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ORIGINAL ARTICLE

Prevalence and Characterization of Multidrug-Resistant Non-Typhoidal Salmonella in Poultry: Implications for Public Health and Food Safety

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ABSTRACT

This study investigates the prevalence of multidrug-resistant non-typhoidal Salmonella species in poultry meat sourced from Karaikudi, Tamil Nadu, India, aiming to address the escalating global issue of foodborne infections associated with antibiotic-resistant pathogens. Through biochemical tests, Salmonella was isolated and identified from flesh, intestine, liver, and spleen samples obtained from fresh retail chicken. Among the 200 samples examined, positive Salmonella isolates were found, with varying prevalence across different poultry organs: 28 from flesh, 42 from intestine, 15 from liver, and 25 from spleen. The results underscore the widespread occurrence of Salmonella in poultry, highlighting a significant public health concern. Moreover, the study reveals a concerning rate of multidrug resistance among the isolated Salmonella strains, exacerbating the gravity of the situation. Urgent measures are required to regulate antibiotic usage in the poultry industry and mitigate the emergence of resistant strains. Effective control strategies for salmonellosis necessitate comprehensive management systems, accurate identification of carrier birds, and precise medication protocols. By addressing these aspects, stakeholders can work towards reducing the prevalence of antibiotic-resistant pathogens in poultry meat, thereby safeguarding public health and promoting food safety.

Keywords*: Salmonella, multidrug resistance, poultry meat, non-typhoid, foodborne infections, antibiotic usage, public health,*

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INTRODUCTION

In last three decades, *Salmonella* spp. has become one of the major food borne pathogenic bacteria leading to high morbidity and mortality worldwide. *Salmonella* spp. causes an intestinal infection in humans known as Salmonellosis (1). The disease is endemic in most developing countries and may cause occasional outbreaks in industrialized countries (2). The global burden of food borne disease is currently unknown but the World Health Organization (WHO) has responded to this data gap by launching a new

initiative to provide better estimates. In last five years it was reported that 600 million, or almost 1 in 10 people in the world, fall ill after consuming contaminated food according to WHO. Poultry is one of the fastest growing agricultural sectors in India. Poultry meat and its products are very popular food in India as well as throughout the world. No wonder since it is delicious, nutritious and considered as a good and cheap source of protein characterized by good flavour and easily digested (www.apeda.gov.in). Poultry is a natural reservoir of *Salmonella* species, constituting the most important source of human infection. There is scope of transmission of *Salmonella* infection from poultry to human through food chain. Several studies have shown that sources of *Salmonella* infection in poultry and poultry products. In developing countries, most chicken meat is produced by integrated broiler operations. These broiler operations control and operate through all phases of the chicken industry, such as breeder flock management, hatchery operation, feed management, broiler slaughter, retail distribution and also handling by consumers (3,4,5). The current emergence of antimicrobial food borne pathogens is a major challenge in both human and veterinary medicine due to over- dependency of antimicrobial agents in the food and livestock sector (6). Poultry, especially broiler chicken, can use antibiotics used for extensively to prevent or treat microbial infections to the birds. Particularly *Salmonella* Spp. has become a concern because of the development of multiple antimicrobial resistance strains, emphasizing the importance of continuous monitoring of the pathogen according to CDC, 2014 (7). In light of their importance, the World Health Organization (WHO) has established risk assessments on *Salmonella* in broiler chickens. Although in India, data regarding food borne diseases associated with resistant pathogenic strains in poultry is lacking, it is considered to be significant. These drugs are used routinely in humans for the treatment of acute and severe diarrhoea, but recent studies have shown that infections with quinolone resistant *Salmonella* tend to be more severe and more often fatal compared with infections with sensitive strains.

MATERIAL AND METHODS

Sampling

The specimens were collected from retail broiler chicken shop in Karaikudi city, Tamilnadu, India. The selected parts of the Samples are intestine flesh liver and spleen of retail chicken were collected from different zones of Karaikudi (East, West, South and North) during the period between December 2022 to April 2023. Sites in the area from which samples were collected include Mathur, Iluppakkudi, Ariyakudi, Kattuthalaivasal, Kalanivasal, Water tank, Five lamps, corporation street and Koviloor.

Cultivation of samples

Bacteriological examine of different organs of retail chicken meat

Using sterile scissors, organs were individually cut into small pieces and enriched with Selenite broth in a ratio of 1:10, the cultures were incubated at 37°C overnight. Twenty-five grams of each sample were pre-enriched in 225 ml of phosphate buffered peptone water (Hi- Media, Mumbai) for 48-hr at 37°C. One-millilitre pre-enriched sample was transferred into 10 ml of tetra thionate broth and selenite cysteine broth and incubated for 48-hrs at 42°C, then a loopful culture was aseptically streaked on modified brilliant green sulphadiazine agar and Xylose- Lysine Deoxycholate agar (XLD). The plates were incubated at 37°C for 24 hrs (APHA 1992). At least five colonies were qualified as presumptive *Salmonella* colonies on modified brilliant green agar and XLD agar plates, (red colonies and red colonies with black centres, respectively) were then picked and sub-cultured on slants of nutrient agar.

Inoculation of plates

A loop of the inoculated selenite-f-broth was streaked on a plate of deoxycholate citrate agar and incubated aerobically at 37º C for 24hours.

Purification and storage of isolates

Non- lactose fermented colonies was purified by repeated subculture on nutrient agar. Pure isolates was stored on nutrient agar slopes in the refrigerator at 4º C.

Microscopic Examine:

Gram's stain

Smears were prepared from the culture by emulsifying a part of a colony in a drop of normal saline on a glass slide, dried and fixed by heating. Then the slides was flooded by crystal violet for 1 minute and then washed with tap water. Iodine solution was applied for 1minute, and then the slide was washed with tap water. The smear was then decolorized with few drops of acetone for seconds and washed immediately with water. Then the smear was flooded with diluted carbolfuchsin for 30seconds and washed with tap water. Slides were then blotted with filter paper and examined under oil immersion lens. Gram-positive bacteria appeared violet in color while that of gram-negative bacteria appeared red.

Biochemical tests for identified bacteria

Primary biochemical tests

Oxidase Test

Strips of filter paper were soaked in 10% solution of tetramethyle–phenylenediaminedihydrochloride in a petridish and then left to dry. Then a fresh young test culture, on nutrient agar, was picked up with a sterile glass rod and streaked on that filter paper. A dark purple color that developed within five to ten seconds was considered positive reaction.

Catalase Test

A drop of 3% aqueous solution of hydrogen peroxide was placed on a clean microscope slide. A colony of test culture, on nutrient agar was then placed on the hydrogen peroxide drop. The test was considered positive when gas bubbles appeared on the surface of the culture material.

