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Prevalence and antibiotic susceptibility of *Salmonella* in chicken eggs from farms in Zaria, Kaduna State, Nigeria

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Abstract

The research was a cross-sectional study conducted from June to December 2021 within Zaria metropolis which aimed to determine the prevalence and antimicrobial susceptibility of *Salmonella* isolated from chicken egg shells and their contents. A total of 240 egg samples were collected from farms that operated on either battery cages or deep litter-rearing systems. The samples were transported in ice packs to the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria for laboratory procedures. *Salmonella* was isolated, identified and confirmed using standard techniques as outlined by the International Organization for Standardization while antimicrobial susceptibility was determined using the disc diffusion method as described by Kirby-Bauer. Out of the 240 eggs sampled, 12 *Salmonella* isolates were confirmed positive by amplifying the genus-specific *inv*A gene. A *Salmonella* prevalence of 4.6% was calculated to be from the egg shells while 0.4% prevalence was from the egg contents. The antimicrobial susceptibility test revealed that there was resistance by *Salmonella* isolates to almost all the 14 antimicrobials used across 8 different classes of antibiotics except for Imipenem in which the isolates exhibited absolute susceptibility. A Fisher's exact test was used to test the association between the variables. There was a significant association (P< 0.0059) between the difference in the prevalence of *Salmonella* on eggshell and egg content but there was no significant association (P>0.7689) between the battery cage and the deep litter systems of rearing. The study highlights the prevalence and the importance of continuous monitoring and surveillance for pathogenic *Salmonellae.*

Keywords: Antimicrobial, Eggs, PCR, Prevalence, *Salmonella*, Susceptibility

Introduction

The bacteria *Salmonella* is widely considered the most prevalent food-borne pathogen worldwide. It has been identified as an important zoonotic pathogen of economic significance in animals and humans, predominantly in developing countries including Nigeria (Tessema *et al.,* 2017). It is interesting to know that food-borne diseases are among the most widespread global public health

problems of recent and their implication for health and the economy is increasingly recognized by experts (Bintsis, 2017).

Epidemiological studies revealed that contaminated chicken eggs and meat are two of the most essential sources of consumer ingestion and contact with the pathogen *Salmonella* (Goncalves *et al.,* 2018). *Salmonella* is a Gram-negative, non-spore-forming, facultative anaerobic, motile rod that belongs to the family *Enterobacteriaceae*. *Salmonella* is comprised of 2 taxonomical species, *Salmonella bongori* and *Salmonella enterica,* with all medically associated *Salmonellae* from the latter. *Salmonella enterica* is a diverse species of bacteria consisting of more than 2,500 different serovars (Divek *et al.,* 2018). Antibiotic resistance is a global public health concern, *Salmonella* is one of the microorganisms in which some resistant serotypes have emerged and the resistant bacteria could be passed to humans through the food chain (Jain *et al*., 2017). The extent of antimicrobial resistance varies across different regions and is usually influenced by the abuse of antibiotics in human and animal populations (Saad *et al*., 2022). *Salmonella* is easily identified through the *inv*A gene, which contains unique DNA sequences and is proven to be a PCR target gene suitable for *Salmonella* detection (Yanestria *et al.,* 2019). The *inv*A gene, according to most researchers, is genus-specific, and the technique has some advantages because it is simple, inexpensive, and rapid (Taskale *et al*., 2018).

