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Original Article

Prevalence and antibiogram of *Salmonella* species isolated from poultry products in Ebonyi State, Nigeria

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ABSTRACT

Objective: This study evaluated the occurrence and antimicrobial susceptibility profile of *Salmonella* species isolated from various poultry products including chicken meat, poultry eggs, poultry bird's drinking water, and poultry feed.

Materials and methods: A total of 79 samples comprising of chicken meat (n=20), egg shell (n=15), poultry egg contents (n=18), drinking water (n=14), and poultry feed (n=12) were bacteriologically and microscopically analyzed for the isolation of *Salmonella* species.

Results: Overall, this study reported a high prevalence of *Salmonella* species (62%) from various poultry products especially in poultry (chicken) meat and poultry egg contents where the percentage occurrence of *Salmonella* species was 100% and 20.4% respectively. The antibiogram conducted on the *Salmonella* species isolated from the various poultry samples reveal that all the isolates were multi-drug resistant to more than 50% of the tested antibiotics especially to tetracycline, gentamicin, tobramycin, nitrofurantoin and imipenem. However, most of the *Salmonella* species were also found to be highly susceptible to ceftriaxone, cefotaxime, ertapenem and ceftazidime. It was also observed in this study that the highest level of resistance to the tested antibiotics was recorded in *Salmonella* species isolated from poultry meat samples.

Conclusion: Salmonellosis due to the consumption of contaminated or infected poultry products could pose serious public health problem to the general public if allowed. Thus, poultry farms and other poultry product outlets should be operated under sanitized conditions that ward-off the incidence of foodborne pathogens such as *Salmonella*. The use of antibiotics as growth promoting agents and prophylaxis in the production of poultry birds in this region should be discouraged – since such practices allowed drug-resistant bacteria to emerge and spread in the community.

KEYWORDS

Antibiotic resistance, Community, Poultry, Salmonellosis

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INTRODUCTION

The food chain especially those that are of poultry origin could be a serious public health concern if they serve as route for the emergence and dissemination of resistant bacteria including *Salmonella* species in the community. According to Marcus et al. (2007), poultry remains an important vehicle of *Salmonella* transmission to humans, and most bacterial infections due to *Salmonella* usually occur via the consumption of contaminated meat. Bacterial infections caused by *Salmonella* species is amongst the most frequent foodborne gastrointestinal infection or disease that occur in the community; and this foodborne pathogen (*Salmonella*) is easily transmitted via poultry products including poultry eggs, meat and water (Hennessy et al., 2004; Kimura et al., 2004). *Salmonella* species is notorious in invading the epithelial cells of the gastrointestinal tract (GIT) during its invasion of a host to cause foodborne illness; and other pathogenic strains of *Salmonella* also cause other bacterial-related infections such as typhoid and paratyphoid fever. When poultry products including eggs and meat and other agricultural products are poorly handled and treated, they could serve as route via which foodborne infection spread in the community (Aarestrup, 2005). *Salmonella sp.* is predominantly associated with poultry products and other products of animal origin, and they are mainly responsible for the zoonotic transmission of salmonellosis in the community (Crump et al., 2008). In some cultures, people eat raw or uncooked eggs. This practice could predispose the people to foodborne infections such as salmonellosis. In most developing countries, the processes involved in the production of poultry birds and their products for human consumption may be unhygienic, and this is a critical public health challenge because of the direct transfer of resistant human pathogens including *Salmonella* species through the food chain. The emergence and spread of antibiotic resistance in foodborne bacteria including *Salmonella* species is well noted; and these organisms portend serious public health challenges since they cause a plethora of community-acquired infections such as salmonellosis (Threlfall et al., 2000; Glynn et al., 2004).

