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Research Paper

PREVALENCE OF MULTIDRUG RESISTANT *Salmonella* FROM CHICKEN RETAIL IN ERODE DISTRICT

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Abstract

The main purpose of this study was to investigate the seasonal prevalence, distribution, growth factors, virulence and characterization of multidrug resistant (MDR) *Salmonella* sp. A total of 200 chicken samples were collected in the four regions of Erode district from December 2013 to March 2014. Among them samples were chosen for this study from intestine (50 samples), flesh (50 samples), gall bladder (50 samples) and spleen (50 samples) respectively. All the samples were screened for the presence of *Salmonella* sp which were confirmed by morphologically examination as gram negative rod shaped motile bacteria and Biochemical characters showed indole negative, methyl red positive, vogue proskeur negative, citrate positive, catalase positive, oxidase negative, urease negative, in triple sugar iron acid by alkaline with gas were observed. The organisms fermented carbohydrates of dextrose, maltose, rhamnose, xylose, trehalose, sorbitol, mannitol, mannose while did not ferment sucrose, lactose and raffinose. An organism has utilized arginine that indicated positive reaction and did not utilized lysine and ornithine thus showed negative reaction. Under microscopic examination gram negative rod shaped bacterium. We found that nineteen isolates of *Salmonella* sp from each part of chicken samples showed resistance to antibiotics. All the *Salmonella* sp were highest resistant to tetracycline 100%, followed by nalidixic acid 89.59%, erythromycin 82.62%, co-trimoxazole 81.28%, amoxicilin 75.18%, oflaxacin 73.04%, ampicillin 71.52%, chloramphenicol 63.33%, rifampicin 61.71%, trimethoprim 58.52%, streptomycin 48.13%, clavulnoic acid 33.33%, ciprofloxcin 31.79%, kanamycin 30.05%, respectively. The lowest levels of resistance were found in amikacin 26.18%, gentamicin 12.55%., cephotaxime (7.72%) and ceftriaxone (2.11%) was highly sensitive to all the isolates tested.

Key words: *Multidrug resistant Salmonella sp, Chicken meat, Ceftriaxone highly sensitive*.

INTRODUCTION

The genus *Salmonella* sp belongs to the family Enterobacteriaceae, are facultative anaerobic, Gram-negative, oxidase-negative rod-shaped bacteria [1]. They are straight rod and have 0.7 to 1.5 x 2.5 diameters. Although members of this genus are motile by peritrichuous flagella,

nonflagellated variants, such as *Salmonella pullorum* and *Salmonella gallinarum* and non-motile strains resulting from dysfunctional flagella [2]. *Salmonella enterica* constitutes a major public health problem it associated with significant morbidity and mortality in those infected with the pathogens [3]. Salmonellosis is most after attributed to the consumption of contaminated foods such as chicken, beef, fish, pork, eggs, milk and fresh products [4]. In additional, recent outbreaks resulting from environmental sources such as, contaminated water, slaughter house waste, hospital waste, hospital waste and sewages contribute to human illness and are consequently now being increasingly investigated as a potentially significant reservoir of *Salmonella* sp [5] transmission also includes changes in agronomic practices, the use of gray water and sewage for irrigating the vegetable fields, changing dietary habits and increased international trade of fresh vegetable produce [6]. It is often an opportunistic bacterium, meaning it infects an animal when its immune system is suppressed, when other competing gut bacteria are absent (common after antibiotic therapy), or when the animal is very young [7]. Each year, millions of people are sickened by *Salmonella* and thousands of them died worldwide due to the consumption of *Salmonella* sp contaminated foods [8]. Drug resistance is of considerable importance to microbiologists and is posing a major therapeutic problem for the public and for public health authorities [9]. The emergence and spread of antimicrobial-resistant pathogens, among them *Salmonella* sp, has become a serious health hazard worldwide and high costs of mortality and morbiding among human and animals [10]. Recently multidrug resistant (MDR) strains have emerged, presumably due to the extensive use of antimicrobial agents both in human and animals [11]. Studies have found antimicrobial resistance in *Salmonella* sp species isolated from food animals and humans, including resistance to relatively new antimicrobial agents such as eryithromcyin, ciproflozacin and chloramphenicol resistant typhoid fever became a major problem [12]. In the past, ampicillin and trimethoprim sulfamethoxazole (SXT) were frequency used to treat such cases, but today, their usefulness is limited because of increasing *Salmonella* sp resistance to these antibiotics [13]. Subsequently ciprofloxacin, ceftriazone nalidixic acid and fluroquinolones was resistant to typhoid fever become a major problem in Asia and other parts of the world [14].

