

1 **Household triclosan and triclocarban exposure impacts the adult intestinal microbiome**  
2 **but not the infant intestinal microbiome**

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21 **Abstract**

22           In 2016, the US Food and Drug Administration banned the use of specific microbicides  
23 in some household and personal wash products. This decision was due to concerns that these  
24 chemicals might induce antibiotic resistance or disrupt human microbial communities. Triclosan  
25 and triclocarban (referred to as TCs) are the most common antimicrobials in household and  
26 personal care products, but the extent to which TC exposure perturbs microbial communities in  
27 humans, particularly during infant development, was unknown. We conducted a randomized  
28 intervention of TC-containing household and personal care products during the first year  
29 following birth to characterize whether TC exposure from wash products perturbs microbial  
30 communities in mothers and their infants. Longitudinal survey of the intestinal microbiota using  
31 16S ribosomal RNA amplicon sequencing showed that TC exposure from wash products did not  
32 induce global reconstruction of either infant or maternal intestinal microbiotas following 10  
33 months of exposure after birth. However, broadly antibiotic-resistant species from the phylum  
34 Proteobacteria were enriched in stool samples from mothers in TC households only after the  
35 introduction of triclosan-containing toothpaste. Despite the minimal effects of TC exposure from  
36 wash products on the gut microbial community of infants and adults, these results suggest  
37 detected taxonomic differences are associated with potential harmful effects on host physiology,  
38 highlighting the need for consumer safety testing of self-care products not subject to the ban on  
39 the human microbiome and health outcomes.

## 40 **Introduction**

41           Triclosan and triclocarban (TCs) are chlorinated, broad-spectrum antimicrobial  
42 chemicals found in thousands of consumer and industrial products. They are present most  
43 notably in personal wash products including toothpaste and liquid soaps (triclosan) and bar  
44 soaps (triclocarban). In 2016, a Food and Drug Administration (FDA) ruling banned the use of  
45 TCs and 17 other antimicrobial chemicals in over-the-counter wash products, driven by the  
46 concern that the use of these products contributed to antibiotic resistance and might negatively  
47 affect human health, either through endocrine disruption or modification of the human  
48 microbiota(1). Notably, many other TC-containing products, such as toothpaste, fabrics and  
49 plastic goods (including toys), were not subjected to the ban.

50           To date, limited data exists regarding the effects of TCs on the human microbiota(2).  
51 The microbes that occupy the human body in niches from the gut to the skin have diverse roles  
52 in human health, ranging from metabolic support to immunomodulation. Imbalances in these  
53 microbial communities are implicated in a wide variety of diseases(3). The extent to which  
54 triclosan exposure may induce microbial perturbations has been studied in fish and rodent  
55 models with conflicting outcomes (reviewed in (4)). Triclosan exposure restructures the juvenile  
56 fish microbiome(5), but results in recoverable alterations following short-term perturbation in  
57 adult fish(6). Adolescent rats receiving oral triclosan at levels comparable to human exposures  
58 develop lower microbial diversity in the gut and more prominent changes in taxonomic  
59 composition than in adult rats(7). While triclocarban exposures are less studied, in pregnant rats  
60 and their offspring less than 10 days old, exposure lead to lowered phylogenetic diversity and  
61 revealed a dominance of the Proteobacteria phylum in the gut(8). In a small, randomized cross-  
62 over human study, TC wash product exposure did not induce major perturbations of the oral and  
63 gut microbiomes(9). This finding supports other studies that have shown minimal impact of  
64 triclosan on dental microbial ecology, despite slowing the progression of periodontitis(10).

65           The core microbiome of humans is established in the first few years of life(11).  
66    Disruptions to the microbiota early in development by extrinsic factors, such as antibiotics, can  
67    have long-term impacts on metabolic regulation(12) and can delay normal microbiota  
68    maturation(13). The impact of TC exposure through household and personal care products on  
69    the developing microbiota is unknown. As a nested, randomized intervention within Stanford's  
70    Outcomes Research in Kids (STORK), a prospective cohort study of healthy mothers and  
71    infants(14), we provided commercially available wash products containing or not containing TCs  
72    (TC and nTC arms, respectively) to evaluate their relative impact on the maternal and infant  
73    intestinal microbiota over the first year of the infant's life.

74

## 75    **Results**

### 76    **Study Demographics.**

77           Thirty-nine households from the STORK cohort met our inclusion criteria (i.e., at least 5  
78    of 6 expected stool samples available from the household). Complete sampling for both infants  
79    and mothers for three time points after birth was available for 26 households, and one sample  
80    was missing for 13 households. Home visits and sample collection occurred on average 74 (14-  
81    124), 200 (135-256) and 317 (241-377) days following birth (Supplementary figure 1). These  
82    days correspond to approximately 2.7, 6.6, and 10.6 months, referred to as 2, 6, and 10 months  
83    hereafter. The average age of mothers in this subset was 34 years and 46% were of Hispanic  
84    origin (Table 1).

85

### 86    **Randomization to TC-containing household and personal products is sufficient to** 87    **increase triclosan exposure after 6 months.**

88           Mothers in TC households had higher spot urinary triclosan levels at 6 months when  
89    compared to those in nTC households, with a median triclosan measurement of 837.05 pg/ $\mu$ L  
90    compared to 76 pg/ $\mu$ L ( $p < 0.001$ ). With one exception, levels in children were uniformly low,

91 with a median of 38.3 pg/ $\mu$ L in TC households and 10.05 pg/ $\mu$ L in nTC households ( $p=0.06$ ,  
92 Figure 1, Supplementary table 2).

