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PREVALENCE OF SALMONELLA SPECIES INFECTION IN POULTRY FARMING SYSTEMS IN ENUGU METROPOLIS NIGERIA

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ABSTRACT

Salmonella infection in poultry has remained a serious community health problem globally. This has resulted to the financial load of not only developed as well as underdeveloped nations. This study investigated the prevalence of salmonella species infection in poultry farming system. Stool samples were collected from different farms in Enugu metropolis. A well structured questionnaire was used to obtain information on clinical pattern of the birds. Stool samples collected were analyzed using selenite F and then subcultured unto deoxycholatecitrate agar (SSA) and salmonella-shigella agar. Those with salmonella growth were identified using motility test, sugar fermentation test, urease test and oxidase test. A total of 120 stool samples were collected from different farms in Enugu metropolis. Out of 120 stool droppings, the bacteriological examination reveals 54 (45%) strains of Salmonella, 63(52.5%) yielded normal intestinal flora [ie E coli] while 3(2.5%) yielded no bacteria growth. Overall Prevalence rate of 45% was observed in the four different groups of broilers the prevalence of 8(14.8%) was observed in Uzo farm, 16(29.6%) was observe in peters farm, 8(14.8%) in Uche's farm and22 (40.7%) observed in Phinoma farm Ngwo . This Phinoma farm had the highest prevalence rate as shown in diagram.

KEYWORDS: - *Prevalence, Salmonella species, Infection in poultry farming.*

INTRODUCTION

Salmonella infection are major public health concern worldwide and Salmonella is caused by non-typhoidal Salmonella enterica serotype (serotype other than s. typhi and s.paratyphi) and is typically characterized by a self limiting gastroenteritis syndrome manifesting as diarrhea, fever and abdominal pain. In Healthy persons, the infection is generally mild but in infants, elderly and immunocompromised, the situation can be severe and life threatening (Chen *et al.*,2013) different serotype have been associated with Salmonella but limited number are responsible for most human infections. S.enterica Enteritidis is the most frequent followed by S. enterica typhimurium (CDC, 2012).

Salmonella Enteriditis is commonly associated with poultry and poultry by product while S.tyhpimurium has a wider species ranges including pigs and cattle as well as poultry, hence foods of Animal origen like poultry productds (eggs and poultry meat)have been considered as the main vehicle of salmonella infection (Butyle *et al.*,2006). Four disease caused by salmonella are significant in poultry, they include Salmonella enteric seroval pollurum, fowl typhoid (FT) caused by S. gallinarium, paratyphoid caused by subspecies of Salmonella like S.typhimurium, S.enteritidis and S. infantis. There are many sources of infection in poultry and this includes Vertical Transmission, Contaminated Feeds and The Environment where they are reared (Pande *et al.*, 2016). Certain factors that can cause loss in poultry farming; includes: The Environment, it is essential that the Environment be favourable since unfavourable Environment can affect production. Excessive cold and heat may also cause production losses and impair bird health and welfare leading to increase bird morbidity and mortality. Another factor is Food Safety- contamination of foods by pathogens is of major concern. Proper attention should be given to the quality of feeds given to the birds. Compliances with health programmes like Vaccination, Cleaning and Disinfection will help in the control of losses in poultry farms (Zhou *et al.*, 2010).

MATERIALS AND METHODS

Study population

This was a cross-sectional survey carried out at Enugu State University Teaching Hospital (ESUT) between November 2018-July 2020.The stool samples were collected from different poultry farms in Enugu metropolis .Different colours of the stool samples were collected from the birds ranging from redish stool, black stool as well as greenish stool. A total of 120 stool samples were collected from the birds.

A structured questionnaire was used to collect data on clinical patterns of these birds.

Sample collection

A total Of 120 stool droppings from broilers were collected from four different farms in Enugu metropolis, The 120 broilers were collected as follows: 60 from phinomar farm Ngwo, one of the biggest farm in southeast , 20 from Uzodimma farm in Trans Ekulu , 20 from Peters farm in Abakpa and 20 from Uche ‘s farm in timber market Kenyatta . The samples were transported to the laboratory for further analysis within one hour from the time of collection.

