

Antimicrobial Resistance Gene of *Salmonella enterica* Isolated from Lab Equine Fecal Samples and Human Diarrheal Samples

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Abstract

Antimicrobial resistance (AMR) of *S. enterica* is a global public health threat. The prevalence, antimicrobial susceptibility, and integron-mediated resistance of *Salmonella enterica* isolated from human diarrheal and laboratory equine fecal samples were investigated. Selective enrichment, culture on *Salmonella–Shigella* agar, and biochemical confirmation were used to process a total of 172 samples (104 human and 68 equine). Susceptibility to eight antibiotics was determined by Kirby–Bauer disc diffusion. Integrins were PCR amplified and typed using *HinfI* restriction enzyme. *Salmonella enterica* was isolated from 47 samples (27.32%): 37/104 (35.57%) human and 10/68 (14.70%) equine. All isolates were resistant to ampicillin (100%) and cefotaxime

(100%) and were also resistant to sulphamethoxazole–trimethoprim (66.6%) and chloramphenicol (66.6%), but were susceptible to ceftazidime, nalidixic acid, and gentamicin. All isolates were subjected to amplification of integron genes (492 bp). Class I integrons were observed at greater frequency (90%), class II (10%), and class III was not observed at all. High-level resistance and carriage of class I integrons are both commonly associated with both host species, suggesting a common reservoir of resistance and a potential for horizontal transmission, making integrated One Health surveillance viable.

Introduction

Diarrheal disease is still a major cause of illness and death in the world, especially in low- and middle-income countries (LMICs) with limited access to safe water and sanitation (Troeger et al., 2018). Despite the reduction in deaths due to diarrhoea, the incidence has remained stable, and repeated enteric infections are still affecting growth and cognition in children (Kosek et al., 2003; Victora et al., 2000). As a result,

enteric infections lead to loss of life as well as long-term disability (Guerrant et al., 2002).

Salmonella enterica belongs to the family Enterobacteriaceae, is a Gram-negative, facultatively anaerobic rod, and is a major cause of food- and waterborne disease (Crump et al., 2015). There are more than 2,500 serovars in the species, with the majority belonging to subsp. *enterica* (Eng et al., 2015). Non-typhoidal *Salmonella* (NTS) usually leads to self-limited gastroenteritis, but can cause invasive disease in children, older adults, and immunocompromised patients (Crump et al., 2015). Typhoidal serovars are restricted to humans and cause enteric fever (Gal-Mor et al., 2014).

Invasive salmonellosis requires antimicrobial therapy, but resistance is reducing the effectiveness of therapy (Murray et al., 2022). Resistance is mediated by mobile genetic elements (MGEs) such as plasmids, transposons, and integrons, which contain resistance determinants and contribute to resistance dissemination (Partridge et al., 2018). Integrons are gene-capture systems that include resistance cassettes on a single expression platform, allowing for the accumulation of resistance to multiple unrelated classes (Gillings, 2014; Mazel, 2006).

There is growing awareness of the importance of equids as a component of the resistance ecology. Salmonellosis is always a problem in equine farms and veterinary hospitals and has led to temporary closure of facilities in the past (Kohnen et al., 2023). *Salmonella* bacteria can be shed by horses over weeks to months and thus contaminate the environment (Simó-Martínez et al., 2025). *Salmonella* resistance determinants are found in humans and animals, and molecular characterisation of equine and human *Salmonella* has direct relevance to One Health.

The purpose of this study was to isolate *S. enterica* from laboratory fecal samples from horses and diarrheal samples from humans, and to identify phenotypic resistance and detect and identify integrons. The goals were: (i) isolation and biochemical identification of *S. enterica* from both sources; and (ii) detection of antimicrobial resistance genes (integrons) and their classes.

Literature Review

Global Burden and Clinical Categories

Diarrheal disease is responsible for about 20% of child mortality in developing countries, particularly in children less than 5 years old (Kosek et al., 2003). Rotavirus, *Cryptosporidium*, enterotoxigenic *Escherichia coli*, and *Shigella* have been identified by multi-country surveillance as the pathogens most strongly associated with moderate-to-severe diarrhoea (Kotloff et al., 2013). In addition to death, diarrheal disease can result in permanent disability in growth, fitness, and brain function (Guerrant et al., 2002; Troeger et al., 2018). There was an improvement in mortality in the late 20th century, mainly due to the use of oral rehydration therapy (ORT) (Victora et al., 2000).

According to WHO, diarrhoea can be defined by frequency (three or more loose stools in 24 hours) or changes in consistency (WHO, 2005). It is better considered as a symptom complex caused by gastrointestinal infection, systemic infection, or non-infectious causes (Keusch et al., 2006). The clinical categories are acute watery diarrhoea (80% of childhood diarrhoea cases, 50% of deaths), persistent diarrhoea (lasting 14 days or more, 10% of cases, high risk of death), and bloody diarrhoea/dysentery (WHO, 2005; Alam & Ashraf, 2003). They are associated with three pathogenetic mechanisms: enterotoxin-mediated fluid secretion, inflammatory/cytotoxic mucosal damage, and microbial penetration and enteric fever (Guerrant et al., 2004).

