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Antibiotics Sensitivity Reaction of *salmonella* Species Isolated from Ready-to-Eat Porridge Beans Sold in Federal Polytechnic of Oil and Gas Bonny of Rivers State

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ABSTRACT: *Salmonella* specie is one of the four key global causes of diarrhoeal diseases. In this research we investigated the frequency occurrence of *salmonella* species isolated from ready-to-eat porridge beans sold from vendors in the proximity of federal polytechnic of oil and gas bonny island. A total of 40 samples were purchased from four different food vendors in four different spots [vendor 1(The first spot), vendor 11(second spot), vendor 111(third spot) and vendor 1V (fourth spot)] between February and May 2024.All samples were processed and analysed using standard culturebased, biochemical methods and antibiotic susceptibility test assays to confirm *salmonella* isolates. The highest total occurrence of heterotrophic bacteria population densities were: vendor1 4.0.0x10^5CFU/g; vendor11 4.4x10^7CFU/g, vendor111 4.5x10^2CFU/g and vendor 1V 4.2x10^9CFU/g. The highest total occurrence of salmonella population density were, for vendor1 3.9x10^2, Vendor11 2.7x10^4 , Vendor111 4.1x10^5,Vendor1V 4.4x10^3 respectively. Antibiotics susceptibility test was performed for the isolates which exhibited that all of them were susceptible to Ciprofloxacin (CPR)-5μg, Nitrofurantoin (NIT)-30μg, Oflotaxin (OFL)-5μg, were susceptible to *Salmonella* species. While Gentamicin (GEN)-10μg, and Cefuroxime (CXM)-5μg, were intermediate and Finally, Augmentin (AUG)-30μg, Cefuroxime (CAZ)-30μg, and Cefuroxime (CRX)-30μg were resistant to *Salmonella* species. Therefore, it can be stated that ready-to-eat porridge beans sold in the proximity of FPOG environment are possible route of transmission for *Salmonella* species. However, due to lack of intense antibiotic resistance among these bacteria, most of them can be treated with the antibiotics available in the market. Nonetheless, strict monitoring and regular surveillance is necessary.

KEYWORDS: Antibiotic Sensitivity, Cooked Porridge Beans, Contamination, RTE food, Salmonella Spp. Antibiotic Resistance.

1.1 INTRODUCTION

Antibiotic Sensitivity Testing

Antibiotic sensitivity testing is pivotal in guiding antibiotic therapy, utilizing culture-basetechniques and genetic assays to determine bacterial susceptibility. Resistance mechanisms, including genetic mutations and horizontal gene transfer, underscore the ongoing need for improved testing methods (Leekha et al., 2011; Kang et al., 2018).

Salmonellosis: A Global Health Threat

Salmonellosis, primarily transmitted through contaminated food, poses a significant public health threat worldwide. The surge in cases during the 1980s, particularly in Africa and Europe, highlights the importance of assessing antibiotic sensitivity in Salmonella strains, with certain foods like porridge beans being particularly susceptible to contamination (Didelot, 2011; Bayer & Bernard, 2014).

Impact of Foodborne Illnesses on Public Health

Foodborne illnesses, often originating from sold foods, represent a substantial burden on public health, with *Salmonella* species being a major contributor. Infections can lead to various health complications, including gastroenteritis and typhoid fever, while the emergence of antibiotic-resistant strains exacerbates the problem, underscoring the need for effective control measures (Rodriguez et al., 2014; Oghenevo et al., 2016).

Role of Food Vendors in *Salmonella* Transmission

Food vendors play a significant role in the transmission of *Salmonella*, contributing to millions of cases of foodborne diseases annually. Contamination can occur at various stages, from production to retail marketing, emphasizing the importance of stringent

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food safety practices. Furthermore, antibiotic resistance in *Salmonella* isolates poses challenges in both veterinary and public health sectors, necessitating comprehensive control strategies (Akinyemi et al., 2017; Zhao et al., 2017).

***Salmonella* Infections in Sub-Saharan Africa**

In sub-Saharan Africa, non-typhoidal *Salmonella* infections are prevalent and associated with high case fatality rates, particularly among vulnerable populations. Contaminated foods, including rice, meat, eggs, and raw milk, serve as common sources of infection, highlighting the urgent need for improved hygiene practices and food safety regulations. Additionally, the increasing proportion of antibiotic-resistant *Salmonella* strains further complicates treatment options and underscores the importance of antibiotic sensitivity testing in guiding therapeutic interventions (Tennant et al., 2010).

