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Emergence and Spread of *Clostridioides difficile* Isolates With Reduced Fidaxomicin Susceptibility in an Acute Care Hospital

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Abstract

Background. There have been several recent reports of *Clostridioides difficile* infection (CDI) due to isolates with reduced fidaxomicin susceptibility (minimum inhibitory concentration [MIC] ≥ 2 $\mu\text{g/mL}$). However, the clinical implications are uncertain because fidaxomicin achieves high concentrations in the intestinal tract.

Methods. In an acute care hospital, we conducted a 3-year cohort study of patients with CDI to determine the frequency of infection with isolates with reduced fidaxomicin susceptibility and the impact on response to fidaxomicin treatment. Stool specimens were cultured for *C. difficile*, and susceptibility testing was performed using agar dilution. Whole-genome sequencing was used to identify mutations associated with reduced fidaxomicin susceptibility and to determine relatedness

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of isolates. For genomically related susceptible and reduced susceptibility isolates from the same patient, we compared rates of growth, sporulation, and toxin production.

Results. Of 108 fidaxomicin-treated patients, 6 (5.6%) were infected with isolates that possessed reduced fidaxomicin susceptibility (MICs 8–32 µg/mL), including 3 with initially susceptible isolates followed by clinical failure with subsequent recovery of genomically related isolates with reduced susceptibility. Isolates with reduced fidaxomicin susceptibility harbored mutations in RNA polymerase associated with reduced susceptibility and exhibited reduced toxin production, and 20% to 40% of isolates tested had reduced growth and/or sporulation in comparison with susceptible isolates. Three patients were infected with genomically indistinguishable ribotype 097 isolates with reduced fidaxomicin susceptibility.

Conclusions. Our findings highlight the potential for the emergence on therapy of clinically relevant reduced fidaxomicin susceptibility in *C. difficile* and its spread via transmission to other patients.

Keywords

Clostridioides difficile ; fidaxomicin; susceptibility; transmission; whole-genome sequencing

Fidaxomicin and vancomycin are the primary antibiotics recommended for treatment of *Clostridioides difficile* infection (CDI) [1, 2]. Although the potential for emergence of resistance to these agents is a concern [3], they have remained effective in achieving clinical cure of CDI, and reduced susceptibility has been rare in recent surveillance studies in the United States and Europe [4–7]. However, recent reports have raised concern that isolates with reduced susceptibility to fidaxomicin and vancomycin may be emerging [8–11]. The clinical implications of reduced susceptibility have been uncertain as these antibiotics achieve high concentrations in the intestinal tract [3]. However, Eubank et al [9] recently reported that *C. difficile* isolates with vancomycin minimum inhibitory concentrations [MICs] >2 µg/mL (range, 4–16) were associated with reductions in initial cure and sustained clinical response.

Fidaxomicin is a macrocyclic antibiotic that targets bacterial RNA polymerase [12, 13]. The Clinical and Laboratory Standards Institute (CLSI) has not established clinical breakpoints for fidaxomicin. The European Committee on Antimicrobial Susceptibility Testing breakpoint for resistance is >0.5 mg/L based on the epidemiological cutoff value [14]. Baines and Wilcox [15] considered isolates with MICs of 2–4 µg/mL to have reduced susceptibility and isolates with MIC ≥16 µg/mL to be resistant. In vitro, selection of mutants with reduced fidaxomicin susceptibility has been associated with mutations in the genes *rpoB* and *rpoC* that encode the RNA polymerase complex β and β' subunits, respectively [12, 16, 17]. To our knowledge, there have been 7 reports of clinical *C. difficile* isolates with reduced fidaxomicin susceptibility (MICs 2 to >64 µg/mL), including several isolates recovered from patients with recurrent infections [11, 18–25]. In 5 reports, mutations were identified in the *rpoB* or *rpoC* gene [11, 16–20, 23]. The potential for strains with these mutations to compromise the efficacy of fidaxomicin is uncertain because the mutations may result in defects in growth, sporulation, and toxin production [11, 16, 17]. Moreover,

no reports have demonstrated that reduced fidaxomicin susceptibility results in failure to achieve clinical cure.

It is unclear if reduced susceptibility to fidaxomicin is emerging as an important concern because surveillance is limited and susceptibility testing is not included in diagnostic algorithms [1, 2]. At our institution, we have occasionally identified CDI patients who failed to respond to fidaxomicin. We therefore examined the frequency of detection of isolates with reduced fidaxomicin susceptibility in all CDI patients and tested the hypothesis that reduced susceptibility may be associated with treatment failure.

METHODS

Setting

The Cleveland VA Medical Center is a 215-bed hospital with an adjacent long-term care facility (LTCF). A 2-step CDI test algorithm is used with an initial nucleic acid amplification test (NAAT) followed by an enzyme immunoassay for toxin in NAAT-positive samples [1]. During the study, fidaxomicin was recommended for initial CDI cases in patients aged ≥ 65 years, immunocompromised patients, and LTCF residents and for first recurrences. The Supplementary Material provides information on CDI epidemiology, diagnosis, treatment, and control measures in the facility.

Study Design

The facility's institutional review board approved the study protocol. Between 1 July 2020 and 30 June 2023, we conducted a cohort study of all patients with a positive NAAT for *C. difficile* who were treated with oral vancomycin or fidaxomicin. For patients considered treatment failures, stool specimens were collected for CDI testing prior to a change in therapy to assess for microbiologic response to the treatment and emergence of resistance. NAAT-positive stool specimens were cultured for toxigenic *C. difficile*, and isolates were tested for fidaxomicin susceptibility. All isolates from patients with 1 or more isolates with reduced susceptibility to fidaxomicin were subjected to whole-genome sequencing. Isolates with reduced susceptibility and paired genomically related susceptible isolates from the same patients were assessed for growth, toxin production, and sporulation. Medical records were reviewed to obtain information on demographics, laboratory results, and response to therapy.

