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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 or more so serving food at the wrong temperature that supports the growth of the potentially harmful microorganisms which may result in food contamination and its attendant consequences. The organisms isolated from the ready-to-eat cooked foods in the restaurants and their prevalence were, Bacillus species (12.50%), Enterobacter species (30.00%), 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 media for growth of many organisms 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; Street et al. 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 (viii) 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 (Souza, 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 (Wanenya 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 10⁴ 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 the washed ready-to-use serving plates and the hands of food handlers in the sampled restaurants. Sampling was done by swabbing 25 cm² 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 37°C 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 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:

\[ \text{Microbial counts} = \frac{\text{number of colonies counted}}{\text{volume of sample inoculated}} \times \text{dilution factor} \]  

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 \((d)\).

\[ \text{Microbial prevalence} = \frac{\text{total number of all microbes isolated from the food samples}}{\text{total number of all microbes isolated from the food samples}} \]  

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

\[ \text{Microbial count} = \frac{\text{number of colonies counted}}{\text{area equivalent of the volume of inoculated sample}} \]  

Where: Microbial count was expressed as colony forming units per square centimeter of contact surface (cfu/cm²).

\[ \text{Area equivalent of the volume of inoculated sample} = \frac{\text{volume of inoculated sample} \times \text{total area of sampled surface}}{\text{total volume equivalent of swabbed area}} \]  

Given that:

Volume of inoculated sample = 0.1 ml; Total area of sampled surface = 25 cm²; Total volume equivalent of swabbed area = 10 ml

\[ \text{Microbial prevalence} = \frac{\text{number of a specific microbe isolated from the food contact surface}}{\text{total number of all microbes isolated from the food contact surface}} \]
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/cm²) | Mean TCC (cfu/cm²) | Mean TSC (cfu/cm²) |            |
| Hands of ready-to-eat food servers | 1         | 12.33 ± 0.33       | 1.33 ± 0.33     | 5.00 ± 0.58        | γ  |
|                     | 2         | 3.33 ± 0.33        | 1.00 ± 0.00     | 3.00 ± 0.00        | β  |
|                     | 3         | 22.67 ± 0.33      | 0.00 ± 0.00     | 20.33 ± 0.33       | γ  |
|                     | 4         | 10.67 ± 0.33      | 0.00 ± 0.00     | 8.00 ± 0.00        | β  |
|                     | 5         | 6.67 ± 0.33       | 0.00 ± 0.00     | 5.00 ± 0.00        | γ  |
|                     | 6         | 14.33 ± 0.33      | 0.00 ± 0.00     | 12.33 ± 0.33       | β  |
| Ready-to-use serving plates | 1         | 14.67 ± 0.33      | 0.67 ± 0.33     | 0.00 ± 0.00        | γ  |
|                     | 2         | 5.67 ± 0.33       | 1.00 ± 0.00     | 2.33 ± 0.33        | γ  |
|                     | 3         | 0.00 ± 0.00       | 0.00 ± 0.00     | 0.00 ± 0.00        | γ  |
|                     | 4         | 0.00 ± 0.00       | 0.00 ± 0.00     | 0.00 ± 0.00        | γ  |
|                     | 5         | 0.00 ± 0.00       | 0.00 ± 0.00     | 0.00 ± 0.00        | β  |
|                     | 6         | 12.67 ± 0.33      | 1.33 ± 0.33     | 10.33 ± 0.33       | β  |

TAVC: Total aerobic viable counts; TCC: Total coliform counts; TSC: Total Staphylococcus counts; SE: Standard error; cfu: colony forming units; cm²: 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      |
|----------------------------|-----------------------|-----------------|
|                            | Enterobacter sp.      | 4/8 (50.00 %)   |
|                            | Bacillus sp.          | 1/8 (12.50 %)   |
| Ready to Eat Food          | Streptococcus sp.     | 1/8 (12.50 %)   |
|                            | Micrococcus sp.       | 1/8 (12.50 %)   |
|                            | Staphylococcus aureus | 1/16 (6.25 %)   |
| Hands of ready – to - eat Food Servers | Enterobacter sp. | 4/11(36.36 %)   |
|                            | Streptococcus sp.     | 1/1 (9.09 %)    |
|                            | Micrococcus sp.       | 1/1 (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/cm² – 22.67 cfu/cm² 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/cm² – 1.33 cfu/cm². 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 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; Iduw, 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 10⁶ CFU/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 Staphylococcus 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 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 microorganisms 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 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 not met. Increased 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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