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Accuracy of the ELITe MGB assays for the detection of carbapenemases, CTX-M, *Staphylococcus aureus* and *mec*A/C genes directly from respiratory samples

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SUMMARY

Introduction: Bacterial lower respiratory tract infections (BLRTI) may represent serious clinical conditions which can lead to respiratory failure, intensive care unit admission and high hospital costs. The detection of carbapenemase- and extended-spectrum β-lactamase (ESBL)-producing Enterobacterales, as well as meticillin-resistant *Staphylococcus aureus* (MRSA), has become a major issue, especially in healthcare-associated infections. This study aimed to determine whether molecular assays could detect genes encoding carbapenemases, ESBL and MRSA directly from respiratory samples in order to expedite appropriate therapy and infection control for patients with BLRTI.

Methods: The carbapenem-resistant enterobacterales (CRE), ESBL and MRSA/SA ELITe MGB assays were performed directly on 354 respiratory specimens sampled from 318 patients admitted with BLRTI. Molecular results were compared with routine culture-based diagnostics results.

Results: Positive (PPV) and negative (NPV) predictive values of the CRE ELITe MGB kit were 75.9% [95% confidence interval (CI) 60.3–86.7] and 100%, respectively. PPV and NPV of the ESBL ELITe MGB kit were 80.8% (95% CI 63.6–91.0) and 99.1% (95% CI 96.6–99.8), respectively. PPV and NPV of the MRSA/SA ELITe MGB kit were 91.7% (95% CI 73.7–97.7)/100% and 98.3% (95% CI 89.8–99.3)/96.8% (95% CI 81.6–99.5), respectively.

Discussion: Validity assessment of molecular assays detecting the main antibiotic resistance genes directly from respiratory samples showed high accuracy compared with culture-based results. Molecular assays detecting the main carbapenemase, ESBL, *S. aureus* and meticillin resistance encoding genes provide an interesting tool with potential to expedite optimization of antibiotic therapy and infection control practices in patients with BLRTI.

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Introduction

Bacterial lower respiratory tract infections (BLRTI), including bronchitis, pneumonia and infectious exacerbations in chronic lung disease, can represent serious clinical conditions which can lead to respiratory failure, intensive care unit admission, prolonged admission and high hospital costs [1–7]. Patients admitted with BLRTI are frequently prescribed broad-spectrum empirical antibiotics; timely identification of pathogens is necessary to support antibiotic stewardship and therefore reduce the risk of selection of antibiotic resistance. Detection of carbapenemase- and extended-spectrum β-lactamase (ESBL)-producing Enterobacterales and meticillin-resistant *Staphylococcus aureus* (MRSA) has important implications for both antimicrobial therapy and infection control, especially in healthcare-associated infections.

A positive microbiological diagnosis in BLRTI may only be made in approximately 30% of cases [8] and, as commensal and colonizing micro-organisms complicate the analysis, conventional phenotypic diagnostics for respiratory samples typically takes approximately 48–72 h, hampering antimicrobial stewardship. Molecular tests for genes encoding carbapenemases, ESBLs and MRSA have been successfully applied directly to blood culture samples [9,10] but there is limited published evidence about their performance on respiratory samples [11–15].

The ELITe InGenius (ELITechGroup Molecular Diagnostics, Turin, Italy) platform 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 enterobacterales (CRE) and ESBL ELITe MGB kits are qualitative multiplex real-time PCR assays for the detection of the most prevalent carbapenemase- and ESBL-encoding genes in Enterobacterales. The CRE ELITe MGB kit detects $bla_{\text{KPC-like}}$, 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 detects $bla_{\text{CTX-M}}$ genes belonging to groups 1 (including CTX-M-15) and 9 (including CTX-M-14). The MRSA/SA ELITe MGB kit is a multiplex assay that simultaneously detects a conserved sequence of the *S. aureus*, *mec*A gene and its homologue $mecA_{\text{LGA251}}$ (*mec*C).

The aim of this study was to evaluate the performance of CRE, ESBL and MRSA/SA ELITe MGB assays directly on respiratory samples, including comparing real-time PCR cycle threshold (Ct) values with bacterial load quantification.

