Original Research Article

Prevalence of *Salmonella* in Sea foods And Its Resistance to Drugs

ABSTRACT

Salmonella are non-spore forming, predominantly motile enterobacteria with peritrichous flagella (all around the cell body). They are intracellular pathogens causing illness. They usually invade only the gastrointestinal tract and cause Salmonellosis. Humans become infected most frequently through contaminated food or water. Sea foods are mostly associated with *Salmonella* infections. This study therefore was carried out to isolate *Salmonella* and determine the antibiotic sensitivity patterns of the *Salmonella* isolates from sea foods associated with food-borne diseases. These sea foods were obtained from three different selected markets within Port Harcourt Metropolis, Rivers State. A total of three (3) sea food samples were collected at random from each of the market. The samples were put through standard microbiological techniques. The result of total heterotrophic bacterial counts showed that Prawn from Mile 1 market had high count of $2.06x10^8$ cfu/g while Crab from Creek Road market had the least count of $1.15x10^8$ cfu/g. The result of total coliform count showed that Crab from Mile 1 market had the highest count of $2.86x10^6$ cfu/g while Periwinkle from Creek Road market had the least count of $2.30x10^6$ cfu/g. Also, the result of salmonella shigella count showed that Periwinkle from Mile 1 market had the least count of $0.76x10^5$ cfu/g while Crab from Creek Road market had the highest count of $2.80x10^5$ cfu/g. Characteristics of bacterial isolates from the sea foods showed that *Proteus sp., Bacillus spp., Pseudomonas sp., Micrococcus sp., Staphylococcus aureus, Escherichia coli,* and *Salmonella spp.* were present and identified from the samples. The result of the antibiotic susceptibility pattern of the *Salmonella* isolates showed that all the isolates were 100% susceptible to Tarivid, Peflacine, Septrine and Ciprofloxacin while they exhibited 100% intermediate sensitivity or resistance to the other antibiotics such as Ceporex, Nalidixic acid, Ampicillin, Gentamycin, Streptomycin, and Augmentin. The result of the multidrug resistance index of 3 (100%) of the 3 *Salmonella* isolates had a MAR index $\geq$ 0.2. The presence of these organisms in sea food could pose threat of food-borne infection as well as resistance to some commonly used antibiotics. Enforcement of existing laws associated with food storage, preparation and hygiene should be done in order to prevent or reduce these food-borne diseases such as salmonellosis.

Keywords: *Salmonella,* Sea foods, Prevalence, Antibiotic Resistance.

INTRODUCTION

"*Salmonella* is a rod-shaped bacillus and are gram-negative bacteria of the family Enterobacteriaceae. It was named after Daniel Elmer Salmon (1850 – 1914), an American Vetinary Surgeon who discovered the bacteria. *Salmonella* species are non-spore forming, predominantly motile enterobacteria with peritrichons flagella (all around the cell body)" (Fabrega *et al*., 2013). "They are chemotrophs, obtaining their energy from oxidation and reduction reactions using organic sources. They are facultative aerobes; capable of generating ATP with oxygen (aerobically) when it is available, using other electron acceptors for fermentation anaerobically" (Hung *et al*., 2017). "*Salmonella* are well known pathogens highly adaptive and capable of causing disease in humans and animals. The three general species of *Salmonella* are *Salmonella enterica, Salmonella typhimurium* and *Salmonella bongori. S. enteric* is further divided into six sub species which are: enterica (serotype I), salamae (serotype II), arizonae (IIIa), diarizonae (IIIb), hontenae (IV) and indica (VI)" (Janda and Abbott, 2006). "The former serotype (V) was bongori, which is now considered its own species" (Su and Chiu, 2007). "It was estimated that *Salmonella species* are the cause of over 70 million of diarrhea-associated diseases in the world with 85% of those cases being linked to food" (Hung *et al*., 2017). "Most infections are due to ingestion of food contaminated by animal feaces, or by human feaces, such as by a food-service worker at a commercial eatery. Sea food sources are predominantly associated with *Salmonella"* (Hurtado *et al*., 2017). "Antibiotic resistance in *Salmonella* is a major concern for public health safety. More focus is required to target them in humans' foods supply" (CDC, 2004). "The mechanism of antibiotic resistance can be due to the modification or destruction of the antimicrobial agent, pumping the antimicrobial agent out from the cell by efflux pumps, modification or replacement of the antibiotic target and decrease in cell membrane permeability" (Hurtado *et al*., 2017). "Thus, *Salmonella* develops resistance mechanisms by developing mutations in the gene locations of target proteins or acquiring mobile genetic elements resistance genes such as plasmid, integrons and transposons, which are readily transferred among *Salmonella* strains and between other bacterial species. Multi-drug Resistance *Salmonella* strains resulting from acquisition of these genetic element have been found worldwide and are a growing concern for public health and food safety" (CDC, 2004). "Although future research efforts on the ecology, epidemiology, and evolution of drug-resistant *Salmonella,* in conjunction with technological advances to allow rapid identification and characterization of antimicrobial resistant *Salmonella* isolates, are needed to address this food safety issue. There are many classes of antimicrobial drugs, but most common antimicrobials that *Salmonella* has developed resistance at the present include; aminoglycosides, β-lactams, chloramphenicol, quinolones, tetracyclines, sulfonamides and trimethoprim" (Walker *et al*., 2001). "Among *Salmonella* isolates, the most commonly encountered resistance type is referred to as Multidrug Resistance Ampicillin C, which indicates resistance to ampicillin, chloramphenicol, amoxicillin, streptomycin, sulfamethoxazole, and tetracycline and reduced susceptibility to ceftriaxone" (CDC, 2004). As a result, this present study examined to investigate the relative frequency of the risk that a person develops *Salmonella*-associated illness with reduced susceptibility that would be isolated from sea foods, and its resistance to drugs.

