The Effect of *Klebs*iella *pnemoniae* on Catfish (*Clarias gariepinus)*\

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The objective of this study is to isolate, identify and evaluate the effect of *Klebs*iella *pnemoniae* in relation to the catfish, by isolating the microorganisms present on the catfish skin and intestine. To find out if *Klebsiella pneumoniae* bacteria isolate can survive in the fish (immunity of fish) and observation of the public health hazard that bacteria i.e. test organism and natural flora exposes the people to, samples of catfish were analyzed using standard techniques. The physicochemical analysis showed that there were changes in the pH, the temperature and the dissolved oxygen of the water samples. The distributions of the bacteria species isolated from the catfish intestine and on the skin showed that *Klebsiella pneumoniae* [12(25.0%)] was the most predominant bacteria isolates. This was followed by *Enterococcus feacalis* [9(18.6%)], *Staphylococcus aureus* [7(14.6%)], *Escherichia coli* [6(12.5%)], *Citrobacter ferundii* [5(10.4%)], *Pseudomonas aeruginosa* [5(10.4%)], and *Bacillus subtilis* [4(8.3%)]. It also showed that *Enterococcus feacalis* and *Citrobacter ferundii* were absent in all the skin samples of the catfish but present in all the intestines. *Klebsiella pneumoniae* was present on all the skin and intestines of the catfish samples. *Escherichia coli* were present in all the skin and intestine except for skin of the catfish samples in Experiment 1. *Bacillus subtilis* was only present in the skin of the catfishes used as control. *Staphylococcus aureus* was absent in all the intestine of the catfishes samples but present in their skins. *Pseudomonas aeruginosa* was absent in all the samples except for the intestines of the catfishes used as control and those in experiment 2. Generally, *Klebsiella pneumoniae* inoculated into catfish *Clarias gariepinus* presented to be virulent in the fishes leading to shedding of skin patches and fading of colour on skin from black to faint black. The findings of this study showed that *Klebsiella pneumoniae* may cause an infection in catfish and can act as a vector of human pathogen. Furthermore, other bacteria were isolated which may be resident flora of the catfish. Therefore, it is important to handle fishes with proper hygiene and best public health measures since fishes are used for consumption.

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1. INTRODUCTION

Fishes are members of the super class Pisces, but those having a conspicuous set of feelers surrounding the mouth are called the catfishes. *Clarias gariepinus* is a highly nutritious fish that contains high amount of vitamins, proteins, minerals and a little or no saturated fat and is low in carbohydrate (Lee, 1991). All catfishes have either smooth or armored naked bodies with bony plate. The dorsal and pectoral fins are often edged with sharp spines that are used for defense. They can inflict severe wounds and are poisonous in some species; this feature is usually for protection from predators (Redmond, 2010).

Fish is one of the most highly perishable food products (Sallam, 2007). Fish is a very perishable, high-protein food that typically contains a high level of free amino acids. Microbes metabolize these amino acids, producing ammonia, biogenic amines such as putrescine, histamine, and cadaverine, organic acids, ketones, and sulfur compounds (Emborg et al., 2005; Olafsdottir et al., 2005; Baixas-Nogueras et al., 2005; Dalgaard et al., 2006; Doyle, 2007). Degradation of lipids in fatty fish produces rancid odors (Haugen and Undeland, 2003; Doyle, 2007). In addition, marine fish and some freshwater fish contain trimethylamine oxide that is degraded by several spoilage bacteria to trimethylamine (TMA), the compound responsible for fishy off odors. Iron is a limiting nutrient in fish, and this favors growth of bacteria such as pseudomonads that produce siderophores that bind iron (Gram and Dalgaard, 2002; Doyle, 2007).

Enterobacteriaceae are a large, diverse heterogeneous group of rod shaped gram negative bacilli that survive under aerobic

conditions and normally inhabit the intestine of man and animals; some are motile while some others are not. The family includes many genera, some of which are part of the normal flora and incidentally cause diseases especially when given the opportunity. They are non-spore forming and some have capsules while others do not (Olayemi *et al.,* 2007).

Bacteria from the genus *Klebsiella* causes numerous infections in human, which are often treated with β–lactam antibiotics (Amin et al., 2009). A variety of nosocomial and community acquired (food borne) infections are caused by *K. pneumoniae*, one of the most deadly pathogens of *Enterobactericeae* (Podschun and Ullman, 1998; Amin et al., 2009). These pathogens possess β-lactamase, therefore they mediate high levels of resistance to β-lactam antibiotics and have become a global threat (Bouchillon *et al*., 2004; Amin et al., 2009).

