Detection of antibiotic resistance genes in clinical samples using T2 Magnetic Resonance (T2MR®)

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Abstract

Background: Antibiotic resistant bacteria are spread through selective pressure from the use of broad spectrum empirical therapies, mobile genetic elements that pass resistance genes between species, and the inability to rapidly and appropriately respond to their presence. Resistance gene identification is often performed with post culture molecular diagnostic tests. The T2Resistance Panel, which detects methicillin resistance genes mecA/C; vancomycin resistance genes vanA/B; carbapenemases blaKPC, blaOXA-48, blaNDM, blaVIM, and blaIMP; AmpC β-lactamases blaCMY and blaDHA; and extended spectrum β-lactamases blaCTX-M directly from patient blood samples, is based on T2 magnetic resonance (T2MR), an FDA-cleared technology with demonstrated high sensitivity and specificity for culture-independent bacterial and fungal species identification. Here we report the clinical performance of T2MR detection of resistance genes directly from patient blood samples.

Methods: Patients with a clinical diagnosis of sepsis and an order for blood culture (BC) were enrolled in the study at two sites. BCs were managed using standard procedures and MALDI-TOF for species identification. Resistance testing with the T2MR assay was performed on a direct patient draw and compared to diagnostic test results from concurrent BC specimen and BC specimen taken at other points in time. Potential impact on therapy was evaluated through patient chart review.

Results: T2MR detected the same resistance genes as detected by post culture diagnostics in 100% of samples from concurrent blood draws. Discordant results occurred when T2MR was taken ≥ 48 h after BC for patients on antimicrobial therapy. The average time to positive result was 5.7 h with T2MR versus 38.9 h with post-culture molecular testing.

Conclusion: The T2Resistance Panel detected antibiotic resistance genes in clinical samples and displayed agreement with post culture genetic testing. T2MR results were achieved faster than culture-dependent diagnostic testing results and may allow for an earlier change from empiric to directed therapy. The use of culture independent diagnostics like T2MR could enable a quicker response to antibiotic resistant organisms for individual patients and developing outbreaks.

Introduction

Principles of T2MR

The technique of T2 magnetic resonance (T2MR) can be used to sensitively detect pathogen DNA in whole blood samples.[1] The FDA-cleared T2Candida and T2Bacteria Panels utilize this technology to detect fungal and bacterial species that cause blood stream infections. Whole blood samples are minimally processed by a T2Dx Instrument with the workflow shown in Fig. 1a, and the released pathogen DNA is amplified using a multiplex amplification procedure. DNA probe sequences, which directly bind to the target DNA, are conjugated to superparamagnetic particles. These particles are mixed with the amplified DNA and become clustered in the presence of their targets as shown in Fig. 1b. Clustered particles can be readily detected by a T2MR reader due to changes in magnetic relaxation. Because of the unique properties of T2MR detection, opaque sample matrices like blood do not influence the measurement. T2MR detection pathogens in whole blood samples has been shown to have high sensitivity (90%) and high specificity (98%) in the T2Candida and T2Bacteria Panels.

Fig 1. a) Workflow for preparing direct from blood samples for T2MR detection. Pathogen cells within a whole blood sample are concentrated, lysed, and amplified directly in the lysate. b) Probe conjugated superparamagnetic particles cluster in the presence of their target DNA amplicon. T2MR measurements readily distinguished between clustered and unclustered particles.

Early Targeted Therapy

Current standard of care for a patient suspected of sepsis is blood culture and subsequent antimicrobial susceptibility testing, which can take 2-5 days. During this time no clinical data is available to support patient treatment, therefore patients are typically treated empirically. A meta-analysis of 70 studies found that empiric antibiotic therapy was inappropriate in 46.5% of patients with blood stream infections.[2] It has also been demonstrated that for every hour of delay in appropriate therapy there is a 7.6% decrease in survival for septic shock patients.[3] A study of patients with blood stream infections caused by carbapenem resistant Enterobacteriaceae (CRE) demonstrated that the median time to appropriate therapy was 47 hours and that 49% of infected individuals died within 30 days.[4] Additionally, blood stream infections treated appropriately within the first 24 hours had significant reduction in hospital length of stay, mortality and cost.[5] With the rate of infections with multi-drug resistance organisms on the rise, the development of a rapid antibiotic resistance gene assay could reduce the time to appropriate therapy, which could decrease patient mortality and over prescription of inappropriate antibiotics.

