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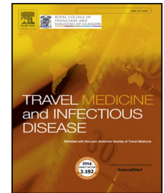

Instances of altered gut microbiomes among Irish cricketers over periods of travel in the lead up to the 2016 World Cup: A sequencing analysis

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A R T I C L E I N F O

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A B S T R A C T

Background: Changes and stresses experienced during travel have the potential to impact the gut microbiome, with travel implicated in the spread of antibiotic resistance genes across continents. The possibility of gut microbiome-mediated negative impacts arising from travel, and consequences for peak performance, would be of particular concern for elite athletes.
Methods: Faecal samples were collected from male (N = 14) and female (N = 7) cricket players during the build-up to the 2016 Cricket World Cup. Baseline and post-travel samples were collected from all participants and subjected to 16S rRNA amplicon sequencing. Samples from a subset of participants (N = 4) were also analysed by shotgun metagenomic sequencing.
Results: Analysis revealed a single travel time point as having the potential to have an impact on the gut microbiome. Reductions in alpha diversity following travel were observed, accompanied by shifts in the taxonomic profile of the gut microbiome. Antibiotic resistance and virulence genes were also identified as undergoing changes following travel.
Conclusions: This study reveals that periods of travel, in particular following gastrointestinal distress, may result in gut microbiome disruption. While this analysis was completed in athletes, the findings are applicable to all travelling individuals and considerations should be made surrounding travel in an attempt to reduce these changes.

1. Introduction

Many aspects of a modern lifestyle including diet, pharmacological agents, exercise, and disease state influence the gut microbiome, which in turn can impact significantly on host health [1–11]. It is hoped that by gaining a better understanding of factors that modulate the gut microbiome, it will be possible to more successfully mitigate undesirable changes and enhance health.

Recently, there has been an increased focus on the gut microbiome of athletes and its contribution to athlete health and performance [4,5,12]. Many athletes travel extensively to train and compete, facing changes in time zone, stress, diet, and gastrointestinal (GI) disturbances, which may in turn result in gut microbiome alterations [13–17]. The 2020 Olympics in Japan is an upcoming occasion of travel for a range of athletes worldwide. The consideration of the potential implications of travel is important for travelling athletes and their coaches [18].

Gut microbiome composition prior to travel may also be important in relation to the impact of travel on antibiotic resistance gene acquisition during travel [19,20]. Some gut microbiota-associated risks that are particularly enhanced as a consequence of travel include an increased exposure to antibiotic resistance [21]. Additionally, traveller's diarrhoea (TD), a disorder commonly experienced by individuals who travel, in particular to Asia, can result in gut microbiota alterations [22,23].

Anecdotal evidence from Cricket Ireland suggested travel was having a major impact on GI wellbeing, which subsequently impacted performance. This study set out to characterise the gut microbiota of

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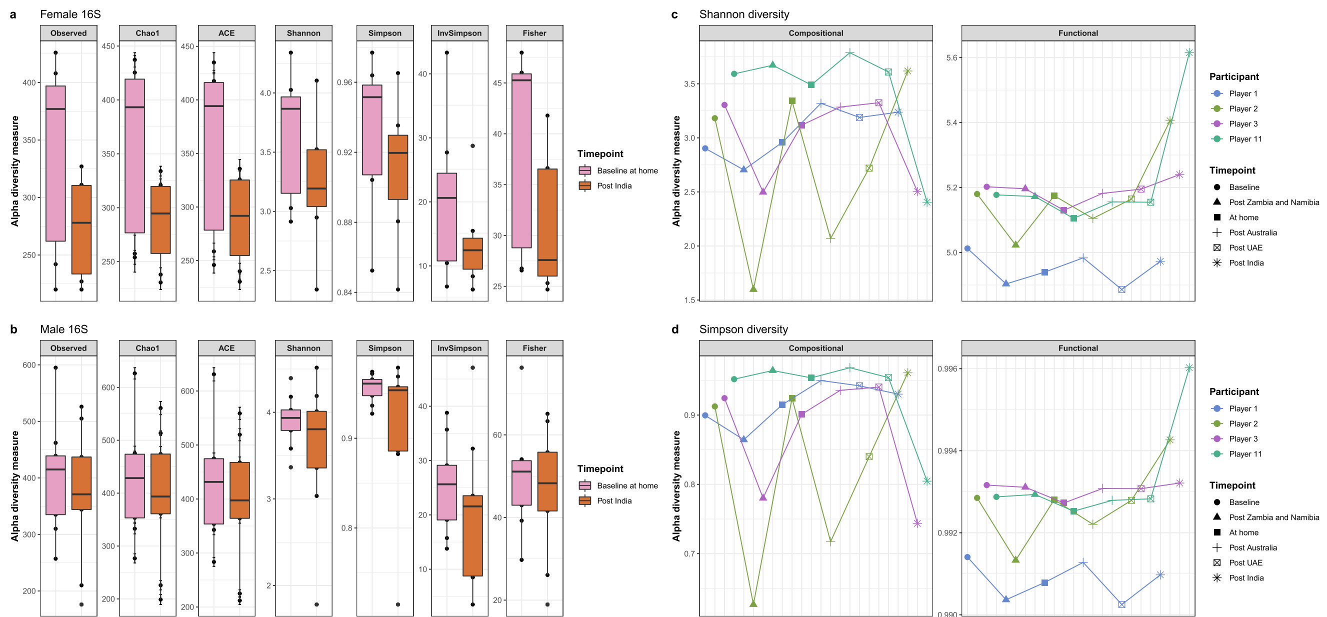