MATERIALS AND METHODS

Description of the Study Area

This study was carried out in three (3) selected markets in Port Harcourt Metropolis, Rivers State, Nigeria.

Collection of Samples

A total of three (3**)** fresh sea foods were randomly purchased from the three (3) selected markets in Port Harcourt Metropolis, Rivers State, namely Mile 1, Mile 3 and Creek Road markets. The fresh sea foods purchased from each market were Crab, Periwinkle and Prawn. The samples were collected in ice packs and immediately transported to Rivers State University Microbiology Laboratory for analyses.

Serial Dilution

There was a sequential ten-fold dilution. To obtain stocks of the samples in various test tubes, one gram (1g) of each sample was weighed into nine milliliters (9ml) of sterile normal saline. One milliliter (1ml) was transferred from the stock into test tubes filled with 9ml of sterile normal saline before being serially diluted with dilution factors of 10^{-1} to 10^{-5} (Cheesbrough, 2005).

Inoculation and Isolation of the Test Organism

Each sample was diluted appropriately, and an aliquot (0.1 ml) of each was placed in duplicate on sterile Nutrient, MacConkey and Salmonella-Shigella (SS) agar plates. The spread plate technique was used to disperse it equally using a sterilized glass spreader. The agar plates were incubated at 37**°**C for 24 hours. The plates were examined after incubation, and the colonies that emerged were counted and noted (Zhou *et al.*, 2002).

Purifying and Preserving of Bacterial Isolates

Following the bacterial isolation using a sterile wire loop, distinct colonies with specific characteristics were selected from the incubated agar plates and sub cultured using the streak plate method on sterile nutrient agar plates. The plates were then incubated at 37 °C for 24 hours to produce pure isolates. A single colony was moved aseptically from the subculture plates to the nutritional agar slants, where it was cultured for 24 hours at 37°C. Following incubation, the slants were housed in well-baffled vials of 10% glycerol storage media and kept in a refrigerator at -4°C. It has become vital to keep the pure cultures free of contamination in order to preserve the viability and purity of the isolates (Cheesbrough, 2006).

Characterization of Bacterial Isolates

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. Morphological studies were carried out on different media plates used for the isolation of the organisms; pure colonies were isolated based on colony size, shape, pigmentation, elevation and texture of the individual organisms after 48 hours of growth at 30^0C. Pure isolates from the respective media were characterized and identified based on their morphological, biochemical and physiological features. A colony of the isolate was selected and streaked on a freshly made

nutrient agar plate, where it was cultured for 24 hours at 37°C. The following morphological characteristics of the isolate colony after incubation were seen visually using a hand lens: shape, size, coloration, edge, texture, and elevation. Gram staining and other biochemical assays were utilized to examine the cell morphology of the overnight pure cultures of the bacterial isolates (Cheesbrough, 2005; Cheesbrough, 2006; Holt *et al.*, 1994).