Etiology

Rotavirus, norovirus, enteric adenovirus, and astrovirus are among the viral causes (Clark & McKendrick, 2004). The bacterial agents are *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, *Campylobacter* spp., *Vibrio* spp., and pathogenic *E. coli* (Alam & Ashraf, 2003; Kaper et al., 2004). Protozoans are responsible for *Giardia*, *Entamoeba*, *Cryptosporidium*, *Cyclospora*, *Isospora*, and microsporidia. Isolating the cause of illness is hard; the rates differ in different areas and, even after thorough testing, no cause is found in about half of cases.

The Genus *Salmonella*

Salmonella are Gram-negative bacilli, typically motile with peritrichous flagella (except for *S. Gallinarum* and *S. Pullorum*), catalase-positive, oxidase-negative, urease-negative, and non-lactose fermenting (Quinn et al., 2002). According to taxonomy, *S. enterica* and *S. bongori* are recognised, and *S. enterica* is subdivided into six subspecies (Eng et al., 2015). The traditional method of serotyping is done by O and H antigens; there are more than 2,500 serovars, the majority of which are subsp. *enterica* (Crump et al., 2015). Typhoidal serovars (*S. Typhi* and *S. Paratyphi*) are responsible for enteric fever, while non-typhoidal serovars are responsible for gastroenteritis (Gal-Mor et al., 2014). NTS is commonly found in poultry, beef, pork, eggs, milk, seafood, and produce (White et al., 2001).

Pathogenesis

Once ingested, *Salmonella* binds to and enters the intestinal epithelium, which then engulfs the bacteria; if the bacteria survive intracellular killing, they replicate and disseminate to the lymph nodes in the mesentery, blood, liver, gallbladder, spleen, and bone marrow. The inflammation that results from the discharge of gallbladder contents into the intestine (Rao, 2009).

Laboratory Isolation and Typing

Isolation from feces, blood, or urine is still the gold standard. *Salmonella*–*Shigella* agar has transparent and black colonies. Recovery is enhanced by the use of selenite or tetrathionate broth. Widal serology is not reliable (Itah & Akpan, 2004). Molecular techniques (RAPD, REP–PCR, 16S–23S ITS, real-time PCR) offer greater detection sensitivity and a shorter turnaround time (Antonio et al., 2000; Kurowski et al., 2002; Yaqoob et al., 2007). The highest resolution is now available from whole-genome sequencing (Partridge et al., 2018).

Equine Salmonellosis

Salmonellosis is an important disease in breeding farms and veterinary hospitals for horses (Kohnen et al., 2023). Temporary closures have been reported in nosocomial outbreaks, and shedding of infected horses has been found to last for 30–300 days (Simó-Martínez et al., 2025). The most important risk factors are stress, temperature changes in the barn, diet changes, colon impaction, nasogastric intubation, surgery, anaesthesia, concurrent diseases, and antimicrobial treatment (Kohnen et al., 2023). Prevalence is variable – a population-based study in the United States had 2.0% fecal prevalence and 7.0% operation-level prevalence, of which most were pan-susceptible (Kohnen et al., 2023). On the other hand, non-symptomatic horses in the Eastern part of Spain were positive for resistant *Salmonella* spp. of public health concern (Simó-Martínez et al., 2025). Microbiological shedding is higher in hospitalised horses and foals, and antimicrobial treatment raises the number of horses shedding by 10- to 40-fold in their faeces.

Antimicrobial Resistance

Antimicrobial resistance is one of the biggest public health threats. In 2019, 4.95 million deaths were attributed to bacterial resistance and 1.27 million directly to

bacterial resistance (Murray et al., 2022). New antimicrobials are introduced, but they often have similar structures to the ones already in use and resistance is predictable (Livermore, 2003). Resistance will occur more quickly when overused (Holloway, 2000). Resistance in developing countries is further exacerbated by poverty, lack of diagnostics, poor sanitation, and poor antimicrobial use (Byarugaba, 2004). Agriculture plays a significant role in the pool of environmental resistance (Sharma et al., 2007). Antibiotic-resistant bacteria and antibiotic residues are discharged into the environment as waste products from sewage, hospital waste products, and agricultural runoff, and are then selected and enriched (Kim et al., 2007). At low concentrations of antibiotics, horizontal transfer boosts gene frequencies and propagates resistance (Shoemaker et al., 2001). Resistance genes have been found in irrigation ditches, river sediments, seawater, dairy lagoons, and wastewater effluents (Henriques et al., 2006; Pruden, 2006). Dynamic resistance occurs with surveillance data; in US animals, *Salmonella* resistance increased from 4.0% to 18.8% in 2003 (Frye & Fedorka-Cray, 2007). However, in India, all 111 *Salmonella* isolates from equids were resistant to one or more antimicrobial drugs, with 75.7% being multidrug-resistant (MDR) (Singh et al., 2009). Antimicrobial residues in the intestine increase intra- and intergeneric transfer of resistance determinants (Edlund & Nord, 2000).

