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MICROBIOLOGICAL QUALITY OF SOME SELECTED POULTRY FEEDS IN OBIO/AKPOR LOCAL GOVERNMENT AREA, RIVERS STATE AND THEIR PUBLIC HEALTH IMPORTANCE

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ABSTRACT

Introduction: Poultry production in general plays a significant role as a major source of animal protein in both developed and developing nations of the world. The quality of poultry feed is of public health importance because it affects the quality of poultry and the wholesomeness of the poultry meat and eggs consumed by human. **Aim:** This study is aimed at evaluating the microbiological quality of some selected poultry feeds in Obio/Akpor Local Government Area, Rivers Sate and their public health Importance. **Methodology:** Poultry feeds sample were randomly selected, aseptically collected and analyses using microbiological techniques to ascertain the total Heterotrophic bacterial count and fungal count. The obtained isolates were further characterized using polymerase chain reaction. **Result:** A total bacteria genera were identified ranging from highest to the least, *Micrococcus* sp incidence of 31.0% followed by *Actinomycetes* 17.2%, *Staphylococcus* sp and *Alcaligens* 13.8% *Corynebacterum* 10% and *Acinetobacter* 6.9%. *Actrobacter* and *Lactobacillus* 3.4%. The fungi isolates are *Aspergillus* 44.4%, *Fusarium* 22.4% and *Rhizopus* 33%. The PCR result shows Isolate 1 as *Proteus* sp. isolate 2 *Proteus mirabilis*, isolate 3 *E. coli*, isolate 4 *Enterobacter cloacca*, isolate 5 *Klebsiella quansipneumoniae*, isolate 6*Bacillus flexus*, isolate 7 *Myrodies*. Isolate 8 *Pseudomonas xiamenenalla* and isolate 9 *Acinebacter* species. The fungi isolates are *Aspergillus torcosus, Pichia kudriavzeril* strain subtsrate and *Leptosharerulira austrians*. **Conclusion;** The results shows the presence of these microorganisms in the poultry feeds which indicates a major source of cross contamination to human and animal and as such a public health threat. Hence there is need to emphasised on proper and improved storage of feeds within the poultry farms.

KEYWORDS: Poultry feeds: Microorganism: Public health importance.

INTRODUCTION

Poultry is normally referred to all avian species with social economic and nutritionally benefits to human[1] Human recently depends largely on poultry as a major source of animal protein. In both developed and under developed countries of the world, poultry production generally plays an essential roles as the main source of animal protein to boast the national economy.[2,3] However, in Nigeria, there is an increase production of poultry products and this now have resulted in the demand of the production of the feeds.

Poultry feeds are usually food materials formulated to include all nutritional needs of the poultry birds for proper growth, good quality meats and eggs production.[4,5] The pattern of poultry feeds formulation allows for easy digestion by the birds to provide the needed nutritional requirements. The sources of the of the materials employed are from both plants and animals origin which consist millets, maize, cassava flour, bean meal, lysine, wheat, oyster shell, fish meat, palm kernel cake, soya bean cake and brewery waste with different compounds with antioxidant behavior among which is primarily minerals and mineral dependents.[6] The quality of the poultry feeds is of public health concern because its affects the quality of the poultry products such as meat and eggs consumed by human. Occasionally, the poultry feeds may be contaminated with different micro flora from numerous environmental factors such soil, water, dust and insects depending on the material, location its origin, climatic conditions involved even during harvesting, processing, storage and transportation.[6]

Poultry feeds are usually classified as starter, grower mash, layer mash and finisher base on the type of poultry birds as well as the purpose of the production[7]). According to[5]) poultry feeds used for both local and commercial farming should be subjected to safety regulation of the products. This is because micro flora

reduces grain values through nutritional changes, physical damages and also lead to the animal health).[8] This harmful effects of micro flora on the poultry industry can as well affect the environment through poor management of manure and litter.[8]

The potential risk of the microorganisms related to the poultry feeds with poultry birds, animal consuming them and human as well has draw the attention of many researchers and this have become a great public health challenge. Conversely, there is inadequate research work done in this regards. To the best of my knowledge this work is the first to capture data on the aforementioned topic in obio/Akpor LGA, Rivers State, Nigeria. This study was designed to evaluate the microbiological quality of some selected poultry feeds in Obio/Akpor Local Government Area, Rivers State and their public health importance.

