Research Article

Prevalence, Conventional and Molecular Characterization of *Salmonella* Isolated from Chicken Farms and Slaughterhouses

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Abstract | The consumers' perspective and expectations on food safety and quality are increased over time as many food stuff and products are exposed to contamination by numerous pathogens concerning *Salmonella* species. The current study aimed to investigate the prevalence of *Salmonella* contributing contamination in chicken samples that were collected from broiler farms and slaughterhouses using conventional culturing, biochemical, and serological identifications versus molecular detection. A prospective study was designed to last for six months from March 2021 to the end of August 2021. A total number of 126 chicken samples (100 samples from five broiler chicken farms and 26 samples from two slaughterhouses) were collected from the Ismailia governorate. Each sample was composed of liver, intestine, and breast and thigh muscles. The study revealed a total prevalence of 35.7% (45 positives out of 126 samples). Slaughterhouse I and II, chicken farms I, II, III, IV, and V revealed prevalence up to 15.3, 23.0, 40.0, 35.0, 50.0, 45.0, and 30% respectively. Initial isolation revealed 14.2% and delayed isolation procedures revealed a prevalence of up to 21.42%. The bacteriological analysis was carried out using conventional cultural and molecular means (cyclic polymerase chain reaction; cPCR) targeting the *invA* gene. The isolated *Salmonella* culture revealed higher resistance incidence up to 100% against amoxicillin-clavulanic acid (AMC; 30 µg), ampicillin (AMP; 10 µg), and nalidixic acid (NAL; 30 µg), 90% against enrofloxacin (ENR; 5 µg), and 80% against doxycycline HCL (DO; 30 µg). The conventional culture method revealed up to 83% sensitivity and 90% specificity while the molecular analysis revealed up to 100% sensitivity and 100% specificity for *Salmonella* detection. The study concluded that the high prevalence of the *Salmonella* with high resistance against 60% of the tested antibiotics reflects a serious problem with the hygienic and biosecurity measures taken in the poultry and slaughterhouses, as well the extensive use of the antibiotic contributed to the recorded high resistance among the isolated strains.

Keywords | Antimicrobial, Broiler chickens, Conventional, Molecular, Prevalence, *Salmonella*

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INTRODUCTION

The poultry industry directed its goals on combating infectious and contagious diseases, sustaining high production, raising the product quality, and achieving the goals with low costs (Cavani et al., 2009; Delpont et al., 2021; Schweitzer et al., 2021). To meet these expectations, good biosecurity measures have to be applied in the poultry facilities to minimize the entrance of pathogenic micro-organisms known as "bioexclusion" and prevent the transmission of the pathogens from one area to another known as "biocontainment" (Hafez, 2005; FAO Statitics, 2020; Delpont et al., 2021). Several actions have been adapted like increasing the self-sufficiency of broiler chickens, monitor and observing to increase the control over the disease's development, increasing the veterinary services to improve the productivity and reduce the disease incidence, improving the vaccination act, and establishment

of legislation programs to control the development of infectious diseases (Attia et al., 2017; Otte et al., 2021).

The consumers perspective on food safety and quality is a continuous issue. Many food stuff and products are exposed to contamination by numerous pathogens concerning *Salmonella* and *Campylobacter* species. The control of these pathogens should involve a deep understanding of the epidemiological triad of these micro-organisms and the commitment to the application of strict preventive and biosecurity measures (Alsultan et al., 2019; Morishita and Derksen, 2021). Many serious actions adapted and approved some actions to prevent the contamination of poultry products with pathogenic micro-organisms like *Salmonella enterica* serovars.

Salmonella is a genus of the family *Enterobacteriaceae* that includes more than 300 serovars and caused many disease problems in chickens (Sheela et al., 2003; Rogers et al., 2021). *Salmonella* is a gram-negative, non-spore-forming rod, motile through flagella but can shift into non-motile onto cultures (Su and Chiu, 2007; Pulford et al., 2021). *Salmonella* is chemotrophs that contain their energy from the oxidation-reduction reactions in the organic sources surrounding those (Rayan et al., 2017; Rosenberg et al., 2021). They are facultative anaerobes that are capable of generating adenosine triphosphate (ATP) from the oxygen once it can be available, otherwise, they use electron acceptors at the end of the transport chain including sulfate, nitrate, or sulfur, or fermentation (Fàbrega and Vila, 2013; Johansson et al., 2021). *Salmonella* is not heat resistant and at the same time doesn't grow at low microclimatic temperatures, but they also may survive in an excellent state in acid foods and can as well resist dehydration. Meaning, while not able to multiply in many processed foods, if contamination is present, it can be difficult to eradicate (Mandal and Kwon, 2017).

The current study investigated the presence/absence information of *Salmonella* serovars in chicken samples collected from chicken farms and slaughterhouses. The investigation was based on conventional culturing means, biochemical, and serological identifications versus the molecular investigation using cyclic polymerase chain reaction (cPCR) targeting the *invA* gene.

MATERIALS AND METHODS

Ethical approval

The materials, methodology, and study design were approved by the Scientific Research Ethics Committee on animal and poultry researches, Faculty of Veterinary Medicine, Suez Canal University, Egypt with approval number (2021030).

