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ANTIBIOTIC SUSCEPTIBILITY STUDY OF *SALMONELLA* SPECIES ISOLATED FROM POULTRY FARMS IN EBONYI STATE, NIGERIA

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ABSTRACT

The emergence of antibiotic resistance among human has prompted concerns about the public health implications of antibiotic use in agriculture. The objective of the study was to determine the occurrence of *Salmonella* and their antimicrobial susceptibility pattern in poultry droppings from different locations within Abakaliki metropolis. About 100 poultry samples were aseptically collected, serially diluted and cultured using pour plate method. Isolation and identification of *Salmonella* were performed according to standard bacteriological protocol. Susceptibility testing of *Salmonella* isolates to 8 different antibiotics was carried out using disc diffusion method on Muller-Hinton agar. The microbial load of the samples ranged between 0.20±0.03 to 3.72±2.80 x10^6 CFU/g. The percentage occurrence of the isolates revealed that 9(15%) for Fidelis farm, 7(11.7%) Kpirikpiri market, 5(8.3%) Aguogboriga, 4(6.7%) Meat market and 2(3.3%) Garage express farm. Antibiotic sensitivity patterns of the bacterial isolates against eight tested antibiotics showed 100% susceptibility to imipenem, 21% was susceptible to tobramycin, amoxicillin 17% and meropenem 3%, but it was 100% resistant to ceftazidime. Overall, improper antimicrobial treatment and overuse of antibiotics for agricultural purposes

which contributed to increase incidence of multiple antibiotic resistance in farm animals must be discouraged.

Keywords: Antibiotic resistance, Abakaiki, Poultry farm, Salmonella

INTRODUCTION

The occurrence of *Salmonella* species has become one of the major health challenges not only in the study area but in several parts of Nigeria. *Salmonellosis* ranges in severity from self-limiting gastroenteritis to septicaemia **[1]**. The severity of the salmonellosis depends heavily on host susceptibility and the virulence of the serovars. It is characterized clinically by one or more of the three major syndromes, septicaemic, acute and chronic enteritis which infects both humans and animals with millions of illness reported worldwide. *Salmonellosis* is one of the leading causes of bacterial gastroenteritis in humans and is responsible for over 1.4 million illnesses annually in the USA **[2]**.

Although, *Salmonella* species also cause clinical diseases in a variety of animal species, many domestic and wild animals become colonized and shed these bacteria in their faeces with apparent sign of illness. If ingested either through direct exposure to faeces or through faecal contamination of food or water, dominant *Salmonella* serovars can subsequently cause disease in humans and other animals **[3]**. The infected birds may serve as transport vehicle for transmission of *Salmonella* species to humans through the consumption of poultry meat and eggs.

In sub-Saharan Africa, non-typhoidal *salmonellae* are the most common causes of bacterial bloodstream infections in both adults and children presented with fever and are associated with case fatality rate of 20–25% **[4]**. Infections can occur most often via ingestion of contaminated meat, eggs, raw milk, fruits, and vegetables. Contamination of these foods can occur during production, processing, distribution, and retail marketing.

Antimicrobial resistance is a global concern causing infections by resistant microorganisms resulting in prolonged illness and death and reduces effectiveness of treatment. An increasing proportion of *Salmonella* isolates is resistant to commonly used antibiotics in both developing and developed countries and this increase is seen in both veterinary and public health sectors **[5]**. Contamination can occur at various levels for example, fecal excretion of *Salmonella* can be a source of contamination both at farm and at abattoir

levels. Contaminated hides and viscera can be sources of contamination at abattoirs. Abattoir workers can spread the contamination during evisceration and handling meat without proper hand disinfection.

The research was aimed to study the antibiotics susceptibility of *Salmonella* species isolated from poultry wastes in different poultry farms within Abakaliki, Ebonyi State, Nigeria.

MATERIALS AND METHODS

Study Area

Abakaliki is the capital city of Ebonyi State in Southeastern Nigeria, located 64 kilometres (40 mi) southeast of Enugu. The inhabitants are primarily members of the Igbo nation. The geographical coordinate of Abakaliki is 6°20′N 8°06′E. It was the headquarters of the Ogoja province before the creation of the Southeastern State in 1967.

Equipment and Instruments

The following equipment and instruments were used in the course of this research; autoclave (Yx-280A, England), incubator (NL-9052-I, England), refrigerator, water bath, hot air oven, petri dishes, sample bottles, test tubes, swab sticks, beakers, morter and pestle, inoculating loop and Bunsen burner.

