Detection of Bacterial Pathogens in Ready-To-Eat Foods: Potential Public Health Hazard

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors SEO and CSJ designed the study and wrote the first manuscript. Authors SEO, CSJ, ICO and DAN carried out the study, analyzed data, read, revised and approved the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The right to obtain safe food is one of the most vital and fundamental human rights that must not be compromised or neglected; this is important because foodborne diseases can lead to prolonged disability and even death. Our study examined 28 samples of ready-to-eat foods, of which 27 samples (96.4%), contained bacterial contaminants. The bacterial pathogens isolated include Escherichia coli (50%), Salmonella spp (75%) and Staphylococcus aureus (85.7%). All the samples of jollof rice (100%), bean porridge (100%) and eba (100%) were contaminated while 85.7% of egusi soup samples contained bacterial contaminants. The presence of these bacterial pathogens in the ready-to-eat foods poses huge risk to public health. It calls for immediate and sustainable action to prevent the possibility of foodborne disease out-break and intoxication capable of harming public health and socio-economic development.

Keywords: Bacterial pathogens; Escherichia coli, public health; safe food; Salmonella; Staphylococcus aureus.

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1. INTRODUCTION
The right to access and obtain safe food is one of the most vital and fundamental human rights that must not be violated, obstructed, compromised or neglected by any responsible individual, group, Government or Non-Governmental Organization involved in food and food-related matters. Citizens of all nations are entitled to have access to sufficient amount of safe food to improve health and promote socio-economic development [1]. Availability and accessibility to sufficient amount of safe food and nutritious food is Paramount to sustaining life, promoting and improving good health [1,2]. Contaminated food harbouring pathogenic bacteria, viruses, parasites or chemical substances has the potential to cause over 200 diseases ranging from diarrhoea to cancers; diarrhoeal diseases cause estimated 230,000 deaths annually with another 550 million people falling ill [1]. Foodborne diseases may lead to prolonged disability and death as well as impeding socio-economic development by straining healthcare systems, and harming national economies, tourism and trade [1]. The consumption of unsafe food could be a source of foodborne disease outbreaks and diverse public health hazards.

Ready-to-eat foods can be raw or cooked, hot or cold foods consumed without further heat treatment [3]. In Nigeria, ready-to-eat foods are usually sold by vendors and hawkers whose hygiene practices and sanitation knowledge are unknown. Others establish selling points where they often cook, package and sell ready-to-eat foods. In most cases, ready-to-eat foods are prepared in environments with compromised sanitation and by individuals who do not possess adequate knowledge of hygiene and sanitation practices. Hence, food handlers may not follow standards thereby creating route for microbial contamination of foods.

The current study was designed and executed to determine the microbial quality of ready-to-eat foods sold within Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria in order to create public health awareness and mitigate the incidence of foodborne diseases while encouraging proper food handling, preparation and distribution.

2. MATERIALS AND METHODS

2.1 Study Area
The study was conducted within Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

2.2 Sample Collection
A total of 28 samples of ready-to-eat foods (including 7 samples each of Jollof rice, Bean porridge, Eba and Egusi soup), were purchased from 5 food selling points (bukaterian), within Michael Okpara University of Agriculture, Umudike, Abia State. These samples were collected into separate sterile plastic containers and transported to the Microbiology laboratory immediately after collection in a cool-box for bacteriological examination. Samples were screened within 3 hours of collection. The research was carried out between June and July, 2021.

2.3 Media Used
The culture media used include Nutrient agar for preservation of pure bacterial isolates, Eosin methylene blue agar (EMB) for Escherichia coli count, Mannitol salt agar for Staphylococcus aureus count, Salmonella-Shigella agar for Salmonella spp count. All culture media used were prepared following Manufacturers' instructions.

