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The association between oral antibiotic use and functional gastrointestinal disorders in
the context of the gut microbiome

by

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ABSTRACT

The association between oral antibiotic use and functional gastrointestinal disorders in the context of the gut microbiome:

Amelia Johnson, MPH, DrPH, 2021

Thesis directed by: David Tribble, MD, DrPH, Preventive Medicine and Biostatistics

Functional gastrointestinal disorders (FGDs) are a group of chronic gastrointestinal conditions with defined symptoms but without clear etiological or pathophysiological characteristics. Some of the most common FGDs are irritable bowel syndrome (IBS), functional diarrhea (FD), functional constipation (FC), and dyspepsia (D). These conditions have a significant impact on productivity, quality of life, and healthcare utilization costs. The risk profile for FGDs involves many factors and the causal pathway between antibiotic use and FGDs remains unresolved; additional research into this relationship is needed to improve understanding and guide prevention efforts.

A systematic review of the literature on antibiotics and FGD in the context of the gut microbiome revealed evidence that antibiotics disrupt the normal gut microbial environment at least temporarily, and that structure and composition of the gut microbial environment differs between FGD cases and healthy controls. However, the current literature does not provide strong epidemiological evidence of an association between antibiotic use and increased FGD risk.

In a nested case-control study of active duty military personnel, risk of FGD was consistently elevated from oral antibiotic use. Additionally, risk of IBS was shown to

increase in a dose-response relationship to number of antibiotic prescriptions. Several individual antibiotic classes were found to be associated with IBS, providing specificity to the relationship.

Finally, the change in host immune response to gut microbiota was explored in relation to IBS cases and non-IBS controls following travelers' diarrhea and antibiotic use exposures in a population of travelers. Specific antigen targets were significantly associated with IBS cases compared to controls and may provide important information in the development of biomarkers for IBS.

Overall, this research contributes support to the association between antibiotics and increased risk of FGD and provides useful information for future studies investigating the host immune response to the gut environment and how it relates to IBS and relevant exposures.

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CHAPTER 1

INTRODUCTION

Background

Functional gastrointestinal disorders (FGDs) consist of a range of chronic conditions that affect the digestive tract and include functional diarrhea (FD), functional constipation (FC), irritable bowel syndrome (IBS), and functional dyspepsia (FD) (34; 142). These conditions cause considerable detriment to quality of life and productivity in the general population while significantly increasing healthcare utilization costs (11; 14; 142). For the same reasons, these chronic GI conditions have extensive impact on the readiness of the U.S. military and costs to the U.S. Military Health System (MHS) (103).

FGDs pose a challenge in terms of diagnosis and treatment due to the lack of structural or biochemical irregularity and an unclear causal pathway. Research attempting to better understand the causes of these disorders often targets disrupting factors in the function of the GI system, which is closely connected with the overall health of the gut microbial ecosystem. The alteration of the normal microbial balance of the GI tract, or gut microbial dysbiosis, is therefore hypothesized to be a main factor in the development of FGDs (22).

Antibiotics are intended to fight infection from harmful pathogens; however, these drugs often have unintended consequences, including targeting symbiotic bacteria that are often essential to normal bodily function (41). Therefore, antibiotics have also been implicated as a causal factor in disrupting the normal gut microbial environment, and, by extension, a causal factor in the development of FGD (82).

Public Health Significance

FGDs have a significant impact on productivity, quality of life, and health care costs. In the military, this translates to reduced readiness, attrition and costs to the MHS and Department of Defense (DoD) for members with these conditions.

Overall, the prevalence and incidence of FGDs are thought to be widely underestimated as many with the disorders do not seek medical care (34). Despite this probable underestimation, these disorders still account for a substantial amount of primary care and specialist visits and are diagnosed frequently. FC is typically the most prevalent FGD seen in both the general population and in the military (60; 87; 102). In the general population, FC prevalence has been found to reach up to 27% and one study found a 12 year cumulative incidence of 17.4% (13). FC typically affects the elderly and females more often (13). Among the most common of the FGDs, and the most studied, is IBS. Prevalence estimates place about 10-20% of the general population with IBS at any one time, and approximately 200 incident cases per 100,000 persons per year (13; 89). IBS patients tend to be disproportionately female and younger in age compared to the other functional GI disorders (13). Prevalence estimates for dyspepsia place about 20-30% of the population with the disorder (87).

In the active component military population, similar trends to the general population are observed, though rates are somewhat lower. In a study using medical encounter data, FC has displayed the highest rates with 376 cases per 100,000 person-years, followed by IBS with 71 cases per 100,000 person-years, D with 14 cases per 100,000 person-years and FD at about 4 cases per 100,000 person-years (60). These reduced incidence rates from what would be expected based on the general population may exist due to differences in study design, diagnostic criteria used, or as a result of a

healthy population effect seen in active duty military personnel-a largely young, healthy, and male population.

Furthermore, among active component military personnel, females had higher FGD rates compared to males, as did blacks compared to whites (60). High rates were seen in personnel aged ≥ 40 years, but surprisingly, the highest rates in the active component population were observed in those aged < 20 years (60). This age category includes a unique subpopulation of the military, that of the recruit population. It is possible that this subpopulation is at higher risk for FGDs due to environmental factors such as crowding, increased stress or mental illness, and increased rates of communicable diseases (120). Both the Army and the Air Force had higher incidence rates compared to the Navy, while rates were reduced in the Marines (60). Additionally, when deployment was assessed as an exposure, higher rates were observed among deployed personnel compared to those without the exposure (60).

FGDs have significant detrimental impact on productivity and quality of life (11). Beyond management of symptoms, there are no specific treatments and there is no cure for the disorders (87). As enigmatic disorders, it can be problematic to effectively manage the symptoms (34). Those suffering from FGDs often score lower on quality of life scores compared to both healthy populations and persons with certain chronic diseases (11; 49).

Linked to increased inpatient and outpatient services as well as non-gastrointestinal related physician visits and health care costs, FGDs exist as a considerable burden on healthcare systems (11; 14; 142). Since 1985, in the general population, rates of ambulatory care visits and hospital admissions have escalated for

FGDs, driven largely by functional constipation (39). The military has seen a similar rise in rates of diagnoses for FGDs, again primarily due to increasing rates of functional constipation (60; 102). In 2004, over 11 million ambulatory care visits were attributable to FGDs in the US; over 13 million prescriptions were filled at pharmacies nation-wide, costing almost three-quarters of a billion dollars (39). Thus, it is clear that FGDs cause a substantial amount of healthcare utilization and burden on the health care system. With increasing rates of FGDs, in both the general US population and military populations, the burden of FGDs on the health care systems of this country and the DoD is only expected to increase as well.

Relevant to the DoD community, FGDs are considered a Gulf War Illness (GWI)-a group of illnesses and syndromes associated with military service during the Gulf War. In the Seabee Health Study, Navy Seabees suffering from GWI were 3.32 times more likely than those without GWI to report having taken ciprofloxacin (unadjusted 95% CI: 2.69, 4.10) and the association remained after inclusion in a multivariate model (adjusted OR: 1.37; 95% CI: 1.08, 1.75) (47). While Seabees with GWI were also more likely to report having taken doxycycline (unadjusted OR: 1.46; 95% CI: 1.20, 1.78), the association did not remain after inclusion in the saturated model (47).

The gut microbial environment is a growing area of interest concerning not only gut functionality, but also the impact it has on the overall health of an individual. In the case of FGDs, it is clear that gut dysfunction can greatly reduce the overall wellness of a patient suffering from an FGD. While there is substantial evidence of acute dysbiosis caused by antibiotics, growing evidence showing long-term dysbiosis caused by antibiotic use, and some epidemiological evidence indicating a potential association, a

definitive link between antibiotic use and FGDs has yet to be confirmed. Meanwhile, there is a well-established connection between AGE and FGDs for at least a portion of those suffering from an acute GI infection (92; 96; 101; 102; 143). Duration and severity of AGE is thought to increase the risk of post-infectious sequelae (7; 87). Antibiotics are currently the recommended treatment for more severe types of traveler's diarrhea or AGE, and are thought to lessen the symptoms and shorten the infection (116). This mitigation is hypothesized to impact the downstream sequelae of AGE and it has therefore also been hypothesized that antibiotic treatment may reduce the risk of post-infectious FGDs via a reduction in length and severity in acute enteritis, though there is diminutive evidence confirming this protective association at this point (50; 69).

Antibiotics are commonly used in the general US population; a study using data from the 2010-2011 National Ambulatory Medical Care Survey and National Hospital Ambulatory Medical Care Survey found that approximately 13% of ambulatory visits resulted in a prescription for antibiotics (40). Furthermore, about one-third of those prescriptions were determined to be inappropriate for the patient's condition (40). Therefore, antibiotics are an important, but often unnecessary exposure that may have detrimental consequences beyond antimicrobial resistance and immediate disruptions to the gut flora.

Need for Study

A clear timeline through which many implicated or hypothesized exposures act on the gut microbial environment and lead to FGDs has yet to be fully established. This timeline is important for epidemiological research as a precise follow-up period allows for a more exact capture of true cases and is necessary to establish a temporal

relationship. For instance, too brief a follow-up period may undercount cases that have not appeared following exposure. Additionally, studies that have investigated the timeline between exposures, such as AGE, to FGD diagnosis, have found that the follow-up time and ‘latency’ period between exposure and diagnosis can make it difficult to directly link exposure with the FGD outcome, when interceding contributory events may potentially occur during that time (28).

This project intends to provide a detailed investigation into the association between antibiotic use and the development of FGDs. This association will be considered in conjunction with other important exposures over time, such as acute enteric infection and non-enteric infection. Furthermore, through these studies we hope to elucidate the mechanism and host response to gut antigens related to FGDs. Traveler’s diarrhea and antibiotics will be investigated in terms of changes in the host immune response to the gut microbiota.

Research Question and Aims

Overall Research Question

What is the relationship between oral antibiotic use and functional gastrointestinal disorders (FGDs) in the context of gut microbial dysbiosis?

Specific Aims

1. Systematically review the literature to assess the accumulated evidence on the relationship between oral antibiotic use, persistent gut microbial dysbiosis, and development of FGDs.

Research Question 1.1:

Does existing literature support a relationship between oral antibiotic use and gut microbial dysbiosis in healthy adults?

Research Question 1.2:

Does existing literature support a relationship between gut microbial dysbiosis and incident FGDs in previously healthy adults?

Research Question 1.3:

Does existing literature support a relationship between oral antibiotic use and incident FGDs in healthy adults?

2. Estimate the risk of incident FGDs associated with oral antibiotic use and enteric infection while adjusting for relevant risk factors/covariates and accounting for time at risk, in the U.S. military active component population.

Research Question 2.1:

What is the risk of incident FGDs following oral antibiotic use (independent of infection type) compared to subjects without oral antibiotic use after adjusting for age, gender, ethnicity, rank, service branch, co-morbidities, and differences in antibiotic class, and number of doses, while taking time at risk into account?

Hypothesis:

Ho: There is no difference in risk of incident FGD following oral antibiotic use versus no oral antibiotic use after adjusting for important covariates and accounting for time at risk.

Ha: There is a difference in risk of incident FGD following oral antibiotic use versus no oral antibiotic use after adjusting for important covariates and accounting for time at risk.

3. Explore changes in systemic host responses (antibody responses to gut microbiome antigens) and differences in travel exposures (antibiotic use or acute gastroenteritis) in a population of travelers with and without incident IBS.

Research Question 3.1

Do changes in systemic host responses to gut microbiota differ between new cases of IBS and controls or between exposure groups (antibiotic use or acute gastroenteritis) following travel?

Hypothesis:

Ho: There is no significant difference between systemic host responses to gut microbiota between IBS cases and controls or between exposure groups following travel.

Ha: There is a significant difference between systemic host responses to gut microbiota between IBS cases and controls and/or between exposure groups following travel.

Literature Review

FGDs occur commonly in the population, yet have an unclear pathophysiology or etiology. As a structural or etiological explanation is unknown at this point, cases of FGD are typically diagnosed using validated symptom-based criteria, such as the Rome Criteria (148). The Rome III Criteria for the four FGDs of interest in this study are as follows:

Functional Constipation*

1. Must include two or more of the following:
 - a. Straining during at least 25% of defecations
 - b. Lumpy or hard stools in at least 25% defecations
 - c. Sensation of incomplete evacuation for at least 25% of defecations

- d. Sensation of anorectal obstruction/blockage for at least 25% of defecations
- e. Manual maneuvers to facilitate at least 25% of defecations (e.g. digital evacuation, support of the pelvic floor)
- f. Fewer than three defecations per week
- 2. Loose stools are rarely present without the use of laxatives
- 3. Insufficient criteria for irritable bowel syndrome

Irritable Bowel Syndrome*

Recurrent abdominal pain or discomfort** at least three days per month in the last three months associated with two or more of the following:

- 1. Improvement with defecation
- 2. Onset associated with a change in frequency of stool
- 3. Onset associated with a change in form (appearance) of stool

Functional Dyspepsia*

Must include one or more of the following:

- 1. Bothersome postprandial fullness
- 2. Early satiation
- 3. Epigastric pain
- 4. Epigastric burning AND no evidence of structural disease (including upper endoscopy) that is likely to explain symptoms

Functional Diarrhea*

Loose (mushy) or watery stools without pain occurring in at least 75% of stools

*Criteria fulfilled for the last three months with symptom onset at least six months prior to diagnosis

**“Discomfort” means an uncomfortable sensation not described as pain. In pathophysiology research and clinical trials, a pain/discomfort frequency of at least 2 days per week during screening evaluation is recommended for subject eligibility.

The currently established risk profile for FGDs is multi-factorial, with genetic, immunological, dietary, lifestyle/behavioral, and external and internal (i.e. microbial dysbiosis) environmental factors known to be related to the development of chronic GI conditions (65; 132; 150).

In particular, acute GI infection is a well-established risk factor for FGDs, and is known to disrupt the gut microbial environment (100; 101; 115). About 25% of patients with IBS are found to have history of acute gastroenteritis (AGE) (45). Additionally, multiple studies have linked numerous pathogens with chronic GI sequelae (45; 59; 100; 115; 122; 155). A review of post-infectious irritable bowel syndrome (PI-IBS) found that risk of IBS is increased across the spectrum of bacterial AGE, regardless of the etiology, and concluded that severity of infection is an important predictor in the development of IBS (133).

Similarly, antibiotics are known to cause changes in the gut environment, both transient, and long-term (29; 30). Diarrhea is a well-documented adverse side effect of antibiotic use, particularly common in patients taking antibiotics with broad antimicrobial activity such as amoxicillin/ampicillin, clindamycin, and cephalosporins (147). Furthermore, there is accumulating evidence from cross-sectional and retrospective studies indicating a relationship may exist between the use of various antibiotics and the development of FGDs and other long-term sequelae (71; 82; 94; 154). Studies investigating PI-IBS detected an increase in risk for IBS after prolonged use of antibiotics; however it is unclear whether the antibiotic use in these studies is a true risk modifier or confounder in the causal pathway of disease (28; 119). Therefore, in the case of post-infectious IBS, and for other FGDs occurring after AGE, the scientific evidence

has not yet clearly defined the relationship between acute infection, antibiotic use and development of FGDs.

The gut microbial environment consists of a complex, mutualistic interplay between bacterial, fungal, and viral organisms, and human host cells. The make-up of this ecosystem can depend on a number of factors, including age, environment, diet and health status (138). Dysbiosis is the disruption of the composition and function of an environment (41). Therefore, gut microbial dysbiosis is characterized by alterations in the structure (i.e. number and distribution of organisms) and function of the gut ecosystem. Gut microbial dysbiosis can be measured in terms of microbial diversity. Specifically, α -diversity concerns the diversity of a single environment alone. It includes richness (a measure of the number of organisms present) and evenness (a measure of the abundance of each organism in an environment) (25). The amount of variation between two or more ecosystems is known as β -diversity (25).

Numerous molecular methods, including genomics, transcriptomics, proteomics, and metabolomics, have been developed that permit identification, quantification and profiling of the human microbiota and the host immune response to the microbiota (15; 42; 138). Genomic techniques, and in particular the sequencing of the 16s rRNA gene of microbiota, are used for the identification of bacterial taxa, and can provide quantification of the groups, species, operational taxonomic units, etc. present in a sample (42). While genomic techniques can lend some insight to possible biochemical pathways and functional profiles of the microbiota, they cannot ultimately reveal the expressed functionality of organisms (6; 138). Other techniques move beyond the use of genomics to analyze the structure and function of the microbiome. Meta-transcriptomics,

proteomics and metabolomics can provide further information on the true functional nature of the microbiota present in a sample (42). In addition to functional profiling, proteomics can reveal the intricacies of host-gut microbiome interactions and host-secreted proteins, allowing for an integrated analysis of host, disease mechanisms and microbial functions (42).

The presence of a normal adaptive immune response to antigens from the gut microbiota from infancy into adulthood has been demonstrated, and this systemic immune response is shown to transform in response to various stages of life, health and disease (15; 156). Increasingly, cytokine biomarkers and antibody response profiles to gut microbiota antigens are being used to differentiate patients suffering from FGDs, and in particular IBS, from those with normal gut function, and even in some cases, to facilitate diagnosis (95). Such studies have identified immunological, functional and microbiological alterations between the IBS disease state and the normal, healthy gut based on the expression of proteins in fecal samples, presence of volatile organic compounds in urine, histological differences in cell populations, and mRNA levels (4; 19; 76). In serum, changes have been detected in specific toll-like receptors, interleukins, tumor necrosis factor- α , leptin, tryptophan, and in the pro-inflammatory chemokine CCL-16 (18; 53; 81). One systematic review found consistently elevated levels of TNF- α , and decreased levels of IL-10 in serum from IBS patients vs. controls, with differences in levels between sexes (4). A study investigating the hypothalamic-pituitary-adrenal axis, an important regulatory circuit linking the brain and gut, in IBS patients and matched controls found elevations in pro-inflammatory cytokines (IL-6, IL-8) and cortisol (32). Additionally, the study investigated the effect of corticotropin-releasing hormone

infusion on IBS patients, finding enhanced release of adrenocorticotrophic hormone and cortisol compared to controls, indicating an exaggerated stress response in IBS patients (32). Tryptophan metabolic pathways were found to be altered in male IBS patients in another study, providing evidence that the brain-gut axis is disrupted in IBS patients as well as further possibilities for biomarkers of IBS (18). Moreover, plasma arachidonic acid (AA) levels in female IBS patients have been found to be elevated compared to healthy controls, providing more evidence of a pro-inflammatory mechanism for IBS as well as a potential biomarker (19). In a separate study, circulating antibodies to cytotoxin distending toxin (CdtB) and vinculin in patients with diarrhea-predominant IBS (IBS-D) indicate that alterations in the enteric nervous system and gut motility, further revealing the mechanisms behind the condition (98). Further evidence for low-grade inflammation in IBS-D was provided by one study which found elevations in IL-8 and decreases in IL-10 levels expressed in tissue as well as significantly more eosinophils present in IBS-D patients compared to controls (27). An inflammatory response was again observed in a study of IBS patients, with elevations in IL-6 and IL-8 observed (31).

Regarding microbiome-directed antibody levels, lower mean differences have been observed in certain antibodies to gut microbiota, with marked differences seen in antibody levels against *E.coli* flagellin (*E. coli* FliC) and *B. vulgatus* protease IV/AppA (rIB13), and slightly lower levels of antibodies against *B. vulgatus* ABC transporter ATP-binding protein (rIB19), salmonella flagellin (SalFlic), and *E. faecalis* Sal A (EF20) (95). Conversely, higher concentrations of antibody levels were observed to the antigens *E. coli* OmpC (95). The findings from this study provides more evidence that both cytokine biomarker profiles and microbiome-directed antibody levels can distinguish between

states of disease and dysbiosis, while also revealing information about the potential biological pathways through which disease is produced.

Consequently, deviations in host responses to microbial epitopes, and whether those epitopes are related to the immune response, gut function, and brain-gut communication, are being increasingly identified as potential biomarkers for active diagnosis of IBS, improving on traditional diagnostic methods that rely mainly on symptom presentation or exclusion of other possible conditions. In some cases, changes in the levels of these proteins is also helping to elucidate the pathophysiological reasons behind these disorders, as these changes indicate aberrations in gut motility, the brain-gut axis, and sub-clinical/low-grade inflammation (32; 97; 140). Mounting evidence shows that both passive (i.e. chronic gut inflammation) and active (i.e. acute enteric infection) dissemination of gut microbiota from the gastrointestinal tract can induce changes in systemic antibody responses in animal models and humans (156). Differences in antibody profiles to gut microbiota between IBS patients and healthy controls have been observed as well, and the evidence of a normal immune response to gut microbiota from infancy to adulthood indicates the potential to use microbiota-directed antibodies to identify patterns in FGD patients, provide insights to the timing of disease and add strength to diagnostic methods (15; 156). Further research on the host immune response to microbiota in FGDs is necessary to increase understanding of the complexity and interaction of these systems (95; 133).

The impact of antibiotics on the human gut microbiota has been investigated by Dethlefsen, et al (29). Ciprofloxacin, a broad-spectrum antibiotic with low anti-anaerobic activity, was observed to have a pervasive effect on microbial composition 4 weeks post-

treatment, but little impact on gut function, possibly the result of functional redundancy in the gut microbial community (29). Another study by Dethlefsen, et al. followed subjects for 10 months after repeated ciprofloxacin treatments in a 6 month time-frame and found that the pervasive changes in gut microbial composition from pre-treatment to post-treatment persisted in the long-term (30). The long-term effect of antibiotics on the gut microbiota has also been assessed by Jernberg, et al (56; 57). Persistent effects on the structure and diversity of the gut microbial community were observed over 2 years following treatment with clindamycin, an antibiotic with anti-anaerobic activity (56). A number of additional studies have also noted the acute and chronic effects that clindamycin has on the gut microbiome (72; 137). Similarly, acute and persistent changes in the microbiome have been detected following amoxicillin treatment as well (26; 137). Furthermore, Jakobsson, et al. observed that following a standard, 1-week treatment with clarithromycin and metronidazole, commonly used for *Helicobacter pylori* infection, the gut microbiota was altered and the effect remained over time (54). The long-term impact was not only seen in the microbial composition, but also in the expression of antimicrobial resistance genes up to 3 years following initial treatment and without any further antibiotic pressure (54). Antunes, et al. studied the effects of streptomycin, vancomycin, tetracycline and metronidazole treatment on the gut microbiome and chemical composition (2). Major changes in metabolic pathways and hormone homeostasis were detected (2).

Consequently, there is mounting evidence indicating that antibiotics not only change the microbial environment in the short term, but could potentially have lasting

effects on microbial composition and gut function. Additionally, these studies display the possibility for greater and more long-term changes after repeat exposure to antibiotics.

Some observational epidemiological studies on FGDs provide evidence of a potential association between antibiotics and FGDs. A study by Ruginomez, et al. investigated modifiers in the relationship between acute gastroenteritis and IBS, noting that prolonged use of antibiotics increased the risk of IBS following AGE episode (119). Antibiotic use was also implicated for increased risk of IBS in adults in an observational survey-based study by Mendall, et al (82). Another survey-based nested case-control study focused on both enteric and non-enteric infections and antibiotic use associated with FGDs (94). The results showed a positive association between antibiotic use for non-enteric infection and development of FGD. Furthermore, broad-spectrum antibiotic use and development of IBS was examined by Villareal, et al (154). The group found that macrolide and tetracycline use were associated with increased risk of developing IBS, and the association with tetracycline remained significant after adjustment for multiple factors (adjusted OR=1.48, p=0.046). Similarly, a number of epidemiological studies have linked antibiotic use to the development of other chronic gastrointestinal diseases, such as inflammatory bowel diseases (125; 128; 151). Thus, there is mounting epidemiological evidence indicating a relationship between antibiotic use and FGDs in addition to studies directly linking antibiotic use to sustained changes in the microbiome and gut function. Still, the level of evidence for this relationship needs to improve before antibiotic use can be linked strongly to development of FGDs. More studies, temporally linking exposure and FGD outcome, as well as those connecting exposure, mechanism

and outcome are needed to clarify the relationship between antibiotic use and development of FGDs.

Acute GI issues and associated healthcare visits are frequent in the general population and in travelers, and have implications for the military as well. Unique exposures, such as crowded living arrangements during training and international deployments may put the military population at increased risk of AGE. Indeed, diarrhea is the most common medical problem for deployed U.S. military personnel (102; 121). Strategies to mitigate the impact of acute enteric infections include various antibiotic regimens (1; 48). Additionally, antibiotics are frequently used for many other reasons, to include non-enteric infection treatment, prophylaxis and dermatologic reasons (127).

While the increased risk of FGDs following acute gastroenteritis has been well established in both the general population and in the military, the relationship between antibiotic use and these chronic GI conditions independent of acute enteritis requires further evaluation. Additionally, this causal pathway has not been examined in the context of the human gut microbial environment and the human immune response to gut microbiota. Further research into the causal pathway has been called for to improve understanding and guide primary prevention efforts (71; 82; 103). New developments in the use of proteomics to detect alterations in host response in FGDs offer a novel method to assess the association between oral antibiotic use and development of FGDs. This project is intended to elucidate the relationship between oral antibiotics and incident FGD, providing a basis for further research in this field and making available information to health providers regarding the prescription of antibiotics.

CHAPTER 2: Systematic Review

INTRODUCTION

Functional Gastrointestinal Disorders (FGDs) are a major cause of morbidity in the United States and are characterized by a multifactorial causal pathway (132). Dysbiosis, or a disruption in the normal intestinal ecological balance, is thought to be a contributing factor in the symptoms displayed in these conditions (22). While associated with many contributing factors and causes, dysbiosis is hypothesized to be associated with the intake of antibiotics (29; 30). A number of studies have investigated the association between dysbiosis and FGDs and the association between antibiotics and gut microbial dysbiosis, yet fewer have examined the relationship between antibiotics and FGDs. This review covers the current level of knowledge on the causal pathway between antibiotic use and FGD, as well as the relationship between antibiotics and dysbiosis and the association between dysbiosis and FGDs separately to provide context and biological plausibility to the antibiotic-FGDs pathway, considering dysbiosis an intermediate factor.

METHODS

Three systematic literature searches, based on three main research questions were performed using PUBMED and EMBASE databases. Only English language articles published from 2000-2017 were included in the results. The questions with PICO eligibility criteria incorporated, corresponding search strategies and dates of search are listed below:

1. What is the relationship in the scientific literature, to include observational studies and clinical trials (study design) between oral antibiotic use (exposure) compared to no use (comparison) and gut microbial dysbiosis (defined as an imbalance in the gut

microbiota or a departure from the normal state-see supplemental information for definition of dysbiosis used to assess the literature) (outcome) in healthy adults (participants)?

PUBMED 09 April 2018:

((("anti-bacterial agents"[Pharmacological Action] OR "anti-bacterial agents"[MeSH Terms] OR ("anti-bacterial"[All Fields] AND "agents"[All Fields]) OR anti-bacterial agent*[All Fields] OR antibiotic*[All Fields]) AND (((“intestinal” OR “gut”) AND (“microbiome” OR “microbiota” OR “bacteria”)) OR ("Dysbiosis"[Mesh] OR dysbios* OR disbios* OR dysbacterios*)) AND (Study Characteristics [PT] OR Epidemiologic Study Characteristics OR Research Design[MH] OR (clinical[TIAB] AND trial[TIAB]) OR random*[TIAB] OR therapeutic use[SH] OR (cohort[TIAB] AND study[TIAB]) OR (cohort[TIAB] AND studies[TIAB]) OR case control*[TIAB] OR case stud*[TIAB] OR case report*[TIAB]) AND ("2000/01/01"[PDAT] : "3000/12/31"[PDAT]) AND "humans"[MeSH Terms] AND English[lang] AND "adult"[MeSH Terms])

EMBASE 25 August 2017:

((('anti-bacterial agents'/exp OR 'anti-bacterial agents' OR ('anti-bacterial' AND 'agents') OR 'anti-bacterial agent*' OR 'antibiotic*') AND ('intestinal' OR 'gut'/exp OR 'gut') AND ('microbiome'/exp OR 'microbiome' OR 'microbiota'/exp OR 'microbiota' OR 'bacteria'/exp OR 'bacteria') OR 'dysbiosis'/exp OR 'dysbiosis' OR 'dysbios*' OR 'disbios*' OR 'dys-symbios*' OR 'dysbacterios*') AND ([young adult]/lim OR [adult]/lim OR [middle aged]/lim OR [aged]/lim) AND [humans]/lim AND [animals]/lim AND [english]/lim AND [2000-2017]/py

2. What is the relationship in the scientific literature, to include observational studies and clinical trials (study design), between gut microbial dysbiosis (see supplemental information for definition of dysbiosis used to assess the literature) (exposure) and functional gastrointestinal disorders (outcome) in previously healthy adults (participants)?

PUBMED 09 April 2018:

("Colonic Diseases, Functional"[Mesh] OR "irritable bowel syndrome" OR "ibs" OR (functional[tiab] AND gastrointestinal[tiab] AND disorder*[tiab]) OR "functional constipation"[tiab] OR dyspepsia OR "functional diarrhea"[tiab]) AND (((("intestinal" OR "gut") AND ("microbiome" OR "microbiota" OR "bacteria")) OR ("Dysbiosis"[Mesh] OR dysbios* OR disbios* OR dysbact*)) AND (Study Characteristics [PT] OR Epidemiologic Study Characteristics OR Research Design[MH] OR (clinical[TIAB] AND trial[TIAB]) OR random*[TIAB] OR therapeutic use[SH] OR (cohort[TIAB] AND study[TIAB]) OR (cohort[TIAB] AND studies[TIAB]) OR case control*[TIAB] OR case stud*[TIAB] OR case report*[TIAB]) AND ("2000/01/01"[PDAT] : "3000/12/31"[PDAT]) AND "humans"[MeSH Terms] AND English[lang] AND "adult"[MeSH Terms])

EMBASE 25 April 2017:

('colonic diseases, functional'/exp OR 'colonic diseases, functional' OR 'irritable bowel syndrome'/exp OR 'irritable bowel syndrome' OR 'ibs' OR (functional AND gastrointestinal AND disorder*) OR 'functional constipation'/exp OR 'functional constipation' OR 'dyspepsia'/exp OR dyspepsia OR 'functional diarrhea'/exp OR

'functional diarrhea') AND (('intestinal' OR 'gut'/exp OR 'gut') AND ('microbiome'/exp OR 'microbiome' OR 'microbiota'/exp OR 'microbiota' OR 'bacteria'/exp OR 'bacteria') OR 'dysbiosis'/exp OR 'dysbiosis' OR dysbios* OR disbios* OR 'dys symbios*' OR dysbact*) AND ([young adult]/lim OR [adult]/lim OR [middle aged]/lim OR [aged]/lim) AND [humans]/lim AND [animals]/lim AND [english]/lim AND [2000-2017]/py

3. What is the relationship of the association in the scientific literature, to include observational studies and clinical trials (study design) between oral antibiotic use (exposure) compared to no use (comparison) and functional gastrointestinal disorders, IBS, FC, FD, and D, (outcome) in healthy adults (participants)?

PUBMED 09 April 2018:

("anti-bacterial agents"[Pharmacological Action] OR "anti-bacterial agents"[MeSH Terms] OR anti-bacterial agent*[All Fields] OR antibiotic*[All Fields]) AND (("functional"[All Fields] AND "gastrointestinal"[All Fields] AND disorder*[All Fields]) OR functional gastrointestinal disorder*[All Fields] OR "irritable bowel syndrome"[All Fields] OR "functional constipation"[All Fields] OR "dyspepsia"[All Fields] OR "functional diarrhea"[All Fields]) AND (Study Characteristics [PT] OR Epidemiologic Study Characteristics OR Research Design[MH] OR (clinical[TIAB] AND trial[TIAB]) OR random*[TIAB] OR therapeutic use[SH] OR (cohort[TIAB] AND study[TIAB]) OR (cohort[TIAB] AND studies[TIAB]) OR case control*[TIAB] OR cohort*[TIAB]) AND ("2000/01/01"[PDAT] : "3000/12/31"[PDAT]) AND "humans"[MeSH Terms] AND English[lang] AND "adult"[MeSH Terms])

EMBASE 25 August 2017:

((('anti-bacterial agents'/exp OR 'anti-bacterial agents' OR 'anti bacterial') AND agent* OR antibiotic*) AND (('functional' AND 'gastrointestinal' AND disorder* OR functional) AND gastrointestinal AND disorder* OR 'irritable bowel syndrome'/exp OR 'irritable bowel syndrome' OR 'functional constipation'/exp OR 'functional constipation' OR 'dyspepsia'/exp OR 'dyspepsia' OR 'functional diarrhea'/exp OR 'functional diarrhea') AND ([young adult]/lim OR [adult]/lim OR [middle aged]/lim OR [aged]/lim) AND [humans]/lim AND [animals]/lim AND [english]/lim AND [embase]/lim AND [2000-2017]/py

Search results were combined with any additional known articles related to the research questions. As a whole, the results for each research question were then combined and duplicates removed. Next, each article underwent title and abstract screening by two independent reviewers (A.J. and E.E.) to exclude articles not meeting the eligibility criteria specified in each research question. At this screening step, if the determination was to include or undetermined for either reviewer, the article was advanced to the next eligibility step. Advancing full text articles were then reviewed by A.J. and E.E. according to the eligibility criteria. At this step, a third reviewer (M.R.) reviewed articles with conflicting inclusion determination and made a final decision to include/exclude.

For question 1, on the association between oral antibiotic use and dysbiosis, a total of 762 articles were identified from scientific databases and of these 20 articles were included in the review following screening and eligibility steps. For question 2, on the association between dysbiosis and FGD, 181 total articles were identified from scientific

databases and of these 24 articles were included in the review after screening and eligibility completion. For question 3, on the association between oral antibiotic use and FGD, 542 total articles were identified from scientific databases and of these 8 articles were included in the review after screening and eligibility completion (84; 126). See Figures 1-3 for detailed flow charts for each study question.

Figure 1: Antibiotics and Dysbiosis PRISMA Flow Diagram

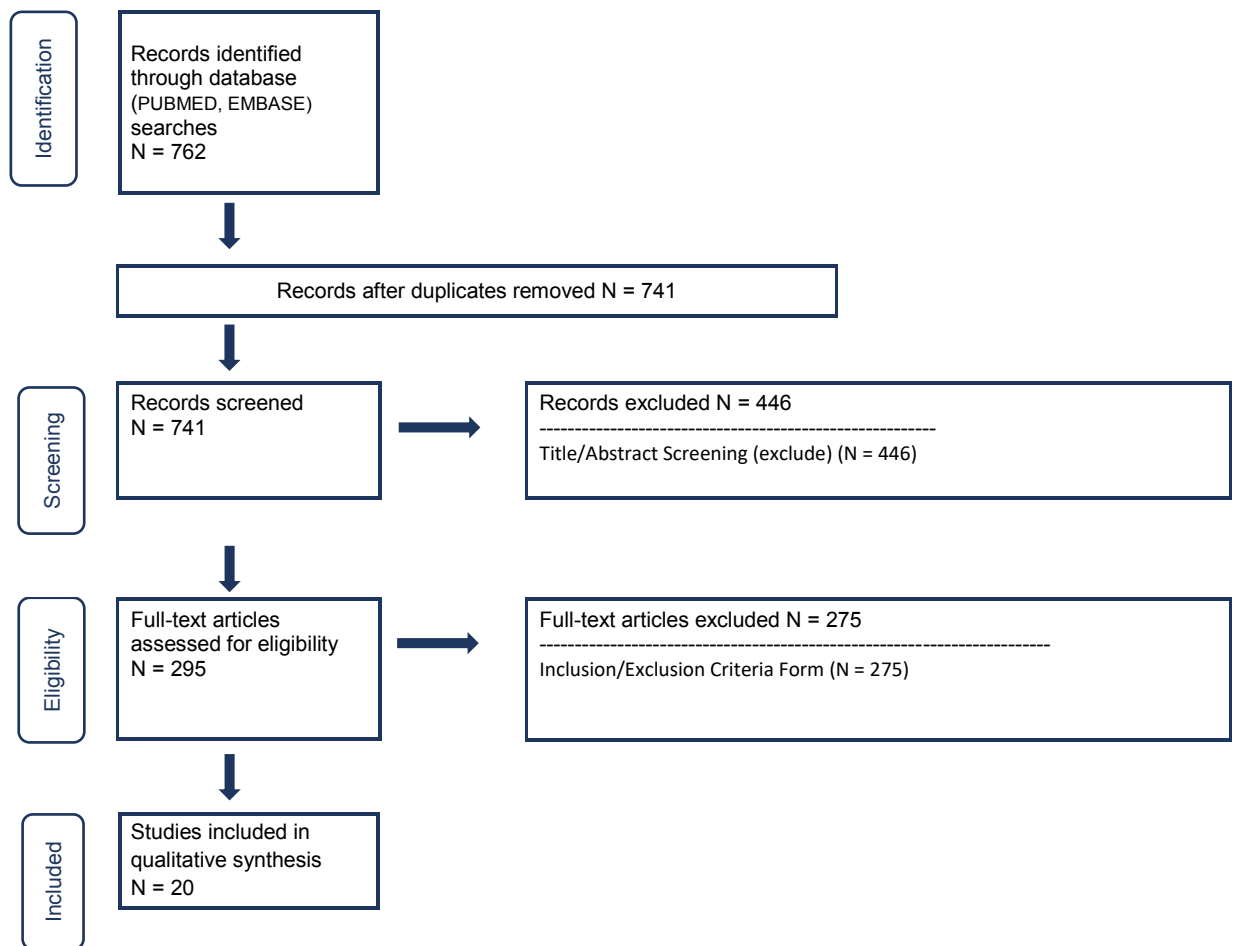


Figure 2. Dysbiosis and FGD: PRISMA Flow Diagram

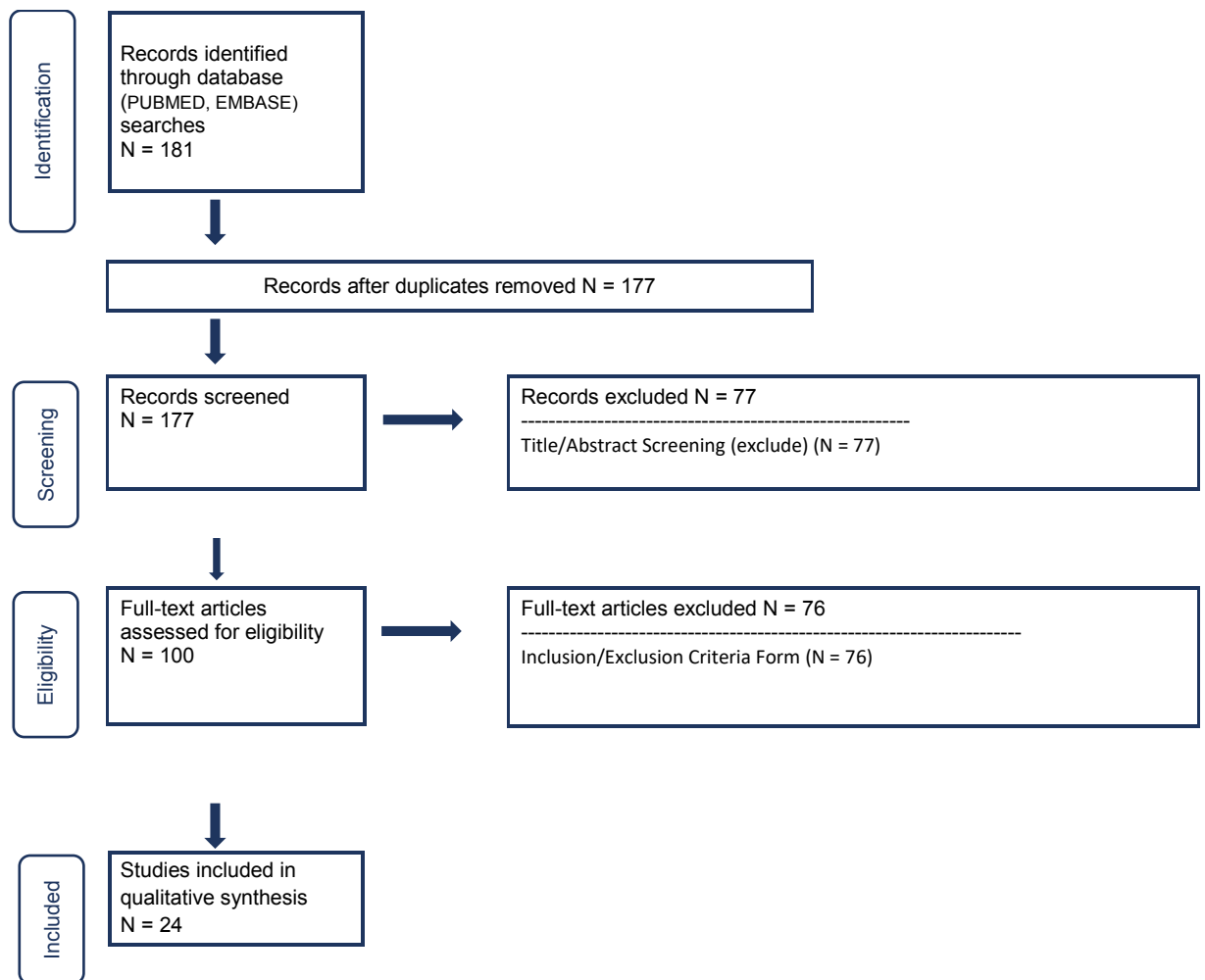
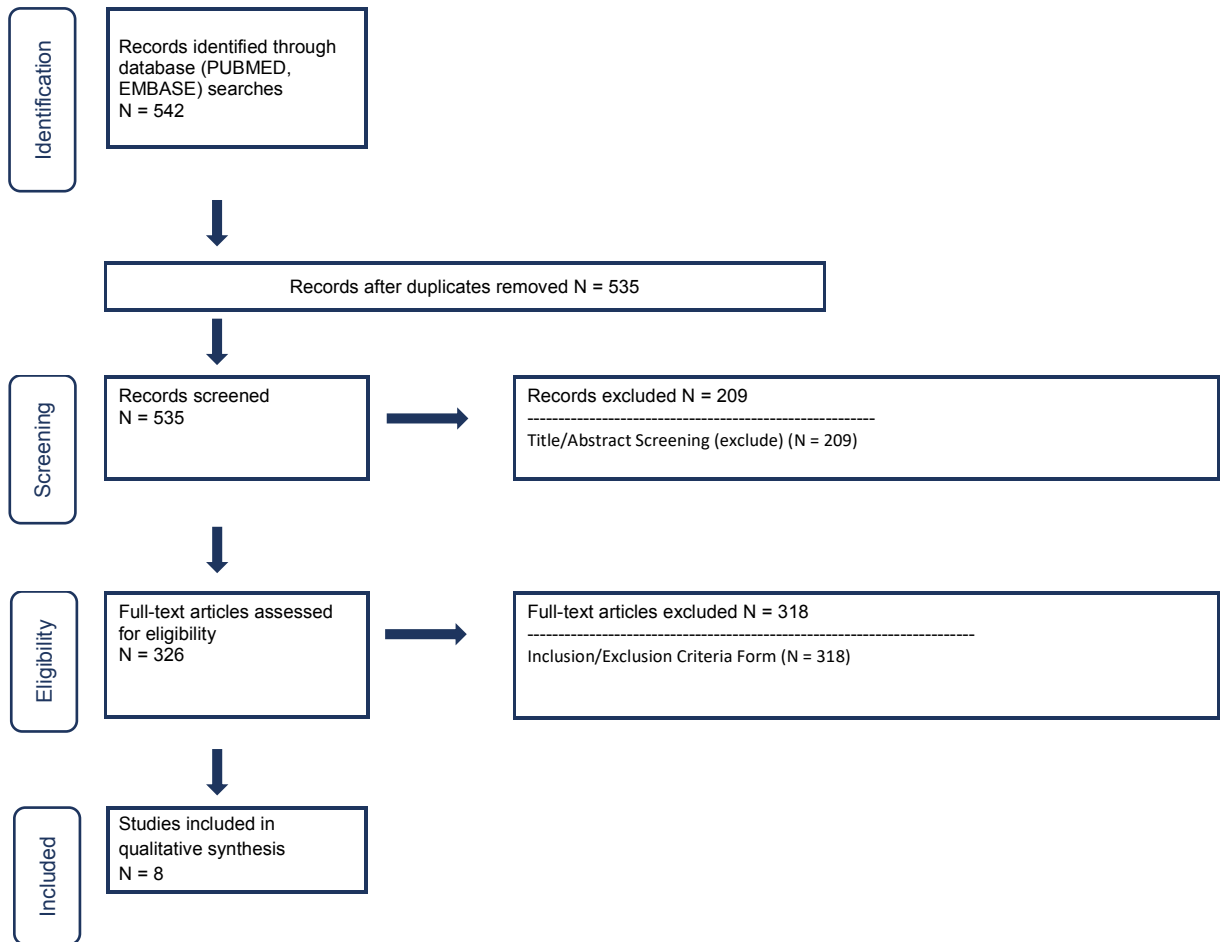


Figure 3: Antibiotics and FGD: PRISMA Flow Diagram



Data abstraction was then performed using a standard form for each question. For the first question, the following information was collected from each article: bibliographic information including year of publication, study design, study setting, study location (region/country), sample size, sex distribution, age distribution, number exposed/unexposed to antibiotics, health status of study population, inclusion/exclusion criteria, definition of antibiotic exposure, antibiotic assessment, co-variate information, length of follow-up, outcome (dysbiosis) definition, outcome assessment, results, main conclusions, study limitations. For the second question, the following information was

collected from each article: bibliographic information including year of publication, study design, study setting, study location (region/country), sample size, sex distribution, age distribution, population health status, inclusion/exclusion criteria, definition of dysbiosis, assessment of dysbiosis, co-variate information, outcome (FGD) definition, outcome assessment, results, main conclusions, study limitations. For the third question, the following information was collected from each article: bibliographic information including year of publication, study design, study setting, study location (region/country), sample size, sex distribution, age distribution, population health status, inclusion/exclusion criteria, definition of antibiotic exposure, antibiotic assessment, co-variate information, length of follow-up, outcome (FGD) definition, outcome assessment, results, main conclusions, study limitations.