Reagents and Chemicals

The following reagents were used; hydrogen peroxide (H_2O_2), safranin, crystal violet, Kovac's reagents, oxidase reagent, Lugols iodine, acetone, peptone water and distilled water.

Culture media

The culture media used include Nutrient agar, Salmonella - Shigela agar (SS), Mueller Hinton Agar (MHA). The culture media was procured from Titan Biotech Ltd., India.

Antibiotics

The antibiotics used in this study include imipenem (10 µg), cefoxitin (30 µg),cefotaxime (30 µg), cefeprime (30 µg), meropenem (10 µg), tobramycin (10 µg) ceftazidime (30 µg) and amoxicillin clavulanic acid (30 µg). All antibiotics were procured from Oxoid limited (Oxoid, UK).

Sample collection and processing

Sixty samples of poultry droppings, swaps from poultry cloacae and poultry intestines were aseptically collected from different poultry farms within Abakaliki metropolis. The samples were immediately taken to Microbiology laboratory where analysis was conducted within 4- 6 hours of collection. Thereafter, 1g each of the samples was aseptically weighed into the sterile peptone water and was incubated for 24 hours for enrichment of the whole samples.

Isolation of Salmonella species

Exactly 1mL each of the enriched samples was aseptically measured into test tubes containing 9 mL of sterile distilled water and shaken thoroughly for even distribution of organisms to make a stock. Then, ten-fold serial dilution was carried out by transferring 1 mL each of the mixture into sterile test tubes containing 9 mL of sterile distilled water. Exactly 1 mL of the 5^{th} diluent was dispensed onto freshly prepared Salmonella-Shigella agar plate using the pour plate method. This was incubated at 37^{o}C for 24 hour for growth to appear. Individual colonies that fermented Salmonella- Shigella agar showed black colonies on the SSA plates. The colonies on the SSA plates were picked, sub-cultured and subsequently identified using standard microbiological procedure.

Antibiotics Sensitivity testing

Susceptibility test was done on Muller Hinton Agar (Oxoid, UK) plates by standard disk diffusion method in conformity to the recommended standard of Clinical and Laboratory Standard Institute (2016). The antibiotic disks used were include; imipenem (10 µg), cefoxitin (30 µg), cefotaxime (30 µg), cefeprime (30 µg), meropenem (10 µg), tobramycin (10 µg) ceftazidime (30 µg) and amoxicillin clavulanic acid (30 µg). All the antibiotics disk were procured from Oxoid limited (Oxoid, UK). These antibiotics were chosen either because they are used in both medicine and human veterinary practice or as a result of previous studies with reports of microbial resistance to them.. Colonies of confirmed *Salmonella* isolates were collected using wire loop and were dispensed into test tubes containing 5 ml distilled water. The cell concentration was adjusted to 0.5 MacFarland standard. Sterile swap stick was used to collect the organisms and these were streaked on freshly prepared Mueller-Hinton agar plates. The plates were allowed to stand for 15 minutes so that the cells will adapt to the environment of the medium. After this, the standard antibiotic disks were placed 15 mm apart and was incubated at 30^{o}C for 24 hours and the zones of inhibition diameter was measured according to CLSI criteria **[6]**.

Determination of multiple antibiotic resistance index (MARI)

Multiple antibiotic resistance (MAR) index was determined for each isolate by using the formula MAR = a/b, where a represents the number of antibiotics to which the test isolate depicted resistance and b represents the total number of antibiotics to which the test isolate has been evaluated for susceptibility. MARI of relative ratio >1 is shown to represent

potential risk source of resistant strain from the environment. If MAR index value is between 0.200 and 0.250 it becomes a very risky case where there are equal chances that MAR may fall in the high risk and low risk phases **[7]**.

Statistical Analysis

Experimental data was presented as mean±standard deviation, while one way ANOVA procedure will be used to analyze statistical difference in the data generated

RESULTS

Microbial load of *Salmonella species* isolated from poultry droppings in different locations within Abakaliki metropolis

The results in **Table 1** showed that *Salmonella* species were high in microbial load from poultry dropping samples from Fidelis farm (3.72±2.80), followed by New market (3.52±4.50), Jossel farm (3.24±0.21), Mile 50_2 (3.14±2.01), Chiboy farm (3.06±1.51) and Jonathan farm (3.01±9.30) while they were low in microbial load among samples from Uchenna Samuel farm (0.20±0.03), followed by Presco campus (0.21±0.03), Nweke farm (0.30±0.02), Daniel farm (0.40±0.02) and Aguogbriga (0.69±0.02) respectively.

