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Isolation, Identification and Characterization of *Salmonella* spp. from Chicken purchased at Wad Madani City, Gezira State, Sudan

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Abstract

Background: *Salmonella* is a potential human pathogen that causes salmonellosis, a food-borne disease. Addressing these major food safety and public health issues with effective monitoring of food-borne pathogens and dietary measures.

Methods: A cross-sectional study was conducted in the local markets of Alsug Alkabeer (AB), Alsug Ashabi (AK), and Alsug Alsageer (AS) sites in Wad Madani state, Sudan to detect and assess *Salmonella* infection in raw and cooked chicken samples.

Results: The results showed that the maximum number of different *Salmonella* species was recovered from raw and cooked specimens of chicken obtained from AB, AK and AS which were 6.5, 4.4, and 4cfu/g, respectively. In addition, *Salmonella* spp. in the locally reared chicken is significantly ($p < 0.05$) higher than that of farm poultry. On the other hand, the highest *Salmonella* count was recorded in AS, AK and AB egg samples which were 5.9, 3.5, and 2.3cfu/g, respectively. Interestingly, eggs from local sources of chicken had significantly ($p < 0.05$) higher numbers of *Salmonella* spp. compared to those from farm sources. Six species of *Salmonella* were described, namely: *Salmonella* typhi, *S. cheers*, *S. arizonae*, *S. enteritidis*, *S. pullorum*, and *S. gallinarum*.

Conclusion: High occurrence of *Salmonella* spp. in this study might be attributed to the low hygienic measures in the poultry retail markets during slaughtering and/or handling processes. Further studies are required and should be conducted within Gezira state to assess food safety.

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Introduction

Irresistible microbial sickness is a major cause of death in so many countries of the continent, particularly the developing countries. The aim of food safety has now been shifted by developed countries from investigative causes of food-borne diseases to proactive food, contamination prevention, and counteractive processes [1]. *Salmonella* has been recognized as a harmful food and waterborne pathogen that can infect humans and animals through extreme pain and mortality. *Salmonella* has three different entry pathways that lead to gastrointestinal disease by infected cooked foods (such as poultry, grains, eggs, and milk), environment litter and fertilizer, and consumption of contaminated raw fruits and vegetables [2-10]. The majority of human salmonellosis is common to both wild and domestic animals, thus, food of animal origin is a source of salmonellosis, typically between unprocessed poultry and prepared food products, i.e. through cross-contamination in food catering or at home. While all *Salmonella* is a non-host-adapted serotypes that cause most of the food-borne *Salmonella* emerge [11, 1, 12]. Handled raw poultry meat naturally host bacteria, most of which provoke poultry meat deterioration. Food-borne pathogens, such as *Salmonella* serotypes, *Campylobacter jejuni*, *Listeria monocytogenes*, *C. perfringens* and *S. aureus* can harbor food-borne pathogens [13, 10]. As disease-related meat, the food-borne disease outbreaks reported in poultry and poultry products to be ranked first and second in most countries around the world, respectively, and third in the United States [14]. Nevertheless, *Salmonella* infections of livestock and poultry products have been reported in Sudan [15, 16, 8]. Many studies have reported *Salmonella* outbreaks in relation to meat or eggs from poultry). A critical manifestation in poultry industry is the vertical transmission of infections from breeding hens to poultry meat as an epidemiology of *Salmonella* species infections [14, 17, 6].

Regardless of the coordinated efforts for the eradication of typhoid, malnutrition and related problems caused by *Salmonella*, it continues a major general medical issue around the world. Because most of *Salmonella* diseases are derived from the ingestion of unsafe food, a possible explanation for the prevalence of safe antimicrobial *Salmonella* is indicated by antimicrobial specialists in the feeding of affected creatures [4].

In Sudan, the broiler chicken population has been estimated to be 22.5 million chicks [18] and Khartoum State generates 90% of Sudan's production [19, 20]. However, the traditional sector (small farms) produced about 60% of total broiler production and the rest was provided by the new sector (companies). However, many people in Sudan rear household chicken and eat their produce (meats and eggs) locally or sell it on the local market. Therefore, the purpose of this study was to isolate, detect and assess *Salmonella* infection in raw and cooked chicken samples from Wad Madani City, Sudan.

