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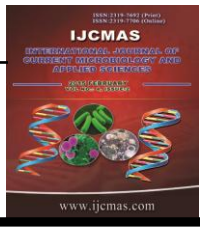

Original Research Article

Antibiotic resistance and plasmid carriage among *Salmonella typhi* isolated from food and hands of food handlers in a Nigeria University

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A B S T R A C T

Hygiene in most eateries in Nigerian campuses is still very poor and not different from what is obtainable in most places outside higher institutions. The prevalence, antibiotic susceptibility of *Salmonella* from the ready-to-eat food and hands of food handlers (HFH)in a Nigeria university was investigated. The food samples and hand swabs from food sellers were collected and screened for *Salmonella typhi*. Disc diffusion method was used to determine the antibiotic susceptibility of the isolates while the carriage of plasmid among ten isolates selected on the basis of antibiotic resistance was also determined. Evaluated, reviewed carefully, and then pre-tested questionnaire interview was used to collect data (socio-demographic and the condition of the food) from both sellers and buyers. A total of 318 isolates of *Salmonella typhi* strains were obtained from food samples (284/453) and the food handlers (34/97). Isolates recovered from the two sources showed similar resistance patterns. The predominant resistance observed (food sample/handlers) were to ofloxacin 62.7/35.3%, gentamicin (50.4/50.0%), and ceftazidime (38.4/38.2%). Multiple antibiotic resistance was observed in 8.5 and 11.8% of the isolates from food and HFHs respectively. All isolates were resistant to three to seven antibiotics, also five phenotypic resistance profiles were observed. The plasmid analysis of the phenotypes revealed that nine out of ten phenotypes harbour a single plasmid with molecular weight of 23.130 kb. All the subjects explained that they patronised the eateries due to their proximity and cheap food items. Two months prior to the survey all the subjects (115) reported to patronise the eatery on the campus at least twice per week. A 39.1 % of the subjects had treated typhoid fever four weeks prior to the survey. It is evident from the results of this study that there is contamination of food by the handlers and this may have responsible for the cases of illnesses observed in this study. Food handlers should be properly educated and monitored to ensure compliance to proper food hygiene guidelines.

Introduction

Food serves as a sources of pathogens and hence a major source of infection to man. Food contaminated with antibiotic-resistant bacterial pathogens is a major threat to

public health (Gajraj*et al.,* 2012). Apart from infecting man they serve as reservoirs of genes for antimicrobial resistance and they (the pathogens) easily transfer the resistant genes to both related and unrelated bacterial species (Okamoto *et al*., 2009; Odu and Akano, 2012).

Globally there is an increase in the prevalence of antimicrobial resistance among foodborne pathogens in the recent year (Threlfall*et al,* 2000). Unregulated use of antibiotics in food-animals and humans, poor sanitary conditions has been reported to be the major conditions for the endemic nature of food-borne diseases in most developing countries (Angulo*et al,* 2000; Van den Bogaard and Stobberingh, 2000; Nma and Oruese, 2013). Africa recorded a third of the global typhoid fever yearly (Ellis *et al*., 1990).

After malaria, typhoid fever has remained a major scourge of most developing nations such as Nigeria. Typhoid fever causes an estimated 16.6 million cases and 600,000 deaths worldwide annually (WHO, 2003). Typhoid fever has continued to plague vulnerable populations in developing countries, mostly as a result of contaminated food and water. Africa recorded a third of the global typhoid fever yearly (Ellis *et al*., 1990).

Most Nigerians consume low cost food sold in bukas, where hygiene conditions are very poor due to poverty as most of them are living on less than $1 per day (Oranusi and Olorunfemi, 2011). Typhoid fever is a bacterial infection that has caused high morbidity and mortality globally (Smith *et al*., 2012). The causative agent of typhoid fever, *Salmonella* has been reported to contaminate different food samples. Improper cooking methods, reheating of food and improper handling of food by food handlers have been identified as major factors responsible for its spread (Goman*et al*, 2002; Nma and Oruese, 2013).

Apparently healthy food handlers, food materials like vegetables, confectionary, meat and meat products, and poultry have been implicated in transmission of bacterial pathogens to man (Okeke*et al*., 2000; Osterblad*et al*., 1999; Schoeder*et al*., 2004). Contamination of food may occur during and after processing of such food. Contamination of ready-to-eat food is of primary concern because such organisms may be pathogenic thereby leading to outbreak of food-borne illness (Oluyege*et al*., 2009).