93

94 **Mother and infants have distinct microbiome compositions not driven by randomization**  
95 **to TC-containing products.**

96 A principle coordinate analysis (PCoA) was performed to identify variability between the  
97 taxonomic structure of the samples. PCoA showed that samples segregated primarily by age,  
98 with 31.9% of variation among samples explained by the first two axes (Figure 2A). By 10  
99 months of age, infant samples clustered more closely to the mothers' samples (Figure 2B).  
100 Individual samples from TC and nTC households were evenly dispersed through the axes for  
101 both infants and mothers. Taxonomic classifications suggest that samples were generally  
102 similar at the phylum level among infants and among mothers, regardless of treatment arm  
103 (Supplementary figure 3). Variations between infants were not driven by factors known to  
104 influence microbial colonization, such as delivery method, breast feeding, and pets in the  
105 household (Supplementary figure 4). Maternal samples from the various time points cluster  
106 more closely by individual than by time point (Supplementary figure 5A), and we did not observe  
107 major variations in taxonomic structure between maternal samples based on TC exposure using  
108 PCoA (Supplementary figure 5B, 5C). Statistical comparisons of treatment arms with  
109 permutational multivariate analyses showed no significant association between TC exposure  
110 and microbiome composition for infants ( $p=0.14$ ), but did demonstrate a significant association  
111 between TC exposure and microbiome composition in the mothers ( $p < 0.002$ ).

112

113 **Randomization to TC-containing products does not decrease gut microbial diversity in**  
114 **infants or mothers.**

115 Randomization to the TC arm was not associated with decreased gut microbiota  
116 diversity for infants or mothers at any of the 2, 6, or 10-month visits after infant birth (Figure 3).

117 Specifically, diversity was not decreased in infants randomized to TC-containing products (p-  
118 values for 2, 6, and 10 months: 0.66, 0.84, 0.49). As expected, microbial diversity increased as  
119 the infants progressed through the first year of life ( $p < 0.001$ )(15), and this effect was not  
120 altered by randomization to TC. Diversity was not statistically significantly decreased in maternal  
121 samples randomized to TC-containing products (Mann-Whitney U-test p-values for 2, 6, and 10  
122 months: 0.73, 0.28, 0.20), however trended to a decrease in stool diversity at the 10-month visit.

123

124 **Intestinal exposure to triclosan through toothpaste, rather than wash products, is**  
125 **associated with Proteobacteria enrichment in TC households.**

126 Given this trend toward decreased diversity with randomization to TC and a statistically  
127 significant difference between maternal gut composition between the TC and nTC arms from  
128 permutation tests, we hypothesized that TC exposure affected a small proportion of taxa within  
129 the community. We identified differentially abundant taxa present in stool samples from TC vs.  
130 nTC infants and mothers by first pooling data from all three visits (Figure 4A, Supplementary  
131 table 3) and separately for each of the three visits (Figure 4B, 4C, Supplementary table 4). A  
132 greater number of taxa were significantly differentially abundant in mothers vs. infants (29 in  
133 mothers, 17 in infants; Figure 4A). Infants did not show an enrichment of specific phyla at any  
134 time point, but *Bacteroides fragilis* was persistently the most enriched species in combined and  
135 time-point analyses at 6 months (Figure 4A, 4B). Mothers showed a strong enrichment of  
136 Proteobacteria in the TC arm after the introduction of triclosan-containing toothpaste (Figure 4C,  
137 Supplementary table 4). A preliminary, unbiased quantification of triclosan resistance in the  
138 mothers using whole shotgun metagenomic sequencing of stool samples for a subset of 12  
139 mothers in each intervention arm at 6 months was conducted. This resulted in low coverage of  
140 triclosan resistance genes; approximately 0.03% of sequenced reads mapped to triclosan  
141 antibiotic resistance genes in the Comprehensive Antibiotic Resistance Database (CARD).  
142 Unsupervised clustering by triclosan resistance gene counts was not sufficient to cluster

143 maternal samples by intervention arm, and this may have been impacted by the limited power to  
144 detect differences between groups due to low coverage (Supplementary figure 6). Differential  
145 gene analysis from CARD showed enrichment of one antibiotic resistance gene, CfxA6, in TC  
146 households at a 10% FDR adjusted p-value threshold.

147

## 148 **Discussion**

149 At the time of the US FDA ruling that banned 19 antimicrobials from wash products, the  
150 extent to which TC exposure perturbed microbial populations in humans, particularly during  
151 infant development, was unknown. To test the hypothesis that exposure to TC-containing wash  
152 products induces a measurable impact on the intestinal microbiota of adults and growing  
153 infants, we assessed the stool microbiome from mothers and infants in households that had  
154 been randomized to TC or nTC wash products during the first year of the infant's life. We  
155 observed that ongoing TC exposure from household products does not contribute to major  
156 reconstruction of either infant or adult intestinal microbiomes after approximately 10 months. TC  
157 exposure did not reduce overall gut microbial diversity in infants or mothers at any visit.  
158 However, there are some notable trends in differential taxa with potential health implications.  
159 The most enriched species in the nTC randomized infants, *Bacteroides fragilis*, has been shown  
160 to direct maturation of the immune system(16) and produce anti-inflammatory  
161 polysaccharides(17). The most enriched organisms in the TC households at the 10-month visit  
162 were *Bacteroides caccae* in infants and *Escherichia coli* in mothers. Strain-specific triclosan  
163 resistance in *E. coli* has been described(18) and may explain its enrichment in TC households  
164 at the late time-point. Given that *B. caccae* was extensively enriched in the infants at the 10-  
165 month visit, it is possible that this organism also harbors interesting strategies for acquired or  
166 innate antimicrobial resistance.

167 In the TC arm of the study, mothers showed a strong enrichment of Proteobacteria, a  
168 phylum associated with broad spectrum antibiotic resistance. The finding that Proteobacteria

169 were enriched in the gut microbiota is consistent with observations in fish following triclosan  
170 exposure(5). The emergence of Proteobacteria was associated with the introduction of triclosan-  
171 containing toothpaste after the 2-month visit. This suggests that the major intestinal exposure to  
172 triclosan is through toothpaste rather than wash products, and that personal care products not  
173 covered by the FDA ban may play a role in the expansion of antibiotic-resistant species in the  
174 intestine.