T2MR Resistance Gene Detection

Here we discuss the development of a two rapid T2MR panels targeted against the genetic mechanisms of antibiotic resistance. The resistance genes in these panels have been identified as major threats to antibiotic resistance by the CDC (Table 1). We began testing with the T2Carba Resistance+ Panel, which include carbapenemase and AmpC β-lactamase genes. A research use only version of this panel was used to test clinical samples from patients with suspected antibiotic resistant infections. Additional resistance genes, including those for methicillin resistance, vancomycin resistance, and extended spectrum β-lactamases, were added to create the T2Resistance Panel.

Table 1: Resistance genes detected by T2MR panels.

Resistance Gene Target	Bacteria Gram Type	Resistance Type	Result reported	Panel	
bla_{KPC}	Negative	Carbapenemases	KPC	T2Carba Resistance+	T2Resistance
bla_{OXA-48}	Negative		OXA-48 Group		
bla_{VIM}	Negative		VIM, IMP, or NDM for T2CR+ MBL (metallo-beta-lactamase) for T2R		
bla_{NDM}	Negative				
bla_{IMP}	Negative				
bla_{DHA}	Negative	AmpC β-lactamases	AmpC (CMY-2, DHA)		
bla_{CMY-2}	Negative				
$bla_{CTX-M-14}$	Negative	Extended spectrum β-lactamases	CTX-M-14/15		
$bla_{CTX-M-15}$	Negative				
vanA	Positive	Vancomycin resistance	*vanA/B*		
vanB	Positive				
mecA	Positive	Methicillin resistance	*mecA/C*		
mecC	Positive				

Results

Clinical Samples

Clinical samples were collected from patients at two sites in Italy, University of Perugia and Policlinico Universitario Agostino Gemelli, under protocols approved by the Institutional Review Boards (IRBs) at both sites. Patients were selected based on suspicion of infection with an antibiotic resistant isolate. Whole blood specimens were detected with the T2Carba Resistance+ Panel (Table 1). Samples were also taken for blood culture (BC); however, in some cases T2MR draws were up to a day after BC draws. The genetic mechanism of resistance was identified from BC isolates using molecular methods.

Fig 2. Clinical samples were tested at two sites in Italy using the T2Dx Instrument. Whole blood vacutainers taken directly from the patient were loaded on the T2Dx as shown.

Table 2: Comparison of BC and T2MR results from clinical samples

Site	ID	BC Species	BC Gene	Time to mechanism result (hh:mm:ss)	T2MR Result	Time to T2MR result (h:mm:ss)	Agreement between BC and T2MR
Perugia	2	*Klebsiella pneumoniae*	KPC	48:36:00†	KPC	6:33:18	✓
Perugia	14	*Klebsiella pneumoniae*	KPC	94:45:00†	KPC	4:40:06	✓
Perugia	12	Negative	Negative	Negative	Negative	4:51:54	✓
Gemelli	23	Negative	Negative	Negative	NDM	4:52:35	T2MR detects NDM
Gemelli	30	*Klebsiella pneumoniae*	KPC	7:00:00	KPC	5:53:37	✓
Perugia	9	*Klebsiella pneumoniae*	KPC	19:09:00†	KPC	6:11:34	✓
Gemelli	33	*Klebsiella pneumoniae*	KPC	24:45:00	KPC	6:55:23	✓

†Time to molecular result calculated as time to BC + 3 h

BCs identified *Klebsiella pneumoniae* in 5/7 samples, and all 5 were found to have bla_{KPC} by molecular testing (Table 2). T2MR results were in agreement with all positive blood culture results (5/5). One of the two samples negative by blood culture was positive by the T2Carba Resistance+ Panel for bla_{NDM}. The time to molecular result for culture dependent tests took on average 38.9 h, largely driven by the time to positive culture. In contrast, T2MR results were obtained in an average 5.7 h.

