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Research Article

Isolation, Identification and Antibiotic Resistance Profiling of *Salmonella* from Chicken and Ducks

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Abstract | Current study was planned to assess the isolation, identification and antibiotic resistance profiling of *Salmonella* in chicken and ducks. A total of 125 feces samples (55 broilers, 45 layers and 25 ducks) were collected in district Hyderabad. Isolation was done according to ISO 6579 standard method. Identification of isolated bacteria was done using the *Salmonella* specific biochemical tests. Antimicrobial resistance patterns were determined using the disk diffusion method. *Salmonella* were detected in 22, 19 and 10 samples, showing prevalence of 40.0, 42.22 and 40.0 percent in broiler, layer and ducks, respectively. Duck isolates exhibited 100 percent resistance against tetracycline, sulfamethazine and doxycycline, followed by ampicillin that showed 70% resistance. In case of layers tetracycline, sulfamethazine and doxycycline showed 100 percent resistance to *Salmonella* isolates, followed by ampicillin and enrofloxacin that exhibited 73.68 and 63.15% resistance. In broiler, sulfamethazine showed 100 percent resistance followed by ampicillin (68.18%). The results shows that out of the 22 *Salmonella* isolates of broiler origin, 20 (90.9%) were recognized as multidrug resistant (MDR). Similarly, out of 19 isolates of layer birds, 17 (89.47%) were recorded as MDR organisms. However, *Salmonella* isolates of ducks exhibited a little bit less number of MDR organisms i.e., 60%. The antibiotic resistance pattern further shows that out of the total 51 poultry (broiler, layer and ducks) isolates 84.31% (n = 43) were recorded as MDR. The resistance pattern further shows that out of the total 51 *Salmonella* isolates, the highest resistance was detected to sulfamethazine 100% (n = 51/51), followed by ampicillin 70.58% (n=36/51), tetracycline 68.73% (n=35/51), doxycycline 56.86% (n=29/51), enrofloxacin 29.41% (n=15/51), norfloxacin 17.64% (n=9/51), ciprofloxacin 11.76% (n=6/51) and gentamicin had 9.80% resistance (n=5/51). These results demonstrated that prevalence of MDR organisms is quite high in chickens (broilers and layers) as compared to ducks. Moreover, gentamicin, followed by ciprofloxacin, norfloxacin and enrofloxacin could be the drug of choice against *Salmonella* of poultry origin.

Keywords | *Salmonella*, poultry farms, Hyderabad, Antimicrobial, Antibiotic resistance

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INTRODUCTION

Salmonella is a Gram negative bacterial pathogen that can cause serious gastrointestinal illness in humans. *Salmonella* belong to the family *Enterobacteriaceae* are short rods that do not produce spores and are not encapsulated. These bacteria are able to live in both aerobic and facultative anaerobic environments (Mondal et al., 2008a).

Poultry is one of the major sources of *Salmonella* contamination. Almost all poultry species including ducks are believed to carry *Salmonella* species in their intestines (Pan et al., 2010). *Salmonella* contamination can be occur at any stage of the production process, including at the farm level, during transport, or in the processing plant (Wang et al., 2023). Antibiotic resistance among *Salmonella* strains is a growing problem that has become a public health concern worldwide. The emergence of antibiotic resistant *Salmonella* strains in the food chain has been linked to the misuse and overuse of antibiotics in animal production, leading to a need for surveillance and identification of antibiotic resistant strains in poultry (Ince & Akan, 2023).

Salmonellosis is the name given to the most prevalent kind of bacterial food poisoning that may be caused by chicken and other poultry products (Ravel et al., 2009; Guo et al., 2011). After *Salmonella* Typhimurium and *Salmonella* Paratyphi, *Salmonella* Enteritidis, a serotype of *Salmonella* enterica, is the third most prevalent *Salmonella* responsible for outbreaks related with poultry (Antunes et al., 2017). Salmonellosis is an infectious illness that may take up to 72 hours to manifest after an individual has been exposed to contaminated food or water. Symptoms of salmonellosis include fever, diarrhea, and severe cramping (Antunes et al., 2017). According to the findings of a research that was carried out by Majowicz et al. (2010) *Salmonella* is known to cause around 155,000 deaths per year as well as 93 billion instances of sickness each year. *Salmonella* outbreaks continue to take place even though several preventative measures and management strategies have been implemented, and the cause of these outbreaks is often the consumption of poultry products that have been contaminated (Williams et al., 2014; Antunes et al., 2017).

