A molecular epidemiological exploration of reduced vancomycin susceptibility in *Clostridioides difficile*

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BACKGROUND

- Although *Clostridioides difficile* infection (CDI) is the most common healthcare-associated infection in the United States, vancomycin is one of only three antibiotics used to treat CDI[1]
- Clinical cure rates with vancomycin have decreased since the early 2000's to ~80% in recent randomized controlled trials[2,3]
- Vancomycin use has increased by 54% following 2018 IDSA/SHEA treatment guideline updates, applying significant selection pressure for antibiotic resistance development[4]
- As susceptibility testing is not routinely performed in *C. difficile*, the clinical significance of vancomycin resistance is not well understood

OBJECTIVE

- To describe the molecular epidemiology of reduced vancomycin susceptibility in clinical isolates during a period of high vancomycin use

METHODS

Study design / Inclusion

- Multicenter cohort study
- Adult hospitalized patients with CDI in Houston, Texas between 2017 – 2021

Statistical analysis

- Descriptive statistics were assessed using SPSS (version 27.0.0.0)

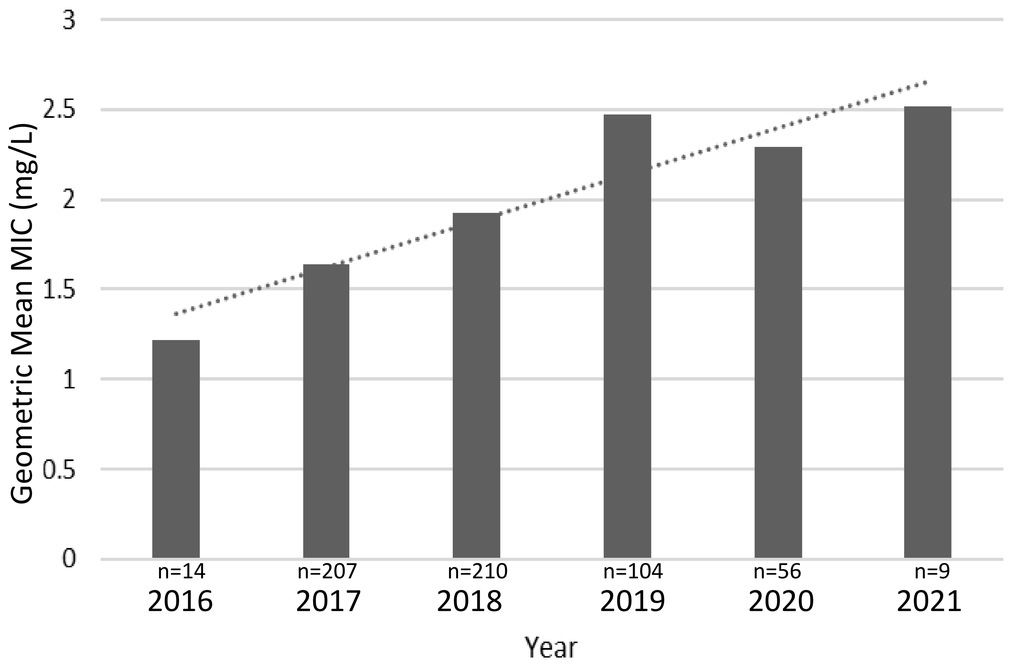
Sample processing / Microbiology:

- Discard stool samples transported to our centralized lab
- Stool plated onto selective cefoxitin-cycloserine-fructose agar (CCFA) plates and anaerobically incubated for 48 – 72 hours for culture
- Fluorescent PCR ribotyping completed
- Vancomycin MIC testing conducted via agar dilution in accordance with CLSI standards
- Reduced vancomycin susceptibility (RS) was defined by MIC >2 mg/L based on epidemiologic cutoff values[6]
- Sanger sequencing conducted on subgroup of isolates with reduced susceptibility

RESULTS

A significant difference was found **in the proportion with reduced susceptibility** based on **collection year** (p<0.001) however no difference for **healthcare system** (25% [46/183] vs 31.2% [130/417]; p=0.15)

Figure 1. Vancomycin MIC distribution over time



A higher proportion of ribotype 027 isolates demonstrated reduced vancomycin susceptibility (78.9% vs 29.3%; p<0.001)

Table 1. Vancomycin susceptibility by ribotype

Ribotype	No. isolates	MIC_{50}	MIC_{90}	MIC range	% RS
All	600	2	4	0.5 - 16	29.3%
F014-020	111	2	2	0.5 - 8	9.9%
F027	95	4	8	1 - 8	78.9%
F106	69	2	4	0.5 - 16	21.7%
F002	48	2	4	1 - 8	27.1%
F255	32	2	4	0.5 - 4	50%
Other	245	2	4	0.5 - 16	19.6%

34 isolates ribotype unavailable and included in 'Other'; Minimum Inhibitory Concentration (MIC); Reduced Susceptibility (RS)

A subgroup analysis revealed **all strains with elevated MICs had mutations in one or both parts of the two-component *vanG* regulator, VanSR**

- 22 (12.9%) isolates had ≥2 mutations in VanR
- 42 (24.6%) isolates had ≥2 mutations in VanS

Table 2. Frequency of *vanSR* mutations

Mutation	No. of isolates (N=171)	Percent with mutation
$vanR_{Cd}$		
Asp46Asn	86	50.3%
Thr115Ala	76	44.4%
Glu37Lys	4	2.3%
Other	5	2.9%
None	11	6.4%
$vanS_{Cd}$		
Ile289Met	28	16.4%
Ser56Ser	23	13.4%
Thr349Ile	20	11.7%
Arg74Arg	20	11.7%
Ser292Ser	7	4.1%
Other	40	23.4%
None	74	43.3%

CONCLUSION

- A high proportion of clinical *C. difficile* isolates exhibited elevated MICs to vancomycin, which was most common in ribotype 027 isolates
- Future research is needed to detail underlying molecular mechanisms and clinical implications of reduced vancomycin susceptibility

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