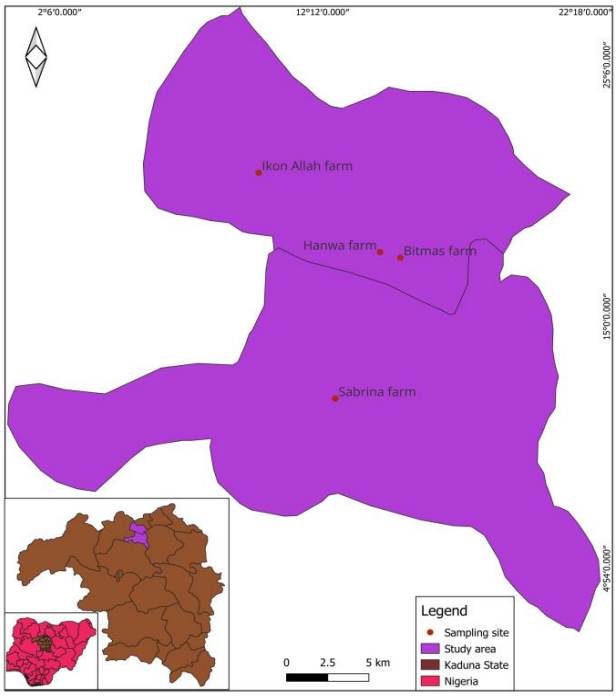

Figure 1: Map of Zaria in Kaduna State showing the geographic locations of the farms where samples were collected for this study

The study aimed to determine *Salmonella's* prevalence and its antimicrobial susceptibility from chicken eggs in farms within Zaria metropolis. This highlights the prevalence of *Salmonella* and its antibiotic susceptibility in chicken eggs. Also, the data provided from the study will support the implementation of surveillance and epidemiological interventions to mitigate *Salmonella* infections arising from chicken eggs.

Materials and Methods

Study area

Zaria is a major city in Kaduna state as well as a local government area located on latitude 11°4'N and longitude 7°42'E Kaduna state (Adamu *et al*., 2016).

Study design

The work was a cross-sectional study within Zaria metropolis from June to December 2021 and 4 farms from the list of registered farms as provided by the Ministry of Agriculture and Natural Resource Kaduna State were conveniently sampled, 2 deep litter and 2 battery cage.

Sampling technique and processing

A random sampling technique was adopted in collecting the eggs from the farms: Ikon Allah (IKA) farm, Bitmas (BT) farm, Sabrina (SB) farm and Hanwa (HW) farm, all within Zaria metropolis. The farms are commercial and a common record with them was the up to date history on vaccination against Newcastle disease and Infectious bronchitis but there was no single record indicating vaccination against *Salmonella* infection inspite of recent cases of *Salmonella* infection reported on the farms. A total of 240 eggs, comprising 60 eggs from each farm was collected and analysed for the presence of *Salmonella.* Sample size of 240 eggs was calculated using a prevalence of *Salmonella* of 18.9% (Fagbamila *et al*., 2017) and an allowable confidence of 5% using Thrusfield (2008) formula. The samples were allocated proportionately to the 4 selected farms. All samples were processed according to the standard guidelines for detecting *Salmonella* on eggshells and contents as outlined by the International Standard Organization (ISO, 2017). A swab from egg shell was collected using a swab stick moist in sterile distilled water and pressed against the walls of the tube to reduce the moisture

content and swabbed around the egg shell then returned into the tube containing the distilled water and was tagged "shell swab". After that, a pipette was used to aspirate 1ml from the shell swab solution into a screw-capped bottle containing 9ml of peptone water for pre-enrichment and incubated at 37^{o}C for 18 hours. After the incubation, 1 ml from the pre-enrichment broth was transferred into a tube containing 10 ml Rappaport-Vassiliadis broth for enrichment and incubated at 37^{o}C for 18 hours. It was later sub-cultured by streaking onto *Salmonella*-Shigella (SS) agar media, and the sub-cultured plate was incubated at 37^{o}C for 18 hours (Gberikon *et al.,* 2019). For the egg contents; yolk and albumin, the pointed end of the eggs was sterilized using 70% alcohol. A gauze-soaked alcohol was scrubbed at the pointed end of the egg for disinfection to remove pathogens before an opening was created on the shell. After the disinfection, a sterile scalpel blade was used to make a sizable hole on the shell where the egg contents were aseptically emptied into a sterile stomacher bag, sealed, and homogenized in a stomacher machine (400R, Seward, England). Thereafter, 1ml of the homogenized egg contents was pre-enriched by inoculating into 9ml of peptone water and incubated at 37^{o}C for 18 hours. After the overnight incubation, 1 ml of each of the pre-enrichment broths was enriched by inoculating into 10 ml Rappaport Vassiliadis broths and incubated at 37°C for 18 hours.