K. pneumoniae is an enteric Gram-negative bacillus causing hospital-acquired infections and infections in debilitated or immuno-compromised patients (Podschun and Ullman, 1998) accounting for up to 10% of all nosocomial bacterial infections (Spencer, 1996). Mostly these infections are treated with β-lactam antibiotics, which are usually hydrolyzed by β-lactamases produced by such microorganisms resulting in failure of therapy (Bush *et al.*, 1995). Because of resistance of many *Klebsiella* sp. strains to β-lactamases; alternative antibiotic therapy can make use of aminoglycosides and quinolone (Sekosawa *et al*., 2002).

The objective of this study is to isolate, identify and evaluate the effect of *Kleb*siella *pnemoniae* in relation to the catfish, by isolating the microorganisms present on the catfish skin and intestine. To find out if *Klebsiella pneumoniae* bacteria isolate can survive in the fish (immunity of fish) and observation of the public health hazard that bacteria i.e. test organism and natural flora exposes the people to.

2. MATERIAL AND METHODS

2.1. CATFISH ACCLIMMATIZATION

This was the first stage of the experiment, where the catfishes where brought in from the pond and introduced into their new environment which was made up of sterile water and big plastic bowls, for them to acclimatize, or get used to their new environment as seen in (plate 3a and 3b) respectively. This lasted for about one week, during which they were fed with 8 seeds of their feed according to their various weights and their water was changed every two days whilst their activity was checked. At the end of this period, some of them didn't survive. Amongst those that survived three were selected. One to represent the control and the other two to represent experiment one and experiment two respectively.

2.2. COLLECTION OF BACTERIAL ISOLATE (*Klebsiella pneumoniae*)

Nutrient agar slant was prepared and introduced into a sterile bijou bottle and autoclaved for 24 hours to make sterile. Then it was taken to the University of Ilorin Teaching hospital (UITH) to obtain a pure bacterial isolate of *Klebsiella pneumoniae.* It was subsequently kept in an incubator to allow for multiplication at $37^{O}C$ for 24 hours and later introduced into nutrient broth, to aid multiplication.

2.3. INOCULATION OF EXPERIMENTAL WATER WITH ISOLATE

The bacterial isolate to be used which was pre-introduced into the nutrient broth and allowed to multiply, it was shared equally amongst two of the three plastic bowls containing fishes in the experimental setup and labeled experiment one and experiment two respectively. The third of them was used as the control experiment and was labeled accordingly.

2.4. PHYSICOCHEMICAL PARAMETERS

Some parameters were considered in the course of this experiment namely; pH of the water, temperature of the water, and dissolved oxygen in the water. The temperature and pH of the water were measured in the mornings and evenings daily for the whole seven days of observation, beginning with the day the water was inoculated with the broth containing the bacterial culture, up until the last day. The amount of dissolved oxygen in the water was taken on the first day, the fourth day and the seventh day on the water to be changed. The pH of the experimental water was determined by using a pH meter. The meter was standardized using buffer solutions of pH 4, pH 7, and pH 9, then placed into each bottle and taking the stable reading on the meter to determine the pH. This was carried out daily for the seven days on samples of water from the three different plastic bowls. The temperature of the water was measured in the morning and

evening daily for the seven days of the experiment, using a mercury thermometer, by swinging it from side to side to normalize it, before and after dipping it in the water, and taking the reading accordingly while the thermometer was still in the water. The dissolved oxygen was determined on the first day, the fourth day and the seventh/last day according to standard method.

2.5. COLLECTION OF CATFISH SAMPLES FOR BACTERIOLOGICAL EVALUATION

The samples of catfish were aseptically removed from the polythene bag and were placed on a sterile trays and with the aid of sterile trays and with the aid of a sterile knife, cuts were made from the edible parts of the fishes and homogenized, and about 10g taken for microbiological analysis.

2.5.1. Skin:

Samples of raw fish from different locations were collected, of which the skin of 150 raw fish was taken by rubbing the sterilized cotton swab over the skin and then inoculating into the nutrient broth.

2.5.2. Intestine:

This was done by cutting a part of intestine and muscle after sterilizing with red hot scalped and inoculation in the media (MacConkey broth tubes). The samples included intestines from 150 raw fish.

