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Full Length Research Paper

Characterization and antibiogram of *Salmonella* spp. from poultry specimens

R. Selvaraj[1], R. Das[1], S. Ganguly[2], M. Ganguli[3], S. Dhanalakshmi[3] and S. K. Mukhopadhayay[3]*

[1]Department of Veterinary Microbiology, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences (WBUAFS), Kolkata 700 037, India.

[2]AICRP on Post Harvest Technology in Department of Fish Processing Technology, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences (WBUAFS), Kolkata 700 094, India.

[3]Department of Veterinary Pathology, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences (WBUAFS), Kolkata 700 037, India.

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The present study was done for characterization and antibiotic sensitivity pattern of the *Salmonella* spp. found as contaminants in chicken carcass. The overall incidence of *Salmonella* contamination of poultry carcass was found to be 4.90% with the higher percentage of *Salmonella* being isolated from chicken meat (8.00%) followed by liver and spleen (6.25%). The isolates were identified as Salmonella *Typhimurium*, Salmonella *Paratyphi* B and *Salmonella* (Rough). Eight *Salmonella* isolates obtained from poultry were confirmed as *Salmonella* spp. according to their biochemical profile and their sensitivity to different antimicrobial agents. Amikacin, kanamycin and ciprofloxacin were found to be the most effective antibiotics against *Salmonella* spp.

Key words: Characterization, *Salmonella* spp., chicken carcass, antibiotic sensitivity.

INTRODUCTION

Salmonellosis is one of the most common infectious diseases in world in both humans and animals. *Salmonella* spp. causes wide range of diseases such as enteric fever, gastroenteritis and bacteriemia. Food borne infections caused by *Salmonella* serotypes occurs at high frequency in industrialized nations and developing countries and is an important public health problem worldwide.

Salmonella spp. is Gram negative, usually motile rods. The bacterial genus *Salmonella* is divided into two species *Salmonella enterica* and *Salmonella bongori*; *Salmonella enterica* itself is comprised of 6 subspecies. They are *S. enterica* subsp. *enterica*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *indica*, *S. enterica* subsp. *houtenae* or I, II, IIIa, IIIb, IV and VI, respectively (Popoff and Minor, 1997).

Approximately, 50% *Salmonella* infections are caused by only three serovars, specifically *Typhimurium*, *Enteritidis* and Newport. In India, *Typhimurium* and *Enteritidis* are the two most common serotypes identified from different sources. *Salmonella* serotype *Enteritidis* is currently the main cause of human Salmonellosis in most industrial countries where human infections are generally associated with the consumption of contaminated food. Salmonellosis, because of its public health significance, has become one of the most important bacterial diseases affecting poultry. Epidemiological studies have shown the outbreaks of Salmonella *Enteritidis* food poisoning to the consumption of contaminated eggs, egg products or meat (St. Louis et al., 1988). For this reason, presence of *Salmonella* in foodstuffs has always been the most significant indicator of their hygienic acceptability or their unsuitability for human consumption. Comparison of *Salmonella* isolates from different sources helps in

*Corresponding author. E-mail: skmpath@gmail.com.

Table 1. *Salmonella* spp. isolated from poultry samples.

S/No.	Source	Number of test sample	Number of positive sample	Number of isolate	Percentage
1.	Intestine and intestinal contents	38	2	2	5.26
2.	Liver and spleen	32	2	2	6.25
3.	Heart	23	ND	ND	ND
4.	Kidney and gall bladder	28	1	1	3.57
5.	Dressed chicken meat	25	2	2	8.00
6.	Egg shell wash	17	1	1	5.88
7.	Total	163	8	8	4.90

ND: No data.

ascertaining the origin of infection.

The present study was done for the isolation and characterization of *Salmonella* spp. from chicken by using enrichment and selective media and their identification by conducting different biochemical tests along with studies on the their antibiotic sensitivity an resistance patterns.

MATERIALS AND METHODS

Intestine and intestinal contents, heart, liver, spleen, kidney, gall bladder of chicken and egg shell wash from poultry eggs were collected as specimens for study from different commercial poultry markets.

