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Phenotypic and Genotypic characterization of Lactose fermenting *Salmonella* isolates from broiler chickens

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ABSTRACT:

The detection of Lactose fermenting *Salmonella* (L.F) refers to the identification and characterization of non-conventional strains, which pose significant concerns for food safety and public health. While *Salmonella* strains are generally non-lactose fermenters (N.L.F), certain isolates have demonstrated lactose fermentation capabilities likely due to genetic mutations or environmental influences affecting metabolic pathways. In this study a total of twelve *Salmonella enterica* isolates were recovered from 300 diseased broiler chickens from farms in Giza, Qalyubia and Fayoum governorates and serotyped as *S. Typhimurium* (3/12; two non-lactose fermenting (NLF) and one lactose fermenting (LF) , *S.* Enteritidis (2/12; one NLF and one LF), *S.* Kentucky (NLF) (2/12), *S.* Montevideo (NLF) (1/12), *S.* Virchow (NLF) (1/12), *S.* Infantis (LF) (1/12), *S.* Havana (LF) (1/12), and *S.* Ruiru (LF) (1/12). The results emphasize the importance of key biochemical tests including lysine decarboxylase (LDC), ortho-nitrophenyl-β-galactoside (ONPG), and triple sugar iron (TSI) agar in the accurate identification of *Salmonella*. Molecular analysis revealed that the *invA* gene, a hallmark of *Salmonella* pathogenicity, was detected in 100% of the examined isolates. All non-lactose-fermenting (NLF) strains tested negative for the lacZ gene, while 3 out of 5 lactose-fermenting (LF) strains (60%) harbored this gene.

Antimicrobial susceptibility testing indicated that LF isolates exhibited a higher level of resistance than NLF counterparts.

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Both groups showed 100% resistance to colistin and amoxicillin. However, LF strains displayed elevated resistance to cefotaxime (80%), sulphamethoxazole-trimethoprim (80%), gentamicin (60%), and neomycin (100%), whereas NLF strains showed comparatively lower resistance rates. Amikacin remained effective against all isolates.
The increased resistance observed in LF *Salmonella* may be attributed to enhanced genetic adaptability and environmental exposure, underscoring the need for ongoing surveillance and the implementation of targeted antimicrobial stewardship strategies.

INTRODUCTION:

Avian Salmonellosis proves to be the most damaging disease globally as poultry rearing and farming is progressing. In poultry, contaminated eggs are mainly associated with the cause of fowl typhoid and pullorum disease via its spread from one generation to the next **(Wigley et al. 2001).**

Avian Salmonellosis is a significant disease due to its ability to cause not only a clinical illness in poultry, but also can be transmitted to humans through food, thereby acting as a major source of food-borne transmission **(Lutful Kabir, 2010).** Avian salmonellosis, due to its infective strains that are lethal to both humans and animals and its ability for zoonotic transmission via food, has made *Salmonella* not only a concern for public health but also a hot topic in several programs of local, national, and international surveillance. Hence, exposure to avian salmonellosis can result in a health risk **(yan et al. 2004).**

Most *Salmonella* strains are characterized as non-lactose fermenters, hydrogen sulfide producers, oxidase-negative, and catalase-positive. Additional biochemical traits useful for their identification include their capacity to utilize citrate as the only carbon source, ability to decarboxylate lysine, and their lack of urease activity **(Abulreesh, 2012).**

Salmonella species are generally non-lactose fermenters, and this characteristic serves as a key laboratory diagnostic marker to differentiate them from other members of the *Enterobacteriaceae* family. Nonetheless, a small proportion less than 1% of *Salmonella* strains are capable of lactose fermentation. These lactose-fermenting variants closely resemble the genus *Escherichia*, with both believed to share a common ancestor **(Doolittle et al. 1996).** Despite this evolutionary link, substantial genetic differences exist, one of the most notable being the presence of the lac operon in *Escherichia coli*, which is absent in S. enterica. The lac operon consists of three genes—lacZ, lacY, and lacA—encoding β-galactosidase, lactose permease, and transacetylase, respectively. Possession of this operon enables *E. coli* to ferment lactose, whereas *S.* enterica lacks this capability due to the operon's absence. In rare cases, lactose-fermenting *Salmonella* strains have been found to carry lactose-metabolizing genes on extrachromosomal elements such as plasmids. These genes may be acquired through horizontal gene transfer mechanisms like conjugation or transduction, and they exhibit strong similarity to the *E. coli* lac genes **(Eswarappa et al. 2009).**

A key diagnostic tool for identifying *Salmonella* is detecting hydrogen sulfide (H_2S) production in media such as Kligler iron agar (KIA) or triple sugar iron agar (TSI). When *Salmonella* fails to produce H_2S, it may escape detection in laboratories that rely solely on this characteristic and lack the resources to employ a comprehensive set of identification techniques.

