Microbiological Safety Evaluation of Ready to Eat Shrimps and Snails Sold Along Lagos-Shagamu Expressway, Nigeria

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Abstract: Vending of ready-to-eat foods (RTFs) along the high ways is a common practice associated to youth unemployment in Nigeria as in some other countries of the world. RTFs sold on streets have been implicated in foodborne illnesses and its attendant economic loses. The microbial quality of ready to eat shrimps (*Penaeus monodon*) and snails (*Achatina maginata*) was investigated in three vending sites along Lagos – Shagamu express road. Sixty samples (30 each of shrimps and snails) were analyzed for microbial counts and for organisms of public health importance. The mean total aerobic plate count cfu/g samples ranged from 1.3×10^4 - $3.5 \times 10^4$and 3.8×10^4 - 5.6×10^5 in abdomen and capitulum of shrimps and 5.6×10^5 - 7.5×10^5 in snails. The samples were contaminated with coliforms and fungi at counts ranging from 1.0×10^2 – 2.3×10^2 and 2.0×10^2 – 8.3×10^3. The microbial isolates identified included species of *Bacillus* (31%), *Staphylococcus aureus* (18%), *Klebsiella* (13%), *Escherichia coli* (6%), *Salmonellae* (2%). Fungal species included *Aspergillus, Mucor, Geotrichum, Fusarium, Paecilomyces, Rhizopus* and *Cladosporium.* The presence of coliforms which are indicator organisms of feacal contamination and *Salmonellae* which are enteric pathogens is a reflection of the sanitary quality of the processing of these food products. This result is informative with respect to public health hazard and calls for urgent improvement in hygiene practices by food processors and vendors. Adoption of hazard analysis and critical control point (HACCP) principles in seafood preparation should be encouraged to prevent possible foodborne illnesses and outbreaks.

***Key words*:** Ready-to-eat foods; foodborne illnesses; coliform; enteric pathogens; HACCP

Introduction

All edible aquatic life may be referred to as seafood. Seafood contributes a significant proportion to the world's food supply and income. Over 70 million tons of seafood is harvested world-wide annually and estimates report consumption averages of 13 kg per person per annum for fish and shellfish [1]. The shrimp landing in Nigeria was estimated at 4,500 to 5,000 tons with annual export earnings of US$29.6M – 50M [2; 3]. Seafood is a good source of protein, it accounts for 14–16% of the animal protein consumed world-wide and over one billion people rely on seafood as their primary source of animal protein [4].

Research has shown that the nutrients and minerals in sea foods can make improvements in brain development and reproduction and has highlighted the role for seafood

in the functions of the human body [5; 6]. According to the U.S. Food and Drug Administration, certain varieties of seafood are excellent sources of vitamins A, C, D and E, calcium, iron and potassium. Eating seafood offers many health benefits, reduces risk of stroke or heart attack largely due to the effects of Omega-3 fatty acids, potentially lower the risk for colon, breast or prostate cancer, and pregnant women can benefit from the high levels of protein, zinc, iodine and iron found in seafood [7; 8; 9].

Seafood is a part of a healthy diet but its consumption is not risk free. Seafood allergy in sensitized individuals could be life threatening [10] and it is responsible for an important proportion of most food-borne illnesses and outbreaks [11-14]. Seafood-associated infections are caused by a variety of bacteria, viruses, and parasites that might have gotten into the food from different sources ranging from rearing or harvesting, processing, transport to food handlers including the consumer. These agents are acquired from three sources: (1) mainly fecal pollution of the aquatic environment, (2) to a lesser extent, the natural aquatic environment, and (3) industry, retail, restaurant, or home processing and preparation [15].

Iwamoto *et al*. [11] reported that 188 outbreaks, 4020 illnesses, 161 hospitalizations and 11 deaths in the United States, from 1973 to 2006 were caused by bacterial, viral or parasitic agents in which seafood was identified as the single food vehicle. Microorganisms implicated in foodborne illness associated with seafood included but not limited to species of *Vibrio*, *Salmonella*, *Shigella*, *Campylobacter*, *Listeria* and pathogenic *E. coli*, *Norovirus/ Norwalk virus*, *Rotavirus*, *Hepatitis* E and A viruses, *Cryptosporidium*, *Cyclospora cayetanensis*, *Giardia lamblia*, *Entamoeba histolytica* and some fungal species [11-14; 16-18].

