The Application of Machine Learning with High-Content Imaging to Infer AMR Phenotypes in *Salmonella* Typhimurium

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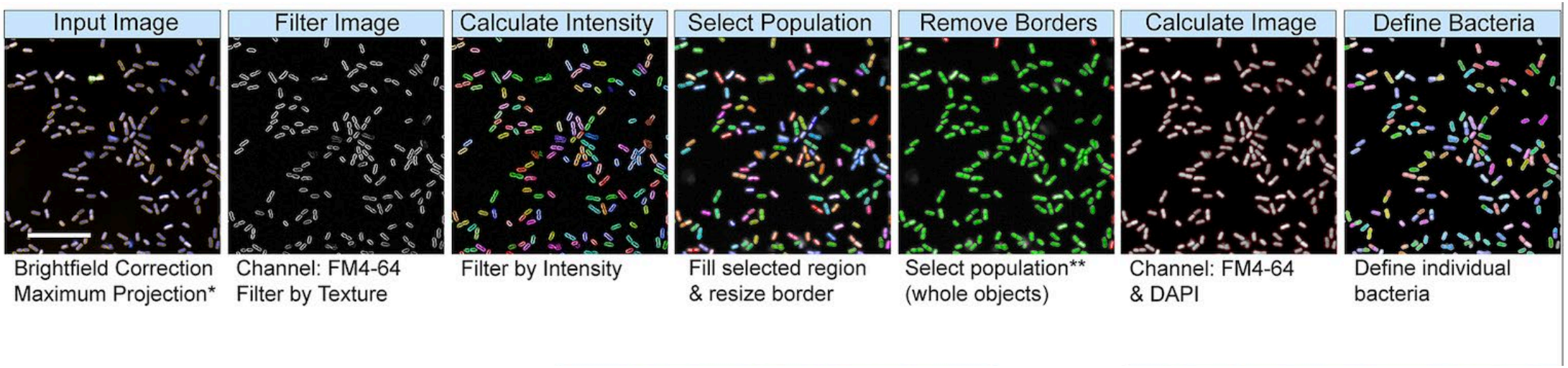
Background

- Ciprofloxacin non-susceptibility is a growing problem in NTS
  - We lack a nuanced understanding of bacterial response to exposure
- Traditional antimicrobial susceptibility testing is time and labor-intensive
  - May be subjective
- High-content imaging allows single-cell resolution microscopy at scale
  - Image analysis captures array of imaging-associated parameters

High content image analysis workflow



Sridhar and Forrest *et al.*, mSystems (2021)

Study questions

- How does *S.* Typhimurium isolate morphology change over 24 h under ciprofloxacin exposure?

- Are there differences between isolates susceptible and resistant to ciprofloxacin?

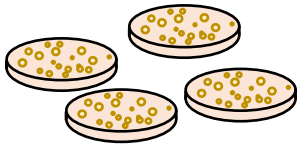
Tuan-Anh Tran

Under review at Nature Communications

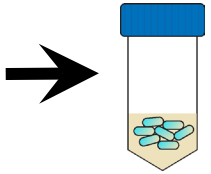
Isolates used

	Isolate	Ciprofloxacin susceptibility	Ciprofloxacin MIC	Resistance mechanism
	SL1344	S	0.015	
	SL1344*gyrA*	R	1.5	GyrA D87Y
iNTS	D23580	S	0.03	
iNTS	VNS20081	R	1.0	GyrA D87N