2.6. BACTERIOLOGICAL EVALUATION

All the chemicals and reagents used were of analytical grade, obtained from Sigma chemical co. Ltd, England. The media used for the bacteriological analysis of catfish was Mac Conkey agar (MCA). Mac Conkey agar was weighed out and prepared according to the manufacturer's specification, with respect to the given instructions and directions and sterilized at 121°C for 15 min. at 15lb pressure. A serial dilution method was used. The sterility of each batch of test medium was confirmed by incubating one or two uninoculated plates along with the inoculated plates. The uninoculated plates were always examined to show no evidence of bacterial growth. Any uninoculated plate that showed evidence of bacterial growth was discarded. All the samples and the test organisms were replicated on different media and the plates were then incubated at 37°C for 24 - 48 h. Discrete colonies were sub-cultured into fresh agar plates aseptically to obtain pure cultures of the isolates. Colonies identifiable as discrete on the Mueller Hinton Agar were carefully examined macroscopically for cultural characteristics. All isolates were subjected to various morphological characterization and gram stained to determine their gram reaction. Biochemical tests were carried out as described by Jolt *et al.* (1994) to determine the identity of the bacteria isolates with reference to Bergey's Manual of Determinative Bacteriology. The isolates were identified by comparing their characteristics with those of known taxa, as described by Jolt *et al.* (1994), Cheesbrough (2006) and Oyeleke and Manga (2008).

3. RESULTS ANALYSIS

The temperature of the water varied with $\pm$ everyday, and it was usually lower in the evenings, when the weather was a bit cool. While the pH of the water varied with $\pm$ 4 everyday there was always a difference with the experimental samples and the control sample. The physicochemical parameters of the water used in the course of seven days, were determined by various means. There were changes in the pH, the temperature and the dissolved oxygen. The results of the physiochemical parameters are shown in Table 1.

The distributions of the isolated bacteria in the catfish intestine and on the skin are shown in Table 2. It showed that *Klebsiella pneumoniae* [12(25.0%)] was the most predominant bacteria isolated from catfish samples used in this study. This was followed by *Enterococcus feacalis* [9(18.6%)], *Staphylococcus aureus* [7(14.6%)], *Escherichia coli* [6(12.5%)], *Citrobacter ferundii* [5(10.4%)], *Pseudomonas aeruginosa* [5(10.4%)], and *Bacillus subtilis* [4(8.3%)]. It also showed that *Enterococcus feacalis* and *Citrobacter ferundii* were absent in all the skin samples of the catfish but present in all the intestines. *Klebsiella pneumoniae* was present on all the skin and intestines of the catfish samples. *Escherichia coli* were present in all the skin and intestine except for skin of the catfish samples in Experiment 1. *Bacillus subtilis* was only present in the skin of the catfishes used as control. *Staphylococcus aureus* was absent in all the intestine of the catfishes samples but present in their skins. *Pseudomonas aeruginosa* was absent in all the samples except for the intestines of the catfishes used as control and those in experiment 2 (Table 2).

Table 1: Physicochemical Parameters of the Water samples

Days	Samples	Control	Experiment 1	Experiment 2	Mean Values
	Weight (G)	98	94	96	96
Day 1	Temperature (°C) Morning	25	25	25	25
	Temperature (°C) Evening	23	23	24	23.3
	pH	6.23	5.22	5.30	5.58
	Dissolved O_2 (mg/l)	2.4	2.4	5.2	3.4
	Observations	Active	Active	Active	
Day 2	Temperature (°C) Morning	25	26	27	26
	Temperature (°C) Evening	25	24	25	24.7
	pH	6.22	5.25	5.73	5.73
	Observations	Active	Active	Active	
Day 3	Temperature (°C) Morning	26	25	25	25.3
	Temperature (°C) Evening	22	24	23	23
	pH	6.54	6.08	5.62	6.08
	Dissolve O2(mg/l)	6.4	2.5	2.5	3.8
	Observation	Active	Not active	Active	
Day 4	Temperature (°C) Morning	25	23	24	24
	Temperature (°C) Evening	22	22	22	22
	pH	6.40	6.42	6.44	6 .42
	Observations	Active	Sluggish	Sluggish	
Day 5	Temperature (°C) Morning	25	22	23	23.3
	Temperature (°C) Evening	24	22	22	22.7
	PH	7.28	7.32	7.32	7.31
	Observations	Active	Weight Loss, Slig colour change	Weight Loss, Slig colour change	
Day 6	Temperature (°C) Morning	25	26	27	26
	Temperature (°C) Evening	22	26	25	24.3
	pH	6.52	6.55	6.56	6.54
	Observations	Active	Shedding of Skin	Shedding of Skin	
Day 7	Temperature (°C) Morning	24	23	24	23.7
	Temperature (°C) Evening	23	24	22	23
	pH	6.30	6.32	6.32	6.31
	Dissolved O_2 (mg/l)	6.8	4.2	4.2	5.1

Table 2: Distribution of the isolated Bacteria in the Intestine and Skin of the Catfish Samples

