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An Epidemiologic Exploration of Vancomycin Resistance in *Clostridioides difficile*

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Clostridioides difficile infection (CDI)

- The most common hospital-acquired infection in the USA and the leading cause of death due to gastroenteritis[1,2]
- Only 2 antibiotics recommended as treatment[3]
  - Oral vancomycin serves as current mainstay of therapy

CDC's *Antibiotic Resistance Threats in the United States, 2019*.

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

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Vancomycin role in CDI

- Culture and susceptibility testing not routinely conducted for *C. difficile*
  - Minimum inhibitory concentration (MIC) of > 2 mg/L considered vancomycin resistant[1,2]
  - *C. difficile* thought to be universally susceptible to vancomycin but has increased by 3.6% since 2012[3]
- **Hypothesis: recent increases in vancomycin use applies a selective pressure expediting resistance development**

1. CLSI M11: Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria. 9th edition.
2. EUCAST. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0, 2022.
3. Saha et al. *Anaerobe.* 2019; 58:35-46..

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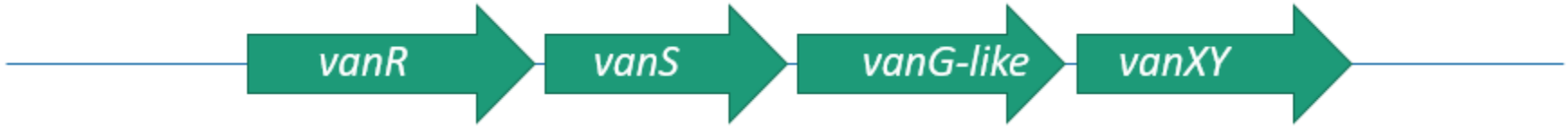
Role of *vanG* in vancomycin resistance

- Vancomycin inhibits cell wall synthesis through binding D-alanine-D-alanine terminal of the growing peptide chain
- Van genes modify the terminal D-ala-D-ala ➡ D-ala-D-x
- 85% of *C. difficile* carry a functional *vanG* operon[1]
  - D-ala-D-ala ➡ D-ala-D-serine, decreasing vancomycin binding by ~7 times[2]
  - Generally silent gene; presence alone of *vanG* does not impact susceptibility[3]



1. Ammam et al. *Can. J. Microbiol*. 2013; 58:547–551.
2. Shen et al. *J Antimicrob Chemother.* 2020; 75(4):859-867.
3. Ammam et al. *Mol Microbiol.* 2013; 89:612-625.

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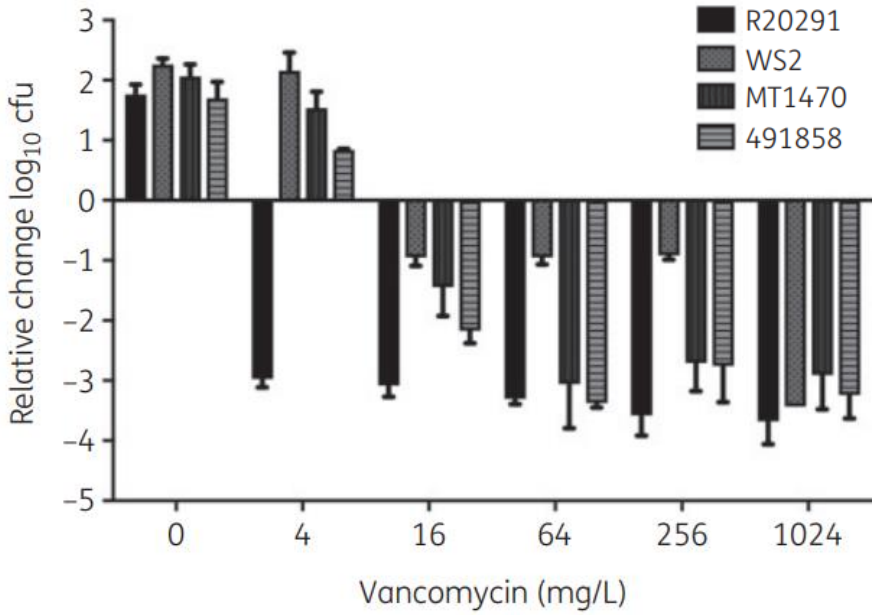
Previous work with *vanG*

- Constitutive expression of *vanG* operon in ribotype 027 strains linked to vancomycin tolerance[1]
- Set of clinical isolates with elevated MICs found to have VanSR mutations leading to constitutive *vanG* expression[1]
  - Strains survived concentrations up to 1,024 mg/L



We hypothesize *vanG* expression in *C. difficile* is higher than appreciated and expression will be predictive of poor clinical outcomes.

