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Molecular Detection of Virulence Genes of *Salmonella* Agona Isolated from Diarrheic Cats in Al-Anbar Province, Iraq

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ABSTRACT

Salmonella infections are a major cause of foodborne infection in both humans and animals. Some serotypes are capable of inducing severe clinical manifestations, including gastroenteritis, septicemia, and abortion. The present study aimed to identify, isolate, and detect *Salmonella* Agona in cats with diarrhea in Al-Anbar Governorate, Iraq, and to evaluate the antimicrobial resistance profiles of the recovered isolates. Between November 2024 and May 2025, 100 fecal samples were collected from cats of different breeds presenting with diarrhea at veterinary clinics in Iraq. These samples were processed using established culture methods, including pre-enrichment in buffered peptone water followed by selective enrichment in tetrathionate broth. The enriched samples were then cultured on selective and differential media, including xylose lysine deoxycholate (XLD) agar and *Salmonella*-Shigella (SS) agar. For isolates identification, biochemical analyses, including catalase, indole, methyl red, Simmons citrate, oxidase, urease, lactose fermentation, triple sugar iron test, and phenylalanine, were performed, and the automated VITEK®2 test was used to confirm the results. The polymerase chain reaction targeted the *invA* gene to identify *Salmonella* species, followed by sequence analysis. Eventually, additional virulence genes were identified, including *Salmonella* enterotoxin (*stn*) and *Salmonella* outer protein E (*sopE*). The current results demonstrated that 4% of the samples (4 out of 100) were positive for *Salmonella* Agona. Antibiotic susceptibility testing against 16 antimicrobial agents demonstrated that the isolates were resistant to eight antibiotics (50%), namely amoxicillin, streptomycin, cefotaxime, oxytetracycline, doxycycline, tetracycline, trimethoprim-sulfamethoxazole, and gentamicin. The virulence gene results indicated that 2 out of 4 isolates (50%) possessed the *sopE* gene, whereas only 1 isolate (25%) carried the *stn* gene. The current findings indicate that *Salmonella* Agona was not prevalent among cats presenting with diarrhea; however, the existence of highly virulent, multidrug-resistant strains represented a vital public health concern for both humans and animals.

Keywords: Feline, *invA* gene, Polymerase chain reaction, *Salmonella* Agona, Virulence gene

INTRODUCTION

The White-Kauffmann classification categorized *Salmonella* (*S.*) species into two groups, including *S. bongori* and *S. enterica*, which are Gram-negative bacilli belonging to the *Enterobacteriaceae* family (Dougan and Baker, 2014). *Salmonella* classification is based on three surface antigens, including the lipopolysaccharide (O) antigen, the flagellar (H) antigen, and the capsular polysaccharide (Vi) antigen (Dougan and Baker, 2014). The genus *Salmonella* includes over 2,659 serotypes, of which approximately 1,547 were classified within the *Enterobacteriaceae* family, including species such as *S. salami*, *S. arizonae*, *S. diazoni*, *S. hotinii*, and *S. indica* (Dougan and Baker, 2014; Yada, 2023).

Salmonella Agona (*S.* Agona) is a non-typhoidal *Salmonella* species known for forming biofilms and surviving in environmental conditions and in dried food products (Song et al., 2025). Chickens, pigs, cattle, and ducks shed *S.* Agona, a pathogen linked to several outbreaks (Song et al., 2025). However, its ability to spread to humans and the role of large-scale genome rearrangements in ongoing infections remain poorly understood (Song et al., 2025). *Salmonella* Agona is a serotype linked to numerous outbreaks of foodborne and waterborne disease worldwide, commonly associated with contaminated animal products and feed (Dhakal et al., 2024). Pet food is considered unsafe for human consumption because it does not meet the same microbial safety standards as human food. This disparity raised concerns about the potential transmission of pathogens, such as *S.* Agona, to humans through direct contact or accidental ingestion. Additionally, due to the close proximity between humans and their pets, individuals often handle pet food with their bare hands and interact with their pets. In some instances, children inadvertently ingest pieces of pet food (Balachandran et al., 2012). Domestic cats can shed pathogens into the environment and contribute to the spread of zoonotic diseases, whether they exhibit clinical signs such as diarrhea or remain asymptomatic carriers (Udainiya et al., 2024).

