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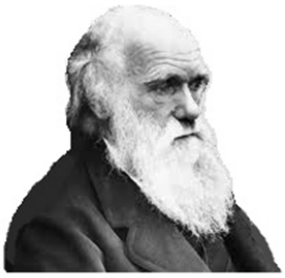

World News of Natural Sciences

An International Scientific Journal

WNOFNS 59 (2025) 430-440 EISSN 2543-5426

Antibiogram of Bacteria Associated with Pig Feeds Sold in Ihitte/Uboma, Imo State, Nigeria

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ABSTRACT

This study investigated antimicrobial resistance in bacteria associated with pig feeds sold in Ihitte/Uboma, focusing on their antibiotic susceptibility profiles. Total of five feed samples were collected and analyzed using standard microbiological methods. Total heterotrophic bacterial counts ranged from 4.0×10^4 to 1.6×10^5, while total coliform counts ranged from 4.0×10^4 to 1.0×10^5. Identified bacterial isolates included *Escherichia coli*, *Pseudomonas* spp., *Staphylococcus aureus*, and *Klebsiella* spp., with varying frequencies. Antibiotic susceptibility testing revealed resistance patterns, notably with *E. coli* resistant to nitrofurantoin and gentamicin, and *S. aureus* resistant to gentamicin and ofloxacin. In contrast, *Klebsiella* spp. were sensitive to all tested antibiotics. These findings underscore the growing issue of multidrug-resistant bacteria in the food chain, driven by antibiotic misuse. In conclusion, the study recommends stricter regulations on antibiotic use, promotion of alternative growth-enhancers like probiotics, and routine surveillance of antimicrobial resistance trends to safeguard food and public health.

Keywords: Pig feeds, microbiological, techniques, isolates, antibiotics, antibiogram

(Received 17 January 2025; Accepted 14 February 2025; Date of Publication 15 February 2025)

1. INTRODUCTION

Demand for animal protein is increasing globally (FAO's 2020; Henchion *et al.,* 2014). The global expansion of intensive farming has led to an increase in antimicrobial use (Van Boeckel *et al.,* 2015) that contributes to the emergence and spread of antimicrobial resistance (Page *et al.,* 2012). The World Health Organization defines AMR as "when bacteria, viruses, fungi, and parasites change over time and no longer respond to medicines, making infections harder to treat, and increasing the risk of disease spread, severe illness, and death". Antimicrobials are an essential component of intensive farming systems and are used to treat and prevent infections, and can also be used in animal feed to increase growth (Lhermie *et al.,* 2018; Prescott 2008).

The pig farming industry in Nigeria, particularly in Ihitte/Uboma, plays an important role in the agricultural economy by providing a vital source of protein. The success of pig farming heavily depends on the quality and safety of feed, which is typically formulated using grains and nutritional supplements. In regions like Ihitte/Uboma, Imo State, pig farming is practiced by small and medium scale farmers. These farms are essential for local food security, generating income, and creating employment. However, many small-scale farmers rely on locally available or poorly regulated feeds, which are often prone to be contaminated by harmful bacteria and toxins. These feeds getting contaminated can further push these pathogens in the food cycle which will become detrimental to human health. Hence, this highlights the need for research on the safety and quality of pig feed in the region.

Feeds in general has been implicated to be a major source for transmission of bacteria and other microorganisms in the agro-based industry. Most animals harbor pathogens which are of food origin which serves as a good source of contamination, which is of significance in the spread of *Escherichia coli* and *Salmonella* species in humans. These bacteria can survive for prolonged periods of time without multiplication on materials with low moisture contents therefore providing the possibilities of the bacteria to be mechanically transmitted from one site to another through fomites, including contaminated feeds. (Mohammed *et al.,* 2021).

Researchers have demonstrated that pigs are *Salmonella* reservoir. Animals can become infected with pathogens through contaminated feed, water, and aerosols and through contact with infected animals. When animals consume contaminated feed or water or have contact with the pathogen indirectly, they can harbor bacteria without manifesting clinical signs (Harrison *et al.,* 2022). The presence of such pathogens in feed not only threatens animal health but also raises public health concerns due to the potential transmission of zoonotic diseases. The prevention of infectious diseases in pigs is important for both animal welfare and economic productivity. Moreover, prevention is also important for food safety and public health when zoonotic pathogens are concerned (Alarcón *et al.,* 2021).