Fig. 1. Alpha diversity fluctuations occur during travel.
Alpha diversity measures for (A) female (N = 7) and (B) male (N = 9) groups at baseline at home (pink) and post-India (orange) time points, from amplicon datasets. (C) Shannon and (D) Simpson alpha diversity measures over time for compositional and functional data for samples sequenced by shotgun metagenomic sequencing (N = 4). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the Cricket Ireland teams during the build-up to the Cricket World Cup of 2016 and, more specifically, to determine if travel was associated with gut microbiota fluctuations. During this period the male team travelled to multiple destinations, i.e., Zambia/Namibia, Australia, United Arab Emirates (UAE), and India, while the female team travelled to India. Faecal samples were collected before and after each journey and subjected to amplicon sequencing. Additionally, samples from a subset of participants underwent shotgun metagenomic sequencing to allow for greater exploration of compositional, as well as functional, changes. While the approach taken does not offer the possibility of discerning between the relative impacts of the many different potential modulating factors involved in long distance travel, it provides an insight into the net impact of such travel on the athlete gut microbiome.

2. Materials and methods

Ethical approval for the study was granted by the clinical research ethics committee of the Cork teaching hospitals. COD, SM, BC, PC, and OOS declare that they have no conflict of interest. Faecal samples were collected from male (N = 14) and female (N = 7) cricket players during the build-up to the 2016 Cricket World Cup using Omnigene-Gut collection kits (DNA Genotek Inc., Ottawa, Canada) to facilitate the stabilisation of the faecal microbiota of samples at room temperature [24,25]. Baseline faecal samples were collected from female participants before travel (Time point LA; N = 7) and following a 30-day tour to India (Time point LB; N = 7). For males, baseline faecal samples were also collected before travel commenced (Time point A; N = 9) and again following travel to Zambia/Namibia (Time point B; N = 11), an extended stay back in Ireland (Time point C; N = 12), and following subsequent travel to Australia (Time point D; N = 12), UAE (Time point E; N = 9), and India (Time point F; N = 11) (supplementary figure A1). Male players stayed at the various destinations, i.e., Zambia/Namibia, Australia, UAE, and India, for 23, 30, 8, and 18 days, respectively.

DNA extraction was completed using the PowerFecal DNA Isolation kit (MoBio laboratories inc., Carlsbad, California, USA) using the altered manufacturer's instructions for use with the Omnigene-Gut collection kits. All samples were prepared for 16S rRNA sequencing on the Illumina Miseq system according to the 16S library preparation workflow. A subset of samples from the 4 male participants who provided samples at all 6 time points (players 1, 2, 3, and 11) were also prepared for shotgun metagenomic sequencing on the Illumina NextSeq system as per modified Nextera XT DNA library preparation protocol (Illumina). All samples were sequenced at the Teagasc sequencing facility. The datasets generated and analysed during the current study are available in the European Nucleotide Archive under the study accession number PRJEB28338.

Resulting FastQ forward and reverse reads from amplicon sequencing were joined using FLASH, clustered into Operational Taxonomic Units (OTU's) and chimeras removed using USEARCH, and taxonomy was assigned using Silva123 [26–28]. Resulting FastQ reads from shotgun metagenomic sequencing were quality checked by first removing contaminating human derived sequences using the NCBI Best Match Tagger, and trimmed and poor quality and duplicate reads were removed using a combination of Picard and SAM tools. Taxonomic classifications were determined using Kaiju [29]. Strain level profiling was completed using StrainEst [30]. Functional profiling was completed using HUMAnN2 [31]. Antibiotic resistance associated genes were determined using the MEGARes pipeline [32]. Reads were also analysed against the 2017 virulence factors marker collection using ShortBRED [33].

Analysis of resulting taxonomic and functional tables was completed in R. Alpha diversities were generated in R using the Phyloseq package for amplicon sequencing data [34]. An UPGMA representation of Bray-Curtis dissimilarities was made using QIIME and visualised using iToL for amplicon sequencing data [35,36]. Bray-curtis distance matrices were computed using the R package vegan for the shotgun dataset [37]. LEfSe was used to determine taxa which characterise specific groups [38]. Binary Jaccard distances were calculated using the R package proxy [39]. Spearman correlations were calculated in R using the RcmdrMisc package (version 1.0–6) and corrected for multiple comparisons using Holms method.

Full materials and methods are available as supplementary document A.1.

3. Results

3.1. Timing of gastrointestinal distress symptoms may influence stability of the gut microbiome

Within sample (alpha) diversity was not found to significantly vary between baseline and final (i.e., post-India) time points (Fig. 1 A and B). Subtle shifts were seen in individuals over time with a mean decrease observed, though not significant (p = 0.1–0.64). Fig. 1 C (Shannon) and D (Simpson) shows shifts in alpha diversity measures across all time points for individuals where shotgun data was available. Fluctuations in alpha diversity measures occurred in all cases, with larger shifts observed within some individuals.

