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Article

Assessment of Microbiological Quality of Ready to Eat Food Served in Ships Along Warri, Koko and Port Harcourt Water Ways, Nigeria

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Abstract: Background: Food borne outbreaks have been associated with sourcing unsafe food. Therefore, the first preventative strategy should be to source safe food. Even if the sourced food is safe, measures need to be put in place to ensure that it remains safe during the transfer, storage, preparation and serving activities that follow. An understanding of the ship food supply and transfer chain will help to illustrate the points at which the food can become contaminated en route to the point of consumption. **Objectives:** The study was conducted in selected sea port in the core Niger Delta to assessed the microbiological quality of food served at different ship galley to crew and passengers and compered it to standard. **Methods:** Samples of food were taken from three (Port Harcourt Area one (PHSP), Warri (WSP) and Koko (KSP)) seaports within the South-South zone for laboratory analysis to uncover food spoilage microorganisms capable of causing disease outbreak among ship which could result to Trans border diseases. Eleven samples of different ready to eat food were collected from the locations, which included cooked rice; fried fish, irish potato porridge, vegetable soup, griki, pepper soup, fried irish potato, salad and bread were collected randomly. The samples were prepared and analyzed using standard procedures. Mean viable counts of aerobic and anaerobic bacteria were determined, ranging from (13×103cfu/g to 78×104 cfu/g) for ready to eat food. **Results:** Based on the finding KSP I, KSP J and KSP K food samples had the highest bacterial contamination on food while WSP F, WSP G and WSP H food samples had the least with the following isolates *Salmonella spp*, *Nocardia spp*, *Shigella spp*, *Listeria spp*, *Bacillus cereus*, *Leuconostoc spp*, *Acinetobacter spp*, *Acetobacter spp*, *campylobacter spp*, *Clostridium spp* and *Vibrio spp* which revealed that the isolates were susceptible to any of these antibiotics Septrin, Chloramphenicol, Gentamycin, Tarvid, Streptomycin, Reflacin, Augumetin, Ceporex, Nalidixic acid, Ampicillin, Ciproflox, Penicillin and Erythromycin. **Conclusion:** Thus, ships operators and regulatory body are expected to take all practicable measures to ensure that they do not receive unsafe or unsuitable food and maintain adequate food temperature at all time.

Keywords: Health Emergency; Seaport; Food Spoilage Microorganisms; Microbiological Quality; Food Temperature; Ships Operators, Core Niger Delta

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1. Introduction

Ships and boats are two of the oldest types of transportation and were first built thousands of years ago. Not only have ships and boats been used for transportation throughout history, they have been used for a number of other reasons including to

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transport cargo, fishing, as a type of defense from armed forces, sports, leisure, and relaxation. The first "ships" were single logs that small cargo was attached to and floated down river for trade. Eventually, logs were tied together to carry bigger cargos. During the 19th century, cruise ships were developed to carry people across the world. In the late 20th century, container shipping developed; leading to the shipping industry we see today [1].

Ships are long acknowledged as semi-closed and densely populated environments with close living and sleeping quarters, and shared water, ventilation and sewage systems. Such environments are accountable to a constant flux of people from over the world and conducive for the spread of communicable diseases [3]. The increased number of people travelling by ship and the increases in itineraries may expose travelers to infectious diseases, such as gastrointestinal and respiratory infections. After disembarkation, travelers may spread acquired infections further in their communities [2, 12, 13, 14, 15]. Approximately 397 million passengers embarked and disembarked in European Union (EU) ports in 2016. It was estimated that 26.6 million tourists spent holidays on cruise ships worldwide in 2017, while a total of 1,647,500 seafarers work on merchant ships operating internationally over the world. The risk of cases and outbreaks of disease among the population on board ships is ever present [3].

Generally, foods raised concern with respect to their potential food poisoning outbreaks due to improper handling and unhygienic practices among food vendor and reports had shown that number of illnesses, hospitalizations trans border diseases and deaths are from contaminated foods on ships, the most common causes of food borne illness are norovirus, *Salmonella enterica*, *Campylobacter spp.*, *Clostridium perfringens* and *Staphylococcus aureus*. Due to its high case fatality rate, *Listeria monocytogenes* has also become a much-noted cause for concern [4, 6].

