UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY TECH

Escuela de Ciencias Biológicas e Ingeniería

TÍTULO:

High prevalence of sulfonamide resistance genes into integrons class 1 and 2 in *Salmonella enterica* in the Andean Region

Trabajo de integración curricular presentado como requisito para la obtención del título de Bióloga

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Urcuquí, diciembre 2021

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Dedicatoria

A la memoria de mis queridos abuelos Gonzalo y Amadita, a su amor incondicional y a sus grandes lecciones de vida.

A mis padres, Carmita y Julio, por siempre cuidar de mí y apoyarme incondicionalmente en cada una de mis metas. A su eterno esfuerzo, amor y paciencia.

A mis queridos hermanos Julio, Ibeth y Nayely, por brindarme afecto y alegrías.

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Lilibeth del Cisne Torres Elizade

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Lilibeth del Cisne Torres Elizade

Resumen

Los genes de resistencia asociados con los integrones pueden movilizarse entre y dentro de las moléculas de ADN. IntFinder, una herramienta bioinformática diseñada para detectar integrones de resistencia en lecturas ensambladas y crudas, se usó para determinar la presencia de estos elementos en secuencias de genoma completo de aislados de *Salmonella enterica*. Una colección de 1688 de estos aislamientos fue extraída de la EnteroBase; este estudio se centró en países de la Comunidad Andina, Colombia, Ecuador, Perú y Bolivia. Se detectó un total de 688 integrones de resistencia, los de clase 2 fueron los más abundantes (93,31%) seguidos por los de clase 1 (6,69%); no se predijo ningún integron de clase 3. Los integrones detectados se relacionaron principalmente con *Salmonella* Infantis (ST32) y con fuentes animales, especialmente pollos de engorde. El gen más común fue *dfrA14* que confiere resistencia a la trimetoprima. También se detectaron otros genes de resistencia, aunque en menor número, incluyendo los genes *aadA* y *bla*. El uso generalizado de antibióticos en la región puede haber influido en la selección de estos serotipos específicos, integrones y genes de resistencia. Estos resultados representan un riesgo para la salud pública debido a la posible propagación de cepas resistentes. La información generada a partir de este estudio *in silico* contribuye a una mejor comprensión de la dinámica de los integrones de resistencia en *Salmonella* spp. patógena. Además, esta información podría utilizarse para diseñar programas de vigilancia destinados a controlar y prevenir la aparición de nuevas cepas resistentes.

Palabras clave: *Salmonella enterica*, integrones clases 1 y 2, genes *dfrA*, IntFinder, Comunidad Andina.

Abstract

Resistance genes associated with integrons can be mobilized between and within DNA molecules. IntFinder, a bioinformatic tool designed to detect resistance integrons in assembled and raw reads, was used to determine the presence of these elements in whole-genome-sequenced isolates of *Salmonella enterica*. A collection of 1688 of such isolates was retrieved from the EnteroBase; this study focused on countries of the Andean community, Colombia, Ecuador, Peru, and Bolivia. A total of 688 resistance integrons were detected, class 2 integrons were the most abundant (93,31%) followed by class 1 integrons (6,69%); no integrons class 3 were predicted. The detected integrons were mainly related to *Salmonella* Infantis (ST32), and to animal sources, especially broiler chickens. The most common gene was *dfrA14* which confers resistance to trimethoprim. Other resistance genes were also detected, although in lower numbers, including *aadA* and *bla* genes. The widespread use of antibiotics in the region may have influenced the selection of these specific serotypes, integrons, and resistance genes. These results represent a public health risk due to the potential spread of resistant strains. The information gathered from this *in silico* study contributes to a better understanding of the dynamics of resistance integrons in pathogenic *Salmonella* spp. In addition, this information could be used when designing surveillance programs aimed at controlling and preventing the appearance of novel resistant strains.

Keywords: *Salmonella enterica*, class 1 and 2 integrons, *dfrA* genes, IntFinder, Andean Community.

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In silico detection of class 1 and 2 integrons reveals high prevalence of *dfrA* resistance genes associated with *Salmonella enterica* in countries of the Andean Community*

* The name of this thesis should be: "*In silico* detection of class 1 and 2 integrons reveals high prevalence of *dfrA* resistance genes associated with *Salmonella enterica* in countries of the Andean Community". Due to complications with submission dates, the official name could not be modified. We apologize for the inconveniences.

ABSTRACT

Resistance genes associated with integrons can be mobilized between and within DNA molecules. IntFinder, a bioinformatic tool designed to detect resistance integrons in assembled and raw reads, was used to determine the presence of these elements in whole-genome-sequenced isolates of *Salmonella enterica*. A collection of 1688 of such isolates was retrieved from the EnteroBase; this study focused on countries of the Andean community, Colombia, Ecuador, Peru, and Bolivia. A total of 688 resistance integrons were detected, class 2 integrons were the most abundant (93,31%) followed by class 1 integrons (6,69%); no integrons class 3 were predicted. The detected integrons were mainly related to *Salmonella* Infantis (ST32), and to animal sources, especially broiler chickens. The most common gene was *dfrA14* which confers resistance to trimethoprim. Other resistance genes were also detected, although in lower numbers, including *aadA* and *bla* genes. The widespread use of antibiotics in the region may have influenced the selection of these specific serotypes, integrons, and resistance genes. These results represent a public health risk due to the potential spread of resistant strains. The information gathered from this *in silico* study contributes to a better understanding of the dynamics of resistance integrons in pathogenic *Salmonella* spp. In addition, this information could be used when designing surveillance programs aimed at controlling and preventing the appearance of novel resistant strains.

Keywords: *Salmonella enterica*, class 1 and 2 integrons, *dfrA* genes, IntFinder, Andean Community.

