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Evaluation of changes in the faecal resistome associated with children's exposure to domestic animals and food animal production

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Abstract

Objectives: The paediatric gut microbiota is a reservoir of antimicrobial resistance genes. Environmental factors such as a child's exposure to faecal contamination and antimicrobial resistance genes of animal origin likely shape the resistome of infants and children. This study measured how different levels of exposure to domestic or food animals affect the structure of the intestinal resistome in children between 1 and 7 years of age.

Methods: One hundred nineteen faecal samples from 39 children were analysed according to the level of exposure to domestic or food animals and categorized into three risk groups. Using high-throughput sequencing with an Illumina NovaSeq 6000 SP platform, we performed faecal resistome analyses using the ResFinder database. Additionally, ResistoXplorer was used to characterize the resistomes of children differentially exposed to domestic animals.

Results: Our data indicated that specific antimicrobial resistance genes such as those that confer resistance to MATFPR (macrolide, aminoglycoside, tetracycline, fluoroquinolone, phenicol, and rifamycin) and tetracyclines were statistically less abundant in the group of children without exposure to animals (group 2), compared with the groups exposed to domestic and food animals (groups 1 and 3). However, the overall resistome structure among the children was not affected by the different levels of exposure to animals.

Conclusions: This study suggests that animal exposure is a risk factor for young children acquiring specific antimicrobial resistance genes from domestic animals or animal production areas. However, the overall resistome structure was not affected.

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Competing interests
None declared

Ethical approval
The Ethics Committee for Research in Human Beings at the Universidad San Francisco de Quito USFQ (code 2017–178M) and the Committee for Protection of Human Subjects (CPHS) at the University of California, Berkeley (Federalwide Assurance #6252) approved the study. The parents or guardians signed the informed consent form, and the children, who were old enough, consented once the research protocol was fully explained.

Keywords

Gut resistome; Children; Domestic animals; Food animal production; Ecuador

1. Introduction

The gut microbiota is a densely populated ecosystem and an essential reservoir of antimicrobial resistance genes (ARGs) [1]. Even children who have not recently been exposed to the selective pressure of antimicrobials are found to have a large and diverse collection of ARGs [2]. The gut resistome (collection of resistance genes in the intestinal microbiota) is enriched and shaped by environmental factors, including diet, certain drugs [3], and the exposure to antibiotics used clinically and during animal production according to the geographical context in which they live [4]. It has been reported that cohabitation of humans with domestic animals in the agricultural context, and the improper handling of excreta, favour the exchange of ARGs between humans and animals in rural areas [5]. Thus, it has been shown that phylogenetic composition and resistance determinants of the human faecal microbiota are more similar to the microbiota of soil close to chicken coops than to the soil of any other place in rural areas [5].

In particular, infants and young children in rural areas in low- and middle-income countries (LMIC) where small-scale animal production is commonly practiced are exposed to zoonotic enteric pathogens due to coprophagia or through various exposures such as hands, toys, cutlery, flies, and food [6]. In semi-rural communities near Quito, Ecuador, a study found that several third-generation cephalosporin-resistant Escherichia coli strains isolated from faeces of young children, dogs, and chickens had acquired different resistance determinants and replicons. However, the authors found clonal transmission of this bacterial species between humans and domestic animals [7]. Therefore, in this analysis, we hypothesized that children who live near animal production areas or own domestic animals have a richer and more diverse resistome than those who live in households that are not exposed to animals.

2. Materials and methods

2.1. Study population and faecal samples

The present study was conducted from August 2018 to September 2021 in peri-urban and rural areas east of Quito. The parishes of Tumbaco, Puembo, Tababela, Pifo, Yaruquí, Checa, and El Quinche are characterized by agricultural activities and small- to large-scale animal production, particularly chickens. Children aged 1 to 7 years were included, and three risk groups for exposure of children to domestic and food animals were established. Risk group 1: children who lived more than 500 meters away from chicken operations and who had domestic animals at home (n = 18, 44 faecal samples); risk group 2: children who lived at a distance greater than 500 meters from chicken operations and did not own domestic animals (n = 15, 38 faecal samples), and risk group 3: children who lived less 500 meters away from chicken operations and had domestic animals at home (n = 13, 37 faecal samples) (Fig. 1A). Thirty-nine children were included in the present study. The

caregivers were instructed to collect and store child faecal samples [7] until field staff could pick them up in a few hours and transport them at approximately 4°C to the laboratory. The caregivers delivered between 1 and 5 serial samples from 5 months to 1 year apart between collections. One hundred nineteen faecal samples were obtained and stored at −80°C until further analysis.

