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# Genomic characteristics of *Salmonella* Montevideo and Pomona: impact of isolation source on antibiotic resistance, virulence and metabolic capacity

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#### ABSTRACT

Salmonella enterica is known for its disease-causing serotypes, including Montevideo and Pomona. These serotypes have been found in various environments, including river water, sediments, food, and animals. However, the global spread of these serotypes has increased, leading to many reported infections and outbreaks. The goal of this study was the genomic analysis of 48 strains of S. Montevideo and S. Pomona isolated from different sources, including clinical. Results showed that environmental strains carried more antibiotic resistance genes than the clinical strains, such as genes for resistance to aminoglycosides, chloramphenicol, and sulfonamides. Additionally, the type 4 secretion system, was only found in environmental strains. Also many phosphotransferase transport systems were identified and the presence of genes for the alternative pathway Entner-Doudoroff. The origin of isolation may have a significant impact on the ability of Salmonella isolates to adapt and survive in different environments, leading to genomic flexibility and a selection advantage.

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*Salmonella*; in silico; antimicrobial resistance; virulence; metabolism

# Introduction

*Salmonella* is a Gram-negative bacterium that causes a wide range of diseases in humans and animals. The diversity within the *Salmonella* genus is vast, with over 2,600 serotypes identified to date (Wray and Wray 2000; Kim and Kim 2021). The Centers for Disease Control and Prevention (CDC) reported that *Salmonella* infections cause approximately 1.35 million illnesses and 420 deaths in the United States (CDC Center for Disease Control and Prevention 2018). This bacterial pathogen is primarily transmitted through consumption of contaminated food, particularly raw or undercooked eggs, poultry, and meat. Other sources of transmission include contaminated water, contact with infected animals, and poor hygiene. *Salmonella* infection, also known as salmonellosis, can cause symptoms, such as fever, diarrhea, abdominal cramps, and vomiting (Coburn et al. 2006; Carrasco et al. 2012; Eng et al. 2015). Surface waters are a common habitat for *Salmonella*, despite

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the fluctuating and potentially unsuitable conditions for its survival (Moore et al. 2003; Medrano-Félix et al. 2017). *Salmonella* encounters stress factors like temperature, pH, and nutrient changes, which can decrease its viability and ability to cause infection (Winfield and Groisman 2003). However, *Salmonella* employs survival strategies, such as the starvation stress response (SSR), to adapt and survive in nutrient-deprived environments by altering its metabolism and gene expression and triggering protective mechanisms like biofilm formation and stress protein production (Kenyon et al. 2002; Spector and Kenyon 2012). The ability of *Salmonella* to adapt under stress conditions is highlighted by its capacity for horizontal gene transfer (HGT), which enables the bacterium to obtain new genetic material from other bacteria, including antibiotic-resistance genes (dos Santos et al. 2021). This process is crucial in the evolution of *Salmonella* (Li et al. 2021), and it can lead to severe health consequences for the host (Pradhan and Devi Negi 2019).

Salmonella enterica, a pathogen with a diverse range of serotypes, has caused numerous outbreaks and incidents of foodborne illnesses worldwide, including Montevideo and Pomona (Jeong et al. 2017). These serotypes have been linked to numerous outbreaks and incidents of foodborne illnesses across the globe, demonstrating the worldwide impact of Salmonella infections (Gieraltowski et al. 2012; Harris et al. 2016; Paradis et al. 2023). Additionally, they exhibit high adaptability, making them challenging to control and prevent. To develop effective measures to curb the spread of this bacterium and reduce the burden of foodborne illnesses, it is essential to comprehend the genetic variations and virulence factors associated with different serotypes. S. Montevideo and S. Pomona are two serotypes that exhibit resistance to various antibiotics and have been associated with human salmonellosis infections (CDC 2012, 2018; Punchihewage-Don et al., 2022). S. Montevideo possesses genes that confer resistance to multiple antibiotics, increasing its virulence, while S. Pomona has been linked to 18% of human salmonellosis infections in the United States due to interactions with reptile pets (Bosch et al. 2015). Genomic studies of S. Pomona have identified genes related to dynamic metabolism, resistance to aminoglycoside antibiotics, and iron acquisition, contributing to its virulence (Burgueño-Roman et al. 2019). The ability of Salmonella to thrive in various environments increases its potential to cause severe disease, especially when it returns to a host (Chakroun et al. 2017; Ramírez et al. 2018; dos Santos et al. 2021). To develop better diagnostic tools and targeted therapies, it is crucial to study the genetic diversity of Salmonella serotypes isolated from different sources (Aguirre-Sanchez et al. 2021; Achtman et al. 2012; Page et al. 2017). In this sense, this study aimed to analyze the genetic content between clinical and environmental strains of S. Montevideo and Pomona, comparing virulence, metabolic capacities, and antibiotic resistance. Understanding the differences and similarities between these strains is essential for improving public health outcomes and addressing knowledge gaps in the epidemiology and pathogenicity of this bacteria.

