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Altered microbiota composition reflects enhanced communication in 15q11-13 CNV mice

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ABSTRACT

Autism spectrum disorder (ASD) is a complex and heterogeneous neurodevelopmental disorder. In addition to the core symptoms of ASD, many patients with ASD also show comorbid gut dysbiosis, which may lead to various gastrointestinal (GI) problems. Intriguingly, there is evidence that gut microbiota communicate with the central nervous system to modulate behavioral output through the gut-brain axis. To investigate how the microbiota composition is changed in ASD and to identify which microbes are involved in autistic behaviors, we performed a 16S rRNA gene-based metagenomics analysis in an ASD mouse model. Here, we focused on a model with human 15q11-13 duplication (*15q dup*), the most frequent chromosomal aberration or copy number variation found in ASD. Species diversity of the microbiome was significantly decreased in *15q dup* mice. A combination of antibiotics treatment and behavioral analysis showed that neomycin improved social communication in *15q dup* mice. Furthermore, comparison of the microbiota composition of mice treated with different antibiotics enabled us to identify beneficial operational taxonomic units (OTUs) for ultrasonic vocalization.

1. Introduction

Autism spectrum disorder (ASD) is considered a heterogeneous set of neurobehavioral diseases which are typified by a range of symptoms, including persistent deficits in social communication and social interaction across multiple contexts and restricted, repetitive behaviors, activities, or interests (Takumi et al., 2019). Apart from the core behavioral symptoms, multiple comorbidities have been reported in patients with ASD, with gastrointestinal (GI) problems being among them (Coury et al., 2012; Doshi-Velez et al., 2014). Comorbid GI symptoms in subsets of ASD patients include diarrhea, constipation, abdominal pain, and gastric reflux. Although the percentage of patients with ASD suffering from GI problems varies from study to study, there is a consensus that GI problems are common in patients with ASD (Xu et al., 2019). Moreover, the severity of GI problems is positively correlated with the severity of ASD symptoms (Adams et al., 2011; Mazurek et al., 2013). Although the causes of GI problems in patients with ASD are unclear, one plausible source is the gut microbiota.

Gut dysbiosis is observed in patients with ASD (Finegold et al., 2010; Adams et al., 2011; Williams et al., 2011; De Angelis et al., 2013). Intriguingly, gut dysbiosis can directly cause behavioral changes, including ASD-like behavior (Hsiao et al., 2013; Desbonnet et al., 2014; Buffington et al., 2016; Coretti et al., 2017; Golubeva et al., 2017; Sgritta et al., 2019). In addition to affecting behavior,

Abbreviations: 15q dup, 15q11-13 duplication; 5-HT, 5-hydroxytryptamine; ACE, Abundance Coverage-based Estimator; Amp, ampicillin; ASD, autism spectrum disorder; BDNF, brain-derived neurotrophic factor; Ctrl, control; CNVs, copy number variations; GI, gastrointestinal; IL-10, interleukin-10; Neo, neomycin; OF, open field; OTUs, operational taxonomic units; SCFAs, short chain fatty acids; USV, ultrasonic vocalizations; UniFrac-PCoA, UniFrac-Principal Coordinate Analysis; WT, wild type.

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gut dysbiosis also mediates changes in immunity (Fung et al., 2017), levels of brain-derived neurotrophic factor (BDNF) (Clarke et al., 2013), serotonin (5-hydroxytryptamine: 5-HT), synaptic-related proteins (Diaz Heijtz et al., 2011), and expression of genes important for synaptic plasticity. Given that these proteins are affected or have roles in ASD (Feyder et al., 2010; Zheng et al., 2016; Takumi et al., 2019), these data provide strong evidence for dynamic interactions between gut microbiota and ASD.

Although many studies have reported differences in microbiota between patients with ASD and control subjects, no gut bacteria species is currently implicated in ASD. Based on the emerging link between gut microbiota and ASD and the lack of consensus on specific causative gut bacteria, we aimed to investigate the microbiota composition in a mouse model of ASD and to identify the specific microbiota community involved in ASD-related behavioral symptoms.

In this study we used *15q dup* mice, a mouse model of ASD with a 6.3-Mb duplication of mouse chromosome 7, which corresponds to duplication of human chromosome 15q11-13 (Nakatani et al., 2009). Duplication of the 15q11-13 region is one of the most frequent chromosomal aberrations or copy number variations (CNVs) found in ASD (Vorstman et al., 2006; Takumi and Tamada, 2018). *15q dup* mice display similar poor social communication and behavioral inflexibility to that that observed in patients with ASD (Nakatani et al., 2009; Tamada et al., 2010). They also show reduced brain 5-HT levels, late-onset obesity, impaired synaptic plasticity, and impaired excitatory/inhibitory balance in the somatosensory cortex (Tamada et al., 2010; Isshiki et al., 2014; Piochon et al., 2014; Kishimoto et al., 2015; Nakai et al., 2017). Moreover, patients with 15q duplication syndrome display GI problems (Shaaya et al., 2015).

To identify a specific microbiota community, we performed fecal microbiota profiling of *15q dup* mice by 16S rRNA gene sequencing. Given that short-term antibiotic treatment has been reported to improve ASD symptoms (Sandler et al., 2000), we also examined the effect of antibiotic treatment to reveal the relationship between gut microbiota composition and ASD-related behavioral abnormalities in these mice.

2. Materials and methods

2.1. Animals

15q dup mice were generated as previously described (Nakatani et al., 2009). They were bred for more than 10 generations on a C57BL/6 J background. *15q dup* mice and wild type (WT) littermates maintained under SPF conditions in the RIKEN animal facility were used. All mice were maintained under a 12-h light/dark cycle with food and water available ad libitum. Mice were randomly assigned to the treatment or control group while ensuring that age and weight were similar between the groups. Male mice were used for all experiments. For antibiotic treatment, 4-week-old mice were treated with either water or 1 g/l ampicillin (Wako Pure Chemical Industries, Ltd, Osaka Japan) or 1 g/l neomycin sulfate (Wako Pure Chemical Industries, Ltd) dissolved in drinking water for a period of 4 weeks. All animal experiments were conducted in accordance with the principles stated in the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, 1996 or the UK Animals (Scientific procedures) Act 1986 and associated guidelines, or the European Communities Council Directive of 24 November 1986 (86/609/EEC). The protocols for animal experiments were approved by the Animal Experimentation Committees of RIKEN Brain Science Institute and were performed in accordance with RIKEN Animal Care and Use Guidelines. The authors further attest that all efforts were made to minimize the number of animals used and their suffering.

2.2. Fecal sample collection

An individual mouse was placed into a clean cage without bedding for feces collection. Newly excreted fecal samples were immediately collected into sterile tubes and immediately snap frozen in liquid nitrogen.

2.3. Microbial DNA extraction

Fecal DNA was isolated and purified according to previously reported methods with minor modifications (Morita et al., 2007). The bacterial pellet was suspended and incubated with 15 mg/ml lysozyme (Sigma-Aldrich Co. LLC, St. Louis, MO, U.S.A.) at 37 °C for 1 h in 10 mM Tris-HCl/1 mM EDTA (TE). Purified achromopeptidase (Wako Pure Chemical Industries, Ltd) was added at a final concentration of 2000 units/ml and the suspension was incubated at 37 °C for 30 min. The suspension was treated with 1 % (wt/vol) sodium dodecyl sulphate and 1 mg/ml proteinase K (Merck, Darmstadt, Germany) and incubated at 55 °C for 1 h. The lysate was treated with phenol/chloroform/isoamyl alcohol (Life Technologies, Carlsbad, CA, U.S.A.). DNA was precipitated by adding ethanol and pelleting by centrifugation at 3300 g at 4 °C for 15 min. The DNA pellet was rinsed with 75 % ethanol, dried, and dissolved in TE. DNA samples were purified by treating with 1 mg/ml RNase A (Wako Pure Chemical Industries, Ltd) at 37 °C for 30 min and precipitated by adding equal volumes of 20 % polyethylene glycol solution (PEG6000-2.5 M NaCl). DNA was pelleted by centrifugation at 8060 *g* at 4 °C, rinsed with 75 % ethanol, and dissolved in TE (Kim et al., 2013).

2.4. Amplicon sequence of 16S rRNA gene

The variable V1-V2 regions of the 16S rRNA gene were amplified by PCR using universal primers (a barcoded 27Fmod and 338R) and multiplexed amplicon sequencing was performed by MiSeq according to the manufacturer's protocol (Illumina, Inc, San Diego, CA, U.S.A.) (Kim et al., 2013).

2.5. Processing of 16S rRNA gene V1-V2 sequence data

The 16S sequence reads were assigned to samples on the basis of their barcode sequence. Reads with an average quality value < 25 and those lacking the primer sequences at both ends were excluded. After trimming both primer sequences from the filtered reads, 3000 reads per sample were randomly selected and used for further analyses.

The high-quality reads were sorted according to quality values and then clustered into operational taxonomic units (OTUs) using a 97 % pairwise-identity cutoff with the UCLUST program v.5.2.32 (http://www.drive5.com/). Representative sequences of the generated OTUs were BLAST-searched against a 16S database we constructed for this study, using the GLSEARCH program to determine the closest taxa. The 16S database was constructed by curating the public databases Ribosomal Database Project (RDP) v.10.31, CORE (http://microbiome.osu.edu/) and a reference genome sequence database obtained from the NCBI FTP website (ftp://ftp.ncbi.nih.gov/genbank/, December 2011). For assignment of OTUs to taxa at phylum, genus, and species levels, sequence similarity thresholds of 70, 94, and 96 % were applied. Analysis of the sequencing data was performed as described in previous studies (Iwasawa et al., 2018; Shibagaki et al., 2017)

2.6. Behavioral tests

All behavioral tests were performed between 9:00 a.m. and 3:00 p.m. The procedures were principally based on those described in

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previous studies (Nakai et al., 2017; Nakatani et al., 2009; Tamada et al., 2010).

2.6.1. Open field test

Locomotor activity was measured using the open field (OF) test. Each mouse was allowed to move freely in the open field apparatus (50 cm (length) × 50 cm (width) × 30 cm (height). The field was illuminated at 100 lx by LEDs. Total distance traveled and time spent in the center area of the open field were recorded using a charge-coupled device (CCD) camera connected to a personal computer. Video images were analyzed using OF image analysis software (O'hara Co. & Ltd., Tokyo, Japan). The center area was defined as a central square of 30 cm x 30 cm. Behavior was monitored for 10 min.

2.6.2. USV test

Ultrasonic vocalizations (USV) were recorded from isolated adult male mice. Before recording, each male mouse was placed individually into a plastic cage for habituation for 10 min. This recording cage was then placed in a dark soundproof box. A wild-type unfamiliar female mouse (8-week-old) was placed into the same cage and the male test mouse was allowed to communicate with the female mouse for 10 min. The USV emitted by the male mouse were recorded using an ultrasonic microphone (UltraSoundGate 416H, Avisoft Bioacoustics, Glienicke, Germany). During the recording session, acoustic data were digitized using an Avisoft signal conditioner and recorded with Avisoft-RECORDER software. The recordings were transformed into spectrograms for analysis of the number of calls as described previously (Nakanishi et al., 2017).

2.7. Statistical analysis

Statistical analysis was conducted using SPSS 23.0 and GraphPad Prism 8.0.1. The observed number of OTUs, and Chao1, Abundance Coverage-based Estimator (ACE), and Shannon's indices were used to evaluate the richness and diversity of the overall microbial community. UniFrac distance was used to assess the dissimilarity (distance) between any pair of samples (Lozupone and Knight, 2005). Unweighted and weighted UniFrac distances were analyzed using permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001). Microbial alpha diversity; microbial relative abundance at the phylum, genus, and OTU level; and behavioral tests were analyzed using Student's *t* test, Mann-Whitney U test, one-way ANOVA, and the Kruskal-Wallis test as appropriate. All post-hoc comparisons were conducted using Dunn's post-hoc test with Bonferroni correction for multiple comparisons. The threshold for statistical significance was set at $p \leq 0.05$ with $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Values in graphs and tables were expressed as mean ± s.e.m. For box plots, the median is shown as the line inside the box with whiskers representing the lowest and highest values within the 1.5 interquartile range (IQR).

