

# Predicting *C. difficile* Infection Severity from the Taxonomic Composition of the Gut Microbiome

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1 **ABSTRACT**    TODO

2 **IMPORTANCE**    TODO

3 **KEYWORDS:** *C. difficile* infection, supervised machine learning

## INTRODUCTION

A few ways to define CDI severity (Figure 1)

## RESULTS

**Model performance** Report median AUROC for trainset and testset, and median AUBPRC for testset (Figure 2).

**Feature importance** Most important OTUs contributing to model performance (Figure 3)

**Clinical value of severity prediction models**

## DISCUSSION

TODO

## MATERIALS AND METHODS

**Sample collection** This study was approved by the University of Michigan Institutional Review Board. All patient samples were collected by the University of Michigan Health System from January 2016 through December 2017. Stool samples that had unformed stool consistency were tested for *C. difficile* by the clinical microbiology lab with a two-step algorithm that included detection of *C. difficile* glutamate dehydrogenase and toxins A and B by enzyme immunoassay with reflex to PCR for the *tcdB* gene when results were discordant. 1,517 stool samples were collected from patients diagnosed with a CDI. Leftover stool samples that were sent to the clinical microbiology lab were collected and split into different aliquots. For 16S sequencing, the aliquot of stool was resuspended in DNA genotek stabilization buffer and then stored in the -80°C freezer. Only the first CDI sample per patient was used for subsequent ML analyses such that no patient is represented more than once, resulting in a dataset of 1,191 samples.

**16S rRNA gene amplicon sequencing** Samples stored in DNA genotek buffer were thawed from the -80°C, vortexed, and then transferred to a 96-well bead beating plate for DNA extractions. DNA was extracted using the DNeasy Power-soil HTP 96 kit (Qiagen) and an EpMotion 5075 automated pipetting system (Eppendorf). The V4 region of the 16S rRNA gene was amplified with the AccuPrime Pfx DNA polymerase (Thermo Fisher Scientific) using custom barcoded primers, as previously described (1). Each library preparation plate for sequencing contained a negative control (water) and mock community control (ZymoBIOMICS microbial community DNA standards). The PCR amplicons were normalized (Sequal-Prep normalization plate kit from Thermo Fisher Scientific), pooled and quantified (KAPA library quantification kit from

KAPA Biosystems), and sequenced with the MiSeq system (Illumina).

All sequences were processed with mothur (v1.43) using the MiSeq SOP protocol (2, 1). Paired sequencing reads were combined and aligned with the SILVA (v132) reference database (3) and taxonomy was assigned with a modified version of the Ribosomal Database Project reference sequences (v16) (4). Samples were rarefied to 5,000 sequences per sample, repeated 1,000 times for alpha and beta diversity analysis.

**Defining CDI severity** IDSA definition of severe CDI based on lab values. CDC definition of severe CDI based on disease-related complications (5).

**Model training and evaluation** mikropml R package (6)

Balanced precision

**Code availability** The complete workflow, code, and supporting files required to reproduce this manuscript with accompanying figures is available at <https://github.com/SchlossLab/severe-CDI>.

The workflow was defined with Snakemake (7) using a custom version of the mikropml Snakemake workflow (8). Dependencies were managed with conda environments. Scripts were written in R (9), Python (10), and GNU bash. In addition to the software already cited above, other packages used in the creation of this manuscript include cowplot (11), ggtext (12), ggsankey (13), schtools (14), the tidyverse metapackage (15), Quarto, and vegan (16).

**Data availability** The 16S rRNA sequencing data have been deposited in the National Center for Biotechnology Information Sequence Read Archive (BioProject Accession no. PRJNA729511).