Salmonella strains that originated in chickens are becoming more resistant to antibiotics, which offers public health hazards comparable to those posed by food poisoning. These risks include diarrhea and severe gastroenteritis. *Salmonella* strains that are present in poultry have been demonstrated in many investigations to have the potential to cause drug-resistant illnesses in humans (Mondal et al., 2008b; Husain, 2010). Antimicrobial resistance is becoming an increasingly pressing issue, which puts *Salmonella* and other food-borne microorganisms in a difficult position. The World Health Organization also has brought attention to the expanding issue of antibiotic-resistant *Salmonella* that are not linked to typhoid fever (McEwen, 2012). To the best of our knowledge, little is known about *Salmonella* prevalence and its' antimicrobial resistance status in poultry particularly in ducks in the Hyderabad region. Therefore, this study aimed to detect prevalence and antibiotic resistance profiling of *Salmonella* in chicken and ducks in district Hyderabad in order to identify the most common types of antibiotic resistance.

MATERIALS AND METHODS

Birds and Sampling

A total of 125 fecal samples were obtained from commercial poultry farms, including 55 faeces samples from broiler farms, 45 faeces samples from layer farms, and 25 faeces samples from backyard ducks in the Hyderabad region. Samples were collected aseptically, put into the plastic zip lock bags and sent to laboratory under refrigerated condition. Fecal samples were processed according to method described by Ansari et al. (2022). In brief, 1 g sample was used to add in 9 mL of 0.9% sterile saline solution. It was vortexed well in a conical flask than diluted subsequently to prepare 10-fold dilution using the 0.85% saline solution. Each 1 mL of diluted sample was used to inoculate onto the various types of culture media.

Isolation and identification of *Salmonella*

ISO 6579 (ISO, 2002) was used as the gold standard for *Salmonella* isolation. The following media were used for inoculation: Muller Kauffmann tetrathionate novobiocin broth, Rappaport-Vassiliadis soya broth (RVS), *Salmonella* Shigella (S.S) agar, Brilliant Green Agar (BGA), Xylose Lysine Deoxycholate (XLD), and MacConkeys' agar. *Salmonella* spp. were cultured on all agars using the streak plate technique.

Colony morphology, Gram staining, motility, oxidase, catalase, indoxyl acetate hydrolysis, triple sugar iron, urease, Simmons citrate, coagulase, methyl red, and Vogues' Proskauer tests, among others, were used for the preliminary identification of isolated bacteria in order to locate the *Salmonella*, as described in Bergey's manual of systematic bacteriology (Holt et al., 1994).

Antibiogram Methodology

In accordance with the criteria established by the CLSI, *Salmonella* isolates were subjected to the disc diffusion method for the purpose of determining their degrees of antibiotic resistance (CLSI, 2012). Antimicrobials such as enrofloxacin (5 µg), norfloxacin (10 µg), flumequine (30 µg), ciprofloxacin (5 µg), ampicillin (10 µg), gentamicin (10 µg), tetracycline (30 µg), tri methoprimsulfamethoxazole (25 µg), doxycycline (30 µg), and chloramphenicol (5 µg) were used.

The in-vitro susceptibility test with different antibiotics from different classes was done in accordance with the protocol that had been developed by the Clinical Laboratory Standard Institute (CLSI, 2012) for the use of the disc diffusion method. Inoculums were produced by mixing pure bacterial colonies that were recovered from fresh culture plates with Muller Hinton broth. The turbidity of each inoculum was adjusted to be equal to 1.5 x 10^8 CFU/ml,

which is equivalent to 0.5 McFarland units. Every inoculum was inoculated with 1 ml of solution, which was then spread out on a plate of Muller-Hinton agar supplemented with 5% sheep blood to generate a confluent lawn of bacterial growth. The plate was then incubated at 37 degrees Celsius for 24 hours. After the addition of the antibiotic discs into the agar, the plates were dried at 37 degrees Celsius for five minutes. The plates were kept in an aerobic condition while being incubated to 37 degrees Celsius for a period of 48 hours. After the incubation process, the size of the inhibitory zones was measured and analyzed.

