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Prevalence and antimicrobial profiles of Salmonella serovars from vegetables in Maiduguri, North eastern Nigeria

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Abstract

This study determined the occurrence of *Salmonella* from vegetables, the prevailing serovars, and the antimicrobial susceptibility profile of the serovars. A total of 300 samples of vegetable comprising spinach (*Amaranthus hybridus* spp.), *Corchorus olitorus* spp., sorrel (*Hibiscus sabdariffa*), bitter leaf (*Vernonia amygadalina* spp.), and water leaf (*Talinum triangulares* spp.) were collected from five different farms and Maiduguri central market from May to August, 2009. Presumptive *Salmonella* isolates were determined by using the conventional biochemical tests, Serovars were confirmed by serotyping, using slide agglutination technique. Antimicrobial susceptibility test was performed with 17 antimicrobial agents using the minimum inhibition concentration tests (MIC) method. Out of the 300 samples analyzed, 19 were positive for *Salmonella,* which represent a prevalence of 6.3%. The serovars obtained were *Salmonella* Hadar, 13(4.3%), *Salmonella* serovar 47: mt:-, 5(1.7%), and *Salmonella* Vinohrady, 1(0.3%). Most of the serovars were susceptible to antimicrobial agents with the exception of *Salmonella* Hadar that exhibited multiple resistances to streptomycin, trimethoprim, sulfamethoxazole, and neomycin. *Salmonella* represents a major contaminant of vegetables consumed in Maiduguri, North-eastern Nigeria. Therefore, vegetables can serve as a route for the transmission of *Salmonella* to humans. This constitutes a serious health risk to the human population, and there is a need for specific *Salmonella* control program to be instituted as part of a national food safety strategy.

Keywords: Antimicrobial profiles, MIC, North eastern Nigeria, Prevalence, *Salmonella* serovars, Vegetables

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Introduction

Foodborne outbreaks caused by *Salmonella* represent a major public health problem worldwide, and developing countries are affected by a wide range of foodborne diseases. More recently, data collected for Pan-American Health Organization (PAHO/WHO) on developing countries indicated that 9,180 foodborne outbreaks were reported for the years 1993 to 2010. From these outbreaks 69% were caused by bacteria, and *Salmonella* was the most frequent agent incriminated for 58.1% of the outbreaks (Pires *et al.,* 2012), and 66.2% of the cases (Franco *et al.,* 2003). Most of the infections produce mild gastroenteritis; life threatening and disseminated infections are common among children, elderly and immunocompromised patients (Le Hello *et al.,* 2011). Salmonellosis is also considered as one of the most widespread foodborne zoonoses in industrialized as well as developing countries, even though the incidence seems to vary between countries (Molla *et al.,* 2003). The majority of human infections are caused by a limited number of the more than 2,600 serovars described to date, and the prevalence of specific serovars differ by geographical region (Hendriksen *et al.,* 2009 ; Lee *et al.,* 2009; Sirichote *et al.,* 2010; Le Hello *et al.,* 2011), but only a limited number of serovars are of public health importance.

The frequency of outbreaks of these infections in association with the consumption of raw or minimally processed fruits and vegetables has increased (De Roever, 1998; Fuzihara *et al.,* 2000; Wegener *et al.,* 2003) mainly from the consumption of lettuce, melon, salads, fruit and vegetable juices and pathogenic bacteria including *Salmonella* are commonly incriminated (Sivapalasingam *et al.,*2004). The factors influencing the increase in salmonellosis due to vegetables are changes in agricultural practices, eating habits and increases in the worldwide commerce of fresh produce (Collins, 1997). Contamination with enteric pathogenic bacteria from horticultural products seems to be the main cause of infection, and two possible routes of contaminations are the use of organic fertilizers of animal origin and irrigation of crops with wastewaters (Natvig *et al.,* 2002; Islam *et al.,* 2004). Thorough anamnesis and microbiological examination are the prerequisites for correct diagnosis, however, quantification of the occurrence of diseases is difficult because many cases, such as gastrointestinal illness, are often not reported, and moreover the symptoms usually do not last long and are self-limiting in healthy people.

