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Characterization of Multi Drug-Resistant *Salmonella* Species in Duck Production Chains from Nile Delta Regions

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Abstract

THIS STUDY provided up-to-date information regarding the circulation of virulent and multidrug- resistant *Salmonella* spp. throughout duck production chains in Egypt. The investigation encompassed 338 samples from both household and commercial duck farms, including cloacal swabs (n = 150), duck organs (n = 60), egg surface swabs (n = 10), egg yolks (n = 10), feed and water samples (n = 30 each), and duck breeder stool specimens (n = 48). Conventional methods were employed to screen all samples for *Salmonella* species. Identified *Salmonella* isolates underwent species-specific PCR and testing for virulence genes (*inv*A and *stn*) and some selected antimicrobial resistance genes (*tet*A, *sul*1, *aad*A1, *qnr*A, and *aac*-6-Ib). Based on the *inv*A gene, *Salmonella* species were isolated at a rate of 14.79% (50/338), with 82% (41/50) of these isolates testing positive for the *stn* gene. The most frequently detected resistance genes were *aad*A1 (28/50), followed by *qnr*A (18/50), *sul*1 (17/50), *aac*-6-Ib (14/50), and *tet*A (13/50). Furthermore, the predominant *Salmonella* serotypes emerged were *S.* Enteritidis, *S.* Typhimurium, and *S.* Kentucky. A significant proportion of *Salmonella* isolates displayed antimicrobial resistance to ceftazidime (98%) and ampicillin (96%). These findings indicate widespread multidrug-resistant *Salmonella* spp. throughout Egyptian duck production chains, potentially posing a significant public health risk due to possible transmission to humans via the food supply.

Keywords: *Inv*A, Enteric pathogens, Eggs, MDR, ducks, Egypt.

Introduction

Salmonella is considered as one of the major pathogens posing threat to public health in most countries [1]. Based on the World Health Organization (WHO) reports, it was estimated that nearly 715,000 population died from diarrheal diseases annually. One-third of these deaths are related to food poisoning, with *Salmonella* being a major contributing factor [2]. In addition in USA consumption of contaminated food such as poultry, pork, beef, eggs, and milk with pathogenic *Salmonella* species leads to 1.35 million infections, 26,500 hospitalizations, and 420 deaths have been reported every year [3]. *Salmonella* is one of the world's most serious zoonotic foodborne enteric pathogen, causing salmonellosis, one of the most common illnesses that lead to significant economic losses in poultry industry. Poultry was the primary cause of numerous Salmonellosis outbreaks in developing countries like India, Egypt, and Zimbabwe [4]. *S.* Typhimurium and *S.* Enteritidis account for 75% of *Salmonella* infections [5]. In Egypt, ducks rank as the second most widely consumed poultry species, after chicken [6]. Despite their widespread distribution, economic significance, ability to support livelihoods and being significant reservoirs and carriers of different zoonotic pathogens including *Salmonella*, which typically presents with subclinical symptoms or silent infections, they have not yet received the interest of scientists and are rarely investigated for *Salmonella* contamination. Consequently, the potential for

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Salmonella transmission from ducks to humans may exceed that from chickens [7]. Globally distinct *Salmonella* serovars have been found in ducks with *S.* Typhimurium and *S.* Enteritidis being the most prevalent serovars [6]. *Salmonella* pathogenicity is associated with several virulence genes, including the *inv*A gene, that allows bacteria to colonize host epithelial cells and is acts as a distinctive marker suitable for identification of *Salmonella strains* [8], while the enterotoxin, *stn* gene generates the protein which responsible for severe diarrhea [6]. Antimicrobial-resistant bacteria like *Salmonella* have emerged as a result of widespread misuse and abuse of antimicrobial agent in animals raised for food, which poses severe worldwide health concerns [9]. In 2019, approximately 1.27 million deaths were linked to infections caused by antimicrobial-resistant this figure could rise to 10 million annually by the year 2050, surpassing the mortality rate due to cancer [10]. Food of animal origin, especially poultry meat may be maintain and disseminate multidrug resistance strains associated with different virulence genes [11]. Despite salmonellosis being the most common zoonotic food-borne infection in Egypt, information about their occurrence, population structure and genomic characteristics across ducks production chain is limited. Thus, this research aimed to evaluate the potential existence of zoonotic *Salmonella enterica* serovars along duck production chain, considering some epidemiological patterns.

Material and methods

Samples collection and preparation:

In the present study, 338 samples collected from duck farms, including cloacal swabs (n = 150), duck organs (n = 60), egg surface swabs (n = 10), egg yolks (n = 10), feed and water samples (n = 30 each), and duck breeder fecal samples (n = 48). The investigation encompassed different duck farms located in the northern Nile Delta including Dakahlia and Damietta governorate, during the period from May 2023 to June 2024**.** The collected samples kept cooled and transferred immediately to the lab.

Ethical approval:

The Animal care and Use committee (ACUC) guidelines were followed for conducting this study (code number, VM.MS.22.09.7).

On duck farms, cloacal swabs were collected aseptically from seemingly healthy ducks according to the method described previously [12]. In brief, cloaca's exterior was initially cleaned using a cotton ball that had been soaked in 70% ethanol. Subsequently, the swabs were gently inserted 22 mm into the cloaca where they rotated five times in a slow clockwise motion around the cloaca, applying moderate pressure to keep the swab-tip in touch. Additionally, the swab-tip was rolled to cover the entire surface of the swab with cloacal material. Finally, the collected swabs placed into sterile tube containing 10 ml buffered peptone water (BPW) and kept in ice box.

Samples of drinking water and duck feed were pooled per sampling site. In brief, samples of duck feed (10 gm each) and water (20 ml each) were collected from each duck house from five different locations and pooled resulting in a composite sample of 100 milliliters. The internal organs (i.e. liver, cecum, and heart) of ducks were collected in sterile cups under aseptic conditions [13]. A total of 10 ducks' eggs were purchased from ducks' farms and farmers' houses. To isolate *Salmonella* from the surface of egg shell, sterile cotton swabs were first moistened with sterile normal saline solution (NSS) and then used to swab egg surface after that the swabs were placed back into a tube containing 10 ml normal saline solution. The contents were transferred to 90 ml of BPW (Oxoid, Basingstoke, UK) and incubated at 37 ^{0}C for 18h [14].

Five milliliters of yolk were combined with 5 milliliters of normal saline solution and then added to 90 milliliters of buffered peptone water, followed by incubation at 37 degrees Celsius for 18 hours [14]. Finally, a total of 48 stool specimens were collected from farm owners, workers and household breeders into sterile cups and transferred to laboratory. All the collected samples were coded and packaged immediately under cooling condition and transported directly to the laboratory for further analyses.

Isolation and identification of Salmonella Spp.

Isolation and identification of *Salmonella* were carried out following the standard procedure outlined in ISO-6579 [15]. All suspected colonies on XLD were selected and cultured onto (XLD) agar and incubated at 37 ◦C for 24 h to for further biochemical identification using triple sugar iron (TSI), urease test and simmon citrate test

Serological characterization of Salmonella strains

Salmonella strains were serotyped by slide agglutination test according to Kauffmann–White scheme [17]. The serology was conducted at Food Analysis Center, Benha University.