Glucose utilization Test

The sugar media were inoculated with the test organism and incubated at 37ºC over night. They were examined daily for 7 days. Acid production was indicated by the development of pink color in the medium, Gas production was indicated by air trapped in the Durham's tube.

Oxidation-Fermentation (O/F) Test

The test was made by growing the test culture in tow tubes of Hugh and Lifeson's medium. A layer of soft paraffin was added to one tube to a depth of about 1 cm. Both tubes were incubated at 37ºC and examined daily. Oxidizer organisms showed acid production in the upper part of medium in the paraffin-covered tube and at the bottom in the open tube.

Motility (Hi-Media, Mumbai)

Motility medium (Semi-solid medium in U- shape tube) was inoculated at the top of one end of the tube with tested organism and incubated at 37ºC for about 4 days. Positive test was indicated by presence of growth in the other sides of the tube.

Secondary biochemical tests

Urease Test

Suspected *Salmonella* colonies were streaked on urea agar slope, incubated 37º C for 2 days. A positive reaction was indicated by a change of color to pink.

Indole Test

The test culture was inoculated into peptone water medium and incubated at 37º C for 48 hours. 1 ml of Kovacs's reagent was rundown to the side of the tube. A pink ring which appeared on the surface within 1 minute indicated positive reaction.

Methyl Red (MR) Test

The test organism was inoculated in glucose phosphate peptone water, incubated 37º C for 2 days. Five drops of methyl red reagent were added. A positive reaction was indicated by appearance of a red color.

Voges Proskauer (V.P) Test

The test organism was inoculated in glucose phosphate peptone water, and then 3 ml of 5% alcoholic solution of α-naphthol and 1ml of 40% KOH aqueous solution was added. A positive reaction was indicated by development of bright pink color within 30 minutes.

Citrate utilization

An isolate colony from nutrient agar was picked up with a straight wire, then inoculated in Simmon's citrate agar and incubated at 37º C and examined daily. A positive test was indicated by change of color from green to blue.

Hydrogen sulphide (H_2S) Production

The test culture was inoculated by stabbing the butt and streaking the slope of triple sugar iron agar in McCarteny bottles and incubated at 37ºC for 2 days. A positive reaction was indicated by development of a black color.

Sugar fermentation test

The sugar media were inoculated with the test organism and incubated at 37ºC overnight. They were examined daily for seven days. Acid production was indicated by development of pink color in the medium, Gas production was indicated by air trapped in the Durham's tube. The sugars used in these tests were lactose, salicin, sucrose, maltose, mannitol, rafinose, inositol, xylose and sorbitol.

Casein hydrolysis test

The skim milk agar plates was prepared and the test organism was streaked. The plates were incubated over night at 37°C. After incubation the plates were observed. The clear zone was formed around the colonies that indicated a positive result.

Starch hydrolysis test

The starch agar plates were prepared and the test organism was streaked. The plates were incubated over night at 37°C. After incubation iodine solution was added over the starch agar plates. The clear zone was formed around the colonies that indicated a positive result.

Antimicrobial sensitivity test

Sensitivity of Salmonella isolates to a number of antimicrobial agents was determined by the standard disk diffusion method. Each isolate was tested to 12 different antimicrobial agents used for Gram-negative bacteria. Colonies from each isolate were emulsified in 2ml nutrient broth and shaken thoroughly to obtain a homogenous suspension of the test culture. The plates were then flooded with the bacterial suspension, tipped in different directions to cover the whole surface with the suspension. Excess fluid was aspirated and the plates were left for 15minutes to dry. The antimicrobial disks were placed on the agar medium by using sterile forceps. The plates were then incubated at 37ºC and examined after 24 hours for zones of inhibition which were measured in mm. The isolates were described as resistant, intermediate and sensitive to different antimicrobial agents according to Bauer *et al*., method.

Table 1: Standard zone of inhibition to different antimicrobial Agents

Antimicrobial agent	Disk potency (mcg)	Zone of Inhibition		
		Resistant	Intermediate	Sensitive
Amikacin	10	14 or less	15- 16	17 or more
Amoxyclav	50/10	12 or less	14- 15	22 or more
Ampicillin	25	11 or less	12- 14	15 or more
Chloramphenicol	10	12 or less	13- 17	18 or more
Ciproflaxacin	10	15 or less	16- 20	21 or more
Co- Trimaxazole	25	13 or less	18- 20	21 or more
Gentamycin	10	13 or less	14- 15	16 or more
Tetracyclin	30	14 or less	15- 18	19 or more
Trimethoprim	10	13 or less	16- 17	20 or more
Nalidixic acid	30	15 or less	13- 14	19 or more
Nitrofurantoin	200	12 or less	14- 15	19 or more
Vancomycin	10	13 or less	15- 16	20 or more

Factor influencing of *Salmonella* isolates

Factor influencing of *Salmonella* isolates different pH

LB broth was prepared and adjusting the following pH 5, 6, 7 and 9 before sterilization. The test organisms were inoculated into the LB broth and measured the OD value at 600 nm for the interval of time 0 hours, 4 hours and 18 hours during the incubation time.

Factor influencing of Salmonella isolates different temperature

LB broth was prepared and autoclaved. The test organisms were inoculated into the LB broth and incubated at various temperatures 28°C, 37°C and 42°C. The test organism were measured the OD value at 600 nm for the interval of time 0 hours, 4 hours and 18 hours during the incubation period.

Factor influencing of Salmonella isolates different concentration of NaCl

LB broth was prepared with different concentration of sodium chloride 0.30%, 0.50% and 0.70%. The test organisms were inoculated into the LB broth and incubated. The test organism were measured the OD value at 600 nm for the interval of time 0 hours, 4 hours and 18 hours during the incubation period.

RESULTS AND DISCUSSION

Isolation of bacteria

A total of 200 samples were subjected to bacteriological examinations. Hundred and ten Gram-negative *Enterobacteriaceae* were isolated from 200 samples. 20 samples showed no bacterial growth, 70 samples did not give typical reactions of *Enterobacteriaceae* with oxidase, Catalase, and glucose fermentation so they were not further identified. A total of 200 chicken samples, the positive isolates were identified. There are 28 samples from flesh, 42 samples from intestine, 15 samples from liver and 25 samples from spleen (Fig 1) and (Table 2).