Statistical analysis

In order to determine whether or not there was a statistically significant difference between the prevalence rate of *Salmonella* in broiler, layer and duck samples, the Fishers' exact test was carried out using JMP statistical package software (version 5.0.1.a, SAS Institute Inc., Cary, NC). The 5% probability level was considered as a level of significance. All results were presented in percentages.

RESULTS

Number and percentage of *Salmonella* positive poultry sample

Different poultry species including broiler, layer and ducks were examined for comparative prevalence of *Salmonella* in fecal samples (Table 1). Out of 55, 45 and 25 samples *Salmonella* were detected in 22, 19 and 10 samples, showing *Salmonella* prevalence of 40.0, 42.22 and 40.0 percent in broiler, layer and ducks, respectively. The *Salmonella* prevalence was slightly ($p > 0.05$) higher in layer, while broiler and ducks exhibited with equal percentage of *Salmonella* prevalence.

Table 1: Number and percentage of *Salmonella* positive poultry sample

Specie	No. of samples	No. of positive samples	Percentage
Broiler	55	22	40%
Layer	45	19	42.22%
Duck	25	10	40%

Antimicrobial resistance profile of broiler isolates

The results presented in Table 2 exhibited that, sulfamethazine showed 100 percent resistance to *Salmonella* isolates of broiler origin, followed by ampicillin that showed 68.18 percent resistance to *Salmonella* isolates. However, tetracycline, norfloxacin, ciprofloxacin, enrofloxacin and gentamicin showed 27.27, 13.63, 9.09, 9.09 and 4.54 percent resistance respectively to *Salmonella* isolates in broiler; while doxycycline showed least resistance to *Salmonella* (0%). In case of susceptibility to *Salmonella* in broiler, doxycycline showed highest susceptibility (68.18%), followed by gentamicin (63.63%), ciprofloxacin (59.09%), norfloxacin (54.54%) and enrofloxacin (50.0%). The antibiotics tetracycline and ampicillin were determined as least susceptible to *Salmonella* isolates in broiler showing 18.18 and 4.54 percent susceptibility, respectively.

Antimicrobial resistance profile of layer isolates

As shown in Table 3, tetracycline, sulfamethazine and doxycycline showed 100 percent resistance to *Salmonella* isolates of layers, followed by ampicillin and enrofloxacin that exhibited 73.68 and 63.15 percent resistance respectively to *Salmonella*. Norfloxacin, and ciprofloxacin showed 21.05, and 15.78 percent resistance respectively to *Salmonella* isolates; while gentamicin exhibited least resistance to *Salmonella* (10%). In case of susceptibility to *Salmonella*, gentamicin, norfloxacin and ciprofloxacin were observed to be susceptible to *Salmonella* showing 73.68, 47.36 and 31.57 percent susceptibility, respectively in layers. The antibiotics showing lower susceptibility to *Salmonella* in layer included enrofloxacin (10.52%), and ampicillin (5.26%); while tetracycline, sulfamethazine and doxycycline did not show susceptibility to *Salmonella* infection in layers.

Antimicrobial resistance profile of duck isolates

Eight antibiotics (enrofloxacin, norfloxacin, ciprofloxacin, ampicillin, gentamicin, tetracycline, sulfamethazine and doxycycline) were tested for their susceptibility or resistance to *Salmonella* (Table 4) isolated from ducks. The data showed that tetracycline, sulfamethazine and doxycycline showed 100 percent resistance to *Salmonella* isolates. Ampicillin also showed high resistance (70%), followed by norfloxacin and gentamicin equally showed 20 percent resistance to *Salmonella* infection; while enrofloxacin and ciprofloxacin were least resistant to *Salmonella* isolates (10%). In case of susceptibility to *Salmonella*, ciprofloxacin, enrofloxacin and gentamicin were highly susceptible to *Salmonella* showing 70, 60 and 60% susceptibility, respectively in ducks. Among the antibiotics, lower susceptibility was exhibited by norfloxacin (30%), and ampicillin (10%); while tetracycline, sulfamethazine and doxycycline showed zero susceptibility to *Salmonella* isolates in ducks.