Isolation and identification of salmonella isolates

The samples collected were inoculated unto selenite F overnight and subcultured into DCA[deoxycholate citrate agar] and SSA[salmonella shigella agar] and incubated aerobically for 24-48 h at 37oC. Suspected colonies on the Salmonella-Shigella agar presenting as non-lactose fermenters, with or without hydrogen sulphide (H2S) production were streaked on MacConkey agar .Suspected colonies of Salmonella were taken for further morphological and biochemical typing which included urease, Indole, motility test, sugar fermentation tests. All biochemically typical Salmonella isolates were serotyped based on reaction with somatic (O), flagellar (H), and capsular (Vi) antisera (Difco, USA). Salmonella O and Vi antigens were identified by slide agglutination test procedure. After the confirmation of the individual Salmonella O antisera, cultures were further characterized for H (phase I and II) antisera based on Spicer-Edwards antisera by tube test procedure.

Antibiotic susceptibilty test

The antibiotic paper disc susceptibility test was performed according to the CLSI method (Kirby-Bauer disk diffusion test) on Muller-Hinton agar (Titan Biotech, India). The isolates were inoculated into Mueller-Hinton broth separately and incubated for 24 h at 37ºC. The broths were matched to 0.5 McFarland standard and streaked using sterile cotton swabs on Mueller-Hinton Agar plates. The antimicrobial Agents used were the following

Ceftazidime 30µg
Cefuroxime 30µg
Gentamycin 10µg
Ceftriaxone 30µg
Ofloxacin 5µg
Ciprofloxacin 5µg
Nitrofurantoin 300µg
Amoxicillin/clavulinate 20/10µg

Pseudomonas aeruginosa ATCC 27853 served as control. Zones of inhibition were evaluated following the recommendations by CLSI, (2007MIC/MBC assay: The following antibiotics powder: (ciprofloxacin, gentamicin, tetracycline and sulphamethoxazoletrimethoprim combinations (cotrimoxazole)), were dissolved in appropriate diluents to obtain a stock solution of 5120 µg/ml each. Subsequent antibiotic dilutions were made in sterile Mueller Hinton broth and equal volume of the standardized inoculums was added to equal volume of an antibiotic concentration in test tubes. Antibiotic ranges were prepared one step higher than the final dilutions range required to compensate for the addition of an equal volume of inoculum. The inoculated tubes were incubated at 37oC for 16- 18 h. Inoculated and uninoculated tubes of antibiotic-free Mueller Hinton broth and Pseudomonas aeruginosa ATCC 27853 served as controls. The MIC corresponds to the lowest concentration of antibiotic at which there is no visible growth of the organism. The MBC was determined by plating out the tubes that showed no sign of growth on antibiotic free- Mueller Hinton agar plates and incubated at 37oC overnight. The MBC corresponds to the lowest concentration of antibiotic that prevented the growth of the test isolates after subculture on antibiotic free-Mueller Hinton agar plates.

Pre-screening test for potential esbls producers

The isolates that exhibites zones of inhibition ≤25mm for Ceftriaxone, ≤22mm for Ceftazidine, etc (all the 3rd generation cephalosporin)were considered potential ESBL Producers as recommended by the CLSI and were further tested by confirmatory methods (double disc synergy test).

Detection of esbl producing strains using double disc synergy test (ddst)

All the suspected ESBL producers were subjected to DDST on Mueller-Hinton agar plates using a disc of amoxicillin-clavulanic acid(20/10µg) and two of the 3rd generation Cephalosporin(Ceftazidine and cefuroxime) were used to confirm ESBLs Production. A sterile swab stick was used to culture the isolates on Muller-Hinton agar, which the after which the Amoxycillin-clavulanic acid was placed on the centre of the plate, two of the 3rd cephalosporin antibiotics were placed on each side of the combined Antibiotics. If the two drugs placed are ESBL producer, they will tend to clear towards the amoxicillin-clavulanic acid since the clavulanic acid are ESBL Inhibitors.

Gene analysis

The ESBL producing salmonella were genetically analyzed using Polymerase chain Reaction (PCR) see procedure below.