The experimental design

A prospective study was designed to last for six months from March 2021 to the end of August 2021. The study was conducted to investigate the presence/absence information of *Salmonella* serovars in chicken meat collected from chicken farms at the marketing time, as well from slaughterhouses as marketable chickens passed for human consumption.

The samples were collected from five broiler chicken farms and two slaughterhouses located in the Ismailia governorate. Ismailia is situated on the west bank of the Suez Canal approximately halfway between the Port Said governorate to the north and Suez governorate to the south with a longitude of 30.5965° N and latitude of 32.2715° E. The climate in Ismailia according to the Köppen-Geiger climate classification system is known to be a hot desert. The hottest recorded temperature was 47°C (117°F) on 14 June while the coldest recorded temperature was 0.2°C (32.4°F) in January.

Sampling and sample preparations

A total number of 126 chicken samples were collected from the Ismailia governorate. The samples were collected at a rate of 100 samples from five broiler chicken farms and 26 samples from two different slaughterhouses. Each sample was composed of liver, intestine, and breast and thigh muscles. The samples were preserved to prevent any further contamination or decaying in an ice-box and transferred to the laboratory as quickly as possible where samples were kept frozen until bacteriological analysis.

In the laboratory, the samples were thawed carefully under complete aseptic conditions, and small pieces of the liver, duodenum of the intestine, and muscle tissues were dissected to be added to pre-enrichment tubes previously set containing 9 ml of buffered peptone water (Thermo Scientific™ Oxoid™ Buffered Peptone Water, CM0509B, 500 g) and incubated at 37°C for 18-24 hours as recommended by American Public Health Association; APHA (2017).

Bacteriological examination

Bacteriological examination was carried out following Herigstad et al. (2001) by transferring one ml from the pre-enriched samples under complete aseptic conditions into clean sterile tubes containing 9 ml fresh Rappaport Vassiliadis broth (RV, Thermo Scientific™ Oxoid™ Rappaport-Vassiliadis Enrichment Broth, CM0669, 500 g) and incubated at 37°C for 18-24 h. Ten µl were dropped onto already solidified Xylose Lysine Deoxycholate (XLD, Thermo Scientific™ Oxoid™ X.L.D. Agar, CM0469, 500 g) agar plates and incubated at 37°C for 18-24 h. The culturing was conducted using the drop plate technique as

recommended by Soliman et al. (2016) and Kim and Lee (2016). The plates were examined morphologically for the growth of the typical black colonies as recommended by Murray et al. (2015).

Negative samples were subjected to delaying protocol through additional enrichment of one ml from buffered peptone water tubes into 9 ml tubes of Rappaport Vassiliadis broth at room temperature for 5-7 days in closed sterile colorless glass bottles with daily renewal of the Rappaport Vassiliadis broth to prevent the desiccation and decaying of the samples. Later, the delayed samples were processed by transferring 1 ml under complete aseptic conditions into clean sterile tubes containing fresh RV broth and incubated at 37°C for 18-24 h. Ten µl were dropped onto already XLD agar plates and incubated at 37°C for18-24 h. The plates were examined for the development of black colonies. The colonies were streaked for biochemical identification, antimicrobial sensitivity testing, and some colonies were preserved into RV broth with glycerol into sterile 2.5 ml Eppendorf tubes for serological identification and RV broth into sterile serum tubes for polymerase chain reaction identification.

Biochemical identification

The biochemical identification was carried out using serious biochemical tests like the triple sugar iron agar test (TSI) indicating gas production and changes in the color from red to yellow. Lysine iron agar test (LIA) to determine the ability of the micro-organism to deaminate lysine aerobically on the slant of the media or anaerobically decarboxylate lysine in the butt of the media.

The urease test to determine the microbial capabilities of hydrolyzing urea to produce ammonia and carbon dioxide. The indole production test was used to measure the ability of micro-organisms to decompose the amino acid tryptophan to indole which accumulates in the medium. Methyl red test (MR) was used to determine the microbial abilities for the production of acid as it identifies bacterial ability to produce stable acid end products through a mixed-acid fermentation of glucose. Voges Proskauer test (VP) determined if an organism produces acetyl methyl carbinol from glucose fermentation.

Serological identification

The isolated *Salmonella* isolates were serotyped using slide agglutination test (stained *Salmonella* antigen Widal latex slide test kit, 8 × 5 mL, Bio Lab® Diagnostics (I) Private Limited) according to Collins et al. (1995). The suspected colonies were sub-cultured on nutrient slopes at 37°C for 24 hours. A slide agglutination test was carried out by re-suspending *Salmonella* colonies into two separate drops of sterile physiological saline on a slide. A drop of *Salmonella* somatic antigen "*Salmonella* O" and *Salmonella* flagellar antigen "*Salmonella* H" were added to the suspended colonies with thorough mixing. Positive results can be detected by the development of agglutinations that can be seen by the naked eyes within a minute. The delayed agglutinations or homogenous drops indicated negative results.