The purpose of this study is to investigate the prevalence, antibiotic susceptibility of *Salmonella* from the ready-to-eat food and (HFH) in a Nigeria university. The carriage of plasmids and antibiotic resistant pattern of the isolates from the screen food samples and the hands of the food handlers were also investigated.

Materials and Methods

Isolation of *Salmonella typhi* from food samples and hands of food handlers (HFHs)

A total 453 food samples were collected for this study from eateries (16), restaurants (7) and canteens (13) in a campus of a Nigeria university. The food samples collected include the following: jollof rice (81), fried rice (87), white rice (45), *eba*(72), *amala* (60) and salad (84). The food samples were taken to the laboratory immediately for analysis. Ten gram of each of the food samples was weighed aseptically into a clean conical flask, containing 90ml of sterile normal saline. The mixture was thoroughly shaken and 1 ml was taken from the mixture and was inoculated into a Bijou

bottle containing sterile peptone water. The Bijou bottle was incubated at 37^{o}C for 6 hours and later sub-cultured on sterile Bismuth sulphide agar (Oxoid) using the streak method. The plates were inoculated at 37 ^{o}C for 18 h.

The hand swabs of the canteen workers were collected in the entire food centres visited and within one hour of collection, they were inoculated directly onto sterile plates of Bismuth sulphide agar. The plates were incubated at 37^{o}C for 24h. Identification was carried out on 18h old cultures using standard methods described by Fawole and Oso (2001) and the result was interpreted according to Holt*et al*. (1994).

Antibiotic Sensitivity Testing:

The isolates were standardized by growing at 37 °C in Mueller-Hinton Broth (Oxoid) for 16 h and adjusted to an optical density of 0.1 (0.5 McFarland Standard) at a wavelength of 625 nm. The disc diffusion method was used for susceptibility testing as described by Clinical and Laboratory Standard Institute (CLSI) (2012). The isolates were tested against eight commercial antibiotics. The commercially prepared antibiotic disks (AbtekBiologicals Limited) and their concentrations in microgram are as follows: ampicilin (10), amoxicillin/clavulanic acid (30), ceftazidime (30), cefuroxime (30), ciprofloxacin (30), gentamicin (10), nitrofurantoin (300) and ofloxacin (5).

Plasmid Analysis

Plasmid profile of *Salmonella typhi* isolated was carried out on ten selected isolates. The bases for isolation are those that are resistant to a minimum of five out of the eight antibiotic tested. Modified alkaline method for plamid extraction described by Birnboim and Doly (1979) was used for plasmid extraction from the isolates. Band produced were separated by 1% agarose gel electrophoresis and observed by ultraviolet light.

Subjects and Questionnaire

A structured questionnaire interview was used to collect data from students that patronize food centres on campus. The questionnaire was previously evaluated, reviewed carefully, and pre-tested. The first part focused on socio-demographic characteristics of respondents while the second part of the questionnaire contained questions on how frequent the subjects visit the eatery and the type of food they consume.

Results and Discussion

Food is a vehicle through which bacterial diseases are transmitted to man. The bacteria gained entry into the food as results of inadequate preparation, poor storage conditions, or unhygienic handling and preparation (Al-Khatib*et al*., 2004; Nanu*et al*., 2007; Nkere*et al*., 2011). In this study similar trends were observed in the resistant patterns of the *Salmonella*Typhi isolated from both HFHs and the food they vended. However, there was a remarkable high resistance to ofloxacin among the isolates from the food samples (62.7 %) compare to those isolated from the food vendors (35.3 %).

This high resistance among the isolates is a serious public health concern because antibiotic resistance among pathogenic bacteria could increase fatality. Helms *et al*. (2003) reported multidrug resistant *Salmonella* strains to be more than 20 times greater in virulence that antibiotic susceptible strains. Highest resistance was observed in the isolates against ampicillin

with 84 % (239 out of 284) and 94.1 % (34 out of 97) respectively for isolates from food samples and from hands of food handlers. The occurrence of gentamicin resistant pathogen in the food sample (50.4 %) and the hand of the HFH (50.0 %) were lower that 90.0 % reported by Putturu *et al*. (2013). Raw materials used for the preparation of the food may be the source of pathogens in the food (Afolabi and Oloyede, 2010; Bayu *et al*., 2013; Karshima *et al*., 2013; Nma ON and Oruese, 2013). Singh *et al*. (2010) reported that the chicken eggs from both poultry farms and marketing channels are the sources of anti-microbial resistance *Salmonella typhi*.

Nitrofuratoin was most effective against the isolates from the HFHs. Relatively; the isolates from HFHs were more resistant to the antibiotics than those from the food samples. For the resistance of the isolates from the two sources the Pr(chi-square) calculated (0.197) was greater than 0.05.

