



IDENTIFICATION OF *SALMONELLA* SEROVARS FROM COMMERCIALY ROASTED CHICKEN CONTACT SURFACES AND UTENSILS IN DUTSE METROPOLIS

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ABSTRACT

The predominant serovars of *Salmonella* associated with environmental contact surfaces and utensils used in processing and selling roasted chicken in Dutse town of Jigawa State was determined. The aerobic plate count of the one hundred (100) cotton swab samples collected from studied surfaces was determined. Cultural Identification of the isolates was done on *Salmonella-Shigella* agar (SSA) followed by microscopy. Biochemical confirmatory tests and serological tests using polyvalent and monovalent sera were conducted. Antibiotic sensitivity pattern of the isolates was determined by the Kirby Bauer disc diffusion method. Thirteen (13) samples yielded growth of *Salmonella* serotypes, giving a prevalence rate of 13.0%. *S. enteritidis* serotype was the most identified (46.0%) while *S. Virchow* (8.0%) was the least. The highest bacterial load was observed on the hands of vendors, with an aerobic plate count of 4.4×10^4 CFU/g while the least was observed on cutting board utensil (2.5×10^4 CFU/g). Rate of contamination was found to be most significant on hand swab samples (38.0%) and least on knife swab samples (8.0%). Isolates were most susceptible to Chloramphenicol and Gentamicin but highly resistant to Septrin, Peflacin, and Tarivid. The hands, contact surfaces and utensils used in the processing of roasted chicken must be adequately sanitized with appropriate sanitizers so as to prevent the dissemination of pathogens such as *Salmonella* to consumers.

Keywords: *Salmonella*, Serotypes, Serovars, Roasted Chicken, Utensils

INTRODUCTION

Salmonellosis is known to be a highly widespread foodborne disease with a global distribution (Bollaerts *et al.*, 2008). It causes significant economic loss due to mortality, morbidity, and losses in terms of product marketability posing a serious challenge to the food industry (Khan *et al.*, 2007). *Salmonella* species are among the leading causes of acute gastroenteritis in both developing and developed countries around the world (Abunna *et al.*, 2017). Foods of animal origin contaminated with fecal matter are the main transmission routes of this pathogen (Swartz, 2002). Salmonellosis is of major public health concern and over several decades there has been a significant shift in predominant *Salmonella* serovars responsible for human infections (Steven *et al.*, 2011). Thousands of other *Salmonella enterica* serotypes may be responsible for infections but *Salmonella Enteritidis* is the most common serotype in human infections (Pang *et al.*, 2007).

Several studies on the effect of microbial cross-contamination via chopping board, cutlery, and hands on the microbiological quality of ready to eat foods have been conducted in household kitchens and other retail outlets (Dawson *et al.*, 2007; Soares *et al.*, 2012). These and many related studies indicate that

contamination of food service environmental surfaces by foodborne pathogens during food preparation and eventual transfer to foodstuff is one of the main causes of foodborne outbreaks. Bacterial cells adhered to those surfaces are not easily removed by normal cleaning procedures (Cogan *et al.*, 1999; De Jong *et al.*, 2008; Van Asselt *et al.*, 2008). Various pathogenic bacteria such as *Salmonella* and *Campylobacter* remain viable on utensils, hands, and cutleries, from where they could cross-contaminate other food substances (Humphrey *et al.*, 2001; Nzouankeu *et al.*, 2010). It is generally estimated that about 40–60% of foodborne disease cases are caused by improper food handling practices (De Jong *et al.*, 2008), especially cross-contamination from cutting boards when ready-to-eat (RTE) meat or other food is handled immediately after handling raw poultry meat (Parry *et al.*, 2005; Lubber, 2009).

