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Emergence of *lmonellaenterica* subspecies *enterica* serovar in Iraqi broiler chiken farmsand humans

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Abstract

Background: worldwide, many domestic and wild animals can serve as a reservoir and harboring various pathogens in their gastrointestinal tracts without exhibiting the signs of illness.

Aim:Isolation and molecular confimation of *S. enterica*subsp. *enterica*in the fecal samples of broiler chicken farms and humans withphylogenetic analysis of some study isolates.

Materials and methods: Totally, 100 fecal samples were collected for the current study including 50 samples obtained from the broiler chicken farms located at different areas inAl-Qadisiyah province, and 50 stools from humans. Initially, all fecal samples were subjected to traditional isolation and biochemical identification of *Salmonella* sp.; and then, the positive isolates were subjected to molecular confirmation of *S.enterica*by the polymerase chain reaction (PCR). Finally, four molecularly positive isolates were selected randomly and sequenced to be submitted in the NCBI-GenBank and examined phylogenetically.

Results: Traditional culture and biochemical testing of totally 100 fecal samples revealed that 32% samples were positive, which comprises 30% of broiler chicken and 34% of human fecal samples. Targeting the *16S rRNA* gene, the results of PCR assay detected that an overall prevalence rate of *S.enterica*among 32 positive isolates was68.75% which identified in 66.67% of broiler chickens and 70.59% of human fecal samples. The sequence data of the 4 study isolates were submitted, named and get specific access numbers in the NCBI-GenBank database as SE-1 (OR563806.1), SE-2 (OR563807.1), SE-3 (OR563808.1), and SE-4 (OR563809.1). Then, multiple sequence alignment, phylogenetic tree analysis and homology sequence identity revealed that three of local study isolates (SE-1, SE-3 and SE-4) were identical to the *S. enterica*subsp. *enterica* Chinese (JF951183.1) and Iraqi (KP420235.1) isolates at a level of similarity ranged from 99.87% to 99.99% and a level of mutation ranged from 0.0001% to 0.0006%; while, the local study isolate SE-2 was identical to the *S. enterica*Iranian (EU118116.1) isolate at 98.5% level of similarity and 0.0007 level of mutation.

Conclusion:This study revealed the high prevalence of *S.enterica* subsp. *enterica* in both chicken farms and humans indicating that control of infections remains a significant challenge as the pathogen demonstrated remarkable ability to adapt and persist in various environments. Therefore, this study suggests that improved biosecurity measures, enhanced food safety practices, and the judicious use of antimicrobials in animal production are all crucial components of a multifacted approach to mitigate the prevalence and impact of *S. enterica*.

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Keywords: Salmonellosis, Feces, Phylogenetic analysis, National Center For Biotechnology Information (NCBI), *16S rRNA* gene

Introduction

Salmonella enterica spp. *enterica* is a Gram-negative, rod-shaped bacterium which is having a significant public heallth concern due to to its ability to cause a wide range of infectious diseases that ranged fron the gastroenteritis to more severe systemic infections (Jajere, 2019; Gast and Porter Jr,2020). This subspecies (enterica) is particularly noteworthy for its remarkable host specificty with some serovars exhibiting a strong preference for a particular animal host, while others are capable to infect a broader range of hosts (Foleyet al., 2013; Andino and Hanning, 2015). This remarkable diversity of the bacterium is largely attributed to the extensive allelic variation within the subspecies (Branchu et al., 2018). Different studies have been conducted to identifythe presence of numerous genetic determinants that contributed to host specificty through biological relevance of many of these associations (Antonelli et al., 2019; Pavlova et al., 2021; Wang et al., 2021). In addition, *S. enterica* spp. *enterica*considers as a highly adaptable pathogen that characertized by its ability to invading and replicating within both phagocytic and non-phagocytic host cells, dendritic cells, and macrophages (Jantschet al., 2011; Anderson and Kendall, 2017). However, the nomenculture of *S. enterica* spp. *enterica* has been a subject of onging debate with some resaerchers perferring the term :serovar" over "serotype" to mentain international consistensy (da Silveira, 2019). Regarding the terminology used, the clinical and veterinary significance of this pathogen underscores the need for continued research and surveillence efforts to better understand and combat the diverse range of infections caused by *S. enterica* spp. *enterica*(Bhunia andBhunia, 2018; Mkangara, 2023).

