International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2023; SP-8(6): 01-03

www.veterinarypaper.com
Received: 01-08-2023
Accepted: 09-09-2023

Sujitha Veeraganti,
B.V.Sc. & A.H,
College of Veterinary Science,
P V Narsimha Rao Telangana Veterinary University,
Rajendranagar, Hyderabad,
Telangana, India

R Sai Kumar Reddy
B.V.Sc. & A.H,
College of Veterinary Science,
P V Narsimha Rao Telangana Veterinary University,
Rajendranagar, Hyderabad,
Telangana, India

A Vijaya Kumar
Assistant Professor & Head,
Department of Veterinary Public Health and Epidemiology,
College of Veterinary Science,
P V Narsimha Rao Telangana Veterinary University,
Rajendranagar, Hyderabad,
Telangana, India

E Kumar,
Assistant Professor,
Department of Veterinary Public Health and Epidemiology,
College of Veterinary Science,
P V Narsimha Rao Telangana Veterinary University,
Rajendranagar, Hyderabad,
Telangana, India

Corresponding Author:
Sujitha Veeraganti,
B.V.Sc. & A.H,
College of Veterinary Science,
P V Narsimha Rao Telangana Veterinary University,
Rajendranagar, Hyderabad,
Telangana, India

A prospective study on prevalence and antibiogram study of *Salmonella* isolated from backyard poultry in rural areas in and around Hyderabad

Sujitha Veeraganti, R Sai Kumar Reddy, A Vijaya Kumar and E Kumar

DOI: https://doi.org/10.22271/veterinary.2023.v8.i6Sa.808

Abstract
Salmonellosis is a bacterial infection caused by *Salmonella*, a member of Enterobacteriaceae family. It is one of the most prevalent diseases in birds, causing high losses in poultry industry and food poisoning in human. There is a limited information about the prevalence of *Salmonella* spp. in backyard chickens. The current investigation was conducted to determine the prevalence and antibiotic resistance of *Salmonella* in backyard chicken. A total of 250 samples were aseptically collected from backyard chicken from rural areas in and around Hyderabad. Samples included cloacal swabs and fecal samples. All the collected samples were tested for *Salmonella* isolates using traditional method and confirmed by ABST. Out of 250 samples 17 (6.8%) isolates of *Salmonella* were formed. Antimicrobial susceptibility of the isolates against 11 antimicrobial agents was determined by the standard disc diffusion method. 100% of the tested *Salmonella* isolates were found to be resistant to oxytetracycline and 82.3% strains showed resistance to tetracycline. Whereas erythromycin and levofloxacin showed 58.8% and 52.9% resistance respectively. No isolate showed resistance to ciprofloxacin. Isolates against ampicillin (76.4%), ceftriaxone (70.5%), and ciprofloxacin (94.1%) showed the highest sensitivity. The isolates exhibited intermediate sensitivity to neomycin (47.05%), chloramphenicol (58.8%), and gentamycin (76.4%). With the increased popularity of keeping backyard chickens, the current study emphasizes the zoonotic risk from *Salmonella* spp. associated with country chicken.

Keywords: *Salmonella*, backyard poultry, antibiotics, prevalence, antibiotic resistant

