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Short report

Detection of antibiotic resistance genes from blood cultures: performance assessment and potential impact on antibiotic therapy management

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SUMMARY

Molecular assays may constitute a valid method for timely prediction of antimicrobial resistance and optimization of empirical antibiotic therapies. This study assessed ELITe MGB assays of blood cultures to detect the main carbapenemase and extended-spectrum beta-lactamase (ESBL) genes, *Staphylococcus aureus* and *mec* genes in less than 3 h. Excellent agreement was found between the results of genotypic and conventional phenotypic approaches.

Retrospective analysis of medical records revealed that approximately 50% of bloodstream infections caused by ESBL-producing Enterobacteriaceae, carbapenemase-producing Enterobacteriaceae or meticillin-resistant *S. aureus* were initially treated with inactive drugs. Overall, 36.3% of patients could have been treated with appropriate therapy at least 24 h earlier if molecular data had been used.

Introduction

Bloodstream infection (BSI) is a serious clinical condition which can lead to sepsis, thereby increasing the burden of morbidity and mortality. Among patients with BSI, the empirical treatment choice is complicated by the growing threat of antimicrobial resistance. The worldwide spread of multi-drug-resistant bacteria has reduced the number of effective drugs, and has increased attributable mortality, length of stay and hospital costs [1–3]. Delaying the administration of appropriate antibiotic therapy has also been associated with an increase in hospital mortality [4,5]. Therefore, the everyday challenge for clinicians who treat patients with BSI is to choose the right antibiotic regimen based on their epidemiological knowledge, avoiding the unnecessary use of broad-spectrum antibiotics. In this regard, the results of microbiological testing play a crucial role in optimizing targeted therapies.

Although matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) has greatly reduced the time needed for bacterial identification, traditional culture-based approaches require 24–72 h before providing antibiotic susceptibility profiles for patients with BSI.

Several molecular diagnostic kits capable of detecting different resistance mechanisms are commercially available. In this regard, the choice of the most appropriate molecular assay for rapid diagnostic work flow from blood cultures should consider laboratory organization as well as local epidemiology of resistance mechanisms.

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ELITe InGenius (ELITechGroup Molecular Diagnostics, Turin, Italy) is an integrated system that automatically performs nucleic acid extraction, real-time polymerase chain reaction (PCR) and interpretation of results in less than 3 h. The carbapenem-resistant Enterobacteriaceae (CRE) and extended-spectrum beta-lactamase (ESBL) ELITe minor groove binder (MGB) kits are qualitative multiplex real-time PCR assays based on MGB technology intended for detection of the most prevalent carbapenemase and ESBL encoding genes in Enterobacteriaceae, respectively. The CRE ELITe MGB kit can detect $bla_{\text{KPC-like}}$, metallo β-lactamase (i.e. $bla_{\text{NDM-like}}$, $bla_{\text{VIM-like}}$, $bla_{\text{IMP-like}}$) and $bla_{\text{OXA-48-like}}$ genes, and the ESBL ELITe MGB kit can identify $bla_{\text{CTX-Ms}}$ genes belonging to group 1 (including CTX-M-15) and group 9 (including CTX-M-14) [6]. The MRSA/SA ELITe MGB kit is a multiplexed assay designed to simultaneously detect a conserved sequence of the *S. aureus*, *mec*A gene and its homologue variant $mecA_{\text{LGA251}}$ (*mec*C).

The goal of this study was to evaluate the diagnostic performance of ELITe MGB assays to detect antibiotic resistance genes directly from blood cultures, and to assess the potential clinical impact of molecular results on antibiotic therapy management.

Methods

Conventional microbiological routine

The Microbiology and Virology Unit of Azienda Ospedaliero Universitaria Città della Salute e della Scienza di Torino, Turin, Italy is routinely open 7 days per week from 08:00 to 18:00 h. FN Plus resin blood culture bottles (bioMérieux, Marcy l'Ètoile, France) were incubated in the BacT/ALERT 3D system at various times each day. Positive blood culture bottles were subjected to Gram staining and subculture on appropriate solid medium when testing positive during routine laboratory working hours. All blood cultures that were positive before 11:00 h had a 5-h subculture on blood agar prior to rapid identification by MALDI-TOF MS. Blood cultures that tested positive at other times were subcultured and MALDI-TOF MS analysis was performed the following day.

