Acquisition of antimicrobial resistance after travel to resource-limited countries: a multi-layer metagenomic epidemiological study (abridged secondary publication)

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KEY MESSAGES

1. Pre- and post-travel resistome and microbiota profiles showed separation in microbial communities and novel antimicrobial resistance genes.
2. Consumption of raw seafood, Actinobacteria richness, Erysipelotrichaceae UCG-003, *Blautia, Butyricicoccus, and Ruminiclostridium 9* were associated with the acquisition of extended spectrum β-lactamase-producing Enterobacteriaceae.
3 Travel to low and middle-income countries was associated with acquisition of antibiotic resistance genes.

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Introduction

Antimicrobial resistance (AMR) acquisition may occur through international travel. The colonisation of extended spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) has been reported among international travellers, regardless of infection status.[1] The human gut microbiota serve as AMR transporters and are associated with AMR acquisition during international travel and therefore are potential biomarkers to predict AMR acquisition. However, previous studies have mostly focused on Western populations.[2] Because ethnicity-associated lifestyle factors strongly influence gut microbiota,[3] evidence from Western populations may not be directly applicable to Asian populations. Therefore, we aimed to establish associations among gut microbiota, travel-associated risk factors, and AMR acquisition in Asian travellers. We also characterised the effects of control measures during the first wave of the COVID-19 pandemic on gut microbiota and resistome profiles in healthy individuals.

Methods

We recruited 269 Hong Kong travellers and asked them to submit two faecal samples and complete two questionnaires within 4 days before and after travel. Among the travellers, 35.7% participated during the first wave of COVID-19 in Hong Kong.

From faecal samples, ESBL-E was isolated and then subjected to antimicrobial susceptibility testing and taxonomic identification; DNA was extracted and aliquoted for real-time polymerase chain reaction quantification of AMR genes, 16S rRNA amplicon sequencing, and shotgun metagenomic sequencing. Sequencing reads for 16S and metagenomic data were analysed. Resistomes were quantified.

We developed a functional metagenomics platform to identify novel AMR genes in post-travel samples. Metagenomic DNA was pooled according to travel region and partially digested; digestion bands with sizes >700 bp were purified. The plasmid vector pZE21-MCS was digested and dephosphorylated. The random digests and plasmid were ligated and transformed into electrocompetent *Escherichia coli* cells. Cells were recovered in Luria-Broth medium and then spread onto Luria-Broth agar with kanamycin and various antibiotics for screening.

Comparisons of ESBL-E carriers and non-carriers were performed using permutational multivariate analysis of variance and principal coordinates analysis. Discriminant genera were identified using the linear discriminant analysis effect size pipeline. Predictors for acquisition of ESBL-E during travel were identified using three models that comprised different sets of predictors. The area under the receiver operating characteristic curve (AUC) was calculated for each model. Distributions of genus predictors were compared using the Wilcoxon rank-sum test.

Results

The pre- and post-travel resistome microbiota profiles showed similar separation in communities

and species richness (P=0.51, Fig 1). In total, 55 novel resistance genes were acquired after travel. Genes conferring resistance to bacitracin, macrolides, mupirocin, polymyxin, quinolones, and sulphonamides increased after travel, although these differences were not significant. The identification of new AMR genes contributed to expansion of antibiotic resistance gene (ARG) databases.

Overall ARG richness and diversity in travellers varied between those visiting low- or middle-income countries and those visiting high-income countries, although the differences were not significant. These findings were supported by the quantitative polymerase chain reaction results; the relative abundances of five common ARGs (*cfxA*, *tetM*, *tetQ*, *ermB*, and *aac(6')-aph(2")*) did not differ significantly between pre-and post-travel samples. To identify potential discriminant ARGs acquired during travel, we analysed changes in ARG abundances and identified 24 ARGs with significant differences (log^2 fold change >1 or <-1, P<0.05) before and after travel. Notably, 23 of these 24 ARGs were enriched after visits to low- or middle-income countries. In contrast, travellers visiting high-income countries exhibited depletion of resistance genes; six of the 24 ARGs were diminished. These findings suggest that travellers visiting high-income countries acquire fewer resistance genes.

