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Fecal Microbiome Among Nursing Home Residents with Advanced Dementia and *Clostridium difficile*

Rafael Araos¹, Nikolaos Andreatos², Juan Ugalde^{3,4}, Susan Mitchell⁵, Eleftherios Mylonakis², and Erika M. C. D'Agata²

¹Instituto de Ciencias e Innovación en Medicina (ICIM), Facultad de Medicina Clínica Alemana, Universidad del Desarrollo, Santiago, Chile

²Infectious Diseases Division, Rhode Island Hospital, Warren Alpert Medical School of Brown University, 593 Eddy Street, Aldrich 720, Providence, RI, USA

³uBiome, Inc, San Francisco, CA, USA

⁴Centro de Bioinformática y Biología Integrativa, Facultad de Ciencias Biológicas, Universidad Andrés Bello, Santiago, Chile

⁵Hebrew SeniorLife Institute for Aging Research, Boston, MA, USA

Abstract

Background/Objectives—Patients colonized with toxinogenic strains of *Clostridium difficile* have an increased risk of subsequent infection. Given the potential role of the gut microbiome in increasing the risk of *C. difficile* colonization, we assessed the diversity and composition of the gut microbiota among long-term care facility (LTCF) residents with advanced dementia colonized with *C. difficile*.

Design—Retrospective analysis of rectal samples collected during a prospective observational study.

Setting—Thirty-five nursing homes in Boston, Massachusetts.

Participants—Eighty-seven LTCF residents with advanced dementia.

Measurements—Operational taxonomic units were identified using 16S rRNA sequencing. Samples positive for *C. difficile* were matched to negative controls in a 1:3 ratio and assessed for differences in alpha diversity, beta diversity, and differentially abundant features.

Results—*Clostridium difficile* sequence variants were identified among 7/87 (8.04%) residents. No patient had evidence of *C. difficile* infection. Demographic characteristics and antimicrobial exposure were similar between the seven cases and 21 controls. The overall biodiversity among

Erika M. C. D'Agata edagata@lifespan.org.

Author's contribution Dr. D'Agata and Araos contributed to the study concept and design, acquisition of subjects, and collection of the relevant data. Dr. Araos, Ugalde, and D'Agata performed the data analysis. All authors contributed to data interpretation and collaborated on the drafting and revision of the manuscript. Dr. Araos is the guarantor and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Compliance with ethical standards

Conflict of interest Juan Ugalde is an employee of uBiome Inc and has received stock options as well as other compensation.

cases and controls was reduced with a median Shannon index of 3.2 (interquartile range 2.7–3.9), with no statistically significant differences between groups. The bacterial community structure was significantly different among residents with *C. difficile* colonization versus those without and included a predominance of *Akkermansia* spp., *Dermabacter* spp., *Romboutsia* spp., *Meiothermus* spp., *Peptoclostridium* spp., and *Ruminococcaceae* UGC 009.

Conclusion—LTCF residents with advanced dementia have substantial dysbiosis of their gut microbiome. Specific taxa characterized *C. difficile* colonization status.

Keywords

Microbiome; Advanced dementia; Colonization; *Clostridium difficile*

Introduction

In the USA, *Clostridium difficile* is responsible for approximately 500,000 infections annually, resulting in 15,000 deaths [1] and is included on the urgent threat list of the Centers for Disease Control and Prevention (CDC) [2]. The burden of *C. difficile* infection (CDI) in long-term care facilities (LTCF) is substantial with a nationwide prevalence rate of 1.85/100 LTCF admissions and increased 3-month mortality [3]. The risk of CDI among residents of LTCFs is sevenfold higher compared to individuals in the community [4]. This increased risk is due to multiple risk factors for CDI in this patient population, including advanced age, frequent antimicrobial exposure, multiple comorbidities, and recurrent hospitalizations [3–5].

Recent advances in the study of the microbiome, the bacteria that colonize the human body, have demonstrated that dysbiosis of this commensal bacterial community structure predisposes to CDI and that restoration to a healthier microbiome, through fecal microbiota transplantation, can prevent disease and recurrences [6–11]. In this study, the diversity and composition of the microbiome were characterized among LTCF residents with advanced dementia, a population whose microbiome has not been previously studied. Furthermore, since *C. difficile* colonization is a prerequisite for infection and is associated with a sixfold higher risk of CDI, [12] this study focused on the microbiome characteristics of LTCF residents with advanced dementia who were colonized with *C. difficile*.

