Using transcriptional profiling and molecular microbiology to better understand and diagnose infections

THE BHATTACHARYYA LAB

Broad Institute of Harvard and MIT
Infectious Disease and Microbiome Program

Meet the lab

The PI:

Roby Bhattacharyya, MD PhD, is an Assistant Professor in the MGH Infectious Diseases Division and Harvard Medical School, and an Associate Member at the Broad Institute in Cambridge, where our lab is. He joined the BBS faculty in July 2023. In addition to leading the lab, he is also a practicing infectious disease physician at MGH.

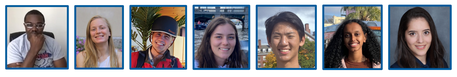
The lab:

Our lab aspires to be a friendly, happy, collaborative, and intellectually rigorous environment in which to study the responses of pathogens to antimicrobials, and of humans to infection, with the ultimate aim of improving the care of infected patients.

We value diversity of thought, experience, and therefore identity, and we believe that this diversity enriches the science we do, the questions we choose to ask, and the people we become.

Lab members include postdoctoral fellows, ID physicians in training, research associates, and undergrads. **We would love to hear from you!**

CURRENT MEMBERS:



ALUMNI:

Contact Information

Bhattacharyya Lab
Broad Institute of MIT and Harvard
415 Main St
Cambridge, MA 02142 U.S.A.

www.bhattacharyyalab.org

@roby_bhatt

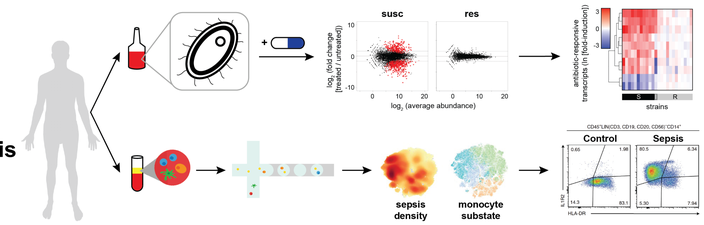
rbhatt@broadinstitute.org

Lab overview: transcriptional profiling for diagnostics and discovery in infectious diseases

Our lab is broadly interested in:

- **Antimicrobial resistance**
- **Microbial genomics & transcriptional profiling**
- **Molecular pathogenesis of sepsis**
- **Molecular diagnostic development**

*Project 1: **g**enotypic and **ph**enotypic **a**ntibiotic **s**usceptibility **t**esting through **R**NA detection (**GoPhAST-R**)*



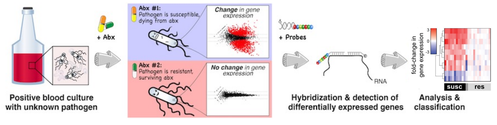
Project 2: single-cell RNA sequencing of circulating immune cells in patients with sepsis

We use standard tools of molecular microbiology, coupled with transcriptional profiling of microbial pathogens and patient immune cells, to address basic and translational research questions in infectious disease, with particular focus on **antibiotic resistance** and **sepsis**.

Gene expression profiles, particularly in response to key perturbations like antibiotic exposure or infection, reflect physiological adaptations by both microbes and patients that we can use to infer mechanisms of disease pathogenesis, and also co-opt for diagnostic purposes.

Project 1: Novel approaches for rapid antibiotic susceptibility testing (AST)

- **Antibiotic resistance** is an urgent, growing threat
- Growth-based antibiotic susceptibility testing is too slow to inform clinical decisions in real time
- Genomics can help, but our knowledge of resistance mechanisms is incomplete
- Our approach: **gene expression** after antibiotic exposure is a rapid, mechanism-agnostic way to assess susceptibility
  - RNA encodes both genotype (sequence) and phenotype (abundance)
  - Dying bacteria look different than non-dying ones, transcriptionally

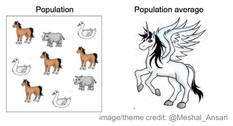


***Gen**otypic and **ph**enotypic **a**ntibiotic **s**usceptibility **t**esting through **R**NA detection (**GoPhAST-R**): transcriptional profiling after antimicrobial exposure can be exploited to distinguish susceptible from resistant pathogens, providing AST in hours instead of days.* Adapted by M. Martinsen from Bhattacharyya et al, *Nat Med* 2019.

