (100)[b]		
Pseudomonas aeruginosa	17 (16.3)	16 (94.1)[c]	1 (5.9)[d]	
Serratia marcescens	2 (1.9)	2 (100)		
Acinetobacter spp.	3 (2.9)	3 (100)[e]		
Enterobacter spp.	5 (4.8)	5 (100)[f]		
Citrobacter spp.	2 (1.9)	2 (100)		
Klebsiella oxytoca	1	1 (100)[g]		
Proteus mirabilis	1	1 (100)		
Pasteurella multocida[h]	1		1 (100)	
Salmonella spp.[h]	1		1 (100)	
Fusobacterium nucleatum[h]	1		1 (100)	
Sphingomonas paucimobilis[h]	1		1 (100)	
Bacteroides fragilis[h]	3 (2.9)		3 (100)	
Pantoea spp.[h]	1		1 (100)	
Total	104	94 (90)	10 (10)	0

[a] Three isolates were detected in polymicrobial blood cultures (one with *Enterococcus faecium*, one with *Staphylococcus epidermidis*, and one with *E. coli*, *Streptococcus gallolyticus*, and *E. faecium*).
[b] One isolate was detected in a polymicrobial culture with *K. pneumoniae*, *S. gallolyticus*, and *E. faecium*.
[c] Two isolates were detected in a polymicrobial culture (one with *E. faecium* and the other with *Candida albicans*).
[d] Encountered in a polymicrobial culture with a *K. oxytoca* isolate.
[e] One *Acinetobacter baumannii* complex isolate in a polymicrobial culture with *Staphylococcus aureus*.
[f] One *Enterobacter cloacae* isolate in a polymicrobial culture with an *Enterococcus faecalis* isolate.
[g] Encountered in a polymicrobial culture with a *P. aeruginosa* isolate.
[h] Species not included in the BC-GN database.

cases, specificity was 100%, evidencing the accuracy of the BC-GN test identification.

Among the 94 isolates detected by the BC-GN assay, 28 (29.8%) featured a resistance marker, with CTX-M detected in 14 (14.9%) isolates and carbapenemases (KPC, OXA, and VIM genes) detected in the other 14 (14.9%) isolates (Table 2). Thirteen (92.9%) out of the 14 CTX-M-positive isolates featured an antibiotic susceptibility profile by pAST compatible with an ESBL-producing phenotype, which was later confirmed by dAST (Table 3). CTX-M genes were confirmed in all isolates by PCR and sequencing, including two isolates featuring a negative DDST result (Table 3). In particular, a single CTX-M-positive isolate by the BC-GN test did not show an ESBL-producing phenotype by dAST or by DDST, although the presence of a CTX-M-9 gene was confirmed by PCR amplification and sequencing (Table 3). Importantly, all nine isolates positive for KPC genes showed an antibiotic susceptibility phenotype by pAST compatible with the production of carbapenemases, which was eventually confirmed by PBA and dAST (Table 3). Similarly, all isolates with OXA (*Acinetobacter* spp.) and VIM (*P. aeruginosa* and *K. oxytoca*) genes featured a multidrug-resistance (MDR) phenotype and were confirmed by PCR and sequencing (Table 3). It is important to underline that three *P. aeruginosa* isolates not featuring any resistance marker (in the BC-GN assay or by PCR) showed a carbapenemase-resistant phenotype. Overall, the positive predictive value (PPV) of the detection of a resistance marker in the isolates belonging to *Enterobacteriaceae* was a combined 95.8% (95% CI, 87.7% to 98.9%), whereas the negative predictive value (NPV) was 100% (95% CI, 93.9% to 100%). The PPV was 100% (95% CI, 75.9% to 100%) for the 16 *P. aeruginosa* isolates, whereas the NPV was markedly lower at 78.6% (95% CI, 51.0% to 93.6%). Absolute concordance was observed for the three isolates of *Acinetobacter*.