Antibiotic Susceptibility Testing

The antimicrobial susceptibility profiles or pattern of the *Salmonella* isolates to antibiotics were determined using the Kirby-Bauer disk diffusion method on sterile Mueller-Hinton agar. Standardization of the isolates in a suspension was carried out by adjusting to 0.5 McFarland turbidity standards containing $x10^8$ cells. A sterile swab was dipped into the bacteria suspension, pressed on the side of the test tubes to allow excess drip off and then used to evenly streak the entire surface of the Mueller Hinton agar and rotating the agar plate 60º each time to ensure even distribution of the inoculum (CLSI, 2017). The plates were left to air dry for 3–5 min. Conventional antibiotics disk impregnated with Tarivid (10mg), Peflacine (10mg), Ceporex (10mg), Ciprofloxacin (10mg), Streptomycin (30mg), Septrine (30mg), Ampicillin (30mg), Augmentin (30mg), Nalidixic Acid (30mg) and Gentamycin (10mg) were aseptically placed on the surface of the inoculated agar plate with sterile forceps. Each disk was pressed down to make full contact with the surface of the agar. The plates were then incubated for 24 hours at 33 to 35ºC in an inverted position. The zones of inhibition were measured in millimetre (mm) using a meter rule and compared to (CLSI, 2017).

Determination of Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic resistance is the resistance of bacterial isolates to three or more antibiotics. Multiple antibiotic resistance (MAR) index was ascertained for each isolate by using the formula MAR = a/b, where ‘’a’’ represent the number of antibiotics to which the test isolates depicted resistance and ‘’b’’ represent the total number of antibiotics to which the test isolate has been tested for susceptibility (Krumperman, 1985).

Data analysis

Statistical Package for Social Sciences (SPPSS) version 22 was used to analyse the data obtained from the measurement of the zones of inhibition. Descriptive statistics were used to summarize all data obtained (Bewick *et al*., 2004).

RESULTS

Results of the total heterotrophic bacterial count for the three sea food samples from the three selected markets as presented in Table 1 revealed that Prawn from Mile 1 market had the highest mean count of $2.06x10^8$ cfu/g while Crab from Creek Road market had the least mean count of $1.15x10^8$ cfu/g. The result of the total coliform count for the three sea food samples from the three selected markets as shown in Table 2 revealed that Crab from Mile 1 market had the highest mean count of $2.86x10^6$ cfu/g while Periwinkle from Creek Road market has the least mean count of $2.30x10^6$ cfu/g. The result of the total salmonella shigella count for the three sea food samples from the three selected markets as shown in Table 3 revealed that Crab from Creek Road market has the highest mean count of $2.80x10^5$ cfu/g while Periwinkle from Mile 1 market had the least mean count of $0.76x10^5$ cfu/g.

Results of the bacterial isolate morphological and biochemical characteristics as shown in Table 4, based on their colonial, morphological, and biochemical traits, isolates' identities were made known based on the comparison with Cheesbrough (2005) and Holt *et al.*(1994), the identities of the bacterial isolates were *Proteus sp., Bacillus spp., Pseudomonas sp., Micrococcus sp., Staphylococcus aureus, Escherichia coli,* and *Salmonella spp.*

Results of percentage occurrence of the bacterial isolates as presented in Tables 5, 6 and 7 revealed that all the isolates were present in all the sampled sea foods from the three selected markets with periwinkle having the highest prevalence (48%) and prawn having the lowest prevalence (20%) from Mile 1 market while crab had the highest prevalence (42%) and prawn having least prevalence (26%) in sea foods from Mile 3 market. Also, crab from Creek road market had the highest prevalence (38%) while prawn had the least prevalence (28%).

Results for the antimicrobial susceptibility of *Salmonella* isolates obtained from the sea foods were presented in Tables 8, 9 and 10. In Table 8, *Salmonella* PK isolate was completely 100% susceptible to Tarivid, Ciprofloxacin, Gentamycin, and Ampicillin while there was a complete 100% resistance to Ceporex and Nalidixic acid only. In Table 9, Tarivid, Peflacine, Ciprofloxacin, Augmentin, and Septrine were 100% sensitive to the isolate of *Salmonella* PR while Gentamycin, Streptomycin, Ceporex, Nalidixic acid and Ampicillin were 100% resistance completely. In Table 10, the result showed that Tarivid, Peflacine, Gentamycin, and Septrine were 100% sensitive to the isolate of *Salmonella* CB, while there was 100% complete resistance to antibiotics such as Augmentin, Ciprofloxacin, Ceporex, Nalidixic acid and Ampicillin.

