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Full Length Research Paper

Antimicrobial resistance patterns and emerging fluoroquinolone resistant *Salmonella* isolates from poultry and asymptomatic poultry workers

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Non-typhoidal Salmonellae have been extensively incriminated worldwide as common causes of bacterial gastroenteritis in humans and animals. This study investigated patterns of resistance of prevailing *Salmonella* serotypes from poultry and asymptomatic poultry workers. A total of 560 stool samples, comprising 200 from non-diarrhoeic workers and 360 from diarrhoeic poultry were randomly collected. Samples were processed and isolates identified by standard methods. The isolates were subjected to antimicrobial susceptibility tests using standard procedures. Sixty-four *Salmonella*e belonging to *Salmonella typhimurium* 32(50%), *Salmonella gallinarum* 28(43.75%) and *Salmonella enteritidis* 4(6.25%) were recorded, with *S. typhimurium* being the most prevalence serotype. There was statistical significance relationship (p>0.05) between the *S. typhimurium* isolated from poultry and healthy poultry workers. *Salmonella* serotypes were 100% resistant to ampicillin, 90.6% to tetracycline and moderately sensitive to nalidic acid (62.5%). Fluoroquinolone resistant *S. typhimurium* strains from poultry were also observed. Seventeen resistance patterns were observed with AmpCotNitStrTet, AmpNitNalStrTet and AmpNitStrTet being the most frequent patterns recorded. The study revealed emergence multiple drug resistant *Salmonella* serotypes from poultry and asymptomatic workers. Continuous use of fluoroquinolones as a growth promoter in animal feeds could be re-examined to reduce the widespread and circulation of emerging strains of this bacterium.

Key words: Poultry workers, poultry, non-typhoidal *Salmonella*, prevalence, antimicrobial resistance.

INTRODUCTION

Salmonella infections in both man and animal have been recognized as a major public health problem (Akinyemi et al., 2007; Bulgin et al., 1982). More than 90% of non typhoidal *Salmonella* infections are food borne and the remaining from nosocomial infections and / or from pets (reptiles and birds), infected persons, contaminated water, eggs, vegetable (those fertilized with untreated (NTS) are continuously isolated from various animals such as cattle, poultry, reptiles and poultry in different poultry faeces), fresh fruits and dry cereal (Hohmann, 2001; Ekdahi et al., 2005). Non-typhoidal *Salmonella* part of the world (Wilson et al., 2003). It is widely spread in Europe and North America (Wright et al., 2005; Takkimen et al., 2002; Ling et al., 2002). Latin America, the Middle East and Africa (Kariuki et al., 2006a; Sirinavin et al., 2001), also in countries such as India (Sahai et al., 2001), Japan (Arii et al., 2001). and the United States, (Voetsch et al., 2004). Several studies had documented isolation of non-typhoidal *Salmonella* from human and poultry in different parts of the Nigeria (Enabulele et al., 2010; Fashae et al., 2010; Raufu et al., 2009; Agbaje et al., 2010). Outbreaks of Salmonellosis caused by *Salmonella gallinum, Salmonella pallorum* and *Salmonella typhimmrium*

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also have been reported (Adenikan et al., 1990; Okoye and Erojikwe, 1986; Akinyemi et al., 2007).

The use of antimicrobials in humans and veterinary medicine as food animal growth promoting agents during the past decade has created enormous pressure for selection of antimicrobial resistance among bacterial pathogens worldwide (Hakanem et al., 2001). Nowadays, there is increasing concern about the development of multidrug resistance in bacteria sp causing zoonosis and having an important animal reservoir, such as *Salmonella* strains (Kariuki et al., 2006b). In Nigeria there is a high rate of consumption of poultry products and increase in the cases of Salmonella-associated gastroenteritis has been frequently noticed. Most individuals with complicated cases usually do not response to conventional or empirical therapy. Besides efficacy of fluoroquinolones drugs have been threatened. Therefore, this study investigated resistance patterns of prevailing *Salmonella* serotypes from poultry and asymptomatic poultry workers

MATERIALS AND METHODS

Study centre

This study was conducted in four organised farms, three of which were private farms located in Lusada, Igbesa and Agbara in Ado-Odo/Ota local government in Ogun-State (Figure 1) and the fourth one is Lagos State government agricultural farm located at Ojo town. Each of these farms possessed a hatchery that has capacity to produce between 1000 and 2500 chickens per day and with staff strength of between 48 and 72.