The verstaility allows the bacterium to evade the innate immune responses of the host and establishing both acute and chronic infections (Hurley et al., 2014).The clinical manifestations of *S. enterica* spp. *enterica* vary widely as ranging from asymptomatic carriage to severe systemic diseases which can be life-threating if left untreated (Lamas et al., 2018). Therefore, the diagnosis of infections relies mainly on a combination of clinical presentation, epidemiological data and laboratory testing (Bula-Rudaset al., 2015; Gast and Porter Jr, 2020). Traditionally, the diagnosis has relied on culture-based methods, which involve the isolation and identification of the bacteria from clinical samples (Andrews and Ryan, 2015). However, these methods can be time-consuming and may not always provide timely results (Lee et al., 2015). In rescent years, advancments in molecular diagnostic techniques have revolutionized the field of Salmonella detection, offering more sensitive, specific and rapid alternatives (Zhuang et al., 2023). One of the benefits of molecular diagnosis is the ability to detect and differentiate various *Salmonella* serotypes, which is crucial for epidemiological surviellence and outbreak investigations (Kitchens et al., 2024). Molecular methods such as PCR assays shown to having a high sensitivity and specificty in detection of bacterial DNA sequences, allowing for the rapid and accurate identification of *Salmonella*species / srains (Deb et al., 2024; Medhi et al., 2024). Moreover, molecular techniques can provide additional information about the virulence factors and antimicrobial resistance profiles of *Salmonella* isolates, enablining clinicians to tailor treatment strategies

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and inform public health interventions (Bahramianfard et al., 2021; Shahrzad et al.,2023). Despite the dvantages of molecular diagnostics, it is important to note that conventional culture-based methods remain essential as they allow for the isolation of *Salmonella* strains, which is neccassry for further characterization and antimicrobial susceptibility testing (McConn et al., 2024). Hence, the current study conducts to isolateand molecularly confim *S. enterica* spp. *enterica* from the fecal samples of broiler chicken farms as well as from human. Phylogenetic analysis of some study isolates was aimed, also.

Materials and Method

Ethical approval

This study gets a license from the Scientific Committee in the Department of Pathology (College of Veterinary Medicine, University of Al-Qadisiyah).

Samples collection

Totally, 100 fecal samples were collected for the current study including 50 samplesobtainedfrom the broiler chicken farms located atdifferent areas inAl-Qadisiyahprovince,and 50 stools from humans. All samples were transported under cooled conditions using of plastic ice-box.InLaboratory of Microbiology (College of Veterinary Medicine, University of Al-Qadisiyah), the collected samples were used for isolation and molecular identification.

Isolation and biochemical identification

Following the steps of Gebeyehu et al. (2022), the samples were inoculated in nutrient agar, and the single pure colony was cultured in Salmonella-Shigella(SS) agar and Xylose lysine deoxycholate (XLD) agar to identify*Salmonella* from lactosefermentedEnterobacteriacea.Citrate utilization,hydrogen sulphide production,indole, lysine decarboxylase,Methyl red, triple sugar iron (TSI), urease, and Vogas-Proskaurtests were used to confirm of study isolates.

Molecular testing

Genomic DNAs of *Salmonella*spp.isolates were extracted using the Mini Genomic DNAKit (Geneaid, Taiwan) fromthe pure bacterial colonies. The purification andconcentration of extracted DNAs were determined by the Nanodrop spectrophotometer. Targeting the *16S rRNA* gene, one set of primers [(F:5´-GGGAGGAAGGTGTTGTGGTT-3´) and (R:5´-CGCTTCTCTTTGTATGCGCC-3´)]wasdesigned based on the NCBI-GenBank database (ID: LC773421.1),provided bythe BioneerCompany (Korea), and used topreparation the PCR Mastermixat a total volume 20µl. For amplification, Thermal Cycler conditions were subjected as5 minute at 95°C for initial denaturation,30 cycles for denaturation at 95°C for30 second, annealing for30 seconds in50°C,and extension for 30 seconds in72°C,and 10 minute for final extension at72°C.The PCR products were analysed in agarose gel (2%)electrophoresis and examined under the UVlight to detect positive samples at 828bp.

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Sequencing

Phylogenetic relationship for the local study *S.enterica*isolateswith the NCBI-GenBank*S. enterica*isolates/ strains was done by the MEGA-11Software (Gharban, 2024a).

Statistical analysis

The *t*-test in GraphPad Prism Software was served to detect significant differences between study values at $p<0.05$ (Gharban, 2024b).

Results

Traditional culture and biochemical testing of totally 100 fecal samples revealed that32% (total no=32) samples were positive, which comprising30% (15/50) of broiler chickensand 34% (17/50) of humans (Figures 1, 2).

