

# Microbiology

Journal

ISSN 2153-0696



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## Prevalence of Multidrug-Resistant (MDR) *Salmonella enteritidis* in Poultry and Backyard Chicken from Tiruchirappalli, India

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### ABSTRACT

This study aimed to investigate the prevalence of multidrug resistant *Salmonella enteritidis* from poultry chicken in comparison with the backyard country chicken along with their seasonal variation in Tiruchirappalli, Tamilnadu, India. A total of 325 rectal swab samples of chicken were included for this study. Among them 157 and 168 were from poultry and backyard chicken, respectively. Samples were randomly collected during each season like monsoon, post monsoon, Winter and Summer from July 2010 to June 2011. The total rate of isolation of *Salmonella* was found to be 26.8% (n = 87). Among them 32.5% (n = 51) were from poultry and 21.4% (n = 36) were from backyard chicken. Highest isolation rate was obtained during summer and the lowest, during winter. All the isolates were found to be highly resistant to  $\beta$ -lactam and macrolide antibiotics (52.9-100%) and highly sensitive to co-trimoxazole and ciprofloxacin (41.2-76.5%). More number of multi drug resistant *Salmonella* isolates was recovered from the poultry chicken from Tiruchirappalli, Tamilnadu. The probability for this may be the frequent exposure of the poultry chickens for various antibiotics in the chicken farms during their cultivation.  $\beta$ -lactam and macrolide antibiotics may be used in these farms routinely for curbing the bacterial infection among the chickens and this was evidenced in comparison with the backyard chicken samples.

**Key words:** Antimicrobial resistance, poultry, backyard chicken, antibiotics, *Salmonella*

### INTRODUCTION

Salmonellosis is an important zoonosis associated with food consumption of animal origin. Poultry eggs, meat and their products are the commonest vehicles for the transmission of human salmonellosis constituting an important threat to public health (Zdragas *et al.*, 2012). Many factors are involved in the transmission of *Salmonella* in broiler flocks, through infected litter, faeces, feed, water, dust, fluff insects, equipment, fomites, diseased chicks and rodents (Poppe, 2000). *Salmonella* is considered to be the most frequent food borne pathogen worldwide with the major source being poultry chicken. Poultry chicken is the main type of chicken consumed in Tamilnadu like many other countries. Apart from this the rearing of backyard chickens is very common, providing a part of nutritional requirement among rural and urban areas of Tamilnadu. The backyard chickens are reared in the countryside and it is also prone to infection with *Salmonella*

through contact with wild animals, domestic mammals and commercial poultry which is a carriers of *Salmonella* and consequently may play a role in the transmission of the organism to other animals and humans.

In addition to their role in food borne diseases, *Salmonella* are also important because it limits the number of available therapeutic options. In addition, most of the fluoro quinolones administered in food-producing animals are frequently the same or belong to the same classes as those used in human medicine (Aarestrup *et al.*, 2008). The excess or overuse of oxytetracycline does not enable microbes to acquire resistance but selective for resistant bacteria (Witte, 2001). Intensive medicated feed production for poultry can potentially provide a suitable environment for the proliferation of antibiotic resistant bacteria (Hayes *et al.*, 2001). The use of antibiotics in poultry production is the causative agent in the establishment of antibiotic-resistant reservoirs within poultry flocks (Price *et al.*, 2007). However, this is not the case with the backyard country chicken, because they are routinely reared in open area of the villages in Tamilnadu, with naturally available feeds.

The aim of this study was to investigate the prevalence of multi drug resistant *Salmonella* from poultry chicken in comparison with the backyard country chicken along with their seasonal variation in Tiruchirappalli, Tamilnadu, India.