It has been reported that the prevalence of antibiotic resistant *Salmonella* is on the increase due to rampant usage of antibiotics in poultry feeds for prophylactic and/or therapeutic purposes, which may encourage the selection of antibiotic-resistant strains and significantly increase the public health risks associated with consuming contaminated animal products (Yang *et al.*, 2010). The objective of this study was to examine the bacterial load and

the various serotypes of *Salmonella* contaminating contact surfaces of RTE roasted chicken sold by roadside vendors in Dutse metropolis. The antibacterial resistance pattern of the *Salmonella* serovars isolated was also examined.

MATERIALS AND METHODS

Study Area

This study was carried out in Dutse, the capital of Jigawa state, Nigeria. It covered twenty five (25) roadside ready-to-eat (RTE) chicken retail outlets in Dutse metropolis, which is located on latitude 11° 46' 39" N, longitude 9° 20' 3" E (Dutse Map, 2016).

Sample Collection

A total of 100 swab samples were collected from equipment and contact surfaces of chicken vendors using sterile swab sticks moistened with sterile normal saline. Samples were obtained from 4 different contact surfaces comprising of wooden table, cutting board, cutting knives and workers' hands. Samples were taken from 25 different chicken vendors using surface swab techniques as described in the Compendium of Methods for the

$$\text{CFU/cm}^2 = \frac{\text{Number of colonies counted} \times \text{Dilution factor}}{\text{Volume Plated}}$$

They were expressed in colony forming units per cm² (CFU/ cm²) (Nesa *et al.*, 2011).

Pre-enrichment and Enrichment of Samples

Each swab sample was incubated at 37°C for 24h in 10 ml of peptone water as pre-enrichment, 1ml from the incubated tubes were transferred to test tubes containing 9ml of selenite F broth for *Salmonella* enrichment.

Isolation and Identification of *Salmonella*

One (1) ml of suspension from each enrichment tube was streaked onto freshly prepared Salmonella-Shigella agar (SSA) plates and incubated in an inverted position at 37°C for 24 hours. Isolates from the selective medium were identified based on colonial morphology, Gram's reaction and a battery of biochemical tests including Catalase test, Motility test, Urease test, Indole test, Glucose fermentation, Lysine decarboxylase test, Citrate and TSI test based on the standard procedures (Cheesbrough, 2006).

Serotyping of *Salmonella* Isolates

Presumptive isolates of *Salmonella* were inoculated onto Triple Sugar Iron (TSI) medium and incubated at 37°C for 24 hours. After incubation, serotypes were determined using polyvalent sera followed by monovalent sera for mixtures with significant agglutination. *Salmonella* agglutinating antiserum poly 'O' and poly 'H' (Pro-lab Richmond Hill, Ontario, Canada) were used according to manufacturer's instructions. Poly 'O' antiserum gives positive agglutination reaction with all serovars during the

Microbiological Examination of Foods (American Public Health, 1992) where 10-15cm² surface area of each contact surface was sampled. The swab sticks were immediately taken to the Federal University Dutse Microbiology Laboratory for analysis (Cheesbrough, 2006).

Aerobic Plate Count (APC)

Each swab stick collected was suspended in 10 ml of buffered peptone water and incubated at 37°C for 24 hours in an incubator. One ml from this initial suspension was added to a series of test tubes containing 9ml of buffered peptone water, giving a dilution ratio of 1:10. A four-fold serial dilution was prepared for each sample giving rise to test tubes containing 10⁻¹-10⁻⁴. 2 ml each from these tubes was dispensed in sterile Petri dishes and a prepared plate count agar (PCA) was poured into the Petri dishes and allowed to solidify. After solidification, the plates were incubated in an inverted position at 37°C for 18-24 hours in an incubator. Distinct colonies on plate count agar were counted manually and colony forming units per cm² (CFU/cm²) of surfaces sampled was determined using the formula below:

preliminary screening of *Salmonella* and poly 'H' antiserum gives specific agglutination reaction for *Salmonella* serovars within 10 seconds (Nesa *et al.*, 2011).

