Direct Eazyplex® Amplex Assay of Blood Culture Broths for Rapid Detection of Carbapenemases, Extended -spectrum ß-lactamases and *mec* Genes

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INTRODUCTION

The spread of multidrug-resistant (MDR) Gram negative bacilli producing extended-spectrum ß-lactamases (ESBLs) and carbapenemases as well as methicillin-resistant *Staphylococcus aureus* represents an emerging public-health concern, with the attendant loss of many previously effective antimicrobial therapeutic agents. As a result, appropriate empirical antibiotic therapy becomes even more necessary with regard to such dangerous isolates . Mortality, particularly in bloodstream infections (BSIs) has been shown to be markedly higher when inadequate or ineffective antimicrobial therapy is administered as empirical therapy (or delayed by 72 h) pending the results of antimicrobial susceptibility testing. The time-consuming process of isolate identification and characterization with conventional culture-based methods has also been improved, thanks to the development of faster technologies allowing identification (ID) of the infecting pathogen directly from BC bottles. Among them, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) emerged as a reliable, cost-effective, time-saving tool for routine ID of bacteria and yeasts causing BSIs (1-4). Various commercial BC-based nucleic acid testing can shorten the ID time of pathogens and resistance genes and have added value for antibiotic stewardship decisions by decreasing time to effective antibiotic therapy (5,6). The eazyplex® system (Amplex Diagnostics GmbH, Germany, Biolife italiana SRL, Milano Italia) is a new commercially available molecular method for rapid detection of carbapenemases, CTX-M ESBL, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *mecA* and *mecC* genes. The clinical experience, to date, shows that this technology correctly identifies the presence of resistance genes with high accuracy when applied directly from single bacterial colonies grown on agar plates (7-9).

Here we evaluated the performance of eazyplex® Amplex assay for detection of *bla* genes belonging to the CTX-M,VIM, OXA and KPC types, and *mecA* and *mecC* genes directly from BC positive bottles in comparison to the conventional culture-based approach.

MATERIALS AND METHODS

Setting. The study was conducted between December 2014 and June 2015 in the clinical microbiology laboratory of the Catholic University Medical Center, Policlinico Gemelli, Rome. Blood samples were inoculated in BACTEC bottles (Becton Dickinson Instrument Systems, Sparks, Md.) or Bact/Alert bottles (bioMérieux, Marcy l'Etoile, France) and incubated up to five days in the BACTEC FX or Bact/Alert automated blood culture (BC) instruments. Broth aliquots from each positive bottle were collected for standard method (Gram staining, culture-based method and direct MALDI-TOF ID of BC broths) and Eazyplex® Amplex assay when *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Staphylococcus aureus* were recognized by direct MALDI BioTyper.

Blood processing. When the growth index of a bottle was positive, broth aliquots were collected for standard ID studies, which entailed Gram staining (the results of which were immediately reported to the patient's physician), direct MALDI-TOF assay with the Bruker Biotyper (MALDI BioTyper, Bruker Daltonik GmbH, Leipzig, Germany) and conventional testing routine subculture. Only a single blood culture bottle was used for each individual patient's organism.

Reference culture-based method. Identification of isolates was obtained by MALDI-TOF analysis of culture samples, supplemented when necessary with additional biochemical methods and/or 16S rRNA gene sequencing. The *in vitro* susceptibility of the isolates was assessed with the Vitek 2 system (bioMérieux) or the Sensititre broth microdilution method (Trek Diagnostic Systems, Cleveland, OH). Results were interpreted in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints (10). Phenotypic testing for ESBL and carbapenemases production was performed according to the recommended method of the EUCAST guidelines (11). The presence of carbapenemase genes of bla_{CTX-M}, bla_{KPC}, bla_{NDM}, bla_{VIM} and bla_{OXA-48} types, and *mec* genes was investigated as previously described (12-16).