Epidemiologic Definitions

CDI was defined as the presence of diarrhea (≥ 3 unformed stools in 24 hours) and a positive test for *C. difficile*. CDI cases were classified as severe versus nonsevere based on standard definitions [1]. Recurrence was defined as a CDI episode occurring 8 weeks or less after the onset of a previous CDI episode, provided that symptoms from the earlier episode resolved [1, 19]. Clinical cure was defined as resolution of diarrhea and no need for further CDI therapy as of the second day after completing ≥ 10 days of therapy. Clinical failure was defined as persistent diarrhea and/or need for additional CDI therapy [1, 26]. Refractory CDI was defined as CDI with no response after 3–5 days of treatment with no alternative explanation for diarrhea and resulting in a change in therapy [27]. Microbiologic

failure was defined as refractory CDI or clinical failure in conjunction with recovery of $>4 \log_{10}$ colony-forming units (CFUs) of *C. difficile* in stool. Hospital ward and non-ward transmission were defined as described previously [28].

Microbiology and Molecular Typing

Stool specimens were cultured for toxigenic *C. difficile* by plating on *C. difficile* brucella agar [29]. Broth enrichment cultures were performed if agar cultures were negative [30]. Isolates recovered on the selective media were plated on nonselective blood agar plates and subjected to identification using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Polymerase chain reaction (PCR) ribotyping was performed [31].

Additional screening was performed for patients with initial fidaxomicin-susceptible isolates followed by recovery of isolates with reduced susceptibility. The initial stool specimens were cultured using *C. difficile* brucella agar and broth that contained 2 $\mu\text{g/mL}$ of fidaxomicin to determine if subpopulations with reduced fidaxomicin susceptibility were present.

Susceptibility Testing

MICs for fidaxomicin, vancomycin, erythromycin, rifampin, and moxifloxacin were determined using the reference agar dilution method in accordance with CLSI recommendations [6, 32]. For fidaxomicin, reduced susceptibility was defined as MIC $\geq 2 \mu\text{g/mL}$ and resistance was defined as MIC $\geq 16 \mu\text{g/mL}$ [15]. Isolates with reduced fidaxomicin susceptibility were sent to 2 laboratories with expertise in *C. difficile* susceptibility testing for confirmation. The Supplementary Material provides detailed methods.

Whole-Genome Sequencing and Bioinformatic Analysis

Whole-genome sequencing was performed as previously described (the Supplementary Material provides detailed methods) [30]. Sequencing was performed on the NextSeq 550 sequencing system (Illumina). De novo assembly of reads was done using SPAdes genome assembler (v.3.9), and a multilocus sequence type was assigned. The *rpoB* and *rpoC* genes were detected using BLAST v2.11.0 using reference genes against the assembled sequences. Trimmed reads were mapped to a *C. difficile* reference genome after dynamic trimming, and a dendrogram of the genome was created using advanced cluster analysis (similarity matrix) on Bionumerics software (v.7.6, Applied Maths). Isolates were considered highly genetically related if they differed by ≤ 2 single nucleotide polymorphisms (SNPs) [19].

Evaluation of Growth, Sporulation, and Toxin Production

We measured growth rates, sporulation rates, and toxin production in strains with reduced fidaxomicin susceptibility in comparison with susceptible isolates from the same patients using previously described methods [11, 17]. Growth curves were performed over 24 hours in brucella broth with 5 mg/L hemin and 1 mg/L vitamin K₁ [17]. Sporulation was assessed after 24, 48, and 72 hours of incubation. The $\log_{10}$ CFU of spores was measured after heat shock for 30 minutes at 65°C [11]. Toxin production was measured after 24, 48, and 72 hours of incubation using a toxin A/B enzyme-linked immunoassay (Techlab TOX A/B

ELISA) with absorbance measurements at OD 450 (SpectraMax M3, Molecular Devices, San Jose, CA).

Data Analysis

For 3 patients with genomically related fidaxomicin-susceptible and reduced susceptibility isolates, linear mixed-effects models were used to compare growth, sporulation, and toxin production for the susceptible and reduced susceptibility isolates.

RESULTS

Figure 1 provides a flow diagram for the study. Of 328 episodes of CDI in 296 patients, 262 (79.9%) were initial diagnoses. Of the 328 CDI episodes, 122 (37.2%) were treated with fidaxomicin and 206 (62.8%) were treated with vancomycin. Clinical cure was achieved in 114 (93.4%) episodes treated with fidaxomicin and 192 (93.2%) episodes treated with vancomycin. Recurrences occurred in 13.2% of cured episodes treated with fidaxomicin and 17.7% of cured episodes treated with vancomycin. Of the 108 patients treated with fidaxomicin, 6 (5.4%) had isolates with reduced fidaxomicin susceptibility, including 3 who achieved clinical cure with fidaxomicin, 2 with clinical failure, and 1 with refractory CDI. One patient treated with vancomycin was infected with an isolate with reduced fidaxomicin susceptibility and was cured with vancomycin.

Characterization of *C. difficile* Isolates

Table 1 shows the characteristics of the 9 *C. difficile* isolates recovered from the 6 patients infected with isolates with reduced fidaxomicin susceptibility. Eight of the 9 isolates were recovered from stool specimens with *C. difficile* NAAT cycle threshold values <30, and 3 were recovered from toxin-positive specimens. All 6 patients had more than 3 unformed stools per day without an alternative explanation for each episode of CDI.

Three patients were infected with ribotype 097 (sequence type 21) isolates (N = 6) with MICs of 8 to 16 µg/mL, including 1 patient (patient 1) initially infected with a fidaxomicin-susceptible ribotype 097 isolate. The ribotype 097 isolates with reduced susceptibility all had A265G mutations in the *rpoC* gene coding for an R89G substitution in the RNA polymerase β' subunit. Two patients were infected with ribotype 014–020 isolates with fidaxomicin MICs of 32 µg/mL with T3428G or T3428A mutations in the *rpoB* gene coding for a V1143G or V1143D substitution in the RNA polymerase β subunit, including 1 patient (patient 2) initially infected with a fidaxomicin-susceptible ribotype 014–020 isolate. One patient (patient 6) was infected with a fidaxomicin-susceptible ribotype 015 isolate; however, a genomically related isolate with a fidaxomicin MIC of 16 µg/mL was subsequently recovered with a T3428A mutation in the *rpoB* gene coding for a V1143D substitution in the RNA polymerase β subunit. None of the isolates had mutations in the *marR* homolog CD22120, which has been associated with reduced fidaxomicin susceptibility [12, 13]. None of the isolates with reduced fidaxomicin susceptibility had mutations previously associated with rifamycin resistance [33], and all were susceptible to vancomycin, rifampin, moxifloxacin, and erythromycin (Supplementary Material).