Following incubation, a loop full of each enrichment broth culture was streaked onto plates of *Salmonella*-Shigella agar and incubated at 37°C for 18 hours. The plates were examined for the presence of transparent or translucent colourless colonies with sometimes black centers depending on the strain which was presumed to be *Salmonella* (Sagar, 2018). Characteristic colonies for *Salmonella* isolates were later transferred onto nutrient agar and incubated aerobically at 37°C overnight. Presumptive *Salmonella* isolates were confirmed phenotypically by subjecting them to other biochemical tests; Gram staining, Sugar fermentation, Catalase, Oxidase, Triple sugar iron, Methyl red, Vogues-proskauer, Urease test, citrate utilization, Sulfide Indole and Motility test in accordance to the method by Cheesebrough (2005). The result was interpreted according to the guidelines set by the International Organization for Standardization (ISO, 2017).

Antibiotic susceptibility

The susceptibility pattern of *Salmonella* isolates to 14 antibiotics commonly used within the study area was performed using the disc diffusion method as described by Biemer (1973) protocol. The concentrations of the antimicrobial agents were; amoxicillin 10µg, ampicillin 10µg, ceftriaxone 30µg, ceftazidime 30µg, enrofloxacin 5µg, nalidixic acid 30µg, ciprofloxacin 5µg, erythromycin 15µg, chloramphenicol 30µg, doxycycline 30µg, oxytetracycline 30µg, gentamicin 10µg, amikacin 30µg, and imipenem 10µg (OXOID) and the positive control was *Salmonella* subspecies NC12684 (England). The zone of inhibition was measured to the nearest millimeter using a meter ruler and interpreted according to the criteria suggested by the Clinical Laboratory Standard Institute (CLSI, 2017).

PCR detection of Salmonella

The extraction of DNA was done using the boiling method procedures according to the method described by Kadry *et al.* (2019). Quantification and purity of DNA were determined using the Nanodrop spectrophotometer (Genoud, 2021) and confirmation of *Salmonella* was by amplifying the *inv*A gene using specific primers as mentioned below, for both the forward and the reverse reactions. The amplification was performed using a uniplex PCR in a total volume of 25µL PCR master mix which consisted of 1.0µL x 10 PCR buffer, 18µL dH_20, 1.0µL dNTPs mix, 1.0µL of MgCl, 1.0µL of each primer, 0.5µL of Taq DNA polymerase (Bioneer) and 0.5µL DNA. The conditions were an initial pre-denaturation at 95^{o}C for 5min, denaturation at 94^{o}C for 40sec, annealing at 54^{o}C for 40sec followed by 35cycles of extension at 72^{o}C for 40sec and final extension at 72^{o}C for 5min.

5'GTGAAATTATCGCCACGTTCGGGCAA 3'

3'TCATCGCACCGTCAAAGGAACC 5'

The PCR products were subjected to 1.5% of 2g agarose (Warwickshire, UK) gel electrophoresis. A 100bp ladder (Promega) was used to determine the length of the bands and visualize them under an ultraviolet transilluminator (Biorad).

Data analysis

The data was entered into Microsoft Excel 2007 and later imported into SPSS version 21 for analysis. Descriptive statistics were employed to depict the data, and associations were tested using Fisher's exact test. Values of $P \leq 0.05$ were regarded as statistically significant.

