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Microbiology

ISOLATION AND IDENTIFICATION OF *SALMONELLA* FROM POULTRY MEAT SAMPLES – A MAJOR FOOD BORNE ZOONOTIC DISEASE

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ABSTRACT Poultry products are easily contaminated by various microbial sources. The main objective of this present investigation is to determine the presence of *Salmonella* in the poultry meat samples. A total of seventy two chicken meat samples were included for the microbiological determination and analysis of *Salmonella* sp. The meat samples that are showing lesions were inoculated into nutrient broth and tetrathionate broth. Further direct broth gram staining, isolation of *Salmonella* in selective media, biochemical tests were done for the confirmation of the bacterial pathogen. Out of seventy two samples, thirty two confirmed the presence of *Salmonella* infection. Antibiotic susceptibility test provided the idea to choose the antibiotics to treat the hen highlighted that gentamycin and ciprofloxacin are effective. Further, the public health department has to take necessary initiative to investigate the poultry samples for the determination of infectious agents for avoiding the spread of such enteropathogenic infections to humans.

KEYWORDS : Poultry, *Salmonella*, antibiotic resistance

Introduction

Due to widespread of importance of consuming poultry meat as a major source of protein, the intake of poultry meat is drastically increased. Infections with *Salmonella* species are playing a very important role in lodging in the animal reservoirs and transmit to humans while consuming such infected animal products (Amelie *et al.,* 2017). The freshly processed hen meat and packed and preserved meat also prone to get *Salmonella* as career to transmit Salmonellosis to humans (Arnold, 2009). *Salmonella* infection is being spread through the handling of raw poultry carcasses and products, together with the consumption of undercooked poultry meat (Panisello *et al.,* 2000).

The non typhoidal *Salmonella* species are associated with food borne infections of animal origin like eggs, milk, poultry meat, pig meat and beef; that are largely reported in developing countries like India (Kaushik *et al.,* 2014). The incidence and more outbreak rate of *Salmonella* infections in chicken meat and milk have been recorded from 6.8 to 97.6% and 0.2 to 28.7% respectively (Ramya *et al.,* 2012; Tajbakhsh *et al.,* 2012).

Among the healthy human subjects, the infectious dose is generally 10^6 to 10^8, but even lesser bacterial counts may cause disease in certain conditions including infants and the elderly who are less immune factors (Artunes *et al.,* 2016). The effective antibiotics are essential to treat such *Salmonella* gastroenteritic cases that are mostly resistant to available antibiotics (Pary and Threlfall, 2008; Chen *et al.,* 2013).

The major preventive measures of infectious diseases through processed foods at primary production are

1. Elimination of *Salmonella* in grandparent and parent flocks;
2. All-in all-out production at the broiler farm, to avoid any carry over during processing;
3. Logistic slaughter planning scheduled to avoid pathogens being transferred from contaminated processing equipment to another flock; and
4. Satisfactory cleaning procedures

At present, the epidemiology is not completely clear, and there are also regional, state and country differences to consider. Thus this study has an objective to determine the prevalence of *Salmonella* in the poultry meat samples.

Materials and Methods

Study area and Samples

The study was done in two districts of Tamilnadu (Pudukkottai and Tiruchirapalli). A simple random method was adopted to select and collect a total of sixty eight fresh chicken meat samples from different vendors between August 2017 and April 2018. Six fresh chicken meat samples were possible to collect from the two hotels, one night restaurant and three street vendors. All the samples were aseptically collected in the chemically cleaned and dried polyethelene bags and labelled appropriately. The samples were maintained on ice for transporting to the laboratory and processed microbiologically within one of collection.

Salmonella enrichment

Ten grams of the chicken meat were chopped into fine pieces aseptically and were pre-enriched in buffered peptone water and incubated at 37°C for 18 hours. After appropriate incubation, one ml of the cultured broth was transferred to selenite cystine broth (SCB) and the set up were incubated at 37°C for 24 hours. We provided the shaking and incubation for better growth and selection. The growth was preliminarily identified by Gram's staining in order to determine the presence of gram negative bacilli.

Selective plating

After 24 hours of shaking incubation of SCB, a loopful of culture was streaked on Nutrient agar, MacConkey agar, Brilliant green agar, Xylose lysine deoxycholate agar (XLD) and *Salmonella-Shigella* agar (SS) and incubated at 37°C for 24 to 48 hours for the determination of *Salmonella* specific colonies. The color of the colony in XLD medium as described in table 1 was impregnated to analyze the species of the *Salmonella*. Further, the same colony colored *Salmonella* species were differentiated by specific biochemical tests.

