REMBRANDT PROTOCOL

Impact of pet contact on antimicrobial-associated dysbiosis and Clostridioides difficile infection

REMBRANDT: REcovery of the MicroBiome fRom Antibiotics for Dental implanTs

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PARTICIPATING STUDY SITES

N/A (single center study)

PRÉCIS

Study Title

Impact of pet contact on antimicrobial-associated dysbiosis and Clostridioides difficile infection

Objectives

Aim 1: To determine how gut microbiota that provide colonization resistance against C. difficile are shared between pet owners and pets.

Aim 2: To assess the effect of pet ownership/contact on disruption and restoration of the gut microbiome following antimicrobial therapy

Aim 3: To determine how pet ownership/contact impacts CD colonization following antimicrobial therapy.

Design and Outcomes

Longitudinal cohort study of individuals age 60 years or older receiving antimicrobial prophylaxis for dental implants to study the disruption and the recovery of the microbiome in pet owners and non-pet owners. Stool samples will be collected from patients, and from the pets of pet owners prior to the start of their antimicrobial regimen, and on days 3, 10, 30 and 90 after their implant procedure.

Interventions and Duration

No intervention. Patients will be followed until 90 days after their implant procedure.

Sample Size and Population

We anticipate being able to enroll approximately 80 pet owners and 120 non-pet owners over the course of four years of recruitment.

1. STUDY OBJECTIVES

1.1 Primary Objective

To assess the effect of pet ownership/contact on disruption and restoration of the gut microbiome following antimicrobial therapy

1.2 Secondary Objectives

To determine how gut microbiota that provide colonization resistance against C. difficile are shared between pet owners and pets.

To determine how pet ownership/contact impacts CD colonization following antimicrobial therapy.

2. BACKGROUND AND RATIONALE

2.1 Background on Condition, Disease, or Other Primary Study Focus

C. difficile infection is the most common healthcare-associated infection and results in significant morbidity and mortality. In the United States, *C. difficile* infection (CDI) is responsible for an estimated 450,000 cases of healthcare-associated infections each year, accounting for 30,000 deaths[1-3]. CDI prolongs hospitalization by 2.8 to 10.4 days[4,5], representing an annual economic burden of more than $6 billion[5]. Recurrent CDI, which occurs in an estimated 30% of patients, is associated with even higher levels of morbidity, mortality, and healthcare costs[6,7].

CDI is associated with disruption of the gut microbiome. *C. difficile (*CD) is an opportunistic pathogen that causes disease in the colon following disruption of the native gut microbiome. Risk factors for CDI include antimicrobial therapy[8-10], older age, immunosuppression, use of proton pump inhibitors, gastrointestinal disease, and exposure to healthcare facilities[11-14], all of which can cause gut dysbiosis. CDI is associated with decreased gut microbial richness and diversity and depletion of key bacterial taxa[15-21] that normally provide colonization resistance via

competitive exclusion, by limiting access to mucosal surfaces, producing antimicrobial molecules, and modulating the intestinal metabolome[13,22-24].

Restoration of a healthy gut microbiome is critical for resolution of CDI and prevention of recurrence. Specific combinations of metabolites (e.g., bile acids) provide a nutritional milieu that compromises CD metabolism and therefore enables successful clearance of CD[17,25-27]. While specific bacterial taxa and species can restore colonization resistance[17,25,28], restoration of a rich and diverse "functionally intact" microbiome in which CD can be outcompeted for growth substrates is sufficient for clearing CD, even if that community differs from the pre-dysbiosis community[27,29-32]. This is underscored by the reported 80-90% success rate of fecal microbiota transplants (FMT) in preventing recurrent CDI[33,34].