Clinical Timeline for Patients Infected With Isolates With Reduced Fidaxomicin Susceptibility

Figure 2 provides a timeline of CDI diagnoses and treatment and infecting ribotypes for the 6 patients infected with 1 or more isolates with reduced fidaxomicin susceptibility. Four of 6 patients (patients 1, 2, 4, and 6) received treatment with fidaxomicin prior to isolation of the isolates with reduced susceptibility. Two patients (patients 1 and 2) were initially infected with fidaxomicin-susceptible isolates and subsequently had clinical failure based on persistent diarrhea that did not improve after ≥ 10 days of fidaxomicin therapy with no alternative explanation, resulting in collection of stool specimens to assess for emergence of resistance. Both were classified as microbiologic treatment failures due to recovery of a high burden of *C. difficile* in stool (patient 1: $6.6 \log_{10}$ CFU/g of stool and PCR cycle threshold value of 21.8 on treatment day 18; patient 2: $4.4 \log_{10}$ CFU/g of stool and PCR cycle threshold value of 28.6 on treatment day 17). For patient 1, diarrhea resolved after CDI therapy was switched to vancomycin. Patient 1 was again diagnosed with CDI 19 months after the CDI episode in July 2021 and was found to again be infected with a ribotype 097 isolate with reduced fidaxomicin susceptibility. Patient 2 underwent colectomy due to fulminant colitis. Three patients (patients 3, 4, and 5) achieved clinical cure with fidaxomicin despite infection with *C. difficile* isolates with reduced susceptibility to fidaxomicin (MICs of 8 to 32 $\mu\text{g/mL}$).

One patient (patient 6) was infected with a ribotype 015 isolate that was initially susceptible to fidaxomicin. He had refractory CDI with no improvement after 4 days of fidaxomicin treatment, and a related ribotype 015 isolate with a fidaxomicin MIC of 16 $\mu\text{g/mL}$ was recovered from stool. His diarrhea subsequently resolved after treatment was switched to oral vancomycin and metronidazole.

For the 4 patients with initial recovery of susceptible isolates followed by recovery of isolates with reduced susceptibility (patients 1, 2, 4, and 6), additional screening of the initial stool specimens did not identify subpopulations of *C. difficile* with reduced fidaxomicin susceptibility (ie, no colonies recovered from media containing 2 $\mu\text{g/mL}$ of fidaxomicin).

Relatedness of the *C. difficile* Isolates by Whole-Genome Sequencing

Figure 3 is a dendrogram that shows the relatedness of all isolates recovered from the 6 patients infected with isolates with reduced fidaxomicin susceptibility. For patient 1, an initially susceptible ribotype 097 isolate (1B, 9 February 2021) differed by only 1 SNP from 2 subsequent isolates with reduced susceptibility (1D, 8 July 2021 and 1E, 29 July 2021) associated with treatment failure. However, when patient 1 again was diagnosed with CDI 19 months later, he was infected with an isolate (1F, 4 April 2023) that had the same RpoC R89G amino acid substitution as the earlier isolates but also had a RpoB D814Y amino acid substitution. This isolate differed by 7 SNPs from the previous 1.D and 1.E isolates (Supplementary Material).

For patient 2, an initially susceptible ribotype 014–020 isolate differed by only 1 SNP from the subsequent isolate with reduced susceptibility. For patient 3, the ribotype 097 isolates

with reduced fidaxomicin susceptibility associated with initial and recurrent CDI episodes were genomically indistinguishable.

Five ribotype 097 isolates from patients 1, 3, and 4 with reduced fidaxomicin susceptibility were genomically indistinguishable (1D, 1E, 3A, 3B, and 4B). Temporally, the first isolate with reduced susceptibility was recovered from patient 4 (4B, 27 April 2021). However, patient 1 had a genomically related (ie, 1 SNP difference) but fidaxomicin-susceptible ribotype 097 isolate recovered several months earlier (1B, 9 February 2021). Patient 1 had multiple hospital and adjacent LTCF admissions between August 2020 and September 2021, with 11 months of total inpatient care. Patients 3 and 4 were not hospitalized, but each had multiple outpatient clinic visits at the hospital during the period when patient 1 was an inpatient.

Patients 2 and 5 were infected with ribotype 014–020 isolates. However, the isolates were genomically distinct with different mutations in the *rpoB* gene.

Evaluation of Growth, Sporulation, and Toxin Production

Figure 4 shows growth rates, sporulation rates, and toxin production for isolates from patients 1, 2, and 6 with reduced fidaxomicin susceptibility in comparison with paired genomically related, susceptible isolates from the same patients. In comparison with susceptible isolates, growth rates were significantly decreased at time points ≥ 10 hours in isolates with reduced fidaxomicin susceptibility for patients 2 and 6 ($P < .001$) but not patient 1 ($P = .07$). In comparison with susceptible isolates, there was no significant decrease in sporulation except in the ribotype 097 isolate, which had mutations in both *rpoC* and *rpoB* (1.F; $P < .01$). In comparison with susceptible isolates, all isolates with reduced susceptibility had significantly reduced toxin production at 48 and 72 hours ($P < .001$).