Methods

Study Population

From 2009 to 2012, the Study of Pathogen Resistance and Exposure to Antimicrobials in Dementia (SPREAD) was conducted in 35 nursing homes [13, 14]. The goals of this study were to quantify and characterize suspected infections, antimicrobial use, and incidence of multidrug-resistant organisms (MDRO). A total of 362 residents with advanced dementia were enrolled. Patient data, including demographics, comorbidities, Bedford Alzheimer Nursing Severity-Subscale (BANS-S), [15] the Test for Severe Impairment (TSI), [16] antimicrobial use, and suspected infections, were recorded at baseline and every 3 months thereafter for 12 months or until death. To determine acquisition rates of MDRO, rectal samples were also collected during these intervals. Results of this study are reported

elsewhere [13]. Suspected infections included the presence of diarrhea. If identified during any interval, data pertaining to clinical symptoms, stool cultures, and *C. difficile* testing were collected. As part of a subanalysis, rectal samples from 87 SPREAD participants, who received antimicrobials prior to sampling (0–9 months), underwent DNA extraction and 16S rRNA analyses (see below) to characterize the microbiome disruption indices (microbiome disruption indices) associated with MDRO acquisition. For the purpose of this study, these rectal samples were also analyzed for the presence of *C. difficile* sequences. The microbiome of each positive *C. difficile* stool sample was then compared to three stool samples in which *C. difficile* was not identified, matched for interval of sample collection.

Specimen Collection and Fecal DNA Extraction

Rectal samples were obtained using a sterile double-tipped swab (Starswab II; Starplex Scientific Inc., Ontario, Canada) inserted into the anus. All samples were placed in a vial containing 20% glycerol within 1–2 h of collection and then stored at – 80 °C for future analyses. Frozen rectal swabs were then thawed and immediately placed into 96-well microtiter plate. Fecal DNA was extracted using the Pow-erSoil DNA Isolation Kit (MOBIO, West Carlsbad CA) according to the manufacturer’s protocol. The concentration of extracted DNA was determined by Nanodrop 1000 (Thermo Scientific, Watham MA), and DNA was stored at – 20 °C until used.

16S rRNA Gene Library Preparation and High-Throughput Sequencing

All samples were amplified and barcoded for multiplex pyrosequencing using primers targeted to the V4 region of the bacterial 16S rRNA gene under uniform PCR conditions that included 3 min at 94 °C and 45 cycles of 45 s at 94 °C, 60 s at 50 °C, and 90 s at 72 °C with final extension for 10 min at 72 °C [17]. We used forward primer (AAT GAT ACG GCG ACC ACC GAG ATC TAC ACT ATG GTA ATT GTG TGC CAG CMG CCG CGG TAA) that includes a 5’ Illumina adaptor, forward primer pad, 2 bp linker, and the 515F 16S rRNA primer and reverse primer (CAA GCA GAA GAC GGC ATA CGA GAT NNNNNNNNNNNN-AGT CAG TCA G-CC-GGA CTA CHV GGG TWT CTA AT) that includes the Illumina 3’ adapter with a 12-nt error-correcting Golay barcode, reverse primer pad, 2 bp linker, and the 806R 16S rRNA primer. We ran PCR in triplicate using 0.2 µM of the primers, 1 µl of template, and 1X HotMasterMix (5 PRIME, Gaithersburg MD) and cleaned the products using a PCR Purification Kit (Qiagen) after pooling. Cleaned PCR products were quantified using the Qubit dsDNA HS Assay Kit (Invitrogen™, Eugene OR) and then adjusted to an optimal molarity, as described [18]. Sequencing was performed using the Illumina MiSeq platform in the New York University Langone Medical Center (NYULMC) Genome Technology Core.

Statistical and Bioinformatic Analysis

Demultiplexed sequences were curated and analyzed using the DADA2 pipeline [19]. Briefly, all the sequences were filtered to truncate the paired reads to 150 nt. in length and eliminate reads with quality values less than 2. Error rates were estimated and corrected by pooling all the reads from the sequencing run, with default parameters. Taxonomy was assigned using the SILVA 123 database. Resulting operational taxonomic units (OTUs) were analyzed using the Phyloseq package in R [20]. Within-sample diversity (alpha diversity)

was estimated by calculating the Shannon diversity index (evenness) and the total number of observed species (richness) and then compared between patients colonized with *C. difficile* or not using nonparametric testing (Wilcoxon rank-sum test). Between-sample diversity (beta diversity) was determined by estimating UniFrac distances between samples. Between-sample distances were visualized using principal coordinates analysis, and sample-clustering patterns were investigated with the ADONIS test. Differentially abundant features between patients colonized with *C. difficile* or not were compared using the linear discriminant analysis (LDA) effect size (LEfSe) method [21]. An LDA score of 2.0 and an alpha level of 0.05 were used for non-parametric testing between groups.