Direct Broth Assay for isolate identification. MALDI ID was obtained using a broth aliquot of 8 ml and a formic acid extraction procedure (FEP) as previously described (Fiori). Mass spectrometry was performed with the Microflex MALDI-TOF MS (Bruker Daltonics GmbH). Captured spectra were analyzed with MALDI BioTyper 3.1 software and compared with those in the BioTyper database (Bruker Daltonics GmbH). Matches were ranked by log identification scores, which ranged from 0 to 3, and the match with the highest score was used for species ID. ID scores of ≥ 1.8 were considered valid to the species level.

Eazyplex assay for spiked blood cultures. Clinical samples were prepared for assay with the protocol described below, which had been defined and validated in preliminary testing of 40 simulated BCs inoculated with 10 ml of fresh, unprocessed blood from healthy volunteers and 10^2 bacterial cells. Clinical strains tested included 10 *K. pneumoniae* strains and 1 *E. coli* strains producing known carbapenemases belonging to ß-lactamase class A (KPC-2 and -3 types), class B (VIM-1 and NDM-1), and class D (OXA-48), 9 CTX-M (CTX-M 1, CTX-M-15, CTX-M-27) producing *Escherchia coli* isolates, 10 *Acinebater baumannii* harboring bla_{OXA-23} genes and 10 *mecA*-positive *Staphylococcus aureus*. Each test was performed in duplicate.

Eazyplex® assay for clinical blood cultures. Testing of BC broths growing *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Staphylococcus aureus* , as determined by MALDI ID, was performed according to the protocol suggested by the manufacturer. Briefly, 25 µl of BC broth was suspended in 500 µl RALF. After the initial step the suspension was heated to 99 °C for 2 min and then centrifugated at 4000 rpm for 30 sec. The supernatant was collected, and a 25-µl sample was applied into each tube of strip. Results for the analysis were provided by the software automatically. The following panels were used: eazyplex® Superbug CRE for *E. coli* and *K. pneumoniae*, eeazyplex® Superbug complete for *A. baumannii*, and eazyplex® MRSA for *S. aureus*.

Data analyses. Eazyplex® Amplex results were compared to those obtained with the comparison molecular method. Results were then classified as *concordant* or *not concordant* (when eazyplex® or furnished no or different results).

RESULTS

Comparison method results. A total of 260 positive BCs containing 211 Gram-negative [*E. coli* (*n*=127), *K. pneumoniae* (*n*=72) and *A. baumannii* (*n*=12)] e 49 *S. aureus* were included in the study. Phenotypic testing revealed that 41 isolates were carbapenem-resistant (25 *K. pneumoniae*, 2 *E. coli* and 11 *A. baumannii*), 59 (7 *K. pneumoniae*, and 52 *E. coli*), all ESBL producers, were not susceptible to one or more oxyminicephalosporins , and 30 *S. aureus* were methicillin resistant. The characterization of the 260 identified pathogens is presented in Table 1.

Table 1. Organisms used for evaluating the sensitivity and specificity of the eazyplex ® Amplex assay

Microorganisms (*n*) [a]	Genes							
	$bla_{CTX-M\ gr.1}$	$bla_{CTX-M\ gr.9}$	bla_{KPC}	bla_{NDM}	bla_{VIM}	$bla_{OXA-48\ like}$	$bla_{OXA-23\ like}$	*mecA*
E. coli (137)	40	21	2	0	0	1		
K. pneumoniae (82)	6	1	28	1	3			
A. baumannii (22)							16	
S. aureus (59)								35

[a] Include 40 simulated and 260 clinical blood cultures

Eazyplex® Amplex results. In preliminary testing, carried out with the 40 spiked BCs, the eazyplex® assay display 100% of concordance with the comparison method. The reproducibility was excellent, with no false negatives and no false positives in the replicate test. As regard the direct clinical BC testing, the Eazyplex® assay correctly detected all isolates with KPC, VIM, OXA-23, and CTX-M enzymes with no false-negatives, even in three samples harboring both KPC and VIM (Figure 1). False positive detection of $bla_{CTX-M\ gr\ 1}$ genes occurred in 4 isolates of *E. coli*. Using Eazyplex® MRSA Panel, the *mecA* gene was correctly identified for 30 methicillin-resistant *S. aureus* organisms. A methicillin-susceptible isolate of *S. aureus* was misclassified as resistant in a polymycrobial BC broth that grew a methicillin-susceptible S. *aureus* isolate and a methicillin-resistant *Staphylococcus hominis* isolate, not identified as such by the panel (Figure 2).