## **MATERIALS AND METHODS**

**Study site and sample collection:** Surveillance on the prevalence of *Salmonellae enteritidis* in chicken was setup at Tiruchirapalli, Tamil Nadu from July 2010 to June 2011. A total of 325 rectal swab samples were analyzed in this study. Of this 325 samples, 157 were from broiler chicken brought to Tiruchirapalli from different farms of the urban areas in Tamilnadu and 168 from Backyard chicken reared in rural areas of Tiruchirapalli. Samples were randomly selected in equal numbers during each season like monsoon (July-September), post-monsoon (October-December), Winter (January-March) and Summer (April-June). The rectum of the chicken was swabbed using a transportable sterile swab (Hi-Media) applied with firm rolling pressure. The swabs were placed immediately in a sterile container with selenite broth and transported under refrigerated conditions to the Medical Microbiology Laboratory, Bharathidasan University.

**Bacteriological analysis:** Specimens collected and transported to the laboratory following standard methods (Cheesbrough, 2006; Winn *et al.*, 2006) were used for bacteriological isolation. The swab was aseptically inoculated into 10 mL of Tetrathionate broth (Hi-Media, Mumbai, India) and incubated at 37°C for 24 h (Maripandi and Al-Salamah, 2010). A loopful of incubated tetrathionate broth was streaked on to *Salmonella-Shigella* agar (SS) plates in triplicate for the isolation of *Salmonella*. The inoculated plates were incubated at 37°C for 18-48 h for the growth of colonies. From each plate a minimum of three presumptive *Salmonella* colonies were subjected to preliminary screening by carbohydrate utilization involving glucose, lactose, sucrose, manitol, salicin, dulcitol and melibiose fermentation tests (Hi-Media, Mumbai, India), which differentiate *Salmonella enteritidis* and *S. typhimurium* (Cox and Williams, 1976; Cappuccino and Sherman, 2001) and further confirmed by slide agglutination test using polyvalent anti-sera (King Institute, India).

**Antibiotic susceptibility tests:** *Salmonella* isolates were tested for susceptibility to 16 various antimicrobial agents by the Kirby-Bauer disc-diffusion technique (CLSI., 2010). A sterile cotton

swab was dipped into overnight culture of bacterial suspension with absorbance adjusted to OD 610 nm and swabbed on Mueller Hinton plates. Commercial antibiotic discs with the following drug contents: Vancomycin (VA<sup>30</sup>), gentamicin (G<sup>50</sup>), amoxicillin (AM<sup>25</sup>), clindamycin (CD<sup>2</sup>), ampicillin (A<sup>10</sup>), penicillin (P<sup>10</sup>), nalidixic acid (NA<sup>30</sup>), fusidic acid (FC<sup>10</sup>), cotrimoxazole (Co<sup>25</sup>), erythromycin (E<sup>15</sup>), rifampicin (RIF<sup>5</sup>), ciprofloxacin (Cf<sup>30</sup>), tetracycline (TE<sup>30</sup>), norfloxacin (NX<sup>10</sup>), methicillin (MET<sup>5</sup>) and amikacin (AK<sup>30</sup>) were placed on the surface of Muller Hinton agar plates. The inoculated plates were incubated at 37°C for 24 h and then the diameters of zones of inhibition were compared to determine the susceptibility or resistant pattern of the isolates to various drugs. The results were interpreted following the National Committee for Clinical Laboratory Standards criteria (CLSI, 2010). *Salmonella enteritidis* MTCC 3219 was used as reference strains.

**Determination of minimum inhibitory concentration for vancomycin:** Minimum inhibitory concentration for vancomycin was determined by broth dilution method as per standard protocols (CLSI, 2010). In brief, two-fold serial dilutions of vancomycin were prepared, with the final concentration of vancomycin ranging from 32-512 µg mL<sup>-1</sup> for the *Salmonella* isolates. Vancomycin, PBS and the medium alone were used as positive, non-treated and blank controls, respectively. A cell suspension of overnight culture of *Salmonella* was prepared and 10% of the cell suspensions were inoculated and incubated at 37°C for 8 h. The MIC is defined as the minimum concentration when there is maximum inhibition of the organism. The MIC was determined by reading the absorbance at OD 610 nm in a biophotometer (Eppendorf, Germany).