Molecular identification

Extraction of DNA (QIAamp DNA Mini Kit, Catalogue no. 51304)

A mix of 20 µl QIAGEN protease, 200 µl of the sample, and 200 µl buffer AL were pipetted into the bottom of a 1.5 ml micro-centrifuge tubes, vortex for 15 sec, and incubated at 56°C for 10 min. About 200 µl of ethanol (96%) were added and mixed by pulse vortex for 15 sec. Then mixtures were carefully transferred to the QIAamp Mini spin column in a 2ml collecting tube and centrifuged at 8000 rpm for one min. The tubes containing the filtrate were discarded and 500 ml from buffer AW1 were added, centrifuged (8000 rpm/ one min), 500 ml buffer AW2 were added and centrifuged at full speed for 3 min, and a 100 µl from buffer AE were added, incubated at room temperature (15-25°C) for one min, and then centrifuged at 8000 rpm for one min.

Preparation of the master-mix

The master-mix was prepared according to Emerald Amp GT PCR master-mix (Takara®) Code No. RR310A kit. The tubes were set with Emerald Amp GT PCR master-mix (2x premix); 12.50 µl, PCR grade water; 4.50 µl, forward primer (20 pmol); 1.00 µl, reverse primer (20 pmol); 1.00 µl, template DNA; 6.00 µl, and the total reaction was optimized at 25.00 µl.

Oligonucleotide primer sequences

The primers were designed (Metabion®, Germany) as follows:

5′-GTGAAATTATCGCCACGTTCGGGCAA-3′.
3′-TCATCGCACCGTCAAAGGAACC-5′.

The primers were targeting the *invA* gene of *Salmonella* (Oliveira et al., 2003). The produced amplified product was 284 bp.

Cycling conditions of the primers during cPCR

The temperature and time conditions of the two primers for detecting the *invA* gene of *Salmonella* during the PCR were as follow: primary denaturation at 94°C/5 min, secondary denaturation at 94°C/30 sec, annealing at 55°C/30 sec, extension at 72°C/30 sec, the total number of cycles was designed to 35 cycles (secondary denaturation, annealing, and extension), and the final extension at 72°C/7 min.

Agarose gel electrophoresis

The gel electrophoresis was carried out according to Sambrook et al. (1989). Ten µl of the required ladder were directly loaded. Electrophoresis grade agarose (1.0 g) was prepared in 100 ml TBE buffer, heated in a microwave to dissolve, allowed to cool at 70°C, then 0.5 µg/ml Ethidium bromide was added, and mixed thoroughly. The warm agarose was poured directly into the gel casting apparatus with the desired comb in apposition and left at room temperature for polymerization.

The comb was then removed and the electrophoresis tank was filled with TBE buffer. Twenty µl of each uniplex PCR product, negative control, and positive control were loaded to the gel. The power supply was run at 1-5 volts/cm of the tank length. The run was stopped after about 30 min and the gel was transferred to the UV cabinet. The gel was photographed by a gel documentation system and the data was analyzed through computer software.

Antibiotic sensitivity

The antibiotic sensitivity test was carried out according to CLSI (Clinical and Laboratory Standards Institute, 2002). A single colony of the suspected colonies of *Salmonella* was inoculated into 5 ml tryptic soy broth and incubated at 37C for 18 hours. The turbidity of the tube was measured against 0.5 McFarland of 1.5 x 10^5 CFU/ml. Few drops of the turbid broth were inoculated onto Muller-Hinton agar plates. Excess of cultural fluid was removed aseptically and the plates were allowed to stand for at 37°C for 15 min for dryness. The inoculated plates were overlaid with antibiotic discs (Amoxicillin and Clavulanic acid - AMC; 30 µg, Ampicillin - AMP; 10 µg; Amikacin - AK; 30 µg; Doxycycline HCL - DO; 30 µg, Meropenem - MEM; 10 µg, Gentamicin - GN; 10 µg, Norfloxacin - NOR; 10 µg; Trimethoprim-sulfamethoxazole - SXT; 25 µg, Nalidixic acid - NAL; 30 µg, and Enrofloxacin - ENR; 5 µg) using sterile forceps considering the distribution of the discs in a manner where the distance among them was optimum and away from the edge of the plate to avoid overlapping of the inhibition zones and gives more wide area for the zone of inhibition.

The inoculated plates were incubated at 37°C for 24 hours. Inhibition zones were measured by caliper and interpretation of the results was carried out in comparison to the interpretative standards of the National Committee for Clinical Laboratory Standards (NCCLS, 1990, MZ-A4).

Sensitivity and specificity

The sensitivity referred to the proportion of those who have the condition that received a positive result on this test (Proportion of true positive). Sensitivity (Sn) was measured as recommended by Powers (2011) and Bénard et al. (2018) according to the following formula:

$$\text{Sn (\%)} = \frac{\text{Number of true positive}}{\text{Number of true positive + number of false negative}}$$

The specificity referred to the proportion of those do not who have the condition that received a negative result on this test (proportion of true negative). Specificity (Sp) was measured as recommended by Powers (2011) and Bénard et al. (2018) according to the following formula:

$$\text{Sp (\%)} = \frac{\text{Number of true negative}}{\text{Number of true negative + number of false positive}}$$

Statistical analysis

The statistical analysis was conducted using a statistical package for social sciences version 20 (IBM Corp, 2016 - IBM SPSS Statistics 20). The obtained data and results were analyzed statistically using One-Way Analysis of Variance (ANOVA) to estimate the prevalence and their statistical differences. The statistical model empathized as follow:

$$Y_{ij} = \mu + \alpha_j + \mathcal{E}_{ij}$$

Where Y_{ij} was the measurement of dependent variables; µ was the overall mean; α_j was the fixed effect of the bacteriological detection of samples positivity, and ε_{ij} was the random error. Nonparametric Kruskal–Wallis was used for detecting the significant differences between the prevalence rates. The results were expressed as highly significant at ($p \leq 0.01$), significant at ($p \leq 0.05$), and non-significant at ($p > 0.05$).