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Salmonella was regarded as an easily treatable infection; however, it has become increasingly difficult to manage due to the global spread of antibiotic resistance (Mohamedtahir Fadlalla, 2025). Several mechanisms contribute to the emergence of antibiotic resistance, including beta-lactamase production, efflux pump overexpression, target-site modifications, and decreased membrane permeability (Mohamedtahir Fadlalla, 2025). Rapid proliferation of antibiotic resistance is occurring due to the excessive use of beta-lactamase production in human and veterinary healthcare settings (Caneschi et al., 2023). Recent surveillance data indicated that multidrug resistance is becoming more prevalent, particularly among isolates obtained from pets (Cardoso et al., 2023). Antibiotic resistance presented significant challenges due to the limited availability of treatment options and the increasing ease of access to antibiotics (Caneschi et al., 2023). Given the limited number of studies on the isolation of *S.* Agona from felines, the present study aimed to isolate *S.* Agona from cats with diarrhea to assess the antimicrobial resistance profiles of these isolates.

MATERIALS AND METHODS

Ethical approval

The present study was approved by the College of Veterinary Medicine at the University of Baghdad, Iraq, in accordance with the research ethics committee (ID: 2968 on 3/12/2025). The present study was conducted at Al-Ramadi Teaching Hospital after obtaining the necessary official documentation (P.G./ 2174 dated 11/2024 and P.G./ 2230 dated 24/11/2024). Pet owners provided their informed consent for the use of their pets' samples in the present study, and the identities of the animals and their owners were maintained confidentially.

Sampling and location

Between November 2024 and May 2025, 100 fecal samples were collected from cats with diarrhea that visited veterinary clinics in Al-Anbar province, primarily in Ramadi, Hit, and Haditha. No samples were collected from cats that had received antibiotics within the previous 10 days. Age, sex, living situation, and breed of cats were recorded. The samples were placed in buffered peptone water (Oxoid, England) and maintained in a cooled transport box at approximately 4°C until their arrival at the Microbiology Laboratory, Ramadi Teaching Hospital, Al-Anbar, Iraq. Subsequently, the samples were stored at –20°C for further analysis.

Isolation and identification of *Salmonella* Agona

Each sample in buffered peptone water was incubated at 37°C for 24 hours, then transferred to tetrathionate broth (TTB; Oxoid, England) at 42°C for 48 hours to promote bacterial growth. Samples were cultured on *Salmonella-Shigella* (SS) agar (Condalab, Spain) and xylose-lysine deoxycholate (XLD) agar (Oxoid, England) and incubated at 37°C for 24 hours. Suspected colonies were subjected to Gram staining and identified using standard biochemical tests, including urease, citrate, phenylalanine deaminase, indole, triple sugar iron (TSI) test, and catalase. The VITEK®2 Compact (GN ID card) was used to confirm the isolates. Pure colonies were obtained from selective media such as XLD agar after incubation at 37°C for 18-24 hours. A single, well-isolated colony was aseptically transferred into a sterile tube containing 3 mL of sterile saline solution (0.45-0.5% NaCl). The bacterial suspension was mixed thoroughly using a vortex mixer, and the turbidity was adjusted to 0.5 McFarland standard using a densitometer. The standardized suspension was then used to inoculate the GN identification card, which was designed for Gram-negative bacteria. The inoculated cards were loaded into the VITEK 2 system according to the manufacturer's instructions. The system automatically performed incubation and biochemical analysis of the isolates. Identification results were produced within 6-8 hours and analyzed using the system's probability percentages (Neyaz et al., 2024).

Antimicrobial susceptibility

The disk diffusion method was employed to assess antibiotic sensitivity, following the protocols established by CLSI VET01 and CLSI VET01S (2024). A total of 16 antibiotics were tested, manufactured by Bioanalyse, Turkey, including amoxicillin (25 µg), ampicillin (10 µg), streptomycin (10 µg), cefotaxime (30 µg), ceftriaxone (30 µg), piperacillin (100 µg), imipenem (10 µg), oxytetracycline (30 µg), doxycycline (30 µg), tetracycline (30 µg), norfloxacin (10 µg), enrofloxacin (5 µg), levofloxacin (5 µg), ciprofloxacin (5 µg), trimethoprim–sulfamethoxazole (SXT [1.25/23.75] 25 µg), and gentamicin (10 µg; Palladini et al., 2023).