Method of DNA extraction

The Genomic DNAs of the bacterial isolates were extracted using the modified boiling method (Ezeonu, 2011)first, the isolates were inoculated into sterile nutrient broth and incubated at 370c for 72hr.five hundred microlitres of the incubated broth was added to an Eppendorf tube and centrifuge at 12,000rpm for 5min to pellet cells.

The supernatant was decanted and 500µl of sterile nuclease-free was added and votexed to wash cells. This was centrifuged at 12,000 rpm for 5min and the supernatant gently decanted. Fifty microlitres (50 µl) of nuclease –free water was added to the cell pellet, heated at 990c for 10mins to lyse the cells and release the DNA. Then it was subjected to cold shock treatment on ice for 10mins and then centrifuged at 1200 rpm.

For 5mins.Fifty microlitres(50 µl) of the supernatant was transferred into a new Eppendorf tube. To the supernatant, 100 µl of ice-cold absolute ethanol was added; mixed to precipitate out the DNA and kept on ice for 30mins.This was centrifuge at 12000 rpm for 2mins. The ethanol was decanted and allowed to air dry. The DNA was dissolved in 50 µl of IX buffer and keep in freezer for PCR use.

Polymerase chain reaction (PCR) for detection of ESBLS genes

Four ESBL markers (blaCTX-M, blaSHV, blaTEM and blaOXA) were used in the PCR. The extraction of dna for PCR was as previously described. The PCR for detection of ESBL genes was carried out using the New English Biolabs(one Taq 2x master-mix) with stranded buffer. Amplification was carried out in a 25µg total volume of PCR mixture containing 12.5µg of IX Master mix (New England biolabs) with standard buffer 0.4 µg of 10 µM each of forward and reverse primers (Inqaba, Biotech, south Africa); 5µg of the extracted DNA and 12.4 µg of sterile nuclease (Norgen, Biotek, corop, Canada) in a DNA thermal cycler. The amplification program was as follows at 35 cycles; for all the four genes initial denaturation was at 94^0c for 5min while denaturation was at 94^0c for 45s.

For blaTEM and blaSHV genes, annealing was at 60^0c for 30s, extension at 72^oc for 3min .for blaCTM and blaOXA annealing was set at 62^0c and 62^0c for 30s while extension was at 72^0c for 2min and a final extension step for all the genes was set at 72^0c for 3min.

RESULTS

Out of 120 stool droppings, the bacteriological examination reveals 54 (45%) strains of Salmonella, 63(52.5%) yielded normal intestinal flora [ie E coli] while 3(2.5%) yielded no bacteria growth. Overall Prevalence rate of 45% was observed in the four different groups of broilers the prevalence of 8(14.8%) was observed in uzo farm, 16(29.6%) was observe in peters farm, 8(14.8%) in Uche's farm and22 (40.7%) observed in Phinoma farm ngwo . This Phinoma farm had the highest prevalence rate as shown below

Table 1: Characteristic/clinical symptoms observed in the poultry farm.

Characteristic	Farm 1	Farm 2	Farm 3	Farm 4
No of birds	100	300	2000	60,000
Symptoms observed				
Cold	Yes	No	No	No
Weakness	Yes	No	No	No
Watery stool	Yes	No	No	Yes
White stool	Yes	Yes	No	No
Green stool	No	Yes	No	Yes
Pasty vent	No	No	Yes	Yes
Loss of appetite	No	No	No	Yes
Cough	Yes	No	No	Yes
High mortality	Yes	No	No	Yes
Measures taken in sick bird				
Sale off	No	No	No	No
Treat with antibiotics	Yes	Yes	Yes	Yes
Antibiotics used for treatment				
Vitamins	Yes	No	No	No
Tetracycline	Yes	No	No	No
Flagyl	Yes	No	No	No
Koleridin	No	Yes	No	No
Bio –E	No	Yes	No	No
Tylocenta	No	Yes	No	No
Dosegen	No	No	Yes	No
Gentatose	No	No	Yes	No
Gentamycin	No	No	Yes	No
Amoxicillin	No	No	Yes	Yes

Colistin	No	No	No	Yes
Enrofloxacin	No	No	No	Yes
Changes in Antibiotic				
Yes	No	No	Yes	Yes
No	No	Yes	No	No

Table 2: Observations in the farm.

