Detection of Antibiotic Resistance of *Salmonella* Species Isolated from Broiler Chicken

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Abstract

Salmonella species are major zoonotic food-borne pathogens which cause outbreaks and sporadic cases of gastroenteritis in human worldwide. Most Salmonella infections in humans result from the ingestion of contaminated foods of animal origin, such as poultry, pigs and cattle. An increase of *Salmonella* strains showing resistance against different antibiotics has been found in isolates from pigs, poultry, and cattle in recent years. This study aimed to compare the prevalence of antimicrobial resistance of *Salmonella* isolated from fresh poultry meat. A total number of sixty fresh chicken meat samples were collected from different locations and isolation was done using conventional methods according to the Bergy's manual. Susceptibility of these isolates to five different antibiotics (cephalexin 30mcg/disc, trimethoprim 5mcg/disc, ciprofloxacin30mcg/mcg, chloramphenicol 125mcg/disc and cotrimazole 25mcg/disc) were assessed by disc diffusion method measuring the inhibition zone. Out of the total samples, 11.7% (7) were positive for *Salmonella*. All the samples (7/7) were resistance to cephalexin and cotrimazole showing lower inhibition zones than the standards of *E.coli* 25922 (ATCC). Three out of seven isolates showed resistance for both trimethoprim and chloramphenicol. Six out of seven isolates were resistant for ciprofloxacin. Multiple drug resistance was also detected in some isolates. Two isolates exhibited the resistance against all five antibiotics and other two isolates had resistance against 4 antibiotics used in the antibiogram. Another two isolates showed resistance to three antibiotics. This study concluded that there is a considerably high prevalence of *salmonella* in fresh poultry meat. Development of antibiotic resistance in *Salmonella* spp. has to be strictly addressed considering its public health significance.

Key words: Antibiotic, Sensitivity, *Salmonella*, Isolation, Resistance

Introduction

Salmonella infections continue to be a major public health problem in both developed and developing countries. Salmonellosis is typically spread through the consumption of contaminated food, water, or through contact with an infected host. There are only two species of *Salmonella;* ie. *S. enterica* and *S. bongori* available with more than 2500 serotypes. Based on the degree of host adaptation *Salmonella* serotypes are divided into three groups; (i) Typhoidal (enteric) *Salmonella*: causing typhoid and paratyphoid fever in humans and generally not pathogenic for animals, (ii) Nontyphoidal *Salmonella*: causing gastroenteritis in a broad range of animals, including mammals, reptiles, birds and insects (iii) *Salmonella* restricted to certain animals like *S.* Abortovis to sheep and *S.* Gallinarum to poultry. The two most common causes of nontyphoidal salmonellosis are *Salmonella typhimurium* and *Salmonella enteritidis.* These two serovars can be colonized in the alimentary tract of animals without causing disease so that their contamination of human food chain can be a significant health concern (Karunasagar, 2012).

Resistance to combinations of several classes of antimicrobials has led to the emergence of multidrug-resistant strains that may pass from food animals to humans. *Salmonella* is one of the important pathogens known to carry resistance factors (Gebreyes and Altier, 2002). Prevalence of the antibiotics resistance in *Salmonella* species is quite high from different places and different isolated species. It depends on several factors such as differences in origin, time period of

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collection and different sampling method that are used. The resistance rate, however, varies with different serotypes and different antibiotics. *Salmonella enteritidis* is one of the most prevalent *Salmonella* serotypes which relatively more susceptible to antimicrobial agents than other serotypes. Because of the increased resistance to conventional antibiotics, extended spectrum cephalosporins and fluoroquinolones have become the drugs of choice for treatment of infections caused by multidrug-resistant *Salmonella* serotypes (Karunasagar, 2012). The emergence and spread of multi-drug resistance including *Salmonella* species have reinforced the need for epidemiological studies describing the prevalence and the patterns of resistance of these strains. There were only few reports available in Sri Lanka regarding the isolation and identification of *Salmonella* spp from fresh broiler chicken and detecting the antibiotic resistance. With this background, the present study was carried out mainly focusing on isolation and identification of *Salmonella* spp. from fresh broiler chicken and detecting the antibiotic sensitivity against these isolates.

Materials and Methods

Sixty chilled broiler chicken meat samples were collected from different broiler chicken meat stalls in kiribathgoda, pettah, Gonawala, Kandy and four processing plants located in kurunagala area in pre sterilized (autoclaved) polythene bags for the study. All the samples were immediately iced and transported to the laboratory for analysis. *Salmonella* was isolated by conventional method as per the protocols recommended by FDA (1992). Briefly, the meat samples were rinsed in sterile lactose broth (pre-enrichment) and the rinse was incubated at 37 °C for overnight. Subsequently the pre enriched samples were inoculated to selective enrichment broths such as tetra thionate broth (TTB), cystein selinite broth (SCB) and Rappaport vassiliadis (RV) broth and incubated overnight. Consequently a loop full of culture from each of these broths was streaked on selective agar media such as Hekton enteric agar (HEA), Bisthmus sulfide agar (BSA) and Xylose lysine deoxycholate (XLD) agar and incubated at 37 ºC for 24 h. The plates were examined for the suspected colonies and they were identified using biochemical tests such as indole, methyl red, vogas proscur and citrate test.