Occurrence and Distribution of the Isolates from different Poultry Dropping Samples from different locations in Abakaliki metropolis

Table 2 revealed that samples from poultry droppings had the highest occurrence and percentage distribution, 30(50.01%) followed by cloacae, 16(26.71%), while samples from poultry intestines had the least occurrence and percentage distribution of 14(23.37%).

Also, poultry dropping samples from Chidubem , Chiboy, New layout market, Aguogboriga , Mile 50^1, Tipper garage express, Unity square close and Mile 50_2 farms had distribution and prevalence of 2(3.33%) respectively, While New market, Fidelis , Ajanwachukwu, CAS campus, Daniel, Uchenna Samu, Presco campus, Ebonyi farm, Sunshine and Mbukobe farms had 1(1.67%) respectively.

Antibiotics susceptibility pattern of the isolates to antibiotics

Table 3 shows the result of the antibiotics susceptibility pattern of *Salmonella* species to the antibiotics used. The results revealed that the isolates were completely resistant to ceftazidime (100%), and 75% resistant to meropenem and amoxicillin/clavulanic acid respectively. Tobramycin (53.33%), cefoxitin (20%), cefepime (18.33%), cefotaxime (13.33%). Meanwhile, the isolates showed complete susceptibility to imipenem (100%), cefotaxime (63.33%), cefepime (51.67%), cefoxitin (50%), tobramycin (16.67%),

amoxicillin/clavulanic acid (13.33%), meropenem (8.33%).

Multiple antibiotics resistance indices of the isolates

The result of the multiple antibiotics resistance index of the isolates as represented in **Table 4**. revealed that Isolates from droppings were found to have highest multiple antibiotics resistance index ranging from 0.6 to 0.9, Isolates from cloacae had range of 0.4 to 0.5 while, the those from intestines had the least MAR index ranging from 0.1 to 0.2.

Table 1: The microbial load of the Salmonella species isolated from different locations in Abakaliki

Location	Colony count x (10^6) CFU/g
Chidubem farm	1.78±5.68
Chiboy farm	3.06±1.51
Jossel farm	3.24±0.21
Newlayout market	2.72±1.51
Kpirikpiri market	2.81±7.30
New market	3.52±4.50
Meat market	1.81±3.53
Fidelis farm	3.72±2.80
Ajanwachukwu farm	1.14±0.20
Aguogboriga farms	0.69±0.02
Odunukwe farm	2.24±0.02
Nweke farm	0.30±0.02
CAS campus farms	2.90±1.41
Mile 50^1 farm	1.84±0.03
Daniel farm	0.40±0.02
Tipper garage express	1.90±0.02
Uchenna Samuel farm	0.20±0.03
Presco campus	0.21±0.03
Ebonyi farm	2.84±1.75
Oroke Onuora farm	1.82±7.64
Unity square close farms	1.35±1.57
Jonathan farm	3.01±9.30
Mile 50^2 farms	3.14±2.01
Sunshine farm	1.76±4.52
Mbukobe farms	0.64±0.03

KEY: CFU = Colony Forming Unit

Table 2: Occurrence and distribution of *Salmonella* species from different poultry dropping samples

SAMPLE LOCATION	DR (%)	CL (%)	IN (%)	TOTAL ISOLATES (%)
Chidubem Farms	2(3.33)	0(0)	2(3.33)	4(6.66)
Chiboy Farms	2(3.33)	0(0)	0(0)	2(3.33)
Jossel Farms	1(1.67)	1(1.67)	1(1.67)	3(5.01)
Newlayout market	2(3.33)	2(3.33)	0(0)	4(6.66)
Kpirikpiri market	2(3.33)	1(1.67)	1(1.67)	4(6.67)
New market	1(1.67)	0(0)	1(1.67)	2(3.34)
Meat market	0(0)	1(1.67)	0(0)	1(1.67)
Fidelis Farm	1(1.67)	0(0)	1(1.67)	2(3.34)
Ajanwachukwu Farms	1(1.67)	1(1.67)	0(0)	2(3.34)
Aguogboriga	2(3.33)	1(1.67)	1(1.67)	4(6.67)
Odunukwe Farms	0(0)	1(1.67)	0(0)	1(1.67)
Nweke Farms	0(0)	1(1.67)	0(0)	1(1.67)
CAS campus Farms	1(1.67)	0(0)	1(1.67)	2(3.34)
Mile 50^1 farms	2(3.33)	0(0)	0(0)	2(3.33)
Daniel Farms	1(1.67)	0(0)	1(1.67)	2(3.34)
Tipper garage express	2(3.33)	1(1.67)	0(0)	3(5.00)
Uchenna Samuel Farms	1(1.67)	1(1.67)	1(1.67)	3(5.01)
Presco campus Farms	1(1.67)	1(1.67)	0(0)	2(3.34)
Ebonyi Farms	1(1.67)	0(0)	1(1.67)	2(3.34)
Oroke Onuora Farms	0(0)	1(1.67)	1(1.67)	2(3.34)
Unity square close Farms	2(3.33)	1(1.67)	0(0)	3(5.00)
Jonathan Farms	1(1.67)	0(0)	0(0)	1(1.67)
Mile 50^2 Farms	2(3.33)	0(0)	1(1.67)	3(5.00)
Sunshine Farms	1(1.67)	1(1.67)	0(0)	2(5.01)
Mbukobe Farms	1(1.67)	1(1.67)	1(1.67)	3(3.34)
Total	30(50.01%)	16(26.71%)	14(23.37%)	60(100%)