Plasmids in most cases carry antibiotic resistance genes and the genes are generally transferable to other pathogens (Nordmann and Poirel, 2005). The selected isolates for plasmid analysis were resistant to Caz, Crx, Gen, Nit and Amp. Six out of the ten selected isolates were resistant to all the antibiotics (Table 3). Except for 10B all the ten selected isolates had a plasmid with 23130 bp size as shown in Plate 1.

The plasmid may have likely coded for the resistance of Amx/clav among other antibiotics. Most antibiotic resistant genes are also located on plasmids. Association between *qnrS1* and a Tn*3*-*like bla*TEM-1-containing transposon has frequently beendetected; on a conjugative plasmid isolated from *Salmonella* sppisolates (Kehrenberg*et al*. 2006, 2007). Avsaroglu*et al*. (2007) also detected plasmid in *Salmonella* spp. isolated from Turkish foods of avian origin. This genetic element has been reported to be transferable (Kehrenberg*et al*., 1998). The presence of the plasmid in the *Salmonella* confers resistance to quinolones (Avsaroglu*et al*. 2007; Cavaco *et al*., 2007).

Salmonella infection ranked among the leading food-borne infection globally (Forshell and Wierup, 2006). *Salmonella* Typhi is a zoonotic pathogen. Of which most of the domestic and food animals are reservoir (EFSA, 2006). Human Salmonellae infection a major health concerns in Africa likewise other resource poor nations of the world.

Salmonella typhi has low doubling period but can survive in most food samples (Dugid and North, 1991). Two months prior to the survey all the subjects interviewed (115) reported to patronise the eatery on the campus at least twice per week. Four weeks prior to the survey, 39.1 % of the subjects reported to have been treated of typhoid fever. All the subjects explained that they patronised the eateries due to their proximity and cheap food items.

The quality of the food samples in the campus of the university studied was similar to the one reported in University of Ghana campus by Yeboah-Manu *et al*. (2010) and that of Oluyege *et al*. (2009). The food samples screened are not microbiologically safe for consumption. In this study, 68% of 68% of the food handlers examined are illiterate that have little or no knowledge about good hygiene practices, while 32% stated that the food retailers are literate.

Most of the food vendors lack proper education and adequate knowledge on how best food could be handled without contamination. All the subjects explained

that they patronised the eateries due to their proximity and cheap food items. The poor personal hygiene of the food handlers has been reported to constitute to the high recovery rate of bacteria pathogen from them (Ojo and Adetosoye, 2009; Esan, 2011). The food handlers in some cases grossly lack formal knowledge of food preparation and hygiene (Omemu and Aderoju, 2008; Adjrah*et al*., 2013). They acquired their knowledge mainly through observation of others or by trial and error methods (Omemu and Aderoju, 2008; Chukuezi, 2010).

In this study, it is evident that *Salmonella* spp transmits through food, and hands of food handlers. The food handlers need adequate training on both personal and environmental hygiene to prevent poor preparation, distribution and serving of food.

Table.1 The food centres visited and the food screened for the presence of *Salmonella typhi*

Food Centres		**Food samples**					
		Jollof rice	**Fried rice**	**White rice**	***Eba***	***Amala***	**Salad**
Eateries	Examined	41	40	18	34	28	37
(n=16)	Positive	27	31	12	25	21	24
Restaurants (n=7)	Examined	14	23	14	13	12	20
	Positive	8	15	10	9	4	16
Canteens (n=13)	Examined	26	24	13	25	20	37
	Positive	17	19	9	11	8	18
Total	**Examined**	81	87	45	72	60	84
	Positive	52	65	31	45	33	58

