# Shiftwork, functional bowel symptoms, and the microbiome

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**Background.** There are about 15 million Americans working full-time on evening, night, or rotating shifts. Between 48% and 81.9% of those working rotating or night shifts report abdominal pain, constipation, diarrhea and other symptoms of functional bowel disorders. The basis for this high prevalence of functional bowel disorders, including irritable bowel syndrome (IBS), among shift workers is unknown. Animal studies, however, suggest that circadian disruption, similar to that in shift workers, may contribute to the development of GI complaints among shift workers by altering the composition and normal diurnal rhythmicity of the resident intestinal microbes. Therefore, the present study was designed to determine if there were differences in 1) composition and diversity of the microbiome of night shift workers compared to day shift workers; and 2) the composition and diversity of the microbiome among shift workers experiencing functional bowel symptoms compared to shift workers who did not experience functional bowel symptoms.

**Methods.** Fifty-one full time staff nurses who worked either 12-hour day or night shifts completed demographic information, and the Rome III IBS module. They also collected two samples of gut microbiota before the beginning and at the end of their last work shift on day 14, using validated field-tested methods consistent with the Human Microbiome Project. After DNA extraction, 16S rRNA sequencing and assignment to the genus level was completed, samples were then compared to determine if there were 1) differences in the diversity and profile of the microbiome by shift type; 2) if there were differences in the microbiome by time of day for collection; and 3) whether there were differences in the diversity and profile of the microbiome of nurses with IBS and those without IBS.

**Results.** There were no differences in alpha or beta diversity of gut microbiota when specimens from day and night shift nurses were compared. There were however marginal differences in beta diversity when specimens collected at the beginning and end of the shifts were compared, with seven OTUs being differentially abundant when collected from day shift workers in the evening. There were also three OTUs to be differentially abundant in participants reporting IBS symptoms.

# Shiftwork, Functional Bowel Symptoms and the Microbiome

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## Abstract

**Background.** There are about 15 million Americans working full-time on evening, night, or rotating shifts. Between 48% and 82% of those working rotating or night shifts report abdominal pain, constipation, diarrhea and other symptoms of functional bowel disorders. The basis for this high prevalence of functional bowel disorders, including irritable bowel syndrome (IBS), among shift workers is unknown. Animal studies, however, suggest that circadian disruption, similar to that in shift workers, may contribute to the development of gastrointestinal complaints among shift workers by altering the composition and normal diurnal rhythmicity of the resident intestinal

34   microbes. Therefore, the present study was designed to determine if there are

35   differences in 1) composition and diversity of the microbiome of night shift workers

36   compared to day shift workers; and 2) the composition and diversity of microbiome

37   among shift workers experiencing functional bowel symptoms compared to shift workers

38   not experiencing functional bowel symptoms.

39

40   **Methods.** Fifty-one full time staff nurses who worked either 12-hour day or night shifts

41   completed demographic information, and the Rome III IBS module. They also collected

42   two samples of fecal microbiota before the beginning and at the end of their last work

43   shift on day 14, using validated field-tested methods consistent with the Human

44   Microbiome Project. After DNA extraction, 16S rRNA sequencing and assignment to the

45   genus level was completed, samples were then compared to determine if there were 1)

46   differences in the diversity and profile of the microbiome by shift type; 2) if there were

47   differences in the microbiome by time of day for collection; and 3) whether there were

48   differences in the diversity and profile of the microbiome of nurses with IBS and those

49   without IBS.

50

51   **Results.** There were no differences in alpha or beta diversity of fecal microbiota when

52   specimens from day and night shift nurses were compared. There were however

53   marginal differences in beta diversity when specimens collected at the beginning and

54   end of the shifts were compared, with seven Amplicon Sequence Variant (ASVs) being

55   differentially abundant when collected from day shift workers in the evening. There were

56   also three ASVs to be differentially abundant in participants reporting IBS symptoms.

57

## Introduction

59

60 There are about 15 million Americans working full-time on evening, night, or rotating

61 shifts, or other irregular employer-arranged schedules; 4.7% on evening shifts, 3.2% on

62 night shifts, 3.1% on irregular schedules, and 2.5% on rotating shifts (United States

63 Department of Labor Bureau of Labor Statistics 2005). Night shift work is associated

64 with increased mortality, higher risk of cardiovascular disease, cancer, diabetes,

65 ⁻ ⁻ hypertension, chronic fatigue, sleep problems and higher body weight (Gu 2015; Jia

66 2013; Myers 2015; Pan 2011; Rajaratnam 2011; Vyas 2012). Night and rotating shift

67 workers also report a higher prevalence of Irritable Bowel Syndrome (IBS), abdominal

68 pain, constipation and diarrhea than do day shift workers (Caruso 2004; Knutsson 2010;

69 Nojkov 2010; Wells 2012). In fact, between 48% and 82% of those working rotating or

70 night shifts report abdominal pain, constipation, diarrhea and other symptoms of

71 functional bowel disorders (Nojkov 2010; Saberi 2010).