3. Results

3.1. Gut microbiota profiles of 8-week-old 15q dup mice and WT littermates

Fecal microbiota profiling of 8-week-old male *15q dup* and WT mice revealed that WT mice had a higher number of OTUs than *15q dup* mice (*t* test; t(7.604) = 2.521, $p = 0.037$) (Fig. 1a). Consistent with this, alpha diversity based on the Chao1, ACE, and Shannon's indices was lower in *15q dup* than in WT mice (Fig. 1b). The lower number of OTUs and alpha diversity suggest less microbiota diversity in *15q dup* mice. Species diversity of an organism typically confers health benefits to its host. Low diversity in *15q dup* mice may be an indicator of disruption to gut microbiota composition in these animals. Given previous findings that the gut microbiota is able to modulate mouse behavior (Diaz Heijtz et al., 2011; Hsiao et al., 2013), this gut dysbiosis may underlie the behavioral symptoms reported in this mouse model.

Phylogenetic distances among samples were assessed using unweighted and weighted UniFrac distance metrics and visualized using UniFrac-Principal Coordinate Analysis (UniFrac-PCoA). Unweighted UniFrac, a qualitative phylogenetic measure which only considers the presence or absence of a taxon, showed no difference between *15q dup* and WT mice (PERMANOVA; $p = 0.3856$). Weighted UniFrac, a quantitative phylogenetic measure which also considers the relative abundance of a taxon, showed a similar result (PERMANOVA; $p = 0.1988$). Consistent with these results, both the unweighted and weighted UniFrac-based 2D PCoA plot showed no clear segregation between *15q dup* and WT mice (Fig. 1c). This similarity in gut microbiota phylogenetic distance between *15q dup* and WT mice indicates a similar gut microbiota structure in the two groups.

Sequencing analysis of fecal samples from *15q dup* and WT mice revealed that the major phyla comprising the gut microbiota was taxonomically classified as Firmicutes (WT: 62.49 % ± 4.07 %; *15q dup*: 54.64 % ± 18.16 %) and Bacteroidetes (WT: 33.61 % ± 5.47 %; *15q dup*: 33.30 % ± 13.55 %) (Fig. 1d and Supplementary Table S1). The relative abundance of most phyla did not significantly differ between *15q dup* and WT mice. Further classification of phyla in *15q dup* and WT mice revealed that the majority of Firmicutes were of the genera *Lactobacillus* (WT: 12.1 % ± 10.57 %; *15q dup*: 16.82 % ± 11.21 %) and *Clostridium* (WT: 9.99 % ± 7.8 %; *15q dup*: 5.89 % ± 6.68 %); and the majority of Bacteroidetes were of the genus *Bacteroides* (WT: 1.12 % ± 0.46 %; *15q dup*: 1.09 % ± 1.09 %) (Fig. 1e and Supplementary Table S2). No significant difference in the relative abundance of each genus was observed between *15q dup* and WT mice. At the species level, 13 OTUs were found to be more abundant in WT mice, among which 7 OTUs were not detected in *15q dup* mice ($p < 0.05$) (Table 1). These OTUs predominantly belonged to the orders Bacteroidales and Clostridiales, with single representatives from Desulfovibrionales. These OTUs were further analyzed in subsequent experiments for their effect on autistic behaviors.

3.2. Neomycin administration increased ultrasonic vocalization (USV) calls in 15q dup mice

Given that short-term antibiotic treatment of autism patients with GI problems reportedly improves behavioral and GI symptoms (Sandler et al., 2000), we performed antibiotic treatment with either ampicillin or neomycin to evaluate the effect of microbiota composition on autistic behaviors in *15q dup* mice. After 4 weeks of antibiotic treatment, behavioral phenotypes, including locomotor activity, anxiety and autistic-related social communication were analyzed in open field and USV tests (Fig. 2a). Ampicillin is known to have a broad antimicrobial spectrum and neomycin is a non-absorbable aminoglycoside antibiotic that acts against Gram-negative bacteria. Therefore, we expected that these antibiotics would alter the microbiota composition in different ways. This was indeed the case, as demonstrated by PCoA plots of the structure of the mouse gut microbiota after ampicillin or neomycin treatment based on unweighted and weighted UniFrac (Fig. 2b).

In the open field test, the total distance traveled was similar between *15q dup* control mice and WT control mice (one-way ANOVA; $F(5,78) = 1.79$, $p > 0.05$), indicating the absence of any locomotor activity anomalies in *15q dup* mice. More importantly, long-term antibiotic treatment also had no effect on locomotor activity (Fig. 2c). In the test for anxiety, *15q dup* control mice spent less time in the center area of the open field than WT control mice, although this difference was not statistically significant (one-way ANOVA; $F(5,78) = 3.081$, $p > 0.05$) (Fig. 2c). Neither ampicillin nor

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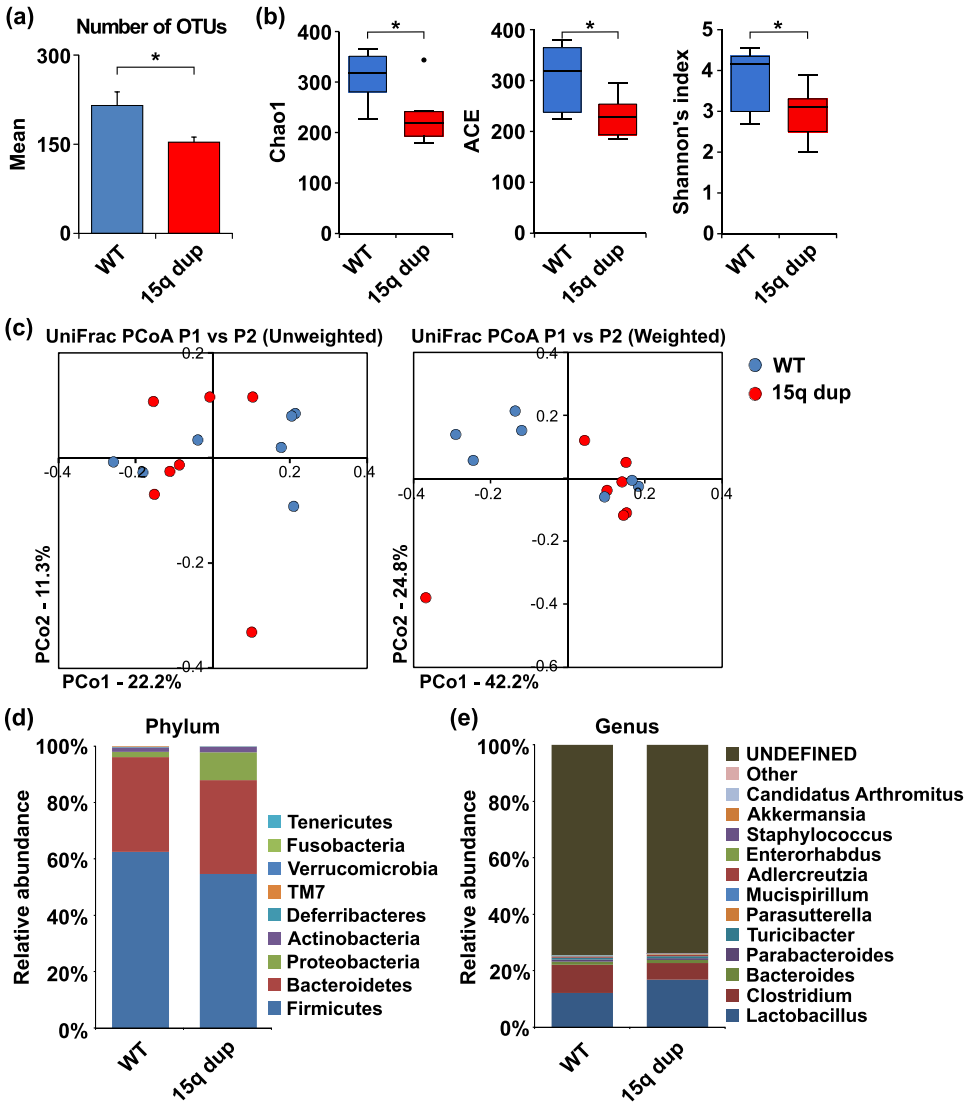

Fig. 1. Profiling of gut microbiota in *15q dup* mice and WT littermates. **(a).** Number of OTUs (3000 reads per sample) in fecal samples from *15q dup* and WT mice. Graphs show mean ± s.e.m. (n = 7 per group; Student's *t* test, $^*p < 0.05$). **(b).** Alpha diversity index (Student's *t* test, $^*p < 0.05$). **(c).** Unweighted (left) and weighted (right) UniFrac-based 2D PCoA plot constructed for all OTUs (n = 7 per group). **(d).** Relative abundance of all identified OTUs classified at the phylum level (n = 7 per group). **(e).** Relative abundance of all identified OTUs classified at the genus level (n = 7 per group).

Table 1
Significantly different OTUs between *15q dup* mice and WT littermates.

Relative abundance (%)		*p* value	Most similar species	Homology (%)*
WT	*15q dup*			
0.095	0.000	0.013685	*Clostridium hathewayi*	89.51
0.029	0.000	0.016690	*Oscillibacter valericigenes*	91.98
0.524	0.210	0.020371	*Barnesiella viscericola*	80.50
0.048	0.005	0.023696	*Anaerotruncus colihominis*	84.62
0.038	0.000	0.03002	*Roseburia inulinivorans*	92.02
0.043	0.005	0.035289	*Fusibacter paucivorans*	88.65
0.033	0.000	0.038245	*Clostridium clostridioforme*	83.38
0.076	0.010	0.042545	*Clostridium* sp. *A9*	98.77
0.029	0.000	0.045256	*Ruminococcus* sp. *5_1_39BFAA*	86.27
0.057	0.010	0.046086	*Desulfocurvus vexinensis*	86.48
0.024	0.000	0.046528	*Hydrogenoanaerobacterium saccharovorans*	87.77
0.038	0.000	0.047246	*Prevotella* sp. *Smarlab 121567*	80.69
0.538	0.157	0.048115	*Clostridium* sp. *ASF502*	99.38

OTUs are listed in ascending order based on the significance of the *p* value.
*Homology (%): the percentage of an OTU sequence which is homologous to that of species from the 16S database used in this study.