Food is the fuel of life. Without food, humans cannot survive as such it is important for us to know where our food actually comes from. The food system is the technical, social, and economic structure that supplies food to the population. It is a complex, dynamic, and international chain of activities that begins with production and harvesting of raw agricultural commodities on farms, ranches and in fishing operations and moves to value added processed and preserved products and then to retail food stores and food service establishments (restaurants and institutions) where these foods are prepared, merchandised and sold to consumers [5, 16, 17, 18]. As such, the focus of this research work was on assessment of microbiological quality of ready to eat food served in ships along Warri, Koko and Port Harcourt water ways, Nigeria.

2. Materials And Methods

2.1. Sample collection

A total of 11 samples of ready to eat food were collected from 3 seaport locations, which included cooked rice; fried fish, irish potato porridge, vegetable soup, griki, pepper soup, fried irish potato, salad and bread. The samples were collected aseptically using sterile gloves, properly labeled and placed in food grade sampling bags. Samples were immediately placed in pre-cooled containers containing ice packs and then transported to the laboratory for analysis.

2.2. Microbial Analysis

2.2.1. Isolation, colony count and identification of food microorganisms

The isolation of bacteria was completed within 24 hours after each food samples collection. This was carried out by mixing 1ml of the food samples into the 9ml of sterile distilled water and diluted serially up to 10^{-10}. This was repeated for all the samples and 0.2ml of the suspension was plated out of nutrient agar, MacConkey agar, potato dextrose agar and mannitol salt agar. The plates were incubated at 35^{0}C for 24 hours. The distinct

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colonies growing on each plate were counted, selected, sub cultured and stored on slants. Pure culture of all the isolates was subjected to biochemical test in order to know the identity of organisms.

2.2.2. Antibiotic susceptibility test

72g of Muller Hinton agar were measured into 1litre of distilled water, the conical flask containing the dissolved Muller Hinton agar was corked with cotton wool, wrapped with aluminum foil and it was then sterilized in the autoclave at 121 ^{0}C for 15 minutes. The susceptibility of the bacteria isolates was assayed using disc diffusion method as described by British Society for antimicrobial Chemotherapy [2008]. A suspension of each isolate in normal saline was compared with 0.5 McFarland standards to standardize the inoculums.

The suspension was used to inoculate MHA plates using sterile swabs sticks and antibiotics disc containing Septrin (30µg), Chloramphenicol (30µg), Gentamycin(10µg), Tarvid (10µg), Streptomycin (30µg), Reflacin (10µg), Augumetin (10µg), Ceporex (10µg), Nalidixic acid (30µg), Ampicillin (30µg), Ciproflox (10µg), Penicillin (20µg), Erythromycin (30µg) was aseptically layered on the surface of the plates. The plates were incubated at 35^{0}C for 24 hours. After incubation, zone of growth of inhibition around each disc was measured and used to classify the organisms as sensitive or resistant to an antibiotic according to the interpretive standard of the Clinical and Laboratory Standard Institute [2005].

3. Results

Table 1 revealed how sampled foods at the selected seaports were coded.

The data in Table 2 shows the bacteria load of ready to eat food served in the ship and the table revealed that rice had the highest aerobic and anaerobic bacteria load were recorded in salad as 78×10^4cfu/g and the least load was observed in fried fish as 13×10^3cu/g.

However, Table 3 showed the different types of bacteria isolated from ready to eat ships food. It also revealed that there were total of twelve genera of bacteria isolated from sampled foods, these include *Salmonella spp, Nocardia spp, Shigella spp, Listeria spp, Bacillus cereus, Leuconostoc spp, Acinetobacter spp, Acetobacter spp, campylobacter spp, Clostridium spp* and *Vibrio spp*.