MATERIALS AND METHODS

3.1 Materials: The materials used for this study includes four (4) different brands of poultry feeds (broiler starter, finisher, grower mash, layer mash and finisher), Lacto phenol cotton blue,microscope,chloramphenicol, gentamycin, universal bottles, test tube, Autoclave, inoculating needle and physiological saline.

3.2 Experimental Design and Technique

An experimental study design was adopted for this study. The poultry feeds sample were collected aseptically using sterile containers adequately labeled to indicate the particular feeds types and conveyed to the Food Research laboratory of the Department of Microbiology, University of Port Harcourt for analysis within 24 hours.

3.3 Study Area: This study was conducted in few selected poultry farms located within Obio/Akpor LGA of Rivers State with it's headquarter at Rumuodomaya. The study was conducted from May to October 2018.

3.4 Sample Size Determination: A convenient sample size of twelve (12) bags of poultry feeds which consist of three (3) bags of broiler starter, four (4) bags of finisher, three (3) bags grower mash and two (2) bags of layer mash was obtained in selected farm in order of 3, 4, and 5 bags per farms used purposively based on the inclusion criteria set for this study.

3.5 Inclusion and Exclusion Criteria

Only poultry farms that has more than three (3) different types of feeds were chosen for the study. Only feeds with standard physical, biological and chemical compositions from manufacturing point, but were not contaminated with micro flora were included in this study. All poultry farms and feeds that do not meet the aforementioned criteria were carefully excluded.

3.6 Ethical Consideration: An ethical approval and permission to conduct this study were obtained from the head of Department of Medical Laboratory Science, Rivers State University, Nkpolu Oroworukwo Port Harcourt and the Management of various farms. The support of the poultry farm staff was properly sought. All information obtained was treated with high level of confidentiality and privacy of the poultry farms maintained and used only for the purpose of this study.

3.7 Methods of Data Collection and Procedures

The poultry feeds sample used for this study were collected using sterile universal bottles by aseptic technique to reduce further contamination during the experiment. The obtained sample were properly labeled and transported in a cello fin bag to the food microbiology laboratory department, University of Port Harcourt. The samples of the feeds were adequately homogenized and one gram (1g) of each sample was dissolved in the 9ml sterile physiological saline to form a stock solution to have 1/10 dilution[9] from this solution, more ($1/10^{-1}$ -10^{-6}) dilution were produced. About 0.1ml of the dilution (10^{-2}-10^{-6}) were plated out in duplicate on already prepared and sterilized agar medium such as MacConkey agar, DeMan Rogasa sharpe agar (MRS), Sabroud dextrose agar (SDA) and Salmonella shigella agar (SSA) to isolate the micro organisms from the dilutions. The inoculated plates were incubated for 48 hours at 37^0C. Sabroud dextrose agar (SDA) plate were supplemented with chloramphenicol at 40ug/ml and gentamycin at 500ug/ml and was incubated at 28^0C for 5 days. All plates with 30 -300 colony forming units (CFU) were documented[10,11] Discreet colonies from each of incubated plates were chosen based on the difference in their colonial morphologies and purified by sub culturing on the freshly prepared sterile media using spread plate technique. The subculture plate were +marked, identified properly and incubated for 24 hours at 37^0C, while the SDA plate were incubated at 28^0C for 5 days. The pure cultures obtained were subjected to screening. The isolates obtained were defined based on their physiological, morphological and biochemical characteristic using aseptic standard techniques[12,13] The microscopic identification of the fungi isolate was done in line with the method already described by)[14] using lacto phenol cotton blue (LPCB) stain on wet mount preparation. The (LPCB) stains is formulated with lacto phenol, which serves as a mounting fluid and cotton blue (an acid dye) which stain chitins present in the cell walls of the fungi. An inoculating needle was used to collect a small piece of the mycelium free of the medium and placed at centre of the a clean grease free microscopic slide with a drop of the lacto phenol cotton blue and covered with a glass slip. The prepared slides were then examined under the microscope at low power (×10) objective lens and the morphology of the fungi was observed and documented. All the isolates from the poultry feeds were subjected to standard protocols of Polymerase chain reaction analysis which includes DNA extraction, Amplication of 16S and 18S rRNA gene, Agarose preparation and Agarose gel electrophoresis in this study.