72

73 The basis for this high prevalence of functional bowel disorders, including IBS, among

74 shift workers is unknown. However, some studies suggest that inappropriate nutrition or

75 irregularity in the timing of meals (Bilski 2006; Lowen 2010), and psychological

76 disorders (Zhen 2006) may contribute to the high prevalence of functional bowel

77 symptoms among workers on rotating or night shifts.

78

79 Other studies strongly suggest that sleep deprivation or sleep disturbances are

80 associated with the presence and severity of functional bowel symptoms reported by

81 resident physicians and nurses (Jarrett 2000; Saberi 2010; Wells 2012). Moreover,

82  animal studies suggest that circadian disruption, similar to that in shift workers,

83  contributes to the development of gastrointestinal (GI) complaints among shift workers

84  by altering the composition and normal diurnal rhythmicity of the resident intestinal

85  microbes (De Bacquer 2009). Interestingly, gut microbiota community composition and

86  diversity are malleable and sensitive to changes in diet and other environmental factors

87  (Voigt 2014), including activity and sleep patterns. For example, Thaiss and colleagues

88  (Thaiss 2014) "jet lagged" a group of mice by subjecting them to an 8-hour advance for

89  three days before allowing them to revert to their usual schedule for three more days,

90  then subjecting them to another 8 hour advance for three days. Mice exposed to 4

91  weeks of this schedule lost their usual pattern of physical activity, and consumed food at

92  irregular intervals. Significantly, this environmentally induced disruption of daily activity

93  patterns (jet lag schedule) was associated with a loss of diurnal rhythmicity of

94  microbiota composition in mice.

95

96  Thaiss and colleagues (Thaiss 2014) also found similar changes in human microbiota

97  composition in two volunteers who flew from the US to Israel (an 8-10 hour advance).

98  Samples collected at baseline (one day pre-flight), during jet lag (one day after landing),

99  and during recovery (2 weeks after landing) showed rapid changes in the composition of

100  the microbiota. During jet lag (the first 24-hours after landing), there was a higher

101  relative representation of Firmicutes, which reversed upon recovery from jet lag (2

102  weeks later). It is unknown it these changes would occur with the chronic circadian

103  disruption experienced by night shift workers. Although some studies have

104  demonstrated no differences in composition of the fecal microbiome when samples from

105 lean and obese individuals were compared (Ley 2006; Turnbaugh 2009), other studies

106 in humans have demonstrated that Firmicutes are associated with a higher propensity

107 for obesity and metabolic disease (Finucane 2014; Ley 2006), conditions that are more

108 common in night and rotating shift workers (De Bacquer 2009; Suwazono 2008).

109

110 Finally, multiple studies have linked reduced microbial diversity and richness in

111 microbial communities to IBS symptoms. For example, Krogius-Kurikka and colleagues

112 (Krogius-Kurikka 2009) reported that fecal samples from patients with diarrhea-

113 predominant IBS were enriched with Proteobacteria and Firmicutes but had reduced

114 Actinobacteria and Bacteroidetes compared to healthy controls. Other studies (Bhattarai

115 Y. 2017; Salonen 2010) have shown an increase in the Firmicutes-to-Bacteriodetes-

116 ratio, a decrease in some types of Firmicutes families (Lactobacilli, Faecalibacterium)

117 and the Actinobacteria population (Bifidobacteria, Collinsella), and an increase in some

118 Firmicutes families (Veillonella, Streptococci, and Ruminococcus spp.) and in

119 Proteobacteria (Enterobacteriaceae spp.). In addition, low microbial richness, an

120 absence of Methanobacteriales, and enrichment with Bacteroides enterotypes are

121 associated with more severe IBS symptoms (Tap 2017). Not only is the composition of

122 the fecal microbiota altered in patients with IBS, these imbalances in the microbial

123 community or dysbiosis, occur more frequently in patients with IBS compared to healthy

124 individuals. Reduced diversity was observed in nearly three-fourths of the IBS patients

125 studied by Casén and colleagues (Casén 2015) compared to 16% in normal individuals

126 (Collins 2014; Jeffery 2012).

127

128  While these studies suggest that shiftwork alters gut microbiota and that alterations in

129  gut microbiota are common in patients with IBS, they do not demonstrate whether these

130  alterations are associated with the somatic symptoms experienced by many rotating

131  and night shift workers. Therefore, the present study is designed as the initial step in

132  determining if there are differences in 1) composition and diversity of the microbiome of

133  night shift workers compared to day shift workers; and 2) the composition and diversity

134  of microbiome among night shift workers experiencing functional bowel symptoms (e.g.,

135  bloating, lower abdominal pain, constipation and diarrhea) compared to night shift

136  workers not experiencing functional bowel symptoms.