Furthermore, the 13 antibiotics used were shown in Table 4. *Salmonella spp* was resistance to ampicillin, chloramphenicol and sensitive to nalidixic acid. Besides, *Listeria spp* and others bacteria were susceptible to the antibiotics mentioned above.

Table 1. Coding of sampled food at the selected seaports

S/N	Ship	Food sampled
1.	PHSP A	Cooked rice
2.	PHSP B	Fried fish
3.	PHSP C	Irish potato porridge
4.	PHSP D	Cooked rice
5.	PHSP E	Vegetable soup
6.	WSP F	Griki
7.	WSP G	Cooked rice
8.	WSP H	Pepper soup
9.	KSP I	Fried Irish potato
10.	KSP J	Salad
11.	KSP K	Bread

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Table 2. Bacteria count of ready to eat ships foods

S/N	Food sample	Result cfu/g
1.	Cooked rice	32×10^3
2.	Fried fish	13×10^3
3.	Irish potato porridge	60×10^4
4.	Cooked rice	23×10^4
5.	Vegetable soup	47×10^4
6.	Gariki	31×10^3
7.	Cooked rice	40×10^3
8.	Pepper soup	55×10^5
9.	Fried irish potato	42×10^3
10.	Salad	78×10^4
11.	Bread	30×10^3

Table 3. Bacteria types isolated from different ready to eat ships foods.

Bacteria species	Ready to eat ships food										
	PHSP A	PHSP B	PHSP C	PHSP D	PHSP E	WSP F	WSP G	WSP H	KSP I	KSP J	KSP K
Bacillus cereus	x	_	_	_	_	_	_	_	_	_	_
Listeria spp	_	X	_	_	_	_	_	_	_	_	X
Campylobacter spp	_	_	X	_	_	_	_	_	_	_	_
Shigella spp	_	_	_	X	_	_	_	_	_	_	_
Salmonella spp	_	_	_	_	X	_	_	_	_	_	_
Legionnaires spp	_	_	_	_	_	X	_	_	_	_	_
Nocardia spp	_	_	_	_	_	_	X	_	_	_	_
Leuconostoc spp	_	_	_	_	_	_	_	X	_	_	_
Vibiro spp	_	_	X	_	_	_	_	_	x	_	_
Acinetobacter spp	_	_	_	_	_	_	_	_	_	X	_

Key x =Bacteria isolated, - =Bacteria not isolated

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Table 4. Bacteria antibiotic sensitivity test

Antibiotics	*Salmonella spp*	*Clostrid-ium spp*	*Campylobac-ter spp*	*Listeria spp*	*Bacillus cereus*	*Shigella spp*	*Nocardia spp*	*Legion-naires spp*
Septrin (SXT)	S	R	S	S	R	R	S	S
Ampicillin (PN)	R	R	S	R	R	S	S	S
Nalidixic acid (NA)	S	R	S	R	S	R	S	S
Penicillin (P)	S	R	S	R	R	S	S	R
Ceporex (CEP)	R	S	R	S	S	R	R	S
Erthromycin (E)	S	S	S	S	S	S	S	S
Chloramphenicol (CH)	R	R	S	S	R	R	S	S
Ciproflox (CPX)	S	R	S	R	R	R	S	R
Streptomycin (S)	R	R	S	R	S	R	R	R
Augumetin (AU)	S	S	S	R	S	R	S	R
Tarvid (OFX)	R	R	S	S	R	R	S	S
Gentamycin (CN)	S	R	S	S	R	R	S	S
Reflacin (PEF)	S	S	S	S	S	R	S	S

Key word R-Resistant, S-Sensitive

4. Discussion

The world we lived in today is highly mobile, interdependent, and interconnected, giving tremendous opportunities for diseases to spread rapidly [12,13,14,15,20]. Besides, the public has been focusing on new health events caused by microbiological, toxic metals concentration and sudden environmental changes in the recent past [21, 22, 23, 24, 25, 26, 27, 28, 29]. Thus, this study revealed that the bacteria load was highest in salad and lowest microbial load in fried fish. Also, the organisms isolated from sampled food were mostly aerobic and anaerobic bacteria such as *Salmonella spp, Nocardia spp, Shigella spp, Listeria sp.* The microbial loads of the sampled foods evaluated in Table 2 were far higher than WHO standards of 10 – 16 cfu/g, total coliform 0 - 10/g and SSB 20 g for ready to eat foods [19].

