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Isolation and antimicrobial resistance of salmonella isolates from commercial layer farms in and around Hyderabad, Telangana

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Abstract
Salmonella is a most important zoonotic pathogen and its prevalence in animals is a global public health concern. Resistance to widely prescribed antibiotics has grown to be a significant issue for managing chickens and preventing illness. The current study was carried out to determine the presence of Salmonella spp. infection in poultry and to look at the antibiogram patterns of isolates from commercial Layer farms in and around Hyderabad, Telangana. A total of 261 samples of faeces (123) and cloaca (138) were collected and processed for the isolation of *Salmonella*. Twenty one (8.04%) of the samples tested were found to be positive for Salmonella. Out of 123 fecal samples and 138 cloacal swabs, the incidence of Salmonella spp. was 7.3% and 8.6% respectively. All the isolates were subjected to antibiotic sensitivity tests against 10 antimicrobials. The results revealed that bacterial isolates had 100% sensitivity for ciprofloxacin and enrofloxacin followed by levofloxacin, gentamycin, ampicillin, neomycin and amoxicillin were found to be the most intermediately sensitive antibiotics. Tetracycline, doxycycline and amikacin were the most resistant antibiotics. The biggest challenge at hand is multiple antibiotic resistance because many antibiotics are ineffective against infections caused by Salmonella. Therefore, the goal of the current study was to determine the pattern of antibiotic resistance exhibited by these Salmonella.

Keywords: Salmonella, isolation, antibiotic sensitivity, poultry

1. Introduction
Poultry is one of the fastest-growing segments of the agricultural sector in India today. The production of eggs and broilers has increased at a rate of 8 to 10 percent annually, compared to the 1.5 to 2 percent annual growth of crops. In 2022-2023, the nation exported 664,753.46 MT of poultry products to the world for a total value of Rs. 1,081.62 crore, or 134.04 USD million (APEDA, 2022) [1]. Over the past forty years, India's poultry industry has experienced a dramatic transformation, moving from traditional farming methods to a commercial production system that incorporates cutting-edge technological advancements. According to the 20th Livestock Census, the total Poultry population in India is 851.81 million. India possesses an abundance of livestock and poultry, which are essential for enhancing the socioeconomic status of the rural people (DHAD, 2022) [2]. The most widely known reservoir of Salmonella is poultry (Cohen *et al*., 1986) [3]. Salmonella species are facultative anaerobic, gram-negative, non-spore-forming, usually motile rods that are found in animals' digestive tracts and belong to the Enterobacteriaceae family (Barrow *et al.*, 2009 [4] and Bell *et al.*, 2007) [5]. Within the Enterobacteriaceae family, Salmonella is a significant genus that comprises only two species: *S. enterica* and *S. bongori* (Brenner, 2000) [6]. Fecal shedding allows *Salmonella* to be transmitted among birds in a flock. *Salmonella* spp. is widespread in poultry production. Prevalence varies considerably depending on country and type of production as well as the detection methods applied. It is known to be the etiological agent responsible for salmonellosis by *Salmonella* spp. in both humans and animals. Food-borne salmonellosis still occurs throughout the world (Bell *et al.*, 2007) [5]. Antibiotics are widely used to control infectious diseases or as growth promoters in the production of poultry (Akond *et al.*, 2009) [7] and excessive use of these antibiotics is thought to be the primary factor influencing bacterial resistance to antibiotics (Moreno, 2000) [8].

Many resistant bacterial strains have emerged in recent years as a result of the widespread use of antibiotics in veterinary medicine. Over the past 20 years, reports of an increase in antibiotic resistance have been made in many countries, including India (Kapil *et al.*, 2004) [9]. Since many antibiotics are ineffective against infections caused by Salmonella, antibiotic resistance is currently the biggest challenge for the pharmaceutical and health sectors. In view of the above facts, the present research work has taken up to know pattern of antibiotic resistance against Salmonella isolates from commercial layer farms in and around Hyderabad.

2. Materials and Methods

2.1 Collection of samples: The current study was conducted in and around Hyderabad, Telangana and a total of 261 fecal samples (123) and cloacal swabs (138) were the samples collected from commercial layer farms. The samples were then transported in sterile wide-mouth screw-capped bottles under refrigeration to the Department of Veterinary Public Health and Epidemiology Laboratory, Rajendranagar, Hyderabad, where they were for further processed for the presence of Salmonella.