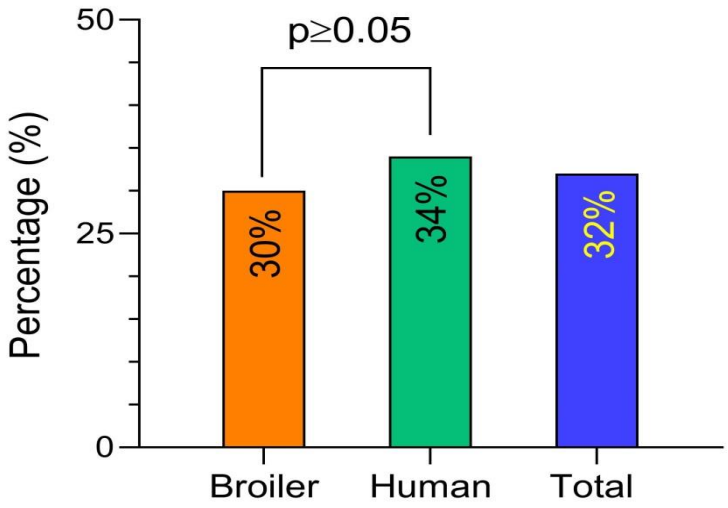

Figure (1): Total positive isolatesof *Salmonella* spp. in totally 100 fecal samples (50 broiler chickens and 50 humans)

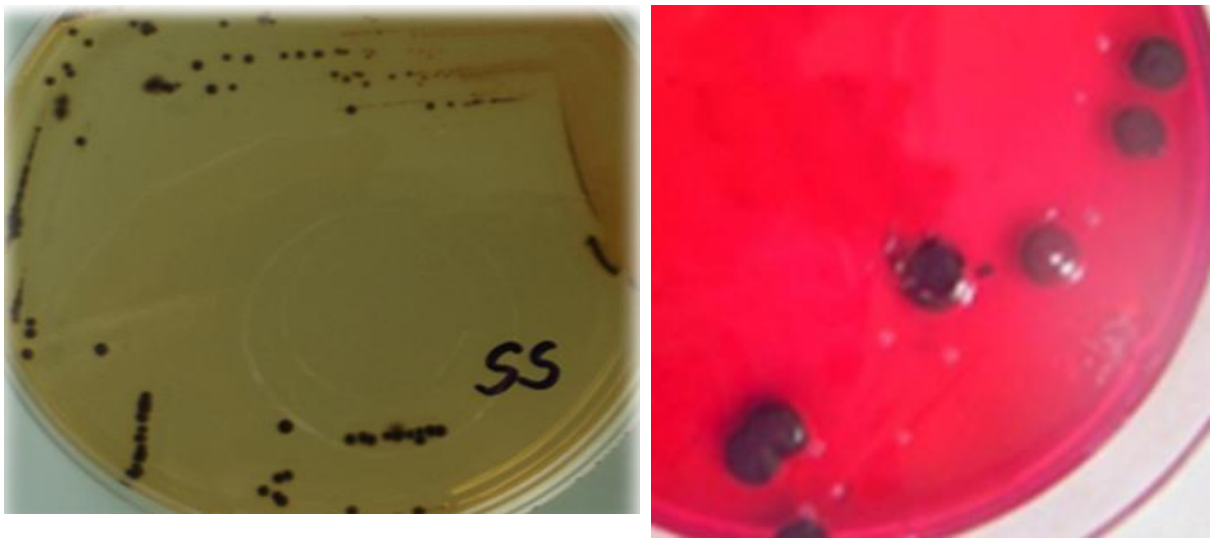

Figure (2): Traditional isolation of*Salmonella*spp. on SS and XLD agars

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Targeting the *16S rRNA* gene, the results of PCR assay detected that an overall prevalence rate of *S.enterica*among the positive isolates (no=32) was68.75% (no=22) which identified in66.67% (10/15) of broiler chickens and 70.59% (12/17) of human fecal samples (Figures 3, 4).

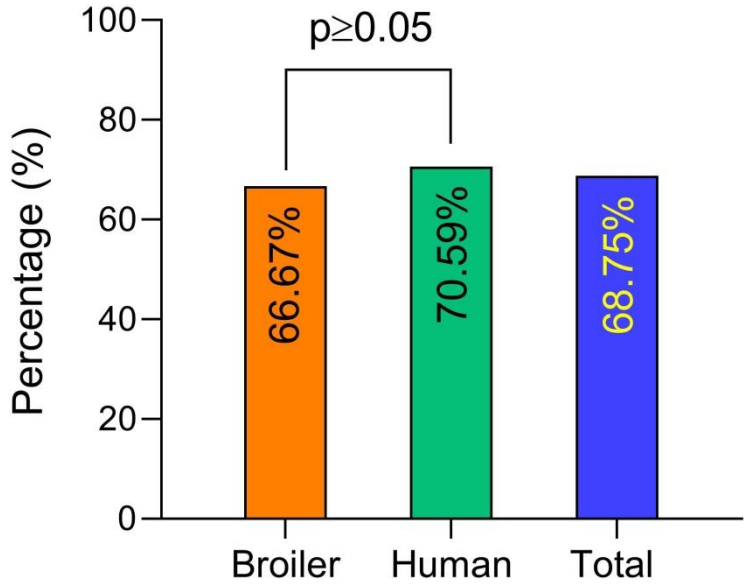

Figure (3):Total positive isolatesof *S.enterica*in totally 100 fecal samples (50 broiler chickens and 50 humans)

