Prevalence of Typhoidal and Non- Typhoidal *Salmonellae* among Poultry Workers in Akwa Ibom State, Nigeria.

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ABSTRACT

Typhoid fever and non-typhoidal salmonellosis remain endemic in most developing countries with large scale transmission through contaminated food and drinking water. Since the early 2000s, poultry has also been found to be a common food source for Salmonella enteritidis infections. A total of 500 blood, stool, cloaca swab and egg shell swab specimens were collected from poultry, non-poultry workers and birds in Akwa Ibom State and screened for Salmonella species using standard cultural and serological techniques. The overall prevalence rate was 117 (23.4%). Salmonella species were distributed in all the three Senatorial Districts of the State. Percent distribution of Salmonella isolates were 42 (25%) for Uyo, 35 (20.8%) for Eket and 40 (23.8%) for Ikot Ekpene Senatorial Districts. Out of 372 human subjects screened, 77 (20.7%) were positive and 38 (20.4%) were isolated from non-poultry workers while 39 (21.0%) were isolated from poultry staff in the three senatorial districts. Out of 128 samples of poultry products analysed, 40 (31.3%) were isolated. 23 (18%) were isolated from cloaca swabs while 17 (13.3%) were isolated from egg shell swabs. In this study all the serological significant typhoid fever cases were confirmed by blood culture. According to sero-grouping and source of sample collection 23.9% S. typhi were isolated from human followed by S. paratyphi A (20.5%) while S. enteritidis and S. gallinarum (19.7%). Among poultry products S.

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gallinarum, S. enteritidis, S pullorum, S. paratyphi A, and S. typhimurium were isolated from bird Cloaca swab and Egg shell swab. S. pullorum and S. typhi were not isolated from human and poultry respectively. High prevalence of Salmonella serovars such as S. gallinarium, S. typhi, S. typhimurium and S. enteritidisis associated with regular consumption of poultry and eggs without proper disinfection and appropriate boiling, and present a serious public health risk in Akwa Ibom State. Generally, all the isolates identified as Salmonella were tested for their susceptibility to antimicrobial agents. The results showed that Augmentin was 100% sensitive to S. gallinarum, S. paratyphi A, 93% sensitive to S. typhi and 80% to S. typhimurium, S. enteritidis and S. pulloru. Salmonella gallinarum were 100% susceptible to Ciprofloxacin and S. pullorum 100% susceptible to Gentamycin whereas all isolates were 100% resistant to Oxacillin and Tylosin antibacterial agents used. We have ascertained the presence of Salmonella serovars in poultry and the non-poultry workers (control group) in Akwa Ibom State, Nigeria, where poultry and their products are major sources of food, also where untreated poultry droppings are used for manure on vegetables commonly eaten raw by the public.

Keywords: Poultry, Salmonella, poultry workers, prevalence.

Introduction

The genus Salmonella is a rod shaped, gram negative, flagellated, facultative anaerobe that belongs to the family *Enterobacteriaceae* (Shrivaprasad, 2000). It consists of two separate species; *Salmonella bongori* and *Salmonella enterica* and encompasses over 2500 known serotypes, all of which are considered potential human pathogen (Baggesen *et al.*, 2000). *Salmonella* causing human disease are divided into human restricted invasive typhoidal serotypes (*S. enterica* var. *Typhi* and *S. enterica* var. *Paratyphi* A) and thousands of non- typhoidal *Salmonella serotypes*- known as NTS *serotypes* (Gordon *et al.*, 2012).

immunity. The O- and H-antigens are used in serological tests (Widal) [named after the French physician Ferdinand Widal, 1862-1929]. Since all *Salmonella* (not only *Salmonella typhi*) and all bacteria related to *Salmonella* possess similar antigens, there are many cross reactions (the test is non-specific). The test also has quite low sensitivity. This means that the contribution of serology is limited in many clinical situations. In animal populations, *Salmonella* was most frequently detected in poultry flocks. Egg and bakery products were the most common sources of *Salmonella* outbreaks, whereas broiler meat was an important source for both *Salmonella* and *Campylobacter* outbreaks (Ibrahim *et al.* 2013).