RESULTS AND DISCUSSION

Prevalence of *Salmonella* in chicken samples

The results in Table 1 revealed a total prevalence of 35.7% (45 positives out of 126 samples). This prevalence was variable among the sources from which samples have been collected. The different locations of sampling; slaughterhouse I and II, chicken farms, I, II, III, IV, and V revealed prevalence up to 15.3 (2 positives out of 13 samples), 23.0 (3 positives out of 13 samples), 40.0 (8 positives out of 20 samples), 35.0 (7 positives out of 20 samples), 50.0 (10 positives out of 20 samples), 45.0 (9 positives out of 20 samples), and 30% (6 positives out of 20 samples). The tissue-specific total prevalence revealed in Table 1 high isolation rates of *Salmonella* from the intestine (17.46%), muscles (11.90%), and liver (6.35%) samples, respectively. The higher isolation rates were detected in chicken farms' samples compared to slaughterhouses samples.

Initial isolation procedures (Table 2) revealed 18 positive out of 126 samples with a prevalence of up to 14.2%.

The prevalence of isolations was nearly zero (0%) in slaughterhouses' samples compared to 15, 20, 25, 15, and 15% isolation rates from the five chicken farms (I, II, III, IV, and V), respectively. The tissue-specific initial culturing prevalence revealed in Table 2 high isolation rates of *Salmonella* from muscles (6.35%), intestine (6.35%), and liver (3.17%) samples, respectively.

Table 1: Prevalence of total and tissue-specific *Salmonella* positive samples during the study.

Source	Tissues	No.	Positive	P (%)
Slaughterhouse I	Liver	13	0	0.00[b]
	Intestine	13	1	7.69[a]
	Muscles	13	1	7.69[a]
	Total	13	2	15.38[D]
Slaughterhouse II	Liver	13	0	0.00[c]
	Intestine	13	2	15.38[a]
	Muscles	13	1	7.69[b]
	Total	13	3	23.07[D]
Chicken farm I	Liver	20	1	5.00[c]
	Intestine	20	4	20.00[a]
	Muscles	20	3	15.00[b]
	Total	20	8	40.00[B]
Chicken farm II	Liver	20	1	5.00[b]
	Intestine	20	3	15.00[a]
	Muscles	20	3	15.00[a]
	Total	20	7	35.00[C]
Chicken farm III	Liver	20	2	10.00[c]
	Intestine	20	5	25.00[a]
	Muscles	20	3	15.00[b]
	Total	20	10	50.00[A]
Chicken farm IV	Liver	20	3	15.00[b]
	Intestine	20	4	20.00[a]
	Muscles	20	2	10.00[c]
	Total	20	9	45.00[B]
Chicken farm V	Liver	20	1	5.00[c]
	Intestine	20	3	15.00[a]
	Muscles	20	2	10.00[b]
	Total	20	6	30.00[C]
Total	Liver	126	8	6.35[c]
	Intestine	126	22	17.46[a]
	Muscles	126	15	11.90[b]
	Total	126	45	35.71

[a,b,c,d,e] Means carrying different superscripts in the same column are significantly different at (P ≤ 0.05) or highly significantly different at (P < 0.01). Means carrying the same superscripts in the same column are non-significantly different at (P < 0.05). [A, B, C, D, E] Means carrying different superscripts in the same column are significantly different at (P ≤ 0.05) or highly significantly different at (P < 0.01). Means carrying the same superscripts in the same column are non-significantly different at (P < 0.05). P= Prevalence (%).

Table 2: Prevalence of total and tissue-specific *Salmonella* positive samples in initial culturing.

Source	Tissues	No.	Positive	P (%)
Slaughterhouse I	Liver	13	0	0.00[a]
	Intestine	13	0	0.00[a]
	Muscles	13	0	0.00[a]
	Total	13	0	0.00[D]
Slaughterhouse II	Liver	13	0	0.00[a]
	Intestine	13	0	0.00[a]
	Muscles	13	0	0.00[a]
	Total	13	0	0.00[D]
Chicken farm I	Liver	20	0	0.00[c]
	Intestine	20	1	5.00[b]
	Muscles	20	2	10.00[a]
	Total	20	3	15.00[C]
Chicken farm II	Liver	20	1	5.00[b]
	Intestine	20	1	5.00[b]
	Muscles	20	2	10.00[a]
	Total	20	4	20.00[B]
Chicken farm III	Liver	20	1	5.00[b]
	Intestine	20	2	10.00[a]
	Muscles	20	2	10.00[a]
	Total	20	5	25.00[A]
Chicken farm IV	Liver	20	1	5.00[a]
	Intestine	20	1	5.00[a]
	Muscles	20	1	5.00[a]
	Total	20	3	15.00[C]
Chicken farm V	Liver	20	1	5.00[a]
	Intestine	20	1	5.00[a]
	Muscles	20	1	5.00[a]
	Total	20	3	15.00[C]
Total	Liver	126	4	3.17[b]
	Intestine	126	6	4.76[b]
	Muscles	126	8	6.35[a]
	Total	126	18	14.28