Antimicrobial Susceptibility Test

Following the guidelines by Clinical and Laboratory Standards (2012), the antimicrobial susceptibility pattern of the isolates was determined using the disk diffusion method on Mueller-Hinton agar. The antibiotics tested were Tarivid, Peflacin, Gentamicin, Amoxicillin, Streptomycin, Spectinomycin, Chloramphenicol, Augmentin, Ciprofloxacin, and Septrin.

Data Analysis

Data were analyzed using SPSS software version 20.0. Chi-square test was used to analyze significant differences in the distribution of *Salmonella* serotypes from samples types analyzed.

RESULTS

Out of the 100 samples analyzed, thirteen (13) samples yielded growth of *Salmonella* spp, which gives a prevalence rate of 13.0%. Four (4) different serotypes were identified and of these, *S. enteritidis* had the highest frequency of isolation (46.0%) while *S. Virchow* recorded the least (8.0%) (Table 1).

Table 1: Percentage distribution of *Salmonella* Serotypes identified from the various samples examined

Serotype	Number Isolated	Percentage (%)
<i>S. Enteritidis</i>	6	46.0
<i>S. Typhimurium</i>	4	31.0
<i>S. Paratyphi B</i>	2	15.0
<i>S. Virchow</i>	1	8.0
Total	13	100

The mean aerobic plate count (APC) of *Salmonella* spp observed was most significant among swab samples collected from the hands of RTE Vendors (4.4×10^4 CFU/g) followed by Knives (4.2×10^4), while the least was observed on swab samples of cutting board utensil (2.5×10^4 CFU/g) (Table 2).

Table 2: Aerobic Plate Count (APC) for Tables, Knives, Hands and Cutting Boards

Materials	Mean Aerobic plate count (APC) CFU/cm ²
Tables	3.6×10^4
Knives	4.2×10^4
Hands	4.4×10^4
Cutting boards	2.5×10^4

The *Salmonella* serotype with the highest occurrence was *S. enteritidis* (46.0%), while *S. Virchow* had the least occurrence rate of 8.0%. Rate of isolation in relation to sample type examined indicate that Hand swab samples yielded the highest recovery rate of 38.0% and the least was observed among Knives (8.0%). *S. enteritidis* isolated from Hand swab samples was the most significant contamination rate observed on sample/*Salmonella* serotype basis (23.0%) (Table 3).

Table 3: Distribution of *Salmonella* Serotypes in relation to Sample types examined among RTE vendors in Dutse

Sample Examined	Serotype (%)				Total (%)
	<i>S. Enteritidis</i>	<i>S. Typhimurium</i>	<i>S. Paratyphi B</i>	<i>S. Virchow</i>	
Tables	2 (15.0)	1 (8.0)	1 (8.0)	0 (0.0)	4 (31.0)
Knives	0 (0.0)	1 (8.0)	0 (0.0)	0 (0.0)	1 (8.0)
Hands	3 (23.0)	1 (8.0)	1 (8.0)	0 (0.0)	5 (38.0)
Cutting board	1 (8.0)	1 (8.0)	0 (0.0)	1 (8.0)	3 (23.0)
Total (%)	6 (46.0)	4 (31.0)	2 (15.0)	1 (8.0)	13 (100)

The antimicrobial susceptibility pattern of the *Salmonella* isolates showed that they were completely susceptible to Chloramphenicol and Gentamicin (100% respectively) followed by Streptomycin (92.0%), Ciprofloxacin and Augmentin (85.0% respectively). Isolates were however, resistant to Septrin (92.0%), Peflacin (70.0%) and Tarivid (85.0%) (Table 4).