S.No	Sources	No. of samples	No. of isolates	Percentage (%)
1	Flesh	50	28	56
2	Intestine	50	42	84
3	Liver	50	15	30
4	Spleen	50	25	50

Table 2. Isolation of salmonella sp from chicken

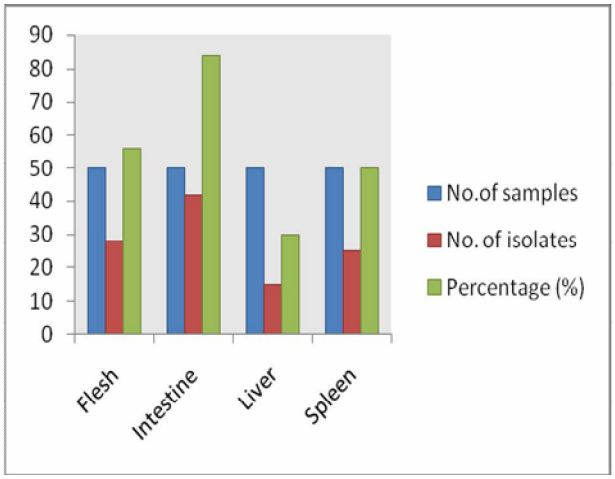


Figure 1. Number of salmonella isolates from chicken

In the present investigation, Out of 110 *Salmonella*, 33 (66%), 19 (38%), 41 (82%), and 17 (34%) were isolated from East, West, South and North zones, respectively. The higher number of *Salmonella* observed in the south zone of Karaikudi (Tab. 3, Fig. 2).

Table 3. Number of *Salmonella* from different Zone

S.No	Selected zone	No. of positive
1	East zone	33
2	West zone	19
3	South zone	41
4	North zone	17

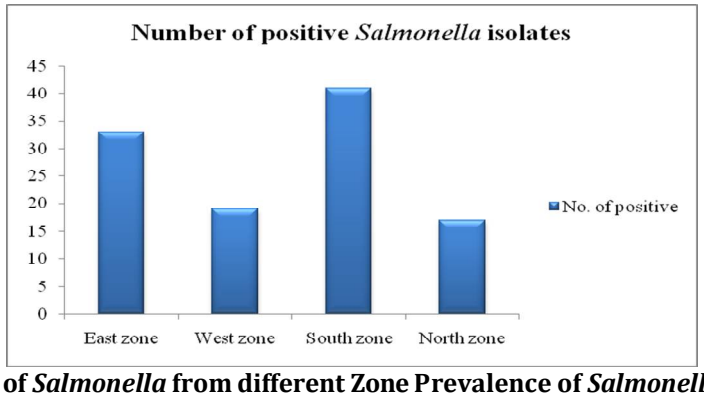


Figure 2. Number of *Salmonella* from different Zone Prevalence of *Salmonella* species of retail broiler chicken sample Site of isolation

The retail broiler chicken samples were collected from the East, West, South and North zones of Karaikudi. Out of 200 samples examined, 55% were positive for *Salmonella*. The present investigation indicated the occurrence of *Salmonella* in intestine (84%) at high levels when compared to the flesh (56%), spleen (50%), and liver (30%) (Fig 3).

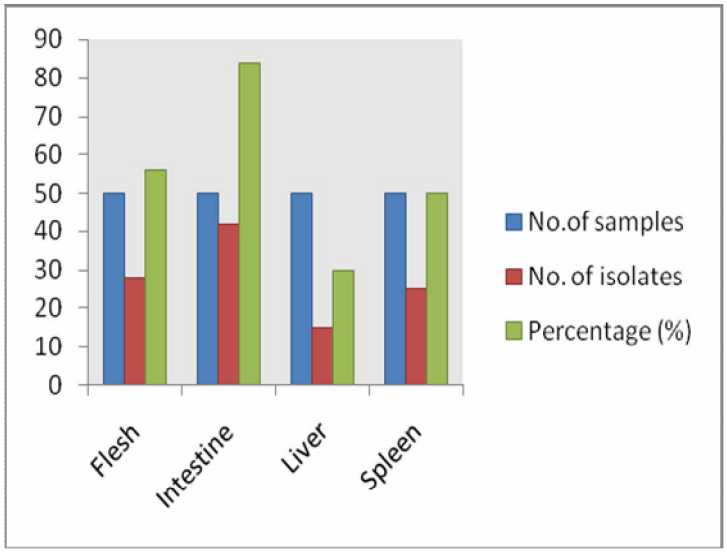


Figure 3. Percentage of *Salmonella* sp from chicken

In the present investigation, Out of 110 *Salmonella*, 33 (66%), 19 (38%), 41 (82%), and 17 (34%) were isolated from East, West, South and North zones, respectively. The higher number of *Salmonella* observed in the south zone of Karaikudi.

Biochemical reactions

Salmonella isolates were Methyl red positive, Indole, VP, urease and gelatinase negative (Fig 4). On triple sugar iron agar *Salmonella* colonies produced hydrogen sulfide which was indicated by black discoloration, gas production causes bubbles in the agar, and pH change was indicated by production of red color in the slant (Fig 5). ONPG test were positive that indicate yellow color zone surrounding the disc (Fig 6), On Oxidase test *Salmonella* isolates do not forms blue color in the disc that indicate the negative result (Fig 7) and Catalase positive (Fig 8).Carbohydrate fermentation test produced gas from glucose and mannitol, while sucrose and lactose were not fermented. Hydrogen sulfide was produced by the isolates (Fig 9). The Table 4 and 5 shown Biochemical confirmation test for *Salmonella* isolates and Interpretation of TSI test.

Table 4. biochemical conformation test

Test	*S. typhi*	*S. paratyphi*	*S. enterica*
Gram strain	-ve	-ve	-ve
Motility	+ve	+ve	+ve
Catalase	+ve	+ve	+ve
Oxidase	-ve	-ve	-ve
Indole	-ve	-ve	-ve
Methyl red	+ve	+ve	+ve
Voges-Proskauer	-ve	-ve	-ve
Citrate	-ve	-ve	+ve
Nitrate	+ve	+ve	+ve
Urease	-ve	-ve	-ve
Ornithine	-ve	+ve	+ve
Dulcitol	-ve	+ve	+ve
Rhamnose	-ve	+ve	+ve
Gelatinase	-ve	-ve	-ve
ONGP	-ve	-ve	+ve

Table 5. Interpretation of TSI test

Test	*Salmonella* strain		
	S. typhi	*S. paratyphi A*	*S. enterica*
TSI Acid from glucose	+	+	+
TSI gas from glucose	-	+	+
TSI acid from lactose	-	-	-
TSI acid from sucrose	-	-	-
TSI H_2S produced	+	-	+

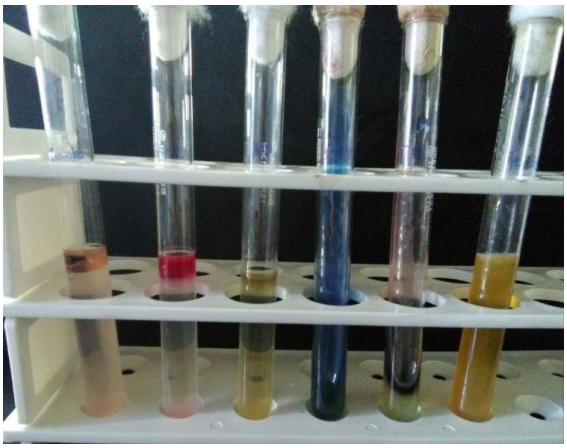


Figure 4. Imvic Test and Gelatinase Test

(Indole Negative, MR Positive, VP Negative, Citrate Positive, TSI - H_2S production and Gelatinase Negative (Left to Right)