Multi-resistance in *Salmonella* isolates

Table 5 revealed the antibiotic resistance pattern of *Salmonella* isolates of various poultry origin. The results shows that out of the 22 *Salmonella* isolates of broiler origin, 20 (90.9%) were recognized as multidrug resistant (MDR). Similarly, out of 19 isolates of layer birds, 17 (89.47%) were recorded as MDR organisms. However, *Salmonella* isolates of ducks exhibited a little bit less number of MDR or

Table 2: *Salmonella* isolates (n=22) from broiler susceptible, intermediate and resistant to various antibiotics

Antibiotic	Disc potency (µg)	Susceptible %	Intermediate %	Resistant %
Enrofloxacin	05	50	40.91	9.09
Norfloxacin	06	54.54	31.81	13.63
Ciprofloxacin	05	59.09	31.81	9.09
Ampicillin	10	4.54	27.27	68.18
Gentamicin	10	63.63	31.81	4.54
Tetracycline	30	18.18	54.54	27.27
Sulfamethazine	25	00	00	100
Doxycycline	30	68.18	31.81	0

Table 3: *Salmonella* isolates (n=19) from layer susceptible, intermediate and resistant to various antibiotics

Antibiotic	Disc potency (µg)	Susceptible %	Intermediate %	Resistant %
Enrofloxacin	05	10.52	26.31	63.15
Norfloxacin	06	47.36	31.57	21.05
Ciprofloxacin	05	31.57	52.63	15.78
Ampicillin	10	5.26	21.05	73.68
Gentamicin	10	73.68	15.78	10.52
Tetracycline	30	00	00	100
Sulfamethazine	25	00	00	100
Doxycycline	30	00	00	100

Table 4: *Salmonella* isolates (n=10) from duck susceptible, intermediate and resistant to various antibiotics

Antibiotic	Disc potency (µg)	Susceptible %	Intermediate%	Resistant %
Enrofloxacin	05	60	30	10
Norfloxacin	06	30	50	20
Ciprofloxacin	05	70	20	10
Ampicillin	10	10	20	70
Gentamicin	10	60	20	20
Tetracycline	30	00	00	100
Sulfamethazine	25	00	00	100
Doxycycline	30	00	00	100

Table 5: Multi-drug resistance (MDR) pattern of various poultry isolates.

Source	No. of isolates	No. of isolates resistant to various antibiotics								No. of isolates resistant to multiple antibiotics				Total NO. (%) of MDR
		Enro	Nor	Cipro	Amp	Gen	Tet	Sulfa	Doxy	0-1	2-3	3-4	>5	
Broiler	22	2	3	2	15	1	6	22	0	2	12	6	2	20(90.9)
Layer	19	12	4	3	14	2	19	19	19	2	5	4	8	17(89.47)
Duck	10	1	2	1	07	2	10	10	10	4	3	3	0	6(60)
Total	51	15	9	6	36	5	35	51	29	8	20	13	10	43(84.31)

Enro: Enrofloxacin, Nor: Norfloxacin, Cipro: Ciprofloxacin, Amp: Ampicillin, Gen: Gentamicin, Tet: Tetracycline, Sulfa: Sulfamethazine, Doxy: Doxycycline

ganisms i.e., 60%. The antibiotic resistance pattern further shows that out of the total 51 poultry (broiler, layer and ducks) isolates 84.31% (n = 43) were recorded as MDR. The resistance pattern further shows that out of the total 51 *Salmonella* isolates, the highest resistance was detected to sulfamethazine 100% (n = 51/51), followed by ampicillin 70.58% (n=36/51), tetracycline 68.73% (n=35/51), doxycycline 56.86% (n=29/51), enrofloxacin 29.41% (n=15/51), norfloxacin 17.64% (n=9/51), ciprofloxacin 11.76% (n=6/51) and gentamicin had 9.80% resistance (n=5/51).