Results

By these methods, 12(2.5%) egg samples were confirmed positive for *Salmonella* out of the 240 eggs sampled as presented in Table 1. Table 2 highlights

the percentage prevalence of deep litter and battery cage systems of rearing birds. Further assessment of the isolates was conducted to determine their carbohydrate fermentation ability and it revealed the production of acid, indicating positive results for glucose, mannitol, sorbitol, xylose, and ornithine. While for Lactose, Sucrose, Dulcitol, and Salicin no change was observed implying no acid production. Also, upon subjecting the isolates to other biochemical tests the reaction observed was typical of *Salmonella enterica* species. The result showed a positive reaction on methyl red, catalase, citrate, hydrogen sulfide, triple sugar iron agar, and motility; however, no reaction was observed on voges-proskauer, indole, oxidase, and urease medium which implies a negative reaction. The susceptibility profile of *Salmonella* isolates was determined against 14 antimicrobial agents that spread across 8 classes of antibiotics: Quinolones, Macrolides, Penicillin, Tetracyclines, Cephalosporins, Chloramphenicol, Carbapenems and Aminoglycosides. Varying reactions of the tests were recorded as presented in Figure 2, with oxytetracycline and amoxicillin recording 91.7% resistance, the highest resistance level recorded for this study. Others include erythromycin and ampicillin recording 83.3% resistance; gentamycin 75%; chloramphenicol, doxycycline, ciprofloxacin, and nalidixic acid recording 58.3% resistance each. However, some of the antibiotics including ceftriaxone, enrofloxacin, amikacin, and ceftazidime recorded lower levels of resistance while for imipenem the isolates displayed absolute susceptibility. The PCR assay was carried out to detect the *Salmonella* invasion virulence gene *invA* which further confirmed 12 isolates to be *Salmonella* as presented in Figure 3 below. The extracted DNA was of pure quality with a mean absorbance ratio of 1.8(A260/A280) which is acceptable for pure DNA. Furthermore, Figure 3 explains the reaction of *Salmonella* isolates where *inv*A gene amplicon 288bp was visualized on 1.5% agarose gel. The M and –VE lanes are the molecular weight marker 100bp DNA ladder and negative control respectively while the lanes IKA2 – BT34 are the tested samples.

Discussion

The study reported an overall prevalence rate of 2.5% for the studied egg samples which constitute both the egg shell and content. The prevalence reported here agrees with that reported by Bata *et al*. (2015) in Nigeria with a prevalence of 1.7% for quail eggs in farms and outlets. The prevalence rate in the study might be attributed to a lack of understanding of the transmission of *Salmonella* infection and improper orientation on biosecurity measures in poultry farms. It could be as a result of a lack of good and sustained hygienic practices on the poultry farms. However, the prevalence reported in this study is lower than the 5.5% obtained for chicken eggs from poultry farms and markets in North India (Singh *et al*., 2010). For the eggshell, the presence of *Salmonella* on egg shell in this study may be due to contamination of egg shells at lay with faeces from the intestinal carrier, dust, litter, and egg collector could also contaminate the egg shells as well as poor hygiene. This study reported a lower prevalence on egg shell compared to 7.8% reported for *Salmonella* isolates from commercial farms in Nigeria (Agada *et al*., 2014) but a bit higher compared to 2.3% reported for *Salmonella* species from chicken eggs in Indonesia (Kassahun *et al*., 2017). The variation in the prevalence reported on the egg shell by other researchers could be a result of differences in the breed of chickens, age, and time the eggs were picked from the floor or cage. Breeds of chickens such as Ross308 and Arbor acres and younger birds are usually more susceptible to *Salmonella* infection (Yaohui *et al*., 2020). Also, the delayed removal of eggs from the litter or cage increases the chances of infection. The study reported a prevalence on egg content which agrees

Table 1: The prevalence of *Salmonella* on eggshells and egg contents from farms within Zaria metropolis, Kaduna State, Nigeria

Source	no. of samples	positive samples	prevalence (%)	p-value
Eggshell	240	11	4.6	
Egg content	240	1	0.4	0.0059
Total	480	12	2.5	

Table 2: Prevalence of *Salmonella* in the rearing system of production in farms within Zaria metropolis, Kaduna State, Nigeria