Figure 5. TSI test - Black Discoloration Indicates The Production Of Hydrogen Sulfide

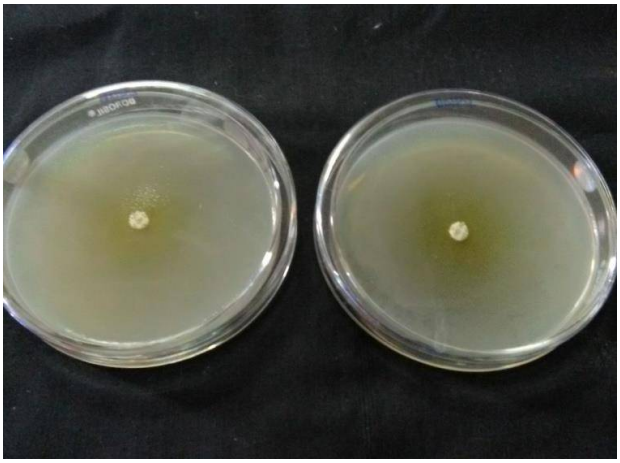


Figure 6. ONPG Disk test- Positive result – yellow color zone surrounding the disc

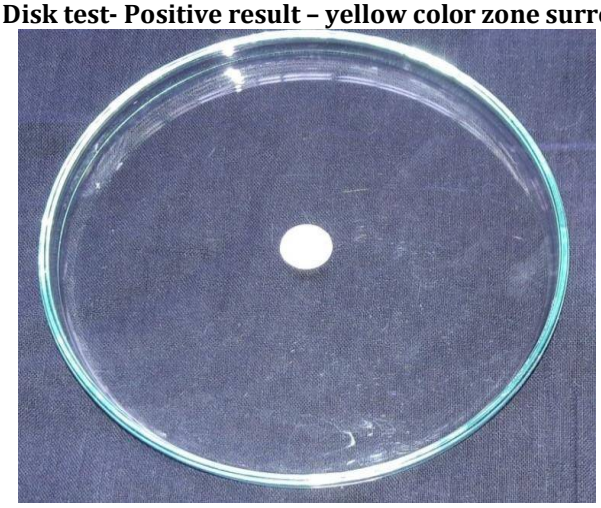


Figure 7. oxidase test -Negative Result – No blue color formation

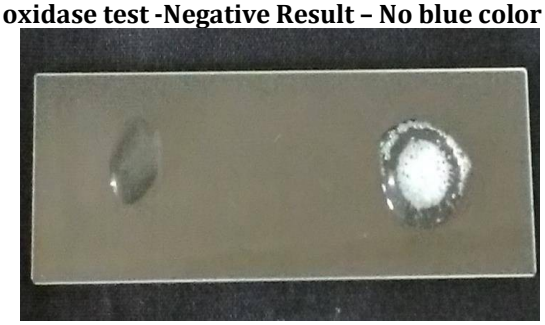


Figure 8. catalase test- Positive result in air bubbles formation

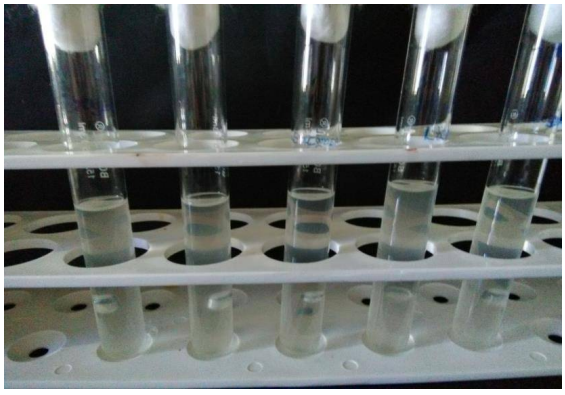


Figure 9. carbohydrate fermentation test- Gas production in Durham's tubes

In Casein hydrolysis test formation of clear zone around the colonies that indicates test organism hydrolysed the caseinolyase enzyme (Fig1.7). In Starch hydrolysis test formation of clear zone around the colonies that indicates test organism hydrolysed the starch (Fig1.8).

Figure 10. Casein test- Positive result – clear zone was formed

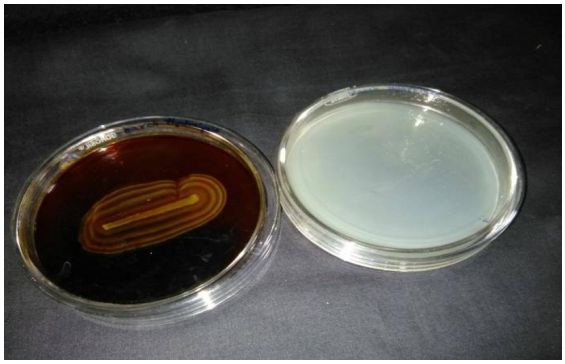


Figure 11. Starch hydrolysis test - Positive result – clear zone was formed

Factors influencing the growth of *Salmonella* isolates

In this study, four highly drug resistant *Salmonella* isolates from Flesh, Intestine, Spleen and Liver samples were used. The results indicated that different level of pH (5, 6, 7 and 9), and Temperature (28° C, 37° C and 42° C), different concentration of sodium chloride (Nacl) (0.30%, 0.50%, 0.70% and 0.90%) all are the important factors influencing the survival of *Salmonella* isolates in laboratory media.

Influence of *Salmonella* growth at different pH

Table 6 shown the isolates exhibited a wide range of growth in different pH. In the present study *Salmonella* isolates were grown well in different level of pH (Fig 12).

3.6 Influence of Salmonella growth at different temperature

The *Salmonella* isolates exhibited a wide range of growth in different level of temperature shown in Table 7 and Figure 13.

Influence of *Salmonella* growth at different concentration of Nacl

In the *Salmonella* isolates were tolerated and grown well in different concentration of sodium chloride (NaCl) shown in Table 8 and Figure 14.

