Gut microbiome and resistome changes during the first wave of the COVID-19 pandemic in comparison with pre-pandemic travel-related changes

Running title: Human gut microbiome and resistome changes during COVID-19 vs international travels

Authors

Ye Peng, MSc[1,2*], Dengwei Zhang, MSc[1,2*], Ting Chen, PhD[3], Yankai Xia, PhD[3], Peng Wu, PhD[2], Wai-Kay Seto, MD[4], Anita L. Kozyrskyj, PhD[5], Benjamin J Cowling, PhD[2], Jincun Zhao, PhD[6,7], Hein M Tun, PhD[1,2,3#]

Affiliations

[1]HKU-Pasteur Research Pole, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong SAR

[2]School of Public Health, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong SAR

[3]School of Public Health, Nanjing Medical University, Jiangning District, Nanjing, China

[4]Department of Medicine, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong SAR

[5]Department of Pediatrics, University of Alberta, Edmonton, Canada

[6]State Key Laboratory of Respiratory Disease, National Clinical Research Center for Respiratory Disease, Guangzhou Institute of Respiratory Health, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China

[7]Institute of Infectious Disease, Guangzhou Eighth People's Hospital of Guangzhou Medical University, Guangzhou, China.

Author Footnotes

*These authors contributed equally.

[#]**Corresponding author**

Hein M. Tun, PhD

Assistant Professor, HKU-Pasteur Research Pole, School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR

Phone: +852-31979944

Email: heinmtun@hku.hk

https://twitter.com/TheTunLab

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Highlight/Teaser

COVID-19-associated measures had a greater impact on gut microbiota, ARGs, and BRGs than did pre-pandemic international travel. During the COVID-19 pandemic, Actinobacteria richness decreased while genes conferring resistance to beta-lactam, polystyrene and phthalate increased. Such alterations may affect both colonisation resistance and acquisition and spread of antimicrobial resistance in future travels.

Our gut microbes are sensitive to environmental changes and exposures. Travel-associated alterations to gut microbial composition and antibiotic resistance gene profiles have been reported. [1, 2] Despite cross-border travel restrictions are in place during the COVID-19 global pandemic, various pandemic control measures including physical distancing, extensive hygiene, and greater disinfectant and antibiotic usage all have the potential to disrupt normal composition of gut microbiota. [3] However, we do not yet fully understand the extent to which gut microbiota and resistance genes against antibiotics and biocides will change in response to the current pandemic control measures.

We performed shotgun metagenomic sequencing on fecal samples collected before (BT) and after (AT) pre-pandemic travels and during the first wave of the COVID-19 pandemic (FW) of 32 Hong Kong adults in a prospective cohort [4] following ethical approval (IRB of the University of Hong Kong/Hospital Authority Hong Kong West Cluster, reference number: UW 18-473). The average interval between fecal samples in the BT and AT periods was 15.0 days (95% confidence interval (CI) 12.5-17.4), and 379.9 days (95% CI 344.5-415.2) between AT and FW samples (Figure 1A). Of the 32 participants, 17 had additional travel

between AT and FW. During the COVID-19 pandemic, two participants experienced COVID-19 related symptoms but were tested negative for SARS-CoV-2 (Table 1).