Sample population

A total number of 200 adult healthy poultry workers within the age range 20 to 35, were randomly recruited with 50 from each of the farms. The workers in this case are those responsible for casual routine works such as feeding of birds, picking and arrangement of eggs into crates, packing and disposing poultry dung and have been on the job for the past 7 to 15 years. At the same time 360 diarrhoeic layer birds, made up of 90 from each farm were randomly selected for this work. Healthy poultry workers are define as those workers that do not have any forms of diseases and have not visited hospital for treatment of any ailments in the last period of three months. Only stool samples were collected from both healthy workers and diarrhoeic layer birds for bacteriological analysis.

Specimen collection and processing

Prior to the enrolment, voluntary and informed consents were obtained from poultry workers. Ethical approval was also obtained. Stool samples were collected aseptically into the sterile universal bottles from the poultry workers. Diarrhoeic stool was collected from the cloacae part of the bird aseptically into sterile tubes with the aid of sterile swab by the veterinary doctor at the respective farms. The samples were transported immediately to Microbiology Laboratory for bacteriological assay. Four grams of the samples were inoculated into 15 ml of Selenite F broth (HIMEDIA LABS. LTD., India) and incubated at 37°C for 24 h. Subcultures were made from Selenite F broth onto Brilliant green agar (UNITED STATE BILOGICALS) and incubated at 37°C for 24 h. The suspected *Salmonella* colonies were gram stained and morphologically studied. The isolates were identified using biochemical reactions as described by Cowan and Steel (1993). Further identification was performed using commercially available API 20E (BIOMERIEUS, France).

Antimicrobial susceptibility test

In-vitro susceptibility of the *Salmonella* isolates to various routine antimicrobial drugs was tested by the standard disc diffusion technique using guidelines established by the NCCLS (2002). Sterile wire loop was used to pick 2 to 3 colonies of each *Salmonella* serotype and emulsified in 3 to 4 ml of sterile physiological saline. Standardization of the suspended colonies was performed by diluting the normal saline suspension until the turbidity matched the 0.5 McFarland Standards. A sterile cotton swab was dipped into the standardized suspension, drained, and used for inoculating 20 ml of Mueller-Hinton agar in a 100 mm disposable plate (STERLIN, UK). The inoculated plates were air dried, and antibiotic discs (HIMEDIA LABS., INDIA and ABTEK BIOLOGICAL LTD., UK) were placed on the agar using sterile forceps and were gently pressed down to ensure contact. The following 9 antibiotic discs were used; ampicillin (AM, 25 ug), cotrimoxazole (COT, 25 ug), gentamicin (10 µg), tetracycline (TET, 25 µg), nalidixic acid (NAL, 30 µg), nitrofurantoin (NIT, 200 µg), streptomycin (STR, 25 µg) levofloxacin (NA, 5ug) and ciprofloxacin (OF, 5 ug) were applied in the test. The plates were incubated aerobically at 37°C for 18 to 24 h. The diameters of the zone of inhibition were measured with a ruler and compared with a zone interpretation chart (Bauer, 1966). *Escherichia coli* (ATCC 25922) was used as control.

RESULTS

Out of the 200 human samples evaluated in this study only 8 (4%) were positive for *S. typhimurium*. A total of 56 Salmonella species made up of 3 serotypes; *S. typhimurium* (24), *S. gallinarum* (28) and *S. enteritidis* (4) were isolated from 360 cloacae swabs from diarrhoeic poultry given an incidence of 15.6% Salmonella isolates in this study (Table 1). The results of antimicrobial susceptibility tests revealed that all the Salmonella serotypes (100%) were resistance to ampicillin, 90.6% to tetracycline, 71.9% to streptomycin and 68.8% to nitrofurantoin. Salmonella isolates were moderately sensitive to cotrimoxazole (59.4%) and nalidixic acid (62.5%) (Table 2). Seventeen resistance patterns were recorded with AmpCotNitroStreTet, AmpNitNaliStreTet and AmpNitStrTet being the most frequently encountered patterns (Table 3). Four strains of *S. typhimurium* were resistant to ciprofloxacin and 2 strains to levofloxacin antibiotics in this study.