Table 6. Influence of Salmonella growth pattern at different pH range

S.No	Time (hrs)	pH	Flesh	Intestine	Liver	Spleen
1	0	5	0.043	0.051	0.024	0.032
		6	0.241	0.907	0.612	0.717
		7	0.243	0.817	0.021	0.121
		9	0.372	0.467	0.421	0.248
2	4	5	0.123	0.289	0.321	0.021
		6	0.415	0.424	0.200	0.321
		7	0.251	0.383	0.172	0.167
		9	0.132	0.241	0.211	0.210
3	18	5	0.216	0.245	0.278	0.311
		6	0.823	0.588	0.286	0.211
		7	0.621	0.518	0.821	0.721
		9	0.543	0.409	0.621	0.217

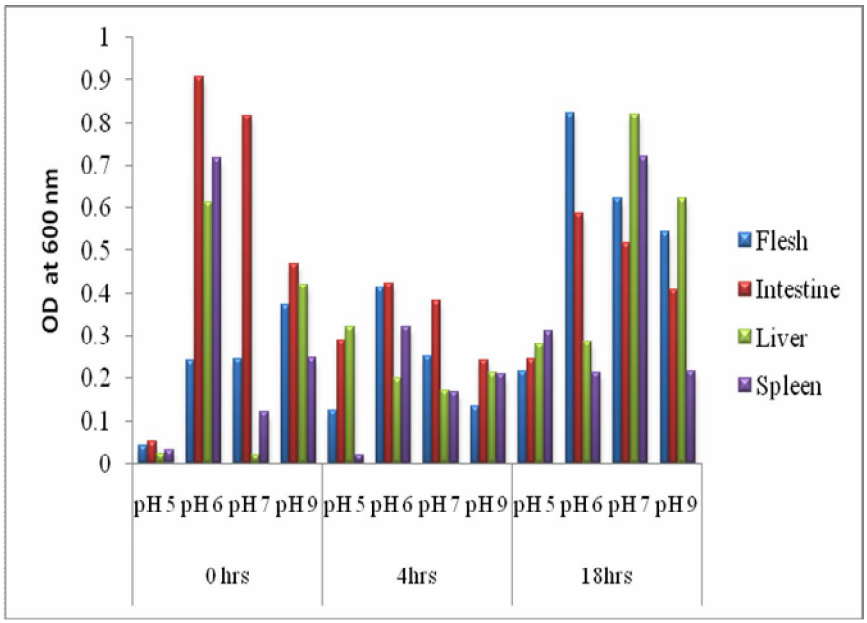


Figure 12. Factors influencing of *Salmonella* species at different pH level

Table 7. Influence of *Salmonella* growth at different temperature

S.No	Time (hrs)	Temperature (℃)	Flesh	Intestine	Liver	Spleen
1	0	28	0.051	0.042	0.017	0.066
		37	0.076	0.051	0.021	0.052
		42	0.081	0.061	0.075	0.125
2	4	28	0.412	0.512	0.471	0.442
		37	0.467	0.321	0.437	0.041
		42	0.135	0.420	0.315	0.361
3	18	28	0.110	0.461	0.310	0.410
		37	0.50	0.601	0.118	0.52
		42	0.341	0.421	0.124	0.51

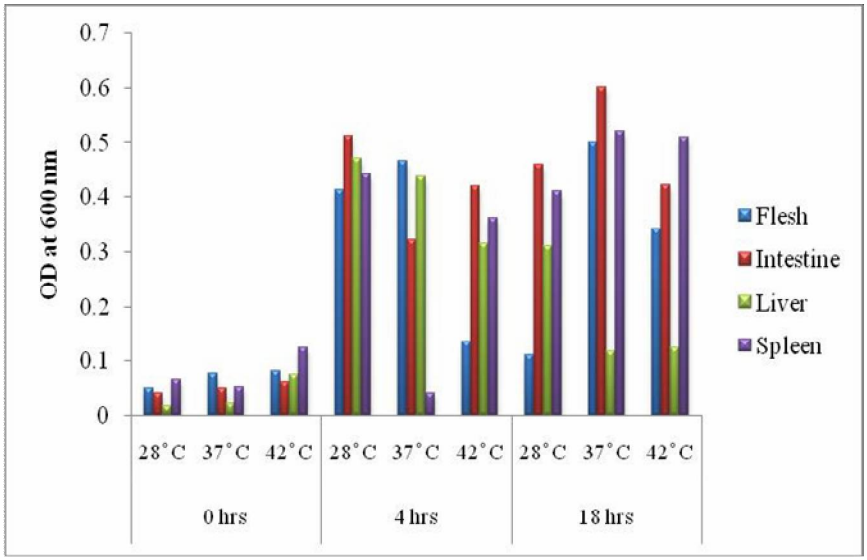


Figure 13. Factor influencing *Salmonella* species at different temperature

Table 8. Influence of *Salmonella* growth at different concentration of NaCl

S.No	Time	Concentration	Flesh	Intestine	Liver	Spleen
1	0	0.30%	0.053	0.071	0.072	0.070
		0.50%	0.060	0.061	0.069	0.073
		0.70%	0.052	0.072	0.065	0.019
		0.90%	0.029	0.031	0.021	0.020
2	4	0.30%	0.22	0.28	0.24	0.26
		0.50%	0.46	0.42	0.45	0.48
		0.70%	0.31	0.23	0.31	0.32
		0.90%	0.32	0.31	0.24	0.25
3	18	0.30%	0.20	0.10	0.25	0.14
		0.50%	0.51	0.21	0.61	0.42
		0.70%	0.32	0.20	0.25	0.25
		0.90%	0.41	0.45	0.35	0.24

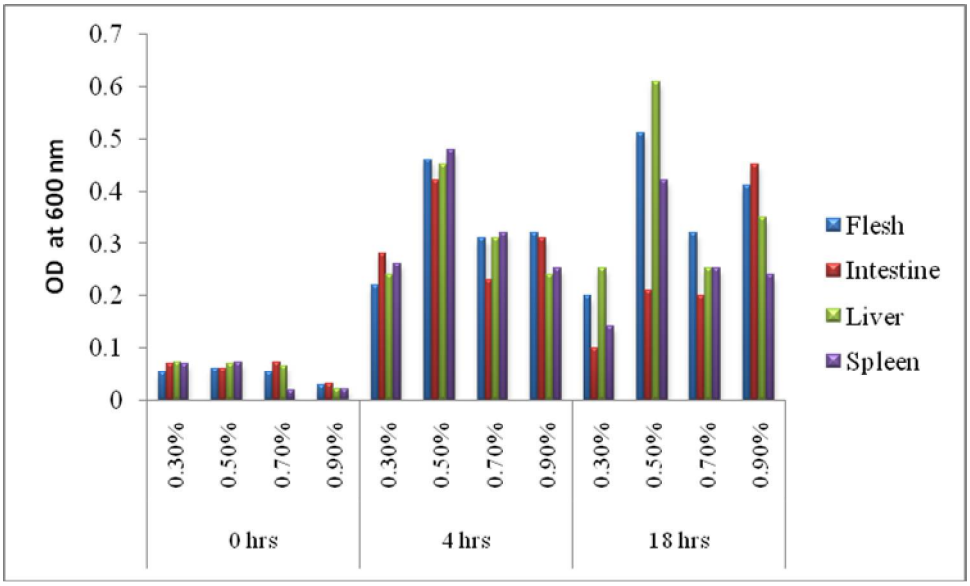


Figure 14. Factor influencing *Salmonella* species at different Nacl

Antimicrobial resistant pattern (%) of *Salmonella* isolated from flesh, intestine, Liver and spleen of broiler chicken.