In recent years, the occurrence of antibiotic-resistant strains of a number of pathogenic bacteria, including those that can be acquired by humans through food has continued to be on the increase and this is of global concerns (Busani *et al.,* 2004). Regrettably, publications addressing the prevalence of *Salmonella* serovars in vegetables in Nigeria do not exist according to the authors' knowledge. This study was conducted to examine vegetable from farms and market, to determine the serovars, the prevalence rate and the antimicrobial susceptibility profiles of the isolates. This information will help to identify the public health significance of vegetables and provide an overview of the role of vegetable as a vehicle for the transmission of foodborne pathogens. The study will equally contribute to the development of national food safety strategies to protect the consumer from salmonellosis associated with consumption of vegetables.

Materials and methods

Study area

The study area (Maiduguri) is located in the arid zone of Nigeria, with an area of about 69,436 km^2 and lies within latitude 11° 50' 46" N and longitude 13° 08' 29" E. It lies within the savannah with low records of rainfall. The area is in the tropical continental north with dry period of 4-8 months, that is, October to May, this is followed by a short rainy season from late June to early October . The state is located within the north eastern corner of Nigeria and has boundaries with Chad to the north east, Cameroon to the east and Adamawa State to the south west. Agriculture and livestock farming are the mainstay of the state economy.

Sample collection and bacterial isolation

A total of 300 samples divided equally among five species of vegetables (60 samples per species) comprising spinach (*Amaranthus hybridus* spp.), *Corchorus olitorus* spp., sorrel (*Hibiscus sabdariffa*), bitter leaf (*Vernonia amygadalina* spp.), and water leaf (*Talinum triangulares* spp.) were collected from five different farms and Maiduguri central market (50 samples per site) from May to August, 2009.

Samples were analyzed at the Microbiology laboratory of the Faculty of Veterinary Medicine, University of Maiduguri, Nigeria on the same day as they were collected. Ten grams of vegetable were enriched in 100ml of Selenite-F broth (Laboratarios Britania, Buenos Aires, Argentina) and incubated at 37^0C for 24 hours. Following enrichment, cultures were inoculated onto Desoxycholate Citrate Agar (Park Scuntif, Northampton, UK) and incubated at 37^0C for 24 hours. Presumptive non-lactose fermenting dark centre colonies were subcultured on Xylosine Lysine Desoxycholate agar (Oxoid Ltd., Hampshire, UK) and incubated for 24 hours at 37^0C. *Salmonellae* were identified using biochemical characterization; Gram staining and microscopy, oxidase test, indole test, methyl red test, Voges-Proskauer test, Simmon’s citrate test, Triple Sugar Iron test, and Urease test (table 1), according to the standard techniques (Cowan & Steel, 1974).

Presumptive *Salmonella* positive isolates were streaked on nutrient agar (Fluka Biochemika, Steinheim, Germany) slants and incubated at 37^0C for 24 - 48 hours and preserved in the refrigerator for serotyping and antimicrobial susceptibility tests.

Serotyping, antimicrobial susceptibility tests.

Presumptive isolates were serotyped at the WHO National Salmonella and *Shigella* Center, Bangkok, Thailand by slide agglutination, Somatic (O) and flagella (H) antigens were characterized by agglutination with hyperimmune sera (S & A Reagents Laboratory, Ltd., Bangkok, Thailand) and serotypes were assigned according to the Kauffmann-White scheme (Popoff and Minor, 2007). Minimum Inhibition Concentration (MIC) determinations were performed on all isolates at DTU-Food, Denmark, by using a commercially prepared, dehydrated panel (Sensititre; TREK

Table 1: Prevalence and biochemical characteristics of *Salmonella* serovars

Salmonella serovars	Nos positive	Prevalence (%)	Biochemical tests: Indole	Methyl red	Vogues-proskauer	Simmon's citrate	Urease	Triple Sugar Iron (TSI): Slant	Butt	H_2S	Gas
Hadar	13	4.3	−	−	−	+	−	K	A	+	+
47:mt:-	5	1.7	−	−	−	+	−	K	A	+	+
Vinohrady	1	0.3	−	−	−	+	−	K	A	+	+