Result of multidrug resistant index was shown in Table 11. The multidrug resistance index of 3 (100%) of the 3 *Salmonella* isolates had a MAR index equal or greater than 0.2.

Table 1: Total Heterotrophic Bacterial Count of the samples from the three selected markets, expressed in Colony Forming Unit per gram (CFU/g)

Sample	Market		
	Mile 1 (cfu/g)	Mile 3 (cfu/g)	Creek Road (cfu/g)
Periwinkle	$1.32x10^8$	$1.26x10^8$	$1.24x10^8$
Prawn	$2.06x10^8$	$1.98x10^8$	$2.02x10^8$
Crab	$1.19x10^8$	$1.21x10^8$	$1.15x10^8$

Table 2: Total Coliform Count of the samples from the three selected markets, expressed in Colony Forming Unit per gram (CFU/g)

Sample	Market		
	Mile 1 (cfu/g)	Mile 3 (cfu/g)	Creek Road (cfu/g)
Periwinkle	$2.39x10^6$	$2.40x10^6$	$2.30x10^6$
Prawn	$2.48x10^6$	$2.35x\ 10^6$	$2.42x10^6$

Crab	**$2.86x10^6$**	**$2.76x10^6$**	**$2.68x10^6$**

Table 3: Total Salmonella Shigella Count of the samples from the three selected markets, expressed in Colony Forming Unit per gram (CFU/g)

Sample	**Market**		
	Mile 1 (cfu/g)	**Mile 3 (cfu/g)**	**Creek Road (cfu/g)**
Periwinkle	**$0.76x10^5$**	**$0.85x10^5$**	**$0.91x10^5$**
Prawn	**$2.74x10^5$**	**$2.68x10^5$**	**$2.65x10^5$**
Crab	**$2.72x10^5$**	**$2.73x10^5$**	**$2.80x10^5$**

Table 4: Biochemical Tests of Bacterial Isolates

Colonial/ Cell Characteristics	Gram Reaction	Catalase	Oxidase	Citrate	Indole	Motility	MR	VP	Sucrose	Glucose	Lactose	Probable organism
Smooth, pale or colorless; rods	-	-	-	-	-	+	+	-	-	-	-	*Proteus sp.*
Flat or slightly convex with irregular edges; rods	+	+	-	-	+	-	+	+	AG	AG	AG	*Bacillus sp.*
Flat, odor, slime and grape-like; rods	-	+	+	-	-	+	+	-	-	-	-	*Pseudomonas sp.*
Spherical, occurring in pairs, tetrads, or irregular clusters; cocci	+	+	+	-	-	+	+	-	A	A	A	*Micrococcus sp.*
Smooth, round and yellow; cocci	+	+	-	+	+	-	+	+	AG	AG	AG	*Staphylococcus aureus*
Smooth, concave and milk white; rods.	+	+	-	-	-	+	+	-	A	A	-	*Bacillus megaterium*
Round, wrinkled and opaque; rods	+	+	-	-	-	+	+	+	A	A	A	*Bacillus licheniformis*
Large, thick, moist, smooth, opaque, grayish white; rods	-	+	+	+	+	-	+	-	A	A	AG	*Escherichia coli*
Smooth, transparent, colorless; rods	-	+		+	+	+	+	+	A	-	A	*Salmonella sp.*
Smooth, transparent, colorless; rods	-	+		+	+	+	+	+	A	-	A	*Salmonella sp.*
Smooth, transparent, colorless; rods	-	+		+	+	+	+	+	A	-	A	*Salmonella sp.*

Table 5: Percentage Occurrence of Probable Organism from each sample in Mile 1 Market

S/N	Probable Organism	Periwinkle	Prawn	Crab	Total (%)	Frequency (%)
1	*Proteus sp.*	0	2	3	5	10
2	*Bacillus cereus*	1	1	2	4	8
3	*Pseudomonas sp.*	2	0	1	3	6
4	*Micrococcus sp.*	3	1	1	5	10
5	*Staphylococcus aureus*	4	0	0	4	8
6	*Bacillus megaterium*	2	0	3	5	10
7	*Bacillus licheniformis*	3	1	1	5	10
8	*Escherichia coli*	2	2	0	4	8
9	*Salmonella sp.*	2	2	1	5	10
10	*Salmonella sp.*	3	0	2	5	10
11	*Salmonella sp.*	2	1	2	5	10
	Total	24	10	16	50	100