1. Introduction
Backyard poultry farming is a traditional method of rearing poultry birds with limited number of country birds by farmers to attain their needs. This provides profit in the very shortest time with low investment and ensures availability of eggs and meat even in remote areas. It is an environmentally friendly method because these are very active in pest control and provide manure. It is a system with low input and output (Sonkar *et al.*, 2020) [1]. Backyard poultry farming is practiced by most of the poor and marginalized rural families all over India. In terms of population, there was a tremendous increase in backyard poultry (45.48%) from 217.49 million to 317.07 million from previous to recent census (20th Livestock Census, 2019) [2]. Eggs and meat of these birds acts as major and cheapest sources of protein for rural Indian people. The eggs produced by country birds may not contribute to the major proportion of total production, it aids the rural people in self-employment and caters much needed animal protein (Singh *et al.*, 2020) [3]. There is a little infrastructure required for backyard poultry farming, and it is easily managed by women, elderly family members, and children. The majority of the constraints of this farming in India are the high mortality rates of young birds due to a lack of good infrastructure and farmers scientific knowledge of bird nutrition or medications (Kumar *et al.*, 2019) [4]. Backyard poultry accounts for nearly 30% of total egg production in the United States. Because this method of rearing involves exposing birds to a variety of infectious agents, these birds are predisposed to severe disease conditions. (Megha *et al.*, 2016) [5]. Backyard chickens can also become infected if they encounter *Salmonella*-carrying wild animals, domestic mammals, or commercial poultry (Jafari *et al.*, 2007) [6]. The poultry industry suffers significant economic losses because of this infection.

Antibiotic use contributes to selection pressures that favour the growth of antibiotic-resistant pathogens (Obi *et al*., 2012) [7]. So, this study was conducted to isolate, characterize, and determine the antibiotic resistance of *Salmonella* in backyard chickens.

2. Materials and Methods

2.1 Sample collection: According to OIE standards, a total of 250 samples i.e., 100 cloacal swabs and 150 fecal samples of country chickens were collected from rural areas such as Budwel, Kismatpur, Rajendranagar, Bairagiguda, and Kothwalguda of Hyderabad. After collecting aseptically, the samples were transported to the Department of Veterinary Public Health and Epidemiology for isolation and characterization of *Salmonella* spp.

2.2 Bacterial isolation and identification: After collection, the clocal swabs and 1 g faeces from each fecal sample were pre-enriched in 9 ml of buffered peptone water. Later, 1ml of pre-enriched samples were placed in the freshly prepared tetrathionate broth for selective enrichment and incubated for 24 hours at 37 ℃. After incubation, a loop full of inoculum was then streaked on to brilliant green agar (BGA), MacConkey agar (MA) and *Salmonella*-Shigella (SS) agar as a selective medium for primary isolation and incubated at 37 ℃ for 24 h. Susceptible colonies were again streaked on Xylose Lysine Dextrose (XLD) agar then incubated for 24 hours and examined for the growth of characteristic colonies of *Salmonella*. The colonies were stained using Gram's method and examined under a microscope to look for Gram-negative rods. Most of the gram-negative bacteria are motile, so the hanging drop method was used to determine the motility of the bacteria. To prepare the hanging drop, one drop of cultured broth was placed on a clean cover slip and placed inversely over the concave depression of the hanging drop slide. Soft Paraffin was applied around the edge of the concave depression slide to improve cover-slip attachment and prevent air current and fluid evaporation. The hanging drop slide was carefully examined under a compound microscope. The motile and non-motile organisms were distinguished by observing motility in contrast to the bacteria's swinging movement. Isolated organisms were subjected to biochemical tests: indole test, nitrate reduction, urease, Voges Proskauer, methyl red and sugar fermentation tests.

2.3 Antibiogram study: On Muller-Hinton agar, 17 isolates were tested for antimicrobial susceptibility against 11 commonly used antibiotics from different groups using the disc diffusion method or the Kirby-Bauer method (Bauer *et al*., 1966) [8]. Antibiotics with the following concentrations and zones of inhibition as determined by the Clinical and Laboratory Standard Institute (CLSI, 2022) [9]. Gentamicin (10 µg), levofloxacin (5 µg), tetracycline (30 µg), ciprofloxacin (5 µg), ampicillin (10 µg), oxytetracycline (30 µg) nalidixic acid (30 µg), chloramphenicol (30 µg), ceftriaxone (30 µg), doxycycline (30 µg), neomycin (30 µg). By using a sterile inoculating loop a single bacterial colony was taken from the surface of SS agar was removed and 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 antimicrobial discs were plated onto Muller Hinton agar using sterile forceps. The plates were incubated overnight at 37 ℃. After incubation, the plates were examined, and the diameters of the inhibition zones were measured. Susceptibility and resistance were determined (Table 2).