#### **Materials and methods**

### Salmonella Montevideo and Pomona isolates and sequencing

A total of 24 genomes each of *S*. Montevideo and 24 genomes of *S*. Pomona were analyzed. This included 10 clinical and 14 environmental strains of *S*. Montevideo, as well as 10 clinical and 14 environmental strains of *S*. Pomona. The dataset was retrieved from the National Center for Biotechnology Information (NCBI) (Agarwala et al. 2017). The accession numbers and corresponding information for these genomes are listed in Supplementary Table S1. In addition, the Laboratorio Nacional para la Investigación en Inocuidad Alimentaria (LANIIA) provided a total of four genomes of *S*. Pomona (JCS-04, JCS-07, JCS-08, and JCS-25) and four genomes of *S*. Montevideo (JCS-06, JCS-27, JCS-28, and JCS-34). The genomes were acquired from strains isolated from river sediment in previous studies. The Whole Genome Shotgun project of the previous *S*. Pomona and Montevideo strains mentioned has been deposited at DDBJ/ENA/GenBank under the accession JAOBPZ000000000, JAOBPY000000000, JAOBPW000000000, JAOBPW000000000, JAOBQD00000000, JAOBQC000000000, JAOBQB000000000, JAOBQA00000000.

#### Assembling and annotation of Salmonella Montevideo and Pomona genomes

Reads quality of the genomes provided by the LANIIA was enhanced using Trimmomatic v0.32, as described by Bolger et al. (2014). The initial 20 bases of each sequence were excluded, and a sliding window of four bases was employed to identify segments with an average Phred quality score of 15 or less. Reads with fewer than 50 bases were excluded. In accordance with the methodology described by Coil et al. (2015), we employed A5-miseq v20160825 to perform the de novo assembly of draft genomes for each river sediment strain.

The amino acid sequences of all genomes in FASTA format were acquired from the RAST seed server and annotated using BlastKOALA (Kanehisa et al. 2016) to examine the metabolic pathways, transport systems, and secretion systems of *Salmonella* in clinical and environmental strains. The metabolic pathways were subsequently recreated with KEGG Mapper (Kanehisa and Sato 2020) based on prior genome annotation.

#### Identification of antimicrobial resistance and virulence genes

To identify antimicrobial resistance genes in the genomes of *S*. Pomona and *S*. Montevideo strains, ResFinder v3.2 program was used (Zankari et al. 2012) to conduct a comprehensive search for both antimicrobial resistance genes and chromosomal mutations. A criterion was created wherein mutations exhibiting a minimum alignment of 70% and identity of 90% or higher were considered. In addition, we utilized the ABRicate software v0.8.13 (https://github.com/tseemann/abricate). This software was used to do a comparative assessment of resistance gene detection using the Comprehensive Antimicrobial Resistance Database (CARD), which can be accessed at: https://card.mcmaster.ca/home. Additionally, this software was utilized to identify virulence genes in the genomes of *S*. Montevideo and *S*. Pomona strains. The identified genes were compared with the virulence factor database VFDB, as described by Liu et al. (2019). The criteria employed were the existence of genes with a similarity level above 90% and a minimum alignment threshold of 70%.