Most ESBL-E isolates (n=55, 91.7%) from post-travel samples were resistant to multiple antibiotics (Fig 2). There was significant separation of post-travel microbial communities between ESBL-E-positive participants and ESBL-E-negative participants (n=34, P=0.038; weighted UniFrac distance, P=0.020). Moreover, we observed significantly lower Actinobacteria richness in ESBL-E-positive participants than in ESBL-E-negative participants (P=0.008).

We constructed a reference model based on 13 pre-travel gut microbial predictors, including Actinobacteria richness and 12 genera. To test whether the combination of travel-related risk factors

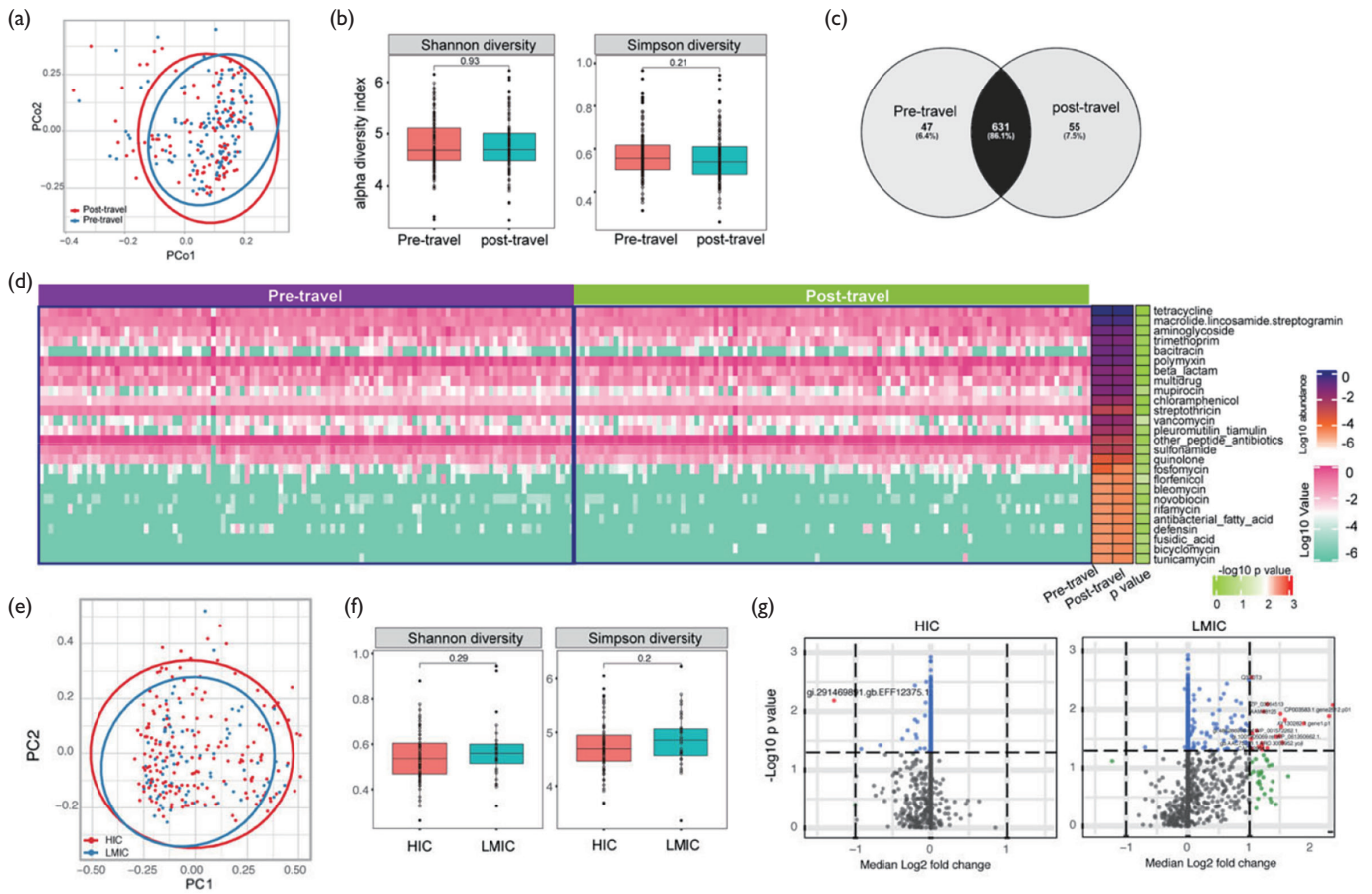

FIG 1. Metagenomic profile of changes in resistome between pre- and post-travel gut microbiota, and between samples collected after visits to high-income versus low- or middle-income countries: (a) principal coordinates analysis of weighted UniFrac distances between pre- and post-travel samples, (b) changes in alpha diversity between pre- and post-travel samples, (c) Venn plot showing the overlap of antibiotic resistance genes between pre- and post-travel samples, (d) heatmap comparing abundances of antibiotic resistance genes between pre- and post-travel samples, and (e, f, and g) changes in alpha diversity, beta diversity, and abundance in the resistomes of travellers who visited low- or middle-income countries versus high-income countries.