GLOSSARY

SPT: Spectinomycin

STR: Streptomycin

TMP: Trimethoprim

AMP: Ampicillin

CTX: Cefotaxime

CAZ: Ceftazidime

TIM2: Ticarcillin/Clavulanic Acid

STX: Co-trimoxazole

1. INTRODUCTION

Antimicrobial agents are used indiscriminately and inappropriately in both the animal industry and the treatment of human infections [1]. As a result, multi-drug-resistant rates of pathogenic and commensal bacteria continue to increase globally [2]. Resistant strains are of concern, particularly those belonging to the *Enterobacteriaceae* family, as they are a significant cause of food-borne infections [3]. Indeed, *Salmonella enteric*a is considered the most frequent food-borne disease worldwide [4–6]; it is mainly associated with the ingestion of contaminated foods [7,8]. *S. enterica* strains resistant to common antibiotics have been reported in countries members of the Andean Community, which includes Colombia, Ecuador, Peru and Bolivia [9–11]. *Salmonella* strains can acquire resistance genes from the environment via mobile genetic elements including plasmids and integrative conjugative elements, which can themselves obtained these genes via mobile elements such as integrons, cassettes, transposons or insertions sequences [12].

Integrons are genetic platforms that play a key role in bacterial adaptability and genome evolution, these structures are capable of capturing gene cassettes and allowing their expression [13]. Integrons are able to associate with conjugative plasmids, allowing the mobilization of their entire platform in a stable way within different bacterial genomes [14]. Its basic structure consists of two highly conserved DNA sequences (CS) at both ends, known as 5´-CS and 3´-CS; in addition, a variable region is present (Fig 1.). In the 5´-CS end, integrons contain a gene with its own promoter encoding for an integrase (*intI*), which is a site-specific recombinase enzyme [15], an *attI* site, which is recognized by the integrase and acts as an acceptor for gene cassettes, this site contains a promoter that ensures the expression of the inserted gene cassettes (Fig 1.) [16]. Gene cassettes are mobile elements of circular DNA that are free and contain an open reading frame (*orf*), generally with its own promoter, and an *attC* recombination site (Fig 1.) [16,17]. The inserted gene cassettes constitute the integron variable region. These elements could harbor several genes in a cassette, but expression diminishes as genes are located far from the promoter (Fig 1.) [16].

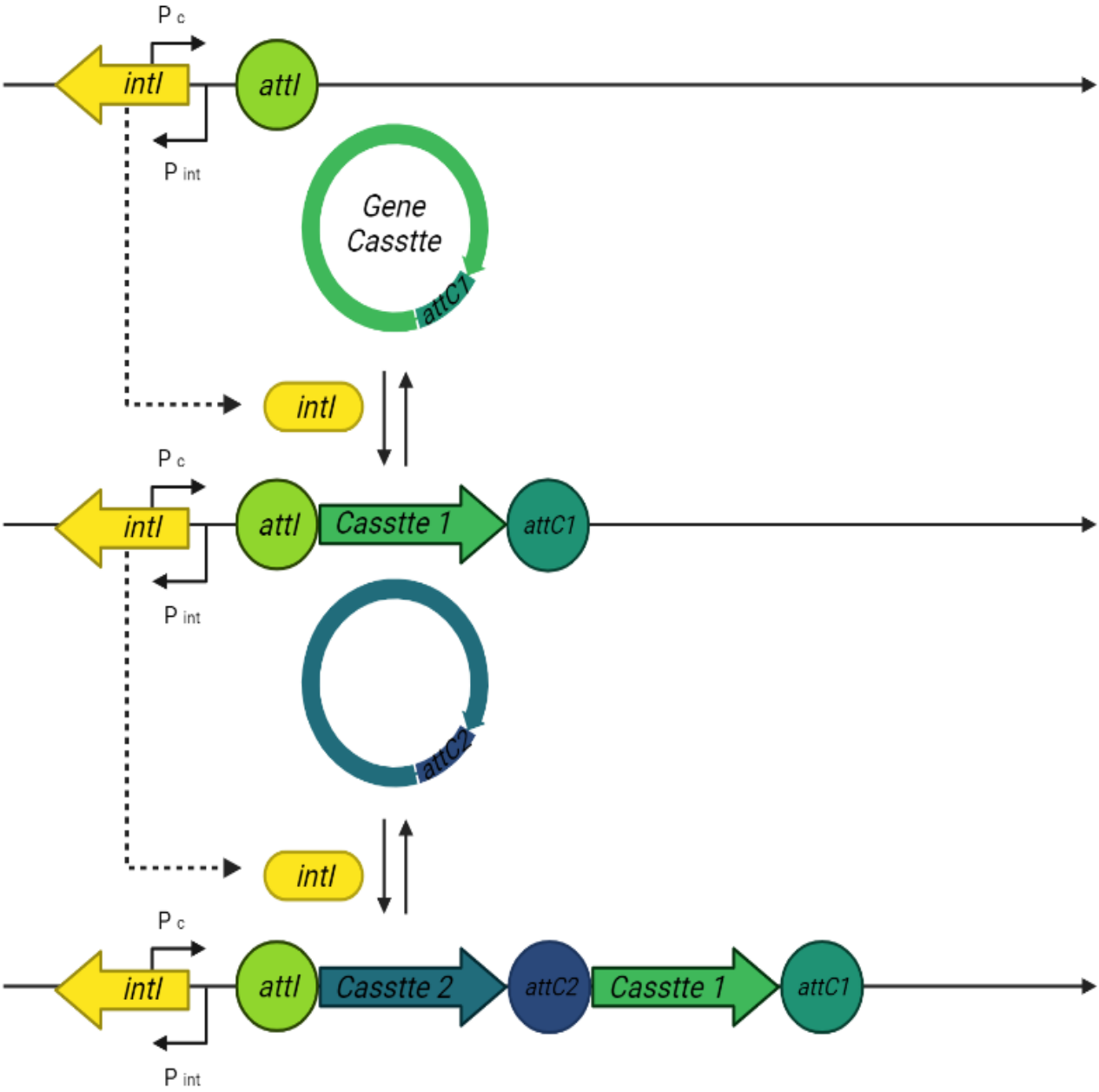

Figure 1. Basic schematic organization of integrons, gene cassettes, and the site-specific recombination mechanism. The functional platform of integrons is constituted by an i*ntI* gene, encoding an integrase, a recombination site *attI*, and cassette promoter *Pc*. The integrase recognizes the *attC* site and catalyzes its recombination at *attI*, bringing the cassette under the control of *Pc* (Modified from [18])