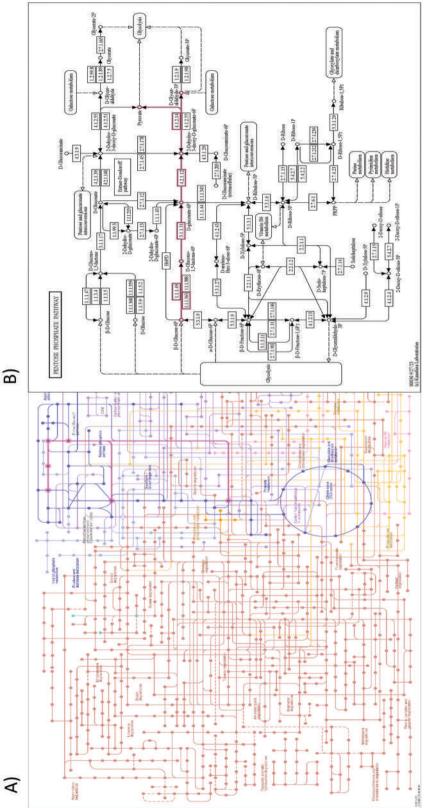
#### Phylogenetic inference

Phylogenetic relationships were established using a core alignment-based phylogenetic tree. The harvest suite alignment and visualization tool were utilized for this purpose (Treangen et al. 2014). The core genome was aligned using Parsnp (Treangen et al. 2014), considering a randomly selected reference. The output obtained was used as an input to create a multi-FASTA file using the HarvestTool software (Pisarenko et al. 2019). A Maximum Likelihood (ML) inference by RAxML was used to construct a phylogenetic tree considering the general time reversible model of nucleotide under the Gamma model of rate heterogeneity (GTRGAMMA) with a statistical support of 100 bootstraps replicates (Stamatakis 2015). Visualization and editing of the resulting tree were performed using the online application iTOL (Letunic and Bork 2021). The analysis was conducted for both the clinical and environmental strains.

# Results

#### Prediction of Metabolic pathways and capabilities using KEGG mapper

The Montevideo and Pomona strains exhibited various metabolic pathways, including carbohydrate, energy, lipid, nucleotide, amino acid, glycan, cofactor and vitamin, and terpenoid and polyketide metabolism. The Embden Meyerhof-Parnas (EMP) pathway was present and complete in both environmental and clinical strains, while the Entner-Doudoroff (ED) pathway, an alternative to the EMP pathway, was also present in all strains (Figure 1). It is suggested that these strains may be capable to utilize various carbon substrates, such as d-glucuronate, galactose, d-galactate, ascorbate, glycogen, trehalose, N-acetyl-D-glucosamine, and glyoxylate, showing similar nutrient





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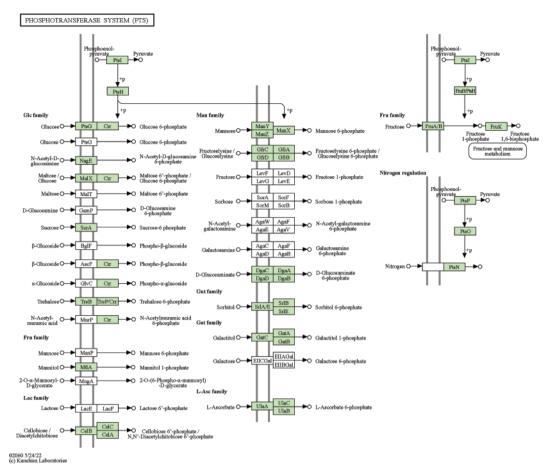


Figure 2. Phosphotransferase systems (PTS) for both environmental and clinical strains of S. Pomona and S. Montevideo. The green-colored blocks serve as an indication of the existence of the gene associated with the corresponding PTS.

acquisition features regardless of isolation source. Additionally, phosphotransferase systems (PTS) for glucose, fructose, lactose, mannose, glucitol, galactitol, l-ascorbate, and nitrogen regulation were detected in both environmental and clinical strains, indicating high metabolic adaptability advantageous for *Salmonella* survival, both outside and inside the host (Figure 2). Interestingly, the presence of genes associated with T1SS, T3SS, T6SS, secretory proteins, and twin arginine targeting proteins was detected in both environmental and clinical strains (Figure 3). Most environmental strains of *S*. Montevideo and Pomona possessed T4SS genes, which are related to membrane proteins and bacterial conjugation. These results indicate that these strains have the potential to adapt to different environments and suggest a high capacity for exchanging genetic material. In contrast, the clinical strains only displayed the presence of these genes in CFSAN023348, CFSAN034931 of *S*. Montevideo, and PNUSAS005642 of *S*. Pomona with only *VirB5* and *VirB6* genes associated with this particular secretion system.

