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Incidence of multidrug-resistant *Salmonella* spp. in local food products sold in Ado-Ekiti, South Western Nigeria

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ABSTRACT

Background: Contaminated foods of animal origin are the primary reservoirs for human non-typhoidal Salmonellae infections. Transmission of Salmonellae to humans typically occurs by ingesting meat, dairy products, and other foods contaminated by animal faeces from foods contaminated with Salmonellae. **Aim:** This work aimed at the detection and incidence of viable *Salmonella* in local food products sold and consumed in Ado – Ekiti. **Methods:** Typing by pulse-field gel electrophoresis (PFGE), polymerase chain reaction (PCR) detection of antimicrobial drug resistance genes, and antibiotic susceptibility testing were done. **Results:** Out 105 samples analyzed, *Salmonella* species was isolated in 77 with highest incidence (100%) observed in kunu, pork meat, egg roll, raw egg and chicken. The antimicrobial drug resistance patterns on the isolates showed that *Salmonella* species were resistant to cotrimoxazole (100%), chloramphenicol (100%), amoxicillin (100%), ampicillin (86%) and ofloxacin (57%) while decreased susceptibility to ciprofloxacin (100%), streptomycin (100%), gentamycin (86%) and pefloxacin (71%) was found. multidrug resistance was observed in about 77% of the isolates. With PFGE, a total of eighty- three (83) patterns were observed and thirty-six 36(43%) isolates had the 3 most common patterns. All isolates from kunu and pork meat contained qnrB2, 6 (86%) isolates from egg roll contained $bla_{CMY-2'}$; 9 (75%) isolates from liquid egg and chicken each contained $bla_{CMY-23'}$. The total isolate of 73% is an indication of high incidence of *Salmonella* spp. in food products obtained in Ado-Ekiti. **Conclusion:** This study showed antimicrobial drug resistance in low resource settings and urgent need for surveillance and control of this phenomenon is recommended.

Introduction

How safe is our food? It sounds a simple question. However getting a reasonable answer is far from simple. The basic problem lies in the fact that only a small fraction of foodborne disease cases get reported through official (or unofficial) reporting systems. Calculating the 'real' rate of foodborne illness requires developments of models that use reported cases as a starting point to estimate underlying disease rates. Given the plethora of pathogens that been transmitted through foodborne routes, this is a complex and somewhat daunting process. It is therefore necessary to access the safety of foods and develop strategies that will prevent disease spread [1,2].

Contaminated foods of animal origin are the primary reservoirs for human nontyphoidal *Salmonellae* infections [3]. Transmission of *Salmonella* to humans typically occurs by eating raw or undercooked meat, poultry, eggs or egg products, which are majorly the sources of *Salmonella* infection. The organisms pass through the food chain from primary production or cross contamination from food and meat products in

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households or food service establishment and institution such as hospital to food consumed by the public [4]. Nontyphoidal *Salmonella* isolates produce a common food-related infection that causes mild and self-limiting diarrhoea and, occasionally, a potentially fatal invasive disease with bacteremia and endovascular infection [5]. They colonize the gastrointestinal tracts of cattle and other animals; many infected cattle are asymptomatic carrier. They can survive for weeks outside a living body [6]. They had been found in dried excrement after two and half years. It is not destroyed by freezing, ultra violet radiation but heat accelerates their demise, and they perish after being heated to 55^{o}C for one hour or 60^{o}C for 30 minutes [3].

Antimicrobial agents such as fluoroquinolone and third-generation cephalosporin are commonly used to treat severe human *Salmonella* infections [7]. Resistance to these and other antimicrobial drugs, as well as multidrug resistance has increased over the last several decades, probably as a consequence of antimicrobial agents in use at intensive animal husbandry and medicine [8]. Antimicrobial resistant strains of *Salmonella* species are now widespread all over the world. In developed countries, it is becoming more and more accepted that a majority of resistant strains are of zoonotic origin and have acquired their resistance in an animal host before being transmitted to human through the food chain [9].

The emergence of antimicrobial drug resistance is a matter of concern. Therefore this research work aims at the detection and incidence of viable *Salmonella* in local food products sold and consumed in Ado – Ekiti, and to determine the rate of multi-drug resistance amongst the isolated *Salmonella*.

Materials and Methods

Collection of samples

Total number of one hundred and five (105) samples; which comprised kunnu (12), yoghurt (12), pork meat (14), chicken (13), turkey (7), egg roll (7), meat pie (7), gala sausage (7), raw egg (12), tin tomatoes (7), and cake (7) were bought from Oja-Oba; a highly patronized market in Ado – Ekiti metropolis, Ekiti State. The food samples were randomly selected and separately collected in sterile tightly covered plastic containers, and then brought to the laboratory within 45 minutes of collection.