Table 6: Percentage Occurrence of Probable Organism from each sample in Mile 3 Market

S/N	Probable Organism	Periwinkle	Prawn	Crab	Total (%)	Frequency (%)
1	*Proteus sp.*	1	1	2	4	8
2	*Bacillus cereus*	0	2	3	5	10
3	*Pseudomonas sp.*	1	1	3	5	10
4	*Micrococcus sp.*	4	0	0	4	8
5	*Staphylococcus aureus*	0	1	2	3	6
6	*Bacillus megaterium*	3	0	2	5	10
7	*Bacillus licheniformis*	1	3	1	5	10
8	*Escherichia coli*	0	2	2	4	8
9	*Salmonella sp.*	2	1	2	5	10
10	*Salmonella sp.*	2	0	3	5	10
11	*Salmonella sp.*	2	2	1	5	10
	Total	16	13	21	50	100

Table 7: Percentage Occurrence of Probable Organisms from each sample in Creek Road Market

S/N	Probable Organism	Periwinkle	Prawn	Crab	Total (%)	Frequency (%)
1	*Proteus sp.*	2	2	1	5	10
2	*Bacillus cereus*	2	0	3	5	10
	Pseudomonas sp.	0	2	2	4	8
4	*Micrococcus sp.*	1	3	1	5	10
5	*Staphylococcus aureus*	3	0	1	4	8
6	*Bacillus megaterium*	1	1	2	4	8
7	*Bacillus licheniformis*	0	1	4	5	10
8	*Escherichia coli*	1	3	1	5	10
9	*Salmonella sp.*	3	0	1	4	8
10	*Salmonella sp.*	2	2	1	5	10
11	*Salmonella sp.*	2	0	2	4	8
	Total	17	14	19	50	100

Table 8: Antibiotic Sensitivity Pattern of *Salmonella* PK and its zone of diameter (mm)

Antibiotics with Concentrations (µg)	% Susceptibility n(%)	% Intermediates n(%)	% Resistance n(%)
Tarivid (10)	1(100)	0	0
Peflacine (10)	0	1(100)	0
Ciprofloxacin (10)	1(100)	0	0
Augmentin (30)	0	1(100)	0
Gentamycin (10)	1(100)	0	0
Streptomycin (30)	0	1(100)	0
Ceporex (10)	0	0	1(100)
Nalidixic Acid (30)	0	0	1(100)
Septrine (30)	0	1(100)	0
Ampicillin (30)	1(100)	0	0

Table 9: Antibiotic Sensitivity Pattern of *Salmonella* PR and its zone of diameter (mm)

Antibiotics with Concentrations (µg)	% Susceptibility n(%)	% Intermediates n(%)	% Resistance n(%)
Tarivid (10)	1(100)	0	0
Peflacine (10)	1(100)	0	0
Ciprofloxacin (10)	1(100)	0	0
Augmentin (30)	1(100)	0	0
Gentamycin (10)	0	0	1(100)
Streptomycin (30)	0	0	1(100)
Ceporex (10)	0	0	1(100)
Nalidixic Acid (30)	0	0	1(100)
Septrine (30)	1(100)	0	0
Ampicillin (30)	0	0	1(100)

Table 10: Antibiotic Sensitivity Pattern of *Salmonella* CB and its zone of diameter (mm)

Antibiotics with Concentrations (µg)	% Susceptibility n(%)	% Intermediates n(%)	% Resistance n(%)
Tarivid (10)	1(100)	0	0
Peflacine (10)	1(100)	0	0
Ciprofloxacin (10)	0	0	1(100)
Augmentin (30)	0	0	1(100)
Gentamycin (10)	1(100)	0	0
Streptomycin (30)	0	1(100)	0
Ceporex (10)	0	0	1(100)
Nalidixic Acid (30)	0	0	1(100)
Septrine (30)	1(100)	0	0
Ampicillin (30)	0	0	1(100)

Table 11: MAR Index of *Salmonella spp* from all the Samples Analyzed

MAR Index	*Salmonella spp* N=3
	n (%)
0.0	0(0.00)
0.1	0(0.00)
0.2	1(33.33)

0.3	**0(0.00)**
0.4	**0(0.00)**
0.5	**2(66.67)**
0.6	**0(0.00)**
0.7	**0(0.00)**
0.8	**0(0.00)**
MAR Index ≥0.2 100%	

