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Antibiotic treatment algorithm development based on a microarray nucleic acid assay for rapid bacterial identification and resistance determination from positive blood cultures

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ABSTRACT

Rapid diagnosis of bloodstream infections remains a challenge for the early targeting of an antibiotic therapy in sepsis patients. In recent studies, the reliability of the Nanosphere Verigene Gram-positive and Gram-negative blood culture (BC-GP and BC-GN) assays for the rapid identification of bacteria and resistance genes directly from positive BCs has been demonstrated. In this work, we have developed a model to define treatment recommendations by combining Verigene test results with knowledge on local antibiotic resistance patterns of bacterial pathogens. The data of 275 positive BCs were analyzed. Two hundred sixty-three isolates (95.6%) were included in the Verigene assay panels, and 257 isolates (93.5%) were correctly identified. The agreement of the detection of resistance genes with subsequent phenotypic susceptibility testing was 100%. The hospital antibiogram was used to develop a treatment algorithm on the basis of Verigene results that may contribute to a faster patient management.

1. Introduction

Sepsis is a leading cause of death in intensive care units (ICUs). Fast diagnosis of bloodstream infections is crucial for the initiation of any targeted antibiotic therapy (O'Brien and Gould, 2013). The most frequent sepsis-causing pathogens *Escherichia coli* and *Staphylococcus aureus* are characterized by the development of increasing antibiotic resistance for which initial empirical antibiotic therapy might fail (Berger et al., 2010; Loonen et al., 2014). Blood culture (BC) as the current standard of microbiological sepsis diagnosis has several limiting factors. Three BC sets should be obtained over a 24-h period to reach a sufficient sensitivity (Weinstein and Doern, 2011). Coagulase-negative staphylococci (CoNS) often overgrow cultures as contaminants from the skin flora. Fastidious bacteria are grown with significant time delay or cannot be always cultured (Loonen et al., 2014). Subcultures from positive BC are needed for phenotypic antibiotic susceptibility testing (AST), resulting in critical time delays for therapy-relevant report generation. Recently, molecular biological approaches based on PCR techniques have been proposed to reduce the time to result of sepsis diagnosis and provide a higher sensitivity, compared to culture (Burdino et al., 2014; Fitting et al., 2012; Skvarc et al., 2013). However, based on several studies, whether the sensitivity of PCR directly conducted on patient blood samples is adequate for routine clinical use remains questionable (Burdino et al., 2014; Fernández-Romero et al., 2014; Skvarc et al., 2013). Negative PCR tests have been reported in cases in which relevant pathogens are isolated from simultaneously BCs (Fitting et al., 2012; Schreiber et al., 2013). High costs and laborious PCR test procedures are not aligned with the demand for faster, easier-to-perform assays that are continuously applicable in routine, time-sensitive diagnostics. At this time, culture-independent PCR tests for sepsis diagnosis have not yet been developed as point-of-care solutions with a minimum of hands-on time. As an alternative solution, the combination of BC with rapid molecular identification of growing pathogens has been introduced to shorten the time to result of sepsis diagnosis (Bhatti et al., 2014; Mancini et al., 2014; Samuel et al., 2013; Wojewoda et al., 2013). The potential of BCs can be improved by supplementation with rapid tests that identify bacterial species as well as resistance marker genes directly from positive BC bottles (Dodémont et al., 2014; Martinez et al., 2014; Southern et al., 2015; Sullivan et al., 2014; Wojewoda et al., 2013). This study was designed to prospectively evaluate the impact of the Verigene Gram-positive and Gram-negative blood culture tests (BC-GP and BC-GN; Nanosphere, Northbrook, IL, USA; purchased from Thermo Fisher Scientific, Wesel, Germany) for rapid identification of pathogens and their resistance determinations, in comparison to conventional identification and AST alone. Verigene BC-GP and BC-GN, which are automated sample-to-result microarray-based assays that detect the most common sepsis-related pathogens and primary resistance genes of staphylococci, enterococci, and Enterobacteriaceae, were implemented in our laboratory as a supplement to conventional microbiology methods in 2014. In this study, the BC diagnosis using the Verigene system and data from the hospital

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antibiogram were integrated into a treatment algorithm to generate appropriate and targeted antimicrobial recommendations.

2. Materials and methods

2.1. Patient samples and BC processing

Clinical samples were BCs submitted as part of routine patient care to the laboratory from ICUs of the University Hospital Jena between February and December 2014. Only data from BCs collected for standard-of care purposes were used for this performance evaluation. Blood samples collected in BD BACTEC Plus Aerobic/F and Lytic/10 Anaerobic/F bottles (BD Diagnostics, Heidelberg, Germany) were incubated on a BACTEC FX instrument (BD Diagnostics). Positive BCs were sampled aseptically; Gram stained; and streaked onto Columbia sheep blood agar, chocolate agar, Drigalski lactose agar, and Schaedler agar (Oxoid, Thermo Fisher Scientific) for overnight incubation at 37 °C. In parallel, an aliquot was prospectively tested using the Verigene BC-GP or BC-GN test according to the results of Gram staining. Only 1 positive BC bottle per patient was tested. Time to positivity, defined as the time between start of incubation and positive signal of growth, was recorded for each BC bottle. Preliminary AST was performed by disk diffusion assay using a 1:50 saline dilution of a sample aliquot.

