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BACTERIOLOGICAL QUALITY AND PREVALENCE OF *Salmonella* IN CHICKEN PRODUCT, FRUITS AND VEGETABLES

Omoigberale, M. N. O.[1], Iyamu, M.I.,[2] & Amengialue, O. O.[3]

[1]Department of Microbiology, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria
[2]Department of Biological Sciences, College of Natural and Applied Sciences, Wellspring University, Benin City, Edo State, Nigeria

ABSTRACT

The bacteriological quality and prevalence of *Salmonella* in chicken products, fruits and vegetables sold in Ekpoma market, Esan West Local Government Area, Edo State was studied. Out of the 20 samples examined, *Salmonella* specie was isolated from 6 samples. The mean total viable count (TVC) of bacterial isolates ranged from 3.6 x 10^{11}cfu/m l to 8. 4 x 10^{11}cfu/ml for chicken sample, 1.2x10 ^{11}cfu/ml to 7.0 x 10^{10}cfu/ml for egg sample, 1.3 x 10^{12}cfu/ml to 3.7 x 10^{12}cfu/ml for egg shell, 2.1 x 10^{11}cfu/ml to 8.9 x 10^{11}cfu/ml for fruits and 1.5 x 10^{10}cfu/ml to 3.5 though 80% of the isolates were susceptible to ciprofloxacin, a high antibiogram resistance was recorded.

Keywords: *Salmonella*, chicken product, antimicrobial test.

INTRODUCTION

The genus *Salmonella* comprises over 2,700 serotypes that are found in different hosts and environments that can cause human illness such as enteric fever, gastroenteritis and septicemia *Salmonella spp* are the most pathogenic bacteria associated with a variety of foods. Although myriad of foods can serve as sources of infection for *Salmonella,* poultry, poultry products and dairy products are significant sources of food borne pathogen infections in humans. Presence of *Salmonella spp* in fresh raw products can vary widely (Harries *et al.,* 2003). Bacterial contamination of whole or minimally processed fresh vegetables can occur at different processing stages; harvesting, trimming, washing, slicing, soaking, dehydrating, blending or packaging (Harries *et al.,* 2003).

Animals infected with *Salmonella* shed the micro-organism in the faeces from where it can spread into soil, water, crops or other animals and fresh fruits are good transmission vehicles (Bouchrif *et al.*, 2009). Factors influencing the rise in *Salmonella* outbreaks linked to vegetables include changes in agricultural practice: and eating habits, as well as greater worldwide commerce in fresh produce (Collins, 1997)

Contamination with *Salmonella* strains from fresh produce apparently stems mainly from horticultural products. The principal contamination routes are probably use of animals waste as organic fertilizers and irrigation with wastewater, humans and other animals (Islam *et al.*, 2004, Natvig *et al.*, 2002). Studies of environmental sources of *Salmonella* contamination indicate that water is an important source, particularly irrigation water containing manure, wild life faeces or sewage effluents (Islam *et al,* 2004).This study is aimed at investigating the bacteriological quality and prevalence of S*almonella* specie in chicken, eggs, egg shell, fruits and vegetables sold in Ekpoma market and to determine the antibacterial susceptibility pattern of isolates.

MATERIALS AND METHOD

COLLECTION OF SAMPLES

Chicken eggs, Chicken meat; fresh fruits (water melon and garden egg) and vegetables (spinach, water leaf, cabbage, carrot and pumpkin leaf) were purchased from Ekpoma market. Samples were collected using sterile polyethene bags, labelled and transported immediately to the laboratory for microbiological investigation. The procedures for isolation of *Salmonella* were carried out according to the techniques recommended by the International Organisation for Standardization (ISO 6579, 2002)

BACTERIOLOGICAL EXAMINATION

450ml of butterfield's phosphate- buffered dilution water was added to 50gm of each food sample and blended using a sterile blender jar for 2mins. These results in a dilution of 10^{-1} and all decimal dilutions were prepared using 90ml of sterile dilute and 10ml of the homogenate. 1ml from each dilution was inoculated into nutrient agar plate using the pour plate technique.

Processing of egg samples

Fresh chicken eggs were mixed well in a sterile polythene bag and 25gm was taken and mixed with 225ml of buffer peptone water (BPW) and incubated at 37^0C for 18 to 24hrs. After pre-enrichment, 0.1ml of pre-enrichment broth was transferred to 10ml of Rappaport- Vassiliades broth (Difco) and incubated at 42^0C for 24hrs. After incubation, one or two loopful of innoculum were streaked in Xylose Lysine Deoxycholate (XLD) agar and incubated at 37^0C for 24 to 48hrs.

