

Research Article

Antibiotic assays of *Salmonella* isolated from poultry chicken of various locations in districts Swat

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Abstract

Poultry meat serves as an important source of protein having different essential amino acids but is associated with various infectious bacteria. The aim of this study was to find out the prevalence and isolation of *Salmonella* from poultry chicken and to check it against antibiotic. About 150 samples were collected from different areas of District Swat. The samples were of three type, i.e. fresh raw meat, cloacal swab sample from slaughter houses and cutting board cloacal swab samples. Based on temperature fluctuation, the sample collection sites were categorized into three regions, i.e. colder, moderate and warm regions. The samples were cultured on different media, e.g. Nutrient Agar (NA), MacConkey Agar (MCA), Deoxycholate Citrate Agar (DCA) and Salmonella-Shigella Agar (S.S). The isolated bacteria were identified after gram staining and Biochemical tests including Indole (I), Methyl Red (M), Vogus Proskeur (Vi), Citrate (C), Urease and Triple Sugar Iron (TSI) Test etc. For the Identification Burjey's Manual was followed. Isolated bacteria included *Salmonella* 22%, *E.coli* 20.66%, *Klebsiella* 15.3 %, *Proteus* 11.3 %, *Pseudomonas* 8.6 % and *Shigella* 7.3%. Raw meat of poultry chicken had about 18% prevalence, slaughter house had 26%, while cutting boards had 22% prevalence of *Salmonella*. *Salmonella* isolated from all the sources was resistant to different antibiotics including tetracycline (89%), neomycin (80%), ampicillin (75%) and novobiocin (74%). It was also revealed in present investigation that *salmonella* was sensitive to ceftriaxone (100%), cephadrine (100%), piperacillin-tazobactam (94%) and cefepime (90%). It is concluded that *Salmonella* is frequently present in chicken and is a major source of Salmonellosis.

Keywords: Antibiotic sensitivity; Poultry chicken; Resistance; *Salmonella*

Introduction

Salmonella spp is an important pathogenic bacterium belongs to the family Enterobacteriaceae. It is gram negative,

facultative anaerobic and rod shaped bacteria. The optimal pH is 7 and best temperature for its growth and multiplication is 35-37°C. It is responsible for causing

gastroenteritis and systemic diseases in human, avian and other animals. The genus salmonella is broadly divided into two species, i.e. *Salmonella enteric* and *Salmonella bongori*. Each species has various serotypes. Some of the *Salmonella* serotypes are host specific, while others are non-host specific and have wider host range [1]. Salmonellosis is the most common disease of humans caused by *Salmonella typhi* and *Salmonella paratyphi* [2]. Typhoidal salmonellosis occurrence has direct relation with socioeconomic conditions of a region or country. It is an important problem of developing countries. A number of diseases were recorded in human history that spread from waste materials and due to unhygienic environmental conditions. According to World Health Organization (WHO) report of 2005, about 17 million cases are reported annually. Depending on region and use of proper antibiotic, the mortality rate of this disease is about 6-7%. In Asia, the data about salmonellosis are scarce, whereas in central and south America and in Africa the mortality rate is about 1-10% of all cases (Hu and Kopecko, 2003). It is also reported that typhoid fever is usually endemic in Asia, Africa and Middle East [2].

Poultry is the quick and main source of nutrition for human population. However in Pakistan large numbers of new poultry industries are facing different infectious diseases, e.g. salmonellosis [3]. *Salmonella* species mainly transmitted to humans from raw meat of chicken and poultry products. 40% clinical cases of salmonellosis are due to consumption of poultry products and eggs [4]. During 1950 to 1970, approximately 40% of different species of *salmonella* were isolated from poultry [5]. Infections can transmit to humans either directly by handling or indirectly by infected pets and food animal [6]. Ingestion of contaminated poultry products such as meat and eggs can

cause salmonella infection in humans and associated with acute and chronic diseases in both poultry and humans [7]. According to several researches, poultry and poultry products are the most important sources of *salmonella's* transmission to the human population [8].

