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Enterococcus colonization impact on *Clostridioides difficile* disease severity

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Outline

- *Clostridioides difficile* – focus on severity scoring
- *Enterococcus* spp. – friend or foe?
- Ongoing research

Clostridioides difficile infection (CDI)

- The most common hospital-acquired infection in the USA and the leading cause of death due to gastroenteritis
- Only 2 antibiotics recommended as treatment
- Limited information for bedside clinician

Hall et al. *Clin Infect Dis.* 2012; 55:216-223.
Lessa et al. *N Engl J Med.* 2015; 372:825-834.
Johnson et al. *Clin Infect Dis.* 2021; 73:e1029–e1044.

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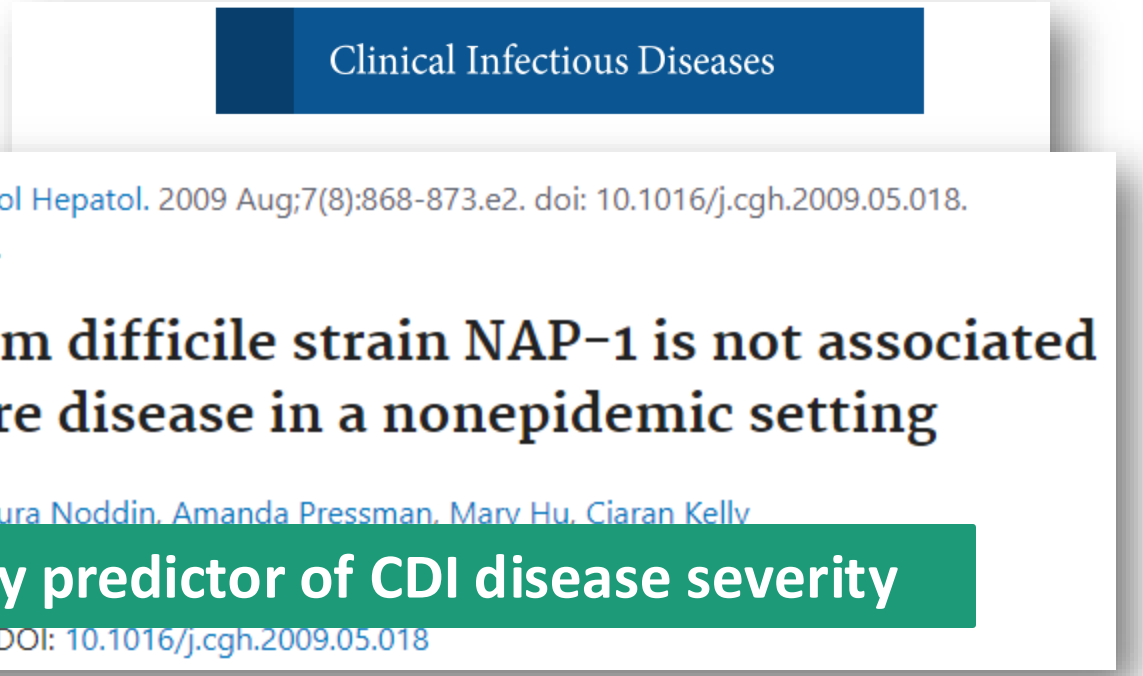
Defining CDI disease severity

- Wide spectrum of disease presentation
- Disease severity categories: non-severe, severe, fulminant
  - Based on patient characteristics and laboratory values

Missing strain type??



Hypothesize the gut microbiome is a key predictor of CDI disease severity

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The Gut Bacterial Community Potentiates *Clostridioides difficile* Infection Severity

Nicholas A. Lesniak,[a] Alyxandria M. Schubert,[a] Kaitlin J. Flynn,[a] Jhansi L. Leslie,[a*] Hamide Sinani,[a] Ingrid L. Bergin,[c] Vincent B. Young,[a,b] Patrick D. Schloss[a]

- Mice colonized with human fecal communities
- Found bacterial population with pathogenic potential were associated with more-severe outcomes

More Severe

Enterococcus, Helicobacter, and *Klebsiella*

Less Severe

Lactonifactor, Blautia, etc
(fiber degradation and bile acid metabolism)

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Another unfriendly gut microbe…

Enterococcus

- Phylum: Bacillota
  - (formerly firmicutes)
- Intrinsically resistant to many antibiotics

Antibiotics associated with *C. difficile* risk	Enterococcus activity
Clindamycin	NO
Cephalosporins	NO
Carbapenems	No – minimal
Piperacillin/tazobactam	Minimal
Quinolones	NO

Freedberg et a. *Intensive Care Med.* 2018; 44(8): 1203-1211.
Berkell et al. *Nat Comm.* 2021; 12:2241.

