




## DATA NOTE

# Data on antibiograms and resistance genes of Enterobacterales isolated from ready-to-eat street food of Ambato, Ecuador [version 1; peer review: 3 approved]

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## Abstract

Foodborne pathogens represent a significant cause of negative impacts on human health and the economy worldwide. Unfortunately, information about epidemiological insights in Latin American countries is scarce. The consumption of ready-to-eat street food in Ecuador is extensive, and information about the presence of foodborne pathogens, their virulence factors, and antimicrobial resistance is negligible. This data includes the occurrence, phenotypic antibiotic resistance profiles, and antibiotic resistance genes of Enterobacterales isolated from ready-to-eat street food in Ambato, central Ecuador during 2020 and 2021. The most common genera detected were *Escherichia coli*, *Klebsiella spp.*, and *Cronobacter spp.* Agar disk diffusion assays were performed to determine their phenotypic resistance. The presence of antibiotic resistance genes conferring resistance against colistin,  $\beta$ -Lactams, aminoglycosides, tetracyclines, sulfonamides, fluoroquinolones, and amphenicols was detected via polymerase chain reaction (PCR) amplification.

## Keywords




antibiotic resistance, enterobacterales, escherichia coli, street food, food microbiology



This article is included in the **Antimicrobial Resistance** collection.

## Open Peer Review

Approval Status 

	1	2	3
<b>version 1</b> 17 Jun 2022	 <a href="#">view</a>	 <a href="#">view</a>	 <a href="#">view</a>

1. **Laura Sala-Comorera** , UCD Earth Institute, Dublin, Ireland  
University College Dublin, Dublin, Ireland
2. **Edgar Gonzalez-Villalobos** , Catalan Institute for Water Research (ICRA), Girona, Spain
3. **Erika A. Rodriguez** , University of Antioquia, Medellín, Colombia

Any reports and responses or comments on the article can be found at the end of the article.

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**Author roles:** **Tubón J:** Data Curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – Original Draft Preparation; **Barragán-Fonseca G:** Formal Analysis, Investigation, Writing – Original Draft Preparation; **Lalaleo L:** Investigation, Writing – Original Draft Preparation, Writing – Review & Editing; **Calero-Cáceres W:** Conceptualization, Data Curation, Funding Acquisition, Investigation, Methodology, Resources, Supervision, Validation, Writing – Original Draft Preparation, Writing – Review & Editing

**Competing interests:** No competing interests were disclosed.

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## Introduction

This data contributes information about the antibiotic resistance profiles of Enterobacterales strains isolated from street food that will facilitate pathogen surveillance in Ecuador and Latin America. This data is useful for the scientific community to determine the presence of pathogenic *Escherichia coli* isolates and antibiotic resistance genes, including mobile colistin resistance genes, carbapenemases, quinolone resistance genes, and extended-spectrum  $\beta$ -lactamases present on Enterobacterales strains isolated from street food. Researchers and policymakers involved with the work related to the One Health initiative could also benefit from this data for retrospective and comparative analysis or epidemiological surveillance projects.<sup>1,2</sup>

## Methods

### Enterobacterales strains

Ready-to-eat street food was obtained in the streets of the city of Ambato, Ecuador, and processed the same day. A sharp sterile blade was used to cut the samples on sterile surfaces. 10 g of each sample was placed in sterile brain heart infusion broth (BHIB) (Merck, Darmstadt, Germany) in 90 ml, shaken on a rotator for 8-10 min, and incubated for 24 h at 37 °C. A large amount of broth was inoculated on MacConkey agar plates (Merck, Darmstadt, Germany), Cromocult Coliforms Agar (Merck KGaA, Darmstadt, Germany), and CHROMagar mSuperCARBA were incubated overnight at 37 °C under aerobic conditions. Further purification was performed on Macconkey agar.