House	no. of samples	positive samples	p-value
Deep litter	120	5 (4.2%)	0.7689
Battery	120	7 (5.8%)	

with the 0% prevalence reported for egg content in a study on eggs in Egypt (Kadry *et al*., 2019). Research revealed that egg contents could be contaminated with droppings from chickens excreting *Salmonella* in such cases, *Salmonella* in droppings can penetrate egg shell pores before the egg cools and before establishing the proteinaceous cuticular barrier (Bata *et al*., 2015). Also, egg shell surface contamination increases the risk of egg contents contamination by penetrating through egg surface cracks (Kadry *et al*., 2019). The study shows that, there is no statistically significant difference (P>0.7689) between the deep litter and the battery cage system of rearing; this further suggests that the level of egg contamination remains similar regardless of the production system. In comparison to related studies, we observed that the prevalence for deep litter and battery cage in the study was slightly higher than the 3.3% for deep litter and 2.3% for battery cage systems reported in Ethiopia (Kassahun *et al*., 2017) but notably lower than the 48.7% reported for poultry rearing system by Shah *et al.* (2012). Access to contaminated feeds, the presence of rodents on the farms, and farm-to-farm service are some of the factors that could account for the spread and circulation of *Salmonella* on poultry farms. Rearing system and stocking density could be another important reason for egg contamination with various micro-organisms (Kadry *et al.,* 2019).

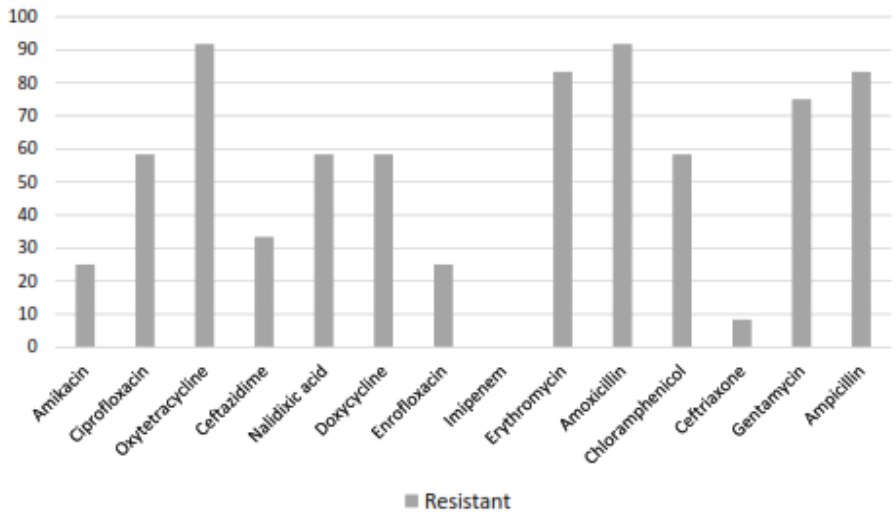

Figure 2: Percentage susceptibility of antimicrobial test among *Salmonella* isolates from chicken eggs in farms within Zaria metropolis, Kaduna State, Nigeria