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1. Shen et al. *J Antimicrob Chemother.* 2020; 75(4):859-867.

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2022 – 2023 specific aims

- SA1: Vancomycin MICs
  - SA1.1 Agar dilution versus broth microdilution
  - SA1.2 Intra-lab comparison of agar dilution standard operating procedures
  - SA1.3 Vancomycin susceptibility by ribotype
- SA2: Epidemiology of *vanG*
- SA3: Clinical outcomes in relation to *vanG* expression

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MIC reproducibility for *C. difficile*

Variable by both method and drug[1-4]

Broth Microdilution (BMD)

- Pros
  - Shorter time commitment
  - Less cumbersome method
- Cons
  - MIC up to 4-dilution difference[1]
  - Typically underestimates MIC[1,2]

Agar Dilution (AD)

- Pros
  - Greater consistency and reproducibility
  - Better identifies resistance
- Cons
  - Time consuming
  - Cumbersome method

1. Hastey et al. *Anaerobe.* 2017; 44:73-77.
2. Citron et al. *Diagn Microbiol Infect Dis.* 2001; 70:554-556.
3. Moura et al. *J Antimicrob Chemother.* 2013; 68:362-365.
4. CLSI M11. Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria. 9th edition.

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SA1: Lab standardization methods

- SA1.1: Agar dilution (AD) versus broth microdilution (BMD)
  - 30 isolates simultaneously evaluated
  - Broth microdilution performed with Brain Heart Infusion (BHI) broth + oxyrase
    - Incubated 24 hours
  - Agar dilution using brucella agar with hemin (5 mg/L), vitamin K1 (10 mg/L) and 5% (v/v) sheep blood
    - Incubated 48 hours

- SA1.2: Intra-lab standardization of AD MICs
  - 18 isolates evaluated by fellows at each lab simultaneously
  - Both fellows blinded to isolates and each read the completed plates
  - Incubated 48 hours

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SA1: Lab standardization results and comparison

SA1.1 Inter-lab Standardization (AD versus BMD)

Method (no. isolates)	MIC (mg/L)			% Resistant	Major error (%)	Very major error (%)	Essential agreement (%)
	Range	MIC_{50}	MIC_{90}				
AD (30)	0.5 - >16	1	1	6.67%	0.0%	6.67%	0.0%
BMD (30)	0.0625 - 0.5	0.125	0.25	0.0%			

SA1.2 Intra-lab Standardization (AD)

Lab (no. isolates)	MIC (mg/L)						Essential Agreement (%)	
	Range		MIC_{50}		MIC_{90}			
Garey lab (18)	2 – 8	1 - 4	4	2	8	2	88.9%	100%
Hurdle lab (18)	1 - 4		2		4			

Definitions: Major error: resistant results by the new method and susceptible results by the gold standard method; **Very major error:** susceptible result by the new method and a resistant result by the gold standard method ; **Essential agreement:** MICs within ± 1 dilution

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SA1.3: Susceptibility per ribotype methods

- Comparison of MICs from 3 locally endemic ribotypes (RT):[1,2] F014-020 (n=73), F027 (n=40), F106 (n=26)
  - MICs performed using AD (max concentration of 16 mg/L)
  - Strain typing by fluorescent PCR ribotyping