The isolates were amplified by polymerase chain reaction (PCR), analysed using agarose gel electrophoresis and visualised with Sybr Safe DNA Gel Stain.<sup>3</sup> For the identification of the isolated Enterobacterales, biochemical tests such as catalase, oxidase, TSI agar, Simmons citrate, lactose test, indole production, urea agar, methyl red test, and Voges-Proskauer were carried out and their interpretation was performed based on Bergey's manual.<sup>4</sup> Additionally, the software for Automated Biometric Identification Systems (ABIS) was used to confirm the biochemical identification results.

### Phenotypic antibiotic resistance profiles

Agar disk diffusion assays (Thermo Scientific Oxoid and Bioanalyse) on Mueller-Hinton Agar (Thermo Scientific Oxoid) were performed. Antibiograms tests were based on the measured diameter of the zones of inhibition and interpreted as sensible, intermediate or resistant by referring to CLSI breakpoints.<sup>5</sup>

### Detection of *E. coli* pathotypes and antibiotic resistance genes detection via PCR

The PCR test was performed according to the standardized protocol of the UTA RAM One Health research group<sup>6,7</sup>: 2.5  $\mu$ L of DNA from each sample and 22.5  $\mu$ L of PCR mix containing 12.5  $\mu$ L DreamTaq PCR Master Mix (ThermoFisher Scientific, USA), 9  $\mu$ L Nuclease-free water, 0.5  $\mu$ L Primer 1 and 0.5  $\mu$ L Primer 2 (final concentration of primers: 0.5  $\mu$ M) were mixed to run PCR. The PCR conditions are reported in Supplementary Table S4. PCR products were analyzed by 1.2% agarose gel electrophoresis stained by Sybr Safe DNA Gel Stain (ThermoFisher Scientific, USA).

### Hierarchical clustering

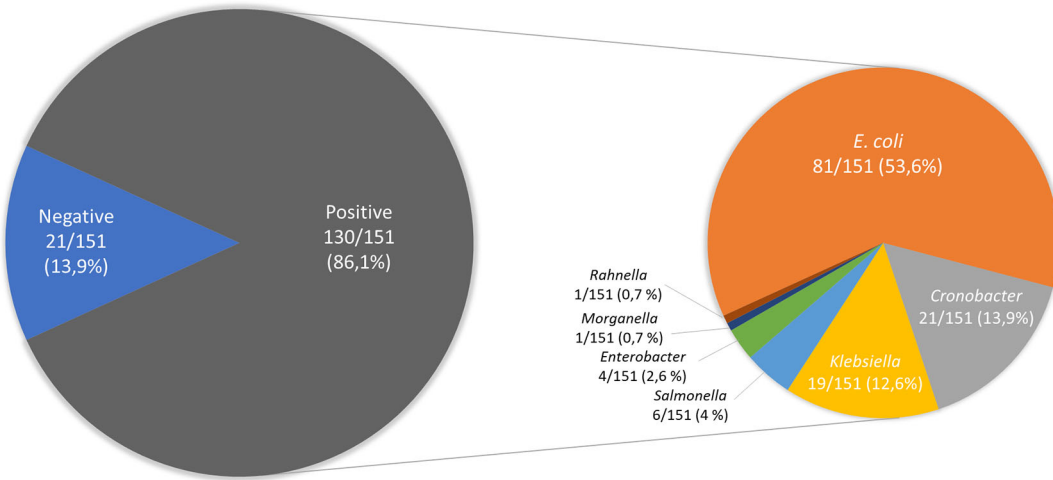
Hierarchical clustering was performed using the Euclidean correlation method and clustered by affinity.<sup>2</sup> The MeV Multiexperiment Viewer software version 4.8.1 was used in this study.