Foodborne illness associated with seafood can be prevented by using safe food handling practices including washing hands, utensils, and cooking surfaces often, cooking seafood to a minimum of 145°F for 15 seconds, keeping raw and cooked seafood separate to avoid cross-contamination, storing seafood in the refrigerator below 40°F or in the freezer below 0°F; all and more of these measures are enshrined in the application of the Hazard Analysis and Critical Control Points (HACCP) system which is one of the most effective tools in food safety, it is a simple and efficient way to ensure food safety, predict, resist and prevent food borne illnesses before they occur [15; 19; 20]. This work was designed to determine the microbial load of ready to eat shrimps and snails sold along the busy Lagos- Shagamu expressway and thus, create awareness for consumers on safety of these RTE seafood purchased along the expressway.

Materials and Methods

Sample sources and collection

Three stop-over terminals for travelers along the Lagos- Shagamu expressway and popularly known for the ready to eat foods (RTFs) vending activities were chosen for sample collection. These spots/ terminals included Berger, on the Lagos axis, Ibafo on the midway and Mowe on the Shagamu end of the highway.

Thirty samples each of ready to eat shrimps and snails were collected (ten samples of each food were randomly purchased from each vending site). The samples were collected as packaged for customers and aseptically placed in sterile polyethylene packs and transported to the laboratory in cold packs for further analyses. Samples were collected and analyzed between January and April, 2014.

Sample analysis

The shrimps were aseptically dissected into capitulum and abdomen with the aid of a sterile scalpel. The capitulum and the abdomen were cultured separately. Twenty five grams of each food sample (capitulum of shrimps, abdomen of shrimps and snails) were blended in Stomacher lab blender and homogenized in 225mL of sterile peptone water (Oxoid, England).

Serial dilutions of the homogenates were made to 10^{-4} and 0.2mL of each dilution was spread inoculated onto triplicate media plates of Nutrient agar (for total aerobic plate count), Sabouraud Dextrose agar (for fungal count), Mannitol Salt agar (for isolation of *S. aureus*), Eosin Methylene blue agar (for coliform count), *B. cereus* medium and Salmonella Shigella agar (for isolation of salmonellae and Shigella following 24h sample pre-enrichment in Selenite-F broth) (all the media were from Oxoid, England). EMB broth (Sigma-Aldrich, USA) in capped test tubes with inverted Durham tubes was inoculated with a gram of samples for coliform test. The culture plates and tubes were incubated for 24 to 48 h at 37°C. A plate of the EMB cultures was however, incubated at 44°C for faecal coliform *E. coli* isolation, while the Sabouraud Dextrose agar (SDA) plates were incubated at laboratory room temperature 28±2°C for 3 to 5 days. Colony counts were made from plates of appropriate dilutions at the end of incubation periods. Cultural characteristics of the colonies were also recorded to aid identification.

Coliform test

Samples with gas formation indicated by the Durham tubes and/ or colour change of dye in the medium were reported as positive for presumptive coliform test. Confirmatory coliform test was carried out by plating out positive presumptive test cultures on EMB agar plates and incubating overnight at 37°C. The presence of characteristic greenish metallic sheen black colonies typical of *E. coli* or brown mucoid colonies

characteristics of *E. aerogenes* and which are Gram negative non-spore forming was considered a positive confirmatory test. The colonial growths were treated for completed test and stored at 4°C for further characterization.

Microbial Colony Count and Identification of Isolates

At the end of incubation time, colonies were enumerated using colony counter (Gallenkamp, England), total counts were expressed as colony forming units per gram of sample (cfu/g). Pure cultures of isolates for characterization were obtained by repeated subculture on appropriate medium. Preliminary identification of bacterial isolates was based on morphological characteristics of colonies, microscopy and biochemical tests including catalase production, indole test, methyl red, Voges-Proskauer, citrate utilization, coagulase, oxidase and urease production, gelatin liquefaction, starch hydrolysis, fermentation of sugars, temperature and salt tolerance tests and motility test. The Biomerieux© sa API system with reference to standard identification manuals was employed in the further identification of the bacterial isolates [21; 22]. Fungal isolates were identified based on morphological characteristics and microscopy with reference to standard identification keys and atlas [23-25].