[a,b,c,d,e] Means carrying different superscripts in the same column are significantly different at (P ≤ 0.05) or highly significantly different at (P < 0.01). Means carrying the same superscripts in the same column are non-significantly different at (P < 0.05). [A, B, C, D, E] Means carrying different superscripts in the same column are significantly different at (P ≤ 0.05) or highly significantly different at (P < 0.01). Means carrying the same superscripts in the same column are non-significantly different at (P < 0.05). Initial culturing included pre-enrichment on RV broth/ day and culturing on XLD agar/ day. P= Prevalence (%)

Delayed isolation procedures in Table 3 revealed a total of 27 positives out of 126 samples with a prevalence up to 21.42%. The isolation rates and prevalence were fluctuating with a minimum of 15% in chicken farm II to a maximum

of 30% in chicken farm IV. The tissue-specific delayed culturing prevalence revealed in Table 3 high isolation rates of *Salmonella* from the intestine (12.70%), muscles (5.55%), and liver (3.17%) samples, chronologically.

Table 3: Prevalence of total and tissue-specific *Salmonella* positive samples in delayed culturing.

Source	Tissues	No.	Positive	P (%)
Slaughterhouse I	Liver	13	0	0.00^{b}
	Intestine	13	1	7.69^{a}
	Muscles	13	1	7.69^{a}
	Total	13	2	15.38^{C}
Slaughterhouse II	Liver	13	0	0.00^{c}
	Intestine	13	2	15.38^{a}
	Muscles	13	1	7.69^{b}
	Total	13	3	23.07^{B}
Chicken farm I	Liver	20	1	5.00^{b}
	Intestine	20	3	15.00^{a}
	Muscles	20	1	5.00^{b}
	Total	20	5	25.00^{B}
Chicken farm II	Liver	20	0	0.00^{c}
	Intestine	20	2	10.00^{a}
	Muscles	20	1	5.00^{b}
	Total	20	3	15.00^{C}
Chicken farm III	Liver	20	1	5.00^{b}
	Intestine	20	3	15.00^{a}
	Muscles	20	1	5.00^{b}
	Total	20	5	25.00^{B}
Chicken farm IV	Liver	20	2	10.00^{b}
	Intestine	20	3	15.00^{a}
	Muscles	20	1	5.00^{c}
	Total	20	6	30.00^{A}
Chicken farm V	Liver	20	0	0.00^{c}
	Intestine	20	2	10.00^{a}
	Muscles	20	1	5.00^{b}
	Total	20	3	15.00^{C}
Total	Liver	126	4	3.17^{b}
	Intestine	126	16	12.70^{a}
	Muscles	126	7	5.55^{b}
	Total	126	27	21.42

^{a,b,c,d,e} Means carrying different superscripts in the same column are significantly different at ($P \leq 0.05$) or highly significantly different at ($P < 0.01$). Means carrying the same superscripts in the same column are non-significantly different at ($P < 0.05$). $^{A, B, C, D, E}$ Means carrying different superscripts in the same column are significantly different at ($P \leq 0.05$) or highly significantly different at ($P < 0.01$). Means carrying the same superscripts in the same column are non-significantly different at ($P < 0.05$). Delayed culturing included pre-enrichment on RV broth / five to seven days and culturing on XLD agar/ day. P= Prevalence (%).

Biochemical identifications

The biochemical profile revealed in Figure 1A positive yellow TSI slants (yellow coloration from the acid formation interrupted with gases and black color from the hydrogen sulfide formation), Figure 1B positive LIA slants (purple slants and purple but accompanied with blackening of the butt by hydrogen sulfide production), Figure 1C positive SC utilization test (blue color), and Figure 1D positive MR test (red color development). Meanwhile, *Salmonella* isolates were negative to all of the urease, indole production, and VP tests.

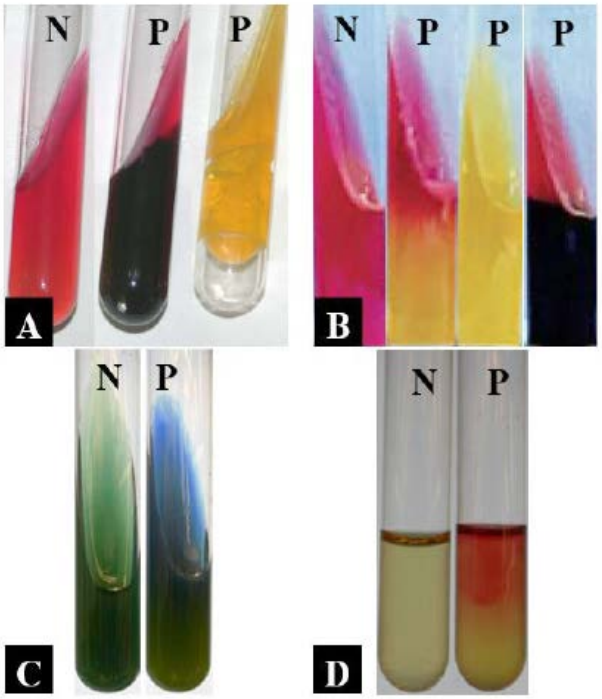

Figure 1: Biochemical positive tests for the isolated *Salmonella*. A. Triple sugar iron agar (N, negative tube; P, positive with the blackening, and P, positive with the fermentation action with gas production only). B, Lysine iron agar (N, negative tube; P, positive tube with the partial fermentation action, positive tube with the complete fermentation action, and P, positive tube with the blackening. C, Simmons citrate (N, negative tube and P, positive tube with the development of the blue color), D, Methyl red (N, negative tube and P, positive tube with the development of the red color).