DISCUSSION

Salmonella are responsible for the transmission of infectious diseases that are spread through sea foods such as cholera, diarrhea, and typhoid (Nassinyama *et al.,* 2000). According to World Health Organization (2011), the permissible limit of total heterotrophic bacteria in a food sample is 100 cfu/g and 0.00 cfu/g for pathogenic bacteria and 0.00 cfu/g for total coliforms, but counts obtained in this study are above limit in all the selected sea food samples which shows that the sea foods are not safe for consumption. The main source of these bacteria in the sea foods can be attributed to human activities and environmental conditions (Isa, 2013). The presence of coliforms in sea food samples in this study is enough grounds for assuming that potential health hazard existed because of the possible presence of pathogens. The possible cause of detecting high level of coliforms in Crab sample from Mile 1 market could be the proximity of poor hygienic conditions of the personnel which may have led to contamination of the food. High total coliform from crab samples can also be originated from environmental sources such as soils. Another possible cause of high coliform from this same market could be poor storage conditions. This is similar to previous findings by Agwa *et al*., (2012) who isolated coliforms from ready to eat foods sold in some markets in Port Harcourt, Rivers State. Similar cases of microbial contamination of ready to eat food samples have also been reported in Malaysia by Alyaaquobi *et al.,* (2009).

Microorganisms in sea foods are a serious threat and concern to public health. The bacteria isolated and identified in this study from the three different sea foods at the three selected markets include *Proteus sp., Bacillus spp., Pseudomonas sp., Micrococcus sp., Staphylococcus aureus, Escherichia coli,* and *Salmonella spp*. Even though most of the microorganisms obtained in this study may not be extremely pathogenic, their presence in sea food samples indicates contamination, and possible presence of other pathogenic organisms (Oranusi and Braide, 2012). *Escherichia coli* are a coliform organism used as an indicator of *fecal* contamination. Although vast majority of *E. coli* are completely harmless, some strains of the bacteria have acquired genetic capabilities which enable them to encode for virulence factors. Pathogenic *E. coli* strains cause diverse forms of bacteria-induced illnesses with symptoms ranging from mild diarrhea to severe complications and even death. Similar results were obtained by Oranusi and Braide, (2012) in a research on foodborne illness incidence rates and food safety risks for population of low socioeconomic states and minority race/ethnicity. *Escherichia coli* have been implicated as one of the leading causes of bloodstream infections (BSI) in humans coupled with its increasing levels of antimicrobial resistance (Oranusi and Braide, 2012). This organism has been a public health concern. Also, the presence of *E. coli* in sea foods is undesirable because it indicates poor hygienic conditions which have led to contamination (Kumar *et al.,* 2009). *Bacillus* genus are spore forming organisms and are able to act as opportunistic pathogens causing illnesses such as cerebrospinal fluid shunt infections, endocarditis, endophthalmitis, meningitis, as well as bacteremia (Alyaaqoubi *et al.,* 2009). *Proteus spp.* is mostly found in the intestinal flora and also, can be found in multiple environmental habitats, including long-term care facilities and

hospitals. When these organisms invade the bloodstream, releases a toxin called endotoxin, a component of gram-negative bacterial cell walls, apparently triggers a cascade of host inflammatory responses and leads to major detrimental effects (Alyaaqoubi *et al.*, 2009). *Pseudomonas sp*. is a multidrug resistant pathogen recognized for its ubiquity, it's intrinsically advanced antibiotic resistance mechanisms and its association with serious illnesses-hospital acquired infections such as ventilator-associated pneumonia and various sepsis syndromes (Hoiby *et al.*, 2010). *Micrococcus sp.* is generally thought to be a saprotrophic or commensal organism, though it can be an opportunistic pathogen, particularly in hosts with compromised immune systems, causing some infections such as recurrent bacteremia, septic shock, arthritis, endocarditis, meningitis, etc. *Staphylococcus aureus* are one of the most common bacterial infections in humans and are the causative agents of multiple human infections, including bacteremia, infective endocarditis, skin and soft tissue infections, osteomyelitis, gastroenteritis, and urinary tract infections (Tony *et al.*, 2015). *Salmonella* are a group of bacteria implicated as the spoilage of variety of foods such as sea foods, poultry eggs, pork, meats and meat products, beef, fruits and vegetables. *Salmonella enterica* has been reported to be the causative agents of gastroenteritis, diarrhea, fever, and urinary tract infections (CDC, 2004; Hung *et al.*, 2017).

