Original Research Article

Microbiological Assessment of Fishes Caught in the Two Major Fishing Ground in Ado Ekiti, Ekiti State (Water works River and Ogbese River)

ABSTRACT

Microbial contamination of food is the main obstacle of ensuring food safety. For this, it's paramount to ascertain the safety of fish caught in the major waters in Ado Ekiti. 22 fish sample was collected from the two major fishing grounds in Ado Ekiti Metropolis. After standard microbiological and biochemical Test. The total bacteria count (TBC) for all the fish samples ranged between 0.46×10^4 and 0.95×10^4 cfu/g . Out of the 22 fish samples analyses for TBC, a sample from Water works dam had the highest number of bacteria with 0.95×10^4 cfu/g. The Ogbese sample 4 had the lowest isolation with 0.46×10^4 cfu/g. The study demonstrated the occurrence of bacterial isolates such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter aerogenes*,*Staphylococcus aureus,Bacillus subtili, Micrococcus luteus* and *Proteus vulgaris* bacteria. This analysis indicated the incidence of fish contamination.

Keywords: Bacteria, Fishing grounds, Safety, Contamination

INTRODUCTION

Majority of the fishes consumed are caught in the wild Traore *et al*.,(2015). Hence, understanding the microbial community associated with fishes caught in the wild could provide useful information about the husbandry requirement, health management, as well as help dictate effective biosecurity measure for these fishes to be raised in captivity. Adebayo *et al*., (2012) had earlier suggested the fact that fishes caught in the wild are grossly understudied with respect to microbial communities, and could be potential sources of pathogenic infections. This is a public health concern in the industry due to the possibility of cross infections (between fishes) and zoonotic infections (between fish and man). The knowledge of the susceptibility of these fish to different microbes in the wild could help dictate measures that would prevent transfer of potentially pathogenic bacteria and fungi from infected fish to others fish group reared together in captivity and vice versa.

In the United States, bacteria was responsible for 20,000 admissions and 380 deaths per year over a three-year period Bae *et al*., (2015). In 2015, also in the US, a *Salmonella* outbreak was identified, with the recall of frozen raw tuna (*Thunnus alalunga*) from an Indonesian industry. This led to 65 people becoming infected with *Salmonella Paratyphi B* and *Salmonella Weltevreden* in 11 American states (CDC, 2015). Another study, conducted on 11,3120 seafood samples imported from different countries over a 9-year period in which they were collected by the Food and Drug Administration (FDA) concluded

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that the overall incidence of *Salmonella* spp. in seafood was of 7.2%, and that incidence rates are higher in Central Pacific and African countries, or developing countries, compared to developed countries, such as countries in Europe, including Russia, and North America, that displayed a significantly lower incidence. Heinitz *et al*., (2000).

Specific Objectives

The specific objectives of this study are to:

I. To identify and isolate micro-organisms caught from the two major fishing grounds in Ado-Ekiti Metropolis

II. To make useful recommendations based on the outcome of the study.

MATERIALS AND METHOD

Materials

Swab sticks, distilled water, test tubes, ethanol, cotton wool, petridishes, antibiotic sensitivity discs(positive and negative), ruler, paper tape, hand glove, beaker, conical flask, stirrer, foil paper, bunsen burner.

Equipment

Weighing balance, oven, autoclave, microscope, refrigerator, incubator.

Media used

Nutrient agar and potatoes dextrose agar.

Sterilization

Glass wares were thoroughly washed with detergent, rinsing with distilled water and were oven dry at 160^0C for 1 hour, and then the work bench was disinfected by swabbing with ethanol. All work in the laboratory was done in sterile environment.

Preparation of media

The media used were nutrient agar and potato dextrose agar; they were all prepared according to manufacturer instructions. The media was dissolved in adequate amount of distilled water, the media were all homogenized and autoclave at 121^0C for 15minutes.

Isolation of microorganisms

22 fresh fish was dissect and remove the intestine 10g of each fish intestine were weight and macerated using mortar and pestle, 1g of the macerated sample was taken and dissolved inside 9mL of distilled water. Series of test tubes were prepared for the isolation of bacteria and fungi. 9mL of sterile distilled water was put into each of the test tubes. To the first test tube, 1mL of the sample was added to give a dilution of 10^{-1}. The contents were shaken properly and 1mL of the solution was taken and added to the next test tube containing 9mL of sterile distilled water to make a concentration of 10^{-2}. The serial dilution was made up to 10^{-6} dilution for the fish samples. 0.1ml of the 10^{-3} and 10^{-4} dilution was cultured on the agar plates using the pour plate technique.

The PDA plates were incubated at room temperature for five days. Counting of the bacterial colony was done by using colony counter ,distinct colonies were sub cultured picked by streaked on fresh nutrient agar plates. The pure culture was preserved on agar slants for further studies.