Between group (beta) diversity measured by UPGMA (an unweighted hierarchical cluster method) analysis (amplicon data; supplementary figure A2 C and D), and MDS analysis (shotgun data; supplementary figure A2 A and B), revealed that samples cluster by individual regardless of when they were collected. However, some exceptions were observed in both datasets, with one example being the samples collected from player 11. More specifically, the composition, of both amplicon and shotgun metagenomic sequencing, and functional potential from player 11 corresponding to the post-India sample diverged from that of other samples collected from the same individual.

To assess stability of the microbiome binary Jaccard distances were computed for each pair of samples from the respective male participants to understand subtle changes to community composition (Fig. 2). From the shotgun dataset, at species level, samples from players 2 and 11 can be seen to be variable. Players 2 and 11 reported GI distress symptoms at 3 and 2 time points, respectively. Both of these individuals reported GI distress symptoms at time point E, leading us to postulate that the timing of GI distress, i.e. before travel to India, could result in instability in the gut microbiome composition.

3.2. Individual taxa are unique between baseline and post-India time points

While individual specific responses to travel could be observed from the amplicon dataset (supplementary figure A.3), 13 taxa were identified as discriminatory for the baseline (7 taxa) or post-India (6 taxa) time points (Fig. 3). Differences based on travel time points were only identified in the amplicon dataset, which may be as a consequence of the small number of samples (N = 4) at each of the time points in the shotgun metagenomic sequencing dataset. Discriminatory taxa were only established for the baseline and post-India time points, indicating that perhaps while fluctuations in individual taxa happen during other time points (i.e. post-Zambia and -Namibia, at home, post-Australia, or post-UAE time points) these two time points are the most distinct in terms of composition. This finding may also have been as a consequence of the comparably lower number of samples available for these time points as only male participants travelled to these destinations. *Staphylococcus*, Staphylococcaceae, *Ruminiclostridium* 5, Incertae Sedis Lachnospiraceae, *Erysipelotrichaceae* UCG003, *Ruminococcus* 1, and *Dorea* were found to be associated with baseline samples, while