Mechanisms of Resistance

Resistance can be acquired through chromosomal mutation, or through the acquisition of plasmids, transposons, or integrons containing resistance genes (Poole, 2001). Hypermutator strains could be better at adhering, growing faster, and/or at cost adaptation of fitness (Livermore, 2003). Intrinsic resistance could be due to evolutionary resistance mechanisms, such as impermeability or absence of the target (Cox & Wright, 2013). Acquired resistance can occur by mutation, horizontal gene transfer, or transformation (Cox & Wright, 2013). Plasmids are circular double-stranded DNA molecules which contain virulence and resistance genes (Kaye et al., 2000). Conjugative transfer is significant because it can span species and may be transferred to organisms that are not related, and single plasmids may carry multiple resistance genes. The role of transduction and transformation in the dissemination of resistance is less significant because of the limit of packaging, the narrow host range, and the nuclease susceptibility of the genes (Cox & Wright, 2013).

Integrons

Integrons are potentially mobile DNA elements that consist of a site-specific recombination system that can add and express genes within "cassettes" (Gillings, 2014). They acquire and transport resistance genes in gene cassettes, while the integrase activity is provided by the integron of the recipient, and not by the cassette (Mazel, 2006). An integron is comprised of a highly conserved region at both ends that includes an integrase gene, and a variable region of resistance gene cassettes between the two highly conserved regions (Partridge et al., 2018). The 5'-CS consists of three essential elements: integrase gene (*intI*), a neighboring *attI* site, and a promoter for expression of the cassette (*P_c*) (Gillings, 2014). Cassettes are promoterless and therefore the expression of these genes must rely on the integron promoter, while arrays may shuffle, expand, and/or shorten, allowing for rapid evolution under selection (Partridge et al., 2018).

Classification and Epidemiology of Integrons

There are four types of integrons defined by different integrase genes. Class 1 integrons are most often described and are most common in clinical isolates, and are closely linked to multidrug-resistant hospital strains of bacteria (Naas et al., 2001). In addition, class 2 integrons are found frequently, class 3 integrons are found rarely, and class 4 integrons are linked to *Vibrio* (Partridge et al., 2018). Class 1 integrons are

frequently found within transposons, e.g., Tn*402*, Tn*21*, and have several dissemination mechanisms (Gillings, 2014). In *Salmonella* and *E. coli* samples from both clinical and food-producing animal sources, surveillance has consistently found class 1 integrons at a higher frequency than class 2 (Dessie et al., 2013; Kim et al., 2007). A recent US genomic survey revealed that 116 (60.1%) out of 193 isolates of non-typhoidal *Salmonella* from food animals contained class 1 and/or class 2 integrons, of which class 1 was present in 105. Some integrons were expressed in more than one copy (Hsu et al., 2025). In a Brazilian pork chain, in silico detection of integrons was shown to be correlated with resistance phenotypes and the detection of integron cassettes conferring resistance to aminoglycosides and trimethoprim (Rodrigues et al., 2024). Antimicrobial resistance genes commonly found in integrons include *aadA*, which encodes resistance to streptomycin/spectinomycin, and *dfrA*, which encodes resistance to trimethoprim, both of which are used in the treatment of humans and animals (Dessie et al., 2013). Detection and classification is thus available as additional information beyond phenotypic testing, and PCR–RFLP is still a viable option in a resource-restricted lab for class-level characterisation (Hsu et al., 2025).

Impact and Knowledge Gaps

The main effect of the evolution of resistance in animal-origin bacteria is loss of effectiveness of antimicrobials. If not sufficiently treated, death rates go up, and if treated, the expense may be too high. *Salmonella* is known to be resistant to first-line agents like ampicillin, chloramphenicol, and trimethoprim–sulphamethoxazole, which is why this is documented on multiple occasions, and resistance to extended-spectrum cephalosporins is emerging (Crump et al., 2015; Frye & Fedorka-Cray, 2007). *Salmonella* containing OXA-48 or third-generation cephalosporin resistance have been classified by WHO as priority pathogens (WHO, 2024). Even with the surveillance data, there remain some gaps that should be addressed: phenotypic testing is unable to differentiate intrinsic from acquired resistance, many studies report frequencies without characterising genes, and there is a shortage of studies comparing the integron content of human and animal isolates from the same area. This study will fill this void as it will provide a characterisation of the phenotype along with the integron class of *S. enterica* recovered from both human diarrheal and lab equine fecal samples.

Materials and Methods

Study Design and Duration

The study was an experimental descriptive study which was carried out during six months in the Microbiology Laboratory, Department of Biosciences, COMSATS Institute of Information Technology, Islamabad.

Sample Size and Sampling

A total of 172 samples were processed, 104 human diarrheal samples and 68 laboratory equine fecal samples. Non-probability sampling was used.

Inclusion and Exclusion Criteria

Isolates of *Salmonella enterica* from laboratory equine fecal and human diarrheal samples were included. Isolates from other animals or non-diarrheal human samples were excluded.