Methodology

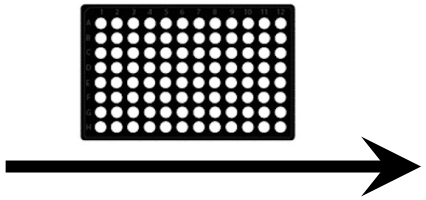


Isolates



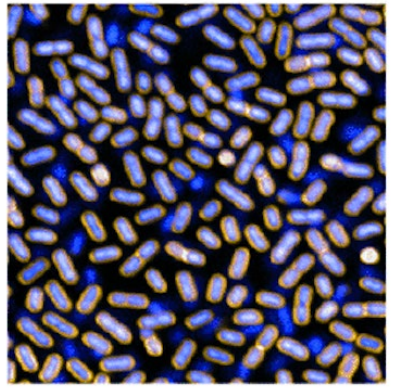
O/N culture

Sub culture with ciprofloxacin at 0x, 1x, 2x, 4x MIC



Grow at 37°C
Sample every 2 hours over 24 h

Fix bacteria



Automated microscopy on Opera Phenix

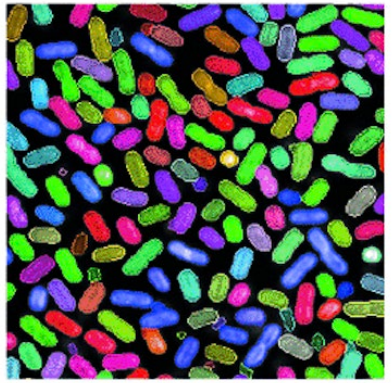
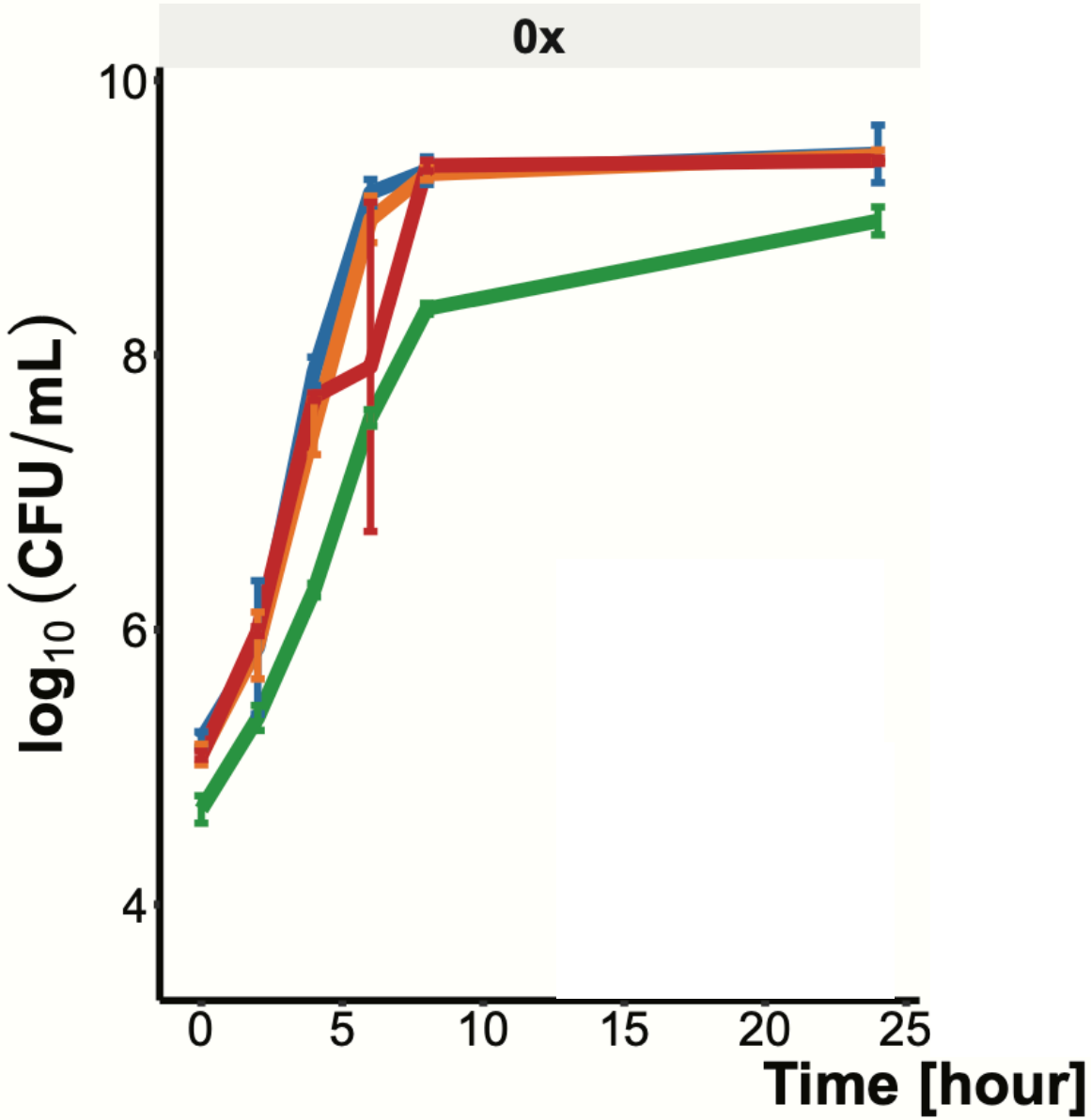