The pattern of resistance of *Salmonella* analyzed in this study is shown in figure 15a. Significant differences were seen in the diffusion zone sizes for all agents. Resistance to tetracycline was present in 100% of the all *Salmonella* isolates.

Antimicrobial resistant pattern in Flesh

Antimicrobial resistance of *Salmonella* isolates in Flesh sample were analysed shown on Table 9. The following these are highly resistant to sensitive of antimicrobial resistant pattern in flesh. Vancomycin (100%) is highly resistance of *Salmonella* isolates. Amoxyclav (95.23%), Nalidixic acid (90.9%), Ampicillin (89.9%), Chloramphenicol (64.72%) are also resistance of *Salmonella.* The intermediate antibiotic resistance of isolates are Tetracycline (45.51%), Trimethoprim (35.58%). Amikacin (17.12%), Gentamycin (15.32%), Nitrofurantoin (15.11%), Co- Trimaxazole (10.9%) and Ciprofloxacin (2.99%) are sensitive to *Salmonella* isolates.

Antimicrobial resistant pattern in Intestine

Antimicrobial resistance of *Salmonella* isolates in intestine sample were observed shown on Table 9. The following these are highly resistant to sensitive of antimicrobial resistant pattern in intestine sample. Vancomycin (100%) is highly resistance of *Salmonella* isolates. Amoxyclav (96.26%), Ampicillin (94.73%), Nalidixic acid (83.1%), Chloramphenicol (72.5%), Tetracycline (70.83%), Trimethoprim (60.53%) are also resistance of *Salmonella.* The intermediate antibiotic resistance of isolates are Co-Trimaxazole (20.83%), Amikacin (20.3%). Nitrofurantoin (16.21%) Gentamycin (13.12%) and Ciprofloxacin (3.62%) are sensitive to *Salmonella* isolate.

Antimicrobial resistant pattern in liver:

Antimicrobial resistance of *Salmonella* isolates in liver sample were noted shown on Table 9. The following these are highly resistant to sensitive of antimicrobial pattern in liver. Vancomycin (100%) is highly resistance of *Salmonella* isolates. Amoxyclav (98.09%), Nalidixic acid (86.25%), Chloramphenicol (80.02%), Tetracycline (65.32%), Trimethoprim (56.66%) are also resistance of *Salmonella.* The intermediate antibiotic resistance of isolates are Ampicillin (50.01%), Ciprofloxacin(34%), Co-Trimoxazole (25.23%), Nitrofurantoin (23.21%). Gentamycin (20.13%) and Amikacin (18.17%) are sensitive to *Salmonella* isolates.

Antimicrobial resistant pattern in Spleen

Antimicrobial resistance of *Salmonella* isolates in spleen sample were observed shown on Table 9. The following these are highly resistant to sensitive of antimicrobial pattern in spleen. Vancomycin (100%) and Chloramphenicol (100%) are highly resistance of *Salmonella* isolates. Amoxyclav (99.09%), Ampicillin (95.32%), Nalidixic acid (81.81%), Trimethoprim (78.28%), Tetracycline (72.56%) are also resistance of Salmonella. The intermediate antibiotic resistance of isolates are Amikacin (50.12%), Nitrofurntoin (21.83%) Gentamycin (16.17%), Co-Trimoxazole (14.56%) and Ciprofloxacin (3.21%) are sensitive to *Salmonella* isolates.

Average of Antimicrobial resistant pattern

Resistance was most commonly observed *Salmonella* isolates from flesh, intestine, spleen and liver to Amoxyclav (97.16%), Nalidixic acid (85.51%), Ampicillin (82.49%), Chloramphenicol (79.31%), Tetracycline (63.55%), Trimethoprim (57.67%), Amikacin (26.42%), Nitrofurantoin(19.09%), Co-Trimoxazole (17.88%), Gentamycin (16.18%) and Ciproflaxacin (3.30%). Vancomycin (100%) is highly resistance of *Salmonella* isolates. In *Salmonella* isolates, Amoxyclav, Nalidixic acid, Ampicillin, Chloramphenicol and tetracycline are highly resistance and very low resistance were observed to Ciprofloxacin and Gentamycin (Fig 15a,b and Fig 16).

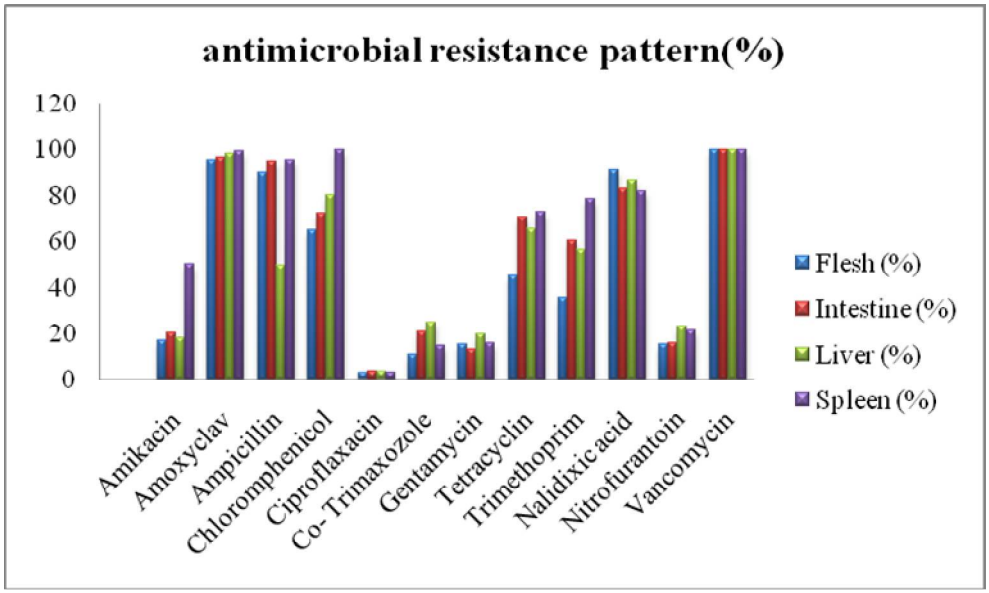
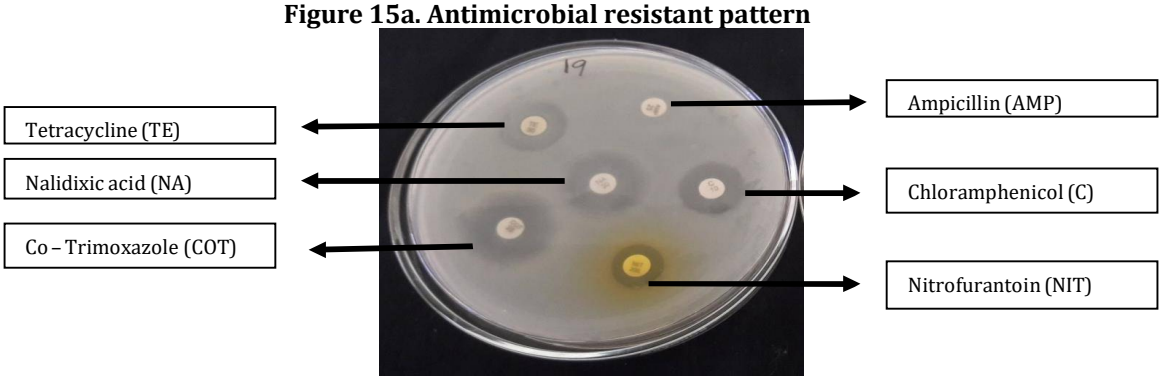


