International Journal of Research in Agriculture, Biology & Environment (IJAGRI)

DOI: 10.47504/IJAGRI.2021.5138

E-ISSN : 2582-6107

Volume 2 (2) 2021
Apr - Jun - 2021

Prevalence of *Salmonella* Enteritidis Infection in Chicken Eggs Collected from Different Poultry Farms in Awka, Anambra State of Nigeria

ABANA CHIOMA C[1] and NWAJIOBI FAVOUR O[2]

[1,2]Department of Applied Microbiology and Brewing

Faculty of Biosciences, Nnamdi Azikiwe University

Awka, Nigeria

ABSTRACT

This study examined the determination of the prevalence of Salmonella Enteritidis in table eggs circulating in Awka, Anambra State. A total of 400 good-looking, undamaged eggs were randomly selected and bought from different poultry farms in Awka and then, screened for Salmonella Enteritidis. The isolation of Salmonella Enteritidis and antibiotic susceptibility testing of the isolates was done using the standard procedures stated in the bacteriological analytical manual (BAM) of the United States Food and Drug Administration (USFDA) and Clinical and Laboratory Standard Institute (CLSI), respectively. Out of 49 (12.3%) isolates positive for Salmonella species, 16 isolates were positive to Salmonella Enteritidis representing 32.7% of the isolated organisms (P>0.05). The prevalence of Salmonella Enteritidis in egg content and eggshell was 32.7% and 0.0% respectively and the difference in their prevalence was not statistically significant (P<0.05). All isolates showed 100% resistance to augmentin and ceftazidime (zone of inhibition <13mm), ceftriaxone, and erythromycin showed 87.5% resistance (zone of inhibition <35mm). Ninety-three point seven percent (93.7%) of the strains were sensitive to ofloxacin (zone of inhibition >21mm), 75% sensitive to gentamicin (zone of inhibition >15mm) and 50%to cotrimoxazole (zone of inhibition >16mm). The strains studied in terms of their antibiotic-resistant pattern could be classified into different groups based on the number of antibiotics they resisted. Even though six of the sixteen isolated displayed the presence of plasmid, one can envisage that other non plasmids containing serovars demonstrating multi-drug resistance attributes have other means of antibiotics resistance other than the plasmid.

KEY WORDS: *Salmonella Enteritidis*, Prevalence, Antimicrobial Susceptibility, Resistance, Plasmid.

1. INTRODUCTION

Salmonella enterica serotype *Enteritidis (SE)* has emerged as a major cause of human egg-associated salmonellosis (non-typhoidal salmonellosis) occurring in high frequency mostly in industrialized nations and developing countries, and also, representing an important public health problem world – wide (Baumler, 2000; De Jong and Ekdahl, 2006; Herikstad *et al.*, 2002). Most of the food – borne salmonellosis pandemic in humans over many years has been observed to be as a result of ingestion of contaminated raw or undercooked shell eggs and food containing raw eggs (Kabir *et al.*, 2010; Marler, 2005). In the 1970s, stringent procedures for cleaning and inspecting eggs were implemented, thus making salmonellosis caused by external fecal contamination of egg shells extremely rare (CDC, 2011; Jean and Hsueh, 2011). Unlike salmonellosis of past decades, the current epidemic is due to intact and disinfected Grade A eggs where *Salmonella* Enteritidis silently infects the ovaries of healthy appearing chickens thereby contaminating the eggs before the shells are formed (vertical transmission) (Drew, 2010; Guard-Petter, 2001; NCIRD, 2005). Also, shell penetration through cracks can cause internal contamination of eggs (Gast and Holt, 2000). This bacterium is implicated in shell eggs, in the passage of the eggs through the cloaca (horizontal transmission) or contamination of the environment (Keller *et. al.*, 2003, Messens *et al.*, 2005). Backyard hens can get infected through contact with either domestic mammals or commercial poultry that are carriers of this bacterium, and consequently play a role in its transmission to other animals and humans (Jafari *et al.*, 2007; Sander *et al.*, 2001).

The risk of infection from raw or undercooked eggs is dependent in part, upon the sanitary conditions under which the hens are kept (Barrow and Wallis, 2000; Roy *et al.*, 2002). A study by the U. S. department of Agric in 2002, suggests that the problem is not prevalent as once thought but only 2.3 million eggs out of 69 billion eggs produced annually were contaminated with *Salmonella*, which is equivalent to just one in thirty thousand eggs (FAO, 2002; Hope *et al.*, 2002). *Salmonella* Enteritidis

