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SHORT COMMUNICATION

THE PREVALENCE OF FOODBORNE PATHOGENS RECOVERED FROM READY-TO-EAT FOOD FROM RESTAURANTS IN OKADA, EDO STATE, NIGERIA

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ABSTRACT

This study assessed the microbiological quality of various ready-to-eat cooked food and their contact surfaces in selected restaurants in the university town of Okada, Edo state, Nigeria. Microbial analysis was carried out on the samples obtained from six restaurants. The restaurants sampled were the ones with the highest patronage within the study area. The results obtained indicated that most of the ready-to-eat food did not meet the required quality standards, and therefore, posed potential risks of foodborne infections to consumers. Exposure assessment was used to evaluate the level of hygiene practices in the selected restaurants with results indicating poor hygiene practice. The organisms isolated from the ready- to-eat cooked foods in the restaurants and their prevalence were, *Bacillus* species (12.50%), *Enterobacter* species (50.00%), *Streptococcus* species (12.50%), *Micrococcus* species (12.50%) and *Staphylococcus aureus* (12.50%), from the food contact surfaces (Hands of ready-to-eat food servers) were, *Enterobacter* species (36.36%), *Streptococcus* (9.09%), *Micrococcus* (9.09%) *Staphylococcus* (45.46%) and ready-to-use serving plates (*Streptococcus* (33.33%) and *Enterobacter* sp. (50%). Haemolysis test was employed as a phenotypic marker of pathogenicity for *Staphylococcus aureus* and *Streptococcus* sp. The prevalence of the pathogenic strains isolated from the ready-to-eat food were *Streptococcus* sp. (9.09%) and *Staphylococcus* sp. (45.46%) and that of the one isolated from the serving plate was *Streptococcus* sp. (33.33%).

Keywords: Microbial quality, Prevalence, Foodborne pathogens, Food poisoning, Food intoxication, Food contamination, Public Health, ready-to-eat food, Nigeria.

INTRODUCTION

Foodborne diseases include an array of illnesses triggered by ingestion of foods contaminated with microorganisms or chemicals **(WHO, 2014).** It has become a major cause of morbidity and mortality, posing a major public health challenge globally and contributing to a marked economic loss, reduction in quality of life and productivity **(Scharff, 2012**). Although there are no reliable estimates for the burden of diarrheal diseases alone, it however makes up a sizeable proportion of foodborne diseases. It is estimated that 1.9 million children die globally every year due to foodborne infections. **(WHO, 2014).** Cooked Foods are ideal culture medium for growth of many organism and these Ready-to- eat cooked food can be contaminated at any stage in the process of preparation or serving by foodborne pathogens capable of causing foodborne illnesses.

Foodborne pathogens are a leading cause of illness and death in developing countries amounting to billions of dollars in medical care and social costs. There is now an increased public health concern as a result of the frequent reports of food poisoning in relation to the potential presence of pathogenic organisms in food. This problem is aggravated by the massive changes in eating habits, mass food preparation, unsafe food storage conditions and poor hygiene practices amongst food handlers.

It is worthy of note that the microbial assessment of restaurants is not carried out as part of the inspection process of restaurants. This is probably due to the time limitations of traditional microbial analyses which takes up to 48 hours for culturing, isolation and subsequent evaluation. This is a major challenge since bacterial and viral contaminations are not detectable by visual assessment. The use of hygiene swabs and agar contact plates have shown that visual inspection is a poor indicator of cleaning **(Griffith *et al.*, 2000; Moore & Griffith, 2002).** Most restaurants even do not consistently clean certain surfaces outside the kitchen particularly true for furniture, equipment and other frequently used items. Some of the factors that contribute to the outbreaks of the foodborne disease are (i) inadequate food manipulation (ii) improper holding temperatures (failing to properly refrigerate food) (iii) inadequate cooking (iv) contaminated equipment (failure to clean and disinfect kitchen or processing plant equipment) and (v) poor personal hygiene (vii) preparing food a day or more before serving with improper holding and reheating (vii) cross contamination (from raw to cooked products) and (viii) adding contaminated ingredients to the previously cooked food. After foods are contaminated, the main factor is the appropriate temperature that supports the growth of the potentially hazardous microorganisms or its toxin production in the food.

