Bacterial Quality and Safety of Commercial Fish and Chicken Feeds Sold at Kisenyi Market, Kampala-Uganda. A Laboratory Based Cross-sectional Study.

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Abstract

Background:

The growth in the poultry and fish industry in Uganda has resulted in heavy dependence on finished feeds supplied by feed millers, the quality of which determines the profit margin of the farmers. The presence of pathogenic microbes, however, tends to deplete the nutritive value of this poultry and fish diets. This study, therefore, focused on assessing the prevalence of micro-organisms in commercial poultry and fish feeds sold at Kisenyi Market, Uganda.

Method:

Poultry and fish feeds were randomly sampled and examined for their microbiological qualities using standard microbiological and analytical methods. The isolates were identified according to their cultural, microscopic, and biochemical properties.

Results:

A total of 42 micro-organisms were isolated, including; *Citrobacter spp* (4.76%), *Corynebacterium spp* (9.52%), *E. coli* (2.38%), and *Enterococcus spp* (35.71%) *Proteus spp* (2.38%) and *S. aureus* (45.2%). Comparing contamination in pelleted and non-pelleted feeds, there was no significant difference in the microbial contamination (feeds (X2 = 7.287, P> 0.05). Their susceptibility pattern revealed major resistance of; *S. aureus* to Gentamycin (78.95%), *Corynebacterium spp* to linezolid (100%), *Enterococcus spp* to Gentamycin (100%), and negative rod enterococcus to Cefoxitin (100%).

Conclusion:

The presence of a high level of pathogenic micro-organisms in the selected feeds offered to poultry and fish predisposes them to health hazards, with resultant economic loss. Therefore, the commercial feeds should be periodically examined for biosafety, to reduce or prevent the risk of cross-contamination of poultry and poultry products with resistant bacterial strains.

Keywords: Chicken feeds, Fish feeds, Commercial, Microorganisms, Date Submitted: 2022-04-01 Date Accepted: 2022-08-30

1. Background

Animal feeds are good and nourishing food supplements with varying constituents of various nutrients i.e., proteins, carbohydrates, minerals, essential amino acids, antibiotics, vitamins, premix, and antioxidants. According to (Tainika, 2019), Uganda's poultry population is estimated to be around 47.6 million while aquaculture production has improved from a tone of 24,382.5 in 1995 to 98,063 metric tones as of 2013 (Safina, 2018). The

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growth in the poultry and fish industry in Uganda has resulted in heavy dependence on finished feeds supplied by feed millers, the quality of which determines the profit margin of the farmers (Pius *et al.,* 2021).

The bacterial quality of feeds is still a challenge and one of the leading causes of infections on farms (Swelum *et al.,* 2021). Poultry feed is considered one of the important sources of contamination to poultry as they are routinely subject to contamination from diverse sources including environmental pollution, activities of insects, and microbes (EFSA *et al.,* 2021). Specifically, some of the additives have been incriminated among the principal sources of bacteria of public health concern (Hoque *et al.,* 2019). Microorganisms that can contaminate poultry feeds include Escherichia coli, Staphylococcus aureus, Salmonella spp, Listeria spp, Streptococcus spp, Klebsiella spp, Pseudomonas spp, Aspergillus niger, Aspergillus flavus, Rhizopus spp, Penicillium spp, Fusarium spp. However, the number and types of microorganisms in poultry feeds vary depending on the function of materials, location of their origin, climate conditions encountered, harvesting, processing, storage, transport technologies employed, and packaging materials (Diaz *et al.,* 2019).

The effects of microorganisms in poultry feeds may include degradation of nutrient value, change in smell and color, caking of the feed, and production of the toxin, an example of these toxins include mycotoxins which differ in their toxicological effect and are usually found in mixed form (Yu *et al.,* 2022). Poultry feeds have been implicated in several poultry diseases of viral, bacterial, and fungal origin, suggesting that such feeds can potentially act as carriers for the farm as well as animal pathogens. Most of the bacterial isolates in these feeds are of poultry health concern, as they reportedly cause such as *omphalitis, aerosacculitis, salpingitis, polyserositis, panophthalmitis, septicaemia,* and other mainly extra intestinal diseases in chickens (Aliyu *et al.,* 2012). *Staphylococcus aureus* has been reported to cause food poisoning Alabi et al., 2018), thus not only extending the risk to the farm animals but also the humans.