Infections with multidrug-resistant bacteria are hard to treat since fewer or no treatment options are available. Consumption of agro-based product of pig origin contaminated with microorganism showing resistance to antimicrobials, probably as a result of ingestion of contaminated feed might be disastrous to humans as there are chances of being infected with these bacteria with multidrug-resistance. Upon infection, these could actually cause problems for the patient resulting in increased duration of illness and prolonged hospital stay, excess costs, number of complications, and deaths (Aika *et al.,* 2022). Therefore, there is need for proper antibiogram study of these pathogens. Antibiogram studies help identify effective treatments for infections and reveal resistance trends and the strategies to mitigate this trend.

In Ihitte/Uboma, where pig farming is a prominent agricultural activity, there is need to assess the antibiotic resistance patterns of bacteria associated with pig feed. Investigating the specific antibiograms of bacteria in pig feed from Ihitte/Uboma is essential for enlightening local farmers on the awareness of these microbes, reducing the prevalence of resistant bacteria in the food chain, and safeguarding both animal and human health.

This study aims to evaluate the bacterial contamination and antibiotic resistance profiles of pig feed sold in Ihitte/Uboma, Imo State. Specific objectives include identifying bacterial species present in pig feed, determining their antibiotic resistance patterns, and assessing the public health risks associated with contaminated feed. By addressing these objectives, this research seeks to provide data that can inform strategies to enhance feed safety, promote responsible antibiotic use, and mitigate the spread of antibiotic-resistant bacteria.

2. MATERIALS AND METHODS

Source of Sample Collection

Five samples of pig feeds were purchased from five different vendors at Isinweke Market in Ihitte/Uboma, Nigeria.

Materials - The materials used include: petri dishes, sterile glass slides, bent glass rod, forceps, pipette, Conical flasks, beakers, inoculating needles and loops, test tubes, test tube rack, meter rules, Agar media, Autoclave, Hot air oven, Bijou bottles, Nose mask, hand gloves, test tubes cover, spatula and Bunsen burner.

Reagents - Distilled water, Nutrient Agar, Methyl red, Kovac Reagent, Crystal violet, Safranine

Collection of Samples: Samples were collected in sterile polythene bags and were sent to the lab for microbiological analysis within 30minutes after collection

Media - The media used included: Nutrient Agar, MacConkey Agar, Muller Hinton Agar, Simmon's Citrate Agar, Peptone Water.

Media Preparations: The method of Ohazuruike *et al.,* 2017 as used by Ohabughiro *et al.,* 2024 was adopted. The solid components of the media were dissolved in a conical flask according to the manufacturer's instructions, the flask was closed with cotton plug and covered with Aluminum foil, placed into an autoclave and sterilized at 121 °C for 15 mins. After sterilization, the media were cooled to 45 °C, the cotton plug was removed and the mouth of the flask was flamed over a Bunsen burner in other to ensure sterility, and the media were poured into sterile, empty petri dishes (15-20 ml into each petri dish). The petri dishes were kept horizontally until the media were completely solidified, then they were turned upside down and stacked for storage. The plates were labeled according to the media and also a sterility test was performed on them by incubating some plates at 37 °C for 24hrs and after which they were examined.

Sterilization: All glass wares used were sterilized after washing with detergent using the hot air oven at 160 °C for 2hours. All the media were sterilized using autoclave as previously described. Wire loops were sterilized by flaming to red hot using Bunsen burner and all laboratory benches were cleaned before and after work with 75% alcohol. Bunsen burner was lit during the work to keep the environment sterile.

Microbiological Analysis of Samples

1g of each feed sample was homogenized with 9 ml of distilled water to make a 10^{-1} dilution. Subsequent dilutions (10^{-2}, 10^{-3}, and 10^{-4}) were prepared using standard microbiological techniques. Inoculation was done on the media using the spread plate method of inoculation.

Isolation and Sub-Culturing: Bacterial isolates were sub-cultured on fresh nutrient agar plates to obtain pure cultures.