137
138  **Materials & Methods**

139
140  **Subjects**

141  Participants in this study included 51 full-time staff nurses who worked 12-hour day or

142  night shifts at a large university hospital. Registered nurses were eligible to participate if

143  they were between the ages of 18 and 65 and did not report a history of inflammatory

144  bowel disease (e.g., Crohn's disease or ulcerative colitis) or other chronic disorder

145  affecting the GI track (e.g., GI cancer). Those with recent antibiotic exposure were

146  asked to delay their enrollment in the study for two weeks after their last dose of

147  antibiotics.

148

149  As expected, the sample was predominantly female (96%), with a mean age of 32.9 ±

150  10.0 years and a range of 21-59 years. Just under half of the participants reported

151  working straight day shifts (47%), with the remainder of the sample working either

152  straight night shifts (51%) or rotating shifts (2%). For purposes of the analysis, the nurse

153   who reported working rotating shifts was categorized as working night shift since she

154   worked straight nights during the two-week data-gathering period. Although only three

155   participants (6%) reported a prior diagnosis of IBS, a total 18 participants (35%) met

156   criteria for the diagnosis of IBS using the Rome III criteria. Participant BMIs ranged from

157   18.2 to 39.5 with a mean BMI of 26.7 $\pm$ 5.4. As illustrated in Table 1, there were no

158   significant differences by shift type in terms of age, BMI, diagnosis of IBS or type of IBS.

159
160   **Instruments**

161   Data for this pilot study was obtained using a variety of subjective and objective

162   measures. A Demographic Questionnaire and Brief Health History was used to collect

163   information about participant age, and the usual shift worked. Participants were also

164   asked to report any previous diagnosis of inflammatory bowel disorders or chronic

165   diseases affecting the GI, and to list current medications and supplements used. The

166   IBS module from the Rome III Questionnaire (2006) consists of 10 questions that ask

167   subjects to rate the frequency of recurrent abdominal pain or discomfort, onset of pain

168   associated with a change in frequency of stools, and the onset of pain associated with a

169   change in the form of stools. This module is considered the gold standard for assessing

170   functional bowel symptoms.

171
172   Samples of fecal microbiota were collected just before the beginning and just after the

173   end participants' work shift at the end of the two week data-collection period using

174   validated, field-tested methods consistent with the Human Microbiome Project (Methé

175   2012). Four specimens (two each time) were collected using the Elution-swap system

176   (Copan). The rectal swabs were stored in 1 mL of Amies transport medium (Copan) and

177   immediately frozen and stored until DNA extraction. Prior to extraction, fecal material

178   (200 mg) was suspended in 500-µl lysozyme (20 mg/ml in 20 mM Tris-HCl pH 8, 2 mM

179   EDTA, 1.2% w/v Triton X-100) and incubated at 37°C for 2 h using the QIAamp® DNA

180   Stool Mini Kit (Qiagen, Inc., Valencia, CA).

181

182   **Procedure**

183   After obtaining approval from the Emory University's IRB (MOD001-IRB00089064) and

184   Emory Healthcare's Nursing Research Council, emails describing the study were sent to

185   all staff nurses. Those interested in participating were instructed to contact the research

186   team to schedule an appointment to provide informed consent, review study

187   procedures, and complete the demographic and Rome III questionnaire. After written

188   informed consent was obtained, the participant's work schedule was then reviewed to

189   determine an appropriate date to collect samples of fecal microbiota at the beginning

190   and end of the participant's shift.

191

192   **Data Analysis**

193   **DNA extraction & 16S rRNA sequencing** DNA extraction and 16S sequencing was

194   performed by Omega Bioservices (Norcross, GA, USA) using a standard protocol. DNA

195   was isolated using Omega Biotek Mag-Bind® Universal Pathogen DNA Kit. The V3-V4

196   region of the bacterial 16S rRNA gene sequences were amplified using the primer pair

197   containing the gene-specific sequences and Illumina adapter overhang nucleotide

198   sequences. The full length primer sequences are: 16S Amplicon PCR Forward Primer

199   (5'- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG)

200   and 16S Amplicon PCR Reverse Primer (5'-

201   GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC)

202   . For amplicon PCR, each 25 µL of polymerase chain reaction (PCR) reaction contained

203   12.5 ng of sample DNA as input, 12.5 µL 2x KAPA HiFi HotStart ReadyMix (Kapa

204   Biosystems, Wilmington, MA) and 5 µL of 1 µM of each primer. PCR reactions were

205   carried with an initial denaturation step performed at 95°C for 3min followed by 25

206   cycles of denaturation (95°C, 30 s), annealing (55°C, 30 s) and extension (72°C, 30

207   sec), and a final elongation of 5 min at 72°C. PCR product was cleaned up from the