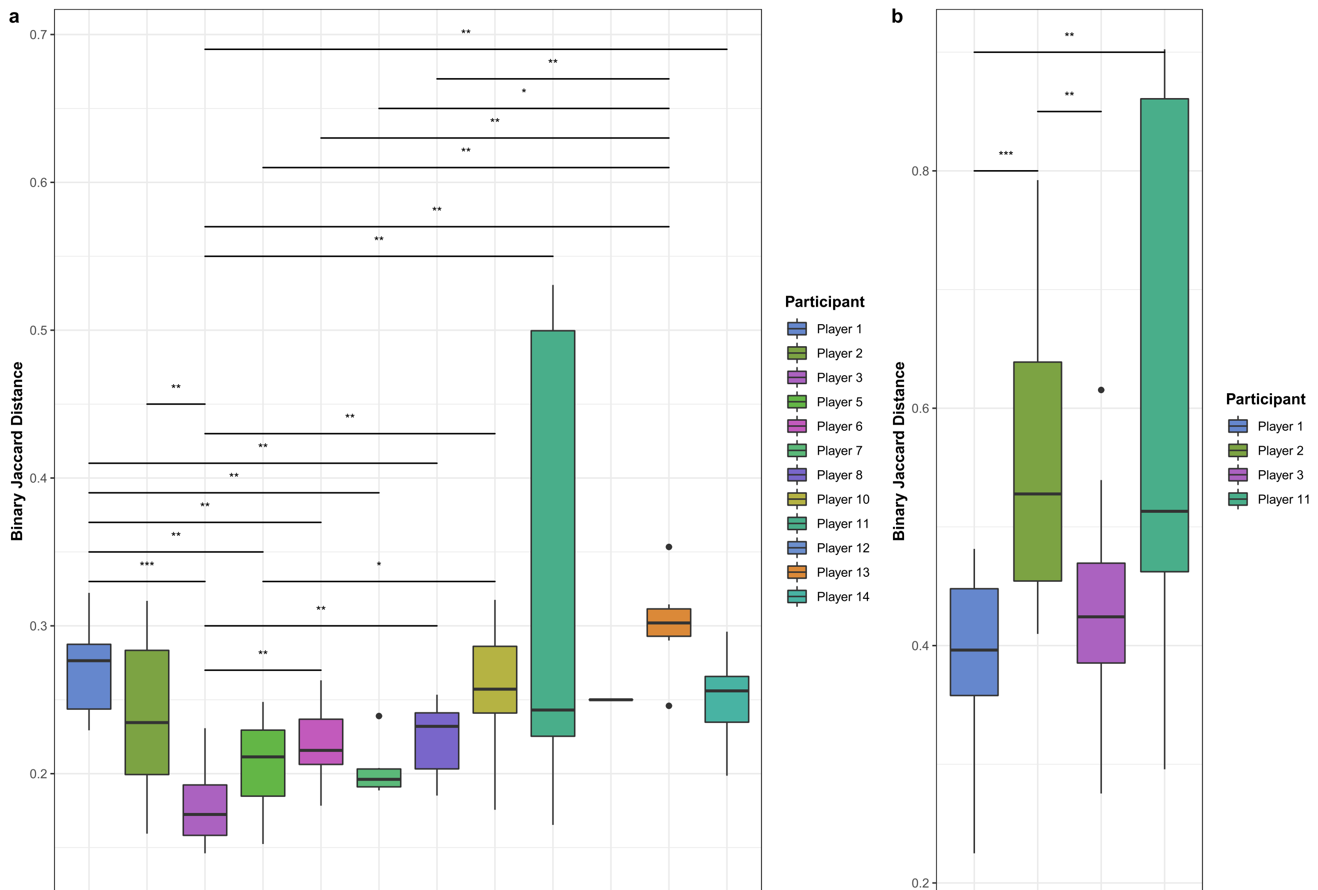

Fig. 2. Individual variability in microbiome stability.
Binary Jaccard distances were calculated between time points available for each individual. Boxplots show distances for each individual in (A) the genera classified in the amplicon sequencing dataset and (B) the species classified in the shotgun metagenomic sequencing dataset. Binary Jaccard distances between individuals were compared using the Kruskal-Wallis test, with a Mann-Whitney *U* test performed for significant results to determine the players between which this difference applied. P values were corrected for multiple comparisons using the Benjamini-Hochberg method. *P $<$ 0.05; **P $<$ 0.01; ***P ≤ 0.001.

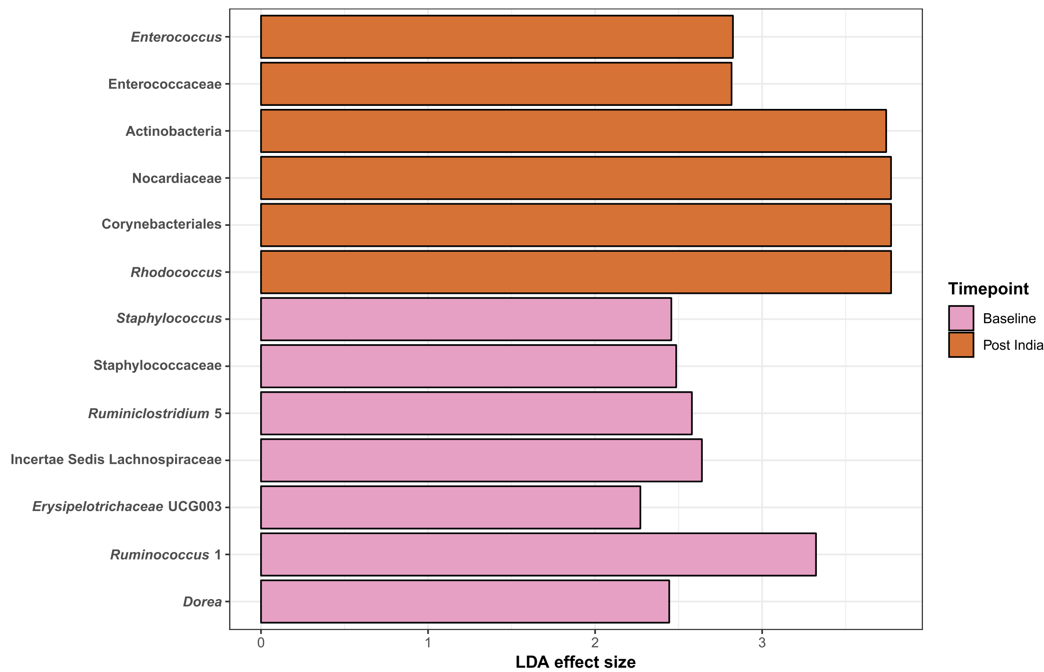

Fig. 3. Significant differences in composition based on time point.
LEfSe plots identifying discriminatory taxa between baseline (N = 16) and post-India (N = 16) time points where both female and male participants are grouped from amplicon sequencing data. An LDA effect size cut off of 2 was used.

Enterococcus, Enterococcaceae, Actinobacteria, Nocardiaceae, Corynebacteriales, and *Rhodococcus* were found to be associated with post-India samples.

Shotgun data allowed a more in-depth analysis of the taxonomic profiles in a subset of samples, with a total of 126 species being identified across these 24 samples (Fig. 4 A). Taxonomic profiles based on shotgun metagenomic sequencing data were found to be individual specific. Of particular interest, *Escherichia coli* was identified in samples F011 and F002 (i.e. post-India samples from players 2 and 11). These samples were of particular interest as they did not cluster with other samples from those individuals on an MDS plot (supplementary figure A2 A). To further analyse this result strain level analysis was completed