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Enterococcus and *C. difficile* play well together?

- Granata et al:
  - *Enterococcus* spp. and *C. difficile* interaction during CDI
  - *Enterococcus* spp. intestinal burden as risk factor for CDI
  - Prevalence of VRE in CDI patients
  - Role of CDI treatment in the occurrence of VRE colonization

Conclusion:
Yes, but…further research needed


Journal of *Clinical Medicine*

MDPI

Systematic Review

***Clostridioides difficile* and *Enterococci*'s Interplay in the Human Gut: Bacterial Alliance or Competition? A Systematic Literature Review**

Guido Granata [1,*], Francesco Schiavone [2], Fabrizio Taglietti [1] and Nicola Petrosillo [3]

VRE = vancomycin-resistant *Enterococcus*

nature

Article | Published: 16 November 2022

Enterococci enhance *Clostridioides difficile* pathogenesis

Alexander B. Smith, Matthew L. Jenior, Orlaith Keenan, Jessica L. Hart, Jonathan Specker, Arwa Abbas, Paula C. Rangel, Chao Di, Jamal Green, Katelyn A. Bustin, Jennifer A. Gaddy, Maribeth R. Nicholson, Clare Laut, Brendan J. Kelly, Megan L. Matthews, Daniel R. Evans, Daria Van Tyne, Emma E. Furth, Jason A. Papin, Frederic D. Bushman, Jessi Erlichman, Robert N. Baldassano, Michael A. Silverman, Gary M. Dunny, ... Joseph P. Zackular + Show authors

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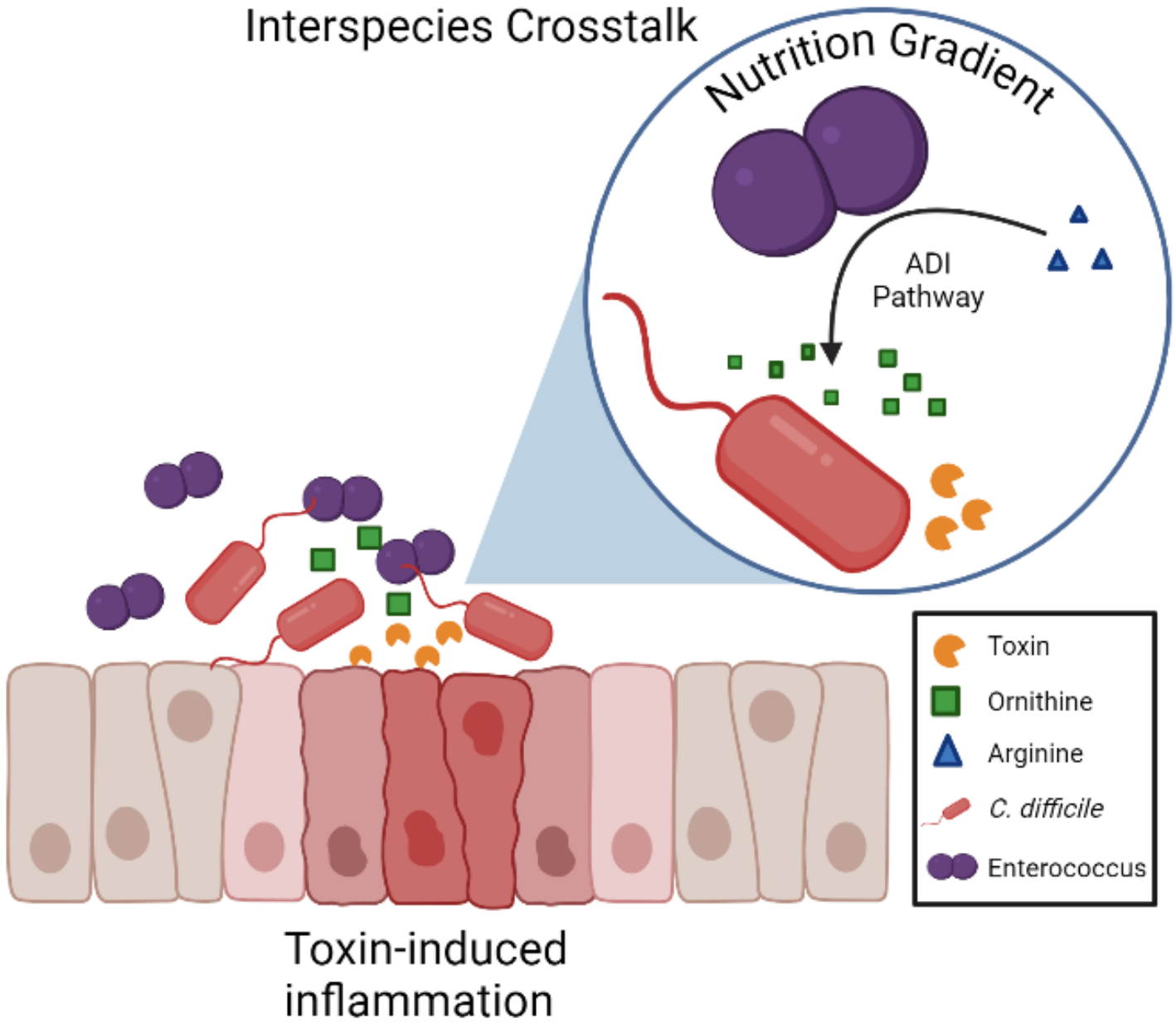
1. ✓ Co-localize with *C. difficile* in the lumen (mouse model)
2. ✓ Readily forms dual-species biofilms (*in vitro*)
3. ✓ Increases toxin production (mouse model)
4. ? Reshapes *C. difficile* metabolic environment (*in vitro*)