Likewise, according to the time of blood culture positivity, antimicrobial susceptibility testing was performed on either early or overnight subcultures using the Microscan WalkAway plus system according to the manufacturer's instructions (Beckman Coulter, Brea, CA, USA). Carbapenemase production in Enterobacteriaceae was screened for using the Mastdiscs combi Carba plus disc system (Mast Group Ltd, Bootle, UK).

All newly available identification and susceptibility results were promptly communicated to the clinicians and uploaded on to the laboratory information system.

Study design

Over a six-month period, routine microbiological practices were performed in parallel with ELITe MGB assays on 192 positive blood cultures sampled from 192 patients with clinical suspicion of BSI admitted to the intensive care unit (N=66), onco-haematology unit (N=24) or internal medicine ward (N=102). Patients with a previous recent BSI (<20 days) were excluded.

The CRE ELITe MGB kit and ESBL ELITe MGB kit were assayed on 112 blood culture bottles positive for Gram-negative bacilli, and the MRSA/SA ELITe MGB kit was tested on 80 blood culture bottles with direct MALDI-TOF MS identification of *Staphylococcus aureus* (N=54), coagulase-negative staphylococci (CoNS, N=23) or Streptococcaceae (N=3).

Molecular results were first compared with conventional results to estimate the agreement between genotypic and phenotypic data. Subsequently, a retrospective analysis of the therapeutic antimicrobial management at the time of positive blood culture and final conventional results was performed in order to evaluate the potential contribution of molecular assays to early optimization of empirical antibiotic therapy among septic patients with carbapenemase- and/or ESBL-expressing Enterobacteriaceae or *S. aureus* infection. In this analysis, empirical therapy was deemed adequate if the causative bacterial strains were susceptible *in vitro* to at least one prescribed drug. A switch in targeted antibiotic therapy was defined as any change in antibiotic regimen, including escalation or de-escalation therapy but not simplification actions.

Results

The mean times to blood culture positivity were 12.1 ± 6.5 and 13 ± 10.1 h for Gram-negative and Gram-positive BSIs, respectively. The average times to obtain antimicrobial susceptibility testing results from the time of blood culture collection were 41 h (range 28–64 h) and 38 h (range 27–76 h) for Gram-positive cocci and Gram-negative bacilli, respectively. Turnaround times ranged from one to two days, depending on the time of detection of blood culture positivity. This length of time further increased in patients with Gram-negative polymicrobial infections. The average ELITe MGB assay test time was 2.5 h.

The comparison between molecular and phenotypic results is provided in Table I. The $bla_{\text{CTX-Ms}}$ gene was detected in 34 of 35 ESBL-producing Enterobacteriaceae positive blood cultures. The only negative case might be explained by the expression of other beta-lactamases (i.e. TEM, SHV, AmpC) or CTX-M variants not targeted by the assay. Furthermore, *bla* genes were detected correctly in 24 of 24 carbapenemase-producing Enterobacteriaceae positive samples, with eight of them also harbouring $bla_{\text{CTX-Ms}}$.

In the non-carbapenemase/non-ESBL-producing Enterobacteriaceae BSI group, no resistance gene expression was detected, but a case of *Klebsiella oxytoca* harbouring $bla_{\text{CTX-M}}$ was found.

Among Gram-positive BSIs, the MRSA/SA ELITe MGB kit correctly identified *S. aureus* and *mec* targets. One case of false-positive *S. aureus* detection was obtained in the CoNS infection group with a real-time cycle threshold >35, suggesting that it was probably the result of contamination or non-specific amplification.

Retrospective analysis of medical records showed that adequate empirical antibiotic therapy rates were 54.3%, 54.2%, 41.7% and 100% for ESBL-producing Enterobacteriaceae, carbapenemase-producing Enterobacteriaceae, meticillin-resistant *S. aureus* (MRSA) and meticillin-susceptible *S. aureus* (MSSA) BSIs, respectively. The most common inadequate empirical antibiotic treatments were: third- or fourth-