Specimen Collection and Culture

Stool specimens were obtained by clean-catch midstream collection and transported without delay; when delayed, they were transported in Cary–Blair medium at 4 °C. Equine fecal samples were collected into sterile containers and stored at 4 °C. Two

grams of each sample were enriched in 15 mL Selenite Cysteine Broth Base at 37 °C for 24 h. A loopful was streaked onto *Salmonella*–*Shigella* (SS) agar and incubated at 37 °C for 24 ± 3 h. Typical *Salmonella* colonies (black centre, transparent periphery) were subcultured onto MacConkey and nutrient agar.

Media preparation: SS agar (6.3 g/100 mL), MacConkey agar (5 g/100 mL), and nutrient agar (2.8 g/100 mL) were prepared per manufacturer instructions (Oxoid, UK), autoclaved at 15 psi/121 °C for 15 min, and poured into 90-mm Petri dishes.

Biochemical Tests

Isolates were confirmed by Gram staining, lactose fermentation, indole, H₂S production, motility, citrate utilisation, catalase, oxidase, methyl red, TSI, and urease tests following standard protocols.

Gram stain: differentiated Gram-positive and Gram-negative bacteria.

Lactose fermentation: white colonies on MacConkey indicated non-lactose fermenters (consistent with *Salmonella*); pink indicated lactose fermenters.

Indole: SIM agar with Kovac's reagent; red = positive, yellow = negative.

H₂S: black along stab line in SIM agar = positive.

Motility: diffuse growth around stab = motile; confined = non-motile.

Citrate: Simon citrate agar; green to blue = positive.

Catalase: 3% H₂O₂; bubbles within 10 s = positive.

Oxidase: filter paper impregnated with reagent; blue = positive, no colour = negative.

Methyl red: MR–VP broth; red immediately = positive.

TSI: alkaline slant, acid butt, gas, H₂S = suggestive of *Salmonella*.

Urease: urea broth; no colour change = negative.

Disc Diffusion Susceptibility Testing

Susceptibility was determined by Kirby–Bauer disc diffusion on Mueller–Hinton agar (Oxoid, UK) according to NCCLS criteria. Antibiotics tested: nalidixic acid (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), trimethoprim (5 µg), ampicillin (25 µg), sulphamethoxazole–trimethoprim (25 µg), gentamicin (10 µg), chloramphenicol (30 µg), and ofloxacin (5 µg). Inocula were adjusted to McFarland standard and spread on dried plates. Discs were placed 15 mm from the edge and 24 mm apart. Zones of inhibition were measured and determined according to manufacturer tables after 24 hours at 37 °C.

DNA Extraction

DNA was extracted by phenol–chloroform. One mL of cell suspension was centrifuged (10,000 rpm, 3 min), washed twice with 400 µL STE buffer (100 mM NaCl, 10 mM Tris/HCl, 1 mM EDTA, pH 8.0), and centrifuged (10,000 × g, 30 min). Pellets were resuspended in 200 µL TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0), mixed with 100 µL Tris-saturated phenol (pH 8.0), vortexed 60 s, and centrifuged (13,000 rpm, 15 min, 4 °C). The upper aqueous layer (160 µL) was transferred, mixed with 40 µL TE buffer and 100 µL chloroform–isoamyl alcohol (24:1) and extracted until the white interface disappeared. The purified DNA was kept at –20 °C.

Agarose Gel Electrophoresis

DNA was run on 1% agarose (0.3 g/30 mL 1× TBE); PCR products on 2% agarose (0.6 g/30 mL 1× TBE). Ethidium bromide (5 µL) was added before pouring. Samples (5 µL DNA/PCR product + 3 µL 10× loading dye) were electrophoresed at 100 V (80 mA) for 30 min in 1× TBE. Images were captured with Gel Doc XR (Bio-Rad) and Quantity One software.

PCR Amplification of Integrons

PCR used 2 µL purified DNA in a 25-µL reaction with 1.5 µL each of Hep35 and Hep36 primers, 0.3 µL Taq polymerase (Fermentas), 0.2 µL dNTPs, and 2 µL 10× buffer with MgCl₂. Conditions: 94 °C 4 min; 10 cycles of 94 °C 45 s, 53 °C 45 s (–1 °C/cycle), 72 °C 1 min; 30 cycles of 94 °C 45 s, 54 °C 45 s, 72 °C 1 min; final 72 °C 10 min. Products were resolved on 2% agarose with ethidium bromide; sizes compared to 1-kb plus ladder (Invitrogen).

Table 1. Primer sequences for integron amplification

Primer	Sequence (5'→3')	Product size	Reference
Hep35	TGCGGGTYAARGATBTKGATTT	492 bp	Gu et al. (2007)
Hep36	CARCACATGCGTRTARAT	492 bp	Gu et al. (2007)

Table 2. PCR reaction components (25 µL)

Component	Volume	Final concentration
10× PCR buffer with 2 mM MgCl ₂	2 µL	1×
dNTPs	0.6 µL	2.5 mM each
Hep35	1.5 µL	10 pmol
Hep36	1.5 µL	10 pmol
Template DNA	2 µL	10–100 ng
Taq DNA polymerase (5 U/µL)	0.3 µL	1 unit
Distilled H ₂ O	17.1 µL	—

RFLP Analysis

PCR products were digested with *Hinf*I: 10 µL PCR product, 2 µL buffer R, 1.5 µL *Hinf*I, 18 µL distilled water; incubated 37 °C for 16 h; inactivated at 65 °C for 20 min. Products were resolved on 2% agarose and visualised under UV. Class I integrons remain at 492 bp (no *Hinf*I site); class II yield 300 bp and 192 bp; class III would yield 272 bp and 220 bp.