Most of the *Salmonella* isolates were resistant to Ceporex, Nalidixic acid, Ampicillin, Gentamycin, Streptomycin, and Augmentin. The resistance of the isolates to some of these antibiotics has been reported by Agwa *et al.*, (2012). When combined, the *Salmonella* isolates recorded 100% resistance to the Augmentin and Ampicillin combination. This point to the fact that since the organism was resistant to the antibiotics singly even when combined in synergy, there was no positive effect.

The susceptibility pattern of the *Salmonella* isolates revealed that Tarivid, Peflacine, Septrine and Ciprofloxacin were more effective drugs and could be considered the drugs of choice for infections caused by this bacterial. A similar report was made by (Wemedo and Robinson, 2018) that Ciprofloxacin, Peflacine, Tarivid and Septrine were effective against bacteria isolated from air in a Public Hospital and Health Centre.

Higher percentage of the *Salmonella spp* had a higher multidrug resistant index greater than 0.2. Today development of multidrug resistance is become natural phenomenon, due to interestingly raise in the number of immunocompromised conditions, blind and improper use of broad spectrum of antibiotics as well as poor infection prevention, beside that patients profile, environmental and geographical factor were among important player determining the bacterial profile and resistance pattern (Lebea and Davies, 2017; Weinstein, 2001).

CONCLUSIONS AND RECOMMENDATIONS

In this study, the microbial loads were high and exceeded the limits of acceptable microbial loads in food. The counts revealed that Crab had the lowest microbial load among the three sea food samples while Prawn had the highest microbial load. Also, seven bacterial genera belonging to the genus; *Proteus, Bacillus, Staphylococcus aureus, Escherichia coli, Pseudomonas, Micrococcus* as well as *Salmonella* were identified from the sea food samples. Furthermore, the antibiotic sensitivity profile of the *Salmonella* isolates revealed that *Salmonella* isolates were resistant to Ceporex, Nalidixic acid, Ampicillin, Gentamycin, Streptomycin, and Augmentin. thereby raising concern of possible multidrug resistance. Because of the increased demand for sea foods due to growing human population, we have become exposed to pathogens present in the sea food sold in markets which poses a risk to food poisoning associated with them. Furthermore, this study support assertions that the presence of *Salmonella* isolated from sea foods may have been contaminated upon handling,

storage, poor hygienic conditions of the sellers and other environmental factors, therefore, good hygiene of personels and storage conditions should be encouraged since it became necessary to maintain food safety which would have reduced the microbial loads to the acceptable limits.

REFERENCES

Agwa, O. K., Ugoigwe, C. I. and Wokoma, E. C. (2012). Incidence and antibiotic sensitivity of *B. cereus* isolated from ready to eat foods sold in some markets in Port Harcourt, Rivers State. *Asian Journal of Microbiology and Biotechnology Environmental Science*, 14(1), 13-18.

Alyaaquobi, S. J. M., Sani, N. A., Abdullah, A. and Abdul Rahman, R. D. (2009). Microbiological Quality of Selected Ready to Eat Food at Hulu Langat District, Malaysia. *Prosiding Seminar Kimia Bersama Ukm-Itb*. 9; 421-433.

Bewick, V., Cheek, L. and Ball, J. (2004). Statistics Review 9: One-way Analysis of Variance. Critical Care, 8 (2), 130 – 136.

Center for Disease Control and Prevention, 2004. National Antimicrobial Resistance Monitoring System for enteric bacteria (NARMS): 2002 human isolates final report. *U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta*.

Cheesbrough, M. (2000). Microbiological test District Laboratory Practice in Tropical Countries. In: Cremer, A. and Evan, G. (eds). *Cambridge University Press*, UK. Pp:1-226.

Cheesbrough, M. (2005). District Laboratory Practice in Tropical Countries, part 2. *Cambridge University Press, Cambridge*. Pp:159-162.

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries. *Cambridge University Press*. Pp:62.

Clinical and Laboratory Standard Institute. (2017). *Performance Standards for Antimicrobial Susceptibility Testing, Twenty-first Informational Supplement.* CLSI document M100-S21 (ISBN1-56238-742-1) Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087 USA, 30(1): 68-70.

Fàbrega, A. and Vila, J. (2013). Salmonella enterica serovar Typhimurium skills to succeed in the host: virulence and regulation. *Clinical Microbiology Reviews.* 26(2): 308–341.