2.2 Study Rationale

Factors that contribute to restoration of the gut microbiome following dysbiosis are not well understood. The microbial and metabolic factors that provide colonization resistance against CD are relatively well known, but those that promote the recovery of the microbiome following dysbiosis are only beginning to be explored. While key groups of bacterial taxa are thought to re-seed the disrupted microbial ecology[35], their identity and the ability of an individual's microbiome to recover appear to vary with host and environmental factors[28]. Longitudinal studies of the microbiome following antimicrobial therapy have documented consistent, reproducible, and profound disruption followed by highly variable extents of and times to recovery[36-41]. While the initial state of the gut microbiome[42], diet[36], and the surrounding environment[36,43] may influence recovery, their relationship to human dysbiosis remains unclear, either because they were performed in animals or appeared underpowered to detect the effect of external factors. Moreover, no such studies have been performed in the elderly, a population particularly at risk of dysbiosis[44] and CDI[14]. Given that more than half of CDI cases are community-acquired[45] and that recurrence tends to occur more in community-acquired CDI than in hospital-acquired CDI[46], a better understanding of the contribution of environmental factors (such as pet cohabitation) to recovery of the gut microbiome following antimicrobial therapy is critically needed.

Animal contact can modulate the gut microbiome in ways that are beneficial to human health, but its role in mitigating dysbiosis is unknown. An expansive body of literature has documented the ability of animal contact to modulate the human microbiome in ways that can be beneficial in preventing certain types of disease, such as asthma, atopy, and cardiovascular disease[47-54]. *Pet ownership/contact was found to be protective against colonization with CD in healthy community-dwelling persons[55,56] and against recurrence of community-acquired CDI[57]*. Further study is needed to understand the mechanism underlying these protective effects and the circumstances in which they apply. Notably, it is unclear whether these effects are due to direct sharing of microbiota between pets and their owners or indirect factors such as reduced stress and increased physical activity associated with pet ownership[58,59]. Sharing of microbiota between pets and their owners[60,61] could be responsible for these effects, but no studies have been performed to substantiate this hypothesis. The proposed research will shed light on the microbial exchanges that occur between pets and their owners and the clinical effects these exchanges produce in the specific context of antimicrobial-associated dysbiosis.

Animal contact, if proven beneficial, could be leveraged as a non-invasive benign therapy against gut dysbiosis and CDI. With increasing interest in the use of alternative, non-pharmacologic treatments for CDI[62,63] and the emerging notion that companion animals could represent a novel microbiome-based therapy[64], more information on the relationship between animal contact and recovery from gut dysbiosis is needed. If animal contact improves the health of the human gut microbiome (Figure 1), increasing animal contact through animal-assisted therapy or close contact with household pets following antimicrobial therapy could be "prescribed". In addition to the myriad psychosocial and physiological benefits associated with pet contact[59,65-69], especially in the elderly[70-73], animal contact could provide a non-invasive, non-pharmacologic form of therapy for patients undergoing gut dysbiosis.

3. STUDY DESIGN

The source population will include patients over 50 years of age receiving prophylactic oral antimicrobials for dental implants (standard regimen, 500 mg of amoxicillin TID for 7 days) at the University of Pennsylvania School of Dental Medicine. This age group is chosen to 1) ensure relative homogeneity of age within the cohort and 2) target the population most at risk of CDI. Approximately 10% of outpatient antimicrobials in the US are prescribed for dental procedures, and CDI following antimicrobial dental prophylaxis occurs at a rate of 1.7/1,000 person-years. This population therefore represents an ideal cohort, as patients receiving dental implants are relatively healthy (and therefore less at risk of experiencing dysbiosis for other reasons) and because antimicrobial prophylaxis occurs so commonly for dental procedures, the generalizability of our results will be maximized. Exclusion criteria for enrollment in our study include: 1) antimicrobial therapy or hospitalization in the prior three months; 2) any gastrointestinal illness or underlying pathology (e.g., Inflammatory Bowel Disease, gastric ulceration); 3) sustained diarrheal disease (i.e., at least 3 episodes of loose or watery stool per day for 3 or more days) in the prior 3 months; 4) prior history of CDI in the prior 2 years; 6) immunomodulating medication (e.g., tumor necrosis factor inhibitors or systemic steroids). These enrollment criteria are wide enough to exclude patients that might experience gut dysbiosis for reasons unrelated to antimicrobial therapy while narrow enough to allow recruitment of enough subjects to test the primary hypotheses and explore interactions between antimicrobial therapy, pet ownership/contact, and underlying health conditions.