Almost all animal feeds still have a high possibility of contamination with both commensal and pathogenic bacteria from harvesting, processing, handling, and marketing of the bagged feeds (Alabi *et al.,* 2018). These microorganisms may probably have originated from the raw materials from which the feeds are being produced or other sources (Swelum *et al.,* 2021). Effective control measures of any infectious disease require the identification of the possible sources of the disease-causing agent. However, few studies have been done to identify the microbial quality of feeds sold in the Kisenyi Market. Investigation of the possibility of microbial contamination in commercial chicken and fish feeds was taken as an ideal step in generating knowledge on feed biosecurity. The investigation involved the overall prevalence and antimicrobial susceptibility of the different isolates.

2. Methodology.

Study design

This was a laboratory-based cross-sectional study that was carried out in animal feed market stalls of Kisenyi Market from January to February 2022. Poultry and fish feeds were collected aseptically and conveyed to the University of Kisubi Microbiology laboratory for microbiological analysis using standard analytical methods.

Study area

The study was conducted in a few selected poultry and fish stalls located within Kisenyi. It ran from January to February 2022.

Sampling technique

Convenient sampling was done during the selection of the chicken and fish feeds from the stalls. This sampling technique was chosen because of the limitations due to the number of available stalls at the study site.

Study selection criteria

Inclusion criteria

- Already formulated chicken and fish feeds.
- Stalls that their owners verbally consented to participate in the study

Exclusion criteria

- Raw materials (unformulated feeds).

Quality assurance and quality control

Data quality was ensured by carrying out analysis following standardized analytical techniques. The data collected was thoroughly checked for completeness. All the steps were guided by a qualified laboratory technician and my institution supervisor. Other quality controls observed were:

Pre-Examination

• Proper sample collection with minimized contamination was observed i.e., sterilization of sample bags, double packaging, and disinfection of hands before sample handling.

• A cool box was used in sample transportation to the laboratory.

• Proper sample labeling for easy identification.

• Proper storage of samples in the refrigerator at 4 to 8°C.

Examination

• Sample preparation was done on arrival at the laboratory or within a time-space of 24hrs.

• Sample verification before the examination.

• Aseptic microbiological techniques were observed during analysis.

• Proper care and maintenance of equipment e.g. incubator temperature maintenance log charts will carefully be observed.

• Following and adhering to protocol SOPs.

Post Examination

• Verification of microbiological test results.

• Recording of results.

• Analysis, interpretation, and inference of results.

• Writing of reports and documentation

Materials

Sterile normal saline to immerse the samples was prepared. Primary media i.e., MAC, MSA, BEA, and XLD onto which the samples were cultured, peptone water, and NA for sub-culturing. Coagulase, Catalase, citrate, TSI, SIM, and Kovac's reagent for biochemicals followed by NA and antibiotic discs for AST (Antimicrobial Susceptibility Testing) were also used.

Sample collection

Samples of 14 bags of different poultry feed ingredients (approximately 5g of each) i.e., small fish, soya bean cake, and fine maize brand among others, that are usually mixed to make a final fortified poultry feed will be collected, and 6 bags of different sizes of fish feed pellets i.e., powder, 1mm-4mm sizes were collected from a randomly selected stall in Kisenyi. Generally, sample collection involved the following steps:

• Sterile polyethylene bags were bought from shops and surface sterilization using 70% ethanol was always done to prevent possible external contaminants.

• The bags were double packaged and transported along to the sample collection site.

• Disinfection of the hands using 70% ethanol was constantly done before handling the specimen which was aseptically double packaged in the sterile polythene bags.

• The samples were then transported back to the laboratory in a cool box at 4°C to 8°C.

Laboratory analysis

Sample preparation

During sample preparation for analysis:

• Using a sterile spatula, a spoonful of each sample was transferred in 10mls of sterile normal saline in sterile tubes.