Preparation of samples, isolation and identification of *Salmonella*

The samples were kept in sterile beakers separately and they were homogenized aseptically to obtain suspension. The homogenates were cultured into Nutrient Broth (MP Biomedicals, USA). The streaked plates were inverted and were incubated at 37°C for 24hrs. A loopful of the suspension was subculture on Shigella–Salmonella agar (Thomas Scientific, USA). The *Salmonella* spp. was identified using conventional methods [10].

Susceptibility testing

Susceptibility testing was carried out by disk diffusion method according to Clinical and Laboratory Standard Institute [11]. A colony from stock was sub-cultured into 5 mL of nutrient broth (LAB) and was incubated at 37^{0}C for 18h. About 0.1 mL of the overnight broth of each organism was pipette into 9.9 mL of the broth to yield a 10^{1} dilution [11]. The procedure was continued to obtain a final dilution of 10^{3}. The bacterial suspension was spread onto a Mueller-Hinton agar (MP Biomedicals, USA) and a multi-disk (Abtek, UK) containing cotrimoxazole (25µg), chloramphenicol (30µg), sparfloxin (10µg), ciprofloxacin (10µg), ampicillin (10µg), amoxicillin (30µg), gentamycin (10µg), pefloxacin (10µg), and streptomycin (30µg) were placed on the agar. Multidrug resistance was defined as non-susceptibility to ≥3 antimicrobial drug classes [11].

Pulsed–field gel electrophoresis (PFGE)

All *Salmonella* isolates were analyzed for genetic relatedness by PFGE by using *Xba*1 according to the CDC PulseNet protocol [12]. Electrophoresis was performed with a CHEF-DR111 system in the Science Technology Research Lab, Federal Polytechnic, Ado-Ekiti, Nigeria by using 1% Seakem agarose in 0.5x Tris-borate-EDTA at 180V. Running conditions consisted of 1 phase from 2.2 to 63.8s for a run of 22h**.**

PCR detection of antimicrobial drug resistance genes

Presence of *qnr* genes was determined by using PCR; using the QIAGEN Plasmid Purification mini kit, with primers QP1 and QP2 for *qnrA*, FQ1 and FQ2 for *qnrB*, and 5'-ATGGAAACCTACAATCATAC-3' and 5'-AAAAACACCTCGACTTAAGT-3' for *qnrS*. The *qnrB* allele was determined by amplification and sequencing with primers FQ1 and FQ2. Screening for *aac(6')-1b-cr* was performed [13]. Primer pairs used for amplification of β-lactamase genes were;

bla$_{cmy}$ (5'-ATGATGAAAAAATCGTTATGC-3') and (5'TTGCAGCTTTTCAAGAATGCGC-3'),*bla*$_{OXA-1}$ (5'AATGGCACCAGATTCAACTT-3') and (5'-CTTGGCTTTTATGCTTGATG-3'); and *bla*$_{SHV}$ (5'-GGTTATGCGTTATATTCGCC-3') and (5'-TTAGCGTTGCCAGTGCTC-3'). *bla*$_{CTX-M}$ genes were screened by using a multiplex PCR assay [14].

Results

The results of this work are shown in tables below. **Table 1** shows the incidence of *Salmonella* spp. in some food and meat product samples. High incidence (100%) of *Salmonella* spp. was observed in kunu, pork meat, chicken, egg roll, and raw egg; while low incidence was observed in tin tomatoes and gala sausage, and cake. Out of 105 samples tested, 77(73%) were positive for the *Salmonella* spp.

Table 2 shows the susceptibility pattern of *Salmonella* spp. to conventional antimicrobial agents. All the isolates were sensitive to ciprofloxacin 77(100%), and cotrimoxazole 77(100%), while 66(86%) and 55(71%) were sensitive to gentamycin and pefloxacin respectively. All isolates were resistant to cotrimoxazole, chloramphenicol and amoxicillin while 66(86%) and 44(57%) were resistant to ampicillin and ofloxacin respectively. With PFGE, a total of eighty- three (83) patterns were observed and thirty-six 36(43%) isolates had the 3 most common patterns. The isolates with these patterns were found to show high resistance to cotrimoxazole, chloramphenicol, ampicillin and amoxicillin.

Table 1. Incidence of *Salmonella species* in some food and meat product samples.