### Dataset validation

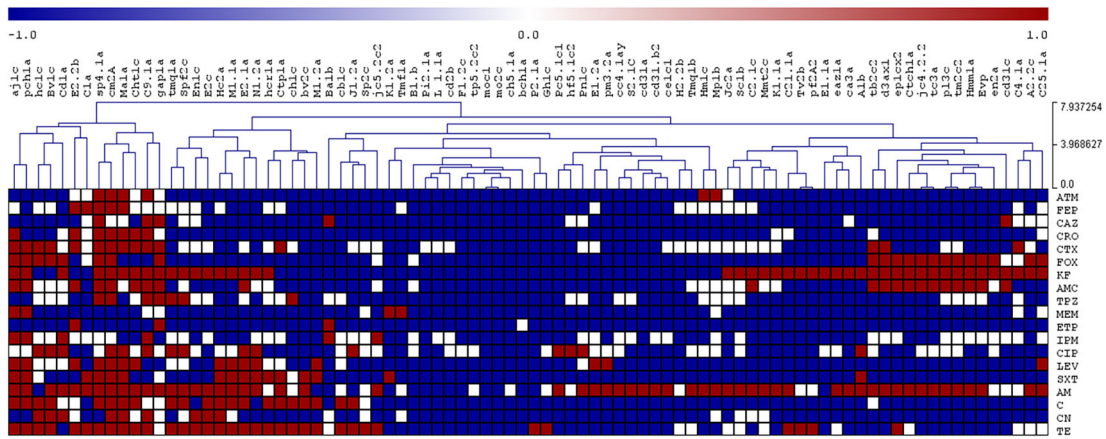
The data presented show the frequency of isolation of Enterobacterales in 151 samples of ready-to-eat street food in Ambato, Ecuador (Figure 1). The specific characteristics (date of sampling, type of street food, location) of the samples were reported in Supplementary Table S1. A total of 145 isolates were analyzed, and the results of the biochemical tests were reported in Supplementary Table S2. Among them, 86 isolates corresponded to *E. coli* and 59 isolates to other Enterobacterales.

To visualize the relative similarity of the antimicrobial resistance patterns of the isolates, a hierarchical cluster analysis was performed using the results of the antibiograms, where the phenotypes 'resistant', 'intermediate', and 'susceptible' were observed as red, white, and blue colors respectively. Dendrograms and clustered data were assembled using the complete linkage method through Pearson correlation and sample leaf organization.<sup>7</sup> For this purpose, the MeV Multiexperiment Viewer software version 4.8.1 was used.<sup>8</sup> Figures 2 and 3 represent the resistance profiles and the hierarchical clustering of *E. coli* and the rest of Enterobacterales, respectively. The complete information is shown in Supplementary Table S3.

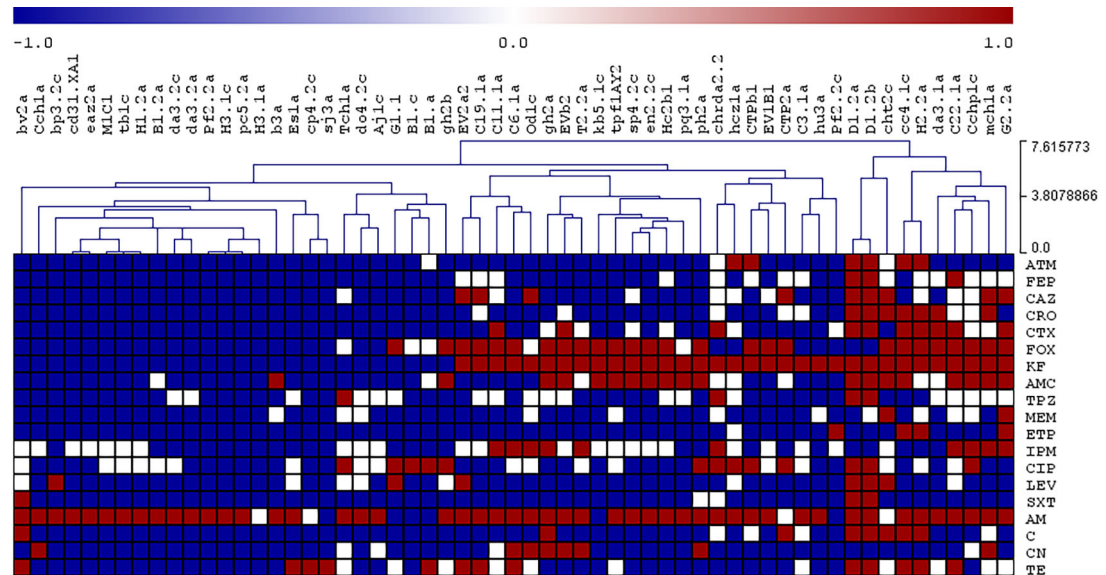
The presence of diarrheagenic *E. coli* pathotypes present in ready-to-eat food was assessed in this study through the analysis of virulence genes related to the pathotypes. Only one isolate (C2.1c) was positive for the eae gene,