Enrolled subjects will submit stool samples or rectal swabs prior to beginning antimicrobial therapy (i.e., on or before day 0), and on days 3, 10, 30 and 90 after their dental implant (Figure 1). Pet owners will be asked to provide stool samples from one of their pets (dogs or cats only) on the same sampling days.

The initial samples will be used to assess sharing of microbiota between pets and their owners and as a baseline from which to assess disruption and recovery of the gut microbiome and CD burden. To assess the extent of gut dysbiosis and CD burden following antimicrobial therapy, subjects will submit stool samples collected at home on day 3 of therapy, when dysbiosis appears to be most profound. Recovery of the gut microbiome will be assessed in samples collected on days 10, 30 and 90 post-impalnt. In all cases, subjects will be provided with home collection kits containing e-swabs, flocked swabs, ice packs, pre-paid addressed insulated mailing envelopes, and detailed instructions on how to collect and send in their samples to the laboratory via UPS pick up. Demographic (age, sex, race, ethnicity) and basic health information (comorbidities, medications) will be collected for all patients at enrollment using medical history forms. Additional information on pet contact will be collected with a previously used form, while recent medical history of both the pet(s) and patient will be collected by patient interview during enrollment.

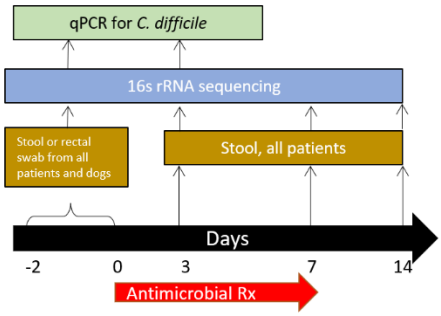

Figure 1: Sample collection and analysis

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria

- 50 years of age or older.
- Receiving a dental implant.
- Ability to understand study procedures and to comply with them for the entire length of the study.

4.2 Exclusion Criteria

Candidates meeting any of the exclusion criteria at baseline will be excluded from study participation.

- Antimicrobial therapy or hospitalization in the prior three months;
- Any gastrointestinal illness or underlying pathology (e.g., Inflammatory Bowel Disease, gastric ulceration)
- Sustained diarrheal disease (i.e., at least 3 episodes of loose or watery stool per day for 3 or more days) in the prior 3 months;
- Prior history of CDI in the prior year;
- Immunomodulating medication (e.g., tumor necrosis factor inhibitors or systemic steroids) or conditions (e.g., leukemia)
- Inability or unwillingness of individual or legal guardian/representative to give written informed consent.

4.3 Study Enrollment Procedures

- Potentially eligible subjects will be identified from the clinic schedule of the periodontal clinic at the Penn School of Dental Medicine and approached for participation in the study at the time of their pre-surgical treatment planning appointment.
- Reasons for ineligibility and for non-participation of eligible candidates will be documented in the Screening Log.
- Informed consent will be obtained from patients interested in participating at the time of their pr-surgical planning visit. A copy of the informed consent will be provided to participants, and they will electronically sign the consent form through a Redcap instrument.
-

5. STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

N/A – no intervention

5.2 Handling of Study Interventions

N/A – no intervention

5.3 Concomitant Interventions

N/A – no intervention

5.4 Adherence Assessment

Adherence will be defined as completing all relevant questionnaires at enrollment and sending in stool samples on each of five sampling dates from themselves, and, if applicable, their pet.

6. STUDY PROCEDURES

6.1 Schedule of Evaluations

Assessment	Screening/Enrollment: Visit-1 (Day-14 to Day -1)	Sampling day 1 (Day -2 to 0)	Sampling day 2 (Day 3 post implant)	Sampling day 3 (Day 10 post implant)	Sampling day 4 (Day 30 post implant)	Sampling day 5 (Day 90 post implant)
Informed Consent Form	X					
Demographics	X					
DXA	X					
Medical History	X					
Current Medications	X					
Inclusion/Exclusion Criteria	X					
Enrollment	X					
Concomitant Medications	X					
Adverse Events		X	X	X	X	X

6.2 Description of Evaluations

6.2.1 Screening Evaluation

Potentially eligible patients will be identified by performing a query of the Axium electronic medical records at Penn Dental. Patients will be further screened for eligibility (i.e., rule out exclusion criteria) at their pre-surgical planning visit by answering screening questions in a questionnaire.