Results

Clinical Characteristics

Clostridium difficile sequence variants were identified in seven patients (7/87, 8.04%), of whom none had diarrhea or a diagnosis of *C. difficile* infection. These seven patients (cases) were matched to 21 patients who did not have *C. difficile* sequence variants (controls). Among these 28 patients, demographics and clinical characteristics were as follows: mean age 89.3 years, male gender 25%, Caucasian 92.9%, diagnosis of diabetes mellitus, chronic obstructive pulmonary disease and congestive heart failure 21.4, 10.7, and 10.7%, respectively, TSI = 0 42.9%, mean BANS-S score 20.8 ± 2.6 , hospice care 14.3%, and do not resuscitate orders 82.1%. There were no statistically significant differences between cases and controls in these characteristics ($P > 0.05$). There were also no differences in types of antimicrobials received between cases and controls (Table 1).

Sequencing Output

A total of seven cases and 21 controls were included in the analysis, yielding a total of 471,689 sequences with a median (IQR) of 17,501.5 (3986). A total of 1602 unique OTUs were identified. *C. difficile* sequence variants retrieved from cases represented an extremely small proportion of the gut microbiome, with a mean relative abundance of $0.09\% \pm 0.29$.

Alpha Diversity

Microbial diversity defined by species richness (number of observed species) and relative abundance within individual samples (evenness) was similar between cases and controls ($P > 0.05$). Figure 1 shows the comparison of the Shannon diversity index and the total number of observed species (evenness and richness indicators, respectively) between the study groups. The overall median Shannon index was 3.2 (interquartile range [IQR] 2.7–3.9). The index was not significantly different between cases (3.47 [IQR 1.93–4.05]) and controls (3.12 [IQR 1.09–4.2], $P = 0.49$).

Beta Diversity

The differences in the fecal microbiome composition among samples belonging to each patient group were similar to the differences observed when comparing the composition of the samples between groups, indicating that no distinct community structure characterized the microbiome of residents colonized with *C. difficile* or the microbiome of controls. As shown in Fig. 2, no specific sample-clustering patterns were observed when comparing

samples grouped according to *C. difficile* colonization status (*P* value for weighted and unweighted UniFrac = 0.56 and 0.97, respectively).

Compositional Assessment and Identification of Differentially Abundant Features

The composition of bacterial taxa at the phylum level did not differ between cases and controls (*P* > 0.05). In both groups, members of the phylum *Firmicutes* dominated the fecal microbiota (cases: 42.7%, controls: 38.8%), followed by *Bacteroidetes* (cases: 20.4%, controls: 26.3%), *Proteo- bacteria* (cases: 20.8%, controls: 24.9%), and *Actinobacteria* (cases: 8%, controls: 6.8%). Among the overall population, the more abundant genera (> 5%) were *Bacteroides* spp. (14.7% ± 15.9), followed by *Proteus* spp. (6.7% ± 15.6), *Campylobacter* spp. (6.2% ± 11.5), *Corynebacterium* spp. (5.9% ± 18.9), and *Escherichia/ Shigella* spp. (5.3% ± 11.4).

The analysis of differentially abundant features showed that patients colonized with *C. difficile* had a significantly higher relative abundance of *Akkermansia* spp., *Dermabacter* spp., *Romboutsia* spp., *Meiothermus* spp., *Peptoclostridium* spp., and *Ruminococcaceae* UGC 009 (Fig. 3). Figure 4 shows the composition of the fecal microbiota by *C. difficile* colonization status at the phylum and genus levels. In the latter, only taxa representing 1% relative abundance are shown.

Discussion

The present study investigated the microbiome diversity and composition of the unique patient population of LTCF residents with advanced dementia, according to *C. difficile* colonization status. The main findings were that among this patient population, the overall microbiome diversity was substantially reduced and specific compositional characteristics of the microbiome were associated with *C. difficile* colonization.

Patients with advanced dementia are characterized by increased frailty, multiple comorbidities, and frequent infections [22]. In turn, these characteristics are associated with microbiome dysbiosis and reduced diversity [23, 24]. Thus, it is not surprising that microbiome diversity, as measured by the Shannon index, was substantially reduced in the study population with a value of 3.2, which is substantially lower than reported values of 4.5 among healthy controls [25]. In addition, antimicrobial exposure further contributes to reduced microbial diversity [26]. LTCF residents with advanced dementia are frequently exposed to antimicrobials, with up to 66% receiving at least one course of antimicrobials over a 1-year period [27]. In this study, all subjects were exposed to antimicrobials, further explaining the reduced diversity in the commensal community structure of the microbiome. Differences in diversity were not detected between subjects who were colonized with *C. difficile* compared to those who were not colonized. The absence of significant differences likely reflects the presence of a severely dysbiotic microbiome in the population of advanced dementia patients who have substantial antimicrobial exposure.