**Determination of virulence factors:** The identified MDR strains were screened for various virulence factors by biochemical-mediated approaches. The strains were screened for the presence of phospholipase, proteolytic, caseinolytic and hemolytic activity on nutrient agar plates impregnated with appropriate substrates, including egg yolk, BSA, casein and blood, respectively. The strains were streaked on the agar plates and incubated for 18-48 h at 37°C for the formation of halo zones around the colonies. The enzyme activities were measured by subtracting the diameter of the colony from the total zone diameter as described earlier (Sathiamoorthi *et al.*, 2011).

**Statistical analysis:** Fisher's two-tailed contingency test was used for significance of correlations (p-value) between two parameters. The p<0.05 was considered to be statistically significant for the resistance among the poultry and backyard chicken.

## RESULTS

The overall *Salmonella* prevalent in chicken rectal swabs, which collected from Tiruchirapalli was 26.8% (n = 87). A difference (p = 0.024) in the rate of isolation of *Salmonella enteritidis* was observed between the backyard and poultry chicken with 21.4% (n = 36) and 32.5% (n = 51), respectively. All the isolates were confirmed as *Salmonella enteritidis* by slide agglutination test using specific antiserum.

The seasonal distribution showed a statistically higher rate of isolation during summer (p = 0.05). During summer 30.9 and 41.5% of the isolates were obtained from backyard and poultry chicken, respectively (Fig. 1).

The multi drug resistant pattern of all the isolates revealed that the isolates were resistant to at least one of the 16 antibiotics we used (Table 1). A statistically significant (p = 0.05) difference

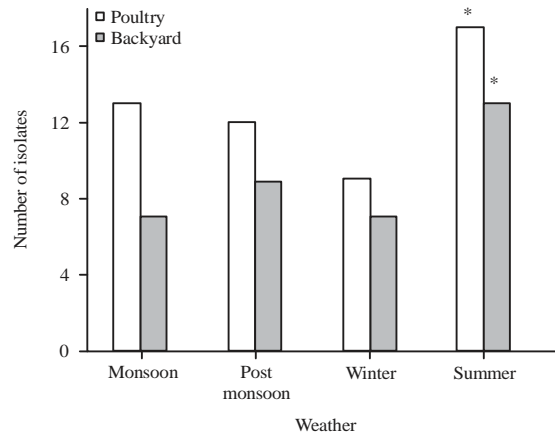


Fig. 1: Seasonal distribution of *Salmonella enteritidis* isolates from poultry and backyard chicken from Tiruchirappalli. The asterisk (\*) shows a significant ( $p < 0.05$ ) difference in the distribution of *Salmonella* isolates between poultry and backyard chicken

Table 1:  $\beta$ -lactam and macrolide antibiotic resistance of *Salmonella enteritidis* isolated from poultry and backyard chicken from Tiruchirappalli

Antimicrobial agents	Resistant isolates				p-value
	Backyard chicken (n = 36)		Poultry chicken (n = 51)		
	No.	%	No.	%	
Vancomycin	12	33.3	51	100.0	<0.001
Gentamicin	15	41.6	31	60.7	0.079
Amoxycillin	16	44.4	27	52.9	0.435
Clindamycin	21	58.3	49	96.1	<0.001
Ampicillin	11	30.5	47	92.1	<0.001
Penicillin-G	19	52.7	51	100.0	<0.001
Nalidixic acid	17	47.2	48	94.1	<0.001
Fusidic acid	11	30.5	30	58.8	<0.001
Co-Trimoxazole	9	25.0	30	58.8	<0.001
Erythromycin	21	58.3	51	100.0	<0.001
Rifampicin	21	58.3	51	100.0	<0.001
Ciprofloxacin	6	16.6	12	23.5	0.436
Tetracycline	20	55.5	47	92.1	<0.001
Norfloxacin	19	52.7	34	66.6	0.191
Methicillin	19	52.7	51	100.0	<0.001
Amikacin	21	58.3	46	90.1	<0.001