**Figure 1. Occurrence of Enterobacteriales on 151 samples of ready-to-eat street food in Ambato, Ecuador.**



**Figure 2. Profiles of antibiotic resistance and hierarchical tree of *E. coli* isolates. Red: resistant, White: intermediate, Blue: sensitive.**



**Figure 3. Profiles of antibiotic resistance and a hierarchical tree of other *Enterobacteriales* isolates. Red: resistant, White: intermediate, Blue: sensitive. Abbreviations: TE: Tetracycline 30 µg, AM: Ampicilin 10 µg, KF: cephalotin 30 µg, C: chloramphenicol 30 µg, CIP: Ciprofloxacin 5 µg, CTX: Cefatoxime 30 µg, LEV: Levofloxacin 5 µg, FOX: Cefoxitin 30 µg, STX: Trimethoprim/sulphamethoxazole 25 µg, AMC: Amoxicillin/ClavulanicAcid 30 µg, CN: Gentamicin 10 µg, CRO: Ceftriaxione 30 µg, FEP: Cefepime 30 µg, ATM: Aztreonam 30 µg, IPM: Imipenem 10 µg, TPZ: Piperacillin/Tazobactam 110 µg, ETP: Ertapenem 10 µg, MEM: Meropenem 10 µg, CAZ: Ceftazidime 30 µg.**

**Table 1. Beta-lactamase resistance genes of *Enterobacteriales* isolated from ready-to-eat food.**

Ready-to-eat food samples	Bacteria	Sample ID	Date	Location market streets	Beta-lactamases	
					<i>bla<sub>TEM</sub></i>	<i>bla<sub>CMY</sub></i>
Cow stomach stew	<i>E. coli</i>	M1.1a	14/12/2020	Mayorista	+	-
Cane juice	<i>E. coli</i>	Jc5.2c1	08/03/2021	Modelo	+	-
Lupine ceviche	<i>E. coli</i>	ch5.1c2	08/03/2021	Modelo	+	-
Cow stomach stew	<i>E. coli</i>	Gap1a	20/04/2021	Sur	-	+
Boiled beans, ulluco and pork rind	<i>E. coli</i>	Hmm1a	10/05/2021	Artisanal	-	+
Chilli sauce	<i>Klebsiella</i> spp	D1.2a	07/12/2020	Mayorista	+	-
Sweet meringue (espumilla)	<i>Salmonella</i> spp	E2.2b	19/01/2021	Primera de Mayo	+	-
Salad from street food	<i>E. coli</i>	N1.2a	20/12/2020	Mayorista	+	-
Total <i>Enterobacteriales</i> isolated					6(145)	2(145)

suggesting the potential presence of enteropathogenic *E. coli* (EPEC) or enterohemorrhagic *E. coli* (EHEC). The  $\beta$ -lactamase resistance genes of Enterobacterales isolated in this study are reported in Table 1. Mobile colistin resistance genes or quinolone resistance genes were not found in the Enterobacterales isolates. The complete information about virulence genes and antibiotic resistance genes are available in Supplementary Table S5. The information about primers and PCR conditions were shown in Supplementary Table S4. The gel electrophoresis images are available at Supplementary figure S6. The disk diffusion assays figures were shown at Supplementary figure S7.