At the phylum level, the composition of the microbiome did not differ with regard to *C. difficile* colonization status, with the majority of taxa belonging to four phyla: *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Proteobac-teria*. These phyla are also the most common

among healthy controls [26]. At the genus level, however, specific differences between subjects who were colonized with *C. difficile* and controls were identified. The gut microbiome among patients with *C. difficile* colonization was characterized by a predominance of *Akkermansia* spp., *Dermabacter* spp., *Romboutsia* spp., *Meiothermus* spp., *Peptoclostridium* spp., and *Ruminococcaceae* UGC 009. These taxa may identify a subset of subjects at higher risk of *C. difficile* colonization. The predominance of *Ruminococcaceae* spp. is an interesting finding as the depletion of these bacteria has been associated with CDI [28, 29]. Members of this genus produce butyrate, and depletion of which may result in epithelial dysfunction and increased susceptibility to CDI [29]. Since colonized subjects in this study did not develop CDI, *Rumi-nococcaceae* spp. may have protected against subsequent infection. Validation of this concept, however, will require a longitudinal study of the microbiome composition among *C. difficile*-colonized subjects, with CDI as the outcome of interest.

Several studies have addressed the microbiome indices associated with *C. difficile* among LTCF residents or hospitalized patients [25, 30–34]. Comparable to the results of this study, reduced biodiversity of the microbiome in these patient populations was reported. Specific compositional signatures were also identified among subjects with CDI, which differed from the present study [25, 30–34]. However, differences in patient populations and antimicrobial exposure, as well as the fact that our study only investigated colonization with *C. difficile*, limit the scope for valid comparisons between the microbial community structure characterized in this study and previous reports. Studies are needed to better characterize the microbiome changes that accompany colonization and infection with *C. difficile* in the high-risk population of LTCF residents with advanced dementia and substantial antimicrobial exposure.

Certain limitations should be considered before interpreting the present findings. First, the sample size was small and data from larger patient cohorts are needed to confirm our findings. Second, *C. difficile* colonization was determined by 16S sequencing, and not by standard testing. This may raise concerns for a possible misclassification bias as the use of 16S sequencing for this indication has not been formally validated. However, 16S sequencing is a well-standardized technique, and sequences were analyzed using the DADA2 pipeline, which has sufficient resolution for detecting taxa at the species level [19]. Third, for the microbiome analyses, rectal samples were used instead of the more commonly used fecal samples. However, several studies have shown that data obtained from either sampling method are comparable [35, 36].

Preventing *C. difficile* colonization and CDI would have important implications toward the improvement of outcomes among all LTCF residents. Reconstituting the fecal bacterial community structure to one which represents a healthy microbiome can reduce the risk of recurrence in patients with CDI [10, 11]. The findings of this study suggest that specific microbiome disruption indices may be able to identify subjects at high risk of *C. difficile* colonization and may assist in the formulation of targeted preventive and therapeutic strategies in the LTCF setting [10, 11].

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References

1. Clostridium difficile infection in 2015. Centers for Disease Control and Prevention (CDC) Available at: <https://www.cdc.gov/media/releases/2015/p0225-clostridium-difficile.html>. Accessed July 3, 2017.
2. Antimicrobial resistance. Centers for Disease Control and Prevention (CDC) Available at: https://www.cdc.gov/drugresistance/biggest_threats.html. Accessed July 3, 2017.
3. Ziakas PD, Joyce N, Zacharioudakis IM, et al. Prevalence and impact of Clostridium difficile infection in elderly residents of long-term care facilities, 2011: a nationwide study. *Medicine (Baltimore)*. 2016;95:e4187. [PubMed: 27495022]
4. Karanika S, Grigoras C, Flokas ME, et al. The attributable burden of Clostridium difficile infection to long-term care facilities stay: a Clinical Study. *J Am Geriatr Soc*. 2017;65:1733–1740. [PubMed: 28306141]
5. Ziakas PD, Zacharioudakis IM, Zervou FN, et al. Asymptomatic carriers of toxigenic C. difficile in long-term care facilities: a meta-analysis of prevalence and risk factors. *PLoS ONE*. 2015;10:e0117195. [PubMed: 25707002]
6. Vincent C, Manges AR. Antimicrobial use, human gut microbiota and Clostridium difficile colonization and infection. *Antibiotics (Basel)*. 2015;4:230–253. [PubMed: 27025623]
7. Theriot CM, Young VB. Interactions between the gastrointestinal microbiome and Clostridium difficile. *Annu Rev Microbiol*. 2015;69:445–461. [PubMed: 26488281]
8. Manges AR, Labbe A, Loo VG, et al. Comparative metagenomic study of alterations to the intestinal microbiota and risk of nosocomial Clostridium difficile-associated disease. *J Infect Dis*. 2010;202:1877–1884. [PubMed: 21050115]
9. Vincent C, Stephens DA, Loo VG, et al. Reductions in intestinal Clostridiales precede the development of nosocomial Clostridium difficile infection. *Microbiome*. 2013;1:18. [PubMed: 24450844]
10. Girotra M, Garg S, Anand R, et al. Fecal microbiota transplantation for recurrent Clostridium difficile infection in the elderly: long-Term outcomes and microbiota changes. *Dig Dis Sci*. 2016;61:3007–3015. [PubMed: 27447476]
11. Seekatz AM, Aas J, Gessert CE, et al. Recovery of the gut microbiome following fecal microbiota transplantation. *MBio*. 2014;5:e00893–00814. [PubMed: 24939885]
12. Zacharioudakis IM, Zervou FN, Pliakos EE, et al. Colonization with toxinogenic C. difficile upon hospital admission, and risk of infection: a systematic review and meta-analysis. *Am J Gastroenterol*. 2015;110:381–390. (quiz 391). [PubMed: 25732416]
13. Mitchell SL, Shaffer ML, Kiely DK, et al. The study of pathogen resistance and antimicrobial use in dementia: study design and methodology. *Arch Gerontol Geriatr*. 2013;56:16–22. [PubMed: 22925431]
14. Mitchell SL, Shaffer ML, Loeb MB, et al. Infection management and multidrug-resistant organisms in nursing home residents with advanced dementia. *JAMA Intern Med*. 2014;174:1660–1667. [PubMed: 25133863]
15. Volicer L, Hurley AC, Lathi DC, et al. Measurement of severity in advanced Alzheimer's disease. *J Gerontol*. 1994;49:M223–M226. [PubMed: 8056941]
16. Albert M, Cohen C. The Test for Severe Impairment: an instrument for the assessment of patients with severe cognitive dysfunction. *J Am Geriatr Soc*. 1992;40:449–453. [PubMed: 1634695]
17. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods*. 2010;7:335–336. [PubMed: 20383131]