Identification of bacteria isolates

The bacterial isolates were identified by using morphological and biochemical tests such as coagulase test, catalase test, oxidase test, indole, voges proskauer and methyred test.

Biochemical test

Gram staining technique

A sterile wireloop was flamed to red hot and it was used to put a drop of sterile water a clean grease free slide, the wireloop was reflamed and was used to pick a distinct colony from the plates and it was emulsified with the water on the slide, the smear was allowed to air dry and heat fixed to fix the smear on the slide. The smear was covered with Crystal violet for 40seconds, the smear was rinsed with water, it was then covered with Lugol's iodine for 60seconds, the stain was washed off with distilled waterand decolourized rapidly with Acetone alcohol for 20seconds. The smear was rinsed with distilled water, cover with Safranin for 30seconds and this stain was washed off with distilled water. The smear was allowed to air-dry and then examined microscopically under oil immersion lens.

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Catalase test

A drop of 3% Hydrogen Peroxide [H_2O_2] was put on a slide and a sterile inoculating loop was used to pick an inoculum and then mixed with the 3% hydrogen peroxide H_2O_2 rapidly, the slide was observed for agglutination.

Indole test

Peptone water was prepared according to the manufacturers instruction ,it was distributed into several test tubes and sterilized in an autoclave at 121^0C for 15minutes.The tubes were inoculated with the isolates and then incubated for 48hours. 2ml of Kovac reagent was added to the test tubes, it was mixed gently ,and allowed to stand for 20minutes.

VogesProskauer (VP) test

MR-BP broth was prepared and poured into several test tubes, the test tubes were autoclaved at 121^0C, the test tubes were allowed to cool and the organisms were inoculated into the medium in the tubes, the tubes are then incubated at 37^0C for 48hours. To the tubes, 1ml of 5% alpha-naphthol was added, 1ml of 40% KOH, the tube were allowed to stain for one hour and the changes were observed.

Methyl red Test

Peptone broth was prepared and distributed into several test tubes, the test tubes were sterilized in an autoclave at 121^0C, the test tubes were allow to cool and the organisms were inoculated into the test tubes , the tubes were incubated at 37^0C for 48hours,five drops of methyl red was added to the tubes and the tubes were shaken and examined after 5minutes,coloured change was observed.

Coagulase test

A loopful of test isolates were picked from a young culture, emulsified with serum placed on a clean grease free slide and rocked for one minute. The presence of agglutination indicates a positive reactions

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Oxidase Test

2 drops of oxidase reagent agent was placed on a filter paper, a colony was smeared across the same area, a positive oxidase test turns the oxidase reagent to dark purple.

RESULTS AND DISCUSSION

Results

Table 1: Bacteria load of fish samples (Water works and Ogbese River)

Sample	Total bacterial count cfu/ml
WW 1	0.74×10^4
WW 2	0.95×10^4
WW 3	0.57×10^4
WW 4	0.48×10^4
WW 5	0.60×10^4
Ogbese 1	0.64×10^4
Ogbese 2	0.58×10^4
Ogbese 3	0.88×10^4
Ogbese 4	0.46×10^4
Ogbese 5	0.87×10^4
Ogbese 6	0.49×10^4
Ogbese 7	0.74×10^4

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Table 2: Morphological and Biochemical characteristics of Bacteria Isolated (Water works and Ogbese River)

	MOPHOLOGICAL CHARACTERISTIC				BIOCHEMICAL TEST								
Sample	**Colour**	**Shape**	**Edges**	**Elevation**	**Indole**	**Methyred**	**v.p**	**Shape**	**Gram s rxn**	**Catalase**	**Oxidase**	**Coagulase**	**Suspected organism**
1	Cream	Round	Entire	Raized	+	-		Cocci	+	+	-	+	*Staphylococcus aureus*
2	Yellow	Round	Irregular	Flat				Rod	-	+	+	-	*Pseudomonas aeruginosa*
3	Pink	Round	Entire	Raized	+	+	-	Rod	-	+	+	-	*Escherichia .coli*
4	Yellow	rhizoid	irregular	Raized	+	+	-	Rod	-	+	-	-	*Proteus vulgaris*
5	Cream	Round	Entire	Flat		+	+	Rod	+	+	+	-	*Bacillus substilis*
6	Brown	Rhizoid	Irregular	Flat	+	-	+	Rod	-	+	+	-	*Enterobacter aerogenes*
7	Yellow	Round	Entire	Raized	-	+	-	Cocci	+	+	-	-	*Micrococcus luteus*

Key: + = Positive,
- = Negative
V.P =
rxn = Reaction

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Table 3: Bacteria Isolated (Water works and Ogbese River)