Methods

Study Area

This study was carried out at Wad Madani, capital of Sudan's Gezira State. The town is situated on the west side of the Blue Nile between 14 ° 24'N- 14.4 ° N longitude and 33 ° 31'E- 33.517 ° E latitude, 136 km southeast to Khartoum.

Samples Collection

Raw and cooked chicken products samples were collected from Alsug Alkabeer (AB), Alsug Ashabi (AK) and Alsug Alsageer (AS) local market at Wad Madani city during the period 2016-2017. The original sources of the chicken and egg samples were either local farms or local houses from where they had been brought to the market. All samples were stored in sterile ice bags and forwarded to Department of Botany and Agricultural Biotechnology, Faculty of Agriculture, Khartoum University, Sudan.

Culture preparations for *Salmonella* Isolations

For preparation of serial dilution, 9 ml of sterile distilled water was poured aseptically into five test tubes each and 1 ml of the initial sample (chicken and egg) was added to the first tube giving 1:10 dilution. Again, 1 ml was transferred from the first tube, added to the second tube, and thoroughly mixed. Procedure continued until the fifth test tube. Each sample was diluted from 10^{-1} to 10^{-5}. Further analyses were carried out on the samples. 25 grams of the sample were sterilized, aseptically weighed, and thoroughly mixed with 225 ml of sterile nutrient broth then was grown at 37 °C for 24 hours. Additionally, 10 ml of solution was added to 100 ml of sterile selenite cysteine broth aseptically drawn. The broth was placed in an incubator at 37°C for 24 hours; a decimal dilution series was prepared in 0.1% peptone solution in the surface covered with 0.1 ml amount of dilution onto pre-poured pre-dried plate of bismuth sulphite agar (BSA). In order to promote *Salmonella* growth, the agar plates were incubated at 37°C for 72 hours. Thereafter the discrete black metallic sheen colonies were the viable colonies of *Salmonella* that were counted by colony counter and the results were expressed as colony forming unit per gram (cfu/g).

Salmonella Isolation and Identification

Salmonella pure colonies were streaked onto sterile agar plates with nutrients and were incubated for 24 hours at 37°C. The pure colonies of *Salmonella* isolates were sub-cultivated in nutrient agar slopes and incubated for 24 hours at 37°C, and then the plates were kept in the refrigerator at 4°C until it was used for biochemical testing. Purified isolates have been identified according to Cowan and Steel [21].

Salmonella Biochemical Identification Tests

For the detection and characterization of *Salmonella* isolates, biochemical measures were used as laid down by Harrigan [22]; Juneja *et al.* [23]. These tests included Gram stain test, catalase test, nitrate reduction test, Vogs- Proskauer (VP) test (acetone production), citrate use test, urease test, indole test, motility test, sugar

fermentation, casein hydrolysis, starch hydrolysis and methyl red test.

Statistical Analysis

Biochemical research results were entered into Microsoft Excel, edited, coded and analyzed using Statistical Software (SPSS version 19.5 for Windows). The collected data was recorded as arithmetic means ± standard mean error (SEM). Then after, Student's *t*-test and the chi-square test were used to make comparisons and correlations between explanatory variables, respectively. Therefore, the mean and percentage of *Salmonella* were used for quantitative testing, and the Student's *t*-test and the chi-square to test variables significance at $P < 0.05$.

Results

Enumeration of *Salmonella*

Tables (1-3) indicate *Salmonella* counts from cooked samples of chicken, eggs and chicken shawarma obtained from three separate locations (Alsouq Alkabeer, Alsouq Alshabi and Alsoug Alsageer).

It has been observed that the highest *Salmonella* spp. count was recorded after 120 days of storage in cooked chicken samples collected from Alsoug Alshabi (6.5 cfu / g), followed by Alsoug Alkabeer samples (4.4 cfu / g) and finally Alsoug Alsageer samples (4 cfu / g mean). There was, however, a significant difference between the different locations (F= 40.95; Fcrit=4.46; P-value=0.0063) with a big difference between the storage times (F= 12.46; Fcrit=3.84; P-vaue=0.001628).

Location	RC0	RC1	RC2	RC3	RC4
Alsoug Alkabeer	2.3	2.3	3.7	2.8	4.4
Alsoug Alshabi	4.3	4.3	5.3	5	6.5
Alsoug Alsageer	3.3	3.3	3.7	3.9	4.2

Table 1: *Salmonella* count (cfu/g) of Cocked chicken samples collected from different locations that were stored for various periods: 0, 30, 60, 90 and 120 days, that represented by RC0, RC1, RC2, RC3 and RC4, respectively.