• The tubes were covered with sterile aluminum foil and incubated at 37°C for 24 hours.

• After, inoculation on primary cultures and sub-culturing on NA was done.

Actual bacteria isolation

This followed the following steps: Different Gram staining from NA sub-cultures and different biochemical tests were carried out to identify the bacterium isolate to the species hierarchy. District Laboratory Practices 2 were used to identify the different bacterial isolated according to their biochemical tests.2: The laboratory analysis of the samples.

(Key: A=Labeling of media prior to inoculation, B= Normal saline, C=Sample transportation in cooler box, D= Inoculated media in incubator).

Antimicrobial Susceptibility Patterns of the isolates

During this process, NA plates were prepared, and the Kirby-Bauer disc diffusion method of AST was used to determine the antimicrobial susceptibility patterns of the different isolates. The

Figure 1: The laboratory analysis of the samples

AST chart was used to interpret the zones of clearance. The different antibiotics included; tetracycline, Cefoxitin, Levofloxacin, Gentamycin, erythromycin, and linezolid.

Data management and analysis

The data collected was entered and cleaned in Microsoft excel 2016 spreadsheet and used to determine the bacterial isolated with the highest prevalence, P, and presented in form of a histogram. The excel data was imported to the STATA software package (version 14.2, 4905 Lakeway Drive, college station, texas 77845 USA) for analysis. Chi tests were used to calculate the p-value to test the hypothesis (H-0: There is no significant association between pelleted/unpelleted feeds and bacterial diversity). All levels of statistical significance were established at $p<0.05$.

3. Results *Staphylococcus aureus.*

Prevalence of bacterial contamination in chicken and fish feeds.

3.1. Comparing contamination pelleted and non-pelleted feeds

There was no significant difference in the bacterial contamination in pelleted and non-pelleted feeds ($X^2 = 7.287$, P=0.2).

3.2. Antimicrobial susceptibility of the isolated organisms.

Susceptibility testing revealed that *Staphylococcus aureus spp* (n=19) were resistant to; tetracycline (57.89%), Cefoxitin (63.16%), Levofloxacin (10.53%), Gentamycin (78.95%) and erythromycin (31.58%).

Susceptibility testing revealed that the isolated *Corynebacterium spp* (n=4) were resistant to; linezolid (100%), Gentamycin (75%) and Erythromycin (50%).

Enterococcus spp. (n=15) were resistant to; Gentamycin (100%) and linezolid (13.33%).

Susceptibility testing revealed that the isolated negative rod *enterobacteria spp.* (n=4) were resistant to; Amoxicillin-Clavunate (50%), and Cefoxitin (100%).