Table 1: Colony color determination of *Salmonella* species

Salmonella Species	Colony
S. enteritidis	Red with black centres
S. paratyphi A	Red
S. paratyphi B	Red with black centres
S. typhi	Red with black centres

Biochemical tests

For further confirmation of the *Salmonella* species biochemical tests including catalase, oxidase, motility, indole, methyl red, Voges-Proskeur, citrate utilization, urease and triple sugar iron agar tests were done and results were interpreted as described in table 2.

Table 2: Biochemical differences of *Salmonella* species

Biochemical test	*S. typhi*	*S. paratyphi A*	*S. paratyphi B*	*S. enterica*
Catalase	+	+	+	+
Citrate	-	-	+	-
Indole production	-	-	-	-
Methyl Red	+	+	+	+

Motility	+	+	+	+
Nitrate reduction	+	+	+	+
Oxidase	-	-	-	-
TSI	K/A with H_2S and no gas	K/A without H_2S and gas	K/A with abundant H_2S and gas	K/A with H_2S and gas
Urease	-	-	-	-
Voges Proskeur	-	-	-	-

[+ positive; - negative; TSI-Triple sugar iron; K-alkaline; A-acid; H2S-hydrogen sulphide]

Antibiotic sensitivity test

After confirmation of the Salmonella species from the chicken meat samples, the isolates were determined for the antibiotic sensitivity test by performing Kirby Bauer method using Mueller Hinton agar and gallery of nine antibiotics and the procedure was followed as per the CLSI guidelines. The table 3 determined the concentration of the antibiotics (Himedia) and interpretive criteria.

Table 3: Clsi Guidelines Of Antibiotic Susceptibility Interpretive Criteria

Antibiotics	Concentration/ disc	Interpretive criteria		
		Sensitive	Intermediate	Resistant
Ampicillin (AMP)	10 µg	≥ 15 mm	12-14 mm	≤ 11 mm
Amikacin (A)	20 µg	≥ 18 mm	15-17 mm	≤ 15 mm
Cefotaxime (CTX)	30 µg	≥ 26 mm	23-25 mm	≤ 22 mm
Ceftazidime (CAZ)	30 µg	≥ 21 mm	18-20 mm	≤ 17 mm
Ceftriaxone (CTR)	30 µg	≥ 23 mm	20-22 mm	≤ 19 mm
Ciprofloxacin (CIP)	5µg	≥ 21 mm	16-20 mm	≤ 15 mm
Imipenem (ERY)	10 µg	≥ 23 mm	20-22 mm	≤ 19 mm
Gentamycin (GEN)	10 µg	≥ 15 mm	13-14 mm	≤ 12 mm
Tetracycline (TE)	30 µg	≥ 15 mm	12-14 mm	≤ 11 mm

Results and Discussion

Out of 74 chicken meat samples collected from vendors, 72 samples were possible to process; sue to some technical and time consuming nature, two samples were discarded. All the samples were underwent for *Salmonella* specific culturing and characterized by biochemical tests. Typical black colored colonies surrounded by narrow green margin colonies were determined and further confirmed by various comparative biochemical characters. On Gram's staining, pink color rods were identified indicating gram negative bacilli. Table 4 described *Salmonella* spp on various culture media.

Table 4: Cultural characteristics of *Salmonella* species

Culture media	Colony morphology (color)	Confirmation
MacConkey agar	Colorless colonies	Lactose non fermenting colonies
Brilliant Green agar	Red colored colonies	*Salmonella* species
Xylose lysine deoxycholate agar	Red color colonies with black centres	*Salmonella* species
Salmonella-Shigella agar	Colorless colonies with black centre	*Salmonella* species

Further based on the biochemical tests, it was further confirmed as *Salmonella* species. Out of seventy two samples, 32 (44.4%) showed positive to *Salmonella* isolation (Figure 1). Among the 32 *Salmonella* isolates, *S. typhimurium* dominated with 14 isolates followed by *S. enterica, S. paratyphi* B and *S. typhi* with 9, 7 and 2 respectively. The detailed description of various species of *Salmonella* was impregnated in figure 2.

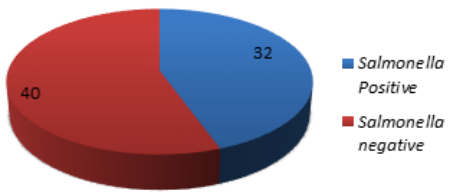

Figure 1: Confirmation of *Salmonella* isolates

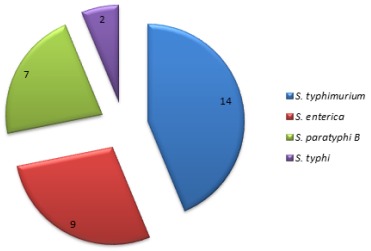

Figure 2: Determination of various *Salmonella* species

As described in detail in the text related to the antibiotic susceptibility test, the antibiotics like ampicillin, amikacin, cefotaxime and tetracycline showed maximum resistance towards all four species of Salmonella isolated. Further, ceftriaxone, ciprofloxacin, imipenem and gentamycin showed high sensitive towards all isolates. Only one antibiotic ceftazidime showed equal determination of sensitivity and resistance. The comparative analysis of various antibiotics verses sensitivity and resistance pattern of four various Salmonella isolates were impregnated in table 5.