In summary, antibiotic sensitivity testing remains essential for effective antibiotic therapy, especially in the context of emerging antibiotic resistance in *Salmonella* strains. The prevalence of *Salmonella* contamination in various food sources underscores the need for robust food safety measures and vigilant monitoring to mitigate the risk of foodborne illnesses. Collaborative efforts between healthcare professionals, food regulators, and the food industry are crucial in addressing this pressing public health issue.

Multiple lines of evidence indicate that food exposed on sale by vendors may become infected by bacteria. There have been claims that some Ready-To-Eat Foods (Porridge Beans) sold in FPOG bonny island proximity is responsible for reported cases of ill-health in the area; most consumers complain of ill-health after consuming (Porridge Beans). Even at this, some vendor's ready-to-eat foods have shown epidemiological links with illness and this has raised concern in respect to their potential for serious food poisoning outbreaks. Evidently, some of the vendors in FPOG are noted to have low hygiene practices. That is, proper hygiene practice is not well followed or established. Consequent upon this challenge, it becomes necessary to investigate the (Porridge Beans). Vendors in these areas for contamination with some bacteria associated with food-related illnesses.

The aim of this research work was to determine the sensitivity of Salmonella species isolated from some ready-to-eat foods in FPOG.

The Objectives of the Study were to:

i. Isolate and characterize Salmonella species from food vendors in proximity of federal polytechnic of oil and gas Bonny Island;
ii. Determine the frequency of occurrence of *Salmonella* species in the foods;
iii. Determine the sensitivity of the isolated *Salmonella* species to some antibiotics.

This study aims to provide valuable information to FPOG members and health authorities for addressing the health risks associated with contaminated foods, benefiting inhabitants by guiding them in mitigating foodborne illnesses, aiding health workers in proper diagnosis, and assisting food vendors in controlling *Salmonella* evasion, particularly in porridge beans, to prevent food poisoning outbreaks.

The project focused on isolating and character 30µg, Nitrofurantoin 30µg, Ciprofloxacin 5µg).

2.1 LITERATURE REVIEW

Salmonella: A Global Foodborne Threat

Salmonella is a major contributor to global foodborne illnesses, transmitted primarily through ready-to-eat foods in developed countries and via contaminated water, vegetables, and human-to-human transmission in developing nations (Heredia et al., 2018). Poor hygiene exacerbates food contamination, emphasizing the urgent need for enhanced food safety management, particularly in restaurants and during food handling (Gizaw Z, 2019).

Salmonella Infection Mechanisms

Salmonella infection mechanisms differ between typhoidal and non-typhoidal serotypes. Non-typhoidal serotypes disrupt tight junctions in the intestinal wall, causing inflammation and diarrhea by entering via M cells (Haraga et al., 2008). Conversely, typhoidal serotypes breach the intestinal barrier through phagocytosis and immune cell trafficking, leading to systemic infection (Haraga et al., 2008). The AvrA toxin, delivered by the SPI1 type III secretion system of S. Typhimurium, suppresses the host's immune system, increasing infection susceptibility (Mittal et al., 2010). Salmonellosis manifests in various clinical patterns, including gastrointestinal infection, enteric fever, bacteremia, and localized infections (Choi et al., 2010). Additionally, the bacteria can remain latent, potentially causing secondary infections over time (Choi et al., 2010).

Salmonella Adaptation and Treatment Challenges

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Salmonella, a facultative intracellular pathogen, primarily spreads through contaminated food or water, exhibiting different host adaptability and disease manifestations (Jantsch et al., 2011). Host adaptation involves genetic changes to evade immune responses, while common adhesions in more pathogenic serovars have evolved through convergent evolution (Kisiela et al., 2012). Horizontal gene transfer and the formation of new serovars contribute to Salmonella's evolution (Baumler et al., 1998). *Salmonella* Newport exhibits signs of adaptation to plant colonization, influencing its association with foodborne illnesses linked to produce (de Moraes et al., 2018). Treatment for *Salmonella*-induced food poisoning varies, with antibiotics recommended for certain vulnerable populations to prevent complications (de Moraes et al., 2018). Carriers of Salmonella, even asymptomatic ones, can spread the infection and may require gallbladder removal and antibiotic treatment (de Moraes et al., 2018).