Foodborne infections are caused by the ingestion of food containing pathogenic microorganisms which multiply within the gastrointestinal tract, producing widespread inflammation and have become a significant public health problem with major economic and social effects **(Altekruse and Swerdlow, 1996).** They have an incubation periods usually from 6 to 24-hour longer after ingestion and the causative organism may be identified by laboratory examination of the vomits, faeces, or blood of the infected individual and the suspected food **(Sudershan *et al.*, 2014).** It is usually characterized by diarrhea (which may be sometimes bloody) and vomiting. Symptoms may include fever and cold, headache, nausea, abdominal pain and cramps, distress, and weakness which in some instances may lead to respiratory arrest; other symptoms include signs of shock which include weak or rapid pulse or shallow breathing, confusion **(Mead *et al.*, 1999).** In developing countries, an estimated 70% of diarrheal episodes are associated with the ingestion of contaminated foods **(WHO, 2008).** Approximately 10 to 20% of foodborne disease outbreaks result from contamination of foods by the food handlers **(Gizaw *et al.*, 2014; Zain and Naing, 2002).**

The surge in urbanization has increased the frequency of eating of meals outside the home and despite this growth, there is little or no effective education or training for the food handlers or hygienic control of the food sold in many restaurants. The contamination of food with pathogens and its persistence, growth, multiplication and/or toxin production is now a significant public health concern. Despite this, only a fraction of all food-borne infections is ever diagnosed and officially reported or can be traced to a definite vehicle **(Lukinmaa *et al.*, 2004).**

The problems of food safety are considerably different between the industrialized and developing countries. The hygienic standard of food is usually assessed by the analysis of indicator microorganisms **(Forsythe and Hayes, 1988).** Some of the major microorganism groups are used alone or together to verify the microbiological characteristics and the hygienic condition of the food. Faecal coliforms like *Escherichia coli* are used as an indicator of the sanitary condition. It is a typical component of the faecal microbiota and its detection may show the potential occurrence of other microorganisms which could be even more pathogenic **(Sousa, 2005).** Several *E. coli* pathotypes have been implicated with the diarrheal illness, a major public health problem worldwide, with over two million deaths occurring each year **(WHO, 2002).**

Cross-contamination during food preparation is identified as a critical factor associated with the foodborne illness **(Wanyenya *et al.*, 2004).** Food handlers also play a role in ensuring food safety in food preparation, processing and storage chain. Complete or partial disregard for the hygiene measures by food handlers may result in food contamination and its attendant consequences. A sizeable proportion of foodborne illnesses could be prevented by the actions taken by the food handlers and there is the need to reduce these risks by taking steps to train the people involved in food handling **(Gilling *et al.*, 2001).** The cleaning procedures for the food contact surfaces should be evaluated with special attention given to the utensils used during the processing (Souza *et al.*, 2003). The U.S. Food and Drug Administration (FDA) recommends that food be

prepared with the least possible manual contact, with suitable utensils, and on the surfaces that prior to use have been cleaned, rinsed and sanitized to prevent cross-contamination **(FDA, 1978).**

Staphylococcus aureus is a major human pathogen with capabilities of causing a wide range of infections. Staphylococcal food poisoning is caused by the enterotoxigenic producing *S. aureus* and an important foodborne disease throughout the world. Many staphylococcal strains produce enterotoxin, the cause of the staphylococcal food poisoning. Enterotoxigenic strains have a high possibility of being implicated in food poisoning **(Bergdoll, 2000).**

Salmonella is a leading cause of human foodborne infections. The risk factors regarding the cross contamination of food include inapt cleaning and disinfection, manipulation of contaminated materials as such and (re)contaminated surfaces **(Berends *et al.*, 1998).** The isolation of microorganisms and subsequent cultivation on culture medium is the first basic step in more detailed investigations of the nature, source, and potential risk of the microbial contamination of the food **(Allen *et al.*, 2004**). This research was therefore carried out to evaluate the prevalence of the foodborne pathogens recovered from ready-to-eat food ad their contact surfaces from restaurants within and around Okada, Edo state, Nigeria.

MATERIALS AND METHODS

Study sites

Six restaurants selected for this study are all located in the university town of Okada headquarters to Ovia North-East Local Government Area of Edo State, Nigeria. It has an area of 2,301 km² and a population of 153,849 at the 2006 census.