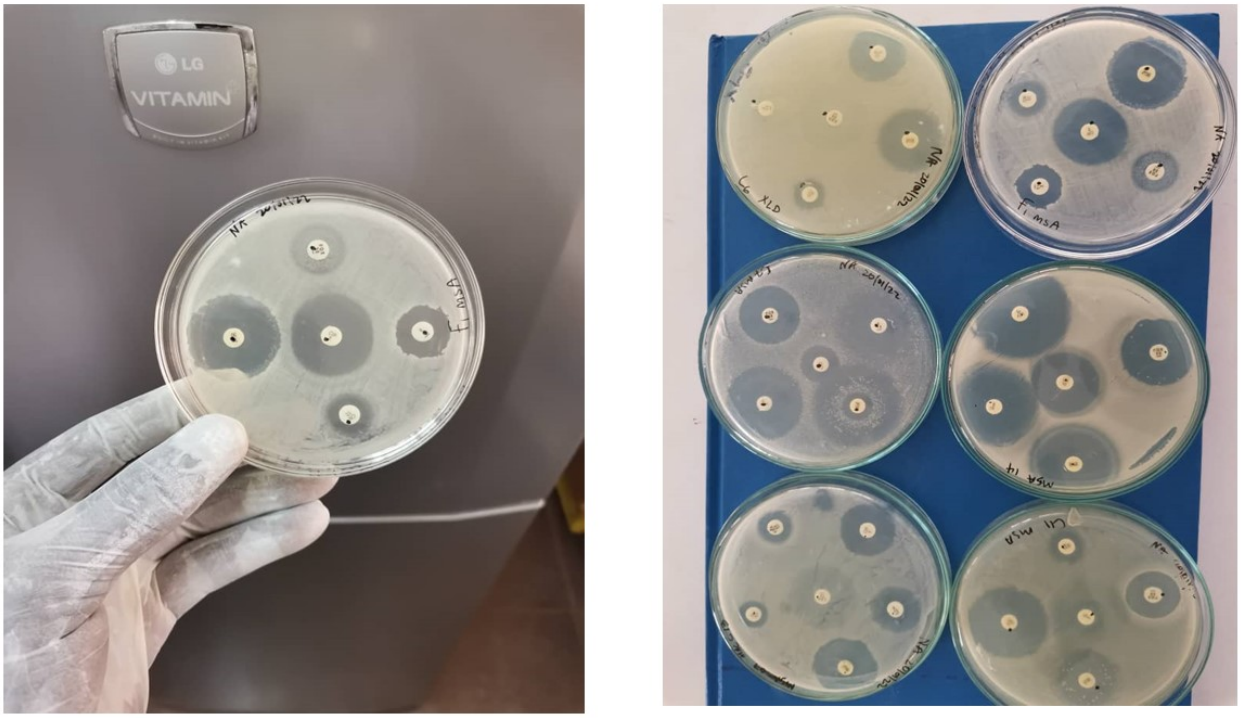

Figure 2: Susceptibility testing using Kirby discs

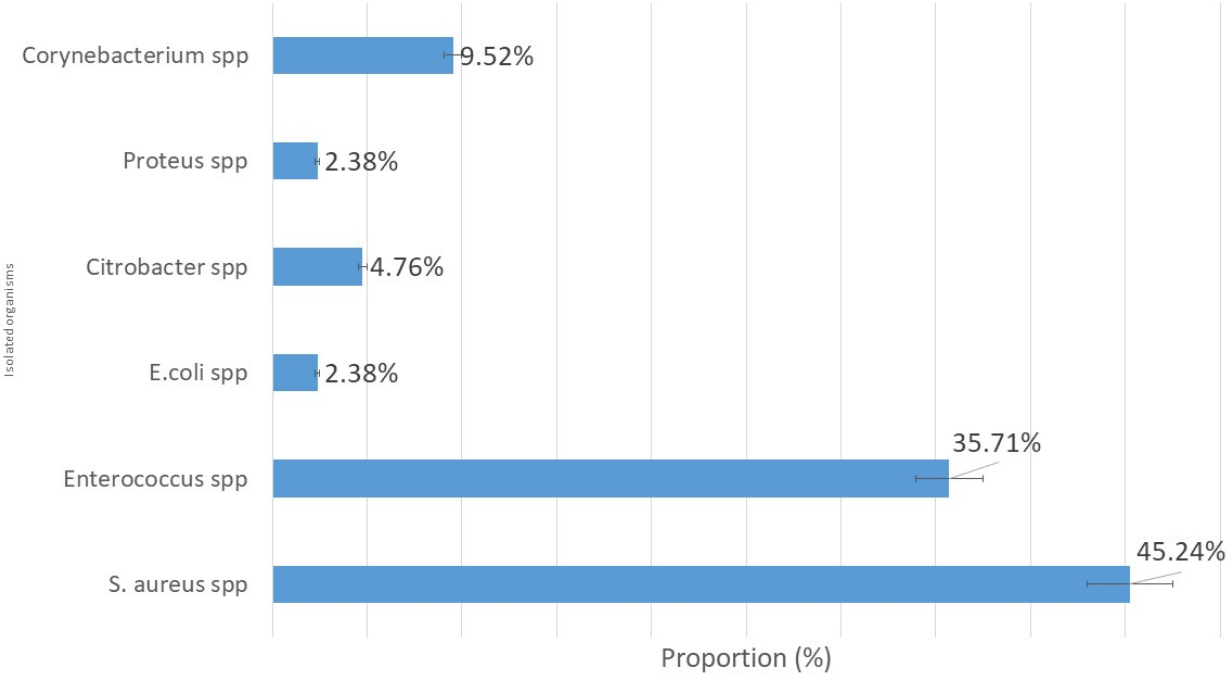

Figure 3: The prevalence of bacterial contaminants in the chicken and fish feeds