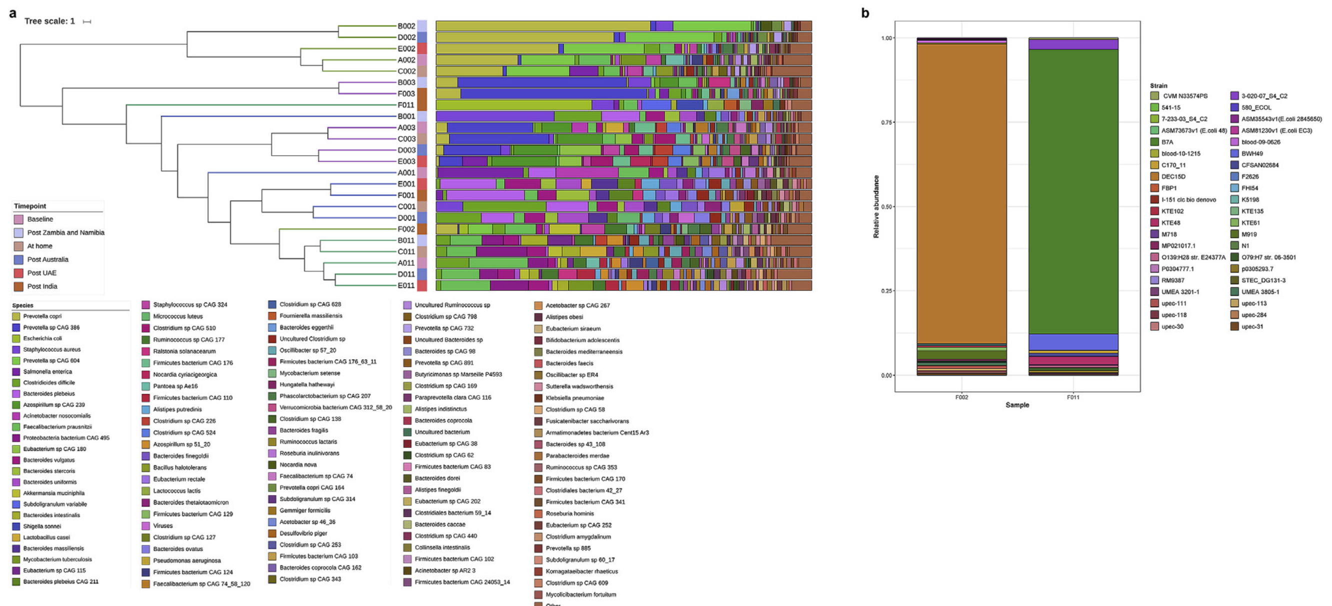

Fig. 4. Shotgun metagenomic sequencing reveals unique *Escherichia coli* strains between participants post-India.
(A) Hierarchical cluster analysis, with species level taxonomic profiles of individual players over time for whom shotgun metagenomic sequencing data was available (N = 4). Individual bars are coloured by participant, while colours of stacked bar alongside tree represent time point at which sample was collected. Individual numbers relate to one player and letters relate to time point (A: male baseline at home, B: male post-Zambia and -Namibia, C: male at home, D: male post-Australia, E: male post-UAE, F: male post-India (see Supplementary Fig. A1 for timeline)). Each bar represents an individual sample. Each colour represents an individual species with only those species which were present at > 1% relative abundance shown, with all others grouped as other. (B) Strain level profiles of *Escherichia coli* in participants 2 and 11 at the post-India time point. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