Molecular diagnostic assay

All the biochemically identified *Salmonella* strains (n=98) underwent DNA extraction utilizing the Gene JET Genomic DNA Purification Kit (Fermentas), following the guidelines provided by the manufacturer.

Conventional PCR assay

PCR reactions were conducted in a total volume of 25 µl, which included 1 µl of each primer (10 pmol), primers used in the study were listed in Table (1), 10 µl of DreamTaq™ Green PCR Master Mix (2X) from Thermo Scientific, USA, 5 µl of template DNA, and 8 µl of nuclease-free water. The cycling conditions were performed in a Mini PCR TM Mini16 Thermal Cycler (Amplyus, Cambridge, MA, USA) for the *inv*A (invasion) gene, starting with an initial denaturation at 94ºC for 3 minutes, followed by 35 cycles consisting of denaturation at 94ºC for 30 seconds, primer annealing at 62ºC for 30 seconds, extension at 72ºC for 30 seconds, and a final extension at 72ºC for 10 minutes. For the *stn* (enterotoxin) gene, the PCR cycle began with an initial denaturation at 94 °C for 5 minutes, followed by 30 amplification cycles with denaturation at 94 °C for 5 seconds, annealing at 68 °C for 10 seconds, and extension at 72 °C for 20 seconds, concluding with a final extension of 7 minutes at 72 °C. The amplified DNA was visualized using 1% agarose gel electrophoresis with a blue gel detection and visualization unit from Amplyus, Cambridge, MA, US [19].

For identification of resistance genes, PCR amplification and primer sequences for *tet*A (tetracycline resistance gene), *sul*1(sulfonamide resistance gene), and *aad*A1(aminoglycosides resistance gene) genes were performed as previously reported by Zishiri et al. [20] while fo*r qnr*A (quinolones resistance gene*)* and *aac-6-ib* (aminoglycosides resistance gene) were done according to Herrera-Sánchez et al [21].

Antimicrobial susceptibility testing

Antibiograms were performed to molecularly confirmed *Salmonella* strains (n=50) according to Clinical and Laboratory Standards Institute guidelines using disk diffusion [24] using different antibiotic classes as Aminoglycosides (Gentamycin CN—120 ug; Streptomycin S—25 ug; Kanamycin K— 30 ug), Quinolones (Nalidixic acid NA—30ug), Polymyxins (CL—Colistin 25 ug), Phenicoles (Chloramphenicol C—30 ug), Cephalosporin (CAZ—Ceftazidime 30 ug), Beta lactam (Ampicillin AMP—10 ug), Fluoroquinolones (CIP—Ciprofloxacin 30 ug), Tetracyclines (Tetracycline TE—30 ug). The Multiple Antibiotic Resistance (MAR) index for each *Salmonella* strain was calculated using this formula: MAR = Number of resistant antibiotics / Total number of antibiotics tested.

Statistical analysis

The analysis of virulence and antimicrobial resistance genes in *Salmonella* strains isolated from different sources was conducted using logistic regression and SPSS software (SPSS Inc., Chicago, IL; version 22). A p-value of less than 0.05 was deemed statistically significant.

Results

In the present study, 98 *Salmonella* spp. out of 338 (28.9%) were biochemically identified in duck cloacal swabs (44/150; 29.3%), liver (2/20; 10%), cecum (2/20; 10%), heart (1/20; 5%), egg surface swab (9/10; 90%), eggs yolk (3/10; 30%), feed (3/30; 10%), water (22/30; 73.3%) and stool specimen (12/48; 25%); while they were molecularly confirmed using *Salmonella* gene marker *inv*A in 50 samples out of 338 (14.8%) with the percentage of 18% (27/150) in duck cloacal swabs; 10% (2/20) in liver; 10% (2/20) in cecum, 0% (0/20) in heart; 50% (5/10) in egg surface swab; 0% (0/10) in eggs yolk; 26.6% (8/30) in feed; 0% (0/30) in water; and 12.5% (6/48) in stool specimens (Table 2; Fig.1). The molecularly confirmed *Salmonella* strains (*n* = 50) using *inv*A gene, were tested for *stn* gene, the results have shown that the gene was detected in 82% (41/50**)** of *Salmonella* isolates (Fig. 2).For antimicrobial resistance genes, the *aad*A1 (Aminoglycoside adenylyltransferase) gene was the most abundant gene identified (28/50; 56%). The gene was identified frequently in human stool specimens (5/6; 83.3%) and in water samples (6/8; 75%); while *tet*A gene was the least identified gene (13/50; 26%**)** as shown in (Fig. 3**).** Furthermore, twelve serovars were characterized and the most prevalent serotypes identified were S. Enteritidis (10/50; 20%)*, S.* Typhimurium (8/50; 16%) and *S.* Kentucky (8/50; 16%) (Fig.4). for antimicrobial susceptibility testing, the data demonstrated that *Salmonella* strains exhibited MDR in 92% (46/50). The isolates were highly resistant to ceftazidime and ampicillin with the percent of 98 (49/50) and 96 (48/50) respectively, while they showed moderate resistance to tetracycline (68%), nalidixic acid (52%)

and streptomycin (50%). Nonetheless, a low resistance was reported to chloramphenicol (18%) and gentamycin (16%) (Table 3; Fig. 5).

The classification of *Salmonella* isolates according to their antibiotic resistance profiles and MAR index showed that among the 50 isolates there were 35 distinct resistance patterns identified. Notably, 46 of the 50 *Salmonella* isolates, accounting for 92%, exhibited resistance to at least three antibiotics (Table 4). Additionally, the majority of the *Salmonella* serovars displayed multidrug resistance. (Table 5). Among the identified 50 *Salmonella* isolates ten were serotyped as *S.* Enteritidis theses isolates were recovered from ducks cloacal swabs, water, cecum, egg samples as well as human stool and harbored *aad*A1, *qnr*A also 8/10 with the percentage of 80% of the isolates had both *inv*A and *stn* genes (Table 6).

For statistical analysis, no significance difference was observed for the prevalence of *tet*A, *sul*1, *aad*A1 and *aac*-6-Ib genes among the human, duck and environmental isolates. Nonetheless, there is a significant difference ($p<0.05$) was noticed for *qnr*A prevalence among the isolates from different identified sources. Interestingly marked variations ($p<0.001$) for *inv*A and *stn* genes prevalence among the different isolates from human, duck and environmental samples.