Lactose fermenting *Salmonella* include urease positive, H_2S negative and lysine decarboxylase negative can be challenging in clinical and food safety microbiology. It might lead to misidentification during isolation of the bac-

terium. **ISO6579:2017** summarized that variant strains shall be detected by using plating media XLD and other complementary medium to facilitate detection of lactose positive (i.e. Bismuth Sulphite) or H_2S negative *Salmonella* (i.e. Briliant Green).

This study planned to highlight on detection of Lactose fermenting *Salmonella* isolated from diseased broiler chickens to avoid misidentification which affect negatively on right intervention and treatment as well as to point out the phenotypic and genotypic variation features between non lactose fermenting and lactose fermenting *Salmonella* serovars.

MATERIAL AND METHOD

Bacterial Isolates:

E.coli ATCC®2522 lactose fermenting isolate used as control positive strain provided by Serology unit and bacterial bank, AHRI, Dokki, Egypt.

Sample collection:

Between late 2022 and early 2024, a total of 300 diseased broiler chickens of various ages were collected from farms located in Giza, Qalyubia, and Fayoum governorates. From each bird, internal organ samples including liver, spleen, and cecum were aseptically obtained, properly labeled, and promptly transported in an ice box to the Serology Unit at the Animal Health Research Institute (Dokki) for *Salmonella* isolation and identification.

Isolation and identification of *salmonella*:

For each broiler chicken, the liver, cecum, and spleen were pooled to form a single sample. *Salmonella* isolation and identification were conducted in accordance with **ISO 6579:2017** protocols. Presumptive *Salmonella* colonies were verified through a series of biochemical assays, including triple sugar iron (TSI) agar, urea hydrolysis, indole production, and lysine iron agar, following **ISO 6579-1:2017** guidelines. Confirmed isolates were then serotyped according to **ISO 6579-3:2014**, which entails detecting somatic (O) and flagellar (H) antigens using SIFIN antisera (Berlin, Germany) at the Serology Department of the Animal Health Research Institute (AHRI), Dokki, Giza.

Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing of the isolated *Salmonella* strains was performed using the disc diffusion method in accordance with the Clinical and Laboratory Standards Institute **(CLSI, 2020)** guidelines. Ten antimicrobial discs representing six antimicrobial categories were selected: penicillins [amoxicillin/ clavulanic acid (AMC, 30 µg)], cephalosporins [cefotaxime (CTX, 30 µg)], aminoglycosides [amikacin (AK, 30 µg), neomycin (N, 30 µg), gentamicin (CN, 10 µg)], sulphonamides [trimethoprim-sulfamethoxazole (SXT, 25 µg)], quinolones [levofloxacin (LEV, 5 µg), ciprofloxacin (CIP, 5 µg), ofloxacin (OFX, 5 µg)], and polymyxins [colistin (CT, 10 µg)]. The inhibition zone diameters were measured and interpreted as susceptible or resistant according to **CLSI (2020)** breakpoints. Isolates resistant to at least one antimicrobial agent in three or more antimicrobial classes were classified as multidrug resistant (MDR). The multidrug resistance index (MARI) was calculated as the ratio of the number of antimicrobials to which resistance was observed to the total number of antimicrobials tested.

DNA extraction:

Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Germany, GmbH) with slight modifications to the manufacturer's protocol. In brief, 200 µl of the sample suspension was mixed with 20 µl of proteinase K and 200 µl of lysis buffer, followed by incubation at 56 °C for 10 minutes. Subsequently, 200 µl of absolute ethanol was added to the lysate. Washing and centrifugation steps were performed according to the kit instructions, and nucleic acids were finally eluted in 100 µl of the provided elution buffer.

Oligonucleotide Primer.

Primers, supplied by Metabion (Germany), are listed in Table (1).

PCR amplification.

Polymerase chain reaction (PCR) was carried out in a 25 µl reaction mixture containing

12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer (20 pmol), 5.5 µl of nuclease-free water, and 5 µl of DNA template. Amplification was performed using an Applied Biosystems 2720 thermal cycler.