DISCUSSION

In a cohort study of 296 CDI patients, isolates with reduced fidaxomicin susceptibility were recovered from 6 of 108 (5.6%) patients who received fidaxomicin. In 3 of the 6 patients, clinical failure or refractory CDI occurred with subsequent recovery of isolates with reduced fidaxomicin susceptibility that were genomically related to initially susceptible isolates. Each isolate with reduced fidaxomicin susceptibility harbored mutations in RNA polymerase previously associated with reduced fidaxomicin susceptibility. Our findings highlight the potential for the emergence on therapy of clinically relevant reduced fidaxomicin susceptibility in *C. difficile*.

We are the first to suggest that elevated fidaxomicin MICs may contribute to treatment failure. The microbiologic basis for CDI treatment failure despite the reported high concentrations of fidaxomicin and vancomycin in stool is uncertain. It has been proposed that factors such as reduced concentrations of drug in the intestinal lumen versus in stool and elevated minimum bactericidal concentrations versus MICs might contribute to clinical failure [8]. Alternatively, some patients may have relatively low drug concentrations in stool. Louie et al [34] reported wide variation in fidaxomicin concentrations in patients after 10 days of treatment (range, 12 to 787 $\mu\text{g/g}$ of stool).

Previous reports have revealed that some mutations that confer decreased fidaxomicin susceptibility reduce the fitness of *C. difficile* isolates based on reductions in growth, sporulation, and toxin production [11, 16, 17]. In our investigation, the mutations reduced toxin production but did not consistently alter growth and sporulation. Moreover, 3 patients infected with isolates with reduced fidaxomicin susceptibility had positive stool toxin assays, and 1 patient developed an episode of CDI with the same ribotype 097 isolate 2 years after an initial infection, suggesting long-term carriage of the isolate. Of 8 CDI episodes due to isolates with reduced fidaxomicin susceptibility, 1 (11%) was classified as severe [1]. Notably, the patient with severe CDI (patient 2) had a negative enzyme immunoassay for toxin in stool and underwent colectomy due to fulminant colitis. Others have reported that a substantial proportion of CDI patients who meet criteria for severe CDI have negative enzyme immunoassays for toxin in stool [35, 36].

Our findings suggest that isolates with reduced fidaxomicin susceptibility may have spread from patient to patient in our facility. Three patients were infected with genomically indistinguishable isolates from ribotype 097 (sequence type 21), a rarely reported *C. difficile* ribotype [7, 37], accounting for only 0.3% of CDI cases from 2012 through 2020 in the Centers for Disease Control and Prevention's CDI Surveillance Program (Amy Gargis, written personal communication, 4 December 2024). There were no ward-level interactions between the patients. However, the presumptive index case had prolonged hospital and LTCF stays, and the other 2 patients had 6 to 7 outpatient visits each in the hospital during that period.

Our study has some limitations. The study was conducted in 1 hospital during a period in which 36% of CDI patients were treated with fidaxomicin. We isolated and performed molecular typing for 1 colony of *C. difficile* per culture; we cannot exclude the possibility that multiple strains were present. We cannot state with certainty whether isolates with reduced susceptibility emerged due to new genetic mutations during fidaxomicin therapy versus due to expansion of preexisting subpopulations due to fidaxomicin selective pressure. However, we did not identify subpopulations of *C. difficile* with reduced susceptibility in stool collected at the start of treatment. Finally, we did not screen asymptomatic patients or patients diagnosed in outside hospitals prior to transfer to our facility.

In conclusion, our findings suggest that *C. difficile* isolates with clinically relevant reduced fidaxomicin susceptibility may emerge during therapy and spread to other patients. Clinicians should be aware that reduced susceptibility should be considered as a potential contributor to fidaxomicin treatment failure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability.

All genome sequences generated by this project have been submitted to National Center for Biotechnology Information (Bioproject ID PRJNA1199382). Reasonable requests for deidentified data will be honored by the corresponding author.

References

1. Johnson S, Lavergne V, Skinner AM, et al. Clinical practice guideline by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA): 2021 focused update guidelines on management of *Clostridioides difficile* infection in adults. *Clin Infect Dis* 2021; 73:755–7. [PubMed: 34492699]
2. Kelly CR, Fischer M, Allegretti JR, et al. ACG clinical guidelines: prevention, diagnosis, and treatment of *Clostridioides difficile* infections. *Am J Gastroenterol* 2021; 116:1124–47. [PubMed: 34003176]
3. Greentree DH, Rice LB, Donskey CJ. Houston, we have a problem: reports of *Clostridioides difficile* isolates with reduced vancomycin susceptibility. *Clin Infect Dis* 2022; 75:1661–4. [PubMed: 35653393]
4. Gargis AS, Karlsson M, Paulick AL, et al. Reference susceptibility testing and genomic surveillance of *Clostridioides difficile*, United States, 2012–17. *Clin Infect Dis* 2023; 76:890–6. [PubMed: 36208202]
5. Snyderman DR, McDermott LA, Thorpe CM, et al. A US-based national surveillance study for the susceptibility and epidemiology of *Clostridioides difficile* isolates with special reference to rifamycin: 2020–2021. *Antimicrob Agents Chemother* 2023; 67:e0034923.
6. Thorpe CM, McDermott LA, Tran MK, et al. U.S.-based national surveillance for fidaxomicin susceptibility of *Clostridioides difficile*-associated diarrheal isolates from 2013 to 2016. *Antimicrob Agents Chemother* 2019; 63:e00391–19.
7. Freeman J, Vernon J, Pilling S, et al. Five-year pan-European, longitudinal surveillance of *Clostridium difficile* ribotype prevalence and antimicrobial resistance: the extended ClosER Study. *Eur J Clin Microbiol Infect Dis* 2020; 39:169–77. [PubMed: 31811507]
8. Darkoh C, Keita K, Odo C, et al. Emergence of clinical *Clostridioides difficile* isolates with decreased susceptibility to vancomycin. *Clin Infect Dis* 2022; 74:120–6. [PubMed: 35016207]
9. Eubank TA, Dureja C, Garey KW, Hurdle JG, Gonzales-Luna AJ. Reduced vancomycin susceptibility in *Clostridioides difficile* is associated with lower rates of initial cure and sustained clinical response. *Clin Infect Dis* 2024; 79:15–21. [PubMed: 38382090]
10. Adler A, Miller-Roll T, Bradenstein R, et al. A national survey of the molecular epidemiology of *Clostridium difficile* in Israel: the dissemination of the ribotype 027 strain with reduced susceptibility to vancomycin and metronidazole. *Diagn Microbiol Infect Dis* 2015; 83:21–4. [PubMed: 26116225]
11. Marchandin H, Anjou C, Poulen G, et al. In vivo emergence of a still uncommon resistance to fidaxomicin in the urgent antimicrobial resistance threat *Clostridioides difficile*. *J Antimicrob Chemother* 2023; 78:1992–9. [PubMed: 37352110]
12. Leeds JA, Sachdeva M, Mullin S, Barnes SW, Ruzin A. In vitro selection, via serial passage, of *Clostridium difficile* mutants with reduced susceptibility to fidaxomicin or vancomycin. *J Antimicrob Chemother* 2014; 69:41–4. [PubMed: 23887866]
13. Leeds JA. Antibacterials developed to target a single organism: mechanisms and frequencies of reduced susceptibility to the novel anti-*Clostridium difficile* compounds fidaxomicin and LFF571. *Cold Spring Harb Perspect Med* 2016; 6: a025445.