Discussion

Ducks are considered the most significant reservoirs of *Salmonella* spp. in poultry production systems. Chronic carriers can transmit bacteria to humans. To date, only few studies have evaluated the prevalence of *Salmonella* spp. in duck production systems. Hence, this research was performed to clarify whether virulent and multidrug resistant *Salmonella* species are present throughout duck production chains in Egypt. Our results highlight the potential health concerns of foodborne *Salmonella* infections by demonstrating the significant incidence of MDR strains. The frequency of *Salmonella* spp. detected in cloacal swabs in this study aligns with findings by Adzitey et al. [25], who reported a 20% (15/75) occurrence rate. However, other researchers have observed varying detection rates. For instance, Saengthongpinit et al. [26] in Thailand and Seleem et al. [27] in Egypt found rates of 4.2% (27/639) and 1.5% (3/197), respectively. In China, researchers noted *Salmonella* in cloacal swabs ranging from 4.29% (3/70) to 44.00% (22/50) [28]. The current study identified an overall *Salmonella* spp. occurrence of 6.66% (4/60) in duck organs. This rate exceeds that reported in an Egyptian study which found *Salmonella* spp. in 3.33% (5/150) of samples [29]. Additionally, research conducted in Iran identified *Salmonella* in 10% (2/20) of duck livers [30]. In contrast, other Egyptian studies reported higher prevalence rates of *Salmonella* in duck organs, with 14.5% (29/200) and 12.36% (11/89) respectively [31,32]. This study found a 50% (5/10) prevalence of *Salmonella* spp. in eggs (shell and content), which exceeded that reported in another study. For comparison, Egyptian studies reported 9.09% (1/11) and 10% (5/50) in duck eggs [32,31], while an Iranian study 16.67% (5/30) was reported in egg content and 0% in eggshells [30]. The hatchery stage (egg samples) had the greatest isolation rate of *Salmonella* (50%) indicating a potential concern to the downstream of the duck production chain [2].

The current investigation detected no *Salmonella* in environmental feed samples, aligning with Nigerian research that found a 0% recovery rate in Edo and Delta states [33]. However, studies from Thailand and Egypt reported *Salmonella* prevalence in poultry feed as 13.3% (2/15) and 8.3% (1/12), respectively [26, 34]. *Salmonella* spp. prevalence in water samples was 26.6%, higher than the 0% reported in Thailand [26], but lower than the 47.1% (8/17) in free-grazing flocks and 33% (5/15) in confined flocks reported in Egypt [34].

The overall *Salmonella* species percentage in stool specimens was 12.5%, consistent with a study that isolated *Salmonella enterica* at 12.07% (7/58) [35]. In contrast, Egyptian studies reported lower detection rates of 4.4% (3/68) and 4% (1/25) [36, 37]. Variations in *Salmonella* prevalence across samples and studies may be attributed to factors as hygienic practices conditions, biosecurity levels, management systems, duck housing arrangements, geographic location, and seasonal influences [38].

Traditional methods identified 98 *Salmonella* isolates out of 338 samples (28.9%), with 51% confirmed as *Salmonella* spp. using the *inv*A gene marker. These results differed from studies in Egypt [32] and China [39], which confirmed *inv*A gene presence in all recovered *Salmonella* serovars. The heat-labile *Salmonella* enterotoxin gene (*stn*), involved in salmonellosis pathogenesis, was detected in 82% (41/50) of examined strains. This contrasts with Egyptian studies reporting 100% *stn* gene detection in *Salmonella* serovars from duckling and duck farms [40, 29], while another Egyptian study

found *stn* gene in 65.8% out the identified strains [6]. The high detection rate of virulence genes underscores these isolates' pathogenic potential and potential public health risks. Variations can arise due to the sensitivity and specificity of the primers, the concentration of the inoculum, the capabilities of the laboratory, and the skills of the individuals involved [41].

The study identified multiple *Salmonella* serotypes, with *S.* Enteritidis, *S.* Typhimurium, and *S.* Kentucky being the most prevalent at 20%, 16%, and 16%, respectively. More than 70% of human infections are caused by nontyphoid *Salmonella* serovars, particularly *S.* Enteritidis and *S.* Typhimurium which are the main causes of salmonellosis epidemics, according earlier research. [42]. These findings align with previous study [36] that identified *S.* Enteritidis and *S.* Typhimurium as the dominant serotypes in human and animal isolates in Egypt. Another Egyptian study [6] detected *S.* Typhimurium, *S.* Kentucky, and *S.* Enteritidis in ducks with prevalence of 31.4% (22/70), 22.9% (16/70), and 20% (14/70), respectively. Additional research reported *S.* Typhimurium and *S.* Kentucky as the most common serotypes isolated from ducks, with incidences of 31.3% (5/16) and 25% (4/16), respectively [29]. In Southeast Asia, *S.* Typhimurium and *S.* Enteritidis were found to be the main serotypes in duck samples from Malaysia, with rates of 29.6% (37/125) and 12% (15/125), respectively [25]. *S.* Kentucky is commonly found in poultry, such as chickens and turkeys, while it is less frequently seen in ducks [2]. In the current investigation, *S.* Kentucky was found more frequently in ducks (cloacal swabs, liver, and eggs), as well as in environmental samples. This suggests that *S.* Kentucky need more study attention because it has become a global human pathogen.

The emergence of antimicrobial resistance in *Salmonella* serovars represents a major public health issue, as food-producing animals often serve as reservoir hosts for *Salmonella*, making eradication challenging. In this study, the two predominant zoonotic serotypes identified were *S.* Enteritidis and *S.* Typhimurium. These serovars were MDR and expressed both virulent and antibiotic resistance genes. They were isolated from human, duck, and environmental samples, highlighting the risk of spreading these serovars through food of duck-origin. Strict monitoring is desperately needed to prevent the spread of these strains.

Excessive use of antibiotics in both humans and animals contributes to the rise of antimicrobial resistance [43]. In veterinary field, antibiotics are widely employed for purposes such as promoting growth, treating illnesses, and enhancing weight gain and feed efficiency. The issue of multidrug resistance is becoming an increasingly serious public health challenge globally. Recent research highlighting the proliferation of multidrug-resistant pathogens from diverse sources underscores the urgent need to optimize antibiotic use in both human and veterinary healthcare [44].

In this study, 92% of *Salmonella* strains were classified as MDR. This percentage is higher than the 68.4%reported in previous study [45]. The *Salmonella* isolates exhibited high resistance to Ceftazidime (49/50, 98%) and Ampicillin (48/50, 96%). These results are consistent with findings from researchers in Morocco [46]. Similarly, studies in China reported high resistance to AMP at 55.1% (27/49) and 97.6% (82/84), respectively [39,13]. Fluoroquinolones, which are very efficient broad spectrum antibiotics to treat human salmonellosis, have a resistance rate of 34% (17/50), which is consistent with other research in Egypt by El-Saeed et al. [43]who report high resistance to ciprofloxacin (63/129) 48.8%. In contrast, this study found low resistance to Chloramphenicol (9/50, 18%) and Gentamycin (8/50, 16%), which aligns with other research [47] reporting low resistance to Gentamycin (1/26, 3.80%). Elshebrawy et al. [6] also reported a resistance rate of 21.5% (34/158) against Chloramphenicol. The current study found that *S.* Enteritidis and *S.* Typhimurium with MDR character were predominate in cloacal swabs and stool specimens, consistent with previous findings [41]. *Salmonella* isolates in our study have demonstrate high levels of resistance to the most therapeutically significant groups of antibiotics, including tetracycline, aminoglycosides, betalactams, cephalosporins, and quinolones. Suggests that these antibiotics are frequently used as growth promoters or therapeutics. This could lead to food-borne AMR *Salmonella* infections in human, potentially creating a significant challenge in treating *Salmonella* infections in both humans and animals in Egypt.