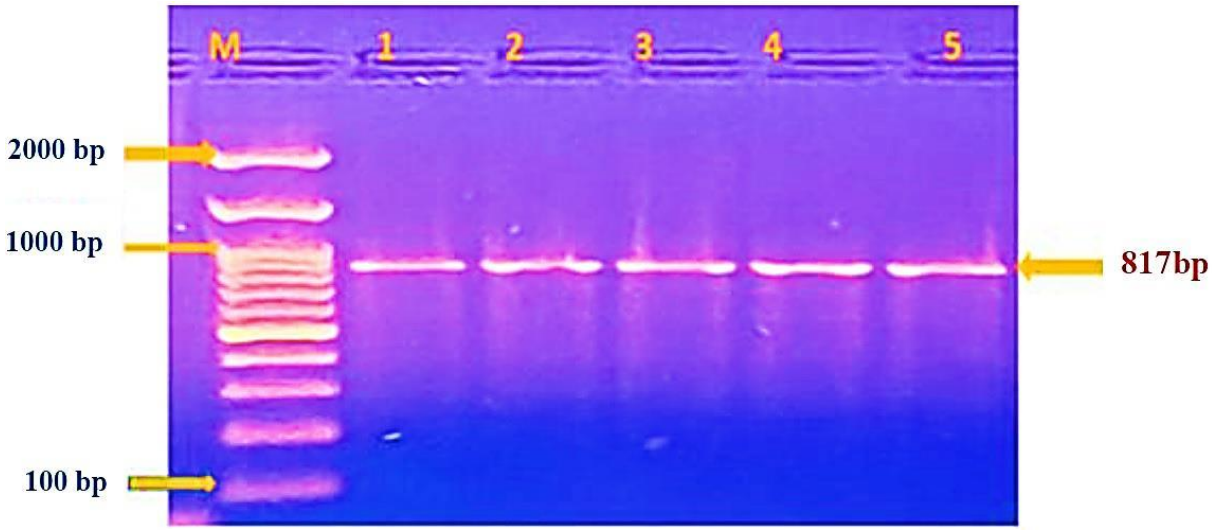

Figure (4): Agarose-gel electrophoresis of positive samples at 817bp to *S. enterica*using the PCR assay

The sequence data of the 4 study isolates were submitted, named and get specific access numbers in the NCBI-GenBank database as SE-1 (OR563806.1), SE-2 (OR563807.1), SE-3 (OR563808.1), and SE-4 (OR563809.1). Then, multiple sequence alignment, phylogenetic tree analysis and homology sequence identity revealed that three of local study isolates (SE-1,

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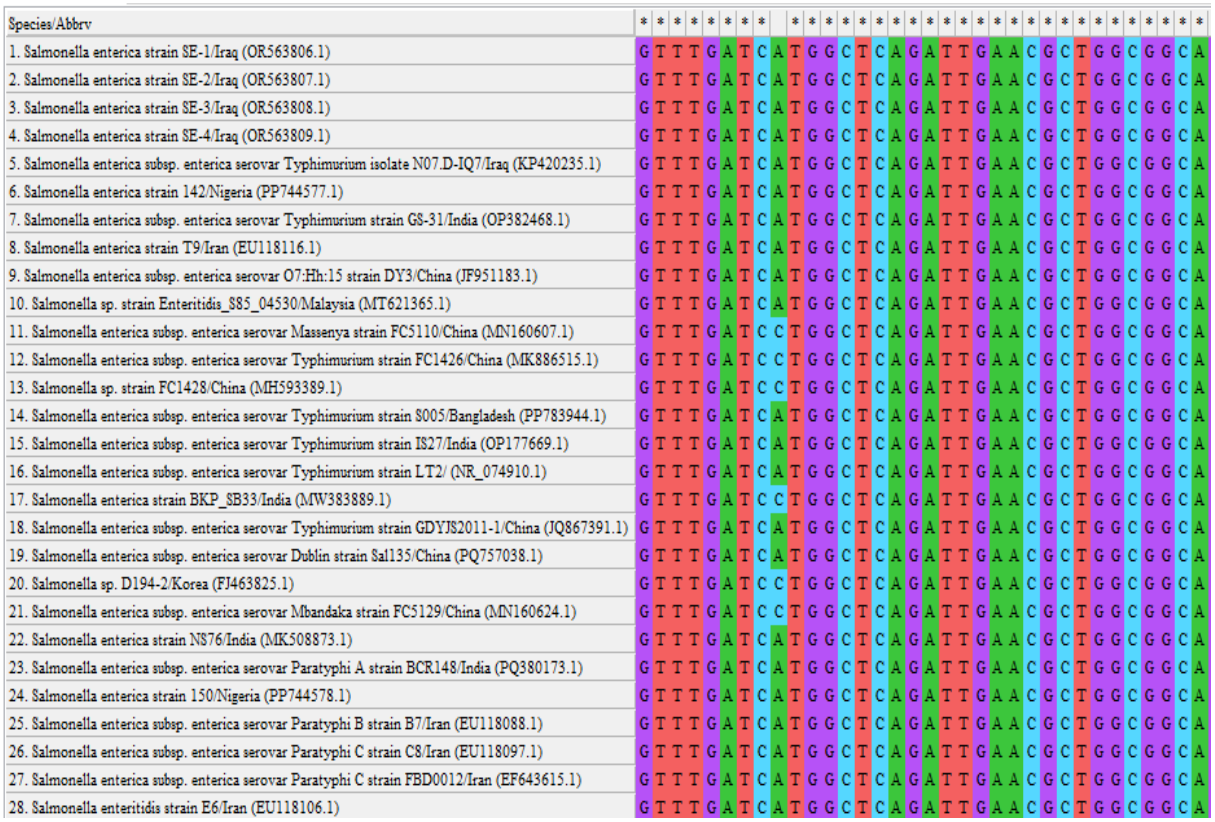
SE-3 and SE-4) were identical to the *S. enterica*subsp. *enterica*Chinese and Iraqi isolates at a level of similarity ranged from 99.87% to 99.99%and a level of mutation ranged from 0.0001% to 0.0006%; while, the local study isolate SE-2 was identical to the *S. enterica*Iranian isolate at 98.5% level of similarity and 0.0007 level of mutation (Table 1, Figures 5-7).