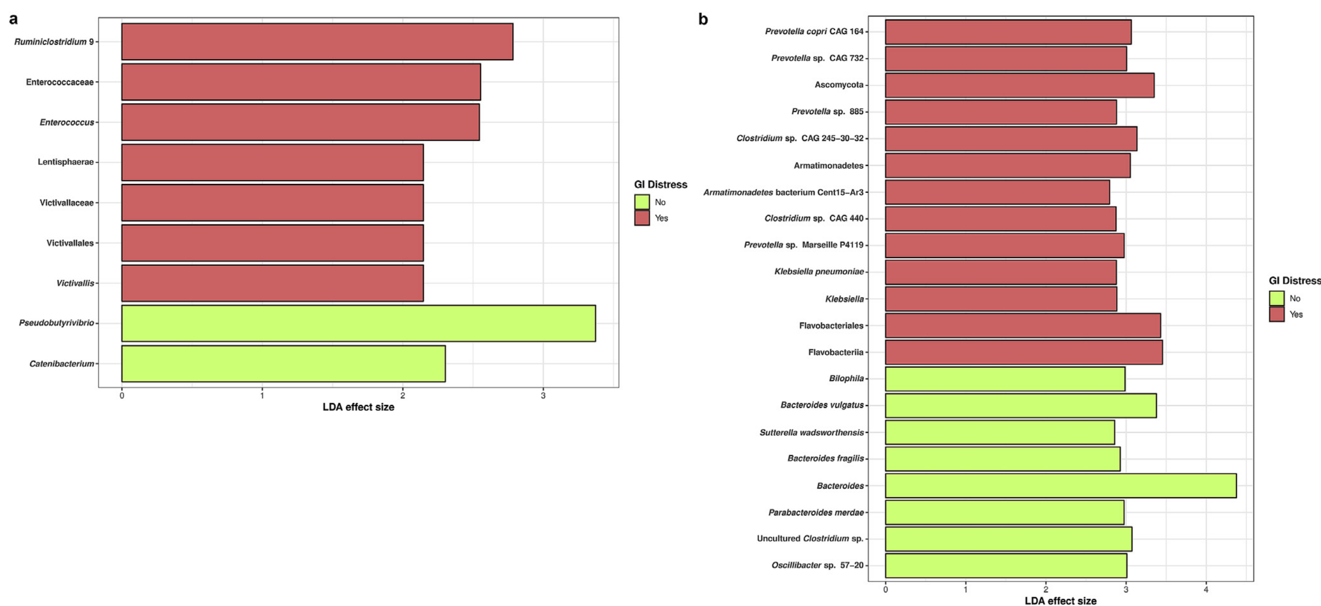

Fig. 5. Significant differences in composition based on reported gastrointestinal distress symptoms.
LEfSe plots identifying discriminatory taxa from (A) amplicon analysis and (B) shotgun metagenomic sequencing, between those who reported (A: N = 10; B: N = 7) and did not report gastrointestinal distress symptoms (A: N = 22; B: N = 17). Taxonomy was assigned to the amplicon dataset using Silva123 and to the shotgun metagenomic sequencing dataset using Kaiju. An LDA effect size cut off of 2 was used.

for *E. coli*. Strain level profiles (Fig. 4 B) reveal that while the *E. coli* population (2.4% of overall relative abundance) of sample F002 (from male participant 2) was dominated by *E. coli* DEC15D (88%), the *E. coli* profile of sample F011 (41.5% overall relative abundance) was dominated by *E. coli* B7A (84%), with only one strain (p0305293.7) shared between these two samples, indicating that while the time point of acquisition may have been shared, the source may have varied between these two individuals.

3.3. Individual taxa are associated with gastrointestinal distress symptoms

LEfSe revealed 20 taxa which were associated with GI distress (Fig. 5), 7 of these were identified from amplicon analysis (A), while 13 were identified from shotgun metagenomic sequencing analysis (B). *Ruminiclostridium* 9, Enterococcaceae, *Enterococcus*, Lentisphaerae, Victivallaceae, Victivallales, and *Victivallis* were found to be associated with reported GI distress from the amplicon dataset. Interestingly, crossover was not observed between those taxa identified as being associated with GI distress from the shotgun metagenomic sequencing dataset; this may be as a result of the variance in the databases used in each of these analyses. Ascomycota, *Clostridium* sp. CAG 245-30-32, Armatimonadetes, *Armatimonadetes* bacterium Cent15-Ar3, *Clostridium* sp. CAG 440, *Klebsiella pneumoniae*, *Klebsiella*, Flavobacteriales, Flavobacteriia, and 4 species of *Prevotella* (namely *P. copri* CAG 164, *P.* sp. CAG 732, *P.* sp. 885, and *P.* sp. Marseille P4119) were identified as being associated with reported GI distress in the shotgun metagenomic sequencing dataset.

3.4. Antibiotic resistance potential is altered as a consequence of travel to India

Overall alterations in gut microbiome functional potential were observed with travel. Further investigations analysed antibiotic resistance and virulence genes, as these are of particular relevance to travel medicine. Players 2 and 11 showed an increase in the number of antibiotic resistance genes identified at the post-India time point (Fig. 6: F002 and F011). Prior to this time point antibiotic resistance remained individual specific across both of these participants. Post-India, resistance potential to 8 antibiotic classes was seen to increase in both of these participants, while in addition resistance potential to a further 4 antibiotic classes was seen to increase in player 11 at this time point.

Notably, players 2 and 11 reported GI distress symptoms at the post-UAE time point (E). While GI distress symptoms were reported at other time points (player 2 at time points B and D; player 3 at time points D and F; and player 11 at time point E), no increase in antibiotic resistance potential was observed following these events. It is possible that the period of GI distress symptoms combined with other factors encountered during travel to India contributed to the acquisition of antibiotic resistance genes. It also is important to note that player 11 had received antibiotic treatment in the three months prior to the collection of sample F. However, no antibiotic usage was reported for player 2. A corresponding investigation of virulence marker genes also identified changes here, in particular in post-India samples (supplementary figure A4 A and B).

4. Discussion

Individuals, who travel on a regular basis, including athletes, are susceptible to stress, time zone changes, alterations in diet, and symptoms such as travellers' diarrhoea (TD) [17,40,41]. While the impacts of these factors on performance have been variable, associations between international travel with injury potential and reduced performance have been identified [42–46]. Identification of undesirable changes that occur within the gut microbiota during travel might result in the development of therapeutics to maintain health. To this end, this pilot study of 21 individuals was carried out and a number of notable changes in microbiome composition, diversity, and functional potential were observed.

The main finding of this research was the establishment that a specific event, in this case travel to India, can have a significant impact on gut microbiome composition and functional potential, with associated increases in antibiotic resistance and virulence genes. Other destinations were not identified in this study as having a significant impact on the gut microbiome; however, this finding may be as a result of a larger number of samples available for baseline and post-India time points (i.e., samples from both female and male groups), in comparison to other time points.