The investigation into antibiotic resistance gene prevalence revealed that the *aad*A1 gene, responsible for aminoglycosides resistance, was the most common, occurring in 56% (28/50) of cases, with stool samples showing the highest incidence at 83.3% (5/6). These outcomes are consistent with

earlier studies [48, 49, 50]. The *aac*-6-Ib gene was identified in 28% (14/50) of samples, and the *tet*A gene in 26% (13/50), although some researchers were unable to detect *tet*A in their *Salmonella* collections [51]. A study by Chen et al. [49] found that 25.9% (21/81) of isolates expressed the *aac*-6-Ib gene. The transmission of resistance genes to consumers via the production chain underscores the need for effective *Salmonella* control measures. Additionally, the detected MDR *Salmonella enterica* serovars represent a potential health hazard to humans. As a result, it is essential to implement strict regulations on antimicrobial agent usage in poultry farming [41].

Conclusion

The study indicates that ducks may serve as a potential zoonotic source for salmonellosis, particularly due to the high prevalence of *Salmonella* found in duck cloacal swabs and environmental samples, as well as in humans. This is especially concerning for ducks carrying multidrug-resistant (MDR) genes. These results underscore the significance of *Salmonella* and shed light on antibiotic usage practices in both human and animal healthcare sectors.

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Conflict of interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of the faculty of veterinary medicine, Mansoura University, Egypt **(code number, VM.MS.22.09.7).**

Author contributions

H.El M: Formal analysis, methodology, writing–original draft. Sh. El B: Conceptualization, data curation, revising original draft. H.R: Conceptualization, methodology, revising original draft. M.G: Conceptualization, methodology, data curation, Writing–review and editing the final version. All the authors approve the final version.

TABLE 1. Primer sequences of virulence and antibiotic resistance genes for *Salmonella* spp.

Target genes	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
*inv*A(F)	**5′** GTG AAA TTA TCG CCA CGT TCG GGC AA**′3**	285	**[18]**
*inv*A(R)	**5′** TCA TCG CAC CGT CAA AGG AAC C**′3**		
stn (F)	5′-CTTAATCGCGCCGCCATGCTGTT-3′	480	**[19]**
stn (R)	5′-CATGAACTGGCGCAGGTGAT-3′		
*tet*A(F)	**5′** GCTACATCCTGCTTGCCTTC **′3**	201	**[22]**
*tet*A(R)	**5′** CATAGATCGCCGTGAAGAGG**′3**		
*sul*1(F)	**5′**TCACCGAGGACTCCTTCTTC**′3**	316	**[23]**
*sul*1(R)	**5′**AATATCGGGATAGAGCGCAG**′3**		
*aad*A1(F)	**5′**TAT CAG AGG TAG TTG GCG TCAT**′3**	484	**[23]**
*aad*A1(R)	**5′**GTT CCA TAG CGT TAA GGT TTC ATT**′3**		
*qnr*A(F)	**5′** CCGCTTTTATCAGTGTGACT **′3**	188	**[21]**
*qnr*A(F)	**5′** ACTCTATGCCAAAGCAGTTG **′3**		
aac-6-Ib(F)	**5′** TTGCGATGCTCTATGAGTGGCTA **′3**	482	**[21]**
aac-6-Ib(F)	**5′** CTCGAATGCCTGGCGTGTTT**′3**		

TABLE 2. Frequent distribution of *Salmonella* serovars from the examined samples based on biochemical and molecular identification

Samples type	NO. of examined		Number of biochemically of confirmed	PCR results	Serotypes
Duck samples (n=170)	Cloacal swabs	150	44(29.3%)	27(18%)	*S.* Enteritidis (n=5, 18.5%), *S.* Kentucky (n=5, 18.5%), *S.* Typhimurium (n=4, 14.8%), *S.* Wingrove (n= 3, 11.1%), *S*. Muenster (n=2, 7.4%), *S.* Larochelle (n=2, 7.4%), *S.* Molade(n=2, 7.4%), *S.* Papuana (n=2, 7.4%), *S.* Inganda(n=1, 3.7%), *S.* Saintpaul (n=1, 3.7%)
	Liver	20	2(10%)	2(10%)	*S*. Kentucky (n=1, 50%), *S*. Typhimurium (n=1, 50%)
	Cecum	20	2(10%)	2(10%)	*S*. Enteritidis (n=1, 50%), *S*. Papuana (n=1, 50%)
	Heart	20	1(5%)	0(0%)	
Duck eggs (n=10)	Egg yolk samples	10	9(90%)	5(50%)	*S.* Wingrove (n=1, 20%), *S.* Inganda (n=1, 20%), *S*. Molade (n=1, 20%), *S*. Kentucky (n=1, 20%), *S*. Enteritidis (n=1, 20%)
	Egg surface swab	10	3(30%)	0(0%)	
Environmental samples	Feed	30	3(30%)	0(0%)	
	Water	30	22(73.3%)	8(26.6%)	*S*. Enteritidis (n=2, 25%), *S*. Kentucky (n=1, 12.5%) *S*. Heidlberg (n=1, 12.5%), *S*. Paratyphi A (n=1, 12.5%), *S*. Typhimurium (n=1, 12.5%), *S*. Larochelle (n=1, 12.5%), *S.* Saintpaul (n=1, 12.5%)
Human samples	Stool	48	12(25%)	6(12.5%)	*S*. Typhimurium (n=2, 33.3%), *S*. Enteritidis (n=1, 16.6%), *S*. Heidlberg (n=1, 16.6%), *S*. Paratyphi A (n=1, 16.6%), *S*. Saintpaul (n=1, 16.6%)
Total		**338**	**98 (28.9%)**	**50 (14.8%)**	

TABLE 3. Antimicrobial susceptibility of confirmed *Salmonella* strains (n=50) recovered from different sources

Antibiotics classes		Sensitive		Resistant	
		NO	%	NO	%
Quinolones	Nalidixic acid	24	48	26	52
Polymyxins	Colistin	36	72	14	28
Aminoglycosides	Gentamycin	42	84	8	16
	Streptomycin	25	50	25	50
	Kanamycin	28	56	22	44
Phenicoles	Chloramphenicol	41	82	9	18
Cephalosporin	Ceftazidime	1	2	49	98
Beta lactam	Ampicillin	2	4	48	96
Fluoroquinolones	Ciprofloxacin	33	66	17	34
Tetracyclines	Tetracycline	12	24	38	76

TABLE 4. Antimicrobial resistance profile and MAR indexes of *Salmonella* serovars isolates (n = 50) from ducks, environment and human sources.