Table (2): Homology Sequence identity (%) of the local and NCBI-BLAST *S. enterica*isolates / strains

Local isolate			**NCBI isolate**			
Name	**Access No.**	**Species**	**Species**	**Country**	**Access No.**	**%**
SE-1	OR563806.1	*S. enterica*	*S. enterica*subsp. *enterica*	China	JF951183.1	99.99
SE-2	OR563807.1	*S. enterica*	*S. enterica*	Iran	EU118116.1	98.5
SE-3	OR563808.1	*S. enterica*	*S. enterica*subsp. *enterica*	Iraq	KP420235.1	99.87
SE-4	OR563809.1	*S. enterica*	*S. enterica*subsp. *enterica*	China	JF951183.1	99.94



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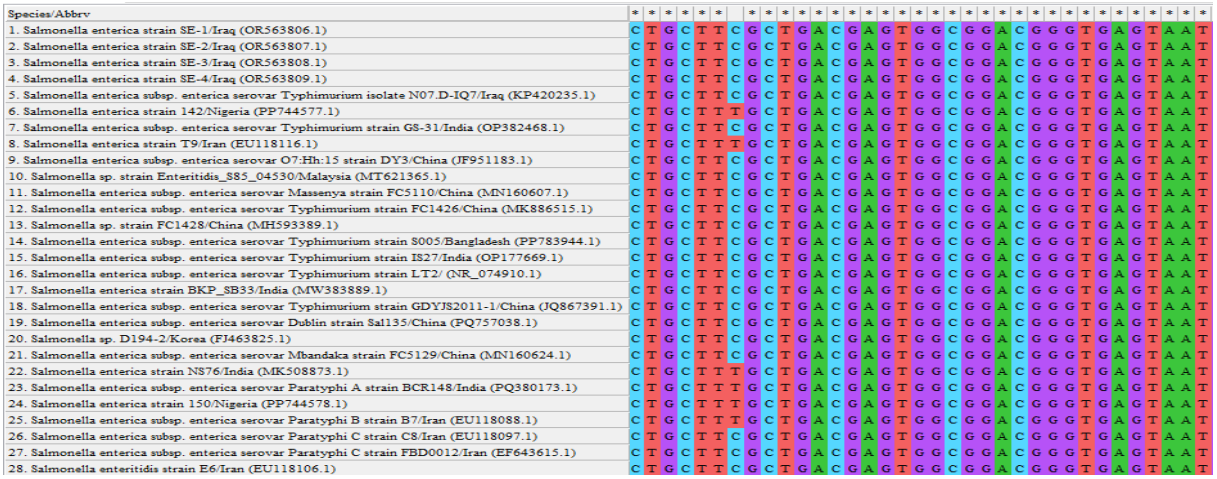