Figure 15a. Antimicrobial resistant pattern



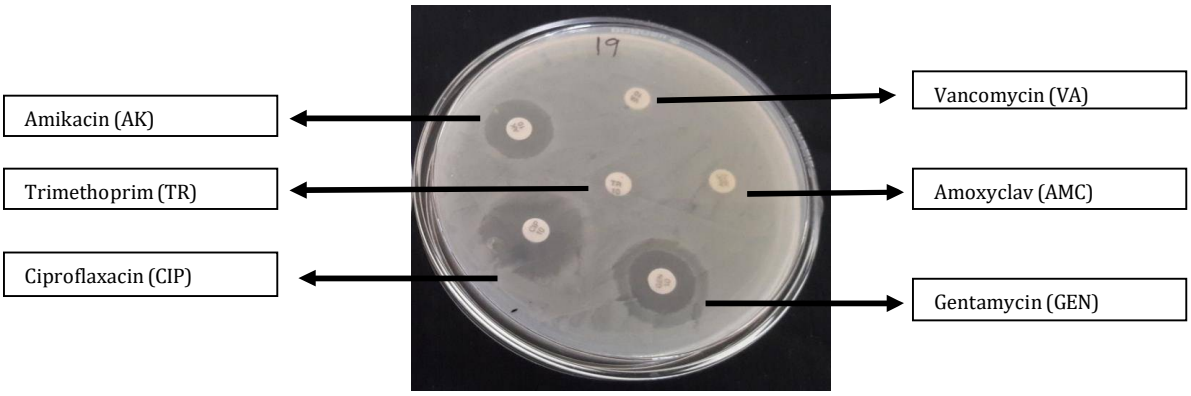


Figure 15b. Antimicrobial resistant pattern

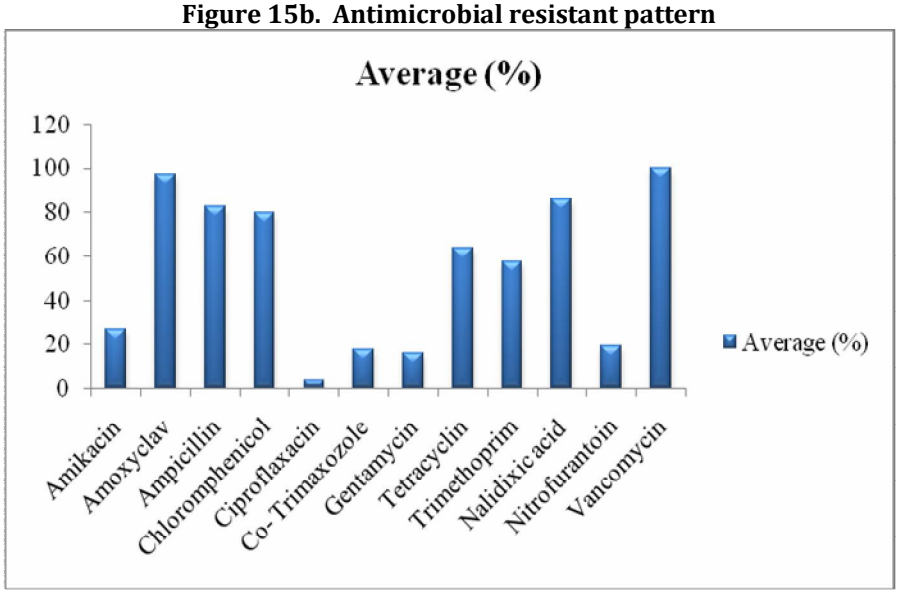


Figure 16. Average of antimicrobial resistance

Table 9. Antimicrobial resistant pattern (%) of *Salmonella* isolated from Flesh, Intestine, Liver and Spleen of broiler chicken

S.No	Antibiotics	Flesh (%)	Intestine (%)	Liver (%)	Spleen (%)	Average (%)
1	Amikacin	17.12	20.3	18.17	50.12	26.4275
2	Amoxyclav	95.23	96.26	98.09	99.09	97.1675
3	Ampicillin	89.9	94.73	50.01	95.32	82.49
4	Chloramphenicol	64.72	72.5	80.02	100	79.31
5	Ciproflaxacin	2.99	3.62	3.4	3.21	3.305
6	Co- Trimoxazole	10.9	20.83	25.23	14.56	17.88
7	Gentamycin	15.32	13.12	20.13	16.17	16.185
8	Tetracycline	45.51	70.83	65.32	72.56	63.555
9	Trimethoprim	35.58	60.53	56.66	78.28	57.7625
10	Nalidixic acid	90.9	83.1	86.25	81.81	85.515
11	Nitrofurantoin	15.11	16.21	23.21	21.83	19.09
12	Vancomycin	100	100	100	100	100