Bacterial resistance to some commonly available antimicrobial agents continues to remain a significant public health concern worldwide; and this phenomenon limits the choice of antibiotic therapy in some bacterial-related infections or diseases. Several studies have shown that the continued use of antibiotics in animal rearing and other agricultural activities including the rearing and production of poultry birds as a major driving force behind the development and dissemination of antimicrobial-resistant *Salmonella* in the community (Mathew et al., 2002; van den Bogaard et al, 2000; Rajashekara et al., 2000; Devasia et al., 2005). The undue use of antimicrobial agents as growth promoting agents and prophylactic agents in the production of food producing animals such as poultry birds and livestock is a major contributing factor to the widespread emergence and dissemination of drug resistant bacteria in the community (van den Bogaard et al., 2000; Threlfall et al., 2000). Thus, this study evaluated the incidence and antimicrobial susceptibility profile of *Salmonella* species isolated from some poultry products in Abakaliki metropolis, Nigeria.

MATERIALS AND METHODS

Collection and processing of samples: Ethical approval was sought for and obtained prior to the commencement of this research project. A total of 79 environmental samples including samples from poultry bird (chicken) meat, poultry egg content, poultry bird's drinking water, egg shell, and poultry feed were used for this study. The samples were aseptically collected into clean zip lock bags using a clean spatula and swab sticks; and each of the sample containers were labeled and transported to the microbiology laboratory unit of Ebonyi State University, Abakaliki for further microbiological analysis.

Bacteriological analysis: Sterile swab sticks was used to swab the surface of the collected samples, and these were inoculated in 5 mL nutrient broth (Oxoid, UK). The drinking water of the poultry birds (1 mL each) was inoculated in 5 mL nutrient broth. All the inoculated samples were incubated at 35°C for 18-24 h. After incubation, all the tubes showing turbidity after incubation were bacteriologically cultured on xylose-lysine deoxycholate (XLD) agar and *Salmonella-Shigella* agar (SSA) (Oxoid, UK) for the selective isolation of *Salmonella* species. The culture plates were incubated at 35°C for 18-24 h (Cheesbrough, 2000; Khan et al., 2005). And each of the plates was macroscopically examined for the presence of colonies that resemble *Salmonella* species. Suspected bacterial colonies on the culture media plates were aseptically subcultured onto freshly prepared SSA and XLD agar plates for the isolation of pure cultures of *Salmonella* species. And the isolated organisms were purified on nutrient agar plates for further bacteriological studies.

Identification of bacteria: Suspected bacterial isolates on the respective culture media plates were identified using standard microbiological identification techniques including motility test, indole, triple sugar iron test, H_2S production test, sugar fermentation test, citrate utilization

test, Voges-Proskauer test, and Methyl-red test (Cheesbrough, 2000).

Antimicrobial susceptibility testing: Antimicrobial susceptibility studies were carried out as per the guideline of the Clinical Laboratory Standard Institute (CLSI) using the Kirby-Bauer disk diffusion method. Single antibiotic disks including ceftriaxone (CRO, 30 µg), penicillin G (P, 10 µg), gentamicin (CN, 30 µg), amoxycillin (AML, 25 µg), nitrofurantoin (F, 300 µg), tetracycline (TE, 10 µg), amoxycillin/clavulanic acid (AMC, 30 µg), sulphametho-xazole-trimethoprim (SXT, 25 µg), ofloxacin (OFX, 5 µg), cefotaxime (CTX, 30 µg), tobramycin (TOB, 30 µg), ceftazidime (CAZ, 30 µg), imipenem (IPM, 10 µg), ertapenem (ETP, 10 µg) and nalidixic acid (NA, 30 µg) (Oxoid, UK) were used for the antibiogram studies as was previously described (CLSI, 2014; Ejikeugwu et al., 2012; Saifullah et al., 2016). Briefly, the test isolate (adjusted to 0.5 McFarland turbidity standards) were aseptically swabbed on the surface of Mueller-Hinton (MH) agar plates, and the antibiotic disks were aseptically inserted into the MH agar plates using sterile forceps. Inoculated plates were incubated at 35°C for 18-24 h, and the inhibition zone diameter (IZD) were recorded and reported as per the CLSI standards based on the organism susceptibility and resistance profile.

RESULTS

A total of 79 poultry samples including chicken meat, poultry egg contents, poultry bird's drinking water, egg shell and poultry feed were bacteriologically examined in this study for the isolation of *Salmonella* species. **Table 1** showed the percentage occurrence of *Salmonella* isolated from the test samples. It was observed in this study that a total of 49 (62%) isolates of *Salmonella* species were recovered from the various poultry samples analyzed. The least number of *Salmonella* species isolated was recovered from poultry feed. However, higher number of *Salmonella* species was isolated from chicken meat; and this was followed by poultry egg content, egg shell and drinking water used for poultry production.