Identification of Isolates

After purification, the isolates were maintained using nutrient agar slant. The bacterial isolates were identified using colonial morphology, Gram Staining, Motility test and biochemical properties. Biochemical test includes Oxidase test, Citrate utilization test, Indole test, Voges-Proskauer test, Methyl-Red test, Coagulase test, Sugar fermentation test and Catalase test.

Antibiotics Susceptibility Testing

The method of Ohazuruike *et al.,* 2017 as used by Ohabughiro *et al.,* 2024 was adopted. After Isolation and Identification, antimicrobial susceptibility test was carried out using the disk diffusion method on Muller Hinton agar medium. The following antibiotics were employed for sensitivity analysis; Ceftazidime, Ceftriaxone, Gentamicin, Ceftriaxone, Erythromycin, Cloxacillin, Ofloxacin, Augmentin, Nitrofurantoin, Ciprofloxacin, Cefuroxime. The growth was standardized by diluting the culture with normal Saline to match the turbidity of 1.0×10^6 cfu/ml (0.5 McFarland standards). The 0.1 ml was collected and spread on the surface of Muller Hinton agar (Oxoid ltd Basingstone, Uk) using sterile glass rod. The antibiotic disc was placed carefully to make good contact with the agar surface using sterile forceps and sufficiently separated from each other in order to prevent overlapping of the zones of inhibition. The agar plates were left on the bench for 30 mins to allow for diffusion of the antibiotics and incubated at 37 °C for 24 hrs and results were interpreted as sensitive and resistant. Isolates resistant to at least three antibiotics were selected as multi-drugs resistance (MDR). Results were be recorded by measuring the zone of inhibition and comparing with the CLSI susceptibility.

3. RESULTS

Bacterial Loads of Samples

Table 1. The bacterial load for each sample is presented in colony-forming units per gram (Cfu/g).

SAMPLES	THPC (Cfu/g)	TCC (Cfu/g)
A	1.6×10^4	1.0×10^5
B	4.0×10^3	4.0×10^4
C	4.0×10^3	7.2×10^4
D	6.0×10^3	9.6×10^4
E	8.0×10^5	4.0×10^3

Key: **THPC** = Total Heterotrophic Plate Count; **TCC** = Total Coliform Count

Colonial and Morphological Characteristics of Bacterial Isolates

Table 2. The colonial and morphological characteristics of bacterial isolates from the samples.

Sample	Colour	Shape	Surface	Arrangement	Probable organism
1	Yellow	Round	Glassy	Cocci in clusters	*Staphylococcus aureus*
2	Pink	Raised growth	Smooth and dry	Rods in singles	*Eschericia coli*
3	Pink	Raised growth	Slimy	Short Rods	*Klebsiella* spp
4	Greenish	Irregular	Smooth	Rods	*Pseudomonas* spp

Identification of Bacterial Isolates

Table 3. Biochemical tests and the probable organism based on the tests.

Isolates	Bacteriological tests			Biochemical tests										Probable organism
	Gram reaction test	Cellular arrangement	Motility test	Catalase test	Citrate test	Indole test	Oxidase test	Coagulase test	Voges Proskuer test	Methyl red test	Glucose test	Lactose test	Sucrose test	
1	-	Rods	+	+	+	-	+	-	-	-	-	-	-	*Pseudomonas* spp.
2	-	Rod	+	+	-	+	-	-	-	+	+	+	+	*Escherichia coli*
3	+	cocci	-	+		-	-	+	+	+	+	+	+	*Staphylococcus aureus*
4	-	Rod	+	+	+	-	-	-	+	-	+	+	+	*Klebsiella* spp.

Key: **+** = Positive
j- = Negative

Frequency of Occurrence of Bacterial Isolates

Table 4. Frequency and percentage occurrence of bacterial isolates.

ISOLATES	FREQUENCY	PERCENTAGE (%)
Staphylococcus aureus	4	19
Pseudomonas spp	5	24
Eschericia coli	7	33
Klebsiella spp	5	24
Total	21	100

Antibiogram for Gram Negative Organisms

Table 5. Antibiotic susceptibility test for Gram-negative organisms.