DISCUSSION

Salmonella is an important zoonotic pathogen and it's prevalence in animals poses a continuous threat to man (Muragkar et al., 2004). In this study 64 (11.4%)

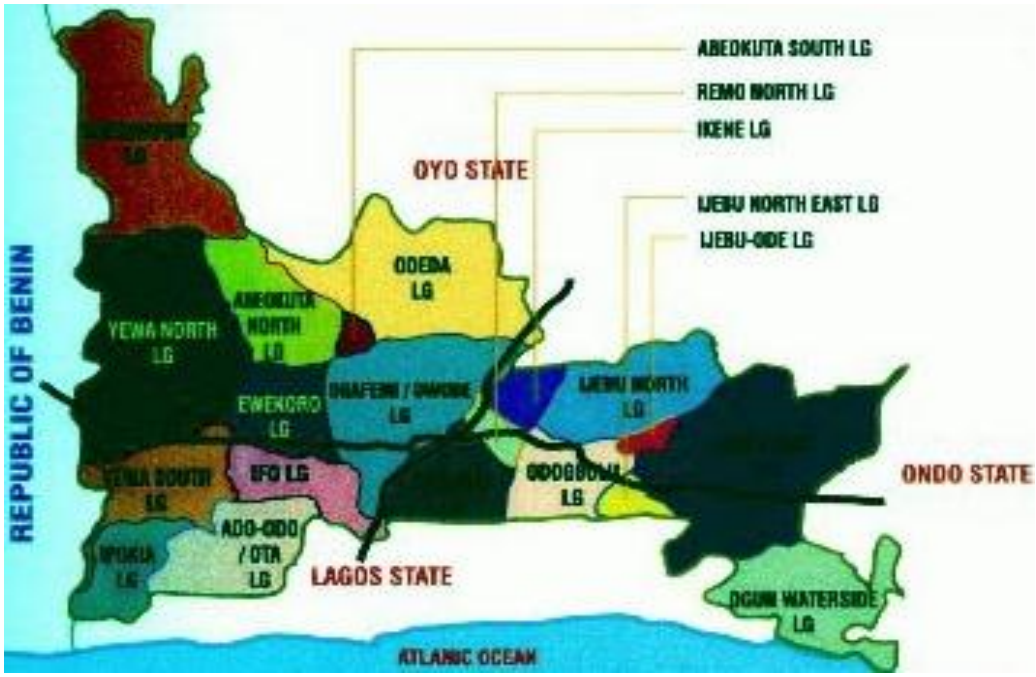

Figure 1. Map of Ogun State, Nigeria.

Salmonella isolates comprises 3 serotypes: *S. typhimurium* 32(50%), *S.gallinarum* 28(43.75%) and *S. enteritidis* 4(6.25%) were isolated. It is important to state that only 8 strains of *S. typhimurium* and none of *S. enteritidis* was isolated from healthy poultry workers. Although, in Nigeria outbreaks of Salmonellosis caused by *S. gallinarium*, *S. pallorum and S. typhimmrium* in poultry animals were reported over two decades ago (Adenikan et al., 1990; Okoye and Erojikwe, 1986), but no *S. Pallorum* was isolated from poultry in this study, an indication of non circulation of this serotype in our environment. *S. typhimurium* was the most prevalent serotype isolated from both diarrhoeic poultry and healthy poultry workers. In a similar study carried out by Muragkar et al. (2004) in India, *S. typhimurium* serotype isolated was higher, although, the higher isolation rate in their work could be attributed to the fact that diarrhoeic stools from poultry and human patients were used. Several reports have implicated *S. typhimurium* and *S. enteritidis* as the most prevalent *Salmonella* serotypes isolated from both human and animal nontyphoidal Salmonellosis (Ekdahl et al., 2005; Velge et al., 2005). For example *S. typhimurium* is mostly prevalent in Europe and America and is of growing importance in the Southeast Asian and Western Pacific (Herikstad et al., 2002). In African countries such as Kenya, Zaire and Rwanda, both invasive and non-invasive *S. typhimurium* strains are common (Kariuki et al., 2006) and in Nigeria, *S. typhimurium* and *S. enteritidis* are increasingly isolated (Akinyemi at al., 2007).