Poultry is an essential component of the Nigerian economy, providing income for small scale farmers and a good source of high quality protein for the ever- growing population of Nigeria. In livestock production, poultry occupies a prominent position in the provision of animal protein and this accounts for about 25% of local meat production in Nigeria (Agbaje *et al.*, 2010). With the great expansion of poultry rearing and farming, *Salmonellosis* has become an important public health problem in Nigeria and other parts of the world, causing heavy economic loses through substantial morbidity and mortality in humans and animals (Muhammed *et al.*, 2010). Horizontal and vertical transmissions are important in the epidemiology of *Salmonellosis* worldwide, especially fowl typhoid and pullorum disease (Zancan *et al.*, 2000; Okwori *et al.*, 2013). Anyone can get a *Salmonella* infection, but the elderly, infants, and persons with impaired immune systems are at increased risk for serious illness. In these persons, a relatively small number of *Salmonella* bacteria can cause severe illness. In

Materials and Methods

Study area and population

The study was conducted in the three senatorial zones, Ikot Ekpene (IK), Eket (EK) and Uyo (UY)Akwa Ibom State. Akwa Ibom State is located on longitude $8^{0}30'$ and latitude $5^{0}30'$ in the Southern region of Nigeria; it covers an area of $455km^{2}$. The majority of people living in Akwa Ibom are civil servant and farmers. Blood and stool samples were collected from poultry workers in each senatorial zone. A total of 372 blood and stool samples were collected from the three zones, one hundred and eighty-six (186) samples of blood and stool were from human subjects and one hundred and twenty-eight (128) samples from poultry, giving a total of 500 samples from the three zones.

Preliminary investigation

The chosen hospitals and poultry farms were visited to obtain official permission to conduct the study and to discuss the feasibility of the study and to seek the cooperation of the Clinicians, Medical Laboratory Scientists and poultry managers or owners.

Ethical approval

Ethical clearance and approval was obtained from the Department of Research and Education Ethical Committee of the State Ministry of Health, to collect specimens from both Government and Private owned poultry facilities and health facilities within the study area.

Inclusion criteria

Subjects included in the study were between 14 years old and above, working as poultry staff or owners and non-poultry workers in the study area. For the purpose of analysis, subjects were divided into two groups: Group 1: Subjects with or without symptoms of

Preparation of pure and stock cultures

Plates were examined after overnight incubation. Non-Lactose-fermenting colonies picked from *Salmonella shigella* Agar and Deoxycholate Citrate Agar, characteristic of *Salmonella* were sub-culturedon nutrient agar to obtain pure isolates. The pure isolates were stored on nutrient agar slants and preserved in refrigerator at about 4°C to 8°C. Transfer onto fresh agar slopes was made at regular interval of one week (Erickson and Doyle, 2012). Plates showing no growth or growth of lactose fermenters were re-incubated for 24hours before discarding as being negative for *Salmonella* organisms (Cheesbrough, 2002; Tam *et al.*, 2008).

Biochemical characterization of the bacterial isolates

Isolates were identified using biochemical tests and API 20E for confirmation; API 20E is a standardized identification system for Enterobacteriaceae and other non-fastidious Gram-negative rods which use 20 miniaturized biochemical tests and a database.

Antimicrobial sensitivity test

Antibacterial sensitivity testing was performed according to Kirby-Bauer disk diffusion method recommended by the NCCLS (2004) and CLSI (2012). The antimicrobial assay was carried out using Mueller Hinton agar and were tested in vitro for susceptibility to fifteen different antibiotics.

of *salmonellosis* have indicated repeatedly that the most common sources of non-typoidal infections in developing countries are undercooked or raw eggs, and contaminated poultry products (Wain and Hosoglu, 2008).