2.2 Isolation and identification of *Salmonella:* *Salmonella* spp. was isolated using the standard microbiological procedures. Ten milliliters of buffered peptone water were used to pre-enrich each cloacal swab, 1 g of faeces were separately inoculated in 9 ml of BPW and incubated at 37 ℃ for 18-24 h. Later, 1 ml of pre-enriched culture was inoculated with 9 ml tetrathionate broth for selective enrichment of Salmonella organisms and incubated at 37 ℃ for 24 h in bacteriological incubator, a loopful of each culture was streaked on to MacConkey agar (MA), brilliant green agar (BGA) and *Salmonella*-Shigella (SS) agar as a selective medium for primary isolation and incubated. The temperature and the period of incubation were standardized at 37 ℃ for 24 h, respectively. The incubated media were then examined for bacterial growth after 24 hours. Morphology and cultural characteristics were used to determine an initial identification of suspected Salmonella cultures. The colonies were then stained using Gram's method and examined under a microscope to look for Gram-negative rods. The non-lactose fermenting colonies of MA were characterized microscopically using Gram's-stain. The organisms from the agar media were sub-cultured into Xylose-lysine-deoxycholate (XLD) agar, Hektoen Enteric Agar plates with the help of inoculating loop in case of gram-negative rods in the smears. Thus, a single pure colony was obtained. The pure isolates that were obtained in this manner were subjected to standard biochemical characterization techniques, including, nitrate reduction, indole test, motility, citrate utilization, methyl red, Voges-Proskauer, and urease.

2.3 Antibiotic sensitivity test (ABST): Every Salmonella isolate was tested to see how sensitive it was to antimicrobial agents and ABST was conducted by the disc diffusion method in Mueller Hinton Agar as per Bauer *et al* (1966) [10]. Each isolate was inoculated in BHI (brain heart infusion) broth, following the 24 h of incubation at 37 ℃, the broth was streaked by using sterile swabs on Mueller-Hinton agar and individual antimicrobial discs were gently pressed down on the agar surface with sterile forceps. The plates were incubated overnight at 37 ℃. The concentrations of antibiotics used zone of inhibition around the disc was measured by a millimeter scale and interpreted as per Clinical and Laboratory Standard Institute (2022) standards (CLSI, 2022) [11]. Antibiotic discs (Hi-Media) of extensively employed antimicrobials such as ciprofloxacin (5 µg), levofloxacin (15 µg), amoxicillin (30 µg), ampicillin (10 µg), enrofloxacin (10 µg), doxycycline (30 µg), gentamicin (10 µg), tetracycline (30 µg), neomycin (30 µg) and amikacin (5 µg) were used. The susceptible, intermediate, and resistant categories were derived from the zone diameters of each antimicrobial agent.

3. Results and Discussion

Upon culture of the samples on various agars, the following observations were made: In the case of XLD agar, red colonies were initially produced after 24 h of incubation, which got blackened at the centre with prolonged incubation. Regarding Hektoen enteric agar, smooth, black-centred colonies have a greenish periphery that resembles a bull's eye, and it was found that colonies on MC agar were translucent and colourless. For BG agar, a pale pink colony was seen against a rose-pink background. On SS agar, colonies that were translucent, colourless, or occasionally black were seen. In the present study, 21 samples, or 8.04% of the 261 samples that were examined for salmonella were contaminated. Of the 138 cloacal samples, 12 samples (8.6%) contained salmonella, while 9 out of 123 faeces samples (7.3%) tested positive for the bacteria (Table 1). Out of 138 cloacal swabs collected from 138 birds in the present study, 8.6% tested positive to Salmonella. The results were close to (Kolhe *et al.*, 2022) [12] with 6.29%. The study conducted by Li, X., *et al.* (2007) [13] reported a higher (30.8%) prevalence rate of Salmonella in faeces collected from layers. Murugkar & Rahman *et al.* (2005) [14] also reported higher isolation rate, 34 (14.7%) of the 231 cloacal swab samples from diarrhoeic birds showed presence of *Salmonella*. The isolation rate was higher than reported in this study, which could be attributed to the fact that only diarrheal birds' swabs were processed in their study.