208   reaction mix with Mag-Bind RxnPure Plus magnetic beads (Omega Bio-tek, Norcross,

209   GA). A second index PCR amplification, used to incorporate barcodes and sequencing

210   adapters into the final PCR product, was performed in 25 µL reactions, using the same

211   master mix conditions as described above. Cycling conditions were as follows: 95°C for

212   3 minutes, followed by 8 cycles of 95°C for 30", 55°C for 30" and 72°C for 30". A final, 5

213   minutes' elongation step was performed at 72°C. The libraries were normalized with

214   Mag-Bind® EquiPure Library Normalization Kit ((Omega Bio-tek, Norcross, GA) then

215   pooled. The pooled library ~600 bases in size was checked using an Agilent 2200

216   TapeStation and sequenced (2 x 300 bp paired-end read setting) on the MiSeq

217   (Illumina, San Diego, CA). Sequence data was submitted to the National Center for

218   Bioinformatic Information Short Read Archive database: accession PRJNA687007.

219

220   **Processing of sequence data and taxonomic assignment to Amplicon Sequence**

221   **Variants (ASVs).**  Data processing, including demultiplexing, QC filtering,

222   contamination and sample mislabeling data checks, ASV representation, taxonomy

223   assignment via a reference database (Caporaso 2010; Wang 2007), and phylogeny and

224   diversity analysis (Lozupone 2007; Lozupone 2006; Lozupone 2005) was done using R

225   packages dada2 and phyloseq (Callahan 2016; McMurdie 2013). After filtering for low

226   quality reads and filtering possible contaminants, we obtained a range of 12,455-

227   119,605 reads per sample with a mean of 33,933 (SD=10,412 and a median of 33, 452

228   (scripts used to generate this data have been included as supplemental files dada2-

229   analysis 1, and dada2-analysis 2)

230

231   **Statistical Analysis**

232   All statistical analyses were conducted using R package 3.6.3. A p-value less than 0.05

233   was considered statistically significant.

234

235   We computed the Chao1 (measuring species richness) and Shannon (measuring both

236   richness and evenness) alpha diversity indices for each sample, since loss of taxonomic

237   diversity in general is an indicator of disease state in many ecological systems. We log-

238   transform Chao1 to be at the same scale as Shannon. We also computed the Bray-

239   Curtis (measuring dissimilarity/distance of each pair of samples based on relative

240   abundance data) and Jaccard (based on presence-absence data) beta diversity

241   distance metrics. We used Principal Coordinates Analysis (PCoA) to visualize clustering

242   of the samples based on these distance metrics.

243

244   Given that this is a pilot study with 51 subjects, the primary focus was analyzing the

245   microbiome profiles (in terms of alpha diversity, beta diversity, and individual genera at

246   the relative abundance and presence-absence scales) for (a) differences between day

247   and night shift workers, (b) differences between the beginning and the end of the shift

248   (main effect of time) and whether the differences depend on the shift type (time and shift

249   type interaction), and (c) differences between subjects with and without IBS symptoms.

250   Since each subject is measured four times (two at the beginning of the shift and two at

251   the end) for the microbiome, this study has a matched-set design. Care should be taken

252   in analyzing such matched-set data because the samples from the same subjects tend

253   to have positive correlations and the samples taken at the same time for the same

254   subjects tend to have even stronger positive correlations. While the analyses in (a) and

255   (c) consist of two-group comparisons ``between" sets, the analyses in (b) consist of two-

256   group comparison ``within" sets for the main effect of time as well as two-group

257   comparison of the change (from the beginning to the end of the shift) ``between" sets.

258   We have developed the Linear Decomposition Model (LDM) method and extended the

259   existing PERMANOVA method that both can handle all of these analyses [Hu and

260   Satten 2020; Zhu et al., in press]. While PERMANOVA performs comparison at the

261   community level based on a beta-diversity distance matrix, the LDM performs the same

262   community-level comparison based on the relative abundance or presence-absence

263   data. In addition, the LDM performs comparison at individual-genus level (e.g., detecting

264   individual genera that are differently "abundant" or "present" between groups) with false-

265   discovery rate control at the nominal level 10%. We also use the LDM for comparing the

266   alpha diversity by treating it as a single genus.

267

268   **Results**

269

270 The PCoA plot in Figure 1 shows that samples obtained from the same participants tend

271 to cluster together and samples from the same time on sample participants tend to

272 cluster even more closely. This observation is consistent with the notion that

273 microbiome samples are homogeneous within subjects and heterogenous across

274 subjects, and thus confirms good quality of our data. Supplemental text (phyloseq

275 analysis) contains plots showing the composition of the samples at taxonomic different

276 levels

277

278 As shown in Figure 2, there were no significant differences in Chao1 (p=0.411 by the

279 LDM) and Shannon (p=0.242) between day and night shift nurses. Nor were there

280 differences in Bray-Curtis (p=0.476 by PERMANOVA) and Jaccard distances (p=0.625)

281 by shift type (Figure 3). Nor were there differences in relative abundance (p=0.489 by

282 the LDM) and presence-absence (p=0.824) data across all genera.