Consenting Procedure

A single informed consent form that describes both the screening and study procedures will be used. Study coordinators will conduct the consent process in person. The study will be described to the potential participant by the coordinators.

The informed consent document will be reviewed periodically by study coordinators and the principal investigator in case changes may be required. Documentation of signed consent will be collected through a Redcap instrument.

Screening

Screening evaluations to determine eligibility must be completed within 60 days before the implant procedure.

Medical records query will identify patients with relevant inclusion criteria.

Absence of exclusion criteria will be confirmed at enrollment via a questionnaire administered to the candidate.

6.2.2 Enrollment, Baseline, and/or Randomization

Enrollment

A participant who has completed the eligibility screening, agreed to participate and provided informed consent in the study will be enrolled. Their contact information will be entered into the Redcap database, and they will be provided with all of the study material needed for at-home sample collection. Upon completion of enrollment, patients will receive a Clincard, onto which $25 will be loaded.

Baseline Assessments

No baseline assessment will be performed at enrollement. The baseline stool sample will be collected 1 to 2 days prior to the patient's implant surgery.

Randomization

N/A

6.2.3 Blinding

- N/A

6.2.4 Followup Visits

No follow-up visits are included in the study.

At each designated sampling date (day 3, day 10, day 30 ±2 days and day 90±2 days), the patient will collect stool samples from themselves and their pet if they are a pet owner and send them in via UPS.

6.2.5 Completion/Final Evaluation

No specific assessment will be performed on the participant at any point in the study following enrollment.

7. SAFETY ASSESSMENTS

Because no clinical interventions will be administered during this study and because patients will have no direct contact with the study team following the enrollment visit, we will rely on patients to report any complications or adverse events associated with the stool collection to us directly.

7.1 Specification of Safety Parameters

Because no clinical interventions will be administered during the study, there are no specific defined safety parameters to monitor.

7.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

Because no clinical interventions will be administered during the study, there are no specific defined safety parameters to monitor.

7.3 Adverse Events and Serious Adverse Events

The only foreseeable adverse event associated with the study is the possibility of injury to the colonic mucosa in the event that a patient needs to swab themself (i.e., they were not able to produce a stool sample on the designated sampling day).

No **serious adverse events (SAE)** are anticipated in this study.

7.4 Reporting Procedures

Any reports of adverse events will be reported within 3 days to the IRB.

7.5 Followup for Adverse Events

If an adverse event occurs, patients will be advised to seek input from their physician.

8. INTERVENTION DISCONTINUATION

Study participation will conclude once the last sample (i.e., day 90 post implant) is collected.

Participants may choose to withdraw from the study at any time.

9. STATISTICAL CONSIDERATIONS

9.1 General Design Issues

This study is a longitudinal cohort study to investigate the effect of pet ownership on the disruption and evolution of the gut microbiome.

Aim 1: The gut microbiome and levels of CD-resistant bacterial taxa will be more similar within a pet/owner pair than within a pet/non-pet owner control pair, and among pet owners, similarity will increase with increasing pet contact.

Aim 2: *Pet owners will experience less gut microbiome disruption (lower drop in alpha diversity) and a more complete return to baseline gut microbiome composition following antimicrobial therapy than non-pet owners.*

Aim 3: Pet owners will be less likely to be colonized with CD following antimicrobial therapy than non-pet owners, and colonized pet owners will have a lower burden of CD than colonized non-pet owners.