2.2. Verigene testing

The Verigene system consists of a sample processor for fully automated magnetic bead–based DNA extraction and hybridization to complementary capture probes on a glass microarray slide (test cartridge). Target genes are detected by a second nanoparticle-conjugated probe. Sample processing took about 2.5 h. The extraction tray, an utility track, and a test cartridge were loaded into the Verigene processor. Depending on the result of Gram staining, a volume of 350 μL or 700 μL medium from BC bottles was pipetted into the sample well of the extraction tray of a BC-GP or BC-GN assay unit, respectively. After processing, the microarray slide was separated from the test cartridge and transferred into the Verigene reader (Version 2.2.1b2/304-0). Array reading and analysis took about 1 minute.

The BC-GP array identifies *S. aureus*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus anginosus*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, and *Enterococcus faecium* on the species level. *Staphylococcus* spp. and *Streptococcus* spp., as well as *Listeria* spp. and *Micrococcus* spp., are detected on the genus level. Resistance determinants include *mecA*, *vanA*, and *vanB*. The BC-GN array identifies *E. coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, and *Serratia marcescens* on the species level and *Acinetobacter* spp., *Citrobacter* spp., *Enterobacter* spp., and *Proteus* spp. on the genus level. As resistance genes, $bla_{\text{CTX-M}}$, bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, and bla_{OXA} are targeted.

2.3. Species identification and AST in subcultures

Isolates sampled from positive BCs were identified by Vitek MS or Vitek 2 (bioMérieux, Nürtingen, Germany). Preliminary resistance patterns by disk diffusion assay were evaluated according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. Final AST was performed using Vitek 2 and MIC interpretation according to EUCAST criteria. Production of extended-spectrum beta-lactamases (ESBLs) was verified by the combination disk method using cefotaxime (CTX) versus CTX + clavulanic acid (CV) and cefpodoxime (CPD) versus CPD + CV. For staphylococci, cefoxitin disk diffusion assay was performed to confirm beta-lactam resistance.

2.4. Statistical analysis on antibiotic resistance

Antibiotic resistance data from ICUs were analyzed using the statistics tool 15.10.0 of the laboratory software Swisslab (version 2.12.3.009992; Berlin, Germany).

3. Results

A total of 275 positive BC were analyzed using the Verigene BC-GP and BC-GN test. Four Verigene tests (1.5%) reported an invalid result. Verigene identification and resistance gene determination were compared with routine diagnosis species identification and AST.

Table 1 summarizes the Verigene results on Gram-positive bacteria. A total of 178/190 (93.7%) of isolates were correctly identified to the species or genus level by Verigene BC-GP, compared to Vitek MS results. Nine bottles (4.7%) had a positive blood culture but negative Verigene BC-GP result, and 3 Verigene BC-GP tests (1.6%) were invalid. Two *S. aureus* cases that were tested positive for *mecA* by Verigene were subsequently confirmed as MRSA by final AST. All *S. epidermidis* isolates with a positive *mecA* signal were confirmed as oxacillin resistant by AST. No information on *mecA* is provided when CoNS are only identified on the genus level. It should be noted that more than 50% of CoNS showed resistance to oxacillin by AST. In 10 cases, Verigene detected *Streptococcus* spp. on the genus level. Subsequent species identification of the isolates by Vitek MS revealed *Streptococcus mitis* (4), *Streptococcus dysgalactiae* (3), *Streptococcus gallolyticus* (1), *Streptococcus anginosus* (1), and *Granulicatella adiacens* (1). Four Verigene signals for *S. pneumoniae* could be confirmed by subsequent microbiological diagnosis. *E. faecalis* and *E. faecium* infections were also correctly identified by Verigene BC-GP. In cases of *E. faecium*, there were 10 positive *vanA* signals and 1 for *vanB*, corresponding to subsequent phenotypic AST. The mean time to positivity of BCs was about 12 h for *S. aureus* and *E. faecium*, 10 h for *S. pneumoniae*, and less than 10 h for streptococci and *E. faecalis*. The mean time to positivity for CoNS was more than 20 h, although the relationship of skin flora contamination in BCs and time to positivity for CoNS was not evaluated. Verigene tests were resulted in 3 h.

From 6 BC bottles with Gram-positive cocci, mixed subcultures were obtained. Mixed infections could be differentiated by Verigene

Table 1
Identification of Gram positives and resistance genes from positive blood cultures by BC-GP array in comparison to subsequent ASTs.

Verigene			Antibiogram		Mean time to positivity of BC (SD, h)
Identification (n)	*mecA*, n (%)	*vanA*/*vanB*, n (%)	FOX resistant, n (%)	VAN resistant, n (%)	
S. aureus (23)	2 (8.7)		2 (8.7)	0	11.5 (6.5)
S. epidermidis (85)	67 (78.8)		67 (78.8)	0	21.3 (9.5)
Staphylococcus spec. (24)	N/A		14 (58.3)	0	21.2 (11.6)
Streptococcus spec. (10)					7.6 (7.1)
S. pneumoniae (4)					10.9 (1.7)
E. faecalis (10)		0		0	8.6 (6.1)
E. faecium (21)		11 (52.4)		11 (52.4)	11.7 (6.2)
L. monocytogenes (1)					7.5

FOX = cefoxitin; VAN = vancomycin; N/A = not applicable.

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