Following isolation and identification, Salmonella isolates were tested for antibiotic susceptibility using the method described by Bauer *et al.* (1966). Cephalexin 30 mcg/disc, trimethoprim 5 mcg/disc, ciprofloxacin 30 mcg/mcg, chloramphenicol 125 mcg/disc and cotrimazole 25 mcg/disc antibiotics were used according to manufacturer's (HiMedia Laboratories Pvt. Ltd., India) guidelines. A young culture of Salmonella grown for 10-12 h in 5 ml Mueller-Hinton broth (HiMedia Laboratories Pvt. Ltd., India) was poured on well-dried Mueller-Hinton agar (HiMedia Laboratories Pvt. Ltd., India) to prepare a lawn. After gently air drying in a laminar flow, the antibiotic discs were placed on the surface of the medium and incubated for 24 h at 37 °C for the appearance of a clear zone. The designation of strains as susceptible or resistant was based on the diameter of inhibition zone around each disc was done according to the manufacturer's instructions (Technical data sheets, Himedia Laboratories Pvt. Ltd, India) compared to the *E. coli* 25922 (ATCC).

Results and Discussion

Out of the sixty total samples, seven (11.7%) were positive for Salmonella. This clearly indicates the alarming situation that has been aroused in the country regarding the public health aspect of the food industry. *Salmonella* organisms belonging to subspecies 1 (Typhoidal) are the most resistance

bacterial pathogens which are wide spread in both developed and developing countries (Karunasagar, 2012). Since early 1990s, the multiresistant strains of *S. enterica* serovar Typhimurium have been displaying resistance to six commonly used antibiotics. The development of resistance to key antimicrobials such as fluoroquinolones, particularly in Asian countries, and also extended-spectrum cephalosporins worldwide is the cause of concern. Epidemiological reports have indicated that antimicrobial-resistant strains of *Salmonella* could cause more prolonged or more severe illness than do susceptible strains (Travers *et al.*, 2002).

Frequent use of antibiotics in agriculture, aquaculture and poultry has led to the development of antibiotic resistance in Salmonella. Multi drug resistant salmonella has gained prominence in many parts of the world and has become a cause of concern (Helms *et al.*, 2005). All the samples (7/7) were resistance to cephalexin and cotrimazole showing lower inhibition zones than the standards (*E. coli* 25922, Technical data sheets, Himedia Laboratories Pvt. Ltd, India) (Table 1). Resistance shown for trimethoprim and chloramphenicol was 3/7 isolates each (Table 1). Six out of seven isolates were resistant for ciprofloxacin. Multiple drug resistance was also detected in some isolates. Two isolates exhibited the resistance against all five antibiotics and other two isolates had resistance against 4 antibiotics used in the antibiogram Another two isolates showed resistance to three antibiotics. One isolate showed sensitivity to chloramphenicol and four other antibiotics used in the study. Meanwhile one isolate (S33) was sensitive to chloramphenicol and trimethomprim whereas the isolate S47 showed sensitivity only to trimethomprim. S37 exhibited the sensitivity to trimethomprim, ciprofloxacin and cholramphenicol and this is the only isolate that showed the sensitivity to ciprofloxacin. Isolate 34 displayed sensitivity to trimethomprim and chloramphenicol and it was resistance to other antibiotics. Both the isolate 43 and 45 had multiple drug resistance having resistance to all the antibiotics used in the study (Table 1).

Conclusions

This study concluded that there is a considerably high prevalence of *Salmonella* in fresh poultry meat. Development of antibiotic resistance in *salmonella* spp. has to be strictly addressed considering its public health significance.

Table 1. Antibiotic resistance pattern and inhibition zones

Isolate number	Inhibition zones (mm) and antibiotic resistance of isolates									
	Cephalexin		Trimethoprim		Ciprofloxacin		Chloramphenicol		Co-trimoxazole	
E.coli 25922	17-22		21-28		30-40		21-27		23-29	
s21	R	10.66 ±0.09	R	17.13± 0.21	R	23.59± 0.31	S	20.91 ± 0.06	R	21.54±0.05
s33	R	15.23± .14	S	22.82 ± 0.17	R	27.53 ± 0.1	S	26.1± 0.08	R	20.78 ±0.02
s47	R	12.66± 0.12	S	23.03± 0.05	R	25.92± 0.03	R	20.4 ±0.01	R	21.92± 0.03
s37	R	14.94 ±0.04	S	20.85 ± 0.07	S	29.72± 0.05	S	24±0.01	R	21.89± 0.04
34	R	9.78± 0.26	S	21.35 ± 0.28	R	26.34 ±0.06	S	20.16± 0.09	R	21.84± 0.09
43	R	8.04± 0.03	R	18.7± 0.09	R	28.70 ±0.09	R	16.34 ± 0.06	R	16.84±0.03
45	R	9.02± 0.01	R	7.15± 0.37	R	26.71± 0.04	R	12.64 ± 0.05	R	6.88± 0.05

R indicates resistance, S indicates sensitive to antibiotics.

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