infection usually results from the consumption of contaminated egg or egg products. This leads to gastroenteritis with clinical symptoms appearing about 8 – 72 hours of contact with the pathogen (USFDA, 2007; Yan *et al.,* 2003). The clinical symptoms include nausea, vomiting, abdominal pain and diarrhea with or without fever (CFIA, 2008; Dupont, 2011). Generally, just about 10^6 bacterial cells are needed to cause infection (Erdğrul, 2004). In elderly persons or individuals that use antacids, low gastric activity is common thus reducing the infective dose to 10^3 cells while in vaccinated persons it could increase to 10^9 cells (Porwollik, 2004; WHO, 2008). In United State of America, between 1985 and 2003, 997 outbreaks of *S.* Enteritidis infection were recorded which resulted in 33,687 illnesses with 3,281 hospitalized and 82 certified dead. The number of reported outbreaks in U. S. increased from 26 in 1985 to about 85 in 1990 with gradual decrease to 34 thereafter in 2003. Also the number of cases decreased from 2,656 in 1990 to as low as 578 cases in 2003. In 44% of the outbreaks, food vehicles were confirmed to be the source of infection and out of outbreaks of common vehicle, 75% of outbreaks were due to consumption of food containing either egg ingredient or primarily egg – based (Braden, 2006).

In Denmark, high incidence of *Salmonella* Enteritidis infection in the 1990s with its epidemic peaks of about 70% per 100,000 populations in 1997 was recorded (Wegener, 2003). The most reported *Salmonella* outbreaks in humans in the last few years have been retraced back to eggs, with the major serotype isolated being *S.* Enteritidis (De Buck *et al.,* 2004; Doug, 2013). In Nigeria, *Salmonella* associated aliments have been documented in different locations in clinical setting (Kabir *et al.,* 2010). There are however only a few reports on the circulating strains of non – typhoidal *Salmonella* and their significance especially *S.* Typhimuruim *and S.* Enteritidis in Awka. Due to death of information regarding the subject matter, this study is aimed at determining the prevalence of *Salmonella enterica* serovar Enteritidis in table eggs circulating in Awka, Anambra State, Nigeria.

2. METHODOLOGY

2.1 Sample Collection

Four hundred good looking, undamaged chicken eggs were randomly collected from different poultry farms located within Awka and transported to the laboratory in sterile nylons for bacteriological examination. Adherent materials on the shell surface of eggs were brushed off and the eggs disinfected by submerging them in a 3:1 solution consisting of three parts of 70% alcohol to one part of iodine/potassium iodide solution. After 10 seconds, the eggs were brought out; air dried and was aseptically broken into a sterile beaker. The egg content was manually mixed until the yolk is completely mixed with the albumen. Gloves were changed in between samples. Samples were incubated at room temperature for 96h ± 2h.

2.2 Isolation of *Salmonella*

Pre-enrichment: After 96h of incubation, 25ml of each incubated sample of pooled eggs was pre-enriched by inoculating it into 225ml of sterile Trypticase Soy Broth (TSB) supplemented with ferrous sulphate (35g ferrous sulphate added to 1000ml TSB and mixed well by swirling and allowed to stand for 60mins at room temperature). The inoculated sample was incubated for 24h at 35°C.

Selective Enrichment: The incubated pre-enriched sample was gently shaken and 0.1ml transferred to 9.9ml of Rappaport-vassiliadis (RV) medium and incubated at 42 ± 0.2°C for 24h in the water bath. Then, another 1ml of the pre-enriched sample was also transferred into 9ml of the Tetrathionate (TT) broth, vortexed and incubated at 35 ± 2.0°C for 24h.

Selective plating: The mixture was vortexed and a loopful of incubated TT broth streaked on Xylose Lysine Desoxycholate (XLD) agar and another on Bismuth Sulfite (BS) agar. A loopful of incubated RV was also streaked on XLD and BS plates. All plates were incubated at 35°C for 24h. Pure cultures were obtained afterwards using nutrient agar. Suspected colonies were Gram stained and viewed with oil immersion under the microscope using x100 lens objective. The shapes and colours observed were recorded. They were further screened using the following biochemical test: sugar fermentation test (), Triple sugar test (TSA), Lysine Iron Agar (LIA) Test, motility test, Indole test, Urease test, e.t.c.

Antimicrobial Susceptibility Testing: Antimicrobial susceptibility testing was done using the Kirby and Bauer disk diffusion method in accordance with the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards) whereby commercially available antimicrobial disks were used. Three to five colonies of the test organisms were inoculated into a tube containing nutrient broth and incubated for 24h at 37°C. Standardization of the inocula was done by diluting the broth cultures until turbidity matched the 0.5 McFarland standards. Using the flooding method, the prepared Mueller – Hinton agar plates were inoculated with the broth cultures. Thereafter, antibiotic disks were placed on the agar and pressed down to ensure contact using sterile forceps. The zone of inhibition was measured with a transparent ruler and compared to a zone – interpretation chart. Dilution techniques were also used to measure the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) (Cheesbrough, 2002).