Isolated Bacteria	No. (%)	Control Skin	Control Intestine	Experiment 1 Skin	Experiment 1 Intestine	Experiment 2 Skin	Experiment 2 Intestine
Citrobacter ferundii	5 (10.4)	Absent	Present	Absent	Present	Absent	Present
Escherichia coli	6(12.5)	Present	Present	Absent	Present	Present	Present
Bacillus subtilis	4(8.3)	Absent	Present	Absent	Absent	Absent	Absent
Staphylococcus aureus	7(14.6)	Present	Absent	Present	Absent	Present	Absent
Pseudomonas aeruginosa	5 (10.4)	Absent	Present	Absent	Absent	Absent	Present
Enterococcus feacalis	9(18.6)	Absent	Present	Absent	Present	Absent	Present
Klebsiella pneumoniae	12(25.0)	Present	Present	Present	Present	Present	Present
Total	**48(100.0)**						

4. DISCUSSION

The result of the physicochemical tests in this study shows that the temperature ranged from 25^0C to 27^0C and the pH ranged from 6.5 – 7.5 which favours the optimum growth of most bacteria as most bacteria as most bacteria grow best at or near 7 (Willey *et al.,* 2008) and the temperature range favours the mesophilic bacteria, a group which include pathogens in man and other animals (Willey *et al.,* 2008). The amount of dissolved oxygen in the water during the experimental period which

is from 2.4 – 6.5 indicate that oxygen was considerable used up.

The microorganisms reported in this study are similar to what has been reportedly isolated in other studies in Nigeria (Okonko et al., 2008, 2009; Adebayo-Tayo et al., 2012a,b,c,d). In a study by Yagoub (2009), *Enterobacteriaceae* were isolated from gills, skin, muscles and the intestine of randomly collected fishes. Gram-negative bacilli (GNB) are associated with bloodstream infections (BSI) resulting in significant mortality, particularly in patients in intensive care units (ICUs) (Della-Latta et al., 2011). Among the most prevalent GNB pathogens are *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Karlowsky et al. 2004; Gaynes et al., 2005; Della-Latta et al., 2011).

The assessment of the effect of certain bacteria on fish in order to know their virulence on the fish and in remain since fishes are used for human consumption. There is a correlation between organisms found in water and in fishes. These organisms could have gotten into water from normal sources such as feacal contamination by man and other animals. Some of the bacteria isolated such as *Bacillus subtilis* could be as a result of agricultural activities, as this bacterium is a resident flora of the soil (Willey *et al.*, 2008). Similar observations were made by (Buras *et al.*, 1987). *Bacillus sp* causes a toxin-medicated disease rather than infection such as diarrhea and emetic illness characterized by nausea and vomiting (Bergdoll, 1981; Adebayo-Tayo et al., 2006, 2009, 2012c,d).

Klebsiella pneumoniae is a ubiquitous microorganism gram negative and posses the ability to cause infections in humans. These organisms were isolated into the catfish sample died before the 7 days Experiment was up and the organism *Klebsiella pneumoniae* was re-isolated. The re-isolation of *Klebsiella pneumoniae* could be the virulent to human. The amount of dissolved oxygen in the water showed that oxygen was used by *Klebsiella pneumoniae up* to low oxygen level (2.4 mgl/c). This situation could result in death of fish; this conforms to the study of Anibeze *et al.* (2000) who reported that low oxygen levels create a toxic environment which could result in the death of the fish.

The presence of other bacteria in the fish put a threat to fish consumers as these organisms have been implicated in a number of diseases. The presence of *Bacillus subtillis* possess a threat of food poisoning and wound infections because they produce toxins which are infectious on the other hand, the presence of *Pseudomonas aeruginosa* could cause general inflammation and sepsis in critical body organs such as lungs, kidney, urinary tract, which can be fatal because it thrives in most surface.

Staphylococcus aureus, a mesophile have been implicated in food poisoning outbreak of some food material (Adebayo-Tayo et al., 2009, 2012c,d). *S. aureus* had been isolated from several clinical specimens from different part of Nigeria (Chigbu and Ezeronye, 2003; Ehinmidu, 2003; Olukoya et al., 1995; Odunsanya, 2002; Kolawole et al., 2005; Obiazi et al., 2007).

The presence of *S. aureus* is an indication of contamination by food handlers and 80% of them are being harbored by man as normal micro flora (Adebayo-Tayo et al., 2009, 2012c,d). *S. aureus* is known for production of heat stable enterotoxin (Stewart, 1974) and potentials for multiple antibiotic resistances when they get into the living tissue (Foster, 1996; Allen and Cowan, 1997; Okuma et al., 2002; Scott, 2002; Klein et al., 2007) makes the product of immense epidemiological danger (Adebayo-Tayo et al., 2009, 2012c,d).