An average time of 3 h 30 min from blood sampling to the loading of the blood culture bottles into the BacT/Alert system was calculated, with an average time to positivity of 15 h 18 min. The average time from positivity to the Gram stain and subculturing on solid medium was 5 h 17 min, making 24 h 5 min the average time from sampling to subculturing. Consequently, the BC-GN assay results were available in an average of 26 h 5 min compared to 40 h 5 min and 46 h 5 min for the pAST and dAST results, respectively (16 h were considered from the time of subculturing to pAST and identification by mass spectrometry, whereas a further ≥6 h were usually necessary for dAST). As evidenced in Table 3, this would have theoretically allowed more targeted antibiotic regimens 14 h earlier (e.g., one working day earlier) than pAST in 55.6% (5/9) of the patients with a KPC-producing *K. pneumoniae* isolate and in 80% of patients (4/5) with an OXA- or a VIM-producing isolate.

In conclusion, the Verigene BC-GN test provided highly accurate identification results and earlier potentially important information on antibiotic susceptibility, both confirming and excluding the presence of an MDR phenotype, especially for *En-*

TABLE 2 Resistance genes detected by the BC-GN assay

Organism	No. of isolates tested	No. (%) of isolates with resistance genes	No. of resistance genes: CTX-M	OXA	KPC	VIM	NDM	IMP
K. pneumoniae	19	11 (57.9)	2		9			
E. coli	45	12 (26.7)	12					
P. aeruginosa	16	2 (12.5)				2		
S. marcescens	2	0						
Acinetobacter spp.	3	2 (66.7)		2				
Enterobacter spp.	5	0						
Citrobacter spp.	2	0						
K. oxytoca	1	1 (100)				1		
P. mirabilis	1	0						
Total	94	28 (29.8)	14	2	9	3	0	0

TABLE 3 Concordance between BC-GN-detected resistance markers and other phenotypic and molecular assays used in this study and results of definitive antibiotic susceptibility testing for each isolate and ongoing empirical therapy

BC-GN test gene product	Organism	Phenotypic test results[a]				dAST[b]									Ongoing empirical therapy[c]
		DDST	EDTA	PBA	PCR	Cephalosporins[d]	Carbapenems[d]	TZP	TGC	GEN	AMK	SXT	CIP	CST	
CTX-M[e]	*E. coli*	−	−	−	CTX-M-14	R	S	S		S	S	R	R		RIF
	E. coli	+	−	−	CTX-M-15/27	R	S	I		R	S	R	R		CIP + MET
	E. coli	+	−	−	CTX-M-15	R	S	S		S	S	R	R		TZP
	E. coli	+	−	−	CTX-M-15	R	S	R		R	R	R	R		MEM
	E. coli	+	−	−	CTX-M-15	R	S	S		S	S	S	R		CIP
	E. coli	+	−	−	CTX-M-15	R	S	S		R	S	R	R		TZP
	E. coli	+	−	−	CTX-M-15	R	S	I		S	S	S	R		LVX + TZP
	E. coli	+	−	−	CTX-M-15/14	R	S	S		S	I	S	R		MEM
	E. coli	+	−	−	CTX-M-15	R	S	S		S	S	R	R		CIP
	E. coli	+	−	−	CTX-M-15	R	S	R		S	I	S	R		CIP + MET
	E. coli	+	−	−	CTX-M-15	R	S	S		R	I	R	R		MEM
	E. coli[f]	−	−	−	CTX-M-9	**S**	S	R		S	S	R	R		TZP + DPC
	K. pneumoniae	+	−	−	CTX-M-15	R	S	R		S	S	S	R		TZP + MET
	K. pneumoniae	+	−	−	CTX-M-15	R	S	I		S	S	R	I		TZP + MET
KPC	*K. pneumoniae*	−	−	+	KPC-2	R	R	R	I	S	R	S	R	S	**TZP**
	K. pneumoniae	−	−	+	KPC-2	R	R	R	I	R	S	R	R	S	**VAN + TZP + AMK**
	K. pneumoniae	−	−	+	KPC-2	R	R	R	I	I	R	S	R	S	GEN + MEM + CST
	K. pneumoniae	−	−	+	KPC-2	R	R	R	I	S	S	S	R	S	**None**
	K. pneumoniae	−	−	+	KPC-2	R	R	R	R	I	R	R	R	S	**TZP**
	K. pneumoniae	−	−	+	KPC-2	R	R	R	R	I	R	R	R	S	**MEM + LZD**
	K. pneumoniae	−	−	+	KPC-2	R	R	R	I	I	R	R	R	R	GEN + MEM + CST
	K. pneumoniae	−	−	+	KPC-2	R	R	R	R	S	R	R	R	S	GEN + MEM + CST
	K. pneumoniae	−	−	+	KPC-2	R	R	R	I	S	R	R	R	S	GEN + MEM + CST
OXA	*Acinetobacter*				OXA-58		R			R		R	R	S	**MEM**
	Acinetobacter				OXA-58		R			R		R	R	S	**LZD**
VIM	*P. aeruginosa*				VIM-1	R	R	R		R	R		R	S	**TZP**
	P. aeruginosa				VIM-1	R	R	R		R	S		R	S	MEM + CST
	K. oxytoca	−	−	−	VIM-1	R	R	R		R	S	R	I	S	**CAZ**