Interspecies Crosstalk
Nutrition Gradient
ADI
Pathway
Toxin
Ornithine
Arginine
C. difficile
Enterococcus
Toxin-induced
inflammation

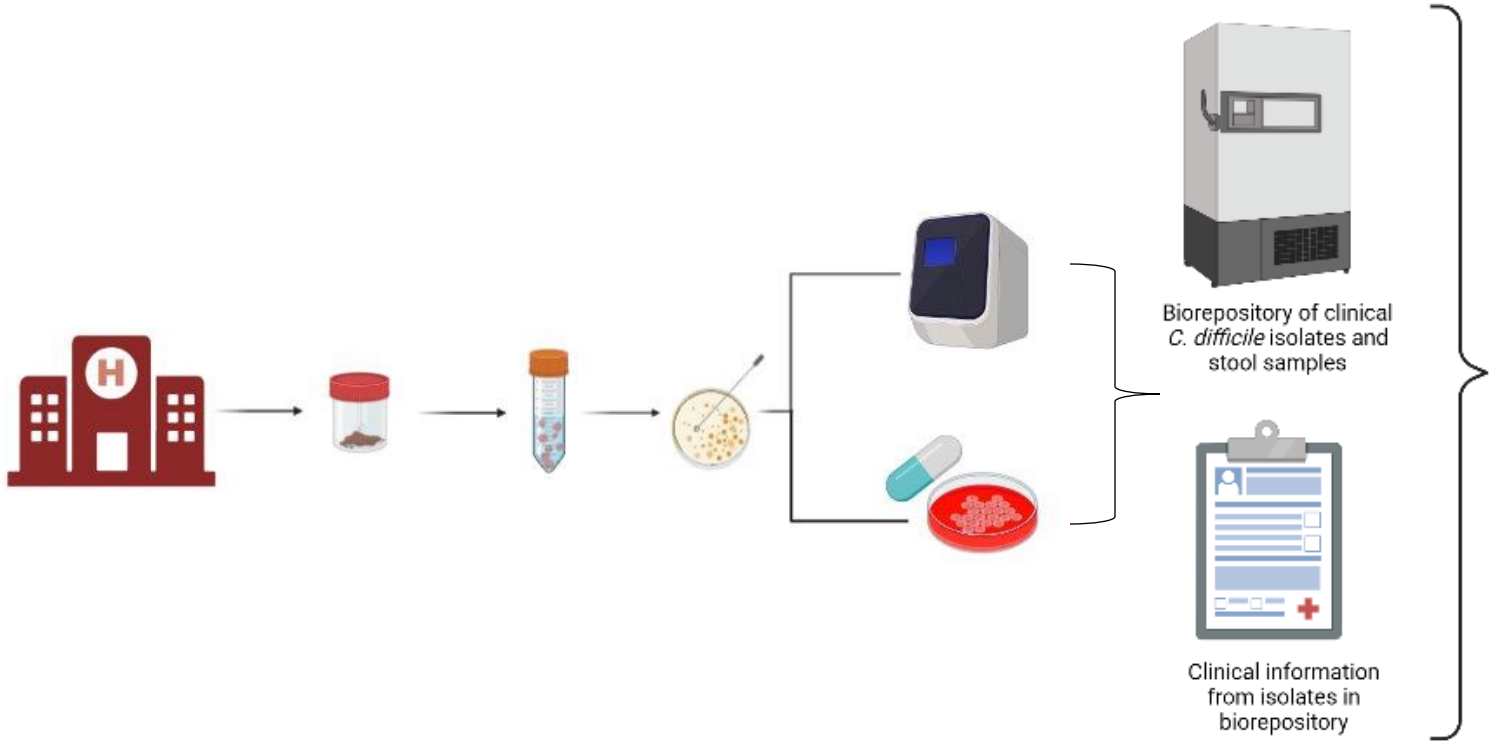
Opportunities

- Focused largely on *E. faecalis*
- Lacking robust human cohort
  - Small pediatric cohort to quantify *Enterococcus* burden
  - Small adult cohort associating *Enterococcus* abundance and WBC
- Lacking clinical implications to disease severity & patient outcomes

WBC = white blood cells

Current study – preliminary results

Cohort study of patients (2016-2024) from 2 health systems (14 hospitals) in the Texas Medical Center



SA1. Determine if Enterococci stool colonization in hospitalized patients with CDI predicts disease severity

16S rRNA sequencing by Illumina MiSeq

qPCR using Qubit 4 Fluorometer

Analysis and statistics using CLC genomics and R studio

SA2. Determine if ornithine stool concentrations in hospitalized patients with CDI predicts disease severity

LC-MS/MS

Ornithine & Arginine

Analysis and statistics using PICRUSt2 and R studio

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Study Objectives

1. Characterize the *Enterococcus* spp. abundance in our cohort
2. Assess association with CDI disease severity

Severity definition (non-severe vs severe)

- Severe = severe + fulminant
- Utilizing 2017 IDSA/SHEA *C. difficile* Clinical Practice Guidelines