Microbial Safety of Ready-to-Eat Foods

Ready-to-eat (RTE) foods are pre-cleaned, precooked, and packaged for immediate consumption without requiring additional preparation (CDC, 2015). The US food code stipulates that RTE foods must undergo sufficient cooking to eliminate harmful microorganisms, particularly those of animal origin like Salmonella spp and Listeria monocytogenes (FDA, 2009). Bacteria play crucial roles in foodborne diseases, responding to environmental pH variations and acid stress by inducing protective responses like the acid tolerance response (ATR) (De Reuk et al., 2018). Preventing Salmonella infection from RTE foods involves adhering to hygiene practices and proper food handling techniques (WHO, 2010). Contaminated water and unhygienic vending practices further exacerbate the risk of RTE food contamination (Al Manun et al., 2013). Quality ingredients are essential for RTE food safety, yet economic constraints often lead to the use of low-quality ingredients prone to bacterial contamination (Alimi, 2016). Addressing factors like personnel hygiene, ingredient quality, and post-production handling practices is essential for mitigating the risk of RTE foodborne illnesses in both developed and developing regions.

3.1 METHODOLOGY

The research was carried out in four selected different spots of food vendors in federal polytechnic of oil and gas in bonny island rivers state (fig 3.1).Bonny is an island town and a local govt. area in river state in south Nigeria. Bonny Island lies between $4^{o}52^{I}$N to $5^{o}02^{I}$N and longitudes $6^{o}56^{I}$E $7^{o}04^{I}$E

A total of 40 porridge beans samples were collected over 10 days from 4 vendors (vendor 1, vendor 2, vendor 3, and vendor 4) under sterile hygienic conditions, using sterile spoon and mouthed sample container and it was put in a sterile polythene bag and transported to Research Laboratory, Federal Polytechnic of Oil and Gas for the isolation of salmonella species. All samples were purchased from the food vendors inside the polytechnic community between February to May 2024.

Microbiological analyses

Isolation and characterization of *salmonella* specie

The microbiological analyses carried out on the samples were; Serial dilution, Inoculation and incubation by spread plate method on selective media Salmonella Shigella Agar(SSA), Enumeration and isolation of colonies on complex medium (Nutrient Agar), Biochemical characterization of isolates, and Antibiotic susceptibility profile.

Identification of the isolates was based on their cultural morphology, microscopic examination and biochemical tests. References were made to Bergey's Manual of Determinative Bacteriology 1992 for identification of bacteria (Holt *et al.*, 1994). Morphological studies were carried out on different media plates used for the isolation of the microorganisms; pure cultures were isolated based on colony size, shape, pigmentation, elevation, and texture of the individual organisms after 48 hours of growth at 30°C. Pure isolates from the respective media were characterized and identified based on their morphological, biochemical and physiological features (Cheesbrough, 2006; Holt *et al.*, 1994).

Colonial Morphology

A colony of the isolate was picked and streaked on a freshly prepared nutrient agar plate and was incubated at 37°C for 24 hours. After incubation, morphological features: shape, size, color, edge, texture, and elevation of the colony of the isolate observed visually with hand lens (Holt *et al.*, 1994).

Antimicrobial Susceptibility Profile

Antimicrobial susceptibility profile of the bacterial isolates was carried out using Kirby-bauer disc diffusion method and readings interpreted by adopting the breakpoints of Clinical and Laboratory Standard Institute (CLSI). Purified isolates were inoculated on 5ml nutrient broth and incubated overnight. The optical density (OD) of the turbidity of the broth was determined to conform with