283

284 Figure 4 shows an increase of the log Chao1 index from the beginning to the end of the

285 shift for day-shift workers while a decrease for night-shift workers, and this difference of

286 change is significant (interaction p=0.034 by the LDM); so does the Shannon index

287 (p=0.008). However, the change among the pooled workers was not significantly

288 different (main effect of time p=0.473 for Chao1 and p=0.236 for Shannon) possibly due

289 to the cancellation of effects with opposite directions. In terms of beta diversity, the

290 change of the beta diversity metric from the beginning to the end of the shift among the

291 pooled workers was marginally significant or significant (main effect of time p=0.056 by

292 PERMANOVA for Bray-Curtis and 0.014 for Jaccard). Marginally significant and

293    significant findings were also noted by the LDM (main effect of time p=0.035 and 0.063

294    based on relative abundance and presence-absence data, respectively). However,

295    there is not enough evidence to confirm that the change was significantly different

296    between day shift and night shift workers possibly due to the small sample size,

297    although there was suggestive evidence (interaction p=0.192 and 0.118 by

298    PERMANOVA based on Bray-Curtis and Jaccard, respectively; p=0.320 and 0.134 by

299    the LDM based on the relative abundance and presence-absence data). In addition, the

300    LDM revealed seven ASVs to be differentially abundant between the beginning and the

301    end of the shift; ASV_455(S5-A14a, more abundant at the beginning),

302    ASV_2527(Ruminoccaceae_NK4A214_group, more abundant at the end),

303    ASV_1304(Ruminococcus_1, more abundant at the end), ASV_221 (Mobiluncus, more

304    abundant at the beginning), ASV_7(Campylobacter), ASV_130(Alistipes, more

305    abundant at the end), and ASV_62(Agathobacter, more abundant at the end).

306

307  Finally, as shown in Figure 5 there were no significant difference in alpha diversity when

308    comparing participants without and with IBS symptoms (p=0.849 for Chao 1, p=0.484

309    for Shannon, by the LDM method). Although there were no differences in beta diversity

310    (p=0.206 for Bray-Curtis and p=0.213 for Jaccard) by whether or not the participant had

311    symptoms of IBS), there were some significant differences based on the LDM results.

312    Specifically, three ASVs, ASV_1160 (Flavonifractor), ASV_1134 (Oscillibacter) and

313    ASV_2379 (Ruminiclostridium_9), were detected to be differentially abundant. Three

314    ASVs, ASV_1160 (Flavonifractor), ASV_2379 (Ruminiclostridium_9) and ASV_47

315   (Escherichia/Shigella), were detected to be significantly more likely to be present in

316   participants reporting IBS symptoms.

317

318   **Discussion**

319

320   The findings of this pilot study suggest that there are no differences in the richness and

321   diversity of species when samples from nurses working day and night shifts were

322   compared. There were however some statistically significant changes in both alpha and

323   beta diversity metrics when specimens collected at the beginning and end of the shifts

324   were compared and there was also some evidence that the changes were different for

325   day shift and night shift workers, with increased alpha diversity noted at the end of the

326   day shift and decreased alpha diversity noted at the end of the night shift.  Seven ASVs

327   were found to be differentially abundant between the beginning and end of the shifts for

328   the entire sample. In addition, there were three ASVs to be differentially abundant in

329   participants reporting IBS symptoms.

330

331   Studies comparing the effects of shift work on the gut microbiome are limited and

332   somewhat contradictory. For example, a study of 10 male security guards who worked

333   both day and night shifts found there were no significant differences in in alpha or beta

334   diversity within and across-subject variation for both shifts (Mortas 2020). In contrast,

335   slight changes in microbial abundance and diversity were noted when 22 subjects, aged

336   20-35 years, delayed their sleep period for 2-4 hours (Liu 2020). Although there have

337   been studies comparing circadian variation in the gut microbiota in mice (Thaiss 2014)

338   and another describing the results samples collected during multiple time points over

339   several days by two subjects (Thaiss 2014), our study is the first to compare the

340    richness and diversity of gut microbiota collected from 51 human participants at two

341    different time points in 24 hours.