18. Iizumi T, Taniguchi T, Yamazaki W, et al. Effect of antibiotic pre-treatment and pathogen challenge on the intestinal microbiota in mice. *Gut Pathog.* 2016;8:60. [PubMed: 27891184]
19. Callahan BJ, McMurdie PJ, Rosen MJ, et al. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods.* 2016;13:581–583. [PubMed: 27214047]
20. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE.* 2013;8:e61217. [PubMed: 23630581]
21. Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. *Genome Biol.* 2011;12:R60. [PubMed: 21702898]
22. Mitchell SL. Advanced dementia. *N Engl J Med.* 2015;373:1276–1277.
23. Claesson MJ, Jeffery IB, Conde S, et al. Gut microbiota composition correlates with diet and health in the elderly. *Nature.* 2012;488:178–184. [PubMed: 22797518]
24. Lewis BB, Pamer EG. Microbiota-based therapies for *Clostridium difficile* and antibiotic-resistant enteric infections. *Annu Rev Microbiol.* 2017;71:157–178. [PubMed: 28617651]
25. Antharam VC, Li EC, Ishmael A, et al. Intestinal dysbiosis and depletion of butyrogenic bacteria in *Clostridium difficile* infection and nosocomial diarrhea. *J Clin Microbiol.* 2013;51:2884–2892. [PubMed: 23804381]
26. Kim S, Covington A, Pamer EG. The intestinal microbiota: antibiotics, colonization resistance, and enteric pathogens. *Immunol Rev.* 2017;279:90–105. [PubMed: 28856737]
27. D'Agata E, Mitchell SL. Patterns of antimicrobial use among nursing home residents with advanced dementia. *Arch Intern Med.* 2008;168:357–362. [PubMed: 18299489]
28. Lee YJ, Arguello ES, Jenq RR, et al. Protective factors in the intestinal microbiome against *Clostridium difficile* infection in recipients of allogeneic hematopoietic stem cell transplantation. *J Infect Dis.* 2017;215:1117–1123. [PubMed: 28498996]
29. Lawley TD, Walker AW. Intestinal colonization resistance. *Immunology.* 2013;138:1–11. [PubMed: 23240815]
30. Schubert AM, Rogers MA, Ring C, et al. Microbiome data distinguish patients with *Clostridium difficile* infection and non-*C. difficile*-associated diarrhea from healthy controls. *MBio.* 2014;5:e01021–01014. [PubMed: 24803517]
31. Zhang L, Dong D, Jiang C, et al. Insight into alteration of gut microbiota in *Clostridium difficile* infection and asymptomatic *C. difficile* colonization. *Anaerobe.* 2015;34:1–7. [PubMed: 25817005]
32. Milani C, Ticinesi A, Gerritsen J, et al. Gut microbiota composition and *Clostridium difficile* infection in hospitalized elderly individuals: a metagenomic study. *Sci Rep.* 2016;6:25945. [PubMed: 27166072]
33. Rea MC, O'Sullivan O, Shanahan F, et al. *Clostridium difficile* carriage in elderly subjects and associated changes in the intestinal microbiota. *J Clin Microbiol.* 2012;50:867–875. [PubMed: 22162545]
34. Chang JY, Antonopoulos DA, Kalra A, et al. Decreased diversity of the fecal Microbiome in recurrent *Clostridium difficile*-associated diarrhea. *J Infect Dis.* 2008;197:435–438. [PubMed: 18199029]
35. Bassis CM, Moore NM, Lolans K, et al. Comparison of stool versus rectal swab samples and storage conditions on bacterial community profiles. *BMC Microbiol.* 2017;17:78. [PubMed: 28359329]
36. Budding AE, Grasman ME, Eck A, et al. Rectal swabs for analysis of the intestinal microbiota. *PLoS ONE.* 2014;9:e101344. [PubMed: 25020051]