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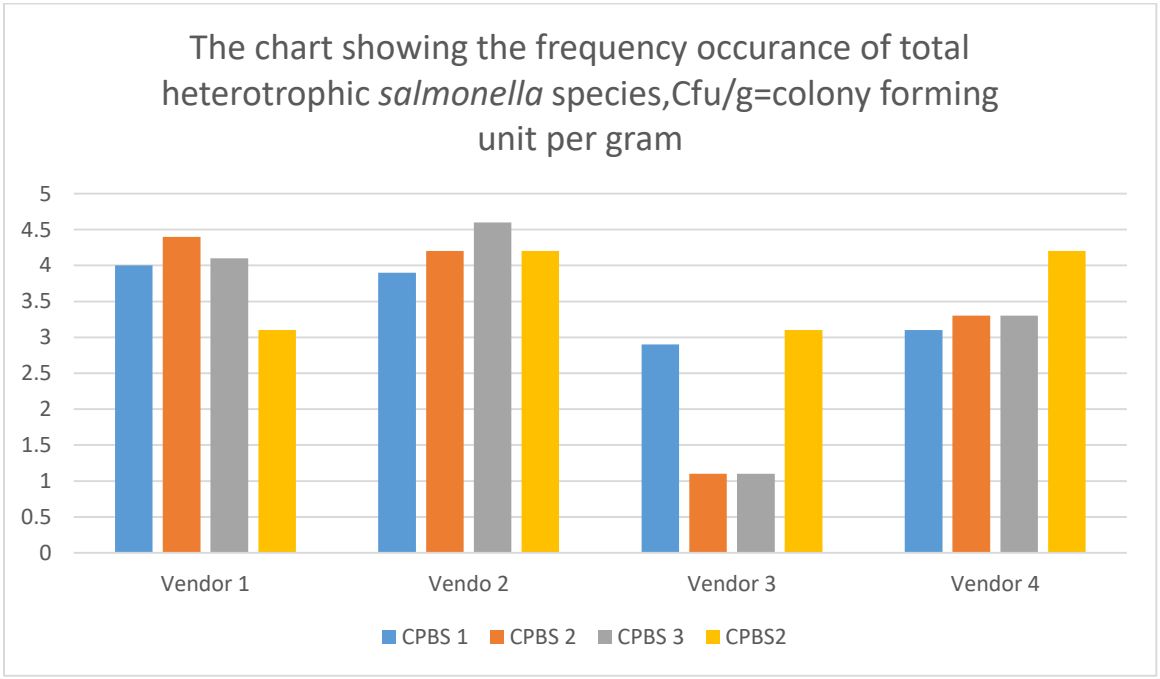
the Optical Density (OD) of the McFerland turbidity standards (that is 0.5 McFerland standard) where the bacterial suspension or cells are equivalent to 1.5 x 10^8 colony forming units (cfu/ml). Using a sterile swab stick, inoculum from the broth (that is, respective standards) were aseptically swabbed on Mueller Hinton Agar. A total of 8 antibiotics disc which includes Gentamycin (10mg), Erythromycin (5μg), Ofloxacin (5μg), Cloxacillin (5μg), Ceftriaxone (30μg), Cefuroxime (30μg), Ceftadidime (30μg), and Augmentin (30μg) were employed. The respective discs were also aseptically impregnated on the agar plates using a sterile forceps. Plates were allowed to stand at room temperature for 5min to allow the media to absorb effectively and incubated at 37^{o}C for 18-24hours. Characterization of the resistance, intermediate and susceptibility profile of the isolates were determined by measuring zone of inhibition and then compared with the interpretative chart to determine the sensitivity, intermediate and resistant nature of the isolates to the antibiotics used using the CLSI (2017) interpretative chart. The resistance of the isolates to some of these antibiotics has been reported by Agwa *et al.*, (2012).

4.1 ANALYSIS AND RESULTS

Table 4.1: Total Heterotrophic Bacterial Count of the samples from the four selected food vendors, expressed in Colony Forming Unit per gram (CFU/g)

	Hawkers			
Sample Codes	**Vendor I**	**Vendor II**	**Vendor 111**	**Vendor IV**
Sample 1	$4.0x10^5$	$4.4x10^7$	$4.1x10^2$	$3.1x10^{10}$
Sample 2	$3.9x10^6$	$4.2x10^7$	$4.6x10^2$	$4.2x10^{10}$
Sample 3	$2.9x10^5$	$1.1x10^6$	$4.0x10^2$	$3.1x10^8$
Sample 4	$3.1x10^5$	$3.3x10^6$	$4.5x10^2$	$4.2x10^9$
p-value			0.05107919632567923	

Key: CPBS=Porridge Beans Sample, THB= Total Heterotrophic Bacteria, Cfu/g=Colony forming unit per gram