According to permutational multivariate analysis of variance, no statistically significant dissimilarity was found between fecal microbiota collected at BT, AT and FW (Figure 1B). Further, additional travel during the first wave of the COVID-19 pandemic had no significant impact on fecal microbiota collected. However, change in microbial beta-diversity between AT and FW was significantly greater than that between BT and AT (Figure 1B), and these changes were not correlated with the length of the intervals between sampling time points (Spearman correlation test, $P > 0.05$). We also observed significantly lower Actinobacteria richness and higher Bacteroidetes richness in the gut microbiome of the participants during the pandemic (Figure 1C); neither the first travel episode nor additional travel changed microbial alpha diversity. Normal inhabitants of the oral cavity as well, members of the phylum Actinobacteria become more diverse in human gut with greater exposure to the natural environment. [5] It is conceivable then, that lowered species richness within the Actinobacteria during the COVID-19 pandemic reflects a reduction in outdoor recreational activities subsequent to pandemic control measures. At the species level, higher abundance of *Lactococcus lactis* (of the Firmicutes phylum) and *Corynebacterium durum* (of the Actinobacteria) was identified following episodes of international travel; the opposite trend was noted during the first wave of the pandemic. On the other hand, higher *Bacteroides thetaiotaomicron* (*B. thetaiotaomicron*) and *Parabacteroides distasonis* (*P. distasonis*), both members of the Bacteroidetes phylum, and lower *Actinomyces oris* (of the Actinobacteria) were observed during the first wave of the pandemic (Figure 1F). Among these, the change of *Actinomyces oris* abundance was negatively correlated with interval length between AT and FW (partial Spearman correlation, $rho = -0.42$, $P = 0.019$). *B. thetaiotaomicron* and *P. distasonis*, mostly resistant to beta-lactam antibiotics, [6] became more plentiful during the

pandemic. Beta-lactam resistant genes harbored by these Bacteroidetes species could be transferred to other microbes in the gut. [7]

Changes to the gut resistome were then investigated. No community differences were identified between BT and AT for either antibiotic resistance genes (ARGs) or biocide resistance genes (BRGs) (Figure 1D, E). However, a significant change in beta-diversity of ARGs was observed during the pandemic (Figure 1D). Similar to microbiota beta-diversity results, changes to ARGs and BRGs between AT and FW were significantly greater than those for BT versus AT (Figure 1D, E). Among ARGs, the abundance of rifamycin resistance genes declined, while that of the beta-lactam resistance genes rose during the COVID-19 pandemic (Figure 1F). Besides, we found statistical enrichment of polystyrenes and phthalate resistance genes in the gut microbiome during the pandemic, findings attributed to two membrane transporter genes, *ttgA* and *ttgB*. Polystyrenes are components of face masks; whereas phthalates are used as plasticizers in food packaging and building materials. Persistent use of masks in Hong Kong, [8] enhanced exposure to food packaging due to pandemic-related in-dining restrictions at restaurants, and prolonged indoor stays due to physical distancing measures might have led to these changes. Given our reported correlations with the *ttgA* and *ttgB* genes, these known antibiotic transporters [9] may co-localize with the beta-lactam-related ARGs on the genomes of *B. thetaiotaomicron* and *P. distasonis*. Notably, class A beta-lactamase, OXA-209 and *ttgB* were all strongly correlated with the abundance of *B. thetaiotaomicron* and *P. distasonis*.

We further explored the impact of COVID-19 pandemic measures on gut microbiota and the resistome using canonical correspondence analyses. Frequency of using alcohol-based hand sanitizer, using sanitizer after defecation, hand washing or sanitization before and after taking off a face mask, and antibiotic use in the past month had significant influences on the gut microbial community ($P < 0.05$, Figure S1A). Several *Collinsella* (*C.*) species,

including *C. intestinalis*, *C. aerofaciens* and *C. stercoris*, as well as *Streptococcus parasanguinis* were positively correlated with frequency of using hand sanitizer. Both *Collinsella* and *Streptococcus* species can produce carcinogenic acetaldehyde from ethanol. [10] Therefore, this possible collateral impact of excessive hand sanitizer use should not be neglected. Furthermore, reusing a face mask and having COVID-19 related symptoms significantly shaped the beta diversity of both ARGs and BRGs ($P < 0.05$, Figure S1B-C) by increasing abundances of beta-lactam and multidrug resistant genes as well as several BRGs.