Table 1: Isolation of salmonella from commercial layers

Sampling group, Source	Samples collected	No of positive samples	Percentage
A, Faeces	41	4	9.7%
B, Faeces	41	2	4.8%
C, Faeces	41	3	7.3%
D, Cloacal	46	5	10.8%
E, Cloacal	46	3	6.5%
F, Cloacal	46	4	8.6%
Total	261	21	8.04%

To determine antibiotic resistance among Salmonella isolates, antibiotic sensitivity tests were performed on 21 Salmonella isolates obtained from fecal and cloacal samples using 10 different antibiotics from different classes (Table-2). Ciprofloxacin and enrofloxacin were found to be 100% effective, whereas other agents disclosed varying degrees of sensitivity: levofloxacin (71.4%), gentamicin (61.9%), ampicillin (47.6%), and amoxicillin (42.8%). The higher sensitivity observed in the current study against ciprofloxacin and enrofloxacin is similar to that reported in previous studies by Suvethika *et al.* (2021) [15], Kolhe *et al.* (2022) [12] and Renu *et al.* (2013) [16]. On the other hand, Tuhin-Al-Ferdous *et al.* (2013) [17] reported 68.75% resistance to Enrofloxacin. However, no resistance found against enrofloxacin indicating that resistance can vary greatly from farm to farm (Yildirim *et al.*, 2011) [18]. Tetracycline had the highest percentage of resistance (76.1%) among 21 isolates, followed by

doxycycline (57.1%), amikacin (52.8%), and ampicillin (42.8%). These findings are consistent with the observations made by Kolhe *et al.* (2022) [12] and Khan *et al.* (2005) [19]. Tetracycline has been used to treat day-old chickens, which might have resulted in the emergence of tetracycline resistant Salmonella in the layer and broiler flocks. A noteworthy portion of the isolates had intermediate resistance to neomycin (66.6%), amoxicillin (57.1%), and gentamycin (38%). Similarities observed in intermediate resistant of Salmonella to neomycin (100%) and amoxicillin (97%) in study performed earlier by Tuhin-Al-Ferdous *et al.* (2013) [17]. The results are in agreement with those of Suresh *et al.* (2019) [20] as Salmonella isolated from commercial poultry birds showed resistance to gentamycin. The occurrence of resistant samples to amikacin, doxycycline, and tetracycline can be attributed to their frequent use in veterinary care. The indiscriminate use of these antibiotics in animal husbandry and livestock production may have contributed to the development of resistance against them (Cohen *et al.*, 1986) [3]. The variation in resistance exhibited by Salmonella across different environments, evaluating the antibiogram of isolates at the farm level is frequently helpful in determining the most effective antimicrobial agent.

Table 2: Antibiogram sensitivity/resistance pattern of Salmonella isolates.

Antibiotics (µg/ disc)	Total no. of Salmonella isolates tested	Resistant No (%)	Intermediate No (%)	Sensitive No (%)
Amikacin-AK (30 µg)	21	11(52.8%)	4 (19.04%)	6 (28.5%)
Ampicillin- A (10 µg)	21	9 (42.8%)	2 (9.5%)	10 (47.6%)
Amoxicillin-AMX (30 µg)	21	1 (4.7%)	12 (57.1%)	8 (38.09%)
Ciprofloxacin-CIP (5 µg)	21	0 (0)	0 (0)	21 (100%)
Doxycycline-DO (30 µg)	21	12 (57.1%)	2 (9.5%)	7 (33.33%)
Enrofloxacin-EN (5 µg)	21	0 (0)	0 (0)	21 (100%)
Gentamicin- GE (10 µg)	21	0 (0)	8 (38.09%)	13 (61.9%)
Levofloxacin-LF (5 µg)	21	2 (9.5%)	4 (19.04%)	15 (71.4%)
Neomycin -N (30 µg)	21	2 (9.5%)	14 (66.6%)	5 (23.8%)
Tetracycline-T (30 µg)	21	16 (76.1%)	3 (14.2%)	2 (9.5%)

4. Conclusion

The indiscriminate use of antibiotics as treatment adjuncts and feed additives causes variation in the antibiogram profile. This variation can be attributed to enzymatic degradation, mutations at binding sites, down regulation of outer membrane proteins, efflux pumps, and transduction of genes in bacterial isolates. As a result, enzymatic degradation, mutation, and transduction of genes do not occur, which may account for the resistance of bacterial isolates to the majority of the antibiotics. Therefore, it is best to limit the indiscriminate use of antibiotics in poultry. The use of various antibiotics for therapy may be the cause of the variations in antimicrobial resistance patterns observed in various locations.

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