**Low bacterial biomass of oral wash samples**

We quantified the bacterial DNA of every oral wash sample in azithromycin group using droplet digital PCR system. The bacterial DNA burden in oral wash samples (the first quartile and the third quartile, 1.1 x 106~4.5 x 106 copies/g) was significantly lower than sputum samples across different timepoints (Wilcoxon signed-rank test, q<0.05 for all Additional file 12: Figure S9).

**Contamination identification on oral wash samples sequencing in azithromycin group**

For successful 16S rRNA gene library preparation, we increased the volume of oral wash samples, up to 3 ml, for DNA extraction. The methods of DNA extraction, 16S rRNA gene sequencing and statistical analysis workflow were consistent with those of sputum samples. One sample was removed because of insufficient sequencing for microbial analysis. The microbial community composition between oral wash samples and control samples was quite distinct based on both unweighted UniFrac and weighted UniFrac distance (PERMANOVA, R2=0.26, 0.50; P=0.001,0.001 Additional file 13: Figure S10a-b). The five Zero-radius Operational Taxonomic Units (ZOTUs, 100% sequence similarity) detected in oral wash samples with the highest abundance comprised 27.13% of all sequences, while comprising 0.97% of all sequences detected in control samples (Additional file 13: Figure S10c). The most abundant ZOTU detected in control specimens (ZOTU29) accounted for only 0.21% of all sequences detected in oral wash specimens (Additional file 13: Figure S10). We performed microbial ecology analysis using the decontam package whose function was to identify contaminants in sequencing data based on statistical approach. We used both the frequency and prevalence methods with threshold 0.1 and 0.5 respectively and confirmed 7 contaminants ZOTUs (ZOTU1093, ZOTU1456, ZOTU1601, ZOTU1729, ZOTU2157, ZOTU2241, ZOTU41) in our data (Additional file 13: Figure S10e-f, Additional file 2: Table S1). The relative abundance of the 7 ZOTUs were very low in both oral wash and negative control samples (comprising 5.05 x 10-4%, 2.26 x 10-4%, 5.22 x 10-5%, 5.39 x 10-4%, 3.48 x 10-5%, 3.48 x 10-5%, 0.12% of all oral wash samples sequences and 0.1%, 0.04%, 0.03%, 0.01%, 0.01%, 0.01%, 25.13% of all negative control samples sequences). After removal of the 7 ZOTUs, a total of 2823 ZOTUs were analyzed.

**Similar but not identical microbial profile between the oral cavity and the airway microbiota**

In azithromycin group, the species richness of baseline oral cavity microbiota of healthy volunteers (D0) was significantly higher than the sputum microbiota (484.33 ± 89.57 vs 414.42 ± 89.63 two-sided paired t-test, q=0.0033 Additional file 14: Figure S11a-b). PERMANOVA testing using both unweighted UniFrac distance and weighted UniFrac distance found that the microbial community composition at D0 between the two niches was quite distinct (R2=0.04, 0.05; P=0.029, 0.033 Additional file 14: Figure S11c-d). As can be seen in the heatmap of detection rate, about 7% of ZOTUs (whose relative abundance was greater than 0.01% and detection rate was greater than 50% at baseline) in the sputum microbiota didn’t appear in the oral wash microbiota (Additional file 17: Figure S14a). Conversely, about 22.6% of ZOTUs were unique in the oral cavity microbiota (Additional file 17: Figure S14a). Although the clustering of taxa with high relative abundance were similar between the oral cavity and airway at ZOTU level (Additional file 17: Figure S14b), the relative abundance of families *Prevotellaceae*, *Veillonellaceae*, *Porphyromonadaceae*, *Micrococcaceae*, *Peptostreptococcaceae* and genera *Prevotella*, *Porphyromonas*, *Veillonella*, *Gemella* were significantly higher in the sputum microbiota while families *Flavobacteriaceae*, *Pasteurellaceae*, *Fusobacteriaceae* and genera *Capnocytophaga*, *Fusobacterium*, *Haemophilus* were higher in the oral wash microbiota (Wilcoxon signed-rank test, q<0.05 for all Additional file 17: Figure S14c-d).