Table 4: Percentage Susceptibility Pattern of *Salmonella* Serotypes identified

Antimicrobial drugs tested	Susceptibility test	
	Sensitive (%)	Resistant (%)
Septrin (SXT)	8.0	92.0
Chloramphenicol (CH)	100	0.0
Spectinomycin (SP)	46.0	54.0
Amoxicillin (AMX)	54.0	46.0
Augmentin (AU)	85.0	15.0
Gentamicin (CN)	100	0.0
Peflacin (PEF)	30.0	70.0
Tarivid (OFX)	15.0	85.0
Streptomycin (S)	92.0	8.0
Ciprofloxacin (CPX)	85.0	15.0

DISCUSSION

The presence of *Salmonella* spp, no matter how negligible, on RTE foods should be of serious public health concern. Because of its low infectious dose, most serotypes when ingested can induce Salmonellosis and as such, making it a pathogen of utmost public health concern. In this study, the prevalence of *Salmonella* serotypes on contact surfaces of RTE roasted chicken was low. The highest contamination rate was observed on the hands of vendors. This agrees with the report of Abdi *et al.* (2017) in a study which involves the detection of *Salmonella* on poultry in Southern Ethiopia. They observed a high contamination rate on the hands of personnel working on the poultry products. The presence of *Salmonella* spp on the hands of vendors is quite worrisome as there is high likelihood of re-contamination of the RTE meat product even after it has been cooked. Such re-contamination could arise when the vendors happen to be asymptomatic carriers shedding the pathogen, and being the final handlers of the meat at the point of sale. Contrary findings were reported by Abunna *et al.* (2017), who observed a low prevalence rate on the hands of workers. They reported an overall prevalence rate of 5.6% which is lower than what was observed in this study. Such differences could be attributable to differences in study design and experimental conditions.

Food and drug administrators (FDAs) have confirmed that the detection of *Salmonella* spp in at least 25g of RTE foods is considered potentially hazardous and is attributable to inadequate processing of raw products, cross-contamination (from contact surfaces including hands of possible asymptomatic carriers; vendors in this case) or contaminated raw materials. They further recommend that collaboration is required with the local food safety regulators to determine if

disposal and/or product recall is warranted so as to safeguard the population (HPA, 2009; FDA, 2012; ICMSF, 1996). Based on results obtained from this study, the level of sanitary standard expected of such critical food processing environments should be stressed, and call for stricter sanitary measures that will minimize the level of contamination with, and proliferation of pathogens such as *Salmonella* spp should be advocated.

S. Enteritidis was the most prevalent serotype observed in this study. It has been reported that 40% to 80% of food poisoning occurring in developed countries have been caused by *Salmonella enteritidis* (Parvej *et al.*, 2016). In another study by Vinuesa-Burgos (2016), *S. Enteritidis* was reported as the second most prevalent serotype of *Salmonella* identified among RTE roasted broiler chickens. Ravishankar *et al.* (2010) also isolated *Salmonella enterica* from cutting boards and knives used for cutting raw chicken. To assess the possibility of cross contamination, after using the utensils to cut raw chicken, the researchers then used the same tools to cut RTE lettuce without cleaning. 2 log cfu/cm² of *S. enterica* was detected on the lettuce. But when the tools were thoroughly sanitized with soap and water, <1 log cfu/g of pathogens was detected on the lettuce. This stresses the importance of good sanitary hygiene on contact surfaces and utensils when preparing meat products and all types of food, especially the RTE foods.

Salmonella serotypes were highly resistant to Septrin, Peflacin, and Tarivid. Resistance to these drugs should be of public health importance due to their frequent use in the treatment of various infectious diseases and if allowed to spread, could pose a significant challenge in the treatment and control of such diseases. This was further corroborated by Abdi *et al.* (2017) who observed that most of the *Salmonella* serotypes identified in their study were resistant to all antibiotics tested, giving rise

to a typical multi-drug resistant (MDR) serotype. They attributed this to abuse, misuse and overuse of antibiotics, which could render them ineffective over time.

CONCLUSION

We report the contamination by *Salmonella* spp of ready to eat (RTE) roasted chicken sold in Dutse. The most significant specie observed was *Salmonella enteridis*. We reveal that the hands of the vendors was the most contaminated and isolates were resistant to Septrin, Peflacin and Tarivid. Washing of hands by vendors using available antiseptics will go a long way in reducing the risk of recontamination of RTE roasted chicken.

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