September 26, 2022

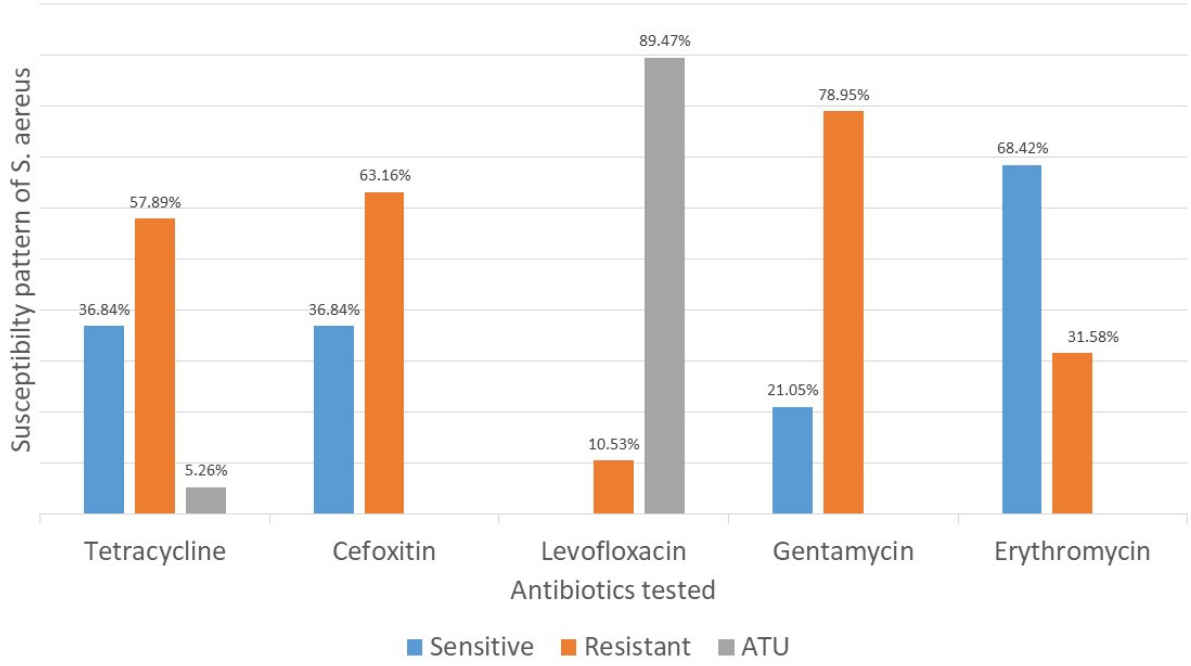

Figure 4: Susceptibility pattern of isolated S. aureus toselected antibiotics

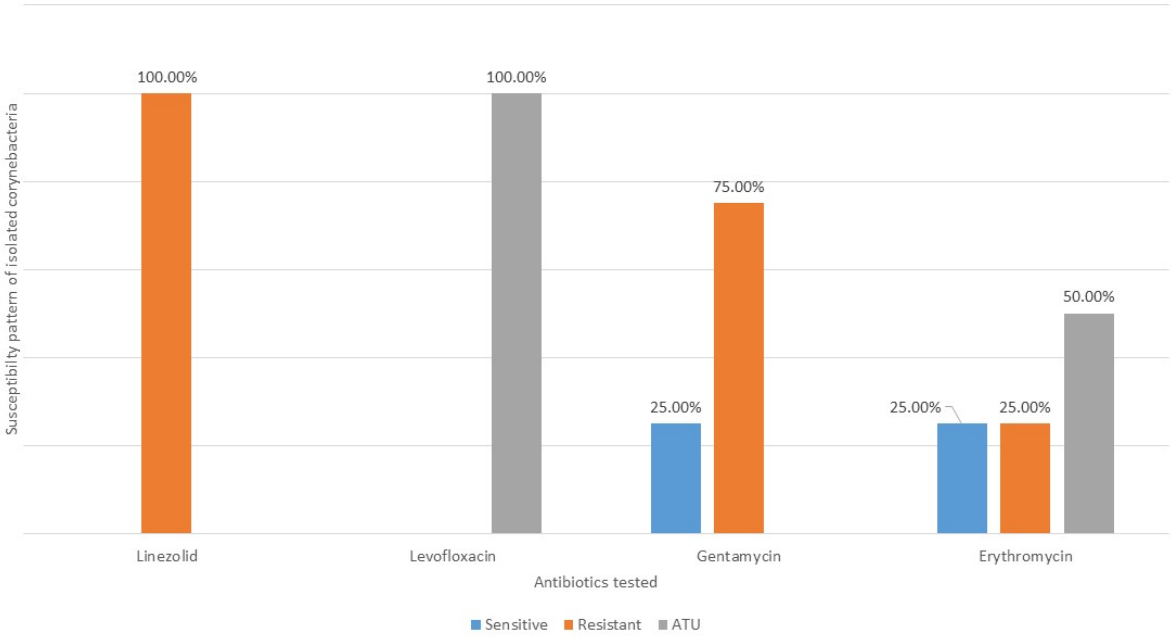