- Process & workflow:
  - RNA-Seq (~4000 genes; days) to define key transcriptional signatures of susceptibility
  - Machine learning to identify best predictors
  - Targeted assay (~10 genes; hours) for AST
- Ongoing projects:
  - **Clinical pilot** at MGH on blood cultures
  - Extending approach to **fungal, TB AST**
  - Adapting to CRISPR/Cas13a-based readout for **global health applications**

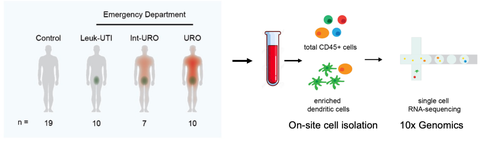
Project 2: Single-cell transcriptional profiling of immune cells in sepsis

- **Sepsis** (infection + immune dysregulation causing organ damage) is common, costly, & deadly
- Due to **heterogeneity** of the clinical syndrome, it is also very challenging to diagnose accurately
- **Single-cell RNAseq** has revolutionized our view of heterogeneity in complex biological systems

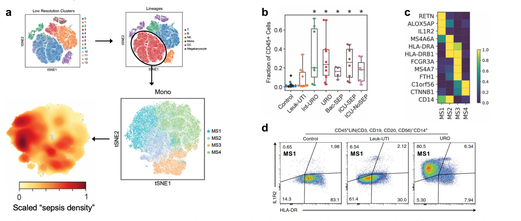


image/theme credit: @Meshal_Ansari

- We applied scRNAseq to circulating immune cells in a cohort of patients with sepsis…



- …and found a novel transcriptional substate of monocyte, **MS1**, enriched in sepsis & critical illness

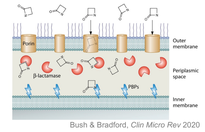


***MS1 monocytes are enriched in sepsis.** **(a)** scRNAseq reveals MS1 substate of monocytes **(b)** enriched in sepsis. **(c)** Top differentially expressed genes in MS1. **(d)** MS1 is detectable by flow cytometry.* From Reyes et al, *Nat Med* 2020.

- Ongoing projects (sepsis cohort actively enrolling):
  - MS1 kinetics vs clinical illness trajectory
  - Goal: decipher heterogeneity for precision Dx, Rx

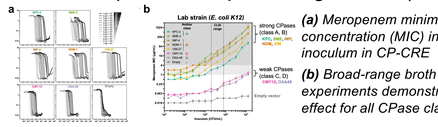
Project 3: Investigating mechanisms of carbapenem resistance

- Carbapenems are the broadest antibiotic class in routine clinical use, yet resistance is growing
- Main mechanisms:
  - Hydrolysis
  - Decreased entry (porin mutation)



Bush & Bradford, *Clin Micro Rev* 2020

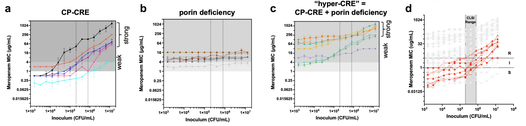
- Better understanding how bacteria evolve resistance to carbapenems is crucial
- We demonstrated inoculum-dependent resistance in all carbapenemase producing strains (CP-CRE)



***(a)** Meropenem minimal inhibitory concentration (MIC) increases with inoculum in CP-CRE*

***(b)** Broad-range broth microdilution experiments demonstrate inoculum effect for all CPase classes*

- This inoculum effect depends on mechanism of resistance, and can complicate clinical diagnosis



***(a)** CP-CRE but not **(b)** porin deficient isolates exhibit inoculum effects; **(c)** the combination exhibits high-level resistance at all inocula. **(d)** This inoculum effect can lead to clinical misclassification of resistant carbapenemase producers (red).*

Bhattacharyya lab, unpublished

- Ongoing projects:
  - We discovered a novel role for a transcription factor in carbapenem resistance & multi-drug resistance
  - We are investigating this transcription factor network in *K. pneumoniae*
  - We helped develop BacDrop, a new method for bacterial scRNAseq