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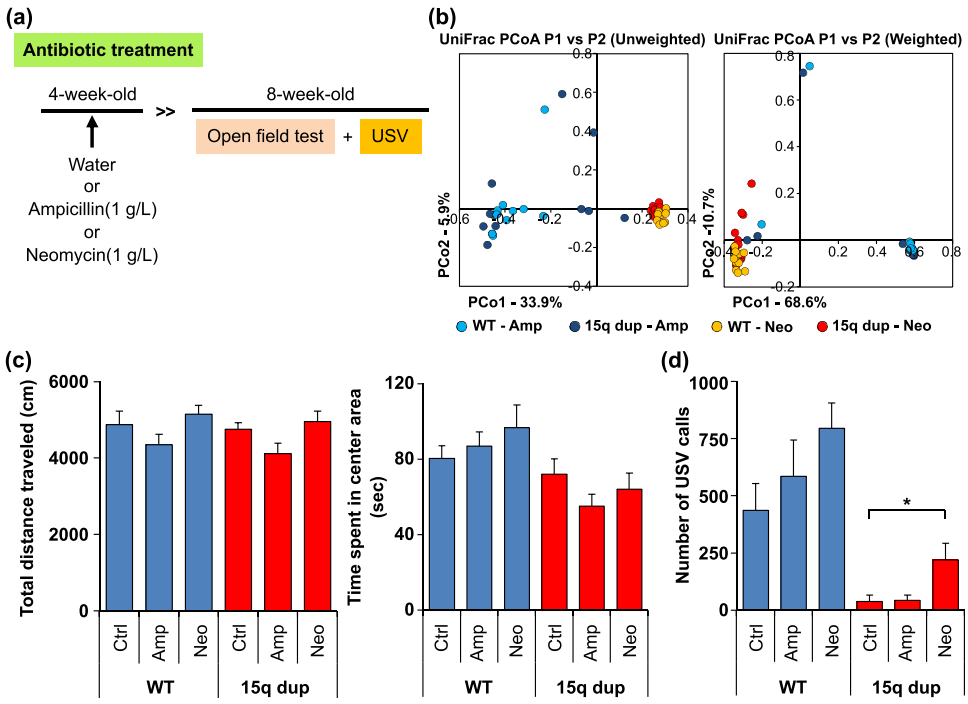

Fig. 2. Behavioral tests in *15q dup* and WT mice after antibiotic treatment. **(a).** Schematic diagram of the experimental design. At 4 weeks old, mice were treated with water or ampicillin (1 g/l) or neomycin (1 g/l). After 4 weeks of antibiotic treatment, mice were subjected to open field and USV tests. **(b).** Unweighted (left) and weighted (right) UniFrac-based 2D PCoA plots constructed for all OTUs in ampicillin- and neomycin-treated mice. **(c).** Total distance traveled (cm) and time spent in the center area of the open field arena (sec) (one-way ANOVA, $^*p < 0.05$). **(d).** Number of USV calls (Kruskal-Wallis with Dunn's post-hoc test, $^*p < 0.05$). **(c–d).** Ctrl: n = 16 (WT), n = 17 (*15q dup*); Amp: n = 10 (WT), n = 13 (*15q dup*); Neo: n = 11 (WT), n = 17 (*15q dup*). Graphs show mean ± s.e.m. Ctrl, control; Amp, ampicillin; Neo, neomycin.

neomycin treatment affected anxiety behavior in *15q dup* and WT mice.

The USV call number of ampicillin-treated *15q dup* mice did not change. In contrast, neomycin treatment increased the USV call number in *15q dup* mice when compared to *15q dup* control mice, although the number did not reach that that observed in WT control mice (Fig. 2d, Kruskal-Wallis test $\chi^2(2) = 33.799$; Dunn's post-hoc test $p = 0.0124$). This result suggests that neomycin improved social behavior in *15q dup* mice, which may be mediated either by the elimination of harmful bacteria or enabling the growth of beneficial bacteria. To determine which gut microbiota was responsible for the behavioral improvement, we profiled the gut microbiota after antibiotic treatment.

3.3. *Gut microbiota profiles of 8-week-old* 15q dup *mice and WT littermates after 4 weeks of antibiotic treatment*

Antibiotic treatment is typically followed by the loss of bacterial species and strain diversity (Willing et al., 2011; Becattini et al., 2016). Nevertheless, post-antibiotic treatment profiling of gut microbiota revealed that WT and *15q dup* mice responded differently to the antibiotics. Ampicillin treatment decreased the number of OTUs in WT but not *15q dup* mice. In contrast, although neomycin treatment had no effect on the number of OTUs in WT mice, it significantly increased that of *15q dup* mice (Fig. 3a). Given that bacteria affected by antibiotic treatment are not limited to those that are targeted by the antibiotic, the increase in the number of OTUs observed in neomycin-treated mice may have caused by a network of co-dependence among the members of the microbiota community (Willing et al., 2011; Vangay et al., 2015). Examination of species richness according to the Chao1 and ACE indices also showed different patterns between WT and *15q dup* mice treated with neomycin (Fig. 3b). Regarding species evenness, a reduction in Shannon's index was observed in ampicillin-treated WT mice but not in ampicillin-treated *15q dup* mice (Fig. 3b). Neomycin had no effect on species evenness in either strain (Fig. 3b). Taken together, these results confirmed the broad antimicrobial spectrum of ampicillin in WT mice and revealed a rescuing effect of neomycin on the species richness of the microbiota in *15q dup* mice only.

Comparison of the microbial structure using unweighted and weighted UniFrac distance analysis between *15q dup* and WT mice showed no differences following ampicillin treatment (PERMANOVA; $p = 0.1239$ (unweighted), $p = 0.7193$ (weighted)) but a significant difference following neomycin treatment (PERMANOVA; $p = 0.00099$ (unweighted), $p = 0.02198$ (weighted)). This is shown in the UniFrac-PCoA plot by the lack of segregation in samples from ampicillin-treated mice (Fig. 3c) compared with the tendency to cluster according to genotype in samples from neomycin-treated mice (Fig. 3d). This suggests that ampicillin treatment of *15q dup* and WT mice yields the elimination of similar bacterial strains. Thus, there is close taxonomical relatedness between bacterial communities in ampicillin-treated *15q dup* and WT mice. In contrast, the microbiota in *15q dup* and WT mice diverged after neomycin treatment, again indicating that the bacterial communities in *15q dup* mice respond differently to neomycin.

Antibiotic treatment changed the microbiota composition at either the phylum or genus level in both *15q dup* and WT mice (Fig. 3e–f, Supplementary Tables S3 and S4). Although the relative abundance of the two dominant phyla, Firmicutes and Bacteroidetes, was changed after ampicillin treatment, there were no statistical differences in either WT or *15q dup* mice. Ampicillin increased the relative abundance of Firmicutes (WT: 62.49 %, control vs 89.66 %, Amp; *15q dup*: 54.64 %, control vs 82.90 %, Amp) and reduced the relative abundance of Bacteroidetes (WT: 33.61 %, control vs 4.72 %, Amp; *15q dup*: 33.30 %, control vs 10.36 %, Amp). This result is consistent with those of previous reports (Grazul et al., 2016; Leclercq et al., 2017). After neomycin treatment, the relative abundance of Firmicutes and Bacteroidetes decreased (WT: 62.49

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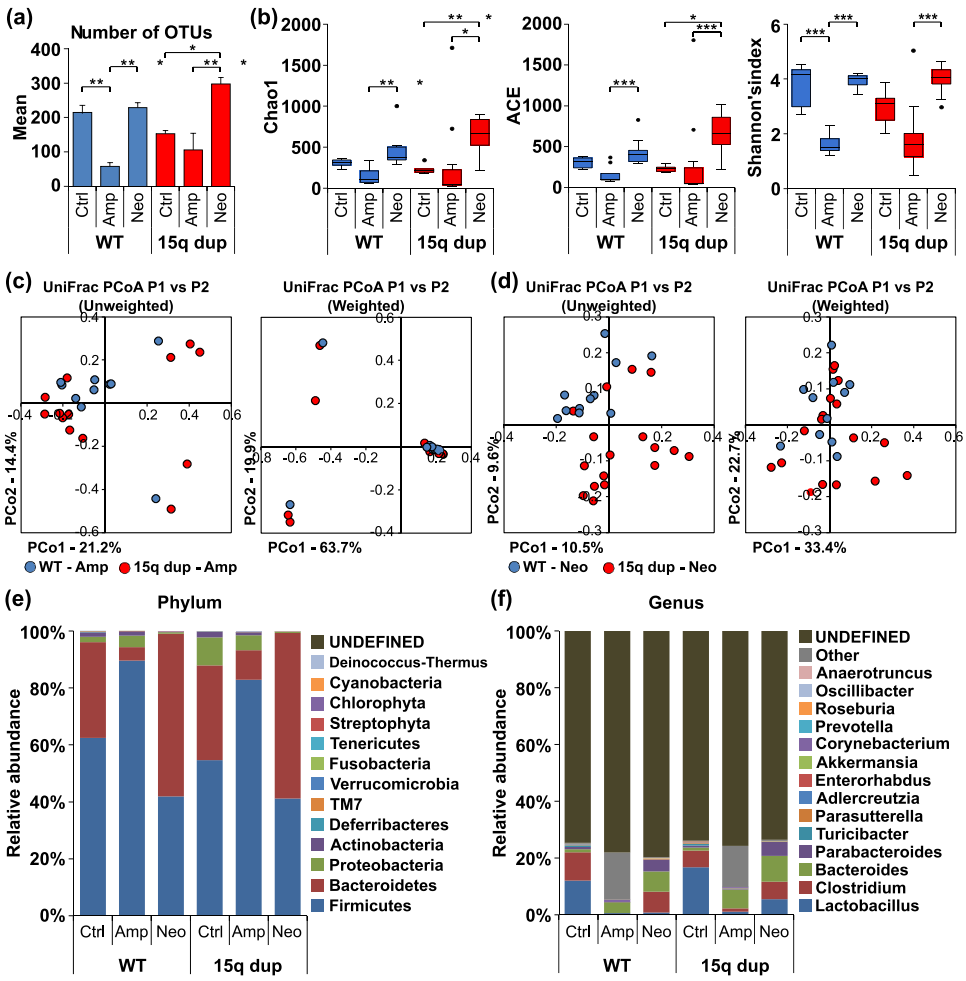

Fig. 3. Profiling of gut microbiota in *15q dup* and WT mice after antibiotic treatment. **(a).** Number of OTUs (3000 reads per sample) in fecal samples from *15q dup* and WT mice. Graphs show mean ± s.e.m. (Kruskal-Wallis test with Dunn's post-hoc test, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). **(b).** Alpha diversity index for species richness and evenness (Kruskal-Wallis test with Dunn's post-hoc test, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). **(c).** Unweighted (left) and weighted (right) UniFrac-based 2D PCoA plots constructed for all OTUs in ampicillin-treated mice (PERMANOVA, $p = 0.1239$ (unweighted), $p = 0.7193$ (weighted)). **(d).** Unweighted (left) and weighted (right) UniFrac-based 2D PCoA plots constructed for all OTUs in neomycin-treated mice (PERMANOVA, $p = 0.00099$ (unweighted), $p = 0.02198$ (weighted)). **(e).** Relative abundance of all identified OTUs classified at the phylum level. **(f).** Relative abundance of all identified OTUs classified at the genus level. **(a–f).** Ctrl: n = 7 (WT), n = 7 (*15q dup*); Amp: n = 10 (WT), n = 13 (*15q dup*); Neo: n = 11 (WT), n = 17 (*15q dup*). Ctrl, control; Amp, ampicillin; Neo, neomycin.

%, control vs 41.97 %, Neo; *15q dup*: 54.64 %, control vs 41.21 %, Neo) and increased (WT: 33.61 %, control vs 57.12 %, Neo; *15q dup*: 33.30 %, control vs 58.15 %, Neo), respectively. At the genus level, ampicillin significantly reduced the relative abundance of *Lactobacillus, Clostridium*, *Turicibacter*, *Parasuterella*, *Adlercreutzia* and *Enterorhabdus* but increased that of *Corynebacterium* in both WT and *15q dup* mice. In *15q dup* mice, neomycin significantly increased the relative abundance of *Bacteroides* (1.09 %, control vs 9.13 %, Neo) and *Parabacteroides* (0.62 %, control vs 4.88 %, Neo), but decreased that of *Parasutterella* (0.41 %, control vs 0.167 %, Neo). In WT mice, neomycin treatment increased the relative abundance of *Roseburia* and *Oscillibacter* and reduced that of *Lactobacillus*. These results further support the notion that the gut microbiota of *15q dup* mice responds differently to neomycin treatment.

3.4. *Candidate OTUs relevant to USV found in* 15q dup *mice after antibiotic treatment*

Treatment with different antibiotics can lead to unique microbiota compositions based on the elimination of target bacteria and dynamic interactions among the remaining bacteria. To identify candidate OTUs/bacteria involved in the USV improvement in neomycin-treated *15q dup* mice, we performed further analysis at the species level. The strategy used is depicted in Supplementary Fig. S1. We hypothesized that the microbiota in neomycin-treated *15q dup* mice should contain either more OTUs with beneficial effects on USV or less OTUs with deleterious effects that hamper USV calls. For this strategy, we compared the microbiota composition of neomycin-treated *15q dup* mice with that of ampicillin-treated *15q dup* mice. A second criterion was added to further verify the candidate OTUs by comparing their relative abundance between *15q dup* control and WT control mice. We expected that there would be fewer beneficial bacteria and more detrimental bacteria in *15q dup* control mice compared to WT control. Ten OTUs belonging to six species had lower relative abundance in neomycin-treated *15q dup* mice. However, after further verification, none of the six species were detected or significantly changed in *15q dup* control mice (Supplementary Table S5). The candidates OTUs for beneficial bacteria are classified according to their most similar species in Table 2 and Supplementary Table S6. OTUs similar to *Barnesiella viscericola, Clostridium hathewayi, Oscillibacter valericigenes, Prevotella* sp. *Smarlab 121567, Clostridium clostridioforme, Hydrogenoanaerobacterium saccharovorans,* and *Roseburia inulinivorans* were identified. Except for *Barnesiella viscericola*, which was less abundant in *15q dup* control mice than in WT control, none of the other six species was detected in *15q dup* control mice (Table 1).

