REVIEW ON IDENTIFICATION AND CHARACTERIZATION OF SALMONELLA SPECIES ISOLATED FROM DOMESTIC CHICKEN SOLD IN KURE ULTRA MODERN MARKET MINNA, NIGERIA.

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Abstract

Salmonella as a group of microorganisms has long been recognized as an important zoonotic pathogen of worldwide economic significance in animals, birds and man. They are intestinal bacteria which give rise to enteritis and typhoid-like disease. The prevalence of *Salmonella* differs depending upon sample types, collection and handling methods, detection techniques and geographic regions and management systems. These differences may mask the impact of other factors such as raising practices, seasonal patterns and processing procedures that are actually causing true changes in the distribution of the bacteria. Salmonella infection causes not only decreased production performance and even death of poultry, but also contamination of the human food chain, leading to serious economic losses in the poultry business, as well as being a threat to public health. This review recommends that: The best preventive and control strategy is vaccination.

Keywords: Identification, Characterization, *Salmonella*, Domestic, Market.

INTRODUCTION

Salmonellae are Gram-negative facultative anaerobic rod-shaped bacteria that measure 0.7-1.5 by 2.0-5.0 μm, non-sporogenic. All are motile with long, peritrichous flagella except two serovars, *Salmonella* serovar *pullorum* and *gallinarum* (Scheleton, 2018). *Salmonella* as a group of microorganisms has long been recognized as an important zoonotic pathogen of worldwide economic significance in animals, birds and man. They are intestinal bacteria which give rise to enteritis and typhoid-like disease. The early observation of the disease was made by Ebert 1880 who described the typhoid bacillus in the tissue of a dead patient,

and the organisms was isolated by Salmon in 1885 (Merchant & packer 2006) and named after him.

Preliminary *Salmonella* research is dated back to 1880, when the bacteria were isolated from a person who died from typhoid fever. Subsequently, in 1886, Daniel E. Salmon and colleagues isolated from swine the organism currently known as *Salmonella choleraesuis*, which was believed to be the causative agent for hog cholera (Le Minor, 2005).

Salmonella is an important zoonotic pathogen that causes infectious diseases in animals and humans. Salmonella infection causes not only decreased production performance and even death of poultry, but also contamination of the human food chain, leading to serious economic losses in the poultry business, as well as being a threat to public health. Although various prevention and control measures, including eradication programs and vaccine and drug use, have been carried out, Salmonella infection is still one of the most important problems worldwide.

Classification:

The scientific classification of *Salmonella* was described by Hafez (2005) as follows:

Domain: *Bacteria*
Kindom: *Monera*
Phylum: *Proteobacteria*
Class: *Gamma Protobacteria*
Order: *Enterobacteriales*
Family: *Enterobacteriaceae*
Genus: *Salmonella*

Recent advances in *Salmonella* taxonomy divide the genus into two species: *Salmonella bongori* and *Salmonella enterica* (Le Minor 2005). *S. bongori* contains less than 10 serovars while *S. enterica* contains more than 2500 serovars and are divided into six subspecies namely *enterica, salamae, arizonae, diarizonae, houtenae and indica.* All centers of disease control and prevention recommended that *Salmonella* species should be named by their genus and serovar e.g. *Salmonella typhi* instead of *Salmonella entrica subspecies enterica serovar Typhi.* Most commonly, the *Salmonella* are classified according to serology. The main division is first by the somatic (O) antigen and by the flagellar (H) antigen. (O) Antigen is of a lipopolysaccharide nature and (H) antigen of protein nature (Kauffmann White Scheme 1960). The genus *Salmonella* can roughly be classified

into 3 groups (Hafez and Jodas 2005). Group I includes highly host adapted and invasive serovars such as *S. gallinarium, S. polurium* in poultry and *S. typhi* in human. Group II includes non-host adapted and invasive serovars such as *S. typhimurium, S. arizonae* and *S. entertidis.* Group III contains non-host adapted and non-invasive serovars, most of these serovares are harmless for animals and human.

Cultural characteristics:

Salmonella are facultative anaerobic. The optimum growth temperature is 37ºC, but some growth is observed in a range from about 5 to 45ºC. *Salmonella* can grow within a pH range of approximately 4.0 to 9.0, with an optimum pH around 7.0. The organisms grow in selective enrichment media such as selenite-F-broth and tetrathionate broth, and on differential plating media such as MacConkey, bismuth sulfite, and brilliant green agars. The optimum incubation times for *Salmonella* enrichment cultures were obtained by inoculation of enrichment broth onto plating media after 24 hours incubation at 37ºC, after 48 hours at 37ºC, after a 3-day delayed secondary enrichment (DSE), and after a 5-day DSE procedure. Inoculations of the enrichment broth onto plating media after 24 hours incubation followed by 5-day (DSE) enable the detection of 96-98% of *Salmonella* positive samples and were the best combination of condition. The *Salmonella* colonies appear with different shapes and colours on different media. On nutrient agar they appear small, smooth, circular and translucent while *S. gallinarum* colonies are blue gray. On macConky agar they are colourless, smooth, round, shiny and up to 2mm in diameter. *S. gallinarum* produce colonies larger than *S. pullorum* and have a characteristic odour. On selenite-F- broth the growth is turbid with heavy flocculent sediment. On desoxycholate citrate agar (DCA) the colonies are slightly opaque, dome shaped with central black spot. *S. pullorum* is a lactose ferementer producing pink colonies with a precipitate in surrounding media. On triple sugar iron agar (TSI) *S. pullorum and S. gllinarum* produce a red slant with a yellow butt that show delayed blackening from H2S production. The objectives of this research are to:

I. To find out Prevalence of *Salmonella* in domestic chickens.
II. To isolate and identify *Salmonella* organism from commercial layers by biological methods and molecular characterization.
III. To determine antibiotic resistance profile of organisms isolated from the fowls.

Methodology

This review was carried out in Niger State, the focal point was mainly Kure Ultra-Modern Market, Minna.

Review on Prevalence of Salmonella, in Domestic Chicken at Kure Ultra-Modern Market, Minna

The major prevalent serotypes of *Salmonella* originating from 2 duck slaughterhouses and 13 chicken slaughterhouses tested were *S. typhimurium* and *S. enteritidis*, respectively (Bawa *et al.*, 2013). (Lawal *et al*., 2013) identified 165 *Salmonella enterica* isolates from 382 samples taken from conventional farms, abattoirs and retail from Kure Ultra-modern market from 2010 to 2011. Among these isolates, *S. enterica* serotypes Derby (76 isolates, 46%) and *S. typhimurium* (16 isolates, 10%) were the most prevalent. A comparison on the prevalence of *Salmonella* infection in layer hens from commercial layer farms with high and low rodent densities was investigated. Out of 280 laying hens sampled from three commercial 6 layer farms with high rodent densities, *Salmonella enterica subsp. enterica serovar Enteritidis* (Salmonella Enteritidis) was isolated from 20 (7.14%) hens and *Salmonella enterica subsp. enterica serovar Infantis* (*Salmonella Infantis*) from three (1.07%) hens. *Salmonella* infections from contact with live poultry (chickens, ducks, turkeys, and geese) continue to be a public health problem. In 2011, two clusters of human *Salmonella* infections were identified through Pulse Net, a molecular subtyping network for foodborne disease surveillance. Standard outbreak and traceback investigations were conducted. Most patients or their parents reported purchasing chicks or ducklings from multiple locations of an agricultural feed store chain that was supplied by a single mail-order (Centre for disease control and prevention, 2012). Isolated strains of *Salmonella spp.* from poultry products in Niger state, Nigeria by (Ahmed *et al.*, 2009). A total number of 114 samples were collected from 63 broiler carcasses derived from two processing plants and two supermarkets and 51 extra samples were collected in broiler farms located in the State which used three live production stages. Each excreta sample considered of a fresh excreta pool from 100 birds. Samples were submitted to microbiological analysis and the isolated Salmonella strains were tested for antimicrobial sensitivity. No *Salmonella* was isolated from excreta samples, while broiler carcass samples showed a high contamination rate of 11.8%. Three serotypes were identified: *S. enterica serovar enteritidis* 50%, S. *enterica serovar panama* 33% and *S. enterica serovar newport* 17%. Studies was carried out on the sero prevalence, isolation and characterization of *Salmonellae*

from layer chickens during the period from January to May 2006. The used materials were blood sample, cloacal and liver swabs from live and dead birds respectively and visceral organs (liver, lungs, spleen and intestine). The detection methods used were serum plate agglutination (SPA) test; necropsy and histopathology; cultural, morphological and biochemical test. The overall prevalence was 43.4%. A total of 33 (21.02%) Salmonellae from live and dead birds were isolated. The isolation rate of Salmonellae was higher in seronegative (31.6%) group than seropositive (3.2%) group. Out of 33 Salmonella isolates, 25 were *S. pullorum*, 3 were *S. gallinarum* and the rest 5 were motile Salmonellae (Islam *et al.*, 2006).

Review on Isolation, Identification and cultural characteristics of *Salmonella spp.*

Salmonella organisms' shows different cultural characteristics in different media. These are turbidity in Tetra Thionate broth, pink white color colonies in Brilliant Green agar, gray white colony in Nutrient agar, slightly grayish color colonies in Salmonella Shigella agar, black color colony in Tripple Suger Iron agar, pale color colonies in MaConkey's agar, well defined glistening colonies in Blood agar and pinkish colonies in EMB agar. Islam *et al*., (2003) reported that **t**he liver of chicken was found to be the most suitable organ for isolation of *S. gallinarum*. Use of pre-enrichment media was better than conventional media for the successful isolation of the bacteria. Isolates revealed moist, pin-sized, circular, non-lactose fermenting colonies on MacConkey, S-S, BGA, and BHI agar media.