Statistical Analysis

All data from colony counts are presented as mean and standard deviation. The level of significance in differences of means was determined by DMR test using SPSS 20.0 software for windows

Results

The mean microbial population for total aerobic plate count, coliform count, and fungal count of the shrimps and snails food samples from the three vending sites reveals that TAPC of snails are significantly different from the TAPC obtained from capitulum and abdomen of shrimps. Similarly, TAPC of samples of snails from Ibafo terminal were significantly different from counts of samples from other sampling terminals (Table 1). The table 1 also shows that coliform counts from shrimp capitulum obtained from Ibafo were significantly different from that of shrimp abdomen and snails from Mowe and Berger. The fungal counts from the capitulum of shrimp samples were significantly different from that of snails and samples of shrimp abdomen. Similarly, fungal counts of shrimps from Ibafo and Mowe were significantly higher than counts from Berger sampling site.

Table 2 shows the counts for *Staphylococcus* and *Salmonellae* in samples from the three terminals. The table revealed that the *S. aureus* from samples of snail obtained from Ibafo were significantly higher than *S. aureus* counts from other samples. The capitulum of shrimp has significant higher *S. aureus* and *Salmonellae* counts compared to

samples of shrimp abdomen and snails.

Fig.1 presents the percentage occurrences of bacterial and fungal isolates from all the food samples. It shows that species of *Bacillus, Staphylococcus* and *Klebsiellae* are the predominant bacteria isolates while *Aspergillus, Geotrichum* and *Mucor have* the highest percentage of occurrences as fungi.

Discussion

All the samples analyzed in this study had microbial loads below 10^6, with a TAPC ranging from 10^4 to 10^5 $cfug^{-1}$ of samples, but for some samples with salmonellae and coliforms, it could be said that most of the ready-to-eat shrimps and snails (sea foods) sold along Lagos-Shagamu expressway are of acceptable microbial quality. The ICMSF [26], Microbiological quality guide for ready-to-eat foods [27] states that ready-to-eat foods with heterotrophic plate counts of 10^3 are acceptable and 10^5 are of tolerable microbial quality. Although in some countries zero (0) bacteria $25g^{-1}$ of sample is the acceptable microbiological level in cooked crustaceans [28].

The aerobic plate counts could be contaminants from foods own flora that escaped destruction by processing techniques or post process contaminants from processing environment, water, utensils, and food personnel [15; 29; 30].

The presence of coliforms in some of the shrimp and snail samples could be explained to mean possible contamination of products by animals or human faecal materials. Coliforms are indicator organisms connoting that their presence could imply possible presence of other enteric pathogens. Contamination with coliforms could be from the personnel (food processors and vendors) as the samples are often packed and arranged with bare hands in white cellophane or hawked in small bowls for customers to select with toothpicks or fork. The water for processing and utensils could be a source for sample contamination with coliforms [31; 32]. The environment of the food vending terminals (Berger, Ibafo and Mowe) could contribute to coliform contamination [30], animals in their flocks is a common scene as cattle, goat, ram, sheep, donkey, chicken, etc. are moved through these terminals to nearby markets for sale. The growth of coliforms at 44-45°C incubation indicates that some of the coliforms are of feacal rather than environmental origin. Effective application of good manufacturing practices and HACCP is necessary to prevent coliform contamination.