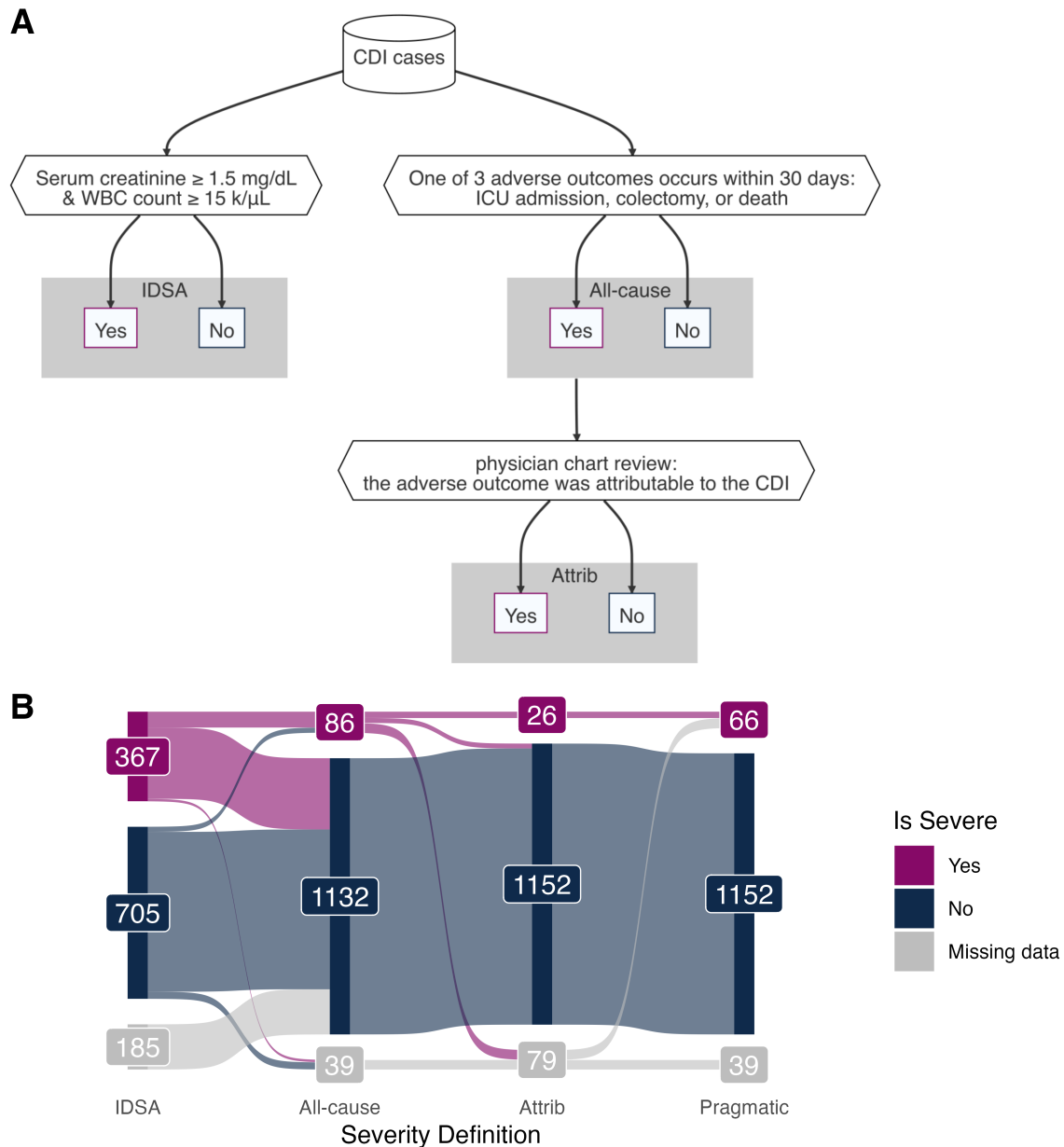
## ACKNOWLEDGEMENTS

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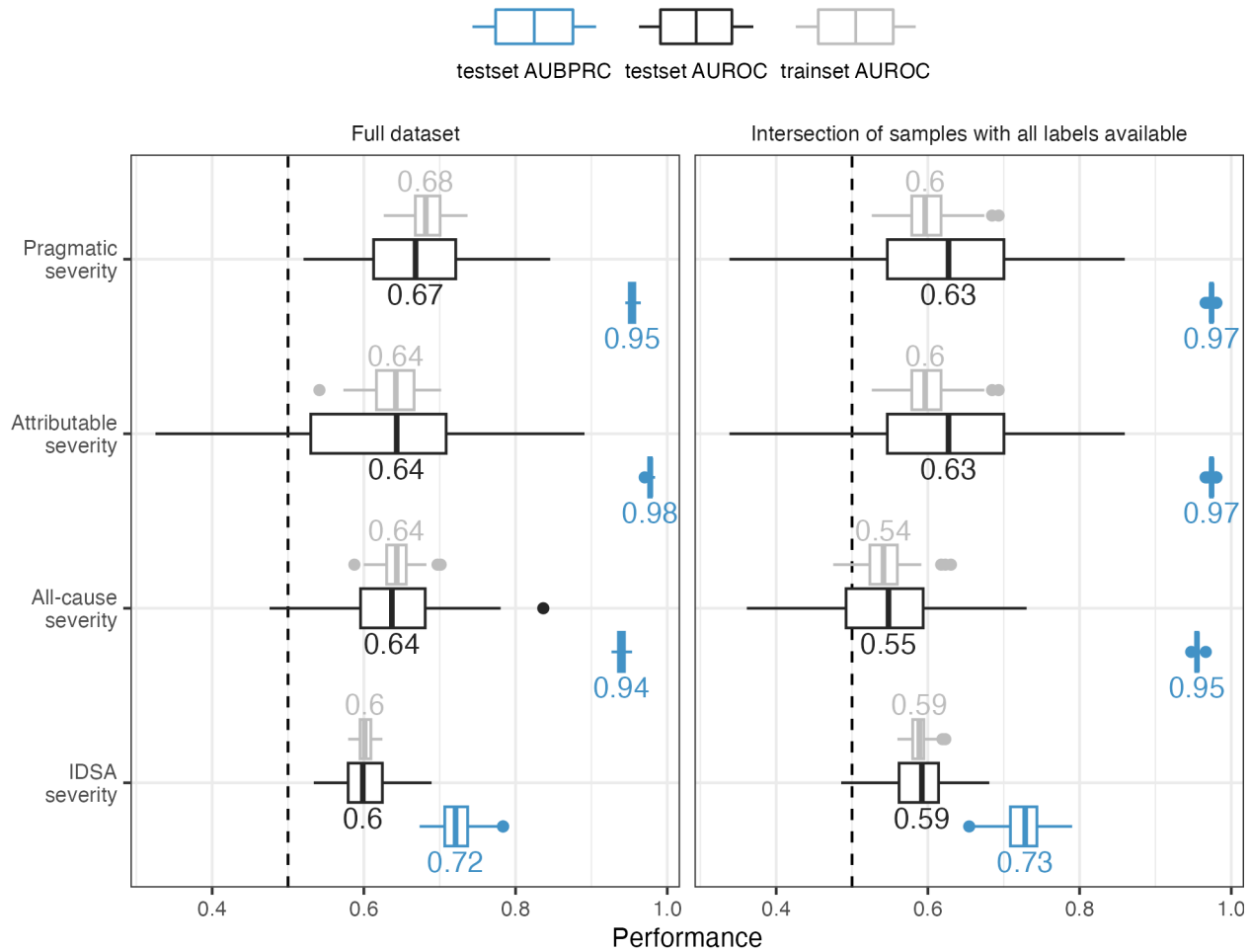
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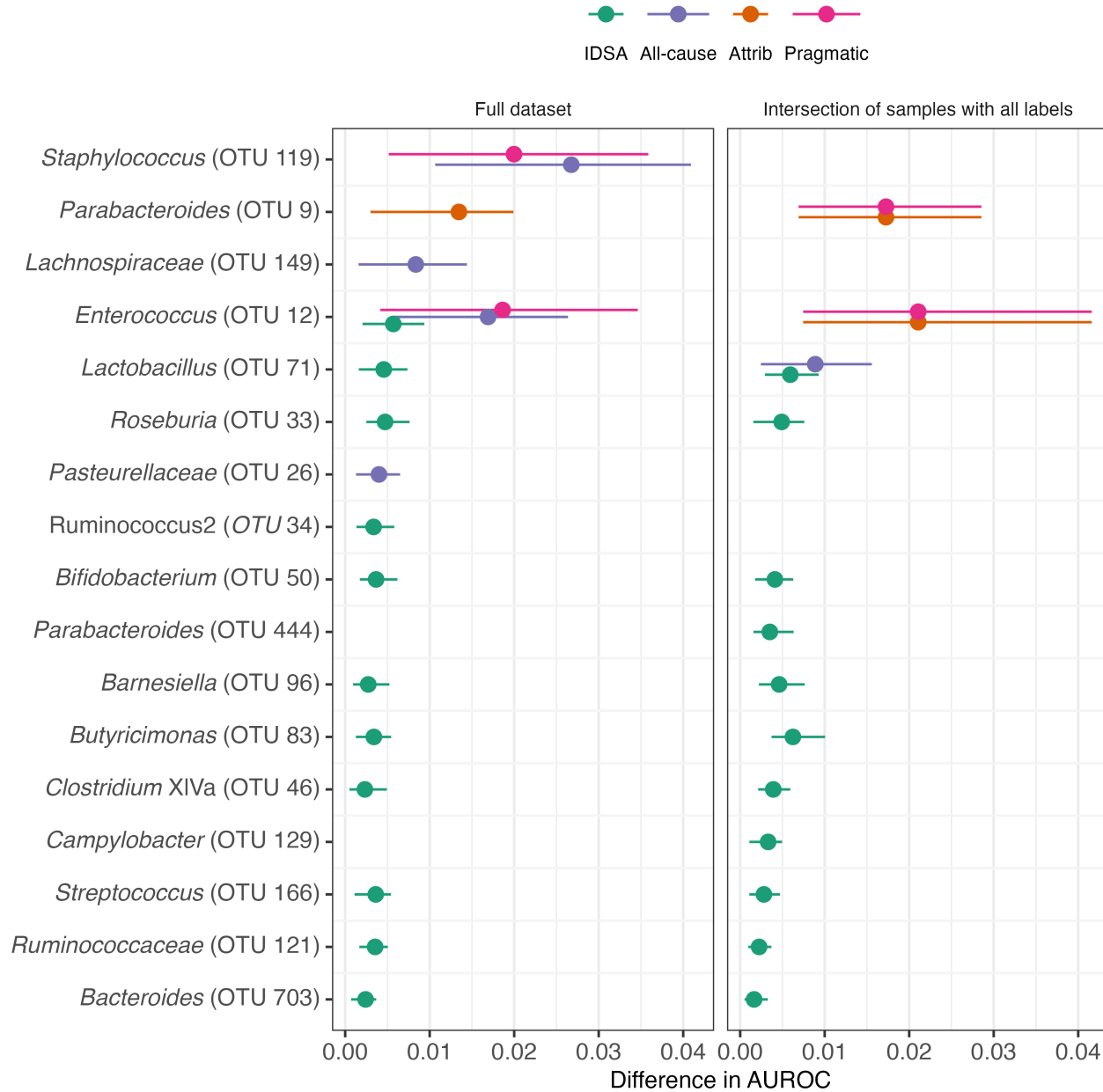
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**FIG 1 CDI severity definitions.** A) Decision flow chart to define CDI cases as severe according to the Infectious Diseases Society of America (IDSA) based on lab values, the occurrence of complications due to any cause (All-cause), and the occurrence of disease-related complications confirmed as attributable to CDI with chart review (Attrib). B) The proportion of severe CDI cases labelled according to each definition. An additional 'Pragmatic' severity definition uses the Attributable definition when possible, and falls back to the All-cause definition when chart review is not available.



**FIG 2 Performance of ML models.** Area Under the Receiver-Operator Characteristic Curve (AUROC) for the testsets and cross-validation folds of the trainsets, and the Area Under the Balanced Precision-Recall Curve (AUPRC) for the testsets. Left: models were trained on the full dataset, with different numbers of samples available for each severity definition. Right: models were trained on the intersection of samples with all labels available for each definition.



**FIG 3 Feature importance.** Feature importance via permutation test. For each OTU, the order of samples was randomized in the test set 100 times and the performance was re-calculated to estimate the permutation performance. An OTU was considered important if the performance decreased when the OTU was permuted in at least 80% of the models. OTUs with a greater difference in AUROC (actual performance minus permutation performance) are more important. Left: models were trained on the full dataset, with different numbers of samples available for each severity definition. Right: models were trained on the intersection of samples with all labels available for each definition.