Multidrug resistance has been linked to three different types of integrons class 1, class 2, and class 3, each integron harbors different *int* genes (*intI1*, *intI2*, and *intI3*) (Fig 2) [19]. For class 1 integrons, the 3´-CS end contains various open reading frames such as the *qacEΔ1* and *sul1* genes, which confers resistance to quaternary ammonium and sulfonamides, respectively [14,20]. For class 2 integrons, the 3´-CS end contains one or more genes involved in transposition processes *tnsA*, *tnsB*, *tnsC*, *tnsD*, and *tnsE* [21]*;* the structure of the 3´-CS end of class 3 integrons remains unknown (Fig 2).

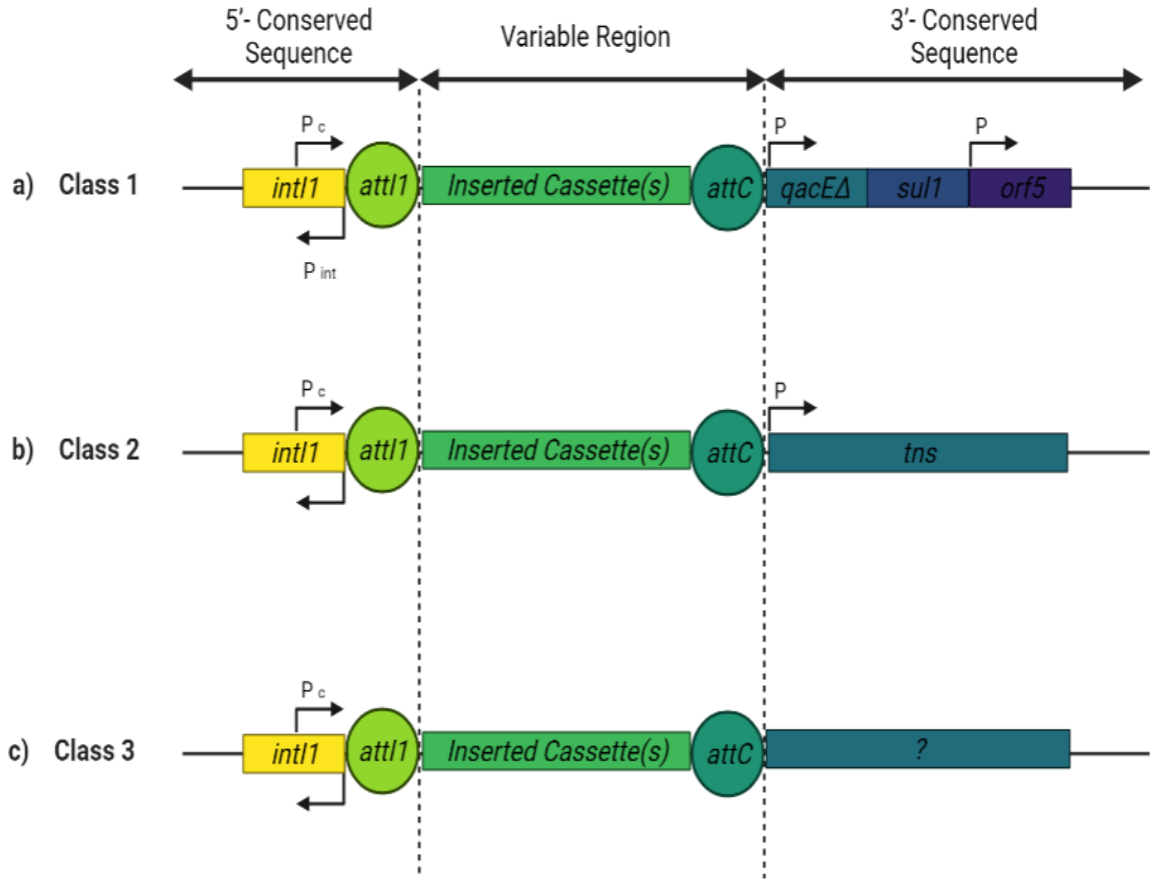

Figure 2. Structure of resistance integrons. a) class 1 integrons; b) class 2 integrons; c) class 3 integrons. All have an *intI* gene, an *attI* site for the integron recombination, an *attC*, a cassette gene recombination site, and a variable region between the conserved ends. Class 2 integrons harbor *tns* genes (transposition module), while class 1 integrons contain the *qacEΔ1* gene conferring to quaternary ammonium, and the *sul1*, conferring resistance to sulfonamides (Modified from [18]).

Classes 1 and 2 are the most frequent integrons distributed among *S. enterica* strains, class 3 have not been commonly detected [22]. Integrons are considered one of the leading drivers in the appearance of *S. enterica* strains, resistant to common antibiotics, including beta-lactams, aminoglycosides, trimethoprim, chloramphenicol, fosfomycin, macrolides, lincosamides, rifampicin, quinolones, disinfectants and heavy metals [12,13]. Undoubtedly, the identification and characterization of integrons are critical for determining the epidemiology of resistance genes, particularly in areas where information is limited. In the Andean community, class 1 and 2 integrons in Multidrug-resistant isolates from *S. enterica* have been uniquely reported in Peru and Colombia [23,24], no information is available from Ecuador and Bolivia. The lack of knowledge about integrons hinders our ability to investigate their dynamics and, more importantly, their relationship with antibiotic-resistant genes.