Transmissions of typhoidal salmonellosis in humans are based on contaminated environment, drinking of contaminated water and ingestion of contaminated food. According to some researchers it is considered as a zoonotic disease as poultry meat and poultry products are the important sources of typhoidal salmonellosis [9]. It is reported that in Pakistan during 1950-70 all form of salmonellosis were isolated from poultry. About 40% of clinical cases of salmonellosis are due to poultry products and eggs consumption [5].

Material and methods

A total of 150 samples (i.e. 50 samples of fresh meat of chicken, 50 cloacal swab samples from the cutting board and 50 cloacal swab samples from slaughter houses) were taken from Swat regions. Sampling sites were divided into three categories on the basis of temperature. The temperature ranges of different locations were 19-25°C, 25-31°C and 32-38°C. The samples were collected in sterilized bags and brought for processing to the laboratory of "Center for Biotechnology and Microbiology", University of Swat within 4 - 6 hours after collections. Meat, cutting board and slaughter swab samples were streaked onto Nutrient agar plates and were allowed to incubate for 48 hours. For further isolation and identification, subcultures were taken on Deoxycholate Citrate Agar (DCA) and Salmonella-Shigella Agar (S.S.A). Moreover Salmonella were biochemically tested by Indole (I), Methyl Red (M), Vogus Proskeur (Vi), Citrate (C), Urease and Triple Sugar Iron (TSI) Test. For the In-vitro evaluation SCLI method was applied. Other species of bacteria were also

accordingly conformed biochemically. Isolates of *Salmonella* were subjected to antibiotic sensitivity test by disk diffusion method. Antibiotics used were from different generations.

Results

Percentage of isolated bacteria

Six different species of bacteria were isolated from all the three samples. These included *Salmonella* 22%, *E.coli* 20.66%, *Shigella* 7.3%, *Proteus* 11.3 %, *Pseudomonas* 8.6 % and *Klebsiella* 15.3 % were isolated from all the three samples (Figure 1). A maximum percentage of *Salmonella* (26%) were isolated from Slaughter house cloacal swab sample, followed by 22% from cutting table

swab samples and 18% from meat samples (Figure 2).

Prevalent bacterial species

The maximum prevalence of *Salmonella* was observed in samples from regions with temperature range of 32 to 38°C (57.57%) followed by 33.33% from 26 to 31°C and regions with 19 to 25°C temperature showed minimum prevalence of 9% (Figure 3).

Isolated *Salmonella* showed resistance of different percentages to most of antibiotics. The highest resistance was found against the Tetracycline 89% followed by Neomycin 80%. However they showed maximum sensitivity against the Ceftriaxone and Cephadrine 100% followed by Sefepime 90% (Table1).