Antimicrobial resistance patterns	Number and (%)of isolates	MAR index	Resistance profile	Number and (%)for each profile
AMP,TE,CAZ,K,NA,CL,CN,S,CIP	1(2%)	0.9	Multidrug-resistant	46(92%)
AMP, TE, CAZ, K, NA, CL, S,CIP	2(4%)	0.8		
AMP, TE, CAZ, K, NA, S, C	1(2%)	0.7		
AMP, TE, CAZ ,K, S, CIP, C	1(2%)	0.7		
AMP, TE, CAZ, K, CN, S, CIP	1(2%)	0.7		
AMP, TE, CAZ, K, NA, CL, CN	1(2%)	0.7		
AMP, TE, CAZ, NA, CL, S, CIP	1(2%)	0.7		
AMP, TE, CAZ, NA, CL, CN, S	1(2%)	0.7		
AMP, TE, CAZ, K, NA, CL	2(4%)	0.6		
AMP, TE, CAZ, NA, S, CIP	2(4%)	0.6		
AMP, TE, CAZ, CL, S, C	1(2%)	0.6		
AMP, TE, CAZ, K, S, C	1(2%)	0.6		
AMP, TE, CAZ, K, NA, CIP	2(4%)	0.6		
AMP, TE, CAZ, NA, CL, S	1(2%)	0.6		
AMP, CAZ, K, NA, CL, CN	1(2%)	0.6		
AMP, CAZ, K, NA, CL, CIP	1(2%)	0.6		
AMP, TE, CAZ, S, C	4(8%)	0.5		
AMP, TE, CAZ, K, NA	3(6%)	0.5		
AMP, TE, CAZ, K, C	1(2%)	0.5		
AMP, TE, CAZ, K, CL	1(2%)	0.5		
AMP, TE, CAZ, NA, S	3(6%)	0.5		
AMP, TE, CAZ, S, CIP	2(4%)	0.5		
AMP, CAZ, K, NA, CIP	1(2%)	0.5		
AMP, CAZ, K, NA, CL	1(2%)	0.5		
AMP, TE, CAZ,CN	1(2%)	0.4		
AMP, TE, CAZ, S	1(2%)	0.4		
AMP, CAZ, K,NA	1(2%)	0.4		
AMP, CAZ, S, CIP	1(2%)	0.4		
AMP, TE, CAZ	3(6%)	0.3		
AMP, TE, NA	1(2%)	0.3		
AMP, CAZ, CN	1(2%)	0.3		
AMP, CAZ, CIP	1(2%)	0.3		
AMP, CAZ	2(4%)	0.2	Low drug-resistant	4(8%)
CAZ, CN	1(2%)	0.2		
CAZ	1(2%)	0.1		

TABLE 5. Classification of *Salmonella enterica* serovars isolates (n = 50) according to their antimicrobial resistance profile against the 10 antimicrobial agents.

Serovars	Number of isolates	Antimicrobial resistance pattern	Antimicrobial resistance classes	MAR Index	Classification of Strains	
					Type of resistance	No. and %
Salmonella enterica subsp. *enterica* serovar Enteritidis (n=10)	1	AMP, TE, CAZ, CL, S, C	Beta lactam, Tetracyclines, Cephalosporin, Polymyxins, Phenicoles, Aminoglycosides Phenicoles	0.6	Multidrug -resistant	9(90%)
	1	AMP, TE, CAZ, K, NA, CIP	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Quinolones, Fluoroquinolones	0.6		
	1	AMP, TE, CAZ,NA, S, CIP	Beta lactam, Tetracyclines, Cephalosporin, Quinolones, Aminoglycosides, Fluoroquinolones	0.6		
	1	AMP, TE, CAZ, S, C	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Phenicoles	0.5		
	1	AMP, CAZ, K, NA, CIP	Beta lactam, Cephalosporin, Aminoglycosides, Quinolones, Fluoroquinolones	0.5		
	1	AMP, TE, CAZ, S	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides	0.4		
	1	AMP, CAZ, S, CIP	Beta lactam, Cephalosporin, Aminoglycosides, Fluoroquinolones	0.4		
	1	AMP, CAZ, K, NA	Beta lactam, Cephalosporin, Aminoglycosides, Quinolones	0.4		
	1	AMP, TE, CAZ	Beta lactam, Tetracyclines, Cephalosporin	0.3		
Average MAR index=0.44	1	CAZ	Cephalosporin	0.1	Low drug-resistant	1(10%)
Salmonella enterica subsp. enterica serovar Typhimurium (n=8)	1	AMP, TE, CAZ, K, CN, S, CIP	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Fluoroquinolones	0.7	Multidrug -resistant	7(87.5%)
	1	AMP, TE, CAZ, K, NA, CL, CN	Beta lactam, Tetracyclines, Cephalosporin, Quinolones, Polymyxins, Aminoglycosides	0.7		
	1	AMP, TE, CAZ, NA, CL, CN, S	Beta lactam, Tetracyclines, Cephalosporin, Quinolones, Polymyxins, Aminoglycosides	0.7		
	1	AMP, TE, CAZ, S, C	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Phenicoles	0.5		
	1	AMP, TE, CAZ, K, NA	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Quinolones	0.5		
	1	AMP, TE, CAZ, CN	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides	0.4		
Average MAR index=0.5	1	AMP, TE, CAZ	Beta lactam, Tetracyclines, Cephalosporin	0.3		
	1	AMP, CAZ	Beta lactam, Cephalosporin	0.2	Low drug-resistant	1(12.5%)

