

Rapid antibiotic susceptibility testing in *Klebsiella pneumoniae* and *Acinetobacter baumannii* using RNA transcriptional signatures

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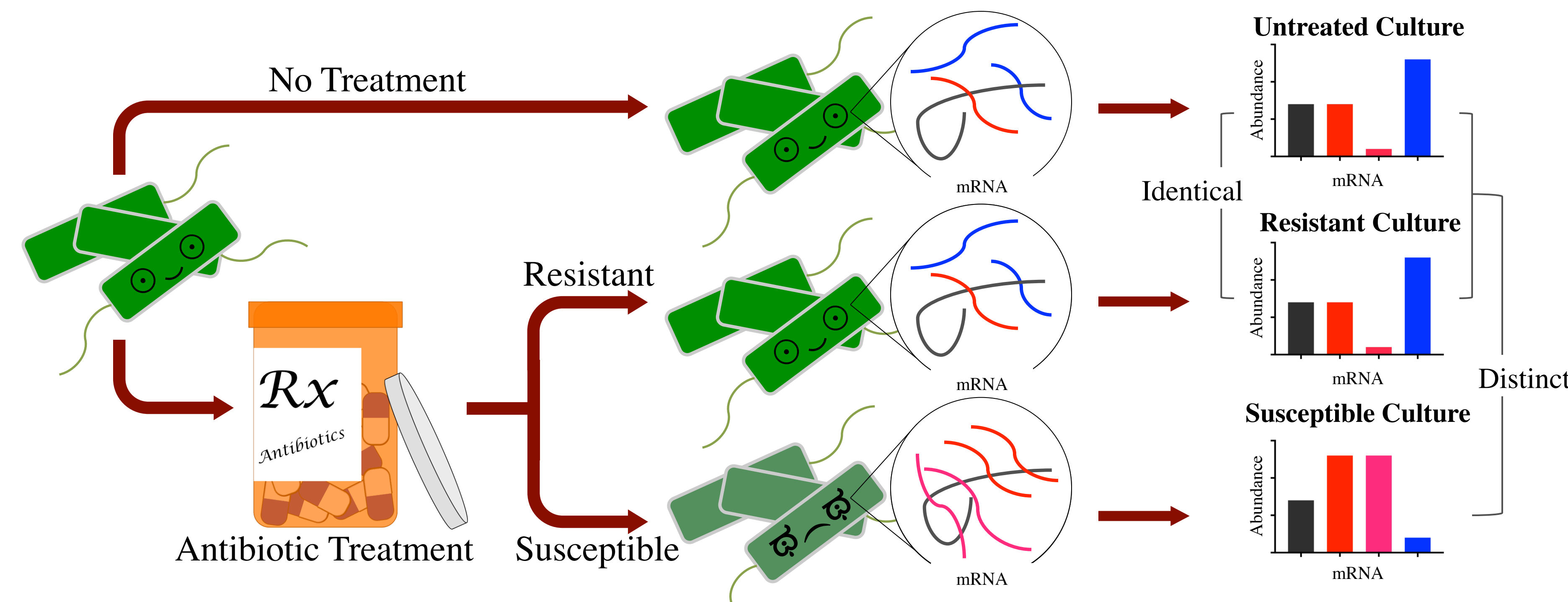
Introduction and Motivation

- Diagnosis of major pathogens continues to depend on slow, culture-based methods to determine antibiotic susceptibility
- Delays in treatment for a patient with sepsis can greatly increase mortality
- Current molecular diagnostics necessitate complete and prior knowledge on all possible genotypes conferring resistance

We propose a “semi-phenotypic” RNA-based assay to probe antibiotic susceptibility rapidly in a manner agnostic to resistance mechanism.

We use RNA-Seq data and an RNA hybridization assay (NanoString) to distinguish susceptible and resistant strains of *Acinetobacter baumannii* and *Klebsiella pneumoniae* for 3 key antibiotics from distinct classes: ciprofloxacin, gentamicin, and meropenem.

Background and Hypothesis



Results, continued

		Culture Determined Susceptibility		
		S	I	R
NanoString Technologies Determined Susceptibility	S	79	1	1
	I	-	1	-
	R	1	3	87

96%
accuracy

- Our assay demonstrated 96% accuracy (167 out of 173).
- Based on the FDA criteria for antimicrobial susceptibility devices, our assay consisted of:

- 4 cases of minor error where an intermediate strain is incorrectly determined as resistant or susceptible (yellow)
- 1 major error where a strain determined as susceptible by culture is called resistant by our assay (orange)
- 1 very major error where a strain determined as resistant by culture is called susceptible by our assay (red)

- Reasons for assay failure are currently being explored

Conclusion and Future Directions

- In previous works, we have demonstrated that:
 - our phenotypic, transcriptional assay can determine antibiotic susceptibility without prior knowledge on the genetic basis for resistance.
 - This assay can be conducted on a positive blood culture with minimal sample preparation.