Salmonellosis is a major public health concern and continues to have a serious economic importance in the poultry industry in all countries (8). With the great expansion of the poultry industry, the wide spread occurrence of the salmonellosis has ranked it as one of the most important bacterial diseases of poultry. The present study was conducted to investigate the prevalence of *Salmonella* from selected parts of retail broiler chicken samples were collected from the East, West, North and South zones of Karaikudi city. In this study *Salmonella* represented the most dominant isolate and 55% were positive. The present investigation indicated the occurrence of *Salmonella* in intestine (84%) at high levels when compared to the flesh (56%), spleen (50%), and liver (30%). The *Salmonella* isolation rate was comparable to that reported in other studies. Yagoub and Mohammed (8) examined 1488 samples and isolated 58 *Salmonellae* which comprise 3.9% of total isolates. In another study Ezdihar (9) examined 610 samples from poultry in the Sudan and isolated 45 *Salmonellae* which counted for 7.4% of the total isolates. The higher isolation rate was obtained from a south zone (82%) of Karaikudi city. This can be due to poor hygiene in this farm. Among the examined samples, the highest rate of isolation was obtained from Intestine. This finding indicates a high shedding of *Salmonella* from the intestinal tracts of birds in this farm. *S. enteritidis* is the most important serovar in poultry flocks and recently it was of high occurrence worldwide (10). Phillips and Optiz (11) showed that *S. enteritidis* could attach to granulose cells in the preovulatory membrane and subsequently infect the ovum during the ovulation. On the other hand, *S. enteritidis* had the ability to penetrate eggs through the shell pores and causes egg contamination. From the view point of public health, human Salmonellosis was reported to increase in France and United States of America due to *S. enteritidis* (12). It was reported to cause food poisoning due to consumption of under cooked egg dishes (13). Isolation of this bacterium from some farms in Karaikudi represents a real threat to the public health. *S. arizonae* was reported to cause arizonae infection in chickens (14). Numerous studies have evaluated the effect of environmental factors such as temperature (15), pH and sodium chloride (16) on microbial growth. *Salmonella* have an optimum growth temperature of 37°C, however they readily adapt to extreme conditions. *Salmonella* growth temperatures low at 5.9°C and high at 54°C have been reported for specific experimental conditions. The antibiogram of the *Salmonella* isolates were encountered in the present study revealed that most of the strains had acquired resistance to more than 4-5 antibiotics. Multiple antibiotic resistant *Salmonella* in chicken and fish in Indian markets has been reported earlier (17, 18,). The resistance to antibiotics was much higher when compared to the resistance patterns from developed countries. This is mainly because of the frequent abuse of antibiotics in our country. Most of the antibiotics can be purchased over the counter without prescription as well as there is no proper follow up to ensure that the patients are completing the course. This results in an increased selection pressure, which could add to the emergence of resistant strains. Also, wide spread use of antibiotics in animal production systems is contributing significantly to the increased antibiotic resistance among pathogenic bacteria of animal origin (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 (20). This is evident in the results from our study, where we observed relatively higher levels of tetracycline resistance among the *Salmonella* isolates from commercial layer hen eggs and non-commercial layer hen eggs. The resistance level was much higher than the level reported by Hatha and Lakshmanaperumalsamy (18). Ampicillin resistance among the *Salmonella* strains encountered in the present study were lower than those reported by Suresh *et al*., (21). Ciprofloxacin is a fluoroquinolone antibiotic that is increasingly and successfully used for the treatment of septicaemia in humans Ciprofloxacin resistance in human and veterinary *Salmonella* isolates has occasionally been found. The *Salmonella* having similar level of resistance and resistance pattern indicates their origin from a common source. The multiplicity of resistance pattern indicates the large pool of resistance plasmids among these bacteria, which would pose a potential threat once they released into the environment. Also the treatments of infections caused by these MAR forms are going to be extremely difficult. A high incidence of multidrug resistant *Salmonella* in the poultry wastes was noticed by with frequent resistance against tetracycline, streptomycin and colistin (22). Almost all strains from different sources in the present study were resistant to Vancomycin. Other studies have also reported similar antibiotic resistance of *Salmonella* strains from different sources against Vancomycin. All strains of *Salmonella* were found sensitive to ciprofloxacin, Gentamycin, Co – Trimoxazole, Nitrofurantoin, Amikacin and Trimethoprim. Resistance to Gentamycin has been reported by Lee *et al*., (23) which determined 10% resistance to this agent from 105 *Salmonella* isolates. Also there was an increasing development of quinolones resistance all over the world. Treatment failure due to a reduced susceptibility to Ciprofloxacin in *Salmonella* is now well established (24). The logical interpretation of the results of the MAR index of all *Salmonella* strains isolated in the study showed that they might have originated from high risk sources of contamination. Poultry is identified as one of the major reservoirs of *Salmonella* species. There is a large body of literature reviewed by Novick (19) demonstrating that the sub-therapeutic use of antibiotics in the

mass production of poultry, eggs and pork has promoted the emergence and maintenance of MAR pathogenic bacteria in the environments of these animals. In general, *Salmonella* is the most important agent implicated in outbreaks in food-borne diseases around the world (25). Effective control or eradication programs for salmonellosis depend on good management system, identification of carrier birds and accurate medication. The prevalence of *Salmonella* in poultry was relatively high than the other environmental factors. The multidrug resistance in most of these *Salmonella* strains adds to the gravity of the problem. Based on the results we again reiterate the need for regulating control over the use of antibiotics in poultry industry. Effective control or eradication programs for salmonellosis depend on good management system, identification of carrier birds and accurate medication.

CONCLUSION

The main purpose of this study was to investigate the prevalence, distribution, factors affecting the growth of *Salmonella* isolates, and antimicrobial activity of multidrug resistant (MDR) *Salmonella* were isolated from chicken samples in different region (East, West, North and South) of Karaikudi city, Sivaganga district, Tamilnadu, India, during the periods from December 2022 to April 2023. A total of 200 samples were examined, 55% were positive for *Salmonella*. The present investigation indicated the occurrence of *Salmonella* in intestine (84%) at high levels when compared to the flesh (56%), and spleen (50%), liver (30%). The higher number of *Salmonella* observed in the south zone (82%) of Karaikudi. Besides, the result indicates the existence and diversity of *Salmonella* species vary temporally and is strongly influenced by seasonal precipitation, turbidity, heterotrophic bacteria and pH. There is a clear seasonal trend for salmonellosis cases, gradually in the winter. Although higher case rates coincide with peak annual temperatures, it remains unclear which factors drive this seasonality regionally or how this pattern might relate to the presence of the pathogen itself in the environment. Antimicrobial agents are used to treat infected human or animals, to protect them from infectious diseases, and to provide a faster growth rate. But, now most of the pathogens are resistant to commonly used drugs. The *Salmonella* genus has been traditionally susceptible to antimicrobial agents. Resistance was most commonly observed *Salmonella* isolates from flesh, intestine, spleen and liver to Amoxyclav (97.16%), Nalidixic acid (85.51%), Ampicillin (82.49%), Chloramphenicol (79.31%), Tetracycline (63.55%), Trimethoprim (57.67%), Amikacin (26.42%), Nitrofurantoin (19.09%), Co- Trimoxazole (17.88%), Gentamycin (16.18%) and Ciprofloxacin (3.30%). In *Salmonella* isolates, Amoxyclav, Nalidixic acid, Ampicillin, Chloramphenicol and tetracycline are highly resistance and very low resistance were observed to Ciprofloxacin and Gentamycin.

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