2.4 Bacteriological Analysis

2.4.1 Preparation of samples for analysis
Exactly 10g of each sample was measured and aseptically introduced into a mixer with a sterile spatula. Thereafter, it was homogenized by adding 90ml of peptone (water 0.1%). About 1ml portion of each homogenate was used to prepare ten-fold serial dilutions up to 10−7 with peptone water.

2.4.2 Escherichia coli count
Escherichia coli was enumerated on Eosin methylene blue (EMB) agar. The inoculated plates were incubated at 37°C for 24-48 hours. Colonies with greenish metallic sheen on Eosin methylene blue agar were counted as Escherichia coli. Biochemical tests such as indole, methyl Red, Voges-Proskauer and Citrate (IMViC) tests were conducted on colonies that demonstrated shiny-metallic green to identify Escherichia coli.

2.4.3 Total Staphylococcus aureus count
Staphylococcus aureus was enumerated on Mannitol salt agar (MSA). Inoculated plates were incubated at 37°C for 24 hours. Yellow colonies
on Mannitol salt agar were taken as *Staphylococcus aureus*. Biochemical tests; catalase and coagulase were performed to identify *Staphylococcus aureus*.

2.5 **Salmonella** spp Count

About 0.1ml of each sample was spread plated on *Salmonella-Shigella* agar plates and incubated at 37°C for 48 hours. Colonies with black centres were assumed to be *Salmonella* spp.

### Table 1. Bacterial contamination of samples

| Sample          | Number Examined | Number Contaminated |
|-----------------|-----------------|---------------------|
| Jollof rice     | 7               | 7 (100%)            |
| Bean porridge   | 7               | 7 (100%)            |
| Eba             | 7               | 7 (100%)            |
| Egusi soup      | 7               | 6 (85.7%)           |
| Total           | 28              | 27 (96.4%)          |

3. RESULTS

Food safe for human consumption should not contain microbial pathogens with great potential to cause public health problems. A total of 28 samples of ready-to-eat foods were thoroughly examined, of which 27 (96.4%) samples were contaminated by bacterial pathogens (Table 1). All the samples of jollof rice (100%), bean porridge (100%), and eba (100%) were contaminated. Of the 7 samples of egusi soup screened, 6 (85.7%) contained bacterial contaminants. Table 2 shows that 50% of the total samples examined contained *Escherichia coli*, 85.7% contained *Staphylococcus aureus* while *Salmonella* spp were isolated from 21 (75%) samples.

*Escherichia coli* ranged from $0.3 \times 10^3$ to $2.3 \times 10^4$ (CFU/g) in jollof rice, *S. aureus* ranged from $0.9 \times 10^3$ to $1.9 \times 10^6$ (CFU/g) while *Salmonella* spp ranged from $3.1 \times 10^5$ to $1.6 \times 10^6$ (CFU/g), (Table 3). In bean porridge, *E. coli* ranged from $0.2 \times 10^3$ to $2.5 \times 10^3$ (CFU/g), *S. aureus* ranged from $2.6 \times 10^3$ to $1.27 \times 10^6$ (CFU/g), while *Salmonella* spp ranged from $2.8 \times 10^3$ to $3.1 \times 10^6$ (CFU/g), (Table 4). Table 5 presents the bacterial load from eba samples; *E. coli* ranged from $0.3 \times 10^3$ to $1.0 \times 10^3$ (CFU/g), *S. aureus* ranged from $3.7 \times 10^4$ to $7.8 \times 10^6$ (CFU/g) while *Salmonella* spp ranged from $1.5 \times 10^3$ to $4.9 \times 10^4$ (CFU/g). *E. coli* ranged from $0.2 \times 10^3$ to $1.8 \times 10^3$ (CFU/g) in egusi soup, *S. aureus* ranged from $1.3 \times 10^3$ to $3.3 \times 10^4$ (CFU/g), while *Salmonella* spp ranged from $1.9 \times 10^4$ to $7.1 \times 10^5$ (CFU/g). In general, *E. coli* ranged from $0.2 \times 10^3$ to $2.5 \times 10^3$ (CFU/g), *S. aureus* ranged from $0.9 \times 10^3$ to $1.27 \times 10^6$ while *Salmonella* spp ranged from $1.5 \times 10^3$ to $1.6 \times 10^7$ (CFU/g).