Hoiby, N., Bjarnsholt, T., Givskov, M., Molin, S. and Ciofu, O. (2010). Antibiotic resistance of bacterial biofilms. *International Journal of Antimicrobial Agents.* 35, 322-332.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Stanley, J. T., and William, S. T., (1994). Bergey's Manual of Determinative Bacteriology. Williams and Wilkins, Baltimore, 786-788.

Hung, Y. T., Lay, C. J., Wang, C. L. and Koo, M. (2017). Characteristics of non-typhoidal gastroenteritis in Taiwanese children: A 9-year period retrospective medical record review. *Journal on Infections to Public Health.* 10: 518–521.

Hurtado, A., Ocejo, M. and Oporto, B. (2017). *Salmonella* spp. and *Listeria monocytogenes* shedding in domestic ruminants and characterization of potentially pathogenic strains. *Veternary Microbiology*. 210: 71–76.

ICMSF (International Commission on Microbiological Specifications for Food). 2000. Microorganisms in Foods 1. Their Significance and Methods of Enumeration, 2nd ed. Toronto: *University of Toronto Press.*

International Society of Automation – (ISA) (2013). ANSI/ISA-95.00.03-2013. Enterprise-Control System Integration – part 3; Activity Models of Manufacturing Operations Management. *Research Triangle Park;* ISA.

Janda, J. M. and Abbott, S. L. (2006). "The Enterobacteria", ASM Press.

Karch, H., Mellman, A. and Bielaszewska, M. (2009). Epidemiology and pathogenesis of enterohemorrhagic *Escherichia coli. Journal of Clinical Microbiology*, 122: 417-424.

Krumperman, P. H. (1985). Multiple Antibiotic Indexing of E. coli to Identify High-Risk Sources of Fecal Contamination of Foods. Applied and Environmental Microbiology. 46, 165–170.

Kumar, S., Otta, S. K. and Karunasagar, I. (2009). Detection of Shiga-toxigenic Escherichia coli (STEC) in fresh seafood and meat marketed in Mangalore India by PCR. *Letters in Applied Microbiology* 33(5): 334–338.

Lebea, M. M. and Davies, V. (2017). Evaluation of culture proven neonatal sepsis at a tertiary care hospital in Johannesburg, South Africa. *South African Journal of Child Health*, 11, 170-173.

Nassinyama, G. W., McEwen, S.A., Wilson, J. B., Waltmer-Tower, D., Gyles, C. L. and Oputa, J. (2000). Risk factors for Acute Diarrhea among inhabitants of Kampala District Uganda. *South African Medical Journal.* 90 (90): 891-898.

Oranusi, S. U. and Braide, W. (2012). Microbiological status of processed fruit juice sold in the commercial city of Onitsha. *Scholarly Journal of Biological Science*, 1 (3). Pp. 25-30. ISN 2315-6147.

Su, L. H. and Chiu, C. H. (2007). *Salmonella*: clinical importance and evolution of nomenclature. *Chang Gung Medical Journal*. 30 (3): 210–219.

Tony, S. Y. C., Davis, S. D., Emily, E., Holland, T. L. and Fowler Jr, V. G. (2015). *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Review.* 28(3): 603-661.

Walker, R. A., E., Lindsay, M. J., Woodward, L. R., Ward, and Threlfall, E. J. (2001). Variation in clonality and antibiotic-resistance genes among multi-resistant *Salmonella enterica* serotype Typhimurium phage-type U302 (MR U302) from humans, animals, and foods. *Microbiology Drug Resistance*. 7:13-21.

Weinstein, R. A. (2001). Controlling antimicrobial resistance in hospitals: infection control and use of antibiotics. Emerging infectious diseases, 7, 188.

Wemedo, S., A. and Robinson, V. (2018). Evaluation of indoor Air for Bacteria Organisms and their Antimicrobial susceptibility Profiles in a Government Health Institution. *Journal of Advances in Microbiology*, 11(3): 1-7.

World Health Organization (2011). Outbreaks of *Escherichia coli* in Europe. *Reviewed* 2018.

Zhou, Z., Nishikawa, Y., and Zhu, P., (2002). Isolation and Characterization of Shiga toxin-producing *Escherichiacoli* O157:H7 from beef, pork and cattle fecal samples in Changchun, China. *Journal of Veterinary Medical Science*, 64(11): 1041-1044.