KEY: 'Dr' = 'Droppings', 'CL'= 'Cloacae', 'IN' = 'Intestine'

Table 3: Antibiotics susceptibility test of the isolates

Antibiotics	Resistance (%)	Intermediate (%)	Susceptibility (%)	Total (%)
IPM	0(0)	0(0)	60(100)	60(100)
FOX	12(20)	18(30)	30(50)	60(100)
CTX	8(13.33)	14(23.33)	38(63.33)	60(99.99)
FEP	11(18.33)	18(30)	31(51.67)	60(100)
TOB	32(53.33)	18(30)	10(16.67)	60(100)
MEM	45(75)	10(16.67)	5(8.33)	60(100)
CAZ	60(100)	0(0)	0(0)	60(100)
AMC	45(75)	7(11.67)	8(13.33)	60(100)

KEY: IPM = Imipenem, FOX = Cefoxitin, CTX = Cefotaxime, FEP = Cefepime, TTOB = Tobramycin, MEM = Meropenem, CAZ = Ceftazidime, AMC = Amoxicillin/Clavulanic acid, % = Percentage.

Table 4: The multiple antibiotics resistance indices (MARI) of the isolates

SAMPLE	LOCATION	MARI	ANTIBIOTICS
DROPPINGS	Chidubem Farms	0.7	FOX FEP TOB MEM CAZ AMC
	Chiboy Farms	0.9	FOX CTX FEP TOB MEM CAZ AMC
	Jossel Farms	0.7	FOX FEP TOB MEM CAZ AMC
	Newlayout market	0.9	FOX CTX FEP TOB MEM CAZ AMC
	Kpirikpiri market	0.6	FEP TOB MEM CAZ AMC
	New market	0.6	FEP TOB MEM CAZ AMC
		0.6	FEP TOB MEM CAZ AMC
	Meat market	0.9	FOX CTX FEP TOB MEM CAZ AMC
	Fidelis Farm		
	Ajanwachukwu Farm	0.7	FOX FEP TOB MEM CAZ AMC
		0.9	FOX CTX FEP TOB MEM CAZ AMC
	Aguogboriga	0.4	FOX TOB CAZ
	Odunukwe Farms		
	Nweke Farms	0.5	CTX TOB MEM CAZ
	CAS campus	0.4	FOX TOB CAZ
	Mile 50[1] Farms	0.5	CTX TOB MEM CAZ
CLOACAE	Daniel Farms	0.4	FOX TOB CAZ
	Tipper garage express	0.4	FOX TOB CAZ
		0.5	CTX TOB MEM CAZ
	Presco campus Farms	0.1	CAZ
	Ebonyi Farms		
	Oroke Onuora Farms	0.2	MEM CAZ
	Mbukobe Farms	0.1	CAZ
	Unity square close	0.1	CAZ
	Jonathan Farms	0.2	MEM CAZ
INTESTINES	Mile 50[2]Farms	0.1	CAZ
	Sunshine Farms	0.2	MEM CAZ

KEY: PD = Poultry dropping, IPM = Imipenem, FOX = Cefoxitin, CTX = Cefotaxime, MEM = Meropenem, TOB = Tobramycin, FEP = Cefepime, CAZ = Ceftazidime, AMC = Amoxicillin/Clavulanic acid, MARI = Multiple antibiotics resistance index