Analysis of the PCR Products.

PCR products were resolved by electrophoresis on a 1.5% agarose gel (Applichem, Germany, GmbH) prepared in 1× TBE buffer, run at a constant voltage of 5 V/cm at room temperature. For gel analysis, 20 µl of the uniplex PCR products were loaded alongside a GelPilot 100 bp ladder (Qiagen, Germany) and a GeneRuler 100 bp ladder (Fermentas, Germany) to estimate fragment sizes. Gels were visualized using a gel documentation system (Alpha Innotech, Biometra), and images were analyzed with dedicated computer software.

Table 1.Target genes Primers sequences, amplicon sizes and cycling conditions.

			Amplification (35 cycles)					
Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Secondary denaturation	Annealing	Extension	Final extension	Reference
lacZ	ATGAAGCAGAACAACTTCAACGCCGT CGCCGATGTCGTGTCCAGCGG	1257 bp	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 1.2 min.	72˚C 12 min.	**Martin et al., 2010**
Salmonella invA	GTGAAATTATCGCCAC-GTTCGGGCAA TCATCGCAC-CGTCAAAGGAACC	284 bp	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	**Oliveira *et al.*, 2003**

RESULTS

Isolation and identification of *Salmonella* serovars

12 *Salmonella* isolates were isolated from 300 diseased broiler chickens from farms in Giza, Qalyubia and Fayoum governorates with an incidence of 4%.

Table 2. Cultural character of isolates:

Isolates	S.S agar	XLD agar	B.S
Lactose fermenting *Salmonella* serovars	Pale pink colonies With or without H_2S	Yellow colonies With or without H_2S	Black with metallic sheen
Non Lactose fermenting *Salmonella* serovars	Yellow colonies with black center (H_2S)	Red colonies with black center (H_2S)	Black with metallic sheen
E.coli ATCC® 2522	Pale pink colonies	Yellow colonies	

Table 3. Biochemical character:

Isolates	ONPG	TSI	LDC	Urea	Citrate
(NLF) *Salmonella*					
*S.*Typhimurium	colorless	Alkaline / Acid + H_2S	+ve	-ve	+ve
*S.*Typhimurium	colorless	Alkaline / Acid + H_2S	+ve	-ve	+ve
S. Enteritidis	colorless	Alkaline / Acid + H_2S	+ve	-ve	+ve
S. Kentucky	colorless	Alkaline / Acid + H_2S	+ve	-ve	+ve
S. Kentucky	colorless	Alkaline / Acid + H_2S	+ve	-ve	+ve
*S.*Montevideo	colorless	Alkaline / Acid + H_2S	+ve	-ve	+ve
*S.*Virchow	colorless	Alkaline / Acid + H_2S	+ve	-ve	+ve
(LF) *Salmonella*					
*S.*Typhimurium	yellow	Acid/Acid	+ve	-ve	+ve
S. Enteritidis	yellow	Acid/Acid+ H_2S	+ve	-ve	+ve
*S.*Infantis	yellow	Acid/Acid	+ve	-ve	+ve
*S.*Havana	yellow	Acid/Acid+ H_2S	-ve	-ve	+ve
*S.*Ruiru	yellow	Acid/Acid	+ve	-ve	+ve
E.coli ATCC®2522	yellow	A / A with gas	-ve	-ve	-ve

API 10E and API 20E was used to differentiate between L.f. *Salmonella* serovars, non L.f. *Salmonella* serovars and *E.coli* ATCC®2522 as control positive in lactose fermentation. Also API used for detection of B-galactosidase activity by ONPG test, **(O'Hara et al .1992).**

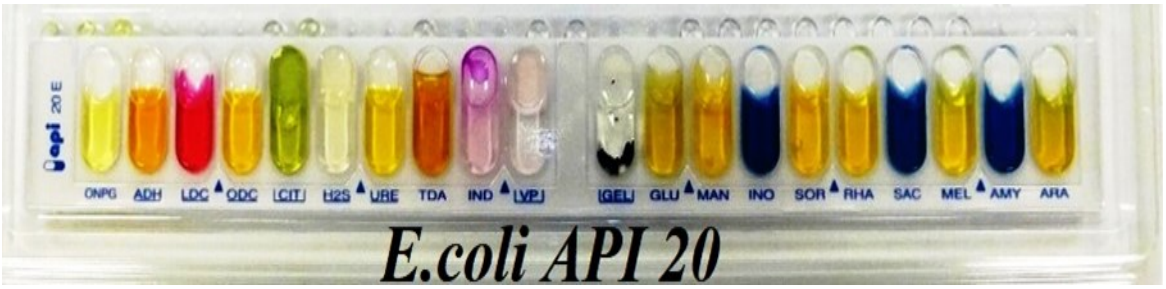


Fig (1): *E.coli* ATCC®2522 showed ONPG, indol and glucose tests positive while citrate, LDC, Urea and H_2S tests negative.