3. Results and Discussion

The current study aimed to isolate, characterize, and determine antibiotic resistance of *Salmonella* spp. isolated from country chicken from various rural areas in and around Hyderabad. Cultural and staining characteristics, motility, and biochemical tests all confirmed that the isolates were *Salmonella*. The antibiotic sensitivity and resistance patterns of the isolates were identified in this study. After 24 hours of incubation at 37 ℃, Cloacal swab samples inoculated into nutrient broth revealed the growth of bacteria. The presence of turbidity and cultural characteristics on different agars indicated bacterial growth. The thin smears prepared with the colony from SS agar, MC agar and BG agar for Gram's staining revealed Gram-negative, pink colored, small rod-shaped appearance, arranged in single or paired under the microscopic examination. When the isolates were examined under a microscope using a hanging drop slide, they were all found to be motile. All the isolates were Methyl-red positive, Voges-Proskauer and Indole negative, fermented dextrose, maltose and mannitol with the production of acid but did not ferment lactose and sucrose. Acid production was indicated by the color change from reddish to yellow.

Eleven (7.3%) out of 150 fecal samples and six (6%) out of 100 cloacal swabs yielded positive results. Overall, *Salmonella* prevalence was found to be 6.8% (Table 1). The results were in accordance with the report of Samanta *et al* (2014) [10]. However, the study by *Das et al* (2017) [11] found 13.24% prevalence, which was nearly twice as high as the current study, and the study by Manning *et al* (2015) [12] found 10.4% isolation rate.

Table 1: Prevalence of *Salmonella* in cloacal swabs and faeces sample of country chicken

S. No	Source	No of samples	No of positive samples	Percentage
1	Cloacal	100	6	6%
2	Faeces	150	11	7.3%
Total		250	17	6.8%

Table 2: Antimicrobial sensitivity pattern of *Salmonella* isolates

S. No	Antimicrobial agents	Symbol	Strength (µg)	Number (%) of sensitive isolate	Number (%) of Intermediate isolate	Number (%) of resistant isolate
1	Ampicillin	AK	10	13 (76.4%)	1 (5.8%)	3 (17.6%)
2	Tetracycline	T	30	1 (5.8%)	2 (11.7%)	14 (82.3%)
3	Chloramphenicol	C	30	5 (29.4%)	10 (58.8%)	2 (11.7%)
4	Ciprofloxacin	CIP	10	16 (94.1%)	1 (5.8%)	0
5	Neomycin	N	30	6 (35.2%)	8 (47.05%)	4 (23.5%)
6	Levofloxacin	LF	5	3 (17.6%)	5 (29.4%)	9 (52.9%)
7	Gentamycin	GE	10	2 (11.7%)	13 (76.4%)	2 (11.7%)
8	Doxycycline	DX	30	4 (23.5%)	6 (35.2%)	7 (41.1%)
9	Oxytetracycline	O	30	0	0	17 (100%)
10	Erythromycin	E	15	3 (17.6%)	4 (23.5%)	10 (58.8%)
11	Ceftriaxone	CI	30	12 (70.5%)	2 (11.7%)	3 (17.6%)

Eleven different antibiotics were employed, each at a different therapeutic concentration. All isolates showed higher resistance (100%) to oxytetracycline, followed by tetracycline (82.3%), erythromycin (58.8%), levofloxacin (52.9%), and doxycycline (41.1%). These findings are consistent with the observations made by Samanta *et al.* (2014) [10] regarding isolates showing resistance to oxytetracycline (100%) and tetracycline. Bhuvaneswari *et al.* (2016) [13] also found nearly sixty percent of the *Salmonella* isolates were resistant to erythromycin. The highest sensitivity was revealed by isolates against ciprofloxacin (94.1%), ampicillin (76.4%), and ceftriaxone (70.5%). The higher sensitivity observed in the current study against ciprofloxacin is similar to that reported in previous studies by Bhuvaneswari *et al.* (2016) [13]. The isolates were intermediately sensitive to gentamycin (76.4%), chloramphenicol (58.8%), neomycin (47.05%). In contrast, Arora *et al.* (2013) [14] reported that most of the isolates were sensitive to gentamycin (76%).

