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Original Article

Microbiological Quality Assessment and Antibiogram of the Bacteria Isolated from Fish Feed, Oyo, South-West Nigeria

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Abstract

Essential nutrient is required for the growth of aquaculture either in the wild or in the artificial environment. Therefore, artificial feed need to contain essential nutrients and free of pathogenic microorganism. Samples of both imported and local fish feed samples were collected and presence of microorganisms was examined using bacteriological media. Morphological and Biochemical identification of the isolates were carried out. Also, antibiotic susceptibility patterns of the bacteria isolated were carried out using Gram negative and positive specific antibiotics (Oxoid) with reference to zones of inhibition observed from the plates. It was observed that fish feed samples possess pH and moisture content ranges from 6.8 to 7.0 and 10% to 17% respectively. The proportion of microorganisms obtained from the sample shows *Bacillus* sp 33%, *Staphylococcus* sp 13%, *Streptococcus* sp 13%, *Pseudomonas* sp 7%, *Klebsiella* sp 7% and *Proteus* sp 6%. Antimicrobial susceptibility was tested and interpreted using NCCLS, 2006. Regulatory authorities on animal (fish) feed should therefore endeavor to ensure pathogen-free animal (fish) feed. This will ensure good and healthy meat or food (fish) production for human population.

Keywords: Fish feed, bacteriological, healthy, susceptibility, human population.

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Introduction

Fish like other animals have a requirement for essential nutrients in order to grow properly. In the wild, natural feeds are available and as the fish forage for these, they are able to meet their body needs. When fish is removed from its natural environment to an artificial one, enough food must be supplied in order to enable them grow. Artificial diets may be either complete or supplemental (Chow *et al.,* 1995). Complete diet supply with all the ingredients (protein, carbohydrates, fats, vitamins and minerals) is necessary for the optimal growth and health of the fish. Supplemental diet do not contain a full complement of nutrients needed but are used to help fortify the naturally available diets. (Riche *et al.,* 2003) reported that fish reared in intensive tank systems requires all nutrients in a complete pelleted diet since natural food is limited and fish cannot forage freely for natural foods. This has the advantage of high quality and consistency of diet. Good nutrition in fish production system is essential to economically produce healthy and high quality fish products. In fish farming, nutrition is critical because feed represents 40-50% of the production costs. Fish nutrition has advanced dramatically in recent years with the development of new, balanced commercial diets that promote optimal fish growth and health (Ruiter *et al.,* 1995). The development of new species-specific diet formulations supports the aquaculture (fish farming) industry as it expands to satisfy increasing demand for affordable, safe, and high-quality fish and sea food products (Riche *et al.,* 2003).

Fish feeds are constantly in contact with environmental organisms and become readily colonized by various microbial species. According to FAO (1987), environmental factors during storage predispose fish feeds to microbial spoilage. The presence of bacteria in feed causes decomposition and subsequently responsible for diseases in fish. Bacteria such as *Salmonella, E. coli* and other bacteria strains have been reported to contaminate fish feeds (Zmyslowska *et al.,* 1999). Also, serological study of *Pseudomonadiasis* revealed the existence of two major O serotypes related to the fish host (Actis *et al.,* 2001). Fungal contamination of fish feed has been reported to result in aflatoxicosis (Ashley, 1970). Aflatoxins are chemical produced by fungi like *Aspergillus flavus* and *A. parasiticus* commonly known as mold (Russso and Yanong, 2006). Mold-infested fish feeds have been reported to impact negatively on the growth of *Heterobranchus bidorsalis* fish (Effiong and Alatise, 2009). Aflatoxins in fish have been known to be capable of having carcinogenic effects on human consumers of contaminated fish (Brown, 2009). The occurrence of these microbial strains in fish feeds has been reported to depend on the storage conditions of the feeds, particularly temperature. The quality of fish feeds and the hygienic levels of technological process employed during feed formulation determine the level of risk of microbial contamination aided by temperature. According to Zmyslowska (2000), storage conditions especially temperature and humidity are important factors affecting microbial quality of fish feeds. Improper storage temperature may prolong survival of the microorganism in fish feeds by enhancing their multiplication and production of toxic substances which may be injurious to fish.