DOI: 10.47504/IJAGRI.2021.5138

PLASMID EXTRACTION (TENS – MINIPREP): Plasmid DNA extraction was carried out using the TENS Method; a combination and modification of the methods described by Kraft *et al.*, 1988, Lech and Brent, 1987 and Maniatis *et al.*, 1982. 1.5mls each of 24h cultures grown on Mueller Hinton broth were centrifuged at 10,000rpm for 1min in a micro-centrifuge to pellet cells. The supernatant was decanted leaving about 50 - 100µl together with the cell pellet which was vortexed to homogenize. 300µl of TENS solution was added to the mixture and the solution mixed by inverting tubes 3-5 times until the mixture becomes sticky. 150µl of 3.0M sodium acetate pH 5.2 was added and the mixture vortexed to mix completely. This was centrifuged at 10,000rpm for 10minutes to pellet cell debris and chromosomal DNA. The supernatant was transferred into a fresh tube and mixed well with 900µl of ice-cold absolute ethanol. This was followed by centrifuging at 10,000rpm for 15minutes. After centrifuging, a white pellet was observed. The supernatant was discarded and the pellet was washed twice with 500µl of 70% ethanol. The pellet was then air dried and re – suspended in 20 – 40µl of TE buffer.

AGAROSE GEL ELECTROPHORESIS: Electrophoresis was carried out to separate the plasmid DNA using a 0.8% agarose gel in a 1X (single strength) concentration of Tris-Borate-EDTA (TBE) buffer. The agarose gel was prepared by heating 0.8% of agarose powder in 100mls of 1X TBE buffer. After heating, the solution was allowed to cool and 10µl of Ethidium bromide was added to the cooled agarose gel solution. This was poured into a casting tray with comb placed across its rim to form wells. Thereafter, the extracted DNA was loaded into the wells after mixing with 2µl of Bromophenol blue. A DNA molecular weight marker (HNDIII digest of λ-DNA) was also loaded into one of the wells. The gel was afterwards electrophoresed in a horizontal tank at a constant voltage of 60°V for about 1h 30mins. After electrophoresis, plasmid DNA bands were viewed by fluorescence of bound ethidium bromide under a short wave ultraviolet light transilluminator and the photograph was taken using a photo documentation system.

PLASMID CURING: Curing of plasmid DNA was done in this study to determine whether the plasmid DNA encodes for the multidrug resistance in *Salmonella* isolates. Tomoeda *et al.*, 1968 was adopted, where Sodium Dodecyl Sulphate (SDS) was used as a curing agent. 10g of SDS was added to 90mls of Nutrient broth, autoclaved and adjusted pH to 7.6 and steamed for 1h. An overnight culture was diluted in complete broth 100 fold. 500µl of diluted culture was added to 30mls of nutrient broth (pH 7.6), and incubated at 37°C for 2h. 3,000µl SDS stock solution was added to give the required final concentration of 1%w/v. After incubation for 72h at 37°C in a water bath with gentle shaking, 100µl of mixture was taken and diluted 10 fold by adding 900µl of nutrient broth and plated out on nutrient agar plates. Confirmation of the plasmid DNA curing was done by subjecting the cured isolates to plasmid extraction and agarose – gel electrophoresis followed by antimicrobial susceptibility testing to know whether the cured plasmid DNA was actually coded for antibiotic resistance.

3. STATISTICAL ANALYSIS

The results obtained in this research were statically analyzed using the social package for social science version 20.

4. RESULTS

The percentage prevalence rate of *Salmonella* Enteritidis was higher in the egg content than in the egg shell (Fig. 1). The antibiotics susceptibility testing results showed all the strains of *Salmonella* Enteritidis isolated having an exceeding total resistance to augmentin, ceftazidime, and also 87.5% resistance to ceftriaxone while 100% of the strains were sensitive to ofloxacin followed by 75% and 50% of gentamicin and cotrimoxazole, respectively. However, 18.8% of gentamicin and cotrimoxazole disclosed some level of partial resistance. Ofloaxcin, at as low as 5µg, had the highest sensitivity (95%) with zero resistance across all isolates while erythromycin had zero sensitivity and 90% resistance (Fig. 2).

The sensitivity index, which is the measure of the ratio of the percentage of sensitive to the percentage resistant organisms, varies in this study from 0.00 to 14.2. It ranges from 0.00 for ofloxacin, augmentin, ceftazidime, and erythromycin through 1.5 and 4.0 for cotrimoxazole and gentamicin respectively to 14.2 for ceftriazone. (Table 1)

In studying the antibiotic resistant pattern, the strains of organism isolated could be classified into different groups based on the number of antibiotics they resisted. They were classified into 3, 4, 5, and 6 different groups. Even though the sensitivity pattern in terms of the antibiotics varied, these groups were still distinct (Table 2).

The minimum inhibitory concentration of the tested isolates varies from one antibiotic to the other representing different levels of inhibition. This variation was found to range from 5 - > 20µg/ml (table 3).

The minimum bactericidal concentration of the tested isolates varies from one antibiotic to the other showing different levels of bactericidal effect on the strains, ranging from 10 - >20µg/ml (table 4).

DOI: 10.47504/IJAGRI.2021.5138

Of the 16 isolates studied, 6 were found harboring plasmids representing 37.5%. Of these six harboring plasmids, some were found possessing one plasmid, some two while others contained four different plasmids (table 5).