3.8 STATISTICAL ANALYSIS

After the laboratory analysis of the isolates, the data were also subjected to statistical analysis using both (mean, standard error of mean range, percentage and inferential (chi square) with statistically significant at ($P < 0.05$). All data were processed using Statistical Analysis System (SAS) version 9.2.

3.0 RESULTS

3.1. Isolated of bacteria and fungi from the feeds in the first month: The results of the total heterotrophic count of bacteria (THBC) and fungi count present in the first month are shown in Table 3.1. The microbial load of twelve feed samples from four different brands showed that the grower mash microbial load ranged from 5.5 $\times 10^4$ to 6.8 $\times$ 10^4cfu/g, the layers mash was between 8.0 x 10^5 to 9.0 $\times$ 10^4cfu/g, while the broiler starter ranged between 1.0x 10^5– 4.0 $\times$ 10^4cfu/g The feed sample was analysed also to show the presence of fungi in all the feed, the fungi level ranged between 1.5 x 10^3 – 4.1 $\times$ 10^3cfu/g while in broiler starter feeds the fungal count ranged from 2.0 $\times$ 10^2– 5.5$\times$ 10^3cfu/g. Broilers finisher vital and layers mash vial had no fungi contamination as shown in (table 3.1).

3.2. Isolation of Total Heterotrophic Bacteria Count and Fungi Count: The results of the total heterotrophic bacteria count of bacteria and fungi count present in the second month are shown in Table 3.2. The microbial load of twelve feed samples from four different source indicates that the grower mash microbial load ranged from 4.9 $\times 10^4$ to 5.8 $\times$ 10^4cfu/g, the layers mash was between 7.3 x 10^5 to 9.4 $\times$ 10^4cfu/g, while the broiler starter ranged between 4.3x 10^5– 9.4 $\times$ 10^4cfu/g.

The feed sample was analysed also to show the presence of fungi in all the feed, the fungi level ranged between 1.5 x 10^3 – 7.7 $\times$ 10^3cfu/g while in broiler starter feeds the heterotrophic fungal count ranged from 3.0 $\times$ 10^2– 6.6$\times$ 10^3cfu/g. Broilers as shown in (table 3.2).

3.4 Colony morphology of bacterial isolates

Table 3.4 shows the colonial and morphological characteristics of the bacterial isolates. Identification of colony on nutrient agar was based on the size, shape, texture, opacity, margin and elevation as shown in (table 3.4).

3.5 Biochemical characteristic of bacterial isolates

Table 3.5 depicts the biochemical characteristics of the isolated bacteria, found in all the poultry feed samples. The morphological and biochemical characteristics of bacteria species isolated were tentatively identified as*: Staphylococcus sp,, Micrococcus sp,. Actinomyces sp,Alcaligenes sp,Acintobacter sp,Corynebacterium sp Arthrobacter sp and Lactobaccillus sp*. Gram reaction was used as a preliminary step for the identification of the isolates as shown in (table 3.5).

3.6. Morphological Identification of Fungi Isolate

Table 3.6 shows the morphological characteristics of the fungi isolates. The isolates were characterized using colonial properties (macroscopy) on Patotoes Detrose Agar (PDA) with reference to medically important fungi). The fungal species tentatively isolated were: *Aspergillus* sp, *Fusarium* sp and *Rhizopus* sp.

Table. 3.1: Total heterotrophic bacteria count and fungi count of different feeds in the first month. Table 3.2 Total heterotrophic bacteria count and fungi count of different feeds in the second month.