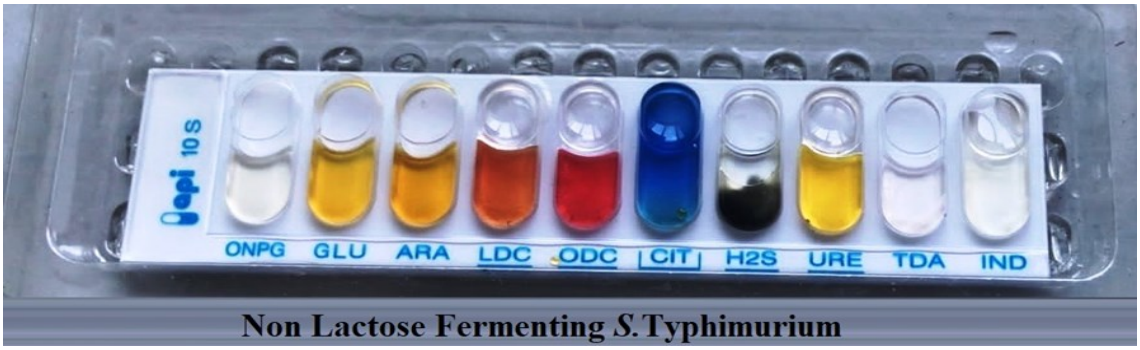


Fig (2): *S.*Typhimurium (non-lactose fermenting) showed ONPG, Indol, urea tests were negative while LDC, Citrate, H_2S tests were positive

Fig (3): *S.*Typhimurium (Lactose fermenting) showed ONPG, LDC, Citrate, H_2S and glucose tests were positive while urea and indol tests were negative

Table 4. *Salmonella* serovars isolated from diseased broiler chickens (No. 300):

Serovars of *Salmonella* isolates	No.
Salmonella Typhimurium (two NLF and one LF)	3
Salmonella Enteritidis (one NLF and one LF)	2
Salmonella Infantis (LF)	1
Salmonella Havana (LF)	1
Salmonella Ruiru (LF)	1
Salmonella Kentucky (NLF)	2
Salmonella Montevideo (NLF)	1
Salmonella Virchow (NLF)	1
Total	12

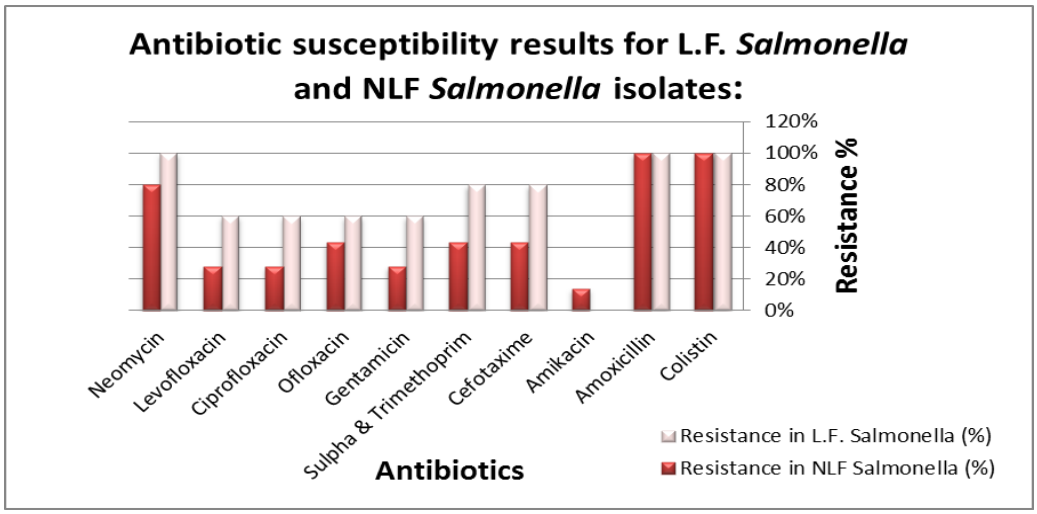
Table 5. Antimicrobial susceptibility pattern of *Salmonella*