175 One caveat of this study is that triclosan has a relatively short half-life (24 hours) and  
176 urinary triclosan results were from only one time point; thus, it is not possible to know the  
177 cumulative dose of triclosan received by the study participants by absorption through the skin or  
178 intestine. Although mothers in the TC arm had higher levels of triclosan detected in urine than  
179 those in nTC households, the median triclosan level we detected in urine of nTC households  
180 was higher than those reported in the National Health and Nutrition Examination Surveys  
181 (NHANES) cohort from 2003-2012 (geometric mean concentration of 13.0 pg/ $\mu$ L, 95<sup>th</sup> percentile  
182 concentration of 459.0pg/ $\mu$ L)( 19). Urinary triclosan levels of mothers in TC households were  
183 approximately half of the levels following a 15 day randomization to household TC-containing  
184 products in a previously published crossover study(9). Some of the differences may be due to  
185 methodological variations in the protocols used for triclosan detection or differences in exposure  
186 due to geography and inconsistent product usage. Infants in TC households and nTC  
187 households often had low triclosan levels with no statistically significant difference observed  
188 between groups. Low levels are unsurprising, as infants are not using adult toothpaste in the  
189 first year of life.

190 Another limitation of the current study is that controlling for household TC use may not  
191 be sufficient to identify the impact of other antimicrobials on the human microbiome. We did not  
192 measure triclocarban levels directly, but exposure from external sources such as clothing and  
193 plastics (such as in children's toys) likely occurred. Exposure to other microbicides, including



194 any antibiotics administered throughout pregnancy and the first year, might have influenced  
195 prenatal and postnatal microbiomes in ways that cannot be experimentally controlled. However,  
196 these perturbations were present in both arms of the study. The Proteobacteria enrichment in  
197 TC mothers but not the infants suggest the small microbiota disruptions were TC dose  
198 dependent.

199         The impact of likely low-dose but long-term (>4 months) household product-based  
200 antimicrobial exposure on the human intestinal microbiome has not been previously described  
201 in either adults or infants during the critical phases of microbiota assembly early in life. While the  
202 impact appears to be minimal, we do identify specific taxa previously associated with anti-  
203 inflammatory properties that are enriched in nTC households, as well as other taxa previously  
204 associated with broad spectrum antibiotic resistance that are enriched in TC households. The  
205 measurable shift in the intestinal microbiome that occurs in mothers between the first and  
206 second visits of this study, which corresponds to the introduction of triclosan through toothpaste,  
207 suggests that toothpaste exposure places more selective pressure on intestinal microbial  
208 species than wash products. While a selective expansion of Proteobacteria is not known to  
209 cause various diseases, Proteobacteria expansion has been proposed as a potential diagnostic  
210 signature of dysbiosis linked to diabetes, colitis, and malnutrition(21). Future studies may  
211 illuminate the impact of these shifts on health-related outcomes. Evidence that antibiotic  
212 resistance develops in diverse bacterial taxa following prolonged triclosan exposure suggests  
213 that triclosan resistance may be mediated by specific genes(22–24), and that these genes may  
214 be horizontally transferred(25). Although we did not identify a significant enrichment of an  
215 antibiotic-resistance genes in a subset of TC-exposed mothers, future *in vitro* and potentially *in*  
216 *vivo* studies will be required to more thoroughly characterize the impact of TCs on antibiotic  
217 resistance in the gut microbiota. Triclosan exposure is known to play a role in allergen and food  
218 sensitization(26, 27); topical skin application of triclosan is sufficient to induce peanut sensitivity  
219 in mice(28). Given the high prevalence of TC exposure on the skin in this study, it will be

220 interesting to study the impact of these wash products on the skin microbiota and related health  
221 outcomes. Despite the minimal effects of TC exposure from wash products on the gut microbial  
222 community of infants and adults, these results suggest detected taxonomic differences are  
223 associated with potential harmful effects on host physiology and highlight the need for consumer  
224 safety testing of consumer antimicrobial products on the human microbiome.

225

## 226 **Materials and Methods**

### 227 **Study Design**

228 Subjects in this study were recruited to participate in Stanford's Outcomes Research in  
229 Kids (STORK), a prospective cohort study of healthy mothers and infants(14). Briefly, pregnant  
230 mothers were enrolled in the study at approximately 20 weeks of gestation from both Lucile  
231 Packard Children's Hospital (Stanford, CA) and the Tully Road Clinic of Santa Clara Valley  
232 Medical Center (San Jose, CA). Enrolled mothers were additionally invited to participate in a  
233 nested, randomized intervention of TC-containing household and personal wash products to  
234 study the effects of these microbicides on illness and the development of the infant microbiome.

235 Participants were provided commercially available wash products (liquid and bar soap,  
236 toothpaste, dishwashing liquid) all either containing or not containing TCs. Bar soap was the  
237 only provided product that contained triclocarban in addition to triclosan. Because of concerns  
238 about potential endocrine disruption, mothers were initially not randomized to toothpaste but  
239 could continue using their preferred product during pregnancy. At the first post-delivery home  
240 visit (~2 months post birth), either triclosan-containing or triclosan-free toothpaste was provided  
241 according to assigned arm. Supplies were replenished every four months as needed during  
242 home visits.

243 Household visits were conducted every 4 months to collect demographic and household  
244 information as well as stool and urine samples. Samples were stored at -80°C until processed.  
245 Automated weekly surveys on breastfeeding, diet, infant illness, including antibiotic use, were

246 conducted, and infant medical records were referenced as available to account for antibiotic use  
247 around the time of sample collection. Of the 39 infants in this study, 34 had medical record  
248 verification of systemic antibiotic administration through the first year of life; 67.6% of infants in  
249 this study received systemic antibiotics (76.4% nTC, 60% TC) but administration did not occur  
250 within one month of sample collection in 95% of cases (104 of 109 infant samples). No antibiotic  
251 data was available for the mothers.