**Different process of variation in oral cavity microbial diversity after antibiotic exposure**

After exposure to azithromycin, the species richness of the oral cavity microbiota was dramatically reduced compared to D0 at D4 (two-sided paired t-test, q=6.826x10-6 Additional file 14: Figure S11a) and didn’t return to the baseline level by the end of observational period (q=0.017 Additional file 14: Figure S11a ). Similarly, pairwise PERMANOVA testing using unweighted UniFrac distance showed significant compositional differences between timepoint D0 and timepoints D4, D14, D30 and D60 (R2=0.096, 0.141, 0.125, 0.101; q=0.01, 0.01, 0.02, 0.01 Additional file 14: Figure S11c). The Shannon index significantly decreased at D4 compared to D0 (two-sided paired t-test, q=2.65x10-6 Additional file 14: Figure S11b), unlike the shifts in the sputum microbiota, and came back to the D0 level at D30 (q=1 Additional file 14: Figure S11b). When we used weighted UniFrac distance perform pairwise PERMANOVA testing, the significant change in microbial community composition compared to D0 was only identified at D4 (R2=0.085, q=0.01 Additional file 14: Figure S11d).

By the timepoints D4, D14, D30 and D60, there weren’t significant difference in species richness and Shannon index between the oral cavity and the airway microbiota (two-sided paired t-test, q>0.05 Additional file 14: Figure S11a-b). The microbial community composition between the two niches were similar at D4 (PERMANOVA testing using unweighted UniFrac distance and weighted UniFrac distance, R2=0.03, 0.03; q=0.14, 0.13 Additional file 14: Figure S11c-d). However, by D14, D30 and D60, PERMANOVA testing showed that the difference of microbial composition between the oral cavity and the airway microbiota became obvious (weighted UniFrac distance, R2=0.04, 0.09, 0.09; q=0.045, 0.001, 0.001 Additional file 14: Figure S11d). These results suggested that the oral cavity and the sputum microbiota of the same group volunteers showed a convergent trend after azithromycin disturbance at D4 while had different change patterns over time.

**Microbial taxonomic variation during 60 days’ follow-up**

We involved 354 ZOTUs of the oral wash microbiota and 294 ZOTUs of the sputum microbiota whose relative abundance was greater than 0.01% and detection rate was greater than 50% at baseline to analyze. After exposing to azithromycin, in the oral cavity microbiota, the detection rate of 127 ZOTUs decreased at D4, including some families, such as *Veillonellaceae* (16.54%), *Leptotrichiaceae* (14.17%), *Fusobacteriaceae* (9.45%), *Neisseriaceae* (7.87%), *Actinomycetaceae* (7.87%), *Pasteurellaceae* (7.09%), *Lachnospiraceae* (5.51%)and *Prevotellaceae* (3.94%), which were similar to the variation in the sputum microbiota (Additional file 18: Figure S15, Additional file 4: Table S2). Most of the ZOTUs returned to the D0 level at D14 but 21.26% of the above mentioned ZOTUs still remained low detection rate at D60, which was higher than the ratio (15.18%) in the sputum microbiota (Additional file 19: Figure S16a, Additional file 6: Table S3). Among 354 ZOTUs of the oral microbiota, the detection rate of 227 ZOTUs returned to the D0 level at D4 and 15.42% of them exhibited significant differences compared to D0 again at D14 (Additional file 19: Figure S16a, Additional file 6: Table S3). The relative abundance of 273 ZOTUs were not significantly different between D0 and D4 and 15.02% of those ZOTUs had novel variation in the relative abundance at D14 (Additional file 19: Figure S16b, Additional file 6: Table S3). Among 294 ZOTUs of the sputum microbiota, the detection rate of 182 ZOTUs returned to the D0 level at D4 and only 8.79% of them exhibited significant differences compared to D0 again at D14 (Additional file 5: Figure S3a, Additional file 6: Table S3). The relative abundance of 227 ZOTUs were not significantly different between D0 and D4 while 13.66% of those ZOTUs had novel variation in the relative abundance at D14 (Additional file 5: Figure S3b, Additional file 6: Table S3).