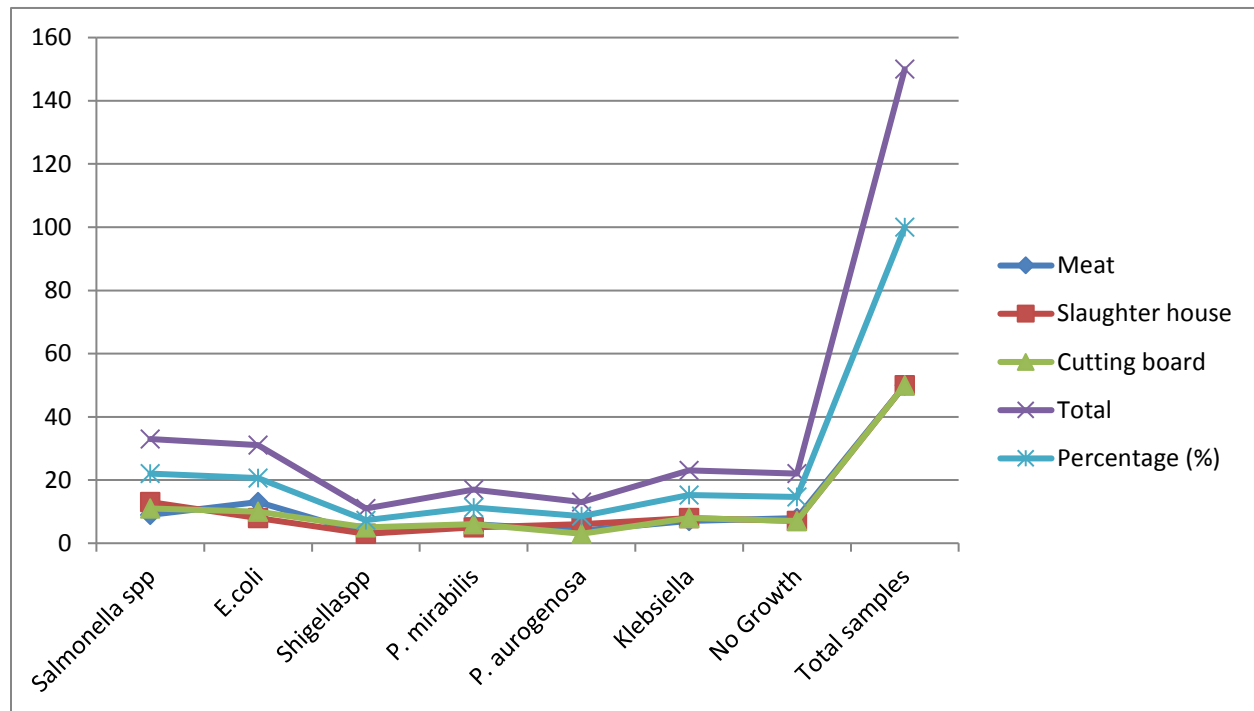


Figure 1. Total frequencies and percentages of bacteria isolated form poultry raw meat, cutting boards and poultry slaughter houses

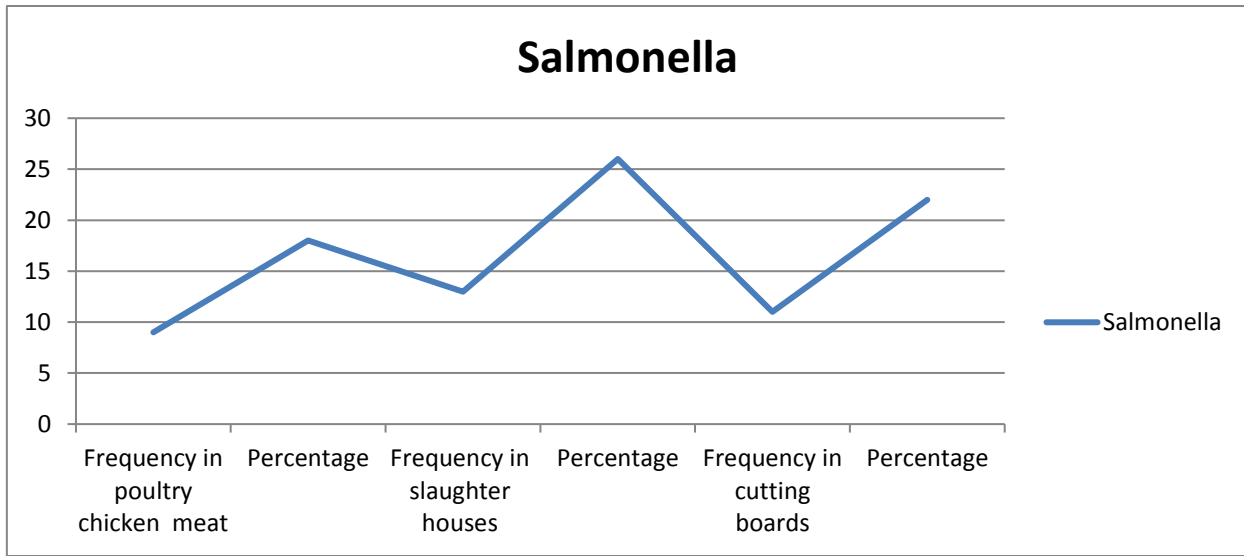


Figure 2. Salmonella’s frequencies and percentage isolated from poultry chicken meat, slaughter houses and cutting boards