Serological identification

The serological identification using agglutination technique was carried out to all the positive samples from the traditional culturing technique (35.7%; 45 positives out of 126 samples) and revealed a majority of *Salmonella typhi* O by 84% and *Salmonella typhi* H by 16%.

Antibiotic sensitivity

The antibiotic sensitivity diffusion test against the isolated *Salmonella* culture revealed in Table 4 higher

sensitivity incidence up to 100% to Amikacin (AK, 30 µg), Meropenem (MEM, 10 µg), and Gentamicin (GN, 10 µg) followed by lower sensitivity incidence up to 80% in Norfloxacin (NOR, 10 µg) and Trimethoprim-sulfamethoxazole (SXT, 25 µg). The isolated *Salmonella* culture revealed in Table 4 higher resistance incidence up to 100% against Amoxicillin- Clavulanic acid (AMC, 30 µg), Ampicillin (AMP, 10 µg), and Nalidixic acid (NAL, 30 µg) followed by a resistance level up to 90% against Enrofloxacin (ENR, 5 µg) and a resistance level up to 80% against Doxycycline HCL (DO, 30 µg).

Table 4: Incidence of antibacterial resistance in the isolated *Salmonella*.

Antibiotic discs	Sensitivity
Amoxicillin- Clavulanic acid (AMC, 30 µg)	R (100%)
Ampicillin (AMP, 10 µg)	R (100%)
Amikacin (AK, 30 µg)	S (100%)
Doxycycline HCL (DO, 30 µg)	R (80%)
Meropenem (MEM, 10 µg)	S (100%)
Gentamicin (GN, 10 µg)	S (100%)
Norfloxacin (NOR, 10 µg)	S (80%)
Trimethoprim-sulfamethoxazole (SXT, 25 µg)	S (80%)
Nalidixic acid (NAL, 30 µg)	R (100%)
Enrofloxacin (ENR, 5 µg)	R (90%)

Molecular identification

The molecular analysis was carried out on a representative sample size from the total collected samples (including the positive and negative samples from the culturing technique). The gel electrophoresis as shown in Figure 2 revealed about 80% positivity from the samples used in the traditional cultures. The positive bands were revealed at 284 bp compared to the ladder, positive, and negative control.

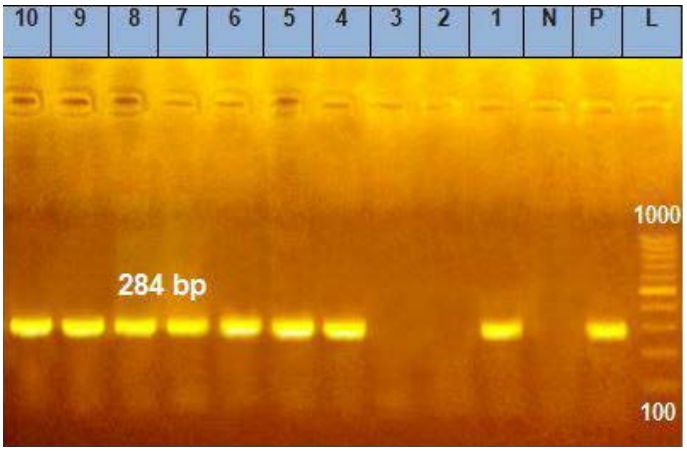

Figure 2: Gel electrophoresis UV images showing representative samples with positive bands (1 and 4: 10) and negative samples (2 and 3) compared to the ladder, positive, and negative controls.

Sensitivity and specificity

The sensitivity (%) calculations revealed in Table 5 a total of 83% sensitivity (9 false positives) for the traditional culture method and 100% sensitivity (zero false positives) for the molecular detection of the *Salmonella* in the admitted samples. The specificity (%) revealed in Table 5 a total of 90% specificity (9 false negatives) for the traditional culture method and 100% specificity (zero false negatives) for the molecular detection of the *Salmonella* in the admitted samples.

Table 5: Sensitivity (Sn) and specificity (Sp) of the characterization methods.

Items	Traditional cultures	PCR
Total no. of samples	126	126
No. of positives	45	38
False positives	9	zero
No. of negatives	81	88
False negatives	9	zero
Sensitivity (Sn)	83%	100%
Specificity (Sp)	90%	100%

DISCUSSION

Salmonella is the most dangerous and worldwide foodborne pathogen and contributed to salmonellosis in animals, poultry, and human due to its zoonotic potential (Antunes et al., 2016; Center for Disease Control and Prevention, 2020). *Salmonella* usually can achieve access to humans via consumption of contaminated food causing non-typhoidal salmonellosis with severe gastro-enteritis manifestation (Braden, 2006). *Salmonella* usually gains access to contaminate poultry meat and products from the initial *Salmonella* infection in poultry farms from feed, water, wild birds, recovering birds, asymptomatic birds, rodents, and flies (Le Bouquin et al., 2010).