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Cohort demographics

	Non-severe (n=50)	Severe (n=50)
Demographics		
Age >65	30 (60%)	36 (72%)
Female sex	34 (68%)	19 (38%)
Race/ethnicity		
White, non-Hispanic	29 (58%)	25 (50%)
Black, non-Hispanic	7 (14%)	12 (24%)
Hispanic	12 (24%)	9 (18%)
Others/not reported	2 (4%)	4 (8%)
CCI, mean (SD)	**5.0 (± 2.7)**	**6.3 (± 2.8)**
ICU upon admission	**5 (10%)**	**13 (26%)**

Unpublished, preliminary data

CDI characteristics

	Non-severe (n=50)	Severe (n=50)
CDI characteristics		
CDI classification		
HO	23 (46%)	27 (54%)
CO-HCFA	13 (26%)	14 (28%)
CO	14 (28%)	9 (18%)
Initial episode	44 (88%)	45 (90%)
Diagnostic test utilized		
NAAT	**30 (60%)**	**21 (42%)**
GDH/EIA	**20 (40%)**	**29 (58%)**

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Prior Antibiotic Exposures

	Non-severe (n=50)	Severe (n=50)
Antibiotic Exposure		
CDI-active antibiotics in past 90 d	9 (18%)	10 (20%)
Previous PO VAN	5 (10%)	6 (12%)
Previous MTZ	7 (14%)	6 (12%)
Previous FDX	2 (4%)	1 (2%)
Previous Rifaximin	1 (2%)	1 (2%)
High-risk antibiotics in past 30 d	**29 (58%)**	**36 (72%)**
Clindamycin	1 (2%)	0 (0%)
Fluoroquinolones	9 (18%)	5 (10%)
Second-generation cephalosporins	2 (4%)	2 (4%)
Third-generation cephalosporins	**9 (18%)**	**16 (32%)**
Fourth-generation cephalosporins	**9 (18%)**	**15 (30%)**
Piperacillin/tazobactam	**5 (10%)**	**12 (24%)**
Ampicillin/sulbactam	1 (2%)	2 (4%)
Carbapenems	**4 (8%)**	**13 (26%)**