# Antimicrobial Resistance (AMR) genes present in environmental and clinical strains of S. Pomona and S. Montevideo

Figure 4 shows the presence of AMR genes in the S. Montevideo strains, which contain AAC(6')-ly, APH(3'')-lb, and APH(6)-ld genes, known for their role in aminoglycoside resistance. The

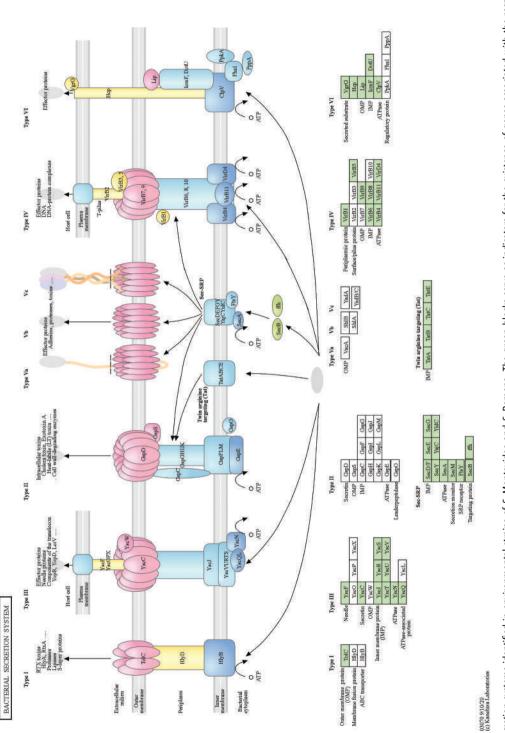


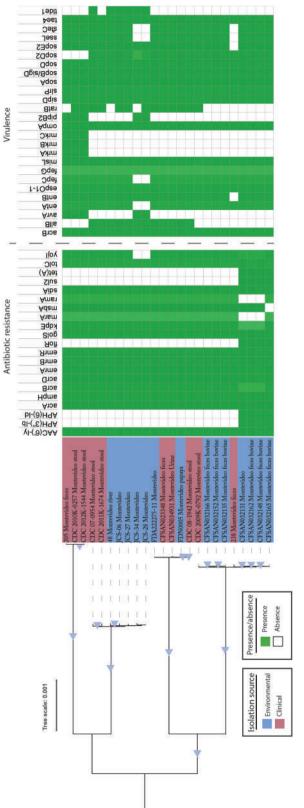
Figure 3. Secretion systems identified in environmental strains of 5. Montevideo and 5. Pomona. The green blocks serve as indicators for the existence of genes associated with the respective secretion system. In this case, the occurrence of genes associated with the T4SS were identified mostly in environmental strains.

AAC(6')-ly gene was found in both environmental and clinical strains, whereas the other two genes, APH(3")-lb and APH(6)-ld, were exclusive to four environmental strains. The presence of ampH in clinical and environmental S. Montevideo strains suggest possible resistance to  $\beta$ -lactams. Furthermore, clinical and environmental strains of S. Montevideo possess acrA, acrB, and acrD genes, which are associated with efflux pumps, as well as emrA, emrB, and emrR genes, which encode drug-binding proteins. The four environmental strains exhibited the presence of the *floR* gene, which is related to resistance to florfenicol. The golS and kdpE genes, which are associated with metal ion resistance and K+ transport, respectively, were detected in all strains of S. Montevideo, including clinical and environmental strains. The marA gene, which regulates an efflux pump, was absent in only three environmental strains. The msbA gene, which is involved in lipopolysaccharide biosynthesis, was present in all strains except one environmental strain. Notably, three environmental strains did not contain the ramA gene which suggest susceptibility to fluoroquinolone antibiotics. All clinical and environmental S. Montevideo genomes contained the sdiA gene, which is related to bacterial quorum sensing, suggesting the potential for this process to occur, which may lead to increased bacterial virulence. Only four environmental strains possessed the sul2 and *tet*(A) genes, which are related resistance to sulfonamide and tetracycline, respectively. The *tolC* gene, which is involved in efflux pumps, was found in clinical and environmental S. Montevideo strains, suggesting the potential to efflux a wide range of antimicrobial compounds and toxins. Finally, the *yojl* gene, which is related to resistance against antimicrobial peptides, was absent in only two environmental strains.

The present study found that all clinical and environmental strains of S. Pomona had the genes AAC(6')-ly, Escherichia coli-ampH, and FosA7. Additionally, the strains had genes related to transcriptional regulation, efflux pumps, and different types of antibiotic resistance. The study also revealed that the environmental and clinical strains shared the *sdiA* and *tolC* genes but lacked the *yojI* gene. These results suggest that there is a similar potential for pathogenicity in both environmental and clinical strains of S. Pomona, regardless of the isolation source.