Interestingly, eight (8) of the healthy poultry workers positive for *S. typhimurium* in this study were involved in routine packing of poultry dung and picking of laid eggs into the crates, some of the eggs were found to be faecally contaminated. In addition, it was observed that protective guidelines and measures such as; hand washing with diluted sodium hypochlorite, hand glove and boot wearing were not adhered to. Studies elsewhere had demonstrated that conditions such as improper hand washing, indiscriminate eating in animal facilities, supply of contaminated feeds and farm to farm service are some of the factors responsible for the spread and circulation of *Salmonella* agent in poultry farms (Wright et al., 2005; Snoeyenbos, 1991), features that were common among the subjects of this study. There was statistical significance relationship ($p>0.05$) between the *S. typhimurim* isolated from poultry and healthy poultry workers in this study. This result is not surprising as *S. typhimurium* has been reported to have a broad host range and can infect both human and animals (Chiu et al., 2004).

The result of antimicrobial susceptibility testing in this study revealed that all *Salmonella* serotypes were 100% resistant to ampicillin, 90.6% to tetracycline, and 71.9% to streptomycin (Table 2). These results were not unusual as increasing resistance of non-typhoidal *Salmonella* to ampicillin, tetracycline, cotrimoxazole and chloramphenicol have been reported in many parts of the world such as the Netherland (Kivi et al., 2005), Singapore (Ling et al., 2002), Finland (Takkinen et al., Kenya (Kariuki et al., 2006b) and Nigeria (Akinyemi et al., 2007).

Table 1. Isolation of *Salmonella* spp. from poultry and poultry workers.

Source	No. of samples	No. of positive (%)	*Salmonella serotypes* (%)			
			S. Typhimurium	***S. Gallinarium***	***S. enteritidis***	**Total isolates**
Human (stool)	200	8(4.0)	8(4.0)	0	0	8 (12.5)
Poultry (cloacal swab)	360	56(15.6)	24(37.5)	28 (43.8)	4 (6.3)	56(87.5)
Total	560	64 (11.4)	32 (50)	28(43.8)	4(6.3)	64 (100)

Table 2. Percentage of resistance *Salmonella* serotypes to various antibiotics.

	No	Amp (%)	Cot (%)	Gen (%)	Levs (%)	Cip (%)	Nit (%)	Nal (%)	Str (%)	Tet (%)
Human										
S. Typhimurium	8	8(12.5)	6 (9.4)	6(9.4)	0	0	8(12.5)	2(3.1)	8(12.5)	8(12.5)
Poultry										
S. Typhimurium	24	24(37.5)	16 (25)	2(3.1)	2(3.1)	4(6.3)	16(25)	4(6.3)	24(37.5)	20(31.3)
S. Gallnarum	28	28(43.8)	2 (3.1)	0	0	0	18(28.1)	18(28.1)	24(37.5)	16(25)
S. enteritidis	4	4(6.3)	2 (3.1)	0	0	0	2(3.1)	0	2(3.1)	2(3.1)
Total	64	64(100)	26(40.6)	8(12.5)	2(3.1)	4(6.3)	44(68.8)	24 (37.5)	58 (90.6)	46 (71.9)

Amp: Amplicillin, Cot: contrimoxazile, Gen: Gentamicin, Levo: Levoflaxacin, Cip: Ciprofloxacin Nit: Nitrofurantoin, Nal: Nalidixic acid, Str: Streptomycin, Tet: Tetracycline

Table 3. Resistance pattern of *Salmonella* serotypes to various antibiotics.