1. Gonzales-Luna et al. *Emerg Microbes Infect*. 2020; 9:341-347.
2. Almutairi et al. Anaerobe. 2021; 72:102440.

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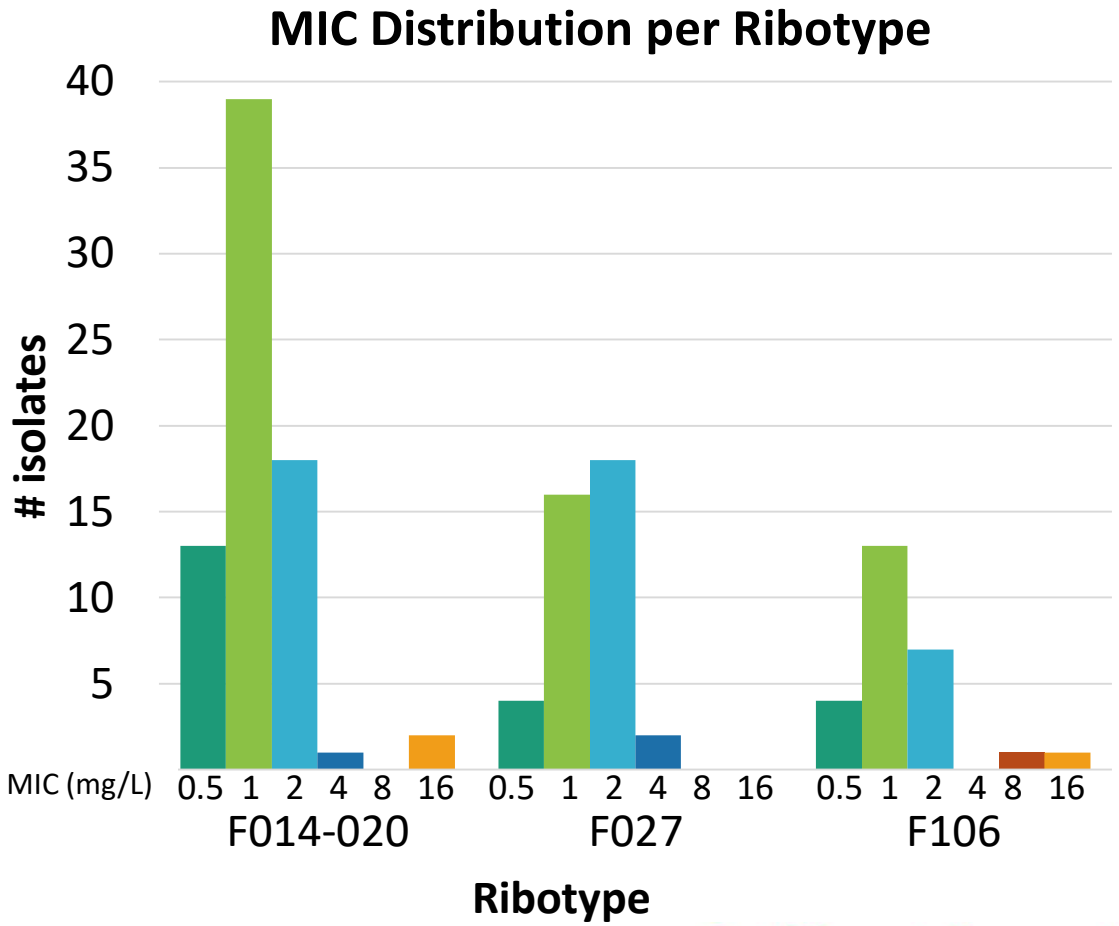
SA1.3: Susceptibility per ribotype results

Ribotype	n	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range (mg/L)	% resistant
F014-020	73	1	2	0.5 - >16	4.1%
F027	40	2	2	0.5 - 4	5.0%
F106	26	1	2	0.5 - >16	7.7%
Total	139	1	2	0.5 - >16	5.0%



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Conclusions

- Agar dilution versus broth microdilution result in variable MICs with broth microdilution underestimating resistance
- Intra-lab comparison of agar dilution method found an essential agreement of 88.9%
- Resistance rates are similar between 3 virulent ribotypes with an overall rate of 5%

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Next steps & future directions

- Expand cohort: goal of N = 600 (200 isolates per ribotype)
- Characterize *vanG* expression
- Investigate clinical outcomes associated with *vanG* expression

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