Materials and Methods

Samples and Sampling

Random sampling technique was used in selecting fish feed samples from different brands. The sampling was done in a space of two months so as to cover different batches of the four brands selected. Serial dilution of the sample was carried out by dissolving 1g in 9 ml of distilled water in a test tube and then 1 ml was pipetted and then introduce into another test tube up to 10^{-5} dilution factor. Inoculation of the sample was done using Nutrient Agar and Plate Count Agar and then incubated at 35 ± 2^{0}C for 24 h while PCA was for 48 h.

Morphological and Biochemical Identification of the Isolates

Pure (isolated) colonies were subcultured onto differential media (MacConkey Agar and Eosin Methlene Blue Agar) for colony identification. Morphological identification was confirmed by carrying out Gram reaction and Endospore staining. Subsequently, biochemically identity was confirmed through catalase, oxidase, indole,

coagulase, Motility, gas & H_2S production, and sugar fermentation (glucose, lactose, sucrose, fructose and maltose) (Harrigan and MeCance, 1993).

Antibiotic Susceptibility Testing

Antibiotic susceptibility were determined by the agar diffusion technique on Mueller-Hinton agar (Kirby-Bauer NCCLS modified disc diffusion technique) using 8 antibiotic discs (gram positive specific) - Ampicillin AMP (10µg), Tetracycline TET (10 µg), Gentamycin GEN (10 µg), Co-trimoxazole COT (25 µg), for Gram positive bacteria and 5 antibiotic discs (gram negative specific) - Imipenem IPM (30 µg), Amikacin AK (30 µg)) for gram negative bacteria (Oxoid). The bacterial isolates were cultured in peptone water for 16 -18 h, swab stick was dipped into the culture, pressed with wall of the test tube to avoid high density of the inoculums and later swabbed on the agar plate. The antibiotic disc is placed on the swabbed bacterial plate with the aid of sterile forcep and antibiotic coated region were pressed on the agar to ensure proper antibiotic contact the swab. The antibiotics used in this study correspond to the drugs mostly used in the treatment of human and animal bacterial infections. The zones of inhibition were measured in millimeter and interpreted using standard of NCCLS, 2006 (Plate i).

Statistical Analysis

The statistical analysis was done by loading the data into the excel work sheet (MS 97) for percentage and plotting of the bar-chart.

Determination of Moisture Content

Three crucibles were dried in the oven at 80^{o}C for 24 h. The crucibles were cooled in the desiccators for 30min and the weight was taken. 2g of fish feed was weighed in the crucible and ground in a mortar. This was then dried in the oven at 80^{o}C for 24 h. After 24 h, the crucibles were brought out of the oven, cooled in the desiccator for 30 min and then weighed. Moisture content was determined by the formula;

$$\text{Moisture content} = \frac{\text{Loss in weight of sample}}{\text{Initial weight of sample}} \times 100\%$$

The pH observed in the fish feed samples collected and analyzed ranges from 6.61 to 6.76 while the moisture content of feed ranges from 10% to 17% (Table 1).

Table 1: Physico-chemical Analysis of Fish Feed Samples.

	Fish Feed Samples	
	Brand 1	**Brand 2**
pH (Mean)	6.76±0.20	6.61±0.20
Moisture Content (%)	10	17

Colony count for bacterial isolates ranges from 4 x 10^3 to 3.6 x 10^4 (Table 2). Morphological and biochemical identification of the bacterial isolates were carried out using standard microbiological methods and Bergey's manual was used for identification.

Table 2: Microbial Count of the Fish Feed Samples.

Sample Code	Bacterial Count Cfu/g
FF 01	4 x 10^3 EST
FF 02	6 x 10^3 EST
FF 03	2 x 10^4 EST
FF 04	1.5 x 10^4 EST
FF 05	4 x 10^3 EST
FF 06	5 x 10^3EST
FF 07	8 x 10^3 EST
FF 08	8 x 10^3 EST
FF 09	2.5 x 10^4 EST
FF 10	2.5 x 10^4 EST
FF 11	3.5 x 10^4 EST
FF 12	3.6 x 10^4 EST
FF 13	2.5 x 10^4 EST
FF 14	2.8 x 10^4 EST
FF 15	3.0 x 10^4 EST
FF 16	2.1 x 10^4 EST

FF – Fish feed.
EST – Estimate.