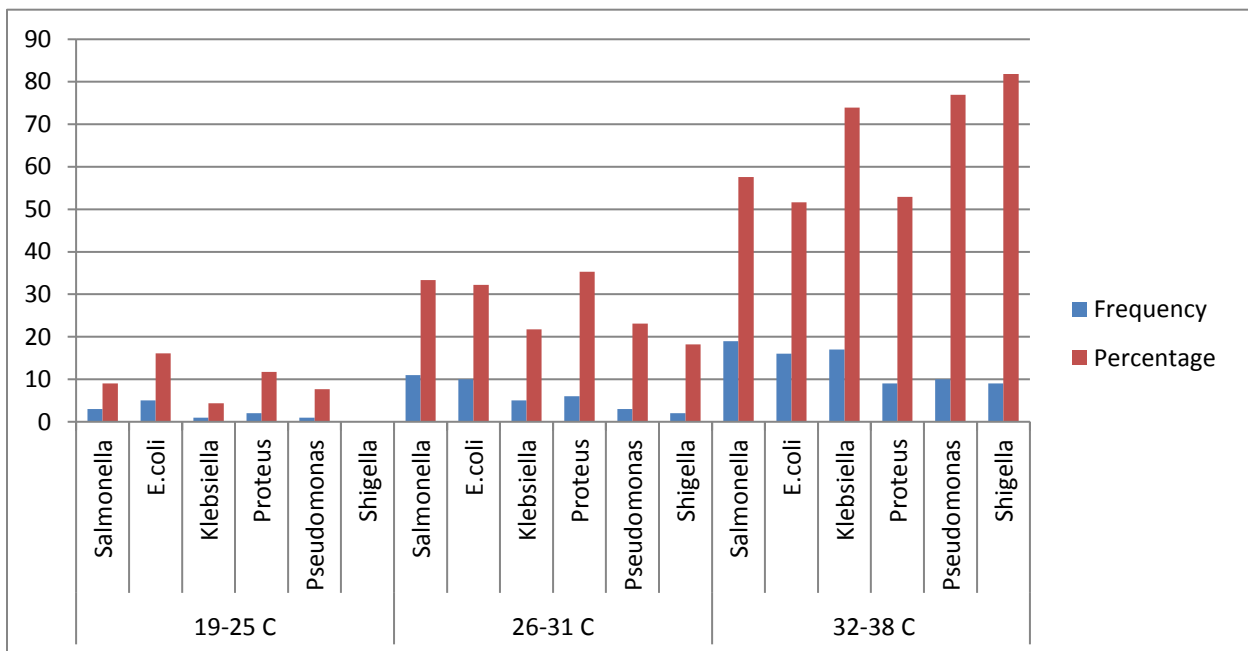


Figure 3. Bacterial isolations from different temperature based areas

Table 1. Antibiotic sensitivity profile of *Salmonella* isolated from poultry chicken, slaughter house and chopping (cutting) board

Antimicrobial drugs	Resistance (%)	Intermediate resistance (%)	Sensitive/Susceptible (%)
Ampicillin	75	20	5
Streptomycin	31	15	54
Kanamycin	8	17	75
Bacitracin	56	19	25
Novobiocin	74	20	6
Nalidixic acid	50	25	25
Erythromycin	40	32	28
Spectinomycin	4	46	50
Neomycin	80	10	10
Tetracyclin	89	5	6
Ceftriaxone	-	-	100
Azithromycin	70	8	22
Piperacillin-tazobactam	-	6	94
Ciprofloxacin	4	20	76
Amoxicillin-clavulanic acid	30	45	25
Chloramphenicol	41	32	9
Cephadrine	-	-	100
Cefepime	-	10	90

Discussion

Poultry is an important agricultural industry in Pakistan, having an investment of more than 732 billion rupees. Poultry meat serves as an important source of protein having different essential amino acids like arginine, histidine, isoleucine, leucin, cystine, cystine, arginine and methionine, etc. [10]. Poultry meat is a valuable commodity for the local consumers in Swat, Pakistan. Typhoidal and non-typhoidal salmonellosis are important food borne diseases, which are major health problems in many regions of the world. *Salmonella* spp. is common pathogenic bacteria associated with different foods including meat, meat products, poultry and poultry products [11]. In the present study fresh meat samples, slaughter house swab samples and cutting board samples were collected from different butcher shops.

