# Predicting *C. difficile* infection severity from the taxonomic composition of the gut microbiome

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## Introduction

- C. difficile infection (CDI) can lead to adverse outcomes including recurrent infections, colectomy, and death (1).
- The composition of the gut microbiome plays an important role in determining colonization resistance and clearance when exposed to C. difficile (2, 3).
- Regression models trained on Electronic Health Records extracted on the day of diagnosis perform modestly well at predicting whether the CDI resulted ICU admission, colectomy, or 30-day mortality (AUROC 0.69) (4).
- Identifying the specific microbiome features that distinguish severe CDI cases would allow clinicians to tailor interventions based on a patient's risk, ultimately leading to better health outcomes.

### Dataset

We have 16S amplicon sequence data from 1,191 CDI patient stool samples, with cases classified as severe or not severe according to three separate definitions:

- **IDSA**: the Infectious Diseases Society of America (IDSA) definition with severe CDI having a white blood cell count ≥ 15 k/µL and serum creatinine level ≥ 1.5 mg/dL (5).
- **Attributable**: the CDC definition of ICU admission, colectomy, or death occurring within 30 days of CDI, and confirmed as attributable to CDI via clinical chart review.
- **All-cause**: ICU admission, colectomy, or death occurring within 30 days of CDI, regardless of the cause.

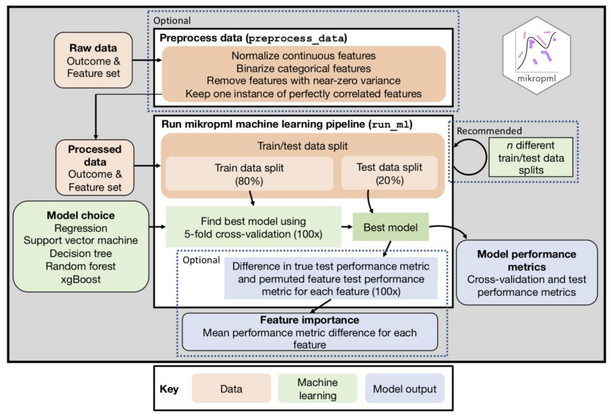
| Severe | IDSA | Attributable | All-cause |
|---|---|---|---|
| no | 649 | 513 | 1059 |
| yes | 342 | 26 | 83 |
| TOTAL | 991 | 539 | 1,142 |

The attributable severity definition requires chart review by physicians, which has been completed for about half of the cases.

## Methods

- Sequences were processed with mothur according to the MiSeq SOP and clustered into de novo OTUs at a 3% distance threshold (6, 7).
- We then trained machine learning (ML) models with OTU abundances as features to predict the IDSA severity, CDI-attributable severity, and all-cause severity of CDI cases using the mikropml R package and accompanying snakemake workflow (8, 9).

### Machine learning pipeline

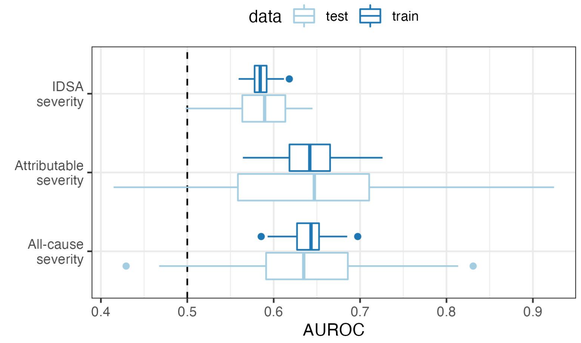



- Prior to model training, the features were pre-processed to scale and center at zero, remove features with near-zero variance, and collapse perfectly correlated features.
- The dataset was randomly split 100 times into training and testing sets with 80% of the data in the training set.
- On each partition, random forest models were trained with 5-fold cross-validation repeated 100 times, and performance as the area under the receiver-operator curve (AUROC) was measured on the held-out testing set for the best model.
- The importances of features for model performance were found using a permutation test.

## Results

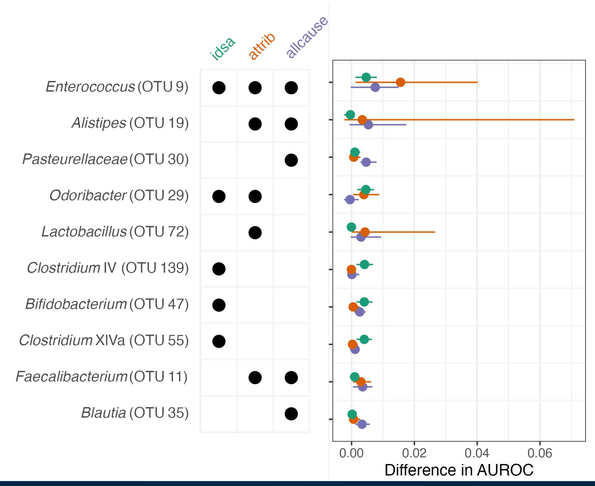
### Model performance

The models predicting CDI-attributable severity performed best (median AUROC 0.65), followed closely by those predicting all-cause severity (median AUROC 0.63). The models predicting IDSA severity performed worst (median AUROC 0.59).




### Feature importance

The top 5 most important OTUs for predicting each outcome were determined with a permutation test.




## Conclusions

- That models predicting CDI-attributable severity performed best implies that chart review by physicians is an important step to filter out other causes of complications.
- The long tails of the performance distributions for CDI-attributable and all-cause severity may reflect the rarity of severe outcomes according to these definitions.
- The poor-to-modest performance of these OTU-based models implies that the taxonomic composition of the microbiome is not the only important factor contributing to severe CDI outcomes.
- Only *Enterococcus* had a feature importance above 0.01 (diff. in AUROC). No single OTU had outsized influence on model performance, perhaps due to the interconnected nature of the microbiome.

### Future directions

- Using the precision-recall curve (AUPRC) may provide a better estimate of model performance than AUROC as the data are imbalanced.
- Training models with both EHR data and OTUs as features may improve model performance.

### Acknowledgements

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