Figure 5: Susceptibility pattern of isolated *Corynebacterium spp* to selected antibiotics

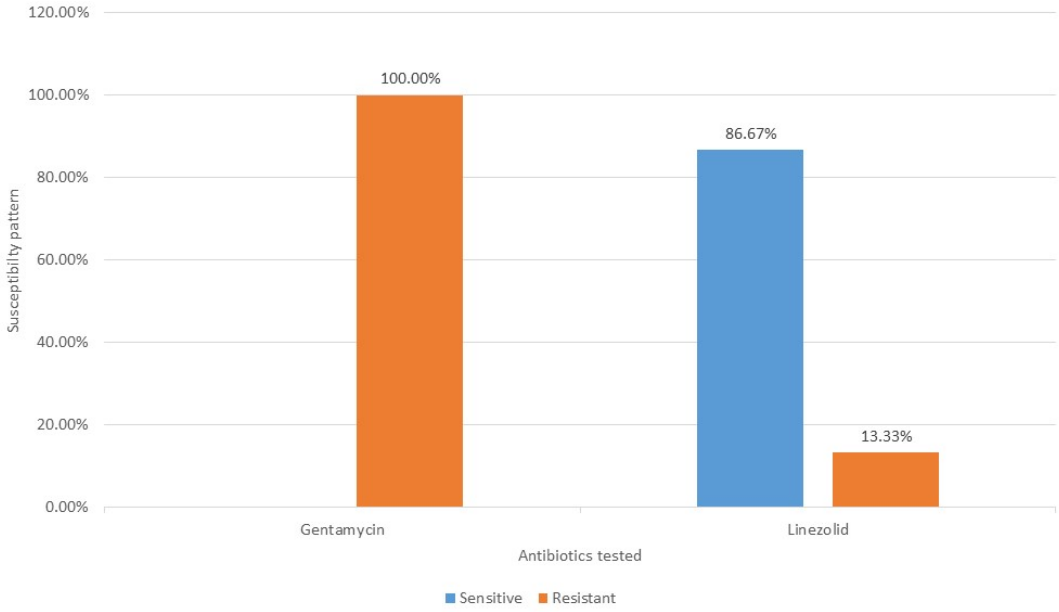

Figure 6: Susceptibility pattern of isolated *Enterococcusto* selected antibiotics