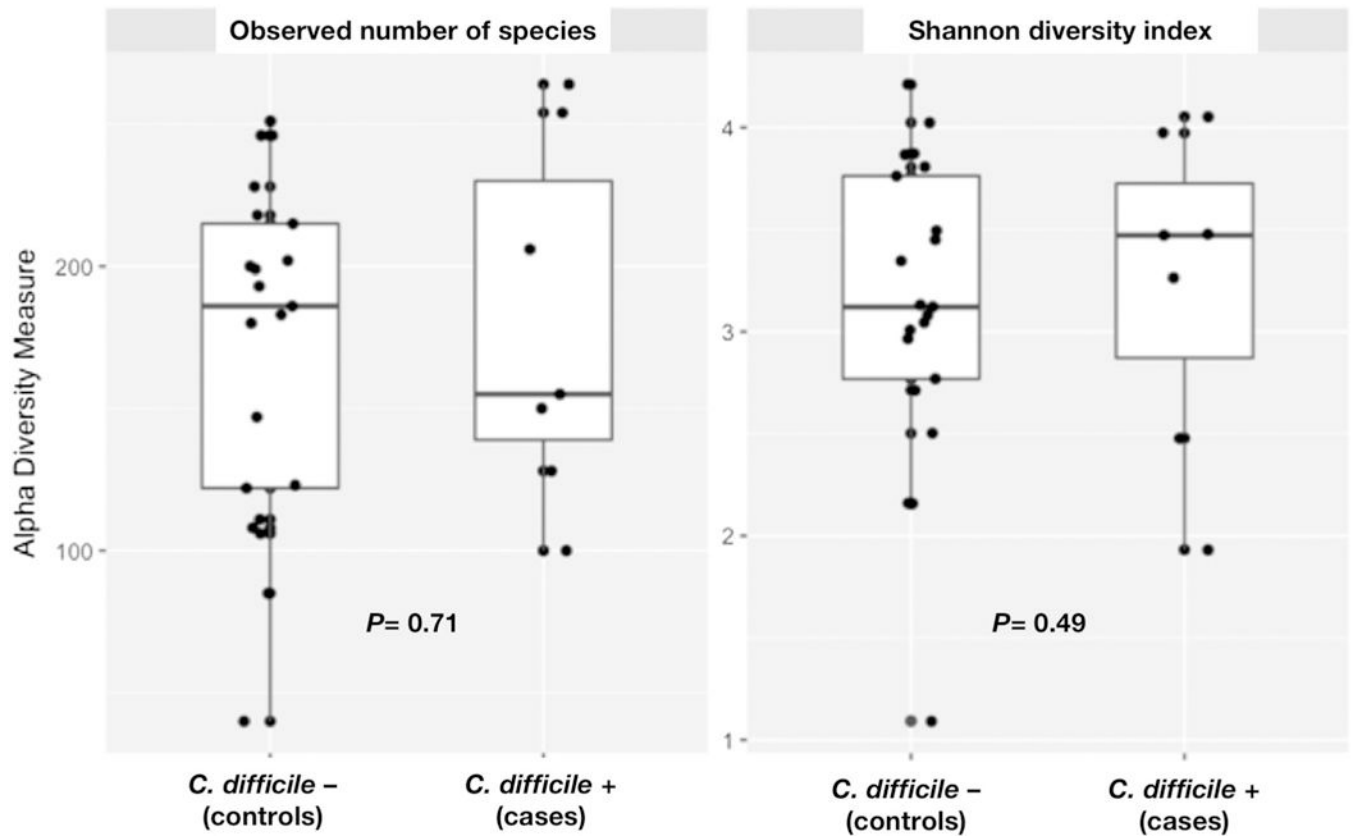


Fig. 1.
Alpha diversity metrics among nursing home residents with and without *Clostridium difficile* colonization