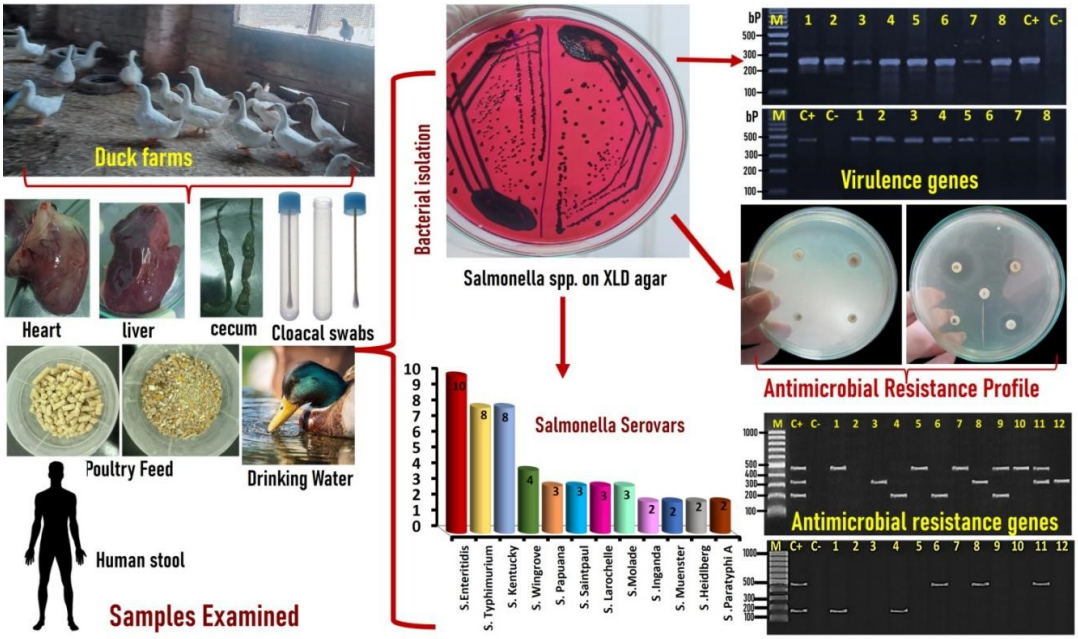
Serovars	Number of isolates	Antimicrobial resistance pattern	Antimicrobial resistance classes	MAR Index	Classification of Strains	
					Type of resistance	No. and %
Salmonella enterica subsp. enterica serovar Kentucky (n=8)	1	AMP, TE, CAZ, K, NA, CL, S, CIP	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Quinolones, Polymyxins, Fluoroquinolones	0.8	Multidrug -resistant	8(100%)
	1	AMP, TE, CAZ, NA, CL, S	Beta lactam, Tetracyclines, Cephalosporin, Quinolones, Polymyxins, Aminoglycosides	0.6		
	1	AMP, TE, CAZ, NA, S	Beta lactam, Tetracyclines, Cephalosporin, Quinolones, Aminoglycosides	0.5		
	1	AMP, TE, CAZ, S, CIP	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Fluoroquinolones	0.5		
	2	AMP, TE, CAZ, K, NA	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Quinolones	0.5		
Average MAR index=0.53	1	AMP, TE, CAZ, K, C	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Phenicoles	0.5		
	1	AMP, CAZ, CN	Beta lactam, Cephalosporin, Aminoglycosides	0.3		
Salmonella enterica subsp. enterica serovar Wingrove (n=4)	1	AMP, TE, CAZ, K, NA, CL, CN, S, CIP	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Quinolones, Polymyxins, Fluoroquinolones	0.9	Multidrug -resistant	4(100%)
	1	AMP, TE, CAZ, K, NA, CIP	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides**,** Quinolones, Fluoroquinolones	0.6		
	1	AMP, TE, CAZ, K, NA, CL	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Quinolones, Polymyxins	0.6		
Average MAR index=0.65	1	AMP, TE, CAZ, S, C	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Phenicoles	0.5		
Salmonella enterica subsp. enterica serovar Papuana (n=3)	1	AMP, TE, CAZ, K, NA, S, C	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Quinolones, Phenicoles	0.7	Multidrug -resistant	3(100%)
	1	AMP, TE, CAZ, K, S, C	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Phenicoles	0.6		
Average MAR index=0.53	1	AMP, TE, CAZ	Beta lactam, Tetracyclines, Cephalosporin	0.3		

Serovars	Number of isolates	Antimicrobial resistance pattern	Antimicrobial resistance classes	MAR Index	Classification of Strains	
					Type of resistance	No. and %
Salmonella enterica subsp. enterica serovar Saintpaul (n=3)	1	AMP, TE, CAZ, K, S, CIP, C	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Fluoroquinolones, Phenicoles	0.7	Multidrug -resistant	2(66.66 %)
	1	AMP, TE, CAZ, S, CIP	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Fluoroquinolones	0.5		
	1	CAZ, CN	Cephalosporin, Aminoglycosides	0.2	Low drug-resistant	1(33.33 %)
Average MAR index=0.46						
Salmonella enterica subsp. enterica serovar Larochelle (n=3)	1	AMP, TE, CAZ, K, NA, CL	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Quinolones, Polymyxins	0.6	Multidrug -resistant	3(100%)
	1	AMP, CAZ, K, NA, CL	Beta lactam, Cephalosporin, Aminoglycosides, Quinolones, Polymyxins	0.5		
	1	AMP, TE, CAZ, S, C	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Phenicoles	0.5		
Average MAR index=0.53						
Salmonella enterica subsp. enterica serovar Molade (n=3)	2	AMP, TE, CAZ, NA, S	Beta lactam, Tetracyclines, Cephalosporin, Quinolones , Aminoglycosides	0.5	Multidrug -resistant	3(100%)
	1	AMP, TE, CAZ, K, CL	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Polymyxins	0.5		
Average MAR index=0.5						
Salmonella enterica subsp. enterica serovar Inganda (n=2)	1	AMP, TE, CAZ, K, NA, CL, S, CIP	Beta lactam, Tetracyclines, Cephalosporin, Aminoglycosides, Quinolones, Polymyxins, Fluoroquinolones	0.8	Multidrug -resistant	2(100%)
	1	AMP, CAZ, K, NA, CL, CN	Beta lactam, Cephalosporin, Aminoglycosides, Quinolones, Polymyxins	0.6		
Average MAR index=0.7						
Salmonella enterica subsp. enterica serovar Muenster (n=2)	1	AMP, TE, CAZ, NA, CL, S, CIP	Beta lactam, Tetracyclines, Cephalosporin, Quinolones, Polymyxins, Aminoglycosides, Fluoroquinolones	0.7	Multidrug -resistant	2(100%)
	1	AMP, CAZ, K, NA, CL, CIP	Beta lactam, Cephalosporin, Aminoglycosides, Quinolones, Polymyxins, Fluoroquinolones	0.6		
Average MAR index=0.65						

Serovars	Number of isolates	Antimicrobial resistance pattern	Antimicrobial resistance classes	MAR Index	Classification of Strains	
					Type of resistance	No. and %
Salmonella enterica subsp. enterica serovar Heidlberg (n=2)	1	AMP, TE, CAZ, NA, S, CIP	Beta lactam, Tetracyclines, Cephalosporin, Quinolones, Aminoglycosides, Fluoroquinolones	0.6	Multidrug -resistant	2(100%)
	1	AMP, CAZ, CIP	Beta lactam, Cephalosporin, Fluoroquinolones	0.3		
Average MAR index=0.45						
Salmonella enterica subsp. enterica serovar Paratyphi A (n=2)	1	AMP, TE, NA	Beta lactam, Tetracyclines, Quinolones	0.3	Multidrug -resistant	1(50%)
	1	AMP, CAZ	Beta lactam, Cephalosporin	0.2	Low drug-resistant	1(50%)
Average MAR index=0.25						

TABLE 6. Molecular characterization and antimicrobial resistance profile of *Salmonella* isolates (n=50)