Nowadays, the development of next-generation sequencing has made genomic analyses more available. Many bioinformatics tools used for detecting mobile genetic elements require previous bioinformatics expertise for operation. In this study we have made use of a friendly software, IntFinder, to identify integrons in raw reads and assembled genomes/contigs [25]. Additionally, we studied the relationship between integrons and antibiotic resistance genes in isolates reported in countries of the Andean Community. Identifying integrons and their associated resistance genes will help to improve our understanding of particular multidrug-resistant strains of *S. enterica*.

2. MATERIALS AND METHODS

2.1 IntFinder

IntFinder is a bioinformatic tool that enables rapid detection of resistance integrons, it was written in Python 3. Initially, it was designed to detect class 1 integrons; however, the version used in this study can also identify integrons class 2 and 3. Its development and validation process is available in a public repository [25]. IntFinder is accessible on a web server https://cge.cbs.dtu.dk/services/IntFinder-1.0/ and it can be downloaded and installed on any computer from the website: https://bitbucket.org/genomicepidemiology/intfinder/src/master/.

IntFinder databases were constructed based on information provided by the INTEGRALL database (http://integrall.bio.ua.pt/). This is an online repository for integrons that comprises information about the molecular diversity of inserted gene cassettes, and the type of integrases [26]. Currently, the INTEGRALL database contains entries from isolates of more than four hundred different bacterial species [27]. A total of 52 entries were linked to the area under investigation, from these entries, only 3.85% were related to *S. enterica*, which have been reported uniquely from Colombia [27]. Each IntFinder database was constructed according to the number assigned by INTEGRALL to each integron (*In*), each *In* includes information about gene cassettes, integron names, and GeneBank accession numbers. These accession numbers were used to retrieve all nucleotide sequences from the gene database of NCBI. Subsequently, all nucleotide sequences were blasted to detect the 5' and 3' (CS) ends of integrons as well as their associated resistance genes. The nucleotide coordinates of integrons were found using ResFinder [28].

IntFinder detects integrons using k-mer alignment (KMA) to match the input sequence with the entry sequences of each IntFinder database; this happens according to a user-defined similarity threshold, in the software the threshold can be adjusted as desired, while in the web version the threshold is 0.5. Based on the sequence of the *intI* gene, IntFinder determines integron classes, thus, if the *intI1* sequence was detected in the input sequence, it is labeled as a potential class 1 integron. The same occurs with class 2 and 3 integrons, sequences of *intI2* and *intI3* were used to detect these classes, respectively. Input sequences carrying similar integrases were considered members of the same class [29]. As a result, IntFinder generated four files. First, results.txt, containing information with regard to name, query/template length, resistance genes, positions, and accession numbers. Second, results.tsv, containing the graphical alignment between the input sequence and the integron entry of the database; moreover, this file showed information about integron length and associated genes. Third, Hit_in_genome_seq.fsa, showing the coverage, identity, and match. Finally, integrons.fsa displaying the information about the sequence of the detected integron.

2.2 Dataset Selection

Whole-genome-sequenced isolates of *S. enterica* were retrieved from the EnteroBase (last accessed in February 2021, http://enterobase.warwick.ac.uk/) in FASTA format according to the listed criteria: i) documented in countries belonging to the Andean Community, including Venezuela as it withdrew from the organization; ii) reported between 1956 and 2021. The final dataset included a total of 1688 sequences of *S. enterica*, nine from Bolivia (0.53%), 466 from Colombia (27.61%), 889 from Ecuador (52.67%), 274 from Peru (2.96%), and 50 from Venezuela (2.96%) (Supplementary materials, S1 Table).

2.3 Characterization of integrons in *S. enterica* isolates

The presence of integrons class 1, 2, and 3 integrons among the screened isolates was carried out using the IntFinder tool, using the script detailed in the supplementary materials (S3 Script). Detection of resistance integrons was conducted under the following parameters, coverage = 1, sequence identity = 1, and depth = 1. Additionally, a maximum likelihood phylogenetic tree based on single nucleotide polymorphism (SNP) was rendered using the CSI Phylogeny version 1.4 [30]. SNP tree was constructed with the integron-positive sequences (688 isolates) and with the *S. enterica* reference genome retrieved from the GenBank database (NC_003197.2). The Phylogenetic tree was visualized and annotated with iTOL v6 [31].

2.4 Classification of integrons associated with different antibiotic resistance genes

Resistance genes were categorized as being associated with different integrons. If a gene was detected within the variable region of an integron, it was considered associated.

3. RESULTS

3.1 Characterization of detected integrons

In total, IntFinder detected 688 integrons (40.76%,) (95% CI, confidence interval). Class 1 integrons were detected in 46 isolates (2.73%) and class 2 integrons in 642 (38.03%). No integrons class 3 were predicted (Figure 3A). Moreover, not a single positive isolate harbored more than one integron. Only Colombian (5.81%) and

Ecuadorian (0.87%) isolates had class 1 integrons, whereas isolates from Ecuador (33.66%), Colombia (11,77%), and Peru (17.88%) had class 2 integrons (Figure 3B). The most common integrons were *In14* and *In33* being detected in 83.87% and 9.45% of the isolates, respectively, both were class 2. Other detected integrons include *In54*, *In167*, and *In573*, all of them were class 1.