The proportion of microorganisms obtained from the sample shows *Bacillus* sp 33%, *Staphylococcus* sp 13%, *Streptococcus* sp 13%, *Pseudomonas* sp 7%, *Klebsiella* 7%, *Proteus* sp 6% and *Aspergillus* sp 20% as shown in (Figure 1).

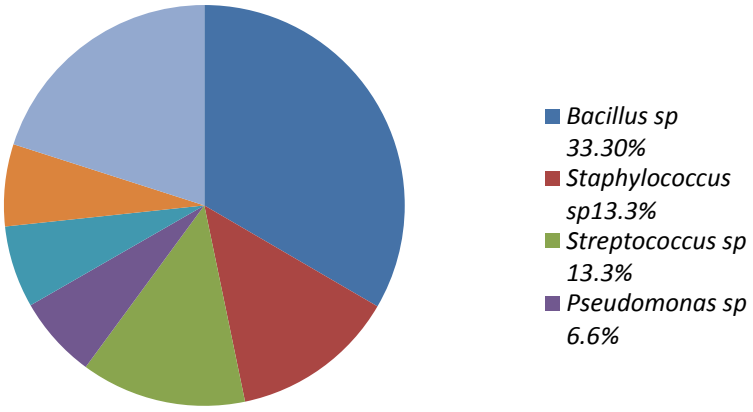

Fig. 1: Frequency of occurrence of isolates.

Aspergillus sp was identified with specialized features observed under microscope at × 40 objectives which include uniseriate and distinct conidiophores terminated by a swollen vesicle bearing flask shaped phialides. The phialides are borne directly to the vesicle. The spores are greenish and hyphae are septate. The identified fungi appeared green on plate. The antimicrobial susceptibility and distribution showed that *Bacillus* was 100% resistant to Augmentin, and Amoxicillin, *Streptococcus* sp grossly resisted all the antibiotics used, (Table 3).

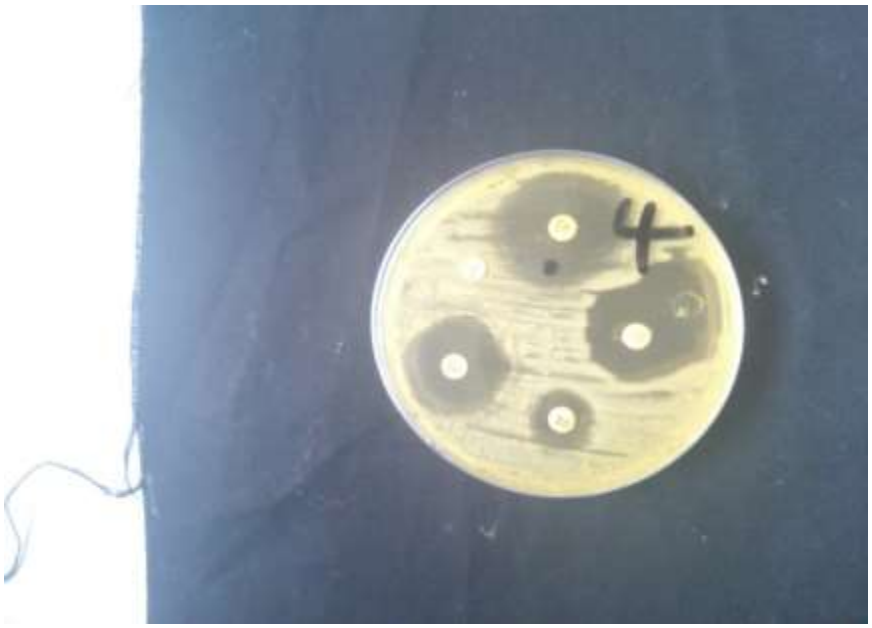

Plate i: Antimicrobial susceptibility plate of *Klebsiella* sp (Kirby- Bauer-NCCLS modified disc diffusion technique).