Staphylococcus aureus has been recognized as a very important virulent and frequently encountered pathogen in clinical practice (Obiazi et al., 2007). It is an endogenous microorganism colonizing the nasal cavity, skin, gastrointestinal, anus and vaginal vulvae of healthy women (Onanuga et al., 2005; Obiazi et al., 2007). The capacity to produce human diseases had not diminished even with the introduction of antibiotics (Obiazi et al., 2007). *S. aureus* has been associated with different clinical conditions. For instance, it is still one of the most frequently encountered single bacterial species in hospitals and continues to be frequent cause of burns and wounds sepsis (Obiazi et al., 2007). It produces pustules, carbuncles, boils and impetigo. It frequently causes septicaemia, osteomyelitis, bacteraemia and otitis (Emmerson, 1994; Obiazi et al., 2007).

Pseudomonas aeruginosa also causes cross infection in hospitals and clinics (Balcht and Smith, 1994). *Pseudomonas sp* on the other hand is prevalent among patients with wounds, burns, cystic fibrosis are likely to have introduced into the environment by swimmers and infected individuals who use these waters were the catfish samples were obtained for recreational purposes (Adebayo – Tayo et al., 2006, 2012c,d).

Citrobacter Ferundii is common causes of bladder gull, kidney, *meningitis* and other body infections. It also produces attaching and effacing lesions in the large intestine and alimentary infection (Stainer *et al.,* 1987). *Enterococcus* spp. is a causative agent of dental plagues and scarlet fever (Willey *et al.,* 2008). *Enterococcus* sp. has been implicated in human infections like pharyngities, scarlet fever and pneumonia (Adebayo-Tayo et al., 2009, 2012c,d).

Escherichia coli strains are the causative agents of gastro–enteritis and urinary tract infection (Strainer *et al*., 1987). *Escherichia coli* is implicated in newborn meningitis and infantile diarrhea, *Salmonella paratyphi* is the causative agent of paratyphoid fever in humans, who are the only reservoir of this organism (Nester et al., 1995; Adebayo-Tayo et al., 2006, 2012c,d).

Generally, *Klebsiella pneumoniae* inoculated into catfish *Clarias gariepinus* presented to be virulent in the fishes leading to shedding of skin patches and fading of colour on skin from black to faint black as earlier reported by Ezenwaji and Inyangi (1998). The presence of highly pathogenic bacterial isolates, like *Bacillus* sp., *Salmonella* sp., *Shigella* sp., *E. coli*, *Pseudomonas* sp. and *S. aureus* are organisms of public health concern. The presence of these microbes is an indication of possible contamination resulting from the use of well water, which is mostly used in local food processing industries are not free from microbial contamination (Potter, 1983; Adebayo-Tayo et al., 2009, 2012c,d).

The presence of the coliform group of bacteria, mainly *Citrobacter, Enterobacter, Escherichia* and *Klebsiella* in fish and fish products presents a health hazard to humans (Caldreich and Clarke, 1966; van DUIJN, 1973; Fapohunda et al., 1994; Ampofo and Clerk. 2010). Allen and Hepher (1979) have stated that most of the epidemics attributed to wastewater sources are from raw sewage gaining access to food eaten directly by man, or from contamination of water supply systems by untreated sewage (Ampofo and Clerk, 2010). Olayemi *et al.* (1991) have reported that the presence of faecal coliform in fish intended for human consumption may constitute a potential danger not only in causing disease, but also because of the possible transfer of antibiotic resistance from aquatic bacteria to human infecting bacteria from nonaquatic sources (Ampofo and Clerk, 2010).

Some human pathogens such as *Aeromonas, Escherichia, Klebsiella, Pseudomonas, Salmonella* and *Vibrio* have been found to survive and multiply in the gut, mucus and tissues of fish and thus render fish a potential vector of human disease over long periods (Allen and Hepher, 1969). All these pathogens have been identified to be present in the tissues of fish that were cultured in the organic waste-fertilized ponds in this study (Ampofo and Clerk, 2010).

5. CONCLUSION

The findings of this study showed that *Klebsiella pneumoniae* may cause an infection in catfish and can act as a vector of human pathogen. The presence of these six genera in the fish is, therefore, a threat to the fish industry as fish, which do not succumb to the attack may still be subjected to spoilage. Furthermore, other bacteria were isolated which may be resident flora of the catfish. Therefore, it is important to handle fishes with proper hygiene and best public health measures since fishes are used for consumption. Also, fish handlers should avoid contact with fish and fish water when they have skin injuries as these microorganisms (both test organism and resident flora) may be present. However, fish should always be handled with utmost care and hygiene.