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Table 2
Candidate species with positive/beneficial effect on USV.

Relative abundance (%)		*p* value	Most similar species
15q dup-Amp	*15q dup*-Neo		
0.333	8.524	$p < 0.001$	*Barnesiella viscericola*
0.000	0.892	$p < 0.001$	*Clostridium hathewayi*
0.008	0.339	$p < 0.001$	*Oscillibacter valericigenes*
0.008	0.331	$p < 0.001$	*Prevotella sp. Smarlab 121567*
0.000	0.092	$p = 0.005$	*Clostridium clostridioforme*
0.000	0.892	$p = 0.010$	*Hydrogenoanaerobacterium saccharovorans*
0.000	0.045	$p = 0.014$	*Roseburia inulinivorans*

OTUs are listed in ascending order based on the significance of the *p* value.

Taken together, these findings suggest that neomycin treatment altered the microbiota in *15q dup* mice, and that the new microbiota composition favors the growth of bacteria with beneficial effects on social communication.

4. Discussion

Although gut dysbiosis has been observed in patients with ASD and ASD model mice, a well-defined ASD-associated microbiota has to date remained obscure. This study showed that the number of OTUs in fecal samples from *15q dup* mice was less than that from WT mice. Similarly, the number of OTUs in cecal samples from BTBR T + tf/J mice, another ASD mouse model, was also less than that from WT mice (Golubeva et al., 2017). However, a similar number of OTUs has been observed in fecal (Hsiao et al., 2013; Coretti et al., 2017) and cecal samples (de Theije et al., 2014) between WT and other ASD mouse models. Studies in children with ASD have also reported differences in species richness and alpha diversity (Williams et al., 2011; De Angelis et al., 2013; Liu et al., 2019). This discrepancy suggests that the etiology of microbiota dysbiosis may vary among autism subtypes. Despite varying observations both within and between human and animal studies, a change in the number of OTUs, which reflects changes in microbiota diversity, is common among patients with ASD. This reinforces the role of gut dysbiosis in the mouse model of 15q duplication.

The gut microbiota is typically dominated by bacteria of the phyla Firmicutes and Bacteroidetes. These two major bacterial phyla are also the dominant phyla found in *15q dup* mice, BTBR mice and VPA-exposed mice (de Theije et al., 2014; Coretti et al., 2017; Golubeva et al., 2017). However, unlike other human or animal studies which found differences in the relative abundance and phylogenetic distance of the microbial community between ASD and control (Finegold et al., 2010; Adams et al., 2011; Williams et al., 2011; De Angelis et al., 2013; Hsiao et al., 2013; de Theije et al., 2014; Coretti et al., 2017; Golubeva et al., 2017), this study found no differences in microbial community at the phylum or genus level. The similar relative abundance and phylogenetic distance in phyla and genera between *15q dup* and WT mice observed in this study may be explained by the fact that *15q dup* and WT mice were co-housed. Supplementary Fig. S2, a modified version of Fig. 1c, that uses symbols of the same color to represent co-housed WT and *15q dup* mice, demonstrates that 4 of 7 pairs showed a similar microbiota composition. In agreement with this study, human studies comparing gut microbiota composition between ASD patients and their healthy siblings revealed no difference in microbiota composition (Gondalia et al., 2012), and that healthy siblings tend to have a gut microbiota composition more similar to patients with ASD than healthy controls (Finegold et al., 2010). Human studies suggest that individuals from the same family and living in the same household have a more similar bacterial community than unrelated individuals (Yatsunenko et al., 2012; Faith et al., 2013; Goodrich et al., 2014b). In mice, microbiota sharing may occur due to co-housing and inoculation at birth (Goodrich et al., 2014a). Overall, those studies, similar to the present study, suggest an effect of cohabitation on microbiota composition. Microbiota studies in mice and humans must therefore be carefully planned and designed due to the numerous confounding factors that can affect the results and interpretations. The use of littermates as control mice in this study was appropriate. However, due to subtle differences in the microbiota of *15q dup* mice and their WT littermates, separating the mice according to their genotype may be a more suitable approach.

It is widely known that antibiotics alter the gut microbiota composition. In this study, ampicillin- and neomycin-treated mice showed altered gut microbiota composition compared to control mice. However, neither of the antibiotics had effects on locomotor activity or anxiety behavior according to distance traveled and time spent in the center area of an open field arena, respectively. A similar result was reported after ampicillin administration during the prenatal to postnatal period (Leclercq et al., 2017). This result suggests that only certain behavioral circuitry is responsive to changes along the gut-brain axis. A more detailed battery of behavioral tests should be performed to investigate the effect of different antibiotics on autism phenotypes.

Our findings showed that treatment with neomycin, but not ampicillin, in *15q dup* mice may enhance USV. Thus, we classified the increased OTUs in neomycin-treated *15q dup* mice as beneficial OTUs. Interestingly, most of these beneficial OTUs belong to Clostridium clusters XIVa and IV. *Clostridium hathewayi*, *Clostridium clostridioforme*, and *Roseburia inulinivorans* belong to Clostridium cluster XIVa; and *Oscillibacter valericigenes* and *Hydrogenoanaerobacterium saccharovorans* belong to Clostridium cluster IV (Atarashi et al., 2013; Furusawa et al., 2013). Clostridium clusters XIVa and IV are known for their high potency for enhancing the abundance of Treg cells in the colon and inducing the production of anti-inflammatory molecules such as interleukin-10 (IL-10). Inoculation with 20 Clostridia species, including *C. hathewayi, O. valericigenes*, *C. clostridioforme*, and *H. saccharovorans*, restores Treg induction in germ-free mice (Atarashi et al., 2013). Furthermore, *Roseburia hominis*, a species closely related to *R. inulinivorans* and implicated in Treg induction (Patterson et al., 2017), was significantly increased in neomycin-treated *15q dup* mice (Supplementary Table S6).

Clostridium clusters XIVa and IV also abundantly produce short chain fatty acids (SCFAs), including butyrate, propionate, and acetate (Furusawa et al., 2013). The remaining two beneficial candidate bacteria, *Prevotella* sp. and *Barnesiella viscericola*, also have SCFA-producing capability and are often associated with healthy microbiota (Shkoporov et al., 2008; Cosorich et al., 2017). Reports have speculated that SCFAs may mediate gut-brain signaling as they can cross the blood brain barrier, exert neuroprotective effects in neurodegenerative disorders, improve spatial learning and memory, have antidepressant properties (Dalile et al., 2019), and attenuate social behavioral deficits in a mouse model of autism (Kratsman et al., 2016). SCFAs may regulate brain function through

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many pathways, including inhibition of histone deacetylase (Davie, 2003) and modulation of peripheral levels of 5-HT (Reigstad et al., 2015; Yano et al., 2015; Dalile et al., 2019). SCFAs may also affect brain 5-HT levels because treatment of a mouse model of chronic unpredictable mild stress with sodium butyrate increased brain 5-HT concentrations (Sun et al., 2016). 5-HT signaling has been associated with USV: administration of 5-HT1a receptor agonists attenuated isolation-induced USV in mouse pups, and lack of brain 5-HT impaired USV (Olivier et al., 1998; Mosienko et al., 2015). Given that the beneficial OTUs identified in this study are SCFA-producing species with the above-described properties, it is possible that these bacteria are capable of modulating 5-HT levels, thereby affecting USV.

Although further studies using human samples or germ-free mice conventionalized with the candidate of beneficial OTUs are required to confirm our results, the findings presented in this study suggest that beneficial OTUs, which regulate Treg development and butyrate production, may enhance USV in the *15q dup* mouse model of ASD.

Author contributions

C.-W.L., K.H. and T.T. conceived the research. D.E.S., R.O., W.S., and H.M. performed the experiments. D.E.S, C.-W.L., N.N., W.S., M.H., and K.T. analyzed the data. D.E.S., C.-W. L., and T.T. wrote the manuscript. T.T. supervised the study.

Declaration of Competing Interest

The authors declare no competing interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.neures.2019.12.010.

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Supplementary Information

Altered microbiota composition reflects enhanced communication in 15q11-13 CNV mice

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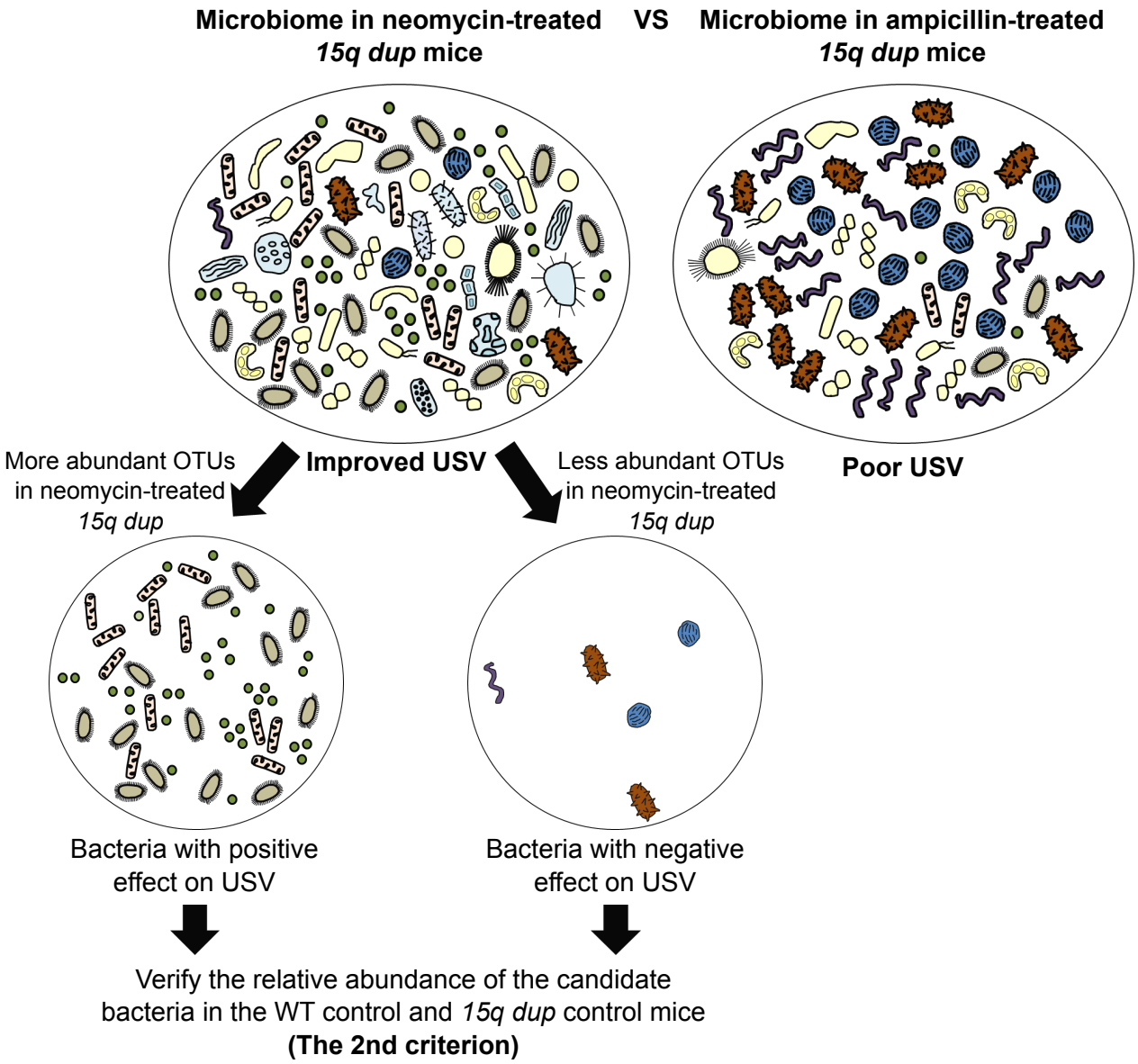
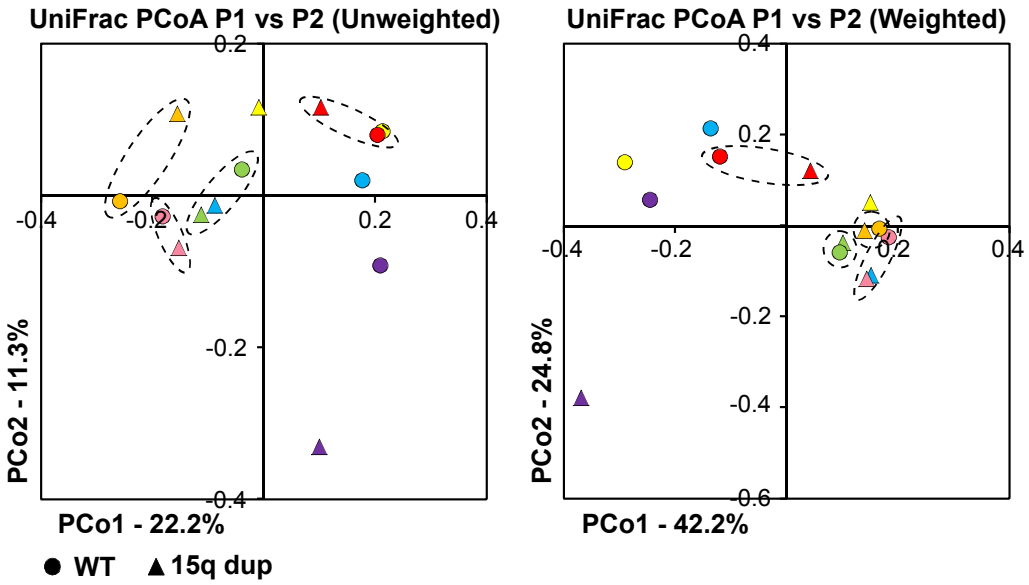