Relative stability in the human gut microbiome is expected over

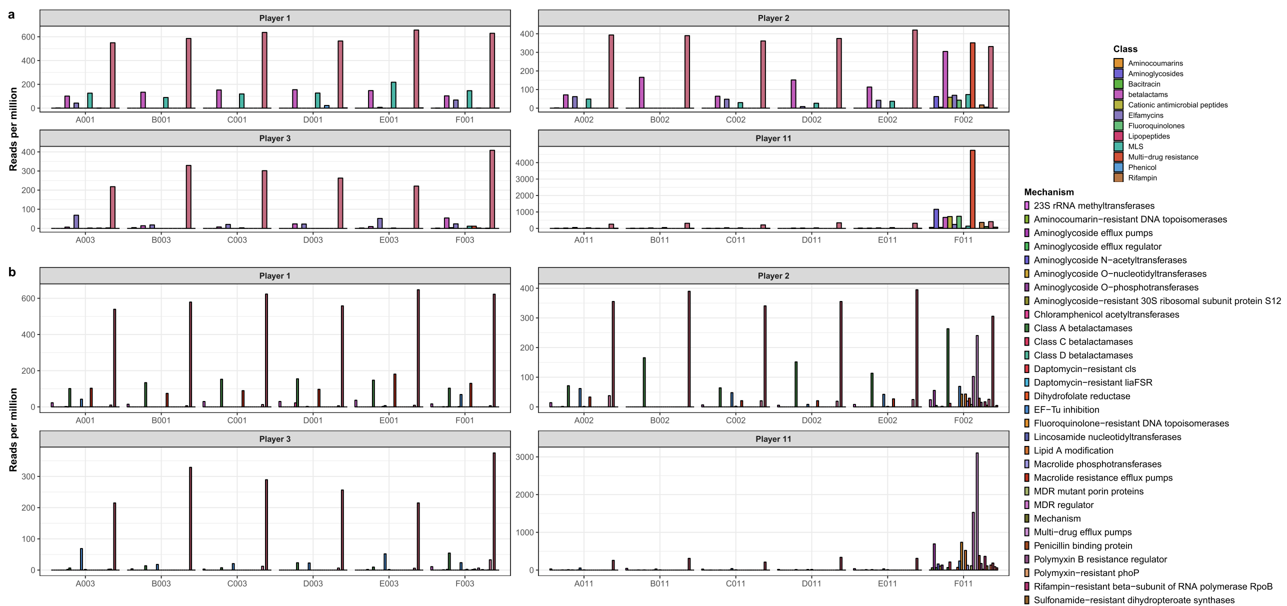

Fig. 6. An increase in antibiotic resistance potential is seen in some individuals post-India.
Antibiotic resistance potential over time characterised using resistome analyser. (A) Resistance to antibiotic classes by participant. (B) Mechanisms of action of antibiotic resistance potential by participant.

time, with perturbations occurring for example with disease states and antibiotic use. However, the microbiome is also resilient allowing for the return to a stable composition in most situations [47]. Here, for the majority, microbial stability is observed, however in certain individuals instability of the gut microbiome was observed. This is consistent with very many studies that have established the stability and resilience of the gut microbiota over time [48–50]. In order to establish an understanding of more subtle, individual shifts in the microbiome over time, binary Jaccard distances (used to estimate stability) were computed. Timing of reported GI distress symptoms could potentially be an important factor in any instability noted in the gut microbiome, with symptoms reported prior to travel to India potentially related to instability post-India. As such a small number of participants fit into these criteria (2 participants), it is possible that any other number of factors is influencing these results. Other influencing factors which cannot be accounted for here include individual variability and dietary impacts. Potential alterations in stability may be associated with the changes observed in taxa and antibiotic resistance profiles, with gut microbiome instability previously associated with diarrhoea, IBS symptoms, and *Clostridium difficile*-associated disease [47].

Although travel was found to only induce minimal shifts in the gut microbiome overall, taxa were identified which were significantly altered at baseline and post-India time points, while another group of taxa were found to be significantly altered with reported GI distress. Actinobacteria, found in this study to be associated with the post-India time point, has previously been identified as increased in IBS [51], potentially indicating this change could put these individuals at risk of post-infectious IBS [52]. Of interest, *Ruminococcus* was identified as being associated previously with the ability to clear antibiotic resistance post travel, while in this dataset it was associated with baseline samples, a potentially beneficial finding [19]. Such a relationship could not be identified in this dataset as it was not possible to collect samples following an extended period at home post-India.

Noteworthy, from the shotgun dataset, was an increase in *E.* coli identified in two participants following travel to India, with these individuals reporting GI distress at the pre-India time point (i.e. post-UAE). *Escherichia* species are among those most frequently identified as causative for TD and so investigation of this was of particular interest in this cohort [23]. Strain level differences in *E. coli* were established between players 2 and 11, with DEC15D and B7A found to be dominating members, respectively. *E. coli* DEC15D was sequenced as part of a collection of diarrheagenic *E. coli* strains which are a major cause of diarrhoea, while *E. coli* B7A is from the B1 subgroup which contains many commensals but also a number of pathogens [53–55]. This indicated that it is possible that these strains may have been related to GI distress symptoms experienced by these individuals.