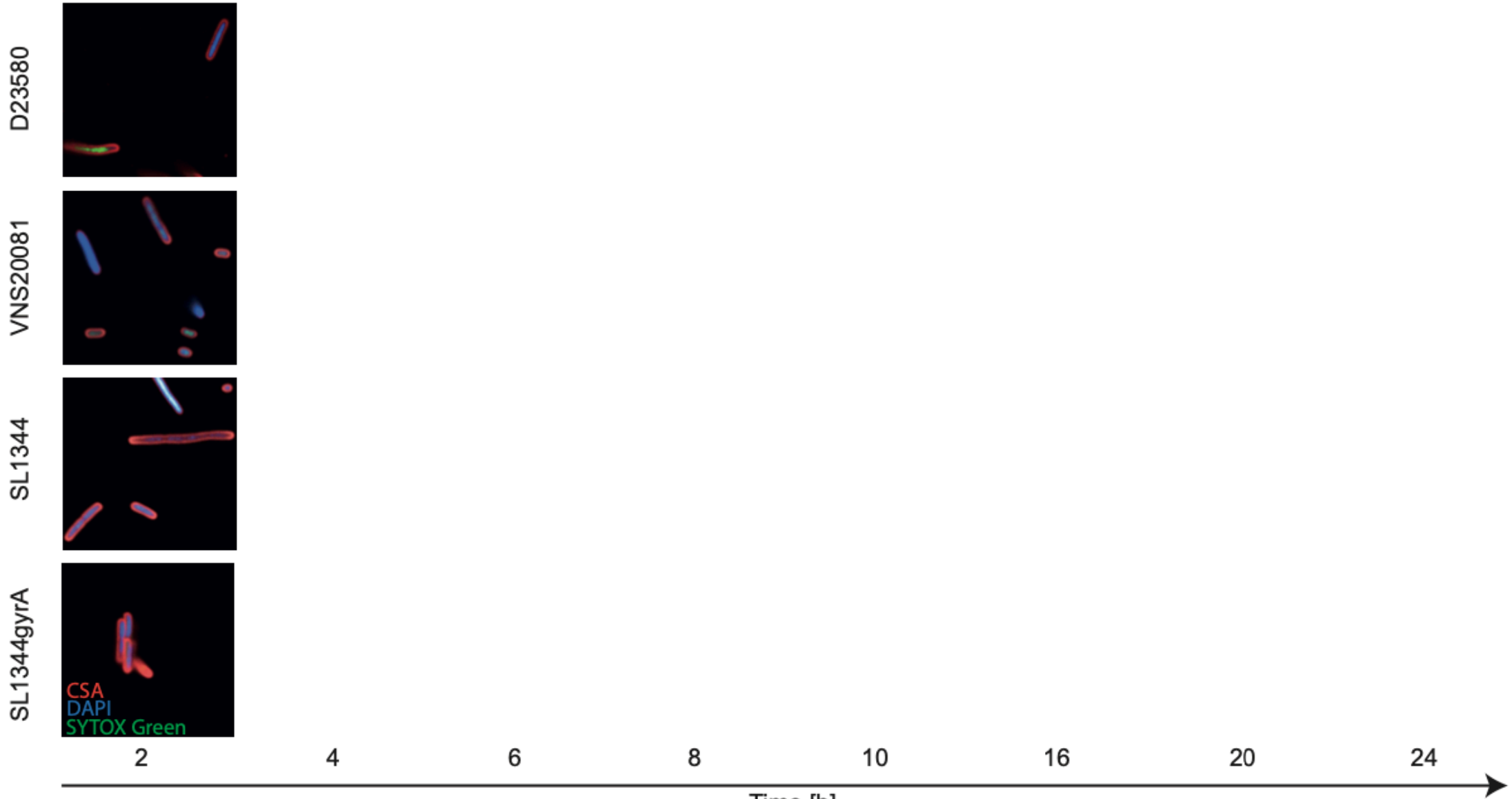
Image analysis

Machine learning analysis on 65 imaging features