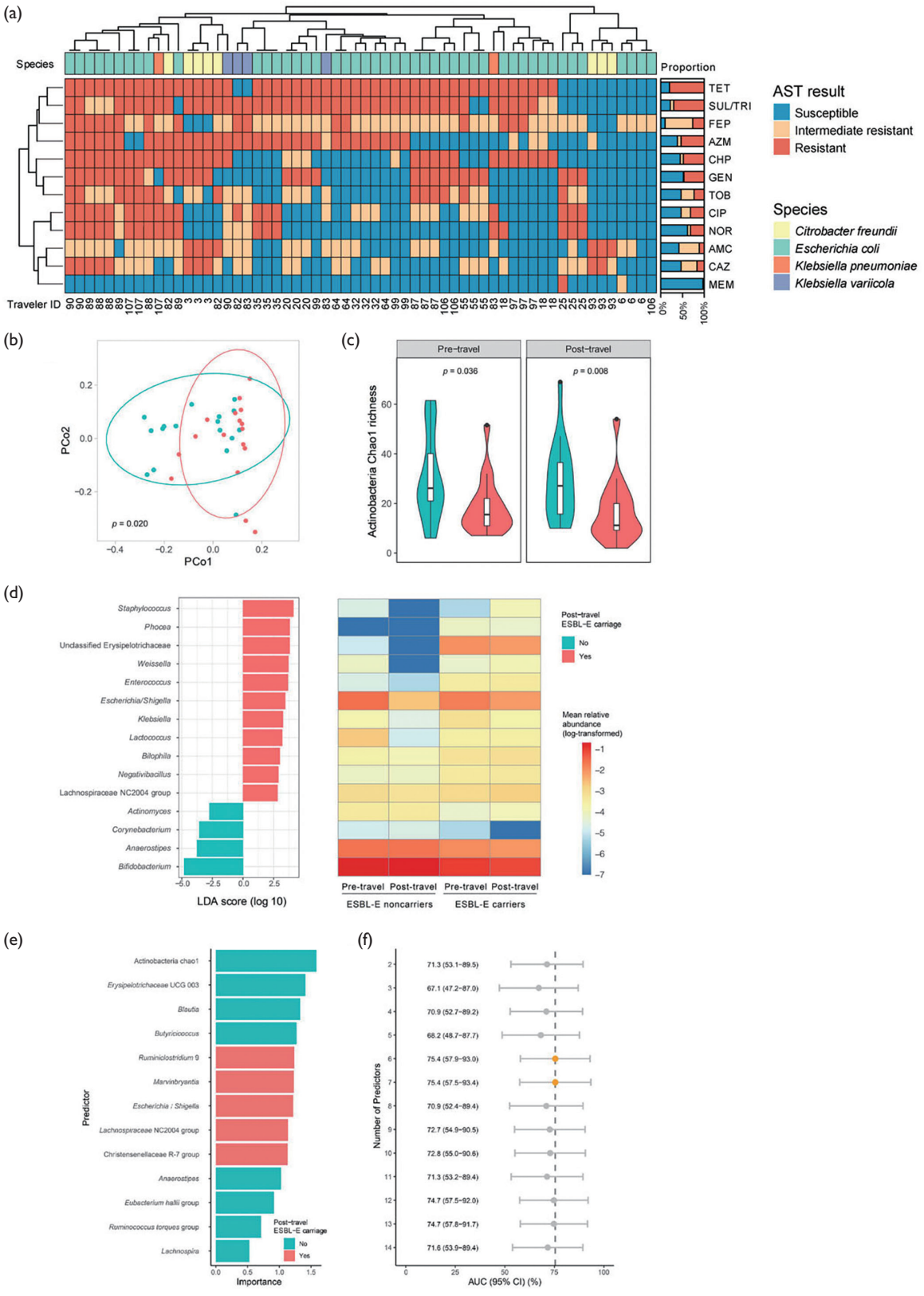

FIG 2. Travellers with extended spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) positivity after travel have distinct gut microbiota: (a) antimicrobial susceptibility testing (AST) of ESBL-E isolates in terms of amoxycillin (AMC), ceftazidime (CAZ), cefepime (FEP), meropenem (MEM), gentamycin (GEN), tobramycin (TOB), azithromycin (AZM), tetracycline (TET), ciprofloxacin (CIP), norfloxacin (NOR), trimethoprim-sulfamethoxazole (SUL/TRI), and chloramphenicol (CHP); (b) principal coordinates analysis of weighted UniFrac distances between ESBL-E-positive and -negative travellers; (c) Actinobacteria richness between ESBL-E-positive and -negative travellers; (d) linear discriminant analysis (LDA) score of genera between post-travel ESBL-E-positive and -negative travellers ($P<0.05$); (e) the twelve most important genus predictors among post-travel ESBL-E-positive and -negative travellers; and (f) areas under the curve (AUC) for combinations of raw seafood consumption and microbial predictors. Dashed line indicates the area under the curve of 75.4% for the reference model using Actinobacteria and 12 genera.
Abbreviation: 95% CI = 95% confidence interval

and microbial predictors could improve prediction, we established a series of models using combinations of raw seafood consumption and the stepwise addition of microbial predictors. A model with raw seafood consumption and five genus predictors (Actinobacteria richness, Erysipelotrichaceae UCG-003, *Blautia*, *Butyricicoccus*, and *Ruminiclostridium 9*) demonstrated an AUC of 75.4% (95% confidence interval=57.9%-93.0%).

Discussion

After travel, compared with ESBL-E-positive participants, ESBL-E-negative participants were associated with higher Actinobacteria richness and higher abundances of short-chain fatty acid producers, along with lower abundances of several opportunistic pathogenic genera. Potential gut acidity secondary to increased levels of short-chain fatty acids could inhibit ESBL-E colonisation.[4] Our findings likely reflect the association between compromised gut homeostasis and ESBL-E colonisation.

Raw seafood consumption during travel is a risk factor for ESBL-E acquisition, which could be attributed to seafood farming near areas of coastal run-off or the use of antibiotics in aquaculture. Thus, we suggest that travellers avoid raw seafood consumption during travel. The predictive power of Actinobacteria richness plus raw seafood consumption was higher than that of raw seafood consumption alone. Therefore, both travel-related risk factors and baseline gut microbiota are important for predicting ESBL-E acquisition during travel.

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Disclosure

The results of this research have been previously published in:

1. Peng Y, Liang S, Poonsuk K, et al. Role of gut microbiota in travel-related acquisition of extended spectrum β-lactamase-producing Enterobacteriaceae. J Travel Med 2021;28:taab022.
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