DISCUSSION

In this study, we aim to detect and profile the antibiotic resistance of *Salmonella* isolated from broiler, layer and duck farms in order to identify the most common types of antibiotic resistance and the factors contributing to this resistance. The results of this study probably valuable for the development of strategies to control and prevent the spread of antibiotic resistant *Salmonella* strains in poultry production.

In our study *Salmonella* was found in 40.0, 42.22, and 40.0 percent of the samples taken from broilers, layers, and ducks, respectively. The *Salmonella* prevalence was slightly ($p > 0.05$) higher in layer, while broiler and ducks exhibited with equal percentage of *Salmonella* prevalence. Poultry are the most important reservoir for *Salmonella*, with prevalence in chicken carcasses ranging from 20-70% in most countries (D'Aoust, 1989). However, Beli et al. (2001) reported the low prevalence (8%) of *Salmonella* in poultry products in Albania. The difference in the prevalence rates may be due to socio-economic factors. Mikanatha et al. (2010) reported a 22.2% prevalence of *Salmonella* spp. in chicken meat sold at retail outlets of central Pennsylvania which is disagree with our study. *Salmonella* spp. were isolated from 39.0% (41/105) of duck cecal contents in Malaysia (Adzitey et al. 2012). In contrast to our study Hyobi et al. (2016) found 20.75% *Salmonella* positive samples from ducks, which is quite lower than prevalence recorded in our current study.

Bacterial resistance to antimicrobial drugs is one of the major risks for global public health, which develops due to many reasons such as misuse of antimicrobials (Okorie-Kanu et al., 2016). In our study eight antibiotics (enrofloxacin, norfloxacin, ciprofloxacin, ampicillin, gentamicin, tetracycline, sulfamethazine and doxycycline) were tested for their resistance to *Salmonella* isolated from broilers, layers and ducks. In ducks, data showed that tetracycline, sulfamethazine and doxycycline showed 100 percent resistance to *Salmonella* isolates. Ampicillin also showed high resistance (70%), followed by norfloxacin and gentamicin equally showed 20 percent resistance to *Salmonella* infection; while enrofloxacin and ciprofloxacin were least resistant to *Salmonella* isolates (10%). Ruichao et al. (2013) observed antimicrobial resistance rates for tetracycline (87%), sulfamethoxazole (73%), nalidixic acid (41%) and spectinomycin (41%) which was closely agree with our study. Our study disagree with Hyobi et al. (2016) those reported 15.7%, 13.3% and 8.4% resistance against tetracycline, streptomycin and ampicillin respectively, which was lower as compared to our study.

In layers tetracycline, sulfamethazine and doxycycline showed 100 percent resistance to *Salmonella* isolates, followed by ampicillin and enrofloxacin that exhibited 73.68 and 63.15 percent resistance respectively. Norfloxacin, and ciprofloxacin showed 21.05, and 15.78 percent resistance respectively to *Salmonella* isolates, while gentamicin exhibited least resistance to *Salmonella* (10%). Analogous pattern of resistance to tetracycline, doxycycline were observed among *Salmonella* isolates from eggs of commercial layer hens by Harsha et al. (2011). Mohamed et al. (2019) tested 12 antimicrobials, 86.4% resistance was found to streptomycin and oxytetracycline followed by neomycin and erythromycin (77.3%), norfloxacin and ampicillin (68.2%) across the study sites which is agreed with our results, while gentamicin remained sensitive by 90.9%, which is higher as compared with our results. Similar values of resistance pattern against gentamycin (3.2%) for *Salmonella* isolates was detected by Bywater et al. (2004). Opposes the results of present study Oluyege et al. (2009) from Nigeria, reported the 71.8% resistance against gentamycin. Ciprofloxacin is a fluoroquinolone antibiotic that is increasingly and successfully used for the treatment of septicemia in humans; and ciprofloxacin resistance in human and veterinary isolates of *Salmonella* has occasionally been found. In our study ciprofloxacin showed 15.78 percent resistance to *Salmonella* isolates. The study of Harsha et al. (2011) reported a little lower levels of ciprofloxacin resistance (6.06%) among *Salmonella* isolates from backyard layer hen eggs.