The highest *Salmonella* spp. count was recorded in samples from shawarma (Table 2) collected from Alsoug Alshabi (mean 6.4 cfu / g) in 120 days storage, followed by Alsoug Alkabeer (mean 3.9 cfu / g) and lastly Alsoug Alsageer (mean 2.3 cfu / g). Table (2) also revealed that the times stored for Shawarma samples were substantially influenced by the *Salmonella* count (F= 12.85; F-crit= 3.84; P-value= 0.00), in addition to the significant differences between locations (F= 169.35; F-crit= 4.46; P-value= 0.00). Contamination of cooked chicken and eggs with *Salmonella* can be caused by the use of contaminated raw materials and/or unhygienic measures during storage, as well as long-term maintenance at room temperature of poultry feed [24].

Regarding Table 3, the highest *Salmonella* spp. count reported in the collected egg samples was from Alsoug Alshabi (mean 5.9 cfu / g) in 120 days storage, followed by Alsoug Alkabeer (mean 3.5 cfu / g) and lastly Alsoug Alsageer (mean 2.3 cfu / g). Overall, eggs can become infected with the penetration of *Salmonella* during or after oviposition [25].

Location	RC0	RC1	RC2	RC3	RC4
Alsoug Alkabeer	2.7	2.7	3	3.4	3.9
Alsoug Alshabi	4.3	4.3	5	5.2	6.4
Alsoug Alsageer	1	1	2	1.2	2.3

Table 2: *Salmonella* count (cfu/g) of Shawarma samples collected from different locations those were stored for various periods: 0, 30, 60, 90 and 120 days, that represented by RC0, RC1, RC2, RC3 and RC4, respectively.

Location	RC0	RC1	RC2	RC3	RC4
Alsoug Alkabeer	2.3	2.3	2.3	3	3.5
Alsoug Alshabi	3	3	4.3	1.2	5.9
Alsoug Alsageer	1	1	1	4.7	2.3

Table 3: *Salmonella* count (cfu/g) of egg samples collected from different locations that were stored for various periods: 0, 30, 60, 90 and 120 days, that represented by RC0, RC1, RC2, RC3 and RC4, respectively.

Table (3) also revealed that the various storage times did not significantly affect the *Salmonella* counts (F= 0.80; F-crit= 3.84; P-value = 0.32668), and there were no significant differences between the various locations (F= 1.29; F-crit= 4.46; 0.32668).

Salmonella spp. isolated from various samples in this study were comparable to the results reported by Zhao *et al.* [11]; Beach *et al.* [1]; and Salsbury [26]. *Salmonella* was isolated in 19–54% of cattle carcasses, 1.9% of beef samples at retail and 4.2% of retail chicken samples [11, 1]. As well as large amounts of human salmonellosis being specifically related to human interaction with wild and domestic animals. Anyway, livestock feeding stuffs are vectors for salmonellosis [27-30].

Salmonella Quality and quantities

A total of 242 *Salmonella* spp. isolates from the samples collected from different locations, and sources (local and farm) are shown in Fig. (1).

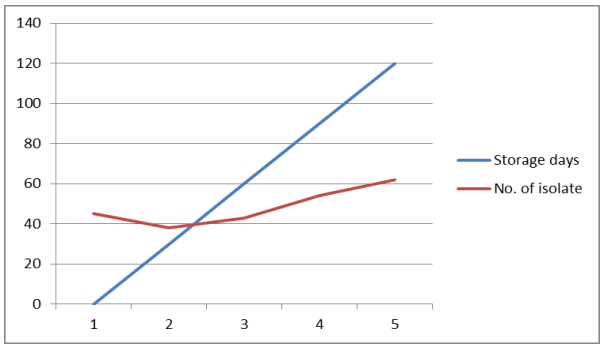

Figure 1: Number of *Salmonella* isolate for samples stored for various periods.