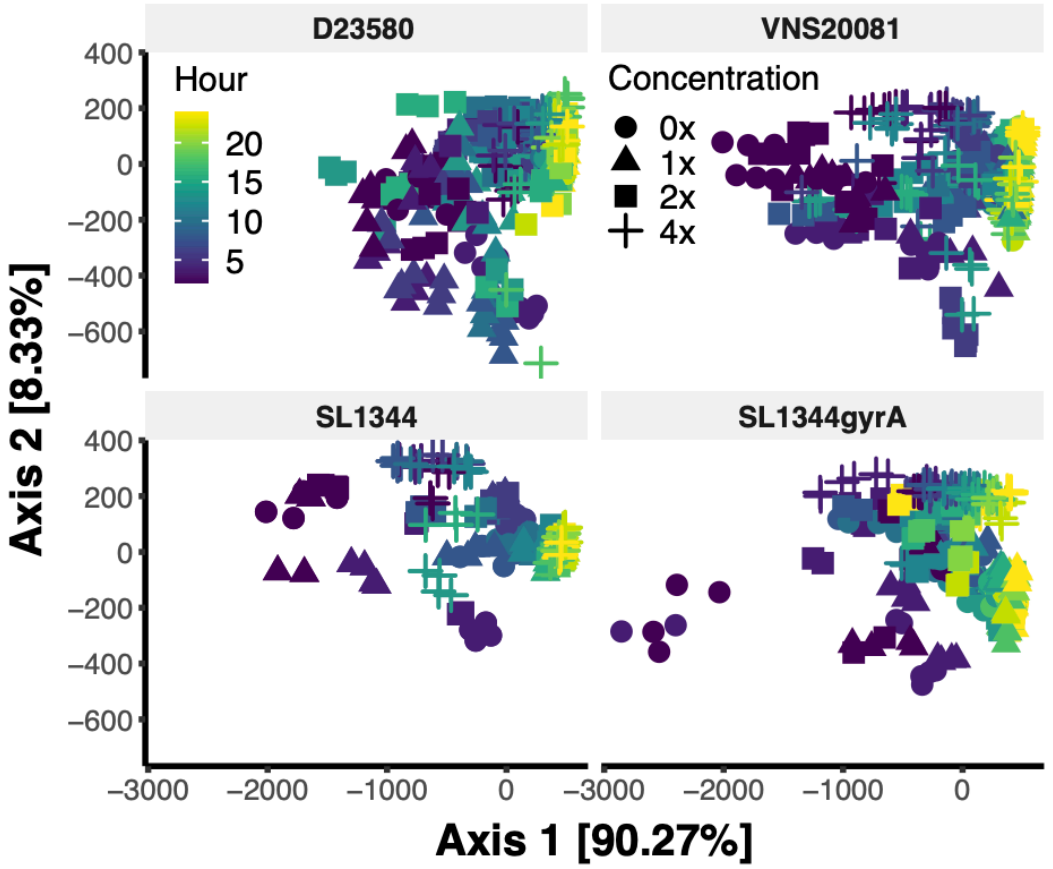
Bacterial growth response to ciprofloxacin