### Table 2. Percentage (%) occurrence of isolates

| Isolates  | Jollof rice | Bean porridge | Eba | Egusi soup | Total |
|-----------|-------------|---------------|-----|------------|-------|
| *E. coli* | 4 (57.1)    | 4 (57.1)      | 3 (42.9) | 3 (42.9) | 14 (50) |
| *S. aureus* | 6 (85.7) | 7 (100)       | 7 (100) | 4 (57.1) | 24 (85.7) |
| *Salmonella* spp | 6 (85.7) | 7 (100)       | 3 (42.9) | 5 (71.4) | 21 (75) |

4. DISCUSSION

The implications of consuming food contaminated by microbial pathogens are enormous; it can lead to numerous abnormal health conditions and even death. Foodborne pathogens are causing a great number of diseases with significant effects on human health and economy [4]. We found that 96.4% of ready-to-eat foods examined were massively contaminated by bacterial pathogens particularly *Escherichia coli* (50%), *Staphylococcus aureus* (85.7%), and *Salmonella* spp (75%). Our finding agrees with existing studies by Temesgen et al [5] and Derbew et al [6]; both which isolated *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., *Proteus* spp., *Klebsiella* spp., *Citrobacter* spp., and *Enterobacter* spp., from street vended foods in Hawassa and Gondar, Ethiopia. Similar studies in Nairobi County, Kenya, and South Africa [7,8,9] isolated pathogenic bacteria from street-vended foods which include *Salmonella*, *E. coli*, *Campylobacter jejuni* and *S. aureus*. 
Table 3. Bacterial contamination of jollof rice (CFU/g)

| Sample No. | E. coli count (CFU/g) | S. aureus count (CFU/g) | Salmonella spp count (CFU/g) |
|------------|-----------------------|-------------------------|----------------------------|
| JR1        | 0.9x10^3              | 1.9x10^6                | 1.02x10^5                  |
| JR2        | -                     | 2.3x10^5                | 1.6x10^7                  |
| JR3        | -                     | 1.2x10^5                | -                         |
| JR4        | -                     | -                       | 2.8x10^4                  |
| JR5        | 1.5x10^3              | 2.7x10^4                | 3.1x10^3                  |
| JR6        | 0.3x10^3              | 0.9x10^3                | 6.7x10^5                  |
| JR7        | 2.3x10^3              | 3.3x10^3                | 3.5x10^5                  |

JR= Jollof rice; - = Not detected

Table 4. Bacterial load on bean porridge (CFU/g)

| Sample | E. coli count (CFU/g) | S. aureus count (CFU/g) | Salmonella spp count (CFU/g) |
|--------|-----------------------|-------------------------|----------------------------|
| BP1    | 2.5x10^3              | 9.8x10^5                | 3.1x10^6                  |
| BP2    | 0.2x10^3              | 1.27x10^6               | 4.2x10^3                  |
| BP3    | -                     | 3.5x10^4                | 2.8x10^3                  |
| BP4    | -                     | 7.2x10^4                | 3.7x10^4                  |
| BP5    | 0.5x10^3              | 4.3x10^4                | 5.6x10^3                  |
| BP6    | -                     | 2.6x10^3                | 2.1x10^4                  |
| BP7    | 1.3x10^3              | 3.9x10^4                | 4.0x10^4                  |