### Data availability

Figshare project: [https://figshare.com/projects/Data\\_on\\_antibiograms\\_and\\_resistance\\_genes\\_of\\_Enterobacterales\\_isolated\\_from\\_Ready-to-eat\\_street\\_food\\_of\\_Ambato\\_Ecuador/137014](https://figshare.com/projects/Data_on_antibiograms_and_resistance_genes_of_Enterobacterales_isolated_from_Ready-to-eat_street_food_of_Ambato_Ecuador/137014)

This collection contains the following underlying data:

Figure 1. Occurrence of Enterobacterales on 151 samples of ready-to-eat street foods in Ambato, Ecuador. figshare. Figure. <https://doi.org/10.6084/m9.figshare.19579087.v1><sup>9</sup>

Table 1. Beta-lactamase resistance genes of Enterobacterales isolated from ready-to-eat food. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.19579099.v1><sup>10</sup>

Figure 2 and 3. Antibiotic resistance profiles and hierarchical trees of Enterobacterales isolated from ready-to-eat street food in Ambato, Ecuador. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.19579267.v1><sup>11</sup>

### Extended data

This collection contains the following extended data:

Supplementary table S1. Characteristics (Sample type, date, treatment type, location, coordinates) of the ready-to-eat food samples. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.19579108.v1><sup>12</sup>

Supplementary table S2. Biochemical tests performed on Enterobacterales isolates from Ready-to-eat Street Food in Ambato, Ecuador. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.19579177.v1><sup>13</sup>

Supplementary table S3. Antibiogram of Enterobacterales isolated from ready-to-eat Street food of Ambato, Ecuador. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.19579189.v1><sup>14</sup>

Supplementary table S4. Primers used in this study and PCR conditions. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.19579198.v1><sup>15</sup>

Supplementary table S5. Antibiotic resistance genes and virulence genes harbored by Enterobacterales isolates from ready-to-eat street food in Ecuador. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.19579207.v1><sup>16</sup>

Supplementary figure S6. PCR results (positive electrophoresis images). figshare. Figure. <https://doi.org/10.6084/m9.figshare.19729618.v1><sup>17</sup>

Supplementary figure S7. Disk diffusion assay images-Antibiotic resistance evaluation of Enterobacterales isolated from ready-to-eat street food of Ambato, Ecuador. figshare. Figure. <https://doi.org/10.6084/m9.figshare.19729630.v1><sup>18</sup>

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### Acknowledgments

The authors would like to thank to the Directorate of Postgraduate Studies of the Department of Food and Biotechnology Science and Engineering, Universidad Técnica de Ambato.

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[Publisher Full Text](#)
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[Publisher Full Text](#)
11. Calero-Cáceres W: **Antibiotic resistance profiles and hierarchical trees of Enterobacteriales isolated from ready-to-eat street food in Ambato, Ecuador.** *figshare.* Dataset. 2022.  
[Publisher Full Text](#)
12. Calero-Cáceres W: **Characteristics (Sample type, date, treatment type, location, coordinates) of the ready-to-eat food samples.** *figshare.* Dataset. 2022.  
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[Publisher Full Text](#)
15. Calero-Cáceres W: **Primers used in this study and PCR conditions.** *figshare.* Dataset. 2022.  
[Publisher Full Text](#)
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[Publisher Full Text](#)
17. Calero-Cáceres W: **PCR results (positive electrophoresis images).** *figshare.* Figure. 2022.  
[Publisher Full Text](#)
18. Calero-Cáceres W: **Disk diffusion assay images-Antibiotic resistance evaluation of Enterobacteriales isolated from ready-to-eat street food of Ambato, Ecuador.** *figshare.* Figure. 2022.  
[Publisher Full Text](#)



# Open Peer Review

Current Peer Review Status:   

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Version 1

Reviewer Report 19 July 2022

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**Erika A. Rodriguez** 

Bacterial Molecular Epidemiology Line, Research Group in Basic and Applied Microbiology (MICROBA), School of Microbiology, University of Antioquia, Medellín, Colombia

The data note by Tubón J et al. aims to give information about the antibiotic resistance profiles of Enterobacterales strains isolated from street food in Ecuador. The work is relevant because, usually in Latin American countries, street food consumption is typical, it has little sanitary regulation, and in some towns, potable water access is limited, which could favor the presence of resistant bacteria in foods. On the other hand, the research gives information about antibiotic resistance in settings different from hospitals and contributes information from Latin American countries where the data is limited. Another relevant point is that the work detects antibiotic resistance Enterobacterales strains in cooked food differently from other studies on lettuce and raw vegetables.