Species/Abbrv	Alignment
1. Salmonella enterica strain SE-1/Iraq (OR563806.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
2. Salmonella enterica strain SE-2/Iraq (OR563807.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
3. Salmonella enterica strain SE-3/Iraq (OR563808.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
4. Salmonella enterica strain SE-4/Iraq (OR563809.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
5. Salmonella enterica subsp. enterica serovar Typhimurium isolate N07.D-IQ7/Iraq (KP420235.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
6. Salmonella enterica strain 142/Nigeria (PP744577.1)	CTGCTTTGCTGACGAGTGGCGGACGGGTGAGTAAT
7. Salmonella enterica subsp. enterica serovar Typhimurium strain GS-31/India (OP382468.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
8. Salmonella enterica strain T9/Iran (EU118116.1)	CTGCTTTGCTGACGAGTGGCGGACGGGTGAGTAAT
9. Salmonella enterica subsp. enterica serovar O7:Hh:15 strain DY3/China (JF951183.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
10. Salmonella sp. strain Enteritidis_S85_04530/Malaysia (MT621365.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
11. Salmonella enterica subsp. enterica serovar Massenya strain FC5110/China (MN160607.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
12. Salmonella enterica subsp. enterica serovar Typhimurium strain FC1426/China (MK886515.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
13. Salmonella sp. strain FC1428/China (MH593389.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
14. Salmonella enterica subsp. enterica serovar Typhimurium strain S005/Bangladesh (PP783944.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
15. Salmonella enterica subsp. enterica serovar Typhimurium strain IS27/India (OP177669.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
16. Salmonella enterica subsp. enterica serovar Typhimurium strain LT2/ (NR_074910.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
17. Salmonella enterica strain BKP_SB33/India (MW383889.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
18. Salmonella enterica subsp. enterica serovar Typhimurium strain GDYJS2011-1/China (JQ867391.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
19. Salmonella enterica subsp. enterica serovar Dublin strain Sal135/China (PQ757038.1)	CTGCTTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
20. Salmonella sp. D194-2/Korea (FJ463825.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
21. Salmonella enterica subsp. enterica serovar Mbandaka strain FC5129/China (MN160624.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
22. Salmonella enterica strain NS76/India (MK508873.1)	CTGCTTTGCTGACGAGTGGCGGACGGGTGAGTAAT
23. Salmonella enterica subsp. enterica serovar Paratyphi A strain BCR148/India (PQ380173.1)	CTGCTTTGCTGACGAGTGGCGGACGGGTGAGTAAT
24. Salmonella enterica strain 150/Nigeria (PP744578.1)	CTGCTTTGCTGACGAGTGGCGGACGGGTGAGTAAT
25. Salmonella enterica subsp. enterica serovar Paratyphi B strain B7/Iran (EU118088.1)	CTGCTTTGCTGACGAGTGGCGGACGGGTGAGTAAT
26. Salmonella enterica subsp. enterica serovar Paratyphi C strain C8/Iran (EU118097.1)	CTGCTTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
27. Salmonella enterica subsp. enterica serovar Paratyphi C strain FBD0012/Iran (EF643615.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT
28. Salmonella enteritidis strain E6/Iran (EU118106.1)	CTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT

Figure (5):Multiple sequence alignment of the local and NCBI-BLAST *S. enterica*isolates/strains using of MEGA-11 software