BP= Bean porridge; - = Not detected

Table 5. Bacterial load on Eba sample

| Sample | E. coli count (CFU/g) | S. aureus count (CFU/g) | Salmonella spp count (CFU/g) |
|--------|-----------------------|-------------------------|----------------------------|
| E1     | 0.3x10^3              | 7.8x10^5                | 4.9x10^4                  |
| E2     | -                     | 5.1x10^5                | -                         |
| E3     | -                     | 4.3x10^5                | -                         |
| E4     | -                     | 3.7x10^4                | -                         |
| E5     | 0.8x10^3              | 6.0x10^5                | 1.5x10^3                  |
| E6     | 1.0x10^3              | 7.2x10^5                | 2.0x10^3                  |
| E7     | -                     | 3.5x10^5                | -                         |

E=Eba sample; - = Not detected

Table 6. Bacterial load on Egusi soup (CFU/g)

| Sample | E. coli count (CFU/g) | S. aureus count (CFU/g) | Salmonella spp count (CFU/g) |
|--------|-----------------------|-------------------------|----------------------------|
| ES1    | 0.4x10^2              | 3.3x10^4                | 7.1x10^5                  |
| ES2    | -                     | -                       | 3.6x10^4                  |
| ES3    | -                     | -                       | 1.9x10^4                  |
| ES4    | 1.8x10^3              | 3.7x10^3                | 6.2x10^4                  |
| ES5    | 0.2x10^3              | 2.4x10^4                | 4.3x10^4                  |
| ES6    | -                     | 1.3x10^3                | -                         |
| ES7    | -                     | -                       | -                         |

ES=Egusi soup; - = Not detected

The high level of bacterial contamination of ready-to-eat foods investigated by this study is worrisome and calls for immediate intervention to prevent foodborne disease out-breaks and intoxications. This is paramount because a great number of students, staff, manual labourers and visitors to the University regularly purchase and consume ready-to-eat foods from these food-selling points. These bacterial contaminants render the foods unsafe and hazardous for human consumption. Studies in India and Nigeria [10-17] had isolated various bacterial pathogens
from ready-to-eat foods; bacterial organisms that were isolated include species of *Proteus*, *Pseudomonas*, *Bacillus*, *Salmonella*, *Klebsiella*, *Enterococcus* and *Penicillium*. *Escherichia coli* and *Staphylococcus aureus* were also isolated of which some exhibited antibiotic resistance. Consumption of unsafe ready-to-eat foods pose great threat to socio-economic development and endangers public health; hence, there is need to ensure that ready-to-eat foods served to the University community are safe and readily accessible.

5. CONCLUSION

This study revealed that some ready-to-eat foods sold within Michael Okpara University of Agriculture, Umudike were widely contaminated by bacterial pathogens thereby making it unsafe for human consumption. Ready-to-eat foods should be microbiologically safe. The source of contamination of these foods can be linked to lack or poor hygiene practices exhibited by food-handlers whether during preparation or selling. The use of contaminated water and containers during food preparation, packaging and distribution can be potential sources of microbial contamination. Touching eba with bare hands during preparation and packaging to check its softness or thickness, using unclean container for preparation as well as using unclean turning-stick could transmit bacterial pathogens into the food sample. Bean porridge and egusi soup could be contaminated by exposure, addition of unclean water to reduce thickness and increase quantity. Stirring with unclean spoon or turning-stick could transmit bacterial pathogens into ready-to-eat foods. Jollof rice could be contaminated by contact with human hands and environmental objects. Adding contaminated water shortly before removing from heat could be a potential source of contamination because some of the bacterial pathogens might survive. Using unclean spoon to fetch jollof rice could be another means of bacterial contamination. Generally, food-handlers who are carriers of any of these bacterial pathogens could transmit it to ready-to-eat foods served to the public. Handling paper currency while serving or handling ready-to-eat foods could be an unrecognized means through which bacterial pathogens can contaminate ready-to-eat foods.

6. RECOMMENDATION

We recommend that food handlers within the University environment should be regularly checked for hygiene practices and level of environmental sanitation should be monitored. Ready-to-eat foods sold to the University community should be monitored to ensure that they are microbiologically safe.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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