RESULTS

Oral antibiotic use and gut microbial dysbiosis

A total of 20 articles were included for this study question. Antibiotics examined included Amoxicillin (5) or Amoxicillin-Clavulanate/Amoxicillin-Clavulanic acid (2 studies), Clarithromycin (2), Clindamycin (4), Ciprofloxacin (2), Doxycycline (1), Linezolid (1), Moxifloxacin (1), Paromomycin (1), Nitrofurantoin (1), Surtomycin (1), Cefprozil (1), SMT19969 (a novel non-absorbable antibiotic) (1), multi-drug (1). Impact to the gut microbial environment by antibiotic studied in this group of articles, as well as information on antibiotic class and spectrum of activity is detailed in Table 1.

Table 1. Antibiotics and Dysbiosis: Antibiotic effects on microbial composition

Antibiotic Studied	Antibiotic Class	Antibiotic Spectrum of Activity	Number of Studies	Decrease from Baseline	Increase from Baseline
Moxifloxacin	Fluoroquinolone	Broad spectrum of antibacterial activity against Gram-positive and Gram-negative bacteria, anaerobes, and atypical organisms	1	Enterococci, <i>E. coli</i>	Candida
Clarithromycin	Macrolide	Broad spectrum activity against a variety of Gram-positive and Gram-negative bacteria, Mycoplasma, Chlamydia and mycobacteria.	2	Enterobacteriaceae, <i>E. coli</i> , Bifidobacteria, bacteroides, lactobacilli, clostridia, total anaerobes	
Linezolid	Oxazolidinone	Broad spectrum activity against Gram-positive bacteria	1	Enterococci, Klebsiella, bifidobacteria, lactobacilli, clostridia, Bacteroides	Klebsiella
Form of Amoxicillin	Beta-lactam	Broad-spectrum activity against different types of infections caused by both Gram-positive and Gram-negative bacteria	6	bifidobacteria, lactobacilli, clostridia, Roseburia, total aerobes	Enterococci, Escherichia, Shigella, Bacterioides, Parabacterioides, total anaerobes
Clindamycin	Cephalosporin	Spectrum of activity includes staphylococci, streptococci and pneumococci, most anaerobic bacteria	3	Bacteroides, enterococcal colonies	Enterococcus species variation
Doxycycline (low dose)	Tetracycline	Broad-spectrum activity	1	Enterococci, <i>E. coli</i>	
Ciprofloxacin	Fluoroquinolone	Broad-spectrum activity against some Gram-positive and many Gram-negative bacteria	3	<i>Faecalibacterium</i> and <i>Ruminococcus</i> (Firmicutes), <i>Allistipes</i> (Bacteroidetes), gram-negative anaerobes, overall gut microbial diversity	<i>Bacteroides</i> (Bacteroidetes), Lachnospiraceae family (Firmicutes)
Nitrofurantoin	Nitrofurantoin-derived antibiotics	Broad-spectrum activity against both Gram-negative and Gram-positive bacteria	1	Minimal impact	Minimal impact
SMT19969 (Ridnilazole)	Novel	Narrow spectrum activity targeting <i>Clostridium difficile</i>	1	Total clostridia	Minimal impact
vancomycin, gentamycin, meropenem	Glycopeptide, aminoglycoside, carbapenem	vancomycin has narrow-spectrum activity against Gram-positive bacteria, gentamycin has broad-spectrum activity usually used for moderate to severe Gram-negative infections, meropenem has broad-spectrum activity against Gram-positive and Gram-negative bacteria	1	Enterococci, coliforms, bifidobacteria, total anaerobic bacteria count	Aerobic bacteria, facultatively anaerobic bacteria
Surtomycin	Novel	Narrow spectrum, selective activity against <i>C. difficile</i> and other Gram-positive bacteria	1	Clostridia, enterococcus, streptococcus	Enterobacteriaceae
Cefprozil	Cephalosporin	Broad-spectrum of activity against Gram-positive and Gram-negative bacteria	1	<i>Bifidobacteriaceae</i> , <i>Coriobacteriaceae</i> , <i>Eubacteriaceae</i> , <i>Oxalobacteriaceae</i> , <i>Pasteurellaceae</i> , <i>Veillonellaceae</i> , overall richness	<i>Flavonifractor</i> , <i>Lactoclostridium</i> , <i>Parabacterioides</i>
Azithromycin	Macrolide	Broad-spectrum of activity against staphylococci, streptococci and a wide variety of Gram-negative bacilli	1	Overall gut microbial diversity, <i>Proteobacteria</i> , <i>Actinobacteria</i> , <i>Fusobacteria</i> , <i>Verrucomicrobia</i>	

Study sizes ranged from 6-56 total subjects. Follow-up time periods ranged from 9 days to 2 years (17; 20; 37; 51; 58; 61; 66; 72; 74; 75; 77; 83; 85; 90; 99; 110-112; 135; 153). Most of the studies followed subjects for less than a year after antibiotic use. Only

four articles had follow-up of over a year (58; 72; 75; 111), and all remaining articles followed subjects for 180 days or less (17; 20; 37; 51; 61; 66; 74; 77; 83; 85; 90; 99; 110; 112; 135; 153). Six articles did not examine a comparison group, only observing inter-individual variation in the intestinal microbiome (20; 37; 51; 74; 83; 99). Three studies evaluated exposure to a probiotic, separately and/or together in treatment with an antibiotic (61; 66; 90). Five studies evaluated the impact of 2 antibiotics on the gut microbiome against each other (37; 74; 77; 111; 135).

All studies collected fecal samples and used both qualitative and quantitative methods to assess gut microbiota composition (17; 20; 37; 51; 58; 61; 66; 72; 74; 75; 77; 83; 85; 90; 99; 110-112; 135; 153). Seven articles specified using 16s rRNA sequencing to characterize the gut microbiota, the preferred method of obtaining detailed taxonomic composition of the microbiome (20; 51; 61; 90; 99; 111; 135). The remaining studies used other genomic analyses (i.e. quantitative PCR) or bacterial culture methods (17; 37; 58; 66; 72; 74; 75; 77; 83; 85; 110; 112; 153). Sampling time-points and study length varied widely between the studies.

Thirteen studies demonstrated an initial disruptive or altering effect of oral antibiotic use on the gut microbiome, that was observed to return to baseline by the end of each study (20; 37; 66; 72; 74; 77; 83; 85; 90; 99; 110; 112; 153). Most of these studies (11) still found a substantial impact during and/or directly following antibiotic administration. The antibiotics studied in these articles included moxifloxacin, clarithromycin, linezolid, amoxicillin-clavulanic acid, azithromycin, amoxicillin, clindamycin, multi-dose (vancomycin, gentamycin, meropenem), cefprozil, ciprofloxacin. Concurrent or initial impacts on the microbiome specified by these studies

included changes in *Enterococcus* (6 studies), *Bacteroides* (4), *Bifidobacteria* (4), *Lactobacillus* (5), Clostridia (3), *E. coli/Escherichia* (4), and *Enterobacteriaceae* (2) (37; 66; 72; 74; 77; 83; 85; 90; 112). Three of the 13 studies emphasized that the observed effects of the antibiotic of interest on the gut microbiome were minimal. One of those studies was an evaluation of a 40-day sub-antimicrobial dose (40mg) of doxycycline (110). The other studies evaluated novel antibiotic drugs that exhibit a narrow spectrum of anti-microbial activity, including a non-absorbable antibiotic, SMT19969, over a 9 day period and Surtoycin (developed to treat *Clostridium difficile*-associated diarrhea) (17; 153). Antibiotics with broad-spectrum activity or activity against anaerobes were observed to have the most disruptive impact on the microbiota (58; 61; 72; 75; 99; 112).

While 13 studies did not identify a persistent disruptive effect, the remaining seven studies observed a lasting impact of antibiotic administration on the gut microbial environment (17; 51; 58; 61; 75; 111; 135). Two of the seven had follow-up periods of less than 1 month (17; 61), but three of the studies followed subjects for 1-2 years (58; 75; 111). Two of these found that the persistent effect was weak or minimal (17; 135). Four articles described the persistent changes as “fundamental”, “substantial”, “strong” or “large” (51; 58; 61; 111). Specific impacts on the microbiome included changes observed in *Faecalibacterium*, *Lachnospiraceae*, *Blautia*, *Bacteroides*, *Escherichia*, *Parabacteroides*, *Enterobacteriaceae*, *Rosburia*, *Enterococcus*, Clostridia, and *Streptococcus* (17; 51; 58; 61; 75; 111).

Additionally, in the three studies evaluating a probiotic in addition to the antibiotic, probiotics were generally observed to temper or reduce the disruptive impact of the antibiotic on the microbiome (61; 66; 90).

Three of the 20 total articles evaluated the impact of antibiotics following infection (85; 99; 135). Monreal *et al.* showed that use of amoxicillin for treatment of respiratory tract infection decreased *Bifidobacterium* and *Lactobacillus* spp. levels, but levels returned to baseline by day 30 (85). Ciprofloxacin and nitrofurantoin were evaluated for the treatment of urinary tract infection by Stewardson, et al., displaying a more significant impact on the microbiota by ciprofloxacin that weakly persisted after 1 month (135). Following challenge with Entero-toxigenic *E. coli*, the impact of ciprofloxacin administration was investigated by Pop *et al.*, finding a more severe impact on the gut microbiota as a result of ciprofloxacin treatment than by diarrheal disease alone. The decrease observed in overall gut microbial diversity appeared to return to baseline by days 28 and 84 of the study (99).

For more details on these studies, see Table 2 and Table S-1.

Table 2. Antibiotics and Dysbiosis: Characteristics of included studies

First author, year of publication	Study Size (N)	Comparison group	Number exposed to antibiotics	Follow-up Period	Antibiotic type(s)	Measurement of Dysbiosis	Sampling Sequence	Dysbiosis Observed	Recovery period
Edlund, 2000	12	none	12	35 days	Moxifloxacin (400mg once daily for 7 days), Clarithromycin (500mg 2x daily for 7 days)	fecal samples; quantitative and qualitative culture methods	days -2 and -1 prior to dosing; days 2, 4, and 7 of drug administration and days 14, 21, and 35 of follow-up	Yes	35 days
Lode, 2001	12	none	12	35 days	Linezolid (600mg 2x daily for 7 days), Amoxicillin/Clav ulanic acid (1000mg once daily for 7 days)	fecal samples; quantitative and qualitative culture methods	days -3 and -1 prior to dosing; days 4 and 8 of drug administration and days 14, 21, and 35 of follow-up	Yes	35 days
Matute, 2002	18	placebo treated	12	42 days	Azithromycin, Clarithromycin	fecal samples; quantitative and qualitative culture methods	day -7 prior to dosing; day 0, day 3 and 7 of drug administration; days 10, 14, 21, 28, and 42 of follow-up	Yes	21 days
De la Cochetiere, 2005	6	none	6	60 days	Amoxicillin (1.5g/day oral for 5 days)	fecal samples; 16s rRNA PCR; TTGE analysis	day 0 (last stool prior to enrollment), day 1 (first stool passed at the beginning of treatment), day 30 and 60 after enrollment	Yes	variable; 30-60 days
Monreal, 2005	42	blood donors at the same hospital	22	30 days	Amoxicillin indicated for treatment of	fecal samples; quantitative and qualitative	prior to treatment (T1), during treatment (T2), 30 days post-	Yes	30 days

					respiratory tract infection	culture methods	treatment (T3); controls only sampled once (T0)		
Lofmark, 2006	8	healthy volunteers not receiving antibiotics	4	2 years	Clindamycin (150mg 4x daily for 7 days)	fecal samples; quantitative and qualitative culture methods	prior to dosing (day 0), last day of dosing (day 7), two weeks (day 21), and 3, 6, 9, 12, 18 and 24 months after administration	Yes	Over 2 years (no return to baseline during study)
Jernberg, 2007	8	healthy subjects not receiving any antibiotics	4	2 years	Clindamycin (150mg oral 4x daily for 7 days)	fecal samples; repetitive sequence-based PCR and T-RFLP	prior to dosing (day 0), last day of dosing (day 7), two weeks (day 21), and 3, 6, 9, 12, 18 and 24 months after administration	Yes	Over 2 years (no return to baseline during study)
Koning, 2008	38	probiotic + antibiotic	38; 19 antibiotic only, 19 antibiotic + probiotic	63 days	Amoxicillin (500mg 2x daily for 7 days)	fecal samples; quantitative and qualitative culture methods	day 0 (baseline), days 7, 14, and 63	Yes	63 days (stabilization by end of study)
Lindgren, 2009	8	healthy subjects not receiving any antibiotics	4	2 years	Clindamycin (150mg capsules orally 4 times daily for 7 d)	fecal samples; quantitative and qualitative culture methods and pyrosequencing	day 0; last day of dosing (day 7); day 21 and 3, 6, 9, 12, 18 months and 2 years after administration	Yes	Over 9 months; recovery by end of study (2 years)
Rashid, 2013	34	placebo treated	17	20 weeks	Doxycycline (sub-antimicrobial dose of 40mg once daily for 16 weeks)	fecal samples; quantitative and qualitative culture methods	prior to administration and at 4, 8, 16, and 20 weeks post-administration	Yes; minor	20 days
Pallav, 2014	22	no treatment group; probiotic group	8	8 weeks	Amoxicillin (250mg 3x daily for 7 days)	fecal samples; 16s rRNA sequencing	control group: day 0, 28, and 56; treatment group: day 0 (prior), days 10 and 14 (during), and days 21, 28, and 56 (after)	Yes	8 weeks
Stewardson, 2015	42	controls w/o antibiotic treatment; household contact of ciprofloxacin patient	20	4 weeks	Ciprofloxacin (500mg 2x daily); Nitrofurantoin macrocrystals (100mg 2x daily) indicated for Urinary Tract Infection	fecal samples; 16s rRNA pyrosequencing	control group: baseline and 7-10 days after baseline; treatment group: baseline (T1), at treatment completion (T2), and 4 weeks after second sample (T3)	Yes	Over 1 month (persistent weak effect through end of study)
Heinsen, 2015	5	none	5	42 days	Paromomycin 4g oral for 3 days	fecal samples; 16s rRNA sequencing	prior to dosing (day 0), following treatment (day 4), 6 weeks post-administration (day 46)	Yes	Approx. 46 days
Vickers, 2015	56	placebo treated	40	9 days	SMT19969, a novel non-absorbable antibiotic (2-2000mg single oral dose or 2 oral doses of 200/500mg)	fecal samples; quantitative and qualitative culture methods	day -1 prior to dosing, day 4 and 9	Minimal	N/A
Rashid, 2015	30	placebo treated	20	1 year	Ciprofloxacin (500mg twice daily for 10 days) or Clindamycin (150mg 4 times daily for 10 days)	fecal samples; 16s rRNA sequencing	prior to administration and at day 11, and 1, 2-, 4- and 12-months post-administration	Yes	Ciprofloxacin: 1-12 months; Clindamycin: 12 months or more

Mikkelsen, 2015	12	none	12	180 days	4-day, 3 drug oral antibiotic course (vancomycin 500mg, gentamycin 40mg, meropenem 500mg once daily)	fecal samples; quantitative and qualitative culture methods	1 day prior to each study visit (day 0, 4, 8, 42, 180)	Minor	by 180 days
Citron, 2016	30	healthy volunteers not receiving antibiotics	24	13-15 days	Surtoymycin (oral doses of 250, 500, 1000 mg twice daily)	fecal samples; quantitative and qualitative culture methods and quantitative PCR	baseline (days 0 and 1), mid-study (days 7-9) and end of treatment (days 13-15)	Minimal	Over 15 days (no return to baseline by end of study)
Raymond, 2016	24	healthy volunteers not receiving antibiotics	18	90 days	Cefprozil 500mg oral for 7 days, a second-generation cephalosporin	fecal samples; shotgun metagenomic sequencing	prior to dosing (day 0), end of dosing (day 7) and 90 days post-administration	Yes	up to 90 days
Pop, 2016	30	none	30	84 days	Ciprofloxacin (500mg twice daily for 3 days following challenge with <i>E. coli</i> (ETEC H10407))	fecal samples; 16s rRNA sequencing	days -1, 0, 1-7, 9, 28, and 84	Yes	24-84 days
Kabbani, 2017	49	probiotic, probiotic + antibiotic, no treatment	12 antibiotic; 12 probiotic + antibiotic	21 days	Amoxicillin-Clavulanate (875/125mg twice daily for 7 days)	fecal samples; 16s rRNA pyrosequencing	control group: days 0, 10, 21; treatment group: prior to screening and day 0, during treatment (days 3, 7, 10, 13) and post-treatment (day 21)	Yes	Over 21 days (full return to baseline not observed by end of study)

Gut microbial dysbiosis and FGD

A total of 24 articles for the second question were included in the review (10; 12; 16; 21; 24; 33; 43; 44; 55; 63; 67; 73; 76; 88; 93; 104-107; 109; 117; 130; 144; 145).

Study size ranged from 26 to 668 total subjects. All of the included articles were of case-control study design, examining FGD cases and healthy controls, with one exception-a retrospective study of a population of small intestinal bacterial overgrowth (SIBO) patients and subjects without SIBO, for which presence of FGD was subsequently obtained (106). Nearly all articles assessed IBS as the sole FGD in the study, except for one study examining cases of chronic constipation (including both functional constipation and IBS with constipation) (93) and one study examining dyspeptic patients (24). Of the 22 articles investigating IBS, 14 examined multiple subtypes of the disorder (i.e. IBS

with diarrhea, IBS with constipation, IBS-alternating, IBS-mixed) (10; 16; 33; 55; 63; 73; 76; 104-107; 117; 130; 145). Thirteen studies diagnosed FGD based on Rome II criteria, 10 diagnosed FGD according to Rome III criteria and one diagnosed FGD according to both Rome II and III criteria (10; 12; 16; 21; 24; 33; 43; 44; 55; 63; 67; 73; 76; 88; 93; 104-107; 109; 117; 130; 144; 145).

Sampling methods used to measure the gut microbiota varied between the included studies. The majority of studies sampled fecal material only (10 studies) (10; 12; 43; 55; 67; 88; 105; 107; 117; 130). or fecal material plus an additional specimen type such as mucosal or tissue biopsy (7 studies) (16; 21; 63; 73; 76; 93; 145). Two studies sampled duodenal aspirates and one sampled jejunal aspirates (44; 104; 106). Two studies used only a Hydrogen Breath test as measurement of intestinal dysbiosis, either to diagnose SIBO or to evaluate the presence of methanogens in the intestinal flora (24; 109). Measurement of dysbiosis differed amongst the studies as well. Twelve studies utilized 16s rRNA sequencing techniques to evaluate the composition of the gut microbiome, performing an in-depth, taxonomic analysis (10; 16; 21; 44; 63; 67; 88; 93; 105; 117; 130; 145). Two of the twelve studies performing a taxonomic analysis only performed it on a subset of the study subjects (44; 130). Other methods included bacterial culture and additional genomic assays. Six remaining studies only assessed the taxonomic composition of the microbiome in a limited manner, either through quantitative and qualitative culture or selective PCR or sequencing methods (12; 63; 88; 104; 106; 144). The remaining six studies did not perform any taxonomic analysis, instead observing differences in DGGE profiles, protein expression, presence of SIBO, or functional groups of bacteria (21; 24; 33; 43; 76; 109).

Evaluation of the gut microbial environment in FGD cases compared to control groups revealed presence of gut microbial dysbiosis associated with the studied disorders in most cases. Indeed, 16 of the articles described at least the presence of increased levels of gut microbial dysbiosis in FGD cases compared to controls (10; 12; 21; 24; 33; 43; 44; 63; 67; 88; 93; 106; 107; 109; 144; 145). Additionally, seven studies presented findings of specific intestinal microbial profiles associated with FGD or IBS specifically (16; 55; 73; 76; 105; 117; 130). Only one study did not find a significant association between FGD and an altered gut microbiome, which only evaluated SIBO in association with IBS (104). Table 3 outlines changes in bacterial taxonomic groups and biomarkers relating to FGD type and related symptoms.

Table 3. Dysbiosis and FGD: FGD types, symptoms and intestinal environment

FGD type or symptom	Increase in bacteria of the intestinal tract or biomarker	Decrease in bacteria of the intestinal tract or biomarker
IBS	Veillonellaceae, Prevotellaceae, Firmicutes, Actinobacteria, Bacilli, Clostridia, Lactobacillales, Holdemania, Lactobacillus, Streptococcus, Shigella, Escherichia, Ruminococcus, Aeromonas, Pseudomonas, Aneurinibacillus, Papillibacter, Dialister, Peptococcus, Anaerovorax, Blautia, Bryantella, Coprococcus, Dorea, Lachnospira, Roseburia, Eubacteria, Sporobacter, Lactobacilli, Veillonella, Gram-negative bacteria, coliforms, miRNA-1999b expression, upregulation of TLR4, TLR5, TLR9, small intestinal bacterial overgrowth (aerobic bacterial overgrowth); metabolites: choline, taurine, glucose, deoxycholate; organic acids: acetic acid, propionic acid, total organic acids	Ruminococcaceae, Lachnospiraceae, Mycobacteriaceae, Neisseriaceae, Erysipelotrichaceae, Bacteroides, Bacteroidia, Bifodobacterium, Oscillibacter, Blautia, Coprococcus, Faecalibacterium, Allistipes, Odoribacter, Parabacteroides, Prevotella, Tannerella, Bacteroidetes, Clostridiales, Barnesiella, Methanogenic flora; metabolites: U2,2-methylbutyrate, isovalerate, isobutyrate
IBS-C	Bacteroides, Pseudomonas, Gram-Negative bacteria, Methanogens, H2-consuming sulphate-reducing bacteria, Enterobacteriaceae, Aneurinibacillus, Lachnospira, Anaerotruncus, Clostridia, Papillibacter, Ruminococcus, Sporobacter, Peptococcus, Anaerovax, Bryantella, Coprococcus, Dorea	Bifidobacteria, Lactobacilli, Allistipes, Bacteroides, Odoribacter, Parabacteroides, Prevotella, Tannerella, Bacteroidetes
IBS-D	Firmicutes, Bacteroides, Prevotella, Pseudomonas, Lachnospira, Clostridia, Peptococcus, Butyrivibrio, Dorea, Roseburia, Ruminococcus, Gram-Negative bacteria, Coliforms, segmented filamentous bacteria, small intestinal bacterial overgrowth (aerobic bacterial overgrowth)	Bacteroidetes, Methanogens, Erysipelotrichaceae, Ruminococcaceae, Odoribacter, Tannerella, Parabacteroides
IBS-M	Firmicutes	Actinobacter, Methanogens, Erysipelotrichaceae, Ruminococcaceae,
IBS-A	Streptococcus, Papillibacter, Ruminococcus, Dialister, Clostridia, Blautia, Bryantella, Dorea,	Allistipes, Bacteroides, Odoribacter, Parabacteroides, Prevotella, Tannerella, Bacteroidetes, Faecalibacterium, Clostridiales
Constipation	Bacteroides, Flavobacterium, Faecalibacterium, Lentisphaerae, Opitutae, Lentisphaeria, Puniceococcales, Victivallales, Puniceococcaceae, Victivallaceae, Desulfahalobiaceae, Odoribacter, Victivallis	Peptostreptococcaceae
Flatulence	Lachnospiraceae	
Abdominal Pain	Bacteroides, Ruminococcus, Prevotella, Catenibacterium, Proteobacteria	Erysipelotrichaceae
Abdominal Distension	Bacteroides, Clostridium, Pseudomonas, segmented filamentous bacteria, Gram-negative bacteria	
Bloating	Clostridium, Cyanobacteria, Gram-negative bacteria	
Severe IBS		Faecalibacterium
Depression		Actinomycetales, Actinomycetaceae

Colonic Transit Time	Euryarchaeota, Methanobacteria, Methanobacteriales, Methanobacteriaceae	
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Specific portions of the microbial ecosystem that were found associated with FGD and/or IBS-types compared to controls included Firmicutes, Actinobacteria, Bacteroidetes, Bacillia, Clostridia, *Bacteroides*, Lactobacillales and Bacteroidales, *Holdemania*, *Parabacteroides*, *Aeromonas* and *Faecalibacterium* genera, *Ruminococcaceae* taxa, *Erysipelotrichaceae*, *Lachnospiraceae*, *Veillonellaceae*, *Prevotellaceae*, *Mycobacteriaceae*, *Neisseriaceae*, *Catenibacterium*, Proteobacteria (*Shigella/Escherichia*), *Bifidobacteria* and *Lactobacilli*, sulfate-reducing bacteria (SRB), *Oscillibacter*, *Blautia*, and *Coproccus* spp., *Pseudomonas aeruginosa*, and methanogenic flora (10; 12; 16; 44; 55; 63; 67; 73; 88; 93; 105; 107; 109; 117; 130; 144; 145).

For more details, see Table 4 and S-2.

Table 4. Dysbiosis and FGD: Characteristics of Included Studies

First author, year of publication	Study Setting	Study Size (N)	Control Group	Measurement of FGD	Number with FGD outcome	Follow-up Period	Measurement of Dysbiosis	Taxonomic Analysis	Presence of Dysbiosis	Conclusions
Dlugosz, 2017	Clinic	36	N=14 HC without FGD or antibiotic/probiotic use 1 month prior to biopsy	Rome II criteria; clinical diagnosis	22 IBS (7 IBS-D, 8 IBS-C, 7 other)	N/A	biopsies of jejunum and colon; quantitative PCR and immunohistochemistry for mRNA and protein expression	No	Yes	Certain proteins indicating gut bacterial patterns were upregulated in IBS cases compared to controls
Labus, 2017	University	52	N=23 HC meeting exclusion criteria	Rome III criteria; clinical diagnosis	29 IBS (10 IBS-D, 1 Alternating, 5 Mixed, 2 unspecified)	N/A	fecal samples; 16s rRNA sequencing	Yes	Yes	Gut microbial composition was found related to IBS subtype compared to controls, but was not found to be associated with IBS symptom presentation
Ringel-Kulka, 2015	Community	76	N=20 HC with no recurring GI symptoms	Rome III criteria; clinical diagnosis	56 IBS (26 IBS Bloating, 6 IBS without Bloating) (21 IBS-D, 21 IBS-C, 14 IBS-M)	N/A	fecal samples; 16s rRNA sequencing	Yes	Yes	Microbiota composition was associated with IBS types, symptoms and bowel habits compared to controls
Chung, 2015	Hospital	47	N=19 HC receiving an endoscopy during check-up	Rome III criteria; questionnaire	28 IBS (14 IBS-D, 7 IBS-C, 7 IBS-M)	N/A	jejunal mucosal biopsies and fecal samples; 16s rRNA sequencing	Yes	Yes	Microbial signatures were found to be associated with IBS and IBS types compared to controls
Mansour, 2016	University	90	N=20 HC with no	Rome III criteria; clinical diagnosis	70 IBS (20 IBS-C, 18 IBS-D, 32 IBS-M)	N/A	fecal samples and quantitative culture of	No	Yes	Increased coliform count and decreased miRNA-199b were

			recurring GI symptoms				coliforms; serum samples and miRNA assay for miRNA-199b expression			observed in IBS cases compared to controls
Parthasarathy, 2016	Clinic	50	N=25 HC female	Rome III criteria	25 Chronic Constipation (13 functional constipation, 6 IBS-C, 6 IBS-M)	N/A	fecal samples and colonic biopsies; lactulose breath test for methane production and colonic transit; 16s rRNA sequencing	Yes	Yes	Microbiota composition was found to differentiate chronic constipation cases from controls
Pozuelo, 2015	Clinic	179	N=66 HC	Rome III criteria; questionnaire	113 IBS (54 IBS-D, 32 IBS-C, 27 IBS-M)	72 IBS (33 IBS-D, 26 IBS-C, 13 IBS-M) and 22 HC provided 2nd stool sample 1 month after the 1st	fecal samples; 16s rRNA sequencing	Yes	Yes	On a global and taxonomic level, differences were observed between IBS, IBS subtypes and certain symptoms compared to controls
Casen, 2015	Hospital	668	N=297 HC without IBS, IBD or other organic GI disorder	Rome II or III criteria	236 IBS (44% IBS-D, 22% IBS-A, 17% IBS-C, 11% IBS-unsubtyped, 4% IBS-M)	For 5 subjects (health status not indicated), fecal samples collected at 1-wk intervals up to 14 wks	fecal samples; 16s rRNA sequencing	Yes	Yes	The majority of IBS patients (73%) observed to have bacterial profiles consistent with a validated dysbiosis test compared to 16% of controls.
Giamarellos - Bourboulis, 2015	Hospital	258	N=21 HC, N=163 non-healthy, non-IBS controls	Rome III criteria; clinical diagnosis	74 IBS	N/A	duodenal aspirates; quantitative PCR for genus-specific bacterial diversity and levels and 16s rRNA sequencing in a subset	Yes; subset	Yes	Certain bacterial genera were increased in the small bowel and a reduction in overall diversity was observed in IBS cases compared to controls
Chassard, 2012	Community	26	N=12 sex-matched HC with no GI symptoms and normal stool frequency	Rome II criteria; questionnaire	14 female IBS all classified as IBS-C	N/A	fecal samples; qualitative and quantitative anaerobic culture methods and fluorescent in situ hybridization of main functional bacterial groups	Limited	Yes	Certain functional bacterial groups were either significantly increased or decreased in IBS-C patients compared to controls
Shukla, 2015	Clinic	77	N=30 HC, age and gender matched	Rome III criteria; questionnaire	47 IBS (20 IBS-C, 20 IBS-D, 7 IBS (unknown))	N/A	fecal samples; quantitative PCR and sequencing in a subset; limited taxonomic analysis	Yes, subset	Yes	Microbiota composition was distinctly associated with IBS type compared to controls
Tap, 2017	Clinic	149	N=39 HC without chronic disease or GI symptoms	Rome III criteria; clinical diagnosis	110 IBS (18 IBS-C, 43 IBS-D, 43 IBS-M, 2 IBS-unknown)	N/A	fecal samples and sigmoid biopsies; 16s rRNA pyrosequencing	Yes	Yes	Machine learning analyses provided identification of differences in the gut microbial composition between IBS cases and controls
Liu, 2016	Clinic	100	N=20 HC	Rome III criteria; clinical diagnosis	40 IBS-D, 15 Depression, 25 co-morbid IBS and Depression	N/A	fecal samples and sigmoid biopsies; 16s rRNA pyrosequencing	Yes	Yes	Specific microbial signatures were associated with IBS-D and depression cases compared to controls

Pyleris, 2012		320	N=258 subjects without SIBO	Rome II criteria; questionnaire	112 IBS (35 IBS-D, 19 IBS-C, 58 IBS-A)	N/A	duodenal aspirates; quantitative and qualitative culture for SIBO	Limited	Yes	SIBO observed more often in IBS and IBS-D compared to controls
Rajilic-Stojanovic, 2011	Clinic/comm unity	108	N=46 HC	Rome II criteria; trial recruitment	62 IBS (25 IBS-D, 18 IBS-C, 19 IBS-A)	N/A	fecal samples; quantitative PCR and phylogenic microarray analysis	Yes	Yes	Composition of the fecal microbiota differed between IBS cases and controls
Jeffery, 2012	Clinic	57	N=20 HC without GI symptoms	Rome II criteria; questionnaire	37 IBS (15 IBS-D, 10 IBS-C, 12 IBS-A)	N/A	fecal samples; 16s rRNA sequencing	Yes	Yes	Characteristics of the microbiota of subjects were found to be associated with IBS cases and IBS symptoms compared to controls
Le Gall, 2011	Clinic	45	N=22 HC	Rome II criteria; clinical diagnosis	10 IBS	2 years	fecal samples; NMR for microbial metabolites and denaturing-gradient gel electrophoresis	No	Yes	Metabolic composition of the gut microbiota and DGGE profiles were related and remained stable over time for subjects but differed distinctly between IBS cases and controls
Noor, 2010	Clinic	66	N=22 HC	Rome II criteria; clinical diagnosis	11 IBS	N/A	fecal samples; denaturing-gradient gel electrophoresis of 16s rRNA gene and sequencing of select bands	Limited	Yes	Reduced diversity observed in IBS cases compared to controls
Kerckhoffs, 2011	Clinic	57	N=20 HC without intestinal complaints/ history of bowel resection	Rome II criteria	37 IBS (13 IBS-D, 11 IBS-C, 13 IBS-A)	N/A	fecal samples and duodenal brush samples; denaturing-gradient gel electrophoresis of 16s rRNA gene and quantitative PCR	Limited	Yes	DGGE profiles differed between IBS cases and controls
Tana, 2010	Hospital/comm unity	52	N=26 HC age and sex-matched; without GI symptoms	Rome II criteria; interview	26 IBS	N/A	fecal samples; culture, quantitative PCR and organic acid analysis	Limited	Yes	Gut microbiota composition differed between IBS cases and controls and IBS cases also displayed unbalanced fecal organic acid levels compared to controls
Codling, 2009	Comm unity	80	N=33 HC	Rome II criteria; clinical diagnosis	47 IBS	N/A	fecal samples and colonic biopsies; denaturing-gradient gel electrophoresis of 16s rRNA gene; only microbial variability measured	No	Yes	DGGE profiles differed significantly between IBS cases and controls
Rana, 2009	Clinic	599	N=254 HC age and gender-matched	Rome II criteria; clinical diagnosis	345 IBS	N/A	H2-lactulose breath test for SIBO	No	Yes	Methanogenic flora significantly reduced in IBS cases compared to controls
Posserud, 2007	Clinic	188	N=26 HC without history of GI symptoms	Rome II criteria; clinical diagnosis	162 IBS (49 IBS-D, 37 IBS-C, 76 IBS-A)	N/A	jejunal aspirate quantitative and qualitative culture and H2-breath test for SIBO	Limited	Unclear	SIBO not significantly associated with IBS cases compared to controls, but a significant subset of IBS cases found with high counts of upper gut bacteria compared to controls

Costa, 2012	Clinic	34	N= 11 non-dyspeptic volunteers	Rome III criteria; questionnaire	23 dyspepsia patients	N/A	H2-lactulose breath test for SIBO	No	Yes	Dyspepsia cases and cases using PPIs presented with SIBO at higher prevalence compared to controls
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Oral antibiotic use and FGDs

A total of 8 studies were included for the third research question in this review. Study size ranged from 91 to 21,364 total subjects (68; 71; 78; 94; 119; 131; 149; 154). Composition of comparison group differed based on study design (see Table 4). Six articles used surveys or questionnaires to assess antibiotic use and/or FGD diagnosis (68; 71; 78; 94; 131; 149). The remaining two used medical records or computerized patient profiles to ascertain FGD diagnosis (119; 154). Five articles explicitly mentioned or referenced use of Rome criteria to diagnose or confirm FGD diagnosis (68; 78; 94; 131; 149). All studies examined IBS as the FGD outcome (68; 71; 78; 119; 123; 131; 154), only two examined any additional FGDs in addition to IBS (94; 149). Follow-up periods ranged from 3 months up to 12 years for non-cross-sectional study designs (68; 78; 94; 119; 149; 154). Antibiotics studied in these articles included ciprofloxacin, azithromycin, macrolides, tetracycline, betalactams, cephalosporin, penicillin, amoxicillin, trimethoprim, doxycycline, aminoglycosides, sulfamethoxazole/trimethoprim and fluoroquinolones (68; 71; 78; 94; 154), though a few articles did not specifically state antibiotic names or classes (119; 131; 149).

Significant or borderline significant associations between oral antibiotic use and FGD outcome were observed in nearly all included studies. Studies that displayed only borderline significant results included a cross-sectional study of general antibiotic use and IBS (OR=1.8; 95%CI: 1.0-3.1) and a retrospective study of general antibiotic use and

IBS (OR= 2.37; 95%CI: 1.07-5.25) (131; 149). Significant findings were as follows:

Paula, et al. showed a positive association between antibiotic use for non-GI infection and development of FGD compared to no antibiotic use for non-GI infection (OR= 1.9; 95%CI: 1.21-2.98) (94); Lee, et al. showed a positive association between doxycycline use and FGD during deployment in a developing country (OR=6.99; 95%CI: 3.19-15.31), doxycycline use during deployment in a developed country (OR=6.93; 95%CI: 1.4-34.39), though the increased risk was insignificant among non-deployed subjects using doxycycline (71); Ruginomez, et al. presented an increased risk of IBS in a cohort of gastroenteritis patients following general antibiotic use when 2-5 or more than 5 prescriptions were documented (OR= 2.0; 95%CI: 1.3-3.0 and OR=2.2; 95% CI: 1.2-4.1, respectively), but the association was not significant for 1 antibiotic prescription;(119) Maxwell, et al reported an increased risk of IBS 4 months after antibiotic use compared to subjects without antibiotic use (OR=3.14; 95%CI: 1.27-7.75) (78); Villareal reported a positive association between tetracycline use and macrolide use and IBS diagnosis compared to non-IBS controls ($p<0.25$ and $p=0.036$, respectively) (154). Lalani, et al. reported no association between antibiotic use in subjects with traveler's diarrhea and subjects with traveler's diarrhea without antibiotic treatment and development of FGD, however the total incidence of FGD in the cohort was only 6 cases ($p=0.6$) (68).

For more details on the described studies, see Table 5 and S-3.

Table 5. Antibiotics and FGD: Characteristics of Included Studies

First author, year of publication	Study Design	Study Setting	Study Size (N)	Comparison Group	Number exposed to antibiotics	Follow-up Period	FGD type	Assessment of FGD	Overall Incidence of FGD	Incidence of FGD among Antibiotic Exposed	Antibiotic type(s)	P-value; OR (95% CI) of the association
Lalani, 2015	Prospective Cohort	Clinic	151	Subjects with traveler's diarrhea with no treatment/antidiarrheal only	46	3, 6, 9, and 12 months	IBS	Modified Rome III criteria; survey	6 (4.0%)	N=1 (2.2%); P=0.60	Ciprofloxacin, azithromycin	p=0.6

Siah, 2016	Cross-Sectional	Community	297	Subjects without IBS determined by self-reported symptoms	Not specified	N/A	IBS	Rome III criteria; questionnaire	62 (20.9%)	Not stated	Not specified	p=0.046; OR=1.8 (1.0-3.1)
Tornblom, 2007	Retrospective	Community	333	Subjects without FGD	45	5 years	IBS, functional dyspepsia, functional diarrhea	Rome II criteria; questionnaire	41 (12%)	N=10 (24%)	Not specified	p=0.05; OR=2.37 (1.07-5.25)
Villarreal, 2012	Retrospective	Hospital/Clinic	21,364	Subjects without IBS diagnosis	Tetracycline N=5479, Macrolide N=5595	up to 12 months	IBS	ICD-9 code 564.1; medical record	115 (0.54%)	Tetracycline N=40 (0.73%), Macrolide N=40 (0.71%)	macrolides, tetracycline, betalactams, cephalosporin, penicillin	tetracycline p<0.025, macrolide p=0.036
Maxwell, 2002	Prospective Cohort	Clinic	91	Subjects without antibiotic use	45	4 months	IBS	Rome criteria; questionnaire	31 (34%)	20 (48%)	Amoxicillin, macrolides, penicillin, tetracycline, trimethoprim, others	p=0.01, OR=3.14 (1.27-7.75)
Ruigomez, 2007	Nested Case-Control	Community	783	Subjects from the same source population without gastroenteritis	587	1-10 years	IBS	IBS code with specific treatment, repeated IBS entries, or IBS dx with related GI symptoms; medical record	241 (30.8%)	197 (33.5%)	Not specified	OR ranged from 1.2 (1 prescription) to 2.2 (greater than 5 prescriptions)
Lee, 2013	Cross-Sectional	Community	1167	Subjects with the same deployment location and no doxycycline intake	189	N/A	IBS	Self-report or questionnaire	41 (3.5%)	21 (1.8%)	Doxycycline	Doxy in developing country = 6.99(3.19-15.31) Doxy in developed country = 6.93(1.40,34.39) Doxy in Non-deployed = 2.81(0.34-23.04)
Paula, 2015	Nested Case-Control	Community	566	Age-matched, non-FGD patients based on self-report	338	~11.5-12 years (mean follow-up period)	IBS, functional dyspepsia	Modified Rome II criteria; survey	316 (55.8%)	207 (61.2%)	Penicillin, aminoglycosides, cephalosporin, macrolides, tetracycline, sulfamethoxazole/trimethoprim, fluoroquinolones,	GI infection, antibiotics = 1.30 (0.43-3.91); Non-GI infection, antibiotics = 1.9 (1.21-2.98)

											antifungal , other	
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DISCUSSION

This review gathered and synthesized the current literature on the association between oral antibiotic use and FGD, together with the established research on dysbiosis as an intermediate in the pathway. Therefore, an examination of the association between oral antibiotic use and gut microbial dysbiosis and the association between dysbiosis and FGD are also included to provide context and plausibility to any observed association between antibiotics and FGD.

Twenty articles published from 2000-2017 were gathered on the association between antibiotics and gut dysbiosis. Specific departures from the baseline microbiome observed in each study were variable, and differed based on antibiotic type, the method of dysbiosis measurement and inter-individual characteristics of the study populations. The majority of studies did not observe persistent departures from a normal gut microbiome following antibiotic therapy through the extent of the study period. However, seven studies did display a sustained impact of antibiotic use on the gut environment, and four articles emphasized that the observed impact was substantial (17; 51; 58; 61; 75; 111; 135). Though the majority of the articles did not find persistent effects, most still observed a clear impact of antibiotic use on the intestinal microbiota, though effects were transient (20; 37; 66; 72; 74; 77; 83; 85; 90; 99; 110; 112; 153). Interestingly, for the majority of the studies that noted a recovery in the microbiome, the recovery appeared to coincide with the length of the study period, therefore, it is unclear whether varying study

length would have produced different conclusions for many of the studies. Only seven of the 20 studies used 16s rRNA sequencing methods to characterize the gut microbiome, which is the preferred method to obtain detailed taxonomic composition of the gut microbial environment. Additionally, sample sizes were very low for most of the studies, which may have reduced the statistical power to detect small but potentially meaningful differences. The strength of evidence for an association between oral antibiotic use and gut microbial dysbiosis would be increased with additional studies on higher numbers of subjects, a thorough and sufficiently long sampling timeframe, evaluation of inter-individual variability and pre-treatment sample comparisons as well as un-treated control comparisons, and well-defined, clinically significant antibiotic exposure and dysbiosis assessment.