Antibiotics	*Escherichia coli* (mm)	*Pseudomonas* spp. (mm)	*Klebsiella* (mm)
CAZ	12.0	10.0	12.0
CRX	10.0	16.5	11.5
GEN	R	17.1	9.3
CXM	9.0	13.0	5.0
OFL	14.6	12.3	9.9
AUG	13.0	15.0	10.7
NIT	R	15.2	15.9
CPR	10.0	R	17.0

Key: R = Resistance (No zone clearance)

Antibiogram Test for Gram Positive Bacteria

Table 6. Antibiotic susceptibility test for Gram-positive organisms.

Antibiotics	***Staphylococcus aureus* (mm)**
CAZ	6.0
CRX	9.0
GEN	R
CTR	10.5
ERY	11.5
CXC	8.0
OFL	R
AUG	15.0

Key: R = Resistance (No zone clearance)
CAZ = CEFTAZIDIME, CRX = CEFTRIAXONE, GEN = GENTAMICIN,
CTR = CEFTRIAXONE, ERY = ERYTHROMYCIN, CXC = CLOXACILLIN,
OFL = OFLOXACIN, AUG = AUGMENTIN, NIT = NITROFURANTOIN,
CPR = CIPROFLOXACIN, CXM = CEFUROXIME

4. DISCUSSION

The antibiogram of bacteria associated with pig feeds sold in Ihitte/Uboma revealed significant insights into the prevalence and resistance patterns of various bacterial species. The identified bacterial isolates included *Escherichia coli, Staphylococcus aureus, Klebsiella spp.*, and *Pseudomonas* spp. The antibiogram results demonstrated resistance in *E. coli* to nitrofurantoin and gentamycin, while *Pseudomonas* spp. exhibited resistance to ciprofloxacin. In contrast, *Klebsiella* spp. showed susceptibility to all tested antibiotics, and *Staphylococcus aureus* was resistant to gentamycin and ofloxacin. These findings emphasize the challenge of antimicrobial resistance in pig feed contamination. Antimicrobial resistance is defined according to Amagliani *et al.,* (2012) as the ability of microorganisms to adapt and survive antimicrobials.

The findings align with those from similar studies. For instance, animal feeds have been identified as a potential source of microorganisms in farmed animals (Uwaezuoke *et al.*, 2008). Uwaezuoke *et al.*, (2008) discovered the presence of four bacteria (*Salmonella* spp., *Shigella* spp., *E. coli*, and *Proteus mirabilis*) in both feed and droppings samples, suggesting possible threat to the well-being of the animals. The detection of these bacterial species, particularly those with implications for public health, raises significant concerns regarding the potential

direct consumption of feed contaminated with these bacteria or their associated toxins by livestock (Umar *et al.*, 2023).

A study from Colombia reported the prevalence and antimicrobial resistance pattern of *E. coli* and Salmonella spp. in animal feed samples. It was discovered that 4.7% samples were positive for *E. coli* and 3.8% for *Salmonella* spp. Among *E. coli* isolates, resistance was observed for beta-lactam drugs and fluoroquinolones (Paredes *et al.*, 2023). Similarly, research in Rajshahi, Bangladesh, reported that *E. coli* showed resistance to bacitracin and sulphamethoxazole, Enterococcus sp. was highly resistant only to sulphamethoxazole while *Salmonella* sp. showed high to moderate resistance to Ampicillin, gentamicin and sulphamethoxazole (Mohanta *et al.*, 2016). Also, according to Ajayi *et al.*, (2023), a closer observation of the overall antibiotic susceptibility shows that resistance to ampicillin was highest 44 (77.2%), followed by resistance to ertapenem 37 (64.9%), tetracycline 36 (63.2%) and meropenem 32 (56.1%). The organisms showed the highest susceptibility to gentamicin 41 (71.9%) and norfloxacin 36(63.2%) which is almost the same with the experiment carried out.