Type of feed Brand of feed		Total Heterotrophic B bacteria Count (CFU/	Fungal Count
Broiler starter	Hybrid	4000 ± 0.004	5500± 0.003
	Vital	10 000000± 0.003	2000±0.004
	Top	10 000000±0.003	5400±0.004
Broiler Finisher	Hybrid	15000±0.004	6500±0.003
	Vital	90000±0.003	-
	Top	140000±0.002	6300±0.003
Grower mash	Hybrid	6400±0.003	3700±0.002
	Vital	55000±0.004	1500±0.003
	Top	68000±0.005	4000±0.004
Layer mash	Hybrid	80000±0.004	4000±0.005
	Vital	80000±0.002	-
	Top	90000±0.005	160±0.004

Table 3.2: Total Heterotrophic Bacterial Count and Fungi Count of different Feed in the Second Month

Type of feed Brand of feed		Bacterial	Fungal
Broiler starter	Hybrid	43000±0.002	6000±0.003
	Vital	1080000±0.003	5000±0.002
	Top	970000±0.003	3000±0.003
Broiler Finisher	Hybrid	161000±0.004	7700±0.003
	Vital	99000±0.003	1500±0.004
	Top	11.31113000±0.002	5200±0.003
Grower mash	Hybrid	4900±0.004	2000±0.004
	Vital	56000±0.003	2000±0.003
	Top	58000±0.003	37000±0.002
Layer mash	Hybrid	84000±0.002	6600±0.004
	Vital	73000±0.003	-
	Top	94000±0.004	1500±0.003

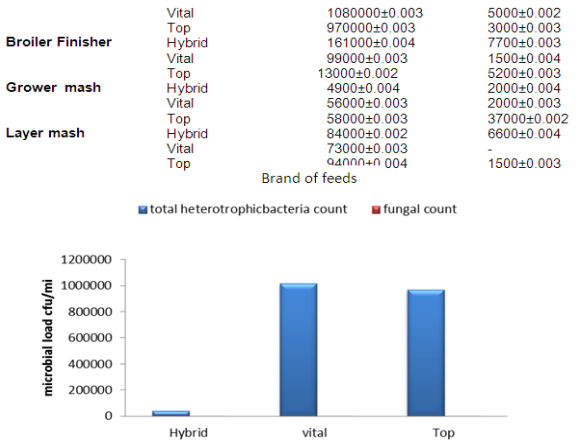

Fig. 4. 1 The Total Heterotrophic Bacterial Count and Fungal Count in Broiler Starter in the First Month.

The result in Figure 3.1 showed the total heterotrophic bacteria (THBC) and fungi count present in different source of feed in the first month. The total heterotrophic bacterial count (THBC) showed a corresponding increase in Vital followed by Top and very lower in Hybrid. Conversely the fungal count was significantly very low in hybrid, top and in vital. It is observed that there was significant difference in the sample at (P <0.05).

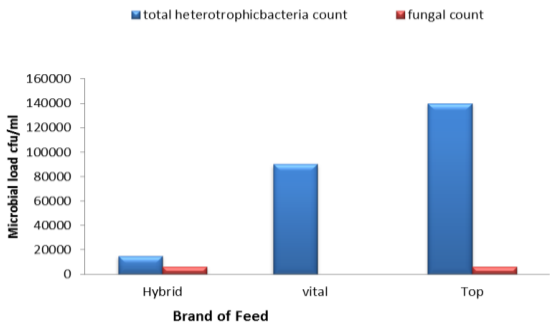

Figure. 3.2: The Total Heterotrophic Bacterial Count and Fungal Count in Finisher

The result in figure 3.2 showed an elevated value of the total heterotrophic bacterial count in Top, Vital and reduction in Hybrid. However the fungal count was in Hybrid and top. This showed that there was significant different in the sample at (P<0.05)

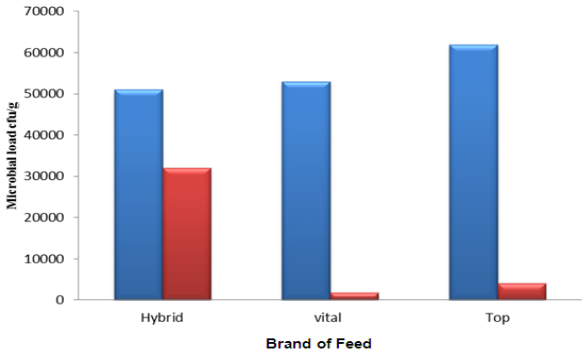

Fig. 3.3Total heterotrophic Bacteria count and Fungal Count in Grower Mash in the First Month

The result in Figure 3.3 revealed highest THBC in Top and subsequently followed by Vital and then Hybrid. Conversely, the fungal count was noticeable in Hybrid, low in top and very low in vital. There was significantly difference at (P<0.05).