From 24 articles assessed in the association between gut microbial dysbiosis and FGD, the majority found a clear link between presence of FGD and abnormal gut environment. Sixteen studies found significant departures of the intestinal microbiota in FGD cases from that of controls (10; 12; 21; 24; 33; 43; 44; 63; 67; 88; 93; 106; 107; 109; 144; 145). Furthermore, seven studies were able to link specific microbial profiles to FGD and IBS-types (16; 55; 73; 76; 105; 117; 130). However, few studies examining the link between dysbiosis and FGD in this review actively measured inter-individual variability in their comparisons and all used separate control groups rather than comparing a baseline/pre-disease sample to a diseased sample from the same individual. Only three studies employed multiple sampling time-points, but the samples did not indicate different health status for an individual (10; 43; 105). Given the importance of inter-individual variability in many microbiome studies, this is a significant limitation of

the articles assessing the association between dysbiosis and FGD (29; 61; 90; 99; 112).

As a whole, the current level of evidence supporting a clear, temporal association between gut microbial dysbiosis and FGD is weak and further research is necessary.

On the overarching association between oral antibiotic use and FGD, a total of 8 articles were eligible for inclusion in this review. The majority of studies found associations between antibiotic use and FGD (71; 78; 94; 119; 154). In some studies this was limited to certain subgroups (i.e. increased number of antibiotic prescription, deployed subjects only, non-GI infection treated patients), however the presence of significant associations for certain subgroups may elucidate specific groups that are at modified risk of chronic complications from the added exposure of antibiotic use.

There were many limitations in the current literature available for this association. Antibiotic type and use were not well assessed in most of the articles for question 3. Additionally, there were many other areas where bias may have influenced study findings, such as in retrospective or cross-sectional design, or indirect assessment of FGD diagnosis through medical record or self-reported symptoms on a questionnaire. It is unclear whether enteric infection, severity of infection, or other important, potentially modifying factors were properly adjusted for in some of the studies (71; 94; 119; 131; 149; 154). Low sample size or low numbers of subjects exposed to antibiotics and/or with diagnosed FGD may have impacted the ability to observe a true association in some of the studies (68; 154). It is also likely that misclassification of either the antibiotic exposure or FGD outcome occurred, obscuring the results of a number of the studies (78; 94; 119).

Consequently, the current literature provides scant quality evidence of an association between oral antibiotic use and FGD. The evidence for a causal pathway between oral antibiotic use and development of FGD is uncertain, with gut microbial dysbiosis as an intermediate being supported by a large number of studies indicating an association between intestinal dysbiosis and FGD, but the association between antibiotic use and sustained gut microbial dysbiosis (as would likely be necessary for development of a chronic GI condition in this pathway) is less supported by the current research. Research on the association between antibiotic use and development of FGDs remains lacking, and therefore improved epidemiological study design is needed to elucidate this relationship. Ideally, future studies should employ larger sample sizes, case-control or cohort study designs, well-defined, validated and clear documentation of FGD outcome and antibiotic exposure, and assessment of modifying factors in the association. In conclusion, the totality of current evidence provided in this review prompts the need for further research on this topic with improved study design, methodology and study populations.

CHAPTER 3: Nested Case-Control Study

INTRODUCTION

Functional gastrointestinal disorders (FGDs) consist of a range of chronic conditions that affect the digestive tract and include functional diarrhea (FD), functional constipation (FC), irritable bowel syndrome (IBS), and functional dyspepsia (34; 142). FGDs are estimated to affect approximately 25% of the population in the United States and cause significant morbidity and increase healthcare utilization and costs (11; 13; 91; 142). For the same reasons, these chronic GI conditions negatively affect the readiness of US military personnel and are associated with substantial costs to the US Military Health System (MHS) (103). Despite the widespread effect of FGDs, the pathogenesis of these disorders remains poorly understood.

Acute infectious gastrointestinal enteritis (AGE) is one established risk factor for FGDs (100-102; 115). However, the use of antibiotic treatment for AGE may modify or confound the association between AGE and FGD (78; 94; 119; 154). Antibiotics can cause drastic changes to the gut microbiome, but the long-term effect on the composition and stability of the microbiome and the ancillary adverse health impacts of antibiotics on the gastrointestinal system are less well-defined (20; 37; 51; 58; 74; 75; 83; 110; 111).

Population-based studies on the association between antibiotic use and FGD are limited in number, scope and quality (71; 78; 94; 119; 123; 131; 149; 154). This study

aimed to assess antibiotic use as a risk factor for FGD, while considering potential modifying exposures and co-morbidities such as AGE, psychological disorders, and the use of other drugs that are observed to alter the gut microbial ecosystem.

METHODS

Study Design and Data Source

A population-based cohort design with a nested case-control analysis was employed. Subjects were selected from a cohort of active component U.S. military service members entering service from 1 January 2005 through 31 December 2014. The source of data was the Defense Medical Surveillance System (DMSS), a U.S. Department of Defense (DoD) administrative and medical records database (118). Specifically, demographic and military service data are located in the Defense Manpower Data Center (DMDC); inpatient, outpatient, and microbiological laboratory data are located in the Defense Health Services System (DHSS) database; prescription medication data are located in the Pharmacy Data Transaction Service (PDTS); and deployment medical encounter data and deployment medication data are located in the Theater Medical Data Store (TMDS and TMDS_MEDS (TMDS_MEDS data only from 2008 onwards)). These data are stored and managed by the Armed Forces Health Surveillance Branch (AFHSB). The research protocol underwent initial review by the Uniformed Services University of the Health Sciences Institutional Review Board and was determined exempt from human

subjects' research protection review. All data were de-identified and protected health information and personally identifiable information were removed from the datasets prior to release by AFHSB.

Subjects were followed through 31 December 2016 to account for a full 24 months of follow-up time for all potential subjects. Subjects were followed until incident FGD diagnosis, exit from military service, or the conclusion of the study period. Subjects were required to have at least 24 months of follow-up before entering the risk set. As the study population consisted of newly accessioned military service members, it was assumed that the subjects were healthy, without pre-existing FGD upon entrance to military service.

Case Definition

The outcomes of interest included following four FGDs (see Table S-4 for ICD-9 and ICD-10 codes): FC, IBS, Dyspepsia, FD. Two case definitions were used for an incident outcome as follows:

1. A possible case was defined as a subject with at least two medical encounters for a given FGD diagnosis within 1 year, with no prior diagnoses for any of the FGDs of interest.
2. A probable case was defined as a possible case with the following additional qualifications: the relevant ICD-9 or ICD-10 codes were in the first two

diagnostic positions. These cases also had medical encounter evidence of a gastroenterology visit or an upper or lower endoscopy procedure (see Table S-5 for CPT codes) for at least one of the qualifying FGD encounters.

The incident diagnosis was designated as the date of first diagnosis regardless of the diagnostic position and the incident diagnosis was required to occur during the study timeframe.

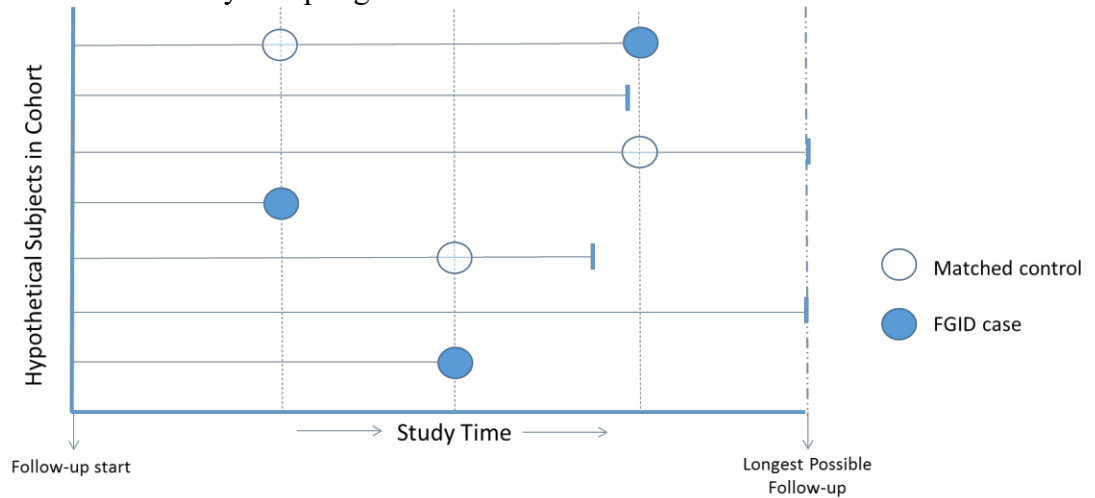
FGD outcome was assessed as one group including all four FGDs (functional constipation, irritable bowel syndrome, dyspepsia and functional diarrhea-Yes/No). Additionally, the four FGDs were assessed individually as there is already a well-established relationship between some FGDs and acute gastroenteritis (23; 50; 92; 101; 102; 143). This is particularly true for post-infectious IBS (PI-IBS) and dyspepsia and less well recognized for the remaining FGDs (50; 96; 143). For the all FGD outcome, an individual could qualify only once. However, for the individual FGD outcomes, an individual was able to qualify for each.

Control Subjects

Four controls were matched to each incident FGD case, and randomly selected from the risk-set at the time of each incident FGD medical encounter. An incidence-density sampling method of selecting cases and controls was used, with cases and

controls matched by calendar time (See Figure 4). Additional matching criteria included age (within 1 year) and sex.

Figure 4: Incidence Density Sampling Illustration



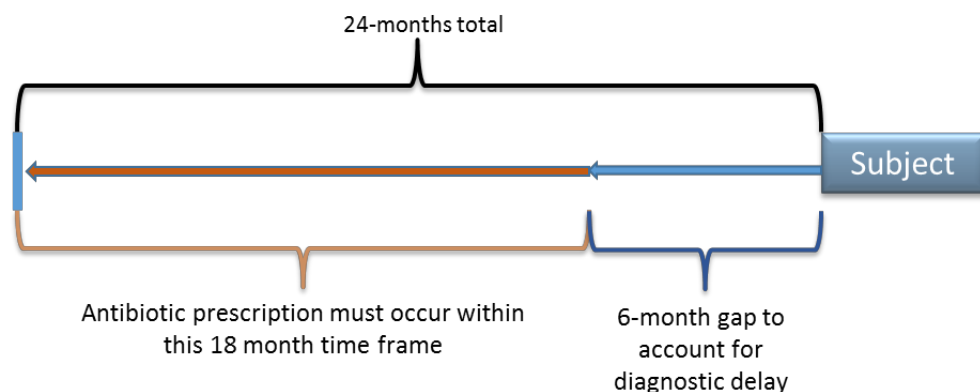
Exposure

The exposure of interest was oral antibiotic use. Antibiotic data extracted from the PDTS and TMDS_MEDS databases included antibiotic name, class, prescription date and dosage form. A complete list of oral antibiotics of interest can be found in Appendix Table S-6. Antibiotic classes included the following: carbapenems, cephalosporins, fluoroquinolones, glycopeptides, lincosamides, macrolides, monobactams, penicillins, sulfonamides, tetracyclines, isoniazid, linezolid, rifampin, and rifaximin (See Table S-6). The primary assessment of antibiotic use included any oral antibiotic use vs. no oral antibiotic use. In a sub-group analysis, the effect of multiple antibiotic prescriptions and

individual classes of antibiotics on IBS outcome only (see Appendix Table S-6 for individual antibiotic class definitions).

A case was considered exposed to antibiotics if any antibiotic prescription was dated 24 to 6 -months before the initial FGD diagnosis. Antibiotic use during the 6 months prior to FGD onset was excluded as an exposure to account for the lag time for condition development. This exclusion increased the plausibility of a temporal relationship between the exposure and disease onset (See Figure 5). The total 24-month follow-up period was an attempt to account for delays in disease onset, patient health care seeking behavior, and provider diagnostic patterns (102). Matched controls were considered exposed if those subjects had a documented antibiotic prescription in the 24 to 6-month period prior to the match date.

Figure 5: Exposure Timeline



Covariates

Covariates of interest included the following: age, sex, race/ethnicity, education level, military branch, military rank, presence of AGE, presence of an acute, non-enteric infection, presence of an axis I or axis II psychological disorder (see the Appendix Table A1 for ICD-9 and ICD-10 codes). Age and sex were matched for in the design and therefore were not assessed in the conditional logistic regression model.

Similar to antibiotic use and for similar reasons, AGE exposures and acute non-GI infections 24 to 6-months prior to the match date were evaluated.

Psychological disorders were considered if a specified medical encounter with one of the diagnostic codes occurred in the 24-months prior to the match date. To ensure specificity of this condition, psychological conditions were included if there was a hospitalization with any of the defining diagnoses in the first or second diagnostic position, if there were two or more outpatient medical encounters within 180 days of each other in the first or second diagnostic position or if a person had a single outpatient medical encounter in a psychiatric or mental health specialty setting (28).

Statistical Analysis

Crude odds ratios of FGD following oral antibiotic use were estimated with 95% confidence intervals using conditional logistic regression. Similarly, crude odds ratios were estimated for all available demographic variables and covariates of interest.

Multivariable conditional logistic regression modeling was used to estimate the adjusted odds ratios, allowing for a number of important variables, confounders, and effect-modification to be considered simultaneously. To be included in the multivariable model, factors either were considered nominally associated ($p < 0.25$) with the outcome at the univariate level and/or previously determined to be important for the relationship. Variables were added to the model one at a time and excluded if their inclusion did not result in a change of $>10\%$ in the main effect size. A similar approach was taken for interaction terms to assess effect modification where both an AGE or non-enteric infection event and antibiotic prescription occurred in the 24 to 6-month period prior to the match date (64). All analyses were performed using SAS version 9.4 for Windows (SAS Institute, Cary, North Carolina).

RESULTS

There were 4038 probable cases of FGD and 16150 controls selected for the study. The majority of subjects were 21-29 years of age (77.3%), with a relatively equal distribution of males ($n=10,075$, 49.9%) and females ($n=10,113$, 50.1%) (see Table 1).

Most of the study subjects were in the Army (44% of cases and 36.4% of controls) of any of the services and of E1-E4 rank (56.9% of cases and 52.7% of controls). A higher percentage of the study population was found to have been diagnosed with an Axis II psychological disorder (38.1% of cases and 16 % of controls) compared

to the proportion of subjects with an Axis I psychological disorder (9.8% of cases and 3.3% of controls). Only 5.4% of cases and 2.8% of controls had an episode of acute gastroenteritis during the exposure period of the study. Non-enteric infections were diagnosed in 69.6% of cases and 53% of controls during the subject's exposure time-frame. Oral antibiotic use was identified in 66.3% of cases and 51.2% of controls (see Table 6).

Table 6. Demographic and Covariate Frequencies by FGD outcome

		FGD Case N FGD Case %		FGD Control N FGD Control %		IBS Case N IBS Case %		IBS Control N IBS Control %		FC Case N FC Case %		FC Control N FC Control %		Dyspepsia Case N Dyspepsia Case %		Dyspepsia Control N Dyspepsia Control %		FD Case N FD Case %		FD Control N FD Control %	
Total		4038	100	16150	100	1401	100	5604	100	1728	100	6910	100	870	100	3480	100	39	100	156	100
Sex	Female	2023	50.1	8090	50.1	552	39.4	2208	39.4	1104	63.9	4414	63.9	359	41.3	1436	41.3	8	20.5	32	20.5
	Male	2015	49.9	8060	49.9	849	60.6	3396	60.6	624	36.1	2496	36.1	511	58.7	2044	58.7	31	79.5	124	79.5
Age (yrs)	18-20	135	3.3	443	2.7	41	2.9	145	2.6	59	3.4	189	2.7	31	3.6	98	2.8	4	10.3	11	7.1
	21-24	1444	35.8	5960	36.9	494	35.3	2028	36.2	593	34.3	2440	35.3	341	39.2	1422	40.9	16	41.0	70	44.9
	25-29	1640	40.6	6553	40.6	582	41.5	2322	41.4	708	41.0	2840	41.1	342	39.3	1355	38.9	8	20.5	36	23.1
	30-34	541	13.4	2105	13.0	189	13.5	741	13.2	246	14.2	962	13.9	100	11.5	383	11.0	6	15.4	19	12.2
	35-39	172	4.3	684	4.2	66	4.7	255	4.6	72	4.2	289	4.2	34	3.9	137	3.9			3	1.9
	40+	106	2.6	405	2.5	29	2.1	113	2.0	50	2.9	190	2.7	22	2.5	85	2.4	5	12.8	17	10.9
Race/Ethnicity	White	2363	58.5	9103	56.4	972	69.4	3260	58.2	877	50.8	3756	54.4	486	55.9	2000	57.5	28	71.8	87	55.8
	Black	706	17.5	2747	17.0	150	10.7	902	16.1	434	25.1	1254	18.1	120	13.8	566	16.3	2	5.1	25	16.0
	Hispanic Asian/Pacific Islander	558	13.8	2272	14.1	158	11.3	745	13.3	244	14.1	1029	14.9	151	17.4	477	13.7	5	12.8	21	13.5
	Native American	107	2.6	650	4.0	33	2.4	207	3.7	38	2.2	278	4.0	34	3.9	155	4.5	2	5.1	10	6.4
		42	1.0	164	1.0	13	0.9	56	1.0	16	0.9	68	1.0	12	1.4	39	1.1	1	2.6	1	0.6

		/Alaskan Native																				
		Other	182	4.5	926	5.7	54	3.9	338	6.0	75	4.3	414	6.0	52	6.0	165	4.7	1	2.6	9	5.8
		Unknown	80	2.0	288	1.8	21	1.5	96	1.7	44	2.5	111	1.6	15	1.7	78	2.2			3	1.9
Education	High School or less																					
		2781	68.9	10822	67.0	968	69.1	3814	68.1	1179	68.2	4462	64.6	605	69.5	2431	69.9	29	74.4	115	73.7	
	Some College	492	12.2	1675	10.4	162	11.6	551	9.8	228	13.2	779	11.3	98	11.3	336	9.7	4	10.3	9	5.8	
		496	12.3	2400	14.9	171	12.2	825	14.7	211	12.2	1093	15.8	111	12.8	463	13.3	3	7.7	19	12.2	
	College Advanced Degree	175	4.3	831	5.1	58	4.1	285	5.1	78	4.5	376	5.4	37	4.3	161	4.6	2	5.1	9	5.8	
		Unknown	94	2.3	422	2.6	42	3.0	129	2.3	32	1.9	200	2.9	19	2.2	89	2.6	1	2.6	4	2.6
Service	Army	1777	44.0	5878	36.4	678	48.4	2080	37.1	799	46.2	2475	35.8	282	32.4	1255	36.1	18	46.2	68	43.6	
	Navy	1014	25.1	4218	26.1	318	22.7	1433	25.6	391	22.6	1875	27.1	298	34.3	884	25.4	7	17.9	26	16.7	
	Air Force	868	21.5	4209	26.1	263	18.8	1444	25.8	396	22.9	1842	26.7	196	22.5	884	25.4	13	33.3	39	25.0	
	Marines	379	9.4	1845	11.4	142	10.1	647	11.5	142	8.2	718	10.4	94	10.8	457	13.1	1	2.6	23	14.7	
Rank	E1-E4	2298	56.9	8539	52.9	795	56.7	2963	52.9	999	57.8	3507	50.8	482	55.4	1978	56.8	22	56.4	91	58.3	
	E5-E9	1262	31.3	4959	30.7	444	31.7	1741	31.1	543	31.4	2207	31.9	264	30.3	969	27.8	11	28.2	42	26.9	
	O1-O5	467	11.6	2620	16.2	159	11.3	891	15.9	185	10.7	1183	17.1	118	13.6	523	15.0	5	12.8	23	14.7	
	O6-O10 Warrant Officer			4	0.0							3	0.0			1	0.0					
		11	0.3	28	0.2	3	0.2	9	0.2	1	0.1	10	0.1	6	0.7	9	0.3	1	2.6			
Axis I Dis-order	No	3642	90.2	15621	96.7	1247	89.0	5429	96.9	1552	89.8	6675	96.6	807	92.8	3370	96.8	36	92.3	147	94.2	
	Yes	396	9.8	529	3.3	154	11.0	175	3.1	176	10.2	235	3.4	63	7.2	110	3.2	3	7.7	9	5.8	
Axis II Dis-order	No	2501	61.9	13573	84.0	815	58.2	4732	84.4	1056	61.1	5756	83.3	603	69.3	2952	84.8	27	69.2	133	85.3	
	Yes	1537	38.1	2577	16.0	586	41.8	872	15.6	672	38.9	1154	16.7	267	30.7	528	15.2	12	30.8	23	14.7	
Acute Gastro-enteritis	No	3820	94.6	15696	97.2	1310	93.5	5457	97.4	1644	95.1	6712	97.1	829	95.3	3374	97.0	37	94.9	153	98.1	
	Yes	218	5.4	454	2.8	91	6.5	147	2.6	84	4.9	198	2.9	41	4.7	106	3.0	2	5.1	3	1.9	
Non-enteric Infect-ion	No	1226	30.4	7592	47.0	459	32.8	2793	49.8	461	26.7	2997	43.4	287	33.0	1710	49.1	19	48.7	92	59.0	
	Yes	2812	69.6	8558	53.0	942	67.2	2811	50.2	1267	73.3	3913	56.6	583	67.0	1770	50.9	20	51.3	64	41.0	

Oral Anti- biotic Use	No	1361	33.7	7883	48.8	469	33.5	2813	50.2	544	31.5	3276	47.4	331	38.0	1712	49.2	17	43.6	82	52.6
	Yes	2677	66.3	8267	51.2	932	66.5	2791	49.8	1184	68.5	3634	52.6	539	62.0	1768	50.8	22	56.4	74	47.4

Conditional Logistic Regression Models

In univariable analyses, the effect of race on FGD risk varied by outcome. Compared to white subjects, those that were black had increased odds of IBS and decreased odds of dyspepsia while those that were Asian/Pacific Islander had lower risks of IBS and FC and those of ‘other races’ had lower odds of IBS and FC. The effect of education also varied over the outcome with an advanced degree having the lowest risk of FC; with minimal significant associations with other outcomes. Relative to Army, personnel in other branches of service had decreased odds of IBS and FC; however, personnel in the Navy had increased odds of dyspepsia. Compared to the lowest enlisted rank category (E1-E4), the O1-O5 rank had significantly decreased risk of all FGD, IBS and FC and the E5-E9 rank had decreased risk of FC.

All covariates-AGE, non-enteric infection, axis I and axis II psychological disorders-were associated with increased risk of all FGD, IBS, FC and dyspepsia on the univariate level. Only subjects with axis II disorders had increased risk of FD on the univariate level. The primary exposure, oral antibiotic use, had increased odds of all FGD, IBS, FC and dyspepsia compared to subjects without oral antibiotic use on the univariate level. See Table 7 for all univariable model results.

Table 7: Univariable Associations by probable FGD Outcome

		All FGD (Crude)			IBS (Crude)			FC (Crude)			Dyspepsia (Crude)			FD (Crude)		
Variable		Odds Ratio	Lower CL	Upper CL	Odds Ratio	Lower CL	Upper CL	Odds Ratio	Lower CL	Upper CL	Odds Ratio	Lower CL	Upper CL	Odds Ratio	Lower CL	Upper CL
Race/Ethnicity	White	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
	Black	0.99	0.90	1.09	0.55	0.45	0.66	1.49	1.30	1.70	0.87	0.70	1.09	0.25	0.05	1.12
	Hispanic	0.95	0.85	1.05	0.70	0.58	0.84	1.02	0.87	1.20	1.31	1.06	1.62	0.74	0.27	2.08
	Asian/Pacific Islander	0.63	0.51	0.78	0.52	0.36	0.76	0.58	0.41	0.83	0.89	0.60	1.32	0.53	0.09	2.99
	Native American/Alaskan Native	0.99	0.70	1.40	0.78	0.43	1.44	1.00	0.58	1.74	1.27	0.66	2.45	2.99	0.18	48.56
	Other	0.76	0.64	0.89	0.53	0.39	0.71	0.78	0.60	1.00	1.30	0.94	1.80	0.36	0.04	2.87
	Unknown	1.07	0.83	1.38	0.72	0.45	1.17	1.70	1.19	2.44	0.78	0.45	1.37			
Education	High School or less	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
	Some College	1.13	1.01	1.26	1.13	0.93	1.38	1.06	0.89	1.25	1.17	0.91	1.52	1.66	0.43	6.37
	College	0.80	0.71	0.89	0.80	0.66	0.96	0.69	0.58	0.82	0.96	0.76	1.23	0.65	0.17	2.53
	Advanced Degree	0.80	0.68	0.95	0.77	0.56	1.05	0.70	0.53	0.93	0.92	0.62	1.37	0.86	0.13	5.61
	Unknown	0.86	0.68	1.08	1.25	0.88	1.78	0.57	0.39	0.83	0.86	0.52	1.43	0.95	0.09	9.64
Service	Army	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
	Navy	0.79	0.73	0.86	0.55	0.46	0.64	0.64	0.56	0.73	1.52	1.26	1.84	1.05	0.40	2.76
	Air Force	0.68	0.62	0.74	0.66	0.54	0.81	0.66	0.57	0.75	1.00	0.81	1.23	1.24	0.53	2.89
	Marines	0.68	0.60	0.76	0.67	0.58	0.78	0.60	0.49	0.74	0.90	0.69	1.18	0.15	0.02	1.24
Rank	E1-E4	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
	E5-E9	0.93	0.86	1.01	0.90	0.78	1.04	0.79	0.69	0.90	1.14	0.95	1.38	1.15	0.44	2.99
	O1-O5	0.65	0.58	0.72	0.61	0.50	0.75	0.48	0.40	0.58	0.94	0.73	1.21	0.96	0.28	3.27
	O6-O10 Warrant Officer	1.43	0.71	2.88	1.24	0.34	4.60	0.30	0.04	2.35	2.78	0.98	7.89			
Axis I Disorder (Y/N)		3.21	2.80	3.67	3.95	3.14	4.98	3.23	2.63	3.97	2.44	1.76	3.37	1.33	0.36	4.93
Axis II Disorder (Y/N)		3.29	3.04	3.55	3.90	3.42	4.45	3.32	2.94	3.75	2.51	2.11	2.99	2.56	1.13	5.78
Acute Gastroenteritis (Y/N)		1.98	1.67	2.33	2.59	1.98	3.40	1.73	1.33	2.24	1.57	1.09	2.28	2.67	0.45	15.96
Non-enteric infection (Y/N)		2.10	1.95	2.27	2.27	1.99	2.59	2.30	2.03	2.61	2.15	1.82	2.54	1.54	0.75	3.15
Oral Antibiotic Use (Y/N)		1.91	1.77	2.05	2.08	1.83	2.36	2.05	1.83	2.30	1.62	1.38	1.89	1.45	0.70	3.00

The effect of race on FGD outcome risk remained mostly unchanged following adjustment for other important demographic factors and covariates; with the exception of black subjects associated with significantly decreased odds of all FGD compared to white subjects after adjustment. In the multivariate analyses, education was excluded from the models for all outcome categories as inclusion did not change the main effect size by 10% or more. Compared to subjects in the Army, service in the Navy remained associated with decreased risk of all FGD, IBS, and FC in multivariable models. Service in the Marines still had decreased odds of all FGD and FC compared to the Army and service in the Navy had increased risk of dyspepsia compared to the Army after adjustment. The O1-O5 officer rank remained significantly associated with lower risk of all FGD and FC compared to the lower enlisted rank, E1-E4, in the multivariable model.

All covariates significantly increased the risk of all FGD and IBS after adjustment. Risk of FC was increased by presence of axis I disorder, axis II disorder and non-enteric infection in subjects after adjustment; risk of dyspepsia was increased by presence of axis I disorder and non-enteric infection and only axis II disorder remained associated with increased odds of FD in the multivariable models. Oral antibiotic use was significantly associated with increased risk of all FGD, IBS, FC, dyspepsia following adjustment for other important variables. Neither interaction variables, AGE*antibiotic use or non-enteric infection*antibiotic use, changed the main effect size of the multivariable model by more than 10% for any of the FGD outcomes and therefore they

were not included in the multivariable model. Refer to Table 8 for all multivariable model results. Univariable and multivariable model results for possible FGD outcomes can be found in Tables S7-S8.

Table 8: Multivariable Associations by probable FGD Outcome

		All FGD (Adjusted)			IBS (Adjusted)			FC (Adjusted)			Dyspepsia (Adjusted)			FD (Adjusted)		
Variable		Odds Ratio	Lower CL	Upper CL	Odds Ratio	Lower CL	Upper CL	Odds Ratio	Lower CL	Upper CL	Odds Ratio	Lower CL	Upper CL	Odds Ratio	Lower CL	Upper CL
Race/Ethnicity	White	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref			
	Black	0.89	0.81	0.98	0.52	0.43	0.64	1.36	1.17	1.56	0.87	0.68	1.10			
	Hispanic	0.94	0.84	1.04	0.73	0.60	0.90	1.03	0.87	1.22	1.31	1.04	1.63			
	Asian/Pacific Islander	0.62	0.50	0.77	0.50	0.34	0.75	0.58	0.40	0.84	0.92	0.61	1.39			
	Native American/Alaskan Native	0.94	0.65	1.34	0.75	0.39	1.44	1.09	0.61	1.96	1.04	0.52	2.07			
	Other	0.81	0.68	0.96	0.59	0.43	0.81	0.94	0.72	1.23	1.21	0.86	1.71			
	Unknown	1.14	0.87	1.48	0.78	0.47	1.30	1.86	1.27	2.72	0.78	0.43	1.39			
Service	Army	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref			
	Navy	1.04	0.95	1.15	0.96	0.81	1.13	0.87	0.75	1.01	1.96	1.59	2.42			
	Air Force	0.78	0.71	0.86	0.64	0.54	0.76	0.78	0.68	0.91	1.18	0.95	1.48			
	Marines	0.86	0.76	0.99	0.82	0.66	1.02	0.78	0.63	0.96	1.20	0.91	1.60			
Rank	E1-E4	ref	ref	ref				ref	ref	ref	ref	ref	ref			
	E5-E9	1.05	0.96	1.14				0.95	0.83	1.10	1.17	0.96	1.43			
	O1-O5	0.83	0.74	0.93				0.70	0.57	0.85	1.19	0.92	1.56			
	O6-O10 Warrant Officer	1.63	0.80	3.36				0.30	0.04	2.48	4.24	1.40	12.77			
Axis I Disorder (Y/N)		1.50	1.29	1.74	1.66	1.28	2.15	1.53	1.22	1.92	2.50	2.07	3.02			
Axis II Disorder (Y/N)		2.53	2.32	2.76	2.93	2.52	3.40	2.53	2.21	2.90				2.56	1.13	5.78
Acute Gastroenteritis (Y/N)		1.58	1.33	1.88	2.01	1.50	2.70	1.30	0.98	1.72	1.44	0.98	2.12			
Non-enteric infection (Y/N)		1.57	1.45	1.71	1.64	1.41	1.90	1.64	1.43	1.88	1.79	1.50	2.15			
Oral Antibiotic Use (Y/N)		1.45	1.34	1.57	1.57	1.36	1.81	1.57	1.38	1.78	1.40	1.17	1.66			

Sub-group Analysis: Antibiotic Exposure and IBS Outcome

Subgroup frequencies of antibiotic dose categories and individual antibiotic classes are listed in Table 9. In an apparent dose response, the number of antibiotic prescriptions during the exposure period increased, the risk of probable IBS increased (Table 10). This effect remained after adjustment for other important variables (Table 11). Individual antibiotic classes found to be significantly associated with probable IBS after controlling for important covariates included cephalosporins, fluoroquinolones, glycopeptides, lincosamide, macrolides, metronidazole, penicillins (amino), and rifaximin (see Table 11).

Table 9: Subgroup Analysis: Frequencies of Antibiotic Categories

Variable	Effect	IBS case		IBS control	
		N	%	N	%
Cumulative antibiotics	0	469	33.48	2813	50.20
	1	376	26.84	1430	25.52
	2-4	438	31.26	1158	20.66
	5-10	110	7.85	192	3.43
	>10	8	0.57	11	0.20
Antibiotic Class (Y/N)	Cephalosporins	462	5.66	1214	5.78
	Fluoroquinolones	1070	13.11	1989	9.47
	Glycopeptides	7	0.09	1	0.00
	Isoniazid	149	1.83	760	3.62
	Lincosamide	319	3.91	735	3.50
	Linezolid	1	0.01	4	0.02
	Macrolides	1714	21.00	4235	20.17
	Metronidazole	727	8.91	1613	7.68
	Penicillins, Amino	1225	15.01	2976	14.17
	Penicillins, Natural	372	4.56	1248	5.94
	Penicillins, Penicillinase	28	0.34	90	0.43

Rifampin	46	0.56	130	0.62
Sulfonamides	810	9.93	2201	10.48
Tetracyclines	1231	15.08	3801	18.10

Table 10: Subgroup Analysis: Univariable associations

Variable	Effect	Odds Ratio	Lower CL	Upper CL
Cumulative antibiotics	0	ref	ref	ref
	1	1.63	1.40	1.90
	2-4	2.44	2.09	2.84
	5-10	3.78	2.91	4.92
	>10	5.62	2.24	14.12
Antibiotic Class (Y/N)	Cephalosporins	1.35	1.16	1.56
	Fluoroquinolones	2.15	1.89	2.45
	Glycopeptides	20.43	2.44	170.80
	Isoniazid	0.78	0.52	1.16
	Lincosamide	1.61	1.36	1.89
	Linezolid	1.00	0.11	8.95
	Macrolides	1.75	1.54	1.99
	Metronidazole	2.36	2.01	2.78
	Penicillins, Amino	1.66	1.46	1.88
	Penicillins, Natural	1.16	1.01	1.34
	Penicillins, Penicillinase	1.19	0.76	1.86
	Rifampin	1.04	0.65	1.67
	Sulfonamides	1.29	1.13	1.47
	Tetracyclines	1.15	1.02	1.31

Table 11: Subgroup Analysis: Multivariable association
(for full model results, see supplemental information, Table S-9)

Cumulative Antibiotics and Antibiotic Class Effects				
Variable	Effect	OddsRatio	LowerCL	UpperCL
Cumulative Antibiotics	0	ref	ref	ref
	1	1.34	1.14	1.58
	2-4	1.85	1.56	2.19

	5-10	2.29	1.72	3.06
	>10	2.67	1.01	7.10
Antibiotic Class (Y/N)	Cephalosporin	1.18	1.01	1.39
	Fluoroquinolones	1.88	1.63	2.16
	Glycopeptides	25.85	2.87	233.31
	Lincosamide	1.42	1.19	1.69
	Macrolides	1.39	1.21	1.60
	Metronidazole	2.18	1.83	2.60
	Penicillins, Amino	1.46	1.28	1.67

DISCUSSION

FGDs are considered to have a multifactorial risk pathway (35). We observed an approximate 30% increased risk of FGD following oral antibiotic use after adjusting for other significant variables. Oral antibiotic use was associated with an approximate 30% increase in risk of incident FGD after adjusting for other significant variables. This pattern was mirrored in the univariable and multivariable associations between antibiotic use and IBS, FC and dyspepsia individually. Further strengthening the evidence behind the association between antibiotic exposure and FGD was the dose-response relationship observed between the number of antibiotic prescriptions and increased probable IBS risk.

Demographic risk factors for all FGD included race/ethnicity, education and military branch of service and rank. These findings are generally consistent with what has been previously reported in this population (60; 101).

Consistent with prior studies, we found an increased risk in FGD following AGE (92; 100-102; 115). AGE is hypothesized to be associated with FGD as a result of a

sustained disruption to the gut microbial environment and chronic inflammation following the infection (28; 143; 146). Of note, the lack of a significant association between AGE and dyspepsia on the multivariate level is inconsistent with the systematic review by Pike, et al. which reported 1.81-fold increased risk of dyspepsia following AGE across studies with medical encounter reported AGE (96). However, the directionality of the association in this study is consistent with the review findings. Some studies have reported an association between non-enteric infection and FGD, suggesting either an alternative mechanism or unmeasured or controlled covariate, such as antibiotic use (41; 94). For non-enteric infections, a significant association was observed for the individual FGDs except for FD on both univariate and multivariate levels. To observe whether these covariates modify the association between antibiotics and FGD, interaction variables were incorporated in the multivariable model. However, when the interaction between either AGE or non-enteric infection and antibiotic use was assessed in this study, it was not found to be significant for either AGE and antibiotic use or non-GI infection and antibiotic use. Thus, the association between antibiotic use and FGD was not shown to be dependent on either AGE or non-enteric infection in this study.

The sub-group analysis of specific categories of antibiotic exposures and development of probable IBS was intended to provide a deeper look at the dose-response and specific effects of antibiotics in the pathway (Tables 4-6). Indeed, on a univariate level, a dose-response effect was observed, with an incremental increase of risk of IBS as

the number of antibiotic prescriptions increased compared to zero prescriptions. Furthermore, individual antibiotic classes were assessed in relation to development of probable IBS. Of note, the following classes were observed to be associated on the univariate level: cephalosporins, fluoroquinolones, glycopeptides, lincosamide, macrolides, metronidazole, penicillins, sulfonamides, and tetracyclines. On the multivariate level, the following classes remained significantly associated with an increased risk of IBS: cephalosporins, fluoroquinolones, glycopeptides, lincosamide, macrolides, metronidazole, and penicillins (amino). Certain antibiotic classes have previously been shown to be related to gut microbial dysbiosis, such as fluoroquinolones, cephalosporins, lincosamides and penicillins and therefore provide plausibility to the relationships observed in this study (37; 66; 72; 74; 77; 85; 90; 112). Additionally, Villareal, et al. previously found an association between macrolide and tetracycline use and elevated IBS risk after adjustment for covariates; the macrolide effect was confirmed for this study, however tetracyclines were not found to significantly increase the risk of IBS in the multivariable model (154). Therefore, this study adds to the evidence that use of several antibiotic classes increase risk of IBS in particular, perhaps as a result of gut microbial dysbiosis.

Psychological disorders are thought to have a bi-directional relationship with FGD (34). We investigated the relationship between axis I and axis II psychological disorders and FGD, and found that both were associated with elevated FGD risk (adjusted

OR: 1.5 and 2.53, respectively). This was true for all FGDs on an individual level except for FD, in which only Axis II disorders were significantly associated. Additional studies are needed to investigate confirm the temporality of this relationship and investigate the association further.

A number of limitations affect the interpretation and generalizability of this study. The study population was restricted to US military service members; this population is younger, healthier and predominately male and therefore these results may not be generalizable to a wider US or international population (60). As a surveillance data analysis using an administrative data set with ICD-9 and ICD-10 coded diagnoses, we cannot confirm that FGD diagnoses were made using Rome criteria. Also, due to the subjective nature of FGD symptom presentation and provider diagnosis, misclassification of some FGD diagnoses cannot be ruled out, although strict case criteria may have reduced the number of false positives. Next, it is possible some ICD-9 or ICD-10 codes were missing or miscoded; missingness is unlikely to be systematic and therefore would likely drive effect measures towards the null. Similarly, prescriptions recorded in the PDTS database may not have indicated actual use of the medication; however, this is not expected to be systematically different between cases and controls. We also did not have information on the indication for antibiotic use in this study. This information may be particularly useful for cases of AGE treated with antibiotics; antibiotic use for AGE can indicate severity of the infection and risk of post-infectious IBS is believed to increase

with AGE severity (23; 68). As a retrospective study, temporality of the antibiotic-FGD relationship could not be fully guaranteed. Additionally, there are many potential confounders that were not available for inclusion: antibiotic use and other exposures prior to military service, stressors during deployment or training, medications beyond those included in the study, BMI and diet (70). Other medications, such as statins, proton-pump inhibitors, opioids, metformin, non-steroidal anti-inflammatory drugs, and antipsychotics have been observed to be related to either gut microbial dysbiosis and/or AGE in previous studies and are therefore of high interest in the development of FGDs (70). While FGDs are exclusionary medical conditions for military service, it is possible that some persons may have entered the service undiagnosed (60). Another variable that was not available for inclusion is administration of IV antibiotics. This may have resulted in misclassification of antibiotic exposure. Since IV antibiotics may be used on patients with more severe infections, and the severity of infection may increase the risk of developing FGD, it is possible that missing antibiotic exposure for these patients limited the ability to detect a true association between antibiotic use and FGD (80).

There are strengths of this study and data set, including the near-complete ascertainment of the population of interest, a large sample size, and the nested case-control study design with matched controls (38; 118; 136). Use of the case criteria, both possible and probable, improved the flexibility to detect cases while providing higher positive predictive value of FGD cases. Requiring multiple medical encounters for a

diagnosis of FGD likely reduced misclassification of the outcome. Additionally, the diagnostic delay from the initiation of antibiotic use likely reduced misclassification. Also, requiring subjects to have 24 months of follow-up before entering the risk-set ensured that risk factor and exposure information was assessed prior to FGD diagnosis or control selection. The use of comprehensive information on antibiotic prescription and indication along with the ability to assess the duration and number of antibiotics prescribed (and a dose-response relationship) is another strength. Furthermore, the inclusion of variables such as age, sex, race, rank and acute GI infection allowed for an extensive consideration of multiple confounders and time-varying covariates.

Due to the size of the active component military cohort over the 10-year study period and the presence of several time-varying covariates in the dataset, the nested-case control design was determined to be the most efficient, manageable and powerful analysis approach (136). The nested case-control design is a hybrid approach combining the advantages of using a well-defined cohort with the efficiency of case-control sampling. With an adequate number of controls selected for each case, the results of this analysis should agree with results that would be obtained from the analysis of the entire cohort (38; 52). Additionally, the incidence density sampling technique helped to reduce the likelihood of selection bias compared to traditional case-control studies.

Using a well-defined cohort of active duty US service members, we found antibiotics to be an independent risk factor for development of FGD. Given the consistent

associations, dose-response relationship, and specific effects of individual classes of antibiotics on risk of FGDs observed in this study, providers should use caution with antibiotic prescription especially in situations where it may not be necessary or where patients may be at increased risk of FGD as a result. Future studies should expand upon this foundation in different populations, and with modified study design, ascertainment and timing of FGD outcomes, antibiotic exposure and use indication, and inclusion of other important microbiome-associated factors such as BMI, diet, co-morbidities and other medication use.

CHAPTER 4: Proteomics Study

INTRODUCTION

The gastrointestinal microenvironment is known to play a role in a variety of conditions and can be altered by various disease states and environmental factors (15; 138). Travelers and deployed military personnel are at high risk for acute gastrointestinal enteritis (AGE) or travelers' diarrhea (TD), particularly in specific regions of the world (101; 116). Additionally, this group is exposed to antibiotics frequently, either for prophylactic (i.e. anti-malarial prophylaxis) or treatment reasons. Both AGE and antibiotics can cause significant alterations in the gut microbiome from a baseline state (2; 20; 99; 112). In some cases, these exposures have long-term effects on the stability and composition of the gut microbiome (58; 75; 111). The host immune response to gut microbiota is hypothesized to be impacted by traveler's diarrhea and host response to gut microbiota is known to differ by health status, suggesting an inflammatory reaction origin could contribute to FGD pathogenesis (15; 28; 95; 98). Irritable bowel syndrome (IBS) is FGD that has been investigated in relation to gut microbial dysbiosis, host response to the gut microbiota, AGE and to a lesser degree, antibiotic use (68; 95; 98). However, the associations, causal pathway, and potential modifying effects of TD and antibiotics in relation to IBS remains unclear (41).