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REFERENCES

1. Adebayo-Tayo AC; Odu NN; Michael MU;Okonko IO. Multi-Drug Resistant (MDR) Organisms isolated from Sea-foods in Uyo, South-Southern Nigeria. Nature and Science 2012a; 10(3): 61-70
2. Adebayo-Tayo BC, Adegoke AA and Akinjogunla OJ. 2009. Microbial and physico-chemical quality of powdered soymilk samples in Akwa Ibom, South Southern Nigeria. African Journal of Biotechnology, 8 (13): pp. 3066-3071

3. Adebayo-Tayo BC, Odu NN, Igiwiloh NJPN, Okonko IO. Microbiological and Physicochemical Level of Fresh Catfish (*Arius hendelotic)* From Different Markets in Akwa Ibom State, Nigeria. *New York Science Journal 2012d;5(4):46-52*
4. Adebayo-Tayo BC, Odu NN, Okonko IO. Microbiological and physiochemical changes and its correlation with quality indices of tilapia fish (*Oreochromis niloticus*) sold in Itu and Uyo markets in Akwa Ibom State, Nigeria. *New York Science Journal 2012c;5(4):38-45*
5. Adebayo-Tayo BC, Onllude AA, Ogunjobi AA. 2006. Bacteriological and Proximate Analysis of Periwinkle from two different creeks in Nigeria. *World Applied Science Journal 1* (2) 87-91.
6. Adebayo-Tayo BC; Odu NN;Anyamele LM; Igwiloh NJPN;Okonko IO. Microbial Quality Of Frozen Fish Sold In Uyo Metropolis. Nature and Science 2012b; 10(3): 71-77
7. Allen GH, Busch RA, Morton AW. Preliminary bacteriological studies on wastewater- fertilized marine fishponds. Humboldt Bay, California Bay, California. In: Advances in Aquaculture Fishing News Books, Oxford, England 1979; pp. 492-8.
8. Allen GH, Hepher B. Recycling of wastes through aquaculture and constraints to wider application. In: Advances in aquaculture Pillay TVR and Dill WA, Eds. Oxford, Fishing News Books, England 1969; pp. 478-87.
9. Allen JL, Cowan ME (1997). Monitoring outbreaks of methicillinresistance *Staphylococcus aureus*. Use of a commercial database and personal computer B.J. Biomed. Sci. 54: 10-12.
10. Amin, A., Ghumro, P.B., Hussain, S. and Hameed, A. 2009. Prevalence of antibiotic resistance among clinical isolates of *Klebsiella pneumoniae* isolated from a Tertiary Care Hospital in Pakistan. Malaysian Journal of Microbiology, Vol 5(2) 2009, pp. 81-86
11. Ampofo JA and GC. Clerk. 2010. Diversity of Bacteria Contaminants in Tissues of Fish Cultured in Organic Waste-Fertilized Ponds: Health Implications. *The Open Fish Science Journal,* 2010, *3,* 142-146
12. Anibeze C.I.P, A. Eze and J.E. Eyo (2000) Incidence of biliary stasis and Hepatoma in Cultural Heterobranchous logitilis (valenciennes). *World Journal of Biotechnology,* 123(2-6).
13. Baixas-Nogueras S, Bover-Cid S, Veciana-Nogues MT, Marine-Font A, and Vidal-Carou MC. 2005. Biogenic amine index for freshness evaluation in iced Mediterranean hake (*Merluccius merluccius*). J Food Prot 68:2433–2438.
14. Balcht, Aldona and Smith Raymond (1994*)* Study of *pseudomonas aeruginosa.(121)16-19.*
15. Bergdoll MS (1981). Bacillus cereus, food borne disease. Clin. Microbiol. Newsl. 3: 85-87.
16. Bouchillon, S. K., Jonson, B. M., Hoban, D. J., Johnson, J. L., Dowzicky, M. J., Wu, D. H., Visalli, M. A. and Bradford, P. A. (2004). Determining incidence of extended spectrum β-lactamase producing *Enterobacteriaceae*, vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* in 38 centers from 17 countries: the PEARLS Study 2001–2002. *International Journal of Antimicrobial Agents* 24, 119-124.
17. Buras N, Duecyl NWS, Herper B and SOW Banke (1987) microbiology. Aspect of from grown in treated Water Res 21:1-10.
18. Bush, K., Jacoby, G. A. and Medeiros, A. A. (1995). A functional classification scheme for β-lactamases and its correlation with molecular structure. *Antimicrobial Agents and Chemotherapy* 39, 1211-1233.
19. Caldreich EE, Clarke NA. Bacterial pollution indicators in the intestinal tract of freshwater fish. J Appl Microbiol 1966; 41: 429-437.
20. Cheesbrough M. 2006. Medical Laboratory Manual for Tropical Countries ELBS 7: 312 - 315.
21. Chigbu CO, Ezeronye OU (2003). Antibiotics resistant Staphylococcus aureus Abia State of Nigeria. Afr. J. Biotechnol. 2(10): 374-378.
22. Dalgaard P, Madsen HL, Samieian N, and Emborg J. 2006. Biogenic amine formation and microbial spoilage in chilled garfish (*Belone belone belone*)— effect of modified atmosphere packaging and previous frozen storage. J Appl Microbiol 101:80–95.
23. Della-Latta P, H. Salimnia, T. Painter, F. Wu, G.W. Procop, D.A. Wilson, W. Gillespie, A. Mender, and B.S. Crystal. Identification of *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* in Blood Cultures: a Multicenter Performance Evaluation of a Three-Color Peptide Nucleic Acid