***Salmonella* serotypes**	**Sources**	**Antimicrobial resistant genes**	**Virulence genes**
***S.* Enteritidis (n=10)**	cloacal swabs n=5, cecum n=1, Egg yolk n=1, water n=2, stool n=1	*aad*A1, *qnr*A	*Inv*A, *stn* (8)
***S.*Typhimurium (n=8)**	cloacal swabs n=4, liver n=1, water n=1, stool n=2	*sul*1, *aad*A1, *aac*-6-Ib	*inv*A, *stn* (7)
***S.* Kentucky(n=8)**	cloacal swabs n=5, liver n=1, egg yolk n=1, water n=1	*tet*A, *qnr*A	*inv*A, *stn*(6)
***S.* Wingrove(n= 4)**	cloacal swabs n=3, egg yolk n=1	*sul*1	*inv*A, *stn*
***S.* Papuana (n=3)**	cloacal swabs n=2, cecum n= 1	*sul*1, *aac*-6-Ib	*inv*A, *stn*
***S.* Saintpaul (n=3)**	cloacal swabs n= 1, water n=1, stool n=1	*aad*A1	*inv*A, *stn*
***S.*Larochelle (n=3)**	cloacal swabs n=2, water n=1	*aad*A1	*inv*A, *stn*(2)
***S.* Molade (n=3)**	cloacal swabs n=2, egg yolk n=1	*tet*A, *aac*-6-Ib	*inv*A, *stn* (2)
***S.* Inganda (n=2)**	cloacal swabs n=1, egg yolk n=1	*sul*1	*Inv*A, *stn*
***S.* Muenster(n=2)**	cloacal swabs n=2	*aad*A1	*inv*A
***S.* Paratyphi A (n=2)**	Water n=1, stool n=1	*tet*A, *aad*A1	*inv*A, *stn*
***S.* Heidelberg (n=2)**	Water n=1, stool n=1		*inv*A, *stn*



Graphical Abstract

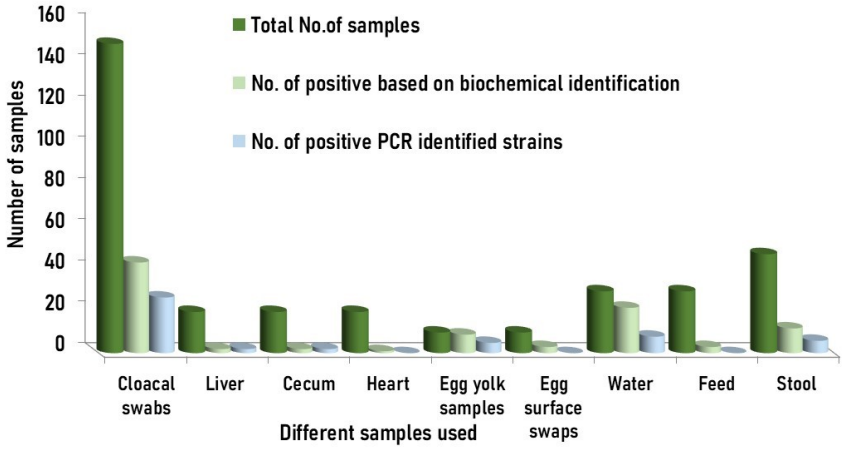


Fig. 1. Number of *Salmonella*-positive samples based on their biochemical and molecular characterization.

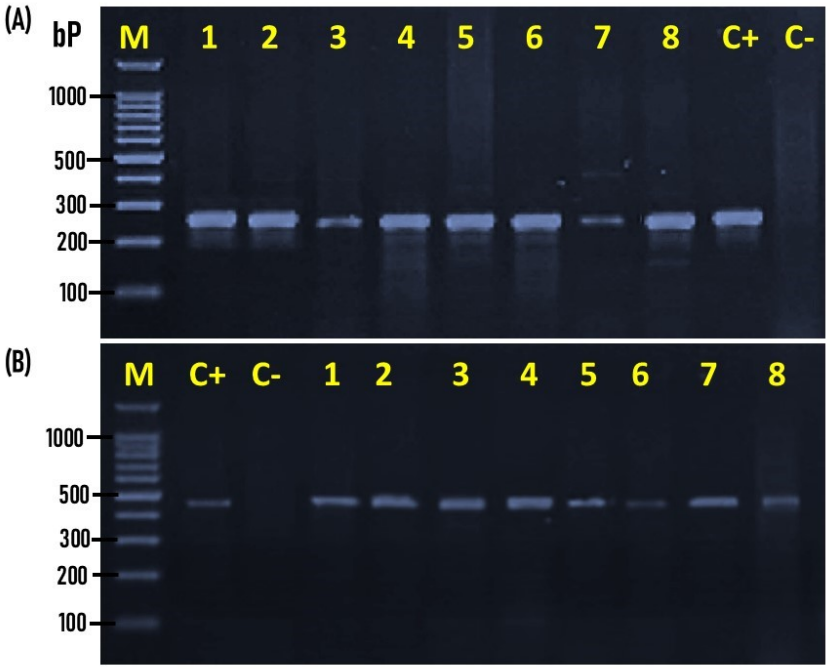


Fig. 2. (A) Representative Agarose gel electrophoresis of *inv*A (285 bp) gene. (B) Representative Agarose gel electrophoresis of *stn* (480 bp) gene detected in *Salmonella* isolates. M: DNA marker (100-bp gene ladder).C+: Control positive. C–: Control negative.