Methods

Routine culture-based microbiological diagnostics

At the Microbiology and Virology Unit of Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino in Turin, Italy, respiratory samples were subjected to Gram staining and culture on appropriate solid medium at the time of arrival at the laboratory. Matrix-assisted laser desorption/ionization–time of flight mass spectrometry analysis was used for bacterial identification, and antimicrobial susceptibility testing was carried out on overnight subcultures using Microscan WalkAway plus system (Beckman Coulter, Brea, CA, USA) according to the manufacturer's instructions. Antimicrobial susceptibilities were interpreted according to EUCAST breakpoints as updated in 2019 [16]. The Total ESBL Confirm kit (Rosco, Taastrup, Denmark) was used to identify ESBL production if cefotaxime and/or ceftazidime minimal inhibitory concentrations (MICs) were > 1 mg/L. The Mastdiscs combi Carba plus disc system (Mast Group Ltd, Bootle, UK) was used to assess carbapenemase producers when meropenem MIC was > 0.125 mg/L. Detection of carbapenem resistance genes was performed using the Xpert Carba-R assay (Cepheid, Sunnyvale, CA, USA).

Specimen collection and study design

Respiratory samples included in the study were those submitted for standard of care bacterial culture from January to June 2019. They were selected at random based on sample type, integrity and amount of remnant specimen. Lower respiratory tract specimens included: sputum, tracheal aspirate (TA), bronchoaspirate (BA) and bronchoalveolar lavage (BAL). The administration of antibiotics before specimen collection was not assessed.

The ELITe MGB assays were performed directly on 354 respiratory specimens sampled from 318 patients. The CRE and ESBL ELITe MGB kits were assayed on sputum (N=7), TA (N=16), BA (N=16) and BAL (N=202), and the MRSA/SA ELITe MGB kit was tested on sputum (N=35), TA (N=25), BA (N=15) and BAL (N=38). Two-hundred microlitres of a 1:4 dilution in dithiothreitol solution (Sputasol, Oxoid Ltd, Basingstoke, UK), previously heated in a thermoblock at 90°C for 5 min, was used for sputum, TA and BA, whereas 200 μL of a 1:2 dilution in dithiothreitol solution was used for BAL. The ELITe MGB kits' internal control and positive and negative controls were used as described previously [10]. The total ELITe MGB assay test run time is 2 h 12 min with data analysis available immediately after the run. The total cost of the ELITe MGB assays includes reagents (approximately £25 per sample including DNA extraction), staff time and platform ELITe InGenius rental.

Molecular results were compared with routine culture-based microbiological diagnostics results to estimate the accuracy of genotypic analysis. Molecular results for the CRE, ESBL and MRSA/SA targets were interpreted as shown in Table I. All cycles with a Ct value >35 were considered negative for detectable signal. Presence of *S. aureus* and *mec*A/C targets at the same relative quantity (ΔCt between the two targets < 2) was considered indicative of MRSA (to mitigate against the potential for detection of meticillin-susceptible *S. aureus* together with meticillin-resistant coagulase-negative staphylococci).

Ct values were also compared with the quantitative culture results in order to maximize the potential clinical impact of the molecular results.

This study was conducted in accordance with the Declaration of Helsinki. Formal ethical approval was not required by the study centre's institutional review board as the samples were anonymized and de-identified before being obtained by the study team.

Statistical analysis

Accuracy, sensitivity, specificity and positive (PPV) and negative (NPV) predictive values of the CRE, ESBL and MRSA/SA

Table I
Interpretation of molecular results on respiratory samples for the detection of carbapenemase, extended-spectrum β-lactamase (ESBL), *Staphylococcus aureus* and meticillin resistance encoding genes

CRE ELITe MGB kit	ESBL ELITe MGB kit	Interpretation	Report
+	−	Carbapenemase encoding gene	KPC, NDM-IMP-VIM, OXA-48 DNA detected
+	+	Carbapenemase and ESBL encoding genes	Both KPC, NDM-IMP-VIM, OXA-48 DNA detected and CTX-M_s DNA detected
−	+	ESBL encoding gene	CTX-M DNA detected
−	−	Neither carbapenemase nor ESBL encoding genes	Neither KPC, NDM-IMP-VIM, OXA-48 nor CTX-M_s DNA detected
MRSA/SA ELITe MGB kit			
S. aureus	*mec*A/C		
+	+	ΔCt < 2, MRSA	MRSA DNA detected
+	+	ΔCt > 2, MSSA	MSSA DNA detected
+	−	MSSA	MSSA DNA detected
−	+	No *S. aureus*	No *S. aureus* DNA detected
−	−	No *S. aureus*	No *S. aureus* DNA detected

CRE, carbapenem-resistant enterobacterales; MSSA, meticillin-susceptible *S. aureus*; MRSA, meticillin-resistant *S. aureus*; Ct, cycle threshold. All cycles with a Ct value >35 were considered negative.