was observed between the antibiotic resistant pattern of the isolates from poultry and backyard chicken. All the isolates from poultry chicken showed resistance to penicillin, methicillin, vancomycin, clindamycin, fusidic acid, erythromycin and rifampicin whereas isolates from backyard chicken showed only ~60% resistance to the same antibiotics. The next higher incidence of resistance among poultry isolates was observed in nalidixic acid (94.1%) and amikacin (90.1%). The isolates from both poultry and backyard chicken were found to be highly sensitive to ciprofloxacin. In general, all the isolates were found to be highly resistant to  $\beta$ -lactam and macrolide antibiotics and highly sensitive to ciprofloxacin and co-trimoxazole.

Enzyme secretions such as phospholipase, proteinase, caseinolytic and hemolytic activity were considered as virulence determinants and their activities were assayed. The results of the variable determinants are tabulated in Table 2. The virulence traits between the *Salmonella* isolates from

Table 2: Enzymatic virulence of *Salmonella enteritidis* isolates

<i>Salmonella</i> spp. (n = 87)	Phospholipase	Proteinase	Caseinolytic	Hemolytic
Backyard (n = 36)	17 (47.2)	23 (63.9)	15 (41.6)	29 (80.5)
Poultry (n = 51)	39 (76.5)	46 (90.2)	35 (68.6)	49 (96.1)
Significance: p	0.005*	0.003*	0.012*	0.019*

\*: Significant

poultry and backyard chicken varied significantly ( $p = 0.003-0.019$ ). Poultry isolates showed 90.2% proteolytic and 96.1% hemolytic activity, whereas the backyard chicken isolates showed 63.9 and 80.7% proteolytic and hemolytic activity evidencing the virulence trait of the poultry isolates.

## DISCUSSION

In our previous study, we have reported a massive outbreak of typhoid fever compared to paratyphoid, among human cases. Such an outbreak implied that the animal products that are locally available would have been contaminated with *S. typhi* envisaging a transmission to humans through the consumption of contaminated animal products (Sathiamoorthi *et al.*, 2011). Thus, one such possible route of transmission can be attributed to the use of poultry chicken from the retail outlets of Tiruchirappalli. *Salmonellae* usually infect their hosts via gastrointestinal tract. In the absence of other microflora, the organisms are apparently able to adhere, multiply and colonize at any point along the GI tract of chicks (Soerjadi *et al.*, 1982). They may be shed in the faeces and form a source of contamination for other animals, humans and the environment (Poppe, 2000). Understanding the epidemiology of *Salmonella* in poultry production is essential to enhance the food safety of poultry products. Rectal swabs are of moderate diagnostic utility for detection of *Salmonella* and may be useful during the collection of faecal samples is not very practical (Kotton *et al.*, 2006). Thus, rectal swabbing offers an easy method of surveying the carrier rate of *Salmonella* among chickens. In Tiruchirappalli, India the chicken retail shops usually bring the chickens from various farms and they process it in the shop itself, or until the birds has to be in small metal cages. Therefore, it is difficult to go with individual faecal material of the particular chicken. Therefore, the cloaca swabs were practiced even though the sensitivity may be slightly less compared to the faecal culture. In this regard, cloaca swabs can be used to provide evidence of persistent intestinal colonization by *Salmonellae* in individual birds (Gast and Beard, 1990). Thus, in the present study collection of rectal swabs was carried out to study the prevalence of *Salmonella* among backyard and poultry chicken.