252 For intestinal microbiome analysis, we included all households with at least 5 of the  
253 expected 6 stool samples; 13 of the 39 households were missing one sample. From 13  
254 households, one sample was missing: 5 were missing a sample from the mother (ID: 1002,  
255 1084, 2360, 2443, 2584) and 8 from the infant (ID: 1009, 2137, 2201, 2274, 2284, 2341, 2421,  
256 2534). To determine if the randomization to TC-containing products was sufficient to increase  
257 TC exposure, urine samples were obtained from mothers and infants at the 6-month visit to  
258 measure urine triclosan levels. This time point was chosen because it followed randomization to  
259 all products, including toothpaste.

260

### 261 **Urinary triclosan detection**

262 Urinary triclosan levels (triclocarban was not assessed) were measured using liquid  
263 chromatography-mass spectrometry at the Stanford University Mass Spectrometry core facility.  
264 Urine samples (1 mL) were subjected to liquid-liquid extraction with ethyl acetate. Stable isotope  
265 labeled triclosan ( $^{13}\text{C}_{12}$ , 99%, Cambridge Isotope Laboratory) served as the internal standard  
266 (IS) and blank urine from subjects with no to minimal exposure to triclosan was used as sample  
267 matrix for calibration curve standards. The upper organic phase was collected and dried under a  
268 stream of nitrogen gas. Samples were reconstituted in 100 $\mu\text{L}$  of 20% methanol and transferred  
269 to auto-sampler vials. The LC-MS/MS analysis was performed on a TSQ Vantage triple  
270 quadrupole mass spectrometer coupled with an Accela 1250 HPLC (Thermo Fisher Scientific).  
271 Injection volume was 10  $\mu\text{L}$ . Reversed phase separation was carried out on a Kinetex C18

272 column (50 mm x 2.1 mm ID, 2.6  $\mu$ m particle size, Phenomenex). Mobile Phase A was water,  
273 and mobile phase B was methanol; flow rate was 350  $\mu$ L per minute. The gradient was as  
274 follows: 0 min. (20% B), 2.5 min. (98% B), 4 min. (98% B), 4.5 min (20% B) and 6 min (20% B).  
275 The mass spectrometer was operated in negative APCI mode, with selected reaction monitoring  
276 (SRM).

277 Three SRM transitions were used for each triclosan and triclosan IS: 250.9 > 159.1,  
278 187.0, 214.9 and, 263.07 > 169.0, 197.9, 226.9, respectively. The calibration curve was linear  
279 from 1 to 40,000 fmol/ $\mu$ L, and the lower limit of quantitation (LLOQ) was around 10 fmol/ $\mu$ L of  
280 triclosan in extracted urine. Samples were measured in triplicate. All results were divided by 10  
281 to account for concentration. Given the skewed distribution of triclosan levels, we used a  
282 nonparametric Mann-Whitney U test to determine triclosan level differences between  
283 intervention arms.

284

### 285 **DNA extraction, 16S ribosomal DNA amplification and amplicon sequencing**

286 Samples were prepared and sequenced in two batches with approximately equal TC and  
287 nTC households per batch (29 households in batch 1, 10 households in batch 2) with identical  
288 methods. Samples were incubated for 10 minutes at 65°C before bead beating. One 20-minute  
289 round of bead-beating was performed at room temperature and samples were mixed by  
290 inversion. DNA was isolated from stool samples using the PowerSoil Isolation Kit (Mo Bio  
291 Laboratories, Inc., Carlsbad, CA) per manufacturer's instructions. The protocol was modified to  
292 use approximately half the suggested weight (125 mg v. 250 mg) of stool per sample. Region-  
293 specific primers, which included Illumina adapter sequences and 12-base barcodes on the  
294 reverse primer, were used to amplify the V4 region of the 16S ribosomal RNA gene. Failed  
295 reactions were rerun and amplicons were cleaned using UltraClean-htp 96-well PCR Clean-Up  
296 Kit (Mo Bio Laboratories, Inc., Carlsbad, CA). Samples were quantified using Quant-iT dsDNA  
297 Assay Kit High Sensitivity (Thermo Fisher Scientific, Waltham, MA) and measured on a

298 FLEXstation II 384 microplate reader in the Stanford High-Throughput Bioscience Center.  
299 Amplicons were then combined in equimolar ratios, ethanol precipitated and gel purified. Paired-  
300 end, 250 bp sequencing was performed on an Illumina MiSeq at the Stanford Functional  
301 Genomics Facility. A median of 47,143 (6,434-315,978) reads were sequenced per sample.

302

### 303 **Sequence processing and classification**

304 We used a non-clustering method for 16S rRNA sequence classification using  
305 BaseSpace Application 16S Metagenomics v1.0 (Illumina, Inc.). Non-clustering implies amplicon  
306 reads are not grouped given a similarity score (typically 97-99%) to account for sequencing  
307 errors prior to classification. One caveat of clustering is that it reduces fine-scale variation that is  
308 biologically important. Briefly, the BaseSpace pipeline trims the 3' ends of non-indexed reads  
309 when the quality score is less than 15. High quality reads were classified using a modified  
310 Ribosomal Database Project (RDP) Classifier (29) with a curated version of the Greengenes  
311 May 2013 reference taxonomy database. The original RDP classifier algorithm uses 8-base  $k$ -  
312 mers, however BaseSpace RDP uses 32-base  $k$ -mers, giving each  $k$ -mer more specificity for a  
313 given species. A curated version of the Greengenes May 2013 reference taxonomy filters those  
314 entries with 16S sequence length less than 1250 bp, more than 50 wobble bases, or those not  
315 classified at the genus or species level. The pipeline does not specifically check for chimeras;  
316 however, if the forward and reverse reads do not map to the same sequence in the reference  
317 database they are excluded from classification. The comparison of the BaseSpace RDP pipeline  
318 to another non-clustering sequence classification method can be found in the supplementary  
319 methods.