The treatment of the plasmid containing strains of *Salmonella* Enteritidis with SDS resulted in total curing of all the isolates (Table 6).

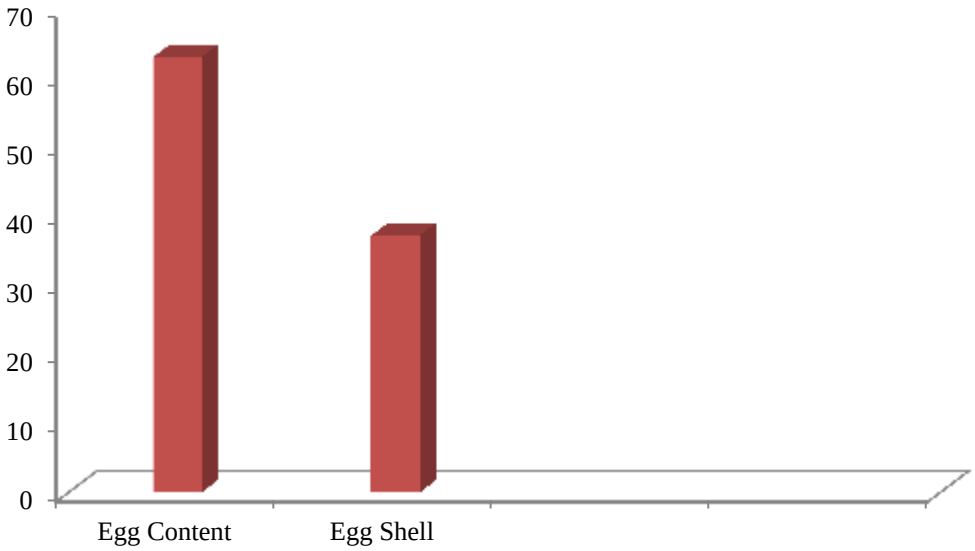

Fig. 1: Prevalence of Egg content and shell

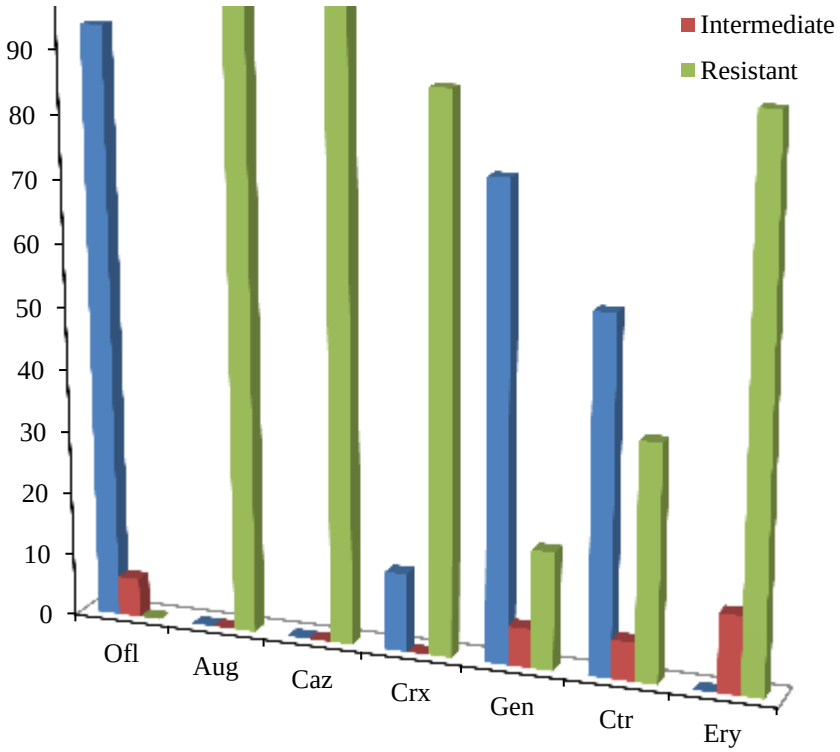

Fig. 2: Antibiotics susceptibility testing for *Salmonella enterica* serovar Enteritidis Antibiotics

KEY: Ofl = Ofloxacin, Aug = Augmentin, Caz = Ceftazidime, Crx= Ceftriaxone, Gen= Gentamicin, Ctr = Cotrimoxazole, Ery = Erythromycin

DOI: 10.47504/IJAGRI.2021.5138

Table 1: Sensitivity index of *Salmonella enterica* Serovar Enteritidis

Antibiotics	Sens (n (%))	Int (n (%))	Res (n (%))	Sensitivity Index
OFL	15(93.8)	1(6.2)	0(0.00)	0.00
AUG	0(0.00)	0(0.00)	16(100)	0.00
CAZ	0(0.00)	0(0.00)	16(100)	0.00
CRX	2(12.5)	0(0.00)	14(87.5)	14.2
GEN	12(75)	1(6.2)	3(18.8)	4.00
CTR	9(56.3)	1(6.2)	6(37.5)	1.5
ERY	0(0.00)	2(12.5)	14(87.5)	0.00

$$\text{Sensitivity Index} = \frac{\%\ \text{Sensitive}}{\%\ \text{Resistant}}$$

KEY: OFL = Ofloxacin, AUG = Augmentin, CAZ = Ceftazidime, CRX= Ceftriaxone, GEN = Gentamicin, CTR = Cotrimoxazole, ERY = Erythromycin, Sens = Sensitive, Res = Resistance, and Int = Intermediate.