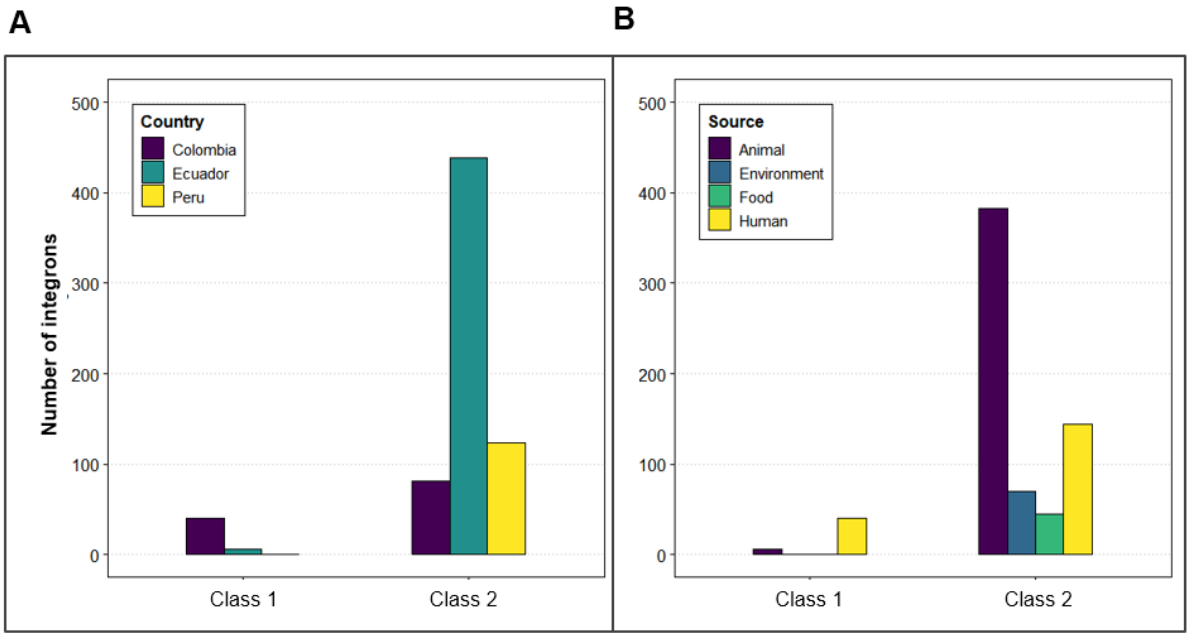

Figure 3. Frequency of detected integrons in whole-genome-sequenced isolates of *S. enterica* in the Andean Community, **(A)** depending on the country of isolation, and **(B)** depending on the source of isolation.

3.2 Differences between MLST types and serovars

Figure 4 shows the frequency of class 1 and class 2 integrons among several *Salmonella* spp. serotypes and MLST types. Class 1 integrons were mostly related to the serotype Typhimurium (ST19) (5.81%), followed by Havana (ST588) (0.87%). They were mainly related to human sources and were present in isolates from Colombia and Ecuador. In contrast, class 2 integrons appeared dispersed through Paratyphi B (ST28) (9.45%) and Infantis (ST32) (83.87%) serotypes. Particularly, the most common integron was *In14*, linked to *S.* Infantis and present in isolates from different sources, especially animals; it was principally found in isolates from Ecuador, Colombia, and Peru.

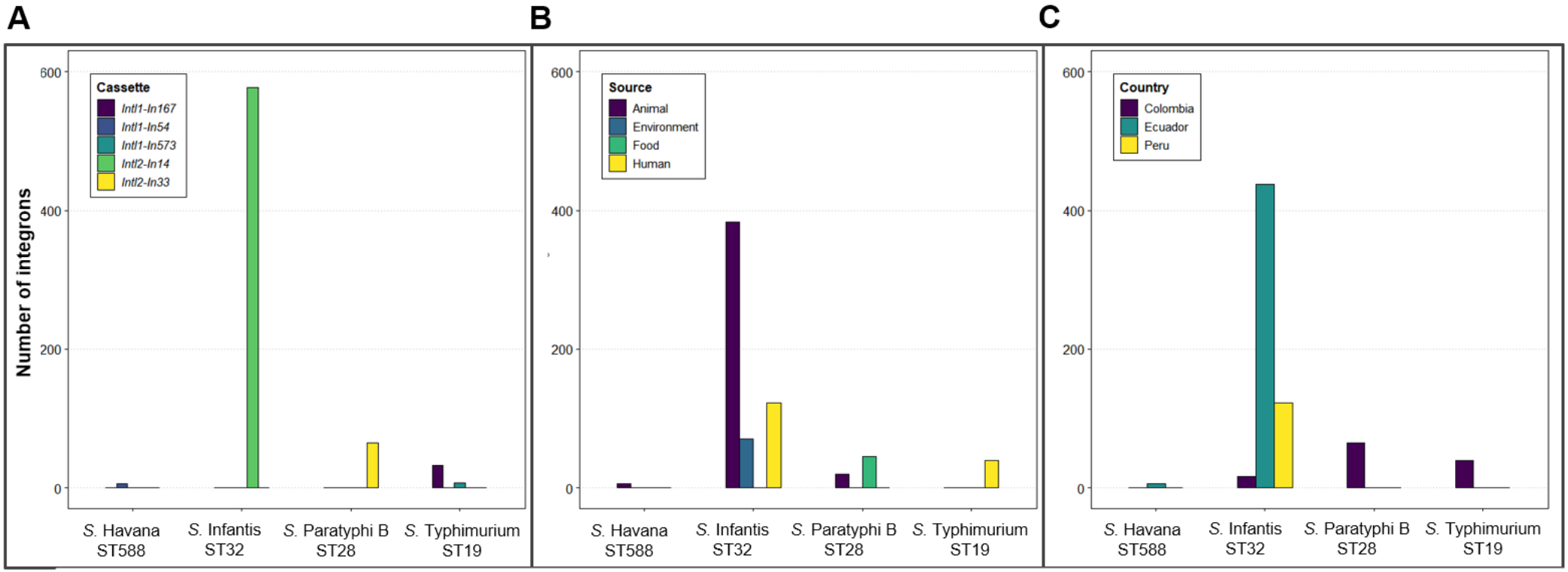

Figure 4. Frequency of integrons according to serovar and sequence type in the Andean Community, **(A)** depending on the integron, **(B)** source of isolation, and **(C)** country.

To achieve a better understanding of the relationship between resistance integrons and *S. enterica* strains, we reconstructed a phylogenetic tree based on SNPs. These strains were found to be genetically related, suggesting a clonal expansion. Indeed, *S.* Infantis strains carried the same integron and only isolates from *S.* Typhimurium carried more than one integron (Figure 5).