342

343    Even though there are numerous studies that have reported increased and/or

344    decreased amounts of various gut bacteria among patients with IBS (Bhattarai Y. 2017;

345    Casén 2015; Pittayanon 2019; Salonen 2010; Tap 2017), a recent systematic review

346    found only nine studies that discussed differences in alpha-diversity in patients with IBS

347    compared to normal controls (Pittayanon 2019). Slightly over half of the studies (55.6%)

348    reported a significant decrease in the richness and diversity in patients with IBS (Carroll

349    2011; Carroll 2012; Liu 2016; Rangel 2015), whereas the remaining four studies (Carroll

350    2012; Durban 2012; Rigsbee 2012; Tap 2017), like our current study, revealed no

351    differences in alpha-diversity compared to healthy controls. A more elaborate study

352    (Pozuelo 2015) comparing fecal and mucosa-associated microbiome samples obtained

353    from the sigmoid colon during colonoscopy, not only found no differences in alpha

354    diversity when the two types of samples were compared, but that there were no

355    differences in richness and diversity when samples obtained from healthy controls and

356    patients with IBS or IBS subtypes were compared.

357

358    Like other studies comparing patients with IBS symptoms to healthy controls, our pilot

359    study found increased Firmicutes (Chong 2019), specifically Flavonifractor,

360    Oscillibacter, and Ruminiclostudium among participants with IBS (Casén 2015).  The

361    increased abundance of E. coli/ Shigella possibly reflects the suspected relationship

362    between Shigellosis and IBS (Youn 2016). In contrast, Pozuelo et al (Pozuelo 2015)

363    reported that Methanobacteriaceae, Ruminococcaceae, Erysipleotrichacea, and one

364    unknown Clostridiales family were all found to be decreased in patients with IBS-M

365    (IBS-mixed type) and IBS-D (IBS-diarrhea), but not in patients with IBS-C (IBS-

366    constipation).

367

368    IBS is estimated to have a world-wide prevalence of 10-15% (Canavan 2014; Sperber

369    2017). Prevalence rates of IBS are typically higher among nurses, with rates ranging

370    from 17.4% in China (Liu 2014) to 45.2% in Nigeria (Akere 2014). The prevalence rate

371    of IBS among study participants was 35%, quite similar to the prevalence rate reported

372    among nurses at the University of Michigan Medical Center (36.6%) (Nojkov et al.

373    2010). However, the study of hospital staff nurses (Nojkov et al. 2010), and other

374    studies of shift workers (Kim 2013), there were no differences in the prevalence of IBS

375    symptoms among day and night shift nurses in our study.

376

377    This study is limited by a number of factors. First, our study population consisted of a

378    convenience sample of nurses that might not be representative of the larger nursing

379    workforce or the larger population of shift workers. The overall participation rate was

380    relatively low, which raises concerns about how representative the participants were of

381    the total population of nurses who were invited to participate. Additionally, given the

382    focus of the study, nurses who experienced IBS symptoms may have been more likely

383    to participate than those who did not experience IBS symptoms. Finally, the severity of

384    IBS symptoms and quality of life was not assessed, two factors which may have been

385    impacted by gut microbiome diversity and richness.

386

## Conclusions

388

389 There were no statistically significant differences in the richness and diversity when

390 samples of the gut microbiome from nurses working day and night shifts were

391 compared. However, when specimens collected at the beginning and ends of the shifts

392 were compared, there were some statistically significant or marginally differences in

393 alpha and beta diversity. Three ASVs were more common in participants reporting IBS

394 symptoms.

395

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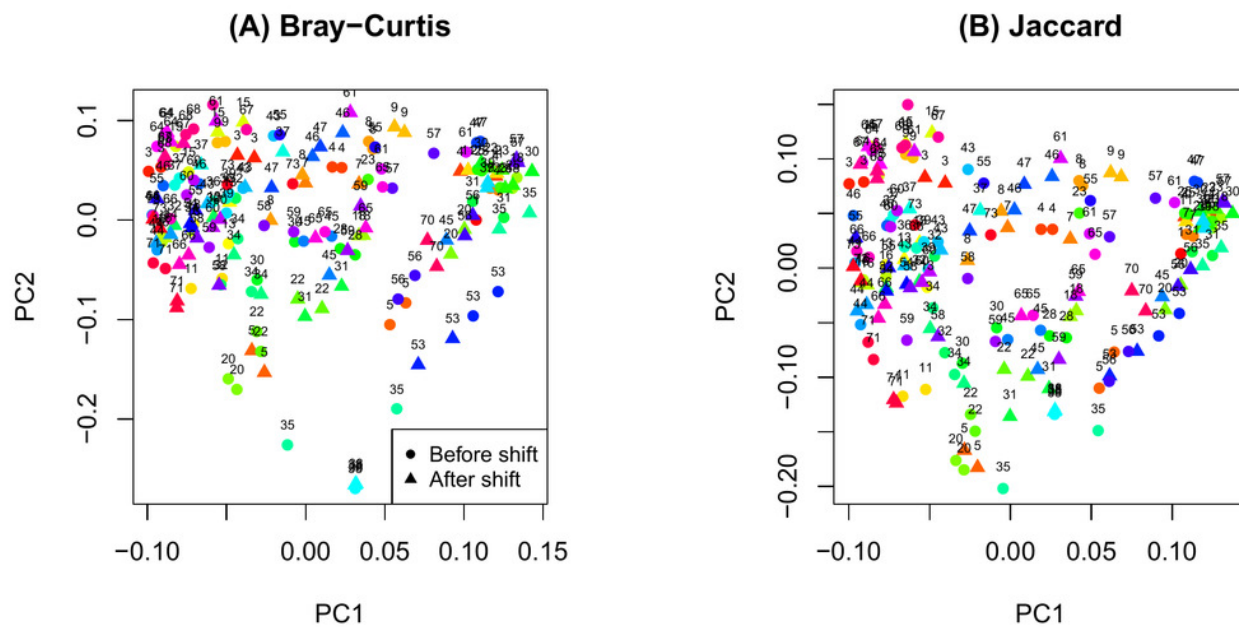
A Comparison of Day and Night Shift Participants

