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Research Article

Antimicrobial Resistance Pattern of *Salmonella* spp. Isolates of Broiler Production System

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ABSTRACT

Broiler's production systems are often found to be a proliferating place for Salmonella spp. The present study was planned with objective to determine the antimicrobial resistance pattern of Salmonella spp. Isolates of broiler production system in and around Parbhani city, Maharashtra in India. A total of 216 samples comprising of 36 samples from each 6 different sources were collected and analysed. A total of 6 isolates were confirmed by biochemical characterization. All the 6 biochemically confirmed isolates were further analysed for their antimicrobial resistance pattern with disk diffusion method against 15 different antibiotics amongst with Erythromycin and Cephalothin were found to be (100%) resistant followed by ceftazidime and Amikacin (66.66%) and Amoxiclav (50%). This resistance pattern of Salmonella spp. Isolates indicates a threat to the public health aspect of the end consumer which is a greater concern for physicians.

Keywords: *Salmonella, Broiler, Antibiotic-resistance, Poultry, Food-borne.*

INTRODUCTION

Poultry meat is an important food and economically inseparable aspect of the food industry. Broiler meat is comparatively cheap and has a rich source of valuable proteins essential for growth, wear and tear of the body. This has led to intensive broiler production on large scale and has also contributed a lot to the nation's growth both economically and nutritionally.

Along with rampant expansion of poultry rearing and farming, horizontal transmission of *Salmonella* is frequently found following consumption of water and food contaminated with droppings of infected birds in a flock along with handlers and environmental sources (Agada, 2014).

Antibiotics readily available to the producers over the counter without prescription and its administration without proper knowledge has already made various bacterial agents resistant. Many resistance genes have been identified on mobile genetic elements such as plasmids, transposons, and integrons.

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Movement of these elements then promotes the dissemination of resistance genes among bacteria (Diarrassouba et al., 2007). Industry has been using various antibiotics conveniently as it suits economically making *Salmonella* resistant. Due to plasticity of these bacteria, they have adapted and developed mechanisms to resist the effects of antibiotics using genetic strategies such as gene mutations or acquisition of resistance genes by horizontal transfer (Herrera-Sánchez et al., 2020). With time, *Salmonella* strains isolated from the broiler farm and its environment are evidently identified as multidrug resistant (Akond et al., 2013). Transmission of resistant bacteria enclosing resistant gene from farm to fork rendering further therapeutic administration to clinically conditions ineffective. Presently antibiotic resistance has been marked as an extremely important public health and food safety disaster. *Salmonella* spp. In the broiler production chain and its, antibiotic resistance pattern is comprehensively studied.

MATERIALS AND METHODS

This Study was carried out in the Department of Veterinary Public Health and Epidemiology, College of Veterinary and Animal Sciences, Parbhani – 431402, Maharashtra Animal and Fishery Sciences University, Nagpur. Maharashtra. Throughout the period of November to January, 2021. A total of 216 samples, 36 from each of the sources (Cloacal swab, Worker hand swab, Utensil swab, feed water and litter) were collected from a total of 5 broiler units randomly selected in and around Parbhani city. Details of the samplings are shown in Table 1.

Isolation & Identification

Salmonella spp. isolation was done on XLD agar (Himedia laboratories, Mumbai) following procedures as per IS-5887 Part 3 (1999). Presence of slightly transparent read halo and a black centred colony was considered as *Salmonella* spp (Plate 2). The isolates were further maintained on nutrient agar (Himedia laboratories, Mumbai) for further studies. Isolates presenting typical morphological characters were subjected to biochemical tests those confirmed were studied for their Antimicrobial resistance pattern.

Antimicrobial resistance pattern study

Antibiotic sensitivity test was done using disc diffusion method (Bauer et al., 1966) using Mueller-Hinton agar (Himedia laboratory Mumbai).

The Mueller-Hinton agar was prepared as per the directions of manufacturer. After autoclaving the media at 121°C for 15 min., it was cooled to 50°C and approximately 30 to 50 ml was poured into the petri dishes. The depth of the agar in the petri dish was maintained approximately at 4 mm.

The Salmonella spp. isolates were grown in nutrient broth and overnight grown was used as inoculum.

The dried surface of a Mueller-Hinton agar plate was inoculated by streaking with inoculated suspension over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure a smooth distribution of inoculum. As a final step the rim of the agar was swabbed. The plate was left open for 3 - 5 min. to allow for any excess surface moisture to be absorbed before applying the drug impregnated disc.

The predetermined battery of antimicrobial discs was dispensed onto the surface of the inoculated agar plate. Each disc was pressed down gently to ensure complete contact with the agar surface. The disc placed in the agar surface was not closer than 24 mm from centre to centre. A total of 5 discs were placed on one 150 mm plate. The inoculated plates were incubated at 16-18 hrs at 37°C.