Table I
Agreement between molecular and conventional results

Identification and phenotypic conventional results	CRE ELITe MGB kit			ESBL ELITe MGB kit	MRSA/SA ELITe MGB kit		Agreement (%)
	bla$_{KPC-like}$	*bla*$_{NDM-IMP-VIM-like}$	*bla* $_{OXA-like}$	*bla*$_{CTX-M}$	*S. aureus* specific sequence	mecA/C	
Ep-EB (*N*=35)	0/35	0/35	0/35	34/35	-	-	97.1
Escherichia coli (*N*=29)							
Klebsiella pneumoniae (*N*=5)							
Proteus mirabilis (*N*=1)							
Cp-EB (*N*=24)	22/22	0/0	2/2	8/24	-	-	100
Klebsiella pneumoniae KPC (*N*=22)							
Klebsiella pneumoniae OXA-48 (*N*=2)							
nCp-EB/nEp-EB/ NFB (*N*=53)	0/53	0/53	0/53	1/53	-	-	98.1
Escherichia coli (*N*=20)							
Klebsiella pneumoniae (*N*=5, 2 in co-infection)							
Pseudomonas aeruginosa (*N*=10, 1 in co-infection)							
Enterobacter cloaceae (*N*=1 in co-infection)							
Proteus mirabilis (*N*=2)							
Serratia marcescens (*N*=3, 1 in co-infection)							
Enterobacter aerogenes (*N*=2, 1 in co-infection)							
Klebsiella oxytoca (*N*=2)							
Morganella morganii (*N*=1)							
Acinetobacter baumanni complex (*N*=1)							
Pseudomonas putida (*N*=1)							
Stenotrophomonas maltophilia (*N*=1, in co-infection)							
Citrobacter freundi (*N*=1, in co-infection)							
MRSA (*N*=24)	-	-	-	-	24/24	24/24	100
MSSA (*N*=30)	-	-	-	-	30/30	30/30	100
CoNS or Streptococcaceae (*N*=26)	-	-	-	-	1/26	Not evaluated	96.1

CRE, carbapenem-resistant Enterobacteriaceae; ESBL, extended-spectrum beta-lactamase; Cp-EB, carbapenemase-producing Enterobacteriaceae; Ep-EB, extended-spectrum beta-lactamase-producing Enterobacteriaceae; nCp-EB, non-carbapenemase-producing Enterobacteriaceae; nEp-EB, non-ESBL-producing Enterobacteriaceae; NFB, non-fermenting bacilli; MSSA, meticillin-susceptible *Staphylococcus aureus*; MRSA, meticillin-resistant *Staphylococcus aureus*; CoNS, coagulase-negative staphylococci.

generation cephalosporins for ESBL-producing Enterobacteriaceae (10/16, 62.5%); beta-lactams or beta-lactams/beta-lactamase inhibitors (BLBLIs) alone, or in combination with other drugs, for carbapenemase-producing Enterobacteriaceae (6/11, 54.5%); and BLBLIs for MRSA (7/14, 50%). Twenty of 28 empirical antibiotic therapies for MSSA BSIs were adequate; two patients were untreated until microbial identification results were available.

Overall, 67 switches in targeted therapy were executed as soon as antimicrobial susceptibility testing results were available (Table II). Most switches for Gram-negative infections involved escalation of therapy, whereas 10 of 20 adequate broad-spectrum regimens were de-escalated for MSSA.

Discussion

Excellent agreement was found between the results obtaining using genotypic and traditional phenotypic approaches: 97.7%, 100% and 98.7% for the ESBL, CRE and MRSA/SA ELITe MGB kits, respectively. However, sensitivities of the CRE and ESBL ELITe MGB assays may differ worldwide, depending on the local predominant mechanisms of resistance.

This study also assessed the utility of integrating molecular assays into routine blood culture work flow to provide timely information about antibiotic resistance. The findings demonstrate the potential for rapid diagnostics to assist with antimicrobial stewardship, both in ensuring that patients with multi-drug-resistant pathogens are treated effectively and that there is de-escalation of treatment for other patients [7]. This study found that 36.3% (41/113) of patients could have been treated with appropriate therapy at least 24 h earlier based on the results of the ELITe MGB assays.

However, there are several important considerations that need to be tested in larger studies. For example, it is not known whether clinicians would have the confidence to change antibiotic therapy (and especially to de-escalate therapy) on the basis of molecular test results rather than the full antibiotic susceptibility profile. Certainly, molecular testing cannot be considered a stand-alone assay that replaces conventional

Table II
Switches in antibiotic therapy after the availability of antimicrobial susceptibility testing results