	Drug resistance pattern	No of antibiotics	*S. typhimurium* (32)	*S.gallinarum* (28)	*S. enteritidis* (4)	Total (%)
1.	Amp Cot Gen Levo Cip Nit Str Tet	8	2	-	-	2(3.2)
2.	Amp Cot Cip Nit Nal/str Tet	7	2	-	-	2 (3.2)
3.	Amp Cot Gen Nit Nal/StrTet	7	2	-	-	2 (3.2)
4.	Amp Cot gen Nit Str Tet	6	4	-	-	4(6.5)
5.	Amp Cot Nit Str Tet	5	8	-	2	10(16.5)
6.	Amp Nit Nal Str Tet	5	2	6	-	812.9)
7.	Amp Nit Str Tet	4	4	4	-	8(12.9)
8.	Amp Nit Nal. Tet.	4	-	2	-	2(3.2)
9.	Amp Nal str Tet	4	-	4	-	4(3.2)
10.	Amp Cot Nal Tet	4	-	2	-	2 (3.2)
11.	Amp Nit Nal Str	4	-	2	-	2(3.2)
12.	Amp Str Tet	3	4	-	-	4(6.5)
13.	Amp Cot Str	3		-	-	4(6.5)
14.	Amp Nit Str	3	-	4	-	4(6.5)
15.	Amp Nal Str	3	-	2	-	2(3.2)
16.	Amp Str	2	-	2	-	2(3.2)
17.	Amp	1	-	-	2	2(3.2)
	Total		32	28	4	64 (100%)

Drug abuse, over the counter sales of antibiotics, use of antibiotics as growth promoter in animal and prophylaxis use of sulphamethoxazole-pyrimethamine for plasmodiasis were some factors linked to the increasing resistance to these antibiotics (Kariuki et al., 2006b). In this study, sixteen resistance patterns were recorded. On

comparisons of resistance patterns of the two prevailing *Salmonella* serotypes, only one common resistance pattern AmpCotGenNitStrTet was observed; an indication of circulation of MDR among *Salmonella* serotypes in our environment. Studies had shown that emerging and re-emerging MDR Salmonella is common to human and animal strains of this bacterium (Akinyemi et al., 2007; Vaagland et al., 2004), an observation that has come to stay in Nigeria. Similar pattern was noticed among the *Salmonella* serotype isolated in poultry in Senegal (Bada-Alambedj et al., 2006).

Moreover, 6 strain of *S. typhimurium* from poultry exhibited resistance to fluoroquinolone. It is worthy of note that the use of fluoroquinolone as a growth promoter in animal has not been observed or reported in Nigeria. The explanation to this is difficult but it might be linked to the fact that fluoroquinolone antibiotics have found their way into feeds as a result of increasing smuggling of animal feeds into Nigeria; it is also possible that the affordable different brands of fluoroquinolone in the market might have been used routinely in the treatment of animals. In Denmark and Taiwan where there have been an increasing use of fluoroquinolones in food animals as growth promoter, this has lead to increased quinolones resistance of NTS isolates (Chiu et al., 2002; Molbak et al., 2002). Strict measure such as, testing of poultry products for the presence of *Salmonella* before importation had been put in place to prevent the transmission of quinolone resistant *Salmonella* (Birgitta and Ekdal, 2006). Furthermore, in Germany transmission of *Salmonella* through food chain to human was reduced by immunization of animals with live attenuated *Salmonella* vaccine on routine bases (Chiu et al., 2004). In Nigeria, *Salmonella* serotypes with reduced fluoroquinolone susceptibility from human had been documented (Akinyemi et al., 2007). However, the emergence of fluoroquinolone resistant *S. typhimurium* strains from both poultry and humans in this study call for serious concern. The implication of this is increasing emergence strains of fluoroquinolones resistant pathogens. Our fear is that if urgent steps are not taken, the efficacy of fluoroquinolones the preferred antibiotics for the treatment of Salmonella-associated diseases will be doubtful thereby increasing the mortality rate, thus put the problems into international perspective.

In conclusion, this study revealed emergence multiple drug resistant *Salmonella* serotypes from poultry and asymptomatic workers. Prudent use of fluoroquinolone antibiotic is essential and its continuous use as a growth promoter might need to be re-examined. The poultry workers should strictly adhere to protective guidelines.

Proper treatment of poultry dung before disposal or use as manure is advocated.

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