14. European Committee on Antimicrobial Susceptibility Testing. Clinical breakpoints—breakpoints and guidance. Available at: https://www.eucast.org/clinical_breakpoints. Accessed 14 October 2024.
15. Baines SD, Wilcox MH. Antimicrobial resistance and reduced susceptibility in *Clostridium difficile*: potential consequences for induction, treatment, and recurrence of *C. difficile* infection. *Antibiotics (Basel)* 2015; 4:267–98. [PubMed: 27025625]
16. Babakhani F, Seddon J, Sears P. Comparative microbiological studies of transcription inhibitors fidaxomicin and the rifamycins in *Clostridium difficile*. *Antimicrob Agents Chemother* 2014; 58:2934–7. [PubMed: 24550338]
17. Kuehne SA, Dempster AW, Collery MM, et al. Characterization of the impact of *rpoB* mutations on the in vitro and in vivo competitive fitness of *Clostridium difficile* and susceptibility to fidaxomicin. *J Antimicrob Chemother* 2018; 73:973–80. [PubMed: 29253242]
18. Goldstein EJ, Citron DM, Sears P, Babakhan F, Sambo SP, Gerding DN. Comparative susceptibilities to fidaxomicin (OPT-80) of isolates collected at baseline, recurrence, and failure from patients in two phase III trials of fidaxomicin against *Clostridium difficile* infection. *Antimicrob Agents Chemother* 2011; 55:5194–9. [PubMed: 21844318]
19. Eyre DW, Babakhani F, Griffiths D, et al. Whole-genome sequencing demonstrates that fidaxomicin is superior to vancomycin for preventing reinfection and relapse of infection with *Clostridium difficile*. *J Infect Dis* 2014; 209:1446–51. [PubMed: 24218500]
20. Schwanbeck J, Riedel T, Laukien F, et al. Characterization of a clinical *Clostridioides difficile* isolate with markedly reduced fidaxomicin susceptibility and a V1143D mutation in *rpoB*. *J Antimicrob Chemother* 2019; 74:6–10. [PubMed: 30247587]
21. Peng Z, Addisu A, Alrabaa S, Sun X. Antibiotic resistance and toxin production of *Clostridium difficile* isolates from the hospitalized patients in a large hospital in Florida. *Front Microbiol* 2017; 8:2584. [PubMed: 29312258]
22. Martínez-Meléndez A, Tijerina-Rodríguez L, Morfin-Otero R, et al. Circulation of highly drug-resistant *Clostridium difficile* ribotypes 027 and 001 in two tertiary-care hospitals in Mexico. *Microb Drug Resist* 2018; 24:386–92. [PubMed: 29485939]
23. Kolte B, Nübel U. Genetic determinants of resistance to antimicrobial therapeutics are rare in publicly available *Clostridioides difficile* genome sequences. *J Antimicrob Chemother* 2024; 79:1320–8. [PubMed: 38598696]
24. Aoki K, Takeda S, Miki T, Ishii Y, Tateda K. Antimicrobial susceptibility and molecular characterisation using whole-genome sequencing of *Clostridioides difficile* collected in 82 hospitals in Japan between 2014 and 2016. *Antimicrob Agents Chemother* 2019; 63:e01259–19.
25. Le TM, Eubank TA, McKelvey AM, Cao X, Hurdle JG, Garey KW. Fidaxomicin resistance in *Clostridioides difficile*: a systematic review and predictive modeling with RNA polymerase binding sites. *Antimicrob Agents Chemother* 2024; 68: e0120624.
26. Louie TJ, Miller MA, Mullane KM, et al. Fidaxomicin versus vancomycin for *Clostridium difficile* infection. *N Engl J Med* 2011; 364:422–31. [PubMed: 21288078]
27. van Prehn J, Reigadas E, Vogelzang EH, et al. European Society of Clinical Microbiology and Infectious Diseases: 2021 update on the treatment guidance document for *Clostridioides difficile* infection in adults. *Clin Microbiol Infect* 2021; 27(Suppl 2):S1–21.
28. Donskey CJ, Sunkesula VCK, Stone ND, et al. Transmission of *Clostridium difficile* from asymptotically colonized or infected long-term care facility residents. *Infect Control Hosp Epidemiol* 2018; 39:909–16. [PubMed: 29848392]
29. Nerandzic MM, Donskey CJ. Effective and reduced cost modified selective medium for isolation of *Clostridium difficile*. *J Clin Microbiol* 2009; 47:397–400. [PubMed: 19073869]
30. Gonzalez-Orta M, Saldana C, Ng-Wong Y, et al. Are many patients diagnosed with healthcare-associated *Clostridioides difficile* infection colonized with the infecting strain on admission? *Clin Infect Dis* 2019; 69:1801–4. [PubMed: 30855075]
31. Martinson JN, Broadaway S, Lohman E, et al. Evaluation of portability and cost of a fluorescent PCR ribotyping protocol for *Clostridium difficile* epidemiology. *J Clin Microbiol* 2015; 53:1192–7. [PubMed: 25631804]