9.2 Sample Size and Randomization

Aim 1: We previously found that the mean (SD) Bray-Curtis intra-litter and inter-litter beta diversity was 0.47 (0.13) and 0.70 (0.14), respectively. In another study, the mean (SD) unweighted UniFrac beta diversity between pets and owners was 0.84 (0.046) units and 0.72 (0.003) between pet owners and non- pet owners[61]. With the projected sample size, will be sufficiently powered (0.80) to detect a difference in Bray-Curtis beta diversity as small as 0.06 units and in unweighted UniFrac beta diversity as small as 0.02 units between pet owners and non-pet owners. With an anticipated enrollment of 80 pet owners, we will have adequate power (80%) to detect a 32% increase in the similarity of the gut microbiome with each 1-point increase in the pet contact score. If an association of this magnitude can be demonstrated, a clinically meaningful beneficial effect of pet contact can be established.

Aim 2: In a prior study, patients taking antimicrobials experienced a drop in alpha diversity of the gut microbiome of 2.8 units (or 5.4 standard deviations) on day 4 of their antimicrobial regimen [37]. If pet ownership can lessen that drop by half (e.g., 1.4 unit change), we will have demonstrated a clinically significant difference. With an anticipated enrollment of 80 pet owners and 120 non-pet owners, we will have 99% power to detect such a difference, and adequate power (80%) to detect a change as small as 0.46 units.

Aim 3: In a previous study, it was found that the proportion of patients that became colonized with CD following oral antimicrobial therapy increased from 6% to 57%, an almost 10-fold increase in prevalence[74]. In the elderly, where the prevalence of CD colonization is higher[75], a similar fold-change would result in an increase from 16.4%[75] to 100%. If pet ownership can decrease the post-antibiotic prevalence of CD colonization by as little as 25%, (i.e., from 16% to

75% prevalence), a clinically meaningful change in risk could be achieved. With an anticipated enrollment of 80 pet owners and 120 non-pet owners, we will have 99% power to detect such a difference between pet owners and non-pet owners, and 80% power to detect a difference as small as 8%.

9.3 Definition of Populations

Pet owners and non-pet owners.

9.4 Interim Analyses and Stopping Rules

N/A

9.5 Outcomes

Aim 1: Beta-diversity, or the degree of difference between the microbiomes of pets and either their owner or a non-pet owner control, as measured by weighted UniFrac distance.

Aim 2: Alpha diversity of the gut microbiome, as measured by the Shannon index

Aim 3: Presence and burden of CD in patient stool.

9.6 Data Analyses

Aim 1: To compare the similarity of the gut microbiota within the pet/owner and pet/control pairs, gut bacterial community beta-diversity characterized by weighted UniFrac metrics for all pet-owner and pet-control pairs will be compared by permutational analysis of variance (PERMANOVA). We will assess clustering of pet-owner samples relative to samples from other patients with principal coordinate analysis. To explore whether specific bacterial taxa tend to be shared between pets and their owners, we will apply analysis of composition of microbiomes (ANCOM).

The presence/absence and relative abundance of CD-resistant bacterial genera will be assessed in samples from pets, their owners, and non-pet owner controls. The presence/absence will be compared using McNemar's test: for each genus, we will compare the number of times a genus is 1) present in the pet and owner but not in the control and 2) present in the pet and control but absent in the owner, and test the hypothesis that the number of cases in the first scenario will be significantly higher than in the latter scenario. Next, the correlation in levels of relative abundance of each genus will be calculated for each pet-owner and pet-control pair using the Spearman correlation and compared using t-test or Wilcoxon rank sum test.

Among pet owners, bivariable analyses will be performed to determine the association between the primary outcomes (beta diversity and relative abundance of bacterial taxa in Table 1) and the pet contact score or number of pets. Similar analyses will be performed to assess for confounding and effect modification by other variables. Multivariable analysis will be performed with generalized estimating equations incorporating patient-level characteristics, and odds ratio and 95% confidence intervals will be calculated to determine the strength of associations.