MATERIALS AND METHODS

Prevalence of Salmonella species of Chicken samples

A total of 200 samples from the chicken were collected from four regions of Erode district in the four surveillance seasons from November 2013 to March 2014. Out of 200 chicken samples were selected for the study from intestine (50), carcasses (50), flesh (50), gall balder (50) and spleen (50).

Bacteriological examination 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 37^0C 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 37^0C. One-milliliter pre-enriched sample was transferred into 10 ml of tetrathionate broth (Difco) and selenite cysteine broth (Oxoid) and incubated for 48-hrs at 42^0C, then a loopfull culture was aseptically streaked on modified brilliant green sulphadiazine agar and Xylose-Lysine Deoxycholate agar (XLD). The plates were incubated at 37^0C for 24 hrs (APHA 1992). At least five colonies were qualified as presumptive *Salmonella* sp colonies on modified brilliant green agar and XLD agar plates, (red colonies and red colonies with black centers, respectively) were then picked and sub-cultured on slants of nutrient agar.

Morphological characterization

Gram staining was performed according to the respective method [2].

Motility Test

Motility agar were prepared and inoculated with a straight inoculating needle making a single stab about 1-2cm down into the medium. The motility was examined after 35-37oC for 24 hour Motility was indicated by the presence of diffuse growth (appearing as colouring of the medium) away from the line of inoculation. With exception of Salmonella pullorum-gallinarum, all Salmonella species are motile [15].

Biochemical Confirmation

Selective plating was performed using [2] Hektoen enteric agar (HEA) (Hi-Media, India) with overnight incubation at 37°C. Typical black color colonies surrounded by narrow green margin on HEA were biochemically tested by Indole, Methyl Red, Vogus Proskeur, Citrate, catalase and oxidase tests. The colonies showing Salmonella specific IMViC pattern (-+-+) were further confirmed for catalase and oxidase tests followed on Triple sugar iron (TSI) slant. Furthermore, the colonies producing alkaline slant (pink) and acidic butt (yellow) with or without H2S production (blackening) were tested for urease production on urea agar slant. All the urease negative isolates were considered as biochemically confirmed.

Triple sugar iron agar (TSI)

At least one of each colony type of the well-isolated colonies was selected on plate using a sterile straight wire loop. The center of the colony was lightly touched and prepared TSI medium were inoculated by stabbing the butt and streaking the slants. These were then incubated at 37oc for 24 hours [2]. A yellow butt (acid) and red or pink (alkaline) slope indicates the fermenting of glucose only. Cracks and bubbles in the medium indicate gas production from glucose fermentation. A yellow (acid) butt indicates the fermentation of lactose. A red or pink (alkaline) slope and butt indicates no fermentation of glucose or lactose. Blackening along the stab line or throughout the slant indicates hydrogen Sulphide (H2S) production. Salmonella forms a red slope (alkaline) and yellow (acid) butt with/out gas or H2S production [2].

Urease test (Christensen's (modified) urea broth)

Urea agars were inoculated heavily over the entire surfaces of the slants in bijou bottles. The cap were loosened and then incubated at 37^{o}C for 3-12 hours. A urease-positive culture produces an alkaline reaction in the medium, evidenced by pinkish-red colour of the Medium. Urease-negative organisms do not change the colour of the medium, which is pale yellow-pink. Salmonella is always urease negative [2].

Carbohydrate fermentation test

The carbohydrate fermentation test was performed [2] by inoculating a loop full of nutrient broth culture of the organisms into the tubes containing different sugar media (sugars such as dextrose, sucrose, lactose, maltose, mannitol, rhamnose, raffinose, xylose, trehalose, sorbitol, mannitol and manose) and incubated for 24 hours at 37^{0}C. Acid production was indicated by the colour change of the medium reddish to yellow and the gas production was noted by the appearance of gas bubbles in the inverted Durham's tube.

Determination of antibiotic resistance patterns

Antibiotic resistance pattern of *Salmonella* sp isolates were determined by disk diffusion methods.