# Virulence genes present in environmental and clinical strains of S. Pomona and S. Montevideo

The virulence gene profiles of the S. Montevideo strains are illustrated in Figure 4. All environmental and clinical strains of S. Montevideo were found to possess acrB, espO, fepG, misL, ompA, sipD, slrp, sopA, sopB, sopD, and tae4. These genes are involved in various functions related to pathogenicity, such as efflux pumps, T3SS, iron acquisition, adhesins, porins, translocations associated with pathogenicity islands, T4SS secretion effectors, and antibacterial amidases. In contrast, mrkA, mrkB, and mrkC, which play a role in fimbriae production, were only detected in three clinical strains of S. Montevideo. The allantoinase gene allB was found in only five clinical strains, whereas it was present in six environmental strains. Furthermore, avrA, which is associated with the T3SS effector, was detected in only three clinical strains and two environmental strains. The entA gene, which is involved in siderophore production, was absent in both environmental strains. Similarly, entB was absent only in one clinical strain. The two environmental strains lacked the *fepC* gene, which is associated with an inner membrane transporter protein. The *pipB2* gene, which is related to a secretion effector protein, was found in three clinical strains and two environmental strains. On the other hand, the ratB gene, which plays a role in the colonization of the human gut, was detected in eight clinical strains and seven environmental strains. Interestingly, the sopD2 gene, which is responsible for encoding the secretory protein of the T3SS, was absent in three clinical strains. Moreover, *sopE2* was not detected in any clinical strain. Additionally, one clinical strain and two environmental strains lacked the protein effector-related gene, sseL. Furthermore, the steC gene, which is also associated with an effector protein, was absent in two environmental strains. Lastly, the *tlde1* gene, which is linked to T6SS, was identified in one clinical strain and five environmental strains. Figure 5 depicts the existence of several virulence





genes in S. Pomona strains, both clinical and environmental. These genes include *acrB*, *fimA*, *fimC*, *fimD*, *fimF*, *fimW*, *fimY*, *fimZ*, *invA*, *invB*, *invC*, *ompA*, *orgA*, *rcsB*, *rpoS*, *sicA*, *sicP*, *sifA*, *ssax*, *sscA*, *sseK1*, *steB*, and *steC*. These genes are responsible for various virulence factors such as efflux pumps, fimbriae production, invasion regulation, porins, oxygen control, response regulation to biofilm development, sigma factors, T3SS, and secretion effector proteins associated with pathogenicity islands. Only two environmental strains had the *allB* gene, which is associated with allantoinase, while one clinical strain had the east1 gene, linked to enteroaggregative heat-stable toxin 1. Four environmental strains had genes such as *galf*, *hcp2*, *ipfE*, *sspH1*, *tlde1*, and *tssL*, and one environmental strains had an absence of *sopD2* and *steA* genes, while one environmental strain lacked the *tae4* gene, linked to antibacterial amidase activity.

### Phylogenetic analysis

Phylogenetic analysis showed that the clinical and environmental strains of *S*. Montevideo and *S*. Pomona were genetically similar, with their core genomes exhibiting > 80% coverage for both species (Figures 4 and 5). This suggests a close evolutionary relationship between the clinical and environmental isolates of the *Salmonella* serotype. The constructed phylogenetic tree revealed distinct clustering of the clinical and environmental strains into four major clades within each serotype, with a combination of clinical and environmental isolates present in each clade. The arrangement of genomes in the clades was not influenced by the source of isolation, suggesting that there is no discernible difference between clinical and environmental strains solely based on their genetic relationship.

### Discussion

The Montevideo and Pomona serotypes are known for their capacity to cause illnesses through contaminated food, water or contact with reptiles and have been implicated in recent outbreaks in the United States, Europe, Australia, and Asia. *S.* Montevideo has been associated with a growing number of cases of illness and outbreaks, while highly pathogenic *S.* Pomona strains are frequently isolated from reptilian species like snakes, lizards, and turtles, which could pose a risk to human health as reptiles are popular pets (Lalsiamthara and Lee 2017; Haendiges et al. 2021; Colon et al. 2022; Lee et al. 2022). This suggests that reptiles may serve as reservoirs for *S.* Pomona and contribute to the spread of the bacteria to humans and other animals (Song et al. 2023). These serotypes can also be found in environments such as in river water and sediments, which indicates a high risk for individuals to be infected by these serotypes via various routes.