The current study showed a total prevalence of isolated *Salmonella* up to 35.7%. The different sampling locations like slaughterhouse I and II, chicken farms, I, II, III, IV, and V revealed prevalence up to 15.3, 23.0, 40.0, 35.0, 50.0, 45.0, and 30% respectively. The isolated *Salmonella* was detected by initial isolation procedures (18 positives out of 126 samples; 14.2%) and delayed isolation procedures (27 positives out of 126 samples; 21.42%). The results were compatible with those recorded by Donado-Godoy et al. (2012) who recorded a prevalence of up to 41% of *Salmonella* in broiler farms compared to the 40.5% median prevalence of *Salmonella* estimated worldwide. Rodriguez et al. (2015) and Kloska et al. (2017) also recorded a 17.4% prevalence of *Salmonella* in broiler production lines. They also strengthen that the authorities must consider the surveys on *Salmonella* status in the farms and ensure

the appropriate biosecurity and control measures to be taken in poultry farms. Althaus et al. (2017) and Cota et al. (2019) showed that the poultry meat contamination might arise from the handling during slaughtering or using contaminated equipment and benches. The outcome ensures poor hygienic conditions and bad biosecurity measures.

Elshebrawy et al. (2021) also determined the prevalence of *Salmonella* serovars isolated from duck, pigeon, and quail carcasses in Egypt. They detected up to 62%, 40%, and 46% *Salmonella* in duck, pigeon, and quail carcasses, respectively with an overall prevalence of 49.3% (148/300). The current results also considered the different contamination sources that might arise from poor bio-security practices, depopulation time, litter recycling, inefficient flaming process, dust material (dust-borne infection), the hatchery machines, contaminated parental flocks, flock to flock infection-transmission, other biological reservoirs (pets or pests), and bad sanitation and disinfection practices as documented by Armwood et al. (2019), McWhorter et al. (2019), and Voss-Rech et al. (2019). Mir et al. (2010) examined 480 samples from adult health birds and tissue samples from four governmental farms in Kashmir from September 2007 to April 2008. They recovered *Salmonella* Gallinarum (84.8%), *Salmonella* Enteritidis (9.09%), and *Salmonella* Typhimurium (6.06%).

The serological identification on the positive colonies (35.7%; 45 positives out of 126 samples) revealed a majority of *Salmonella typhi* O by 84% and *Salmonella typhi* H by 16%. The results were in agreement with Rodriguez et al. (2015) who recorded 17.4% *Salmonella* that was serotyped as *Salmonella* paratyphi B with a rate up to 36.17% from the isolated serotypes in broiler production lines. Schneid et al. (2006) also evaluated the usage of an indirect enzyme-linked immune-sorbent assay (ELISA) based on a monoclonal antibody specific for *Salmonella enterica* serovar Enteritidis on 154 chicken meat samples. They revealed that 23% of the samples were contaminated according to the conventional cultures and 26% according to ELISA. They revealed higher sensitivity and specificity (94%) of ELISA compared to the conventional means.

The isolated *Salmonella* culture revealed higher resistance up to 100% against AMC (30 µg), AMP (10 µg), and NAL (30 µg) followed by a resistance level up to 90% against ENR (5 µg), and a resistance level up to 80% against DO (30 µg). The results were synchronized with those reported by Matsui et al. (2021) who recorded an increase in the proportion of *Samonella* Schwarzengrund resistant to kanamycin by a rate of 51.4–89.7%. Uddin et al. (2021) elucidate the molecular mechanisms, genetic relationships, and phenotype correlations of colistin-resistant *Salmonella* and found that the majority of the tested *Salmonella* isolates were found resistant to colistin (92.68%), ciprofloxacin (73.17%), tigecycline (62.20%) and trimethoprim/sulfamethoxazole (60.98%). Al-Ansaria et al. (2021) also reported that *Salmonella* zoonosis and infection presented hazardous effects on consumers and also recorded that the isolated serovars contributed to a high genotypic resistance pattern to antibiotics. That is why they recommended the restrictions of using antibiotics in poultry farms.

Obe et al. (2021) determined the antimicrobial tolerance of 25 *Salmonella* isolates recovered from poultry handling equipment and recorded minimum inhibitory concentration values between 500 and 1,000 parts per million for chlorine or 3 to 25 parts per million for quaternary ammonium compounds. Besides, the recorded isolates were resistant to multiple antibiotics, and 64% exhibited resistance to aminoglycosides and β-lactams. Yu et al. (2021) recorded high antimicrobial resistance of *Salmonella enterica* subsp. enterica serotype Enteritidis against nalidixic acid (97.6%) and ampicillin (74.2%) and they contributed this resistance to the genome structure of the *Salmonella* that arboured single mutations in gyrA, possessed the plasmid-mediated quinolone resistance genes qnrS (0.8%), oqxAB (2.4%), and the blaTEM-1 (67.7%), as well the extended-spectrum beta-lactamase (ESBL) genes blaCTX-M-55 were detected in 2.4% of the strains.