Table 2 showed the antimicrobial susceptibility profile of the *Salmonella* species isolated from poultry feed. The *Salmonella* species isolated from poultry feed samples showed varying levels of susceptibility and resistance to the tested antibiotics. The inhibition zone diameter of the *Salmonella* species to the tested antibiotics was in the range of 6-26 mm (**Table 2**). The isolates were least susceptible to penicillin, tetracycline, amoxycillin-clavulanic acid, nitrofurantoin and amoxycillin but they showed better susceptibility to the other tested antibiotics especially to cefotaxime, gentamicin, ceftazidime, imipenem and ertapenem. **Table 3** showed the antimicro-bial susceptibility profile of the *Salmonella* species isolated from the drinking water of the poultry birds. The *Salmonella* species from the drinking water of poultry birds had inhibition zone diameter that ranged from 6-28 mm.

Table 4 showed the inhibition zone diameter of the *Salmonella* species isolated from egg shell. The inhibition zone diameter of *Salmonella* species isolated from egg content is shown in **Table 5**. The *Salmonella* species isolated from the egg shell samples were very susceptible to the tested antibiotics especially to ceftriaxone, imipenem, ertapenem, ofloxacin, cefotaxime, gentamicin and ceftazidime. However, the *Salmonella* species isolated from the egg shell samples were least susceptible to penicillin, amoxycillin, nitrofurantoin, and tobramycin. Table 6 show the inhibition zone diameter of the *Salmonella* species isolated from poultry meat. The *Salmonella* species isolated from poultry meat were most susceptible to ceftriaxone, cefotaxime, ertapenem and ceftazidime. However, the isolates were highly resistant to tetracycline, nalidixic acid, penicillin, gentamicin, tobra-mycin, nitrofurantoin, imipenem, sulphameth-oxazole-trimethoprim, amoxycillin-clavulanic acid and amoxy-cillin.

DISCUSSION

Salmonella species are common bacterial pathogens implicated in most foodborne infections. The organism causes salmonellosis and it is also the leading cause of gastroenteritis in humans. Salmonellosis can be transmitted through the food chain especially those of poultry origin when proper hygiene and infection control practices are not imbibed. This study evaluated the prevalence and antimicrobial susceptibility profile of *Salmonella* species isolated from various poultry products including chicken meat, poultry eggs, poultry bird's drinking water, and poultry feed. A total of 49 isolates of *Salmonella* species were isolated from the various poultry product samples. Only 4 (8.2%) samples were found to be positive for *Salmonella* species isolated from 12 poultry feed samples. And this was the least isolation of *Salmonella* species from the test samples. All the chicken meat samples were positive for the isolation of *Salmonella* species. Out of the 18 samples of poultry egg content analyzed in this study, *Salmonella* species was isolated from only 10 (20.4%) samples of egg content. However, a total of 8 (16.3%) samples and 7 (14.3%) samples were positive for *Salmonella* species isolation from the egg shell samples and poultry drinking water respectively. The overall prevalence of *Salmonella* species (62%) in this study is high. The prevalence of *Salmonella* species in

Table 1. Isolation rate of *Salmonella* species

Sample source*	Number of sample	No (%) of *Salmonella* isolated
Poultry egg content	18	10 (20.4)
Chicken meat	20	20 (100)
Eggshell	15	8 (16.3)
Drinking water	14	7 (14.3)
Poultry feed	12	4 (8.2)

**All the samples were sourced from poultry products and they were aseptically obtained for this study*

Table 2. Inhibition zone diameter of *Salmonella* species isolated from poultry feed

SL	Organism	CN	TE	CAZ	AML	F	AMC	P	CRO	TOB	SXT	ETP	NA	IPM	OFX	CTX
1	*Salmonella* sp.	17	10	10	09	06	06	06	06	16	15	10	12	17	14	06
2	*Salmonella* sp.	24	11	23	15	11	14	06	24	06	18	22	14	14	17	26
3	*Salmonella* sp.	19	09	16	06	11	09	06	11	14	06	09	11	14	05	12
4	*Salmonella* sp.	16	07	23	09	14	08	06	09	16	20	12	06	16	09	15