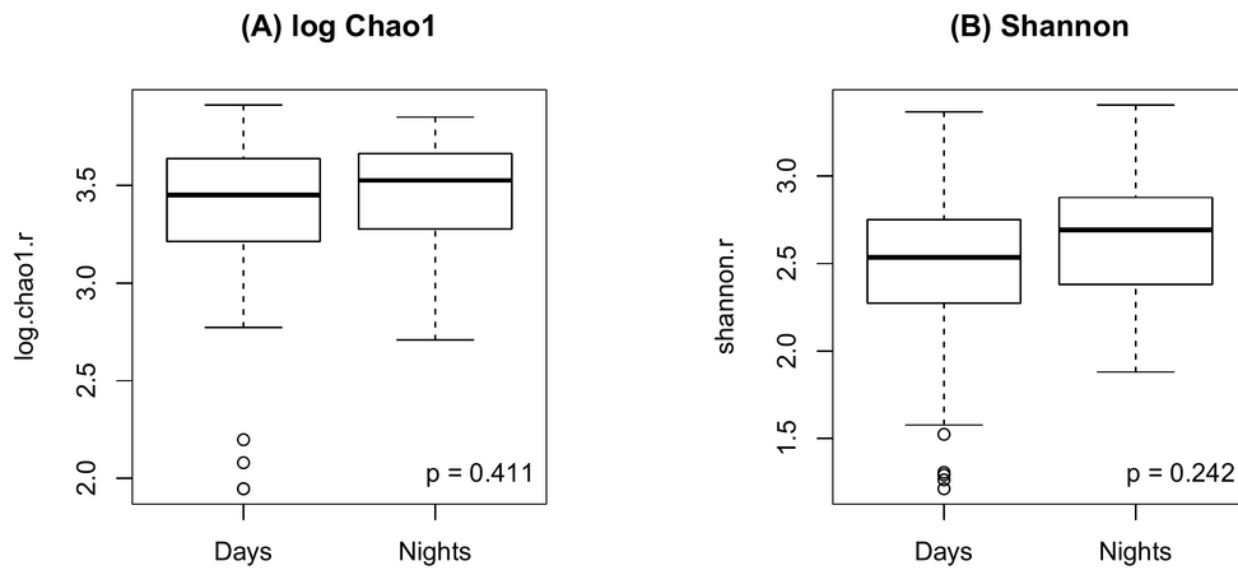
1   Table 1 Sample Description

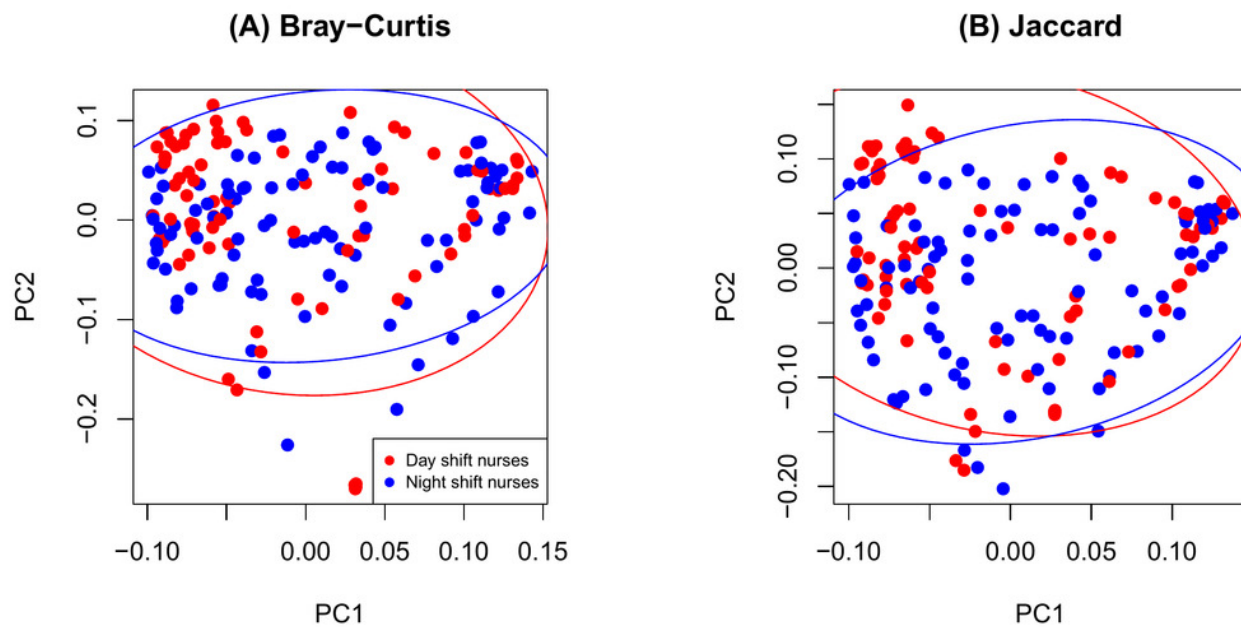
| | Day Shift (n=24) | Night Shift (n=27) | P Value |
|---|---|---|---|
| Age (mean) | 32.4 | 33.3 | 0.73* |
| BMI (mean) | 27.1 | 26.3 | 0.60* |
| BMI | | | |
| <20 | 1 | 3 | 0.41** |
| 20-24.9 | 10 | 8 | |
| 25-29.9 | 5 | 10 | |
| >30 | 8 | 6 | |
| IBS (Rome III criteria) | | | |
| No | 17 | 16 | 0.56** |
| Yes | 7 | 11 | |
| IBS Symptoms | | | |
| IBS with diarrhea | 1 | 2 | 1.0** |