In the present study the analysis 24 genomes of clinical and environmental *S*. Montevideo and *S*. Pomona strains revealed the presence of the alternative metabolic pathway Entner-Doudoroff in environmental and clinical strains of both serotypes, indicating its possible role in the survival and persistence of these strains. This pathway allows for the utilization of a broader range of carbon sources, enhancing the bacterium's ability to thrive in diverse environments (Patra et al. 2012; Flamholz et al. 2013). Understanding this metabolic pathway could aid in the development of targeted interventions to control the spread of these strains and alleviate their impact on public health. The versatility in sugar utilization, enabled by the presence of multiple PTS systems, allows these strains to adapt to different environments and exploit a wide range of ecological niches, enhancing their survival and persistence in different environments (Barabote and Saier 2005; Comas et al. 2008; Lim et al. 2019; Jeckelmann and Erni 2019). The PTS system involving N-acetyl-D-glucosamine was detected in all strains. Previous research has shown that environmental *Salmonella* strains in aquatic environments often use this alternative carbon source (Medrano-Félix et al. 2017; Gonzáles-López et al., 2021; Chaidez et al. 2020). In addition, the N-acetyl-D-glucosamine PTS has been associated with the induction of the *mdtEF* genes, which

Virulence

Antibiotic resistance

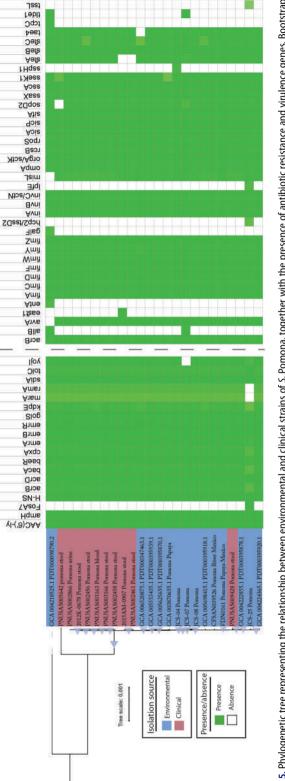


Figure 5. Phylogenetic tree representing the relationship between environmental and clinical strains of 5. Pomona, together with the presence of antibiotic resistance and virulence genes. Bootstrap values greater than 85% are shown in blue triangles.

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provide resistance to many antibiotics (Hirakawa et al. 2006). This finding highlights the potential of targeting this specific system to develop targeted therapies against these strains. Further investigation into the regulation of these PTS systems could provide valuable insights into how their expression can be manipulated to limit their ability to use various carbon sources and ultimately hinder their survival. Overall, studying these PTS systems has the potential to contribute to understanding their ecological adaptation and support the development of effective intervention strategies.

Several AMR genes were identified in both environmental and clinical *S*. Montevideo and Pomona strains. Notably, only the environmental strains of *S*. Montevideo contained the APH(3'')-lb and APH(6)-ld genes, which are responsible for aminoglycoside antibiotic resistance (de Melo et al. 2021)). Additionally, the environmental strains of Montevideo were the only ones with the *floR* gene, which confers resistance to florfenicol and chloramphenicol. The presence of this gene in environmental strains indicates that antibiotic resistance can be transmitted between environmental and clinical settings (Cloeckaert et al. 2000; Nasim et al. 2015; Mei et al. 2021). The presence of specific genes such as *sul2* and *tet*(A) was exclusively observed in the environmental strains of *S*. Montevideo. These genes confer resistance to sulfonamide and tetracycline, two widely used antibiotics in human and animal medicine, respectively, because of their effectiveness against various types of bacteria (Pavelquesi et al. 2021). Multiple factors contribute to the acquisition of tetracycline resistance, such as mobile genetic elements, ribosome-binding site modifications, and chromosomal mutations (Adesoji et al. 2015; Sheykhsaran et al. 2019). These mechanisms lead to the spread and persistence of multidrug-resistant strains, which are highly adaptable and challenging to control.