Recent research in IBS has attempted to characterize associated biological markers in order to improve understanding and diagnosis of the disorder (18; 19; 31; 32; 81; 95). The host immune response to gut microbiota antigens is one such target that has potential to serve as a proxy for the status and function of the gut microbiome as well as a method to track destabilization or normalization of bowel function (15; 95). Precedent to this study, Pike, et al. investigated the host seroreactivity to gut microbial antigens in IBS subjects versus healthy controls (95). However, the host response in IBS cases has yet to be observed on a longitudinal basis, comparing healthy to diseased states over time and in the context of important exposures. Therefore, this exploratory study aimed to assess changes in the host response to gut microbial antigens in traveling or deploying subjects that developed incident IBS after travel and non-IBS controls by comparing the levels of serum biomarkers for gut microbiota in pre- and post-travel/deployment samples. Additionally, relevant travel exposures including oral antibiotic use and TD were assessed in relation to IBS and changes in levels of serum biomarkers.

METHODS

Study Population

The study population consisted of subjects enrolled in two established study protocols: the TravMil cohort study and the Trial Evaluating Ambulatory Treatment of

Travelers' Diarrhea (TrEAT TD) clinical trial. Population characteristics, inclusion and exclusion criteria have been stated in previous publications (68; 114).

For subjects from TravMil and TrEAT TD to be included in this study they were required to have available serum for both a pre-travel/deployment timepoint (day 0/baseline serum sample for TrEAT TD) and a post-travel/deployment timepoint (day 21 for TrEAT TD). Subjects were excluded if there was evidence of IBS or other functional gastrointestinal disorder at baseline or prior to travel.

The research protocol underwent initial review by the Uniformed Services University of the Health Sciences Institutional Review Board and was determined exempt from human subjects' research protection review. All data were de-identified and protected health information and personally identifiable information were removed from the datasets prior to release by the Infectious Disease Clinical Research Program.

Sampling

Archived serum samples collected at 2 timepoints for TravMil and TrEAT TD study subjects were used in this study. For TravMil subjects, the baseline sample was collected prior to deployment/travel. For TrEAT TD subjects, the baseline sample was taken at Day 0, the time of presentation/enrollment in the trial. Post-exposure samples were taken following deployment/travel for TravMil subjects and at day 21 of a TrEAT TD subject's enrollment in the trial.

Microbiota Antigen Microarray

Seroreactivity to gut microbiota antigens was assessed through a custom serum microarray developed by the University of Alabama Birmingham (UAB) as previously described (15). Microbiota antigens were diluted in TRIS buffer (pH 8.0) with 0.5% sodium dodecyl sulfate (SDS) at 0.2mg/mL and printed on FAST 16 nitrocellulose pad slides (Whatman; GE Healthcare Life Sciences, Pittsburgh, PA) in quadruplicate using a MicroGrid II robot (Genomic Solutions, Marlborough, MA). The slides were air-dried overnight then blocked with a Protein Array Blocking Buffer (Whatman) and then probed with human serum samples at 1:100 dilution, washed and incubated with Alexa Fluor 647- or Alexa 546-labeled goat anti-human immunoglobulin G (IgG) or IgA (KPL, Gaithersburg, MD). The assay measured mean fluorescent intensity (MFI) corresponding to the relative proportion of IgG and IgA bound to the slide. The MFI values were averaged across the four printed array features for a mean MFI value. The antigens evaluated in this study are listed in Table 12. This assay was previously used to assess the IgG-adaptive immune response to the intestinal microbiota in human subjects across spectrums of age (children and adults) and health status (healthy subjects, allergic disease, and PI-IBS) (15; 95).

Table 12: Microarray antigens

Phylum	Class	Primary Species	Name	Protein ID	Function
Bacteroides	Bacteroidia	<i>Bacteroides vulgatus</i>	CBir19	ABC transporter ATP-binding protein	Other/unknown
		<i>Alistipes shahii</i>	CBir23	Elongation factor 1A	Transc/transl

		<i>Bacteroides eggerthii</i>	CBir45	Glycosyl hydrolase	Metabolism
		<i>Tannerella sp.</i>	CBir8	Elongation factor thermo unstable	Other/unknown
		<i>Bacteroides fragilis</i>	P3	HSP90	Other/unknown
		<i>Bacteroides fragilis</i>	Keto	transketolase	metabolism
		<i>Bacteroides vulgatus</i>	rIB13	Protease IV/SppA	Other/unknown
		<i>Bacteroides thetaiotaomicron</i>	Tetraco	tetratricopeptide repeat protein	Other/unknown
Firmicutes	Bacilli	<i>Lactobacillus salivarius</i>	CBir14	Glyceraldehyde-3-phosphate dehydrogenase	Metabolism
		<i>Enterococcus faecalis</i>	EF20	Sal A	Other/Unknown
	Clostridia	<i>Eubacterium rectale</i>	AgF1	Flagellin	Motility
		<i>Roseburia inulinivorans</i>	CBir11	Flagellin	Motility
		<i>Roseburia intestinalis</i>	CBir63	Ig-like surface protein	Other/unknown
		<i>Roseburia inulinivorans</i>	FlaX	Flagellin	Motility
		<i>Clostridium bolteae</i>	MDR247	Adenine deaminase	Metabolism
		<i>Roseburia intestinalis</i>	MDR90	collagen adhesion protein	Other/unknown
		<i>Clostridium citroniae</i>	rIB1	RecN	transc/transl
		<i>Roseburia intestinalis</i>	rIB10	Nucleotidyltransferase/hypothetical	Transc/transl
		<i>Flavonifractor plautii</i>	rIB12	Homoserine dehydrogenase	Metabolism
		<i>Ruminococcus sp.</i>	rIB14	Helicase	Transc/Transl
		<i>Flavonifractor plautii</i>	rIB16	Pyruvate synthase	Metabolism
		<i>Flavonifractor plautii</i>	rIB17	NlpC/P60	Other/unknown
		<i>Lachnospiraceae3-1-57</i>	rIB8	Glycosyltransferase	Metabolism
		<i>Roseburia intestinalis</i>	rIB9	Methyl-accepting chemotaxis protein	Motility
		<i>Clostridium asparagiforme</i>	rIB4	Relaxase	transc/transl
Firmicutes and Bacteroides		<i>Blautia hansenii</i>	rIB2	SAM domain protein	transc/transl
Firmicutes and Proteobacteria	Clostridia	<i>Erysipelotrichaceae 3-1-53</i>	rIB5	Hypothetical protein-cytoplasmic	Other/Unknown

Firmicutes flagellin	Clostridia	<i>Lachnospiraceae</i>	28-4band	Flagellin	Motility
		<i>Lachnospiraceae</i>	3-1-57Fla	Flagellin	Motility
		<i>Roseburia intestinalis</i>	A4Fla2	Flagellin 2 from A4 isolate	Motility
		<i>Roseburia inulinivorans</i>	A4Fla3	Flagellin 3 from A4 isolate	Motility
		<i>Butyrivibrio fibrisolvens</i>	CBir1	Flagellin	Motility
		<i>Roseburia intestinalis</i>	CBir66	Flagellin	Motility
		<i>Flavonifractor plautii</i>	MDR254	Flagellin	Motility
		<i>Roseburia faecis</i>	RfaeF3	Flagellin	Motility
		<i>Roseburia hominis</i>	RhomF1	Flagellin	Motility
		<i>Roseburia hominis</i>	RhomF2	Flagellin	Motility
		<i>Roseburia intestinalis</i>	RintF1	Flagellin	Motility
		<i>Roseburia inulinivorans</i>	RinuF5	Flagellin	Motility
Proteobacteria	Beta	<i>Verminephrobacter eiseniae</i>	rIB20	ABC transporter	Other/Unknown
	Delta/epsilon	<i>Helicobacter cinaedi</i>	CBir5	Methyl-accepting chemotaxis protein	Motility
		<i>Helicobacter canadensis</i>	CBir56	Methyl-accepting chemotaxis protein	Motility
	Epsilon	<i>Campylobacter showae</i>	rIB18	Surface array protein	other/unknown
	Gamma	<i>Escherichia coli</i>	FtsZ	FtsZ protein - homologue of tubulin (putative pANCA)	Other/unknown
		<i>Salmonella dublin</i>	SalFliC	Salmonella dublin flagellin	Motility
		<i>Escherichia coli</i>	UNCfliC	FliC from UNC101 <i>E. coli</i>	motility
		<i>Escherichia coli</i>	OmpC	OmpC from UNC <i>E. coli</i>	Other/Unknown
		<i>Gram-negative bacteria (e.g. C.jejuni)</i>	CdtB	Cytolethal Distending toxin	DNase activity
Chordate	Mammal	<i>Homo sapien</i>	Vinc	Vinculin	Cytoskeletal

Gut microbiota antigens (plus CdtB and Vinculin) cloned from murine cecal microbiota were searched against sequences from the described human microbiota in the

MetaHit, HMB, and NCBI databases. Each sequence matched to the phyla, class, and primary species in the human microbiota to which it was tracked (15).

Exposures

Travel exposures of interest included incident TD during travel/deployment and reported antibiotic use during travel/deployment. For TravMil subjects, TD was ascertained from responses in travel-illness diaries or post-travel surveys (68). TD was defined as presentation with 3 or more stools within a 24-hour period or 2 loose stools plus one associated symptom (nausea, vomiting, fever, blood in stool, or abdominal cramps) within a 24-hour period during the study period. All TrEAT TD subjects presented with TD to be included in the trial (114). TD was defined as ≥ 3 loose stools in 24 hours or ≥ 2 loose stools in 24 hours with associated symptoms (nausea, vomiting, abdominal cramps, or tenesmus) of ≤ 96 hours duration in the trial.

Antibiotic use during travel/deployment for TravMil subjects was assessed during clinic visits or infectious disease consult, a follow-up clinical visit, record in a study medications table, or from travel-illness diaries. Antibiotic exposure was defined as any documented antibiotic use during the study travel/deployment period. Antibiotic class information was not included in this study. For TrEAT TD subjects, all were treated with antibiotics under 2 main experimental arms. The first arm included subjects with acute watery diarrhea and assigned to one of three single dose interventions: rifaximin 1650

mg, azithromycin 500mg or levofloxacin 500mg all adjunct with an initial dose of loperamide (4mg) followed by 2mg of loperamide after each unformed stool, not to exceed 16mg/day for 2 days. The second arm included subjects with acute dysentery or febrile diarrhea and assigned the following two interventions: single dose azithromycin 1000 mg plus loperamide 4 mg initially followed by 2 mg after each unformed stool, not to exceed 16 mg/day for 2 days; single dose azithromycin 1000mg alone.

In addition to documented use of antibiotics, the prescription of antibiotics was assessed in this study. For TrEAT TD, all subjects were prescribed antibiotics as part of the treatment regimen in each study arm. For TravMil, prescription of antibiotics was determined through records from pre-travel medical visits, ill-subject clinic visits or infectious disease consults. Prescription of antibiotics for travel was defined as any documented prescription of antibiotics for use during travel/deployment, either for diarrhea treatment or malaria prophylaxis.

IBS Outcome and Control selection

The base population from which TravMil subjects were selected for this study included subjects that completed a travel diary, post-survey and follow-up surveys and had paired pre- and post-travel serum samples available, that met inclusion and exclusion criteria (no baseline IBS). For TrEAT TD, subjects were required to have paired baseline

(day 0) and post-exposure (day 21) samples, met inclusion and exclusion criteria and completed follow-up surveys.

For both TravMil and TrEAT TD subjects, IBS cases were identified through self-reported follow-up survey responses and IBS was defined using modified Rome III criteria as recurrent abdominal pain or discomfort (uncomfortable sensation not described as pain) for at least 3 days/month in the last 3 months and symptom onset at least 6 months prior and associated with two or more of the following: improvement with defecation; onset associated with a change in the frequency of stool; onset associated with a change in form (appearance) of stool (34). Subjects could also self-report a clinical diagnosis of IBS. For control selection, subjects without reported IBS at any time during the study were randomly selected from the eligible study population.

Statistical Methods

Demographic characteristics and travel exposures were compared across all study groups using a Pearson's Chi-Square analysis (where >20% of cells contained counts less than 5, Fisher's Exact and Freeman-Halton tests were used). Logistic regression for risk of IBS outcome was performed on demographic variables and travel exposures of interest.

Antibody levels to select gut microbial antigens on the microbiome panel were expressed in mean fluorescence units (MFI) units. Negative values were converted to

zero and natural log transformed after the addition of 1 to each value. Pre- and post-exposure serum samples for each subject were paired to assess changes in anti-microbiota antigen antibody levels following travel exposures of interest. The mean difference between post and pre-deployment anti-microbiota antibodies for each exposure (antibiotics or TD) group was also compared to assess the variation in magnitude of change between the exposure groups.

Two-tailed paired t-tests were used to compare pre and post samples. The mean differences in antibody levels in pre- and post-travel/deployment samples were compared between cases and controls and between exposure groups using two-tailed t-tests and false discovery rate (FDR) p-values to adjust for multiple comparisons. An unrotated principal components analysis (PCA) was performed to assess the correlation between microbiome biomarkers for exposures and outcomes and to determine if specific clusters of antigens could be combined to reduce the dimensions of the data. Top contributing factors were those in the upper 90th or lowest 10th percentile for either PC1 or PC2. Additionally, the top two principal components were compared across the outcome and exposure groups to determine if there was a significant difference between the groups for each dimension.

The data analysis for this study was performed using SAS 9.4 software version 6.2.9200 of the SAS System for Windows, Copyright 2002-2012 by SAS Institute Inc., Cary, NC, USA.

RESULTS

Demographics

A total of 41 IBS cases and 48 non-IBS controls were selected from TravMil and TrEAT TD base populations (see Figure 6 for a break-down of subject selection by study and by outcome and exposure status). The majority of subjects were male (58.4%), less than 50 years of age (61.8%) and had traveled to Asia (41.6%), Africa (33.7%), or Central/South America or the Caribbean (21.3%). Distribution of sex, age categories and region of travel did not differ significantly between cases and controls (Table 12).

Figure 6: Subject Selection Flow Diagram

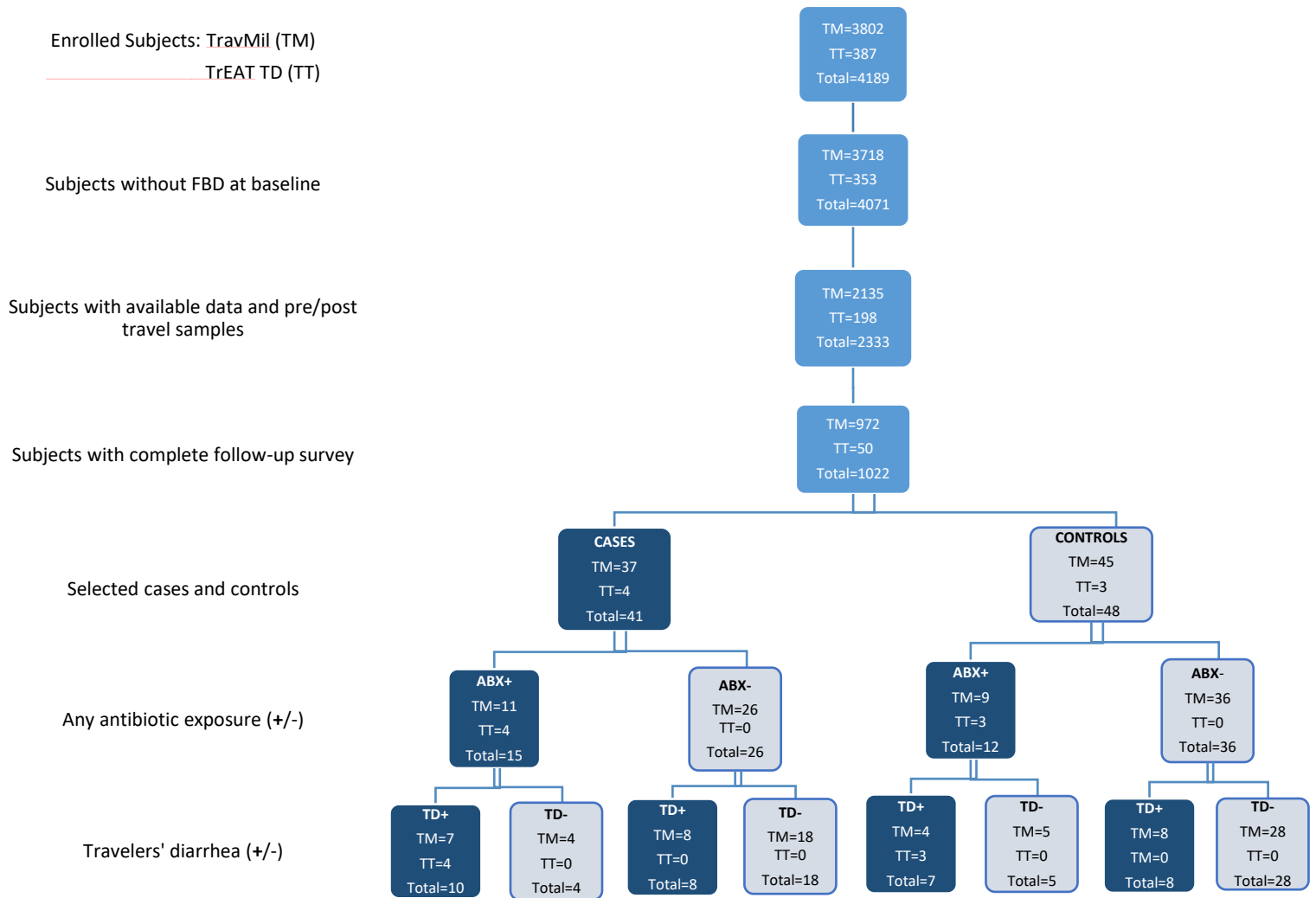


Table 12: Demographic and study characteristics

		IBS Cases (%)	Controls (%)	Total	P-value*
Sex	Male	24 (58.54%)	28 (58.33%)	52 (58.4%)	0.99*
	Female	17 (41.46%)	20 (41.67%)	37 (41.6%)	
Age Category	< 50 years	26 (63.41%)	29 (60.42%)	55 (61.8%)	0.77*
	>= 50 years	15 (36.59%)	19 (39.58%)	34 (38.2%)	
Region of travel	Africa	15 (36.59%)	15 (31.25%)	30 (33.7%)	0.35**
	Asia	14 (34.15%)	23 (47.92%)	37 (41.6%)	
	Central and South America and Caribbean	9 (21.95%)	10 (20.83%)	19 (21.3%)	
	Multiple Regions (Africa and Asia)	1 (2.44%)	0	1 (1.1%)	
	North American and Oceania	2 (4.88%)	0	2 (2.2%)	
Travelers' diarrhea	Yes	19 (46.34%)	15 (31.25%)	34 (38.2%)	0.14*
	No	22 (53.66%)	33 (68.75%)	55 (61.8%)	
Reported antibiotic use for TD during travel	Yes	10 (24.39%)	6 (12.50%)	16 (17.90%)	0.14*
	No	31 (75.61%)	42 (87.50%)	73 (82.10%)	
Any Reported Antibiotic use during travel	Yes	15 (36.59%)	12 (25%)	27 (30.34%)	0.23*
	No	26 (63.41%)	36 (75%)	62 (69.66%)	
Antibiotics prescribed for TD during travel	Yes	39 (95.12%)	44 (91.67%)	83 (93.3%)	0.68***
	No	2 (4.88%)	4 (8.33%)	6 (6.7%)	
Total		41	48	89	

*Pearson's chi-square; **Freeman-Halton Test; *** Fisher's Exact test

IBS = Irritable Bowel Syndrome

Travel Exposures and IBS

TD during travel was no more likely to be reported in IBS cases than in controls (OR=1.90; 95%CI: 0.80, 4.52). Additionally, any antibiotic use or antibiotic use for TD only was not significantly associated with increased risk of IBS (OR= 1.73; 95%CI: 0.70,4.31 and OR=2.26; 95%CI: 0.74,6.88, respectively). There was also no significant difference in the proportion of subjects prescribed antibiotics prior to travel across IBS status (Table 2) (OR=1.77; 95%CI: 0.31,10.21).

Host Response to Gut Microbial Antigens

IBS outcome

Between only post-travel samples of IBS cases versus controls for the IgG response, *Lachnospiraceae* flagellin protein (3-1-57Fla), *Bacteroides eggerthii* glycosyl hydrolase protein (CBir45), and *Roseburia intestinalis* collagen adhesin protein (MDR90) had significantly different log MFI values at the $p < 0.05$ level (Table S-10). For the IgA response, *Lachnospiraceae* flagellin proteins (28-4band, 3-1-57Fla), *Roseburia hominis* flagellin protein (RhomF1), *Salmonella dublin* flagellin protein (SalFliC), *Clostridium citroniae* RecN protein (rIB1), *Flavonfractor plautii* homoserine dehydrogenase protein (rIB12), *Bacteroides vulgatus* protease protein (rIB13), *Flavonfractor plautii* NIpC/P60 protein (rIB17), and *Clostridium asparagiforme* relaxase protein (rIB4) were significantly different between post-travel samples of cases and controls (Table S-11).

In IBS cases, the log change MFI between pre- and post-travel antibody response to microbiota antigens was not significantly different from that of controls for the IgG response. IgG Mean MFI values for pre- and post-travel IBS case and control samples and p-values for differences with FDR correction can be found in Table 13. For the IgA response, the following antibodies were found to have significantly different change in MFI: *Lachnospiraceae* flagellin protein (3-1-57Fla), *Roseburia inulinivorans* flagellin protein (CBir11), *Roseburia hominis* flagellin protein (RhomF1), *Salmonella dublin* flagellin protein (SalFliC), *Escherichia coli* FliC protein (UNCFliC), *Clostridium citroniae* RecN protein (rIB1), *Roseburia intestinalis* nucleotidyltransferase protein (rIB10), *Flavonfractor plautii* NIpC/P60 protein (rIB17), and *Clostridium asparagiforme* relaxase protein (rIB4). Mean IgA MFI values for pre- and post-travel IBS case and control samples and p-values for differences with FDR correction can be found in Table 14.

Table 13. IgG MFI Change-IBS Cases and Controls
Change in IgG serological targets' mean fluorescence intensities between paired baseline and post- samples for IBS cases and controls

Target	Phylum	Class	Protein ID	IBS Cases		Controls		FDR P-value Cases v. Controls
				IgG Mean Change	SD	IgG Mean Change	SD	IgG Mean Change
CBir19	Bacteroides	Bacteroidia	ABC transporter ATP-binding protein	0.43	1.76	-0.07	1.65	0.60
CBir23			Elongation factor 1A	-0.04	0.39	-0.08	0.34	0.86
CBir45			Glycosyl hydrolase	-0.04	0.40	-0.16	0.36	0.60
CBir8			Elongation factor thermo unstable	-0.08	1.07	-0.01	1.37	0.96
P3			HSP90	-0.02	1.45	-0.01	1.30	0.99
Keto			transketolase	0.02	0.52	0.03	0.54	0.98
rIB13			Protease IV/SppA	0.09	1.11	0.14	1.47	0.96
Tetraco			tetratricopeptide repeat protein	0.07	0.90	-0.15	1.57	0.73

CBir14	Firmicutes	Bacilli	Glyceraldehyde-3-phosphate dehydrogenase	0.39	1.24	-0.14	1.15	0.28
EF20			Sal A	0.14	1.90	-0.21	1.47	0.68
AgF1		Clostridia	Flagellin	0.24	1.36	-0.22	1.80	0.60
CBir11			Flagellin	0.00	0.49	-0.25	0.37	0.13
CBir63			Ig-like surface protein	0.02	0.51	-0.04	0.73	0.86
FlaX			Flagellin	0.03	0.56	-0.14	0.61	0.60
MDR24			Adenine deaminase	0.12	1.12	-0.17	1.39	0.68
7			collagen adhesion protein	-0.06	1.48	0.08	1.28	0.86
MDR90			RecN	0.15	0.47	-0.10	0.45	0.16
rIB1			Nucleotidyltransferase/ hypothetical	-0.03	0.64	-0.31	0.58	0.26
rIB10			Homoserine d ehydrogenase	0.20	1.08	0.14	1.59	0.96
rIB12			Helicase	-0.02	0.55	-0.13	0.53	0.68
rIB14			Pyruvate synthase	0.05	0.42	-0.02	0.34	0.68
rIB16			NlpC/P60	0.25	1.10	-0.01	1.32	0.68
rIB17			Glycosyltransferase	-0.15	1.04	0.02	1.14	0.73
rIB8			Methyl-accepting chemotaxis protein	0.28	1.31	-0.01	1.56	0.68
rIB9			Relaxase	0.12	1.51	0.12	1.62	1.00
riB4			SAM domain protein	0.10	1.14	0.01	1.24	0.90
rIB2	Firmicutes and Bacteroides		Hypothetical protein-cytoplasmic	0.12	1.08	-0.11	1.30	0.68
rIB5	Firmicutes and Proteobacteria	Clostridia	Flagellin	0.11	0.67	-0.01	0.66	0.72
28-4band	Firmicutes flagellin	Clostridia	Flagellin	0.21	1.12	-0.24	0.81	0.26
3-1-57Fla			Flagellin 2 from A4 isolate	-0.03	0.46	-0.13	0.70	0.73
A4Fla2			Flagellin 3 from A4 isolate	-0.03	0.60	-0.45	0.58	0.09
A4Fla3			Flagellin	-0.08	0.63	-0.19	0.61	0.72
CBir1			Flagellin	-0.05	0.46	-0.19	0.50	0.60
CBir66			Flagellin	0.06	1.59	-0.44	1.10	0.48
MDR25			Flagellin	0.09	1.22	-0.08	1.08	0.75
4			Flagellin	0.09	1.24	0.00	1.32	0.90
RfaeF3			Flagellin	0.23	1.15	-0.07	1.10	0.63
RhomF1			Flagellin	0.14	0.99	-0.08	1.19	0.68
RhomF2			Flagellin	0.17	0.93	-0.15	1.02	0.60
RintF1			Flagellin	-0.07	1.01	-0.09	0.62	0.96
RinuF5			ABC transporter	0.32	0.96	-0.19	0.67	0.11
rIB20	Proteobacteria	Beta	Methyl-accepting chemotaxis protein	0.17	0.56	-0.03	0.45	0.43
CBir5		Delta/epsilon	Methyl-accepting chemotaxis protein	-0.11	1.27	-0.12	1.38	0.99
CBir56			Surface array protein	0.22	1.17	-0.06	1.21	0.68
rIB18	Proteobacteria	Epsilon	FtsZ protein - homologue of tubulin (putative pANCA)	0.35	1.06	-0.24	0.69	0.10
FtsZ		Gamma	Salmonella dublin flagellin	0.07	0.68	-0.10	0.64	0.66
SalFliC			FliC from UNC101 <i>E. coli</i>	-0.02	1.36	0.18	1.42	0.76
UNCFliC			OmpC from UNC <i>E. coli</i>					
OmpC								

CdtB			Cytolethal Distending toxin	-0.03	1.66	-0.09	1.55	0.96
Vinc	Chordate	Mammal	Vinculin	-0.01	1.02	-0.12	1.36	0.89

Table 14. IgA MFI Change-IBS Cases and Controls
Differences in IgA serological targets' mean fluorescence intensities between paired baseline and post- samples for IBS cases and controls, and IBS cases versus controls post-samples and change between pre- and post- samples.

Target	Phylum	Class	Protein ID	IBS Cases		Controls		FDR P-value IBS Case v. Control
				IgA Change Mean	SD	IgA Change Mean	SD	Change
CBir19	Bacteroides	Bacteroidia	ABC transporter ATP-binding protein	0.36	2.39	-0.15	2.18	0.41
CBir23	Bacteroides	Bacteroidia	Elongation factor 1A	-0.05	0.61	-0.17	0.47	0.41
CBir45	Bacteroides	Bacteroidia	Glycosyl hydrolase	-0.02	0.49	-0.20	0.44	0.17
CBir8	Bacteroides	Bacteroidia	Elongation factor thermo unstable	-0.01	0.87	-0.14	0.75	0.53
P3	Bacteroides	Bacteroidia	HSP90	-0.09	2.37	-0.23	1.98	0.82
Keto	Bacteroides	Bacteroidia	Transketolase	-0.06	0.93	-0.24	0.60	0.41
rIB13	Bacteroides	Bacteroidia	Protease IV/SppA	0.08	0.50	-0.08	0.47	0.21
Tetraco	Bacteroides	Bacteroidia	tetratricopeptide repeat protein Glyceraldehyde-3-phosphate dehydrogenase	-0.29	1.27	-0.35	1.56	0.85
CBir14	Firmicutes	Bacilli	Sal A	0.20	1.84	-0.80	1.79	0.05
EF20	Firmicutes	Bacilli	Sal A	-0.08	2.67	-1.13	1.89	0.1
AgF1	Firmicutes	Clostridia	Flagellin	0.20	1.37	-0.37	1.66	0.17
CBir11	Firmicutes	Clostridia	Flagellin	0.13	0.49	-0.34	0.73	0.02*
CBir63	Firmicutes	Clostridia	Ig-like surface protein	0.15	0.91	-0.39	1.01	0.05
FlaX	Firmicutes	Clostridia	Flagellin	0.05	0.59	-0.27	1.02	0.17
MDR247	Firmicutes	Clostridia	Adenine deaminase	0.10	1.19	-0.50	0.83	0.05
MDR90	Firmicutes	Clostridia	collagen adhesion protein	-0.14	1.63	-0.53	1.48	0.38
rIB1	Firmicutes	Clostridia	RecN	0.17	0.65	-0.25	0.53	0.02*
rIB10	Firmicutes	Clostridia	Nucleotidyltransferase/ hypothetical	0.06	0.84	-0.52	0.95	0.03*
rIB12	Firmicutes	Clostridia	Homoserine dehydrogenase	0.20	0.51	-0.01	0.69	0.21
rIB14	Firmicutes	Clostridia	Helicase	-0.03	0.90	-0.37	0.78	0.14
rIB16	Firmicutes	Clostridia	Pyruvate synthase	0.13	0.64	-0.14	0.49	0.08
rIB17	Firmicutes	Clostridia	NlpC/P60	0.13	0.61	-0.39	0.72	0.02*
rIB8	Firmicutes	Clostridia	Glycosyltransferase	0.01	1.07	0.02	1.23	0.97
rIB9	Firmicutes	Clostridia	Methyl-accepting chemotaxis protein	0.11	2.36	-0.61	1.92	0.21
rIB4	Firmicutes	Clostridia	Relaxase	0.35	0.64	-0.14	0.76	0.02*
rIB2	Firmicutes and Bacteroides		SAM domain protein	0.11	0.57	-0.11	0.45	0.13
rIB5	Firmicutes and Proteobacteria	Clostridia	Hypothetical protein-cytoplasmic	0.08	0.71	-0.12	0.66	0.27
28-4band	Firmicutes	Clostridia	Flagellin	0.08	0.80	-0.09	0.77	0.42
3-1-57Fla	Firmicutes	Clostridia	Flagellin	0.13	1.47	-0.70	1.30	0.04*

A4Fla2	Firmicutes flagellin	Clostridia	Flagellin 2 from A4 isolate	-0.05	0.47	-0.01	1.05	0.85
A4Fla3	Firmicutes flagellin	Clostridia	Flagellin 3 from A4 isolate	0.08	1.16	-0.48	0.97	0.06
CBir1	Firmicutes flagellin	Clostridia	Flagellin	-0.16	0.73	-0.29	0.56	0.43
CBir66	Firmicutes flagellin	Clostridia	Flagellin	0.08	0.68	-0.27	0.73	0.07
MDR254	Firmicutes flagellin	Clostridia	Flagellin	-0.23	2.35	-0.99	2.07	0.21
RfaeF3	Firmicutes flagellin	Clostridia	Flagellin	0.06	2.01	-0.29	0.94	0.41
RhomF1	Firmicutes flagellin	Clostridia	Flagellin	0.10	0.55	-0.26	0.53	0.02*
RhomF2	Firmicutes flagellin	Clostridia	Flagellin	0.18	1.13	-0.22	0.69	0.13
RintF1	Firmicutes flagellin	Clostridia	Flagellin	-0.11	1.38	-0.32	0.57	0.43
RinuF5	Firmicutes flagellin	Clostridia	Flagellin	-0.07	1.70	-0.32	0.66	0.43
rIB20	Proteobacteria	Beta	ABC transporter	0.13	0.94	-0.07	0.98	0.43
CBir5	Proteobacteria	Delta/epsilon	Methyl-accepting chemotaxis protein	0.23	1.57	-0.47	1.10	0.06
CBir56	Proteobacteria	Delta/epsilon	Methyl-accepting chemotaxis protein	0.18	0.89	-0.26	0.68	0.05
rIB18	Proteobacteria	Epsilon	Surface array protein	0.35	2.18	-0.29	0.85	0.17
FtsZ	Proteobacteria	Gamma	FtsZ protein - homologue of tubulin (putative pANCA)	0.09	0.83	-0.25	0.47	0.08
SalFliC	Proteobacteria	Gamma	Salmonella dublin flagellin	0.35	1.37	-0.62	1.16	0.02*
UNCFliC	Proteobacteria	Gamma	FliC from UNC101 <i>E. coli</i>	0.34	1.04	-0.33	0.95	0.02*
OmpC	Proteobacteria	Gamma	OmpC from UNC <i>E. coli</i>	-0.23	1.33	-0.01	0.96	0.43
CdtB			Cytolethal Distending toxin	0.03	0.87	-0.32	0.52	0.08
Vinc	Chordate	Mammal	Vinculin	-0.28	1.53	-0.16	0.83	0.70

Travel exposures

Post-travel response to microbiota antigens was not significantly different between subjects with any reported antibiotic use compared to no use, nor was it different for subjects with antibiotics use for TD compared to no antibiotic use for TD for both IgG and IgA response. Similarly, for both reported antibiotic use categories during travel, the mean difference between pre-and post-travel levels of IgG and IgA antibodies to microbiota antigens was not significantly different. Mean MFI values for antibiotic

exposed and unexposed subjects' pre- and post-travel samples and p-values for differences with FDR correction can be found in Tables S-12 through S-15.

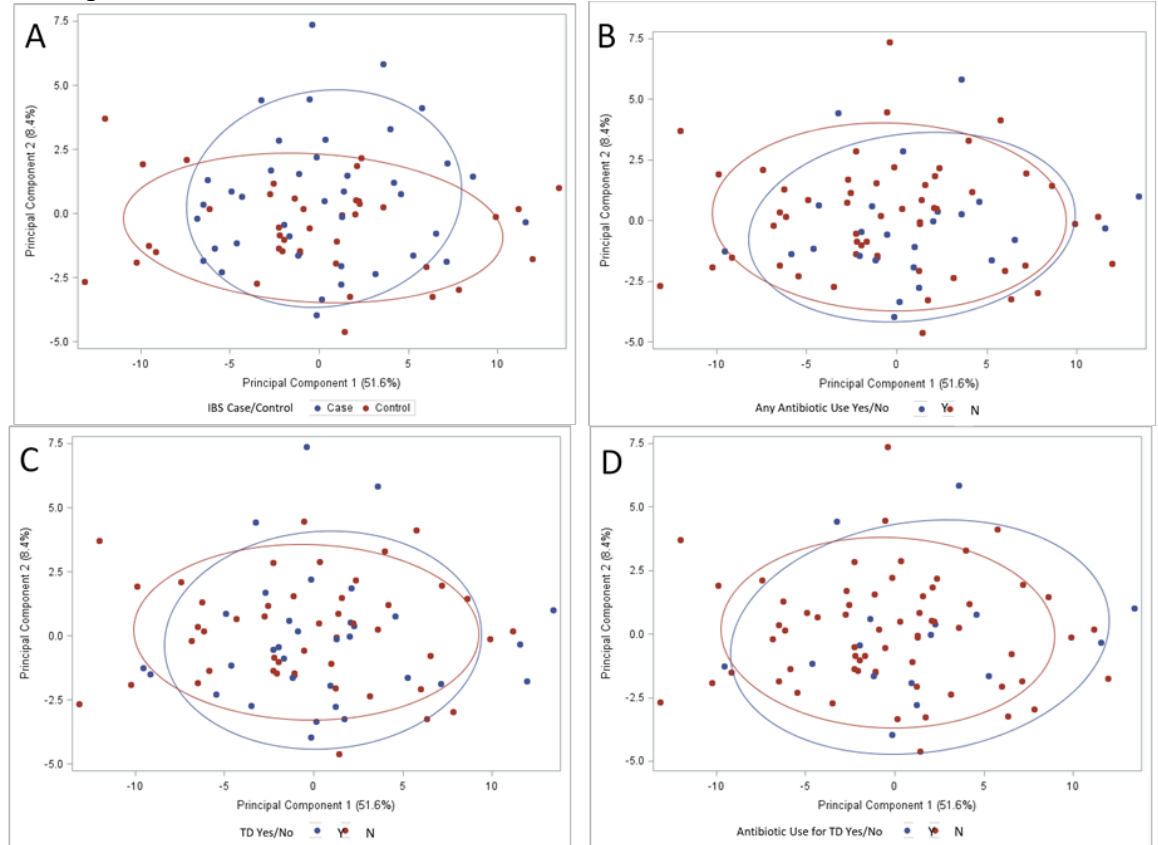
Likewise, no significant difference was observed between the post-travel antibody response to microbiota antigens in subjects with TD versus those that did not report TD, nor was there a significant difference in the change between pre-and post-travel samples for TD+ subjects versus TD- in any of the measured antigens. Mean MFI values for antibiotic exposed and unexposed subjects' pre- and post-travel samples and p-values for differences with FDR correction can be found in Tables S-16 and S-17.

Principal Components Analysis

IgG antibodies to gut microbial antigens

The first component of the PCA (PC1) for IgG targets explained 51.56% of the variation in the data and principal component 2 (PC2) explained 8.43% (see Figure 7).

Figure 7: PCA plots for change in IgG host response
Principal Component 1 v. Principal Component 2; (A) IBS Cases and controls with 75% prediction ellipse; (B) Subjects with any antibiotic use v. without any antibiotic use with 75% prediction ellipse; (C) Subjects with TD v. subjects without TD with 75% prediction ellipse; (D) Subjects with antibiotic use for TD v. no antibiotic use for TD with 75% prediction ellipse



Subjects with similar antibody responses clustered together on the PCA plots. A distinct pattern was not visible for any of the study groups for PC1, however for PC2, subjects appeared to cluster significantly by outcome status (see Figure 7 and Tables 15-16).

Table 15. IgG PCA-PC1 Comparison by Study Group
PCA for change in host response to IgG Microbiota Antigens: Comparison of Principal Component 1 for IBS cases v. controls, antibiotic exposure, and traveler's diarrhea

Principal Component 1 - IgG							
Variable	Category	N	Mean	Std Dev	Min	Max	P-value
Outcome	Case	40	0.31	4.61	-6.86	11.55	0.62
	Control	40	-0.31	6.35	-13.15	13.44	
Any antibiotic use	No	55	-0.39	5.72	-13.15	11.95	0.35
	Yes	25	0.87	5.06	-9.55	13.44	
Antibiotic use for TD	No	64	-0.36	5.47	-13.15	11.95	0.25
	Yes	16	1.43	5.69	-9.55	13.44	
TD	No	47	-0.39	5.73	-13.15	11.17	0.46
	Yes	33	0.55	5.25	-9.55	13.44	

Table 16. IgG PCA-PC2 Comparison by Study Group
PCA for change in host response to IgG Microbiota Antigens: Comparison of Principal Component 2 for IBS cases v. controls, antibiotic exposure, and traveler's diarrhea

Principal Component 2 - IgG							
Variable	Category	N	Mean	Std Dev	Min	Max	P-value
Outcome	Case	40	0.57	2.53	-3.97	7.36	0.02
	Control	40	-0.57	1.74	-4.63	3.69	
Any antibiotic use	No	55	0.13	2.26	-4.63	7.36	0.45
	Yes	25	-0.28	2.19	-3.97	5.83	
Antibiotic use for TD	No	64	0.03	2.19	-4.63	7.36	0.78
	Yes	16	-0.14	2.46	-3.97	5.83	
TD	No	47	0.12	2.04	-4.63	4.46	0.55
	Yes	33	-0.18	2.51	-3.97	7.36	

The top contributing targets-those with influence scores in the bottom 10th or

upper 90th percentile in magnitude for IgG PC1 and PC2 are shown in Tables 17 and 18,

respectively. The antigen target phylum contributing most to either PC1 or PC2 was Firmicutes. All component scores can be found in Table S-18.

Table 17: Top contributing targets to PC1 for IgG host response

Name	Principal Component 1	Phylum	Class	Protein_ID	Function
Cdtb	0.271			Cytolethal Distending toxin	DNase activity
CBir19	-0.028	Bacteroides	Bacteroidia	ABC transporter ATP-binding protein	Other/unknown
riB13	0.230	Bacteroides	Bacteroidia	Protease IV/SppA	Other/unknown
AgF1	0.229	Firmicutes	Clostridia	Flagellin	Motility
CBir14	-0.013	Firmicutes	Bacilli	Glyceraldehyde-3-phosphate dehydrogenase	Metabolism
riB10	-0.009	Firmicutes	Clostridia	Nucleotidyltransferase/ hypothetical	Transc/transl
riB4	0.266	Firmicutes	Clostridia	Relaxase	transc/transl
MDR254	-0.036	Firmicutes flagellin	Clostridia	Flagellin	Motility
UNCfliC	-0.007	Proteobacteria	Gamma	FliC from UNC101 <i>E. coli</i>	motility

Table 18: Top contributing targets to PC2 for IgG host response

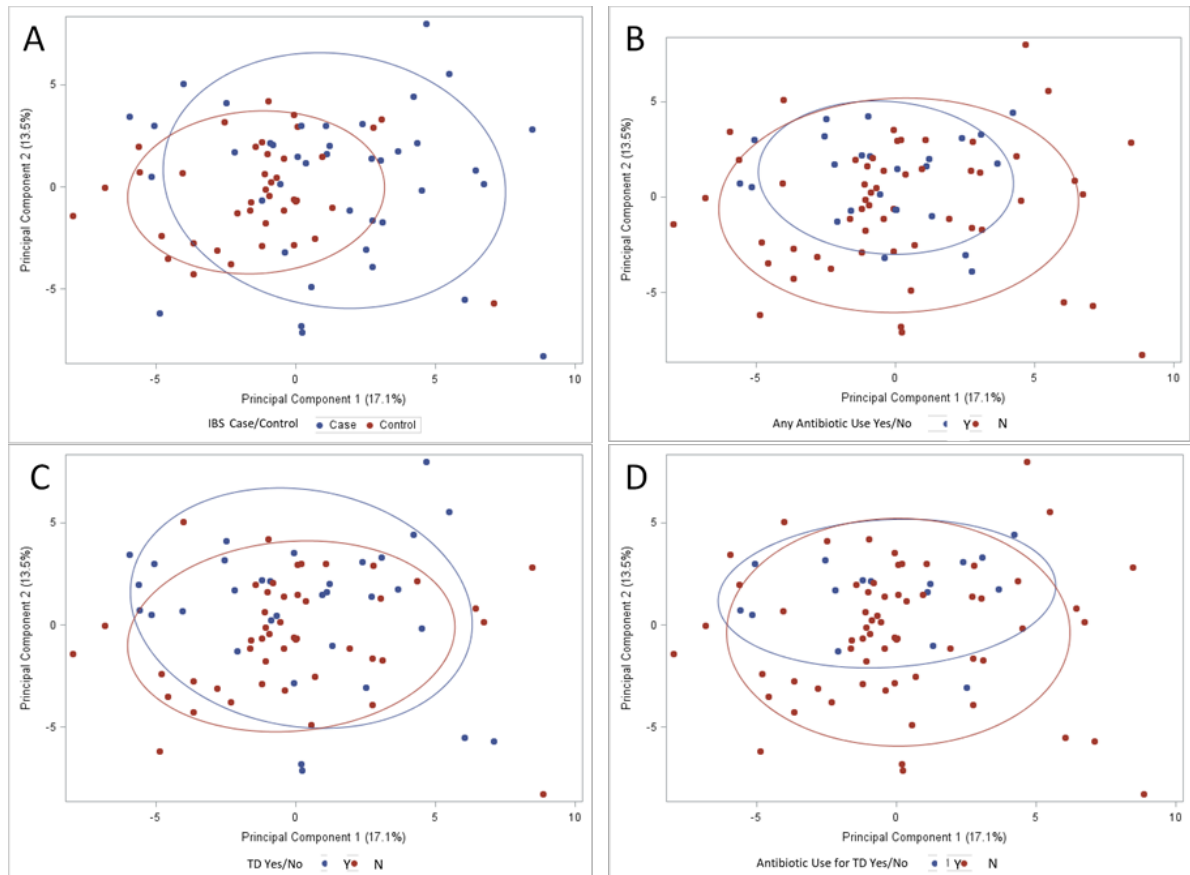
Name	Principal Component 2	Phylum	Class	Protein ID	Function
CBir19	0.517	Bacteroides	Bacteroidia	ABC transporter ATP-binding protein	Other/unknown
Vinc	-0.089	Chordate	Mammal	Vinculin	Cytoskeletal
CBir14	0.324	Firmicutes	Bacilli	Glyceraldehyde-3-phosphate dehydrogenase	Metabolism
EF20	-0.223	Firmicutes	Bacilli	Sal A	Other/Unknown
MDR90	-0.232	Firmicutes	Clostridia	collagen adhesion protein	Other/unknown
riB17	-0.088	Firmicutes	Clostridia	NlpC/P60	Other/unknown
CBir5	0.268	Proteobacteria	Delta/epsilon	Methyl-accepting chemotaxis protein	Motility
SalFliC	0.263	Proteobacteria	Gamma	Salmonella dublin flagellin	Motility
ompC	-0.081	Proteobacteria	Gamma	OmpC from UNC <i>E. coli</i>	Other/Unknown

IgA antibodies to gut microbial antigens

The PCA for IgA targets revealed a PC1 that explained 17.05% of the variation and PC2 explained 13.5% of the variation in the data (see Figure 8).