Preliminary Analytical Sensitivity

Methicillin resistance, vancomycin resistance, and extended spectrum β-lactamases were added to the multiplex amplificiation to create the T2Resistance Panel (Table 1). Analytical sensitivity was tested using isolates containing antibiotic resistance genes added to human whole blood at 3, 5, and 7 CFU/mL (n=8 each concentration). The preliminary limit of detection was assigned to the lowest concentration with 100% positive detection (Table 3).

Table 3: Preliminary limit of detection of T2Resistance Panel

Target	Channel	Species	Strain	Preliminary Limit of Detection (CFU/mL)
KPC-3	KPC	*Klebsiella pneumoniae*	CDC AR-0112	3
OXA-48	OXA	*Klebsiella pneumoniae*	CDC AR-0848	5
VIM-1	MBL	*Klebsiella pneumoniae*	CDC AR-0076	3
NDM-1		*Acinetobacter baumannii*	CDC AR-0037	7
IMP-1		*Pseudomonas aeruginosa*	CDC AR-0103	7
DHA-2	AmpC	*Klebsiella pneumoniae*	CDC AR-0079	5
CMY-2		*Escherichia coli*	CDC AR-0081	3
CTX-M-14	CTX-M	*Klebsiella pneumoniae*	CDC AR-0079	5
CTX-M-15		*Klebsiella pneumoniae*	CDC AR-0848	5
mecA	mecA/C	*Staphylococcus aureus*	CDC AR-0215	5
mecC		*Staphylococcus aureus*	ATCC BAA-2312	5
vanA	van	*Enterococcus faecium*	BEI NR-31928	3
vanB		*Enterococcus faecium*	JMI 1031982	5

Preliminary Analytical Specificity

The T2Resistance Panel was tested against a set of 50 hematology discards from the Lahey Clinic. The samples were negative for all resistance genes. Cross-reactivity testing with high titer (1000 CFU/mL) isolates in blood demonstrated that each detection channel is specific for its intended target (Fig. 3).

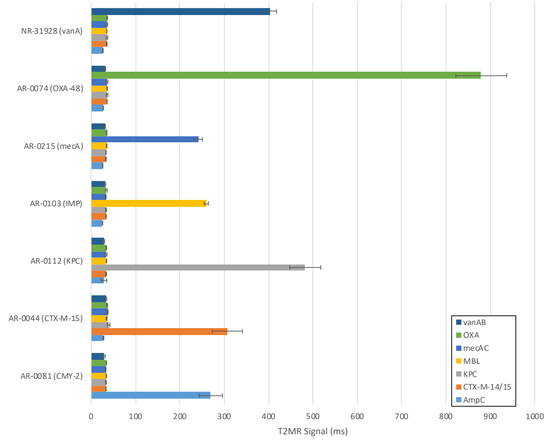

Fig. 3. Cross reactivity testing with 1000 CFU/mL of isolates carrying antibiotic resistance genes in blood. High T2MR signals are obtained only for the channels that detect the gene carried by each isolate.

Conclusions

- Agreement was found between positive BC and T2MR results
- T2MR results were obtained in an average 5.7 h while genetic testing after BC required an average 38.9 h
- The T2Resistance Panel detects both Gram negative and Gram positive resistance genes with high analytical sensitivity and specificity

References

1.Snyder, et al. *Encyclopedia Analytical Chemistry* 2019.
2.Paul, et.al., *Antimcro Agents Chemo* 2010.
3.Kumar, et.al. *Crit Care Med* 2006.
4.Satlin, et.al. *Antimicro Agents Chemo* 2017.
5.Perez, et. al. *J. Infect* 2014.

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