The manuscript is well structured and written. The protocols are appropriate and provide acceptable methods and details to allow replication.

I have provided comments/suggestions as follows:

- I suggest to future reports, the author gives information about the bacteria typification, such as ERIC, PFGE, MLST, or WGS. This information could be help to understand what clones to type of strains are circulating in the region.
- What is the resistance behavior in the Ambato hospitals where the project was carried out? The results could help to understand the resistance problem in the city?
- Any limitations related to this manuscript and/or methods you want to bring up to the reader?
- In the profiles of antibiotic resistance, there are Enterobacterales isolates resistant to carbapenems, and you did not detect carbapenemases. Could you give information about the reason to this resistance, for example, intrinsic mechanisms or other beta-lactamases not detected?



**Is the rationale for creating the dataset(s) clearly described?**

Yes

**Are the protocols appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and materials provided to allow replication by others?**

Yes

**Are the datasets clearly presented in a useable and accessible format?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Bacterial Antibiotic Resistance, Molecular Microbiology, Molecular Epidemiology, Medical Microbiology.

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Reviewer Report 05 July 2022

<https://doi.org/10.5256/f1000research.128952.r141528>

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**Edgar Gonzalez-Villalobos** 

Catalan Institute for Water Research (ICRA), Girona, Spain

In Latin American countries the consumption of street food represents in the majority the alimentary base, due to the conditions that present the most countries belonging to Latin American, for this reason, the information generated by Turbón et al., could facilitate the epidemiology surveillance and allow action before the emergence of foodborne pathogens outbreak.

The authors carry out a complete characterization of a significant number of strains (n=151), highlighting the distribution of pathogens, antibiotic resistance profiles, presence of antibiotic resistance genes.

I suggest reviewing next points:

1. The sentence that refers to "*The isolates were amplified by polymerase chain reaction (PCR)*" confuses a bit since later it is specified that the identification is carried out with conventional biochemicals, just to clarify well, why PCR was used in this section.
2. Add information about how to DNA was obtained for PCR assays.

3. Was there any pathogen that predominated in the sampling areas? If so, the results could show a strain that is already distributed in the community with outbreak potential.

Both articles (that are referenced below) are related with the importance of a good epidemiological surveillance system in a possible outbreak of foodborne pathogens. These are closely related to the study presented by the authors, where they carry out the isolation and complete characterization, anticipating of the emergence of foodborne pathogens in an area such as Latin America where epidemiological data usually are limited.

### References

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Yes

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Yes

**Are the datasets clearly presented in a useable and accessible format?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** antibiotic resistance, microbiology, phages

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Reviewer Report 23 June 2022

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**Laura Sala-Comorera** 

<sup>1</sup> UCD School of Biomolecular and Biomedical Science, UCD Earth Institute, Dublin, Ireland

<sup>2</sup> UCD Conway Institute, University College Dublin, Dublin, Ireland

In this data note article, Turbón and colleagues examined the antibiograms and resistance genes profiles of Enterobacterales order strains isolated in ready-to-eat street food in Ecuador. A larger number of samples and types of food have been selected, resulting in a solid database of Enterobacterales strains (n=151).

I suggested a few points to be clarified.

- Methods
  - Enterobacterales strains- Section
    - “A larger amount of broth”. The authors can specify the volume used for the analysis.
    - Add the reference for the “software for Automated Biometric Identification Systems (ABIS)”.
  - Phenotypic antibiotic resistance profiles – Section.
    - Include the full reference for these companies (Thermo Scientific Oxoid and Bioanalyse) following the same style as the previous ones.
  - Detection of *E. coli* pathotypes and antibiotic resistance genes detection via PCR - Section.
    - Include the method/kid used for the DNA extraction.

**Is the rationale for creating the dataset(s) clearly described?**

Yes

**Are the protocols appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and materials provided to allow replication by others?**

Yes

**Are the datasets clearly presented in a useable and accessible format?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** water microbiology, food microbiology, microbial source tracking

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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