The overall isolation rate was found to be 26.8% with 32.5 and 21.4% isolation rates from the rectal samples of poultry and backyard chicken, respectively. The rate of isolation was higher in poultry, compared with backyard chicken. This definitely poses a risk to industrial chicken farms and seems to be a serious public health concern. Therefore, any prophylactic program aimed at controlling *Salmonella* infections must be taken into account with poultry chicken. The possible reason for increased prevalence of *Salmonella* isolates may be the poultry chicken are reared in crowded closed environments in farm conditions and they also have the exposure to various antibiotics, periodically. In India, backyard chickens are freely grown in the surroundings of the villages, therefore their natural immune mechanism may be efficient than that of the poultry chicken. Due to the natural innate immunity of the backyard chicken, their intestine may not have much colonization with pathogenic *Salmonella*. Five hundred cloacal swabs were assessed, as 100 pooled samples, taken from village chicken in 50 different farms in Morocoto isolate only three cultures (Bouzoubaa *et al.*, 1992). Out of that two were *S. pullorum* and *S. gallinarum*. Since, there is not much information about *Salmonella* infection among the backyard chicken, this may be the first of its kind in comparison with the poultry chicken.



The seasonal distribution showed that during summer the prevalence of *Salmonella* was higher among the poultry (41.5%) and backyard chicken (30.9%). This was found to be statistically significant. Early observations showed broiler carcasses to be nearly 11 times (Odds ratio 10.62) more likely to yield *Salmonella* in the hot season, compared to winter season (Ellerbroek *et al.*, 2010). These differences in isolation might be because *Salmonella* is more prevalent in the hotter season (Fossler *et al.*, 2005; Liljebjelke *et al.*, 2005). Similarly in a study from Nepal, the prevalence of *Salmonella* was found to be high during the months of April and May (Maharjan *et al.*, 2006), which compares with our results of higher *Salmonella* prevalence during the month of April-June.

The resistance to antibiotics was higher with MDR strains in poultry chicken compared to that of the backyard chicken. This may be due to the frequent use of antibiotics in the poultry farms for the control of various bacterial infections. This probably might have a role for the horizontal gene transfer mechanism for evolution and colonization of multidrug resistant *Salmonella* in the intestine of poultry chicken. There have been a number of studies showing transmission of MDR strains from retail chicken meat (Maripandi and Al-Salamah, 2010) environment (Jacobs-Reitsma *et al.*, 1995), drinking water (Solomon and Hoover, 1999) and vertical transmission (Genigegorgis *et al.*, 1986; Pearson *et al.*, 1996) as possible sources of flock colonization. Transport vehicles and crates may be an additional source of contamination between batches of birds and farms (Mead *et al.*, 1994). Further, the increased resistance to antibiotics may be due to the increased activity of the virulence factors among these MDR *Salmonella* isolates.

The MDR strains were shown to be positive for hemolytic and proteolytic activity. Thus, the mechanism of pathogenicity can be attributed to the strong fimbrial adhesion to the host cells and secretion of enterotoxins and virulence enzymes including protease, phospholipase, hemolysin ( $p < 0.05$ ) and thus find their way into the host cells with ease for colonization and propagation.

## CONCLUSION

This study has shown that the frequency of isolation of *Salmonella enteritidis* from poultry chicken differed significantly in comparison with backyard chicken. The isolates from poultry chicken were found to be MDR with significant resistance against  $\beta$ -lactam and macrolide antibiotics. This may be due to the frequent exposure of the poultry chicken to various antibiotics used to control bacterial infections in the cultivating farms. The significantly increased isolation of MDR strains can be attributed to the circulation of genetically similar strains among the poultry chicken and the uncontrolled use of anti microbials in poultry production even when there are use as growth promoters in live stock industry is also highly banned. Therefore, there is an urgent need to minimize the risk of spreading  $\beta$ -lactam and macrolide antibiotic resistance between animal and human populations, especially in the summer when risk factors are much higher than in winter.

## ACKNOWLEDGMENT

KNS are thankful to the University Grants Commission (UGC), New Delhi, India for providing funds to carry out this research work.

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