Bacillus and fungal species are known to be spore formers and common environmental contaminants; this could explain their presence in the shrimp and snail samples. *B. cereus,* a common food borne pathogen, was not isolated from the samples analyzed. Majority of *Bacillus* and fungal species are food spoilage organisms

or opportunistic pathogens, thus, in the absence of known pathogens like *B. cereus* and *B. anthracis* the presence of other *Bacillus* species must be controlled to reduce possible spoilage activities. Fungi such as *Aspergillus* and *Fusarium* species are known to produce deleterious mycotoxins under favourable conditions, their presence in RTE shrimps and snails must not be treated with levity considering the fact that some of the products (shrimps and snails) are hawked for days until they are sold and these products are nutritionally rich to support the proliferation of these fungi in growth and possible mycotoxin(s) production. Some species of *Rhizopus* and *Mucor* have been implicated as opportunistic agents of infections, specifically, in the immunocompromised [33].

Staphylococcus aureus and *S. epidermidis* are normal flora of human; this could explain the possible contamination of the samples from personnel. Cross contamination from equipment and food contact surfaces are likely avenues through which shrimp and snail samples could have been contaminated. Enterotoxin producing strains of *S. aureus* are known to cause food poisoning [34-38], there is therefore need to control RTEFs from S. *aureus* contamination.

The presence of *Salmonellae, Klebsiella, E. coli* and other enterobacteria is an indication of faecal contamination of some of the samples [39]. *Salmonellae* are causative agents in salmonellosis often associated with consumption of contaminated foods and drinks [13; 16; 17]. Pathogenic strains of *E. coli,* specifically, *E. coli* O157: H7 have been implicated in food borne infection outbreaks [40]. *Klebsiellae, Proteus, Pseudomonas* and some other enterobacteria have been implicated as opportunistic pathogens specifically in the immunocompromised [41; 42]. The presence of these enterobacteria in some of the RTE shrimps and snails is a cause for concern. Education and training of the personnel, effective application of GMP and HACCP are imperative to making these products completely safe for human consumption.

The capitulum of shrimps had significantly higher levels of contamination compared to the abdomen; this could be attributed to the organism's feeding mode of straining out small particles including bacteria from water [26]. Though the counts recorded for the samples in this study are generally $<10^6$ $cfug^{-1}$ of samples, the presence of more *salmonellae, S. aureus* and coliforms in the capitulum suggest that it might be safer to consume only abdomen of shrimps while the capitulum be channeled for other useful products [43-45].

Conclusion

Ready-to-eat shrimps and snails sold along the highway had microbial counts $<10^6$ indicating safe level of contaminants for human consumption. However, the presence

of coliforms, *Salmonellae, S. aureus*, and some other enterobacteria signify faecal contamination of some samples and thus they are not fit for human consumption. Food safety enlightenment campaigns for the vendors and processors of RTEFs is necessary, the consumers should demand for better handling and packaging of product, by so doing these personnel will improve on hygiene measures in dealing with the products and thus make it safe for consumption.

Table 1: Mean microbial counts of RTE shrimps and snails ($cfug^{-1}$) sample

Sample Site	Capitulum of shrimps			Abdomen of shrimps			Snail		
	TAPC	Coliform count	Fungal count	TAPC	Coliform count	Fungal count	TAPC	Coliform count	Fungal count
Berger	3.8×10^{4a}	1.0×10^{2a}	6.5×10^{2a}	1.3×10^{4b}	7.0×10^{1a}	2.0×10^{2b}	5.9×10^{5c}	1.2×10^{2a}	2.0×10^{2b}
Mowe	3.9×10^{4a}	1.3×10^{2a}	8.0×10^{2c}	1.5×10^{4b}	NG	5.5×10^{2d}	5.6×10^{5c}	1.3×10^{2a}	2.8×10^{2b}
Ibafo	5.6×10^{5c}	2.3×10^{3b}	8.3×10^{3e}	5.3×10^{4d}	1.2×10^{2a}	5.6×10^{2d}	7.5×10^{5e}	1.2×10^{2a}	2.7×10^{2b}

abcde: Values with different alphabet superscript down the column and across the row for same count are significantly different

Table 2: Mean *S. aureus* and *salmonellae* counts of RTE shrimps and snails ($cfug^{-1}$) sample.