Disk diffusion method

Antimicrobial susceptibility test was determined by Kirby-bauer's disc diffusion method as per CLSI recommendations [16]. For this test, Mueller- Hinton agar plates were prepared. Sterile cotton swabs were dipped in the culture broth 0.5 McFarland standard turbidity and then soaked swabs were rotated against the upper inside wall of the tube to remove excess cultural fluid. The entire agar surface of the plate was streaked with the swab three times, turning plate at 60 degree angle between each streaking. The medium was allowed to dry for 60 minutes. The turbidity of the suspension was adjusted to match 0.5 McFarland standard turbidity (Oxoid). Using antibiotic disc dispense following discs Amoxycilin, Ampicillin, Ceftriaxone, Cephotaxime, Ciprofloxacin, Chloramphenicol, Co-Trimoxazole, Clavulonic acid, Erythromycin, Gentamicin, Kanamycin, Nalidixic acid, Streptomcyin, Tetracyline, Trimethoprim were released on to the surface Mueller- Hinton agar plates and were incubated at 37^{0}C for 24 hours. Zones of inhibition were interpreted as resistant or sensitivity using the interpretative chart of the zone sizes of the Kirby – Bauer sensitivity test method as per CLSI recommendations.

RESULT

Morphological Characters

The organisms were identified as *Salmonella* sp on the basis of morphology, staining and biochemical test. The under microscopic examinations the organisms were found gram negative

rod shaped and motile bacteria (Table 1). The organisms produced black, translucent, round, raised and smooth colonies on Hektoen enteric agar.

Biochemical Characters

Biochemical characters showed indole negative, methyl red positive, vogue proskeur negative, citrate positive, catalase positive, oxidase negative, urease negative, in triple sugar iron acid by alkaline with gas were observed. The organisms fermented carbohydrates of dextrose, maltose, rhamnose, xylose, trehalose, sorbitol, mannitol, mannose while did not ferment sucrose, lactose and raffinose (Table 1). An organism has utilized arginine that indicated positive reaction and did not utilized lysine and ornithine thus showed negative reaction.

Antimicrobial resistant pattern

Antimicrobial resistant pattern (%) of *Salmonella* sp isolated from carceases, intestine, flesh and spleen of broiler chicken. The pattern of resistance of *Salmonella* sp analyzed in this study is shown in Figure 1. Significant differences were seen in the diffusion zone sizes for all agents. Resistance to tetracycline was present in 100% of the all *Salmonella* sp isolates. Resistance was most commonly observed *Salmonella* isolates from rectal carceases, intestine, flesh, and spleen, to erythromycin 98.16%, 96.71% 98.23%, 97.12%, (97.55%), nbalidixic acid 90.00%, 83.33%, 90.9%, 81.81%, (86.51%), ampicillin 43.33%, 95.83%, 90.9%, 100%, (82.51%), chloramphenicol 90%, 62.5%, 72.72%, 100%, (81.3%), amoxicillin 53.33%, 95.83%, 63.63%, 100%, (78.19%), cotrimoxazole 50%, 70.383%, 90.9%, 100% (77.93%), oflaxacin 53.33%, 75%, 72.72%, 72.72%, (68.44%), rifampicin 50%, 70.83%, 63.63%, 72.72%, (64.29%), trimethoprim 66.66%, 70.83%, 45.45%, 81381%, (68.18%), Kanamycin 36.24%, 34.54%, 32.71%, 33.45%, (34.23%) clavulnoic acid 33.25, 34.5%, 32.13%, 34.1%, (33.48%), amikacin 20%, 20.83%, 18.18%, 54.54%, (28.38%), respectively are presented in Table 2.

Table 1. The morphological, biochemical conformation test of *Salmonella* sp

Test	Reaction	Test	Reaction	Test	Reaction
Gram Staining	Negative	Oxidase	Negative	Trehalose	Fermented
Motility	Positive	Triple sugar iron	A/AK^{g+}	Sorbitol	Fermented
Indole	Negative	Urease	Negative	Mannitol	Fermented
Methyl red	Positive	Dextrose	Fermented	Mannose	Fermented
Voges- Proskauer	Negative	Maltose	Fermented	Raffinose	Not Fermented
Citrate	Positive	Rhamnose	Fermented	Lactose	Not Fermented
Catalase	Positive	Xylose	Fermented	Sucrose	Not Fermented