32. Clinical and Laboratory Standards Institute. Methods for antimicrobial susceptibility testing of anaerobic bacteria. 9th ed. CLSI standard M11. Wayne, PA: Clinical and Laboratory Standards Institute, 2018.
33. Dang UT, Zamora I, Hevener KE, Adhikari S, Wu X, Hurdle JG. Rifamycin resistance in *Clostridium difficile* is generally associated with a low fitness burden. *Antimicrob Agents Chemother* 2016; 60:5604–7. [PubMed: 27381389]
34. Louie T, Miller M, Donskey C, Mullane K, Goldstein EJ. Clinical outcomes, safety, and pharmacokinetics of OPT-80 in a phase 2 trial with patients with *Clostridium difficile* infection. *Antimicrob Agents Chemother* 2009; 53:223–8. [PubMed: 18955525]
35. Hecker MT, Son AH, Zuccaro P, Conti J, Donskey CJ. Real-world evaluation of a two-step testing algorithm for *Clostridioides difficile* infection. *Infect Control Hosp Epidemiol* 2023; 44:1494–6. [PubMed: 36750229]
36. Miller R, Morillas JA, Brizendine KD, Fraser TG. Predictors of *Clostridioides difficile* infection-related complications and treatment patterns among nucleic acid amplification test-positive/toxin enzyme immunoassay-negative patients. *J Clin Microbiol* 2020; 58:e01764–19.
37. Rotimi VO, Jamal WY, Mokaddas EM, Brazier JS, Johnny M, Duerden BI. Prevalent PCR ribotypes of clinical and environmental strains of *Clostridium difficile* isolated from intensive-therapy unit patients in Kuwait. *J Med Microbiol* 2003; 52:705–9. [PubMed: 12867566]

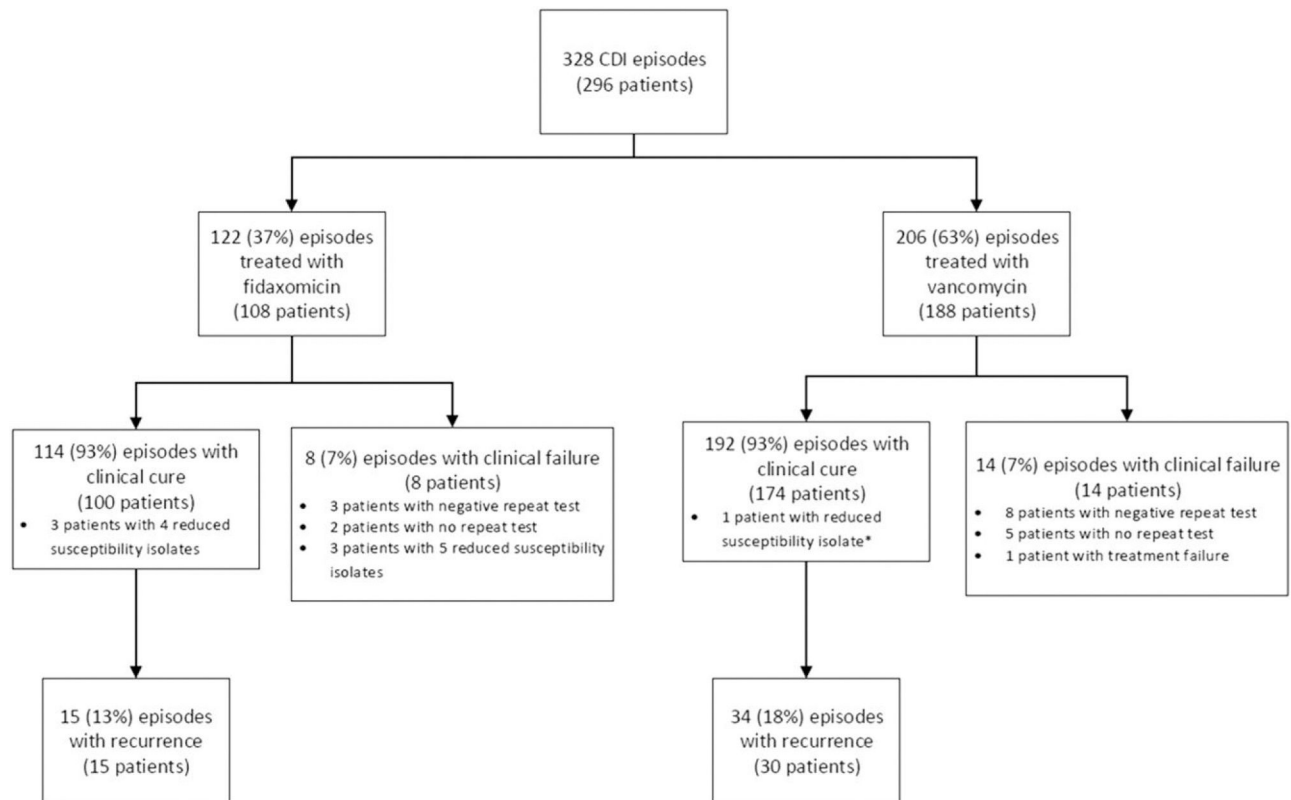


Figure 1.

Flow diagram for the study participants, including 6 patients infected with isolates with reduced fidaxomicin susceptibility. * indicates that the patient was clinically cured with vancomycin and had a subsequent infection with the same strain that was cured with fidaxomicin. Abbreviation: CDI, *Clostridioides difficile* infection.