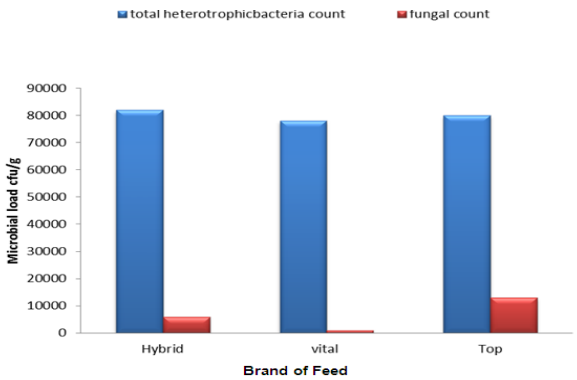

Fig. 3.4 The Total Heterotrophic Bacteria Count and Fungal Count in Layer Mash in the First Month

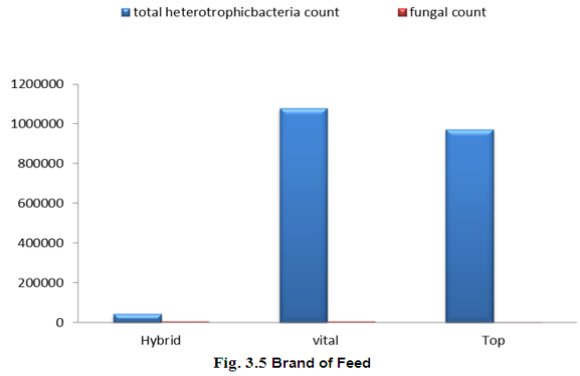

Fig. 3.5 Brand of Feed

The THBC and the Fungal Count in the Broiler Starter in the Second

The result in figure 3.5 revealed that the THBC and fungal count in broiler starter in the second month was highest in t he Vital, subsequently followed by Top and the least in Hybrid. conversely, the fungal count was low in the Hybrid, Vital and in Top. Also, it showed a significant difference as (P< 0.05).

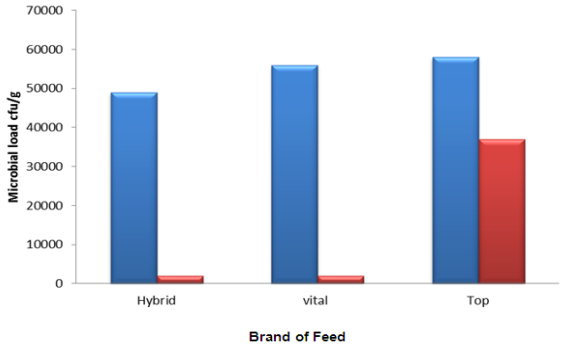

Fig. 3.6 The THBC and the Fungal Count in Grower Mash in the Second Month

From the figure 3.6 above, this showed that THBC was highest in the Top which was followed by Vital and Hybrid respectively. Furthermore, the fungal count revealed highest in the Top and in Hybrid and Vital respectively. There was significant difference at (P<0.05)

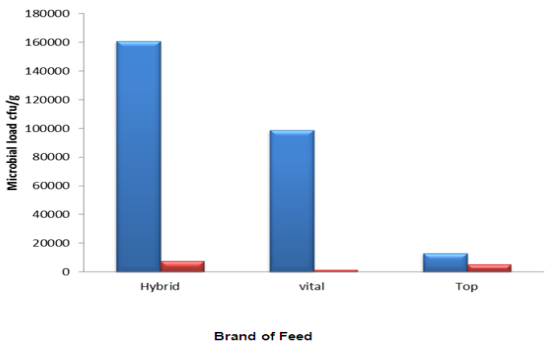

Fig. 3.7 The THBC and the Fungal Count in the Finisher in the Second Month

The result in figure 3.7 indicated that the THBC was very high in the Hybrid, followed by Vital and Top respectively. The fungal count was lowin Hybrid, Top and very low in the Vital. Conversely there was significant different at (P<0.05).