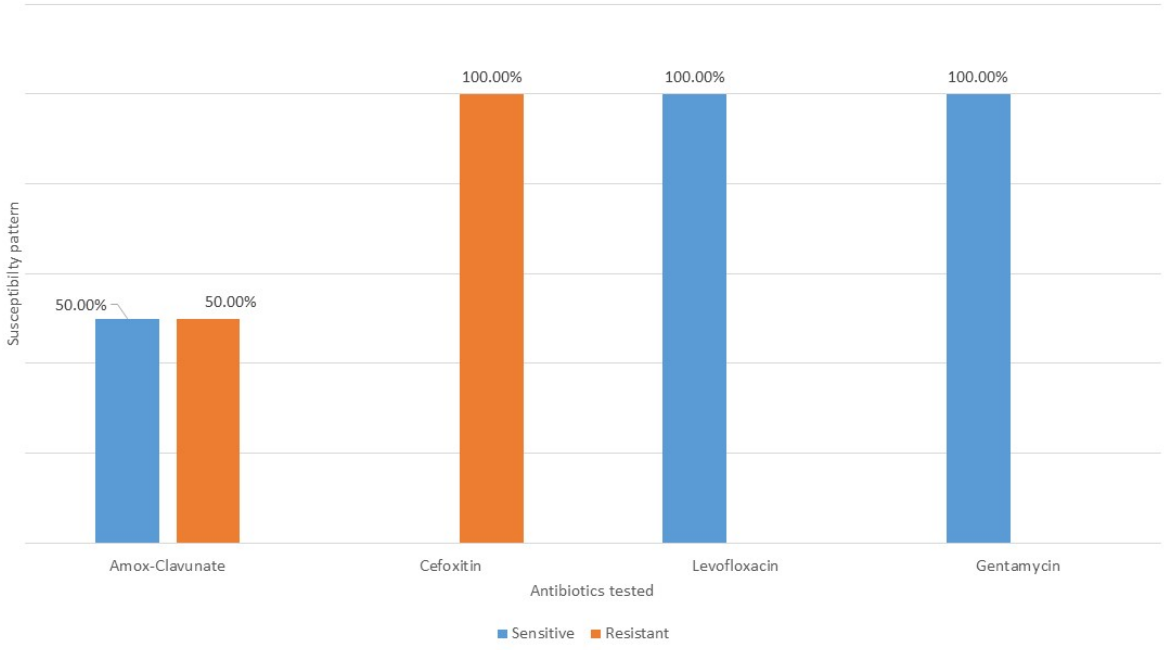

Figure 7: Susceptibility pattern of isolated negative rod *enterobacteria spp* to selected antibiotics

Table 1: Table: Two-way table showing bacterial contamination in pelleted and non-pelted feedsStaphylococcus aureussppCefoxitin (63.16%), Levofloxacin (10.53%), Gentamycin (78.95%) and erythromycin (31.58%).

Isolated organisms	**Nature of sample**			
	Pelted	**Non-pelleted**	**Chi value**	**P-value**
Citrobacter spp.	0	2		
Corynebacterium spp.	2	2		
E. coli	1	0		
Enterococcus spp.	3	12	7.287	0.2
Proteus spp.	1	0		
S. aureus	5	14		
TOTAL	12	30		

4. Discussion:

Prevalence of bacterial contamination in chicken and fish feeds

A total of 42 organisms were isolated, 45.24% were Staphylococcus aureus. All samples of feeds had at least one form of bacteria. The presence of these microorganisms in the poultry feeds suggests that the feeds contain sufficient nutrients for the growth of these microorganisms. The activities of this organism which we studied may cause degradation, thereby reducing the nutrients that would have been wholly available for the livestock to feed on. Our finding was in agreement with that of Alabi *et al.,* (2018) in a study conducted on poultry feeds and a study by Mdemu *et al.,* (2016) who also reported bacterial contamination in chicken feeds in the Ilala district. The variations in the prevalence of the organisms in other studies might be due to the difficulty in detection as well as differences in sampling and testing methods (Soria *et al.,* 2011).