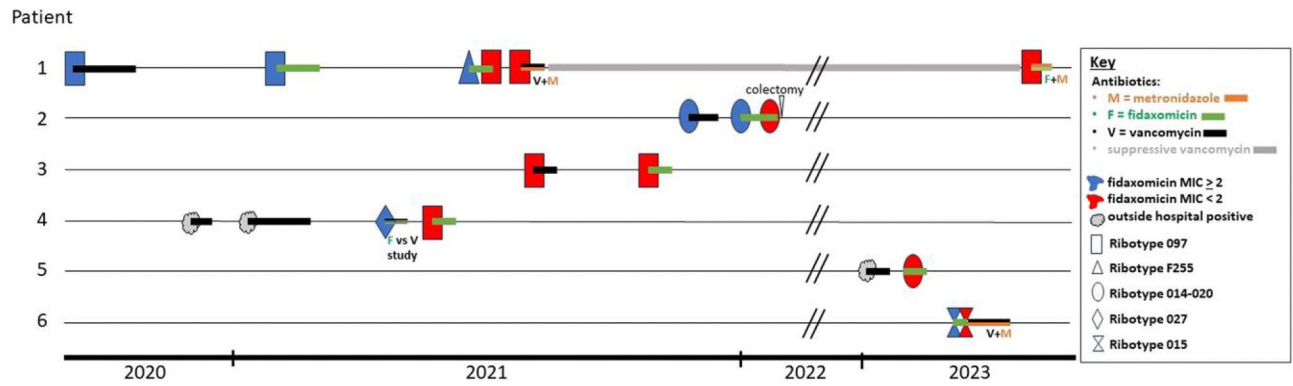
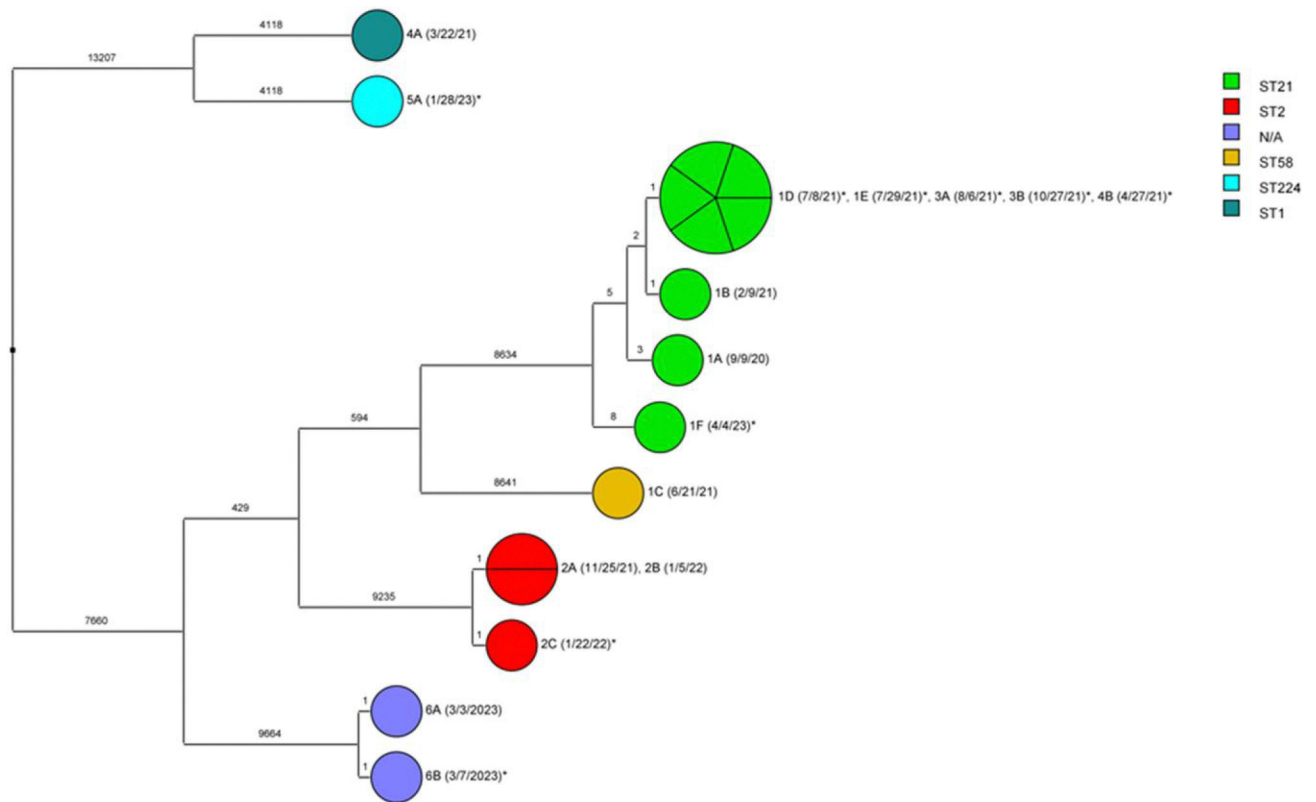
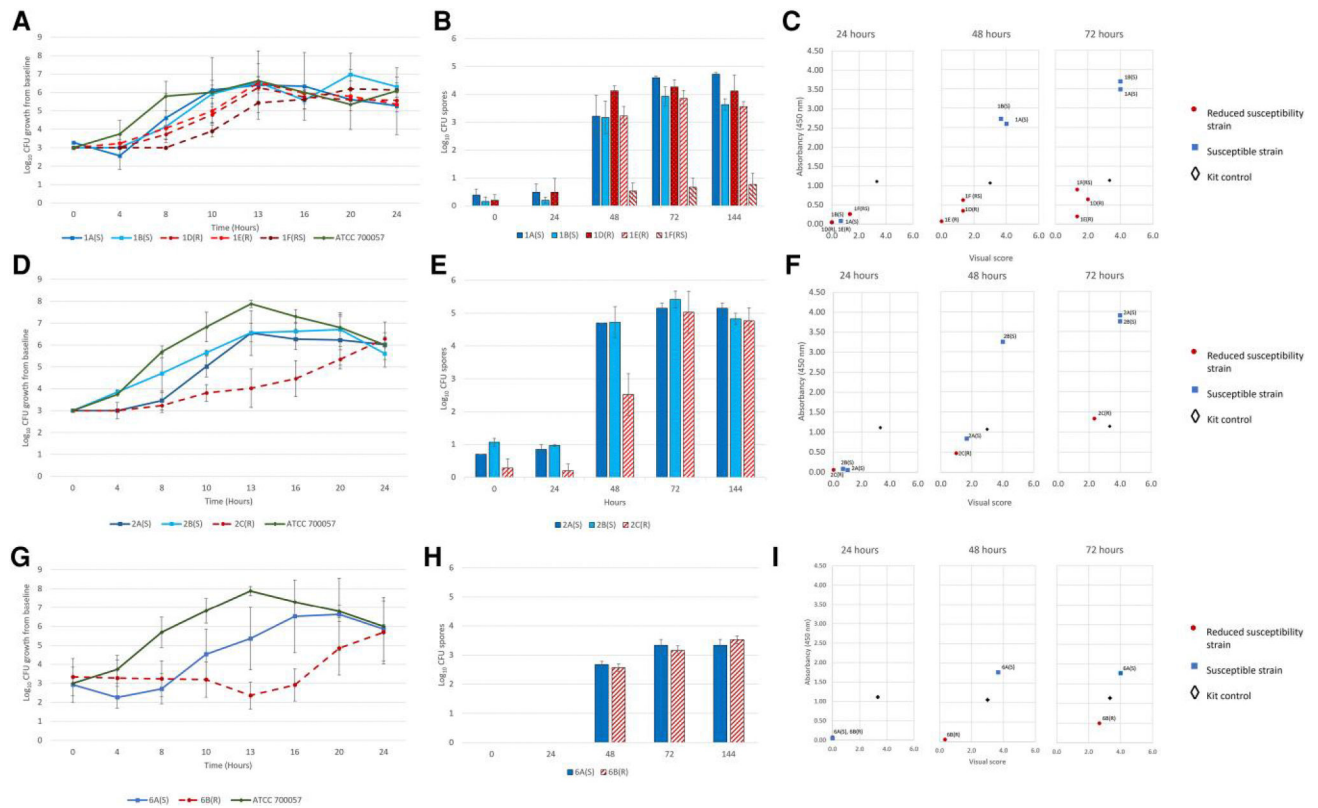