Polymerase chain reaction

The Presto™ DNA extraction kit (Geneaid Biotech Ltd, Taiwan) was used for DNA extraction. Bacterial DNA was extracted using the Genomic DNA Mini Kit (Geneaid). One ml of an overnight culture in brain heart infusion broth was added to an Eppendorf tube (1.5 mL), centrifuged at 13000 rpm for 60 seconds to pellet the cells, and the supernatant

was discarded. A total of 180 µl of GT buffer (lysis solution) was added to the tube, the cells were gently pipetted to resuspend, and the mixture was incubated at room temperature for five minutes. In this step, 20 µl of Proteinase K was added to the sample, and the tube was incubated at 60˚C for 10 minutes, with the tube inverted every three minutes. Subsequently, 200 µL of lysis buffer (GB buffer) was added to the sample, and the mixture was shaken for five seconds. The mixture was then incubated at 70˚C for ten minutes, with the tube inverted every three minutes. Meanwhile, the TE buffer (Elution buffer) was incubated at 70˚C in a water bath (Hearn and Arblaster, 2010). A total of 200 µL of absolute ethanol was added to the sample lysate, and the mixture was vortexed immediately until a precipitate formed. At this time, a genomic DNA (GD) binding column in a collection tube (2 mL) was placed, and the whole mixture was transferred to the GD column, centrifuged at 13000 rpm for two minutes. The GD column was transferred to a new collection tube, and the first collection tube containing the flow-through was removed (Hearn and Arblaster, 2010). A washing solution (400 µL) was added to the GD column, centrifuged at 13000 rpm for 30 seconds, and the column was returned to the collection tube after the flow-through was removed. Then, wash buffer (600 µL) was added to the GD column, centrifuged at 13000 rpm for 30 seconds, and the flow-through was removed. The GD column was placed back into the collection tube (2 mL) and centrifuged for three minutes at 13,000 rpm to dry the column matrix (Menchhoff et al., 2020). Subsequently, 100 µL of preheated elution solution (TE buffer) was added to the center of the matrix in the GD column after placing it in a clean Eppendorf tube. After three minutes, the matrix absorbed the added solution, and the purified DNA was finally eluted by centrifuging at 13000 rpm for 30 seconds and stored at -20˚C until use (Menchhoff et al., 2020).

According to the method of Rahn et al. (1992), primers were designed to detect the *invA* gene, alongside the virulence genes *sopE* and *stn* (Table 1). The PCR reactions were performed in a final volume of 25 µL, consisting of 2.5 µL of 10x PCR buffer, 2.0 µL of magnesium chloride (25 mM), 0.5 µL of dNTP mix (10 mM each), 1.0 µL of each primer (10 µM), 0.2 µL of Taq polymerase (5 units/µL), 2.0 µL of DNA template, and 15.8 µL of sterile distilled water. The PCR was performed using the following thermal cycling settings. An initial denaturation was conducted at 94˚C for five minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, primer annealing at 55°C for 30-60 seconds (depending on the primers' melting temperatures), and extension at 72°C for one minute. The final extension was at 72°C for 10 minutes. Agarose gel (1.5%) was prepared by mixing agarose with 1x Tris-borate-EDTA (TBE) buffer and heating until completely dissolved. Ethidium bromide (2 µL of 10 mg/mL) was added, and the solution was cooled to about 50˚C before being poured into the gel tray. Following this procedure, 5 µL of the DNA samples and 2 µL of loading dye were added to the wells. Electrophoresis was performed for 45 minutes at 5 V/cm², and the DNA bands were visualized under ultraviolet light; then, the results were documented using a gel documentation system.

Table 1. Primers for the *invA*, *sopE*, and *stn* genes used in the present study

Gene	Primer sequence (5'-3')	Product size (bp)	Reference
invA	F: GTGAAATTATCGCCACGTTCGGGC AA R: TCATCGCACCGTCAAAGGAACC	284	Rahn et al. (1992)
sopE	F: GCAACACACTTTCACCGAGG R: TTGCTGTGGAGTCGGCATAG	485	*Primers selected from GenBank
stn	F: ATTCGTAACCCGCTCTCGTC R: GGTCGGTCCCACTGTCTTTT	420	*Primers selected from GenBank

F: Forward primer, R: Reverse primer, *Primers for *sopE* and *stn* were designed based on conserved sequences available in GenBank.