Furthermore, research in Ruiru Sub-County, Kenya, illustrates Salmonella sp. and *Escherichia coli* which were isolated from poultry feeds. Out of analyzed samples, 58% and 28% contained *Escherichia coli* and Salmonella sp. respectively. Bacterial load ranged between 3.1×10^5 and 3.0×10^6 cfu/g. Highest resistance was against ampicillin (41%) for Salmonella sp. and (62%) for *E. coli* isolates. Ampicillin resistant isolates carried TEM and SHV genes. In addition, strB and Dfr resistance genes associated with streptomycin and cotri-moxazole were detected. All the isolates were susceptible to chloramphenicol and ciprofloxacin. The study reveals high bacterial contamination, presence of beta-lactamase, aminoglycoside and sulphoneamide resistance genes across isolates from poultry feeds (Ngai *et al.*, 2021).

Acquisition of resistance to antibiotics by bacteria is one of the most important problems of modern medicine. A particularly disturbing phenomenon is the prevalence of very high percentage, in many cases even 100% of multi-drug-resistant foodborne pathogens in developing countries, mainly in Africa and Asia (Urban-Chmiel *et al.*, 2022). In Nigeria, the pig sector of the livestock industry remains underdeveloped compared to the poultry or cattle sectors. The abuse of antimicrobials by pig farmers occurs as an alternative disease preventive measure, oftentimes without veterinary consultation and prescriptions. Such indiscriminate use of antimicrobials in livestock portends a threat to human health globally because most of the antimicrobials involved are also used for controlling human infections (Adebowale *et al.*, 2020).

The findings from Stanley *et al.*, (2022) underscores the importance of addressing antimicrobial resistance in pig feeds. Resistant bacteria in animal feeds can act as reservoirs for MDR genes, increasing the risk of transmission to humans through direct contact, consumption of contaminated meat, or environmental exposure. The potential zoonotic implications highlight the need for stringent measures to mitigate the spread of antimicrobial resistance and ensure food safety. Actions taken by several European governments to provide transparency on the use of antibiotics that allows the consumer to select more sustainable product are exceptional.

However, these actions are strongly variable on the global scale, and ignoring the antimicrobial resistance issue in any part of the world may impact the global dissemination of antimicrobial resistance. In addition, antibiotics have been used routinely in farm animal production since the 1950s, in particular during intensive farming, in order to keep animals healthy and to increase productivity.

The use of antibiotics in animals has raised concerns that the selective pressure on the bacteria population promotes antibiotic resistance (Lekagul et al., 2019).

Lastly, the climatic weather in Nigeria is warm and humid and Salmonella organisms can, under these circumstances, multiply in the feed especially during farm storage and administration. Contamination may also occur during processing, transport and distribution of compounded feed mixture as most farmers, in a bid to save cost, would either compound their own feed or purchase from a local un-hygienic feed mill (Fagbamila et al., 2017). It is quite difficult at times to maintain the freshness of the feed, where high temperature and oxidation destroy certain vitamins. Feed spoilage is caused by the growth of undesirable molds and bacteria (Onajobi et al., 2023). It is well known that the content of bacteria affects the quality of feed and consequently animal health and production (Zadravec et al., 2023). Producing a pathogen-free feed may be difficult because the pathogens are invisible to the unaided eye, and they can be transferred through the feed mill to the potential animal feeds. At the feed mill, improved microbial control can be accomplished by following good manufacturing practices, employee training, cleaning/sanitation and quality assurance (Mgbeahuruike et al., 2023).

5. CONCLUSION

This study highlights the alarming antimicrobial resistance patterns among bacterial species isolated from pig feeds sold in Ihitte/Uboma, including *Escherichia coli*, *Pseudomonas* spp., *Staphylococcus aureus*, and *Klebsiella* spp. These findings contribute to the global body of evidence linking animal feed contamination to the emergence and spread of resistant pathogens, reinforcing the need for urgent intervention to curb the spread of resistance and ensure food safety.

Hence, strict regulations on the use of antibiotics in pig farming should be enforced to limit the overuse and misuse of these drugs, which contribute to the development of resistant strains. Also improved surveillance systems should be established to regularly monitor resistance patterns in both animal feeds and farm environments. Furthermore, farmers, veterinarians, and other stakeholders should be educated about the risks of antimicrobial resistance and the importance of responsible antibiotic use in agriculture.