As indicated in Fig. (1) and (2), the prevalence of *Salmonella* was the highest among cooked eggs (23.96%) followed by cooked chicken (20.66%), local chicken (11.57%), local egg (10.33%), farm eggs (5.37%) and farm chicken (4.95%). From the 242 *Salmonella* isolates, 39 isolates (16.1%) were identified

as *S. enteritis,* 70 (28.9%) were *S. pullorum,* 42 (17.3%) *S. gallinarum,* 48 (19.7%) were *S. typhi,* 15 (6%) were *S. Arizona* and 28 (12%) *S.* paratyphi. Compare to the present study, *S. pullorum* (28.9 per cent) was the most frequently species of *Salmonella* among the samples (Fig. 2). As well as, *S. Arizona* has been isolated from 5 out of 7 examined sources, with the remaining *Salmonella* species isolated from all sample sources. However, *S. Pullorum* was the most common species among cooked chicken, cooked eggs, farm chicken, farm eggs and local eggs.

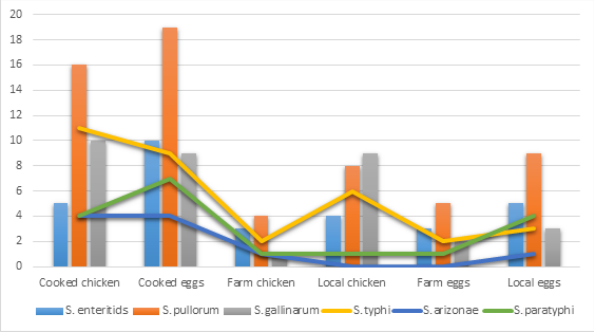

Figure 2: Number of *Salmonella* isolate from different food samples.

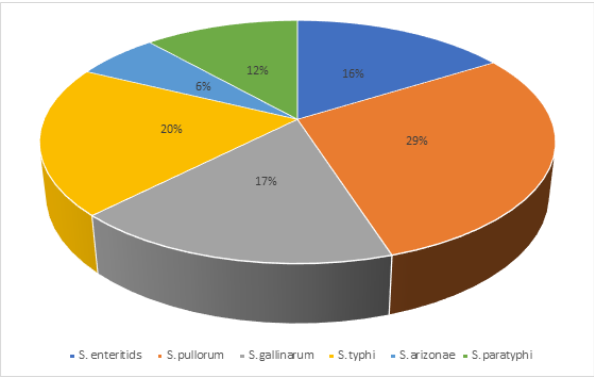

Figure 3: Percentage of *Salmonella* isolate from different food samples.

Discussion

As for the species *Salmonella* isolated from local and farm poultry, five species of *Salmonella* (*S.* typhi, *S. enteritids*, *S.* paratyphi, *S. pullorum* and *S. gallinarum*) have been distributed and found in farm poultry, while four species (*S.* typhi, *S. enteritids*, *S. pullorum* and *S. gallinarum*) have been found in local chicken. However, more than 95 per cent of *Salmonella* cases have been recorded as foodborne diseases. From the results obtained, in Alsug Alkabeer and Alsug Alshabi, the six species of *Salmonella* isolates (*S.* typhi, *S. enteritids*, *S. arizona*, *S.* paratyphi, *S. pullorum* and *S. gallinarum*) were collected from cooked chicken while all species, except *S. enteritids* have been noted in Alsug Alsageer. As for the samples of cooked eggs, the various species of *Salmonella* were isolated.

Chicken contamination with *Salmonella* (Fig. 2) was still far higher than that recorded by El Hussein *et al.* [31], Yagoub [16] and Elsafi *et al.* [8] , which were 9.2%, 6.2%, 3.4%, respectively. In addition, our findings were significantly higher than reports from other countries, such as Nepal 14.5% [32], Canada 14% [7], and South Africa 19.2% [33], and Turkey 12% from [34].

Many developing countries have shown a comparatively higher prevalence of *Salmonella* in humans, food, and animals such as 73.3% in Egypt [31] , 68.2% in Ethiopia, 51.2% in Argentina, 25.9% in Korea, and 72% in Thailand [5]. It is vital to perceive that the prevalence and distribution of *Salmonella* serovars varies from location to location [35] and isolation rates vary depending on the location in which the research was conducted; the sampling program and the limit detections for the methodologies [36].

The high occurrence of *Salmonella* spp. in our study could be observed due to the low hygienic measures noticed in the poultry retail markets of Wad Madani (Sudan) during slaughtering, de-feathering, gutting, cadaver cutting, scalding, and handling. Such methods can lead to the cross contaminations among the safe and clean ones. Furthermore, the absence of veterinary supervision may lead to the slaughtering of diseased birds. Therefore, to irradiate contamination with *Salmonella* and other foodborne pathogens, it is strongly recommended to enhance hygienic practices during chicken rearing, processing and handlings. Furthermore, it also advised to investigate the health status of food handlers on premises that may have had spreaders of foodborne illnesses or asymptomatic organisms.