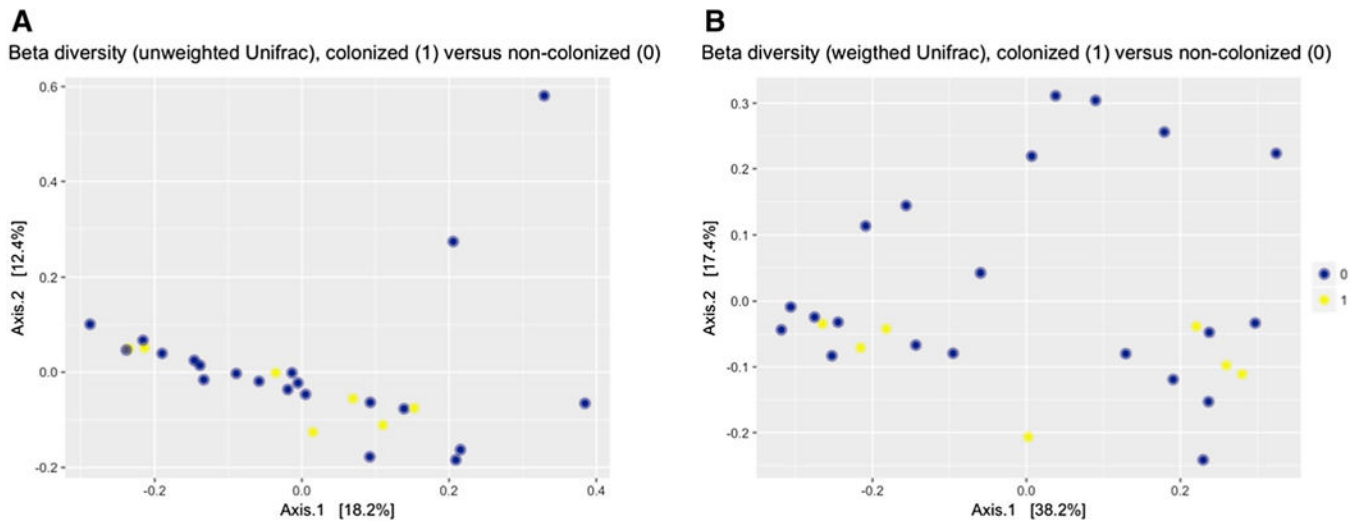


Fig. 2. Beta diversity metrics (unweighted [qualitative] **a**, weighted **b** [quantitative]), among nursing home residents with and without *Clostridium difficile* colonization