The samples collected were inoculated into two enrichment media *viz.* Tetrathionate broth and Selenite F broth. These tubes were incubated for 18 h at 37°C. Later, the cultures from enrichment media were streaked on to the brilliant green agar (BGA) and Mc Conkey's agar plates and were incubated at 37°C for 24 - 48 h. The typical colonies of *Salmonella* spp. were picked up from brilliant green and Mc Conkey agar plates and further streaked on Mc Conkey's agar plates for obtaining only single type of colonies. The colorless, translucent to opaque colonies with generally irregular edges were suggestive of pure *Salmonella* colonies and these were transferred to Nutrient agar slants. Two slants of each isolate were kept at 4°C in refrigerator for further studies.

The isolated colonies were identified on the basis of morphology, cultural characters and their biochemical profile according to Edwards and Ewing (1972).

Gram's staining: The test organisms were stained by Gram's method to determine their staining characteristics and purity of the culture. By this method all isolates were observed for Gram negativity, shape, size, conformation, arrangement patterns, capsule, spore formation etc.

Isolates of *Salmonella* were identified by IMViC reaction, TSI reaction, urease test, H_2S production test, Nitrate reduction test and other fermentative or non-fermentative sugar tests as per methods described by Cruickshank et al. (1975).

All the isolates of *Salmonella* strains were serotyped by using polyvalent O sera in the laboratory. ABST of test isolates was performed according to Edwards and Ewing (1972).

RESULTS

Out of a total of 163 samples collected from different parts of poultry carcasses, only eight samples were positive for *Salmonella* spp. The incidence of *Salmonella* contamination of chicken carcass was 4.90%. The incidence of contamination of poultry carcasses by *Salmonella* spp. are presented in Table 1. The higher percentage of *Salmonella* spp. were isolated from chicken meat (8.00%) followed by liver and spleen (6.25% each), egg shell wash (5.88%), intestine and intestinal contents (5.26%), kidney and gall bladder (3.57%). The heart samples did not show any *Salmonella* contamination. A total of 155 specimens from chicken were negative for *Salmonella* contamination.

Total eight positive *Salmonella* isolates were found from the poultry specimens. It was observed that out of the 8 isolates, only two were non- motile and all the isolates of *Salmonella* spp. fermented glucose within 24 h with the production of acid and gas. Sucrose, lactose, salicin and adonitol were not fermented by any of the strains. All the *Salmonella* isolates were indole negative, MR test negative and VP test negative, but were positive to nirate reduction and citrate utilization tests as similar to those of *Salmonella* spp. The results of biochemical profile of the isolates were confirmed to belong to *Salmonella* spp. according to Edwards and Ewing (1972). Biochemical test profile of the eight isolates is presented in Table 2.

All the eight isolates were serotyped and are presented in Table 3. Out of the eight isolates serotyped, 12.50% samples were found to be *S. Paratyphi* B, 50.00% samples were *S. Typhimurium* and 37.50% samples were rough culture of *Salmonella*. The Salmonella serovars found in chicken are presented in Table 3.

All the eight *Salmonella* isolates were tested for antibiotic sensitivity test to 7 different antibiotics which are presented in Table 4. Amikacin, kanamycin and ciprofloxacin were the most effective (100% effectivity of each) followed by chloramphenical (87.50%), oxytetracycline and ceftriaxone (62.50% each) and cafataxime (50.00%). Intermediate sensitivity was found towards cefotaxime, oxytetracycline and ceftriaxone (37.50% each) and chloramphenicol (12.50%). Resistance was found against cefotaxime only. The

Table 2. Biochemical profile of *Salmonella* isolates.

S/No.	Biochemical test	Positive	Percentage	Negative	Percentage
1.	Indole	0	ND	8	100.00
2.	Methyl red	8	100.00	0	0.00
3.	Voges Proskuer	0	ND	8	100.00
4.	Citrate	8	100.00	0	0.00
5.	TSI	8	100.00	0	0.00
6.	H_2S production	8	100.00	0	0.00
7.	Nitrate reduction	8	100.00	0	0.00
8.	Glucose	8	100.00	0	0.00
9.	Sucrose	0	ND	8	100.00
10.	Lactose	0	ND	8	100.00
11.	Salicin	0	ND	8	100.00
12.	Adonitol	0	ND	8	100.00
13.	Motility	6	75.00	2	25.00

Table 3. Isolated and identified *Salmonella* serovars.

S. no.	Serovar	Antigenic structure	Number of isolate	Percentage of isolate
1.	*S. Paratyphi* B	4,5,12:b:1,2	1	12.50
2.	*S. Typhimurium*	4,5,12:i:1,2	4	50.00
3.	*Salmonella*	Rough	3	37.50
	Total		8	100.00

Table 4. Antibiogram of *Salmonella* isolates.