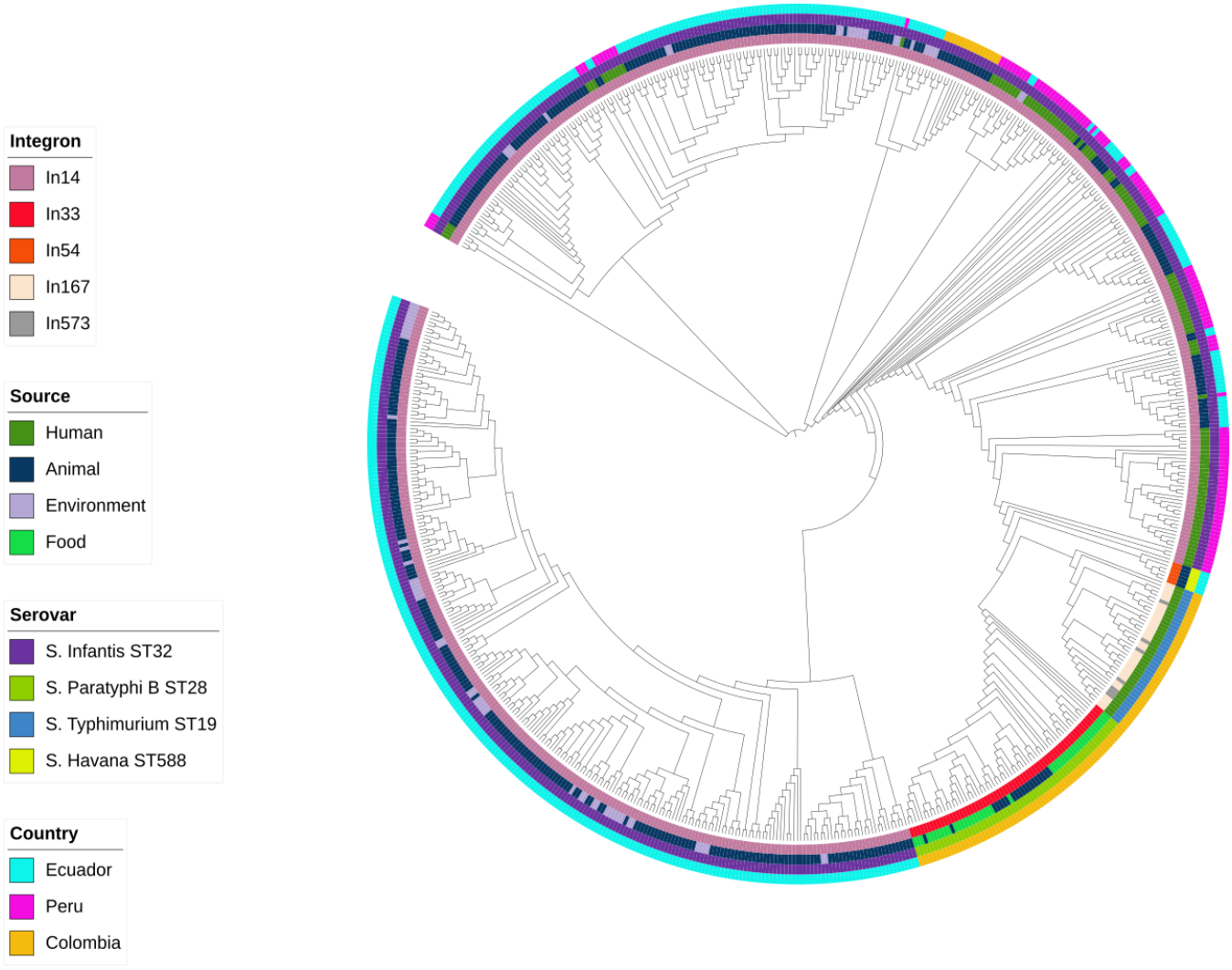

Figure 5. SNP-based phylogenetic tree composed of integron-positive strains. This figure was generated with iTOL v.6. The color bars (inside to outside) covering detected integrons, sampling source, serovar, and country.

3.3 Association of integrons and antibiotic resistance genes

The majority of integrons carried a trimethoprim resistance gene (95.20%), these genes were present in isolates from Ecuador, Colombia and Peru (Figure 6. B). On the other hand, 10.32% of integron-positive isolates harbored spectinomycin/streptomycin resistance genes, which were associated with isolates of animals and food origin (Figure 6A). In addition, such isolates have been mainly reported in Colombia and, to a lesser extent, in Ecuador. Resistance genes related to other antimicrobials (ampicillin, cefotaxime, ceftazidime, and ticarcillin/clavulanic acid) were less common, with the environment being their unique source (Figure 6A). Integrons associated with these genes were only predicted in Colombian isolates (Figure 6B).