Aim 2: The change in alpha diversity between day 0 (pre-antimicrobials) and each sampling day (days 3, 10, 30, and 90) will be calculated and compared by t-test or Wilcoxon rank sum test between pet owners and non-pet owners. Comparisons on day 3, when dysbiosis is most pronounced [36,42] will be used to determine in which group the greatest disruption of the gut microbiome occurs. Comparisons on day 10, 30, and 90 will be used to assess in which group a more complete return to baseline alpha diversity occurs. We will also model alpha diversity over time using a mixed-effects linear regression model incorporating repeated exposures to determine which factors affect the change. The model will incorporate pet ownership, and relevant patient factors (age, sex, co-morbidities) as fixed effects and the patient as a random effect. Subgroup analyses among pet owners will consider number of pets and the pet contact score as fixed effects. Additionally, because the restoration of the gut microbiome will likely vary with the extent of its disruption, the difference in alpha diversity between days 0 and 3 (i.e., the degree of initial perturbation) will be incorporated into the model as a fixed effect to adjust for the degree of dysbiosis experienced by the subject. This will also allow us to account for the differential gut-disrupting effects of different types of antibiotics. We will also perform ANCOM analyses to compare the composition of patient microbiomes at different time points of analysis among and between pet owners and non-pet owners.

Aim 3: The presence/absence and burden of CD will be determined on day 0 (pre-antibiotics) and on day 3, when gut dysbiosis tends to be the greatest[36,42], and compared between pet owners and non-pet owners by chi-squared and t-test, respectively. We will then model the presence/absence of CD over time using longitudinal mixed-effects logistic regression incorporating pet ownership or pet contact score, and relevant patient factors (age, sex, antimicrobial class) as fixed effects and the patient as a random effect.
For the secondary analysis, considering CDI as an outcome, we will compare the cumulative incidence among pet owners and non-pet owners using the chi-square test or Fisher's exact test, as appropriate.

10. DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

All data will be collected at enrollment and entered into Redcap by the study coordinator (e.g., concurrent medications, health conditions) or entered directly into Redcap by the patient (e.g., pet-related information, travel history, household information).

10.2 Data Management

All information will be collected via Redcap surveys, which are then compiled in a Redcap database.

Members of the study team at the dental school and the PI will have access to the Redcap database. Redcap data will be inspected regularly for errors by the PI and study coordinator.

16s rRNA sequencing data will be produced by the PennCHOP sequencing center and stored on university servers.

10.3 Quality Assurance

10.3.1 Training

All personnel involved in recruiting and interacting with patients will complete CITI Human Subjects training and be trained by the PI.

10.3.2 Quality Control Committee

Study coordinators will regularly review data collection forms and compile recruitment reports, which will be sent to the PI.

10.3.3 Metrics

All study outcomes will be analyzed by the PI in coordination with biostatistician collaborators.

10.3.4 Protocol Deviations

Protocol deviations will be captured, documented in protocol deviations forms, reviewed by the PI, and reported to the IRB.

10.3.5 Monitoring

The PI will accompany members of the recruitment team for the first series of enrollments to ensure compliance with the protocol. Once the study team is well versed in the protocol, monthly meetings will be held with the PI to ensure protocol compliance and check for quality control.

Any updates to the protocol and consent forms will be shared with the study team, and the most up-to-date forms will be kept in a Box account that is accessible to study team members.

11. PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 Institutional Review Board (IRB) Review

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the IRB or ethics committee responsible for oversight of the study. The consent form will be separate from the protocol document.

11.2 Informed Consent Forms

A signed consent form will be obtained from each participant. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy will be given to each participant or legal guardian and this fact will be documented in the participant's record.

11.3 Participant Confidentiality

Any data, specimens, forms, reports, video recordings, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done using PIDs only. Information will not be released without written permission of the participant, except as necessary for monitoring by the IRB or NIAID.

11.4 Study Discontinuation

The study may be discontinued at any time by the IRB, the NIAID or other government agencies as part of their duties to ensure that research participants are protected.

12. COMMITTEES

N/A

13. PUBLICATION OF RESEARCH FINDINGS

Any presentation, abstract, or manuscript will be made available for review by the sponsor and the NIAID prior to submission.

14. REFERENCES

Provide the citations for all publications and presentations referenced in the text of the protocol.

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15. SUPPLEMENTS/APPENDICES