Data Analysis

Data were analysed by graphical and tabular representation.

Results

Distribution of *Salmonella*

Among 172 samples, *Salmonella* was isolated from 47 (27.32%). Isolation was 35.57% (37/104) in human samples and 14.70% (10/68) in equine samples (Table 3, Figure 1).

Table 3. Distribution of samples and *Salmonella* isolation

Source	Total	Positive	Negative	Prevalence (%)
Human	104	37	67	35.57
Equine	68	10	58	14.70
Total	172	47	125	27.32

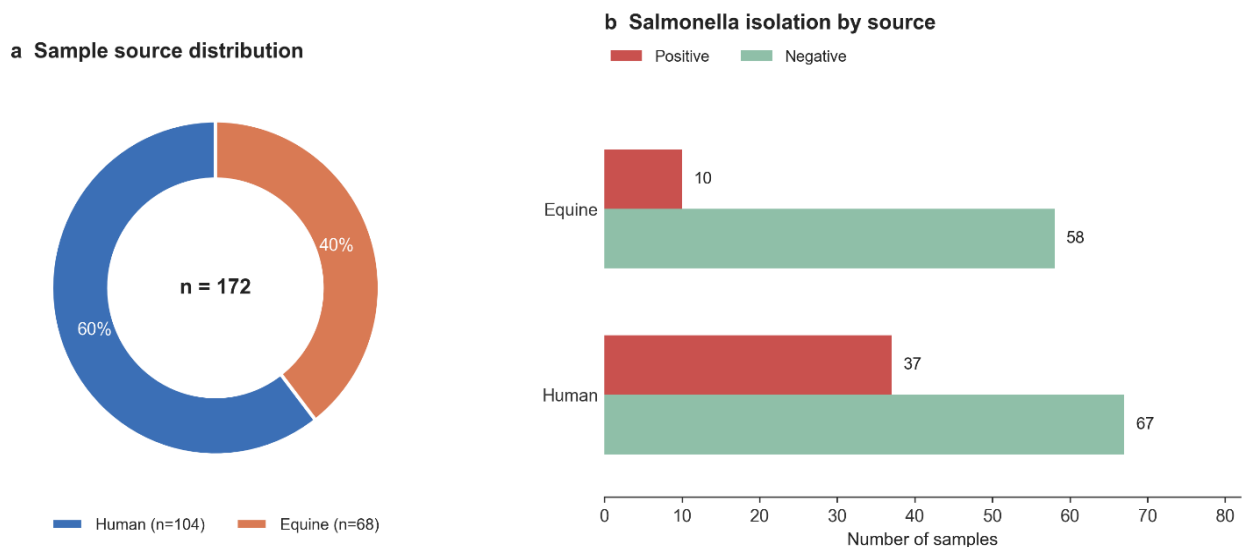
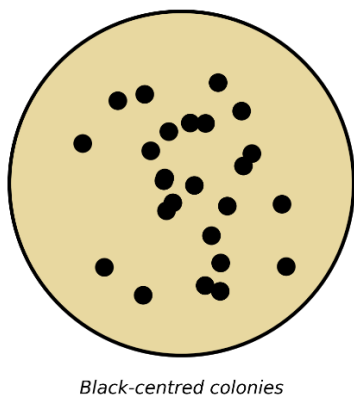


Figure 1. Frequency distribution of samples by source (human vs. equine) and *Salmonella* isolation rates.

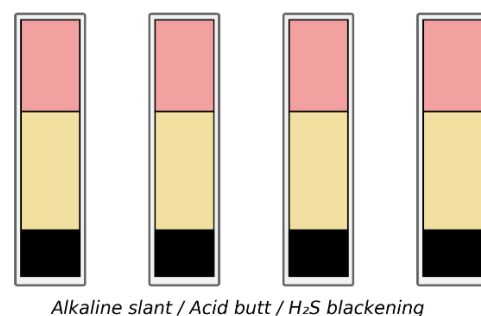
Biochemical Characterisation

All 47 isolates were Gram-negative rods, non-lactose fermenting on MacConkey, and produced alkaline slants/acid butts with H_2S on TSI. Methyl red was positive; urease, indole, and oxidase were negative; catalase and motility were positive; citrate was utilised (Figure 2).