CPBS: Cooked Porridge Beans Sample

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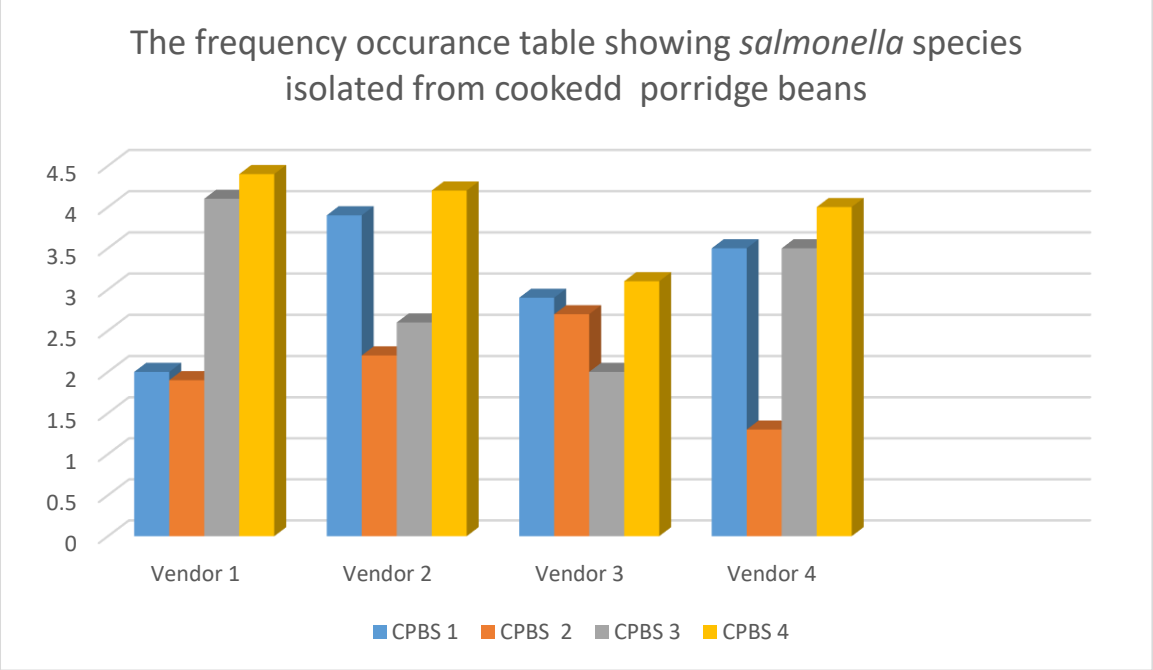
The details of *Salmonella* Species Isolated from food vendors

Table 4.2: Total Salmonella Shigella Count of the samples from the four selected food vendors, expressed in Colony Forming Unit per gram (CFU/g)

			Hawkers	
Sample Codes	**Vendor I**	**Vendor II**	**Vendor 111**	**Vendor IV**
Sample 1	$2.0x10^2$	$1.9x10^3$	$4.1x10^5$	$4.4x10^3$
Sample 2	$3.9x10^2$	$2.2x10^4$	$2.6x10^4$	$4.2x10^5$
Sample 3	$2.9x10^2$	$2.7x10^4$	$2.0x10^5$	$3.1x10^4$
Sample 4	$3.5x10^2$	$1.3x10^6$	$3.5x10^3$	$4.0x10^5$
p-value			0.05417904106253781	

Key: PBS=Porridge Beans Sample, TSSC=Total Salmonella Shigella Count.



CPBS: Cooked Porridge Beans Sample

Details showing the characteristics of *Salmonella* species

Table 4.3: Morphological Characteristics of *Salmonella* species

Color	Size	Elevation	Margin	Shape	Gram Reaction
Translucent	Small	Flat	Undulate	Rod	-Ve

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TABLE 4.4: Biochemical Test and Sugar Fermentation Test

SC	GLU	MAL	LAC	MAN	CAT	OXI	H_2S	CIT	ST	URS	VP	MR	IND	MOT	RESULT
CPB	AG	A	N	A	+	-	+	+	-	-	-	+	-	+	Salmonella spp
CPB	A	A	N	A	+	-	+	+	-	-	-	+	-	+	Salmonella spp
CPB	AG	A	N	AG	+	-	+	+	-	-	-	+	-	+	Salmonella spp
CPB	AG	A	N	AG	+	-	+	+	-	-	-	+	-	+	Salmonella spp

KEY:
SC-Sample code, **CIT-Citrate,**
ST-Salt tolerance, **URS-Urease,**
CBS-Cooked porridge beans sample, VP- Vogues proskauer,
GLU-Glucose, **MR-Methyl red,**
MAL-Maltose, **IND-Indole,**
LAC-Lactose, **MOT-Motility,**
MAN-Mannitol, **AG-Acid/Gas,**
CAT-Catalase, **A-Acid,**
OXI-Oxidase, **N-Neutra/Negative.**
H_2S-Hydrogen sulphide,

Table 4.5: Details of the drug reference zone of inhibition based on clinical laboratory standards on Porridge Beans.