TABLE 2: ANTIBIOTICS RESISTANT PATTERN OF *SALMONELLA ENTERICA* SEROVAR ENTERITIDIS

Strains	Resistance pattern	Antibiotics
1	ACCRE	4
2	ACCRE	4
3	ACCRE	4
4	ACE	3
5	ACCRE	4
6	ACCRE	4
7	ACCRCTE	5
8	ACCR	3
9	ACCR	3
10	ACCRE	4
11	ACCRCTE	5
12	ACCRGCTE	6
13	ACCTE	4
14	ACCRE	4
15	ACCRCTE	5
16	ACCRCTE	5

KEY: A= Augmentin, C=Ceftazidime, CR= Ceftriaxone, G=Gentamicin, CT=Cotrimoxazole, E= Erythromycin.

DOI: 10.47504/IJAGRI.2021.5138

TABLE 3: MINIMUM INHIBITORY CONCENTRATIONS OF SOME SELECTED ANTIBIOTICS AGAINST *SALMONELLA ENTRICA* SEROVARS ENTERITIDIS

Strains	MIC(µg/ml)						
	OFL	AUG	CAZ	CRX	GEN	CTR	ERY
1	5	>20	>20	>20	5	10	>20
2	10	>20	>20	>20	5	10	>20
3	5	>20	>20	>20	5	10	>20
4	5	>20	>20	>20	5	5	>20
5	>20	>20	>20	>20	5	10	>20
6	10	>20	>20	>20	5	10	>20
7	10	>20	>20	>20	5	>20	>20
8	5	>20	>20	>20	5	>20	>20
9	5	>20	>20	>20	10	5	>20
10	5	>20	>20	>20	>20	>20	>20
11	5	>20	>20	>20	>20	>20	>20
12	5	>20	>20	>20	>20	>20	>20
13	5	>20	>20	10	>20	>20	>20
14	5	>20	>20	>20	10	5	>20
15	5	>20	>20	>20	10	>20	>20
16	5	>20	>20	>20	10	10	>20

KEY: OFL = Ofloxacin, AUG = Augmentin, CAZ = Ceftazidime, CRX= Ceftriaxone, GEN= Gentamicin, CTR= Cotrimoxazole, ERY=Erythromycin

Table 4: Minimum Bactericidal Concentration of some selected antibiotics against *Salmonella enterica* Serovars Enteritidis

Strains	MBC (µg/ml)						
	OFL	AUG	CAZ	CRX	GEN	CTR	ERY
1	10	>20	>20	>20	10	20	>20
2	20	>20	>20	>20	10	20	>20
3	10	>20	>20	>20	10	20	>20
4	10	>20	>20	10	10	20	>20
5	>20	>20	>20	>20	10	20	>20
6	20	>20	>20	>20	20	20	>20
7	20	>20	>20	>20	20	>20	>20
8	20	>20	>20	>20	20	>20	>20
9	20	>20	>20	>20	20	20	>20
10	20	>20	>20	>20	>20	>20	>20
11	20	>20	>20	>20	>20	>20	>20

DOI: 10.47504/IJAGRI.2021.5138

12	20	>20	>20	>20	>20	>20	>20
13	20	>20	>20	>20	20	>20	>20
14	10	>20	>20	>20	>20	10	>20
15	10	>20	>20	>20	>20	>20	>20
16	10	>20	>20	>20	>20	20	>20

KEY: OFL = Ofloxacin, AUG = Augmentin, CAZ = Ceftazidime, CRX= Ceftriaxone, GEN= Gentamicin, CTR= Cotrimoxazole, ERY=Erythromycin

Table 5: Plasmid profiling of the Isolated *Salmonella enterica* Serovar Enteritidis

Strains	Plasmid size
1	28487
2	24387, 12121
3	388602, 6024, 2372, 586
4	388602, 6024, 2372, 502
5	NIL
6	NIL
7	NIL
8	24387
9	NIL
10	NIL
11	2030
12	NIL
13	NIL
14	NIL
15	NIL
16	NIL

Table 6: Plasmid profiling after treatment of isolates with Sodium Dedosile Sulphate (SDS)

Strains	Plasmid Size
1	NIL
2	NIL
3	NIL
4	NIL
5	NIL
6	NIL
7	NIL
8	NIL
9	NIL
10	NIL
11	NIL
12	NIL
13	NIL
14	NIL
15	NIL
16	NIL

DOI: 10.47504/IJAGRI.2021.5138

5. CONCLUSION

In this study, the prevalence of Salmonella Enteritidis in egg components (contents and shell) was studied and it was observed that the prevalence of *Salmonella enterica* Serovar Enteritidis was found to be 62.9% and 37.1% for egg content and shell respectively. These findings fall above the range reported earlier by Assefa *et al*., (2011), where the rate of *Salmonella* Enteritidis isolation from chicken eggs varied between 6.3% and 6.8% for egg shell and contents respectively and Betancor, (2010) who reported that the prevalence of *Salmonella* in chicken eggs ranged to be 24.4%.