Water sources for poultry				
Well water	Yes	No	No	No
Tank water	Yes	No	Yes	No
Bore hole	No	Yes	No	Yes
Tap/treated water	No	No	No	No
Stream water	No	No	No	No
Income loss	Yes	Yes	Yes	Yes
Duration of birds in poultry				
6 weeks for broiler	No	No	Yes	Yes
2 weeks for broiler	No	Yes	No	No
3 months	Yes	No	No	No
9o weeks for layer	No	No	No	Yes
Method of waste Disposal				
Waste bin	Yes	Yes	No	No
Deep burial	No	No	No	Yes
Sale off	No	No	Yes	No
Vaccination given to bird				
Gomborelie	No	Yes	Yes	No
Lasota	No	Yes	Yes	No
Salmabic injection	No	No	No	Yes
Biovac SGP695 oral	No	No	No	Yes
Movement of flocks				
Yes	Yes	Yes	Yes	No
No	No	No	No	Yes
Method of feed Storage				
Bags	Yes	Yes	Yes	Yes
Feed boxes	No	No	No	Yes
Silos	No	No	No	Yes
Challenges faced in the farm				
High cost of feed	Yes	Yes	Yes	Yes
Lack of fund	Yes	Yes	Yes	No

Table 3: Salmonella isolates from 120-feacal samples in poultry.

Poultry farms	**S. typhimurium (%)**	**S.enteritidis (%)**	**S.pollorum (%)**	**S.gallinarum (%)**	**Total (%)**
Farm 1	3 (2.5%)	2 (1.7%)	1 (0.8%)	2 (1.7%)	8 (6.7%)
Farm 2	5 (4.2%)	5 (4.2%)	4 (3.3%)	2 (1.7%)	16 (13.3%)
Farm 3	3 (2.5%)	3 (2.5%)	1 (0.8%)	1 (0.8%)	8(6.7%)
Farm 4	7 (5.8%)	8 (6.7%)	4 (3.3%)	3 (2.5%)	22(18.3%)
Total	18 (15%)	18 (15%)	10 (8.3%)	8 (6.7%)	54(45%)

Table 4: Antibiotic susceptibility patterns.

Isolates	**Sensitivity%**	**Resistance%**	**Total%**
S pollorum	4(3.3%)	6(5%)	10(8.3%)
S. gallinarum	6(5%)	2(1.7%)	8(6.7%)
S. enteritidis	14(11.7%)	4(3.3%)	18(15%)
S. typhinorium	16(13.3%)	2(1.7%)	18(15%)

Table 5: Gene analysis of poultry isolates.

SALMONELLA ISOLATES	blaCTM-M	BlaTEM	BlaSHV	BlaOXA-1
S.gallinarum	-	+	-	-
S.pollurum	-	+	-	+
S.typhimorum	+	+	_	_
S.enteritidis	-	+	-	+
S.enteritidis	+	+	-	-
S.galinarum	+	+	-	-
S.typhimorum	+	+	+	-
S.pollorum	-	+	-	+
S.typhimorum	+	+	+	_
S.tphimorum	-	+	+	+

DISCUSSION

Salmonellosis occurs worldwide in both developed and developing countries and is a major contributor to morbidity and mortality with resultant economic costs (Antoine *et al.,* 2008). In the present study, bacteriological screening of samples (120 each from intensively reared chickens.) from apparently healthy chickens showed that 54 samples harboured Salmonella spp. Isolation rate of 6.67% in chickens was significantly higher ($p<0.05$). The low rate of Salmonellae isolation observed in intensively reared chickens could be attributed to improved hygienic conditions in the poultry environment, and possibly, the incorporation of antimicrobials in poultry feeds. Similar rate of Salmonellae isolation (2%) in intensively reared chickens was reported in northern Thailand. Isolation rate obtained in the present study was higher compared to 0%, 0.8% and 0.83% rates reported by Curtello *et al.* (2013) in Jamaica, Aragaw *et al.* (2010) in Ethiopia respectively.

CONCLUSION

The direct plating out method of the samples without enrichment and improved poultry hygienic conditions reported in these studies could have contributed to the lower rates.

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