Supplementary Figure S1.
Schematic strategy used to identify the candidate OTUs with positive or negative effect on USV.
OTUs in neomycin-treated *15q dup* mice were compared to OTUs in ampicillin-treated *15q dup* mice. OTUs with higher relative abundance in neomycin-treated *15q dup* mice were proposed as OTUs with positive effect on USV while OTUs with lower relative abundance in neomycin-treated *15q dup* mice were those that have negative effect on USV. The validity of these candidate OTUs were further verified by comparing their relative abundance in WT control and *15q dup* control.



Supplementary Figure S2.
Unweighted and weighted UniFrac-PCoA of WT and *15q dup* mice. Symbols of the same color indicate co-housed pairs of WT and *15q dup* mice.

Supplementary Table S1.
Comparison of the relative abundance of phyla between WT and *15q dup* mice using Student's *t* test.

Phylum	Relative abundance (%)		*p* value
	WT	*15q dup*	
Firmicutes	62.4905	54.6429	0.30360824
Bacteroidetes	33.6095	33.3048	0.95735736
Proteobacteria	1.87619	9.85238	0.41939845
Actinobacteria	1.55714	1.90952	0.54834727
Deferribacteres	0.25714	0.04762	0.08169740
TM7	0.19524	0.15714	0.62990352
Verrucomicrobia	0.01429	0.08571	0.18173816
Fusobacteria	0	0	> 0.999
Tenericutes	0	0	> 0.999
Streptophyta	n.a	n.a	-
Chlorophyta	n.a	n.a	-
Cyanobacteria	n.a	n.a	-
Deinococcus-Thermus	n.a	n.a	-

n.a, not analyzed.

Supplementary Table S2.
Comparison of the relative abundance of genera between WT and *15q dup* mice using Student's *t* test.

Genus	Relative abundance (%)		*p* value
	WT	*15q dup*	
Lactobacillus	12.10476190	16.8238095	0.43365049
Clostridium	9.985714286	5.89047619	0.31235806
Bacteroides	1.123809524	1.09047619	0.94255365
Parabacteroides	0.719047619	0.62380952	0.68979634
Turicibacter	0.638095238	0.68571429	0.89746451
Parasutterella	0.319047619	0.41428571	0.53436024
Mucispirillum	0.257142857	0.04761905	0.08169740
Adlercreutzia	0.138095238	0.14761905	0.86318926
Enterorhabdus	0.066666667	0.21904762	0.30282349
Staphylococcus	0.019047619	0.00476190	0.37305503
Akkermansia	0.014285714	0.08095238	0.17372206
Candidatus Arthromitus	0.014285714	0.07142857	0.46047955

Supplementary Table S3.
Comparison of the relative abundance of phyla among WT-Ctrl/WT-Amp/WT-Neo and *15q dup*-Ctrl/*15q dup*-Amp/*15q dup*-Neo using one-way ANOVA.

Phylum	WT: Ctrl v Amp	WT: Ctrl v Neo	WT: Amp v Neo
Firmicutes	62.49±4.07; 89.66±26.09 $^{n.s}$	62.49±4.07; 41.97±9.99 $^{n.s}$	89.66±26.09; 41.97±9.99 ***
Bacteroidetes	33.61±5.47; 4.72±14.49 $^{n.s}$	33.61±5.47; 57.12±9.90 *	4.72±14.49; 57.12±9.90 ***
Proteobacteria	1.88±2.23; 4.01±11.88 **	1.88±2.23; 0.52±0.51 $^{n.s}$	4.01±11.88; 0.52±0.51 $^{n.s}$
Actinobacteria	1.56±1.10; 1.57±3.28 $^{n.s}$	1.56±1.10; 0.04±0.05 ***	1.57±3.28; 0.04±0.05 *
Deferribacteres	0.257±0.261; 0 ***	0.25±0.261; 0 ***	0; 0 $^{n.s}$
TM7	0.195±0.138; 0.010±0.023 ***	0.195±0.138; 0.064±0.131 *	0.010±0.023; 0.064±0.131 $^{n.s}$
Verrucomicrobia	0.014±0.018; 0 $^{n.s}$	0.014±0.018; 0.103±0.127 $^{n.s}$	0; 0.103±0.127 *
Fusobacteria	0; 0.007±0.021 $^{n.s}$	0; 0.003±0.010 $^{n.s}$	0.007±0.021; 0.003±0.010 $^{n.s}$
Tenericutes	0; 0 $^{n.s}$	0; 0 $^{n.s}$	0; 0 $^{n.s}$
Streptophyta	0; 0.020±0.0281 $^{n.s}$	0; 0 $^{n.s}$	0.020±0.0281; 0 *
Chlorophyta	0; 0 $^{n.s}$	0; 0.003±0.010 $^{n.s}$	0; 0.003±0.010 $^{n.s}$
Cyanobacteria	0; 0 $^{n.s}$	0; 0 $^{n.s}$	0; 0 $^{n.s}$
Deinococcus-Thermus	0; 0 $^{n.s}$	0; 0 $^{n.s}$	0; 0 $^{n.s}$

Phylum	*15q dup*: Ctrl v Amp	*15q dup*: Ctrl v Neo	*15q dup*: Amp v Neo
Firmicutes	54.64±18.16; 82.90±33.44 $^{n.s}$	54.64±18.16; 41.21±14.27 $^{n.s}$	82.90±33.44; 41.21±14.27 ***
Bacteroidetes	33.30±13.55; 10.36±21.80 $^{n.s}$	33.30±13.55; 58.15±14.23 $^{n.s}$	10.36±21.80; 58.15±14.23 ***
Proteobacteria	9.852±24.28; 5.277±12.18 $^{n.s}$	9.852±24.28; 0.388±0.354 $^{n.s}$	5.277±12.18; 0.388±0.354 $^{n.s}$
Actinobacteria	1.91±1.03; 0.89±1.39 $^{n.s}$	1.91±1.03; 0.04±0.10 ***	0.89±1.39; 0.04±0.10 **
Deferribacteres	0.048±0.086; 0.010±0.037 $^{n.s}$	0.048±0.086; 0 **	0.010±0.037; 0 $^{n.s}$
TM7	0.157±0.150; 0.008±0.020 ***	0.157±0.150; 0.028±0.038 *	0.008±0.020; 0.028±0.038 $^{n.s}$
Verrucomicrobia	0.086±0.125; 0.026±0.083 $^{n.s}$	0.086±0.125; 0.057±0.118 $^{n.s}$	0.026±0.083; 0.057±0.118 $^{n.s}$
Fusobacteria	0; 0 $^{n.s}$	0; 0.004±0.011 $^{n.s}$	0; 0.004±0.011 $^{n.s}$
Tenericutes	0; 0.39±0.94 $^{n.s}$	0; 0 $^{n.s}$	0.39±0.94; 0 $^{n.s}$
Streptophyta	0; 0.021±0.040 $^{n.s}$	0; 0 $^{n.s}$	0.021±0.040; 0 $^{n.s}$
Chlorophyta	0; 0 $^{n.s}$	0; 0 $^{n.s}$	0; 0 $^{n.s}$
Cyanobacteria	0; 0.003±0.009 $^{n.s}$	0; 0 $^{n.s}$	0.003±0.009; 0 $^{n.s}$
Deinococcus-Thermus	0; 0.003±0.009 $^{n.s}$	0; 0 $^{n.s}$	0.003±0.009; 0 $^{n.s}$

Relative abundance is shown as mean ± SD.
*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$. n.s, not significant. Ctrl, control. Amp, ampicillin. Neo, neomycin.

Supplementary Table S4.
Comparison of the relative abundance of genera among WT-Ctrl/WT-Amp/WT-Neo and *15q dup*-Ctrl/*15q dup*-Amp/*15q dup*-Neo using one-way ANOVA.