The pooling sampling technique was found to be more efficient in the isolation of *Salmonella* species generally than the single sampling method ($t= 37$, $p<0.05$). Also, table eggs study revealed an apparent higher infection rate than the local eggs.

The biochemical identification demonstrated that *Salmonella enterica* Serovars Enteritidis was the most predominant amidst other *Salmonella* species. This observation is not surprising as the growth and survival of *Salmonella species* in eggs and its products has long been advocated (Akhtar *et al*., 2010). *Salmonella* Enteritidis represents 32.7% of the isolated organisms. *S*. Enteritidis continues to be an important cause of human food borne disease throughout the world. This Serovar accounted for the highest number of *Salmonella* associated food borne disease in recent years (Weill *et al.,* 2004). High prevalence of *Salmonella* Enteritidis in table eggs supports the notion that table eggs could be a major reservoir of human infection by *S*. Enteritidis.
Drug resistance pattern of *Salmonella enterica* serovars Enteritidis varied depending on their source. Of the 16 strains studied, all were found to be resistant to Augmentin and Ceftazidine. This observation is an indication that this serovar may be harboring an ESBL gene, (Thomas *et al;* 2012). Extended Spectrum beta lactamases (ESBL) have emerged as serious nosocomial pathogens throughout the world (Padmini *et al;* 2008; Thomas *et al.,* 2012). The fact that 87.6% of the *Salmonella enterica* serovars Enteritidis also resisted both ceftriazone and erythromycin may be representing a cross class resistance attribute for these isolates (Paterson and Bonomo, 2005; Pitout *et al.,* 2005). Cross class resistance refers to the ability of an isolate to resist antibiotics belonging to different classes (Pitout *et al.,* 2005; Thomas *et al.,* 2012). The resistance pattern of the studied *Salmonella* Enteritidis grouped the isolated organisms into four different resistance class based on the number of antibiotics they resisted. The reason for this observation hitherto may be due to variation in the strains of organisms studied.

The plasmid profiling of the studied *Salmonella* Enteritidis serovars indicated that six of the sixteen serovars harbored plasmids. This outcome is not new in this serovars, as many authors had previously documented similar findings. Plasmids are extra chromosomal DNAs that are capable of replicating independently of the bacterial chromosome. The presence of plasmid in clinical bacterial isolates may confer on them resistance attributes. The molecular weight of the plasmid containing serovars varied according to the source of organism. This result may further buttress the fact that sources of organism and the environmental condition of an organism may subsequently be a determinant factor for the weight of the plasmid present in them.

All the plasmid containing serovars became susceptible after treatment with Sodium Dedosile Sulphate (SDS), indicating that the multidrug resistant gene of the studied isolates may be located on the plasmids (Rooney *et al*., 2009). However, the fact that, other isolates found not to be harboring plasmid also showed significant level of resistance, clearly disclosed that such isolates may have other mechanisms of antibiotics resistance.

REFERENCES

Akhtar, F. I., Hussain, A. K. and Rahman, S. U. (2010). Prevalence and antibiogram studies of *Salmonella* Enteritidis isolated from human and poultry sources. *Pakistan Veterinary J*., **30**: 25-28.

Assefa, E., Akafete, T. and Haileleul, N. (2011). The Prevalence and Public Health Importance of *Salmonella* from Chicken Table Eggs. *J. Agric. & Environ. Sci.* **11 (4):** 512-518.

Barrow, P. A. and Wallis, T. S. (2000). *Salmonella in domestic animals*: Vaccination against *Salmonella* infections in food animals, rational, theoretical basis and tropical application. CABI publishing, U.S.A. Pp: 323-340

Baumler, A. J., Hargis, B. M. and Tsolis, R. M. (2000). Tracing the origins of *Salmonella* outbreaks. *Science*, **287**: 50 - 52.

Braden, C. R., (2006). *Salmonella enterica* serotype Enteritidis and eggs: A national epidemic in the United States. *Clin Infect Dis*, **43**:512-517.