This research has revealed the presence of Typhoidal and non-typhoidal *Salmonella* species in poultry products, (egg shells and cloaca) and among the workers with overall isolation rate of 117 (23.4%). This finding in itself, is not surprising since *Salmonella* is reported to be a poultry persistent pathogens capable of surviving and proliferating in diverse environments including eggs, eggs shells and gastrointestinal tract of poultry (Wain, *et al.*, 2001). Their prevalence is higher than 10.9% reported by Muhammed *et al.*, (2010) in Jos, plateau. *Salmonella* isolates were found in all the sample sources used in this study; including poultry workers, poultry products and also in the control subjects. This is attributed to poor hygienic practices by this class of subjects. This is in support of the report of Ibrahim *et al.*, (2013) that *Salmonella* species were isolated from farmers, egg swab and bird's cloaca in Egypt. However, the study showed no significant difference at ($p<0.05$) in the rate of infection between the poultry workers and non-poultry subjects, but showed significant level between the isolates from poultry products and poultry workers (human) in all the senatorial districts studied. This is in support of the recent report that the prevalence of *Salmonella* species in poultry products and farmers are up to 2.2% higher in Korea (Ibrahim *et al.* 2013). That *S. typhimurium* (10.3%), (19.7%) *S. enteritidis* and *S. gallinarum* samples were isolated from human and poultry products could be attributed to close contact between the farm workers, poultry and other animals raised in the farms, as the organisms also have a broad host range and the ability to adapt to condition in different animal and environment. This is in agreement with Fasure *et al.*, (2012) who reported that *S. typhimurium* and *S. enteritidis* were isolated from poultry and asymptomatic poultry workers in Ogun State. Investigations have also shown that the prevalence of *S. enteritidis,* in infected chickens and eggs in poultry farms of 18.5% (Wain and Hosoglu, 2008) is an earlier finding that infected raw egg shells or chickens were the major sources of *salmonellosis* to

Only 23.9% occurrence of *S. typhi* was obtained from humans. *S. typhi* isolation may be due to poor sanitary conditions and unhygienic practices. Human can also serve as asymptomatic carriers for typhoidal infections. This study has also revealed that humans were mostly infected with five serogroups (A, B, C, D and F), while poultry had serogroups A, B, D, E and F (Parry *et al.*, 2002). Serogroups F was the most prevalent in both humans and poultry, followed by serogroups A and D. Serogroups B and E remain the major pathogens for foodborne salmonellosis worldwide and their isolation in this study pose a serious public health risk.

Isolation of the causative agent of typhoid by cultural technique has remained the gold standard for diagnosis of enteric fever. The definitive diagnosis of typhoid fever depends on the isolation of *salmonella typhi* from blood, stool, urine and other body fluids. Blood culture has remained the gold standard for diagnosis of typhoid fever. Although culture are associated with a lag period of at least 48hr. for preliminary confirmation of infection, they remain an essential investigation considering the recent emergence of drug resistance among *S. typhi*.

The results of antibiogram revealed multidrug resistant in *Salmonella serovars* incriminated in both human and animal *salmonellosis*. Overall results showed that all *serovars* were 100% resistant to *Tylosin* and *Oxacillin,* 87.2% to Ampicillin, 71.8% to *Ceftazidine*, 66.7% to oxytetracycline and 60% to *Ceftriaxone*. In addition, five *serovars* were 100% resistant to over six different antibiotics. This could be attributed to misuse of drugs in both human and veterinary medicine, most especially for prophylaxis and as a growth promoter in poultry feeds. However, the antibacterial susceptibility test revealed 88% of the isolates were susceptible to *Ciprofloxacin,* 87.2% to Amoxacillin-clavulanic acid, 70.9% to *Gentamycin* and 67.5% to Chloramphenicol. This shows that some *Fluoroquinolones* and *Aminoglycosides* still remain drug of choice for treatment of *salmonellosis*.

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