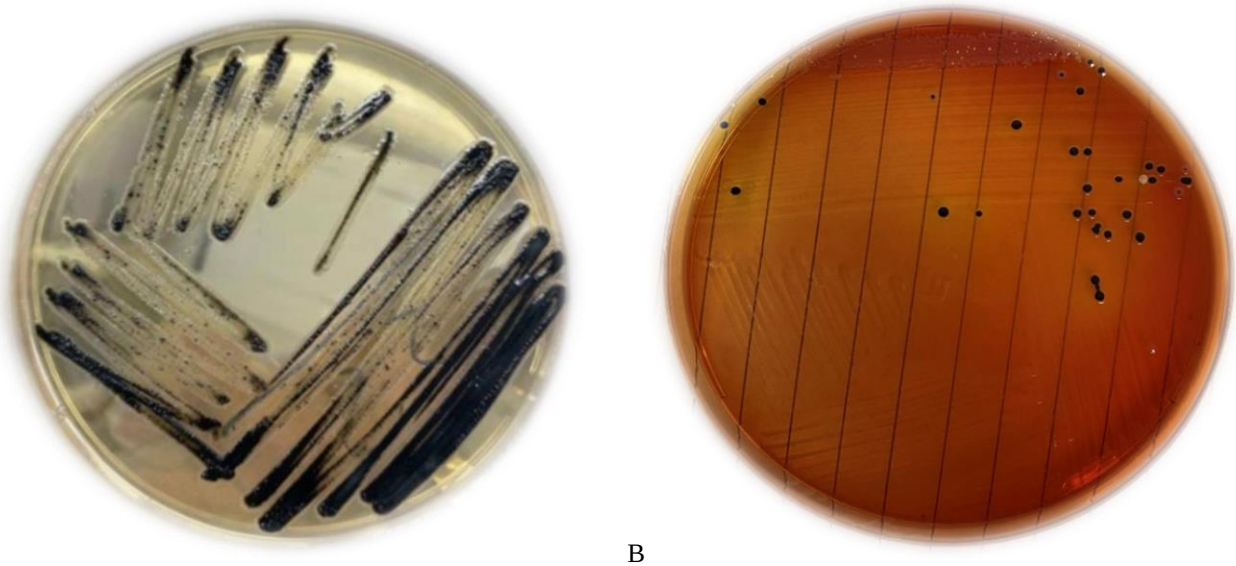
DNA sequencing and data analysis

The *invA* gene products obtained from PCR were sequenced to verify the uniformity of the *Salmonella* isolates. Four strains resembling the PCR products were purified and sent to Macrogen in Korea for sequencing. The same primers used for PCR amplification were employed for DNA sequencing. Sequencing was conducted in forward and reverse directions utilizing the Sanger method. The BioEdit, Chromas, and MEGA software applications were used to edit, assemble, and analyze the nucleotide sequences obtained, thereby generating consensus sequences. The BLASTn tool from the National Center for Biotechnology Information (NCBI, USA) was utilized to analyze the new sequences and evaluate their similarity to reference sequences available in the GenBank database. The NCBI GenBank database subsequently acquired the validated *invA* gene sequences from two isolates for inclusion.

RESULTS

Bacteriological examination

Out of 100 fecal samples, four isolates exhibited *Salmonella* colony morphology, appearing as transparent colonies with a dark center attributable to hydrogen sulfide (H_2S) production on SS agar. The colonies appeared red with a black core or, alternatively, yellow with a black center on XLD (Figure 1). The isolates tested positive for citrate and catalase activity, demonstrated an alkaline/acid TSI reaction with H_2S, and produced gas. However, the samples tested negative for urease, phenylalanine, and indole assays.



A B

Figure 1. *Salmonella* colonies isolated from cats with diarrhea in Al-Anbar province, Iraq, on selective culture media. **A**: Colonies with a black center on *Salmonella-Shigella* (SS) agar. **B**: Colonies with black center on xylose-lysine-deoxycholate (XLD) agar

Confirmation by the VITEK®2 system

All four isolates were classified as *Salmonella* group by the VITEK®2 compact system, exhibiting a high recognition probability of 98% (Table 2).

Table 2. The VITEK®2 compact system results for *Salmonella* species isolated from cats with diarrhea, in Al-Anbar province, Iraq

Number of samples	Source	Identified organism	Probability (%)
1	Cat feces	*Salmonella* spp.	98
2	Cat feces	*Salmonella* spp.	98
3	Cat feces	*Salmonella* spp.	99
4	Cat feces	*Salmonella* spp.	100

Antimicrobial resistance profile

Antibiotic susceptibility testing against 16 antibiotics revealed that all four isolates (100%) demonstrated identical resistance patterns, with resistance to eight agents (50%), including amoxicillin, streptomycin, cefotaxime, oxytetracycline, doxycycline, tetracycline, trimethoprim-sulfamethoxazole, and gentamicin. Additionally, three of the 16 antibiotics (18.75%) exhibited moderate susceptibility, including ampicillin, ceftriaxone, and levofloxacin. The remaining five antibiotics, including piperacillin, imipenem, norfloxacin, enrofloxacin, and ciprofloxacin (31.25%), were fully susceptible (Table 3).

Molecular detection of *Salmonella* species

The PCR amplification of the *invA* gene confirmed *S. enterica* subsp. *enterica* in 4 of the 30 suspected isolates, as evidenced by four bands of approximately 284 bp visualized on the agarose gel (Figure 2).