ELITe MGB kits with 95% confidence intervals (95% CI) were computed.

The Shapiro–Wilk test was performed to verify the normality of distribution of quantitative variables. Analysis of variance with Bonferroni's correction was carried out to assess whether significant differences in Ct values could be detected between quantitative culture groups [negative, 1000–10,000 colony-forming units (cfu)/mL and >25,000 cfu/mL for carbapenemase- and ESBL-producing Enterobacterales; negative, 1000–10,000 cfu/mL, 10,000–50,000 cfu/mL and >50,000 cfu/mL for *S. aureus*). *P*-values <5% were considered significant. All analyses were performed using Stata 14 (Stata Corp., College Station, TX, USA).

Results

Detection of carbapenemase, CTX-M and S. aureus and mecA/C genes

Table II shows the comparison between molecular and conventional phenotypic results. Among the 241 clinical specimens, $bla_{KPC\text{-}like}$ was detected in 29 (12%) specimens by the CRE ELITe MGB kit. Twenty-two (75.9%) of these samples were confirmed by culture. Five of the seven false-positive samples were from patients who became culture-positive for *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* (TA *N*=2; BA *N*=1; urine culture *N*=1; rectal swab *N*=1) in the subsequent 7 days. $bla_{CTX\text{-}M\text{-}like}$ was detected in 26 (10.8%) specimens by the ESBL ELITe MGB kit, of which 21 (80.8%) were confirmed by culture. Two of the five false-

Table II
Performance of the carbapenem-resistant enterobacterales (CRE), extended-spectrum β-lactamase (ESBL) and meticillin-resistant *Staphylococcus aureus* (MRSA)/*S. aureus* (SA) ELITe MGB kits on respiratory samples compared with conventional phenotypic results

CRE and ESBL ELITe MGB kits		Conventional phenotypic results: Respiratory samples *N*=241		Accuracy	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
		Positive	Negative					
$bla_{KPC\text{-}like}$	Positive	22	7	97.1%	100% (85.1–100)	96.8% (93.6–98.4)	75.9% (60.3–86.7)	100%
	Negative	0	212					
$bla_{CTX\text{-}M\text{-}like}$	Positive	21	5	97.1%	91.3% (73.2–97.6)	97.7% (94.7–99)	80.8% (63.6–91)	99.1% (96.6–99.8)
	Negative	2	213					
MRSA/SA ELITe MGB kit		Respiratory samples *N*=113		Accuracy	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
		Positive	Negative					
S. aureus	Positive	82	0	99.2%	98.8% (93.5–100)	100% (88.7–100)	100%	96.8% (81.6–99.5)
	Negative	1	30					
*mec*A/C	Positive	22	2	96.4%	95.7% (79–99.2)	96.7% (88.6–99.1)	91.7% (73.7–97.7)	98.3% (89.8–99.3)
	Negative	1	58					

PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

positive samples were from patients who had ESBL-producing *K. pneumoniae* (blood culture *N*=1; urine culture *N*=1) isolated in the subsequent 7 days. The corresponding cultures of the two false-negative samples grew ESBL-producing *K. pneumoniae* and ESBL-producing *Klebsiella oxytoca* at quantities of 25,000–50,000 cfu/mL. No other ESBL- or carbapenemase-producing Gram-negative bacteria were detected by either molecular or culture-based testing.

ELITe MGB kit detected *S. aureus* in 82 of 113 (72.6%) specimens, all of which were confirmed by culture. There was one false-negative sample which gave a Ct value of 38.2 and a semi-quantitative culture result of 10,000–50,000 cfu/mL. Among the 83 culture-positive *S. aureus* samples, the *mec*A/C target was found in 23. The two presumed false-positive samples by PCR may reflect the limitations of phenotypic detection of meticillin resistance [17] or the presence of mixed populations of bacteria. The false-negative sample showed ΔCt=2.61, and culture-based diagnostics showed a mixed population of MRSA and meticillin-resistant coagulase-negative staphylococci.