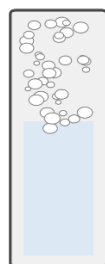
A. Growth on SS Agar



B. TSI Agar Test



C. Catalase Test (Positive)



D. Oxidase Test (Negative)

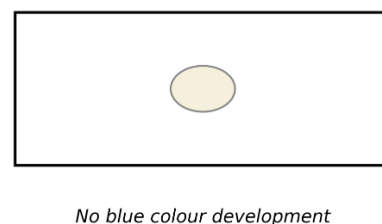


Figure 2. Biochemical characterisation of *Salmonella enterica* isolates. (A) Growth on SS agar showing black-centred colonies; (B) TSI agar showing alkaline slant, acid butt, and H_2S production; (C) Catalase test showing bubble formation; (D) Oxidase test showing negative result.

Antimicrobial Susceptibility

All isolates were resistant to ampicillin (100%) and cefotaxime (100%). Resistance to sulphamethoxazole–trimethoprim was 66.6% (33.4% intermediate), chloramphenicol 66.6%, and ofloxacin 33.4%. All were susceptible to ceftazidime, nalidixic acid, and gentamicin (Table 4, Figure 3).

Table 4. Antimicrobial resistance profile (n = 47)

Antibiotic	Resistant (%)	Intermediate (%)	Susceptible (%)
Ampicillin (25 µg)	100	0	0
Cefotaxime (30 µg)	100	0	0
Sulphamethoxazole–trimethoprim (25 µg)	66.6	33.4	0
Chloramphenicol (30 µg)	66.6	0	33.4
Ofloxacin (5 µg)	33.4	0	66.6
Ceftazidime (30 µg)	0	0	100
Nalidixic acid (30 µg)	0	0	100
Gentamicin (10 µg)	0	0	100

Antimicrobial resistance pattern of *S. enterica* (n = 47)

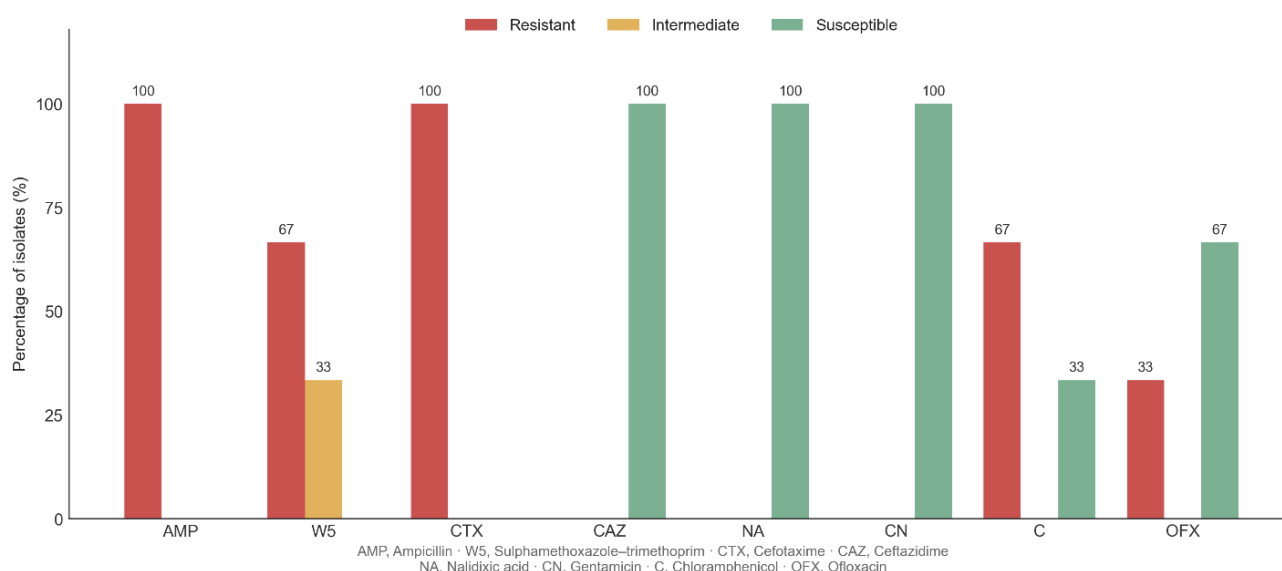


Figure 3. Antimicrobial resistance pattern of *Salmonella enterica* isolates (n = 47). AMP = ampicillin; W5 = sulphamethoxazole–trimethoprim; CTX = cefotaxime; CAZ = ceftazidime; NA = nalidixic acid; CN = gentamicin; C = chloramphenicol; OFX = ofloxacin.

Molecular Detection of Integrons

PCR with Hep35/Hep36 amplified the expected 492-bp integron fragment from all 47 isolates (Figure 4). RFLP with *Hinf*I showed class I integrons in 90% (42/47) and class II in 10% (5/47); no class III integrons were detected (Table 5, Figure 5).