Isolates	col-istin CT 10	amoxi-cillin AMC 30	Cefotax-ime CTX 30	Trime-thopri m SXT 25	Neo-myci n N 30	Gen-tamy cin CN 10	ciprof loxaci n CIP 5	Levofl oxacin LEV 5	Amika-cin AK 30	Ofloxa-cin OFX 5	MD R %
(NLF) *Salmonella*											
*S.*Typhimurium	R	R	R	R	R	S	S	S	S	S	0.5
*S.*Typhimurium	R	R	R	S	R	S	S	S	S	R	0.5
S. Enteritidis	R	R	S	S	R	R	S	S	S	S	0.4
S. Kentucky	R	R	R	R	S	S	R	R	S	R	0.7
S. Kentucky	R	R	S	R	R	S	R	R	S	R	0.7
*S.*Montevideo	R	R	S	S	R	S	S	S	R	S	0.4
*S.*Virchow	R	R	S	S	R	R	S	S	S	S	0.4
% of resistance	100%	100%	43%	43%	80%	28%	28%	28%	14%	43%	
(LF) *Salmonella*											
*S.*Typhimurium	R	R	R	R	R	R	S	S	S	S	0.6
S. Enteritidis	R	R	R	R	R	S	R	R	S	R	0.8
*S.*Infantis	R	R	R	S	R	R	S	R	S	R	0.7
*S.*Havana	R	R	S	R	R	R	R	S	S	S	0.6
*S.*Ruiru	R	R	R	R	R	S	R	R	S	R	0.8
% of resistance	100%	100%	80%	80%	100%	60%	60%	60%	0%	60%	

The results of antibiotic susceptibility testing of ***L.F.salmonella*** and ***NLF.Salmonella*** isolates among the ten antibiotics used showed a high resistance against Colistin and amoxicillin 100% for each while ***L.F. salmonella*** and ***NLF. Salmonella*** isolates showed high sensitivity to Amikacin (100% - 86%) respectively. Other antibiotics showed a variable degree of resistance against ***L.F. salmonella*** *and* ***NLF. Salmonella***. Briefly Cefotaxime (80%-43% respectively), Sulpha & Trimethoprim (80%-43% respectively), Gentamicin (60%-28% respectively), Ofloxacin (60%-43% respectively), Ciprofloxacin (60%-28% respectively) Levofloxacin (60%-28% respectively) , Neomycin, (100%-86% respectively).The analysis of health risk of the MAR index found that 100% of ***L.F.s almonella*** and ***NLF. Salmonella*** showed a MDR index ≥ 0.2, which indicated a high risk of antimicrobials. So most of ***L.F.salmonella*** *and* ***NLF. Salmonella*** isolates showed a multidrug resistance profile

% calculated according to the total number of tested isolates: *L.F. salmonella* serovars (NO. 5), *NLF. Salmonella* serovars (NO. 7)

Fig (4)**:** Antibiotic susceptibility results for **L.F. *Salmonella*** and **NLF *Salmonella*** isolates:



% calculated according to the total number of tested isolates: (LF) Serovars (NO. 5) and (NLF) serovars (NO. 7).

Table 6. Results of PCR for detection of *invA* gene and *LacZ* gene

Isolates	*invA* gene	*LacZ* gene
NLF *Salmonella*		
*S.*Typhimurium	+ve	*-ve*
S. Enteritidis	+ve	*-ve*
S. Kentucky	+ve	*-ve*
S.Montevideo	+ve	*-ve*
S.Virchow	+ve	*-ve*
LF *Salmonella*		
*S.*Typhimurium	+ve	*+ve*
S. Enteritidis	+ve	*-ve*
*S.*Infantis	+ve	*-ve*
*S.*Havana	+ve	+ve
*S.*Ruiru	+ve	+ve

All lactose-fermenting and non-lactose-fermenting *Salmonella* isolates showed 100% positivity for the *invA* gene. Out of five lactose-fermenting strains, three (60%) were found to harbor the lacZ gene and it is not found in NLF *Salmonella* isolates.