Antibiotics/Disc potency(μg)	Diameter of zone of inhibition (n%) % Susceptibility	% Intermediates	% Resistance
CPR $5\mu g$	4 (80)	0 (00)	0 (00)
NIT $30\mu g$	5 (100)	0 (00)	0 (00)
OFL $5\mu g$	2 (40)	0 (00)	0 (00)
CXM $5\mu g$	2 (40)	I(20)	0 (00)
GEN $10\mu g$	1 (20)	I(20)	0 (00)
AUG $30\mu g$	0(00)	0 (00)	-
CAZ $30\mu g$	0 (00)	0 (00)	-
CRX $30\mu g$	0 (00)	0 (00)	-

KEY:PB=PorridgeBeans;(CPR)=Ciprofloxacin,(NIT)=Nitrofurantoin , (OFL)=Oflotaxin,(GEN)=Gentamycin,(CXM)=Cefuroxime,(AUG)=Augmentin, (CAZ)=Ceffazidine, (CRX)=Cefuroxime.

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Table 4.6: Details of the drug reference zone of inhibition based on clinical laboratory standards on

Antibiotics/Disc potency(μg)	Diameter of zone of inhibition (n%) % Susceptibility	% Intermediates	% Resistance
CPR 5μg	5 (100)	**0**	**0**
NIT 30μg	5 (100)	**0**	**0**
OFL 5μg	4 (80)	**0**	**0**
CXM 5μg	1 (20)	I (20)	**0**
GEN 10μg	3 (60)	I (20)	**0**
AUG 30μg	0	**0**	-
CAZ 30μg	0	**0**	-
CRX 30μg	0	**0**	-

Table 4.7: Details of the drug reference zone of inhibition based on clinical laboratory standards on African salad.

Antibiotics/Disc potency(μg)	Diameter of zone of inhibition (mm) % Susceptibility	% Intermediates	% Resistance
CPR 5μg	5 (100)	**0**	**0**
NIT 30μg	5 (100)	**0**	**0**
OFL 5μg	4 (80)	**0**	**0**
CXM 5μg	2 (40)	1 (20)	**0**
GEN 10μg	1 (20)	I (20)	**0**
AUG 30μg	0	**0**	-
CAZ 30μg	0	**0**	-
CRX 30μg	0	**0**	-

Key: IVD 100 Sensitivity Ring N253N

CAZ – Ceffazidine 30μg
CRX – Cefuroxime 30μg
GEN – Gentamacine 10μg
CXM – Cefixime 5μg
OFL – Oflotaxin 5μg
Aug – Augumentin 30μg
NIT – Nitrofuran toin 30μg
CPR – Ciprofloxacin 5μg

Total **Heterotrophic Bacterial Count (THBC)**

The results of the total heterotrophic bacterial count food samples from the four selected food vendors are presented in Table 4.1Porridge beans from Vendor 111 had the highest THB count of $4.5x10^2$ cfu/g while vendor 11 had the least THB count of $1.1x10^6$ cfu/g.

Total Salmonella Shigella Count (TSSC)

The results of the total salmonella shigella count for the four food samples from the four selected food vendors are shown in Table 4.2.Porridge beans from Vendor 1V had the highest count of $4.4x10^3$ cfu/g while Porridge beans from vendor 11 had the least count of $2.1x10^7$ cfu/g.

Antibiotic Susceptibility Testing of *Salmonella* Isolates

The results for the antimicrobial susceptibility of *Salmonella* isolates obtained from the sold foods are presented in Tables 4.5, 4.6 and 4.7 respectively. In Table 4.5, the isolates of *Salmonella* species were completely susceptible to Ciprofloxacin (CPR)-5μg,

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Nitrofurantoin (NIT)-30μg, Oflotaxin (OFL)-5μg, While Gentamycin (GEN)-10μg, and Cefuroxime (CXM)-5μg, were intermediate and Finally, Augmentin (AUG)-30μg, Cefuroxime (CAZ)-30μg, and Cefuroxime (CRX)-30μg were resistant to *Salmonella* species.

Morphological Characteristics of *Salmonella* species

The result for Morphology Characteristics of *Salmonella* Species are present in Table 4.2.