Betancor, L., Pereira, M., Martinez, A., Giossa, G., Fookes, M., Flores, K. and Vignoli, R. (2010). Prevalence of *Salmonella enterica* in Poultry and Eggs in Uruguay during an Epidemic Due to *Salmonella enterica* Serovar Enteritidis. *Journal of Clinical Microbiology*. **48:** 2413–2423

DOI: 10.47504/IJAGRI.2021.5138

Canadian Food Inspection Agency (CFIA), (2008). *Salmonella. http://www.inspection.gc.ca/english/fssa/concen/cause/salmonellae.html*.

Centers for Disease Control, (2011). CDC Prevention Guidelines Database. *http://www.cdc.gov/preventionguidelines*. Assessed on May, 2012

Cheesbrough, M. (2002). *District Laboratory practice in tropical countries: part 2*. Low price editions. Cambridge university press, United Kingdom. Pp: 35-143.

De Buck J., Van-Immerseel, F. and Haesebrouck, F. (2004). Colonization of the chicken reproductive tract and egg contamination by *Salmonella*. *Journal of Applied Microbiology,* **97:** 233–245.

De Jong, B. and Ekdahl, K. (2006). The comparative burden of salmonellosis in the European Union member states, associated and candidate countries. *Public Health*, **6**: 4-12

Drew, F. (2010). Food Poison Outbreaks: Eggs and *Salmonella*, a long lasting public health problem: *Salmonella* Enteritidis and eggs. *Food Poison Journal, **2:*** 10 - 13

DuPont, H. L., (2011). *Cecil Medicine : Approach to the patient with suspected enteric infection.* In: Goldman L, Schafer AI, eds. 24th ed. Saunders Elsevier, Philadelphia, U.S.A. pp: 291.

Erdğrul, Ö. (2004). Listeria monocytogenes, *Yersinia enterocolitica* and *Salmonella* Enteritidis in Quail eggs. *Vet Anim Sci.*, **28:** 597-601.

Food and Agriculture Organization of the United Nations/ World Health Organization (FAO), (2002). Risk assessments of *Salmonella* in eggs and broiler chickens. *http://www.fao.org/docrep/005/y4392e/y4392e00.htm#contents*. Accessed on August 2008.

Gast, R. K. and P. S. Holt. (2000). Deposition of phage type 4 and 13a *Salmonella E*nteritidis strains in the yolk and albumen of eggs laid by experimentally infected hens. *Avian Dis.,* **44:** 706-710.

Guard-Petter, J. (2001). The chicken, the egg and *Salmonella* Enteritidis. *Environ Microbiol*, **3:** 421 – 30.

Herikstad, H., Motarjemi, Y. and Tauxe, R. V. (2002). *Salmonella* surveillance: a global survey of public health serotyping. *Epidemiology Infection*, **129**:1–8.

Hope, B., Baker, A. and Edel, E. (2002). An overview of the *Salmonella* Enteritidis risk assessment for shell eggs and egg products. *Risk Analysis,* **22**: 203-217.

Jafari, R. A., Ghorbanpour, M. and Jaideri, A. (2007). An investigation into *Salmonella* infection status in Backyard chickens in Iran. *Int J Poult Sci*, **6**: 227-229.

Jean, S. S. and Hsueh, P. R. (2011). High burden of antimicrobial resistance in Asia. *Int. J. Antimicrob. Agents;* **37**: 291–295.

Josh, K. (2010). Eggs Benedictus: Medical Risks. *http://foody.org/index.html*.

Kabir, O. A., Werner, P., Wolfgang, B. and Reinhard, B. (2010). Application of phage typing and pulsed-field gel electrophoresis to analyze *Salmonella enterica* isolates from a suspected outbreak in Lagos, Nigeria. *J Infect Dev*, **155**: 547-551.

Keller, L. H., Schifferli, D. M., Benson, C. E., Aslam, S. and Eckroade, R. J. (2003). Invasion of chicken reproductive tissues and forming eggs is not unique to *Salmonella* Enteritidis. *Avian Dis*, **41**: 53- 539.

Marler, C. (2005). *Food Borne Illness:* Common Bacteria and Viruses that cause food poisoning outbreak. Inc., Seattle, WA; Pp: 1301.

DOI: 10.47504/IJAGRI.2021.5138

Messen, W., Grijspeert, K. and Herman, L. (2005). Egg penetration by *Salmonella*: a review. *World's poultry science journal*, **61**: 71-85.

National Center for Immunization and Respiratory Diseases (NCIRD), (2005). Division of Bacterial Diseases. *http://www.cdc.gov/ncidod/dbmd/disease/salmnet_g.htm9*. Accessed on February, 2012

NCCLS (National committee for Clinical Laboratory Standard). (2003). Performance Standards for Antimicrobial Susceptibility Testing: 11th Informational Supplement. Approved Standard M2-A7 and M7-A5 National Committee for Clinical Laboratory Standards, Wayne, PA, USA. *http://www.nccls/antibioticsusceptibilitytesting/*.

Padmini, S. B. and Appala, B. R. (2008). "Evaluation of CIVA agar for rapid detection of extended spectrum [beta]-lactamases (ESBL) among isolates of Enterobacteriaceae." *Indian Journal of Medical Research*, **127(**2): 195.