SA1. Enterococcus colonization

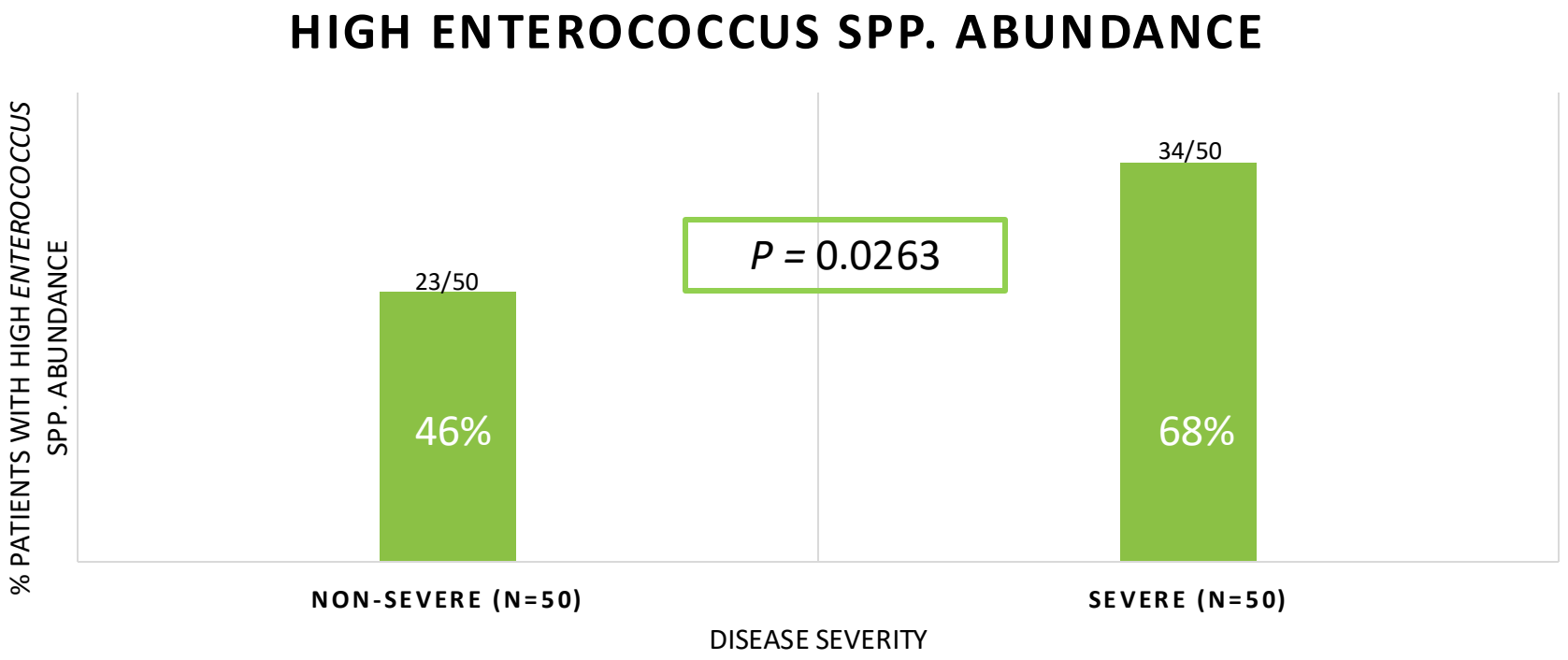
- All patients colonized with *Enterococcus* spp.
- Categorized from high to low colonization
- 57% highly colonized

Grouping	N (%)	Enterococcus DNA Quantity
0	25 (25%)	$<10^4$
1	18 (18%)	$10^4 – 10^6$
2*	44 (44%)	$10^6 – 10^8$
3*	9 (9%)	$10^8 – 10^9$
4*	4 (4%)	$>10^9$