Processing of Egg shell, fruits, vegetables and chicken samples

450ml of butterfield's phosphate- buffered dilution water was added to 50gm of each food sample and blended using a sterile blender jar for 2mins. 25gm of the mixture was taken and mixed with 225ml of buffer peptone water (BPW) and incubated at 37^0C for 18 to 24hrs. After pre-enrichment, 0.1ml of pre-enrichment broth was transferred to

10ml of Rappaport- Vassiliades broth (Difco) and incubated at 42^{0}C for 24hrs. After incubation, one or two loopful of innoculum were streaked in Xylose Lysine Deoxycholate (XLD) agar and incubated at 37^{0}C for 24 to 48hrs.

Biochemical identification of *Salmonella*

All presumptive *Salmonella* colonies (red colonies with black centers) were confirmed by some biochemical tests as recommended by the guidelines of the ISO 6579 (2002)

Antimicrobial susceptibility

This was done using the multi – discs diffusion method according to the CLSI guidelines (Clinical and Laboratory Standards Institute, 2005). The antimicrobial used were Septrin (30µg), Chloramphenicol (30µg), Sparfloxacin (10µg), Ciprofloxacin (10µg), Amoxacillin (30µg), Augumentin (30µg), Gentamycin (10µg), Pefloxacin (30µg), Tarivid (10µg) and Streptomycin (30µg).

RESULTS/DISCUSSION

The total viable counts of bacterial isolated from chicken, chicken products (eggs and egg shell), fruits and vegetables are shown in Table 1.

The least count of 3.0 x 10^{10} cfu/ml was obtained from pumpkin leaf sample while the highest count of 3.7x10^{12} cfu/ml was obtained from egg shell sample 2. The mean total viable count (TVC) of bacterial isolates ranged from 3.6 x 10^{11}cfu/m l to 8. 4 x 10^{11}cfu/ml for chicken sample, 1.2x10 ^{11}cfu/ml to 7.0 x 10^{10}cfu/ml for egg sample, 1.3 x 10^{12}cfu/ml to 3.7 x 10^{12}cfu/ml for egg shell, 2.1 x 10^{11}cfu/ml to 8.9 x 10^{11}cfu/ml for fruits and 1.5 x 10^{10}cfu/ml to 3.5 x 10^{11}cfu/ml for vegetable samples. The chicken and water melon samples recorded the highest bacterial counts. Table 2 shows the prevalence of *Salmonella* specie isolated from chicken, chicken products, fruits and vegetables while Table 3 shows susceptibility pattern of isolates to ten (10) antimicrobial agents. Statistical analysis using t – test showed no significant difference in the mean value of bacterial count obtained from the various samples.

Out of the 20 food samples analyzed, six showed growth of *Salmonella* and this is shown in Table 3.Salmonellosis is one of the major courses of foodborne illnesses worldwide (Gatto *et al*., 2006). The main source of infection in human has been found to be through consumption of contaminated food from animal origin (Ahmed *et al.*, 2000; D'Aoust, 1994; Threlfall, 2000). The predominant serotypes change over time and differ from one geographical area to another (Zerrin *et al.*, 2007).

This study revealed that chicken; chicken product (egg and eggshell), fruits and vegetables sampled had high bacteria count. This can be attributed to the health status of the chicken before it was killed and contamination of samples with faeces and other contaminant.

Chicken product, fruits and vegetable samples showed presence of *Salmonella*. This result is in line with the work of Ait Melloul who sampled egg content and chicken intestine and stated that *Salmonella* is the most prevalent bacteria in the environment and may be found in water , soil, intestinal tract, humans skin, animals and birds. Presence of *Salmonella* in these products can be attributed to the mode of cultivation of fruits and vegetables as they always have contact with the soil from planting to the harvesting stage. Also fruits and vegetables are often displayed in Nigeria markets under poor hygienic conditions and may get contaminated by human or animal feaces.

TABLE 1: Mean Total viable counts (cfu/ml) of bacteria isolated from chicken, chicken product, fruits and vegetables.

SAMPLES	cfu/ml
E 1	1.8X10^{11}
E2	1.2X10^{11}
E3	1.4X10^{11}
E4	1.6X10^{11}
E5	7X10^{10}
C1	4.8X10^{11}
C2	3.6X10^{11}
C3	8.4X10^{11}
ES1	1.3X10^{12}
ES2	3.7X10^{12}
ES3	1.8X10^{12}
Wm1	2.2X10^{11}
Wm2	8.9X10^{11}
Wm3	2.1X10^{11}
GE	2.5X10^{11}
SP	1.5X10^{10}
CA	3.5X10^{11}
CAB	3.2X10^{11}
PL	3X10^{10}
WL	3.0X10^{11}