As shown in Table 2 , specificities and sensitivities of 100% were recorded for bla_{VIM}, bla_{KPC}, and bla_{OXA-23} genes, whereas for the $bla_{CTXM\ gr\ 1}$ gene, they were 100% and 97.7%, respectively. The eazyplex® assay furnished results in 1 h, compared with at least 18 h for antimicrobial susceptibility testing.

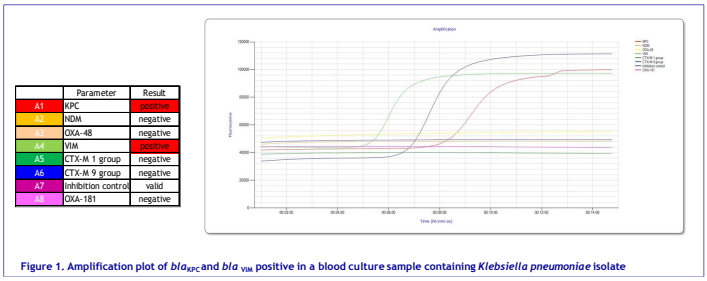

Figure 1. Amplification plot of bla_{KPC} and bla_{VIM} positive in a blood culture sample containing *Klebsiella pneumoniae* isolate

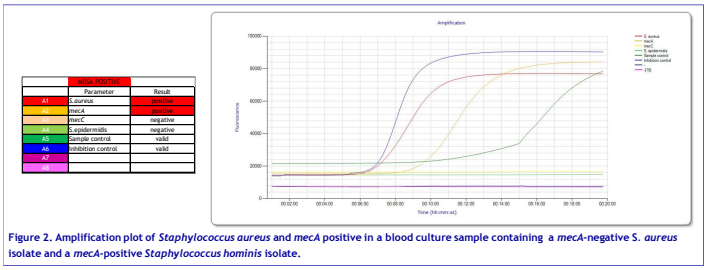

Figure 2. Amplification plot of *Staphylococcus aureus* and *mecA* positive in a blood culture sample containing a *mecA*-negative *S. aureus* isolate and a *mecA*-positive *Staphylococcus hominis* isolate.

Table 2. Comparison of eazyplex® Amplex assay results to those of reference method

Eazyplex® results	Comparison with reference results			
	Sensitivity (%) (TP/TP+ FN)	Specificity (%) (TN/TN+FP)	PPV (%) (TP/TP+FP)	NPV (%) (TN/TN+FN)
$bla_{CTX-M\ gr.1}$	100	97.7	92.0	100
$bla_{CTX-M\ gr.9}$	100	100	100	100
bla_{KPC}	100	100	100	100
bla_{VIM}	100	100	100	100
$bla_{OXA-23\ like}$	100	100	100	100
mecA	100	94.7	97.6	100

CONCLUSIONS

Infections caused by MDR organisms have a significant impact on mortality rates and hospital costs. Therefore, microbiological data must be reported to the physician as soon as possible, especially in high-risk cases. The application of rapid molecular diagnostic techniques to the ID and characterization of pathogens promises to improve outcomes as a result of more timely and effective antibiotic therapy. Although further studies are needed to evaluate its overall performance, our experience with this large series of BCs indicates that the eazyplex® Amplex system is a reliable time-saving tool for routine ID of genes conferring resistance to ß-lactams directly from BCs after species ID is furnished by MALDI BioTyper.

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