Sample Site	Capitulum of shrimps		Abdomen of shrimps		Snails	
	Staphylococcal count	*Salmonellae* count	Staphylococcal count	*Salmonellae* count	Staphylococcal count	*Salmonellae* count
Berger	2.9×10^{3a}	1×10^{1a}	4.0×10^{1b}	NG	3.8×10^{2c}	NG
Mowe	1.8×10^{2d}	1×10^{1a}	1.6×10^{3e}	NG	1.7×10^{2d}	NG
Ibafo	5.7×10^{2f}	1.1×10^{2b}	2.9×10^{2g}	1.1×10^{1a}	3.6×10^{3h}	1.0×10^{1a}

abcdefgh: Values with same alphabet superscript down the column and across the row for same count are not significantly different

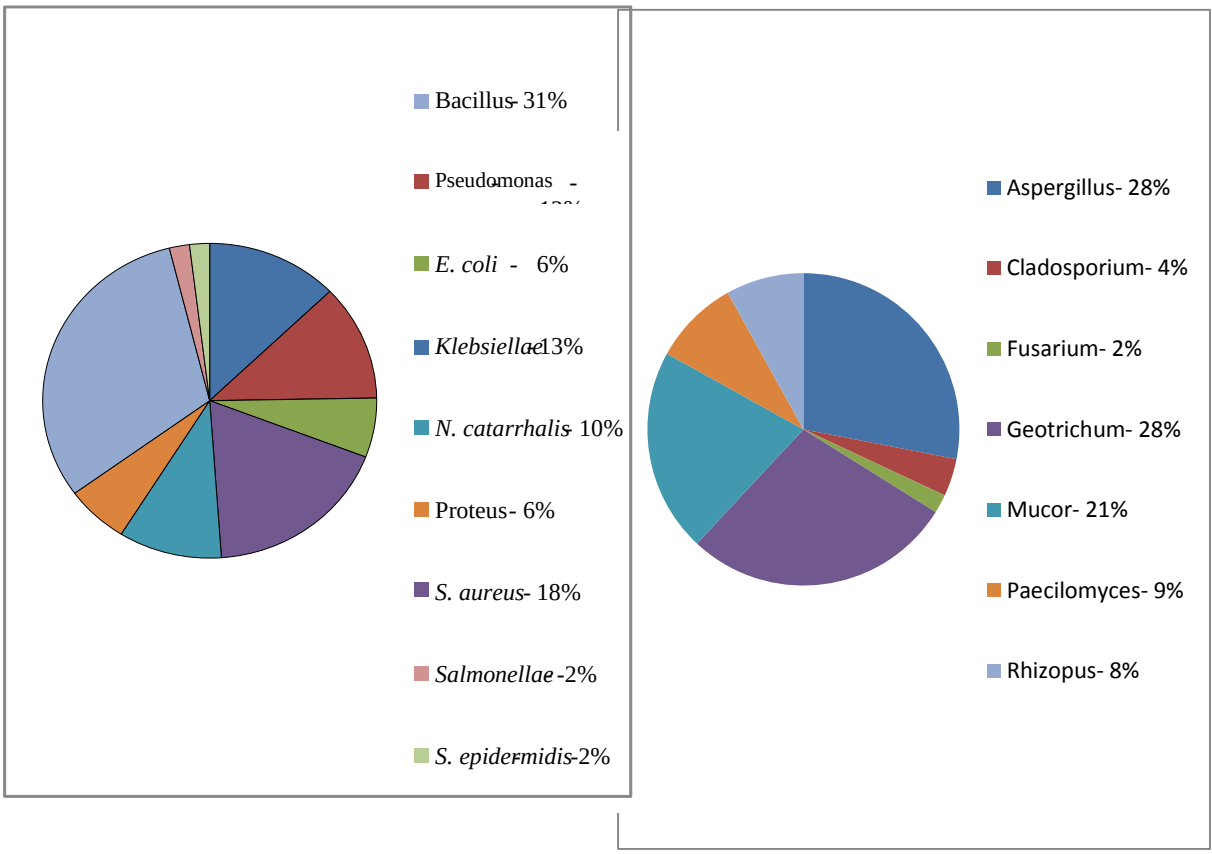

Figure1: Percentage occurrences of Bacterial and Fungal isolates from shrimp and snail samples

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