2.2. DNA extraction and sequencing

Five hundred milligrams of faecal material from each sample was used for the FastDNA™ SPIN Kit for Soil (MP Biomedicals) to obtain 60 μL of DNA stored at −20°C until further analysis. The quality and quantity of the DNA were measured with a Nanodrop (NanoDrop ND-2000, Thermo Scientific) using the absorption ratios of 260/280 and 260/230. The DNA was lyophilized and sent to the high-throughput sequencing facility at the University of North Carolina in Chapel Hill, NC, where the library preparation (KAPA libraries using the Mantis system) and the control quality were carried out. The Illumina NovaSeq 6000 SP platform was used for sequencing and generated 2 × 150 bp paired-end sequences.

2.3. Bioinformatic analysis

The child faecal resistome analysis protocol followed the AM-rPlusPlus pipeline (https://megares.meglab.org/amrplusplus/latest/html/). Briefly, the raw sequences were processed to improve their quality and remove contamination using the FastQC and Trimmomatic tools, respectively [8,9]. We removed the host genome to conduct the resistome analysis using the Resistome Analyzer tool (https://github.com/cdeanj/resistomeanalyzer) together with the ResFinder database [10]. This last step generated tables of short read counts assigned to a specific resistance gene. Only reads with 80% or more identity threshold in the gene fraction [11] were included in the subsequent analyses.

2.4. Statistical analysis

The graphical representation and statistical analyses were carried out using the web tool ResistoXplorer [12]. Before applying any statistical tests, the data were filtered to remove low-quality data. Subsequently, the cumulative sum scaling (CSS) method [13], popularly used in analysing metagenomic data, was used. We applied the diversity measures with the Chao1, Pielou's evenness, and Shannon indices applying the Kruskal-Wallis statistical method for the alpha diversity analysis. We used the nonmetric multidimensional scaling (NMDS) ordination method with the Bray-Curtis index and the permutational multivariate analysis of variance (PER-MANOVA) statistical method for the beta diversity analysis. For the differential abundance analysis of ARGs, we used edgeR [14] with the Trimmed Mean of *M* values (TMM) method [15] to normalize the data. Significant differences were declared with a *P* value <0.05, and in the case of differential abundance analysis, adjusted *P* values (false discovery rate [FDR]) <0.05 were used by applying the Benjamini–Hochberg method [16].

3. Results and discussion

In the 119 faecal samples analysed, 339 different ARGs were found that confer resistance to 8 classes of antibiotics (Fig. 1B). The most abundant ARGs conferred resistance to

tetracyclines (75%), MLS_B (macrolide, lincosamides, and streptogramin B) (9%), and beta-lactams (8%). The relative abundance profile showed that ARGs conferring resistance to tetracyclines, lincosamides, and MATFPRs (macrolide, aminoglycoside, tetracycline, fluoroquinolone, phenicol, and rifamycin) were less abundant in risk group 2 compared to the other two risk groups. In risk group 2, however, resistance to macrolides, MLS_B, and MS_B (macrolide and streptogramin B) was more abundant compared to the risk groups 1 and 3. A differential abundance analysis confirmed that ARG alleles conferring resistance to MATFPR (*mdf*(A)*_1_ Y08743*, FDR <0.001) and to tetracyclines (*tet*(O/W)*_1_AM889118*, FDR <0.05, and *tet*(32)*_2_EF626943*, FDR <0.05) were statistically less abundant in group 2, compared with group 1 and 3. On the other hand, the ARG allele against MLS_B (*erm*(G)*_1_M15332*, FDR <0.001) was significantly more abundant in the group of risk 2 compared to the other two risk groups.

The alpha diversity analysis showed no significant differences in ARGs' richness, evenness, and diversity between the three risk groups (Fig. 2A). Also, when we carried out an ordination analysis to compare the dissimilarities between faecal samples, it was observed that there were no significant differences between faecal samples based on the children's faecal resistome (Fig. 2B).