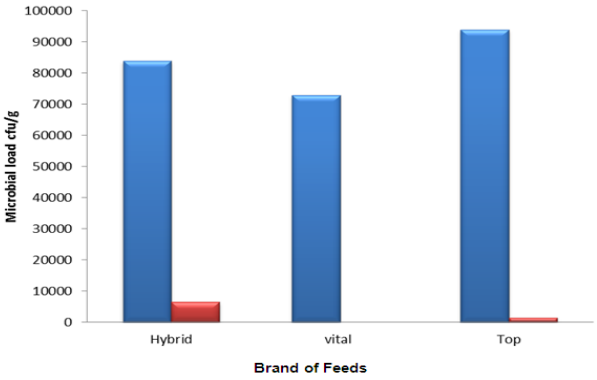

Fig. 3.8 The THBC and Fungal Count in the Layer Mash in the Second Month

On comparing the result in figure 3.8, the THBC was highest in the Top, followed by Hybrid and then slightly low in the Vital The fungal count was very low in Hybrid and lowest in the Top and Vital. Therefore, there was significant different at (P<0.05).

3.7 Occurrence of Bacteria Isolates from the Poultry Feed: The percentage occurrence of bacterial isolates of the poultry feeds is shown in table 3.7. The *result showed thatMicrococcus sp has the highest incidence of 31.0% followed Actinomycetes* with of incidenceof 17.2%, *Staphylococcus* sp and *Alcaligens* with incidence of 13.9% *Corynebacterumwith* 10.3% and *Acinetobacter* 6.9%. The bacterium with the least incidence was *Actrobacter* sp *and Lactobacillus* sp with an incidence of 3.4%.

3.8 Occurrence of Fungi Isolates from the Poultry Feed: The percentage occurrence of fungi isolates of the poultry feeds is shown in table 3.8. The result showed that *Aspergillus* sp has the highest incidence of 44.4% followed *Rhizopus* with of incidence of 33.2%, and *Fusarium* with incidence of 22.4%

Table. 3.6: Occurrence of Bacteria and Fungi Isolates from the Poultry Feed.

Bacteria Isolates n=8	Frequence of Occurrences	Percentage of Occurrences (%)
Staphylococcus sp	4	13.8
Micrococcus	9	31.0
Actinomycetes	5	17.2
Alcaligens	4	13.8
Corynebacterum	3	10.3
Actrobacter	1	3.4
Acinetobacter	2	6.9
Lactobacillus	1	3.4
Total	29	100%.

Table 3.7: Occurrence of Fungi Isolates from the Poultry Feed.

Fungi Isolates n=3	Frequency of Occurrences	Percentage of Occurrences (%)
Aspergillus sp	4	44.4
Fusarium sp	2	22.4
Rhizopussp	3	33.2
Total	9	100%.

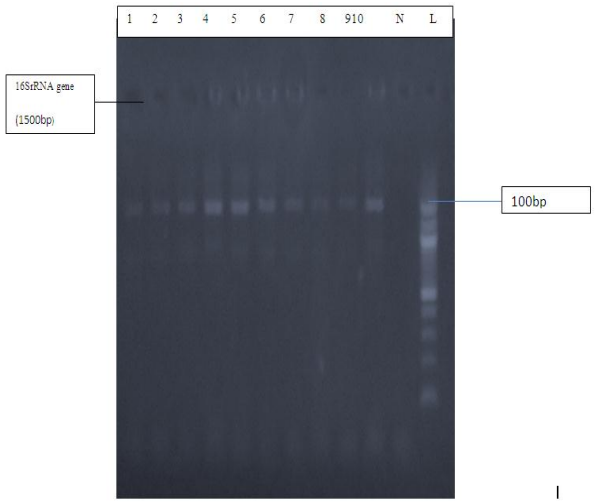

Plate 3.1: PCR amplification images of the 16S rRNA gene bands of the bacteria isolate used in the study. (Lane 1-10 represents 18S rRNA (ribosomal RNA) of the isolates. Lane N represents the negative control; lane L represents the 100 bp molecular ladder).