Table 3. Antibiotic susceptibility testing of *Salmonella* Agona isolated from cats in Al-Anbar province, Iraq

Antibiotic	ug/ disk	Sensitive (%)	Intermediate (%)	Resistant (%)
Amoxicillin	(AMX 25 ug)	0	0	100
Ampicillin	(AMP 10 ug)	0	100	0
Streptomycin	(ST 10 ug)	0	0	100
Cefotaxime	(CTX 30 ug)	0	0	100
Ceftriaxone	(CRO 30 ug)	0	100	0
Piperacillin	(PRL 100 ug)	100	0	0
Imipenem	(IPM 10 ug)	100	0	0
Oxytetracycline	(OXT 30 ug)	0	0	100
Doxycycline	(DO 30 ug)	0	0	100
Tetracycline	(TE 30 ug)	0	0	100
Norfloxacin	(NOR 10 ug)	100	0	0
Enrofloxacin	(ENR 5 ug)	100	0	0
Levofloxacin	(LEV 5 ug)	0	100	0
Ciprofloxacin	(CIP 5 ug)	100	0	0
Trimethoprim-sulfamethoxazole	(SXT [1.25/23.75] 25 ug)	0	0	100
Gentamicin	(CN 10 ug)	0	0	100
Total sensitivity (%)		31.25	18.75	50

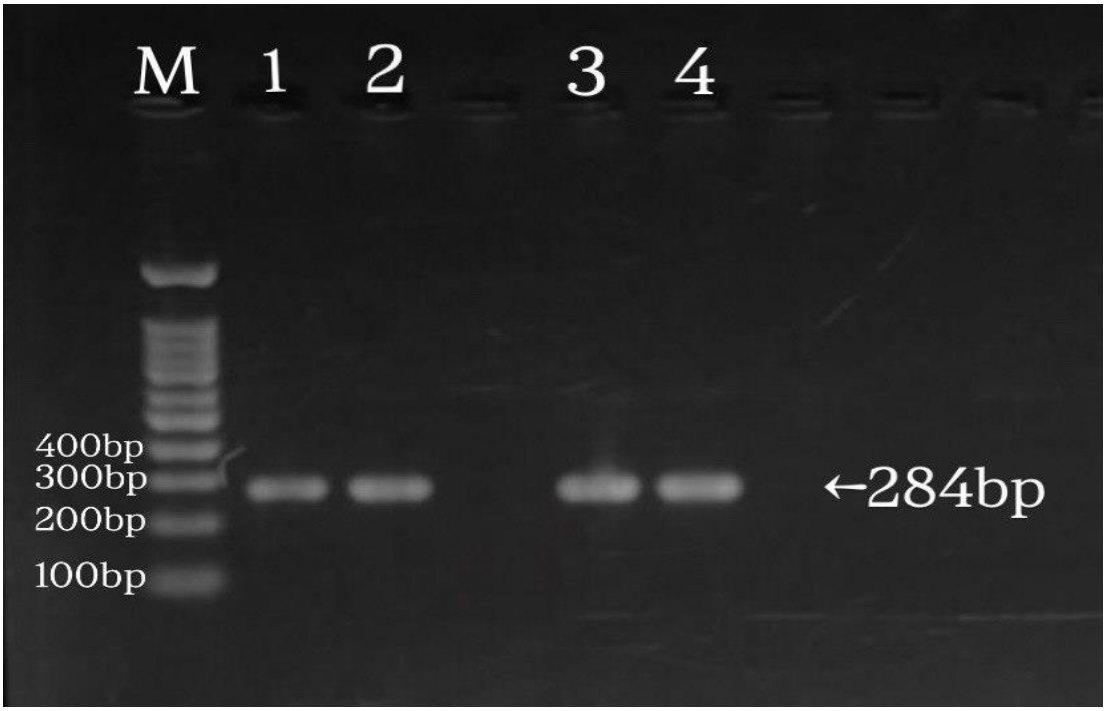


Figure 2. Agarose gel electrophoresis of PCR-amplified *invA* gene fragments from *Salmonella* spp. isolated from cats with diarrhea, in Al-Anbar province, Iraq. The PCR amplification of the *invA* gene yielded partial products of 284 bp, which were resolved by electrophoresis on a 1.5% agarose gel at 5 V/cm² for 45 minutes and visualized under ultraviolet (UV) light. Lane M: DNA ladder (1200 bp), Lanes 1-4: Positive *invA* gene amplicons from *Salmonella* spp.