In this study, we explored whether cohabitation with domestic animals and closeness to animal production areas were related to changes in the intestinal resistome of children between 1 and 7 years of age. Our findings indicated that whole children's faecal resistome structure did not change due to different levels of exposure to domestic and food animal production. However, there were specific resistance genes (*mdf*(A), *tet*(O/W), *tet*(32), *erm*(G)) whose abundance were significantly different between risk groups.

Similarly, a previous report that evaluated the faecal resistome of 1-year-old children shows that the ARGs that confer resistance to tetracyclines and beta-lactams are the most frequent and abundant [17]. However, the authors also frequently found ARGs that confer resistance to fluoroquinolones, unlike our finding where we found ARGs against MLS_B. Genes that confer resistance to tetracyclines (*tet*(O/W) and *tet*(32)) were significantly less abundant in the group of children who do not own domestic animals and live far from chicken operations. It can be explained by the fact that tetracyclines are one of the most used antibiotics worldwide in veterinary medicine for the prevention and treatment of diseases and as growth promoters [18,19]. Furthermore, the ARGs *tet*(O), *tet*(W), and *tet*(32) are genes frequently found in the gut of chickens and cattle [18,20]. The relative abundance of *Escherichia coli* was reduced in the risk group 2 compared to groups 1 and 3 (data not shown). Due to *mdf*(A) being a membrane transporter frequently found in *E. coli,* it could explain that *mdf*(A) gene was significantly less abundant in risk group 2 compared with the other two groups.

We have shown that the whole structure of the faecal resistome was not modified by animal–human cohabitation or its proximity to animal husbandry areas. Nevertheless, the proximity of young children to domestic animals poses a risk to the acquisition of certain ARGs. The faecal contamination of the environment where young children live is possibly the most likely source of ARGs of animal origin [7].

Further research is needed to address antimicrobial resistance transmission from a One Health perspective. This preliminary study is the starting point for using metagenomics to study the evolution and transmission dynamics of antimicrobial-resistant bacteria in the human–animal–environment triad in this geographic area.

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Data availability

The sequences were deposited in the European Nucleotide Archive under accession project number PRJEB50568.

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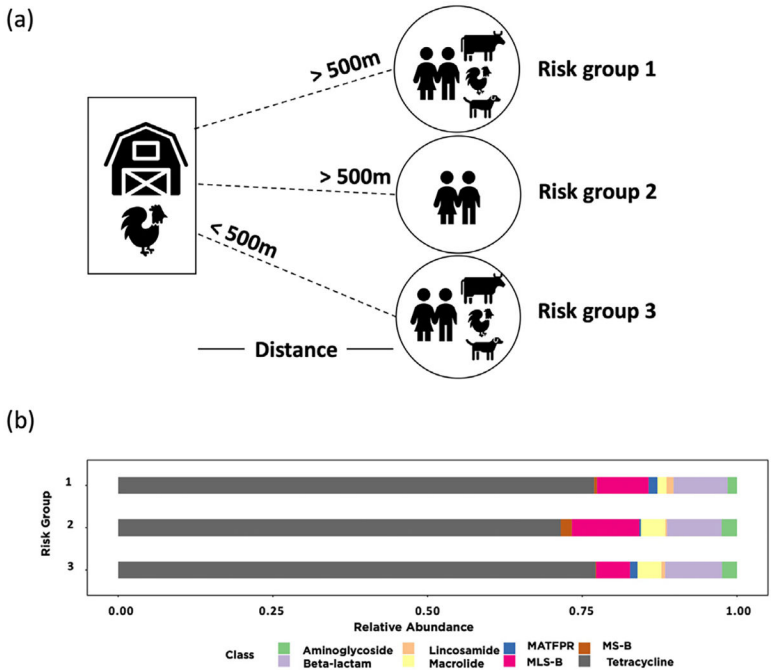

Fig. 1.

Faecal resistome of children in different exposure groups to domestic and farmed animals. (A) Study design and risk groups. (B) Relative abundance in the proportion of faecal antimicrobial resistance genes (ARGs) (classified by class of antimicrobials) grouped in different risk groups.