The results were in accordance with previous works of several authors which revealed that *Listeria spp* have been recovered at greater than 10^3cfu/g. Foods containing 100cfu/g or greater should be considered adulterated. It can be validated that the level of *Listeria monocytogenes* in ready-to-eat food will not exceed 10^2 or 100cfu/g within the food's expected shelf life [6, 7]. For ready-to-eat food *Listeria monocytogenes* is satisfactory at <10cfu/g, borderline at 10-<100cfu/g and unsatisfactory at >100cfu/g (Microbiological Guidelines for Food-Centre for Food Safety [8]. But in this study (23×10^4cfu/g and 60×10^4cfu/g) of *Listeria spp* were revealed in food sampled which indicates an unacceptable

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level of contamination has occurred which were far higher than WHO standard (1.6×10cfu/g), [6].

In comparison with other studies the total coli form count ranged from 2.6×10^3cfu/g to 5.2×10^4cfu/g which is much higher than 3×10^2 to 6.4×10^3cfu/g reported in Gondar and 2.8×10^2cfu/g to 3.99×10^3cfu/g in Tirumala, India [6]. However, this finding revealed that the microbial load index in food sampled ranged from (13×10^3cfu/g to 78×10^4 cfu/g) which was to some extent comparable with 2.6×10^3 to 1.9×10^5cfu/g documented in Addis Ababa [6]. Then, the microbial loads of the ready to eat foods evaluated in Table 2 were far higher than WHO standards of 10 – 16 cfu/g, total coliform 0 - 10/g and Salmonella-Shigella bacteria 20g for street foods and what is set for microbiological quality of ready-to-eat street foods were found to be beyond the acceptable level (below 10^5cfu/g) [6, 9].

Listeria spp were resistance to nalidixic acid was the commonest finding (85.7%), followed by resistance to penicillin (47.6%), and tetracycline (33.3%). This finding was in line with previous reports that *Listeria spp* was susceptible to most antibiotics. The study also reported 71.4% of isolates resistance towards penicillin and the possible explanation was that penicillin is the drug of choice and widely used in listeriosis treatment [10, 11].

While this study revealed that *Listeria spp* was sensitive to 7 antibiotics (Ceporex, Erythromycin, Septrin, Chloramphenicol, Reflacin, Tarvid, Gentamycin) out of 13 used and the percentage sensitivity was 55.9% and it was resistant to 6 antibiotics (Augumetin, Ciproflox, Streptomycin, Penicillin, Nalidixic acid, Amplicin) with 44.1% resistant to the antibiotics which was in agreement with (Saha *et al.,* 2015) that *Listeria spp* is resistant to Nalidixic acid and other antibiotics.

Salmonella spp showed 100% resistance to ampicillin and 88.9% to chloramphenicol. However, it was 89% sensitive to nalidixic acid which is in agreement with study findings in Bahir Dar, Ethiopia where 100% sensitive to the former and 78% to the later drugs was reported [9]. In this study *Salmonella spp* revealed 88% sensitive of nalidixic acid and 91% resistance to Chloramphenicol and 95% to Ampicillin, indicating that those drugs will no longer be used for treatment of salmonella infection. Hence the two findings collaborate that *Salmonella spp* had resistant to Chloramphenicol and sensitive to Nalidixic acid.

5. Conclusions

This work extends our knowledge on distribution of food pathogens and provides a further argument that consumers may be ingesting food borne bacteria or their byproducts on foods they already eat. Furthermore, a detailed risk assessment can be used to identify critical gaps in our knowledge base, characterize the most important risk factors in the production to consumption in the food chain, help identify strategies for risk reduction, and provide guidance for determining priorities in maritime health and food safety research programs.

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