Samples	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Proteus vulgaris*	*Bacillus substilis*	*Enterobacter aerogenes*	*Micrococcus luteus*
WW 1	+	+	-	+	+	-	-
WW 2	+	+	-	+	+	+	+
WW 3	+	-	-	+	+	-	-
WW 4	-	-	+	-	+	-	-
WW 5	+	-	-	+	-	-	-
Ogbese 1	+	+	+	+	+	-	-
Ogbese 2	+		+	-	+	+	+
Ogbese 3	+	+	+	+	+	+	-
Ogbese 4	+	-	+	-	-	-	+
Ogbese 5	+	+	+	+	+	+	-
Ogbese 6	+	+	+	-	+	-	+
Ogbese 7	+	+	+	+	+	+	+
Control	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Key
Positive = +
Negative = -

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Table 4: Cumulative frequency of bacteria isolated from wild fresh fish sample

Bacteria isolate	Number of occurrence	Frequency in percentage %
Pseudomonas aeruginosa	7	13
Micrococcus luteus	5	9
Enterobacter aerogenes	5	9
Bacillus subtilis	10	19
Escherichia coli	8	15
Proteus vulgaris	8	15
Staphylococcus aureus	11	20
Total	**54**	**100%**

DISCUSSION

In this study, the total bacteria count (TBC) for all the fish samples ranged between 0.46 x 10^4 and 0.95 x 10^4 cfu/g as shown in Table 1. Out of the 22 fish samples analysed for TBC, a sample from Water works dam had the highest number of bacteria with 0.95 x 10^4 cfu/g. The Ogbese sample 4 had the lowest isolation with 0.46 x 10^4 cfu/mL. Ogbese had the lowest total bacteria counts might be attributed to the design which allows water to flow out gradually and is not stagnant as it is used to irrigate, drinking etc.

Table 2 revealed the morphological and biochemical characteristics of bacteria isolate from fresh fish sample bought from different location (Water Works, and Ogbese) all in ado-Ekiti indicating the colour, shape, edges, elevation, for the morphological characteristic while Indo, methyred, grams reaction, catalase, oxidase and coagulase for biochemical test.

It was observed in table 3 that *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris, Bacillus substilis, Enterobacter aerogenes* and *Micrococcus luteus* in most of the sample analyze in this study.

Table 4 shows the cumulative frequency of occurrence of bacteria isolate from fresh fish sample in all the location, *Staphylococcus aureus* 11(20.00%)*, Pseudomonas aeruginosa* 7(13.00%)*, Escherichia*

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coli 8(15.00%), *Proteus vulgaris* 8(15.00%), *Bacillus substilis* 10(19.00%), *Enterobacter aerogenes* 5(9.00%) and *Micrococcus luteus* 5(9.00%).

The study demonstrated the occurrence of bacterial isolates such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter aerogenes*, *Staphylococcus aureus,Bacillus subtili, Micrococcus luteus* and *Proteus vulgaris* bacteria.This analysis indicated the incidence of fish contamination. These isolates are potential pathogens and their presence can pose health risks to human in general and immunocompromised individuals in particular when the levels of *Staphylococcus aureus* in the fish. The presence of high numbers of potentially pathogenic *Staphylococci* was emphasized as a negative phenomenon.

Staphylococcus aureus, Escherichia coli and *Pseudomonas aeruginosa,* were the common pathogenic bacteria found associated with fish from the sample analyze with integrated farming systems. Their presence was attributed to the contamination of the fish ponds and stream by animal waste Okonta. *et al*., 2005).The isolation of *Escherichia coli* from the fish samples indicates fecal contamination of the ponds and stream resulting from the human activity and manure that they add to the fish ponds as feed.

The fish and shellfish are highly perishable and prone to vast variations in quality due to differences in species, environmental habitats, and feeding habits. They can also function as carriers of several microbial and other health hazards. Therefore maintenance of quality is most important in production and trade of fishery products. Although only a few infectious agents in fish are able to infect humans, some exceptions exist that may result in fatalities. However, the greatest risk to human health due to the consumption of raw or insufficiently processed fish and fish products.

In this study, *Pseudomonas* sp was isolated from 13.00 % of collected fish samples is of highly importance because this bacterium plays a considerable role as potential pathogenic bacteria for human and as an indicator of food quality as spoilage organism. Apart from the enteric- organisms, *S. aureus* with 20.00% encountered in this study are known enterotoxin producing agent and a microorganism which is poisonous. This is in agreement with the previous study by some authors in Nigeria and outside Nigeria (Okonko *et al*. 2008).

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CONCLUSION

The result of this study revealed tha fish caught in these water are considerably contaminated with bacteria this may be as a result of certain factors like not maintaining personal hygiene, contaminated water taken in by the fishes which may contain faecal matter in their ecosystem which resulted in the isolation of enteric organism like bacteria. Contaminated fish could be dangerous, especially for sensitive populations as children, elderly and immune compromised people, fish caught in these waters should be properly processed before consumption. Public education on the need for proper environmental sanitation to reduce bacteria load should be emphasized. Good hygienic practice aimed at minimizing the microbial load of fish must be ensured. Greater attention should therefore be paid to the microbiological standard activities to reduce microbial load.

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