Sampling

Ready-to-eat cooked foods were collected in sterile containers from the selected restaurants in Okada and were transported to the Laboratory of Igbinedion University, Okada for analysis within 6 hours of sample collection. One-gram portion of the food samples was used to prepare a 10-fold serial dilution to 10^{-5} in 1.5 % W/V sterile peptone water, after which 0.1 ml of each diluted suspension was subsequently spread on triplicate petri-plates containing sterile solidified media.

Collection of samples

The samples were collected from washed ready-to-use serving plates and the hands of food handlers in the sampled restaurants. Sampling was done by swabbing 25 cm^2 area of the contact surfaces with 5 sterile swab sticks according to the method specified by ISO 18593:2004 **(ISO, 2004).** The area of sampling was delimited by sterile templates (an improvised wire that was used to properly define the area of sampling). After swabbing, the swab sticks were put into a sterile container containing 10 ml of 2 % W/V sterile peptone water and was then transported to the Microbiology Laboratory in Igbinedion University, Okada, Edo State, Nigeria where they were analyzed within 6 hours of sampling. 0.1 ml of each suspension was subsequently spread on triplicate petri-plates containing sterile solidified media.

Isolation of microbes from food samples and contact surfaces of foods was performed by spread plating method **(APHA, 1998),** using both general purpose medium (nutrient agar) and selective/differential media (MacConkey agar complemented with crystal violet, and mannitol salt agar). After incubation of agar plates at 37ºC for 18 to 24 hours, distinct colonies seen on the plates were then enumerated and identified.

Identification and characterization of microbes

Phenotypic identification of microbes was performed according to standard methods **(Barrow and Feltham, 2003).** Expressed microbial morphological traits examined include the orientation, size, and pigmentation which were performed by visual inspection of microbial isolates on petri-plates, as well as cell wall characteristics which was performed by Gram staining of the isolates. Expressed biochemical traits examined include: the production of coagulase enzyme (coagulase test); the production of catalase enzyme (catalase test); the production of urease enzyme (urease test); biodegradation of tryptophan to produce indole (indole test); utilization of citrate as a sole carbon source (citrate test); production of stable acids from glucose fermentation (methyl red test); production of acetoin as the main end product with small quantities of mixed acids from glucose metabolism (Voges Proskauer test); and production of haemolysins (hydrolysis test)

Exposure assessment

Exposure assessment was used to quantitatively evaluate the impact of hygiene practices by restaurants situated in Igbinedion University and environs **(Cassini *et al.*, 2016).** The probability of exposure of consumers to pathogenic microbial species isolated from ready-to-eat food samples and food contact surfaces such as ready-to-use serving plates and hands of food handlers was deduced from the prevalence of pathogenic species present in these samples, while the concentration of the microbial isolates in the examined samples was used to deduce the extent of contamination in the restaurants. Parameters such as total aerobic viable counts, total coliform counts, and total *Staphylococcus* counts were also determined.

The microbial counts were performed using the spread plate method **(APHA, 1998).** Total aerobic viable counts (TAVC), total coliform counts (TCC), and total halophilic counts (THC) were carried out by spread plating on sterile nutrient agar, MacConkey agar supplemented with crystal violet, and mannitol salt agar respectively.

The microbial counts and prevalence (the presence of a single microbial isolate in a given sample) of the food samples were then calculated using the following equations:

$$Microbial\ counts = \frac{number\ of\ colonies\ counted}{volume\ of\ sample\ inoculated} \times dilution\ factor \quad (1)$$

Where:

Microbial counts were expressed as colony forming units per gram of food samples (cfu/g); Dilution factor (*d*) is expressed as the reciprocal of specific dilution counted ($\frac{1}{d}$).

$$Microbial\ prevalence = \frac{Number\ of\ a\ specific\ microbe\ isolated\ from\ the\ food\ samples}{Total\ number\ of\ all\ microbes\ isolated\ from\ the\ food\ samples} \quad (2)$$

The microbial counts and prevalence in the food contact surfaces were deduced using the following equations:

$$Microbial\ count = \frac{Number\ of\ colonies\ counted}{Area\ equivalent\ of\ the\ volume\ of\ inoculated\ sample} \quad (3)$$