AML = Amoxycillin, P = Penicillin, AMC = Amoxycillin/Clavulanic acid, TE = Tetracycline, CN = Gentamicin, TOB = Tobramycin, ETP = Ertapenem, IPM = Imipenem, CAZ = Ceftazidime, CTX = Cefotaxime, CRO = Ceftriaxone, F = Nitrofurantoin, OFX = Ofloxacin, NA = Nalidixic acid, SXT = Sulphamethoxazole/Trimethoprim.

Table 3. Inhibition zone diameter of *Salmonella* species isolated from poultry drinking water

SL	Organism	CN	TE	CAZ	AML	F	AMC	P	CRO	TOB	SXT	ETP	NA	IPM	OFX	CTX
1	*Salmonella* sp.	20	06	21	06	06	08	06	14	14	19	12	06	09	14	09
2	*Salmonella* sp.	20	06	22	06	09	10	06	19	09	06	10	14	16	15	28
3	*Salmonella* sp.	18	06	18	06	09	06	06	21	13	06	18	06	14	16	21
4	*Salmonella* sp.	15	06	19	06	11	10	06	24	10	06	14	11	14	13	21
5	*Salmonella* sp.	08	06	14	06	14	09	06	24	09	06	17	06	17	14	22
6	*Salmonella* sp.	19	06	06	06	08	06	06	13	13	14	16	09	10	15	06
7	*Salmonella* sp.	19	11	13	06	06	09	06	08	09	14	12	06	11	20	11

AML = Amoxycillin, P = Penicillin, AMC = Amoxycillin/Clavulanic acid, TE = Tetracycline, CN = Gentamicin, TOB = Tobramycin, ETP = Ertapenem, IPM = Imipenem, CAZ = Ceftazidime, CTX = Cefotaxime, CRO = Ceftriaxone, F = Nitrofurantoin, OFX = Ofloxacin, NA = Nalidixic acid, SXT = Sulphamethoxazole/Trimethoprim.

Table 4. Inhibition Zone Diameter of *Salmonella* Species Isolated from Egg Shell

SL	Organism	CN	TE	CAZ	AML	F	AMC	P	CRO	TOB	SXT	ETP	NA	IPM	OFX	CTX
1	*Salmonella* sp.	18	11	19	06	11	14	06	21	15	16	19	14	14	15	23
2	*Salmonella* sp.	24	06	24	06	06	08	06	19	16	11	20	16	15	20	19
3	*Salmonella* sp.	15	21	14	15	08	16	13	24	09	14	24	11	19	15	16
4	*Salmonella* sp.	17	09	06	07	15	09	06	22	19	11	08	06	20	29	15
5	*Salmonella* sp.	24	14	28	19	11	16	14	17	21	14	16	06	23	19	14
6	*Salmonella* sp.	16	09	22	20	09	24	06	14	06	17	17	09	14	20	24
7	*Salmonella* sp.	20	06	12	06	10	14	06	14	14	10	09	06	19	15	14
8	*Salmonella* sp.	18	14	24	08	06	07	06	09	15	09	06	14	29	29	13

AML = Amoxycillin, P = Penicillin, AMC = Amoxycillin/Clavulanic acid, TE = Tetracycline, CN = Gentamicin, TOB = Tobramycin, ETP = Ertapenem, IPM = Imipenem, CAZ = Ceftazidime, CTX = Cefotaxime, CRO = Ceftriaxone, F = Nitrofurantoin, OFX = Ofloxacin, NA = Nalidixic acid, SXT = Sulphamethoxazole/Trimethoprim.