Source	No of samples	Total number of isolates	Percentage of isolate (%)
Kunu	12	12	100
Yogurt	12	11	92
Pork meat	14	14	100
Chicken	13	13	100
Turkey	7	2	29
Egg roll	7	7	100
Meat pie	7	5	71
Gala sausage	7	1	14
Raw egg	12	12	100
Tin Tomatoes	7	0	0
Cake	7	0	0
	105	77	73

Table 2. Susceptibility pattern of *Salmonella* sp. to conventional antibiotics.

Antibiotics	Sensitive (%)	Resistance (%)
Cotrimoxazole	Nil	77 (100)
Chloramphenicol	Nil	77 (100)
Ofloxacin	33 (43)	44 (57)
Ciprofloxacin	77 (100)	Nil
Ampicillin	11 (14)	66 (86)
Amoxicillin	Nil	77 (100)
Gentamycin	66 (86)	11 (14)
Pefloxacin	55 (71)	22 (29)
Streptomycin	77(100)	Nil

Discussion

The high incidence of the isolated *Salmonella* spp. in these food products may be due to the preparation processes which may be done under unhygienic environmental conditions. Contamination also could result from the carrying and selling environment, cross-contamination from dust, no cognizance of shelf-life of the products, packaging and sale containers and from the hands of vendors. It has been detected that non typhoidal

Salmonella spp. are leading causes of foodborne illnesses in England, Australia and United States [2].

All isolates from kunu and pork meat samples contained *qnrB2*, 6(86%) isolates from egg roll contained bla_{CMY-2}; 9 (75%), isolates from raw egg and chicken each contained bla_{CMY-23} which is the mechanism for extended-spectrum cephalosporin resistance. The genes that code for this resistance have proven to be remarkably mobile and widely distributed within and between species. Integrons are widely distributed among *Salmonella* spp. and are potentially capable of transmitting drug resistance [15]. Similarly **Dallal et al.** [16] reported *Salmonella* spp. from beef sample as 100% resistant to erythromycin and tetracycline; 60% resistant to sulphamethoxazole, and all isolates were susceptible to ciprofloxacin and streptomycin.

Multidrug resistance is evidenced in this study and this can be ascribed to the wide spread use of antibiotics both inside and outside of medicine, selling of the antibiotics over the counter without prescription, misuse and over use of antibiotics by doctors as well as patients, inappropriate prescription and most significantly for this study, addition of antibiotics to the feed of livestock. The multi-drug resistant result in this study is higher than that of **Al-Salauddin et al.** [17] who reported 16.67% isolates of *Salmonella* originated from broiler meat as multidrug resistant. These findings showed that multi-drug resistant in *Salmonella* spp. is prone to increase with the time due to indiscriminate use of antibiotics in dairy and poultry industry.

The emergence of *Salmonella* strains that are resistant to commonly used antibiotics is important to clinicians, microbiologists and those responsible for the control of communicable diseases and also food industries. It is also important to farmer who may sustain economic losses when consumer confidence in their products is lost. Because use of antimicrobial agents contributes to increasing resistance and facilitates transmission of multidrug – resistant salmonellae, promoting guidelines aimed at improving appropriate use of antimicrobial agents may help prevent transmission of multidrug – resistant *Salmonella* infections in food and meat products. Control of multi-drug resistant *Salmonella typhimurium* DT 104 requires reducing infection in foods, meats and lowering the risk of contamination at all stages in the production chain [18]. In addition, the avoidance of unnecessary antibiotics usage in animal feeds should be combined with good husbandry, good abattoir practice and good hygiene at all stages in the food production chain from processing plants to kitchens and food service establishments.

The need for thorough cooking of food prior to consumption should be emphasized. Educating farmers regarding the risk of occupationally acquired infections is of great significance. From the result of the susceptibility test, there is increase in the incidence of *Salmonella* resistance to antimicrobial agent. This suggests that there should be a compulsory reassessment of the pathogen reduction e.g. increase product testing, more efficient cleaning and sanitization, better microbiological quality control and maintaining of micro flora at a low level during packaging, handling and storage of products, avoiding excess use of antibiotics in feeds and medication believing such intervention might concurrently reduce *Salmonella* contamination of food and meat products. Integration of human public sanitary surveillance system is of utmost importance in our public health infrastructure. Since 1994, an increasing number of isolates with additional resistance to trimethoprim and a few to ciprofloxacin have been reported [5].

Conclusion

The *Salmonella* spp. isolated from the local food products sold in the study area were susceptible, and as well resistant to antibiotics. The findings of this study can be used to assist in the direction of policy and interventions; to conduct other analyses like the evaluation of economic cost of *Salmonella* infections while attributing it to various food and meat products sold in Ado-Ekiti. It enables the populace to be advised on what food product should be allowed for sale and consumption in the community.

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Conflict of interest

The authors declare no conflict of interest.

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