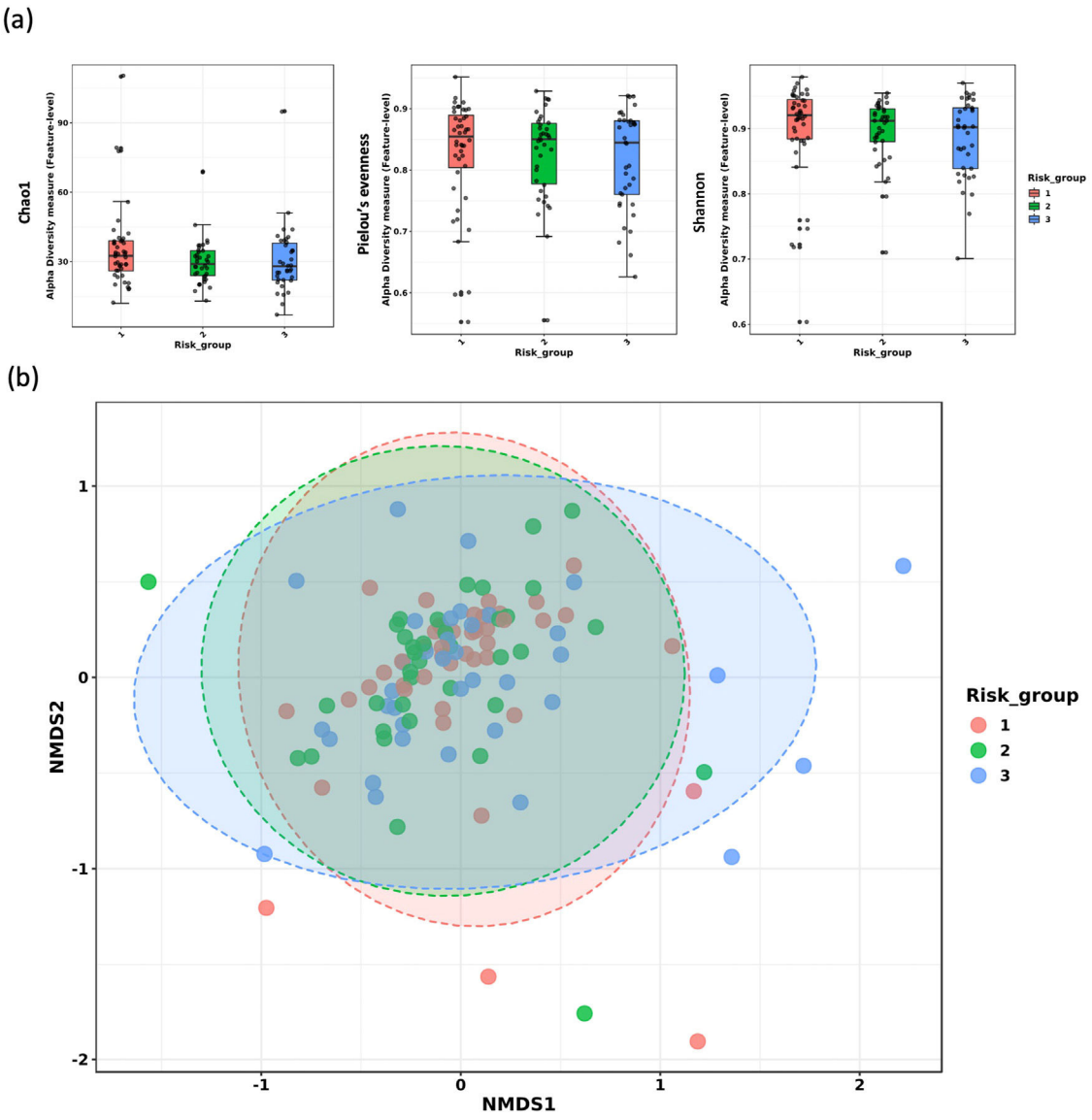

Fig. 2.

Structure of the faecal resistome among the three risk groups based on exposure levels to animals (A). Alpha diversity was measured with the Chao1 (richness), Pielou's evenness (evenness), and Shannon (diversity) indices. Central black horizontal lines indicate median values; the 25th and 75th percentiles are indicated (boxes), and the whiskers extend from each end of the box to the most extreme values. *P* values were obtained using the Kruskal-Wallis test, with $P < 0.05$ as the significance threshold. (B) Nonmetric multidimensional scaling (NMDS) plot based on a Bray-Curtis dissimilarity matrix; significance was obtained using the permutational multivariate analysis of variance (PERMANOVA) test.