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Conflict of Interest Statement

The authors declare that there is no conflict of interest.

Author Contributions

All authors designed the experiments. A.M.E, and F. E. D performed the experiments. E. H. A and A.M.E analyzed the data. N.I. A, A.M.E and S. A .S. wrote the manuscript. All authors read and approved the manuscript.

References

1. Khalil AT, Iqrar I, Bashir S, Ali M, Khalil AH, Shinwari ZK. Preemptive and Proactive Strategies for Food Control and Biosecurity. In Food Safety and Preservation (2018); 39-58. Academic Press.
2. Beach JC, Murano EA, Acuff GR. Prevalence of *Salmonella* and Campylobacter in beef cattle from transport to slaughter. Journal of food protection, (2002); 65(11): 1687-1693.
3. Domınguez C, Gomez I, Zumalacarregui J. Prevalence of *Salmonella* and *Campylobacter* in retail chicken meat in Spain. International Journal of Food Microbiology, (2002); 72(1-2): 165-168.
4. Antunes P, Réu C, Sousa JC, Peixe L, Pestana N. Incidence of *Salmonella* from poultry products and their susceptibility to

antimicrobial agents. International journal of food microbiology, (2003); 82(2):97-103.
5. Cardinale E, Gros-Claude P, David J, Tall F, Cisse M, Guèye EH, Salvat G. Prevalence of *Salmonella* and Campylobacter in retail chicken carcasses in Senegal. Revue Elv. Med. Vet. Pays. Trop. (2003); 56 (1-2): 13-16
6. Gast, R. K., Shivaprasad, H. L., & Barrow, P. A. *Salmonella* infections. Diseases of poultry, (2003): 11, 567-613.
7. Arsenault J, Letellier A, Quessy S, Boulianne M. Prevalence and risk factors for *Salmonella* and *Campylobacter* spp. carcass contamination in broiler chickens slaughtered in Quebec, Canada. Journal of food protection, (2007); 70 (8): 1820-1828.
8. Elsafi HH, Elmadiena MN, El Hussein AA, Siddig MM, Muckle CA, Cole L, Mistry K. *Salmonella* Umbadah: A new *Salmonella serovar* isolated from cattle in Sudan. Tropical animal health and production, (2009); 41(7): 1605.
9. Yagoub SO, Oshi NA, Ibtisam EM. Isolation and susceptibility to antimicrobial agents of *Salmonella paratyphi* from cheese in Khartoum (Sudan). Research Journal of Microbiology, (2010); 5(8): 740-744.
10. Ibrahim SM, Abdelgadir MA, Sulieman AM. Impact of Halal and Non-halal Slaughtering on the Microbiological Characteristics of Broiler Chicken Meat and Sausages. Food and Public Health, (2014); 4(5): 223-228.
11. Zhao C, Ge B, De Villena J, Sudler R, Yeh E, Zhao S, White D, Wanger D, Meng J. Prevalence of Campylobacter spp., Escherichia coli, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, DC, area. Appl Environ Microbiol. (2001); 67(12): 5431-6.
12. Woldemariam E, Molla B, Alemayehu D, Muckle A. Prevalence and distribution of *Salmonella* in apparently healthy slaughtered sheep and goats in Debre Zeit, Ethiopia. Small Ruminant Research, (2005); 58(1):19-24.
13. Waldroup AL. Contamination of raw poultry with pathogens. World's Poultry Science Journal, (1996); 52(1): 7-25.
14. Mishu B, Koehler J, Lee LA, Rodrigue D, Brenner FH, Blake P, Tauxe RV. Outbreaks of *Salmonella enteritidis* infections in the United States, 1985-1991. Journal of Infectious Diseases, (1994); 169(3): 547-552.
15. Mamoun IE, Khalafalla AI, Bakhiet MR, Agab HA, Sabiel YA. *Salmonella* enteritidis infection in the Sudan. Revue d'élevage et de médecine vétérinaire des pays tropicaux, (1992); 45(2): 137-138.
16. Yagoub SO. Isolation of *Enterobacteriaceae* and Pseudomonas spp. from raw fish sold in fish market in Khartoum state. African Journal of Bacteriology Research, (2009); 1(7): 085-088.
17. Keller LH, Schifferli DM, Benson CE, Aslam S, Eckroade RJ. Invasion of chicken reproductive tissues and forming eggs is not unique to *Salmonella enteritidis*. Avian diseases, (1997): 535-539.