Table 5. Integron classes among isolates (n = 47)

Class	Frequency	Percentage
I	42/47	90
II	5/47	10
III	0/47	0

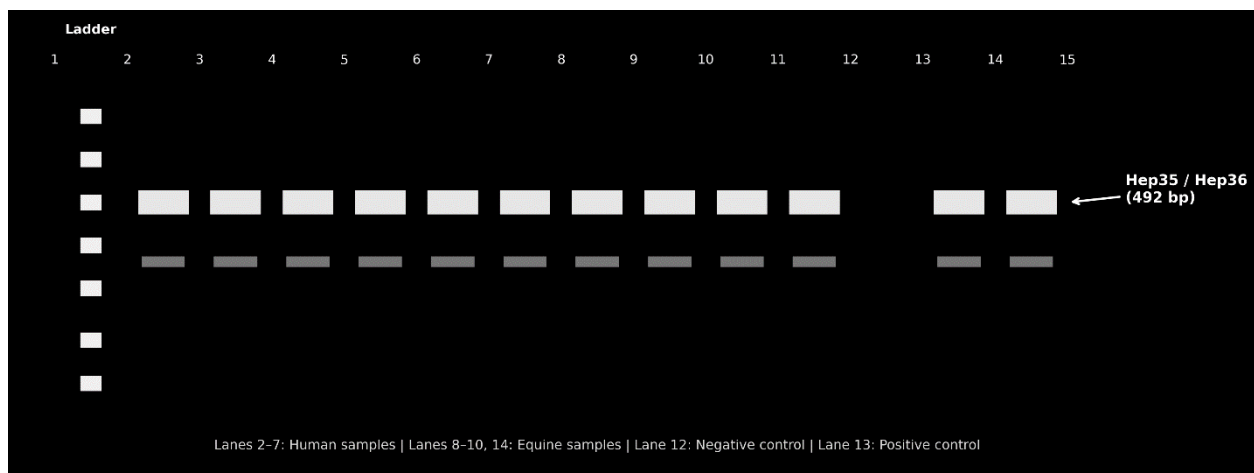


Figure 4. Amplification of integron genes of *Salmonella enterica* (Hep35/Hep36, 492 bp). Lanes 1–6: human samples; lanes 7, 8, 9, and 13: equine samples; lane 11: negative control; lane 12: positive control; ladder: 1-kb plus DNA ladder.

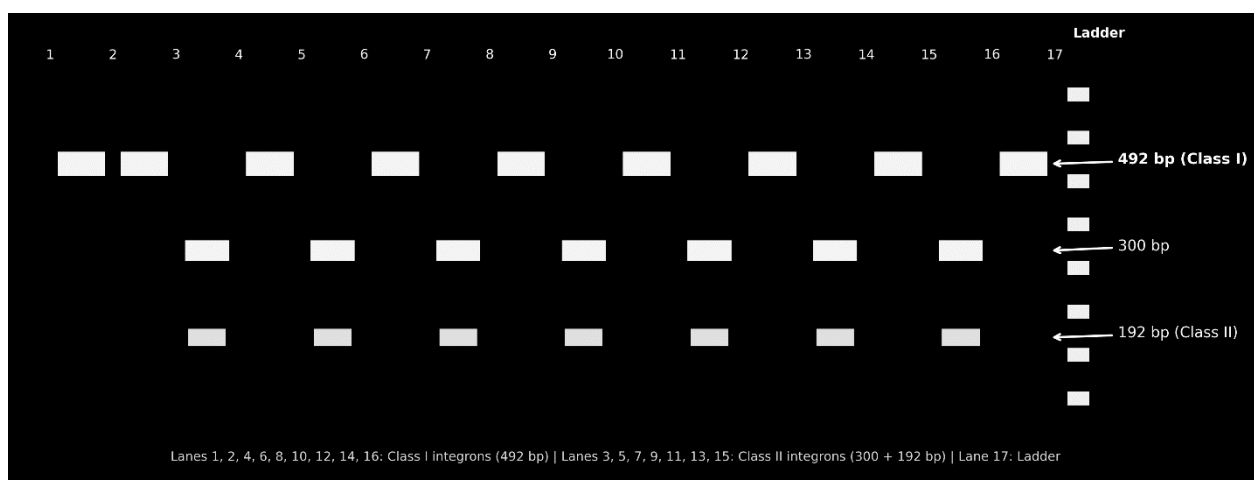


Figure 5. RFLP analysis of class I integrons (492 bp) and class II integrons (300 bp and 192 bp). Lanes 1–17 show restriction fragment patterns; ladder on the right.

Discussion

The overall isolation rate was 27.32%, which is higher than the population-based equine surveys (Kohnen et al., 2023), possibly due to selective enrichment and sampling of people with symptoms and laboratory equids that were confined. This is consistent with the recovery from human samples (35.57%) being higher than the recovery from equine samples (14.70%), suggesting a higher shedding rate in diarrheal patients.

Resistance is a problem: universal resistance to ampicillin and cefotaxime, and high resistance to sulphamethoxazole–trimethoprim and chloramphenicol. This is similar to worldwide data on the increase in resistance in *Salmonella* spp. (Crump et al., 2015; Frye & Fedorka-Cray, 2007) and the WHO classification of *Salmonella* spp. as priority pathogens (WHO, 2024). This resistance pattern is similar to African and Asian studies (Diarra et al., 2024; Singh et al., 2009). One-third of the isolates had intermediate susceptibility to sulphamethoxazole–trimethoprim, indicating that reduced susceptibility is more prevalent than resistance.