Key: + = positive test K = alkaline (red)
− = negative test A = acid (yellow)

Table 2: Distribution and antimicrobial resistance profiles among *Salmonella* serovars isolated from vegetables in Maiduguri, Nigeria

Salmonella Serovar	Origin	No. of isolate	No. (%) of isolates resistant to various antimicrobial agents at the indicated breakpoints (µg/mL)[a]																	
			AUG ≥32	AMP >8	APR >32	FOT >0.5	CHL >16	CIP 0,064-1	>1	COL >2	FFN >16	GEN >2	NAL >16	NEO >4	SPE >64	STR >16	SMX >256	TET >8	TMP >2	XNL >2
Hadar	Vegetable	13	0	1(8)	0	0	0	0	1(8)	0	1(8)	0	1(8)	2(15)	0	5(38)	3(23)	1(8)	4(31)	0
47:mt:-	Vegetable	5	0	0	0	0	0	0	0	0	0	0	1(20)	0	0	0	0	0	0	0
Vinohrady	Vegetable	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a]Abbreviations: AMP, ampicillin; AUG, amoxicillin-clavulanic acid; APR, apramycin; XNL, ceftiofur; CHL, chloramphenicol; CIP, ciprofloxacin; COL, colistin; FFN, florfenicol; GEN, gentamicin,; FOT, cefotaxime; NAL, nalidixic acid; NEO, neomycin; SPE, spectinomycin; STR, streptomycin; SMX, sulfamethoxazole; TET, tetracycline; TMP, trimethoprims

Diagnostic Systems Ltd., East Grinstead, England).
The following antimicrobials and resistance cut-off values breakpoints were used in the study: ampicillin, AMP (R> 4 mg/mL); amoxicillin-clavulanic acid, AUG (R>4 mg/mL); apramycin, APR (R>4 mg/mL); ceftiofur, XNL (R>2 mg/mL); chloramphenicol, CHL (R>16 mg/mL); ciprofloxacin, CIP (R>0.06 mg/mL); colistin COL (R>8 mg/mL); florfenicol, FFN (R>2 mg/mL); gentamicin, GEN (R>2 mg/mL); nalidixic acid, NAL (R>18 mg/mL); neomycin, NEO (R>8 mg/mL); spectinomycin, SPE (R>64 mg/mL); streptomycin, STR (R>16 mg/mL); sulfamethoxazole, SMX (R>256 mg/mL); tetracycline, TET (R>8 mg/mL); and trimethoprim, TMP (R>2 mg/mL).
Epidemiological cut-off values were interpreted according to current harmonized breakpoints by EUCAST (2014). Exceptions were made to interpretation of APR, AUG, COL, FFN, SPE, and XNL where Clinical and Laboratory Standards Institute (CLSI, 2008) and clinical breakpoints were utilized. NEO and STR were interpreted according to research conducted at DTU-Food. Quality control using *Escherichia coli* ATCC25922 on a weekly basis according to CLSI.

Results

Out of the 300 samples analyzed, 19 were positive for *Salmonella* representing a prevalence of 6.3%. Three different *Salmonella* serovars were incriminated; *Salmonella* Hadar, 13(4.3%), *Salmonella* serovar 47: mt:-, 5(1.7%), and *Salmonella* Vinohrady, 1(0.3%) as shown in table 1. Majority of the serovars were susceptible to the antimicrobials with the exception of *Salmonella* Hadar that exhibited multiple resistance to streptomycin (5), trimethoprim (4), sulfamethoxazole (3), and neomycin (2), with one isolate each showing resistance to ampicillin, ciprofloxacin, florfenicol, nalidixic acid and tetracycline. *Salmonella* serovar 47: mt:- and *Salmonella* Vinohrady were susceptible to all the antimicrobial agents except one isolate belonging to *Salmonella* serovar 47:mt:- that exhibited resistance to nalidixic acid (table 2).