Figure 8. PCA plots for change in IgA host response

PCA plots for IgA host response to microbiota antigens, Principal Component 1 v. Principal Component 2; (A) IBS Cases and controls with 75% prediction ellipse; (B) Subjects with any antibiotic use v. without any antibiotic use with 75% prediction ellipse; (C) Subjects with TD v. subjects without TD with 75% prediction ellipse; (D) Subjects with antibiotic use for TD v. no antibiotic use for TD with 75% prediction ellipse



Again, a distinct pattern was not readily observed for the study groups, however in a t-test comparison, subjects clustered significantly for PC1 by outcome and for PC2 by both antibiotic exposure categories and TD (see Figure 8 and Tables 19-20).

Table 19. IgA PCA-PC1 Comparison by Study Group
PCA for change in host response to IgA Microbiota Antigens: Comparison of Principal Component 1 for IBS cases v. controls, antibiotic exposure, and traveler's diarrhea

Principal Component 1 - IgA							
Variable	Category	N	Mean	Std Dev	Min	Max	P-value
Outcome	Case	40	1.40	3.65	-5.94	8.87	<0.01
	Control	40	-1.40	2.74	-7.96	7.10	
Any antibiotic use	No	55	0.15	3.83	-7.96	8.87	0.57
	Yes	25	-0.33	2.68	-5.57	4.21	
Antibiotic use for TD	No	64	0.08	3.59	-7.96	8.87	0.68
	Yes	16	-0.32	3.23	-5.57	4.21	
TD	No	47	-0.15	3.47	-7.96	8.87	0.65
	Yes	33	0.21	3.60	-5.94	7.10	

Table 10. IgA PCA-PC2 Comparison by Study Group
PCA for change in host response to IgA Microbiota Antigens: Comparison of Principal Component 2 for IBS cases v. controls, antibiotic exposure, and traveler's diarrhea

Principal Component 2 - IgA							
Variable	Category	N	Mean	Std Dev	Min	Max	P-value
Outcome	Case	40	0.29	3.73	-8.28	7.98	0.41
	Control	40	-0.29	2.38	-5.70	4.21	
Any antibiotic use	No	55	-0.45	3.34	-8.28	7.98	0.05
	Yes	25	1.00	2.35	-3.92	4.42	
Antibiotic use for TD	No	64	-0.38	3.26	-8.28	7.98	0.03

TD	Yes	16	1.51	1.95	-3.06	4.42	0.05
	No	47	-0.57	2.76	-8.28	5.06	
	Yes	33	0.81	3.46	-7.10	7.98	

Top contributing targets with bottom 10th or upper 90th percentile influence scores for IgA PC1 and PC2 are listed in Tables 21 and 22, respectively. Again, the top contributing targets were mostly attributable to Firmicutes phyla antigens for both PC1 and PC2. All component scores can be found in Table S-19.

Table 21: Top contributing targets to PC1 for IgA host response

Name	Principal Component 1	Phylum	Class	Protein ID	Function
CBir19	0.324	Bacteroides	Bacteroidia	ABC transporter ATP-binding protein	Other/unknown
P3	0.218	Bacteroides	Bacteroidia	HSP90	Other/unknown
riB13	0.052	Bacteroides	Bacteroidia	Protease IV/SppA	Other/unknown
CBir14	0.290	Firmicutes	Bacilli	Glyceraldehyde-3-phosphate dehydrogenase	Metabolism
MDR90	-0.036	Firmicutes	Clostridia	collagen adhesion protein	Other/unknown
riB2	0.052	Firmicutes and Bacteroides		SAM domain protein	transc/transl
MDR254	0.375	Firmicutes flagellin	Clostridia	Flagellin	Motility
RinuF5	0.033	Firmicutes flagellin	Clostridia	Flagellin	Motility

Table 22: Top contributing targets to PC2 for IgA host response

Name	Principal Component 2	Phylum	Class	Protein ID	Function
CBir19	-0.416	Bacteroides	Bacteroidia	ABC transporter ATP-binding protein	Other/unknown
P3	0.411	Bacteroides	Bacteroidia	HSP90	Other/unknown
keto	-0.091	Bacteroides	Bacteroidia	transketolase	metabolism
Vinc	0.173	Chordate	Mammal	Vinculin	Cytoskeletal
EF20	0.523	Firmicutes	Bacilli	Sal A	Other/Unknown
MDR90	0.278	Firmicutes	Clostridia	collagen adhesion protein	Other/unknown
riB9	0.265	Firmicutes	Clostridia	Methyl-accepting chemotaxis protein	Motility
MDR254	-0.156	Firmicutes flagellin	Clostridia	Flagellin	Motility

3_1_57Fla	-0.097	Firmicutes flagellin	Clostridia	Flagellin	Motility
CBir5	-0.159	Proteobacteria	Delta/epsilon	Methyl-accepting chemotaxis protein	Motility

DISCUSSION

IBS cases and non-IBS controls with and without important, travel-related exposures were examined in this exploratory study. The travel-related exposures of interest were oral antibiotic use and TD, due to the evidence that these exposures impact the gut microbial environment and have potential to disrupt the function of the gastrointestinal system as a result, leading to chronic consequences such as IBS (78; 92; 94; 100-102; 115; 149; 154). Therefore, this analysis also employed a microbiome protein microarray to explore changes in the host immunological response to gut microbial antigens over time, with baseline and post-exposure sampling.

In the nested case-control analysis, neither antibiotic use nor TD was found to increase the odds of IBS on the univariate level. Therefore, this study does not provide additional support to the hypothesis that antibiotic use during travel is a significant factor in the causal pathway for IBS (78; 94; 149; 154). Much of the literature supports an independent association between TD and IBS and numerous studies have reported a consistent association between AGE caused by various pathogens as well as some evidence of a biological basis for this association (92; 100-102; 115). This epidemiological relationship was not observed in this study. It is likely the low sample

size reduced the power of this analysis, warranting the need for additional studies investigating the association of these important travel exposures with IBS.

Predominantly, the changes in host response to the gut microbiota observed between the outcome and exposure variables were not deemed significant after FDR adjustment. However, there were some notable differences; for the IgA antibody response, several protein targets from predominately Firmicutes phyla with motility functionality were significantly elevated from pre- to post-exposure samples for IBS cases compared to controls. The microarray used in this study was intended to represent a diverse set of antigens from 3 major phyla in the human microbiome (15). Firmicutes flagellin proteins comprise a large portion of the antigens in the panel and were chosen primarily due to known immunogenicity with Crohn's disease, inflammatory bowel syndrome and colitis (15). The observed elevations in response to Firmicutes phyla antigens in this study may indicate that similar mechanisms are affecting IBS cases. The IgA response to *E. coli* FliC protein was observed to be elevated from pre- to post- travel samples for cases but decreased in controls. A significant difference in host response to this protein was also observed in a study using this microarray by Pike, et al, suggesting that this marker may be important in the mechanisms behind IBS pathogenesis and potentially as a diagnostic biomarker (95). Still, many of the significant differences between IBS cases and controls in the antibody responses to gut microbiota that were

identified in this study appear to be largely attributed to substantial changes in pre- to post-travel samples of control subjects.

The study by Pike, et al. also identified a number of serologic markers that were associated with PI-IBS, including pathogen-specific IBS (95). This small pilot study did not observe any significant markers with TD exposure compared to subjects without TD; however overall, subjects with TD displayed more increases in target response over the study compared to subjects without TD. Therefore, the findings of this study may indicate a trend of elevated immunogenicity to particular targets on this microarray that can be investigated further. Additionally, severity of TD can have important implications for IBS risk. While severity of infection was not directly analyzed, antibiotic use for TD can serve as a proxy for increased TD severity. Subjects that used antibiotics for TD were compared to those without antibiotic use for TD; still, no significant differences were observed between the change in host response to any of the antigens for exposed versus unexposed subjects. It is possible a significant change in the host response to serological markers was not detectable due to small group sizes in the study or due to the indirect measure of TD severity.

A PCA analysis was performed to reduce the dimensions of the data by determining which IgG or IgA targets were correlated with one another in relation to outcome or exposure status. PC1 for IgG antigen targets explained much of the variation in the data, and therefore would be a good candidate for reducing the dimensions of the

IgG data, however none of the study groups clustered significantly by PC1 for IgG. Subjects did cluster significantly for IgG PC2 by IBS outcome status, however there was not a strong pattern observed among the top contributing antigens to PC2. Likewise, while there was significant clustering between some of the study groups for IgA PC1 and PC2, a clear pattern was not visible among the top contributing targets to those components.

There are number of limitations to this exploratory study including a small sample size and the use of subjects from two separate studies that differed in methodology, especially in terms of sampling timeline and enrollment requirements. In particular, the use of a baseline sample for TrEAT TD subjects on the same day those subjects presented with TD might have impacted the immune response in the baseline sample and differentiate those samples from true pre-exposure samples. The duration between pre- and post-exposure samples also differed between TravMil and TrEAT TD (92 and 21 days, respectively). This may have limited the ability to properly assess host responses by IBS outcome status, especially for TrEAT TD subjects, due to the truncated timeline. The inclusion of subjects from TrEAT TD was only a small subset of the study and it ensured a greater number of subjects with antibiotic use and TD exposures. Therefore, despite some characteristic departures, TrEAT TD subjects were still included in the study. Still, the small sample size likely impacted the ability to detect a true association between the exposures of interest and development of IBS. As an exploratory study, many of the

study groups had few subjects which might have contributed to many of the insignificant trends observed. Prior research on antibodies to some markers, such as CdtB and vinculin, have shown differential levels and positivity between specific sub-types of IBS (113). Consequently, the non-specificity of the IBS definition in this study might have reduced the ability to detect a significant difference in certain antibody levels in cases compared to controls.

Additionally, the microbiome microarray used in this study is not intended to provide a comprehensive measure of the gut microbiome. A stool microbiome analysis is commonly the assay of choice to analyze the structure and composition of the microbiome (42). A more in-depth analysis of the composition of the microbiome in tandem with the microbiome microarray is necessary to better ascertain the effect of travel exposures on the microbiome and host response and observe the combined relationship with IBS. Additionally, this study did not measure the composition or changes in the gut virome and fungome, both aspects of the gut environment that have functional implications and effect the bacterial communities as well. However, in this case, a proteomic approach is useful to detect in detection of the immune response to gut microbial antigens and some gut alterations (42). Furthermore, a proteomic analysis provides some insight into mechanisms of IBS pathogenesis that otherwise would be harder to ascertain from a stool microbiome analysis alone (42). A cytokine analysis

could also be employed to improve the basis of proteomic biomarker-profiles associated with the travel exposures and IBS outcome (95).

There are a multitude of factors that impact the composition, stability and resilience of the gut microbiome that were not measured or directly accounted for in this study. These include but are not limited to, demographics, diet, health status, BMI, and other non-antibiotic medication use. There is potential for unmeasured confounding in this study as a result. Confounding may have driven the effect estimates towards the null. Additionally, there is potential for misclassification of both exposure and outcome variables in this study, due to the use of self-reporting and surveys to obtain information on the variables. Many subjects had incomplete and missing surveys and study information. It is likely that this misclassification was non-differential and therefore would bias associations towards the null. Ideally, future research in this field should consider both larger sample sizes, improved ascertainment of both exposure and outcome status, and sub-grouped definitions of IBS-types. Also, future studies should measure factors that are known to be associated with the gut microbiome such as diet, severity of TD, antibiotic use indication and timing of antibiotic exposure, health status, and other medication use.

This exploratory study provides a basis for further research into the causal pathway of IBS and the important-and related-association between antibiotics and TD in this pathway. Additionally, the targets found to be significant in this study may provide

important information for the development of clinical biomarkers or exposure-specific biomarkers for IBS. Future studies that employ a larger, prospective study design with improved outcome and exposure ascertainment and expanded measurement of the gut microbiome can provide additional insight on these associations.

CHAPTER 5: Conclusion

FGDs are the most common gastrointestinal conditions in the general population and constitute a considerable burden to the health and quality of life for those experiencing the disorders (9; 11; 35). Despite their frequency, the natural history of FGDs as well as the structural or biological basis of the disorders is not definitively characterized (35; 36). This dissertation expands upon evidence of a post-infectious origin of FGD, evaluating the role of antibiotic use and assessing the effect of antibiotic use and AGE on the gut microbial environment and the host immune response to gut microbiota.

Numerous hypotheses exist concerning the processes preceding FGDs, including: gastrointestinal disruptions, gut microbial dysbiosis, host immune responses, intestinal barrier disruption, functional abnormalities, genetic predisposition, brain-gut microbiome interactions or combinations of these factors (3; 8; 28; 35; 36; 79; 89; 95; 143). There is a recognized relationship between AGE episodes and several biological risk factors for FGD (28; 96; 134). For example, increased levels of anti-microbiota antibodies in IBS cases versus controls in a number of human and animal studies indicate an immunological origin of FGD caused by AGE (95; 97; 113). Furthermore, AGE may disrupt the composition of the gut microbiota directly (28). Antibiotics are used for AGE (usually more severe cases) and are known to influence the composition of the

microbiome, with potential downstream chronic gastrointestinal effects (41; 69).

Similarly, antibiotic use may confound or modify the association between AGE and FGD (28). Additionally, antibiotic use may be an independent risk factor for FGDs (41; 94).

A systematic review was performed to address three separate but related research areas. First, among 20 studies assessing the association between oral antibiotic use and gut microbial dysbiosis, most noted changes from a baseline state in the composition of the gut microbiome after antibiotic administration; however the effects were largely transient (17; 20; 37; 51; 58; 61; 66; 72; 74; 75; 77; 83; 85; 90; 99; 110-112; 153).

Among 24 included studies evaluating the association between gut microbial dysbiosis and development of FGDs, the majority identified an association between dysbiosis in FGD cases compared to healthy controls (10; 12; 21; 24; 33; 43; 44; 63; 67; 88; 93; 106; 107; 109; 144; 145). Additionally, a number of studies pinpointed specific microbial profiles associated with FGDs (16; 55; 73; 76; 105; 117; 130). However, few addressed potential temporal variation in the microbiome. Importantly, there was variability in sample location (upper versus lower GI tract) and type (biopsy, aspirates, and fecal) among these studies, which has implications for interpretation and comparability of these studies. Studies measuring the flora of the small intestine may reveal more detail about the direct causes of symptoms of FGDs such as IBS and dyspepsia, which can manifest in the upper intestinal tract (62; 139). Additionally, mucosal samples may reveal more information regarding the intestinal barrier integrity, inflammation, and function of the

GI tract at the site of the disease (86; 152). Ten studies incorporated mucosal samples of either the small intestine or colon; each of those studies was able to distinguish FGD cases from controls based on either the microbial composition of the samples or inflammatory characteristics (16; 21; 33; 44; 73; 93; 104; 106; 144). Three studies examined colonic mucosal and fecal samples to compare the microbial characteristics in these differing sample types between FGD patients and healthy controls (21; 93; 145). Two of those studies found that analysis of mucosal samples was either different from the fecal samples (145), or had improved ability to distinguish between FGD and healthy controls (93). One study did not find a difference between the colonic mucosal and fecal microbiota (21). Three other studies examined small intestine mucosal samples in addition to either fecal samples or colonic mucosal samples (16; 33; 63). Two of those studies found a distinction between small intestine versus colonic samples for FGD cases compared to controls (16; 33), and one observed similar differences in the small intestine and colon between FGD cases and controls (63). Therefore, analyzing a variety of sample types or locations can provide more information about FGD subjects and more studies are recommended to determine whether small intestine samples and mucosal samples are able to better distinguish FGD versus healthy subjects, FGD types, and FGD severity.

A total of 8 studies evaluated the association between oral antibiotic use and development of FGDs (68; 71; 78; 94; 119; 123; 131; 149; 154). While a positive association between antibiotic use and FGD was observed in the majority of the studies, a

number of limitations lowered the strength of evidence in this association and warrant further research (71; 78; 94; 119; 154).

A nested-case control study from a cohort of active duty US military personnel was performed to examine the association between oral antibiotic use and FGDs. A number of important demographic and military characteristics were assessed as well as the important co-variables AGE, non-GI infection, and axis I and axis II psychological disorders from a complete cohort of active duty military personnel available in the Defense Medical Surveillance system. Due to the sheer size of the active component military cohort over the 10 year study period and the presence of several time-varying covariates in the dataset, the nested case-control design was decidedly the most efficient, technically manageable and powerful analysis approach(136). The nested case-control design is a hybrid approach combining the advantages of using a well-defined cohort with the efficiency of case-control sampling. Since cases and controls are derived from the same cohort population there is likely to be greater comparability between the groups than in traditional case-control study design (46; 141). While there is still potential for loss of power from control sampling, this is minimal; with an adequate number of controls (four) selected for each FGD case, the observed results should agree with results that would have been obtained from analysis of the entire cohort (38; 52). Additionally, incidence density sampling of controls from the risk-set at the time of FGD diagnosis was employed to reduce the likelihood of selection bias compared to traditional case-control

studies; this sampling design eliminated the need for the rare disease assumption for unbiased estimation of the relative risk through the odds ratio effect measure (141).

Antibiotic use was consistently associated with an increased risk of IBS, FC and dyspepsia. Additionally, the risk of IBS increased with a greater number of antibiotic prescriptions during the observation period. Furthermore, individual antibiotic class effects were observed for IBS risk, with an increased risk associated with the following antibiotic classes: cephalosporins, fluoroquinolones, glycopeptides, lincosamide, macrolides, metronidazole, penicillins (amino and natural types), sulfonamides and tetracyclines. For antibiotics known to broadly target gut microbes such as fluoroquinolones, penicillins, this positive association may indicate a disruption of the gut microflora with potential implications for chronic gut function and subsequent elevated risk of FGD. However, it may be that certain antibiotics were used for FGD treatment, if FGD symptoms existed prior to diagnosis. The study was designed to allow for a delay between the antibiotic exposure and incident FGD diagnosis, however the possibility exists that the FGD was either undiagnosed or misclassified at the time of antibiotic exposure. Prospective study designs and information on antibiotic indication could provide more insight into these associations. In addition to antibiotic use, AGE and psychological disorders were significantly associated with the studied FGDs. The positive association between AGE and FGD observed in this study is consistent with the literature, providing external validity to these results (28; 45; 146). We found no

statistically significant interaction between AGE and antibiotic use, indicating that antibiotic use may not modify the relationship between AGE and FGDs. However, given the close relationship between antibiotic use for AGE and severity of infection, these findings warrant further investigation. The positive association between psychological disorders and FGD also provides additional evidence that gut-brain interactions may play a role in FGD development and suggest the utility of future investigation into the relationship (36; 79).

Key travel exposures, antibiotic use and TD, and post-travel IBS related to host immune response profiles to gut microbiota were explored in the final aim of this dissertation. Human seroreactivity to gut microbial antigens has already been investigated in IBS cases and healthy controls at a single time point (95). To build upon those findings, baseline and post-exposure samples were compared to observe changes in the host response following TD and antibiotic use and leading to incident IBS. The false discovery rate (FDR), or the expected proportion of falsely rejected hypotheses, was used to control for multiple comparisons rather than the family-wise error rate. Correction for the FDR avoids unnecessarily stringent control for the possibility of error, with attention paid to the overall between group differences so that a small number of errors do not alter the overall validity of the results (5). Therefore, the FDR method allowed for higher power in detection and for increased adaptability and stability in the findings (5).

It was hypothesized that some correlation may exist between microbiome biomarkers for the subjects in the study; a principal component analysis (PCA) was performed as a relatively simple, exploratory method to reduce the high dimensionality of the log-normalized microarray data (108; 129). A PCA enables the transformation of a large number of correlated variables into a small number of uncorrelated variables (principal components) (129). If correlations exist, this allows for dimension reduction of the data, and improved interpretability of biomarker profiles associated with study factors such as FGD case status and the travel exposures of interest. There are some limitations to PCA, such as loss of information and the assumption that the components with the largest variance will also contain the greatest amount of information about the data groupings; since the data is transformed without considering the groupings, this is not guaranteed (129). However, PCA has advantages over other ordination analyses such as PCoA or NDMS because the results provide a direct link between the components and the original values, therefore the results have improved interpretability and usefulness (108).

While a significant epidemiological link was not confirmed in this exploratory study, the directionality of the associations between the exposures was consistent with the literature (78; 92; 94; 100; 102; 115; 149; 154). Additionally, the change in the host immune response for several antigen targets was determined to be significantly associated with IBS cases versus controls, indicating a departure in the host immune

response that might be a foundation for the pathogenesis of IBS. In particular, the significant increase from baseline in host response to the antigen *E. coli* Fli C in IBS cases compared to controls is consistent with the elevated response seen in IBS cases compared to controls in Pike, et al (95). Additionally, the majority of antigens showing a significant increase in post-travel IBS cases versus controls were Firmicutes flagellins. These antigens are known to be associated with other chronic GI conditions, suggesting these markers may play a role in IBS (15). Similar findings have been found in a previous study by Schoepfer, et al, displaying an elevated host immune response to Firmicutes flagellins in IBS cases compared to healthy controls (however the specific antigens-A4-Fla2 and Fla-X- were not found significantly increased in IBS cases compared to controls in this study) (124). Further research is needed to investigate these targets, in a larger population, and to provide more biological evidence on these associations through additional measurements of accompanying immunological biomarkers such as cytokines and with simultaneous assessment of the composition of the microbiome.

Future studies can build upon this dissertation in several ways: (1) Use of larger sample size and prospective study design (prospective cohort or randomized controlled trial) to delineate temporality of important exposures such as antibiotic use and TD and the FGD outcomes; (2) Sub-typed definitions for FGDs, such as IBS with constipation, diarrhea or mixed to improve significance of specific associations related to sub-type disease presentations; (3) Measurement of all known important covariates related to the

exposures, FGD outcomes and the gut microbial environment; (4) Improved ascertainment of exposures (such as antibiotic type and class), covariates and outcomes; (5) Longitudinal sampling to examine inter-individual variability over time and in relation to important exposures; (6) Assessment of fecal samples as well as the mucosal microbiome throughout the GI tract to assess the impact of antibiotic exposure on a variety of GI environments and the implications for different FGD types; (7) Assessment of the microbiome and host immune response simultaneously over time.

Therefore, an improved study design to investigate the association between antibiotics and risk of FGD while providing information on mechanisms and causation would incorporate a randomized controlled trial sourced from the general population, with extensive demographic information and medical history to measure important factors such as BMI, diet, history of medication use and mental health. The randomized design should balance the effect of unknown confounders. To address the independent effect of antibiotic use on risk of FGD compared to the effect of antibiotic use for AGE or non-GI infection and FGD risk, the study should have multiple arms (antibiotics, AGE + antibiotics, non-GI infection + antibiotics, and control/unexposed). These study groups would permit analysis and comparison of these varying exposure effects. The following antibiotic classes should be considered in particular, due to the specific antibiotic class effects observed in this study: fluoroquinolones, glycopeptides, lincosamide, macrolides, metronidazole, and amino penicillins. Multiple sampling time points, both pre and post

exposure and with a sufficient follow-up time frame to account for disease onset and progression and a variety of sample types from different locations in the GI system (small intestine and colon) and analytic methodologies, such as both 16s rRNA sequencing of fecal and mucosal sampling, plus serological analysis of microbiome antigens, cytokines and other markers of the host immune response would allow for broad surveillance of the microbial environment and host response simultaneously with exposure and disease timeline. These improvements would permit a comprehensive analysis of the natural history of FGD and what role antibiotic use plays in the development of these conditions.

FGDs contribute significantly to morbidity and health care costs in the general population and the US military. This work contributes to a public health and military need to increase the understanding of these disorders and highlights the need for primary and secondary prevention. A gap in the literature, on the epidemiological link between antibiotic use and risk of FGD, was identified and addressed. In a population of active duty military members, a consistent and specific relationship between antibiotic exposures and risk of FGD was observed. These findings raise the level of available evidence on the relationship and suggest further reason for providers to use caution when prescribing antibiotics in situations where they may be unnecessary or counterproductive. Additionally, an exploratory analysis of the host response to gut microbial antigens over a timeline of travel exposures and post-travel IBS provided some clues to important biomarkers, such as Firmicutes flagellins and *E. coli* FliC, that may play a role in IBS

pathogenesis or have potential to serve as diagnostic markers. There is a need for additional investigation into the host response to these antigens together with analysis of the structure and composition of the entire microbiome in the context of incident IBS, and relevant exposures such as AGE and antibiotic use.

SUPPLEMENTAL INFORMATION

Table S-1. Antibiotics and Dysbiosis: Full Characteristics of included studies

First author, year of publication	Study Size (N)	Comparison group	Number exposed to antibiotics	Follow-up Period	Measurement of Dysbiosis	Dysbiosis described*	Conclusions	Antibiotic type(s)
Edlund, 2000	12	none	12	35 days	Fecal samples; culture methods used to characterize the microbiota	Moxifloxacin, during treatment, compared to pre-treatment levels: ↓enterococci ↓E.coli ↑Candida Clarithromycin, during treatment, compared to pre-treatment levels: ↓E.coli (stabilization by day 35) ↓Bifidobacteria (stabilization by day 35) ↓total anaerobes ↓bacteroides ↓lactobacilli ↓clostridia	Administration of moxifloxacin causes selective reduction in normal microflora affecting primarily Enterobacteriaceae and enterococci; in contrast to clarithromycin, moxifloxacin did not cause significant reduction in the total number of total anaerobic microflora	Moxifloxacin (400mg once daily for 7 days), Clarithromycin (500mg 2x daily for 7 days)
Lode, 2001	12	none	12	35 days	Fecal samples collected, analyzed using culture methods and identified to genus level by morphological, biochemical and molecular analyses	Linezolid, during treatment, compared to pre-treatment levels: ↓enterococci ↑Klebsiella ↓bifidobacteria ↓lactobacilli ↓clostridia ↓Bacteroides (all stabilized by day 35) Amoxicillin/Clavulanic acid, during treatment, compared to pre-treatment levels: ↑Enterococci ↑E.coli ↓bifidobacteria ↓lactobacilli ↓clostridia (all stabilized by day 35)	Administration of linezolid mainly impacted the gram-positive bacteria and thus linezolid displays a different ecological profile from amoxicillin/clavulanic acid	Linezolid (600mg 2x daily for 7 days), Amoxicillin/Clavulanic acid (1000mg once daily for 7 days)
Matute, 2002	18	placebo treated	12	42 days	Bacterial species from fecal samples and throat washes were identified and quantified	Azithromycin and Clarithromycin ↓Enterobacteriaceae family ↔ total anaerobes (all stabilized by day 21)	No significant differences in the effect on the gut microbiota between azithromycin and clarithromycin treated groups were observed; microflora levels returned to normal 21 days after therapy for all groups	Azithromycin, Clarithromycin
De la Cochetiere, 2005	6	none	6	60 days	Fecal samples were collected and underwent 16s rRNA gene amplification and TTGE analysis to define temporal similarity of the microbiome	TTGE profiles compared to day 0, ↓73% similarity on day 3, 88% similarity by day 30, 89% similarity by day 60. 2 subjects had values < 90% at day 60 (one as low as 66%)	This study demonstrated resilience in the human gut microbiome following short-course antibiotic treatment, but persistence of long-term alterations were observed in a small subset	Amoxicillin (1.5g/day oral for 5 days)
Monreal, 2005	42	blood donors at the same hospital	22	30 days	Fecal samples were collected and culture methods used to characterize the microbiota	↓cfu of <i>Bifidobacterium</i> and <i>Lactobacillus</i> spp. (all stabilized by day 30)	This study showed that the use of amoxicillin following bacterial respiratory tract infection influences the intestinal microbiota with recovery after 30 days	Amoxicillin indicated for treatment of respiratory tract infection
Lofmark, 2006	8	healthy volunteers not receiving antibiotics	4	2 years	Fecal samples were collected and culture, biochemical and genotyping methods were used to identify strains of <i>Bacteroides</i> spp.	↓ <i>Bacteroides</i> number and variety; alterations in species diversity and enrichment of resistant strains remained up to 2 years post-treatment	The results indicate that short-term antibiotic administration can cause long-term alterations in the commensal microbiota of individual subjects, detectable 2 years after dose	Clindamycin (150mg 4x daily for 7 days)
Jernberg, 2007	8	healthy subjects not receiving any antibiotics	4	2 years	Fecal samples were collected and clonal typing was performed by repetitive sequence-based	Large and persistent variations were observed in the antibiotic group compared to controls; the <i>Bacteroides</i> community never stabilized to original composition during the study period	Long after selection pressure of a short term antibiotic exposure is removed, there are persistent long term impacts on the intestinal microbiota that remain up to 2 years post-treatment	Clindamycin (150mg oral 4x daily for 7 days)

					PCR and T-RFLP (molecular fingerprinting technique)			
Koning, 2008	38	probiotic + antibiotic	38; 19 antibiotic only, 19 antibiotic +probiotic	63 days	Fecal samples were collected and underwent bacteriological culture to examine microbiota composition	Amoxicillin only: ↑enterococci ↓lactobacilli ↓spore-forming clostridia (stabilized by end of study) Amoxicillin + probiotic: ↓total aerobes ↑total anaerobes ↑ <i>Bacteroides</i> spp. (stabilized by end of study)	No significant differences in bacterial counts between groups was observed except for an increase in enterococci, however changes over time were present in both groups that indicated an amoxicillin effect that differed between groups	Amoxicillin (500mg 2x daily for 7 days)
Lindgren, 2009	8	healthy subjects not receiving any antibiotics	4	2 years	Fecal samples were collected and analyzed using culture methods	↓enterococcal colonies ↑ <i>Enterococcus</i> species variation (severe intestinal disturbances lasted over 9 months, but stabilization by study end)	Short-course treatment of clindamycin had a prolonged impact on both persistence of erm(B)- mediated MLSB resistance and species variation in the intestinal enterococci.	Clindamycin (150mg capsules orally 4 times daily for 7 d)
Rashid, 2013	34	placebo treated	17	20 weeks	Fecal specimens were collected and culture techniques were used to evaluate the microbiome	There were changes in numbers of enterococci and <i>E.coli</i> during doxycycline use but aerobic microflora returned to normal by week 20; there were no significant changes in anaerobic intestinal microflora over the duration of the study	A minor effect of sub-antimicrobial dose of doxycycline was observed on the intestinal microflora	Doxycycline (sub-antimicrobial dose of 40mg once daily for 16 weeks)
Pallav, 2014	22	no treatment group; probiotic group	8	8 weeks	Stool specimens were collected and 16s sequencing was used to analyze the microbiome	↑ <i>Escherichia/Shigella</i> and changes in prevalence of several bacterial genera (recovery towards baseline evident by end of study)	Amoxicillin alters the microbiome and recovery from this disruption can take several weeks	Amoxicillin (250mg 3x daily for 7 days)
Stewardson, 2015	42	controls w/o antibiotic treatment; household contact of ciprofloxacin patient	20	4 weeks	Fecal samples were collected and 16s rRNA pyrosequencing was used to characterize microbiota	Ciprofloxacin: ↓ <i>Faecalibacterium</i> ↓ <i>Ruminococcus</i> (phylum Firmicutes) ↓ <i>Allistipes</i> (Bacteroidetes) ↑ <i>Bacteroides</i> (Bacteroidetes) ↑ Lachnospiraceae family (Firmicutes)	Ciprofloxacin was observed to have a significant, though reversible impact on the intestinal microbiota in patients with UTI, whereas nitrofurantoin had minimal impact of the intestinal microbiota. The data suggest that a weak effect of ciprofloxacin persists after 1 month.	Ciprofloxacin (500mg 2x daily); Nitrofurantoin macrocrystals (100mg 2x daily) indicated for Urinary Tract Infection
Heinsen, 2015	5	none	5	42 days	Fecal samples and sigmoidal biopsies were collected; 16s rRNA and cDNA sequencing used to evaluate the luminal and mucosal-associated microbiota	↓ <i>Blautia</i> ↓ Lachnospiraceae ↓ <i>Ruminococcaceae</i> ↓ <i>F.prausnitzii</i>	The results display fundamental changes in composition of both the luminal and mucosa-associated gut microbiota under antibiotic therapy at the potential activity and abundance level	Paromomycin 4g oral for 3 days
Vickers, 2015	56	placebo treated	40	9 days	Fecal samples were collected and culture methods used for gut microbiota analysis	↓total clostridia in 2 study groups ↑bacteroides ↓lactobacilli in one study group ↔ bifidobacteria ↔ total anaerobes	With the exception of total clostridia, minimal disruption to the gut microbiota was observed in this trial	SMT19969, a novel non-absorbable antibiotic (2-2000mg single oral dose or 2 oral doses of 200/500mg)
Rashid, 2015	30	placebo treated	20	1 year	Fecal samples were collected for microbiota analyses at 6 time points and 16s rRNA sequencing was used to evaluate	Ciprofloxacin: ↔gram-negative anaerobic bacteria ↔gram-positive aerobic bacteria ↓gram-negative anaerobes at day 11; pyrosequencing showed compositional changes of the microbiota from month 1-12. Clindamycin: ↔gram-negative aerobic ↔anaerobic bacteria ↓gram-positive aerobic ↓anaerobic bacteria (stabilized between month 1-12); pyrosequencing showed changes in the microbial	Normalization of gut microbiota took 1-12 months after antibiotic administration, with the most pronounced effect at day 11; time for normalization varied between bacterial species; there was a strong effect on the diversity of the microbiome with changes observed until the 12th month and the most pronounced microbial shift at 1 month. Pyrosequencing indicated clindamycin had a higher impact than ciprofloxacin	Ciprofloxacin (500mg twice daily for 10 days) or Clindamycin (150mg 4 times daily for 10 days)

						composition persisted until month 12		
Mikkelsen, 2015	12	none	12	180 days	Fecal samples were collected and enumeration of bacteria was performed by plate counting methods	↓total anaerobic bacterial count ↓enterococci ↓coliforms, ↓bifidobacteria ↑aerobic bacteria ↑facultatively anaerobic bacteria (stabilization 180 days after antibiotic course)	A broad-spectrum 4-day antibiotic course induced shifts in gut microbiota composition that had no clinically relevant short or long term effects	4 day, 3 drug oral antibiotic course (vancomycin 500mg, gentamycin 40mg, meropenem 500mg once daily)
Citron, 2016	30	healthy volunteers not receiving antibiotics	24	13-15 days	Fecal specimens were collected and analyzed using culture methods and SYBR green quantitative PCR analyses to quantify the fecal microbiota	Minimal disruptive impact; Post-exposure counts of ↓clostridia ↓enterococcus ↓streptococcus ↔enterobacteria ↔ <i>B. fragilis</i> ↑ <i>Enterobacteriaceae</i>	Surtoymycin has modest effects on gut microbiota that persisted to the end of the study (13-15 days)	Surtoymycin (oral doses of 250, 500, 1000 mg twice daily)
Raymond, 2016	24	healthy volunteers not receiving antibiotics	18	90 days	Fecal samples were collected and analyzed using shotgun metagenomic sequencing to characterize the gut microbiome	↓overall richness ↓levels of 6 bacterial families (40 species) ↑3 genera (11 species) ↓marginal in Simpson diversity ↑ <i>L. bolteae</i> (most consistent effect) ↑ <i>E. cloacae</i> complex in subset of 5 subjects initially associated with low diversity <i>Bacteroides</i> -rich cluster. Variability of microbiota was comparable to controls by day 90	Three effects of the antibiotic course were observed in subjects, 1) a reproducible effect on the microbiome in almost all subjects involving an increase in specific bacterial genera and a decrease in several bacterial families 2) in a subset of subjects, there was an observed increase in <i>E. cloacae</i> complex bacteria observed in participants that initially had lower diversity in the <i>Bacteroides</i> enterotype 3) resistance genes that were undetectable before treatment appeared in some cases	Cefprozil 500mg oral for 7 days, a second generation cephalosporin
Pop, 2016	30	none	30	84 days	Fecal samples were collected and 16s rRNA gene sequenced to characterize the microbiome	↓overall gut microbial diversity (stabilized by days 28 and 84) ciprofloxacin treatment appeared to have a more severe impact on overall diversity than diarrheal disease	The study demonstrated that a short-term, single course of ciprofloxacin for infection is unlikely to result in long-term and large-scale microbiota perturbations	Ciprofloxacin (500mg twice daily for 3 days following challenge with <i>E. coli</i> (ETEC H10407))
Kabbani, 2017	49	probiotic, probiotic + antibiotic, no treatment	12 antibiotic; 12 probiotic + antibiotic	21 days	Fecal samples were collected and underwent 16S rRNA pyrosequencing to evaluate differences in the microbiome	Amoxicillin-Clavulanate: ↑ <i>Parabacteroides</i> ↑ <i>Enterobacter</i> ↓ <i>Roseburia</i> ↑ <i>Escherichia</i> (positively correlated with antibiotic associated diarrhea) Amoxicillin-Clavulanate + probiotic: ↑ <i>Odoribacter</i> ↑ <i>Stenotrophomonas</i>	The result show that antibiotic therapy with Amoxicillin-Clavulanate led to substantial alterations of the gut microbiota that reverted towards baseline but were still not completely resolve 2 weeks after completion of the course; when a probiotic was administered together, microbiota changes were lessened	Amoxicillin-Clavulanate (875/125mg twice daily for 7 days)

*Where a directional effect on the microbiota was indicated the following symbols are used: ↓ Decrease ↑ Increase ↔ Stability

Table S-2. Dysbiosis and FGD: Full Characteristics of Included Studies

First author, year of publication	Study Setting	Study Size (N)	Control Group	Measurement of FGD	Number with FGD outcome	Follow-up Period	Measurement of dysbiosis	Dysbiosis described in IBS patients	Conclusion
Dlugosz, 2017	Clinic	36	N=14 HC without functional gastrointestinal disorder or antibiotic/probiotic use for 1 month prior to biopsy	Patients meeting Rome II criteria for IBS from Karolinska University Hospital, Huddinge Outpatient Clinic were recruited as cases	22 IBS (7 IBS-D, 8 IBS-C, 7 other)	N/A	Mucosal biopsy of the proximal jejunum and distal sigmoid colon; measurement of mRNA and protein expression of TLR2, TLR4, TLR5, TLR9-the primary mucosal sensors of bacterial patterns in the small and large bowel mucosa	Upregulation of TLR4, TLR5 and TLR9 in the small bowel in IBS patients compared to HC	Altered expression of TLRs in the small bowel may be involved in the pathogenesis of IBS