Fluorescence *In Situ* Hybridization Assay. J Clin Microbiol. 2011 June; 49(6): 2259–2261
24. Doyle EM. 2007. FRI BRIEFINGS: Microbial Food Spoilage: Losses and Control Strategies. *A Brief Review of the Literature*. Food Research Institute, University of Wisconsin–Madison http://fri.wisc.edu/docs/pdf/FRI_Brief_Microbial_Food_Spoilage_7_07.pdf
25. Ehinmidu JO (2003). Antibiotics susceptibility patterns of urine bacterial isolates in Zaria, Nigeria. Trop. J. Pharm. Res. 2: 223-228.
26. Emborg J, Laursen BG, and Dalgaard P. 2005. Significant histamine formation in tuna (*Thunnus albacares*) at 2°C—effect of vacuum- and modifiedatmosphere- packaging on psychrotolerant bacteria. Int J Food Microbiol 101:263–279.
27. Emmerson M (1994). Nosocomial Staphylococcal outbreak. Scandinavian J. Infect. Dis. Suppl. 93: 47-54.
28. Ezenwaji H.M.S. and Inyang N.M. (1998). Observations on the biology of *Clarias agboylences*, Syndenham 980, In the Anambra (Lood System, Nigeria Fisheries Research)36;47-60.
29. Fapohunda AO, MacMillan KW, Marshall DL, Waites WM. Growth of selected cross-contaminating bacterial pathogens on beef and fish at 15 and 350C. J Food Protect 1994; 57: 337-40.
30. Foster T (1996). Staphylococcus. In: Barron's Medical Microbiology, 4th ed., University of Texas Medical Branch. ISBN 0-9631172-1-1
31. Gaynes R., Edwards J., and the National Nosocomial Infectious Surveillance System 2005. Overview of nosocomial infections caused by gram-negative bacilli. Clin. Infect. Dis. 41:848–854.
32. Geldreich E.E and Clarke N.a (1966) bacteria (pollution indivators in the intestine tract of fresh water fish App1 microbial 14,429-437.
33. Gram L and Dalgaard P. 2002. Fish spoilage bacteria— problems and solutions. Curr Opin Biotechnol 13:262–266.
34. Haugen JE and Undeland I. 2003. Lipid oxidation in herring fillets (*Clupea harengus*) during ice storage measured by a commercial hybrid gas-sensor array system. J Agr Food Chem 51:752–759.
35. Jolt JG, Krieg NR, Sneath PHA, Stanley JT, Williams ST. 1994. Bergey's manual of systematic bacteriology, 9th edn. Williams & Wilkins Co. Baltimore, Maryland, p786
36. Karlowsky J. A., et al. 2004. Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. Ann. Clin. Microbiol. Antimicrob. 3:7.
37. Klein E, Smith DL, Laxminarayan R (2007). Hospitalizations and Deaths Caused by Methicillin-Resistant *Staphylococcus aureus* in United States, 1999–2005. Emerg. Infect Dis. 13(12): 1840-1846
38. Kolawole DO, Bisi-Johnson MA, Shittu AO (2005). Epidemiological analysis of clinical isolates Staphylococcus aureus in Ile- Ife, Nigeria. Pakistan J. Bio. Sci. 8(7): 1016-1020.
39. Lee, J.S (1991). Commercial catfish farming (3rd edition) interstate pub, inc. Pp3-15, 293-297.
40. Nester, E.W., C.E. Roberts and M.T. Nester, 1995. Microbiology, A Human perspective WM.C Brown publishers. Oxford, England.
41. Obiazi H. A. K., Nmorsi, O. P. G., Ekundayo A.O. and Ukwandu N.C.D. 2007. Prevalence and antibiotic susceptibility pattern of *Staphylococcus aureus* from clinical isolates grown at 37 and 44oC from Irrua, Nigeria. African Journal of Microbiology Research pp. 057-060,
42. Odunsanya OO (2002). Antibiotic susceptibility of Micro organisms at a general hospital in Lagos. Nig. J. Nat. Med. Assoc. 94(11): 994-998.
43. Okonko IO, Ogun AA, Adejoye OD, Ogunjobi AA, Nkang AO, Adebayo-Tayo BC. 2009. Hazards analysis critical control points (HACCP) and Microbiology qualities of Sea-foods as affected by Handler's Hygiene in Ibadan and Lagos, Nigeria. African Journal of Food Sciences, 3(1):035-050
44. Okonko, L.O., A.A. Ogunjobi, E.A. Fajobi, B.A. Onaja, E.T. Babalola, A.O. Adedeji (2008). Comparative Studies and Different Assessment of Ready-to-Eat (RTE) Frozen Sea Foods Processed in Ijola-Olopa Lagos State, Nigeria. J. Afr. BioTechnol. 16:2898 – 2901.
45. Okuma K, Iwakawa K, Turnidge J (2002). Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. J. Clin. Microbiol. 40(11): 4289-4294.
46. Olafsdottir G, Jonsdottir R, Lauzon HL, Luten J, and Kristbergsson K. 2005.