Table 5. Inhibition zone diameter of *Salmonella* species isolate from egg content

SL	Organism	CN	TE	CAZ	AML	F	AMC	P	CRO	TOB	SXT	ETP	NA	IPM	OFX	CTX
1	*Salmonella* sp.	13	06	20	06	10	10	06	24	06	06	20	14	16	20	20
2	*Salmonella* sp.	16	06	20	06	10	14	06	10	10	06	20	06	15	14	14
3	*Salmonella* sp.	16	06	10	06	11	11	06	22	06	06	20	14	16	15	13
4	*Salmonella* sp.	14	06	06	06	10	09	06	07	07	06	30	06	15	14	24
5	*Salmonella* sp.	16	06	17	06	08	10	06	22	14	06	14	12	13	20	24
6	*Salmonella* sp.	16	06	24	06	13	14	06	24	12	06	24	16	15	22	24
7	*Salmonella* sp.	19	12	20	06	10	15	20	29	15	14	24	14	19	21	29
8	*Salmonella* sp.	20	14	20	06	12	13	06	14	17	15	16	19	20	23	14
9	*Salmonella* sp.	20	12	24	06	14	14	06	19	17	11	24	19	20	24	29
10	*Salmonella* sp.	22	14	19	06	14	16	06	24	15	14	23	18	18	24	23

AML = Amoxycillin, P = Penicillin, AMC = Amoxycillin/Clavulanic acid, TE = Tetracycline, CN = Gentamicin, TOB = Tobramycin, ETP = Ertapenem, IPM = Imipenem, CAZ = Ceftazidime, CTX = Cefotaxime, CRO = Ceftriaxone, F = Nitrofurantoin, OFX = Ofloxacin, NA = Nalidixic acid, SXT = Sulphamethoxazole/Trimethoprim.

poultry products is well documented (Hennessy et al., 2004; Kimura et al., 2004; Rajashekara et al., 2000). A recent report in Bangladesh reported a higher prevalence of *Salmonella* isolation from poultry products (Mahmud et al., 2015). Elsewhere in Imo State, southeast Nigeria, *Salmonella* species have been reported to be prevalent in poultry products as observable in this study (Okoli et al., 2006). Egg contents of poultry birds have been reported to be low in *Salmonella* incidence due to the protected barrier of the shell and the antibacterial components of the albumen as was reported by (Mahmud et al., 2015). And this usually account for the low isolation of *Salmonella* species from the egg content of poultry birds. But in this study, the isolation of *Salmonella* species from the egg content samples was high (20.4%) compared to previous reports which show that *Salmonella* isolation from egg shell content is usually low (Mahmud et al., 2015). Mahmud et al. (2015) reported 3% occurrence rate

Table 6. Inhibition zone diameter of *Salmonella* species isolates from poultry meat.

SL	Organism	CN	TE	CAZ	AML	F	AMC	P	CRO	TOB	SXT	ETP	NA	IPM	OFX	CTX
1	*Salmonella* sp.	10	06	19	06	10	10	06	12	06	06	20	06	15	06	15
2	*Salmonella* sp.	10	06	19	06	09	10	06	19	06	06	19	06	14	06	19
3	*Salmonella* sp.	14	06	21	06	14	08	06	23	07	06	20	14	15	19	23
4	*Salmonella* sp.	09	06	18	06	11	06	06	19	07	06	16	09	13	10	21
5	*Salmonella* sp.	14	06	22	06	09	09	06	24	06	06	21	06	15	11	28
6	*Salmonella* sp.	06	06	20	09	13	14	06	24	06	06	22	06	15	06	24
7	*Salmonella* sp.	06	06	22	06	14	09	06	19	06	06	20	06	18	09	23
8	*Salmonella* sp.	09	06	21	06	12	09	06	26	06	06	22	06	15	14	24
9	*Salmonella* sp.	19	06	18	08	11	16	06	19	09	06	23	09	18	14	20
10	*Salmonella* sp.	09	06	22	09	13	18	06	25	06	06	20	06	11	06	24
11	*Salmonella* sp.	10	06	22	06	13	14	06	21	12	06	16	06	14	13	24
12	*Salmonella* sp.	06	06	19	06	13	09	06	12	06	06	19	06	14	06	21
13	*Salmonella* sp.	14	06	19	06	10	14	06	24	06	06	20	06	13	09	25
14	*Salmonella* sp.	10	06	22	09	11	09	06	22	06	06	22	06	14	06	19
15	*Salmonella* sp.	06	06	24	06	13	09	06	29	06	06	23	06	19	06	24
16	*Salmonella* sp.	14	06	19	06	09	09	06	16	06	06	19	06	16	09	21
17	*Salmonella* sp.	09	06	19	20	09	09	06	19	06	06	19	06	14	06	13
18	*Salmonella* sp.	06	06	06	06	09	13	06	12	06	06	06	06	16	06	14
19	*Salmonella* sp.	06	06	24	06	13	12	06	15	06	06	24	06	15	06	17
20	*Salmonella* sp.	10	06	12	06	09	09	06	09	06	06	11	06	17	06	10

