Rapid Antibiotic Susceptibility Testing based on tmRNA Detection

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Introduction

Conventional antimicrobial susceptibility testing (AST) typically delivers results 2 to 5 days after sample collection, which is often too late to have a meaningful impact on treatment. The present study demonstrates a new, rapid, phenotypic approach to AST with Qvella's FAST-AST™ process to assess pathogen susceptibility to antibiotic exposure by monitoring transfer messenger RNA (tmRNA). TmRNA functions as a quality control system to monitor cellular protein synthesis by rescuing stalled ribosomes from non-stop protein translation of damaged mRNA. Therefore, tmRNA reflects bacterial response to various environmental stresses.

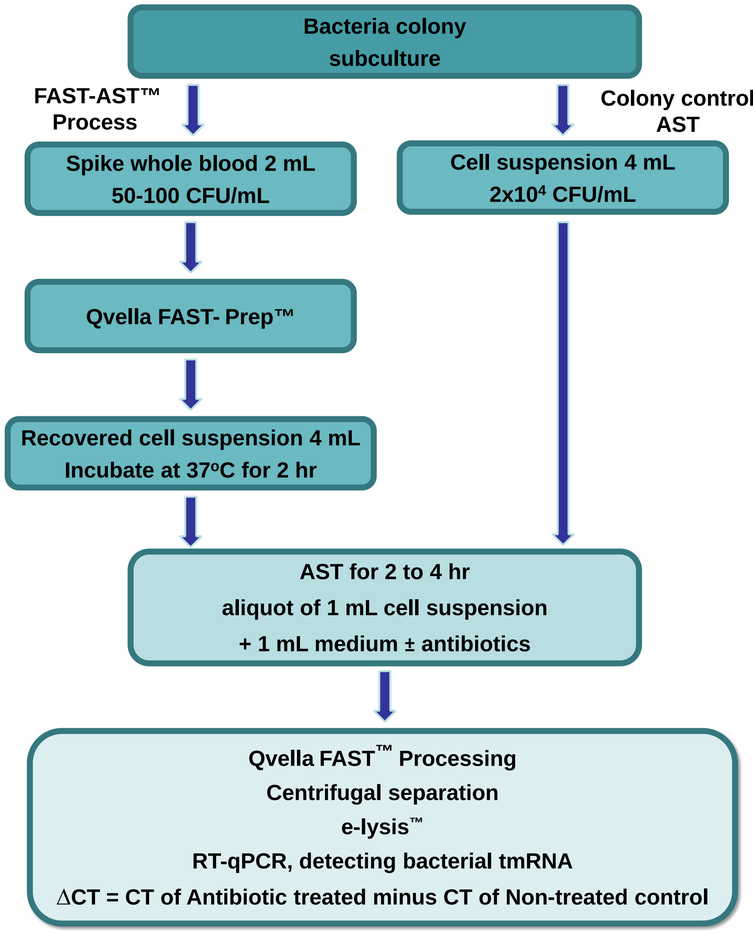
Objective

The objective of this study was to demonstrate proof of principle using tmRNA as a suitable marker for the real-time assessment of pathogen viability and susceptibility to antibiotics.

FAST-AST™ Process

Microbial cells were spiked into 2 mL of EDTA-treated whole blood at 100 CFU/mL. Blood cells were selectively lysed and microbial cells isolated using Qvella's FAST-Prep™ centrifugal separation process. Cells were re-suspended in 4 mL of TSB growth medium followed by pre-incubation at 37° C for 2 hours. This cell suspension was divided into one control aliquot and three aliquots having antibiotics at breakpoint concentrations. The aliquots were incubated at 37° C for 2 hours and then subjected to Qvella's FAST-ID™ process involving centrifugal separation, electrical lysis (e-lysis™), and RT-qPCR for tmRNA targets.

Method



Results

Qvella FAST-AST™ Process

Gram-negative 2 hr

Antibiotics	*K. pneumoniae*	*E.coli*
NFX 8 ug/mL	5.3	5.3
TET 8 ug/mL	3.0	3.2
OXA 4 ug/mL	0.4	0.1

Colony control AST

Antibiotics	*K. pneumoniae*	*E.coli*
NFX 8 ug/mL	5.7	7.8
TET 8 ug/mL	4.7	5.7
OXA 4 ug/mL	1.3	-0.2

Gram-negative 3 hr

Antibiotics	*K. pneumoniae*	*E.coli*
NFX 8 ug/mL	9.8	11.9
TET 8 ug/mL	4.1	4.5
OXA 4 ug/mL	-0.6	-0.6

Antibiotics	*K. pneumoniae*	*E.coli*
NFX 8 ug/mL	8.6	10.9
TET 8 ug/mL	6.6	9.1
OXA 4 ug/mL	-0.3	0.1

While ΔCT for the non-susceptible antibiotics was close to one cycle, it was over 3 cycles for the susceptible antibiotics at 3 hrs. Thus, tmRNA is a good marker for quantifying susceptibility.

Gram-positive 2 hr

Antibiotics	*S. aureus*	*E. fecalis*
VAN 8 ug/mL	0.4	-1.3
TET 8 ug/mL	-0.1	-1.3
GEN 8 ug/mL	1.5	0.0

Antibiotics	*S. aureus*	*E. fecalis*
VAN 8 ug/mL	-3.6	-1.5
TET 8 ug/mL	1.9	0.9
GEN 8 ug/mL	4.5	1.8

Gram-positive 4 hr

Antibiotics	*S. aureus*	*E. fecalis*
VAN 8 ug/mL	1.9	3.9
TET 8 ug/mL	5.3	2.6
GEN 8 ug/mL	5.3	5.5

Antibiotics	*S. aureus*	*E. fecalis*
VAN 8 ug/mL	1.6	0.5
TET 8 ug/mL	5.5	3.3
GEN 8 ug/mL	10.1	2.1

ΔCT is close to 3 cycles for the susceptible antibiotics at 4 hrs. Thus, tmRNA is a good marker for quantifying susceptibility.

Conclusion

This study highlights the utility of tmRNA as a marker of pathogen susceptibility during antibiotic exposure, demonstrating the feasibility of tmRNA-based rapid phenotypic AST.

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