ANTIBIOGRAM OF POTENTIALLY PATHOGENIC MICROBES ISOLATED FROM VENDED SEA FOODS (CARIDEA)

Emmanuel, Morka[1], **Emmanuel-Akerele,** Hilda Abiola[2]

[1]Department of Microbiology, Delta State University, Abraka, Nigeria
[2]Department of Biological Sciences, Anchor University, Lagos, Nigeria.
Corresponding author email: akatahhilda@gmail.com

Abstract

The aim of the study was to evaluate the presence of pathogenic microbes in seafood and determine the isolate's antimicrobial susceptibility. Thirty (30) samples of seafood (shrimps) were randomly purchased from Abraka and Eku markets in February to March 2020. Microbiological analysis and antibiogram was carried out using standard methods. Microorganisms isolated include; Escherichia coli, Klebsiella sp., Salmonella sp., Proteus sp., Serratia sp., Candida albicans and Saccharomyces cerevisae. Among the bacterial isolates, Escherichia coli (26.9%) had the highest prevalence, followed by Proteus sp. (19.2%) and Salmonella sp. (11.5%), while Klebsiella sp. (7.6%) and Serratia sp. (7.6%) were the least dominant strains. Candida albicans (19.2%) was the most prevalent fungus followed by Saccharomyces cerevisae (3.8%). Results of the antimicrobial susceptibility test showed that Chloramphenicol (100%), Ciprofloxacin (100%) and Augmentin were the most susceptible drugs analyzed in the study compared to Gentamycin (20%) and Augmentin (20%) with the least susceptibility. Hence, from the data obtained, it was observed that shrimps were contaminated with pathogenic microbes and if not properly managed and processed, there could be risk of health hazard. It is therefore recommended that measures should be taken in other to prevent contaminations when handling and processing sea-foods.

Key words: Antibiotics, Contamination, Hazards, Microorganisms, Shrimps.

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INTRODUCTION

Food are generally one of the basic necessities of man and its relevance cannot be jettison or outlawed, consequent on this fact includes seafood. Seafood are any form of sea life regarded as food by humans, which includes fish and shellfish (Cordain *et al*., 2015). Shell fish include various species of mollusk (bivalve) such as clams, oysters, and mussels and cephalopods such as octopus and squid, crustaceans (*e.g* shrimps, crabs and lobster) and echinoderms (e.g. sea cucumbers and sea urchins). Seafood is currently accepted as an essential food for humans (FAO, 2018). Seafood is highly regarded for its abundance of high quality proteins, n-3 polyunsaturated fatty acids (PUFAS) and other nutrients, such as minerals, trace elements and vitamins (FAO, 2018). These nutrients are essential for bodily functions and are beneficial to growth, brain functions and the nervous system, they also have anticancer properties.

Sea foods harbor many pathogenic microorganisms such as B*acillus*, *Clostridium*, *Escherichia*, *Serratia*, and *Vibrio*. Gram-negative aerobic rods and facultative anaerobic rods and coli forms constitute the major spoilage bacterial flora (Thompson *et al*., 2018). Pathogens may be present at low levels when fish or shellfish are harvested, and others may be introduced during handling and processing / by unsanitary practices. Bacteria in food may cause illness in humans by infection or intoxication (Cerchietti *et al*., 2017). From 1973 to 2006, *Vibrio* species accounted for 38% of the outbreaks associated with seafood and 54% of the illnesses, *Salmonella* and *Shigella* each were associated with about 10% of the reported illnesses, and *Listeria monocytogenes* approximately 1% (Hwu *et al*., 2018). Foodborne intoxications occur when patients consume pre-formed toxins that are produced by certain types of bacteria when they grow and multiply in the food. *Clostridium botulinum* can produce a

potent neurotoxin during growth under anaerobic condition usually associated with vacuum packed, improperly canned, or fermented products (Osibote *et al*., 2014). *Staphylococcus aureus* can produce enterotoxins that cause foodborne illness, but less than 5% of the seafood associated outbreaks and illnesses were associated with this pathogen over the past three decades (Linseiser *et al*., 2019). Preventing the growth of these bacterial pathogens is important to prevent infection or intoxication when seafood is eaten (Cordain *et al*., 2015).