After incubation, each plate was examined for the development of zone of inhibition surrounding the antimicrobial disc. The diameters of the zones of complete inhibition (judged by the unaided eye) were measured, including the diameter

of the disc. Zones were measured using sliding calipers, which were held on the back of the inverted petri plate. The petri plate was held a few inches above a black, non-reflecting background and illuminated with reflected light. Transmitted light from the colony counter was used to examine the zones for light growth wherever indicated, within apparent zones of inhibition. The zone margin was taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, was ignored. The size of zones of inhibition was interpreted by zone diameter of standard Salmonella and Enterobacteria as per chart given in Hi Media laboratory manual; Mumbai based on (Clinical & Laboratory Standards Institute, 2015) and organisms were reported as resistant, intermediate and sensitive to the antimicrobial agents that have been tested.

RESULTS AND DISCUSSION

A total of six morphologically and biochemically confirmed Salmonella spp. isolates were screened against all major groups of antibiotics commonly used. Diameters of zone of inhibition were measured and classified into sensitive, intermediate and resistant categories as per (Clinical & Laboratory Standards Institute, 2015). The results are presented in Table 2 and Plate 2

In present study a total of 15 commonly used antibiotics were screened by disc diffusion method against all six Salmonella spp. isolates. All six (100%) isolates were found to be sensitive to Levofloxacin, Tetracycline, Chloramphenicol and Ciprofloxacin followed by Amoxicillin/sulbactam (83.33%), Gentamycin (50%), Enrofloxacin (33.33%) and Nalidixic acid (16.66%). Earlier many workers found similar pattern of sensitivity against these antibiotics (Diarrassouba et al., 2007; M. E., 2014; Mridha et al., 2020; Rayamajhi et al., 2010; Shang et al., 2018; Sohan Rodney Bangera et al., 2019; Waghamare et al., 2018; & Zhao et al., 2016) Table 3.

All six isolates were found to be intermediately sensitive to Nalidixic acid (83.33%), Cefotaxime (83.33%), Enrofloxacin (66.66%), Ampicillin/sulbactam (66.66%), Amoxiclav (50%), Gentamycin (50%), Ceftazidime (33.33%), Amikacin (33.33%), Ciprofloxacin (16.66%), Amoxicillin/ sulbactam (16.66%). Earlier (Akond et al., 2013) while studying drug resistance pattern of Salmonella spp. isolated from poultry production system also reported presence of intermediate antibiotic sensitivity of Salmonella spp. isolates from 10 to 30 % against commonly used antibiotics. The results in present study are also in conformity of earlier findings.

Antibiotic resistance in Salmonella spp. isolates is a emerging phenomenon. Multidrug resistance in about 14.5% Salmonella isolates was reported by (Diarrassouba et al., 2007). In present study also multidrug resistance was observed in all six (100%) isolates against Erythromycin and Cephalothin whereas 66.66% isolates showed against Ceftazidime and Amikacin. About 50% isolates showed resistance against Amoxicillin/sulbactam. Earlier (Rayamajhi et al., 2010) also reported that 19.78% isolates were resistant to three or more antibiotics and 42.8% were resistant to two antibiotics. Similar type of resistance pattern was observed by many workers (Diarra et al., 2014; Dogru et al., 2010; Herrera-Sánchez et al., 2020; Shang et al., 2018; Thakur et al., 2013; & Waghamare et al., 2018). The observation of present study are on similar lines. Development of antibiotic resistance amongst Salmonella spp. Isolates in broiler production systems in and around Parbhani city is important from public health point of view.

Table 1: Details of samples collected from various sources for *Salmonella* spp. Isolation

Sr.no	Sources	Farms						Total No of samples
		I	II	III	IV	V	VI	
1	Cloacal Swabs	6	6	6	6	6	6	36
2	Litter	6	6	6	6	6	6	36
3	Feed	6	6	6	6	6	6	36
4	Water	6	6	6	6	6	6	36
5	Workers hand Swabs	6	6	6	6	6	6	36
6	Utensils	6	6	6	6	6	6	36
	Total	36	36	36	36	36	36	216

Table 2: Details of antibiotic sensitivity pattern of *Salmonella* spp. isolates

Sr. No.	Isolate	Antibiotic sensitivity*														
		LE	TE	C	E	CIP	A/S	GEN	AMS	EX	NA	CAZ	AK	AMC	CEP	CTX
1	FE/LT/03	S	S	S	R	S	I	S	S	S	S	I	I	I	R	I
2	FE/CS/05	S	S	S	R	S	I	S	S	S	I	R	R	R	R	R
3	FE/CS/06	S	S	S	R	S	I	S	S	I	I	R	I	R	R	I
4	FA/CS/03	S	S	S	R	I	R	I	I	I	I	R	R	I	R	I
5	FC/LT/01	S	S	S	R	S	R	I	S	I	I	R	R	I	R	I
6	FA/LT/01	S	S	S	R	S	I	I	S	I	I	I	R	R	R	I
	Total No. of Sensitive isolates	6	6	6	0	6	0	3	5	2	1	0	0	0	0	0
	% Sensitive isolates	100	100	100	0	100	0	50	83.33	33.33	16.66	0	0	0	0	0
	Total No. of Intermediate sensitive isolates	0	0	0	0	1	4	3	1	4	5	2	2	3	0	5
	% Intermediate sensitive isolates	0	0	0	0	16.66	66.66	50	16.66	66.66	83.33	33.33	33.33	50	0	83.33
	Total No. of Resistant isolates	0	0	0	6	0	2	0	0	0	0	4	4	3	6	1
	% Resistant isolates	0	0	0	100	0	33.33	0	0	0	0	66.66	66.66	50	100	16.66