Hyeon et al. (2011) recorded that 18 *Salmonella* strains with *Salmonella* London and *Salmonella* Montevideo predominating in chicken meat revealed high resistance to erythromycin (100%), streptomycin (22.2%), tetracycline, and chloramphenicol (16.7%). Yildirim et al. (2011) detected *Salmonella* in 34% of the examined 200 packaged fresh raw chicken samples between April 2005 and March 2006 and identified ten *Salmonella* serovars including predominating *Salmonella* Typhimurium, Infantis, and Heidelberg. They also recorded high resistance against penicillin (100%), oxacillin (97%), clindamycin (97%), vancomycin (92.6%), erythromycin (89.7%), ampicillin (85.2%), tetracycline (67.6%), streptomycin (61.7%), neomycin (55.8%), and cephalothin (52.9%). They concluded that strict hygienic practices have to be enforced to reduce the high contamination levels. Thai and Yamaguchi (2012) examined 283 samples from retail meat and isolated 118 *Salmonella* isolates including Infantis, Anatum, Rissen, Reading, Emek, Typhimurium, Blockley, London, Newport, Derby, Weltevreden, Albany, and Hadar. They revealed tetracycline (54.2%), sulfonamides (52.5%), streptomycin (41.5%), trimethoprim (36.4%), chloramphenicol (35.6%), and ampicillin (33.1%).

The current results revealed the positive bands in the gel electrophoresis of the molecular outcome at 284 bp compared to the ladder, positive, and negative control. The

traditional culture method revealed up to 83% sensitivity and 90% specificity while the molecular analysis (targeting the *invA* gene) revealed up to 100% sensitivity and 100% specificity for *Salmonella* detection in the admitted samples. The results were in agreement with those reported by Rodríguez-Hernández et al. (2021) who collected a total of 135 samples from 15 broiler farms (cloacal, feed, water, environmental, and farm operator feces samples) and they carried molecular confirmation of *Salmonella* isolates by amplification of the *invA* gene and they identified the isolates as *Salmonella* paratyphi B. Gand et al. (2019) also validated the molecular confirmation and characterization for 178 *Salmonella* paratyphi B with accuracy up to 100% compared to biochemical testing and 98% compared to the serological identification.

Davanzo et al. (2021) confirmed molecular characterization as a highly sensitive technique for the detection and characterization of *Salmonella* in poultry slaughterhouses. Kagambega et al. (2021) recorded the efficiency of the used technique for determining 111 strains of *Salmonella* isolated from poultry feces in Burkina Faso using a multiplex assay for rapid typing (SMART) polymerase chain reaction (PCR).

Hyeon et al. (2011) identified 18 *Salmonella* strains as *Salmonella* London and *Salmonella* Montevideo predominating in chicken meat in a study on the prevalence of *Salmonella* in chicken, beef, and pork meat from wholesale markets, retail stores, and traditional markets in Seoul, South Korea, in 2009 using rep-PCR except in *Salmonella* London and Montevideo. Abd El-Aziz (2013) examined 100 retail raw meat and giblets samples in Assiut governorate-Egypt and recorded *Salmonella Typhimurium* at a rate of 44%, 40%, and 48% in chicken meat, liver, and heart, respectively using duplex PCR amplification of DNA using *rfbJ* and *fliC* genes. Schneid et al. (2006) recorded nearly similar results when compared to the traditional cultures to the serological detection of *Salmonella enterica* serovar Enteritidis on 154 chicken meat samples, they revealed higher sensitivity and specificity (94%) of ELISA compared to the conventional means.

CONCLUSION AND RECOMMENDATIONS

A high prevalence of up to 35.7% (14.2% from the initial enrichment and 21.5% from the delayed procedures) of the isolated *Salmonella* was detected in the study period among the five broiler farms and the two slaughterhouses. The isolated *Salmonella* revealed high resistance up to 80: 100% against many of the tested antibiotics (AMC 30 µg, AMP 10 µg, and NAL 30 µg, ENR 5 µg, DO 30 µg). The conventional culture method revealed up to 83% sensitivity and 90% specificity while the molecular conformation revealed up to 100% sensitivity and specificity.

The recorded high resistance of the isolated *Salmonella* reflects a serious problem attributed to the extensive use of the antibiotic. Strategies should be enforced for applying strict hygienic and biosecurity measures in poultry farms and reducing the usage of chemical antibiotics in the poultry farms, as well the use of alternatives like *Nigella sativa* Linn, clay, probiotics, synbiotics, phytobiotics, magnetic water, Tilapia bone, modified egg-shell, activated wheat/rice straw, and *Eichhornia Crassipes* that produce strong antimicrobial actions and enhance the immunity levels in poultry.

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NOVELTY STATEMENT

The study resides the prevalence rates and antimicrobial resistance of Salmonella isolated from chicken farms and slaughterhouses. As well, the study reported a judgment on the hygienic and biosecurity measures installed in the farms and slaughterhouses understudy based on the detected prevalence of *Salmonella*.

AUTHOR'S CONTRIBUTION

ESS designed the experimental design, participated in the execution of the bacteriological analysis experiment, and took part in the writing of the manuscript. MHG collected the chicken samples, participated in the execution of the bacteriological analysis experiment, and took a part in the writing of the manuscript. HME supervised the laboratory study and took a part in the writing of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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