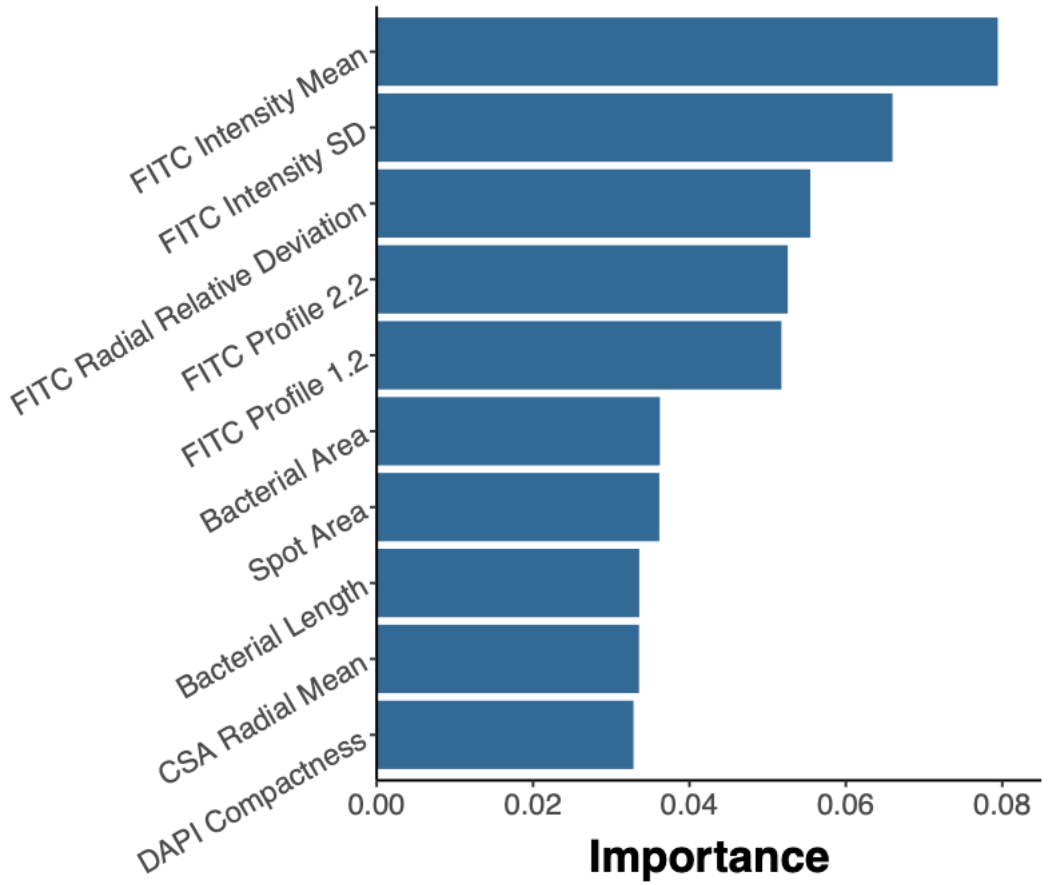
Change in bacterial morphology in cipro 1x MIC over 24 hours