DISCUSSION

The study revealed that poultry dropping samples collected from different locations in Abakaliki, Ebonyi state had high microbial load of 3.72±2.80 CFU/g and low microbial load of 0.20±0.03 CFU/g which is in contrast to **[8]** who reported the microbial load ranging between 7.58 x 10^{-5} and 6.36 x 10^{-5} CFU/g for feed samples prepared using poultry droppings produced by local industries. However, the recovery of these species of *Salmonella* of such public health concern may indicate certain potential hazard to animals and in return to any final consumer of the animal products if not properly handled. Findings

from this study revealed a high microbial load higher than what was recorded for other poultry droppings often used as feed samples as corroborated by work of **[9]** who reported the contamination of poultry feeds with *Salmonella* sp which likely might have resulted from the manufacturer or the ingredients used in compounding feeds using these poultry droppings.

The prevalence of *Salmonella* species in this study was high from isolates obtained from droppings, while those from cloacae had

lower percentage distribution and intestines had the least occurrence which is higher than what was obtained by **[10]** who reported that the frequency of *Salmonella* species isolated from poultry farm in Asella, Ethiopia, was 4.4% **[10]** and in Hawassa, Southern Ethiopia, 2.7% **[11]**. This variation might be attributed to numerous factors, such as topographical location, period, size of the farm, environment sanitation, farm management practices, variation in types of samples evaluated and differences in detection methodologies used. However, in spite of the variation, all of these studies proved quite clearly that poultry droppings can be a significant source of foodborne pathogens of human health significance **[11]**. In line with this, **[12]** in Cameroon reported a higher prevalence (27%) of *Salmonella* among poultry birds than the current study. This might also be due to the difference in the study area. This is also comparable with the result conducted in USA **[13]**.

The total of 60 *Salmonella* species (N=60) isolated were tested against eight commonly used antibiotics. The result of antimicrobial susceptibility pattern of *Salmonella* isolates as shown in **Table 3** revealed that isolates were highly resistance to ceftazidime which is similar with the study conducted in Addis Ababa **[14]** and Harrer **[15]**. *Salmonella* species isolated in this study were highly susceptible to imipenem (100%) which closely agrees with the work carried out in Zaria, Nigeria **[16]**, but is however contradicted by the work carried out in Cameroon **[17]**. Such differences could result from the difference in the geographical location as well as the habitual drug administration standards which differ with different regions. This is followed by cefotaxime, cefepime , cefoxitin having 38(63.33), 31(51.67) and 30(50) respectively. These findings are supported by the results of previous study conducted in some other areas **[18]**. Meanwhile, the resistance rate of *Salmonella* spp to amoxicillin was (13.33%), is lower than what was obtained from the study done in Gonder (88.2%) **[19]**. Meropenem and amoxicillin also showed high resistance to the isolates which is in agreement with the reported findings in Addis Ababa, Ethiopia **[14]**. This might be due to the use of this antibiotic for long period of time in the community because it is relatively cheap and easily available **[20]**.

The result of the MAR index showed that Isolates from droppings had multi-drug resistance to used antibiotics which means that there is high risk of contamination of food materials and farm animals by *Salmonella* species. Meanwhile, isolates

from cloacae had close range of multiple resistances to those from droppings but samples from intestines showed no risk of contamination since the result showed that they are less than 0.2 in the MAR index. The proportion of multidrug-resistant Salmonella isolates found in this study is lower than the previous reports **[21]**. More so, the multiple antibiotics of the isolates was 90% resistant to amoxicillin and 100% resistant to ceftazidime which is in agreement with reports gotten from other areas that were previously studied **[22]**. This might be due to differences in awareness of the people about personal and environmental hygiene.

CONCLUSION

The results of this study revealed 50% prevalence of *Salmonella* species among the poultry dropping samples in the study area. Antimicrobial susceptibility test results showed that *Salmonella* species isolated during this study were highly resistant to ceftazidime. *Salmonella* species were highly sensitive to imipenem and cefotaxime. The occurrence of Salmonella species could be as a result of their high pathogenicity trends. These microorganisms although on their own can cause several poultry and farm animal infections; they also produce toxins that are also of public health importance to both human and the farm animals. The socioeconomic and health implication of these findings are enormous. With the high colonization of bacteria of public health concern in poultry feeds, good manufacturing practice, handling and retailing methods need to be improved to enhance the microbiological quality of these products. Regular microbiological analysis should be carried out to determine the quality of poultry animals in ensuring both human and animal safety.

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