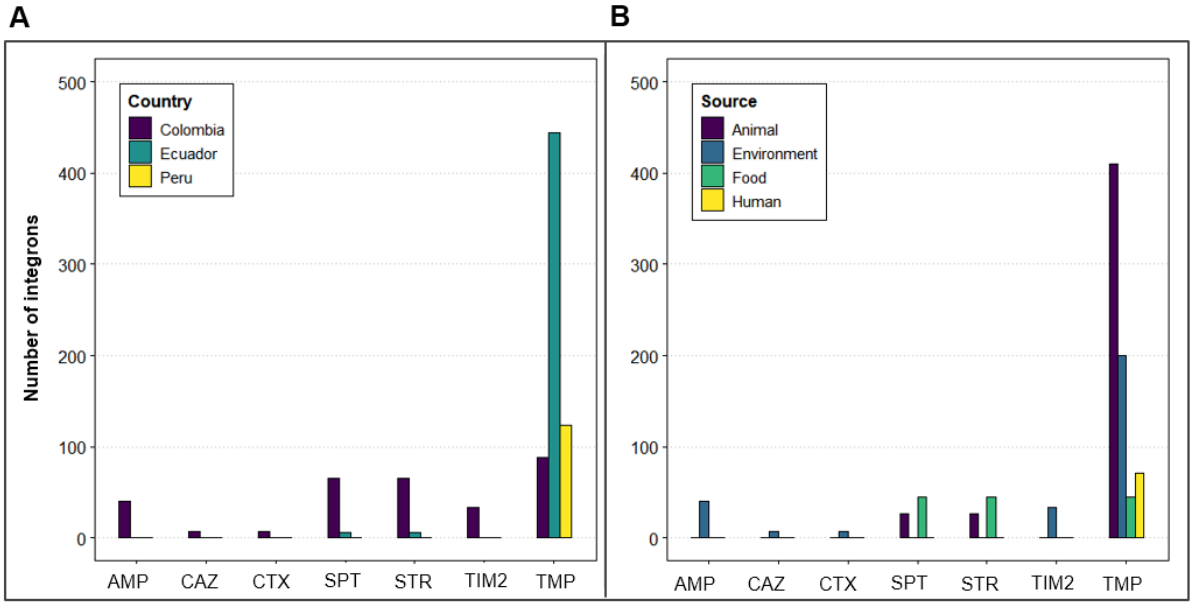

Figure 6. Frequency of integrons associated with antimicrobial resistance genes in the Andean Community, **(A)** depending on the country and **(B)** on the source of isolation. SPT, spectinomycin; STR, streptomycin; TMP, trimethoprim; AMP, ampicillin; CTX, cefotaxime; CAZ, ceftazidime; TIM2, ticarcillin/clavulanic acid.

A total of five different gene cassettes were identified among integron-positive isolates (Table 1). The most frequent were those coding for variants of dihydrofolate reductases (*dfr*) conferring resistance to trimethoprim. *dfr* genes were present in all cassettes except for the one associated with *In167*. Other genes found on cassettes

encoded for variants of aminoglycoside transferases (*aadA*) and β-lactamases (*bla*), they were related to *In54*, *In33*, *In573*.

Table 1. *In silico* detection of resistance integrons in different whole-genome-sequenced isolates of *S. enterica* using IntFinder

Class of integron	Name of integron	Size (bp)	MDR genes	MDR Class			MDR pattern	Accession Number
				Aminoglycoside	Beta-Lactam	Trimethoprim		
Class 1	*In54*	474-789	2	*aadA5*		*dfrA17*	TMP - SPT - STR	AF220757
	In167	866	1		$bla_{\text{CARB-2}}$		AMP - TIM2	AF221899
	In573	472-828	2		$bla_{\text{OXA-2}}$	*dfrA29*	TMP - AMP - CTX - CAZ	AM237806
Class 2	*In14*	483	1			*dfrA14*	TMP	EU780012
	In33	475	2	*aadA1*		*dfrA1*	TMP - SPT - STR	FJ914220

SPT, spectinomycin; STR, streptomycin; TMP, trimethoprim; AMP, ampicillin; CTX, cefotaxime; CAZ, ceftazidime; TIM2, ticarcillin/clavulanic acid

4. DISCUSSION

In the Andean Region, studies focusing on the identification and characterization of integrons in *S. enterica* are scarce. Few studies from Colombia, Peru and Venezuela have shown the presence of integrons in multidrug-resistant isolates of *S. enterica* [24,32,33]. There are no current reports regarding resistance integrons in Bolivia and Ecuador. Clearly, this information could be deemed insufficient to make inferences about the role of integrons in the development of resistant *S. enterica* strains.

Previous studies have demonstrated the advantages of the use of bioinformatics tools as techniques to detect mobile elements and determine their relationship to antimicrobial resistance genes in zoonotic bacteria [34,35]. IntFinder is a software that can find integrons using both assembled genomes/contigs and raw reads as input [25], which is a significant improvement over earlier tools that utilize assembled genomes/contigs uniquely [36,37].

In this study, we determined and characterized the presence of class 1, 2, and 3 integrons in 1688 of whole-genome-sequenced isolates of *S. enterica* collected from food, human, animal, and environmental samples from countries of the Andean Community. In total, 688 integrons were detected. Class 2 integrons were the most abundant followed by class 1. No class 3 integrons were observed. These integrons were mostly found in animals and human isolates, they were mainly associated with *S.* Infantis (ST32). The initial dataset revealed that Ecuador (52.67%) had the largest number of the *S. enterica* sequences reported in the region; as a consequence, it was the country with more integrons, followed by Colombia, and Peru. All of the detected integrons contained resistance genes to different antimicrobial agents.

The majority of class 2 integrons were found in *S.* Infantis (ST32) and in *S.* Paratyphi B (ST28) strains, these strains were collected from animals, human, and environmental sources. *S.* Infantis (ST32) and *S.* Paratyphi B (ST28) strains have previously been related to such sources [36] and to class 2 and 1 integrons [36,38–43]. *S.* Paratyphi B (ST28) strains have been associated with class 1 integrons [44], although we found that these strains were related to class 2 integrons. Class 1 integrons were associated with *S.* Typhimurium (ST19) and *S.* Havana (ST588) strains, they were related to human and animal sources. Indeed, *S.* Typhimurium (ST19) and *S.* Havana (ST588) have been previously linked to class 1 and class 2 integrons [39,44–48], and with the aforenoted sources [47,49,50].