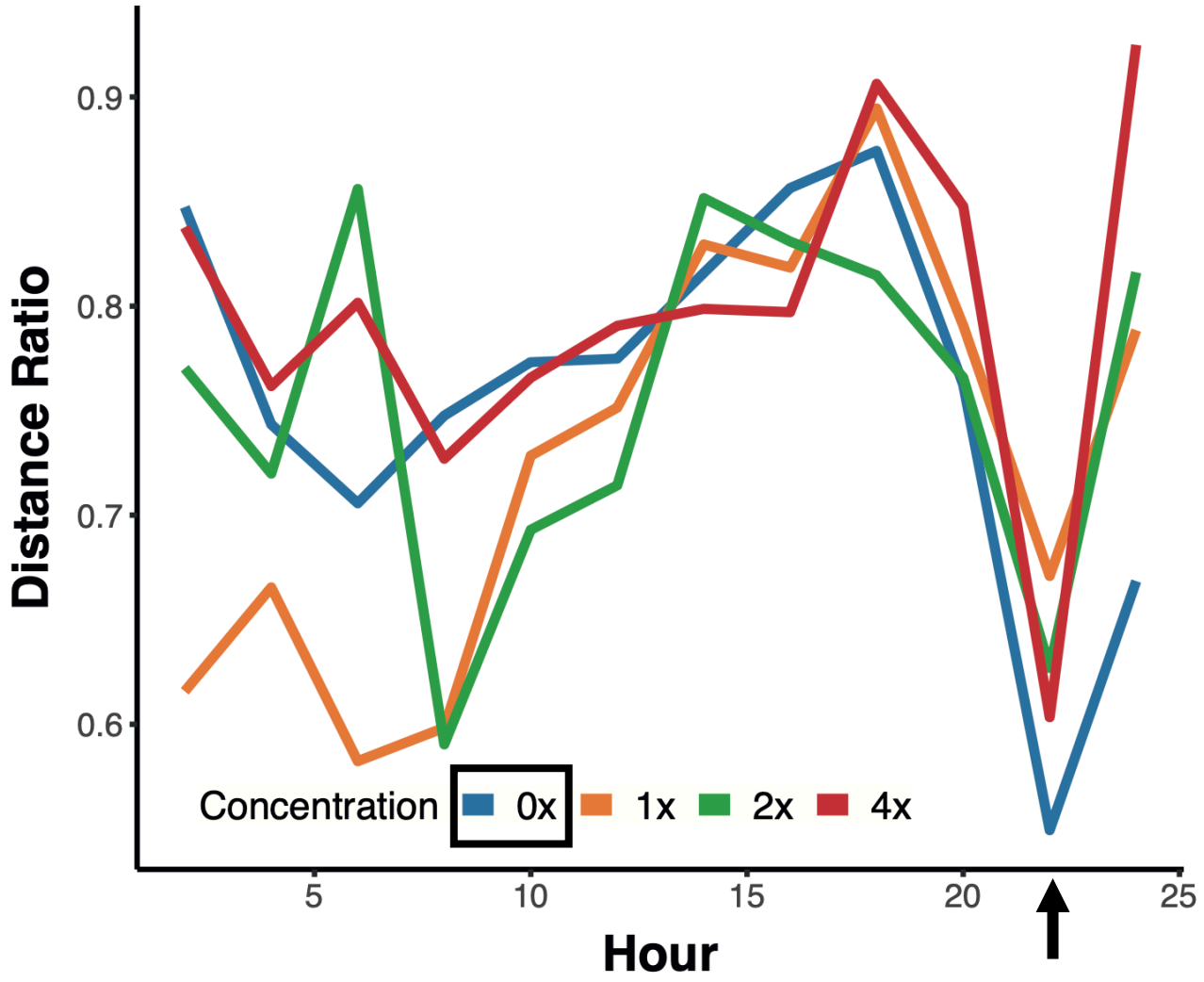
Principal coordinate analysis shows greatest variance due to cipro concentration and time



