Implementation of a Rapid Microarray Assay for the Detection of Gram-Positive, Gram-Negative and Resistance Determinants: Assessing Performance in a Clinical Laboratory in Bulgaria

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Abstract: Two hundred and seven positive blood cultures from two blood culture systems (Bactec and BacTAlert) between May 2012 and February 2014 were enrolled in this study. We present the results of prospectively tested, non-duplicate Gram-positive and Gram-negative organisms tested by the BC-GP (Verigene® Gram-positive) and BC-GN (Gram-negative) Blood Culture Assays (Nanosphere, Inc.) for the first time in Bulgaria. These assays represent new tools for the rapid detection of pathogens and resistance markers in blood stream infections. Results of the Verigene System were compared with conventional testing methods. Of the 207 isolates, 180 (87.0%) were targets on the blood culture panels. Correct identification of Gram-positive organisms was 99.2% and of Gram-negative organisms was 98.2% for organisms that could be detected with BC-GP and BC-GN. Additionally, staphylococci tested for the presence of the *mecA* gene showed a 75.7% correlation with the cefoxitin test and identification of methicillin-resistance by Vitek 2. ESBLs (extended-spectrum beta-lactamases) for Gram-negative organisms were detected by both methods, showing 100% agreement.

Key words: Microarray, verigene, blood cultures.

1. Introduction

BSI (bloodstream infections) and sepsis result in high morbidity and causes high economic burden. The mortality rate associated with BSIs ranges from 10% to 40% and aggregate healthcare costs approach approximately $16 billion per year (http://www.mlo-online.com/articles/201306/rapid-methods-for-pathogen-detection-in-bloodstream-infections.php). Every hour appropriate treatment is delayed has been shown to increase mortality by 7.6%; therefore, earlier detection of etiological agents has the potential to greatly benefit patient care [1]. After the causative pathogen has been identified, the most appropriate antimicrobial agent should be administered as quickly as possible [2].

New diagnostic technologies have been developed which significantly reduce the time required to identify the pathogen(s) causing BSI. FISH (fluorescent in situ hybridization), PCR, MALDI-TOF and sequencing have been implemented for more than a decade [3-6]. Micro-array panels for BSI investigation have been recently developed to identify various Gram-positive, Gram-negative and antibiotic resistance targets in blood cultures [5, 7-9].

The aim of this study is to present, for the first time in Bulgaria, the data obtained by implementing the Verigene® System (Nanosphere, Northbrook, IL, USA) in our laboratory. The assays run on the Verigene System to obtain the data for this study were

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BC-GP and BC-GN. These new tests for the rapid detection of Gram-positive and Gram-negative pathogens in cases of BSI were compared with the conventional methods currently used in our laboratory.

2. Material and Methods

In total, 207 blood culture sets (aerobic and anaerobic bottles) were positive by the Bactec 9050 and BacTAlert systems. Of the 207 positive BC (blood culture) samples investigated in this study, 150 BC were Gram-positive and 57 BC were Gram-negative. After the BC bottle was flagged positive and a Gram stain completed, the appropriate Verigene assay was performed, according to the Gram stain results. The Verigene BC-GP and BC-GN assays were performed according to the manufacture's package insert instructions, using 350 µL of blood culture media from the positive BC bottle for Gram-positive microorganisms and 700 µL of blood culture media for Gram-negative microorganisms [2]. At the same time that the sample aliquot was tested on the Verigene System, the sample was subcultured on the appropriate agar plates. After overnight incubation, the microorganism isolated was prepared for identification and susceptibility testing by Vitek 2 system.

3. Results

Of the 150 Gram-positive organisms recovered, 123 were organisms that are inclusive to BC-GP. Not detected results obtained by BC-GP include 27 blood cultures (16.3%) that contained 15 organisms not belonging to the BC-GP panel, including *Candida* and *Corynebacterium*. Compared to biochemical identification, the BC-GP results show concordance to genus and species level in 99.2 % (122/123) of the Gram-positive bacteria detectable by BC-GP in this study (Table 1). Eight samples of coagulase-negative staphylococci were not identified to the genus level as *Staphylococcus* spp. by BC-GP (detected as *Staphylococcus* spp.) and in 4 polymicrobial Gram-positive samples tested, BC-GP identified 1 of 2 organisms present. The BC-GP assay correctly predicted oxacillin resistance in 56 (75.7%) Staphylococcal strains with the *mecA* gene (Table 1). BC-GP can only report oxacillin resistance (mecA gene) in *S. aureus* and *S. epidermidis*. BC-GP correctly predicted oxacillin resistance in these organisms 100% (56/56) of the time.

Table 1   Verigene results for Gram-positive blood culture identification.

Bacterial targets/antibiotic targets	N of the strains identified by	
	Verigene	VITEK-2
S. aureus	35	35
S. epidermidis	30	30
Staphylococcus spp.	35	35
E. faecalis	11	11
E. faecium	6	6
Streptococcus anginosus	1	1
Streptococcus dysgalactiae	1	*Streptococcus*
Sreptococcus	1	*S. pluranimalium*
Sreptococcus	1	*S. gallolyticus*
Streptococcus thermophilus	1	*Streptococcus*
Staphylococcus	1	*Leuconostoc mesenteroides*
Not detected	27	27[a]
Oxacillin R	56[b]	74[c]

[a]The 15 isolates identified by Vitek-2 are not targets on the BC-GP panel. (See the text).
[b]Verigene detects the presence of *mecA*.
[c]Positive by the cefoxitin screen.