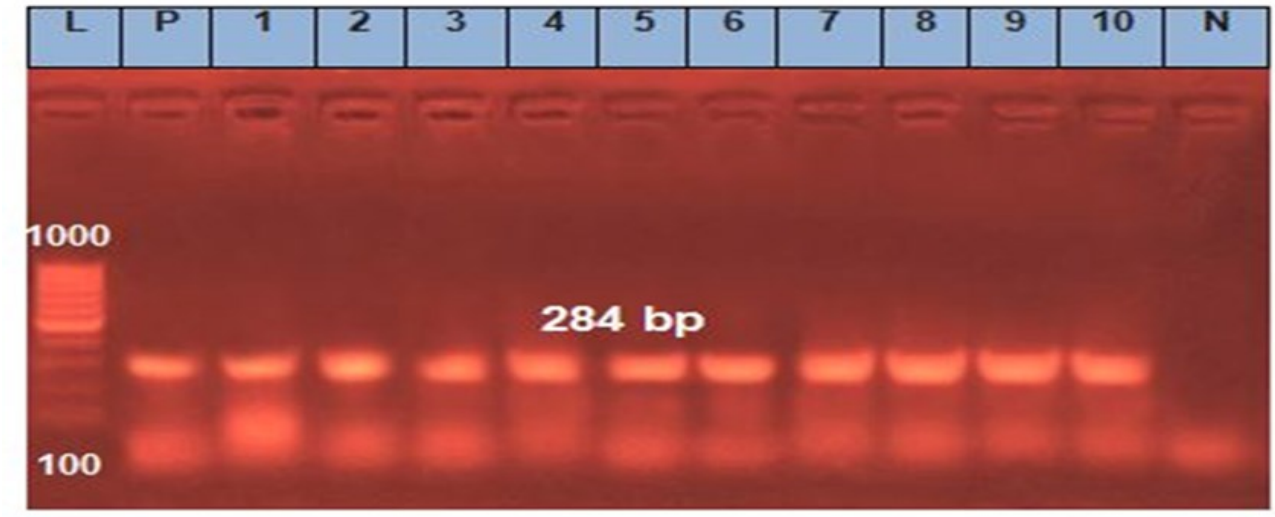


Fig (5): Agarose gel electrophoresis of PCR products after amplification of: invA gene virulence genes for *Salmonella* strains, L: MWM-molecular weight marker (100 – 1000 bp DNA ladder), P: control positive N: control negative and N.L.F strains of *Salmonella* species from (1:*S.*Typhimurium, 2: *S.* Enteritidis, 3: *S.* Kentucky, 4: *S.* Montevideo, 5: *S.* Virchow). L.F strains of *Salmonella* species from (6:*S.*Typhimurium, 7: *S.* Enteritidis, 8: *S.*Infantis, 9: *S.*Havana, 10: *S.*Ruiru). (*InvA* gene products at 284 bp)