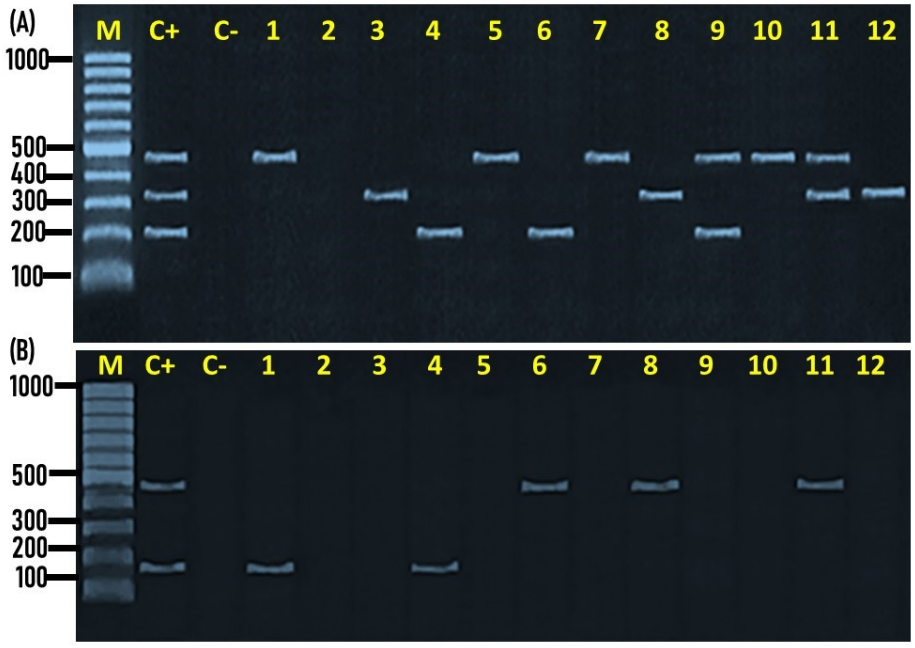


Fig. 3. (A) Representative Agarose gel electrophoresis of *tet*A (201 bp), *sul*1 (316 bp), and *aad*A1 (484 bp) antimicrobial resistant genes for characterization of *Salmonella* species. M: DNA marker (100-bp gene ladder). C+: Control positive. C–: Control negative.Lanes 4 (*S.* Kentucky), 6 (*S.* Molade) and 9 (*S.* Paratyphi A): Positive strains for *tet*A gene.Lanes 3 (*S.* Inganda), 8 (*S.* Papuana), 11 (*S.* Typhimurium) & 12 (*S.* Wingrove): Positive strains for *sul*1 gene.Lanes 1 (*S.* Enteritidis), 5 (*S.* Larochelle), 7 (*S.* Muenster), 9 (*S.* Paratyphi A), 10 (*S.* Saintpaul) & 11 (*S.* Typhimurium): Positive strains for *aad*A1 gene.Lanes 2 (*S.* Heidelberg): Negative strains for *tet*A, *sul*1 and *aad*A1 genes. Lanes 1 (*S.* Enteritidis) & 4 (*S.* Kentuckey): Positive strains for *qnr*A gene. (B) Agarose gel electrophoresis of *qnr*A (188 bp) and *aac*-6-Ib (482 bp) antimicrobial resistant genes for characterization of *Salmonella* species. Lanes 6 (*S.* Molade), 8 (*S.* Papuana) & 11 (*S.* Typhimurium): Positive strains for *aac*-6-Ib gene.Lanes 2 (*S.* Heidelberg), 3 (*S.* Inganda), 5 (*S.* Larochelle), 7 (*S.* Muenster), 9 (*S.* Paratyphi *A*), 10 (*S.* Saintpaul) and 12 (*S.* Wingrove): Negative strains for *qnr*A and *aac*-6-Ib genes.

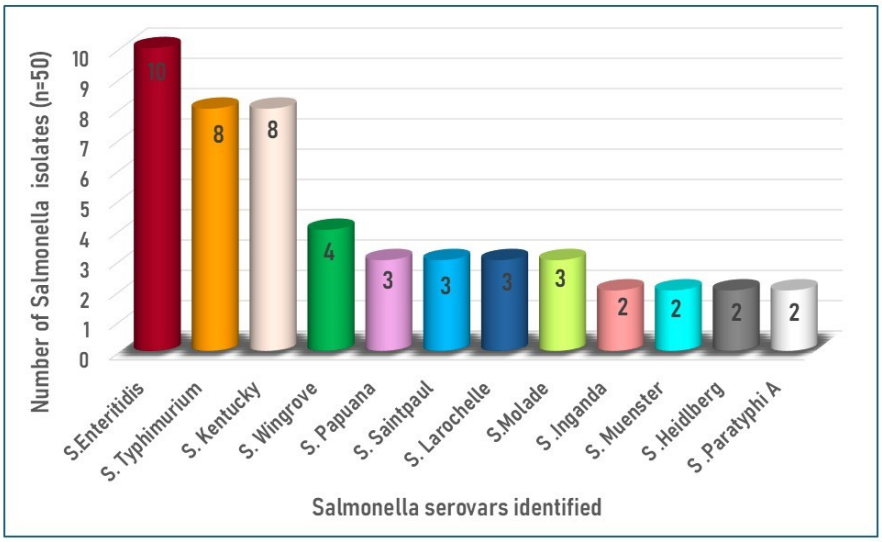


Fig. 4. *Salmonella* identified serovars (n= 50) from different samples

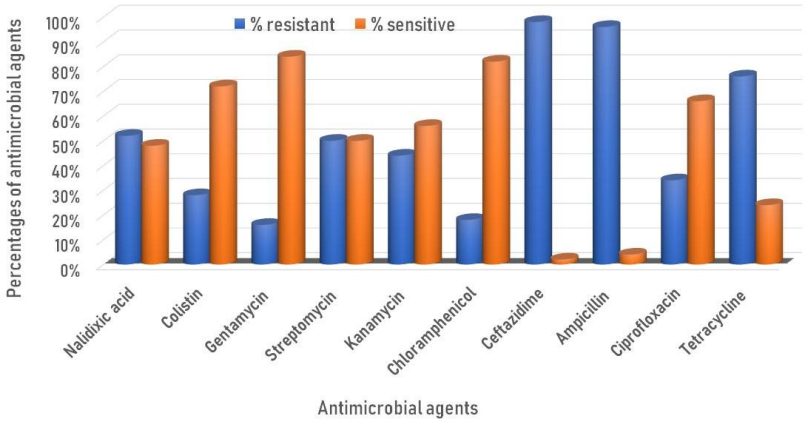


Fig. 5. Percentage of resistant and sensitive *Salmonella* isolates to different antibiotics (n=10)

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توصيف سلالات السالمونيلا المعوية المقاومة للمضادات الحيوية المتعددة من سلاسل انتاج البط فى مناطق دلتا النيل

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الملخص

قدمت هذه الدراسة معلومات حديثة حول انتشار أنواع السالمونيلا المقاومة للعديد من المضادات الحيوية في سلاسل إنتاج البط في مصر. وشملت دراسة علي 338 عينة من مزارع البط والبط المنزلي ، بما في ذلك مسحات من فتحة الشرج (n=150)، وأعضاء البط (n=60)، ومسحات من سطح البيض (n=10)، وصفار البيض (n=10)، وعينات الأعلاف ومياة الشرب (n=30 لكل منهما)، وعينات براز مربي البط (n=48). تم استخدام طرق لفحص جميع العينات بحثًا عن أنواع السالمونيلا. خضعت عزلات السالمونيلا المحددة لتفاعل البوليميراز المتسلسل الخاص بالأنواع واختبار جينات الضراوة مثل *inv*A و *stn* وبعض جينات مقاومة مضادات الميكروبات مثل (*tet*A و *sul*1 و *aad*A1 و *qnr*A و *aac*-6-Ib). استنادًا إلى جين*inv*A ، تم عزل أنواع السالمونيلا بمعدل 14.79% (50/338)، و 41 من 50 عزلة كان ايجابي لجين *stn* بنسبة 82%. كانت جينات المقاومة للمضادات الحيوية الأكثر نسبة هي *aad*A1 (28/50)، تليها(18/50) *qnr*A ، و(17/50) *sul*1، و(14/50) *aac*-6-Ib، و .(13/50) *tet*A علاوة على ذلك، كانت السلالات السائدة للسالمونيلا هي *S*. Enteritidis و *S*. Typhimurium و*S*. Kentucky. أظهرت نسبة كبيرة من عزلات السالمونيلا مقاومة لمضاد الميكروبات السيفتازيديم بنسبة (98%) يليه الأمبيسلين بنسبة (96%). تشير هذه النتائج إلى انتشار السالمونيلا المقاومة للعديد من مضادات الميكروبات على نطاق واسع في جميع سلاسل إنتاج البط المصرية، مما قد يشكل خطرًا كبيرًا على الصحة العامة بسبب انتقاله إلى البشر عبر تناول الغذاء.

الكلمات المفتاحية: السالمونيلا، *Inv*A، سلالات السالمونيلا، MDR، البط، مصر.