The findings in the present study showed that environmental and clinical strains of *S*. Pomona shared similar AMR gene profiles, suggesting the possibility of resistance gene transmission between them. However, the environmental strain JCS-25, obtained from river sediments, exclusively displayed *FosA7*, a gene providing resistance to fosfomycin. Acquiring this gene is linked to the transmission of plasmids, implying the potential transfer of mobile elements to the environment where the strains were isolated. This environment may contribute to the spread of antibiotic resistance, and the potential transfer of plasmids carrying the gene implies that horizontal gene transfer is a crucial factor in the dissemination of antibiotic resistance in the environment (Rehman et al. 2017; Wang et al. 2021).

The analysis of virulence genes in *S*. Montevideo strains revealed that only clinical strains isolated from human feces contained *mrkA*, *mrkB*, and *mrkC* genes. The identification of these genes as components of the *mrk* operon and their association with type 3 fimbriae, which promote biofilm formation, suggests that clinical strains possess a high potential for persistence and colonization (Ong et al. 2008). On the other hand, environmental strains of *S*. Montevideo mostly carry the *allB* gene, which is involved in allantoinase synthesis and helps the bacterium adapt and survive in diverse environments using allantoin as a nitrogen source (Cusa et al. 1999; Hafez et al. 2017). The presence of these virulence and adaptive genes indicates that both clinical and environmental strains of *S*. Pomona have the potential to cause various illnesses in humans.

Both environmental and clinical strains of *Salmonella* possess T1SS, T3SS, and T6SS, which are critical for the delivery of virulence factors into host cells and contribute to the pathogenicity of *Salmonella*. The T4SS, which is found in many bacteria including *Salmonella*, was only observed in the environmental strains of *S*. Montevideo and Pomona. It plays a role in the direct delivery of proteins into host cells, promoting infection and survival (Backert and Meyer 2006; Galán and Waksman 2018; Bao et al. 2020). The presence of T4SS in environmental strains of *Salmonella* indicates their ability to infect both humans and other organisms. T4SS is also involved in the process of conjugation, a crucial mechanism for bacterial gene transfer. The presence of T4SS in these strains enhances their capacity to exchange genetic material with other bacteria, which may lead to the acquisition of antibiotic resistance genes (Alvarez-Martinez and Christie 2009; Christie et al. 2016; Bao et al. 2020). Additionally, T4SS plays a crucial role in *Salmonella*'s ability to persist

within macrophages and epithelial cells by inhibiting the host's innate immune response (Khajanchi and Foley 2022). Understanding the mechanisms of T4SS in *Salmonella* pathogenesis can provide valuable insights for developing targeted therapies against this persistent drug-resistant pathogen. Phylogenetic analysis demonstrated that both clinical and environmental strains exhibited genetic similarities. This suggests that genetic factors contributing to *Salmonella* virulence, antibiotic resistance and metabolism are likely to be conserved across various environments (Zakaria et al. 2021). Furthermore, the phylogenetic tree revealed that certain clinical isolates were closely related to environmental isolates, suggesting the potential transmission of the pathogen between different reservoirs (Pornsukarom et al. 2018). Additionally, the short branches in the tree indicate a high degree of genetic relatedness between the strains, suggesting a recent common ancestor (Zhang et al. 2006). Elucidating the mechanisms by which *Salmonella* adapts to different environments can provide valuable insights into its evolutionary history and potential future threats. By examining the genetic similarities and differences between clinical and environmental strains, it is possible to identify the key genetic determinants that drive *Salmonella* pathogenicity and drug resistance.

# Conclusion

In summary, analysis of the genomes of *S*. Montevideo and Pomona strains from various sources demonstrated substantial dissimilarities in their resistance to antibiotics and their capacity to transfer genetic material, emphasizing the need to comprehend the genomic attributes of these serotypes to control and prevent infections. Future research should focus on elucidating the mechanisms underlying the genomic versatility and selection advantage of *S*. Montevideo and *S*. Pomona strains in different environments, as well as exploring potential interventions to restrict the propagation of antibiotic-resistant strains.

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Visualization, Writing – Review & Editing Jose Andrés Medrano-Félix: Conceptualization, Investigation, Supervision, Writing – Review & Editing. Berenice Gonzáles-Torres: Data Curation, Writing – Review & Editing, Software. Cristóbal Chaidez-Quiróz: Writing – Review & Editing. Osvaldo López-Cuevas: Writing – Review & Editing. Nohelia Castro-Del Campo: Writing – Review & Editing.

#### Data availability statement

The data that support the findings of this study are available on request from the corresponding author, JAMF. The data are not publicly available due to restrictions of the repository.

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