Genus	WT: Ctrl v Amp	WT: Ctrl v Neo	WT: Amp v Neo
Lactobacillus	12.10±10.57; 0.67±1.49 **	12.10±10.57; 0.88±1.26 **	0.67±1.49; 0.88±1.26 n.s
Clostridium	9.99±7.80; 0.03±0.06 ***	9.99±7.80; 7.35±4.83 n.s	0.03±0.06; 7.35±4.83 ***
Bacteroides	1.12±0.46; 3.81±11.86 n.s	1.12±0.46; 7.16±2.58 n.s	3.81±11.86; 7.16±2.58 ***
Parabacteroides	0.72±0.41; 0.003±0.011 n.s	0.72±0.41; 4.19±1.96 n.s	0.003±0.011; 4.19±1.96 ***
Turicibacter	0.638±0.652; 0.003±0.011 ***	0.638±0.652; 0 ***	0.003±0.011; 0 n.s
Parasutterella	0.32±0.23; 0.067±0.211 **	0.32±0.23; 0.246±0.415 n.s	0.067±0.211; 0.246±0.415 n.s
Adlercreutzia	0.138±0.106; 0 ***	0.138±0.106; 0 ***	0; 0 n.s
Enterorhabdus	0.067±0.082; 0 ***	0.067±0.082; 0 ***	0; 0 n.s
Akkermansia	0.014±0.018; 0 n.s	0.014±0.018; 0.103±0.127 n.s	0; 0.103±0.127 *
Corynebacterium	0; 0.91±2.41 ***	0; 0 n.s	0.91±2.41; 0 ***
Prevotella	0; 0.02±0.042 n.s	0; 0 n.s	0.02±0.042; 0 n.s
Roseburia	0; 0.003±0.011 n.s	0; 0.097±0.169 *	0.003±0.011; 0.097±0.169 *
Oscillibacter	0; 0 n.s	0; 0.142±0.205 **	0; 0.142±0.205 **
Anaerotruncus	0; 0 n.s	0; 0.012±0.027 n.s	0; 0.012±0.027 n.s

Genus	*15q dup*: Ctrl v Amp	*15q dup*: Ctrl v Neo	*15q dup*: Amp v Neo
Lactobacillus	16.82±11.21; 1.19±2.46 ***	16.82±11.21; 5.59±3.91 n.s	1.19±2.46; 5.59±3.91 **
Clostridium	5.89±6.68; 1.13±3.41 *	5.89±6.68; 6.11±5.92 n.s	1.13±3.41; 6.11±5.92 ***
Bacteroides	1.09±1.09; 6.662±16.71 n.s	1.09±1.09; 9.13±3.13 *	6.662±16.71; 9.13±3.13***
Parabacteroides	0.62±0.46; 0.33±0.96 n.s	0.62±0.46; 4.88±2.04 *	0.33±0.96 ; 4.88±2.04 ***
Turicibacter	0.686±0.701; 0.110±0.258 **	0.686±0.701; 0 ***	0.110±0.258; 0 n.s
Parasutterella	0.41±0.32; 0.097±0.217 *	0.41±0.32; 0.167±0.304 *	0.097±0.217; 0.167±0.304 n.s
Adlercreutzia	0.148±0.096; 0.013±0.046 ***	0.148±0.096; 0 ***	0.013±0.046; 0 n.s
Enterorhabdus	0.219±0.352; 0.008±0.028 ***	0.219±0.352; 0 ***	0.008±0.028; 0 n.s
Akkermansia	0.081±0.114; 0.026±0.083 n.s	0.081±0.114; 0.061±0.121 n.s	0.026±0.083; 0.061±0.121 n.s
Corynebacterium	0.005±0.013; 0.282±0.365 *	0.005±0.013; 0 n.s	0.282±0.365; 0 ***
Prevotella	0; 0.005±0.018 n.s	0; 0.006±0.013 n.s	0.005±0.018; 0.006±0.013 n.s
Roseburia	0.005±0.013; 0 n.s	0.005±0.013; 0.059±0.128 n.s	0; 0.059±0.128 **
Oscillibacter	0; 0.005±0.018 n.s	0; 0.110±0.242 n.s	0.005±0.018; 0.110±0.242 n.s
Anaerotruncus	0; 0 n.s	0; 0.014±0.029 n.s	0; 0.014±0.029 n.s

Relative abundance is shown as mean ± SD.

*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$. n.s, not significant. Ctrl, control. Amp, ampicillin. Neo, neomycin.

Supplementary Table S5.
OTUs with lower relative abundance in neomycin treated-*15q dup* mice and with lower or no abundance in *15q dup* control mice.

	Relative abundance (%)		*p* value	Most similar species	Homology (%)	Relative abundance in *15q dup*-Ctrl
	15q dup-Amp	*15q dup*-Neo				
total_OTU00749	1.62308	0.00196	**0.026998**	*Acetivibrio cellulolyticus*	80.72	not significant
total_OTU01318	1.43077	0.02745	**0.020795**	*Acetivibrio cellulolyticus*	81.05	
total_OTU00010	4.36410	0.00196	**0.005941**	*Clostridium sticklandii*	79.41	not found
total_OTU00066	0.25385	0.00000	**0.021777**	*Corynebacterium mastitidis*	98.70	not significant
total_OTU01150	6.70256	0.00000	**0.011873**	*Eubacterium desmolans*	83.18	not significant
total_OTU01575	2.86410	0.00000	**0.012812**	*Lachnobacterium sp. wal 14165*	77.65	not significant
total_OTU00001	27.00769	0.01373	**0.001596**	*Sporobacter termitidis*	81.11	not found
total_OTU00313	1.85897	0.01569	**0.006823**	*Sporobacter termitidis*	80.78	
total_OTU01406	0.08974	0.00000	**0.027157**	*Sporobacter termitidis*	82.74	
total_OTU01492	0.25385	0.00000	**0.043399**	*Sporobacter termitidis*	83.39	

Ctrl, control. Amp, ampicillin. Neo, neomycin.

Supplementary Table S6.
OTUs that were significantly increased in neomycin-treated *15q dup* mice that were (top table) or were not (bottom table) significantly different between WT control and *15q dup* control mice.

	Relative abundance (%)		*p* value	Most similar species	Homology (%)
	15q dup-Amp	*15q dup*-Neo	Mann-Whitney test		
total_OTU00028	0.048	0.582	**6.82141E-05**	*Barnesiella viscericola*	81.39
total_OTU00190	0.106	1.569	**5.57010E-06**	*Barnesiella viscericola*	80.06
total_OTU00200	0.112	3.665	**0.000107285**	*Barnesiella viscericola*	81.19
total_OTU00237	0.030	0.724	**0.000216634**	*Barnesiella viscericola*	78.68
total_OTU00262	0.000	0.625	**0.010465363**	*Barnesiella viscericola*	81.25
total_OTU00474	0.000	0.051	**0.006889921**	*Barnesiella viscericola*	80.50
total_OTU00628	0.000	0.147	**0.000125809**	*Barnesiella viscericola*	79.01
total_OTU00677	0.003	0.031	**0.003695344**	*Barnesiella viscericola*	81.00
total_OTU00767	0.000	0.043	**0.003065704**	*Barnesiella viscericola*	81.23
total_OTU00786	0.000	0.024	**0.001671149**	*Barnesiella viscericola*	79.75
total_OTU00918	0.003	0.031	**0.005429553**	*Barnesiella viscericola*	79.00
total_OTU00931	0.000	0.008	**0.041310345**	*Barnesiella viscericola*	80.06
total_OTU00972	0.000	0.061	**0.028675263**	*Barnesiella viscericola*	78.19
total_OTU01118	0.000	0.057	**0.001223090**	*Barnesiella viscericola*	80.37
total_OTU01183	0.058	0.153	**0.033306982**	*Barnesiella viscericola*	82.35
total_OTU01188	0.003	0.071	**0.000168626**	*Barnesiella viscericola*	83.28
total_OTU01234	0.000	0.012	**0.028919289**	*Barnesiella viscericola*	82.97
total_OTU01246	0.006	0.049	**0.005348085**	*Barnesiella viscericola*	78.19
total_OTU01275	0.000	0.008	**0.041310345**	*Barnesiella viscericola*	80.88
total_OTU01378	0.000	0.010	**0.020060862**	*Barnesiella viscericola*	79.18
total_OTU01390	0.000	0.008	**0.041310345**	*Barnesiella viscericola*	79.81
total_OTU01447	0.000	0.016	**0.006763909**	*Barnesiella viscericola*	80.80
total_OTU01457	0.000	0.010	**0.020060862**	*Barnesiella viscericola*	79.94
total_OTU01542	0.000	0.008	**0.041310345**	*Barnesiella viscericola*	79.94
total_OTU01609	0.006	0.141	**6.52081E-05**	*Barnesiella viscericola*	81.73
total_OTU01712	0.000	0.076	**0.003220063**	*Barnesiella viscericola*	81.82
total_OTU01736	0.000	0.014	**0.014362618**	*Barnesiella viscericola*	79.44
total_OTU01898	0.000	0.027	**0.006063194**	*Barnesiella viscericola*	79.44
total_OTU02126	0.003	0.204	**5.30765E-07**	*Barnesiella viscericola*	81.70
total_OTU02136	0.000	0.008	**0.041310345**	*Barnesiella viscericola*	81.00
total_OTU02659	0.000	0.010	**0.020060862**	*Barnesiella viscericola*	80.76
total_OTU03038	0.000	0.024	**0.003445984**	*Barnesiella viscericola*	81.07
total_OTU03135	0.015	0.059	**0.041551429**	*Barnesiella viscericola*	79.03
total_OTU00100	0.000	0.073	**0.004539739**	*Clostridium clostridioforme*	94.75
total_OTU00917	0.000	0.020	**0.036713795**	*Clostridium clostridioforme*	93.21
total_OTU00038	0.000	0.665	**0.009991176**	*Clostridium hathewayi*	90.74

total_OTU00122	0.000	0.208	**0.007516320**	*Clostridium hathewayi*	90.74
total_OTU00226	0.000	0.020	**0.046029002**	*Clostridium hathewayi*	90.77
total_OTU00316	0.000	0.047	**0.010274241**	*Hydrogenoanaerobacterium saccharovorans*	87.16
total_OTU00196	0.009	0.229	**3.65871E-05**	*Oscillibacter valericigenes*	89.51
total_OTU00205	0.000	0.094	**0.028553115**	*Oscillibacter valericigenes*	90.43
total_OTU00363	0.000	0.016	**0.006763909**	*Oscillibacter valericigenes*	86.77
total_OTU00149	0.000	0.045	**0.014262602**	*Roseburia inulinivorans*	83.33
total_OTU00592	0.000	0.020	**0.007639210**	*Prevotella sp. Smarlab 121567*	81.82
total_OTU00601	0.000	0.018	**0.007758552**	*Prevotella sp. Smarlab 121567*	79.50
total_OTU00737	0.003	0.037	**0.000303453**	*Prevotella sp. Smarlab 121567*	79.75
total_OTU01026	0.006	0.235	**0.000445234**	*Prevotella sp. Smarlab 121567*	79.81
total_OTU01765	0.000	0.008	**0.041310345**	*Prevotella sp. Smarlab 121567*	79.75
total_OTU03283	0.000	0.014	**0.048574889**	*Prevotella sp. Smarlab 121567*	77.92

	Relative abundance (%)		*p* value	Most similar species	Homology (%)
	15q dup-Amp	*15q dup*-Neo	Mann-Whitney test		
total_OTU00057	0.000	0.192	**0.011834401**	*[Eubacterium] tortuosum*	85.36
total_OTU00536	0.012	0.114	**0.007791728**	*[Ruminococcus] gnavus*	85.58
total_OTU02547	0.000	0.008	**0.041310345**	*[Ruminococcus] gnavus*	86.81
total_OTU00799	0.012	0.000	**0.039519052**	*Acetivibrio cellulolyticus*	79.74
total_OTU00091	0.000	0.157	**0.007049565**	*Alistipes finegoldii*	94.87
total_OTU00580	0.012	0.202	**0.003076048**	*Alistipes finegoldii*	90.79
total_OTU00256	0.000	0.065	**0.000529100**	*Alistipes indistinctus*	87.62
total_OTU02276	0.000	0.008	**0.041310345**	*Alistipes onderdonkii*	81.56
total_OTU00246	0.009	0.114	**7.65733E-06**	*Alistipes putredinis*	85.35
total_OTU00086	0.003	0.410	**0.006512736**	*Alistipes senegalensis*	91.08
total_OTU00946	0.000	0.094	**0.002929789**	*Alistipes senegalensis*	91.43
total_OTU01458	0.000	0.029	**0.003275458**	*Alistipes senegalensis*	82.45
total_OTU00431	0.000	0.239	**1.52611E-06**	*Alistipes shahii*	91.08
total_OTU02064	0.000	0.067	**0.001462623**	*Alistipes shahii*	86.98
total_OTU00217	0.000	0.033	**0.002863881**	*Alistipes sp. AP11*	93.97
total_OTU00005	0.003	0.324	**0.000293007**	*Alistipes sp. JC136*	90.88
total_OTU01191	0.000	0.008	**0.041310345**	*Alistipes sp. JC136*	88.54
total_OTU00006	0.021	8.051	**0.035356554**	*Allobaculum stercoricanis*	89.80
total_OTU00888	0.003	0.076	**0.035810647**	*Allobaculum stercoricanis*	86.84
total_OTU00017	0.003	0.031	**0.000641181**	*Anaerofustis stercorihominis*	91.64
total_OTU00024	0.009	0.324	**0.000451896**	*Bacteroides acidifaciens*	100.00
total_OTU00051	0.948	2.953	**0.015316471**	*Bacteroides acidifaciens*	86.08
total_OTU00058	0.039	1.492	**0.000139119**	*Bacteroides acidifaciens*	100.00
total_OTU00279	0.279	1.784	**6.51286E-05**	*Bacteroides acidifaciens*	96.84
total_OTU00370	0.000	0.008	**0.041310345**	*Bacteroides acidifaciens*	96.20