4. Conclusion

The presence of *Salmonella* isolates in native chickens in and around Hyderabad was determined in present study. *Salmonella* isolates have demonstrated higher resistance to oxytetracycline and tetracycline. This indicate that *Salmonella* had developed antibiotic resistance. So one should be careful regarding selection of antibiotics in case of backyard poultry. All things considered, it is clear that antibiotic resistance and *Salmonella* prevalence in chickens are serious issues. Furthermore, Salmonellosis is extremely important for public health.

5. Acknowledgement

The authors would like to sincerely thank the University authorities for providing necessary facilities to carry out the present research work.

6. References

1. Sonkar N, Singh N, Santra AK, Verma LP, Soni A. Backyard poultry farming: A source of livelihood and food security in rural India. Pharm. Innov. J; c2020. p. 28-32.
2. DHAD. 20th Livestock census - Ministry of Fisheries, Animal Husbandry and Dairying, India; c2019.
3. Singh KD, Kumar S, Pramanik PS, Kashyap SS, Srivastava AK, Kendra KV, *et al*. Backyard poultry farming – a sustainable tool for rural livelihood.
4. Kumar M, Dahiya SP, Ratwan P. Backyard poultry farming in India: A tool for nutritional security and women empowerment. Biological Rhythm Research. 2021;52(10):1476-1491.
5. Megha SB, Madhavaprasad CB, Karabasanavar N, Achur RN, Shilpa AG, Bagalkote PS, *et al*. Isolation and characterization of *Salmonella*e from backyard poultry. Frontier Journal of Veterinary and Animal Sciences. 2016;5(1):21-23.
6. Jafari RA, Ghorbanpour M, Jaideri A. An investigation into *Salmonella* infection status in backyard chickens in Iran. Int J Poult Sci. 2007;6(3):227-229.
7. Obi OJ, Ike AC. Prevalence and antibiogram profile of *Salmonella*e in intensively reared and backyard chickens in Nsukka Area, Nigeria. Nigerian Journal of Biotechnology. 2015;30:18-25.
8. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am. J Clin. Pathol. 1966;45(4):493-496.
9. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 32th ed. Wayne, PA: CLSI Supplement M100, Clinical and Laboratory Standards Institute; c2022.
10. Samanta I, Joardar SN, Das PK, Sar TK, Bandyopadhyay S, Dutta TK, *et al*. Prevalence and antibiotic resistance profiles of *Salmonella* serotypes isolated from backyard poultry flocks in West Bengal, India. Journal of Applied Poultry Research. 2014;23(3):536-545.
11. Das D, Panda SK, Jena B, Nanda H. Conventional and molecular detection of *Salmonella* species in backyard poultry of Odisha state in India. J Entomol Zool Stud. 2017;5:837-840.
12. Manning J, Gole V, Chousalkar K. Screening for *Salmonella* in backyard chickens. Preventive veterinary medicine. 2015;120(2):241-245.
13. Bhuvaneswari M, Santhanam Shanmughapriya, Natarajaseenivasan, Kalimuthusamy. Prevalence of Multi drug-Resistant (MDR) *Salmonella* enteritidis in Poultry and Backyard Chicken from Tiruchirappalli, India. Microbiology Journal. ISSN 2153-0696. 2015;5:28-35. 10.3923/mj.2015.28.35.
14. Arora D, Kumar S, Singh D, Jindal N, Mahajan NK. Isolation, characterization and antibiogram pattern of *Salmonella* from poultry in parts of Haryana, India. Adv Anim Vet Sci. 2013;1(5):161-163.