Identification of Bacterial Isolates

The microscopic and biochemical characteristics of the bacterial isolates are presented in Table 4.4 based on the comparison with Cheesbrough (2005) and Prescott *et al.* (1999), the identities of the bacterial isolates were all *salmonella* spp.

4.2 DISCUSSION

Studies on the antibiotics sensitivity reaction of *salmonella* species isolated from cooked porridge beans in proximity of FPOG Bonny Island are scarce. The presence of several *salmonella* serotypes that can cause disease in both animals and humans was isolated and identified from the food samples. Several antibiotics classes were used that dictate the need for continued surveillance of *Salmonella* in foods and the environment.

Ready-to-eat food do not need to be reheated before consumption. The data revealed that bacteria isolated from all food samples collected from the vendors in FPOG Bonny Island were *salmonella* species by comparing their morphological and biochemical characteristics with standard reference organisms (Okonko *et al.*, 2018).

The Total Heterotrophic count of Salmonella ranged from 4.0 x 10^5cfu/g to 3.1 x 10^6cfu/g, for ready –to-eat vendor 1,while 4.4 x 10^5cfu/g to 3.3 x 10^6cfu/g for vendor11. 4.1 x 10^2cfu/g to 4.5 x 10^2cfu/g for vendor111 and 3.1x10^{10}-4.2x10^{10} cfu/g for vendorIV ready to eat beans porridge samples. This is an indication of recontamination in food handling and hygiene techniques (Mboto *et al.*, 2012).

In this study, there was presence of *Salmonella* species in porridge beans on both NA plates and SSA plates, and as well as in the vendor 2, 3, 4 on both NA and SSA plates collected from FPOG Bonny Island that was analyzed. It was also found that *Salmonella* occur much on SSA plates of heterotrophic counts containing beans porridge in vendor 3 than NA plates. While total *salmonella* species count occur more also in SSA than NA plates for vendor 4.Microorganisms (*Salmonella*) isolated from ready-to-eat (RTE) food samples in this study have been earlier found in foods, environment and other places and their pattern is similar to previous reports by (Mboto *et al.*,2012). The presence of *salmonella* in ready to-eat (RTE) foods depicts a deflorable state of poor hygienic and sanitary practices employed in the food catering, food handling, processing and packaging of foods (Baluka SA *et al.*, 2015).

5.1 CONCLUSION, RECOMMENDATION, CONTRIBUTION TO KNWOLEDGE

5.1.1 Conclusion

The findings of this study revealed that ready-to-eat (RTE) foods sold at FPOG bonny island proximity, are contaminated with pathogenic gram negative bacteria. The possible sources of these contaminants are due to the unhygienic manner of handling food in the place of preparatory. This implies that ready-to-eat foods are viable source of various diseases. Irrespective of the presence of this gram negative (*Salmonella)* bacteria in ready-to-eat foods analyzed, it is believed that cooking processes and hygiene could greatly reduce the microbial load to harmless level (Agnes, 2015; Heredia et al., 2018).

Conclusively, the presence of *Salmonella* species in ready-to-eat food courses food spoilage and food poisoning. Food should not only be nutritionally balanced, but should be microbiologically safe as well. From the result gotten, it was indicated that these ready-to-eat food samples did not meet the bacteriological quality standard (WHO, 2017).

5.2 RECOMMENDATION

The following recommendations were made as course of this study:

1) Ready-to-eat (RTE) food processors and consumers should be educated on the adverse effect of using untreated or polluted water for processing as these could serve as sources of faecal contamination.
2) Food processors and consumers should observe strict hygienic measures so that they will not serve as source of chance inoculation of microorganisms and contamination of these processed ready-to-eat foods.
3) The need for microbial assessment of water for production of food and food drinks should be emphasized to reduce possible contamination (Al Mamun et al., 2013).

4) Finally, closer supervision should be made on these vendors around the FPOG Bonny island proximity communities by relevant authorities, and further studies should be carried out on other food samples sold in the community, to ensure proper food quality standard.

5.3 CONTRIBUTION TO KNOWLEDGE

To the best of my knowledge, proper hygiene of foods consumed on daily basis should be employed to reduce the risk of pathogens that may have gained entry into the food upon handling, storage, poor hygienic conditions of the sellers and other environmental factors. Furthermore, this study support assertions that the presence of *Salmonella* isolated from some food vendors may have been contaminated upon handling, storage, poor hygienic conditions of the sellers and other environmental factors since it became necessary to maintain food safety which would have reduced the microbial loads to the acceptable limits.

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