DNA sequence analysis

BLAST analysis of the partial *invA* gene sequences demonstrated high genetic similarity between the *Salmonella* isolates recovered from cat fecal samples and several reference strains deposited in GenBank; however, only two sequences were formally registered. The current results demonstrated that the isolates were highly similar to each other and to reference strains of *Enterococcus enterica* serotype Agona. Isolate 1 (sequence ID: LC901557.1) had a perfect sequence match with *S.* Agona, and isolate 2 (sequence ID: LC901560.1) exhibited 100% similarity to *S.* Agona.

Detection of virulence genes

The *sopE* gene, which encodes a secreted effector protein essential for epithelial cell invasion, was identified in two of the four isolates, indicating a high prevalence of this virulence factor (Figure 3). The *stn* gene, which is associated with enterotoxin synthesis, was identified in only one isolate (Figure 4).

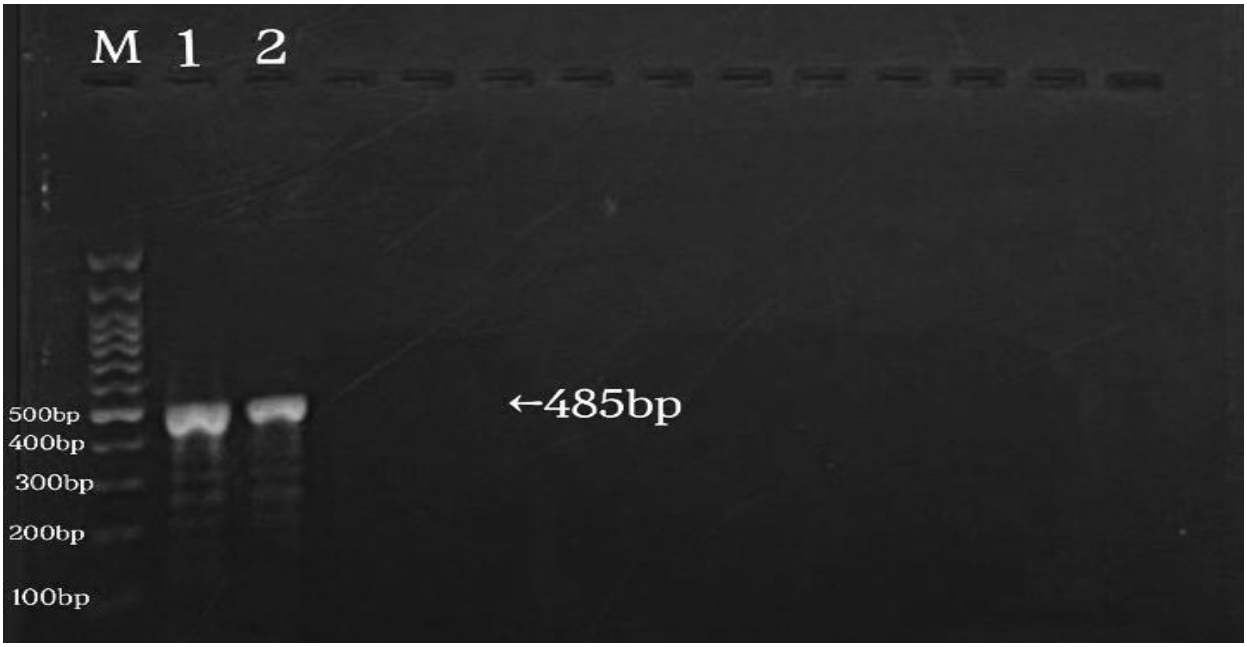


Figure 3. Agarose gel electrophoresis of *sopE* gene fragments from *Salmonella* Agona isolated from cats with diarrhea in Al-Anbar province, Iraq. The *sopE* gene was partially amplified by PCR, yielding a 485 bp product. Amplicons were resolved by electrophoresis on a 1.5% agarose gel at 5 V/cm² for 45 minutes and visualized under ultraviolet (UV) light. Lane M: DNA ladder (1200 bp), Lanes 1 and 2: *Salmonella* Agona isolates testing positive for the *sopE* gene.