| IBS with constipation | 1 | 1 | |
| IBS mixed type | 5 | 7 | |
| IBS un-subtyped | 0 | 1 | |

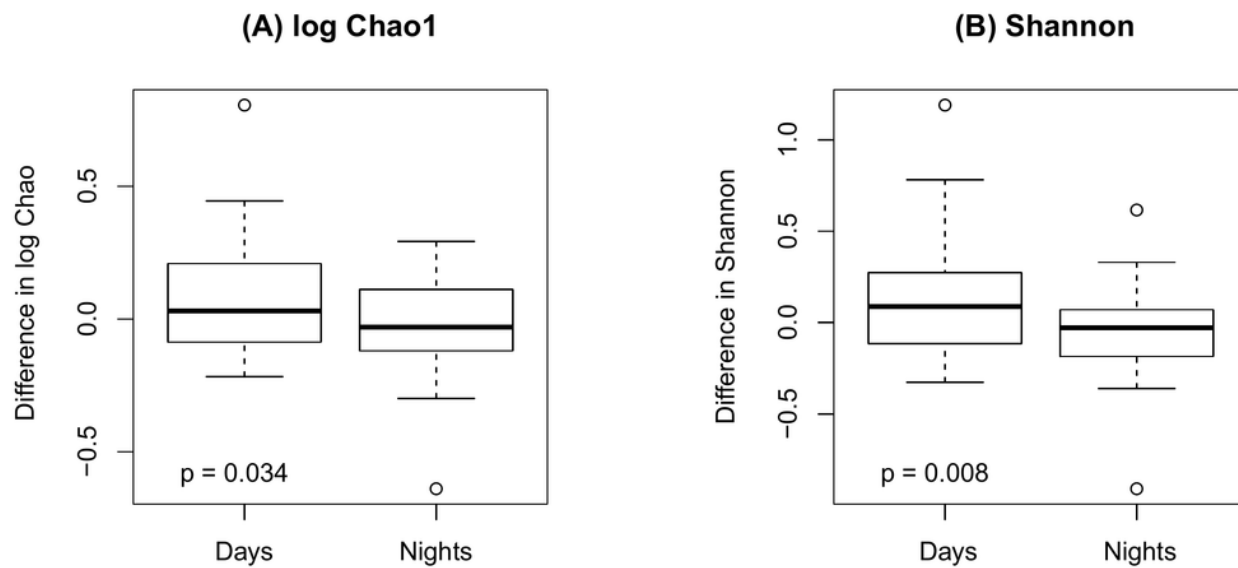
3   *Welsh two-sample t-test
4   ** Fisher's exact test

# Figure 1

Principal Components Analysis Plot by ID

# Figure 2

Alpha Diversity by Shift Type

# Figure 3

PCoA Plot Comparing Beta Diversity by Shift Type

# Figure 4

Changes in Alpha Diversity from the Beginning to the End of the Shift by Shift Type

# Figure 5

Alpha Diversity by Presence of Absence of IBS