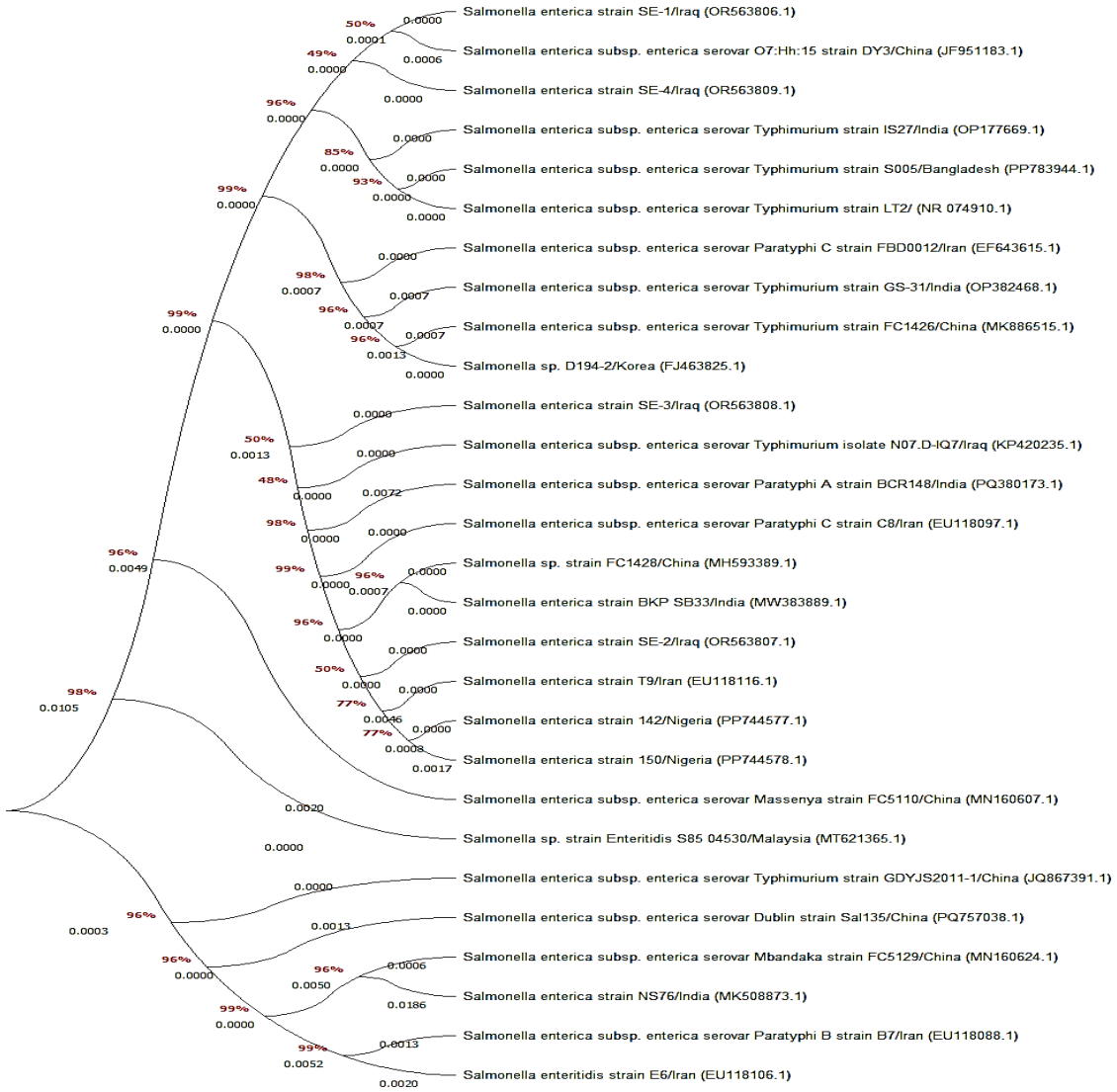

Figure (6): Phylogenetic tree analysis of the local and NCBI-BLAST *S. enterica*isolates/strains using of MEGA-11 software

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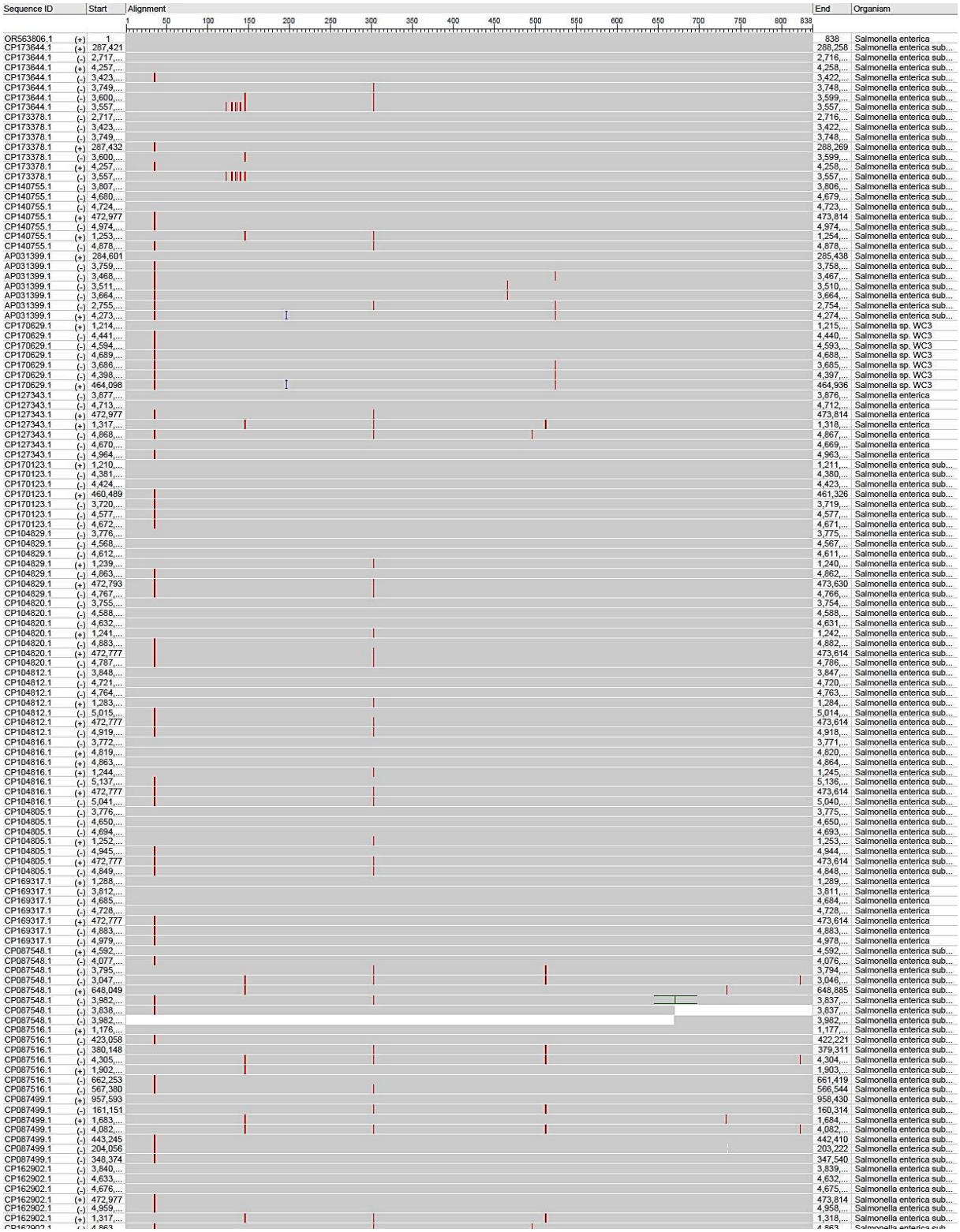