Labus, 2017	University	52	N=23 HC meeting exclusion criteria	Subjects were evaluated by medical history and physical exam by gastroenterologist or nurse practitioner to confirm diagnosis of IBS according to Rome III symptom criteria	29 IBS (10 IBS-D, 1 Alternating, 5 Mixed, 2 unspecified)	N/A	Stool sample; 16s rRNA gene sequencing to profile stool microbial communities and quantitate microbial composition, abundance, and diversity	Increased abundance of Firmicutes (phylum level), Bacilli and Clostridia (class level), Lactobacillales (order level), Holdemania (genus level) and decreased abundance of Bacteroides (phylum level), Bacteroidia (class level), Bacteroidales (order level), Parabacteroides (genus level) compared to HC. Increased F-B ratio compared to HC. The observed gut microbial signatures were not found to be associated with IBS type or symptom presentation	IBS gut microbial composition was not found associated with IBS type or IBS symptom presentation
Ringel-Kulka, 2015	Community	76	N=20 HC with no recurring GI symptoms	Subjects were evaluated for their clinical symptoms using the Rome III IBS module	56 IBS (26 IBS Bloating, 6 IBS without Bloating) (21 IBS-D, 21 IBS-C, 14 IBS-M)	N/A	Stool sample; 16s rRNA gene sequencing to profile stool microbial communities and quantitate microbial composition, abundance, and diversity	IBS with bloating and IBS without bloating groups showed elevation of the genera Lactobacillus and Streptococcus versus HC. Decreases among Ruminococcaceae taxa in IBS without bloating compared to IBS bloating and HC. All IBS subjects displayed significant depletion of Lachnospiraceae members. Actinobacteria phyla were significantly elevated in IBS-C subjects. Clostridiales members (Firmicutes phyla) were significantly decreased in IBS-D compared to all other groups and IBS-M compared to HC and IBS-C subjects.	In IBS, intestinal microbiota differences were found to be associated with clinically relevant IBS types, symptoms and bowel habits
Chung, 2015	Hospital	47	N=19 HC receiving an endoscopy during a health check-up	Patients were evaluated for IBS using Rome III criteria	28 IBS (14 IBS-D, 7 IBS-C, 7 IBS-M)	N/A	Jejunal mucosal biopsies and fecal samples, analyzed using 16s rRNA sequencing methods	IBS patients had higher abundance of Veillonellaceae in stool and Prevotellaceae in small intestine and lower abundance of Mycobacteriaceae and Neisseriaceae in small intestine compared to HC. The ratio of Firmicutes to Actinobacter in the small intestine of IBS-M patients was 9-fold higher than health controls and the ratio of Firmicutes to Bacteroidetes was 3-fold higher in IBS-D patients than HC.	Microbiota signatures were found associated with IBS compared to healthy controls and also found associated with certain IBS types
Mansour, 2016	University	90	N=20 HC with no recurring GI symptoms	Patients were evaluated through complete medical history taking and physical examination for IBS using Rome III criteria	70 IBS (20 IBS-C, 18 IBS-D, 32 IBS-M)	N/A	Fecal samples taken to measure coliform count and serum samples for measurement of miRNA-199b expression	Inverse relationship between coliform count and miRNA-199b expression; increased coliform count in IBS compared to HC (IBS-D highest count and HC lowest); decreased expression of miRNA-199b in IBS compared to HC	This study found an increased count of Coliform and a decreased expression of serum miRNA-199b in IBS patients and IBS subtypes compared to HC
Parthasarathy, 2016	Clinic	50	N=25 HC female	Patients met Rome III criteria for chronic constipation	25 Chronic Constipation (13 functional constipation, 6 IBS-C, 6 IBS-M)	N/A	Stool samples and colonic mucosal biopsies were performed; 16s rRNA gene sequencing assessed overall composition and OTUs of the microbiota; lactulose breath test for methane production identification and colonic transit were also measured	Abundance of Phylum Bacteroides (primarily due to the abundance of Flavobacterium) was 1.5 times higher in constipated patients than HC. Genus Faecalibacterium was also elevated in constipation versus HC.	Microbiota profiles in the colonic mucosa were found to differentiate patients with constipation from HC; fecal microbiota profiles were found associated with colonic transit and methane production but not with constipation
Pozuelo, 2015	Clinic	179	N=66 HC	Patients met Rome III criteria for IBS based on answers to a clinical questionnaire	113 IBS (54 IBS-D, 32 IBS-C, 27 IBS-M)	72 IBS (33 IBS-D, 26 IBS-C, 13 IBS-M) and 22 HC provided second stool	Fecal samples were taken and 16s rRNA sequencing was performed to characterize and quantify the microbiota	At a global level, there were observed differences between IBS-D and IBS-M and IBS-C and HC. At the taxonomic level there were depleted levels of Methanogens, Butyrate-producing bacteria (Erysipelotrichaceae; Ruminococcaceae) in IBS-D and IBS-M and increased abundance of Methanogens in IBS-C. There were correlations between IBS symptoms, including positive correlation	Subjects with IBS and based on IBS type clustered separately from HC at a global level, with major differences at phylogenetic levels, differences between IBS subtypes, correlation of groups of bacteria with

						sample 1 month after the first		between Lachnospiraceae and sensation of flatulence, positive correlation between Bacteroides and Ruminococcus and Prevotella and Catenibacterium and abdominal pain and negative correlation between an unknown Erysipelotrichaceae and abdominal pain.	certain symptoms and an effect of medication on the gut microbiota
Casen, 2015	Hospital	668	N=297 HC with no current and no history of IBS, IBD or other organic GI-related disorder	Patients were diagnosed with IBS using Rome II or III criteria from previous prospective studies	236 IBS (44% IBS-D, 22% IBS-alternating, 17% IBS-C, 11% IBS-unsubtyped, 4% IBS-M)	For 5 individuals (health status not indicated), fecal samples were collected at 1-week intervals for up to 14 weeks	Fecal samples were taken and 16s rRNA sequencing was performed to characterize and quantify the microbiota	73% of IBS patients were determined to be dysbiotic based on the dysbiosis index test; the predominant bacteria contributing to dysbiosis in IBS patients (showing increased signal intensity) were Firmicutes (Bacilli), Proteobacteria (Shigella/Escherichia), Actinobacteria and Ruminococcus gnavus	This study provided evidence that a dysbiosis index test can prove clinically useful in determining dysbiosis and monitoring treatment regimens/evolution of new approaches to treatment
Giamarellos-Bourboulis, 2015	Hospital	258	N=21 HC, N=163 non-healthy, non-IBS controls	Subjects meeting Rome III criteria following baseline assessment and upper GI tract endoscopy	74 IBS	N/A	Duodenal aspirates were taken during endoscopy and largescale qPCR analyses performed to analyze bacterial diversity and compare levels of bacteria. 16s amplicon sequencing analysis was performed in a random subset of 5 IBS patients and 5 HC	Escherichia/Shigella and Aeromonas genera were enriched in IBS patients compared to HC	Patients with IBS display an overabundance of microbes in the small bowel and reduction in overall diversity
Chassard, 2012	Community	26	N=12 sex-matched HC with no GI symptoms and normal stool frequency	Subjects were classified as IBS-C by a questionnaire with Rome II subgrouping criteria	14 female IBS all classified as IBS-C	N/A	Fecal samples were taken and culture methods were used to enumerate functional groups of microbiota	Population levels of Enterobacteriaceae were increased 10-fold in IBS-C compared to HC; bifidobacteria and lactobacilli were present in significantly lower numbers in IBS-C compared to HC; methanogen-harboring microbes and H2-consuming sulphate-reducing bacteria (SRB) were observed in higher amounts in IBS-C compared to HC	A major functional dysbiosis is observed in IBS-C patients compared to HC when taking a function based, culture approach to analyze the microbiota
Shukla, 2015	Clinic	77	N=30 HC, age and gender matched	Patients were classified using a validated Rome III diagnostic questionnaire	47 IBS (20 IBS-C, 20 IBS-D, 7 IBS (unknown))	N/A	Fecal samples were taken and fecal microbiota were quantified using qPCR and relative difference in 16s rRNA copy numbers	In IBS compared to HC, the relative difference of Bifidobacterium species was lower, while that for <i>Bacterioides thetaiotamicron</i> , <i>R. productus</i> , <i>C. coccoides</i> , <i>Veillonella sp.</i> , <i>P. aeruginosa</i> and Gram-negative bacteria were higher. Copy numbers of <i>Lactobacillus</i> species were lower but copy numbers of <i>Bacteroides thetaiotamicron</i> and SFB were higher among IBS-D than IBS-C. <i>P. aeruginosa</i> was frequently detected and higher in number in IBS than HC. Patients with visible abdominal distension had increased number of <i>Bacteroides thetaiotamicron</i> , <i>Clostridium coccoides</i> , <i>P. aeruginosa</i> , Gram negative and SFB and abdominal bloating/discomfort was associated with greater number of <i>Clostridium coccoides</i> and SFB.	The findings support the hypothesis that different phenotypes of IBS were associated with distinct gut microbiota
Tap, 2017	Clinic	149	N=39 HC without chronic disease or current GI symptoms	Patients met Rome III criteria for IBS based on typical clinical presentation and additional	110 IBS (18 IBS-C, 43 IBS-D, 43 IBS-M, 2 IBS-unsubtyped)	N/A	Fecal samples and biopsy samples were taken and 16s rRNA sequencing was performed to assess the microbial composition	When using classic ecologic approaches, no difference observed between HC and IBS patients or between IBS subtypes. No difference regarding alpha diversity or beta diversity, but a trend toward	Using classic ecological approaches, no differences were observed between IBS and HC. With a machine learning

				investigation if considered necessary by gastroenterologist				reduction in richness of fecal microbiota was observed in IBS. Prevalence of Prevotella enterotype decreased and Bacteroides enterotype increased as symptom severity increased in subjects with severe IBS. OTUs including <i>Faecalibacterium</i> , <i>Oscillibacter</i> , <i>Blautia</i> , and <i>Coprococcus</i> spp. were more prevalent in HC than in severe IBS.	approach, identification of a gut microbial signature for IBS severity was possible (detected in both fecal microbiota and intestinal mucosa)
Liu, 2016	Clinic	100	N=20 HC	Patients were evaluated by a gastroenterologist according to Rome III criteria	40 IBS-D, 15 Depression, 25 co-morbid IBS and Depression	N/A	Fecal samples and tissue biopsy samples were evaluated using 454 pyrosequencing to characterize the gut microbiome	Microbial diversity according to the Shannon index was significantly lower in IBS-D and depression patients compared to HC. Microbial communities were dominated by high levels of Bacteroides and Prevotella compared to HC.	This study identified defined microbial signatures associated with abdominal symptoms in IBS-D and depression. This indicates that IBS-D and depression are involved in microbiota-brain-gut interaction disorders and provides insight into understanding their high co-morbidity rate
Pylaris, 2012		320	N=258 subjects without SIBO	Patients were classified as IBS cases according to a symptom questionnaire and Rome II criteria	112 IBS (35 IBS-D, 19 IBS-C, 58 IBS-A)	N/A	Aspirated duodenal fluid from upper GI tract endoscopy was quantitatively cultured	SIBO was observed more often in IBS and in up to 60% of patients with IBS-D	This study demonstrates a higher prevalence of aerobic bacterial overgrowth in the small intestine of IBS patients
Rajilic-Stojanovic, 2011	Clinic/community	108	N=46 HC	Patients with IBS fulfilled Rome II criteria determined during prior clinical trial recruitment	62 IBS (25 IBS-D, 18 IBS-C, 19 IBS-A)	N/A	Fecal samples were collected, qPCR and phylogenetic microarray analysis was performed to assess microbiota composition	A 2-fold decrease in levels of Bacteroides, a 10-fold decrease in Actinobacteria, a 1.5-fold decrease in Bifidobacteria and a >5% increase in the level of Firmicutes was observed in IBS compared to HC.	The composition of the total fecal microbiota of IBS subjects and HC was found to be strongly influenced by the health status of the subjects. Robust signatures were identified that differentiate the fecal microbiota in HC and IBS patients.
Jeffery, 2012	Clinic	57	N=20 HC without current GI symptoms	Patients completed questionnaires to assess GI symptoms and cases fulfilled Rome II symptom criteria	37 IBS (15 IBS-D, 10 IBS-C, 12 IBS-A)	N/A	Fecal samples were collected and pyrosequencing methods were used to determine the fecal microbiota composition	An increase in the ratio of Firmicutes to Bacteroides (FB ratio) was associated with over half the IBS samples. There was an increased abundance of Clostridium cluster XIVa associated with the high FB ratio in IBS.	This study defined patient subgroups with microbiota displaying markedly different characteristics and associated microbial signatures to clinical variables within those subgroups
Le Gall, 2011	Clinic	45	N=22 HC	Patients diagnosed by their clinician according to Rome II criteria	10 IBS	2 years	Fecal water samples were assessed using NMR and PCR-DGGE to investigate the metabolic composition of gut microbiota	Increased bile acid and decreased branched chain fatty acids were observed in IBS relative to HC. DGGE profiles showed profiles from same individuals remained stable over time, but separated IBS from HC, revealing a correlation between gut microbiota profile and metabolite composition	The difference in metabolic profiles observed in this study was related to a distinct difference in the composition of the gut microbiota
Noor, 2010	Clinic	66	N=22 HC	Patients diagnosed by their clinician according to Rome II criteria	11 IBS	N/A	Fecal material was collected and PCR amplification of the 16s rRNA gene and DGGE profile analysis was used to analyze the fecal microbiota	IBS patients showed reduced diversity compared to HC. There were reductions in Bacteroides and Parabacteroides in IBS compared to HC.	There were significant alterations in the gut microbiota in IBS patients compared to HC.
Kerckhoffs, 2011	Clinic	57	N=20 HC without intestinal complaints or history	IBS patients fulfilled Rome II criteria	37 IBS (13 IBS-D, 11 IBS-C, 13 IBS-A)	N/A	Duodenal mucosal brush and fecal samples were collected and analyzed via 16s rRNA amplification and PCR-DGGE	In IBS compared to HC, a higher prevalence and levels of P. aeruginosa were observed in the small intestine and fecal samples	There was no apparent difference in the diversity of predominant microbiota profiles between IBS and HC.

			of bowel resection						but DGGE analysis revealed IBS-specific bands that were predominantly members of the genus <i>Pseudomonas</i> ; q-PCR analysis confirmed <i>P. aeruginosa</i> was more frequently found in IBS than HC.
Tana, 2010	Hospital /community	52	N=26 HC age and sex-matched and without GI symptoms	IBS patients were diagnosed through a medical interview based on Rome II criteria	26 IBS	N/A	Fecal samples were analyzed using qPCR and bacterial culture analysis and organic acid analysis	IBS patients had higher counts of <i>Lactobacilli</i> and <i>Veillonella</i> than controls, higher levels of acetic acid, propionic acid and total organic acids than controls. Levels of acetic acid and propionic acid were associated with GI symptoms, impaired QOL and negative emotion.	This study showed altered intestinal microbiota profiles and unbalanced fecal organic acid levels are associated with IBS
Codling, 2009	Community	80	N=33 HC	IBS was diagnosed according to Rome II criteria	47 IBS	N/A	Fecal samples (all subjects) and colonic biopsy samples (9 patients) were collected and analyzed using DGGE of the V1-V3 region of the 16S rRNA gene	There was significantly more variation observed in the gut microbiota of HC compared to IBS	The study findings provide additional evidence of a significant difference in the distal gut microbiota between IBS and HC
Rana, 2009	Clinic	599	N=254 HC age and gender-matched	IBS diagnosed according to Rome II criteria	345 IBS	N/A	A 10g lactulose hydrogen breath test was administered to subjects to determine the presence of methanogenic flora	The prevalence of methanogenic flora was significantly lower in IBS patients than in healthy controls	Methanogenic flora, especially the predominant methanogenic flora, was significantly decreased in IBS patients than in HC
Posserud, 2007	Clinic	188	N=26 HC without history of GI symptoms	IBS diagnosed clinically based on Rome II criteria	162 IBS (49 IBS-D, 37 IBS-C, 76 IBS-A)	N/A	Small bowel monometry and culture of jejunal aspirate were performed to assess prevalence of SIBO	SIBO was observed in only a small subset of IBS patients, a proportion no different than that observed in HC. However, cultures with higher cfu/ml counts were more frequently seen in IBS than HC.	The results do not support a strong association between SIBO and IBS, however a significant subset of IBS appeared to have higher counts of bacteria in the upper gut compared to HC
Costa, 2012	Clinic	34	N= 11 non-dyspeptic volunteers	Dyspepsia diagnosed by questionnaire applied based on Rome III criteria	23 dyspepsia patients	N/A	H2-lactulose breath test was administered to assess the presence of SIBO	13 of 23 (56.5%) of dyspeptic patients were positive for SIBO, compared to 0 control subjects (P=0.0052). 12 of the dyspeptic patients were taking PPIs, and 9 (75%) of those subjects were SIBO positive. No controls used PPIs	Patients with dyspepsia and dyspeptic patients using PPIs presented with SIBO at a much higher prevalence than non-dyspeptic controls

Table S-3. Antibiotics and FGD: Full Characteristics of Included Studies

First author, year of publication	Study Design	Study Setting	Study Size (N)	Comparison Group	Number exposed to antibiotics	Follow-up Period	Assessment of FGD	Overall Incidence of FGD	Incidence of FGD among Antibiotic Exposed	Antibiotic type(s)	P-value; OR (95% CI) of the association
Lalani, 2015	Prospective Cohort	Clinic	151	Subjects with traveler's diarrhea with no treatment/anti diarrheal only	46	3, 6, 9, and 12 months	Surveys using modified Rome III criteria	6 (4.0%)	N=1 (2.2%); P=0.60	Ciprofloxacin, azithromycin	p=0.6
Siah, 2016	Cross-Sectional	Community	297	Subjects without IBS determined by self-reported symptoms	Not specified	N/A	Survey questionnaire using Rome III criteria	62 (20.9%)	Not stated	Not specified	p=0.046; OR=1.8 (1.0-3.1)
Tornblom, 2007	Retrospective	Community	333	Subjects without FGD	45	5 years	Questionnaire using Rome II Criteria	41 (12)	N=10 (24%)	Not specified	p=0.05; OR=2.37 (1.07-5.25)

Villarreal, 2012	Retrospective	Hospital/ Clinic	21,364	Subjects without IBS diagnosis	Tetracycline N=5479, Macrolide N=5595	up to 12 months	Use of ICD-9 dx code 564.1 in medical record	115 (0.54%)	Tetracycline N=40 (0.73%), Macrolide N=40 (0.71%)	macrolides, tetracycline, betalactam, cephalosporin, penicillin	tetracycline p<0.025, macrolide p=0.036
Maxwell, 2002	Prospective Cohort	Clinic	91	Subjects without antibiotic use	45	4 months	Questionnaire using Rome Criteria	31 (34%)	20 (48%)	Amoxicillin, macrolides, penicillin, tetracycline, trimethoprim, others	p=0.01, OR=3.14 (1.27-7.75)
Ruigomez, 2007	Nested Case-Control	Community	783	Subjects from the same source population without gastroenteritis	587	1-10 years	Review of computer patient profiles: IBS code together with specific treatment, repeated IBS entries, or IBS dx with related GI symptoms	241 (30.8%)	197 (33.5%)	Not specified	OR ranged from 1.2 (1 prescription) to 2.2 (greater than 5 prescriptions)
Lee, 2013	Cross-Sectional	Community	1167	Subjects with the same deployment location and no doxycycline intake	189	N/A	Voluntarily self-reported on questionnaire or inferred by investigators from symptoms reported	41 (3.5%)	21 (1.8%)	Doxycycline	Doxy in developing country = 6.99(3.19-15.31) Doxy in developed country = 6.93(1.40,34.39) Doxy in Non-deployed = 2.81(0.34-23.04)
Paula, 2015	Nested Case-Control	Community	566	Age-matched, non-FGD patients based on self-report	338	~11.5-12 years (mean follow-up period)	Survey using modified Rome II criteria	316 (55.8%)	207 (61.2%)	Penicillin, aminoglycosides, cephalosporin, macrolides, tetracycline, sulfamethoxazole/trimethoprim, fluoroquinolones, antifungal, other	GI infection, antibiotics = 1.30 (0.43-3.91); Non-GI infection, antibiotics = 1.9 (1.21-2.98)

Table S-4. ICD Codes for Conditions of Interest

Condition	ICD-9	ICD-10
Functional GI Disorder Outcome		
Functional Constipation	564.0.00, 564.01, 564.02, 564.09	K59.00, K59.01, K59.02, K59.09
Irritable Bowel Syndrome	564.1	K58
Dyspepsia	536.8	K30
Functional Diarrhea	564.5	K59.1
Acute Gastroenteritis		
Cause Unspecified		

Infectious colitis, enteritis, gastroenteritis	009.0, 009.1	A09
Infectious Diarrhea	009.2	A09
Diarrhea of presumed infectious origin	009.3	A09
Viral		
Norovirus	008.63	A0811
Rotavirus	008.61	A080
Enterovirus	008.67	A08.39
Adenovirus	008.62	A082
Other viral enteritis	008.64-008.69	A0819, A0831, A0832, A0839, A0839
Other not elsewhere classified	008.8	A088
Bacterial		
<i>Campylobacter</i> spp.	008.43	A045
<i>Salmonella</i> spp.	003.0-003.9	A0220-A0224, A0229
<i>Shigella</i> spp.	004	A030-A033, A038, A039
<i>Escherichia coli</i>	008.0	A040-A044
<i>Yersinia enterocolitica</i>	008.44	A046
<i>Vibrio cholera</i>	001	A000, A001, A009
<i>Vibrio parahaemolyticus</i>	005.4	A053
<i>Clostridium difficile</i>	008.45	A047
Other/unspecified bacteria	008.1-008.5 (excluding 008.43-008.45)	A048
Other bacterial food poisoning	005.0-005.9	A050-A055, A058, A059

Protozoan		
<i>Giardia</i>	007.1	A071
<i>Cryptosporidium</i>	007.4	A072
Amoeba	006.0-006.2; 006.9	A060-A062, A069
Other protozoal	007	A070-A073, A078, A079
Psychological Disorders		
Axis I Disorders	295.xx-298.xx	
Schizophrenic disorder	295	F200-F203, F205, F209, F2081, F2089
Affective psychoses	296	F39, F302-F304, F308, F312, F314, F315, F319-F325, F328-F333, F339, F348, F3010-F3013, F3110-F3113, F3130-F3132, F3341, F3342, F3160-F3164, F3173-F3178, F3181
Paranoid states	297	F22-F24
Other non-organic psychoses	298	F4489
Axis II Disorders	300.xx-302.xx, 308.xx-314.xx	
Neurotic disorder	300	F42, F99, F341, F408-F411, F418, F419, F440, F441, F444, F446, F449-F451, F458, F459, F488, F489, F688, F4001, F4002, F4010, F4489, F6811, F4521, F4522, F40218, F40240, F40241
Personality disorder	301	F21, F340, F341, F600-F607, F609, F6081, F6089, F6812
Sexual disorder	302	F66, F641, F642, F560-F54, F659, F6589, F520, F521, F524, F526, F528, F5221, F5231, F5232, F6551, F6552, F6581, F6589, R37, Z87890
Acute reaction to stress	308	F430, R457
Adjustment reaction	309	F438, F930, F948, F4310, F4312, F4320-F4325, F4329

Non-psychotic brain syndrome	310	F09, F070, F482, F0781, F0789
Depressive disorder not elsewhere classified	311	F329
Conduct disturbance not elsewhere classified	312	F630-F633, F639, F6381, F6389, F911, F918, F919
Emotional disturbance child/adolescent	313	F913, F938-F941, F948, F988, F989
Hyperkinetic syndrome	314	F900-F902, F908, F909
Non-enteric infections		
Upper Respiratory Infections	034.0,460,461.9,462,463, 465.9, 472,473.9,786.2	J02.1,J00,J01.90,J03.90,J31.0, J32.9,R05
Lower Respiratory Infections	033.9,079.6,466.0,496,511.0, 786.05,786.09,795.51	A37.90,B97.4,J20.9,J18.9,R09.1, R06.2,R06.89,R76.11
Liver	070.32, 070.51, 070.54, 070.70, 305.90, 571.5, 571.8, 573.3	B18.1,B17.10,B18.2,B19.20, F15.10,F18.10,F19.10,K74.60, K72.10, K72.11,K76.0,K76.9, K75.9
Skin/Wound Infections	041.12,053.9,110.1,682.6,682.9, 686.9,691.8,782.1,919.5	B96.62,B02.9,B35.1,L02.419, L03.119,L02.91,L03.90,L08.9, L20.89,R21,T07,L08.89
Women's Health	112.1,599.0,616.0,616.10,616.9, 625.9,627.9,628.9	B37.3,N39.0,N72,N76.0,N72, N76.0,N76.89,N94.9,N95.9, N97.9,009.893,009.93
Urinary System	595,595.9,599.0,599.7,599.72, 788.1,788.3,788.41,788.42, 788.63	N30.00,N30.01,N30.90,N30.91, N39.0,R31.9,R31.0,R31.2, R30.0, R32,R35.0,R35.8,R39.15
Men's Health	599.9,601.9,602.9	N36.9,N39.9,N41.9,N42.9
Vector-Borne Diseases	061.0,065.4,066.40,082.0,083.0, 084.6,088.81,323.1	A90.0,A91,A92.0,A92.30,A77.0, A78.0,B54.0,A69.20,G05.3

Tuberculosis	010.90,011.90,012.80,013.00, 015.90,017.0,018.90,137.0	A15.7,A15.0,A15.8,A17.0,A18.01, A18.02,A18.09,A18.4,A19.9,B90.9
Veneral Diseases	042,054.10,054.9,079.4,079.98, 097.9,098.0,V01.6,V15.85,V69.2, V74.5	A60.9,B00.9,A63.8,A74.9, A53.9, A51.0,A54.00,Z20.2,Z77.21, Z72.51,Z72.52,Z72.53,Z11.3
Other	079.99,136.9,322.9,780.6,995.91, 999.39	B34.9,B89,G03.9,R50.9,A41.9, T80.29XA,T88.0XXA

Table S-5. CPT Codes for FGD diagnosis and ultrasound endoscopy

GI CPT CODE for DX and Ultrasound Endoscopy	Procedure
43191	Esophagoscopy, rigid, transoral; diagnostic
43193	Esophagoscopy, rigid, transoral; with biopsy
43197	Esophagoscopy, flexible, transnasal; diagnostic
43198	Esophagoscopy, flexible, transnasal; with biopsy
43200	Esophagoscopy, flexible, transoral; diagnostic
43202	Esophagoscopy, flexible, transoral; with biopsy
43235	Esophagogastroduodenoscopy, flexible, transoral; diagnostic
43239	Esophagogastroduodenoscopy, flexible, transoral; with biopsy
44360	Small intestinal endoscopy, enteroscopy beyond second portion of duodenum, not including ileum; diagnostic
44361	Small intestinal endoscopy, enteroscopy beyond second portion of duodenum, not including ileum

44376	Small intestinal endoscopy, enteroscopy beyond second portion of duodenum, including ileum; diagnostic
44377	Small intestinal endoscopy, enteroscopy beyond second portion of duodenum, including ileum
44380	Ileoscopy, through stoma; diagnostic,
44382	Ileoscopy, through stoma; with biopsy
44388	Colonoscopy through stoma; diagnostic
44389	Colonoscopy through stoma; with biopsy
45330	Sigmoidoscopy, flexible; diagnostic
45331	Sigmoidoscopy, flexible; with biopsy,
45378	Colonoscopy, flexible; diagnostic
45380	Colonoscopy, flexible; with biopsy
43231	Esophagoscopy, flexible, transoral; with endoscopic ultrasound examination
43237	Esophagogastroduodenoscopy, flexible, transoral; with endoscopic ultrasound examination limited to the esophagus, stomach or duodenum, and adjacent structures
43259	Esophagogastroduodenoscopy, flexible, transoral; with endoscopic ultrasound examination, including the esophagus, stomach, and either the duodenum or a surgically altered stomach
44406	Colonoscopy through stoma; with endoscopic ultrasound examination, limited to the sigmoid, descending, transverse, or ascending colon and cecum and adjacent structures

45341	Sigmoidoscopy, flexible; with endoscopic ultrasound examination
45391	Colonoscopy, flexible; with endoscopic ultrasound examination limited to the rectum, sigmoid, descending, transverse, or ascending colon and cecum, and adjacent structures

Table S-6. Oral antibiotics of interest

Antibiotic Class	Included in Sensitivity Analysis for ABX classes and IBS outcome (Yes/No)*	Study Class Categorization	Antibiotic Name
Carbapenems	N		Doribax
Carbapenems	N		doripenem
Carbapenems	N		ertapenem
Carbapenems	N		imipenem/cilastatin
Carbapenems	N		Invanz
Carbapenems	N		meropenem
Carbapenems	N		Merrem IV
Carbapenems	N		Primaxin
Cephalosporins, 1 st generation	Y	Cephalosporins	cefadroxil
Cephalosporins, 1 st generation	Y	Cephalosporins	cefazolin
Cephalosporins, 1 st generation	Y	Cephalosporins	cephalexin
Cephalosporins, 1 st generation	Y	Cephalosporins	Duricef
Cephalosporins, 1 st generation	Y	Cephalosporins	Keflex
Cephalosporins, 1 st generation	Y	Cephalosporins	Panixine Disperdose
Cephalosporins, 1 st generation	Y	Cephalosporins	Ultracef
Cephalosporins, 2 nd generation	Y	Cephalosporins	Ceclor
Cephalosporins, 2 nd generation	Y	Cephalosporins	cefaclor

Cephalosporins, 2 nd generation	Y	Cephalosporins	cefotetan
Cephalosporins, 2 nd generation	Y	Cephalosporins	cefoxitin
Cephalosporins, 2 nd generation	Y	Cephalosporins	cefoxitin hcl
Cephalosporins, 2 nd generation	Y	Cephalosporins	cefprozil
Cephalosporins, 2 nd generation	Y	Cephalosporins	Ceftin
Cephalosporins, 2 nd generation	Y	Cephalosporins	cefuroxime
Cephalosporins, 2 nd generation	Y	Cephalosporins	cefuroxime axetil
Cephalosporins, 2 nd generation	Y	Cephalosporins	Cefzil
Cephalosporins, 2 nd generation	Y	Cephalosporins	Raniclor
Cephalosporins, 2 nd generation	Y	Cephalosporins	Zinacef
Cephalosporins, 3 rd generation	Y	Cephalosporins	Avycaz
Cephalosporins, 3 rd generation	Y	Cephalosporins	Cedax
Cephalosporins, 3 rd generation	Y	Cephalosporins	cefdinir
Cephalosporins, 3 rd generation	Y	Cephalosporins	cefditoren
Cephalosporins, 3 rd generation	Y	Cephalosporins	cefditoren pivoxil
Cephalosporins, 3 rd generation	Y	Cephalosporins	cefixime
Cephalosporins, 3 rd generation	Y	Cephalosporins	Cefizox
Cephalosporins, 3 rd generation	Y	Cephalosporins	cefotaxime
Cephalosporins, 3 rd generation	Y	Cephalosporins	cefpodoxime
Cephalosporins, 3 rd generation	Y	Cephalosporins	cefpodoxime proxetil
Cephalosporins, 3 rd generation	Y	Cephalosporins	ceftazidime
Cephalosporins, 3 rd generation	Y	Cephalosporins	ceftazidime/avibactam
Cephalosporins, 3 rd generation	Y	Cephalosporins	ceftibuten

Cephalosporins, 3 rd generation	Y	Cephalosporins	ceftizoxime
Cephalosporins, 3 rd generation	Y	Cephalosporins	ceftriaxone
Cephalosporins, 3 rd generation	Y	Cephalosporins	ceftriaxone in is-osm dextrose
Cephalosporins, 3 rd generation	Y	Cephalosporins	ceftriaxone na/dextrose,iso
Cephalosporins, 3 rd generation	Y	Cephalosporins	Claforan
Cephalosporins, 3 rd generation	Y	Cephalosporins	Fortaz
Cephalosporins, 3 rd generation	Y	Cephalosporins	Rocephin
Cephalosporins, 3 rd generation	Y	Cephalosporins	Spectracef
Cephalosporins, 3 rd generation	Y	Cephalosporins	Suprax
Cephalosporins, 3 rd generation	Y	Cephalosporins	Tazicef
Cephalosporins, 3 rd generation	Y	Cephalosporins	Tazidime
Cephalosporins, 3 rd generation	Y	Cephalosporins	Vantin
Cephalosporins, Other	N		cefepime
Cephalosporins, Other	N		cefpime
Cephalosporins, Other	N		ceftaroline
Cephalosporins, Other	N		ceftolozane/tazobactam
Cephalosporins, Other	N		Maxipime
Cephalosporins, Other	N		Teflaro
Cephalosporins, Other	N		Zerbaxa
Fluoroquinolones	Y	Fluoroquinolones	Avelox
Fluoroquinolones	Y	Fluoroquinolones	Cipro
Fluoroquinolones	Y	Fluoroquinolones	Cipro XR
Fluoroquinolones	Y	Fluoroquinolones	ciprofloxacin
Fluoroquinolones	Y	Fluoroquinolones	ciprofloxacin hcl
Fluoroquinolones	Y	Fluoroquinolones	ciprofloxacin/ciprofloxacin hcl

Fluoroquinolones	Y	Fluoroquinolones	Factive
Fluoroquinolones	Y	Fluoroquinolones	Floxin
Fluoroquinolones	Y	Fluoroquinolones	gemifloxacin
Fluoroquinolones	Y	Fluoroquinolones	gemifloxacin mesylate
Fluoroquinolones	Y	Fluoroquinolones	Levaquin
Fluoroquinolones	Y	Fluoroquinolones	levofloxacin
Fluoroquinolones	Y	Fluoroquinolones	Levofloxacin Systemic
Fluoroquinolones	Y	Fluoroquinolones	moxifloxacin
Fluoroquinolones	Y	Fluoroquinolones	moxifloxacin hcl
Fluoroquinolones	Y	Fluoroquinolones	Moxifloxacin Systemic
Fluoroquinolones	Y	Fluoroquinolones	norfloxacin
Fluoroquinolones	Y	Fluoroquinolones	Norfloxacin Systemic
Fluoroquinolones	Y	Fluoroquinolones	Noroxin
Fluoroquinolones	Y	Fluoroquinolones	ofloxacin
Fluoroquinolones	Y	Fluoroquinolones	ProQuin XR
Glycopeptides	Y	Glycopeptides	dalbavancin
Glycopeptides	Y	Glycopeptides	Dalvance
Glycopeptides	Y	Glycopeptides	Orbactiv
Glycopeptides	Y	Glycopeptides	oritavancin
Glycopeptides	Y	Glycopeptides	telavancin
Glycopeptides	Y	Glycopeptides	Vancocin
Glycopeptides	Y	Glycopeptides	vancomycin
Glycopeptides	Y	Glycopeptides	vancomycin hcl
Glycopeptides	Y	Glycopeptides	Vibativ
Isoniazid	Y	Isoniazid	Isoniazid

Linezolid	Y	Linezolid	Linezolid
Lincosamides	Y	Lincosamides	Cleocin
Lincosamides	Y	Lincosamides	Cleocin Pediatric
Lincosamides	Y	Lincosamides	ClindaMax Vaginal
Lincosamides	Y	Lincosamides	clindamycin
Lincosamides	Y	Lincosamides	clindamycin hcl
Lincosamides	Y	Lincosamides	clindamycin palmitate hcl
Lincosamides	Y	Lincosamides	clindamycin phosphate
Lincosamides	Y	Lincosamides	Clindesse
Lincosamides	Y	Lincosamides	Lincocin
Lincosamides	Y	Lincosamides	lincomycin
Macrolides	Y	Macrolides	azithromycin
Macrolides	Y	Macrolides	Biaxin
Macrolides	Y	Macrolides	Biaxin XL
Macrolides	Y	Macrolides	clarithromycin
Macrolides	Y	Macrolides	Dificid
Macrolides	Y	Macrolides	E.E.S.
Macrolides	Y	Macrolides	Ery-Tab
Macrolides	Y	Macrolides	EryPed
Macrolides	Y	Macrolides	Erythrocin Lactobionate
Macrolides	Y	Macrolides	Erythrocin Stearate
Macrolides	Y	Macrolides	erythromycin base
Macrolides	Y	Macrolides	erythromycin ethylsuccinate
Macrolides	Y	Macrolides	erythromycin lactobionate
Macrolides	Y	Macrolides	erythromycin stearate
Macrolides	Y	Macrolides	fidaxomicin
Macrolides	Y	Macrolides	PCE Dispertab
Macrolides	Y	Macrolides	Zithromax
Macrolides	Y	Macrolides	Zmax
Metronidazole	Y	Metronidazole	Metronidazole
Monobactams	N		Azactam
Monobactams	N		aztreonam

Monobactams	N		aztreonam inhalation
Monobactams	N		Cayston
Penicillins, Amino	Y	Penicillins	amoxicillin
Penicillins, Amino	Y	Penicillins	amoxicillin/clavulanate
Penicillins, Amino	Y	Penicillins	amoxicillin/potassium clav
Penicillins, Amino	Y	Penicillins	Amoxil
Penicillins, Amino	Y	Penicillins	Ampi
Penicillins, Amino	Y	Penicillins	ampicillin
Penicillins, Amino	Y	Penicillins	ampicillin/sulbactam
Penicillins, Amino	Y	Penicillins	ampicillin trihydrate
Penicillins, Amino	Y	Penicillins	Augmentin
Penicillins, Amino	Y	Penicillins	Augmentin ES-600
Penicillins, Amino	Y	Penicillins	Augmentin XR
Penicillins, Amino	Y	Penicillins	Moxatag
Penicillins, Amino	Y	Penicillins	Omnipen
Penicillins, Amino	Y	Penicillins	Penglobe
Penicillins, Amino	Y	Penicillins	Principen
Penicillins, Amino	Y	Penicillins	Trimox
Penicillins, Amino	Y	Penicillins	Unasyn
Penicillins, Extended-Spectrum	N		piperacillin
Penicillins, Extended-Spectrum	N		piperacillin/tazobactam
Penicillins, Extended-Spectrum	N		piperacillin-tazo-dextrose,iso
Penicillins, Extended-Spectrum	N		Pipracil
Penicillins, Extended-Spectrum	N		Ticar
Penicillins, Extended-Spectrum	N		ticarcillin
Penicillins, Extended-Spectrum	N		ticarcillin/clavulanate
Penicillins, Extended-Spectrum	N		Timentin
Penicillins, Extended-Spectrum	N		Zosyn

Penicillins, Natural	Y	Penicillins	Bicillin C-R
Penicillins, Natural	Y	Penicillins	Bicillin C-R 900/300
Penicillins, Natural	Y	Penicillins	Bicillin LA
Penicillins, Natural	Y	Penicillins	Crystapen
Penicillins, Natural	Y	Penicillins	Pen Vee K
Penicillins, Natural	Y	Penicillins	penicillin G aqueous
Penicillins, Natural	Y	Penicillins	penicillin G benzathine
Penicillins, Natural	Y	Penicillins	penicillin G benzathine/penicillin G procaine
Penicillins, Natural	Y	Penicillins	penicillin G potassium
Penicillins, Natural	Y	Penicillins	penicillin G procaine
Penicillins, Natural	Y	Penicillins	penicillin G sodium
Penicillins, Natural	Y	Penicillins	Penicillin V
Penicillins, Natural	Y	Penicillins	penicillin VK
Penicillins, Natural	Y	Penicillins	penicillin v potassium
Penicillins, Natural	Y	Penicillins	Permapen
Penicillins, Natural	Y	Penicillins	Pfizerpen
Penicillins, Natural	Y	Penicillins	Veetids
Penicillins, Penicillinase-Resistant	Y	Penicillins	Bactocill
Penicillins, Penicillinase-Resistant	Y	Penicillins	dicloxacillin
Penicillins, Penicillinase-Resistant	Y	Penicillins	dicloxacillin sodium
Penicillins, Penicillinase-Resistant	Y	Penicillins	Nafcil
Penicillins, Penicillinase-Resistant	Y	Penicillins	naftillin
Penicillins, Penicillinase-Resistant	Y	Penicillins	Nallpen
Penicillins, Penicillinase-Resistant	Y	Penicillins	oxacillin
Rifampin	Y	Rifampin	Rifampin
Sulfonamides	Y	Sulfonamides	Bactrim
Sulfonamides	Y	Sulfonamides	Bactrim DS
Sulfonamides	Y	Sulfonamides	Cotrim
Sulfonamides	Y	Sulfonamides	cotrimoxazole

Sulfonamides	Y	Sulfonamides	Ery-Tab
Sulfonamides	Y	Sulfonamides	erythromycin base
Sulfonamides	Y	Sulfonamides	PCE Dispertab
Sulfonamides	Y	Sulfonamides	Septra
Sulfonamides	Y	Sulfonamides	Septra DS
Sulfonamides	Y	Sulfonamides	sulfadiazine
Sulfonamides	Y	Sulfonamides	Sulfatrim
Sulfonamides	Y	Sulfonamides	sulfisoxazole
Sulfonamides	Y	Sulfonamides	trimethoprim/sulfamethoxazole
Sulfonamides	Y	Sulfonamides	sulfamethoxazole/trimethoprim
Tetracyclines	Y	Tetracyclines	Achromycin V
Tetracyclines	Y	Tetracyclines	Acticlate
Tetracyclines	Y	Tetracyclines	Actisite
Tetracyclines	Y	Tetracyclines	Adoxa
Tetracyclines	Y	Tetracyclines	Atridox
Tetracyclines	Y	Tetracyclines	Avidoxy
Tetracyclines	Y	Tetracyclines	Declomycin
Tetracyclines	Y	Tetracyclines	demeclocycline
Tetracyclines	Y	Tetracyclines	Doryx
Tetracyclines	Y	Tetracyclines	Doxy
Tetracyclines	Y	Tetracyclines	Doxycin
Tetracyclines	Y	Tetracyclines	doxycycline
Tetracyclines	Y	Tetracyclines	doxycycline calcium
Tetracyclines	Y	Tetracyclines	doxycycline hyclate
Tetracyclines	Y	Tetracyclines	doxycycline monohydrate
Tetracyclines	Y	Tetracyclines	Dynacin
Tetracyclines	Y	Tetracyclines	Minocin
Tetracyclines	Y	Tetracyclines	Minocin Kit
Tetracyclines	Y	Tetracyclines	minocycline
Tetracyclines	Y	Tetracyclines	minocycline hcl
Tetracyclines	Y	Tetracyclines	Monodox
Tetracyclines	Y	Tetracyclines	Ocudox
Tetracyclines	Y	Tetracyclines	Oracea
Tetracyclines	Y	Tetracyclines	Periostat
Tetracyclines	Y	Tetracyclines	Solodyn

Tetracyclines	Y	Tetracyclines	Sumycin
Tetracyclines	Y	Tetracyclines	tetracycline
Tetracyclines	Y	Tetracyclines	tetracycline hcl
Tetracyclines	Y	Tetracyclines	Vibramycin
Tetracyclines	Y	Tetracyclines	Ximino

Table S-7. Univariate Associations by possible FGD Outcome

		All FGD (Crude) OddsRatio			IBS (Crude) OddsRatio			FC (Crude) OddsRatio			Dyspepsia (Crude) OddsRatio			FD (Crude) OddsRatio		
		Low	Upper		Low	Upper		Low	Upper		Low	Upper		Low	Upper	
Race/Ethnicity	White	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
	Black	1.18	1.13	1.23	0.61	0.55	0.68	1.45	1.38	1.52	0.96	0.83	1.10	0.40	0.18	0.86
	Hispanic	0.95	0.90	0.99	0.69	0.62	0.77	1.04	0.98	1.10	1.10	0.95	1.26	0.52	0.28	0.95
	Asian/Pacific Islander	0.69	0.63	0.77	0.55	0.44	0.68	0.71	0.63	0.80	0.93	0.72	1.20	0.72	0.27	1.95
	American Indian/Alaskan Native	0.76	0.64	0.90	0.70	0.48	1.00	0.77	0.62	0.95	0.86	0.54	1.35	0.62	0.07	5.40
	Other	0.79	0.73	0.86	0.58	0.48	0.69	0.88	0.80	0.97	0.79	0.63	0.99	0.95	0.41	2.20
	Unknown	1.04	0.91	1.18	0.82	0.61	1.08	1.13	0.96	1.32	1.17	0.82	1.67	0.61	0.13	2.76
Education	High School or less	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
	Some College	1.10	1.04	1.16	1.16	1.03	1.31	1.03	0.96	1.10	1.25	1.06	1.47	0.86	0.44	1.68
	College	0.76	0.72	0.80	0.81	0.72	0.91	0.69	0.64	0.74	0.87	0.74	1.02	0.71	0.37	1.39
	Advanced Degree	0.67	0.61	0.73	0.84	0.69	1.01	0.56	0.50	0.64	0.71	0.53	0.95	0.60	0.17	2.19
	Unknown	0.68	0.60	0.76	0.90	0.72	1.13	0.54	0.46	0.64	0.90	0.66	1.23	0.47	0.10	2.11
Service	Army	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
	Navy	0.60	0.57	0.62	0.66	0.60	0.72	0.53	0.51	0.56	0.86	0.76	0.97	0.79	0.45	1.38
	Air Force	0.82	0.79	0.85	0.99	0.91	1.08	0.73	0.69	0.76	0.98	0.87	1.10	2.26	1.43	3.59
	Marines	0.62	0.59	0.66	0.64	0.56	0.72	0.62	0.58	0.66	0.57	0.48	0.68	0.43	0.19	0.95
Rank	E1-E4	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
	E5-E9	0.81	0.78	0.85	0.85	0.78	0.93	0.73	0.69	0.76	0.95	0.84	1.07	0.88	0.54	1.45
	O1-O5	0.54	0.51	0.57	0.62	0.55	0.69	0.43	0.40	0.47	0.73	0.62	0.86	0.77	0.39	1.52
	O6-O10															59.3
	Warrant Officer	0.56	0.36	0.86	1.01	0.49	2.05	0.24	0.11	0.52	0.92	0.37	2.28	3.59	0.22	6
Axis I Disorder (Y/N)		3.46	3.24	3.69	3.92	3.41	4.50	3.34	3.08	3.62	3.58	2.91	4.40	2.16	0.95	4.90

Axis II Disorder (Y/N)	3.32	3.20	3.44	4.37	4.04	4.73	3.07	2.94	3.21	3.19	2.86	3.56	3.91	2.52	6.08
Acute Gastroenteritis (Y/N)	1.91	1.77	2.06	2.45	2.09	2.86	1.72	1.57	1.89	2.17	1.72	2.72	1.71	0.66	4.46
Non-enteric infection (Y/N)	2.23	2.15	2.31	2.49	2.30	2.69	2.30	2.20	2.41	2.19	1.98	2.43	2.01	1.34	3.00
Oral Antibiotic Use (Y/N)	1.94	1.88	2.01	2.18	2.02	2.35	1.95	1.86	2.03	1.78	1.61	1.96	1.94	1.31	2.87