*Categorized as high Enterococcus colonization

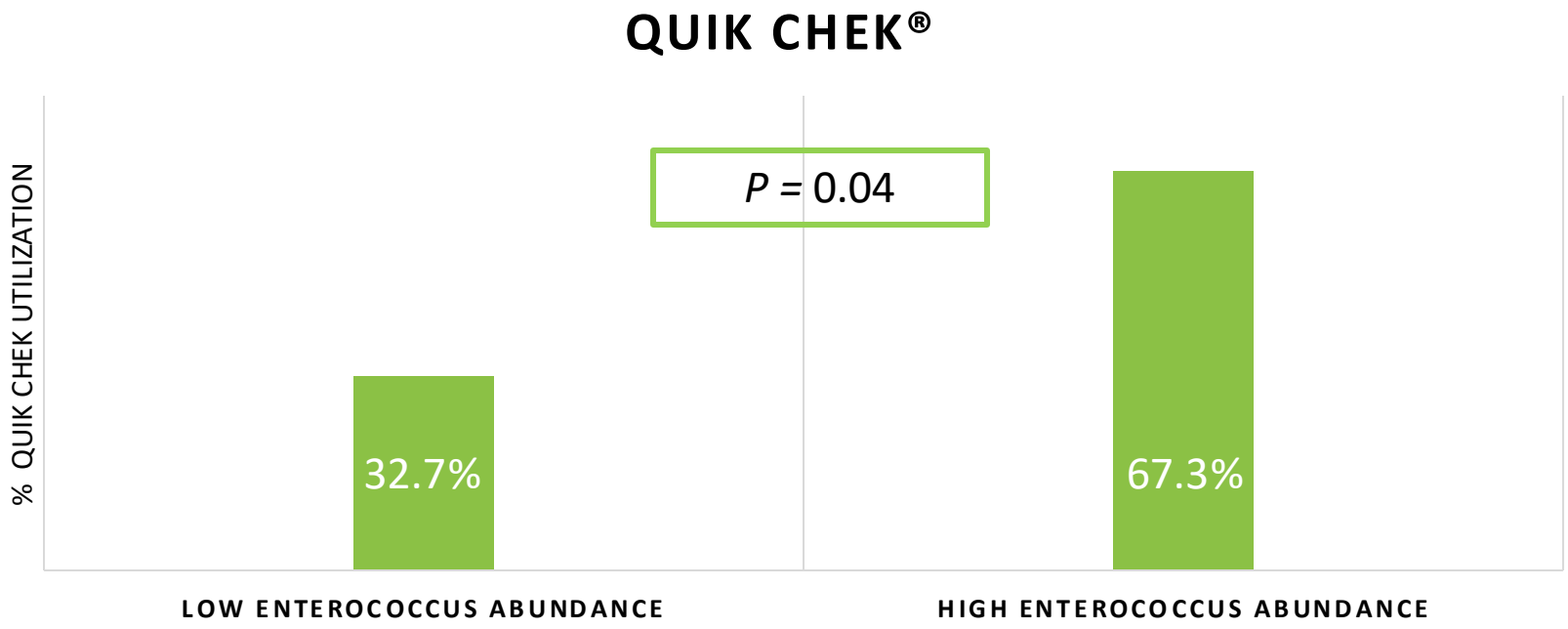
Unpublished, preliminary data

SA2. High *Enterococcus* spp. abundance associated with severe CDI



Unpublished, preliminary data

Unexpected finding:
C. DIFF QUIK CHEK® utilization linked with high *Enterococcus* spp. abundance



Unpublished, preliminary data

Discussion

***Enterococcus* spp. abundance**

- Includes *E. faecalis* and *E. facium* – common pathogenic *Enterococcus* spp.
- Categorization will need further validation

CDI Severity

- *Enterococcus* abundance demonstrated preliminary success with severity prediction

Quik Chek®

- Further evidence of higher likelihood of true disease and dysbiosis

Future Steps

- 16S sequencing underway for further microbiome analysis

- How do we apply to clinical practice?
  - Surrogate biomarkers
    - Ornithine or other metabolites (LC-MS/MS)
    - *vanA* (qPCR)

- Goal: Strategize therapy plan at time of diagnosis for CDI patients

Conclusions

- 57% of the cohort had high *Enterococcus* spp. colonization
- Patients with severe CDI were significantly more likely to be highly colonized with *Enterococcus* spp. than patients with non-severe disease
- Further investigation into rapid disease severity identification/diagnostics

Unpublished, preliminary data

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