Spoilage of food products may be due to chemical, enzymatic and/ microbial activities. The fresh fish spoilage is a very rapid process and the spoilage process generally starts within 12 h of storage at high ambient temperatures (Berkel *et al*., 2014). The oxidative spoilage of seafood is mainly concerned with lipid oxidation of fishes and other seafood with high oil/fat content. With the initiation of spoilage, a sweet odor develops followed by stale-fish odor which is due to the formation of trimethylamine. At the end there is generation of ammonia odor followed by putrid odors due to H_2S and indole compounds (Erkmen and Bozoglu, 2016).

In recent decades, antimicrobial resistance by pathogenic bacteria have become a major public health problem in many countries (Rabbi *et al*., 2014). Many studies have reported the presence of multi drug resistance bacteria in different types of food (Nipa *et al*., 2014). Drug resistance is spreading day by day mainly due to the overuse of antibiotics, incomplete and underuse of medications and wide spread practice of feeding livestock with low levels of antibiotics to promote growth (Sultana *et al*., 2018). Due to the high demand and importance of seafood in our diet and the world at large, coupled with man-made and microbiological contamination alongside the multidrug resistance ability of some pathogenic microbes, there is need to continuously investigate the presence of pathogenic microbes in seafood which are major components of our diet. Hence, the current study was undertaken to isolate pathogenic microorganisms present in seafood and determine the pathogens antimicrobial susceptibility level.

MATERIALS AND METHODS

Study Area

The study was conducted in Abraka, in Ethiope East Local Government Area, Delta State. Abraka. Abraka is located 5^0 47′ 0″ North and $6^06'0''$ East of Delta State, Nigeria.

Sample Collection

Thirty (30) Samples (Seafood) were purchased from Eku market and Abraka environs in Ethiope East Local Government Area of Delta State and was used for study. The samples were placed in a sterile polythene bag and transported in cold ice packs to the Microbiology laboratory for analysis.

Processing of Seafood

1 gram of the seafood samples was cut with a sterile knife. The cut samples were crushed into small pieces in a sterile blender with about 10ml sterile water to get a homogenized mixture. 1ml aliquot volume was measured out and homogenized in a clean, dry sterile beaker containing 9ml of distilled water giving a 1:10/10 dilutions. This was done for all the samples

Estimation of Total Viable Bacteria (TVB)

Pour plate method was used for estimating the total viable bacteria. 1ml of the serially diluted samples were introduced into sterile Petri plates and molten agar (Nutrient agar (NA) (Himedia, India), Eosin methylene blue agar (Himedia, India), Thiosulphate citrate bile salt agar (TCBS) (Himedia, India) cooled to 40-45^{o}C was added for enumerating total viable bacteria (TVB). The plates were incubated at 37^0C for 24 h (Lindstrom and Korkeala, 2016).

Isolation of Fungi

1ml of the serially diluted samples were introduced into sterile Petri plates and molten potato dextrose agar (PDA) (Himedia, India) cooled to 40-45^{o}C was added for enumerating the fungal population. The plates were incubated at 28-30^0C for 3-7 days.

Purification and storage of the isolates

All the isolates were purified by sub-culturing on nutrient agar plates before transferring onto nutrient agar and potato dextrose agar slants for bacteria and fungi respectively. These isolates were stored at 4^0C in the refrigerator as stock cultures for characterization and identification.

Characterization and Identification of Fungi Isolates

The colonial and microscopic characteristics of the fungi isolate were determined using the lactophenol cotton blue stain. A solution of lactophenol cotton blue was prepared. Using a sterile wire, a fragment of a fungal isolate was placed on a clean grease-free slide. Two drop of the lactophenol cotton blue solution were added and the stain allowed to penetrate. The slide was then viewed under the microscope. The isolate were identified following the description of Lindstrom and Korkeala, (2016)

Characterization and Identification of Bacterial Isolates

The isolated bacterial colonies were identified on the basis of the morphological, physiological and biochemical characteristics. These cultures were subjected to various biochemical tests such as gram staining, motility test, catalase test, indole test, oxidase test, urease citrate utilization and triple sugar iron test (TSI), hydrogen sulphide production tests as described in Kirby's manual of systematic bacteriology ((Lindstrom and Korkeala, 2016).