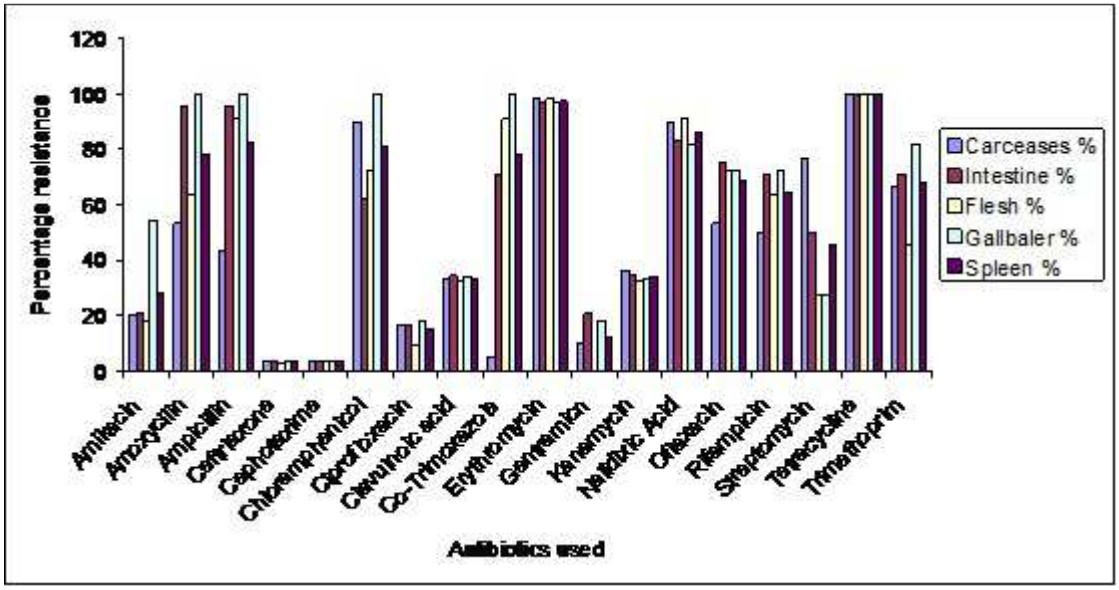

Figure 1 Antimicrobial resistant pattern (%) of *Salmonella* sp isolated from intestine, Carceases, flesh and Gallblader spleen of broiler chicken.

Table 2. Antimicrobial resistant pattern (%) of *Salmonella* sp isolated from intestine, Carceases, flesh and Gallblader spleen of broiler chicken**.**

Antibiotic	Intestine %	Carceases %	Flesh %	Gallblader %	Spleen %
Amikacin	20.83	20	18.18	54.54	28.38
Amoxycilin	95.83	53.33	63.63	100	78.19
Ampicillin	95.83	43.33	90.9	100	82.51
Ceftriaxone	3.71	3.51	2.99	3.21	3.35
Cephotaxime	3.33	3.4	3.6	3.24	3.39
Chloramphenicol	62.5	90	72.72	100	81.3
Ciprofloxacin	16.66	16.66	9.09	18.18	15.14
Clavulnoic acid	34.5	33.21	32.13	34.1	33.48
Co-Trimoxazole	70.83	5	90.9	100	77.93
Erythromycin	96.71	98.16	98.23	97.12	97.55
Gentramicn	20.83	10	0	18.18	12.25
Kanamycin	34.54	36.24	32.71	33.45	34.23
Nalidixic Acid	83.33	90.00	90.9	81.81	86.51
Oflaxacin	75	53.33	72.72	72.72	68.44
Rifampicin	70.83	50	63.63	72.72	64.29
Streptomycin	50	76.66	27.27	27.27	45.3
Tetracycline	100	100	100	100	100
Trimethoprim	70.83	66.66	45.45	81.81	68.18