* Classified as per CLSI standard

S- Sensitive, I- Intermediate and R- Resistant

LE - Levofloxacin, TE - Tetracycline, C – Chloramphenicol, E – Erythromycin, CIP – Ciprofloxacin, A/S – Ampicillin/Sulbactam, GEN – Gentamycin, AMS - Amoxicillin/Sulbactam, EX – Enrofloxacin, NA - Nalidixic Acid, CAZ – Ceftazidime, AK – Amikacin, AMC- Amoxiclav, CEP – Cephalothin, CTX – Cefotaxime

Table 3: Frequency of antibiotic resistance among *Salmonella* spp. isolates by disc diffusion method

Sr. No.	Group	Antibiotics	Disc content (mcg)	Percentage of resistant *Salmonella* spp. isolates (%) (n=6)
1	ß Lactam	Ampicillin/Sulbactam	10/10	33.33
		Amoxicillin/Sulbactam	30/15	0
		Amoxiclav	30	50.00
2	ß Lactamase inhibitors	Tetracycline	30	0
3	Aminoglycosides	Gentamycin	10	0
		Amikacin	30	66.66
4	Fluoroquinolones	Levofloxacin	5	0
		Ciprofloxacin	5	0
		Enrofloxacin	5	0
		Nalidixic Acid	30	0
5	Cephalosporins	Ceftazidime	30	66.6
		Cephalothin	30	100
		Cefotaxime	30	83.33
6	Macrolide	Erythromycin	15	100
7	Chloramphenicol	Chloramphenicol	30	0

Plate 1: Typical Salmonella spp. Colonies with red halo and black centre on XLD agar

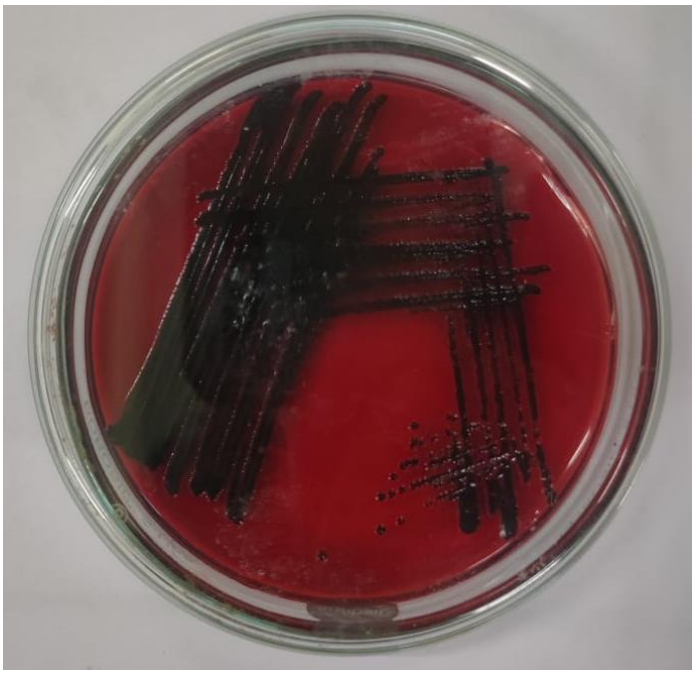
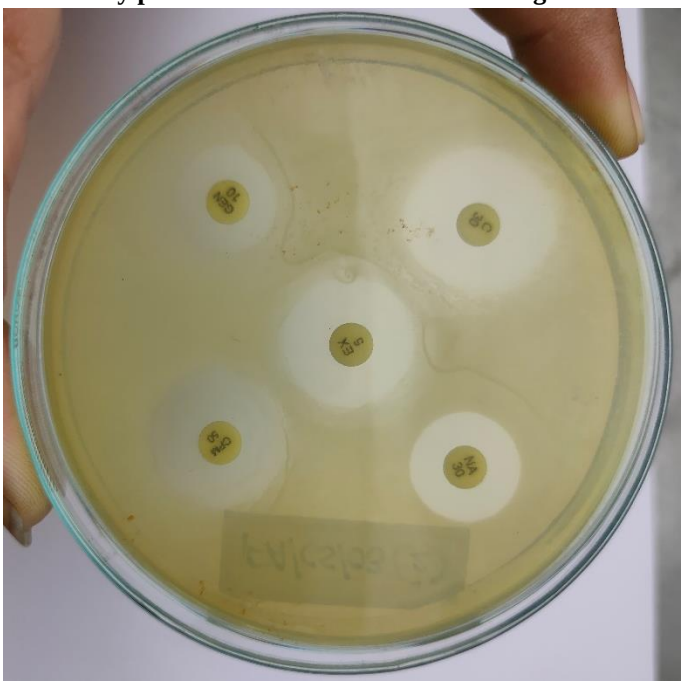


Plate 2: Antibiotic sensitivity pattern of the selected antibiotics against Salmonella spp. Isolates



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