total_OTU00408	0.000	0.014	**0.004096206**	*Bacteroides acidifaciens*	85.13
total_OTU00486	0.000	0.012	**0.028919289**	*Bacteroides acidifaciens*	87.34
total_OTU00487	0.009	0.084	**5.61451E-05**	*Bacteroides acidifaciens*	95.89
total_OTU00533	0.000	0.016	**0.006763909**	*Bacteroides acidifaciens*	94.94
total_OTU00600	0.003	0.039	**0.000221461**	*Bacteroides acidifaciens*	90.25
total_OTU00652	0.000	0.022	**0.011246498**	*Bacteroides acidifaciens*	93.67
total_OTU00843	0.000	0.022	**0.003701534**	*Bacteroides acidifaciens*	94.62
total_OTU00925	0.000	0.012	**0.028919289**	*Bacteroides acidifaciens*	93.67
total_OTU01089	0.000	0.012	**0.028919289**	*Bacteroides acidifaciens*	93.10
total_OTU01158	0.000	0.008	**0.041310345**	*Bacteroides acidifaciens*	94.32
total_OTU01238	0.000	0.024	**0.003445984**	*Bacteroides acidifaciens*	95.89
total_OTU01680	0.000	0.084	**0.000553751**	*Bacteroides acidifaciens*	95.57
total_OTU02113	0.000	0.055	**0.000257888**	*Bacteroides acidifaciens*	96.84
total_OTU02219	0.000	0.008	**0.041310345**	*Bacteroides acidifaciens*	95.22
total_OTU02378	0.003	0.043	**0.002101598**	*Bacteroides acidifaciens*	94.30
total_OTU01294	0.000	0.008	**0.041310345**	*Bacteroides nordii*	84.62
total_OTU01220	0.000	0.069	**0.017689186**	*Bacteroides salanitronis*	81.07
total_OTU02045	0.000	1.010	**4.09064E-05**	*Bacteroides salanitronis*	80.56
total_OTU00463	0.000	0.027	**0.001427023**	*Bacteroides sartorii*	95.25
total_OTU00449	0.000	0.012	**0.028919289**	*Bacteroides sp. D8*	87.70
total_OTU00040	0.012	0.655	**8.18934E-07**	*Bacteroides sp. Smarlab 3302398*	91.35
total_OTU00334	0.009	0.149	**0.000234668**	*Bacteroides sp. TP-5*	97.13
total_OTU00708	0.000	0.008	**0.041310345**	*Bacteroides sp. TP-5*	95.58
total_OTU01297	0.000	0.008	**0.041310345**	*Bacteroides sp. TP-5*	93.99
total_OTU02823	0.000	0.014	**0.014362618**	*Bacteroides sp. TP-5*	96.50
total_OTU00555	0.000	0.024	**0.013366294**	*Bacteroides uniformis*	96.82
total_OTU00771	0.000	0.188	**0.005463732**	*Bacteroides uniformis*	97.15
total_OTU00008	0.024	0.951	**0.009466071**	*Bacteroides vulgatus*	92.74
total_OTU00529	0.000	0.012	**0.028919289**	*Bacteroides vulgatus*	89.03
total_OTU01121	0.000	0.027	**0.014362618**	*Bacteroides vulgatus*	89.59
total_OTU00135	0.003	0.357	**0.001021220**	*Barnesiella intestinihominis*	82.66
total_OTU00761	0.000	0.018	**0.023858231**	*Barnesiella intestinihominis*	81.93
total_OTU00820	0.000	0.496	**2.53744E-05**	*Barnesiella intestinihominis*	83.59
total_OTU00831	0.000	0.020	**0.007639210**	*Barnesiella intestinihominis*	84.52
total_OTU00879	0.000	0.059	**0.006121408**	*Barnesiella intestinihominis*	83.12
total_OTU00891	0.000	0.018	**0.045675773**	*Barnesiella intestinihominis*	81.50
total_OTU00941	0.000	0.088	**0.004781026**	*Barnesiella intestinihominis*	83.59
total_OTU01016	0.006	0.045	**0.000734720**	*Barnesiella intestinihominis*	78.82
total_OTU01233	0.000	0.014	**0.014362618**	*Barnesiella intestinihominis*	83.39
total_OTU01587	0.003	0.035	**0.015614357**	*Barnesiella intestinihominis*	82.04
total_OTU01589	0.000	0.029	**0.038740158**	*Barnesiella intestinihominis*	78.19
total_OTU01622	0.000	0.016	**0.001671149**	*Barnesiella intestinihominis*	80.50
total_OTU02321	0.000	0.047	**0.000323863**	*Barnesiella intestinihominis*	79.38
total_OTU02391	0.000	0.018	**0.023858231**	*Barnesiella intestinihominis*	80.50

total_OTU02637	0.000	0.012	**0.028919289**	*Barnesiella intestinihominis*	81.62
total_OTU02989	0.000	0.043	**0.000184828**	*Barnesiella intestinihominis*	81.56
total_OTU03001	0.000	0.224	**0.000197500**	*Barnesiella intestinihominis*	83.59
total_OTU00114	0.006	0.086	**0.009736978**	*Blautia producta*	92.94
total_OTU00444	0.003	0.143	**0.009224670**	*Blautia producta*	92.64
total_OTU01115	0.000	0.008	**0.041310345**	*Butyricimonas sp. 180-3*	79.38
total_OTU00016	0.000	2.063	**0.003906906**	*Butyrivibrio fibrisolvens*	86.53
total_OTU00315	0.000	0.069	**0.021289420**	*Butyrivibrio fibrisolvens*	84.36
total_OTU01126	0.000	0.039	**0.018234478**	*Butyrivibrio hungatei*	85.59
total_OTU00115	0.003	0.027	**0.012911670**	*Clostridium bolteae*	92.90
total_OTU00603	0.000	0.216	**0.000454225**	*Clostridium indolis*	86.81
total_OTU00140	0.030	0.782	**0.004289114**	*Clostridium leptum*	86.01
total_OTU00945	0.000	0.084	**0.003295849**	*Clostridium polysaccharolyticum*	85.19
total_OTU02492	0.000	0.029	**0.008746890**	*Clostridium polysaccharolyticum*	83.33
total_OTU00278	0.000	0.067	**0.000796998**	*Clostridium saccharolyticum*	92.90
total_OTU00319	0.003	0.043	**0.010722503**	*Clostridium saccharolyticum*	92.28
total_OTU00166	0.015	0.090	**0.041464742**	*Clostridium sp.*	91.41
total_OTU00492	0.000	0.029	**0.047091962**	*Clostridium sp. 6-44*	84.76
total_OTU00259	0.000	0.075	**0.009206315**	*Clostridium sp. 826*	84.44
total_OTU00267	0.000	0.008	**0.041310345**	*Clostridium sp. Clone-3*	92.90
total_OTU00268	0.000	0.018	**0.034276469**	*Clostridium sp. Clone-3*	85.49
total_OTU00104	0.027	0.424	**0.034516941**	*Clostridium sp. Clone-16*	89.57
total_OTU00212	0.000	0.175	**0.004303813**	*Clostridium sp. Clone-17*	99.38
total_OTU00298	0.000	0.078	**0.004251387**	*Clostridium sp. Clone-17*	96.59
total_OTU00479	0.012	0.716	**0.048316614**	*Clostridium sp. Clone-26*	95.06
total_OTU00467	0.003	0.053	**0.012705190**	*Clostridium sp. cTPY-17*	93.25
total_OTU01463	0.000	0.008	**0.041310345**	*Clostridium sp. cTPY-17*	89.20
total_OTU00221	0.000	0.008	**0.041310345**	*Clostridium sp. Culture Jar-8*	94.26
total_OTU01186	0.006	0.104	**0.034578798**	*Clostridium sp. Culture Jar-56*	99.69
total_OTU00277	0.003	0.090	**0.032400411**	*Clostridium sp. Culture-23*	97.84
total_OTU00679	0.003	0.137	**0.010951147**	*Clostridium sp. Culture-27*	83.88
total_OTU00186	0.003	0.039	**0.010593877**	*Clostridium sp. Culture-46*	99.69
total_OTU00209	0.009	0.139	**0.032556053**	*Clostridium sp. Culture-46*	95.99
total_OTU00018	0.027	0.294	**0.000643888**	*Clostridium sp. Culture-54*	94.55
total_OTU00108	0.012	2.980	**0.043741028**	*Clostridium sp. ID4*	99.35
total_OTU00123	0.000	0.400	**0.011537384**	*Clostridium sp. ID5*	86.53
total_OTU01436	0.000	0.008	**0.041310345**	*Clostridium sp. ID5*	87.20
total_OTU00477	0.000	0.008	**0.041310345**	*Clostridium sp. ID6*	91.72
total_OTU00782	0.000	0.012	**0.028919289**	*Clostridium sp. L2-50*	83.65
total_OTU00036	0.003	0.086	**0.048802648**	*Clostridium sp. strain Z6*	79.50
total_OTU00069	0.003	0.104	**0.042356504**	*Clostridium sp. strain Z6*	80.32
total_OTU00685	0.000	0.076	**0.001034880**	*Clostridium sp. strain Z6*	85.53
total_OTU02460	0.000	0.012	**0.028919289**	*Clostridium symbiosum*	87.73
total_OTU00027	0.000	0.175	**0.008438516**	*Clostridium xylanolyticum*	92.28

total_OTU00087	0.006	0.063	**0.014966901**	*Dorea longicatena*	89.20
total_OTU00184	0.006	0.108	**0.032483275**	*Eubacterium coprostanoligenes*	84.71
total_OTU02642	0.006	0.055	**0.049207717**	*Eubacterium coprostanoligenes*	85.02
total_OTU00684	0.000	0.022	**0.016506552**	*Eubacterium eligens*	86.23
total_OTU00120	0.000	0.137	**0.006063194**	*Eubacterium xylanophilum*	85.42
total_OTU00116	0.000	0.022	**0.001569348**	*Flavonifractor plautii*	85.02
total_OTU00421	0.000	0.012	**0.028919289**	*Kopriimonas byunsanensis*	80.81
total_OTU00042	0.433	2.449	**0.000581903**	*Lactobacillus intestinalis*	100.00
total_OTU00738	0.000	0.014	**0.048574889**	*Lactobacillus intestinalis*	95.48
total_OTU00030	0.209	1.422	**0.001014884**	*Lactobacillus johnsonii*	99.71
total_OTU00425	0.000	0.020	**0.007639210**	*Lactobacillus johnsonii*	91.74
total_OTU00142	0.018	0.184	**0.006911715**	*Lactobacillus reuteri*	98.25
total_OTU00271	0.018	0.159	**0.019863417**	*Lactobacillus reuteri*	99.13
total_OTU01129	0.085	1.133	**0.008964192**	*Lactobacillus reuteri*	99.13
total_OTU03176	0.006	0.078	**0.013659649**	*Lactobacillus reuteri*	97.67
total_OTU00657	0.006	0.159	**0.000195708**	*Limibacter armeniacum*	80.50
total_OTU00089	0.006	0.178	**6.72698E-05**	*Lysobacter sp. 107-E2*	78.59
total_OTU01323	0.003	0.067	**0.000252902**	*Marinifilum fragile*	81.06
total_OTU00198	0.003	0.024	**0.036325975**	*Odoribacter splanchnicus*	91.64
total_OTU01891	0.000	0.008	**0.041310345**	*Oscillibacter sp. G2*	89.51
total_OTU00081	0.000	0.014	**0.029882676**	*Oscillospira guilliermondii*	89.51
total_OTU00076	0.015	0.129	**0.012152215**	*Paludibacter propionicigenes*	82.19
total_OTU00460	0.000	0.178	**0.017300960**	*Paludibacter propionicigenes*	81.13
total_OTU00465	0.000	0.016	**0.041310345**	*Paludibacter propionicigenes*	80.43
total_OTU01021	0.000	0.008	**0.041310345**	*Paludibacter propionicigenes*	77.46
total_OTU01256	0.000	0.024	**0.013366294**	*Paludibacter propionicigenes*	82.45
total_OTU01276	0.003	0.100	**0.000835671**	*Paludibacter propionicigenes*	79.50
total_OTU01329	0.024	2.455	**4.41103E-05**	*Paludibacter propionicigenes*	81.82
total_OTU01399	0.000	0.014	**0.048574889**	*Paludibacter propionicigenes*	85.03
total_OTU01933	0.000	0.020	**0.020060862**	*Paludibacter propionicigenes*	82.08
total_OTU02758	0.000	0.147	**0.000342500**	*Paludibacter propionicigenes*	82.39
total_OTU00029	0.330	1.498	**0.001301584**	*Parabacteroides distasonis*	99.04
total_OTU00249	0.000	0.018	**0.045675773**	*Parabacteroides distasonis*	95.30
total_OTU00330	0.003	0.014	**0.029335228**	*Parabacteroides distasonis*	95.89
total_OTU00419	0.000	0.027	**0.004096206**	*Parabacteroides distasonis*	78.95
total_OTU00647	0.003	0.018	**0.026603049**	*Parabacteroides distasonis*	94.55
total_OTU00692	0.003	0.037	**0.002236616**	*Parabacteroides distasonis*	97.12
total_OTU00901	0.000	0.012	**0.009328704**	*Parabacteroides distasonis*	91.51
total_OTU00926	0.000	0.024	**0.023249219**	*Parabacteroides distasonis*	80.56
total_OTU01055	0.000	0.057	**0.000593288**	*Parabacteroides distasonis*	78.70
total_OTU01103	0.000	0.016	**0.015627489**	*Parabacteroides distasonis*	91.51
total_OTU01685	0.003	0.033	**0.002792969**	*Parabacteroides distasonis*	81.96
total_OTU02129	0.000	0.016	**0.015627489**	*Parabacteroides distasonis*	94.62
total_OTU02625	0.003	0.033	**0.041308131**	*Parabacteroides distasonis*	81.21