Table S-8. Multivariate Associations by possible FGD outcome

		All FGD (Adjusted)			IBS (Adjusted)			FC (Adjusted)			Dyspepsia (Adjusted)			FD (Adjusted)		
		Odds Ratio	Low erCL	Upp erCL	Odds Ratio	Low erCL	Upp erCL	Odds Ratio	Low erCL	Upp erCL	Odds Ratio	Low erCL	Upp erCL	Odds Ratio	Low erCL	Upp erCL
Race/Ethnicity	White	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref			
	Black	1.08	1.03	1.13	0.58	0.52	0.65	1.35	1.28	1.42	0.93	0.81	1.08			
	Hispanic	0.97	0.92	1.02	0.68	0.61	0.77	1.09	1.02	1.16	1.16	1.00	1.34			
	Asian/Pacific Islander	0.74	0.67	0.82	0.62	0.49	0.78	0.75	0.66	0.85	1.12	0.86	1.46			
	American Indian/Alaskan Native	0.87	0.73	1.04	0.87	0.59	1.29	0.90	0.72	1.12	0.88	0.55	1.42			
	Other	0.90	0.83	0.97	0.63	0.52	0.76	1.06	0.96	1.17	0.79	0.62	1.00			
	Unknown	1.16	1.01	1.32	0.90	0.66	1.22	1.27	1.07	1.50	1.30	0.89	1.90			
Education	High School or less	ref	ref	ref	ref	ref	ref				ref	ref	ref			
	Some College	1.07	1.01	1.13	1.16	1.02	1.32				1.24	1.05	1.48			
	College	1.11	1.02	1.19	1.32	1.12	1.55				1.07	0.91	1.27			
	Advanced Degree	1.08	0.96	1.23	1.56	1.20	2.02				0.85	0.63	1.16			
	Unknown	0.99	0.86	1.14	1.33	1.02	1.74				0.94	0.67	1.31			
Service	Army	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
	Navy	0.79	0.75	0.82	0.97	0.87	1.08	0.70	0.66	0.75	1.14	1.00	1.31	1.21	0.66	2.21
	Air Force	0.94	0.90	0.98	1.17	1.06	1.29	0.85	0.80	0.89	1.14	1.00	1.29	2.96	1.77	4.96
	Marines	0.80	0.75	0.85	0.86	0.75	0.98	0.80	0.75	0.87	0.73	0.61	0.88	0.60	0.26	1.37
Rank	E1-E4	ref	ref	ref	ref	ref	ref	ref	ref	ref						
	E5-E9	0.94	0.90	0.98	0.98	0.89	1.07	0.87	0.82	0.92						
	O1-O5	0.67	0.61	0.73	0.63	0.52	0.76	0.63	0.58	0.69						
	O6-O10															
	Warrant Officer	0.65	0.42	1.02	1.12	0.52	2.40	0.30	0.14	0.65						
Axis I Disorder (Y/N)		1.60	1.49	1.72	1.53	1.31	1.78	1.65	1.51	1.81	1.67	1.33	2.10			

Axis II Disorder (Y/N)	2.50	2.40	2.60	3.37	3.08	3.69	2.29	2.17	2.41	2.60	2.30	2.95	4.28	2.61	7.00
Acute Gastroenteritis (Y/N)	1.46	1.35	1.58	1.74	1.46	2.07	1.36	1.23	1.50	1.70	1.33	2.17			
Non-enteric infection (Y/N)	1.64	1.57	1.70	1.72	1.58	1.88	1.72	1.63	1.81	1.71	1.53	1.92			
Oral Antibiotic Use (Y/N)	1.41	1.36	1.47	1.57	1.44	1.71	1.42	1.35	1.49	1.35	1.21	1.50	1.58	1.03	2.41

Table S-9. Subgroup Analysis: Multivariable models Full Results

		Cumulative Antibiotics			Antibiotic Class Effects																				
					Cephalosporin			Fluoroquinolones			Glycopeptides			Lincosamide			Macrolides			Metronidazole			Penicillins, Amino		
	Effect	Od dsR atio Est	Lo w er CL	U p pe rC L	Od dsR atio Est	Lo w er CL	U p pe rC L	Od dsR atio Est	Lo w er CL	U p pe rC L	Od dsR atio Est	Lo w er CL	U p pe rC L	Od dsR atio Est	Lo w er CL	U p pe rC L	Od dsR atio Est	Lo w er CL	U p pe rC L	Od dsR atio Est	Lo w er CL	U p pe rC L	Od dsR atio Est	Lo w er CL	U p pe rC L
Race/ Ethnic ity	White	ref	re f	re f	ref	re f	re f	ref	re f	re f	ref	re f	re f	ref	re f	re f	ref	re f	re f	ref	re f	re f	ref	re f	re f
	Black	0.5 4	0. 44	0. 66	0.5 5	0. 45	0. 68	0.5 8	0. 47	0. 72	0.5 5	0. 45	0. 68	0.5 5	0. 45	0. 68	0.5 6	0. 45	0. 68	0.5 0	0. 41	0. 62	0.5 6	0. 45	0. 69
	Hispanic	0.7 6	0. 63	0. 93	0.7 6	0. 62	0. 94	0.7 8	0. 63	0. 96	0.7 5	0. 61	0. 93	0.7 6	0. 62	0. 94	0.7 6	0. 61	0. 93	0.7 3	0. 59	0. 91	0.7 6	0. 62	0. 94
	Asian/Pa cific Islander	0.5 6	0. 37	0. 84	0.5 2	0. 34	0. 78	0.5 4	0. 36	0. 83	0.5 1	0. 34	0. 78	0.5 2	0. 34	0. 79	0.5 0	0. 33	0. 77	0.5 2	0. 34	0. 79	0.5 3	0. 35	0. 80
	American Indian/Al askan Native	0.8 1	0. 42	1. 56	0.9 3	0. 47	1. 84	0.9 0	0. 45	1. 81	0.9 2	0. 46	1. 83	0.9 2	0. 47	1. 83	0.9 5	0. 48	1. 88	0.9 0	0. 45	1. 81	0.9 1	0. 46	1. 81
	Other	0.5 7	0. 41	0. 77	0.5 5	0. 40	0. 77	0.5 7	0. 41	0. 79	0.5 5	0. 40	0. 77	0.5 5	0. 40	0. 76	0.5 7	0. 41	0. 79	0.5 4	0. 39	0. 75	0.5 7	0. 41	0. 79
	Unknow n	0.7 3	0. 44	1. 22	0.7 3	0. 42	1. 27	0.7 2	0. 42	1. 25	0.7 4	0. 43	1. 28	0.7 4	0. 42	1. 28	0.7 5	0. 43	1. 29	0.6 9	0. 40	1. 21	0.7 2	0. 41	1. 25
Axis I Disorder (Y/N)		1.6 7	1. 29	2. 17	1.5 9	1. 22	2. 08	1.5 4	1. 18	2. 02	1.5 9	1. 22	2. 07	1.5 9	1. 22	2. 07	1.6 2	1. 24	2. 11	1.5 7	1. 20	2. 05	1. 16	1. 23	2. 09
Axis II Disorder (Y/N)		3.0 2	2. 60	3. 49	3.0 1	2. 58	3. 50	3.0 1	2. 58	3. 51	3.0 5	2. 62	3. 55	3.0 0	2. 57	3. 49	2.9 0	2. 49	3. 38	2.9 5	2. 53	3. 44	2.9 9	2. 57	3. 48
Acute Gastroenteritis (Y/N)		1.9 3	1. 44	2. 59	1.9 9	1. 47	2. 69	1.9 4	1. 43	2. 63	1.9 9	1. 47	2. 70	2.0 0	1. 47	2. 71	1.9 5	1. 44	2. 65	1.8 9	1. 39	2. 57	1.9 5	1. 43	2. 65
Non-enteric infection (Y/N)		1.5 5	1. 33	1. 79	1.6 5	1. 42	1. 92	1.5 5	1. 33	1. 81	1.6 6	1. 43	1. 93	1.6 4	1. 42	1. 91	1.5 9	1. 37	1. 84	1.5 9	1. 36	1. 84	1.5 9	1. 37	1. 85
Cumul ative Antibi otics	0	ref	re f	re f																					
	1	1.3 4	1. 14	1. 58																					
	2-4	1.8 5	1. 56	2. 19																					
	5-10	2.2 9	1. 72	3. 06																					
	>10	2.6 7	1. 01	7. 10																					
Antibiotic Class (Y/N)					1.1 8	1. 01	1. 39	1.8 8	1. 63	2. 16	25. 85	2. 87	23 3. 31	1.4 2	1. 19	1. 69	1.3 9	1. 21	1. 60	2.1 8	1. 83	2. 60	1.4 6	1. 28	1. 67

Table S-10: IgG MFI differences-IBS cases and controls
Differences in IgG serological markers' mean fluorescence intensities between paired baseline and post- samples for IBS cases and controls, and IBS cases versus controls post-samples and change between pre- and post- samples.

Target	IBS Case (N=41)							Control (N=48)							FDR P-value Cases v. Controls	
	IgG Baseline Mean (N=41)	SD	IgG Post Mean (N=41)	SD	IgG Change Mean (N=41)	SD	FDR P-value (pre/post)	IgG Baseline Mean (N=44)	SD	IgG Post Mean (N=47)	SD	IgG Change Mean (N=43)	SD	FDR P-value (pre/post)	Post	Change
28-4band	5.94	0.57	6.05	0.57	0.11	0.67	0.91	5.91	0.66	5.9	0.63	-0.01	0.66	0.99	0.50	0.72
3-1-57Fla	5.10	0.98	5.31	0.90	0.21	1.12	0.89	5.01	0.71	4.65	1.05	-0.24	0.81	0.31	0.04*	0.26
A4Fla2	6.54	0.58	6.50	0.57	-0.03	0.46	0.91	6.54	0.72	6.42	0.44	-0.13	0.7	0.79	0.62	0.73
A4Fla3	5.95	0.73	5.93	0.70	-0.03	0.60	0.92	5.96	0.47	5.57	0.61	-0.45	0.58	<0.01*	0.09	0.09
AgF1	4.46	0.95	4.70	1.04	0.24	1.36	0.91	4.75	1.12	4.55	1.19	-0.22	1.8	0.88	0.66	0.60
CBir1	6.12	0.76	6.05	0.71	-0.08	0.63	0.91	6.26	0.55	6.07	0.41	-0.19	0.61	0.31	0.88	0.72
CBir11	6.46	0.78	6.46	0.76	0.00	0.49	0.99	6.58	0.5	6.31	0.56	-0.25	0.37	<0.01*	0.52	0.13
CBir14	4.86	1.12	5.25	0.67	0.39	1.24	0.62	5.02	0.91	4.84	1.08	-0.14	1.15	0.88	0.14	0.28
CBir19	4.98	1.82	5.40	1.11	0.43	1.76	0.89	5.16	1.5	5.13	1.37	-0.07	1.65	0.93	0.54	0.60
CBir23	6.41	0.33	6.37	0.37	-0.04	0.39	0.91	6.5	0.35	6.43	0.31	-0.08	0.34	0.50	0.59	0.86
CBir45	6.99	0.55	6.95	0.50	-0.04	0.40	0.91	6.81	0.36	6.65	0.37	-0.16	0.36	0.07	0.04*	0.60
CBir5	5.34	0.78	5.66	0.66	0.32	0.96	0.62	5.71	0.56	5.57	0.59	-0.19	0.67	0.34	0.66	0.11
CBir56	7.46	0.72	7.63	0.76	0.17	0.56	0.62	7.37	0.54	7.33	0.53	-0.03	0.45	0.88	0.14	0.43
CBir63	6.01	0.50	6.03	0.53	0.02	0.51	0.92	5.98	0.52	5.94	0.53	-0.04	0.73	0.88	0.59	0.86
CBir66	6.88	0.92	6.83	0.94	-0.05	0.46	0.91	6.86	0.61	6.67	0.67	-0.19	0.5	0.15	0.55	0.60
CBir8	5.28	0.87	5.20	0.70	-0.08	1.07	0.91	5.34	1.04	5.36	0.93	-0.01	1.37	0.99	0.55	0.96
Cdtb	5.40	0.98	5.37	1.22	-0.03	1.66	0.97	5.79	1.04	5.73	1.21	-0.09	1.55	0.88	0.39	0.96
EF20	4.44	1.47	4.58	1.39	0.14	1.90	0.91	5.24	1.18	5.15	1.28	-0.21	1.47	0.88	0.17	0.68
FlaX	6.83	0.71	6.86	0.76	0.03	0.56	0.92	6.88	0.62	6.75	0.47	-0.14	0.61	0.55	0.60	0.60
FtsZ	5.17	0.77	5.39	0.85	0.22	1.17	0.89	5.43	0.88	5.38	0.93	-0.06	1.21	0.90	0.97	0.68
MDR247	4.84	0.77	4.95	0.93	0.12	1.12	0.91	5.39	0.89	5.26	1.12	-0.17	1.39	0.88	0.39	0.68
MDR254	4.93	1.32	4.99	1.13	0.06	1.59	0.92	5.05	0.87	4.62	0.98	-0.44	1.1	0.11	0.28	0.48
MDR90	4.43	1.08	4.37	1.28	-0.06	1.48	0.92	5.16	0.93	5.31	0.9	0.08	1.28	0.88	0.01*	0.86
P3	4.74	0.98	4.71	1.01	-0.02	1.45	0.97	4.87	0.9	4.87	1.05	-0.01	1.3	0.99	0.64	0.99
RfaeF3	4.47	0.87	4.57	0.91	0.09	1.22	0.91	4.93	0.77	4.9	0.81	-0.08	1.08	0.88	0.22	0.75
RhomF1	5.70	0.75	5.79	0.98	0.09	1.24	0.91	5.89	0.91	5.92	0.95	0	1.32	0.99	0.68	0.90
RhomF2	5.14	1.09	5.37	0.84	0.23	1.15	0.89	5.47	0.88	5.44	0.89	-0.07	1.1	0.88	0.81	0.63
RintF1	5.18	0.72	5.32	0.74	0.14	0.99	0.91	5.46	0.82	5.41	0.96	-0.08	1.19	0.88	0.69	0.68
RinuF5	5.20	0.86	5.38	0.73	0.17	0.93	0.89	5.57	0.74	5.46	0.82	-0.15	1.02	0.88	0.69	0.60
SalFliC	5.90	1.12	6.25	0.82	0.35	1.06	0.62	6.04	0.91	5.8	1.12	-0.24	0.69	0.18	0.14	0.10
UNCFlIC	6.69	1.05	6.76	1.02	0.07	0.68	0.91	6.62	1.16	6.48	1.19	-0.1	0.64	0.88	0.44	0.66
Vinc	5.02	0.85	5.01	0.75	-0.01	1.02	0.97	5.54	0.91	5.47	1.1	-0.12	1.36	0.88	0.14	0.89
keto	5.56	0.43	5.58	0.41	0.02	0.52	0.92	5.73	0.53	5.76	0.37	0.03	0.54	0.88	0.14	0.98

ompC	4.60	0.99	4.59	1.05	-0.02	1.36	0.97	4.88	0.98	5.07	1.03	0.18	1.42	0.88	0.14	0.76
riB1	6.08	0.46	6.23	0.46	0.15	0.47	0.62	6.07	0.43	5.98	0.5	-0.1	0.45	0.58	0.09	0.16
riB10	5.71	0.52	5.68	0.48	-0.03	0.64	0.92	5.74	0.44	5.39	0.57	-0.31	0.58	0.02*	0.09	0.26
riB12	5.99	0.75	6.19	0.81	0.20	1.08	0.89	6.05	1.43	6.21	0.94	0.14	1.59	0.88	0.95	0.96
riB13	6.30	0.75	6.40	0.77	0.09	1.11	0.91	6.41	1.08	6.58	1.05	0.14	1.47	0.88	0.56	0.96
riB14	6.13	0.53	6.12	0.39	-0.02	0.55	0.95	6.35	0.48	6.22	0.42	-0.13	0.53	0.50	0.44	0.68
riB16	7.18	0.53	7.24	0.53	0.05	0.42	0.91	7.11	0.47	7.07	0.41	-0.02	0.34	0.88	0.28	0.68
riB17	4.65	0.85	4.90	0.85	0.25	1.10	0.89	5.14	0.91	5.15	0.93	-0.01	1.32	0.99	0.44	0.68
riB18	4.56	0.88	4.45	0.86	-0.11	1.27	0.91	5.02	0.86	4.97	1.01	-0.12	1.38	0.88	0.09	0.99
riB20	6.19	0.45	6.12	1.09	-0.07	1.01	0.91	6.11	0.53	6.02	0.48	-0.09	0.62	0.88	0.69	0.96
riB8	5.50	0.66	5.35	0.79	-0.15	1.04	0.91	5.5	0.87	5.55	0.82	0.02	1.14	0.99	0.44	0.73
riB9	4.82	0.99	5.10	0.96	0.28	1.31	0.89	5.45	1.05	5.53	1.14	-0.01	1.56	0.99	0.18	0.68
riB2	6.07	0.75	6.17	0.82	0.10	1.14	0.91	5.86	0.89	5.88	0.91	0.01	1.24	0.99	0.31	0.90
riB4	5.64	0.92	5.76	1.06	0.12	1.51	0.91	5.72	1.19	5.88	1.05	0.12	1.62	0.88	0.69	1.00
riB5	5.15	0.67	5.27	0.80	0.12	1.08	0.91	5.39	0.79	5.3	1.08	-0.11	1.3	0.88	0.93	0.68
tetraco	5.24	0.9	5.3	0.7	0.07	0.9	0.91	4.93	1.35	4.8	1.05	-0.15	1.57	0.88	0.09	0.73

IBS = Irritable Bowel Syndrome; IgG = Immunoglobulin-G; SD = Standard Deviation; FDR = False Discovery Rate.

*Significant at P<0.05 level

Table S-11: IgA MFI differences-IBS cases and controls
Differences in IgA serological markers' mean fluorescence intensities between paired baseline and post- samples for IBS cases and controls, and IBS cases versus controls post-samples and change between pre- and post- samples.

Target	IBS Case (N=41)							Control (N=48)							FDR P-value IBS Case v. Control	
	IgA Baseline Mean (N=41)	SD	IgA Post Mean (N=41)	SD	IgA Change Mean (N=41)	SD	FDR P-value (pre/post)	IgA Baseline Mean (N=44)	SD	IgA Post Mean (N=47)	SD	IgA Change Mean (N=43)	SD	FDR P-value (pre/post)	Post	Change
28-4band	6.47	0.85	6.55	1.05	0.08	0.80	0.84	5.94	0.9 3	5.84	1.0 2	-0.09	0.77	0.54	0.01*	0.42
3-1-57Fla	4.63	1.13	4.76	0.97	0.13	1.47	0.84	4.53	1.0 5	3.74	1.5 2	-0.7	1.1 3	0.01*	0.01*	0.04*
A4Fla2	5.79	0.55	5.74	0.71	-0.05	0.47	0.84	5.7	1.0 7	5.69	0.5 7	-0.01	0.05	0.97	0.75	0.85
A4Fla3	5.03	1.00	5.11	0.88	0.08	1.16	0.85	5.21	0.6 4	4.73	0.9 6	-0.48	0.0	0.01*	0.12	0.06
AgF1	5.06	1.69	5.26	1.34	0.20	1.37	0.78	4.97	1.0 6	4.6	1.2 3	-0.37	0.1	0.20	0.07	0.17
CBir1	5.32	0.66	5.16	0.83	-0.16	0.73	0.78	5.62	0.6 0.9	5.35	0.7 7	-0.29	0.0	0.01*	0.32	0.43
CBir11	5.51	0.73	5.64	0.79	0.13	0.49	0.78	5.68	2 1.2	5.37	4 1.8	-0.34	0.73	0.01*	0.16	0.02*
CBir14	4.36	1.23	4.56	1.53	0.20	1.84	0.84	4.57	3 3	3.71	8 8	-0.8	0.79	0.01*	0.07	0.05

CBir19	4.34	2.02	4.71	1.58	0.36	2.39	0.78	3.9	1.5	3.81	1.7	2.	0.72	0.06	0.41
CBir23	5.80	0.52	5.75	0.68	-0.05	0.61	0.84	5.97	0.6	5.79	0.6	-0.17	47	0.81	0.41
CBir45	6.26	0.61	6.24	0.72	-0.02	0.49	0.88	6.16	0.5	5.95	0.5	4	44	0.09	0.17
CBir5	4.99	1.01	5.22	0.89	0.23	1.57	0.78	5.31	0.7	4.85	0.9	-0.2	1.	0.12	0.06
CBir56	5.97	0.68	6.16	0.97	0.18	0.89	0.78	5.31	1	4.85	3	-0.47	1	0.06	0.05
CBir63	4.85	0.74	5.00	0.84	0.15	0.91	0.78	5.95	0.8	5.69	0.8	-0.26	68	0.07	0.05
CBir66	5.79	0.83	5.87	0.91	0.08	0.68	0.84	4.91	0.7	4.56	8	-0.39	01	0.12	0.07
CBir8	5.39	0.71	5.38	0.77	-0.01	0.87	0.97	5.8	0.8	5.55	2	-0.27	73	0.13	0.53
Cdtb	7.09	0.71	7.12	0.90	0.03	0.87	0.88	5.25	0.6	5.1	8	-0.14	75	0.06	0.08
EF20	5.26	2.52	5.18	2.44	-0.08	2.67	0.88	6.98	1	6.64	2	-0.32	52	0.12	0.10
FlaX	5.91	0.71	5.95	0.88	0.05	0.59	0.84	5.28	1.4	4.23	2	-1.13	89	0.24	0.17
FtsZ	5.65	0.65	5.74	0.79	0.09	0.83	0.84	5.9	0.7	5.67	8	-0.27	02	0.17	0.08
MDR247	4.86	0.88	4.96	0.90	0.10	1.19	0.84	5.76	0.5	5.5	5	-0.25	47	0.43	0.05
MDR254	4.01	1.37	3.78	1.69	-0.23	2.35	0.84	5.29	0.5	4.77	0.9	-0.5	83	0.24	0.21
MDR90	4.64	1.51	4.50	1.57	-0.14	1.63	0.84	4.2	1.3	3.25	2	-0.99	07	0.69	0.38
P3	3.99	1.84	3.90	1.76	-0.09	2.37	0.88	5.12	8	4.68	1	-0.53	48	0.53	0.82
RfaeF3	5.07	1.30	5.14	1.85	0.06	2.01	0.88	4.41	1.2	4.17	3	-0.23	98	0.81	0.41
RhomF1	6.11	0.55	6.22	0.70	0.10	0.55	0.78	5.3	0.9	5.05	5	-0.29	94	<0.01	
RhomF2	5.27	1.00	5.45	0.81	0.18	1.13	0.78	5.84	0.4	5.58	9	-0.26	53	*	0.02*
RintF1	5.44	1.09	5.33	1.27	-0.11	1.38	0.84	5.31	0.5	5.1	0.7	-0.22	69	0.09	0.13
RinuF5	5.09	1.44	5.03	1.08	-0.07	1.70	0.88	5.53	0.4	5.19	1	-0.32	57	0.62	0.43
SalFliC	4.94	1.18	5.28	1.03	0.35	1.37	0.78	5.32	0.4	5	8	-0.32	66	0.90	0.43
UNCFliC	5.62	1.45	5.96	1.55	0.34	1.04	0.74	5.23	9	4.59	1	-0.62	16	0.01*	0.02*
Vinc	5.21	1.07	4.92	1.46	-0.28	1.53	0.78	5.57	1.1	5.21	1	-0.33	95	0.03*	0.02*
keto	4.99	0.70	4.94	1.01	-0.06	0.93	0.87	5.28	0.7	5.11	8	-0.16	83	0.07	0.70
ompC	4.88	1.11	4.64	0.79	-0.23	1.33	0.78	5.25	0.6	4.98	7	-0.24	6	0.59	0.41
rlB1	5.47	0.46	5.64	0.54	0.17	0.65	0.78	4.87	0.6	4.8	1	-0.01	96	0.81	0.43
rlB10	5.02	0.57	5.08	0.68	0.06	0.84	0.85	5.52	5	5.25	5	-0.25	53	0.43	0.43
rlB12	6.31	0.59	6.50	0.51	0.20	0.51	0.45	5.2	0.6	4.63	4	-0.52	95	0.01*	0.02*
rlB13	6.83	0.50	6.91	0.56	0.08	0.50	0.78	6.23	0.7	6.2	5	-0.01	69	0.07	0.03*
rlB14	5.23	0.66	5.20	0.79	-0.03	0.90	0.88	6.64	0.4	6.56	7	-0.08	47	0.03*	0.21
								5.64	0.8	5.29	6	-0.37	78	0.01*	0.21
									3					0.67	0.14

riB16	6.31	0.60	6.44	0.53	0.13	0.64	0.78	6.38	0.6	3	6.22	1	-0.14	49	0.09	0.12	0.08
riB17	5.33	0.59	5.47	0.54	0.13	0.61	0.78	5.39	0.6	1	5	5	-0.39	72	0.01*	0.01*	0.02*
riB18	4.82	1.72	5.16	1.55	0.35	2.18	0.78	5.29	0.8	3	5.06	1	-0.29	85	0.05	0.77	0.17
riB20	5.17	0.69	5.30	1.00	0.13	0.94	0.78	5.26	0.7	9	5.19	5	-0.07	98	0.72	0.65	0.43
riB8	5.65	0.57	5.66	0.87	0.01	1.07	0.97	5.33	1.0	1	5.35	8	0.02	23	0.97	0.12	0.97
riB9	4.21	1.80	4.33	1.51	0.11	2.36	0.88	4.15	0.6	1.5	3.69	2	-0.61	92	0.07	0.12	0.21
riB2	6.51	0.61	6.62	0.64	0.11	0.57	0.78	6.39	0.7	7	6.26	1	-0.11	45	0.19	0.06	0.13
riB4	6.39	0.61	6.73	0.59	0.35	0.64	0.07	6.19	0.5	9	6.05	6	-0.14	76	0.30	<0.01	0.02*
riB5	5.43	0.53	5.51	0.72	0.08	0.71	0.84	5.34	0.5	7	5.21	9	-0.12	66	0.30	0.09	0.27
tetraco	5.21	1.08	4.93	1.09	-0.29	1.27	0.78	4.84	1.3	4	4.49	0	-0.35	56	0.20	0.13	0.85

IBS = Irritable Bowel Syndrome; IgA = Immunoglobulin-A; SD = Standard Deviation; FDR = False Discovery Rate.

*Significant at P<0.05 level

Table S-12. IgG MFI differences-any antibiotic use

Differences in IgG serological markers' mean fluorescence intensities between paired baseline and post- samples for subjects with and without any antibiotic use, and any antibiotic use versus no use post-samples and change between pre- and post- samples.

Target	Any ABX+ (N=62)							Any ABX- (N=27)							FDR P-value Any ABX+ v. Any ABX-	
	IgG Baseline Mean (N=59)	SD	IgG Post Mean (N=61)	SD	IgG Change Mean (N=58)	SD	FDR P-value (pre/post)	IgG Baseline Mean (N=26)	SD	IgG Post Mean (N=27)	SD	IgG Change Mean (N=26)	SD	FDR P-value (pre/post)	Post	Change
28-4band	5.87	0.80	6.09	0.75	0.22	0.66	0.72	5.95	0.52	5.92	0.53	-0.03	0.66	0.99	0.83	0.87
3-1-57Fla	4.67	1.03	4.89	1.21	0.29	1.25	0.93	5.22	0.7	4.99	0.95	-0.16	0.83	0.74	1.00	0.87
A4Fla2	6.27	0.89	6.28	0.50	0.01	0.85	0.97	6.66	0.47	6.54	0.49	-0.12	0.43	0.33	0.38	0.87
A4Fla3	5.79	0.69	5.57	0.63	-0.24	0.78	0.72	6.03	0.56	5.81	0.69	-0.24	0.55	0.07	0.70	0.97
AgF1	4.50	1.24	4.99	0.83	0.50	1.48	0.72	4.66	0.95	4.46	1.19	-0.22	1.62	0.96	0.38	0.87
CBir1	5.95	0.85	5.92	0.49	-0.03	0.75	0.97	6.3	0.53	6.12	0.59	-0.18	0.55	0.23	0.63	0.87
CBir11	6.33	0.65	6.14	0.68	-0.19	0.57	0.72	6.61	0.63	6.49	0.63	-0.1	0.38	0.37	0.38	0.87
CBir14	4.98	0.76	5.08	0.68	0.10	0.88	0.94	4.92	1.11	5.01	1.03	0.13	1.34	0.99	1.00	0.96
CBir19	5.26	1.30	5.00	1.29	-0.21	1.75	0.94	4.99	1.8	5.37	1.23	0.34	1.68	0.74	0.83	0.87
CBir23	6.37	0.39	6.35	0.41	-0.01	0.43	0.97	6.5	0.31	6.42	0.31	-0.09	0.33	0.37	0.99	0.87
CBir45	6.82	0.57	6.74	0.42	-0.08	0.44	0.93	6.93	0.41	6.81	0.47	-0.11	0.36	0.23	0.99	0.96
CBir5	5.58	0.75	5.64	0.59	0.05	0.88	0.96	5.51	0.67	5.6	0.64	0.06	0.86	0.99	1.00	0.96
CBir56	7.18	0.79	7.29	0.79	0.13	0.57	0.93	7.52	0.52	7.55	0.58	0.04	0.49	0.99	0.71	0.88
CBir63	5.81	0.57	5.84	0.51	0.03	0.68	0.96	6.07	0.46	6.05	0.52	-0.03	0.61	0.99	0.60	0.96

CBir66	6.67	0.77	6.48	0.66	-0.18	0.53	0.72	6.96	0.76	6.86	0.84	-0.09	0.47	0.74	0.38	0.87
CBir8	5.19	1.25	5.33	0.78	0.15	1.33	0.94	5.36	0.8	5.26	0.86	-0.13	1.18	0.99	1.00	0.87
Cdtb	5.46	0.90	5.60	1.30	0.15	1.54	0.94	5.66	1.07	5.54	1.2	-0.16	1.63	0.99	1.00	0.87
EF20	4.76	1.35	5.00	1.34	0.20	1.57	0.94	4.89	1.41	4.83	1.37	-0.15	1.75	0.99	1.00	0.87
FlaX	6.63	0.80	6.60	0.70	-0.02	0.82	0.97	6.95	0.57	6.89	0.57	-0.07	0.46	0.89	0.53	0.96
FtsZ	5.28	0.79	5.39	0.96	0.12	1.16	0.94	5.31	0.85	5.39	0.86	0.06	1.22	0.99	1.00	0.96
MDR247	5.16	0.74	5.04	1.20	-0.14	1.23	0.94	5.11	0.94	5.15	0.97	0.01	1.29	0.99	1.00	0.96
MDR254	5.27	0.57	4.58	1.41	-0.70	1.51	0.72	4.87	1.25	4.89	0.87	0.03	1.27	0.99	0.83	0.87
MDR90	4.60	1.02	5.05	0.96	0.43	1.09	0.72	4.9	1.08	4.8	1.27	-0.17	1.45	0.99	0.83	0.87
P3	4.61	0.95	4.80	1.00	0.18	1.33	0.94	4.89	0.93	4.79	1.05	-0.11	1.39	0.99	1.00	0.87
RfaeF3	4.81	0.92	4.77	0.87	-0.05	1.04	0.96	4.67	0.82	4.73	0.87	0.03	1.2	0.99	1.00	0.96
RhomF1	5.61	0.93	5.82	1.07	0.22	1.30	0.94	5.88	0.78	5.87	0.91	-0.04	1.26	0.99	1.00	0.87
RhomF2	5.31	1.01	5.38	0.92	0.06	1.20	0.96	5.31	1	5.42	0.84	0.08	1.1	0.99	1.00	0.96
RintF1	5.26	0.88	5.36	1.07	0.11	1.13	0.94	5.35	0.74	5.37	0.77	-0.01	1.08	0.99	1.00	0.96
RinuF5	5.34	0.87	5.41	0.85	0.07	0.99	0.96	5.41	0.8	5.43	0.74	-0.02	0.98	0.99	1.00	0.96
SalFliC	5.79	1.20	5.84	0.86	0.06	1.29	0.96	6.06	0.91	6.08	1.06	0.04	0.73	0.99	0.83	0.96
UNCFlIC	6.28	1.18	6.21	0.87	-0.08	0.75	0.94	6.82	1.04	6.79	1.18	0.02	0.62	0.99	0.38	0.93
Vinc	5.10	0.93	5.30	1.05	0.19	0.98	0.93	5.38	0.9	5.24	0.95	-0.18	1.28	0.94	1.00	0.87
keto	5.58	0.63	5.63	0.48	0.06	0.57	0.94	5.68	0.42	5.69	0.36	0.01	0.51	0.99	1.00	0.96
ompC	4.73	1.07	4.98	0.99	0.24	1.15	0.93	4.75	0.95	4.78	1.09	0.01	1.49	0.99	0.99	0.87
rlB1	6.00	0.56	6.02	0.49	0.00	0.57	1.00	6.11	0.38	6.13	0.49	0.03	0.43	0.99	0.83	0.96
rlB10	5.59	0.60	5.48	0.48	-0.09	0.69	0.94	5.78	0.41	5.54	0.57	-0.21	0.6	0.20	1.00	0.87
rlB12	5.73	1.60	6.23	0.94	0.49	1.55	0.72	6.15	0.87	6.19	0.86	0.03	1.25	0.99	1.00	0.87
rlB13	6.23	0.97	6.49	0.93	0.26	1.15	0.93	6.41	0.92	6.49	0.93	0.05	1.36	0.99	1.00	0.87
rlB14	6.14	0.64	6.09	0.51	-0.05	0.68	0.96	6.29	0.44	6.21	0.35	-0.09	0.47	0.77	0.83	0.96
rlB16	7.00	0.53	7.06	0.53	0.08	0.38	0.93	7.21	0.47	7.19	0.44	-0.01	0.38	0.99	0.83	0.87
rlB17	4.86	0.92	5.14	0.88	0.29	1.06	0.84	4.92	0.92	4.99	0.9	0.04	1.29	0.99	0.99	0.87
rlB18	4.71	0.87	4.83	0.96	0.09	1.15	0.96	4.84	0.92	4.68	0.98	-0.21	1.39	0.89	1.00	0.87
rlB20	6.07	0.59	6.16	0.41	0.09	0.50	0.93	6.18	0.45	6.02	0.94	-0.16	0.93	0.80	0.90	0.87
rlB8	5.41	1.04	5.40	0.99	-0.04	1.34	0.97	5.54	0.62	5.49	0.71	-0.08	0.97	0.99	1.00	0.96
rlB9	5.09	1.10	5.54	1.07	0.44	1.37	0.72	5.17	1.06	5.24	1.07	-0.01	1.46	0.99	0.83	0.87
riB2	5.94	0.93	6.03	0.91	0.13	1.25	0.94	5.97	0.79	6.01	0.87	0.03	1.16	0.99	1.00	0.96
riB4	5.38	1.25	5.76	1.16	0.39	1.73	0.93	5.82	0.96	5.85	1.01	0	1.47	0.99	1.00	0.87
riB5	5.29	0.72	5.21	1.13	-0.09	1.27	0.96	5.27	0.75	5.32	0.87	0.05	1.17	0.99	1.00	0.96
tetraco	5.06	0.61	5.05	0.81	-0.01	0.84	0.98	5.09	1.34	5.03	0.99	-0.06	1.46	0.99	1.00	0.96

Any ABX = Any antibiotic use; IgG = Immunoglobulin-G; SD = Standard Deviation; FDR = False Discovery Rate.

*Significant at P<0.05 level

Table S-13. IgA MFI differences-any antibiotic use
Differences in IgA serological markers' mean fluorescence intensities between paired baseline and post- samples for subjects with and without any antibiotic use, and any antibiotic use versus no use post-samples and change between pre- and post- samples.

Target	Any ABX+ (N=27)								Any ABX-(N=62)								FDR P-value Any ABX+ v. Any ABX-	
	IgA Baseline Mean (N=26)	SD	IgA Post Mean (N=27)	SD	IgA Change Mean (N=26)	SD	FDR P-value (pre/post)		IgA Baseline Mean (N=59)	SD	IgA Post Mean (N=61)	SD	IgA Change Mean (N=58)	SD	FDR P-value (pre/post)		Post	Change
28-4band 3-1-57Fla	6.43	0.91	6.50	1.23	0.04	0.94	0.88		6.09	0.92	6.03	1.01	-0.02	0.71	0.91		0.93	0.94
A4Fla2	4.42	1.61	4.30	1.41	-0.05	1.89	0.93		4.65	0.76	4.18	1.38	-0.4	1.19	0.22		0.93	0.89
A4Fla3	5.51	1.26	5.62	0.63	0.11	1.26	0.82		5.85	0.59	5.75	0.64	-0.09	0.52	0.59		0.93	0.91
AgF1	4.90	1.14	4.82	0.92	-0.08	1.41	0.87		5.23	0.64	4.94	0.95	-0.26	0.93	0.30		0.93	0.91
CBir1	4.44	1.80	4.99	1.33	0.56	1.76	0.47		5.26	1.1	4.87	1.32	-0.39	1.35	0.30		0.93	0.58
CBir11	5.42	0.68	5.15	0.76	-0.28	0.67	0.44		5.5	0.63	5.31	0.68	-0.21	0.64	0.22		0.93	0.91
CBir14	5.51	0.60	5.35	0.66	-0.20	0.51	0.45		5.64	0.92	5.55	0.81	-0.07	0.72	0.76		0.93	0.89
CBir19	4.74	1.17	4.19	1.91	-0.57	1.97	0.47		4.35	1.24	4.07	1.71	-0.19	1.83	0.75		0.93	0.89
CBir23	4.74	1.36	4.12	2.04	-0.55	2.21	0.49		3.84	1.9	4.28	1.6	0.39	2.28	0.59		0.93	0.73
CBir45	5.84	0.59	5.69	0.70	-0.14	0.63	0.52		5.91	0.59	5.8	0.61	-0.1	0.5	0.48		0.93	0.95
CBir5	6.35	0.72	6.18	0.81	-0.15	0.47	0.47		6.15	0.5	6.04	0.55	-0.1	0.48	0.48		0.93	0.91
CBir56	5.19	1.02	5.13	0.83	-0.02	1.38	0.95		5.14	0.82	4.97	0.97	-0.17	1.4	0.74		0.93	0.91
CBir63	5.71	0.74	5.83	0.88	0.11	0.70	0.68		6.07	0.74	5.94	0.93	-0.11	0.86	0.74		0.93	0.87
CBir66	4.98	0.75	4.88	0.74	-0.11	0.78	0.69		4.84	0.73	4.71	1.02	-0.14	1.08	0.74		0.93	0.96
CBir8	5.79	0.78	5.62	0.82	-0.20	0.72	0.47		5.8	0.9	5.74	0.83	-0.05	0.73	0.80		0.93	0.89
Cdtb	5.50	0.64	5.33	0.71	-0.15	0.83	0.60		5.23	0.66	5.19	0.74	-0.04	0.81	0.86		0.93	0.91
EF20	7.01	0.51	6.94	0.91	-0.07	0.62	0.75		7.04	0.75	6.83	0.88	-0.18	0.78	0.44		0.93	0.91
FlaX	5.40	2.47	5.54	2.23	0.15	1.93	0.82		5.21	1.81	4.29	2.33	-0.96	2.46	0.17		0.93	0.58
FtsZ	5.86	0.72	5.70	0.76	-0.16	0.74	0.52		5.92	0.71	5.85	1.01	-0.09	0.9	0.75		0.93	0.92
MDR2	5.76	0.54	5.46	0.81	-0.30	0.69	0.44		5.69	0.62	5.67	0.61	0.01	0.66	0.91		0.93	0.68
MDR2 47	5.17	0.67	4.64	1.06	-0.53	1.25	0.44		5.04	0.8	4.95	0.81	-0.06	0.94	0.80		0.93	0.74
MDR2 54	4.46	0.87	3.42	1.94	-1.03	2.11	0.44		3.96	1.52	3.53	1.7	-0.43	2.28	0.53		0.93	0.87
MDR9 0	4.53	1.70	4.78	1.60	0.22	1.30	0.65		5.05	1.03	4.51	1.51	-0.59	1.61	0.17		0.93	0.58
P3	3.90	1.93	4.11	1.51	0.22	2.09	0.78		4.34	1.36	4.02	1.57	-0.33	2.19	0.61		0.93	0.89
RfaeF3	5.29	0.87	4.85	1.45	-0.44	1.52	0.47		5.14	1.15	5.2	1.42	0.02	1.57	0.91		0.93	0.87
RhomF 1	5.96	0.46	5.84	0.65	-0.13	0.50	0.47		5.98	0.56	5.89	0.69	-0.06	0.6	0.75		0.93	0.91
RhomF 2	5.54	0.48	5.32	0.86	-0.22	0.82	0.47		5.18	0.87	5.24	0.73	0.06	0.99	0.81		0.93	0.87
RintF1	5.45	1.21	5.14	1.29	-0.33	1.59	0.53		5.5	0.57	5.31	0.75	-0.17	0.7	0.41		0.93	0.91
RinuF5	5.40	0.56	5.03	0.91	-0.37	1.01	0.47		5.12	1.21	5.01	0.82	-0.12	1.38	0.79		0.98	0.89
SalFliC	5.06	1.25	4.76	1.11	-0.26	1.74	0.69		5.1	1.03	4.98	1.21	-0.1	1.15	0.79		0.93	0.91
UNCfli C	5.18	1.23	5.22	1.33	0.05	1.23	0.88		5.78	1.29	5.71	1.58	-0.03	0.97	0.91		0.93	0.93

Vinc	5.21	0.85	5.03	1.34	-0.18	1.27	0.69	5.26	0.97	5.02	1.06	-0.24	1.21	0.52	0.99	0.95
keto	5.17	0.84	4.92	1.16	-0.22	0.84	0.47	5.11	0.58	4.98	0.6	-0.12	0.76	0.61	0.93	0.91
ompC	5.10	0.77	4.75	0.78	-0.34	1.07	0.47	4.78	0.94	4.72	0.74	-0.02	1.19	0.91	0.93	0.87
rlB1	5.47	0.56	5.41	0.52	-0.05	0.67	0.82	5.51	0.55	5.44	0.6	-0.04	0.61	0.80	0.93	0.96
rlB10	5.04	0.70	4.75	0.88	-0.27	1.17	0.52	5.14	0.59	4.88	1.02	-0.22	0.83	0.33	0.93	0.96
rlB12	6.11	1.00	6.32	0.51	0.21	0.80	0.47	6.33	0.42	6.35	0.5	0.04	0.52	0.80	0.93	0.89
rlB13	6.73	0.56	6.67	0.56	-0.06	0.46	0.71	6.74	0.47	6.74	0.54	0.02	0.5	0.89	0.93	0.91
rlB14	5.42	0.73	5.19	0.87	-0.24	0.79	0.47	5.45	0.8	5.27	0.73	-0.19	0.89	0.48	0.93	0.94
rlB16	6.28	0.77	6.40	0.46	0.15	0.66	0.52	6.38	0.54	6.29	0.56	-0.08	0.53	0.61	0.93	0.80
rlB17	5.45	0.72	5.19	0.68	-0.25	0.59	0.44	5.33	0.54	5.23	0.63	-0.08	0.76	0.75	0.93	0.89
rlB18	4.91	1.75	4.94	1.52	0.02	2.22	0.95	5.13	1.14	5.18	1.05	0.02	1.36	0.91	0.93	0.99
rlB20	5.26	0.70	5.28	0.93	0.04	0.78	0.87	5.2	0.76	5.22	0.85	0.03	1.04	0.91	0.93	0.96
rlB8	5.60	0.74	5.49	0.95	-0.10	1.22	0.82	5.43	0.88	5.49	0.63	0.06	1.12	0.82	0.99	0.91
rlB9	4.27	1.62	4.00	1.72	-0.31	2.16	0.69	4.14	1.66	3.98	1.63	-0.23	2.19	0.75	0.99	0.96
rlB2	6.48	0.60	6.35	0.61	-0.13	0.47	0.47	6.43	0.66	6.46	0.74	0.06	0.54	0.75	0.93	0.80
rlB4	6.25	0.67	6.44	0.55	0.18	0.64	0.47	6.3	0.58	6.34	0.84	0.06	0.79	0.80	0.93	0.91
rlB5	5.50	0.58	5.33	0.82	-0.17	0.80	0.53	5.33	0.53	5.36	0.60	0.05	0.62	0.80	0.97	0.87
tetraco	5.02	1.02	4.62	1.28	-0.41	1.14	0.47	5.02	1.32	4.73	1.12	-0.28	1.53	0.53	0.93	0.92

Any ABX = Any antibiotic use; IgA = Immunoglobulin-A; SD = Standard Deviation; FDR = False Discovery Rate.