Where:

Microbial count was expressed as colony forming units per square centimeter of contact surface (cfu/cm^2).

$$Area\ equivalent\ of\ the\ volume\ of\ inoculated\ sample = \frac{Volume\ of\ inoculated\ sample\ x\ Total\ Area\ of\ sampled\ surfaces}{Total\ volume\ equivalent\ of\ swabbed\ area} \quad (4)$$

Given that:

Volume of inoculated sample = 0.1 ml; Total area of sampled surface = 25 cm^2; Total volume equivalent of swabbed area = 10 ml

$$Microbial\ prevalence = \frac{Number\ of\ a\ specific\ microbe\ isolated\ from\ the\ food\ contact\ surface}{Total\ number\ of\ all\ microbes\ isolated\ from\ the\ food\ contact\ surface} \quad (5)$$

RESULTS

Table 1 Concentration of microbes on Contact surfaces (hands of ready- to - eat food servers and ready to use serving plates) in selected restaurants.

Sources of Microbes	Restaurant	Concentration of Microflora			*Hm	Confirmed Isolated Microorganism
		Mean TAVC (cfu/cm^2)	Mean TCC (cfu/cm^2)	Mean TSC (cfu/cm^2)		
Hands of ready- to - eat food servers	1	12.33 ± 0.33	1.33 ± 0.33	5.00 ± 0.58	γ β	*Enterobacter* species *Streptococcus* species
	2	3.33 ± 0.33	1.00 ± 0.00	3.00 ± 0.00	β γ	*Staphylococcus aureus* *Enterobacter* species
	3	22.67 ± 0.33	0.00 ± 0.00	20.33 ± 0.33	γ β	*Enterobacter* species *Staphylococcus aureus*
	4	10.67 ± 0.33	0.00 ± 0.00	8.00 ± 0.00	β γ	*Staphylococcus aureus* *Enterobacter* species
	5	6.67 ± 0.33	0.00 ± 0.00	5.00 ± 0.00	γ γ	*Enterobacter* species *Micrococcus* species
	6	14.33 ± 0.33	0.00 ± 0.00	12.33 ± 0.33	β β	*Streptococcus* species *Staphylococcus aureus*
Ready-to-Use serving plates	1	14.67 ± 0.33	0.67 ± 0.33	0.00 ± 0.00	γ	*Enterobacter* species
	2	5.67 ± 0.33	1.00 ± 0.00	2.33 ± 0.33	γ	*Enterobacter* species
	3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	γ	*Enterobacter* species
	4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	γ	*Saccharomyces* species
	5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	β	*Streptococcus* species
	6	12.67 ± 0.33	1.33 ± 0.33	10.33 ± 0.33	β	*Streptococcus* species

TAVC: Total aerobic viable counts; TCC: Total coliform counts; TSC: Total *Staphylococcus* counts; SE: Standard error; cfu: colony forming units; cm^2: square centimeter. Hm: Haemolysis test; β represents complete haemolysis; γ represents no haemolysis.

Table 2 Prevalence (%) of each microbe found in the Six Restaurants sampled in the Area

Source	Isolated Microbes	Prevalence
Ready to Eat Food	*Enterobacter* sp.	4/8 (50.00 %)
	Bacillus sp	1/8 (12.50 %)
	Streptococcus sp.	1/8 (12.50 %)
	Micrococcus sp.	1/8 (12.50 %)
	Staphylococcus aureus	1/8 (12.50 %)
Hands of ready – to - eat Food Servers	*Enterobacter* sp.	4/11(36.36 %)
	Streptococcus sp.	1/11(9.09 %)
	Micrococcus sp.	1/11(9.09 %)
	Staphylococcus aureus	5/11(45.46 %)
Ready-to -use Serving Plates	*Streptococcus* sp.	2/6(33.33 %)
	Enterobacter sp.	3/6(50.00 %)
	Saccharomyces sp.	1/6(16.67 %)