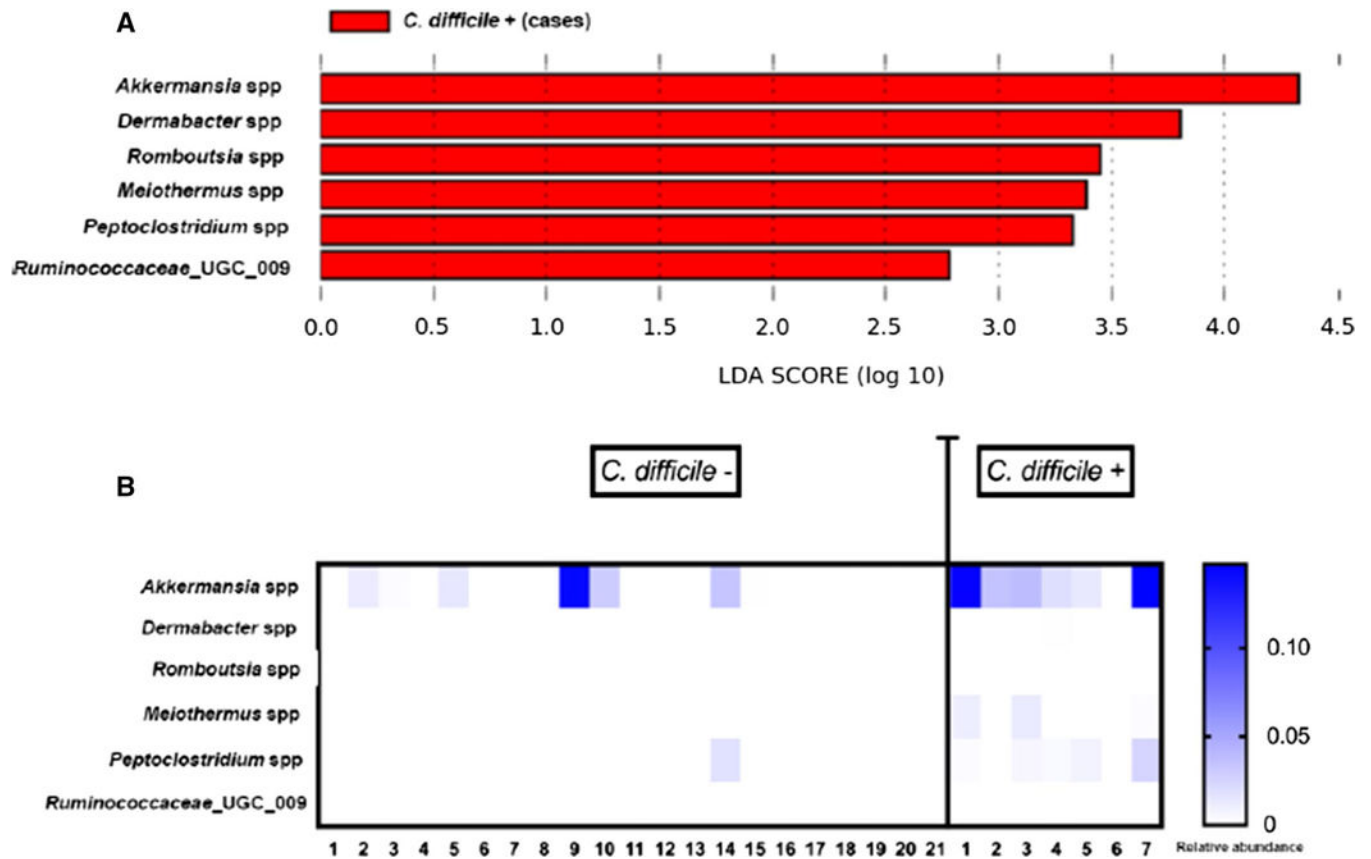


Fig. 3. Analyses of differentially abundant features among nursing home residents with and without *Clostridium difficile* colonization (LDA score **a**, heat map **b**)

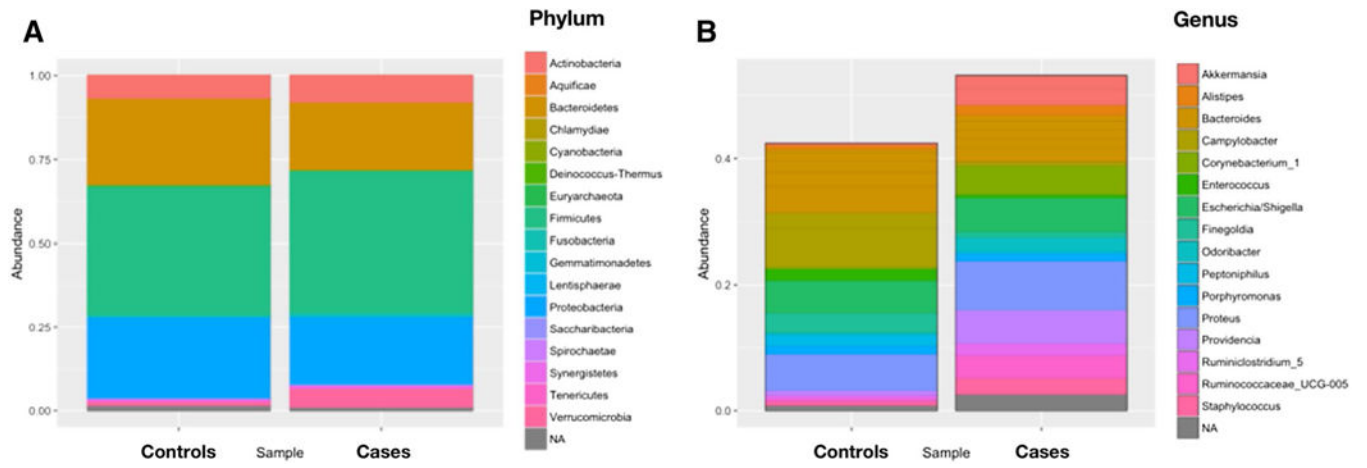


Fig. 4. Comparison of microbiome composition among nursing home residents with and without *Clostridium difficile* colonization (phylum level **a**, genus level **b**)

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Table 1Antimicrobial exposure among residents colonized and not colonized with *Clostridium difficile*

Antimicrobial group N (%)	Controls (<i>C. difficile</i> -) N = 21	Cases (<i>C. difficile</i> +) N = 7	P value
Penicillins	1 (5)	0	1.00
Extended spectrum penicillins	3 (14)	0	0.55
First-generation cephalosporins	3 (14)	1 (14)	1.00
Other cephalosporins	4 (19)	3 (43)	0.32
Carbapenems	1 (5)	1 (14)	0.44
Quinolones	7 (33)	0	0.14
Azithromycin	1 (5)	0	1.00
Doxycycline	0	0	–
Trimethoprim-sulfameth-oxazole	2 (10)	3 (43)	0.08
Nitrofurantoin	4 (19)	1 (14)	1.00
Gentamicin	0	0	–
Vancomycin	2 (10)	0	1.00
Linezolid	0	0	–