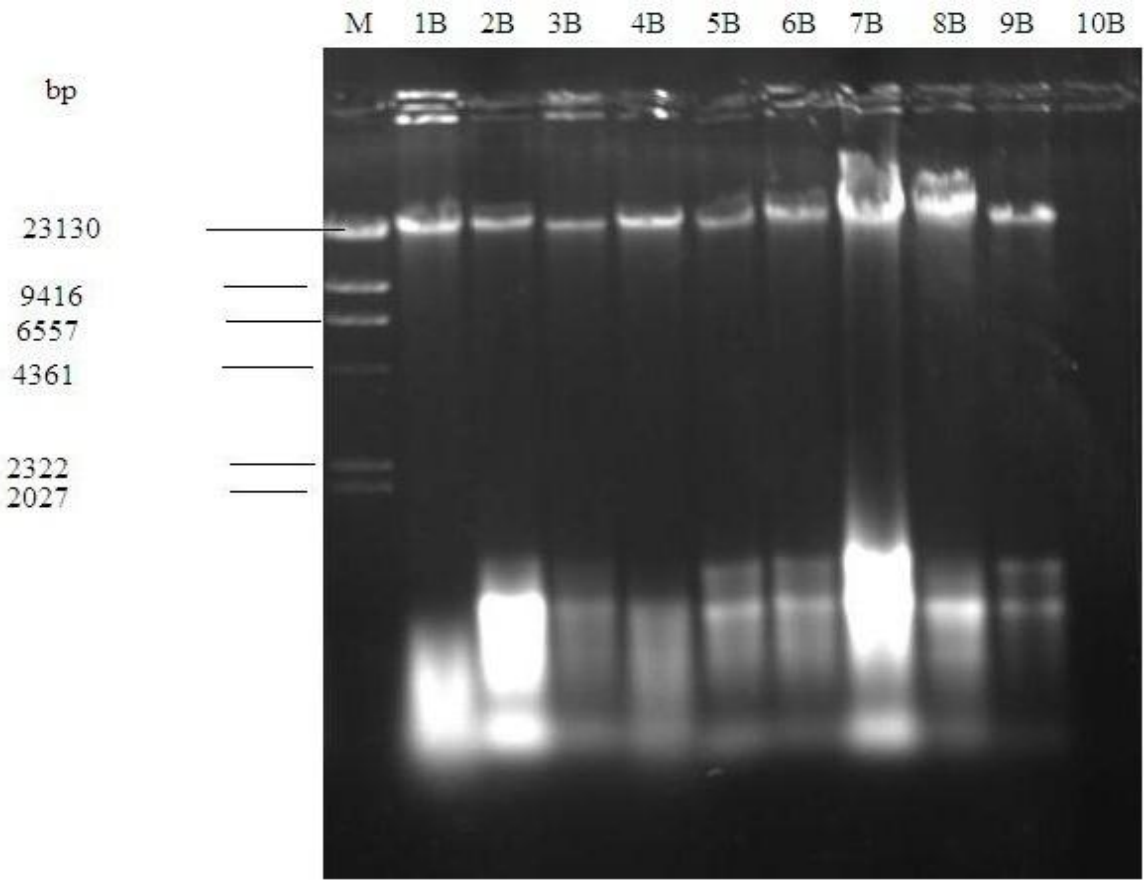
Table.2 Antibiotic susceptibility pattern of Salmonella Typhi isolates from food samples and hands of food handlers

Antibiotics	**Source**			
	Food samples		**Hands of food handlers**	
	Number	**percentage**	**Number**	**percentage**
Caz	10	36.0	13	38.2
Crx	16	57.0	21	61.8
Gen	14	50.4	16	50.0
Ctx	10	38.4	13	38.2
Ofl	17	62.7	12	35.3
Amp	23	84.2	23	94.1
Amox/Clav	14	52.1	27	79.4
Nit	13	46.8	11	32.4

Table.3 Resistant phenotype of selected Salmonella Typhi screened for plasmid carriage

Sources	Code	Antibiotics resistant pattern
Food Samples	1 B	Caz, Crx, Gen, Ctx, Amp, Amx/clav, Nit
	2 B	Caz, Crx, Gen, Ctx, Ofl, Amp, Amx/clav, Nit
	3 B	Caz, Crx, Gen, Ctx, Ofl, Amp, Amx/clav, Nit
	4 B	Caz, Crx, Gen, Ofl, Amp, Amx/clav, Nit
	5 B	Caz, Crx, Gen, Ctx, Ofl, Amp, Amx/clav, Nit
	6 B	Caz, Crx, Gen, Ctx, Ofl, Amp, Amx/clav, Nit
Hand swabs	7 B	Caz, Crx, Gen, Ctx, Ofl, Amp, Amx/clav, Nit
	8 B	Caz, Crx, Gen, Ctx, Ofl, Amx/clav, Nit
	9 B	Caz, Crx, Gen, Ctx, Ofl, Amp, Amx/clav, Nit
	1 0 B	Caz, Crx, Gen, Ctx, Ofl, Amp, Nit

Plate.1 The plasmid profiles of selected Salmonella Typhi isolated from food samples and hands of food handlers.M = HIND III digest of λ-DNA (DNA molecular weight marker). Lanes 1B - 4B = Salmonella Typhi from food samples, Lanes 5B to 10B = Salmonella Typhi from hand of food handlers



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