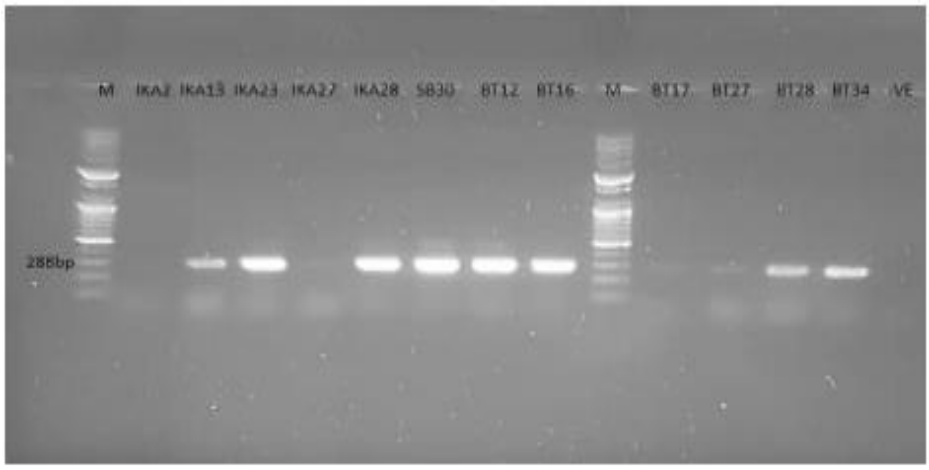

Figure 3: Agarose gel electrophoresis results of the *inv*A gene for identifying *Salmonella* from eggshell and content. Lane M; 100bp ladder, lane IKA2,IKA13,IKA23,IKA27,IKA28,SB30,BT12,BT16,BT17,BT27,BT28 and BT34 are positive *Salmonella* habouring *inv*A gene., Lane -ve control

For the antibiotic test the *Salmonella* isolates recorded the highest level of resistance for oxytetracycline and amoxicillin. Others in which the isolates demonstrated notable resistance were ampicillin, erythromycin, gentamycin, ciprofloxacin, nalidixic acid, doxycycline, and chloramphenicol. The percentage resistance for oxytetracycline in the study was higher than the 63.3% for oxytetracycline reported in Nigeria (Agada *et al*., 2014) and 46% for oxytetracycline reported in Senegal (Bata *et al*., 2015). Oxytetracycline stands out as the most widely used antibiotic for production animals, with day-old chicks and broiler chickens being consistently exposed to antimicrobial drugs throughout their growth phase. Consequently, the development of resistance to antibiotics like oxytetracycline should not be a surprise, given its approval for use in broiler feeds to promote growth (Chinchilla & Rodriguez,

2017). In this study the resistance level observed for oxytetracycline and ampicillin agrees with the 96.4% oxytetracycline and 39.0% ampicillin resistance reported in Ethiopia (Abunna *et al*., 2017). The resistance to ciprofloxacin corresponds with findings in *Salmonella* isolates from a farm as reported in the analysis of antibiotic sensitivity patterns of *Salmonella* species for quail eggs (Bata *et al*., 2015). The resistance recorded for nalidixic acid and gentamicin partially agrees with the 85% nalidixic acid and 70% gentamicin resistance reported in Nigeria for the detection of resistance genes in *Salmonella* from poultry (Nwiyi *et al*., 2018). For others like ceftazidime, enrofloxacin, amikacin, and ceftriaxone the *Salmonella* isolates exhibited a lower level of resistant while there was absolute susceptibility demonstrated for imipenem. Imipenem, classified as carbapenem is effective in treating severe infections in poultry, and maintained its effectiveness possibly due to its infrequent use in poultry. It is important to note that the microbial culture method is presumptive and must be supplemented with genomics and molecular methods for accurate identification and subsequent characterization of microbial species. Final confirmation was achieved through PCR method, with positive results obtained by amplifying the genus-specific biomarker; the *inv*A. The gene serves as the International Standard for *Salmonella* identification, providing an effective, rapid, and accurate method for detecting *Salmonella*. The study revealed that the *inv*A gene was present in 12 *Salmonella* isolates as earlier indicated by the culture test. The result was in line with the report by Kadry *et al*. (2019) who reported 50% of *inv*A gene for *Salmonella* strains isolated from egg samples in Egypt and Sharma & Das (2016) who reported 55% of *inv*A gene detected from *Salmonella* isolates from poultry samples. However, the result differs from the 12.5% reported for *Salmonella* isolates by Yanestria *et al*. (2019) in Indonesia. The difference in the percentage of the *inv*A gene in *Salmonella* may be due to the different species of *Salmonella* in the genus.