All of the detected integrons were associated with resistance genes that not only confer resistance to antimicrobial agents used as the first line of defense to treat salmonellosis, such as trimethoprim and ampicillin, but also confer resistance to third-generation cephalosporins and to at least two different aminoglycosides [51]. In their, majority, integrons harbored trimethoprim resistance genes (*dfrAs*), also some

contained cassettes that confer resistance to aminoglycosides (*aadA* genes), and beta-lactams (*bla* genes). The presence of these resistance genes may not come as a surprise giving the widespread use of antimicrobials agents in food animal production and human infections in the Andean Community [52]. Despite the relatively large number of resistance genes, low diversity of genes and gene cassettes were observed, with only eight different coding sequences associated with five cassettes. The class 2 integron *In14* containing the *dfrA14* gene was most common and was found in isolates from animal, human, and environmental samples. It encodes for a type A TMP-resistant dihydrofolate reductase; *dfrA*s are expressed along with TMP-sensitive *folA*, which permits folate to be produced and so bacterial survival [53]. It was detected in *S*. Infantis (ST32) strains from Peru, Ecuador, and Colombia. Indeed, the *dfrA14* gene has been reported in TMP/SUL-resistant *S.* Infantis animal isolates in Ecuador and Peru [10,54,55], but no relation with resistance integrons was documented. Class 2 integron, *In33*, which contains the *dfrA1-aadA1* array, encoding for a TMP-resistant dihydrofolate reductase and for an aminoglycoside adenylyltransferase, that *O*-adenylates position 3″ of streptomycin and position 9 of spectinomycin [56]. This array was found in *S.* Paratyphi B (ST28) strains in Colombia, as shown by some studies; however, no association with class 2 integrons was assessed [10,57]. There are reports documenting the association of the *dfrA1-aadA1* array with class 1 integrons [58,59] and with class 2 integrons, in the latter, the cassettes have been observed to contain a *sat* gene that encodes for a streptothricin acetyltransferase; such cassettes have been reported in *Escherichia coli*, *Shigella sonnei*, and *Vibrio cholerae* [60–63].

Class 1 integrons that harbored the *bla*$_{\text{CARB-2}}$, *dfrA29-bla*$_{\text{OXA-2}}$, and *dfrA17-aadA5* arrays were detected in human and animal sources. The *dfrA17-aadA5* array was found in Ecuadorian *S.* Havana (ST588) strains. This cassette was previously related to the class 1 integrons [64], there are no reports of the presence of this cassette in *S.* Havana but it has been reported in other serovars such as *S.* Typhimurium and *S.* Enteritidis [41,65]. *S.* Havana (ST588) was identified in Ecuador; however, it did not exhibit resistance to most common antibiotics [66]. The gene *bla*$_{\text{OXA-2}}$ gene encodes for an OXA-type β-lactamases (OXA-15, OXA-2) that confers resistance to ampicillin, cefotaxime, and ceftazidime [67]. The gene *bla*$_{\text{CARB-2}}$ encodes for a class A β-lactamase, which is known for providing resistance to ampicillin and ticarcillin [68]. The latter two cassettes were found in Colombian strains. Actually, *S.* Typhimurium strains resistant

to trimethoprim and ampicillin have been documented in Colombia and linked to the presence of *dfr*s, *bla*$_{\text{CARB-2}}$, and *bla*$_{\text{OXA-2}}$ genes, although no associations with integrons were assessed [9,69].

The synergistic combination between sulfonamides and trimethoprim is widely used in livestock animals [8,71] and it is marketed under the name co-trimoxazole (STX) [7,70]. Undeniably, in the studied region, co-trimoxazole is widely used, some studies have reported the presence of STX resistant strains of *S.* Infantis collected from poultry production [10,11,71], such resistance proved to be associated with the presence of *dfr* genes [9], this fact has been also reported in other countries [40,43,72–74]. It seems that the extensive use of STX might play a selective pressure that favors the spread of integrons associated with dihydrofolate reductases. The observed STX resistance in *S.* Havana and *S.* Typhimurium isolates, could be attributed to *dfr* genes associated with class 1 integrons. Being class 1 integrons, these also contain *sul* genes in their 3' CS providing resistance to sulfonamides [75]. On the other hand, *S.* Infantis and *S.* Parathyhi B serovars proved to contain class 2 integrons, which do not contain *sul* genes. although reports have shown that *S.* Infantis isolates from Ecuador and Peru are in fact associated with *sul* genes [10,11]. In addition, studies have also linked *S.* Paratyphi B with *sul* genes [76]. None of the aforementioned studies has assessed the association between these genes and integrons. Undoubtedly, the presence of these resistance integrons might have favored the spread of resistant strains to the bactericidal drug co-trimoxazole. These integrons also carried genes for resistance to other antibiotics including aminoglycosides and β-lactams [7,70]. Integrons could be part of mobile structures, whereby their associated resistance genes could be spread among bacterial populations [77]. Indeed, some of the integrons detected in this study have been related to plasmids [68,78,79]. In particular, pESI-like plasmids have been found in *S.* Infantis isolated from broilers and chicken feeding products. The *dfrA14* and *aadA1* genes have been related to pESI-like plasmids and have been reported in Peru [32] and Ecuador [10]. However, none of those studies evaluated their association with integrons.

5. CONCLUSIONS

IntFinder served as a valuable tool for detecting integrons in isolates of *S. enterica* reported in countries of the Andean Community. Class 2 integrons were the most common, these genetic structures were related to *S.* Infantis (ST32) coming mainly from animal isolates. Integrons were mainly dependent on serovar/ST rather than country or source. Trimethoprim (*dfrA*) resistance genes were the most abundant, but other resistance genes were also found, including *aadA*, and *bla*. It could be argued that the presence of trimethoprim resistance genes might be a consequence of the widespread use of antibiotics in the region, co-trimoxazole in particular. Given that integron-associated resistance genes are often encoded on transferable genetic elements such as plasmids, dissemination of resistance genes could occur among bacterial species, this fact represents a public health concern due to novel resistant strains might emerge. The information gathered by *in silico* studies could be useful when designing antibiotic surveillance program in the region, especially those targeting pathogenic *Salmonella*.

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7. SUPPLEMENTARY MATERIALS

7.1 S1 Table.

The metadata of all the sequences used is available on https://osf.io/js9xp/?view_only=c2a77c8512844eaab644ef73470cb9b3

7.2 S2 Table.

All integrons detected in this study are available on https://osf.io/js9xp/?view_only=c2a77c8512844eaab644ef73470cb9b3

7.3 S3 Script.

The scripts used in this study are available on https://osf.io/js9xp/?view_only=c2a77c8512844eaab644ef73470cb9b3