Top imaging features contributing to concentration-time difference



Biggest difference between susceptible and resistant isolates at 0x-22h

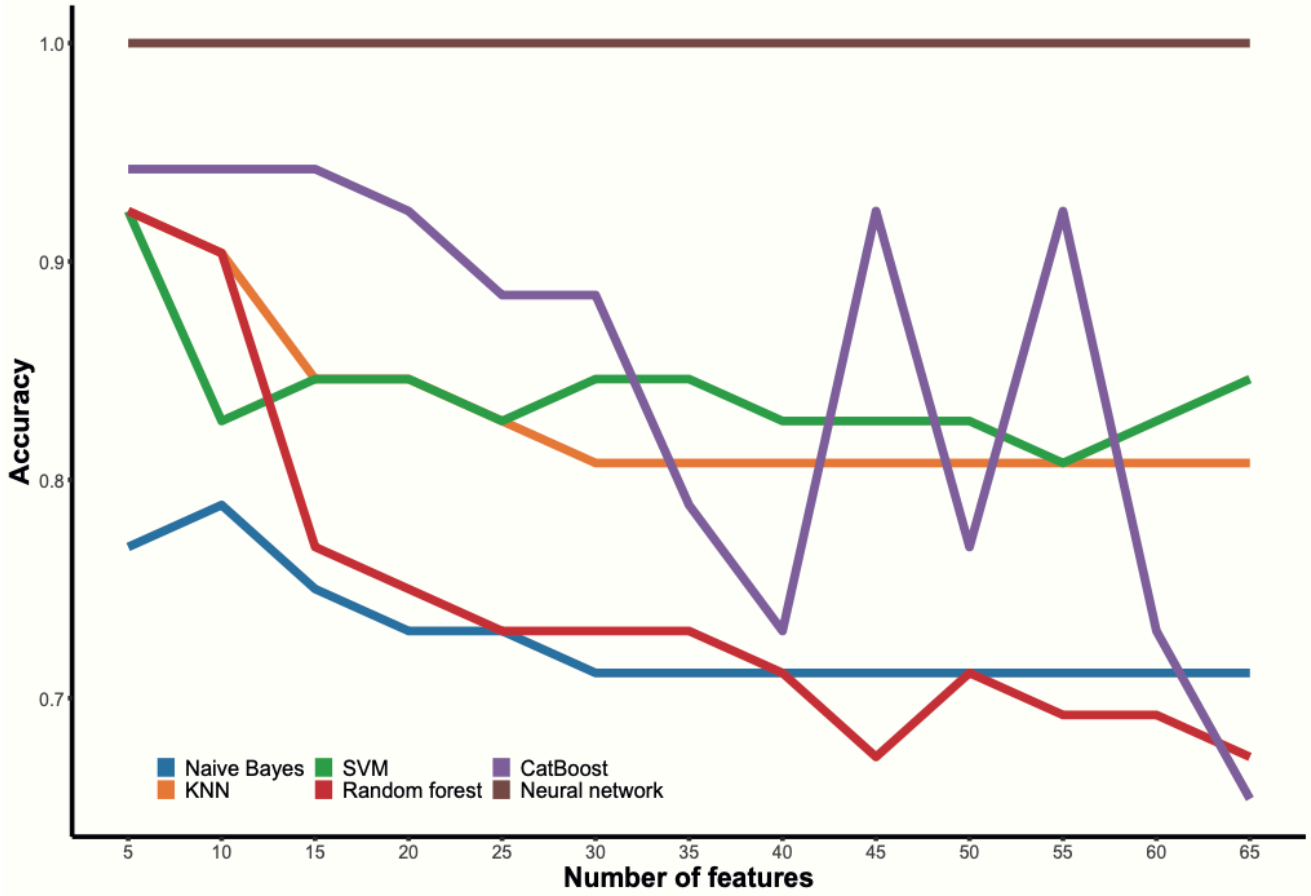


Ten most important features for cipro resistance selected by RF at 0x-22h

CSA Profile 2.2
FITC Profile 1.2
FITC Threshold Compactness 50
DAPI Intensity StdDev
DAPI Intenstiy Mean
CSA Profile 1.2
FITC Radial Relative Deviation
FITC Symmetry 15
FITC Intensity Mean
FITC Profile 2.2

Neural network performs best prediction of isolate susceptibility using 5 features

Tested 13 other iNTS isolates with varying ciprofloxacin susceptibility



Conclusions

- Individual *S.* Typhimurium isolates have different morphological trends
- Ciprofloxacin susceptibility may have morphological signatures that can be identified using imaging
- Machine learning algorithms can differentiate these
- We can predict ciprofloxacin susceptibility of *S.* Typhimurium without exposure to ciprofloxacin using ML algorithms
  - Only 5 imaging features required

Future questions and directions

- Is this still robust when looking at more diverse isolates?
- Do these findings extrapolate to other antimicrobials and bacteria?
- What mechanisms and/or protein changes in resistant isolates explain the imaging feature differences?
  - Can we model GyrA structure to better understand this?
- Can this approach inform future AST or diagnostic approaches?

Acknowledgements

University of Cambridge Department of Medicine

Steve Baker
Sally Forrest
Gordon Dougan
Josefin Bartholdson Scott
Mailis Maes
Ben Warne
Sandra Van Puyvelde
Stephen Reece

Tuan-Anh Tran

Collaborators

Octavie Lunguya
Jan Jacobs
Florian Marks
Rob Kingsley
Binh Nguyen
Pham The Bao

Wellcome Sanger Institute

Nicholas Thomson