Two different selective broths, and two different selective and differential agars should be used in culture isolation of *Salmonella spp.* Tetrathionate broth with 24 hours incubation is not a reliable medium for *Salmonella spp.* isolation from the faeces (Habrun and Mitak 2003).

Molecular Characterization of Salmonella spp.

To assess diversity of *Salmonella enterica* serotypes present in poultry and their environment from Kure Ultra-modern market, the Kauffman-White-LeMinor (KWL) scheme was used to serotype a total of 155 isolates. Isolates were then re-examined with nested PCR and sequencing of the dkgB-linked Intergenic Sequence Ribotyping (ISR) region that assesses single nucleotide polymorphisms occurring around a 5S ribosomal gene. Serotypes identified were Heidelberg (40.6%), Enteritidis (34.2%), Hadar (8.4%), Typhimurium (3.9%), Gallinarum (3.2%), Agona (1.3%), Cerro (1.3%), Livingstone (1.3%), Infantis (0.6%), Isangi

(0.6%), Mbandaka (0.6%), Montevideo (0.6%), and Senftenberg (0.6%), (Pulido-Landínez *et al.,* 2013). (Temelli *et al.,* 2012) evaluated the capability of the Vitek immunodiagnostic assay system easy *Salmonella* (VIDAS ESLM) method and a specific real-time PCR system (Light Cycler), in detecting Salmonella from a total of 105 naturally contaminated samples comprised of poultry meat and poultry meat products. Twelve (33.33%), 11 (30.55%), and 18 (50.00%) out of 36 poultry meat samples were positive for Salmonella by ISO, VIDAS ESLM, and LCPCR, respectively. *Salmonella* detection rates from poultry meat products were 5.80% for ISO and 8.69% for LCPCR, whereas none of these products tested positive by VIDAS ESLM.

Primer was used speF for the detection of *S. gallinarum.* A forward primer, s*pe*F-1 (5'- TTA GCC GTC ATT GCC CGG ATT -3') and a reverse primer, s*pe*F-4 (5'-ACG AGG TTT AAT GAC GTA GC -3') were used. Amplification reaction mixtures contained 30 µL X-mix (916 µL H2O milli-Q, 120 µL 10X buffer, 120 µL dNTP (2 mM), 36 µL MgCl); 0,5 µL of each primer 1 e 4 (*spe*C or *spe*F) and 0,4 µL taq DNA polymerase (Invitroven 10342-020).

Review on Antibiotic Susceptibility test

Reports shows that **o**ver the years 2007-2011, the reports of salmonellosis caused by *Salmonella enterica* serovar has significantly increased. A high prevalence of multidrug-resistant isolates, mainly showing an ampicillin-streptomycin-sulfonamide-tetracycline resistance pattern (ASSuT), was observed. In addition, four extended spectrum beta lactamase (ESBL) (CTX-M- 55)-producing isolates were found, Gallati *et al.,* (2013). A total of 165 *Salmonella enterica* isolates from 382 samples taken from conventional farms, abattoirs and retail markets from 2010 to 2011 in Kure Ultra-modern market were identified. Among these isolates, *S. enterica* serotypes *derby* (76 isolates, 46%) and *typhimurium* (16 isolates, 10%) were the most prevalent, and high antimicrobial resistance observed for tetracycline (77%), nalidixic acid (41%) and spectinomycin (41%). Li *et al.*, (2013). Generally, resistance for 13 different antimicrobial drugs was recognized. The most common resistance was to streptomycin (24/32, 75%), ampicillin (19/32, 59.4%), tetracycline (15/32, 46.9%). Imad *et al.,* (2012) collected 150 chickens from eight retail markets in minna, and 90 (60%) tested positive for *Salmonella*. The isolates were tested for their susceptibilities to amoxicillin, amoxicillin/clavulanic acid, cefoxitin, cefotaxime, gentamicin, streptomycin, tetracycline, chloramphenicol, sulfonamides, nalidixic acid, ciprofloxacin, and trimethoprim/sulfamethazole by disk diffusion assay. Minimum inhibitory

concentrations of ampicillin, streptomycin, tetracycline, sulfonamides, and nalidixic acid were determined for the resistant strains by agar dilution method. Eleven isolates (10.7%) of the 103 tested were susceptible to all antimicrobials. Resistance was most observed to tetracycline (84.5%), streptomycin (44.7%), and nalidixic acid (34%). Forty-one isolates (39.8%) were multidrug resistant (resistant to three or more antimicrobials from different classes).

CONCLUSION AND RECOMMENDATIONS

The prevalence of *Salmonella* differs depending upon sample types, collection and handling methods, detection techniques and geographic regions and management systems. These differences may mask the impact of other factors such as raising practices, seasonal patterns and processing procedures that are actually causing true changes in the distribution of the bacteria. This review recommends that:The best preventive and control strategy is vaccination.

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