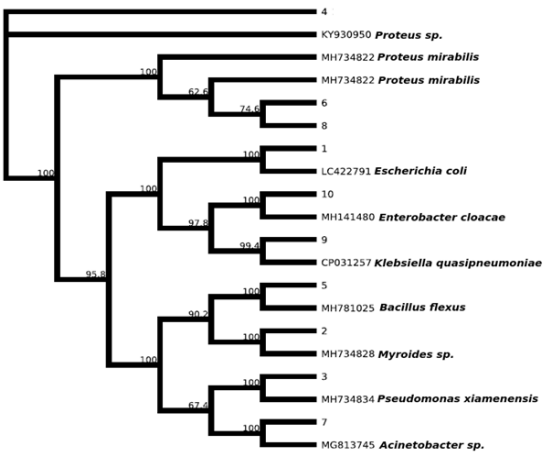

Figure 3.8 Neighbour-joining phylogenetic tree of isolate 1-10.

Bootstrap values of > 50% (based on 500 replicates) are given in the nodes of the tree.

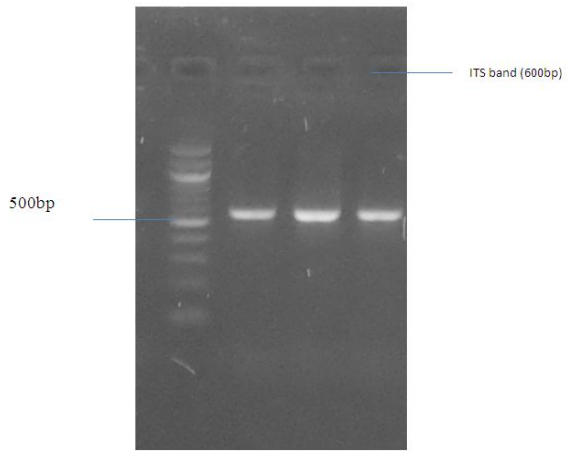

Plate 3.2: Agarose gel electrophoresis showing the amplified ITS sequence. Lane L represents the 100bp DNA ladder while lane 1-3 represent the ITS bands.

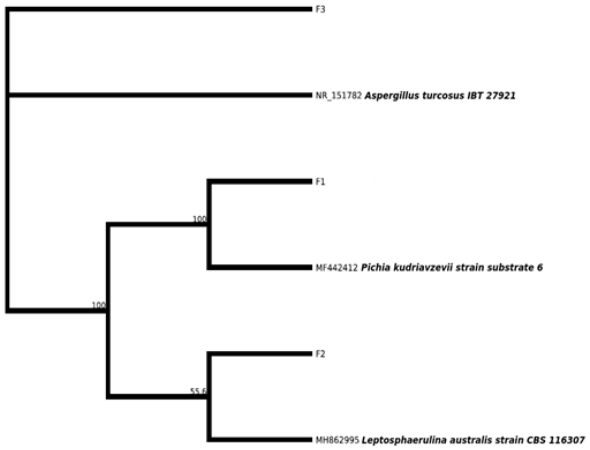

Figure 3.9. Neighbour-joining phylogenetic tree of isolate F1, F2 and F3 Phylogenic tree of the fungi isolate Th e phylogeny tree of the fungi isolates used for the study is shown.

4.0 DICUSSION

All the samples analysed in this study indicated the presence of microorganism, this may suggest that poultry feeds are major source of growth for most microbes found in the feeds due to their nutritional quality The result of this study reveals that both bacteria and fungi are potential risk for public health challenges and this study agrees with report by[15] that these microbes emanated from different sources. This may be attributed to climatic factors uncounted during processing, storage, transportation as well as the technologies used. The most frequently isolated bacteria species are *Micrococcus* sp, *Actinomycetes* sp, *Staphylococcus* sp, *Alcalegen* sp,*Corynebacterium* sp,*Acinetobacter* sp,*Actrobacter and Lactobacillus* sp while the fungal general are *Rhizopus* sp,*Fusarium* sp *and Aspergillus* sp. The samples were inoculated and incubated using the various media,Nutrient agar,MacConkey agar,De ManRogosa Sharpe agar (Mrs),Sabouraud dextrose agar (SDA) and Salmonella Shigella agar (SSA). The following biochemical test such Gram stain, Indole, catalase and citrate test were carried on the bacterial isolates while