This study was limited by lack of repeated sampling between AT and FW, as well as continuous follow-up during second and third waves of the pandemic due to biosafety concerns. Despite these limitations, we present the first scientific evidence for the impact of current pandemic control measures and practices on the gut microbiota and resistome of healthy individuals. It prompts the necessity to collect samples from uninfected subjects in a time-matching manner as a qualified control group in studies of microbiome-SARS-CoV-2 interactions. Our study also informs the need for careful monitoring of unwanted consequences to our gut microbiota and associated health outcomes during the current COVID-19 pandemic. Notably, we reported previously having low Actinobacteria richness in the gut microbiota before travel increased the risk of acquiring extended spectrum β-lactamase-producing Enterobacteriaceae during travel. [4] Given Actinobacteria richness decreased while resistance genes against beta-lactam antibiotics, polystyrene, and phthalate increased during the first wave of the pandemic, special attention should be paid to avoid acquisition and spread of antimicrobial resistance in future travels both during and after the pandemic.

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Author Contributions:

H.M.T. designed research; Y.P. and D.Z. performed research and analyzed data; Y.P. and D.Z. wrote the paper; H.M.T., T.C., Y.X., P.W., W.S., A.K., B.C. and J.Z. provided critical revisions.

Conflict of interest

None declared.

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Tables

Table 1. Selected characteristics/exposures of 32 participants

Characteristics/exposures	**Distribution (n=32)[a]**
High (cm)	162.8 ± 7.2
Weight (kg)	57.1 ± 9.6
BMI	21.5 ± 2.9

Travelled again after first travel	17 (53.1)
Dietary preference	
Normal diet	30 (93.8)
Taking probiotics/prebiotics/synbiotics	10 (31.2)
Drinking	18 (56.2)
Currently having health problem(s)	5 (15.6)
Currently taking medication(s)	4 (12.5)
Diarrhea	
No	25 (78.1)
Diarrhea in the past 3 months	5 (15.6)
Diarrhea at this moment	0 (0)
Diarrhea at this moment and in the past 3 months	2 (6.2)
Antibiotic use in the past month[b]	2 (6.2)
COVID-19-related symptoms[c]	2 (6.2)
Frequency of using alcohol-based hand sanitizer per day	
Never	2 (6.2)
Occasionally to 1-3 times	14 (43.8)
3-5 times	9 (28.1)
5-10 times	6 (18.8)
More than 10 times	1 (3.1)

Using hand sanitizer	
before meal	23 (71.9)
after meal	9 (28.1)
after using toilet	7 (21.9)
after using public transport	22 (68.8)
in other scenarios	27 (84.4)
Cleaning hands using other products than sanitizer	13 (40.6)
Using disinfectant cleaners to clean belongings	22 (68.8)
Using masks	31 (96.9)
Frequency of changing a mask per day	
No mask changing	10 (31.2)
Changing when the mask is dirty, wet or damaged	6 (18.8)
Changing after 8 hours of use	12 (37.5)
Changing after 4 to 6 hours of use	4 (12.5)
Hand washing or sanitization before and after taking off the mask	20 (62.5)
Reused a mask	14 (43.8)
Frequency of washing hands with soap per day	
Occasionally to 1-3 times	8 (25)
3-5 times	5 (15.6)
5-10 times	18 (56.2)

More than 10 times	1 (3.1)
Scenarios for hand washing with soap	
before meal	24 (75)
after meal	9 (28.1)
after using toilet	31 (96.9)
after using public transport	11 (34.4)
in other scenarios	4 (18.8)

[a]Categorical data were shown as Count (proportion (%)); normally distributed continuous data were shown as Mean ± s.d.; other continuous data were shown as Median (first quantile, third quantile).

[b]One participant took lymecycline; another took levofloxacin.

[c]Tested negative for SARS-CoV-2.