	Empirical therapy	Targeted therapy
Ep-EB BSIs (*N*=23)	Inadequate (*N*=13)	
	TZP (*N*=4), CRO (*N*=3), CRO/DAP (*N*=2), CRO/AZM (*N*=1), FEP (*N*=1), AMC (*N*=1), CIP (*N*=1)	MEM (*N*=10), ETP (*N*=2), TZP (*N*=1)
	Adequate (*N*=10)	
	TZP (*N*=7), TZP/AMK (*N*=1), TZP/VAN (*N*=1), TZP/CIP (*N*=1)	MEM (*N*=7), ETP (*N*=1), TZP/AMK (*N*=1), SXT (*N*=1)
Cp-EB BSIs (*N*=15)	Inadequate (*N*=11)	
	TZP (*N*=2), TZP/DAP (*N*=1), CRO (*N*=1), SAM/DAP (*N*=1), FEP/LVX (*N*=1), CAZ/TMN (*N*=1), IPM/LZD (*N*=1), CZT/TMN/LZD (*N*=1), MEM (*N*=1), DAP (*N*=1)	MEM/TGC/AM (*N*=3), MEM/TGC (*N*=2), MEM/ETP/AMK (*N*=1), GEN/TGC/FOS (*N*=1), CST/TMN/FOS (*N*=1), MEM/GEN (*N*=1), GEN/FOS (*N*=1), TGC/FOS (*N*=1)
	Adequate (*N*=4)	
	MEM/TGC/CST (*N*=1), MEM/GEN/TGC/LVX (*N*=1), MEM/AMK (*N*=1), TGC/GEN (*N*=1)	CZA (*N*=1), MEM/GEN/CST/LVX (*N*=1), CZA/GEN (*N*=1), MEM/GEN (*N*=1),
MRSA BSIs (*N*=17)	Inadequate (*N*=13)	
	TZP (*N*=5), CRO (*N*=3); TZP/LVX (*N*=2), TZP/CIP (*N*=1), CRO/LVX (*N*=1), AMC (*N*=1)	VAN (*N*=3), DAP (*N*=2), DAP/TZP (*N*=3), VAN/TZP (*N*=1), VAN/GEN (*N*=1), TEC/TZP (*N*=2), TEC/MEM (*N*=1)
	Adequate (*N*=4)	
	VAN/TZP (*N*=2), VAN/MEM (*N*=1), VAN (*N*=1)	LZD (*N*=1), DAP/MEM (*N*=1), DAP (*N*=1), DAL (*N*=1)
MSSA BSIs (*N*=12)	Adequate broad spectrum (*N*=11)	
	MEM/VAN (*N*=5), TZP/VAN (*N*=3), VAN (*N*=2), MEM/DAP (*N*=1)	OXA (*N*=5), CRO (*N*=3), AMC (*N*=1), AMC/LZD (*N*=1), CZL (*N*=1)
	Adequate narrow spectrum (*N*=1)	
	LVX (*N*=1)	LVX/VAN (*N*=1)

Cp-EB, carbapenemase-producing Enterobacteriaceae; BSIs, bloodstream infections; Ep-EB, extended-spectrum beta-lactamase-producing Enterobacteriaceae; MSSA, meticillin-susceptible *Staphylococcus aureus*; MRSA, meticillin-resistant *Staphylococcus aureus*; TZP, piperacillin/tazobactam; CRO, ceftriaxone; DAP, daptomycin; AZM, azithromycin; FEP, cefepime; AMC, amoxicillin-clavulanate; CIP, ciprofloxacin; MEM, meropenem; ETP, ertapenem; AMK, amikacin; VAN, vancomycin; SXT, trimethoprim-sulphamethoxazole; DAP, daptomycin; SAM, ampicillin/sulbactam; LVX, levofloxacin; TMN, tobramycin; IMP, imipenem; LZD, linezolid; CZT, ceftolozane/tazobactam; AM, aminoglycoside; TGC, tygecicline; GEN, gentamycin; FOS, fosfomycin; CST, colistin; CZA, ceftazidime/avibactam; TEC, teicoplanin; DAL, dalbavancin; OXA, oxacillin; CZL, cefazolin.

antibiotic susceptibility testing. First, resistance genes can be inactivated or downregulated, and consequently are not necessarily associated with phenotypic resistance. Second, phenotypic resistance in Gram-negative bacteria is complicated further by the potential impact of non-beta-lactamase-mediated resistance mechanisms such as efflux pumps and porin loss. Thus, for Gram-negative bacteria, molecular assays have more potential for ensuring adequate antimicrobial cover than for facilitating de-escalation of treatment. Another consideration around implementation of molecular assays in blood culture diagnostics is the need for cost—benefit analysis.

In conclusion, these findings confirm that molecular assays may constitute a valid approach for effective prediction of antimicrobial resistance and optimization of empirical antibiotic therapies; a fast-track work flow for blood cultures could be readily implemented into all laboratories provided that they have relevant expertise, but further studies of clinical and cost-effectiveness are required.

Conflict of interest statement
None declared.

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