18. Khalifa AK. Studies on *Salmonella* spp. and *Escherichia coli* Contamination in Poultry Meat Carcasses at an Automatic Slaughter house in Khartoum State. Sudan (Doctoral dissertation, Sudan University of Science and Technology, (2015).
19. Sirdar MM. The Sudanese History Statistics and future Investment Challenges University of Pretoria Department in Studies Avi Africa June (2014.
20. Sirdar MM. Antibiotic residues in commercial layer hens in Khartoum state, Sudan, 2007-2008 (Doctoral dissertation, University of Pretoria, (2014).
21. Cowan ST, Steel KJ. Manual for the identification of medical Bacteria. X und 217 S., 10 Tab. Cambridge University Press 1965. 50.
22. Harrigan WF. Laboratory methods in food microbiology. Gulf Professional Publishing. Published by Academic Press 1998-09-28 (1998). ISBN 10: 0123260434 I
23. Juneja VK, Eblen BS, Ransom GM. Thermal inactivation of *Salmonella* spp. in chicken broth, beef, pork, turkey, and chicken: Determination of D-and Z-values. Journal of Food Science, (2001); 66 (1): 146-152.
24. IDPH, Illinois Department of Public Health . *Salmonella*. Available at: http://www. idph.state.il.us/public/hb/hbsam.htm. Accessed by 15 Nov., (2019).
25. Van Hoorebeke SV. The Effect of Different Housing Systems on *Salmonella* and Antimicrobial Resistance in Laying Hens. (PhD), Ghent University. Faculty of Veterinary Medicine (2010).
26. Salsbury P. Progress Reports from the Workgroups; Proceedings of Partnership for Food Protection; Washington, USA. (2012); 21 March 2012: p. 7.
27. Hilbert F, Smulders FJ., Chopra-Dewasthaly R, Paulsen P. *Salmonella* in the wildlife-human interface. Food Research International, (2012): 45(2): 603-608.
28. Ejo M, Garedew L, Alebachew Z, Worku W. Prevalence and antimicrobial resistance of *Salmonella* isolated from animal-origin food items in Gondar, Ethiopia. BioMed research international, (2016). Article ID 3714785.
29. Kebede A, Kemal J, Alemayehu H, Habte Mariam S. Isolation, identification, and antibiotic susceptibility testing of *Salmonella* from slaughtered bovines and ovines in Addis Ababa Abattoir Enterprise, Ethiopia: a cross-sectional study. International journal of bacteriology, (2016).
30. Heredia N, García S. Animals as sources of food-borne pathogens: A review. Animal nutrition, (2018); 4(3): 250-255.
31. Hassan AR, Salam HS, Abdel-Latef GK. Serological identification and antimicrobial resistance of *Salmonella* isolates from broiler carcasses and human stools in Beni-Suef, Egypt. Beni-Suef University Journal of Basic and Applied Sciences, (2016); 5(2): 202-207.
32. Maharjan M, Joshi V, Joshi DD, Manandhar P. Prevalence of *Salmonella* species in various raw meat samples of a local market in Kathmandu. Annals of the New York Academy of Sciences, (2006); 1081(1): 249-256.
33. Van Nierop W, Duse AG, Marais E, Aithma N, Thothobolo N, Kassel M, Stewarta R. Potgietera A, Fernandesa B, Galpinb JS, Bloomfield SF. Contamination of chicken carcasses in Gauteng, South Africa, by *Salmonella, Listeria monocytogenes* and *Campylobacter*. International Journal of Food Microbiology, (2005); 99(1): 1-6.
34. Hoelzer K, Switt AI, Wiedmann M. Animal contact as a source of human non-typhoidal salmonellosis. Veterinary research, (2011); 42(1): 34.
35. Ozbey G, Ertas HB. *Salmonella* spp. isolation from chicken samples and identification by polymerase chain reaction. Bulgarian Journal of Veterinary Medicine, (2006); 9(1): 67-73.
36. Uyttendaele MR, Debevere JM, Lips RM, Neyts KD. Prevalence of *Salmonella* in poultry carcasses and their products in Belgium. International Journal of Food Microbiology, 1998; 40(1-2): 1-8.