Lactose phenol cotton blue were used for fungi macroscopic and microscopic. The PCR result shows isolate 1 as *proteus* sp, isolate 2 *proteus mirabilis*, isolate 3 *E. coli*, isolate 4 *Enterobacter cloacca*, isolate 5 *Klebsiella quansipneumoniae*, isolate 6*Bacillus flexus*, isolate 7 *Myrodies*. Isolate 8 *Pseudomonas xiamenenalla* and isolate 9 *Acinebacter* species fungi isolates are *Aspergillus torcosus*, *Pichia kudriavzeril* strain subtsrate and *Leptosharerulira Austrians* The occurrence of bacterial and the fungal species are of serious public health significant which may be critical health hazard in terms of direct consumption of bacteriological or fungal contaminated feed or their toxins by farm animals.[16] The ubiquity of the microbes in the environment is of serious health concern, because poultry diseases with various clinical sign and symptom of different origin such as Newcastle diseases, Avian influenza, infectious coryza and Aspergillosis. Most of the bacterial isolated are highly pathogenic in poultry industry for example staphylococcus and micrococcus have been reported in microbial infection outbreak in poultry farm[17] Interestingly, *Corynebacterium* sp have been implicated in Nigeria according to[18] and was classified as pathogenic organism especially in the compromised individuals. The occurrence of this microbes in the feeds revealed a significant health implication whenever this feeds are consumed without proper hygiene or their toxin by farm animals as well as human.[19] Animal feeds have been known worldwide as one of the source of microbes of poultry and other livestock's. Therefore, the prevalent bacterial and fungal recovered may indicates high risk hazards to the animals. The study revealed a high heterotrophic bacterial count of $16.1x10^4$ -4.0 $x10^4$ and fungal count of $7.7x10^3$-1.0 $x10^3$.This study is in consonance with[6] who obtained a similar range of value of 6.6 $x10^4$ -2.5 $x10^4$ and 7.4 $x10^3$ -1.5 $x10^3$ cfu/g for bacterial and fungal respectively. Furthermore, this study is in agreement with[20], who reported that poultry feeds samples from Ogbomosho, South–West Nigeria were associated with *Coliforms* heterotrophic bacteria and fungi. The result of this research also showed that both bacteria and fungi are potential risk for public health challenges in the environment. This agrees with a report by[15]), that these microbes depend on the feeds for their growth and metabolism, of which this microbe emanates from different sources. However, this may be attributed to climatic factors encountered during processing, storage,transportation as well as technologies used. This study disagree with[19]) who reported the presence of *Listeria*, *Bacillus*, *Pseudomonas*, *Escherichiacoli* and *Salmonella species* as a result of environmental contamination. Also not in consonance with the statistical analysis of the isolates which showed no significance association between feeds types and the isolation rate of each microbes cultivated from the poultry feeds.In the same vein,the study is not in agreement with a report that bacteria loads of 20 samples of poultry feeds ranges from $1\text{-}03x10^8$ cfu/g to 1.232 $x10^9$ cfu/g with prevalent bacteria as *Bacillus*, *Escherichia coli*, *Nocardia*, *Salmonella*, *Proteus*, *Pseudomonas*, *Staphylococcus* and *Streptococcus* species[21] Moreover, this finding does not agrees with a study by[19], whose isolated frequency were *Aspergillus* sp 49,1%,*Penicillium* 18.4%,*Rhizopus* 13.36%,*mucor* 11.55% and *Fusarium* 7.58%. The present study disagrees with a study by[1] who isolated *mucor*,*yeast Aspergillus* and *Rhizipus* as the common moulds found in poultry feeds raw materials sold in Imo state Nigeria.

5. CONCLUSION

This study showed poultry feeds mixtures can be contaminated with numerous types of microbes depending on the function of material, location of its origin, climatic conditions encountered, even at the time of harvesting, processing, storage condition, transportation, packaging materials.

6. RECOMMENDATIONS

Poultry feeds from different source should be examined for bio safety periodically so as to prevent cross contamination to feeds products and that Government should reduce the cost of poultry feeds to enable expansion in poultry production.

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