References

[1] Adebowale, O., Adeyemo, F., Bankole, N. O., Olasoju, M., Adesokan, H., Fasanmi, O., Adeyemo, O., Awoyomi, O. J., Kehinde, O. O. & Fasina, F. O. (2020). Farmers' Perceptions and Drivers of Antimicrobial Use and Abuse in Commercial Pig Production, Ogun State, Nigeria. *International Journal of Environmental Research and Public Health*, 17 (10): 3579. doi:10.3390/ijerph17103579

[2] Aika, I. N. & Enato, E. (2022). Antibiogram of clinical isolates from primary and secondary healthcare facilities: A step towards antimicrobial stewardship. *PLOS Glob Public Health* 2 (12): e0000644. https://doi.org/10.1371/journal. pgph.0000644

[3] Ajayi, A. O., Samuel-Osamoka, F. C., Adejoh, O. P. & Popoola, O. A. (2023). Antibiotic Resistance among Bacteria isolated from Pigs in Selected farms in Ekiti State, Nigeria. *Dutse Journal of Pure and Applied Sciences* 9 (4b): 337-346

[4] Alarcón, L.V., Allepuz, A. & Mateu, E. (2021). Biosecurity in pig farms: a review. *Porcine Health Management*, 7 (5): 2055-5660. https://doi.org/10.1186/s40813-020-00181-z

[5] Amagliani, G., Brandi, G. & Schiavano, G. F. (2012). Incidence and role of Salmonella in seafood safety. *Food Research International*, 45 (2): 780-788. https://doi.org/10.1016/j.foodres.2011.06.022

[6] Fagbamila, I. O., Barco, L., Mancin, M., Kwaga, J., Ngulukun, S. S., Zavagnin, P., Lettini, A. A., Lorenzetto, M., Abdu, P. A., Kabir, J., Umoh, J., Ricci, A. & Muhammad, M. (2017). Salmonella serovars and their distribution in Nigerian commercial chicken layer farms. *PloS ONE* 12 (3): e0173097. https://doi.org/10.1371/journal.pone.0173097

[7] FAO's Animal Production and Health Division: Meat & Meat Products. [(accessed on 15 September 2020)]; Available online: http://www.fao.org/ag/againfo/themes/en/meat/backgr_sources.html

[8] Harrison, O. L., Gebhardt, J. T., Paulk, C. B., Plattner, B. L., Woodworth, J. C., Rensing, S., Jones, C. K. & Trinetta, V. (2022). Inoculation of Weaned Pigs by Feed, Water, and Airborne Transmission of Salmonella enterica Serotype 4,[5],12:i:-. *Journal of Food Protection*, 85 (4): 693-700. https://doi.org/10.4315/JFP-21-418

[9] Henchion, M., McCarthy, M., Resconi, V.C. & Troy, D. (2014). Meat consumption: Trends and quality matters. Meat Sci. 98: 561-568. doi: 10.1016/j.meatsci.2014.06.007

[10] Lekagul, A., Tangcharoensathien, V. & Yeung, S. (2019). Patterns of antibiotic use in global pig production: A systematic review. *Veterinary and Animal Science*, 7, 100058. https://doi.org/10.1016/j.vas.2019.100058

[11] Lhermie G, Tauer LW, Gröhn YT (2018) The farm cost of decreasing antimicrobial use in dairy production. *PLoS ONE* 13(3): e0194832. https://doi.org/10.1371/journal.pone.0194832

[12] Mgbeahuruike, A. C., Agina, O. A., Anyanwu, M. U., Emejuo, N. T., Ekere, S. O., Ugwu, P. C., Uju, C. N. & Andong, F. A. (2023). Microbial Contamination of Poultry Feed and the Effects on Birds' Performance. *Animal Research International*, 20 (1): 4834-4861

[13] Mohammed, S.S.D., Al-Hassan, S., Wartu, J.R. & Abdul, R. (2021). Occurrence of Escherichia coli and Salmonella species in Some Livestock (Poultry) Feeds in Mando, Kaduna, Nigeria. *Journal of Pure and Applied Microbiology*, 15 (2): 1016-1025