Table 2   Verigene results for Gram-negative blood culture identification.

Bacterial targets/antibiotic targets	N of the strains identified by	
	Verigene	VITEK-2
Acinetobacter spp.	14	14
E. coli	15	16
Proteus spp.	9	9
Enterobacter spp.	4	4
K. pneumoniae	4	4
K. oxytoca	1	1
S. marcescens	2	2
P. aeruginosa	5	5
Not detected	3	2[a]
ESBL	9	9
VIM	11	7[b]
NDM	2	2[b]
OXA	10	10[c]

[a]Both isolates were *Salmonella paratyphi* A.

[b]Metallo-beta-lactamases.

[c]Metallo-beta-lactamases or OXA.

Separate Verigene test identifying Gram-negative bacteria in BCs (BC-GN), developed recently, demonstrated concordance to genus and species level in 98.2% (54/55) of the Gram-negative isolates tested in this study (Table 2) that are capable of being detected with Verigene BC-GN. "Not detected" results were obtained by Verigene in 2 (3.5%) blood cultures that were *Salmonella paratyphi* A, a target not inclusive to the BC-GN panel. ESBLs were detected in 9 of the strains showing 100% concordance with traditional laboratory methods. Additionally, in 11 samples (6 *P. mirabilis*, 4 *P. aeruginosa* and 1 *Enterobacter cloacae*), VIM gene was found by Verigene. Two stored isolates (1 *P. mirabilis* and 1 *E. cloacae*) were confirmed for VIM production by PCR. In 10 samples positive for *Acinetobacter* spp., OXA genes were detected. In two *E. coli* samples NDM gene together with an ESBL (CTX-M) was detected using the Verigene System.

There were 9 polymicrobial Gram-positive samples tested during this study. In 2 of 9 blood cultures containing two organisms, BC-GP correctly identified both organisms; in four cultures, BC-GP identified 1 of 2 organisms present. The remaining 3 cultures reported a result of "not detected" by Verigene when there was a target organism present. There were no polymicrobial Gram-negative BCs observed during this study.

There were 29 (14.0%, 29/207) blood cultures that yielded a correct "not detected" result obtained by Verigene, all of which contained one of the following organisms not included on the BC-GP or BC-GN panel: *Candida* spp., *Corynebacterium* spp., *Deramacoccus*, *Salmonella paratyphi* A and *Aeromonas* spp.

4. Discussion

The identification of pathogens involved in blood stream-infections is a priority in Clinical Microbiology, particularly in large hospital complexes. For example, in 2013 in the Military Medical Academy territory in Sofia, out of the 773 blood cultures that were collected, 210 of them (27.2%) were positive [10]. The most frequently isolated microorganisms from blood during that time period were *S. aureus*, Coagulase-negative staphylococci, *E. coli* and *Acinetobacter baumannii* [10]. The gold standard method for pathogen identification in BSI, used also in our laboratory, requires culturing patient blood in an automated continuous monitoring system,

followed by Gram stain, sub-culturing and identification using an automated identification and susceptibility testing instrument. A significant drawback to this method is that the time for positive organism identification may take from 12 hours to more than 72 hours [5]. Therefore, new multiplex, automated nucleic acid technology, such as microarray technology, for identification to the genus and/or species level and genetic antimicrobial resistance determinants for a broad panel of the most common Gram-positive and Gram-negative blood culture bacteria, providing results within 2.5 hours of blood culture positivity, has recently been developed [9]. The results obtained by the Verigene BC-GP and BC-GN panels showed 99.2% concordance to genus and species level for Gram-positive bacteria and 98.2% (1/55; *E. coli* result was "not detected," a false-negative result) for the Gram-negative bacteria when compared with Vitek-2 results, which is in agreement with the literature data [4, 7, 11, 12]. Since this technology is very new in the clinical setting, the results obtained in this study, especially for BC-GN implementation, are probably one of the first in the literature. Buchan et al., reported 3 cases (5%) resulted as "not detected" which is three times less, compared with the data, obtained in our hands (4). In regards to the oxacillin resistance, Verigene *mecA* detection and Vitek-2 correlation was reported to be 97-100% [7, 8], which is differs from the results obtained in our study, 75.7%.

Rapid molecular testing of blood cultures denotes a clear advantage when compared to conventional practice for Gram-positive and Gram-negative bacteria identification and resistant targets in positive blood cultures, which makes it an effective tool to improve patient care by reducing the time to provide optimal antimicrobial therapy to patients with BSI.

5. Conclusions

The Verigene BC-GP and BC-GN tests are very usefully tools for the detection and identification of potentially pathogenic Gram-positive and Gram-negative bacteria which may cause bloodstream infections. This type of technology significantly reduces the time, by approximately about 48 hours, required for pathogen identification and susceptibility testing. However, the Verigene technology is indicated in conjunction with other clinical and laboratory findings for the diagnosis of BSI. It is not to be used to monitor these infections. Sub-culturing of positive blood cultures is necessary to recover organisms for susceptibility testing, differentiation of mixed growth, determination of antimicrobial resistance genes and for epidemiological typing. We estimate that the potential is high for using the Verigene in ESBL and carbapenemase detection in Gram-negative bacteria, which is important for correct administration of targeted antimicrobial therapy in as little as 2-3 hours after the blood cultures becomes positive in patients with BSI.

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