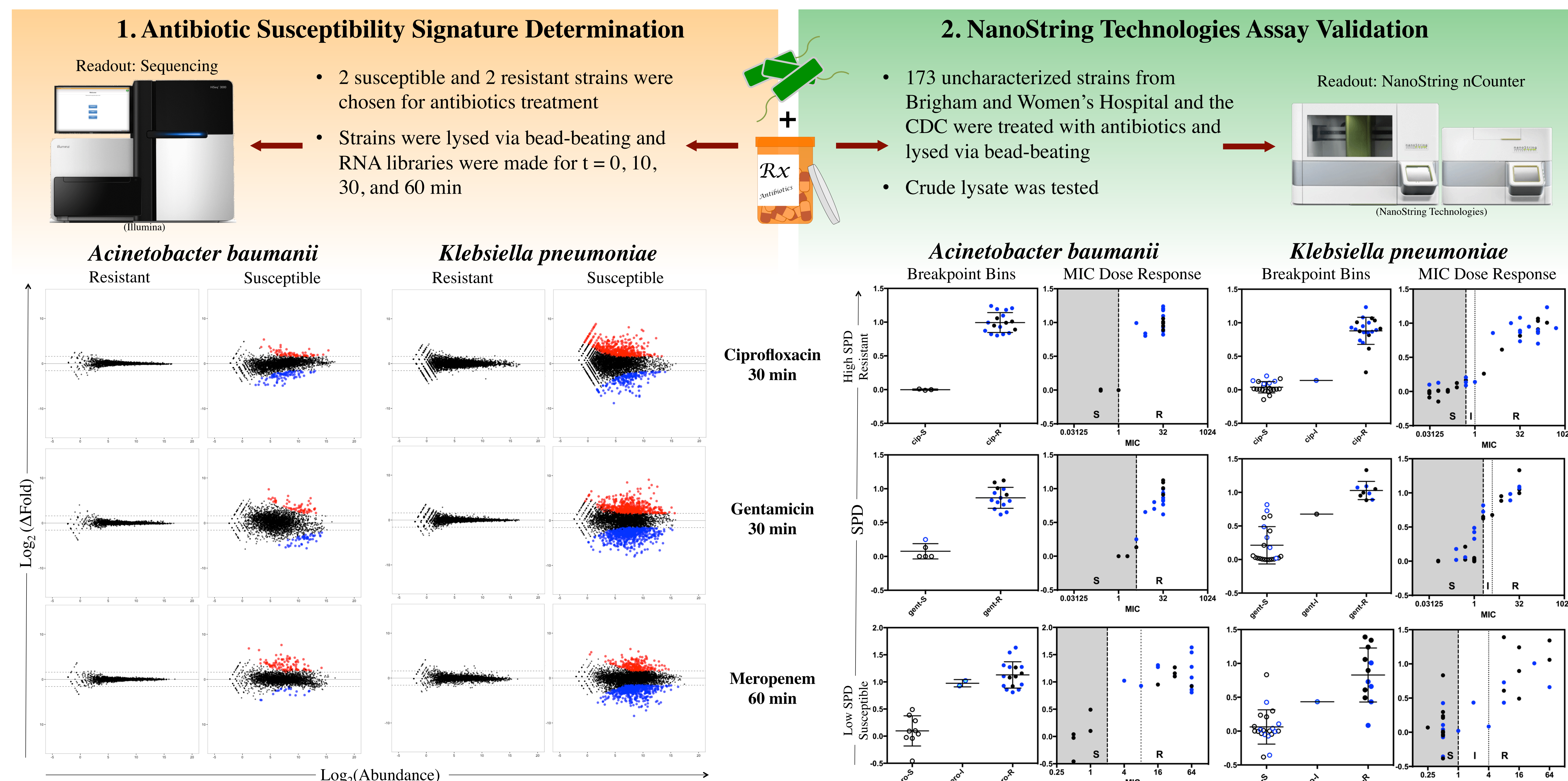
We suggest that our 8-hour transcriptional assay may be further generalized to other pathogen-antibiotic pair to rapidly determine antibiotic susceptibility while remaining agnostic to the resistance mechanisms.

Acknowledgements

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Experiment Schematic, Methods and Results



MA plots depicting log transcript abundance versus log-fold change of susceptible strains and resistant strains where each point represents one transcript. Red dots denote statistically significant up-regulation while blue dots represent down-regulation in expression level compared to untreated strains. Data shows average of 2 unique clinical isolates.

Each species was treated the clinical breakpoint for each antibiotic as dictated by the Clinical and Laboratory Standards Institute (CLSI). *Acinetobacter baumannii* was treated at 1 mcg/mL ciprofloxacin, 4 mcg/mL gentamicin, or 4 mcg/mL meropenem while *Klebsiella pneumoniae* was treated at 2.5 mcg/mL ciprofloxacin, 4 mcg/mL gentamicin, or 2 mcg/mL meropenem.