Table 5: Comparative analysis of antibiotics verses sensitivity and resistance pattern

Antibiotic	Antibiotic susceptibility test of *Salmonella* species vs sensitivity/ resistance			
	S. typhimurium (n=14)	*S. enterica* (n=9)	*S. paratyphi* B (n=7)	*S. typhi* (n=2)
Ampicillin (AMP)	2/12 (14.3/85.7)	1/8 (11.1/88.9)	0/7 (0/100)	0/2 (0/100)
Amikacin (A)	1/13 (7.1/92.9)	0/9 (0/100)	0/7 (0/100)	0/2 (0/100)
Cefotaxime (CTX)	2/12 (14.3/85.7)	1/8 (11.1/88.9)	1/6 (14.3/85.7)	0/2 (0/100)
Ceftazidime (CAZ)	6/8 (42.8/57.2)	3/6 (33.3/66.7)	2/5 (28.6/71.4)	1/1 (50/50)
Ceftriaxone (CTR)	14/0 (100/0)	9/0 (100/0)	7/0 (100/0)	2/0 (100/0)
Ciprofloxacin (CIP)	13/1 (92.9/7.1)	8/1 (88.9/11.1)	7/0 (100/0)	2/0 (100/0)
Imipenem (ERY)	12/2 (85.7/14.3)	8/1 (88.9/11.1)	7/0 (100/0)	2/0 (100/0)
Gentamycin (GEN)	14/0 (100/0)	9/0 (100/1)	7/0 (100/0)	2/0 (100/0)
Tetracycline (TE)	2/12 (14.3/85.7)	1/8 (11.1/88.9)	1/6 (14.3/85.7)	1/1 (50/50)

[Figure in parenthesis denoted percentages]

Salmonellosis in poultry products especially chicken meat are found high in this study thereby it is considered as the important public health issue thereby human get infection through food borne infections. Previously large number of outbreaks was noticed related to human Salmonellosis by which increased number of infections described (Kumar *et al.,* 2010; Taddele *et al.,* 2012; Sudheer *et al.,* 2018). By this study, it was found that mainly the food of poultry origin is most common source of human salmonellosis, further hygienic practices and consumption of completely cooked chicken meat may reduce the infections (Kaushik *et al.,* 2014).

In the present study, *S. typhimurium* and *S. enteritidis* were able to

colonize the intestinal tract and to invade in the spleen after oral inoculation without causing clinical signs. The course of these *Salmonella* infection in poultry is mostly influenced by virulence, phage type, dose, route of infection and species of bird (Bierer, 1960; Brownell *et al.,* 1969; Hafez and Stadler, 1997; Langkabel *et al.,* 2014).

The present study demonstrated a considerably higher prevalence of *Salmonella* in poultry meat samples. These rates were comparably lower than the previous reports (Ivic Kolevska and Kocic, 2009; Eyigor *et al.,* 2010; Suo *et al.,* 2010) but higher than the reports (Aoust *et al.,* 2007; Regan *et al.,* 2008; Patel and Bhagwat, 2008). The absence of *Salmonella* were also observed in the non chicken meat samples was also observed (Patel and Bhagwat, 2008). From this study we have some conclusionary identification that many contributing factors, the differences in the detection rates of *Salmonella* between this study and other reports are as follows

1. Differences in the detection methods used in the studies
2. Strictness of hygiene and biosecurity policies used at the various sampling locations and
3. Sample type and product processing technology.

Moreover, antimicrobial resistance in non-typhoidal *Salmonella* is considered as the major public health threats that are related to food –animal production including poultry meat which is to be controlled at the earliest. With the increasing globalization of foodstuffs like poultry meat, new health problems and challenges might arise related to infection control, making integrated intervention strategies necessary for arresting the food chain (Antunes *et al.,* 2016). The prevalence of *Salmonella* in raw, precooked, partially cooked and cooked poultry meat as well as freeze thawed meat also observed high (Yaser *et al.,* 2017).

Further, this study will be extended to analyze the presence of cooked poultry meat samples which provide the information whether all the processing units processed perfectly to reduce the bacterial load and avoid the spread of *Salmonella* either direct or cross contamination.

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