Most of the bacterial isolates in these feeds are of poultry health concern. For example, E*. coli* infections have been reported to cause diseases such as omphalitis, aerosacculitis, salpingitis, polyserositis, panophthalmitis, septicemia, and other mainly extra intestinal diseases in chickens (Aliyu *et al.,* 2012). *Staphylococcus aureus* is known to cause food poisoning and has been implicated in osteomyelitis, arthritis, synovitis, septicaemia, and cellulitis (Hazaariwala as cited by Alabi *et al.,* 2018).

These microorganisms may probably have originated from the raw materials from which the feeds are being produced. In addition, most of the microorganisms have their origin in the air and soils (Sule & Lori *et al.,* 2017). The contamination of the feeds could have also been due to poor hygienic practices during the process of production. The presence of an S. aureus, normal flora of the skin, and nose present improper handling practices of the feeds (Dweba *et al.,* 2018). On the other hand, the presence of *E. coli* may suggest fecal as well as environmental. For instance, *E. coli* known as coliform bacteria are normal inhabitants of the digestive tract and are abundant in the poultry environment, some of them are implicated in disease conditions such as colibacillosis occurring in various forms such as enteric and septicemic colibacillosis that cause increased mortality and performance of birds.

Alabi *et al.,* (2018) reported microbial contamination of poultry feeds to be a result of climatic conditions encountered, harvesting, processing storage, and transport technologies employed. However, package and packaging materials, environment, and handling circumstances, including the nature and extent of the quality control measure greatly influence the degree and source of contamination (Mitra, 2016). Most of the bacteria isolated are highly pathogenic in the poultry industry. Bacteria such as S. aureus has been reported in microbial infection outbreak in poultry farming.

Antimicrobial susceptibility of the isolated organisms

Susceptibility testing revealed some level of resistance of the isolates to antibiotics such as Tetracycline, Cefoxitin, Levofloxacin, Gentamycin, Erythromycin, and Linezolid. Our finding was in agreement with the report by Gieraltowski *et al.,* (2016) that reported that the isolates from the poultry products exhibited resistance to various combinations of ampicillin, chloramphenicol, gentamicin, kanamycin, streptomycin, sulfisoxazole, and tetracycline. This can be explained by the fact that resistant organisms in feeds can pass to the poultry and fish when fed on.

This study recorded 100% resistance to Gentamycin by enterococcus. This finding did not agree with a recent study that reported 100% resistance of the isolates to ampicillin, amoxicillin, meropenemand tetracycline, but not gentamycin. They reported a much lower resistance to gentamycin of only 40% (Sebastian *et al.,* 2021).

The increasing resistance could have originated from increased exposure of these organisms to antimicrobials which are added to the feeds during production. A recent study by Carrique *et al.,* (2020) in Vietnam found a discrepancy between the existing legislation and the types and quantities of antimicrobial growth promoters added to chicken feeds. Despite these limitations, the well-designed sample collection procedure and analysis limited the possibility of cross-contamination. Thus the contamination shown was from the respective sampled feeds.

The irrational use of antibiotics for treatment and prevention of infection in poultry and as growth promoters in feed might have led to that. Genetic exchange is yet another way by which antibiotic-resistant plasmids can move between bacteria (Cabello *et al.,* 2016). Some bacteria acquire antibiotic-resistant plasmids, intra- and inter-species transfer of resistance, irrespective of the environment (i.e., whether or not antibiotics are present) (Davis *et al.,* 2018).

Study limitations

There were limitations to this investigation. This study did not attempt to assess the quantities of antimicrobial growth promoters added to the feeds. We also did not assess the factors associated with the contamination

5. Conclusion and Recommendations

Conclusion

This study revealed the presence of a high number of pathogenic microorganisms in both poultry and fish feeds investigated. This tends to reflect the level of biosecurity and hygienic practices in the handling and storing of the feeds.

Recommendation

These findings emphasize the need for constant quality assessment of these commercial feeds on sale to maintain the production of microbiologically stable poultry and fish feeds for the farmers. Besides, feed mill management should improve the hygienic practices on the feed mill premises. We also discourage the incorporation of antibiotic additives into the feeds as a way of preventing microbial contamination, as this may be a likely source of resistance to common antibiotics. Further studies should also be conducted to assess the quantity of growth promoter additives added to the feeds.

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