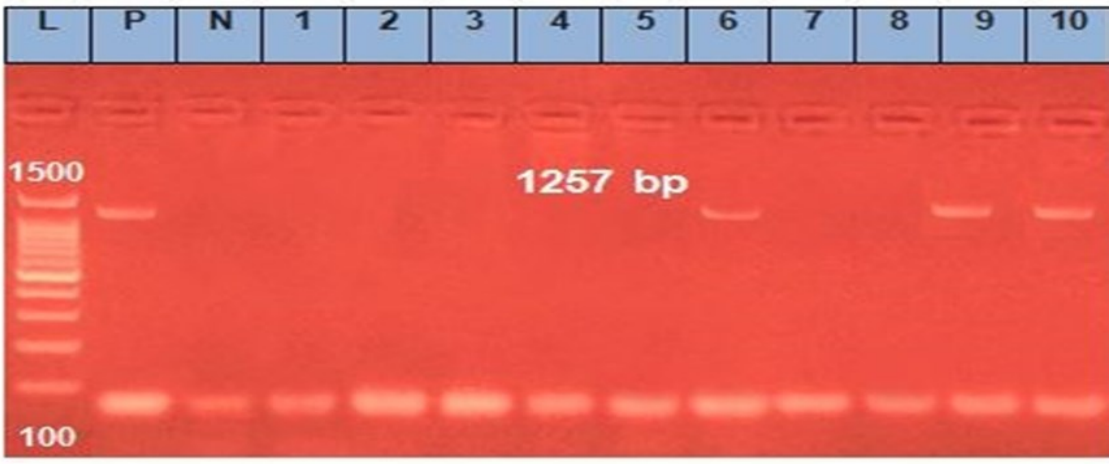


Fig (6): Agarose gel electrophoresis of PCR products after amplification of: *LacZ* gene virulence genes for *Salmonella* strains, L: MWM-molecular weight marker (100 – 1500 bp DNA ladder), P: control positive N: control negative and N.LF strains of *Salmonella* species from (1:*S.*Typhimurium, 2: *S.* Enteritidis, 3: *S.* Kentucky, *4: S.* Montevideo, *5: S.* Virchow). L.F strains of *Salmonella* species from (6:*S.*Typhimurium, 7: *S.* Enteritidis, 8: *S.*Infantis, 9: *S.*Havana, 10: *S.*Ruiru). (*LacZ* gene products at 1257 bp)

DISCUSION

The increased risk of zoonotic *Salmonella* transmission to humans through the food chain is attributed to its ability to spread horizontally and vertically within avian communities, occasionally resulting in subclinical infections or remaining completely asymptomatic **(Antunes et al. 2016).**

In the present study, the incidence of *Salmonella* from 300 diseased broilers was 4%. This prevalence is in line **with Liu et al. (2010),** who found 4.5% prevalence in samples of chicken, and is marginally greater than the 2.5% noted by **Mohamed et al. (1999).** However, the isolation rate was markedly less than that documented by **Shahjada et al. (2017),** who detected *Salmonella* with percentages of 38% of broiler chicks in Bangladesh. Such discrepancies in prevalence are likely attributable to variations in farm management, biosecurity measures, sampling methods, and geographic conditions.

Eight *Salmonella* serotypes were identified in this study: *S.* Typhimurium, *S.* Enteritidis, *S.* Kentucky, *S.* Montevideo, *S.* Virchow, *S.* Infantis, *S.* Havana, and *S.* Ruiru. Both lactose-fermenting (LF) and non-lactose-fermenting (NLF) isolates were detected. The serotype profile aligns partially with findings of (**Mashayekh et al. 2022),** who also identified *S.* Typhimurium, *S.* Enteritidis, *S.* Infantis, and *S.* Havana from broilers, but contrasts with **Shalaby et al. (2022**), who reported a predominance of *S.* Enteritidis (37%).

The most important tests appeared in the API in case of N.L.F *Salmonella* serovars are LDC, Citrate, H_2S and glucose tests were positive while ONPG, urea and indol tests were negative. But in this study we detected H_2S - negative *Salmonella* isolates (*S.* Typhimurium, *S.* Infantis and *S.* Ruiru) these results agree with (**Sakano et al. 2013)** who detected non-H_2S producing *S.* Infantis and *S.* Typhimurium isolates due to a nonsense mutation in the *phsA* gene as the impairment of the *phs* locus is associated with the absence of H_2S production and misidentification of *Salmonella* .Hydrogen sulfide (H_2S) production is a characteristic feature of non-lactose fermenting *Salmonella* and is commonly used as a diagnostic approach for differentiate *Salmonella* from other enteric bacteria **(Boadi et al. 2010).**

In this study, one lactose-fermenting *Salmonella* Havana strain was identified that tested negative for lysine decarboxylase (LDC).

lysine decarboxylase positive phenotype is a key feature of *Salmonella* spp. and is applied in simplified detection assays (**Wilson, 2004).** However, an unusual rise in LDC-negative *S. enterica* serovar Enteritidis strains was reported in Japan in 2003, attributed to a naturally acquired mutation in the *cadC* gene. This mutation is believed to impair the function of the CadC protein as a sensor, resulting in atypical biochemical traits such as LDC negativity in *Salmonella* spp. **(Morita et al. 2006).**

Lactose fermenting *Salmonella* possess both β-galactosidase, lactose permease, two enzymes responsible for the positive lactose fermentation test while Lactose permease enables the transport of lactose molecules into the bacterial cell, the β-galactosidase can hydrolyzes the glycosidic linkage producing glucose and galactose thus ONPG test was detecting β-galactosidase and it was positive in case of presence of this enzyme. (LF *Salmonella*) appear yellow. N.L.F *Salmonella* are lack both β-galactosidase and lactose permease and are unable of producing acid from lactose so they were negative ONPG test and appear colorless (**Boadi et al. 2010) and (Khider 2012).**