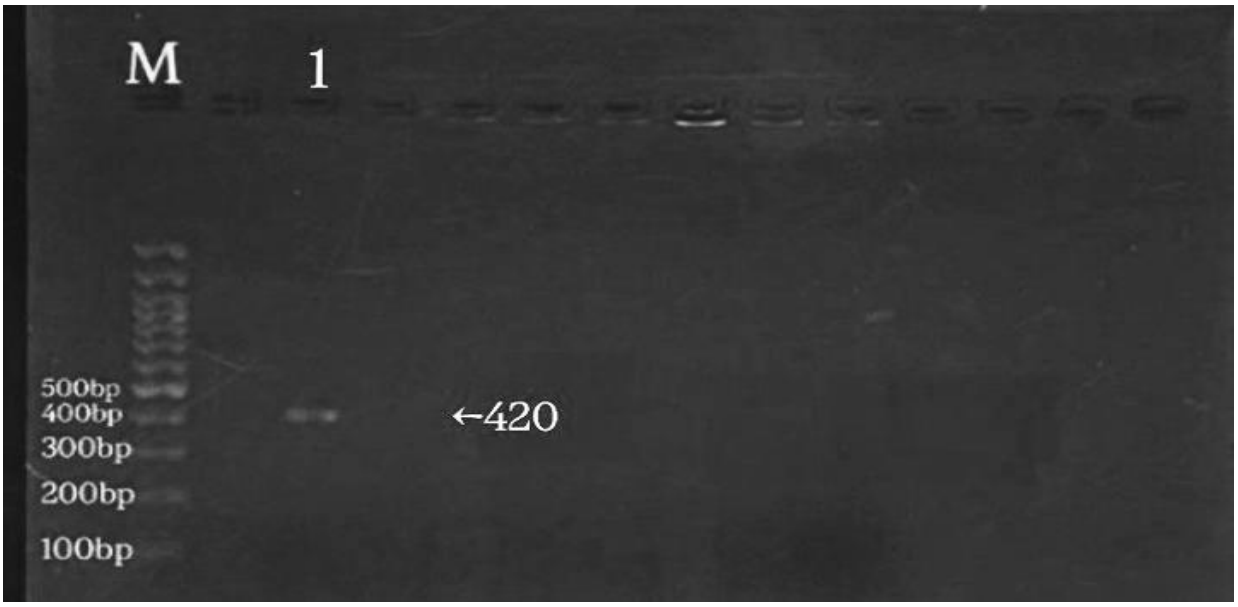


Figure 4. Agarose gel electrophoresis of the *stn* gene fragment from *Salmonella* Agona isolated from cats with diarrhea, in Al-Anbar province, Iraq. The *stn* gene was partially amplified by PCR, yielding a 420 bp product. The amplicon was resolved by electrophoresis on a 1.5% agarose gel in 1× TBE buffer at 5 V/cm² for 45 minutes and visualized under ultraviolet (UV) light. Lane M: DNA ladder (1200 bp), Lane 1: *Salmonella* Agona isolates testing positive for the *stn* gene.

DISCUSSION

Epidemiological importance and prevalence

The present study found that 4% of diarrheic cats in Al-Anbar Province, Iraq, tested positive for *S.* Agona. The findings of the current study were consistent with previous reports, as Barber et al. (2002) in Illinois, USA, identified a single *S.* Agona isolate among 26 feline-derived *Salmonella* isolates (3.8%), and Zhao et al. (2024) in Chongqing, China, detected *S.* Agona in 2 of 90 feline isolates (2.2%), representing similar incidence rates. Although the time and location of these findings differed, all support the fact that *S.* Agona is a rare serovar within the feline population. However, from an epidemiological perspective, the low incidence should not be regarded as insignificant. Cats live in close proximity to humans and may serve as silent carriers of zoonotic *Salmonella*, particularly multidrug-resistant strains. Therefore, the detection of *S.* Agona in cats, although infrequent, warrants investigation due to its potential public health implications.

Worldwide trends in antimicrobial resistance

The antimicrobial susceptibility profile observed in the present study indicated that *S.* Agona isolates exhibited resistance to 50% of the antibiotics tested (8 out of 16), including β-lactams, aminoglycosides, tetracyclines, and sulphonamides. Recent studies collecting samples from humans and food-producing animals have revealed a global increase in the prevalence of multidrug-resistant non-typhoidal *Salmonella* species (Zahra et al., 2023; Lamichhane et al., 2024). Few studies have directly examined whether *S.* Agona isolates from cats exhibit antibiotic resistance. Consistent with the present study, Song et al. (2025) reported that *S.* Agona isolates from avian and human infections exhibited resistance to multiple antibiotic classes, including β-lactams, aminoglycosides, tetracyclines, and sulphonamides. These findings may indicate the possible dissemination of resistance genes among different host species through horizontal gene transfer and environmental exposure, highlighting the epidemiological importance of antimicrobial resistance spread. The widespread use of antibiotics eradicates susceptible bacteria, but resistant bacteria survive and proliferate, thereby increasing resistance over time. The widespread use of antibiotics in animals and humans, combined with horizontal gene transfer, probably promotes the spread of resistance genes (Caneschi et al., 2023).