Figure (7): Multiple sequence alignment of the local and NCBI-BLAST *S. enterica* isolates / strains using NCBI MSA Viewer

Discussion

Salmonella enterica is a major food-borne pathogen, which continues to pose a significant public health concern worldwide (Jajere, 2019). The findings of this study revealed that the prevalence rate of salmonellosis in broiler chicken farms and humans was 30% and 34%,

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respectively; while, molecular results confirmed that *S.enterica*wasfound in 66.67% of broiler chickens and 70.59% of humans.Since *Salmonella* is a ubiquitous organism, the patterns and number of infections have exhibited dynamic changes over the last few decades due to factors, including antibiotic-resistant strain production and contamination of foods of animal origin (Barrow et al., 2012; Besser, 2018). Epidemiological trends have been influenced by two significant events; the first event is that the appearance of different strains of antibiotic resistance to multiple Salmonella in the populations of food animals has become a problem in animal and human health (Akinyemi and Ajoseh, 2017; Vidovic and Vidovic, 2020). Secondaly, most *Salmonella* infections has arisen as one of the most common pathogens linked with eggs and results in large number of human illnesses (Threlfallwt al., 2014; Gast et al., 2024). These changes have be caused by a complex interplay of factors including the intensification of animal agriculture, the widespread use of antimicrobials in livestock and lapses in food safey practices (Iskandar et al.,2020; Miller et al., 2022; Al-saari et al., 2024).

Many wild and domestic animals are colonized by various *Salmonella* species with the bacteria often found in their gastrointestinal tracts without causing apparent ilness (Sanchez et al., 2002). Therefore, *Salmonella* contaminated feces easily contaminate raw foods of animal origin during production and processing which leads to the transmitting pathogen to humans occasionally (Demirbilek, 2017; Dhakal et al., 2024). *Salmonella* exists in other environmental sources like water and soil and hence, shows that there is require comprehensive way of controlling the pathogen because of the fact that it is multiply transmission (Ammendola et al., 2023; Huang, 2024). Global status of *S. enterica* in poultry farming system has made contamination common from farm level to the consumer level which increase the need to establish the prevalence and the resistance patterns of the pathogen to antimicrobial in the broiler chicken population.

There is a great interest in understanding the epidemiology of *S. enterica* in broiler chickens at different stages throughout the poultry production chain (Van Immerseel et al., 2009; Foley et al., 2011; Shivaning Karabasanavar et al., 2020; Shang et al., 2021). During processing, broiler chickens affected with S. enterica may contain large numbers of the organism in their intestines and on the skin surface and thus represent a potent source of contamination(Gastand Porter Jr, 2020; Mkangara, 2023). In many studies, examining range of sample types from hatchery to the end of processing across integrated broiler operations found that *Salmonella* was present in all types of sampleswith hatchery transport pads, flies, drag swabs and boot swabs being the most frequent sources of isolation (Bailey et al., 2001; Kim et al., 2007; Ha et al., 2018). In a separate study, the prevalence of *Salmonella* in broiler chicken crcasses was evaluated with 25 out of 260 samples testing positive for the pathogen, and *S.enterica*and *S. enteritidis* were the most common serovar identified (Jung et al., 2019). Another study reported that 94.7% of *Salmonella* isolates tested were resisteant to at least one antimicrobial agent with the prevalence of resistance to streptomycin, nitrofurantoin, tetracycline, and nalidixic acid (Wang et al., 2020). The persistant and widespread presence of *S. enterica* in broiler chickens, coupled with alarming trends in antimicrobial resistance, underscores the urgant need for control measures (Yamba, 2023; Hameed et al., 2024).

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Conclusion

Our findings revealed the high prevalence of *S. enterica* subsp. *enterica* in both chicken farms and humans indicating that the control of *Salmonella* infections remains a significant challenge as the pathogen has demonstrated a remarkable ability to adapt and persist in various environments. Therefore, this study suggests that improved biosecurity measures, enhanced food safety practices, and the judicious use of antimicrobials in animal production are all crucial components of a multifacted approach to mitigate the prevalence and impact of *S. enterica*.

Funding

No external funds were received.

Conflict of interest

No.

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