AML=Amoxycillin, P=Penicillin, AMC=Amoxycillin/Clavulanic acid, TE=Tetracycline, CN=Gentamicin, TOB=Tobramycin, ETP=Ertapenem, IPM=Imipenem, CAZ=Ceftazidime, CTX=Cefotaxime, CRO=Ceftriaxone, F=Nitrofurantoin, OFX=Ofloxacin, NA=Nalidixic acid, SXT=Sulphamethoxazole/Trimethoprim.

of *Salmonella* species from 103 poultry egg samples analyzed in a similar study in Bangladesh. Studies have shown that the penetration of the egg shell by pathogenic bacteria such as *Salmonella* is attributed to fecal contamination of the egg shell (Schoeni et al., 1995; Detha and Datta, 2016). The antibiotic susceptibility test conducted on the *Salmonella* species isolated from the various poultry samples reveal that all the isolates were multiple resistant to more than 50% of the tested antibiotics. The *Salmonella* species showed reduced susceptibility to tetracycline, nalidixic acid, penicillin, gentamicin, tobramycin, nitrofurantoin, imipenem, sulphamethoxazole-trimethoprim, amoxy-cillin-clavulanic acid and amoxycillin. However, most of the *Salmonella* species were also found to be highly susceptible to ceftriaxone, cefotaxime, ertapenem and ceftazidime. It was also observed in this study that the highest level of resistance to the tested antibiotics was recorded in *Salmonella* species isolated from poultry meat samples; and the resistance of these isolates was high to tetracycline, amoxycillin, penicillin, tobramycin, sulphamethoxazole-trimethoprim, ofloxacin and nalidixic acid in which the test *Salmonella* species had lower inhibition zone diameter. The level of antimicrobial resistance recorded in the isolated *Salmonella* species is similar to the study of Okoli et al. (2006) who showed that *Salmonella* species from poultry origin is multiple resistant to some commonly available antibiotics. The resistance of the *Salmonella* species isolated in this study to some commonly available antibiotics also substantiates previous findings that *Salmonella* from poultry origin are multidrug resistant in nature (Rajashekara et al., 2000; Mathew et al., 2002; Devasia et al., 2005; Khan et al., 2005). The development of antimicrobial resistance genes in bacterial from the community such as is observable in poultry farms is usually attributed to inducible antimicrobial usage which allow organisms to develop resistance under antibiotic selective pressure (Threlfall et al., 2000; Aarestrup, 2005; van den Bogaard et al., 2000). The consumption of animal-based foods especially those that are of poultry origin have usually been attributed to the incidence of some foodborne infections such as salmonellosis which is caused by *Salmonella* species in humans. Salmonellosis due to the consumption of contaminated or infected poultry products could pose serious public health problem to the general public if allowed.

CONCLUSION

This study reported a high prevalence of *Salmonella* species (62%) from various poultry products especially in poultry meat and poultry egg contents. And the *Salmonella* species were multiple resistant to some conventional antibiotics especially to gentamicin, ofloxacin, tetracycline, nitrofurantoin, amoxycillin-clavulanic acid, penicillin, sulphamethoxazole-trimethoprim and amoxycillin. We therefore recommend that those involved in the rearing and production of poultry birds and their products ensure that their premises and other materials used in their enterprise are maintained in a sanitized state that excludes feacal contamination of poultry products. The continuous monitoring of poultry farms and other outlets where poultry products are sold to the public by public health personnel is very vital to the containment of salmonellosis outbreak in this region.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Nothing to declare.

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