Paterson, D. L. and Bonomo, R. A. (2005). Extended-spectrum beta-lactamases: a clinical update. *Clin. Microbiol. Rev.* **18:** 657–686.

Pitout, J. D. and Laupland, K. B. (2008). Extended-spectrum beta-lactamase-producing *Enterobacteriaceae*: an emerging public-health concern. *Lancet Infect. Dis.;* **8**: 159–166.

Popoff, M ., Bockemuhl, J. and Gheesling, L. (2004). Supplement 2002 (no. 46) to the Kauffmann-White scheme. *Research in Microbiology*, **155**: 568-570.

Porwollik, S., Boyd, E. F., Choy, C., Cheng, P., Florea, L., Proctor, E. and McClelland, M. (2004). Characterization of *Salmonella enterica* subspecies I genovars by use of microarrays. *Journal of Bacteriology*, **186(17)**: 5883-5898.

Rooney, C. M., Sheppard, A. E., Clark, E., Davies, K., Hubbard, A. T. and Sebra, R. (2009). Dissemination of multiple carbapenem resistance genes in an in vitro gut model simulating the human colon. *J. Antimicrob. Chemother.* **74** :1876–1883.

Roy, P., Dhillon, A. S., Lauerman, L. H., Schaberg, D. M., Bandli, D. and Johnson, S. (2002). Results of *Salmonella* isolation from poultry products, poultry, poultry environment, and other characteristics. *Avian Dis*, **46**:17- 24.

Ryan, K. J., and Sherris, C. G. (2001). *Medical Microbiology: An Introduction to Infectious Disease.* Fourth Edition. McGraw-Hill, New York: 362–8.

Sander, J., Hudson, C. R. and Dufour-Zavala, L. (2001). Dynamics of *Salmonella* contamination in a commercial Quail operation. *Avian Dis*, **45**:1044-1049.

Soyer Y., Moreno S. A., Davis M. A., Maurer J., Mcdonough P. L., Schoonmaker-Bopp D. J., Dumas N. B., Root T., Warnick L. D., Gröhn Y. T. and Wiedmann M. (2009). *Salmonella enterica* serotype 4,5, 12:i:-, an emerging *Salmonella* serotype that represents multiple distinct clones. *J. Clin. Microbiol.*, **47**: 3546–3556.

Su, L. H., Chu, C., Cloeckaert, A. and Chiu, C. H. (2005). An epidemic of Plasmids? Dissemination of extended spectrum cephalosporinases among *Salmonella* and other Enterobacteriaceace FEMS. *Immunol. Med. Microbiol*., **52**: 155 – 168.

Thomas, E., Lurdes, C., Manageiro, V., Ferreira, E., Jones-Dias, D., Correia, I., Themudo, P., Albuquerque, T. and Caniça, M. (2012). Occurrence of extended-spectrum β-lactamases among isolates of *Salmonella enterica* subsp. *enterica* from food-producing animals and food products, in Portugal. *International Journal of Food Microbiology*. **167(2):** 221-228

Tomoeda, M., Inuzuka, M., Kubo, N. and Nakamura, S. (1968). Effective elimination of drug resistance and sex factors in Escherichia coli by sodium dodecyl sulfate. *J Bacteriol*; **95(3):**1078-89. Assessed from *https://pubmed.ncbi.nlm.nih.gov/4868353/*

United States Food and Drug Administration (USFDA). (2007). Bad Bug Book: *Salmonella* spp. *http://www.cfsan.fda.gov/~mow/chap1.html*.

DOI: 10.47504/IJAGRI.2021.5138

Wallace, H. A., Jacobson, A. and Hammack, T. (2011). Bacteriological analytical manual (BAM - 8): *Salmonella.* *http://www.bacteriological-anlytical-manual(BAM)- iSalmonella-i.mht*

Weill, F. X., Lailler, R., Praud, K., Kerouanton, A., Fabre, L., Brisabois, A., Grimont, P. A. D. and Cloeckaert, A. (2004). Emergence of extended-spectrum-beta-lactamase (CTX- M-9)- producing multiresistant strains of *Salmonella enterica* serotype Virchow in poultry and humans in France. *J. Clin. Microbiol.,* **42**: 5767–5773.

Wegener H., Hald, T. and Wong, D. (2003). *Salmonella* control programs in Denmark. *Emerging Infectious Diseases.* **9**:774-780.

World Health Organization (WHO), (2008). Food safety measures for eggs and foods containing eggs. *http://www.who.int/foodsafety/publications/consumer/en/eggs.pdf*.

Wray, C. and Wray, A. (2000). *Salmonella in Domestic Animals.* CAB International, Wallingford, Oxon, UK.

Yan, S., Pendrak, M. and Abela-Ridder, B. (2003). An overview of *Salmonella* typing public health perspectives. *Clinical and Applied Immunology Reviews.* **4**:189–204.

DOI: 10.47504/IJAGRI.2021.5138