S/No.	Antimicrobial agents	Symbol	Strength (mcg)	Number of sensitive isolate	Percentage of sensitive isolate	Number of Intermediate isolate	Percentage of intermediate isolate	Number of resistant isolate	Percentage of resistant isolate
1.	Amikacin	AK	30	8	100.00	0	ND	0	ND
2.	Kanamycin	K	30	8	100.00	0	ND	0	ND
3.	Oxytetracycline	O	30	5	62.50	3	37.50	0	ND
4.	Chloramphenicol	C	30	7	87.50	1	12.50	0	ND
5.	Ciprofloxacin	CF	5	8	100.00	0	ND	0	ND
6.	Ceftriaxone	CI	30	5	62.50	3	37.50	0	ND
7.	Cefotaxime	CFX	5	4	50.00	3	37.50	1	12.50

ND: no data and mcg: microgram.

antibiotic sensitivity of the *Salmonella* isolates is presented in Table 4.

DISCUSSION

In the present study, the incidence of *Salmonella* contamination of poultry carcasses (4.90%) was much lesser than as reported by Zivkovic et al. (1997). The level of contamination of liver and spleen (6.25%) was significantly lower than the level (23.11%) as reported by Zivkovic et al. (1997) but higher than the reports (2.80%) by Ozbey et al. (2006). Purushotaman et al. (1996) had reported that *S. Typhimurium* was the commonest serotype isolated from poultry and its environment which is in accordance to the present investigation.

The isolates showed highest antibiotic sensitivity to ciprofloxacin (100.00% sensitivity) which was in correlation to the reports of Zahrei et al. (2005). The next highest sensitivity was found to chloramphenical as reported by Hui and Das (2001). Sensitivity found to oxytetracycline (62.50%) was similar to that of reports of Anjanappa et al. (1995).

Conclusion

From the present study it was concluded that *Salmonella* spp. were isolated from 4.90% of the 163 samples comprising of different poultry organs. The majority of the isolates belonged to *S. Typhimurium* serotype. Antibiogram suggested that amikacin, kanamycin and ciprofloxacin showed the maximum potential to be used as promising antimicrobial agents against *salmonella* infections.

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REFERENCES

Anjanappa M, Verma JC, Harbola PC (1995). *In vitro* antibacterial sensitivity of *Salmonella Gallinarum* isolates. Indian J. Comp. Microbial. Immunol. Infect. Dis., 16: 79-81.

Cruickshank R, Duguid JP, Marmion BP, Swain RHA (1975). Medical Microbiology, 12th edn, Churchill Livingstone, London, 2: 236, 432-434.

Edwards PR, Ewing WH (1972). Identification of Enterobacteriaceae (3rd edn.), Burgess Pub. Co., Minneapolis.

Hui AK, Das R (2001). Studies on isolation, serotyping and antibiotic sensitivity of *Salmonella* isolated from ducks. Indian Vet. J., 78(11): 1058-1059.

Ozbey G, Ertas HB (2006). *Salmonella* spp. iaolation from chicken samples and identification by polymerase chain reaction. Bulgerian. J. Vet. Med., 9(1): 67-73.

Popoff MY, Minor LL (1997) Antigenic formulas of the *Salmonella* serovars, 7th revision. W.H.O. Collaborating Centre of Reference and Research on *Salmonella* Institute Pasteur, Paris, France.

Purushotaman V, Premkumar DB, Venkatesan RA (1996). Comparison of plasmid profile analysis, serotyping, biotyping and antimicrobial susceptibility testing as epidemiological tools in the strain identification of *Salmonella* isolates from avian source. Indian J. Anim. Sci., 66(5): 419-430.

St Louis ME, Morse DL, Potter ME, DeMelfi TM, Guzewith JJ, Tauxe RV, Blake PA (1988). The emergence of grade A eggs as a major source of *Salmonella enteritidis* infections. New implications for the control of Salmonellosis, J. Am. Med. Assoc., 259: 2103-2107.

Zahrei ST, Mahzounish M, Saeedzadeh (2005). The isolation of antibiotic resistant *Salmonella* from intestine and liver of poultry in Shiraz province of Iran. Int. J. Poult. Sci., 4(5): 320-322.

Zivkovic J, Jacsic S, Miolovic B (1997). *Salmonella* serovars in chicken meat and chicken meat products in Zagrep, Croatia. Vet. Archive, 67(4): 169-175.