Among the various diagnostic approaches for *Salmonella*, polymerase chain reaction (PCR) offers a powerful and reliable option because of its high sensitivity, specificity, and rapid detection capability. It has been reported that the *invA* gene, found exclusively in *Salmonella* spp., is regarded as a key diagnostic molecular marker for their identification **(O'Regan et al. 2008).** In our study, this gene was detected in all examined *Salmonella* serovars (both NLF and LF) with a prevalence of 100%., high prevalence rates of *invA* virulence gene in *Salmonella* serovars has also been reported by **Chaudhary et al. 2015).**

In this study, the presence of the lacZ gene, which encodes β-galactosidase and is responsible for lactose fermentation, was investigated. All non-lactose-fermenting *Salmonella* strains show negative result for lacZ gene and present in 60% of LF strains, while two LF isolates lacked detectable *lacZ*, suggesting that lactose fermentation in these strains could be due to silent *lacZ* expression under laboratory conditions as some genes are only expressed in the presence of specific inducers or environmental signals, such as lactose itself or low-glucose environments. If such conditions are not replicated during PCR testing, gene expression may go undetected **(Harwani, 2014)** which support our finding as two LF *Salmonella* were negative for lacZ gene.

Also lacZ gene may be present but altered due to mutations that prevent its detection by standard PCR assays or render it non-functional or due to alternative metabolic pathways as some LF *Salmonella* strains may harbor alternative or horizontally acquired metabolic genes enabling lactose fermentation without relying on the canonical lacZ gene. Genes from other *Enterobacteriaceae* may be transferred via plasmids or transposons, allowing fermentation of lactose through novel or less common pathways **(Leonard et al. 2015)** may allow lactose fermentation in its absence.

Generally speaking *Salmonella* spp. are known to be non-lactose fermenters, a key characteristic used to differentiate them from coliform bacteria such as *Escherichia coli* on selective media like MacConkey agar.

Antimicrobial susceptibility profiling revealed a worrisome pattern of multidrug resistance (MDR) in both LF and NLF isolates. A notably high level of resistance was observed for both groups against colistin and amoxicillin, with 100% of isolates resistant. This finding is consistent with previous reports indicating the rising prevalence of colistin resistance among *Salmonella* strains, which is particularly alarming given that colistin is often considered a last-resort antibiotic for multidrug-resistant Gram-negative infections **(Kempf et al.2016).**

Similarly, widespread resistance to amoxicillin among *Salmonella* has been attributed to the extensive and often unregulated use of beta -lactam antibiotics in livestock production. Conversely, both L.F. and NLF *Salmonella* isolates exhibited high sensitivity to Amikacin, with susceptibility rates of 100% and 86%, respectively. This is in agreement with prior studies demonstrating that aminoglycosides, such as Amikacin, retain significant activity against *Salmonella* species, likely due to their

limited use in veterinary medicine compared to other classes of antibiotics **(Lo et al. 2014).**

Resistance to other antibiotics varied. Notably, Cefotaxime resistance was detected in 80% of LF and 43% of NLF isolates, consistent with the global emergence of extended-spectrum β-lactamase (ESBL)-producing *Salmonella* **(Dandachi et al. 2018).** Sulphamethoxazole/trimethoprim resistance was also higher in LF isolates (80%) compared to NLF (43%), possibly due to differential selective pressure. Resistance to gentamicin and fluoroquinolones (Ofloxacin, ciprofloxacin, levofloxacin) was more frequent among LF strains (up to 60%) than NLF strains (28–43%). Neomycin resistance was universal in LF isolates (100%) and slightly lower in NLF isolates (80%). The higher resistance levels in LF strains align with observations by **Khider (2012)** and may reflect distinct genetic backgrounds or antimicrobial exposure histories.

Conclusion and Recommendation:

Accurate detection of lactose-fermenting *Salmonella* is of critical importance in clinical microbiology and public health. These strains, often misidentified as *Escherichia coli* due to their lactose-fermenting phenotype, can lead to misdiagnosis, inappropriate treatment, and unnoticed outbreaks. The significance of lactose-fermenting *Salmonella*, especially those expressing the lacZ gene, extends to the poultry industry, where it raises concerns regarding foodborne illness and antimicrobial resistance. Their ability to cause infection clusters emphasizes the necessity of enhanced diagnostic vigilance and the continual refinement of laboratory protocols to ensure effective disease control and epidemiological monitoring. The findings of this study underline the urgent need for implementing strict antibiotic stewardship programs in poultry farms to mitigate the spread of multidrug-resistant *Salmonella* strains. Continuous surveillance and molecular monitoring are also recommended to track resistance trends and inform public health interventions.

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