By D4, 35 ZOTUs (31.25%) had variation in the detection rate in the airway microbiota while remained stable in the oral cavity microbiota. By timepoints D14, D30 and D60, the ratio was 32.22% (29), 34.62% (18) and 39.29% (11) respectively (Additional file 19: Figure S16c). The unique changes in the relative abundance were seen in 24 ZOTUs (35.83%), 29 ZOTUs (43.28%), 33 ZOTUs (54.10%) and 20 ZOTUs (55.56%) of the sputum microbiota at D4, D14, D30 and D60 respectively (Additional file 19: Figure S16d). We included the ZOTUs whose detection rate or relative abundance had shifts only in the sputum microbiota across different timepoints for PERMANOVA testing. The results showed that the individual changes of the ZOTUs owned by the sputum microbiota had the ability to cause the variation in the airway microbiota composition after exposure to azithromycin (Table S5).

Table S5. PERMANOVA testing with unique ZOTUs

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | The number of unique ZOTUs in detection rate variation | The number of unique ZOTUs in relative abundance variation | Total number of ZOTUs of both | Based on unweighted UniFrac distance | |  | Based on weighted UniFrac distance | |
| R2 | P |  | R2 | P |
| D0\_D4 | 35 | 24 | 54 | 0.115 | 0.001 |  | 0.05 | 0.035 |
| D0\_D14 | 29 | 29 | 53 | 0.127 | 0.001 |  | 0.045 | 0.042 |
| D0\_D30 | 18 | 33 | 48 | 0.108 | 0.001 |  | 0.085 | 0.006 |
| D0\_D60 | 11 | 20 | 29 | 0.096 | 0.001 |  | 0.058 | 0.021 |

**Dissimilar effects of azithromycin on the oral cavity microbiota network**

The distance-based redundancy analysis (db-RDA) was performed to find the association between the environmental stresses and the oral cavity microbiota. Humidity and the level of PM2.5 were significantly associated with the oral cavity microbiota at D4 and D30 respectively, but not antibiotic uses (R2=0.0415, 0.033, -0.0005, 0.0039; q=0.004, 0.004, 1, 0.984 Additional file 20: Figure S17). We thought that azithromycin administration still served as an important factor on the oral wash microbiota shifts at D4 because of the obvious changes immediately after antibiotic exposure. Besides, dissimilar to the sputum microbiota, oral cavity microbiota can bear the high burden of PM2.5 pollution and remain stable microbial community composition at D30 (PERMANOVA testing using weighted UniFrac distance, R2=0.18; q=1 Additional file 14: Figure S11d), suggesting that the oral cavity microbial interactions within the microbial community might be different from those of the sputum microbiota after azithromycin exposure. We performed bacterial community network analysis on the oral cavity microbiota. After azithromycin administration, the oral cavity microbiota structure network became less complicated as well. Compared to the sputum microbiota structure network, the topological parameters of the oral cavity microbiota began to increase at D14 (Additional file 15: Figure S12). ZOTUs belonging to families *Streptococcaceae*, Lachnospiraceae, *Prevotellaceae* and *Veillonellaceae* played the important roles at D0. After exposure to antibiotics, families *Veillonellaceae* still set a central position while families *Streptococcaceae, Lachnospiraceae, Prevotellaceae* superiority in the network declined. By D14, families *Streptococcaceae, Lachnospiraceae, Prevotellaceae* had returned to their original position (Additional file 16: Figure S13a). The number of overlapped edges were large and the closeness of shared nodes was similar between the oral microbiota network and the sputum microbiota network at timepoint D0 (Additional file 16: Figure S13b). Despite having a few overlapped edges between the two niches’ networks at timepoint D4 or D14, the closeness of shared nodes was quite different. By D14, the closeness of the ZOTUs in the oral wash microbiota was higher than those in sputum microbiota, suggesting that the interactions among the species recovered earlier than the sputum microbiota (Additional file 16: Figure S13c).