total_OTU03421	0.000	0.027	**0.006063194**	*Parabacteroides distasonis*	70.02
total_OTU00021	0.003	1.188	**0.002904246**	*Parabacteroides goldsteinii*	99.68
total_OTU00173	0.000	0.014	**0.029882676**	*Parabacteroides goldsteinii*	95.27
total_OTU00297	0.000	0.033	**0.014271069**	*Parabacteroides goldsteinii*	94.30
total_OTU00338	0.000	0.024	**0.028919289**	*Parabacteroides goldsteinii*	95.19
total_OTU01051	0.000	0.008	**0.041310345**	*Parabacteroides goldsteinii*	94.32
total_OTU00687	0.003	0.020	**0.022620582**	*Parabacteroides johnsonii*	91.51
total_OTU00061	0.042	1.937	**1.37771E-06**	*Parabacteroides merdae*	94.87
total_OTU00247	0.000	0.014	**0.014362618**	*Parabacteroides merdae*	94.94
total_OTU00703	0.000	0.016	**0.015627489**	*Parabacteroides merdae*	93.27
total_OTU00855	0.000	0.008	**0.041310345**	*Parabacteroides merdae*	92.48
total_OTU01626	0.000	0.018	**0.007758552**	*Parabacteroides sp. D13*	81.33
total_OTU00175	0.064	0.780	**1.90197E-05**	*Paraprevotella xylaniphila*	80.56
total_OTU01491	0.000	0.012	**0.028919289**	*Paraprevotella xylaniphila*	79.94
total_OTU02202	0.000	0.022	**0.044222167**	*Paraprevotella xylaniphila*	79.43
total_OTU00119	0.036	0.335	**0.011023002**	*Parasporobacterium paucivorans*	85.37
total_OTU00344	0.000	0.012	**0.028919289**	*Prevotella baroniae*	82.13
total_OTU02323	0.033	0.622	**0.000299940**	*Prevotella baroniae*	82.45
total_OTU01002	0.006	0.045	**0.037792272**	*Prevotella brevis*	82.97
total_OTU01441	0.000	0.012	**0.028919289**	*Prevotella brevis*	79.81
total_OTU00585	0.006	0.114	**8.19307E-05**	*Prevotella dentasini*	79.75
total_OTU01124	0.000	0.012	**0.009328704**	*Prevotella dentasini*	78.06
total_OTU00163	0.045	0.520	**2.76533E-05**	*Prevotella denticola*	79.19
total_OTU00832	0.021	0.141	**0.000902396**	*Prevotella denticola*	78.26
total_OTU00210	0.000	0.302	**0.033324149**	*Prevotella genomosp. C1*	81.33
total_OTU00223	0.048	0.527	**1.66093E-06**	*Prevotella genomosp. C1*	81.82
total_OTU00291	0.000	0.024	**0.003445984**	*Prevotella genomosp. C2*	85.17
total_OTU00382	0.000	0.108	**2.12903E-05**	*Prevotella genomosp. C1*	77.50
total_OTU00557	0.000	0.008	**0.041310345**	*Prevotella genomosp. P4*	80.25
total_OTU00285	0.136	0.451	**0.005001322**	*Prevotella maculosa*	80.75
total_OTU01410	0.000	0.041	**0.010254506**	*Prevotella maculosa*	81.31
total_OTU00034	0.221	0.527	**0.043824390**	*Prevotella oris*	79.50
total_OTU00101	0.148	5.759	**3.29335E-06**	*Prevotella oris*	79.81
total_OTU01586	0.000	0.016	**0.015627489**	*Prevotella oris*	83.02
total_OTU02811	0.000	0.008	**0.041310345**	*Prevotella oris*	80.13
total_OTU03015	0.000	0.016	**0.006763909**	*Prevotella oris*	77.29
total_OTU00776	0.000	0.012	**0.028919289**	*Prevotella oulorum*	77.92
total_OTU01678	0.000	0.012	**0.028919289**	*Prevotella pleuritidis*	82.24
total_OTU02265	0.000	0.012	**0.028919289**	*Prevotella pleuritidis*	79.75
total_OTU02293	0.000	0.010	**0.020060862**	*Prevotella pleuritidis*	81.73
total_OTU01152	0.000	0.008	**0.041310345**	*Prevotella shahii*	84.81
total_OTU01120	0.006	0.029	**0.011121595**	*Prevotella sp. canine oral taxon 226*	86.39
total_OTU00574	0.000	0.035	**0.001520557**	*Prevotella sp. ICM33*	81.39

total_OTU00090	0.055	0.353	**8.15505E-05**	*Prevotella sp. oral taxon 317*	79.94
total_OTU00208	0.000	0.016	**0.027463554**	*Prevotella sp. oral taxon 317*	78.55
total_OTU00282	0.245	1.531	**0.000634305**	*Prevotella sp. oral taxon 317*	80.38
total_OTU00331	0.000	0.096	**0.000582546**	*Prevotella sp. oral taxon 317*	78.86
total_OTU00983	0.003	0.143	**0.000288901**	*Prevotella sp. oral taxon 317*	80.70
total_OTU01221	0.000	0.092	**0.000479784**	*Prevotella sp. oral taxon 317*	80.19
total_OTU01796	0.000	0.016	**0.015627489**	*Prevotella sp. oral taxon*	80.88
total_OTU01849	0.009	0.094	**0.000108576**	*Prevotella sp. oral taxon 317*	80.76
total_OTU01988	0.000	0.020	**0.013192084**	*Prevotella sp. oral taxon 317*	81.50
total_OTU03019	0.000	0.024	**0.001671149**	*Prevotella sp. oral taxon 317*	79.43
total_OTU03462	0.000	0.008	**0.041310345**	*Prevotella sp. oral taxon 317*	61.69
total_OTU00049	0.079	1.367	**0.000229975**	*Prevotella sp. P2A_FAAD4*	85.27
total_OTU00307	0.003	0.018	**0.026603049**	*Prevotella sp. P2A_FAAD4*	87.11
total_OTU02465	0.006	0.033	**0.022295895**	*Prevotella sp. P2A_FAAD4*	84.28
total_OTU00096	0.003	0.096	**0.002689047**	*Pseudoflavonifractor capillosus*	89.23
total_OTU00099	0.000	0.010	**0.020060862**	*Pseudoflavonifractor capillosus*	91.38
total_OTU00105	0.024	0.067	**0.048778322**	*Pseudoflavonifractor capillosus*	90.46
total_OTU00305	0.000	0.024	**0.034964280**	*Pseudoflavonifractor capillosus*	88.00
total_OTU00424	0.000	0.018	**0.014856864**	*Pseudoflavonifractor capillosus*	90.15
total_OTU00950	0.000	0.025	**0.027540469**	*Pseudoflavonifractor capillosus*	84.80
total_OTU00996	0.000	0.016	**0.041310345**	*Pseudoflavonifractor capillosus*	88.31
total_OTU00302	0.000	0.247	**5.89450E-09**	*Rikenella microfusus*	82.04
total_OTU00796	0.018	0.447	**0.008597675**	*Rikenella microfusus*	81.53
total_OTU02078	0.003	0.035	**0.001330182**	*Rikenella microfusus*	81.42
total_OTU02830	0.000	0.025	**0.022766770**	*Rikenella microfusus*	79.44
total_OTU00060	0.006	0.345	**0.001635033**	*Roseburia faecis*	85.42
total_OTU02066	0.000	0.014	**0.029882676**	*Roseburia faecis*	88.07
total_OTU00054	0.000	0.267	**0.048285570**	*Roseburia hominis*	91.41
total_OTU00943	0.003	0.022	**0.005848110**	*Roseburia hominis*	92.94
total_OTU00651	0.006	0.204	**0.004853400**	*Ruminococcus sp. M-1*	91.38
total_OTU02075	0.000	0.045	**0.025354424**	*Ruminococcus sp. M-1*	93.87
total_OTU03036	0.000	0.043	**0.017953945**	*Ruminococcus sp. M-1*	92.64
total_OTU00308	0.000	0.139	**0.000549450**	*Subdoligranulum sp. 4_3_54A2FAA*	84.64
total_OTU00147	0.021	0.575	**2.30668E-10**	*Tannerella forsythia*	82.28
total_OTU00378	0.009	0.337	**4.90473E-07**	*Tannerella forsythia*	82.48
total_OTU01257	0.000	0.025	**0.001597852**	*Tannerella forsythia*	82.80
total_OTU01292	0.000	0.014	**0.029882676**	*Tannerella forsythia*	80.70
total_OTU02508	0.003	0.022	**0.027096465**	*Tannerella forsythia*	80.62
total_OTU00082	0.009	3.024	**4.52604E-05**	*Tannerella sp. 6_1_58FAA_CT1*	83.07
total_OTU00374	0.000	0.014	**0.048574889**	*Tannerella sp. 6_1_58FAA_CT1*	81.00
total_OTU00478	0.000	0.022	**0.006940245**	*Tannerella sp. 6_1_58FAA_CT1*	79.94
total_OTU00755	0.000	0.012	**0.028919289**	*Tannerella sp. 6_1_58FAA_CT1*	81.42
total_OTU01111	0.000	0.018	**0.045675773**	*Tannerella sp. 6_1_58FAA_CT1*	82.65

total_OTU01180	0.000	0.012	**0.028919289**	*Tannerella sp. 6_1_58FAA_CT1*	82.24
total_OTU01424	0.000	0.010	**0.020060862**	*Tannerella sp. 6_1_58FAA_CT1*	79.75
total_OTU01913	0.000	0.016	**0.015627489**	*Tannerella sp. 6_1_58FAA_CT1*	79.50
total_OTU02475	0.000	0.010	**0.020060862**	*Tannerella sp. 6_1_58FAA_CT1*	80.80
total_OTU00836	0.003	0.024	**0.028546900**	*TM7 oral taxon 351*	95.42

Amp, ampicillin. Neo, neomycin.