KEY: E1 – E5 = Egg sample 1-5, C1 – C3 = Chicken sample 1-3, Wm1- Wm3 = water melon sample 1-3, GE = Garden egg shell, SP = Spinach sample, CA = Carrot sample, CAB = Cabbage sample, PL = Pumpkin leaf sample, WL = Water leaf sample ES1 - ES3 = Eggshell sample 1-3

This results is in line with the report by the center of disease control (CDC, 2008; 2009), which states that *Salmonella* has been isolated from unpasteurized juice, fresh salad fruits and vegetables. These results also corresponds with similar results obtained by Beuchat, 2006; Natvig, 2002 in Jos, Plateau State, Nigeria. The prevalence of

Salmonellosis and enteric fever in Ekpoma and it's environ may not be unrelated to consumption of contaminated chicken, eggs, fruits and vegetables sold in the market.

Table 2: Prevalence of *Salmonella* spp. in chicken products, fruits and vegetables

Samples	Prevalence of *Salmonella* specie
C1	Negative
C2	Negative
C3	Positive
E1	Negative
E2	Positive
E3	Negative
E4	Negative
E5	Negative
ES1	Positive
ES2	Negative
ES3	Negative
Wm1	Negative
Wm2	Positive
Wm3	Negative
GE	Negative
SP	Negative
CA	Positive
CAB	Positive
PL	Negative
WL	Negative

KEY: E1 – E5 = Egg sample 1-5, C1 – C3 = Chicken sample 1-3, Wm1- Wm3 = water melon sample 1-3, GE = Garden egg shell, SP = Spinach sample, CA = Carrot sample, CAB = Cabbage sample, PL = Pumpkin leaf sample, WL = Water leaf sample ES1 - ES3 = Eggshell sample 1

TABLE 3: Antimicrobial susceptibility pattern of bacterial isolates

	Antibiotics									
Bacteria isolates	**Septrin**	**Chloramphenicol**	**Sparfloxacin**	**Ciprofloxacin**	**Amoxacillin**	**Augmentin**	**Gentamycin**	**Pefloxacin**	**Tarivid**	**Streptomycin**
E1	R	R	R	S	R	R	R	R	R	S
E2	R	R	R	R	R	R	R	R	R	R
E3	R	R	S	S	S	R	R	S	S	R
E4	R	R	R	R	R	R	R	R	R	R
E5	R	R	R	S	R	R	R	R	R	S
C1	R	R	S	S	R	R	R	S	R	R
C2	R	R	S	S	R	R	R	R	R	S
C3	R	R	R	S	R	R	R	R	R	S
ES1	R	R	R	R	R	R	R	R	R	R
ES2	R	R	R	S	R	R	S	R	R	R
ES3	R	R	R	S	S	R	S	R	R	S
Wm1	R	R	S	S	S	R	R	S	S	S
Wm2	R	R	R	S	S	R	R	R	R	R
Wm3	R	R	R	S	R	R	R	R	S	S
GE	R	R	R	R	R	R	R	R	R	R
SP	R	R	R	S	R	R	R	R	R	S
CA	R	R	R	S	R	R	R	R	S	S
CAB	R	R	S	S	S	R	R	R	S	S
PL	R	R	S	S	R	R	S	R	R	R
WL	R	R	S	S	R	R	S	R	R	R

KEY:

E1 – E5 = Egg isolates, C1 – C3 = Chicken isolates, Wm1- Wm3 – water melon isolates, GE = Garden egg isolate, SP = Spinach isolate, CA = Carrot isolate, CAB = Cabbage isolate, PL = Pumpkin leaf isolate, WL = Water leaf isolate, ES1 - ES3 = Eggshell isolates. **S =** Sensitive, R = Resistance

Though antimicrobial therapy is not essential for most *Salmonella* infection, it is necessary for treatment of invasive infections and infections in the immunocompromised patients (Bakeri *et al.,* 2003). In this study, the percentage susceptibility of *Salmonella* to ten (10) antimicrobial agents revealed 80% susceptibility to ciprofloxacin. None of the isolates was sensitive to septrin, augmetin and chloramphenicol Table 3. The isolates showed high antibiotics resistant pattern which is of serious health concern.

CONCLUSION

Poultry products (chicken, egg and egg shell), fruits and vegetables as a source of protein, vitamins and minerals cannot be over emphasized and its consumption is on the increase. Fom this study, it is evident that *Salmonella* is associated with poultry product (Egg shell and chicken), fruits and vegetables and could be responsible for food poisoning.

Salmonella can enter the food chain at any point; from livestock feed and food manufacturing process. Thus there is the need to take adequate measures such as washing of fruits and vegetables properly before consumption. Also food vendors should maintain high personal hygiene to help reduce transmission.

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