The Ct values determined by CRE, ESBL and MRSA/SA ELITe MGB kits were compared with the bacterial loads obtained by conventional culture-based approach (Figures 1 and 2, see online supplementary material). The mean Cts obtained by CRE and ESBL ELITe MGB kits were significantly different ($P<0.05$) between the culture-negative and >25,000 cfu/mL groups. With the MRSA/SA ELITe MGB kit, mean Cts for each quantitative culture group were significantly different ($P<0.05$), except between the 1000–10,000 cfu/mL and 10,000–50,000 cfu/mL groups.

Discussion

Conventional culture-based diagnostics has limitations in tackling the dissemination of multi-drug-resistant pathogens and in optimizing antibiotic therapy in patients with BLRTI in a timely manner. Molecular assays have the potential to perform a role as a more accurate and sensitive decision-making tool, expediting infection control practices and supporting efforts to curtail inappropriate antibiotic use [12,18,19].

This study represents one of the largest performance assessments of molecular assays detecting the main antibiotic resistance genes directly from clinical respiratory samples. High NPV but more variable PPV were found. Several molecular assays of microbiological respiratory diagnostics have focused on rapid pathogen identification but few data on rapid antibiotic resistance have been reported, other than for MRSA [15,20–22]. The CRE and ESBL ELITe MGB kits were particularly suited for the Italian and European epidemiology, as the selection of these samples represents a picture of the most prevalent carbapenemase- and ESBL-producing Enterobacterales [23]. However, the limited number of enzymes tested for would need to be considered if these tests were implemented into clinical practice. The study data indicate that the MRSA/SA ELITe MGB kit, when *S. aureus* and *mec* genes are detected at the same relative quantities in the presence of clinical signs of BLRTI, could be of value in guiding the need for anti-MRSA therapy.

The potential role of molecular assays in surveillance, infection control practices and early optimization of antibiotic therapy is well known [12,24,25]. In particular, rapid availability of molecular results can not only facilitate early appropriate antibiotic therapy for patients with multi-drug-resistant bacterial infection, but can also guide earlier de-escalation of antibiotic therapy for patients with negative results. Accuracy rates of the ELITe MGB assays confirm that a molecular approach together with knowledge of local epidemiology susceptibility patterns could be used to expedite optimization of empirical antibiotic therapy and infection control practices in patients with BLRTI, especially when providing positive results for targeted antibiotic resistance genes. However, conventional culture-based antimicrobial susceptibility testing continues to be required to confirm molecular results and to detect other antibiotic resistance mechanisms.

Nucleic acid amplification techniques cannot distinguish between living and dead bacteria [26]. The degree of correlation between bacterial load and Ct values could be conditional on factors affecting the viability of bacteria at the time of sampling (prior antibiotic treatment, immune-mediated bacterial death [27]), as well as, potentially, the presence of multiple copies of the same gene on mobile genetic elements [28,29]. In spite of these limitations, this study showed how Ct analysis may deliver some information about bacterial load.

This study has several limitations, the main one being the lack of clinical data and prospective assessment of direct implications of molecular results on antimicrobial stewardship and clinical outcome. The administration of antibiotics before sampling was not known, and this factor might have hampered the overall evaluation of the molecular false-positive results. Nevertheless, the authors believe that this study shows the potential for the use of CRE, ESBL and MRSA/SA ELITe MGB kits to support infection control and antibiotic stewardship programmes in patients with a high suspicion of multi-drug-resistant BLRTI. Any future studies of the effectiveness of this approach will need to consider the feasibility of producing results in a timely manner (testing requires approximately 30 min of laboratory hands-on time and a test run time of approximately 3 h), as well as the willingness of clinicians to respond to the results. All of these results would, in turn, have to feed into an assessment of cost effectiveness.

In conclusion, in the smart era of resistance profiling, the ELITe MGB assays showed reasonable accuracy for the detection of carbapenemase- and ESBL-producing Enterobacterales and MRSA in respiratory samples. These tests might be a useful complementary tool for expediting optimization of empirical antibiotic therapy and infection control practices in patients with BLRTI, depending on local prevalence rates of antibiotic resistance. However, further studies are required to confirm these results, to determine robust Ct cut-off values for colonization vs infection, and to determine their clinical and cost effectiveness in routine clinical practice.

Conflict of interest statement
None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2019.12.025.

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