In conclusion, the study was able to reveal the presence of *Salmonella* in the sampled eggs after the culture test and it was confirmed through the polymerase chain reaction. The presence of *inv*A gene in the isolates was a further indication that the organism studied was *Salmonella* since it is the universal marker gene for the identification of the bacteria *Salmonella*. The sensitivity test shows that most of the antimicrobials used were resistant except for Imipenem in which *Salmonella* was completely susceptible. Salmonellosis should be treated with *Salmonella* susceptible antimicrobials, the dosage and administration should be based on a Veterinarian's prescription in order to save the few still effective antimicrobials. Eggs should be properly washed to remove the bacteria *Salmonella* from the shell before cooking or storage to avoid ingesting *Salmonella* or spreading the organism where the eggs are stored. Public health authorities and industries can organize campaigns to enlighten poultry farmers on the mode of spread and transmission of *Salmonella* as this will help reduce the prevalence of *Salmonella*.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

Abunna F, Ashenafi D, Beyene T, Dinka A, Bedaso M & Duguma R (2017). Isolation, identification and antimicrobial susceptibility profiles of *Salmonella* isolates from dairy farms in and around Modjo town, Ethiopia. *Ethiopia Veterinary Journal*, **21**(2): 92-108.

Adamu G, Usman AK, Sawa BA & Abdulkareem B (2016). Analysis of the partial distribution of global system of mobile communication base stations in Zaria urban area, Kaduna State, Nigeria. *Zaria Geographer*, **23**(1): 111-125

Agada GOA, Abdullahi IO, Aminu M, Odugbo M, Chollom SC, Kumbish PR & Okwori AEJ (2014). *Salmonella* isolates from commercial poultry and poultry farm-handlers in Jos, Plateau State, Nigeria. *British Microbiology Research Journal,* **4**(4): 462-479.

Bata SI, Karshima NS & Ogbu KI (2015). Isolation and antibiotic sensitivity patterns of *Salmonella* species from raw beef and quail eggs from farms and retail outlets in Jos, Plateau State,

Nigeria. *Journal of Veterinary Medicine and Animal Health*, **8**(4):29-34.

Biemer JJ (1973). Antimicrobial susceptibility testing by the Kirby Bauer disc diffusion method. *Annals of Clinical and Laboratory Science,* **3**(2): 135-140.

Bintsis T (2017). Foodborne pathogens. *AIMS Microbiology*, doi.10.3934/microbiol.2017.3.529.

Cheesebrough M (2005). District Laboratory Practice in Tropical Countries, Part 2, Second Edition. *Cambridge University Press*: Pp 159-162.

Chinchilla GF & Rodriguez C (2017). Tetracycline in food and feeding stuff: From regulation to analytical methods, bacterial resistance, and environment and health implications. *Journal of Analytical Methods in Chemistry*. doi.10.1155/2017/1315497.

CLSI (Clinical and Laboratory Standards Institute) (2017). Performance Standards for Antimicrobial Susceptibility Testing, 31st edition. CLSI Suppliement M100 ISBN 978-1-68440-105-5. Clinical Laboratory Standard Institute, Wayne, Pennsylvania USA. Pp 118-121.

Divek VTN, Kumar V & Anup KP (2018). Antibiotics resistant *Salmonella* in the food supply and the potential role of antibiotic alternatives for control. *Foods (Basel, Switzerland),* doi.10.3390/foods7100167.

Fagbamila IO, Barco L, Mancin M, Kwaga J, Ngulukun SS, Zavagnin P, Lettini AA, Lorenzetto M, Abdu PA, Kabir J & Umoh J (2017). *Salmonella* serovars and their distribution in Nigerian commercial chicken layer farms, *PLoS One,* doi.101371/journal.pone0173097.

Gberikon, GM, Olabode VB & Ogbonna IO (2019). Determination of antibiotic resistance of *Salmonella* and molecular confirmation of drug resistance *Salmonella* species isolated from raw chicken and quail eggs from selected farms in Jos, Nigeria. *International Journal of Innovative Research and Development*, doi.10.24940/ijird/2019/v8/i1/JAN19012.