DISCUSSION

Other published studies reported similar rates for meat products in the Italy 37.3% [17]. Much higher rates (36 to 60%) have been reported in poultry in studies conduted in Belgium, Spain and Portugal [18]. The prevalence rate of *Salmonella* sp from chickens in our study (52.17%) was lower than reported rates of 57% from chickens in Thailand. Although our results were in this range but it should be considered that they have been deduced from a pilot study. The results also depend on the methods applied. In addition to the high prevalence of *Salmonella* species in both meats and humans, our study also found that the rates of antimicrobial resistance is Switzerland (12%) and Italy (19%) were significantly higher than rates in industrialized countries [19]. Chicken is one of the most nutritious foods with high food value. However most of the people consume due to their nutritional value, cheap and availability. Salmonellosis is common and affects higher numbers of chicken than the incidence of clinical disease would suggest [20]. *Salmonella* species are one of the most prevalent enteric pathogens encountered in chicken in India [21]. This observation was in agreement with similar previous findings. Precise knowledge of *Salmonella* infection in food animals is required for understanding the epidemiology of Salmonellosis and control of the infection in food animals [22]. *Salmonella* sp being zoonotic in nature and prevalent in pet animals might modulate the disease prevalence in other animals and human beings [23]. However, these differences could be due in part to the types of samples analyzed and the step of the food chain samples. Variations observed between the reported *Salmonella* sp prevalence results to other authors, several factors must be taken into consideration, including the different origin initial pre-slaughter *Salmonella* sp load of the birds, sanitation within the slaughterhouse, possible contamination during poultry processing steps, the amount of cross or post contamination of chicken carcasses by fecal material during or after slaughter, and the sensitivity and specificity of different isolation methods applied to detect *Salmonella* sp [24]. Previous studies in India on the *Salmonella* species isolated from humans and animals show prevalence rates similar to or moderately higher than those found in this study in isolated from human sources, while prevalence rates among isolates from animal origin were higher [25]. In an earlier study conducted in India, the higher incidence of *Salmonella* species in the food animals like chicken and fish origins [26]. All the chickens and fish purchased for this study were contaminated with *Salmonella* species. This may be due to the fact that *Salmonella* sp is part of the normal enteric

flora of chickens and fish [27]. Foods and water still play the main roles in the transmission of *Salmonella* sp with human via direct or indirect contact, though wastewater is particularly important. On the other hand, the world is faced with problems related to the management of wastewater due to the extensive industrialization, increasing population density and highly urbanized society [28]. Recycling municipal and industrial wastewater is therefore essential for reducing the negative impact of pollution on the freshwater reserves and also for protecting public health by safeguarding water supplies against the spread of water borne disease [29]. Of the 45 strains studied, 21 came from raw drinking water and 24 from sewage. In the present study, the prevalence of *Salmonella* sp in raw drinking water and sewage was 21 (38.1%) and 24 (50%) respectively. These findings are significantly higher than those reported [11]. Thus, the water and sewages as a public health risk in the transmission of salmonellosis, as already confirmed by other authors [7]. The isolation frequency of *Salmonella* sp (43.2%) in this study is lower than the 65% isolation frequency recorded previously [30]. The lower frequency seen in this study may be as a result of changes in the incidence of *Salmonella* in Erode [6]. The present study has demonstrated a considerable increase in the prevalence rate of *Salmonella* sp species in chicken. This is because potential relationships, associations, correlations and interaction of microbial species found throughout the beef and chicken production chain are not well known, and therefore, the presence or absence of a specific microorganism should not be used as an index or indicator of the presence or absence of others, including pathogens. Additional safety measures should include training in personal hygiene, sanitation and ensuring water quality. In accordance with other studies, we confirmed correlation between high prevalence of antibiotic resistance to animal foods such, as chicken. The results obtained from chicken showed that there was significant development or resistant during the study periods. The results of present study were contrary to the result [7] who reported 100% resistance towards tetracycline. In chicken samples over all resistance to erythromycin 98.16%, 96.71%, 98.23%, 97.12%, 97.55%), nalidixic acid 90.00%, 83.33%, 90.09%, 81.81%, (86.51%), ampicillin 43.33%, 95.83%, 90.9%, 100%, (82.51%), chloramphenicol 90%, 62.5%, 72.72%, 100%, (81.3%), amoxicillin 53.33%, 95.83%, 63.63%, 100%, (78.19%), co-trimoxazole 50%, 70.83%, 909%, 100%(77.93%), oflaxacin 53.33%, 75%, 72.72%, 72.72%, (68.44%), rifampicin 50%, 70.83%, 63.63%, 72.72%, (64.29%), trimethoprim 66.66%, 70.83%, 45.45%, 81.81%, (68.18%), respectively. *Salmonella* isolates from chicken samples were highly resistance to following antibiotics, nalidixic acid 90.6%, tetracycline 71.9%, trimethoprim 56.6%, and streptomycin 25% respectively [4].

CONCLUSION

In this study it was concluded that chicken isolates of *Salmonella* sp showed 100% resistance to tetracycline and lowest level resistance to amikacin, gentamicin, cephotoxime, ceftraxome. The ceftraxome showed highly sensitive to chicken isolates of *Salmonella* sp. The consumption chicken meat infected with *Salmonella* sp could be fatal to human health. To overcome that problem more attentaion should be paid to keep the chicken retail shops very hygienic conditions especially in slaughtering site. The food and water in poultry form should be more hygienic. The effected chicken should be treated with ceftriaxone which kills the *Salmonella* sp

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