320

### 321 **Prevalence taxonomy filtering**

322 To filter rare (i.e. noisy) taxonomically classified reads, we calculated a prevalence  
323 threshold based on taxa found in at least 7 samples. This threshold was chosen to include taxa

324 that constitute a “core” microbiome, which suggests a taxon is persistent within at least two  
325 mothers throughout the study or a taxon during development is found in at least 20% of infants  
326 at one visit. This filtering resulted in the inclusion of 1115 taxa from a pool of 1892 taxa.

327

### 328 **Metagenomic characterization of antibiotic resistance profiles**

329 DNA archived from the previously described stool extraction was processed for shotgun  
330 DNA sequencing using the Nextera XT DNA Library Preparation Kit (Illumina Inc.) per  
331 manufacturer’s instructions on a subset of 24 maternal samples from the 6-month visit. Paired-  
332 end, 101 bp sequencing was performed on an Illumina HiSeq 4000 at the Stanford Sequencing  
333 Service Center with an average of 21,660,032 (12,825,854–31,271,994) reads per sample.

334 Low quality read ends with a Phred score less than 20 were trimmed using TrimGalore  
335 v. 0.4.1 ([http://www.bioinformatics.babraham.ac.uk/projects/trim\\_galore/](http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/)), and PCR duplicates  
336 were removed using Super-Deduper v. 1.40 (<http://dstreett.github.io/Super-Deduper/>). High  
337 quality reads were then aligned to the Comprehensive Antibiotic Resistance Database  
338 (CARD)(30) using Burrows-Wheeler Aligner v. 0.7.10 (<http://bio-bwa.sourceforge.net/>). A  
339 median of 25,064 (8200-72,464) reads mapped to CARD per sample, with a median mapping  
340 percent of 0.11% for both intervention arms after adjusting for number of sequenced reads.

341 Genes known to harbor triclosan resistance (reviewed in (31) mapped a median of 5,476 (763-  
342 22,205) reads per sample. TC samples had a median of 7,043 mapped reads (relative %  
343 adjusted for sequencing depth: 0.030%) compared for 5,330 reads (relative % adjusted for  
344 sequencing depth: 0.027%) for nTC samples. Euclidean distance was calculated between  
345 samples, then clustered with a hierarchical agglomeration method using base R functions.

346

### 347 **Statistical Analyses**

348 Analyses were performed in ‘R’ v. 3.2.4 (<http://www.R-project.org>) with accompanying  
349 packages on non-rarified unique taxonomic classifications(32). Principle coordinate analyses

350 (PCoA) were performed using non-metric Bray-Curtis dissimilarity for combined analyses using  
351 'phyloseq' v. 1.14(33). Levels of triclosan in household products are intended to inhibit bacterial  
352 growth rather than kill bacteria(34); Because we hypothesized that the suppression of growth  
353 would alter taxa abundances rather than the presence/absence of taxa, Bray-Curtis, a non-  
354 phylogeny based method that takes abundance into account, was chosen for PCoA. Sample  
355 distances for maternal-only analyses were calculated using the Canberra distance, which is best  
356 used for centroid type patterns since maternal points on the overall PCA were centralized  
357 (Figure 1).

358         Alpha diversity measured by the Shannon diversity index was calculated using 'vegan'  
359 v. 2.4(35). Nonparametric Mann-Whitney U tests were used to determine statistical differences  
360 in diversity between intervention arms at each time point given the low sample sizes per  
361 comparison. To statistically test treatment effects on the homogeneity of microbial community  
362 composition, we performed permutational multivariate analysis of variance (PERMANOVA)  
363 analyses on distance metrics with 'vegan' v. 2.4; 1000 permutations were performed for mother  
364 and infant groups, stratified by the visits to account for infant development and maternal  
365 toothpaste introduction after the first visit as confounders. Differential taxa abundance and gene  
366 analyses were performed using 'DESeq2' v. 1.10(36). A 1% FDR adjusted p-value threshold  
367 was selected for the treatment comparisons across mothers and infants pooling all three visits.  
368 This threshold is relaxed to 5% for comparisons at each time visit given lowered power from  
369 smaller sample size.

370

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385

386 **Author Contributions**

387           J.P. and C.L. conducted the cohort study and nested intervention from which the  
388 specimens were collected and oversaw collection and coordination of all samples and  
389 metadata. T.D.H. coordinated processing of stool and urine samples and extracted and  
390 amplified DNA from the stool samples. E.T. prepared the shotgun sequencing libraries. A.S.B.,  
391 J.V.R. designed the data analysis; J.V.R. performed the computational and statistical  
392 analysis; J.V.R., A.S.B., J.P. participated in data analysis and manuscript preparation; all  
393 authors edited the manuscript.

394

395 **Conflict of Interest**

396 The authors have no conflicts of interests to report.

397



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506  
507

508 **Figure 1: Urinary triclosan levels are elevated in TC mothers following 6 months of**  
509 **exposure.** Urinary triclosan measurements are available for 38 mothers (17 TC, 21 nTC) and  
510 33 infants (15 TC, 18 nTC). Mothers in TC households have statistically significant higher  
511 triclosan levels than nTC mothers (Mann-Whitney U-test,  $p < 0.0001$ ). Infant TC levels between  
512 treatments are not significant (Mann-Whitney U-test,  $p=0.06$ ).

513  
514 **Figure 2: Mother and infants have distinct microbiome compositions not driven by**  
515 **household TC exposure.** (A) PCoA of Bray-Curtis dissimilarity for all ( $n=221$ ) samples shows  
516 that gut communities cluster by mothers and infants. (B) PCoA separated by time and  
517 treatment.

518  
519 **Figure 3: TC randomization does not decrease gut microbial diversity in infants or**  
520 **mothers.** Shannon diversity measures are plotted as interquartile range with median for each  
521 TC exposure class and time point grouping for infants and mothers. Diversity is not decreased  
522 by exposure to TC containing products in infants (Mann-Whitney U-test p-values for 2, 6, and 10  
523 months: 0.656, 0.842, 0.486). The combined infant cohort shows an increase in diversity as  
524 colonization occurs through the first year (Mann-Whitney U-test for 2 to 12 months,  $p$ -value  $<$   
525 0.001). Diversity is not decreased by exposure to TC-containing products in mothers (Mann-  
526 Whitney U-test p-values for 2, 6, and 10 months: 0.729, 0.280, 0.201).