Antimicrobial Susceptibility Testing

Discrete colonies of the isolates were inoculated into 5ml of normal saline standardized with 0.5 McFarland suspensions. Sterile cotton wool swab was used for the inoculation of the bacterial suspension to freshly prepared Mueller-Hinton agar (Oxoid, United Kingdom) plates prepared according to manufacturer's instructions. The antibiotic discs used were SXT; Septrin (30µg), CH; Chloranphenicol (30µg), SP; Sparfloxacin (10µg), CPX; Ciprofloxacin (30µg), AM; Amoxacillin (30µg); AU; Augmentin (10µg), PEF; Pefloxacin (30µg), OFX; Tarivid (10µg). The antibiotic discs were applied to each plate with sterile forceps with lower concentration towards the center of agar plate. The plate were inoculated at 37^0C for 24 h. The zone of inhibition of growth was measured in millimeter using Kirby-Bauer disc diffusion method (Nipa *et al*., 2014).

RESULTS AND DISCUSSION

A total of thirty (30) different samples of seafood were purchased randomly from Abraka main market and Eku main market. A total of five (5) bacterial isolates and two (2) fungal isolates were obtained from the food samples collected. The bacterial isolates were identified using their cultural morphology and biochemical characteristics as presented in Table 1. The bacterial species isolated were *Escherichia coli, Salmonella sp*., *Klebsiell*a *sp*., *Serratia sp.* and *Proteus sp. Escherichia coli* had the highest occurrence, followed by *Proteu*s sp. and *Salmonella sp*., while *Klebsiella sp.* and *Serratia sp* had the least occurrence with prevalence rates of 26.9%, 19.2%, 11.5%, 7.6% and 7.6% respectively as shown in Table 3.

The fungal isolates were identified using their colonial morphology and microscopic features as presented in Table 2. The fungal species were *Candida albicans* and *Saccharomyces cerevisae* with *Candida* having the highest occurrence with a prevalence percentage of 19.2% and *Saccharomyces cerevisae* having a prevalence percentage of 3.8% as presented in Table 4.

The antibiotic susceptibility test of commercial antibiotics against bacterial isolates is presented in Table 5. The test revealed that chloramphenicol (100%), ciprofloxacin (100%) and Augmentin (100%) were the most susceptible antibiotic while Gentamycin (20%) and Septrin (20%) appeared to be the least susceptible tested antibiotic.

Table 1: Identification of Bacterial Isolates

Tentative genera	Shape	Gram stain streaction	Catalase	Oxidase	Citrate	Indole	H_2S	Acid	Gas	Lactose	Glucose	Motility
Serratia sp.	Rod	-	+	-	+	+	+	-	-	+	+	-
Escherichia coli	Rod	—	+	—	+	+	—		+	+	+	+
Klebsiella sp.	Rod	—	+	+	+	—	—		+	_	+	+
Proteus sp.	Rod	—	+	—	—	—	+		+	—	+	+
Salmonella sp.	Rod	—	+	—	—	+	+		—	—	+	—

Key: + = Positive; − = Negative

Table 2: Identification of Fungal Isolates

S/N	Isolate	Microscopic	Macroscopic
1	*Candida albicans*	Spherical and subspherical budding, 2.7-3 x 8.3µm in size.	White to cream coloured, globotous and yeast like.
2	*Saccharomyces cerevisae*	Spherical to elongated budding, yeast-like cells or blastocnidia, 2.5-6.5 x 14.0 µm in size.	Coal pink, usually pink sometimes, reticulated / corrugated moist to mucoid yeast-like colonies.