Table 3: Percentage of antimicrobial resistant pattern of gram positive isolates.

Antibiotic	Disc Potency(µg)	*Bacillus* sp (%)	*Staphylococcus* sp (%)	*Streptococcus* sp (%)
AUG	30	100	100	100
AMX	25	100	100	100
ERY	15	100	100	100
TET	30	100	100	100
CXC	5	100	100	100
GEN	10	80	100	100
COT	25	40	0	100
CHL	30	80	100	100

AUG: Augmentin (30 µg). AMX: Amoxicillin (25 µg). ERY: Erythromycin (15 µg). TET: Tetracycline (30 µg). CXC: Cloxacillin (5µg). GEN: Gentamycin (10 µg). COT: Co-trimoxazole (25 µg). CHL: Chloramphenicol (30 µg) (NCCLS, 2006).

However, from Gram negative bacterial isolates, *Klebsiella* sp was 100% resistant to Amikacin and Ampicilin – sulbactam while *Proteus* sp was 100% resistant to all the antibiotics as shown in Table 4.

Table 4: Percentage of antimicrobial resistant pattern of gram negative isolates.

Antibiotic	Disc Potency(µg)	*Klebsiella* sp (%)	*Proteus* sp (%)	*Pseudomonas* sp (%)
AK	30	100	100	100
CTR	30	0	100	0
IPM	10	0	100	0
NOR	10	0	100	100
SAM	30	100	100	100

AK: Amikacin (30 µg). CTR: Ceftrazone (30 µg). IPM: Imipenem (10 µg). SAM: Ampicilin – sulbactam (30 µg). NOR: Norfloxacin (10 µg) (NCCLS, 2006).

Discussion

The percentage estimate of moisture content of the feed in this study was observed to be as high as 17%. However, Ashley (1970) reported that feed exposed to moisture level above 14% is liable to contamination by microorganism. Also, the pH observed in this study was about 6.7 which is similar to Zmyslowska (2000) that documented pH close to neutral (7) as standard for fish feed. Colony count for bacterial isolates range from 1.5 x 10^4 to 4 x 10^4 which is significantly high.

A total of twenty-eight bacterial isolates identified as *Bacillus* sp, *Staphylococcal* sp, *Streptococcal* sp, *Klebsiella* sp, *Proteus* sp, and *Pseudomonas* sp isolated from the fish feed samples. The frequency of *Bacillus* sp was observed to occur more than the other bacterial isolates in the fish feed which conform to the work of (Actis *et al*., 2001) that reported the contamination of fish feed by *Bacillus* sp. *Bacillus* sp, *Streptococcus* sp and *Staphylococcus* sp were found to be grossly resistant to all the antibiotics used in this study. *Bacillus* sp and *Staphylococcus* sp were found susceptible to Co-trimoxazole. The Gram negative bacteria identified in this study were susceptible to Ceftrazone and Imipenem. Gross resistance to Amikacin and Ampicilin-sulbactam exhibited by *Klebsiella, Proteus and Pseudomonas* spp was observed in this study. *Klebsiella* sp was found susceptible to Norfloxacin. However, *Proteus and Pseudomonas* spp were resistant to Norfloxacin. *Proteus* sp found in this study was resistant to all the Gram negative-specific antibiotics. The incidence of *Aspergillus* sp in this study is an indication of fungal contamination and subsequently chance of mycotoxin production by mold.

Conclusion

The findings in this study revealed that fish feed has not been properly monitored either during processing or storage thereby causing pathogen-contaminated fish feed. However, pathogen-contaminated fish feed could constitute disease to the aquatic life (fish) and also present public health problems to human population who consume infected fish or fish that habour pathogenic and multiple antibiotic resistant bacteria. Consumption of such contaminated fish can stimulate multiple antibiotic resistant problems in human population. Incidence of antibiotic resistant isolates in fish feed is a call for major concern because some of these antibiotics are used of clinical purposes. Therefore, fish feed production should follow standard process and storage so as to ensure pathogen-free fish feed.

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