[a] DDST, double-disk synergy test; EDTA, EDTA synergy test; PBA, phenylboronic acid synergy test.
[b] dAST, definitive antibiotic susceptibility testing; TZP, piperacillin-tazobactam; TGC, tigecycline; GEN, gentamicin; AMK, amikacin; SXT, trimethoprim-sulfamethoxazole; CIP, ciprofloxacin; CST, colistin; R, resistant; S, susceptible; I, intermediate.
[c] In bold type are all the cases of empirical regimens that could have been changed earlier based on BC-GN resistance marker detection. RIF, rifampin; MET, metronidazole; MEM, meropenem; LVX, levofloxacin; DPC, daptomycin; VAN, vancomycin; LZD, linezolid; CAZ, ceftazidime.
[d] All isolates but one *E.* coli showed a perfect concordance of susceptibility results to the different cephalosporins (ceftazidime and cefotaxime) and carbapenems (imipenem and meropenem) tested.
[e] All CTX-M-positive isolates were resistant to ciprofloxacin, highlighting the risk of empirical use of a quinolone against CTX-M-producing organisms in our center.
[f] CTX-M-positive *E. coli* isolate not showing an ESBL-producing phenotype in DDST or in dAST.

terobacteriaceae and *Acinetobacter* spp. isolates. A more complex scenario was observed for *P. aeruginosa*, in which an MDR marker was always associated with an MDR phenotype usually limited to colistin as the only possible antibiotic choice; conversely, the absence of genetic markers did not exclude resistance to carbapenems or an MDR phenotype due to concurring resistance factors not regulated by a single gene (16).

The BC-GN assay has two theoretical advantages favoring its routine use: a random-access format with a very limited hands-on-time and the possibility of giving important therapeutic information in a "clinically useful" time. Our pilot study certainly needs to be supported by larger prospective studies to confirm our results and to address other important aspects influencing the routine use of this assay. For example, we could not focus our attention on the cost-effectiveness of the BC-GN assay, which is an important point for investigation, especially considering that expensive molecular assays have already been associated in previous studies on sepsis with an overall economic saving (17–19). In our opinion, the Verigene BC-GN assay is a good compromise between a more laborious direct-on-blood molecular assay not giving information on antibiotic susceptibility (20–27) and the standard clinical flowchart of blood cultures. However, we are convinced that a real revolution in the microbiological diagnosis of sepsis will occur only when an assay with the potential of the one investigated in this study is performed directly on blood samples.

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