Zhao et al. (2024) in Chongqing, China, found that *Salmonella* strains isolated from pets exhibited greater resistance to antibiotics, particularly ampicillin, tetracyclines, and sulphonamides. Similarly, the current study found that all *Salmonella* isolates obtained from cats demonstrated complete resistance to tetracycline, doxycycline, and trimethoprim-sulfamethoxazole, indicating a consistent pattern of resistance, particularly to tetracyclines and sulfonamides. The multidrug resistance observed among the isolates in the present study may be attributed to the widespread, often uncontrolled use of antibiotics in both veterinary and human settings, which promoted the selection and persistence of resistant bacterial strains. Furthermore, horizontal gene transfer may facilitate the spread of resistance genes across diverse bacterial populations. The high resistance to tetracyclines and sulphonamides observed in the present study aligned with the findings of Zhao et al. (2024), who documented comparable resistance patterns in *Salmonella* isolates derived from companion animals such as cats and dogs. The susceptibility of the isolates to fluoroquinolones and carbapenems indicated that these antibiotics are still effective treatment options. The susceptibility of the isolates to fluoroquinolones and carbapenems indicated that these antibiotics are still effective treatment options, in line with previous reports of Zhao et al. (2024). The findings of the present study suggest that the bacterial isolates have not yet developed high levels of resistance against these antimicrobial agents. Fluoroquinolones act by inhibiting bacterial DNA replication, whereas carbapenems interfere with bacterial cell wall synthesis, making them highly effective against a broad range of Gram-negative bacteria (Aurilio et al., 2022). The observed sensitivity may be attributed to the limited spread of resistance genes associated with these antibiotic classes among the tested isolates. Similar findings were reported by Zhao et al. (2024), who demonstrated that fluoroquinolones and carbapenems remained among the most active antimicrobial agents against multidrug-resistant bacterial strains. Therefore, the fluoroquinolones and carbapenems may continue to serve as reliable therapeutic options for the treatment of infections caused by the studied isolates.

Distribution of pathogenic potential and virulence genes

Identifying virulence genes is a vital step in understanding the potential hazards posed by *S.* Agona from feline sources. All isolates in the present study possessed the *invA* gene, thereby confirming their classification as *Salmonella* spp. and indicating their capacity to invade intestinal epithelial cells, a marker of *Salmonella* pathogenicity (Rahman et al., 2004; Al-Zubaidy et al., 2015). In the present study, 50% of the isolates carried *sopE*, a gene encoding an effector protein of the type III secretion system, believed to contribute to host cell invasion and the induction of inflammation. Although the *sopE* gene was found in multiple isolates, suggesting increased virulence, the *stn* gene, which encodes an

enterotoxin, was present in only one isolate, indicating limited distribution. Differences in virulence gene profiles indicated that the *S*. Agona strains infecting cats are genetically distinct.

CONCLUSION

Salmonella Agona was identified in 4% of cats presenting diarrhea in Al-Anbar, Iraq. All isolates were subsequently confirmed by molecular analysis using *invA* gene sequencing, which demonstrated a high degree of similarity to reference strains. Whole-genome sequencing was used to examine genetic diversity and resistance. The isolates exhibited multidrug resistance to several commonly used antibiotics while carrying important virulence genes, including *sopE* and *stn*, indicating a potential public health risk despite their low prevalence. The prevalence of *Salmonella* species in Iraqi cats requires further investigation. Monitoring the development of antimicrobial resistance is recommended for future studies.

DECLARATION

Acknowledgments

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Authors' contributions

Ola Adnan Ismail conducted sample collection and laboratory investigations, including bacterial isolation and identification, Vitek analysis, and PCR assays, as well as data analysis and manuscript preparation. Asmaa Hamoody Abdullah supervised the study and provided scientific guidance. All authors read and approved the final edition of the manuscript prior to publication in the present journal.

Availability of the data and materials

All data generated during the study are relevant and included in this manuscript. The data supporting the findings of this study are available upon reasonable request from the corresponding author.

Competing interests

The authors declared no conflict of interest related to the present study.

Ethical considerations

All ethical aspects of the present study, including plagiarism, data integrity, consent to publish, and avoidance of misconduct or duplication, were carefully considered and approved by the authors. Artificial intelligence tools, such as Grammarly, were used only to support language improvement and understanding, without influencing the scientific content of the study. The authors have revised the manuscript using Grammarly and have taken full responsibility for its use. All scientific content, data interpretation, and conclusions were developed entirely by the authors.

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