In broilers, sulfamethazine showed 100 percent resistance to *Salmonella* isolates, followed by ampicillin that showed 68.18 percent resistance. However, tetracycline, norfloxacin, ciprofloxacin, enrofloxacin and gentamicin showed 27.27, 13.63, 9.09, 9.09 and 4.54 percent resistance respectively to *Salmonella* isolates of broiler origin; while doxycycline showed least resistance (0%). Aijaz et al. (2010) observed the 94.73% resistance against tetracycline in isolates of broiler chickens, which is higher than reported in our findings. On the other hand, no resistance to ciprofloxacin was observed by Cardoso et al. (2006). In previous study higher resistance for *Salmonella* isolates of broiler chickens were recognized against oxytetracycline (96.3%) as well as penicillin group (92.6%) (Kamboh et al., 2018). Ampicillin resistance was observed in all the isolates which is in agreement with the findings of Suresh et al. (2006). Harsha et al. (2011) reported that ampicillin resistance among the *Salmonella* strains is the 12.12% which was lower than our findings. This inconsistency may be attributed to the antimicrobial drug usage pattern in their study areas, which may be varied from that in the present study.

Multi-drug resistance is a major clinical problem in food borne pathogens. Multi-drug resistance is also one of the major threats to humans and animals which limit therapeutic selection of antibiotics (Kurincic et al., 2005). Mul-

ti drug resistance pattern of *Salmonella* isolates in present results shows that out of the 22 *Salmonella* isolates of broiler origin, 20 (90.9%) were recognized as multidrug resistant (MDR). Similarly, out of 19 isolates of layer birds, 17 (89.47%) were recorded as MDR organisms. However, *Salmonella* isolates of ducks exhibited a little bit less number of MDR organisms i.e., 60%. The antibiotic resistance pattern shows that out of the total 51 *Salmonella* isolates, the highest resistance was detected to sulfamethazine 100% (n = 51/51), followed by ampicillin 70.58% (n=36/51), tetracycline 68.73% (n=35/51), doxycycline 56.86% (n=29/51), enrofloxacin 29.41% (n=15/51), norfloxacin 17.64% (n=9/51), ciprofloxacin 11.76% (n=6/51) and gentamicin 9.80% (n=5/51). Adabara et al. (2012) detected the multiple drug resistance strain of *S.* typhi. Similarly, to our study Tesfaw et al. (2013) confirmed the resistance to one or more of the antimicrobial drugs used against all the isolates. Parvej et al. (2016) observed contrast results of multidrug resistance *Salmonella* against six drugs, they revealed that 54.54% isolates were sensitive to ciprofloxacin and 81.81% isolates were resistant to amoxycillin, doxycycline, kanamycin, gentamycin, and tetracycline.

CONCLUSIONS

Based on current study it is concluded that *Salmonella* are prevalent in broiler, layer and ducks in the study area. *Salmonella* isolates of duck and layer chicken exhibited 100% resistance against tetracycline, sulfamethazine and doxycycline, while broiler isolates showed 100 percent resistance to sulfamethazine. Multi-resistance pattern revealed that broiler and layer isolates were more MDR as compared to duck isolates. These results suggested the strict control over abuse of antibiotics particularly in food-producing animals.

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CONFLICT OF INTEREST

There is no conflict of interest.

NOVELTY STATEMENT

This is first report from Hyderabad district of Sindh, Pakistan that have investigated antimicrobial susceptibility profile of Salmonella isolated from broilers as well layers and ducks.

AUTHORS CONTRIBUTION

VK performed the experiments for the research project that was conceived by his supervisor (AAK). NAK and AL helped with analytical procedures and manuscript writing. FMK did the proofreading of the manuscript, and RAL helped with sample collection and analysis. KRB acted as co-supervisor and supervised the lab experiments.

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