527  
528 **Figure 4: Enrichment of Proteobacteria is observed in the mothers of TC households.** (A)  
529 Differentially abundant taxa between nTC and TC households. Values left of the grey line  
530 indicate an enrichment in nTC household and values to the right indicate an enrichment in TC  
531 households. (A) Analyses are separated by mothers and infants for all samples across the 3  
532 time points (FDR adjusted  $p$ -value  $< 0.01$ ). Differentially abundant taxa are displayed for (B)  
533 infants and (C) mothers at per visit (FDR adjusted  $p$ -value  $< 0.05$ ).

534

535

536 **Table 1: Selected characteristics of study sample (N=39 households).** Demographics are  
 537 self-reported at the time of enrollment. Age, individuals in the household, and the sample  
 538 collection windows are reported as the median and range. Sample collection times are relative  
 539 to the infant birth date. Not applicable for cleaning products at work indicates the mothers were  
 540 unemployed at the time, and not receiving additional TC exposure similar to mothers that do not  
 541 work with cleaning products.  
 542

	<b>TC (N=17)</b>	<b>nTC (N=22)</b>
<b>Individuals residing in household</b>	4 (3-10)	3 (2-11)
<b>Maternal age (years)</b>	33 (26-41)	34 (28-42)
<b>Ethnicity</b>		
Hispanic	11 (64.7%)	7 (31.8%)
Non-Hispanic	6 (35.3%)	15 (68.2%)
<b>Bathing habits</b>		
Less than daily	5 (29.4%)	3 (13.6%)
At least daily	12 (70.6%)	19 (86.4%)
<b>Pets</b>		
Yes	6 (35.3%)	5 (22.7%)
No	11 (64.7%)	17 (77.3%)
<b>Mother's use of cleaning products at work</b>		
Yes	5 (29.4%)	6 (27.3%)
No	5 (29.4%)	11 (50.0%)
Not applicable	7 (41.2%)	5 (22.3%)
<b>Days after delivery for each sample collection window</b>		
2 months	88 (14-124)	74 (27-122)
6 months	199 (135-256)	199 (146-255)
10 months	330 (259-375)	319 (241-277)

543

544 **Supplementary table 1: Summary of number of sequenced and classified reads per**

545 **sample.** Classified reads reported have been filtered to remove rare, i.e. noisy, taxa.

546

547 **Supplementary table 2: Urinary triclosan levels at 6 months.** Triclosan was measured using

548 liquid chromatography-mass spectrometry in urine samples collected at the second visit (~6

549 months). Summaries are provided as the median (range) of TC molecules in a

550 picogram/microliter (pg/ $\mu$ L).

551

552 **Supplementary table 3: Differentially abundant taxa for infants and mothers aggregated**

553 **across 3 visits.** Differentially abundant taxa using DESeq2 (adjusted p-value < 0.01) with 1115

554 unique taxonomic classifications.

555

556 **Supplementary table 4: Differentially abundant taxa in TC and nTC households by group**

557 **and visit.** Differentially abundant taxa were determined using DESeq2 (adjusted p < 0.05) with

558 1115 unique taxonomic classifications. No species were differentially abundant for mothers at 2

559 months.

560

561 **Supplementary figure 1: Distribution of sample collection relative to infant birth.**

562 Households for each visit time point are sampled approximately within one month, and are

563 balanced between treatments in the study.

564

565 **Supplementary figure 2: DADA2 and BaseSpace RDP algorithms comparably capture**

566 **microbiome variance.** Principal coordinates analyses of Bray-Curtis dissimilarity of “core” or

567 “developmental” taxa show that gut communities cluster by mothers and infants. **(A)** DADA2

568 and **(B)** BaseSpace RDP are comparable in first and second axis variance.

569

570 **Supplementary figure 3: Individuals in mother and infant groups have similar relative**  
571 **microbiome compositions at the phylum level throughout the first year of life**  
572 **independent of TC exposure. (A)** TC households (n=17) and **(B)** nTC (n=22) households have  
573 stable relative abundance of species at 2, 6, and 10 month visits. Analyses are complete, with  
574 only 13 missing samples total out of 234 proposed for the study (94% inclusion). Phyla present  
575 in less than 2% abundance are condensed for visual clarity.

576

577 **Supplementary figure 4: Infant intestinal microbiome variability is not defined by known**  
578 **external factors by 2 months of age (n=34).** PCoA with Bray-Curtis dissimilarity of the infants  
579 at 2 months of age suggests factors known to impact the microbiome, such as **(A)** birth method  
580 and **(B)** formula, **(C)** maternal ethnicity, and **(D)** pets in the household are not the main drivers  
581 of microbiome variance in infants at 2 months of age.

582

583 **Supplementary figure 5: Maternal samples within households are more similar than**  
584 **between households. (A)** Hierarchical clustering of Canberra distances shows that maternal  
585 samples from a given individual throughout the first year of life are more self-similar than other  
586 household mothers at the same visit. The colors are representative of a single household,  
587 labeled by household and visit (B1 = 2 months, B2 = 6 months, B3 = 10 months), and node  
588 colors reflect TC grouping (red=nTC, blue = TC). **(B)** PCoA of Canberra distances shows 9.3%  
589 of the variance is explained by the first two axes and the absence of TC treatment clustering for  
590 any visit among mothers. **(C)** PCoA projection variability between visits occurs similarly between  
591 TC and nTC households when compared to the initial 2-month visit.