Results of the present study demonstrated that 18% *Salmonella* are isolated from meat sample while 26% from slaughter houses and 22% from the cutting boards in butcher shops. Similar results were obtained in another study conducted in North East India shows that raw meat samples from the local market have 20% prevalence of *Salmonella* [12]. However a study conducted in Hyderabad Pakistan shows 38% prevalence of *Salmonella* in poultry chicken raw meat, in contrast to the present study which had revealed 18% prevalence in meat sample. These differences in the prevalence of *salmonella* in poultry chicken raw meat may be due to many environmental factors like temperature of the area, hygienic condition of the poultry farm and water supply to the poultry chicken. Keeping all these factors in mind, current study results should not

necessarily be the same for results from different ecological site.

Study revealed that most of the butcher shops have failed to maintain safe and clean environment. Moreover, use of the same cutting knives for infected and un-infected chicken raw meat, further increases the chances of cross-contamination. Consumption of undercooked and contaminated meat by the consumer results in *Salmonella* infection including typhoid, paratyphoid or other non typhoidal diseases or *non-salmonella* infections. May also be able to result in infection with other pathogenic bacteria like *Shigella*, *Escherichia coli*, *Klebsiella* etc. Such infections can result in enteritis or intestinal infections.

The current study also demonstrated that *Salmonella* spp. is resistant to different antibiotics, mostly resistant to tetracycline 89% followed by neomycin 80%, ampicillin 75%, novobiocin 74%, azithromycin 70%. A study conducted in Dubai United Arab Emirates showed 87.8% resistance of *Salmonella* towards tetracycline, which is consistent with our study [13]. In another study it was determined that 77% *Salmonella* in poultry feed is resistance to tetracycline, which is much closer to the present study findings [14].

Our study showed that *Salmonella* is sensitive to different antibiotics, e.g. 100% to ceftriaxone and cephadrine, followed by tazobactam 94%, cefepime 90% and ciprofloxacin 76%. As in contrast a study revealed that *Salmonella* isolated from poultry chicken showed 52.4% sensitivity to ceftriaxone [14]. These variations in results may be due to changes in environmental and geographic condition. Similarly in another study conducted by shows that tazobactam sensitivity was about 100%, which is in agreement with the present study [15].

The current study further revealed that most of the isolated bacteria were from urban areas

like Mingora, Rahim abad, and Qambar, etc. Whereas less number of bacterial organisms were isolated from rural areas like Marguzar, Islampur and Madyan, etc. The reason may be that urban areas are much crowded and polluted than the rural areas and their chances of bacterial contamination to poultry or agriculture industry is more than rural areas.

Conclusions

Poultry meat serves as an important source of protein having different essential amino acids but is associated with different infectious bacteria. During current study about 150 meat samples were collected from various areas of District Swat. The samples were cultured on different medias and Identified by Burjey's Manual. The Isolated bacteria included *Salmonella* 22%, *E.coli* 20.66%, *Klebsiella* 15.3 %, *Proteus* 11.3 %, *Pseudomonas* 8.6 % and *Shigella* 7.3%. *Salmonella* isolated from all the sources was found resistant to different antibiotics including tetracycline (89%), neomycin (80%), ampicillin (75%) and novobiocin (74%) while found sensitive to ceftriaxone (100%), cephradine (100%), piperacillin-tazobactam (94%) and cefepime (90%). It is concluded that *Salmonella* is frequently present in chicken and is a major source of Salmonellosis.

Authors' contributions

Conceived and designed the experiments: MN Uddin & M Farooq, Performed the experiments: M Waqas, NU Khan & WA Khan, Analyzed the data: Muhammad Rizwan, Contributed reagents/ materials/ analysis tools: I Khan & N Karim, Wrote the paper: Muhammad.

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