Klebsiella was identified in the shotgun metagenomic sequencing dataset as being associated with reported GI distress, which has previously been identified in TD [23]. *Prevotella* species were identified as being associated with reported GI distress, with *Prevotella* previously associated with diarrhoea predominant IBS, TD, and more abundant with loose stool [19,56,57]. Of interest, *P. copri*, one of the species identified in this dataset as associated with GI distress, has previously been identified as more abundant in those individuals who experienced TD even before travel [19]. This pattern was evident to some extent in our study (i.e. player 2) but would require a shotgun metagenomic investigation of a larger cohort to establish its significance.

Acquisition of antibiotic resistance potential and virulence marker genes were explored in the shotgun data, as previous studies have outlined the potential of acquiring antibiotic resistance during travel, while the presence of virulence marker genes could reveal regions where acquisition of potential pathogens is particularly increased [21,22,50,58]. Acquisition of antibiotic resistance related genes seemed to be individual specific, with players 2 and 11 acquiring resistance to a number of new antibiotic classes and also an array of mechanisms of antibiotic resistance activity. Previous studies into the acquisition of antibiotic resistance during travel have mainly focused on extended spectrum β-lactamases (ESBLs) producers, with an increase seen post-travel across a number of studies [59–63]. Individuals have been found to be at increased risk of acquiring antibiotic resistance genes if travel destination is in Asia (particularly India), antibiotics are used during travel, TD persists after return from travel, and/or as a consequence of consuming certain foods during travel such as ice-creams and pastry [60–62]. Travel to India, GI distress, and antibiotic usage could have been contributing factors in the acquisition of antibiotic resistance potential in this cohort. Antibiotic resistance is a major global health problem and the acquisition of antibiotic resistance genes during travel could impact on global carriage of antibiotic resistance genes [19].

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Carriage of antibiotic resistance genes has the potential to persist for years and have the potential to transfer to pathogenic bacteria, increasing the risk of acquiring and harbouring an antibiotic resistant infection [64].

Analysis of virulence marker genes revealed an increase in the alpha diversity of these marker genes in players 2 and 11 post-India, with a notable shift in beta diversity also observed for these samples. Interestingly, in this subset of individuals travel to India resulted in an increase in nine, individual virulence marker genes. Although the presence of these genes doesn't necessarily represent functionality or pathogenicity, increases in proportions of virulence and antibiotic resistance genes following travel is a concern and merits further investigation in the future. Presence of virulence factors in the GI tract could result in intestinal infections including diarrhoea [55], and thus presence of these virulence genes could be related to reported GI distress.

While this study only includes a small number of participants, owing to the number of travelling members of the Cricket Ireland teams available and willingness to participate, it further explores the influence of travel on the gut microbiome. While in this study post-Zambia and -Namibia, at home, post-Australia, or post-UAE time points were not found to be associated with major alterations this could be as a result of the numbers available and so further studies with a larger number to these destinations may offer differing results. This study identifies travel to India, and in particular travel to India following GI distress symptoms, as associated with disturbances in the gut microbiome from both a compositional and functional potential perspective. Previously, travel to India has been identified as having a major impact on the gut microbiome, as has GI distress during travel [60–62].

GI disturbances pre-travel to high risk destinations (e.g. India) could potentially place individuals at an increased risk of gut microbiome alterations, including the acquisition of antibiotic resistance genes, and so individuals falling into this category should in particular take care. Probiotics have been shown to be beneficial in the prevention of TD if taken 4 weeks prior to travel. However, as studies vary by strain(s) used it is not yet possible to recommend one overall option [46,65]. However, none of the investigations to date have been completed in travelling athletes. Although not investigated in this study, diet changes during travel may impact on the gut microbiome. To this end, travelling individuals should take care to avoid those foods associated with the development of TD including but not limited to any raw and unpeeled produce, local water, uncooked meats, and unpasteurised dairy products [46,66].

5. Conclusion

Here we have identified one travel period, travel to India, as having a significant impact on gut microbiome composition, as well as an alteration in the antibiotic resistance and virulence genes present. While individual fluctuations were identified, taxa associated both to pre- and post-travel time points, as well as experienced GI distress, could be identified. Individual, pre-travel insults to the GI tract, such as reported distress or antibiotic usage, could potentially influence the impact of travel on the gut microbiome. These changes identified during travel are of particular relevance and interest to athletes travelling for competition, such as for the upcoming 2020 Olympics [18]. Consideration should be given to potential gut microbiome changes during travel to reduce potential risk factors and the potential negative impact of these identified changes. Ultimately, it is hoped that a greater appreciation of the negative impacts of travel on the gut microbiome of athletes, and other travellers, can reveal interventions that can minimise undesirable health consequences.

CRediT authorship contribution statement

Ciara M. O' Donovan: Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Brendan Connor:** Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing - review & editing. **Sharon M. Madigan:** Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing - review & editing. **Paul D. Cotter:** Conceptualization, Methodology, Validation, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Orla O' Sullivan:** Conceptualization, Methodology, Software, Validation, Investigation, Data curation, Writing - review & editing, Supervision, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2020.101553.

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