592

593 **Supplementary figure 6: Triclosan resistance gene abundances do not distinguish TC**  
594 **and nTC maternal samples following 6 months of exposure.** Metagenomic reads were  
595 aligned to the CARD Database. Known triclosan resistance genes had a median mapping

596 percent of ~0.03% for both intervention arms after adjusting for number of sequenced reads.  
597 Euclidean distance was calculated between samples, then clustered with  
598 a hierarchical agglomeration method. Mothers of both treatment arms have a low number of  
599 reads in triclosan resistance genes (~6,000 reads) suggesting we limited power to detect  
600 difference with this approach and require targeted sequencing or culturing studies to further  
601 understand resistance profiles in the stool.  
602



Figure 1

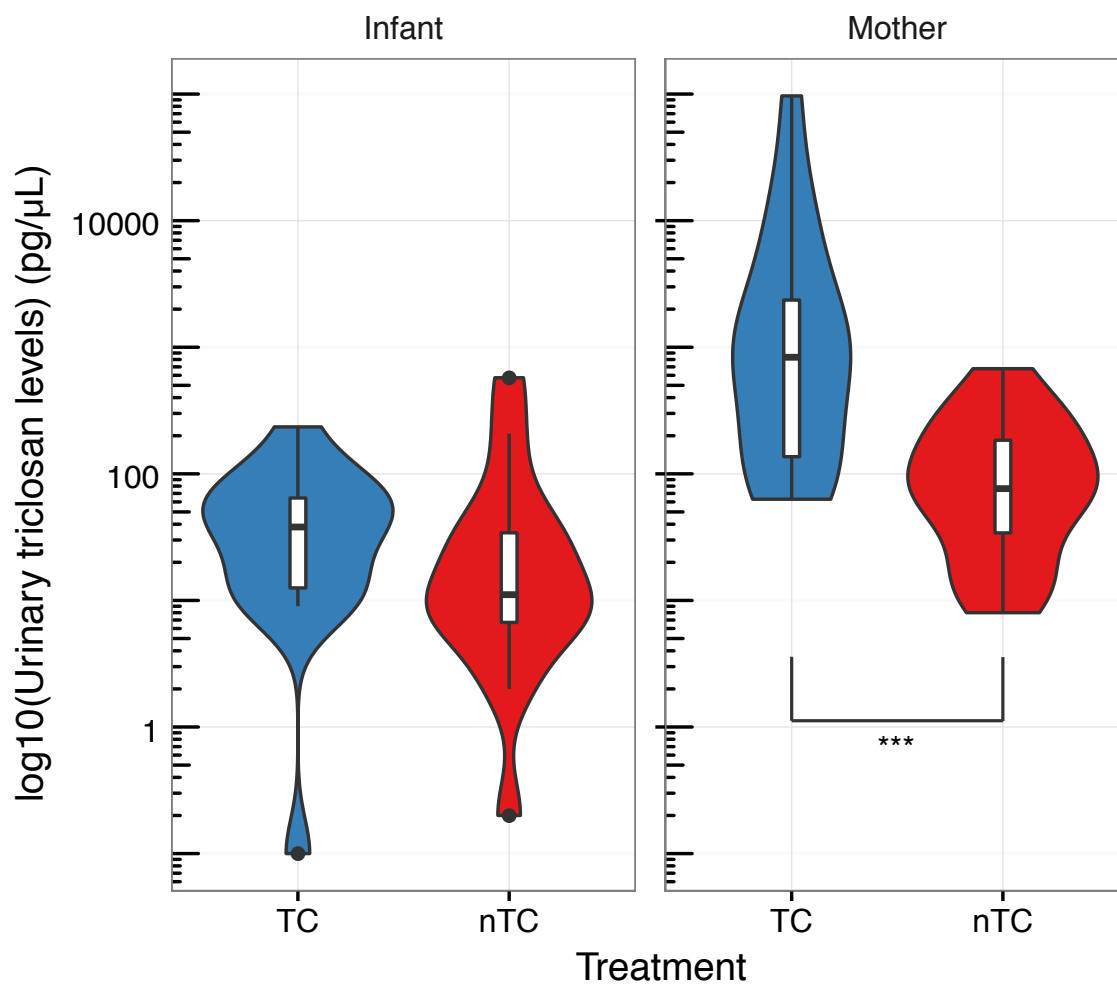


Figure 2

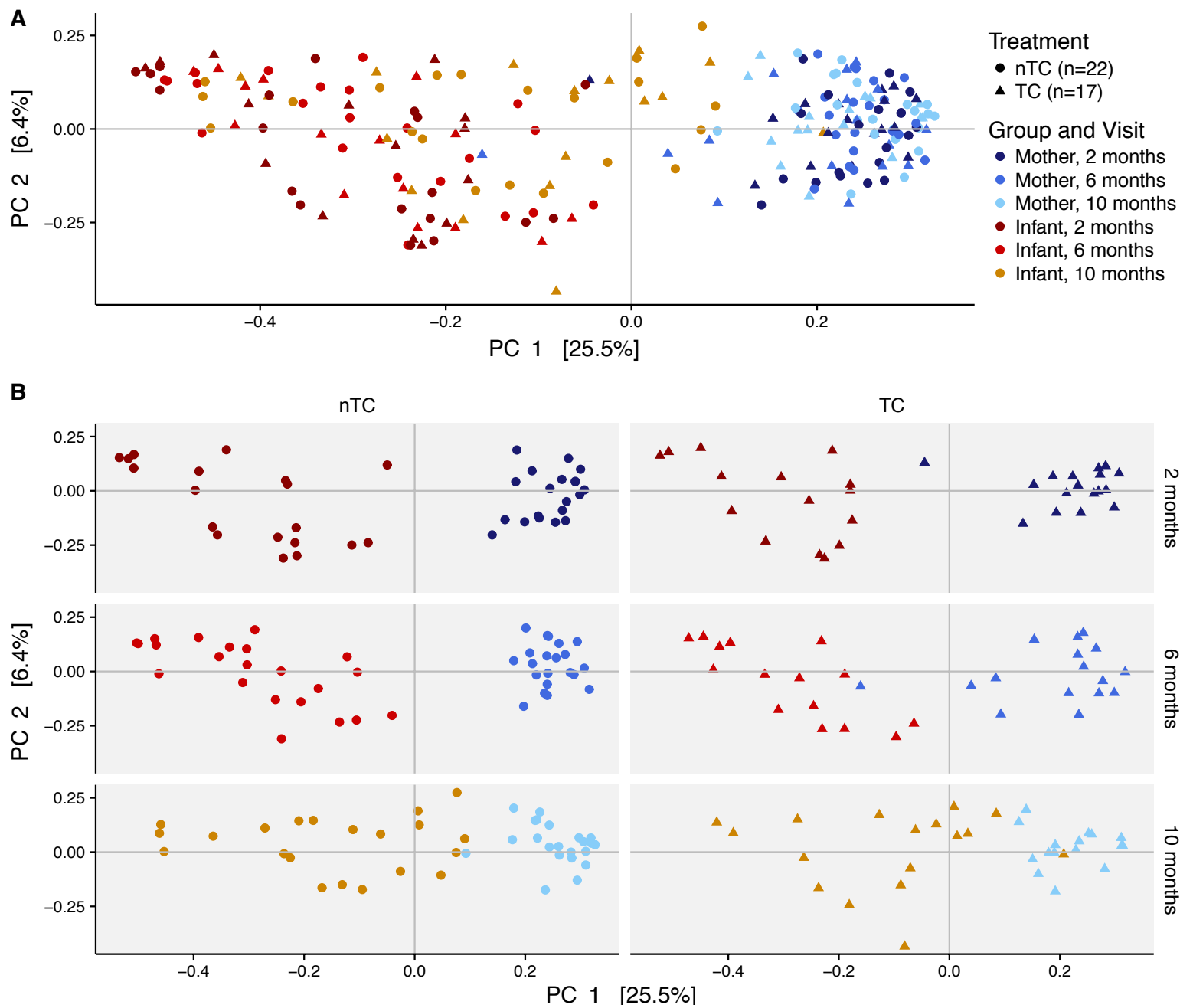


Figure 3

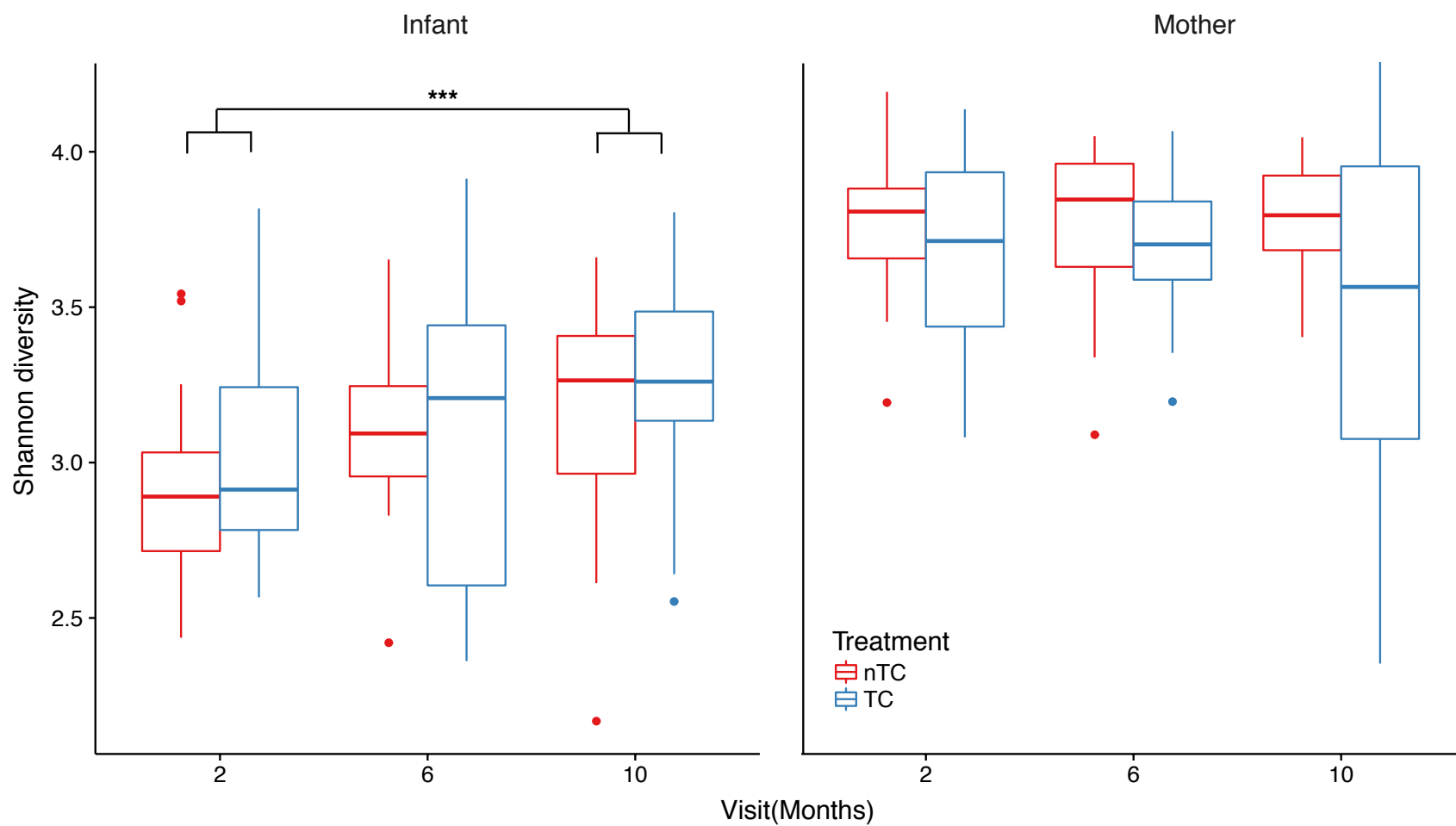


Figure 4