Figure 2.

Timeline of *Clostridioides difficile* infection (CDI) diagnoses and treatments and infecting ribotypes for the 6 patients infected with isolates with reduced fidaxomicin susceptibility. Green rectangles indicate fidaxomicin treatment; black rectangles indicate vancomycin treatment; orange rectangle indicates metronidazole treatment. Blue shapes indicate isolates with fidaxomicin MIC < 2 $\mu\text{g/mL}$; red shapes indicate isolates with fidaxomicin MIC ≥ 2 $\mu\text{g/mL}$. Patient 4 was enrolled in a randomized trial of vancomycin versus fidaxomicin for treatment of his second CDI episode in 2021. Abbreviation: MIC, minimum inhibitory concentration.

**Figure 3.**

Dendrogram showing the relatedness of all isolates recovered from the 6 patients infected with isolates with reduced fidaxomicin susceptibility. *, isolate with reduced fidaxomicin susceptibility. Ribotypes corresponding to STs: ST 21, ribotype 097; ST 58, ribotype 255; ST 2 and 224, ribotype 014–020; ST 1, ribotype 027. Abbreviations: N/A, not assigned; ST, multilocus sequence type.

**Figure 4.**

Growth rates (*A, D, G*), sporulation rates (*B, E, H*), and toxin production (*C, F, I*) for strains from patients 1 (*A–C*), 2 (*D–F*), and 6 (*G–I*) with reduced fidaxomicin susceptibility in comparison with paired susceptible isolates from the same patients. Abbreviation: CFU, colony-forming unit.

Table 1.

Characteristics of the Isolates Recovered From Patients Infected With *Clostridioides difficile* With Reduced Fidaxomicin Susceptibility in an Acute Care Hospital

Patient Number	Isolate Date	Multilocus Sequence Type	Ribotype	RpoB Mutation (Amino Acid Substitution)	RpoC Mutation (Amino Acid Substitution)	Fidaxomicin Minimum Inhibitory Concentration (µg/mL)	Nucleic Acid Amplification Test Cycle Threshold of Stool Specimen	Toxin Enzyme Immunoassay Result of Stool Specimen
1A	9/9/2020	21	097	None	None	0.25 (S)	19.2	+
1B	2/9/2021	21	097	None	None	0.25 (S)	26.6	-
1C	6/21/2021	58	255	None	None	0.25 (S)	23.5	+
1D	7/8/2021	21	097	None	A265G (R89G)	16 (R)	21.8	-
1E	7/29/2021	21	097	None	A265G (R89G)	16 (R)	31.6	-
1F	4/4/2023	21	097	G2440T (D814Y)	A265G (R89G)	8 (RS)	22.8	-
2A	11/25/2021	2	014-020	None	None	0.25 (S)	27.1	+
2B	1/5/2022	2	014-020	None	None	0.25 (S)	29.2	-
2C	1/22/2022	2	014-020	T3428G (V1143G)	None	32 (R)	28.6	-
3A	8/6/2021	21	097	None	A265G (R89G)	8 (RS)	24.8	+
3B	10/27/2021	21	097	None	A265G (R89G)	8 (RS)	24.5	+
4A	3/22/2021	1	027	None	None	0.25(S)	29.8	-
4B	4/27/2021	21	097	None	A265G (R89G)	8 (RS)	22.9	+
5A	1/28/2023	224	014-020	T3428A (V1143D)	None	32 (R)	23.9	-
6A	3/3/2023	UN	015	None	None	0.26 (S)	21.1	-
6B	3/7/2023	UN	015	T3428A (V1143D)	None	16 (R)	25.3	-

The study was conducted between 1 July 2020 and 30 June 2023.

Abbreviations: R, resistant; RS, reduced susceptibility; S, susceptible; UN, unassigned.