DISCUSSION

Exposure assessment was used to evaluate the level of hygiene practices by the restaurants sampled. The probability of exposure of consumers to pathogenic microbial species isolated from ready-to-eat food samples and food contact surfaces such as ready-to-use serving plates and hands of food handlers was calculated from the prevalence of pathogenic species present in these samples. The concentration of the microbial isolates reflected the degree of contamination in the restaurants. The total aerobic viable counts (TAVC) is an indicator of the overall hygiene status in the restaurants ranged from 5.67 cfu/cm^2 – 22.67 cfu/cm^2 and represented in Table 1. The total coliform counts (TCC) is an indicator of potential faecal contamination of ready-to-eat and contact surfaces in the restaurants ranged from 0.67 cfu/cm^2 – 1.33 cfu/cm^2. The total *Staphylococcus* counts (TSC) indicated the unsanitary food handling in the restaurants. These parameters are extensive measures of the degree of contamination in these restaurants. The ready to eat food sampled across the six restaurants were fried rice, soup and jollof rice. These menus were picked because they were high in demand in these restaurants as well being available in almost the restaurants. These ready-to-eat foods yielded a high mean concentration of TAVC and TCC, with only three of these restaurants having no TSC. The organisms isolated from the ready to eat foods in the restaurants and their prevalence were *Bacillus* species (12.50%), *Enterobacter* species (50%), *Streptococcus* species (12.50%), *Micrococcus* species (12.50%) and *Staphylococcus aureus* (12.50%) and presented in Table 2. *Bacillus* sp and *Staphylococcus* sp have also been isolated from similarly prepared ready- to- eat-foods in previous studies **(Nichols *et al*., 1999; Mensah *et al*., 2002; Idowu, 2006; Taulo *et al*., 2008 and Oranusi *et al*., 2013).**

The food contact surfaces (Hands of ready-to-eat food servers and ready-to-use serving plates) also had high microbial loads. The prevalence of the organism isolated from the hands of ready to eat food servers were *Enterobacter* sp. (36.1%), *Streptococcus* sp. (9.09%), *Micrococcus* sp. (9.09%) and *Staphylococcus aureus* (45.46%). This was in agreement with the result of **Almeida *et al*., (1995)** who worked on the microbiological analyses of food workers' hands and revealed a high possibility of cross contamination of food pathogens by food handlers. In their study, the food workers' hands showed aerobic mesophilic plate counts of up to 10^7CFU/hand and the presence of *S. aureus and Clostridium perfringens*. The ready-to-use serving plates had the following isolates *Streptococcus* (33.3%), *Enterobacter* (50%) and

Saccharomyces (16.1%). *Enterobacter* sp. and *Streptococcus* were isolated from all the three-sample source while *Staphylococcus* and *Micrococcus* were isolated from only the food and the hands of the food servers and handlers. The haemolysis test was employed as a phenotypic marker of pathogenicity. The prevalence of isolated organism positive for pathogenicity in the ready-to-eat food were *Staphylococcus aureus* (12.50%), *Streptococcus* spp. (12.50%), hands of the food handlers were *Streptococcus* sp (9.09%) and *Staphylococcus sp.* (45.46%) and the one isolated from the serving plate was *Streptococcus* sp. (33.33 %). The isolation *Enterobacter* an enteric is an indication of a possible faecal contamination of the food, their food contact surfaces and poor hygiene practices **(Little *et al*., 1998; Tambekar *et al*., 2007).** High concentrations of coliforms in food is often associated with food poisoning **(WHO, 1993).** Ready-to-eat foods cooked food must be free from microorgaisms as well as other contaminants as much as possible. The presence of any of these isolates establishes a potential health risk as these organisms are pathogenic and are often implicated in foodborne diseases **(Granum, 2005; Wagner, 2009; CFIA, 2009).**

CONCLUSION

The obtained results in this study reveal a high possibility of cross contamination of food from food handlers and contact surfaces sampled. Organisms like *Enterobacter* sp., *Bacillus* sp, *Streptococcus* sp., *Micrococcus* sp., *Staphylococcus aureus*, *Streptococcus* sp. were isolated from the food and contact surfaces. It is recommended that some preventive measures be adopted to avoid contamination of cooked food like the routine examination of cooked food in restaurants by the relevant authorities and consequent sanctions if minimum standard are violated, hygiene awareness for personnel who prepare and handle ready to eat cooked food, training on the adoption on the integration of Hazard Analysis Critical Control Point (HACCP) procedures into the food preparation and production process.

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