*Significant at P<0.05 level

Table S-14. IgG MFI differences-antibiotic use for TD
Differences in IgG serological markers' mean fluorescence intensities between paired baseline and post- samples for subjects with and without antibiotic use for TD, and antibiotic use for TD versus no use post-samples and change between pre- and post-samples.

Target	TD ABX+ (N=16)							TD ABX- (N=73)							FDR P-value TD ABX+ v. TD ABX-	
	IgG Baseline Mean (N=16)	SD	IgG Post Mean (N=16)	SD	IgG Change Mean (N=16)	SD	FDR P-value (pre/post)	IgG Baseline Mean (N=69)	SD	IgG Post Mean (N=72)	SD	IgG Change Mean (N=68)	SD	FDR P-value (pre/post)	Post	Change
28-4band	5.76	0.80	6.00	0.74	0.24	0.71	0.85	5.96	0.56	5.96	0.58	0	0.65	0.98	0.99	0.89
3-1-57Fla	4.81	1.22	5.00	1.26	0.19	1.47	0.85	5.11	0.74	4.95	0.98	-0.07	0.85	0.98	0.99	0.89
A4Fla2	6.23	0.75	6.22	0.52	-0.01	0.72	0.99	6.61	0.61	6.51	0.49	-0.1	0.57	0.94	0.65	0.93
A4Fla3	5.68	0.84	5.49	0.62	-0.18	0.75	0.85	6.02	0.53	5.79	0.68	-0.26	0.6	0.04*	0.65	0.95
AgF1	4.22	1.43	5.04	0.97	0.83	1.63	0.85	4.7	0.92	4.53	1.13	-0.19	1.55	0.98	0.65	0.89
CBir1	5.83	0.84	5.84	0.47	0.01	0.67	0.99	6.28	0.59	6.11	0.58	-0.17	0.61	0.27	0.65	0.89
CBir11	6.29	0.61	6.13	0.70	-0.17	0.56	0.85	6.58	0.65	6.44	0.64	-0.12	0.42	0.27	0.65	0.95

CBir14	5.13	0.81	5.19	0.71	0.05	0.94	0.99	4.9	1.05	4.99	0.97	0.13	1.27	0.98	0.97	0.95
CBir19	5.13	1.58	5.20	1.38	0.07	1.94	0.99	5.06	1.69	5.27	1.23	0.2	1.67	0.98	0.99	0.95
CBir23	6.40	0.42	6.35	0.45	-0.06	0.42	0.85	6.47	0.32	6.41	0.31	-0.06	0.35	0.94	0.99	0.99
CBir45	6.77	0.69	6.70	0.48	-0.08	0.49	0.85	6.93	0.4	6.81	0.45	-0.11	0.35	0.27	0.97	0.95
CBir5	5.61	0.69	5.49	0.46	-0.13	0.76	0.85	5.52	0.7	5.64	0.66	0.1	0.88	0.98	0.93	0.89
CBir56	7.04	0.92	7.19	0.97	0.16	0.73	0.85	7.5	0.52	7.53	0.56	0.04	0.45	0.98	0.92	0.89
CBir63	5.78	0.49	5.86	0.44	0.09	0.63	0.85	6.04	0.5	6.01	0.54	-0.04	0.63	0.98	0.92	0.89
CBir66	6.54	0.71	6.38	0.70	-0.16	0.55	0.85	6.95	0.76	6.82	0.81	-0.11	0.47	0.51	0.65	0.95
CBir8	5.06	1.55	5.30	0.92	0.23	1.65	0.85	5.36	0.76	5.28	0.82	-0.11	1.11	0.98	0.99	0.89
Cdtb	5.24	0.98	5.52	1.42	0.28	1.66	0.85	5.68	1.02	5.57	1.18	-0.14	1.59	0.98	0.99	0.89
EF20	4.47	1.41	4.76	1.55	0.29	1.62	0.85	4.94	1.37	4.91	1.32	-0.12	1.71	0.98	0.99	0.89
FlaX	6.46	0.80	6.49	0.80	0.03	0.91	0.99	6.94	0.59	6.87	0.56	-0.07	0.49	0.98	0.65	0.95
FtsZ	5.13	0.91	5.38	1.16	0.25	1.35	0.85	5.34	0.82	5.39	0.82	0.04	1.17	0.98	0.99	0.89
MDR247	5.04	0.81	4.92	1.41	-0.11	1.48	0.96	5.15	0.89	5.16	0.94	-0.01	1.22	0.98	0.99	0.95
MDR254	5.26	0.50	4.52	1.67	-0.74	1.74	0.85	4.93	1.19	4.85	0.88	-0.07	1.26	0.98	0.98	0.89
MDR90	4.56	1.02	4.85	1.07	0.29	1.25	0.85	4.87	1.07	4.88	1.21	-0.05	1.4	0.98	0.99	0.89
P3	4.40	1.07	4.71	1.18	0.32	1.60	0.85	4.9	0.89	4.82	1	-0.1	1.31	0.98	0.99	0.89
RfaeF3	4.59	1.05	4.64	1.05	0.05	1.23	0.99	4.74	0.8	4.77	0.83	0	1.13	0.98	0.99	0.97
RhomF1	5.41	1.03	5.73	1.27	0.31	1.52	0.85	5.89	0.76	5.89	0.88	-0.02	1.21	0.98	0.99	0.89
RhomF2	5.10	1.18	5.36	1.14	0.27	1.41	0.85	5.36	0.95	5.42	0.79	0.03	1.06	0.98	0.99	0.89
RintF1	5.01	1.00	5.18	1.25	0.17	1.35	0.85	5.4	0.71	5.41	0.76	-0.01	1.03	0.98	0.99	0.93
RinuF5	5.14	0.96	5.35	1.02	0.21	1.20	0.85	5.45	0.77	5.44	0.72	-0.04	0.93	0.98	0.99	0.89
SalFliC	5.77	1.53	6.05	1.00	0.28	1.59	0.85	6.02	0.86	6	1.02	-0.01	0.71	0.98	0.99	0.89
UNCFluC	6.34	1.17	6.15	0.92	-0.20	0.79	0.85	6.72	1.08	6.71	1.14	0.03	0.63	0.98	0.65	0.89
Vinc	4.79	0.98	5.16	1.17	0.36	1.12	0.85	5.41	0.86	5.28	0.93	-0.17	1.2	0.98	0.99	0.89
keto	5.63	0.67	5.64	0.50	0.01	0.60	0.99	5.65	0.45	5.68	0.37	0.03	0.52	0.98	0.99	0.97
ompC	4.70	1.06	4.97	0.98	0.27	1.26	0.85	4.76	0.97	4.81	1.08	0.04	1.42	0.98	0.99	0.89
riB1	5.96	0.60	5.97	0.44	0.01	0.66	0.99	6.11	0.4	6.13	0.5	0.03	0.43	0.98	0.92	0.99
riB10	5.66	0.69	5.45	0.42	-0.21	0.66	0.85	5.74	0.42	5.54	0.57	-0.17	0.62	0.27	0.99	0.96
riB12	5.37	1.93	6.16	1.16	0.79	1.87	0.85	6.17	0.83	6.21	0.81	0.03	1.17	0.98	0.99	0.89
riB13	6.04	1.10	6.39	1.07	0.35	1.27	0.85	6.43	0.88	6.51	0.9	0.06	1.31	0.98	0.99	0.89
riB14	6.12	0.73	6.03	0.50	-0.09	0.64	0.85	6.27	0.44	6.21	0.38	-0.07	0.52	0.98	0.92	0.97
riB16	6.95	0.65	7.02	0.65	0.07	0.44	0.85	7.19	0.45	7.18	0.43	0	0.37	0.98	0.97	0.91
riB17	4.80	0.98	5.20	0.88	0.40	1.03	0.85	4.93	0.9	5	0.9	0.05	1.26	0.98	0.97	0.89
riB18	4.58	0.99	4.75	1.06	0.17	1.40	0.85	4.85	0.87	4.72	0.96	-0.18	1.3	0.98	0.99	0.89
riB20	6.02	0.68	6.15	0.40	0.14	0.58	0.85	6.18	0.44	6.05	0.88	-0.13	0.87	0.98	0.98	0.89
riB8	5.21	1.27	5.25	1.16	0.04	1.65	0.99	5.57	0.59	5.51	0.71	-0.09	0.92	0.98	0.97	0.95
riB9	4.96	1.26	5.54	1.13	0.58	1.51	0.85	5.19	1.02	5.29	1.07	0.03	1.41	0.98	0.97	0.89
riB2	5.74	1.07	6.01	1.10	0.27	1.52	0.85	6.01	0.76	6.02	0.83	0.01	1.1	0.98	0.99	0.89
riB4	5.04	1.42	5.74	1.29	0.71	1.92	0.85	5.84	0.92	5.84	1	-0.02	1.44	0.98	0.99	0.89
riB5	5.10	0.81	5.10	1.38	0.00	1.50	1.00	5.31	0.72	5.33	0.84	0.01	1.12	0.98	0.99	0.99
tetraco	5.18	0.57	5.27	0.76	0.10	0.94	0.89	5.06	1.26	4.98	0.96	-0.08	1.37	0.98	0.92	0.89

TD ABX = Antibiotic use for TD; IgG = Immunoglobulin-G; SD = Standard Deviation; FDR = False Discovery Rate.

*Significant at P<0.05 level

Table S-15. IgA MFI differences-antibiotic use for TD
Differences in IgA serological markers' mean fluorescence intensities between paired baseline and post- samples for subjects with and without antibiotic use for TD, and antibiotic use for TD only versus no use for TD post-samples and change between pre- and post- samples.

Target	TD ABX+ (N=16)							TD ABX- (N=73)							FDR P-value TD ABX+ v. TD ABX-	
	IgA Baseline Mean (N=16)	SD	IgA Post Mean (N=16)	SD	IgA Change Mean (N=16)	SD	FDR P-value (pre/post)	IgA Baseline Mean (N=69)	SD	IgA Post Mean (N=72)	SD	IgA Change Mean (N=68)	SD	FDR P-value (pre/post)	Post	Change
28-4band	6.23	0.76	6.13	1.32	-0.10	1.10	0.90	6.19	0.96	6.18	1.05	0.02	0.7	0.92	0.98	0.90
3-1-57Fla	4.59	1.66	4.39	1.41	-0.21	2.05	0.89	4.57	0.92	4.18	1.39	-0.31	1.28	0.29	0.98	0.91
A4Fla2	5.64	0.70	5.58	0.77	-0.06	0.61	0.89	5.77	0.89	5.74	0.61	-0.02	0.86	0.92	0.98	0.91
A4Fla3	4.77	1.42	4.76	1.07	-0.01	1.73	0.98	5.21	0.61	4.94	0.91	-0.25	0.9	0.29	0.98	0.90
AgF1	3.99	2.12	4.95	1.63	0.96	2.09	0.63	5.25	1.05	4.9	1.25	-0.34	1.28	0.29	0.98	0.47
CBir1	5.35	0.71	5.02	0.94	-0.33	0.68	0.63	5.51	0.63	5.31	0.64	-0.21	0.64	0.17	0.98	0.89
CBir11	5.49	0.66	5.35	0.71	-0.14	0.40	0.63	5.62	0.87	5.52	0.78	-0.1	0.71	0.53	0.98	0.90
CBir14	4.89	1.27	4.32	2.16	-0.57	2.09	0.63	4.37	1.2	4.06	1.68	-0.25	1.83	0.56	0.98	0.89
CBir19	4.72	1.65	4.04	2.40	-0.68	2.58	0.63	3.97	1.81	4.27	1.57	0.29	2.19	0.58	0.98	0.89
CBir23	5.80	0.69	5.58	0.88	-0.22	0.76	0.63	5.9	0.56	5.81	0.57	-0.09	0.48	0.42	0.98	0.89
CBir45	6.18	0.49	6.01	0.68	-0.17	0.52	0.63	6.22	0.6	6.1	0.64	-0.1	0.46	0.31	0.98	0.90
CBir5	5.33	0.79	5.11	0.98	-0.21	1.34	0.82	5.12	0.9	5	0.92	-0.11	1.41	0.79	0.98	0.90
CBir56	5.40	0.74	5.57	1.04	0.17	0.87	0.71	6.09	0.7	5.98	0.87	-0.09	0.8	0.61	0.98	0.89
CBir63	4.99	0.91	5.02	0.81	0.03	0.80	0.95	4.86	0.7	4.71	0.96	-0.17	1.04	0.53	0.98	0.89
CBir66	5.75	0.79	5.47	0.93	-0.29	0.82	0.63	5.81	0.88	5.75	0.79	-0.05	0.7	0.79	0.98	0.89
CBir8	5.48	0.71	5.30	0.73	-0.18	0.90	0.71	5.28	0.65	5.22	0.74	-0.05	0.79	0.83	0.98	0.90
Cdtb	6.90	0.50	6.87	1.02	-0.02	0.71	0.95	7.06	0.71	6.86	0.86	-0.18	0.74	0.29	0.98	0.89
EF20	4.87	2.42	5.29	1.81	0.42	1.81	0.63	5.36	1.92	4.53	2.46	-0.87	2.41	0.14	0.98	0.45
FlaX	5.73	0.81	5.47	0.88	-0.26	0.86	0.63	5.94	0.68	5.87	0.94	-0.08	0.84	0.72	0.98	0.89
FtsZ	5.64	0.58	5.41	0.90	-0.23	0.74	0.63	5.72	0.6	5.65	0.62	-0.05	0.67	0.79	0.98	0.89
MDR247	5.17	0.69	4.59	1.03	-0.59	1.41	0.63	5.06	0.78	4.92	0.87	-0.11	0.95	0.61	0.98	0.89
MDR254	4.38	1.00	3.36	2.02	-1.02	2.23	0.63	4.05	1.45	3.53	1.72	-0.52	2.24	0.29	0.98	0.89
MDR90	4.07	1.91	4.58	1.87	0.51	1.26	0.63	5.08	1.02	4.6	1.46	-0.54	1.56	0.14	0.98	0.35
P3	3.72	2.34	3.87	1.82	0.16	2.50	0.94	4.32	1.31	4.08	1.48	-0.24	2.09	0.61	0.98	0.89
RfaeF3	5.19	0.96	4.66	1.78	-0.53	1.89	0.63	5.19	1.1	5.18	1.34	-0.02	1.47	0.92	0.98	0.89
RhomF1	5.88	0.49	5.81	0.80	-0.08	0.60	0.88	5.99	0.54	5.89	0.65	-0.09	0.56	0.53	0.98	0.96
RhomF2	5.55	0.50	5.27	0.94	-0.28	0.83	0.63	5.23	0.83	5.26	0.74	0.03	0.97	0.92	0.98	0.89

RintF1	5.31	1.52	4.84	1.56	-0.47	1.97	0.63	5.53	0.55	5.35	0.73	-0.16	0.69	0.29	0.98	0.89
RinuF5	5.39	0.66	4.89	0.90	-0.50	1.01	0.63	5.17	1.14	5.04	0.84	-0.13	1.33	0.72	0.98	0.89
SalFliC	5.00	1.52	4.98	1.31	-0.02	2.07	0.98	5.11	0.98	4.9	1.15	-0.18	1.14	0.53	0.98	0.90
UNCFliC	5.29	1.42	5.24	1.60	-0.05	1.41	0.95	5.67	1.27	5.63	1.5	0.01	0.95	0.96	0.98	0.92
Vinc	4.98	0.96	4.83	1.67	-0.15	1.57	0.89	5.31	0.92	5.07	1	-0.24	1.14	0.34	0.98	0.91
keto	5.21	0.96	4.88	1.48	-0.33	0.96	0.63	5.11	0.59	4.98	0.57	-0.11	0.73	0.53	0.98	0.89
ompC	5.13	0.80	4.77	0.85	-0.37	1.14	0.63	4.82	0.91	4.72	0.73	-0.06	1.16	0.84	0.98	0.89
rlB1	5.46	0.67	5.36	0.63	-0.10	0.80	0.88	5.5	0.52	5.45	0.56	-0.03	0.58	0.84	0.98	0.90
rlB10	5.06	0.79	4.68	0.90	-0.37	1.30	0.63	5.12	0.58	4.87	0.99	-0.2	0.84	0.29	0.98	0.90
rlB12	5.79	1.13	6.23	0.63	0.44	0.92	0.63	6.37	0.43	6.37	0.47	0.01	0.5	0.92	0.98	0.89
rlB13	6.59	0.62	6.62	0.67	0.02	0.47	0.95	6.77	0.46	6.74	0.51	-0.01	0.5	0.92	0.98	0.90
rlB14	5.27	0.80	4.99	1.04	-0.28	0.79	0.63	5.48	0.77	5.31	0.69	-0.19	0.87	0.33	0.98	0.90
rlB16	6.13	0.90	6.33	0.54	0.20	0.83	0.63	6.4	0.52	6.32	0.53	-0.06	0.5	0.61	0.98	0.89
rlB17	5.35	0.88	5.21	0.76	-0.14	0.58	0.63	5.37	0.52	5.22	0.62	-0.13	0.75	0.46	0.98	0.96
rlB18	4.48	1.99	5.10	1.25	0.62	2.45	0.63	5.19	1.12	5.11	1.21	-0.12	1.4	0.77	0.98	0.89
rlB20	5.17	0.84	5.06	1.10	-0.10	0.90	0.89	5.23	0.72	5.28	0.82	0.06	0.98	0.83	0.98	0.89
rlB8	5.62	0.72	5.45	1.09	-0.17	1.35	0.88	5.45	0.87	5.5	0.65	0.05	1.1	0.84	0.98	0.89
rlB9	4.20	1.85	4.23	1.68	0.03	1.84	0.98	4.18	1.61	3.93	1.65	-0.33	2.24	0.53	0.98	0.89
rlB2	6.31	0.65	6.28	0.70	-0.03	0.50	0.94	6.48	0.64	6.46	0.7	0.01	0.53	0.92	0.98	0.90
rlB4	6.02	0.69	6.38	0.70	0.36	0.66	0.63	6.35	0.57	6.37	0.78	0.04	0.75	0.84	0.98	0.89
rlB5	5.45	0.67	5.26	0.90	-0.20	0.89	0.66	5.37	0.52	5.37	0.61	0.02	0.63	0.92	0.98	0.89
tetraco	5.09	0.98	4.66	1.61	-0.43	1.14	0.63	5.00	1.29	4.70	1.06	-0.30	1.48	0.36	0.98	0.90

TD ABX = Antibiotic use for TD; IgG = Immunoglobulin-G; SD = Standard Deviation; FDR = False Discovery Rate.

*Significant at P<0.05 level

Table S-16. IgG MFI Differences-TD

Differences in IgG serological markers' mean fluorescence intensities between paired baseline and post- samples for subjects with and without TD, and TD versus no TD post-samples and change between pre- and post- samples.

Target	TD+ (N=34)							TD- (N=55)							FDR P-value TD+ v. TD-	
	IgG Baseline Mean (N=33)	SD	IgG Post Mean (N=34)	SD	IgG Change Mean (N=33)	SD	FDR P-value (pre/post)	IgG Baseline Mean (N=52)	SD	IgG Post Mean (N=54)	SD	IgG Change Mean (N=51)	SD	FDR P-value (pre/post)	Post	Change
28-4band	5.89	0.72	5.99	0.62	0.10	0.68	0.96	5.95	0.54	5.96	0.6	0.01	0.66	0.96	0.95	0.92
3-1-57Fla	5.02	0.97	4.87	1.03	-0.09	1.19	0.96	5.07	0.77	5.01	1.04	0.03	0.85	0.96	0.95	0.92
A4Fla2	6.39	0.72	6.33	0.57	-0.06	0.59	0.96	6.63	0.59	6.54	0.45	-0.09	0.6	0.96	0.95	0.92
A4Fla3	5.84	0.79	5.68	0.72	-0.16	0.62	0.96	6.04	0.46	5.77	0.65	-0.29	0.63	0.09	0.95	0.92

AgF1	4.61	1.23	4.89	1.05	0.27	1.61	0.96	4.61	0.92	4.46	1.13	-0.17	1.6	0.96	0.95	0.92
CBir1	6.02	0.81	6.00	0.61	-0.01	0.54	0.96	6.31	0.52	6.1	0.54	-0.21	0.65	0.32	0.95	0.92
CBir11	6.43	0.80	6.33	0.75	-0.09	0.49	0.96	6.59	0.53	6.41	0.6	-0.15	0.42	0.23	0.95	0.92
CBir14	5.07	0.92	5.14	0.63	0.07	0.97	0.96	4.86	1.07	4.96	1.08	0.15	1.35	0.96	0.95	0.92
CBir19	4.99	1.59	5.02	1.20	0.06	1.88	0.96	5.12	1.72	5.41	1.27	0.24	1.61	0.96	0.95	0.92
CBir23	6.41	0.38	6.37	0.36	-0.03	0.39	0.96	6.49	0.31	6.42	0.33	-0.08	0.35	0.77	0.95	0.92
CBir45	6.77	0.56	6.69	0.48	-0.08	0.45	0.96	6.98	0.38	6.85	0.43	-0.12	0.34	0.23	0.95	0.92
CBir5	5.46	0.72	5.46	0.49	-0.02	0.85	0.96	5.58	0.68	5.71	0.68	0.1	0.87	0.96	0.95	0.92
CBir56	7.22	0.76	7.40	0.79	0.19	0.65	0.96	7.53	0.51	7.51	0.57	-0.01	0.39	0.96	0.95	0.92
CBir63	5.92	0.51	5.90	0.39	-0.01	0.51	0.96	6.04	0.51	6.03	0.59	-0.01	0.7	0.96	0.95	0.98
CBir66	6.60	0.72	6.45	0.71	-0.15	0.51	0.96	7.04	0.76	6.93	0.81	-0.1	0.47	0.93	0.27	0.92
CBir8	5.31	1.24	5.39	0.78	0.09	1.34	0.96	5.31	0.72	5.22	0.87	-0.13	1.15	0.96	0.95	0.92
Cdtb	5.54	1.05	5.58	1.24	0.04	1.54	0.96	5.64	1.01	5.55	1.22	-0.13	1.65	0.96	0.95	0.92
EF20	4.57	1.56	4.87	1.50	0.27	1.68	0.96	5.03	1.24	4.89	1.28	-0.24	1.69	0.96	0.95	0.92
FlaX	6.71	0.79	6.67	0.76	-0.03	0.68	0.96	6.94	0.55	6.88	0.51	-0.07	0.53	0.96	0.95	0.92
FtsZ	5.31	0.91	5.42	0.94	0.11	1.28	0.96	5.3	0.79	5.37	0.85	0.05	1.16	0.96	0.95	0.92
MDR247	5.08	0.89	5.16	1.16	0.08	1.30	0.96	5.16	0.88	5.09	0.96	-0.1	1.25	0.96	0.95	0.92
MDR254	4.99	0.98	4.69	1.34	-0.30	1.43	0.96	5	1.19	4.86	0.86	-0.14	1.35	0.96	0.95	0.92
MDR90	4.74	1.05	4.85	1.20	0.09	1.32	0.96	4.85	1.07	4.89	1.18	-0.03	1.42	0.96	0.95	0.92
P3	4.70	1.01	4.74	1.03	0.03	1.56	0.96	4.87	0.89	4.84	1.04	-0.05	1.25	0.96	0.95	0.92
Rfaef3	4.79	0.96	4.69	0.90	-0.10	1.21	0.96	4.66	0.78	4.77	0.85	0.08	1.11	0.96	0.95	0.92
RhomF1	5.79	0.98	5.93	1.01	0.15	1.28	0.96	5.81	0.73	5.81	0.93	-0.03	1.28	0.96	0.95	0.92
RhomF2	5.18	1.30	5.54	0.95	0.36	1.20	0.96	5.4	0.74	5.32	0.8	-0.11	1.05	0.96	0.95	0.92
RintF1	5.29	0.93	5.33	0.96	0.04	1.12	0.96	5.35	0.69	5.39	0.81	0.02	1.09	0.96	0.95	0.98
RinuF5	5.47	0.96	5.47	0.78	0.00	1.01	0.99	5.34	0.71	5.39	0.77	0.02	0.97	0.96	0.95	0.98
SalFlIC	5.94	1.28	6.09	1.03	0.16	1.15	0.96	6	0.81	5.96	1	-0.03	0.76	0.96	0.95	0.92
UNCFliC	6.49	1.15	6.38	1.07	-0.12	0.72	0.96	6.75	1.07	6.76	1.13	0.06	0.62	0.96	0.95	0.92
Vinc	5.19	0.98	5.33	0.95	0.13	1.13	0.96	5.36	0.87	5.21	1	-0.19	1.24	0.96	0.95	0.92
keto	5.59	0.60	5.63	0.41	0.05	0.60	0.96	5.69	0.41	5.7	0.39	0.01	0.48	0.96	0.95	0.92
ompC	4.80	1.04	4.99	0.97	0.19	1.38	0.96	4.71	0.96	4.75	1.12	0.01	1.4	0.97	0.95	0.92
rlB1	6.00	0.52	6.07	0.47	0.05	0.53	0.96	6.13	0.38	6.12	0.51	0.01	0.44	0.96	0.95	0.92
rlB10	5.73	0.58	5.56	0.48	-0.15	0.53	0.96	5.72	0.41	5.5	0.59	-0.2	0.69	0.45	0.95	0.92
rlB12	5.81	1.53	6.21	0.92	0.39	1.49	0.96	6.15	0.81	6.2	0.86	0.03	1.25	0.96	0.95	0.92
rlB13	6.31	1.01	6.48	0.89	0.17	1.17	0.96	6.39	0.89	6.5	0.96	0.08	1.38	0.96	0.95	0.92
rlB14	6.16	0.67	6.11	0.42	-0.05	0.58	0.96	6.3	0.38	6.21	0.4	-0.09	0.52	0.96	0.95	0.92
rlB16	7.01	0.54	7.09	0.53	0.09	0.39	0.96	7.23	0.45	7.19	0.44	-0.03	0.37	0.96	0.95	0.92
rlB17	4.97	0.94	5.07	0.79	0.11	1.14	0.96	4.86	0.9	5.01	0.96	0.12	1.28	0.96	0.95	0.98
rlB18	4.64	0.90	4.80	0.87	0.14	1.18	0.96	4.9	0.89	4.68	1.04	-0.28	1.39	0.93	0.95	0.92
rlB20	6.09	0.56	6.11	0.46	0.02	0.63	0.96	6.19	0.45	6.04	0.98	-0.15	0.93	0.96	0.95	0.92
rlB8	5.46	1.04	5.45	0.87	-0.03	1.33	0.96	5.53	0.54	5.47	0.77	-0.09	0.91	0.96	0.95	0.92
rlB9	5.12	1.19	5.51	0.99	0.39	1.39	0.96	5.16	0.99	5.22	1.12	-0.03	1.47	0.96	0.95	0.92
riB2	5.97	1.00	6.11	0.88	0.17	1.24	0.96	5.95	0.71	5.96	0.88	-0.02	1.15	0.96	0.95	0.92

riB4	5.52	1.29	5.84	1.07	0.32	1.67	0.96	5.79	0.9	5.81	1.05	-0.01	1.48	0.97	0.95	0.92
riB5	5.32	0.85	5.30	1.04	-0.03	1.30	0.96	5.25	0.67	5.28	0.9	0.03	1.14	0.96	0.95	0.92
tetraco	5.07	0.68	5.07	0.76	0	0.93	0.99	5.09	1.39	5.01	1.04	-0.07	1.49	0.96	0.95	0.92

TD = Travelers' Diarrhea; IgG = Immunoglobulin-G; SD = Standard Deviation; FDR = False Discovery Rate.

Table S-17. IgA MFI Differences-TD

Differences in IgA serological markers' mean fluorescence intensities between paired baseline and post- samples for subjects with and without TD, and TD versus no TD post-samples and change between pre- and post- samples

Target	TD+ (N=34)							TD- (N=55)							FDR P-value TD+ v. TD-	
	IgA Baseline Mean (N=33)	SD	IgA Post Mean (N=34)	SD	IgA Change Mean (N=33)	SD	FDR P-value (pre/post)	IgA Baseline Mean (N=52)	SD	IgA Post Mean (N=54)	SD	IgA Change Mean (N=51)	SD	FDR P-value (pre/post)	Post	Change
28-4band	6.10	0.75	6.16	1.14	0.03	1.00	0.98	6.26	1.02	6.18	1.08	-0.02	0.63	0.87	0.94	1.00
3-1-57Fla	4.53	1.29	3.97	1.57	-0.52	1.77	0.72	4.6	0.94	4.37	1.25	-0.14	1.18	0.59	0.89	0.90
A4Fla2	5.66	0.74	5.63	0.84	-0.03	0.53	0.95	5.8	0.92	5.77	0.48	-0.03	0.96	0.87	0.89	1.00
A4Fla3	4.93	1.17	4.78	1.12	-0.15	1.33	0.93	5.25	0.5	4.99	0.81	-0.24	0.92	0.22	0.89	1.00
AgF1	4.58	1.92	4.85	1.74	0.27	2.07	0.93	5.28	0.84	4.94	0.97	-0.33	1.03	0.14	0.89	0.67
CBir1	5.28	0.83	5.10	0.91	-0.20	0.68	0.72	5.6	0.46	5.36	0.53	-0.25	0.63	0.12	0.89	1.00
CBir11	5.36	1.06	5.42	0.93	0.03	0.74	0.98	5.75	0.62	5.54	0.65	-0.2	0.6	0.13	0.89	0.67
CBir14	4.51	1.50	4.16	2.04	-0.36	2.07	0.85	4.44	1.03	4.07	1.59	-0.28	1.76	0.47	0.89	1.00
CBir19	4.15	1.99	3.87	1.98	-0.22	2.74	0.93	4.09	1.67	4.45	1.54	0.31	1.93	0.47	0.89	0.91
CBir23	5.74	0.75	5.68	0.87	-0.06	0.67	0.93	5.98	0.44	5.82	0.43	-0.15	0.44	0.13	0.89	0.91
CBir45	6.11	0.54	6.01	0.69	-0.10	0.48	0.85	6.27	0.6	6.14	0.61	-0.12	0.47	0.22	0.89	1.00
CBir5	5.17	0.97	4.95	0.89	-0.20	1.36	0.93	5.15	0.82	5.07	0.95	-0.08	1.42	0.77	0.89	1.00
CBir56	5.67	0.83	5.80	1.01	0.12	0.95	0.93	6.14	0.66	5.97	0.84	-0.15	0.71	0.34	0.89	0.68
CBir63	4.77	0.93	4.84	0.88	0.06	0.76	0.93	4.95	0.58	4.71	0.98	-0.25	1.11	0.32	0.89	0.67
CBir66	5.57	0.89	5.49	0.81	-0.11	0.79	0.93	5.94	0.82	5.83	0.81	-0.09	0.69	0.52	0.89	1.00
CBir8	5.44	0.70	5.26	0.84	-0.17	0.83	0.85	5.24	0.63	5.21	0.66	-0.01	0.8	0.89	0.89	0.91
Cdtb	7.08	0.72	6.91	0.93	-0.17	0.82	0.85	7	0.66	6.83	0.86	-0.13	0.68	0.35	0.89	1.00
EF20	4.90	2.36	4.78	2.16	-0.13	2.30	0.95	5.51	1.75	4.6	2.5	-0.94	2.35	0.12	0.89	0.67
FlaX	5.81	0.90	5.69	0.97	-0.12	0.67	0.85	5.96	0.56	5.87	0.92	-0.11	0.95	0.61	0.89	1.00
FtsZ	5.68	0.67	5.65	0.84	-0.02	0.76	0.98	5.73	0.55	5.58	0.57	-0.13	0.63	0.35	0.89	0.91
MDR247	5.04	0.66	4.92	0.92	-0.11	1.17	0.93	5.11	0.83	4.81	0.9	-0.27	0.99	0.22	0.89	0.91
MDR254	3.90	1.57	3.30	1.93	-0.61	2.25	0.72	4.24	1.23	3.62	1.67	-0.62	2.24	0.22	0.89	1.00
MDR90	4.63	1.49	4.66	1.60	0.01	1.42	0.99	5.06	1.12	4.55	1.5	-0.57	1.62	0.13	0.89	0.67
P3	4.11	1.84	3.99	1.51	-0.12	2.20	0.95	4.27	1.36	4.08	1.57	-0.19	2.16	0.72	0.89	1.00
RfaeF3	5.08	1.20	4.93	1.68	-0.14	1.75	0.93	5.26	0.99	5.19	1.26	-0.1	1.44	0.77	0.89	1.00
RhomF1	5.99	0.60	5.97	0.80	-0.02	0.58	0.98	5.96	0.48	5.82	0.59	-0.12	0.56	0.32	0.89	0.91
RhomF2	5.29	1.05	5.38	0.84	0.09	1.18	0.93	5.29	0.57	5.19	0.73	-0.1	0.76	0.52	0.89	0.91

RintF1	5.45	1.15	5.22	1.26	-0.25	1.46	0.85	5.51	0.52	5.29	0.68	-0.2	0.69	0.22	0.89	1.00
RinuF5	5.38	0.79	5.03	0.98	-0.35	1.04	0.72	5.1	1.2	5	0.76	-0.1	1.41	0.77	0.93	0.91
SalFliC	4.89	1.32	4.86	1.28	0.00	1.62	0.99	5.21	0.91	4.95	1.12	-0.25	1.16	0.34	0.89	0.91
UNCFliC	5.39	1.31	5.26	1.77	-0.11	1.39	0.93	5.73	1.28	5.75	1.31	0.06	0.75	0.73	0.89	0.91
Vinc	5.04	1.02	5.07	1.36	0.03	1.47	0.98	5.37	0.85	5	1	-0.38	1.01	0.12	0.89	0.67
keto	5.01	0.85	4.91	1.10	-0.08	0.85	0.93	5.2	0.51	4.99	0.54	-0.2	0.73	0.22	0.89	0.92
ompC	4.86	1.13	4.77	0.73	-0.08	1.39	0.95	4.88	0.72	4.7	0.77	-0.14	0.99	0.52	0.89	1.00
rlB1	5.37	0.68	5.32	0.67	-0.05	0.68	0.93	5.57	0.44	5.5	0.5	-0.04	0.59	0.77	0.89	1.00
rlB10	5.07	0.76	4.72	1.20	-0.33	1.08	0.72	5.14	0.52	4.91	0.8	-0.18	0.84	0.34	0.89	0.91
rlB12	6.05	0.89	6.33	0.58	0.28	0.75	0.72	6.4	0.4	6.35	0.45	-0.03	0.49	0.77	0.89	0.67
rlB13	6.67	0.62	6.73	0.65	0.06	0.50	0.93	6.78	0.41	6.72	0.47	-0.04	0.48	0.72	0.94	0.91
rlB14	5.21	0.98	5.15	0.96	-0.08	0.85	0.93	5.59	0.57	5.31	0.62	-0.29	0.85	0.13	0.89	0.89
rlB16	6.08	0.73	6.24	0.57	0.17	0.66	0.72	6.51	0.46	6.37	0.5	-0.13	0.49	0.22	0.89	0.58
rlB17	5.36	0.69	5.19	0.71	-0.16	0.63	0.72	5.36	0.54	5.24	0.6	-0.11	0.77	0.50	0.89	1.00
rlB18	4.80	1.66	5.25	1.14	0.45	1.85	0.76	5.23	1.09	5.02	1.25	-0.26	1.48	0.45	0.89	0.67
rlB20	5.07	0.88	5.06	1.05	-0.01	0.91	0.99	5.31	0.63	5.36	0.73	0.06	1	0.77	0.89	1.00
rlB8	5.45	1.14	5.55	0.84	0.11	1.48	0.93	5.5	0.58	5.46	0.68	-0.05	0.88	0.77	0.89	0.96
rlB9	3.97	2.04	4.45	1.72	0.46	2.36	0.85	4.32	1.34	3.7	1.55	-0.73	1.91	0.12	0.89	0.58
riB2	6.44	0.89	6.54	0.94	0.11	0.60	0.85	6.45	0.41	6.36	0.49	-0.07	0.45	0.49	0.89	0.67
riB4	6.15	0.64	6.44	0.66	0.28	0.60	0.57	6.37	0.57	6.33	0.82	-0.02	0.8	0.89	0.89	0.67
riB5	5.42	0.65	5.40	0.78	-0.02	0.78	0.98	5.36	0.48	5.32	0.59	-0.02	0.63	0.87	0.89	1.00
tetraco	4.89	0.99	4.47	1.57	-0.44	1.33	0.72	5.10	1.36	4.84	0.80	-0.25	1.47	0.46	0.89	0.92

TD = Travelers' Diarrhea; IgA = Immunoglobulin-A; SD = Standard Deviation; FDR = False Discovery Rate.

Table S-18. IgG PCA-Component Scores
PCA for change in host response to IgG Microbiota Antigens Eigenvectors for top 5
Principal Components

Target	IgG Eigenvectors/Component Scores				
	Prin1	Prin2	Prin3	Prin4	Prin5
_28_4band	0.094	0.057	0.016	0.052	-0.005
_3_1_57Fla	0.020	0.240	0.144	-0.015	0.228
A4Fla2	0.008	0.091	0.167	-0.088	0.008
A4Fla3	0.002	0.118	0.145	-0.065	0.043
AgF1	0.229	0.021	-0.060	0.236	0.489
CBir1	0.007	0.108	0.157	-0.125	0.074
CBir11	0.013	0.108	0.072	-0.020	-0.025
CBir14	-0.013	0.324	-0.036	0.141	0.058
CBir19	-0.028	0.517	-0.547	0.287	-0.172

CBir23	0.016	0.066	0.061	-0.013	0.054
CBir45	0.024	0.085	0.061	-0.066	0.044
CBir5	0.019	0.268	0.057	0.109	-0.024
CBir56	0.029	0.066	0.025	0.006	0.044
CBir63	0.000	0.079	0.157	0.035	0.121
CBir66	0.011	0.131	0.088	0.024	0.017
CBir8	0.192	0.041	0.001	-0.205	0.119
Cdtb	0.271	0.088	-0.013	-0.130	-0.185
EF20	0.221	-0.223	0.161	0.392	-0.010
FlaX	0.032	0.112	0.135	-0.014	0.009
FtsZ	0.206	-0.009	0.028	0.037	-0.139
MDR247	0.191	-0.029	0.084	0.132	-0.291
MDR254	-0.036	0.123	0.519	0.093	-0.414
MDR90	0.179	-0.232	0.104	0.046	0.068
P3	0.196	-0.071	0.059	-0.140	0.118
RfaeF3	0.158	0.081	-0.016	0.211	-0.078
RhomF1	0.222	0.080	-0.035	-0.100	-0.099
RhomF2	0.156	0.071	-0.056	-0.249	0.020
RintF1	0.180	0.011	0.054	0.047	-0.139
RinuF5	0.137	0.027	0.045	0.106	-0.037
SalFliC	0.004	0.263	0.135	-0.158	0.121
UNCFliC	-0.007	0.163	0.113	0.070	0.009
Vinc	0.204	-0.089	-0.006	-0.028	0.058
keto	0.006	0.117	0.125	0.037	0.068
ompC	0.198	-0.081	-0.056	0.165	0.164
riB1	0.004	0.110	0.074	-0.015	0.034
riB10	-0.009	0.071	0.079	-0.065	0.122
riB12	0.218	0.069	-0.139	-0.206	0.127
riB13	0.230	0.016	-0.026	-0.122	-0.076
riB14	0.016	0.105	0.029	-0.049	0.059
riB16	0.012	0.070	0.051	-0.037	0.042
riB17	0.189	-0.088	0.009	0.184	0.054
riB18	0.191	-0.021	-0.031	0.191	0.088
riB20	0.009	0.049	0.118	-0.184	0.198
riB8	0.170	0.040	0.032	-0.031	-0.120
riB9	0.215	-0.061	-0.108	0.068	0.032
riB2	0.188	0.082	-0.034	-0.206	-0.062

riB4	0.266	0.121	-0.066	-0.220	-0.060
riB5	0.198	0.064	0.042	-0.001	-0.222
tetraco	0.006	0.204	0.294	0.275	0.228

Table S-19. IgA PCA-Component Scores
PCA for change in host response to IgA Microbiota Antigens: Eigenvectors for top 5 Principal Components

IgA Eigenvectors/Component Scores					
Target	Prin1	Prin2	Prin3	Prin4	Prin5
_28_4band	0.079	0.049	0.107	-0.003	0.085
_3_1_57Fla	0.142	-0.097	-0.152	-0.030	-0.091
A4Fla2	0.058	-0.025	-0.018	0.020	-0.059
A4Fla3	0.138	-0.006	0.055	0.003	-0.088
AgF1	0.115	0.149	-0.253	-0.099	0.226
CBir1	0.093	-0.044	-0.025	0.085	-0.078
CBir11	0.131	-0.055	0.021	0.016	-0.027
CBir14	0.290	-0.009	0.173	0.059	-0.092
CBir19	0.324	-0.416	-0.108	0.181	-0.038
CBir23	0.086	-0.023	0.028	0.058	-0.001
CBir45	0.062	-0.002	0.045	0.049	-0.054
CBir5	0.203	-0.159	0.030	0.168	-0.155
CBir56	0.144	-0.003	0.084	0.112	-0.068
CBir63	0.114	-0.076	0.002	0.070	-0.152
CBir66	0.129	-0.066	0.016	0.076	-0.006
CBir8	0.088	0.071	0.077	0.081	0.099
Cdtb	0.071	0.106	0.036	-0.042	0.140
EF20	0.183	0.523	-0.325	-0.233	-0.250
FlaX	0.072	-0.040	0.035	0.098	-0.021
FtsZ	0.071	0.099	0.118	0.022	0.128
MDR247	0.108	0.023	0.230	0.037	0.210
MDR254	0.375	-0.156	-0.175	-0.297	0.052
MDR90	-0.036	0.278	-0.104	0.318	0.087
P3	0.218	0.411	-0.241	0.444	-0.090
RfaeF3	0.207	0.004	0.117	-0.200	0.385

RhomF1	0.082	0.044	0.051	-0.007	0.083
RhomF2	0.112	0.077	0.111	0.026	0.135
RintF1	0.070	0.080	0.177	0.027	0.183
RinuF5	0.033	0.128	0.189	0.171	0.296
SalFliC	0.214	-0.051	-0.141	-0.042	-0.044
UNCfliC	0.138	-0.044	0.076	-0.008	-0.077
Vinc	0.060	0.173	0.114	0.240	-0.062
keto	0.128	-0.091	-0.014	0.051	-0.058
ompC	0.067	0.073	0.130	0.023	0.052
riB1	0.108	-0.029	0.016	0.012	-0.058
riB10	0.091	-0.025	0.049	0.084	-0.162
riB12	0.071	0.081	-0.009	0.017	0.098
riB13	0.052	0.052	0.059	0.010	0.092
riB14	0.136	-0.039	0.011	0.061	-0.053
riB16	0.105	-0.001	-0.058	-0.012	0.003
riB17	0.094	0.059	0.098	-0.016	0.057
riB18	0.166	0.080	-0.228	-0.383	0.188
riB20	0.141	-0.024	0.050	0.078	0.007
riB8	0.131	0.005	0.134	0.030	0.150
riB9	0.101	0.265	0.552	-0.356	-0.456
riB2	0.052	0.066	0.083	0.006	0.041
riB4	0.091	0.039	0.030	-0.025	0.128
riB5	0.093	0.032	0.088	0.009	0.162
tetraco	0.214	-0.009	-0.006	-0.029	-0.055

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