Characterization of volatile compounds in chilled cod (*Gadus morhua*) fillets by gas chromatography and detection of quality indicators by an electronic nose. J Agr Food Chem 53:10140–10147.
47. Olayemi A.B., Eniola K.I.T. and Kolawole O.M. (2007). Bacteriology today (WI Laboratory Manual)Unilorin Press Ilorin PP 22-52.
48. Olayemi AB, Adebayo O, Ojo AO. Microbial flora of six freshwater fish species from Asa River, Ilorin, Nigeria. Rev Biol Trop 1991; 39: 165-7.
49. Olukoya DK, Asielue JO, Olasupo NA, Ikea JK (1995). Plasmid profiles and antibiotic susceptibility patterns of *Staphylococcus aureus* isolates from Nigeria. Afr. J. Med. Sci. 24(2): 135-138.
50. Onanuga A, Oyi AR, Onaolapo J.A. (2005). Prevalence and susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolates among healthy women in Zaria, Nigeria. Afr. J. Biotechnol. 4(11): 1321-1324.
51. Oyeleke SB, Manga SB. Essentials of Laboratory Practicals in Microbiology. Tobest publisher, Minna, Nigeria, 2008; pp.36-75.
52. Podschun, R. and Ullman, U. (1998). *Klebsiella* sp. as nosocomial pathogens Protein. *Journal of Antimicrobial Agents and Chemotherapy* 41, 563-569.
53. Potter NN (1983). Food science. 3rd ed. AVI Publishing company incorporated, Westport.
54. Prescott L.M., Harley J.P and Klein D.A. (2005). Microbiology 7th edition McGraw-Hill companies-inc. Pp: 822-940.
55. Redmond W.A. (2010). Microsoft Encarta Dictionaries,(104)12-16.
56. Sallam KI. 2007. Chemical, sensory and shelf life evaluation of sliced salmon treated with salts of organic acids. Food Chem. 2007; 101(2): 592–600
57. Salle AJ. Fundamental principles of bacteriology, 5th ed. New York McGraw-HillBook Co. 1964.
58. Scott K (2002). Epidermiological and Microbiological Characterization of Infection causes by *Staphylococcus aureus* with Reduced Susceptibility to Vancomycin, United State, p. 1997.
59. Sekowska, A., Janika, G., Klyszejko, C., Wojda, M., Wroblewskiand, M and Szymankiewicz, M. (2002). Resistance of *K. pneumoniae* strains producing and not producing ESBL (extended spectrum β-lactamase) type enzyme to selected non β-lactam antibiotics. *Medical Science Monitor* 8, 100-104.
60. Stainer R.Y., Ingritan J.L., Wheelis M.I. and Panter P.R. (1987). Bacterial general microbiology (15th) edition Prentile, New Jersey.
61. Stewart FS (1974). Bigger's Bacteriology and Immunology for students of medicine 9th edn. ELBS and Bailliere, Tindal and Casell.
62. van DUIJN JC. Diseases of fishes, 3rd ed. Butterworth Co. Ltd. London 1973.
63. Wiley M.S, Sherwood L.M and Wolverton C.J. (2008). Prescott, Harley and Klein's Microbiology. McGraw Hill, New York, pp413-930.
64. Yagoub S.O. 2009. Isolation of *Enterobacteriaceae* and *Pseudomonas spp*. from raw fish sold in fish market in Khartoum state. Journal of Bacteriology Research, 1(7): 085-088.

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