[14] Mohanta, M., Islam, M. S., Saha, A., Khatun, S. & Khatun, Z. (2016). Antibiogram Profiles of Bacterial Isolates from Poultry Feeds and Moribund Hens in Rajshahi, Bangladesh. *Agricultural and Food SciencesImperial Journal of Interdisciplinary*, 2 (5): 41-46

[15] Ngai, D.G., Nyamache, A.K. & Ombori, O. (2021). Prevalence and antimicrobial resistance profiles of Salmonella species and Escherichia coli isolates from poultry feeds in Ruiru Sub-County, Kenya. *BMC Research Notes*, 14 (41): 1-6. DOI:10.1186/s13104-021-05456-4

[16] Ohabughiro, N. B. (2023). Antifungal activities of moulds associated with selected stored grains and legumes. *World News of Natural Sciences* 50: 52-59

[17] Ohazuruike, N. C., Ohalete, C. N., Njoku-Obi, T. N., Uwaezuoke, J. C., Obiukwu, C. E., … & Nwachukwu M. I. (2017). *Seminar Presentation and Research Practicals*. Henco Printing Press, Owerri Imo Nigeria, Pp 175-250.

[18] Onajobi, I. B., Adebanjo, O. L., Samson, O. J., Abiona, T. O., Ogunmoye, A. O., Makanjuola, S. O. &Adebajo, L. O. (2023). Microorganisms Associated with Poultry Feeds in South West, Nigeria. *Journal of Poultry Research*, 20 (1): 1-12. https://doi.org/10.34233/jpr.1302416

[19] Page, S.W.& Gautier, P. (2012). Use of antimicrobial agents in livestock. *Rev. Sci. Tech. Int. Off. Epizoot*, 31: 145-188. doi: 10.20506/rst.31.1.2106

[20] Paredes R, Damme M, Mantilla J, Castellanos LR, Clavijo V, Celis Y, et al. Prevalence and antimicrobial resistance of Escherichia coli and Salmonella spp. in animal feed in Colombia. *Rev Panam Salud Publica.* 2023; 47: e57. https://doi.org/10.26633/RPSP.2023.57

[21] Prescott, J.F. (2008). Antimicrobial use in food and companion animals. *Anim. Health Res. Rev.* 9: 127-133. doi: 10.1017/S1466252308001473

[22] Stanley, D., Batacan, R. Jr. & Bajagai, Y. S. (2022). Rapid growth of antimicrobial resistance: the role of agriculture in the problem and the solutions. *Applied Microbiology and Biotechnology*, 106 (21), 6953-6962

[23] Umar, M. M., Onuoha C. C., Udofia, E. V., Ojo, O. H., Asibe, G. A., Adekplorvi, G., Sani, A. H., Ahmad, B. M., Surajo, A. A., Wankan, B. A., Jasseh, M., Mbahi, M. A., Akerele, Y. E. &Godwin, F. (2023). Prevalence and Antimicrobial Susceptibility of Enteric Bacteria from Poultry Farms in Kano State, Nigeria. *UMYU Journal of Microbiology Research* 8 (2): 92-98

[24] Urban-Chmiel, R., Marek, A., Stępień-Pyśniak, D., Wieczorek, K., Dec, M., Nowaczek, A. & Osek, J. (2022). Antibiotic Resistance in Bacteria-A Review. *Antibiotics (Basel, Switzerland)*, 11 (8): 1079. https://doi.org/10.3390/antibiotics11081079

[25] Uwaezuoke, J. C. and Ogbulie, J. N. (2008). Microbiological quality of commercially available poultry feeds sold in parts of Eastern Nigeria. *Journal of Applied Sciences and Environmental Management*, 12 (1): 113-117. doi:10.4314/jasem.v12i1.55587

[26] Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., Teillant, A.& Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proc. Natl. Acad. Sci. USA.* 112: 5649. doi: 10.1073/pnas.1503141112

[27] Zadravec, M., Mikuš, T., Pradervand, N. & Vrhovnik, I. (2023). Microbiological Quality of Feed. *The Open Agriculture Journal*, 17: 1-8. http://dx.doi.org/10.2174/18743315-v17-e230418-2022-66