Table 3: Prevalence of Bacterial Isolates

Isolates	Prevalence (%)
Escherichia coli	26.9
Klebsiella sp.	7.6
Salmonella sp	11.5
Proteus sp	19.2
Serratia sp	7.6

Table 4: Prevalence of Fungal Isolates

Isolates	Prevalence (%)
Candida albicans	19.2
Saccharomyces cerevisae	3.8

Table 5: Antibiotics Susceptibility for the Bacterial isolates

Organisms	ZONES OF INHIBITION (mm)									
	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
Escherichia coli	8(R)	16(S)	18(S)	16(S)	8(R)	16(S)	18(S)	20(S)	00(R)	00(R)
Proteus sp.	00(R)	15(S)	8(R)	15(S)	18(S)	15(S)	00(R)	00(R)	00(R)	8(R)
Salmonella sp	00(R)	18(S)	16(S)	15(S)	18(S)	18(S)	00(R)	16(S)	18(S)	15(S)
Serratia sp	8(R)	16(S)	8(R)	16(S)	15(S)	15(S)	00(R)	14(R)	14(R)	16(S)
Klebsiella sp	18(S)	15(S)	16(S)	16(S)	18(S)	16(S)	00(R)	16(S)	14(R)	16(S)

KEY: SXT= Septrin, **CH**= Chloramphenicol, **SP**= Sparfloxacin, **CPX**= Ciproflaxacin, **AM**= Amoxacillin, **AU**= Augmentin, **PEF**= Pefloxacin, **CN**= Gentamycin, **PEF**= Pefloxacin, **OFX**= Tarivid, **S**= Streptomycin

Table 6: Percentage antimicrobial susceptibility profile of bacterial isolates

SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
20%	100%	60%	100%	80%	100%	20%	60%	80%	60%

Discussion

The result of the study showed that five (5) bacterial species were isolated from the study which include: *Serratia sp*, *Escherichia coli*, *Klebsiella sp*, *Proteus sp*, and *Salmonella sp*. These organisms were also isolated by Erkmen and Bozoglu, (2016) who reported on the screening, isolation, identification and anibiogram study of bacteria in ready to cook, chilled food product. The bacterial species isolated from this study could be due to poor sanitary condition of the retailers and improper handling during storage and transportation which may have enhanced the proliferation of the microbes. *Serratia* constitutes a public health hazard which cause diarrhea and gastroenteritis. *Candida albicans* and *Saccharomyces cerevisae* were the two fungi isolated from the study. Similar organisms were isolated by Abosode *et al.* (2013) on the isolation and characterization of yeast strains from local food crops. Species of *Candida* have been reported as causal agent of various diseases in man (Abosede *et al.*, 2013).

The highest level of prevalence was observed among *E. coli* (26.9%) strain while *Klebsiella* sp (7.6%) and *Serratia* sp (7.6%) were the least dominant bacterial strain. The presence of *E.coli* and *Klebsiella* sp. suggests faecal contamination and may be responsible for gastrointestinal disorders (Anthony and Karaparnar, 2020). *Candida albicans* (19.2%) was the most dominant fungus, species of *Candida* are mostly pathogenic whereas the report of *Saccharomyces* as a pathogenic organism has not been widely studied except its application in food production.

Result of antibiotic susceptibility revealed that chloramphenicol (100%), ciprofloxacin (100%) and Augmentin (100%) were the most susceptible antibiotic while Gentamycin (20%) and Septrin (20%) appeared to be the least susceptible tested antibiotic. This was similar to the report made by Okoh, (2012) who reported that ciprofloxacin was the most susceptible drug compared with other tested antibiotic in his study. Thus, ciprofloxacin, chloramphenicol and Augmentin are highly recommended drugs for possible treatment of bacterial infections arising from consumption of sea foods.

CONCLUSION

The findings of the study revealed that sea food (shrimp) can be contaminated with pathogenic microbes. Hence, if not properly managed and processed, when consumed can lead to risk of health hazards. It is therefore recommended that safety measures should be taken into consideration in other to avoid contamination when handling or processing these sea foods.

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