The susceptibility to ceftazidime alongside universal cefotaxime resistance is noteworthy, as both drugs are third-generation cephalosporins but differ in their susceptibility to certain β -lactamases. The pattern indicates a particular resistance mechanism as opposed to a generalised carbapenemase. Susceptibility to nalidixic acid suggests that fluoroquinolone resistance has not yet occurred, as nalidixic acid

resistance is the earliest marker. The fact that gentamicin susceptibility is retained is encouraging.

All isolates contained integron genes, the majority of which were class I (90%), followed by class II (10%), and none were class III. This is the same as the literature worldwide, with *Salmonella* and *E. coli* from clinical and food-animal sources showing the highest prevalence of class 1 integrons (Dessie et al., 2013; Kim et al., 2007; Partridge et al., 2018). The high percentage of class I integrons implies that these are well established locally. Class 1 integrons are frequently located in conjugative plasmids within transposons, which means their dominance may lead to the transfer of integrons between strains, species, and hosts (Gillings, 2014). Recent genomic studies have demonstrated that class 1 integrons are ubiquitous in food-animal non-typhoidal *Salmonella* (NTS), and may be present in more than one copy (Hsu et al., 2025). This study reports the same comparison in a human–equine context within one geographical setting.

The lack of class III integrons is in line with their low prevalence in *Salmonella* (Partridge et al., 2018). The preponderance of class I integrons in human and equine isolates suggests that the gene pool of resistance is common to both sectors, that interventions in one can have a positive impact on the other, and that resistance acquired in one can be transmitted to the other. A study of asymptomatic horses in Spain reached the same conclusion (Simó-Martínez et al., 2025).

The limitations are the small scale, lack of serotyping, single laboratory and short time frame, class-level integron typing (no cassette sequencing or whole-genome sequencing), and no clinical outcome information. However, the good correlation between the presence of integrons and phenotypic resistance in all isolates is strong evidence of the involvement of mobile elements in resistance in this population.

Implications

The findings have four practical implications. First, resistance to ampicillin and cefotaxime was universal, and these drugs should not be used for empirical therapy of suspected *Salmonella* infections here; therapy should be guided by culture and susceptibility results. Second, integron carriage in all isolates (90% carrying class I integrons) implies that resistance is carried on mobile elements, and therefore the three remaining drugs (gentamicin, ceftazidime, and nalidixic acid) are also potentially at risk of being lost. Antimicrobial stewardship in human and veterinary practice is therefore crucial (Teklemariam et al., 2023; WHO, 2024). Third, identical classes of integrons are found in human and equine isolates, indicating a shared resistance gene pool; interventions in one sector should benefit the other, and surveillance should include both sectors (Simó-Martínez et al., 2025; Teklemariam et al., 2023). Fourth, PCR–RFLP proved adequate to classify integrons, so laboratories without sequencing facilities can still provide valuable surveillance data on integron distribution (Hsu et al., 2025).

Conclusion

In this study, *Salmonella enterica* was found to be highly prevalent in human diarrheal samples (35.57%) as well as in laboratory equine fecal samples (14.70%), and the overall prevalence rate was 27.32%. Ampicillin and cefotaxime resistance was 100%, sulphamethoxazole–trimethoprim and chloramphenicol resistance was 66.6%, and all isolates were susceptible to ceftazidime, nalidixic acid, and gentamicin. All isolates contained integron genes, with class I integrons being the most common (90%) and class II (10%); class III integrons were not found. High-level multidrug resistance and carriage of class I integrons in both host species suggest a common gene pool of resistance determinants, as class 1 integrons are often harboured on conjugative plasmids and transposons that are mobile across strains, species, and genera (Gillings, 2014; Partridge et al., 2018).

There are important clinical and public health implications. Universal resistance to ampicillin and cefotaxime makes it difficult to use these drugs as empirical therapy, and the high proportion of resistance to sulphamethoxazole–trimethoprim and chloramphenicol further limits treatment choices, with gentamicin, ceftazidime, and nalidixic acid being the only agents tested that remained effective. However, this susceptibility should not be assumed, as resistance can quickly develop under sustained selective pressure, and the presence of class I integrons in all isolates suggests that resistance to these remaining agents may be acquired (Murray et al., 2022; WHO, 2024). Limitations include the small sample size, the single-laboratory nature and short time period, the absence of serotyping, and integron characterization at the class level only, without cassette or whole-genome sequencing. Future studies are needed to address these limitations and fully characterize the resistance mobilome and define the transmission routes (Hsu et al., 2025).

The overall conclusion of this study is that *Salmonella enterica* from human and equine sources in this environment is highly resistant to antimicrobial agents, and widespread carriage of class I integrons is a common threat that requires a coordinated response between sectors. The results support integrated One Health surveillance programmes, the judicious use of antimicrobials in both clinical and agricultural settings, and the routine detection of integrons in resource-limited laboratories (Simó-Martínez et al., 2025; Teklemariam et al., 2023). To ensure the efficacy of last-line agents and to prevent further spread of resistance determinants in *Salmonella* and other foodborne pathogens, continuous surveillance is needed, along with rational use of antimicrobials.

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