Figure Legend

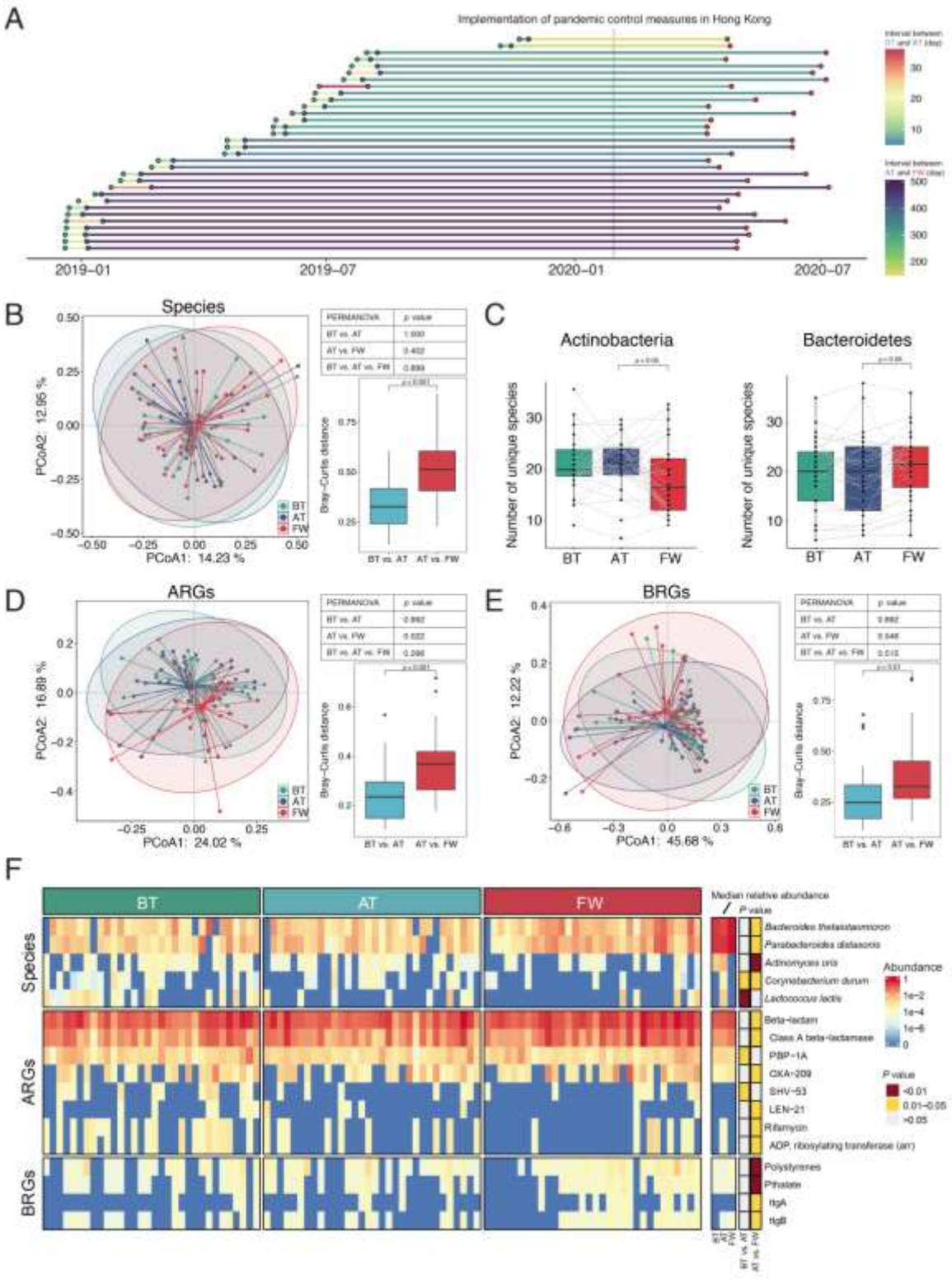

Figure 1. (A) Sampling time for 32 participants of the study. (B, C, D, and E) alpha and beta diversity changes of microbiota, ARGs, and BRGs. P values for comparisons between Bray-

Curtis distance, species richness and abundances were given by paired Wilcoxon rank-sum tests. (F) Heat map showing differences in the abundances of microbiota species (relative abundances), and ARGs and BRGs (copies per cell) between time points.