Discussion

In recent years, the production and sale of ready-to-eat vegetables has increased. Vegetables can be contaminated by agronomic systems employed on the farm (irrigation with contaminated water, organic fertilizers, manure, and soil) during cultivation or during processing, handling and marketing (Kakiomenou *et al.,* 1998). The prevalence of *Salmonella* from vegetable (6.3%) revealed the possible health risk involved in the consumption of fresh or undercooked vegetable. This result is comparable to the study carried out in Mexico by Quiroz-Santiago *et al.* (2009) and other authors, with high isolation rates for *Salmonella* in fresh vegetables; 7.5% prevalence was reported in Spain by Rude *et al.* (1984) and Garcı´a & Ga´lvez (1987) while 9 to 10% prevalence was reported in New Jersey (Nzouankeu *et al.,* 2010).
It is evident from this study that consumption of raw vegetables directly or as part of dishes, with minimal processing, poses a risk, for direct infection of the consumer or cross-contamination of other foods. This represents a severe health risk if proper care is not taken in preparation. Knowledge of the epidemiology of salmonellosis remains the major tool for control of this disease.
Contamination of vegetable has often been linked to the agricultural practices being employed by farmers. Farmers in developing countries especially Nigeria occasionally utilize cattle and poultry faeces as fertilizer on vegetable farms, especially irrigated vegetable farms, resulting in food and environmental contamination. These pathways result in a complex epidemiology. It is therefore, important to determine the source of infection in order to estimate the burden, and implement preventive and control measures.
Salmonella Hadar has been an important serotype frequently isolated from chickens. It is also one of the major serovars recovered from humans in Europe and Africa (Hendriksen *et al.,* 2011). Its occurrence in this study may be linked to environmental contamination emanating from the use of faeces from poultry house to fertilize vegetable farms leading to cross contamination. This scenario might be a significant contributory factor for the dissemination of diverse serovars with humans as consumers. It equally detailed a widespread cross contamination in the country agricultural production system. Isolation of *Salmonella* serovar 47: mt:- and *Salmonella* Vinohrady which are rare serovars being reported for the first time is a cause for concern.
In recent years, worldwide health concern has been on the occurrence of antibiotic-resistant strains of a number of pathogenic bacteria that can be acquired by humans through food (Busani *et al.,* 2004). The use of antimicrobial agents both for chemotherapeutic and prophylactic purposes is

considered as the most important factor for the emergence, selection, and dissemination of antimicrobial- resistant bacteria*.*

This study reports a low level of antibiotic resistance and absence of resistance to the fluoroquinolones and cephalosporins which are especially important for evaluating the risk to human health. However, but the inappropriate application of antibiotics in poultry results in the development of resistance to the antimicrobial agents by the bacteria and this can subsequently be transferred to the vegetable through the use of faeces from poultry houses as manure on vegetable farms. It is obvious from the study that consumption of contaminated vegetables can instigate public health problem, the development of antimicrobial resistance could result from indiscriminate use of antimicrobial agents in the human population, resulting to the transfer of resistance genes or plasmids, thereby increasing the rate of resistance in both man and animals. It is therefore, suggested that vegetable should be properly processed prior to consumption.

In conclusion, The three *Salmonella* serovars, *Salmonella* Hadar, 13 (4.3%), *Salmonella* serovar 47: mt:-, 5(1.7%), and *Salmonella* Vinohrady, 1(0.3%) are contaminants of vegetables obtained from farms and central market in Maiduguri, Nigeria. This study showed *Salmonella* serovar Hadar to be the major contaminant of vegetables, they constitute a serious health risk for human population, and there is therefore a need for specific control programmes. There is also a need to educate the public on the danger associated with consumption of raw or improperly cooked vegetables. This information will enable the policy makers to identify the risks of human exposure to vegetables and guide in decisions of whether vegetables should be included in routine food surveillance for *Salmonella*. The study will also contribute towards the development of national food safety strategies, to protect the consumer from salmonellosis. It is therefore recommended that concerted efforts should be made towards regular surveillance of vegetables sold for public consumption to ascertain the microbial loads and the antimicrobial resistance profiles. This will guide in formulating prompt and effective control measures.

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