Genoud C (2021). Tools to make volume – SEM techniques easier and faster on EM facilities. *European Microscopy Congress*, doi.10.22443/rms. emc2020.708.

Gonçalves-Tenório A, Silva BN, Rodrigues V, Cadavez V & Gonzales-Barron U (2018). Prevalence of pathogens in poultry meat: a meta-analysis of European published surveys. *Foods*, doi 10.3390/foods7050069.

ISO (International Organization for Standardization) (2017). Microbiology of the Food Chain-Horizontal Method for the Detection, Enumeration and Serotyping of *Salmonella*. https://standards.iteh.ai/catalog/standards/sist/6ccc484a-Oac8=4d1f-aef5-167d9fc52e0/iso-6579-1-2017, retrieved 01-02-2017.

Jain AK & Yadav RA (2017). Study of antibiotic resistance in bacteria isolated from table egg. *International Journal of Pharma and Biology Science*, **8**(1): 668-674.

Kadry M, Nader SM, Dorgham SM & Kandil MM (2019). Molecular diversity of the invA gene obtained from human and egg samples. *Veterinary World*, **12**(7): 1033-1038.

Kassahun T, Hussen B, Mebrat E & Adem H (2017). Prevalence and antibiotic resistance of Salmonella species isolated from chicken eggs by standard bacteriology methods. *Journal of Veterinary Sciences and Technology*, doi.10.4172/2157-7579.10000421.

Nwiyi P, Chah K & Shoyinka SV (2018). Detection of some resistance genes in *Salmonella* isolated from Poultry Farms in Abia and Imo States, Southeastern Nigeria. *Nigerian Veterinary Journal*, **39**(2): 124-132.

Saad S, Jubara A, Wani A, Munyeme M, Taha & Kwenda G (2022). Antimicrobial susceptibility of *Salmonella* isolated from chickens and humans in Wau, South Sudan. *International Journal of Microbiology.* doi.10.1155/2022/8570081.

Sagar A (2018). *Salmonella* Shigella (SS) Agar Culture Media. https://microbiologyinfo.com>salmonella-shigella-ss-a, retrieved 10-08-2018.

Shah AH & Korejo NA (2012). Antimicrobial resistance profile of *Salmonella* serovars isolated from chicken meat. *Journal of Veterinary Animal Science*; **2**: 40-46.

Sharma I & Das K (2016). Detection of invA gene in isolated *Salmonella* from marketed poultry meat by PCR assay. *Journal of Food Processing Technology,* **7**(3): 1–3.

Singh S, Yadav AS, Singh SM & Bharti P (2010). Prevalence of *Salmonella* in chicken eggs collected from poultry farms and marketing channels and antimicrobial resistance. *Food Resource International*. **43**: 2027-2030.

Taşkale KN, Yüksel FN, Akçelik N & Akçelik M (2018). Genetic diversity of food-originated *Salmonella* isolates. *Biotechnology and Biotechnological Equipment*, **32**(3): 638-645.

Tessema K, Bedu H, Ejo M & Hiko A (2017). Prevalence and antibiotic resistance of *Salmonella* species isolated from chicken eggs by standard bacteriological method. *Journal of Veterinary Science and Technology,* doi.10.4172/2157-7579.100042.

Thrusfield M (2008). Veterinary Epidemiology. *John Wiley and Sons,* UK. Pp227-247.

Yanestria SM, Rahmaniar RP, Wibisono FJ & Effendi MH (2019). Detection of invA gene of *Salmonella* from milkfish (Chanoschanos) at Sidoarjo wet fish market, Indonesia, using polymerase chain reaction technique. *Veterinary World*, **12**(1): 170-175.

Yaohui X, Xiao Z, Zenghai J, Yaru Q, Abdelaziz E & Min Y (2020). Epidemiological investigation and antimicrobial resistance profiles of *Salmonella* isolated from breeder chicken hatcheries in Henan, China. *Front Cell Infection Microbiology*, doi:10.3389/fcimb.2020.00497.