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Isolation of Enteric Bacteria from Various Sources in Selected Poultry Farms in Kaduna State

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Abstract: This study was designed to isolate enteric bacteria from various sources in selected poultry farms in Kaduna state. One hundred and fifty samples of poultry feed, water and droppings from five poultry farms in Kafanchan, Zaria, Gonin-gora, Kamazou and Ungwan Television were examined for the presence of enteric bacteria. 30 samples were collected from each farm and were analysed using spread plate method. The culture media used were Selenite Feaces (SF) broth) and Bismuth sulphite agar. The contaminants isolated include Escherichia coli, Salmonella spp and Proteus mirabilis. The distribution of the isolates based on sample type were droppings (1.33%) and feed (2.67%). There was no significant difference in each of the samples ($p>0.05$). The percentage distribution of isolates based on location were: Kafanchan (0.00%), Zaria (0.00%), Kamazou (33.33%) for Salmonella and (16.67%) for Proteus mirabilis, Gonin-gora (50%), and Television (0.00%). The results showed that the poultry feeds and droppings from the poultry farms visited in Kaduna, Nigeria had bacteria contaminants. The presence of these bacteria may be a serious health concern as these organisms are involved in causing various diseases. Therefore, hygienic measures should be taken in processing and handling of the poultry products being sold to general public. The national and local health authorities should enforce the food hygiene regulations to reduce the spread of diseases caused by these enteric bacteria. Public enlightenment programmes on the modes of transmission of Salmonella, E. coli and P. mirabilis should be conducted by Human and Veterinary Public Health services. Further studies should be conducted to know the extent of distribution of these organisms in different areas of Kaduna State and the country at large.

Keywords: Poultry, Feed, Water, Droppings, Enteric Bacteria, Escherichia Coli, Salmonella Spp, Proteus Mirabilis

1. Introduction

The term 'poultry' used in agriculture generally refers to all domesticated birds kept for egg laying, meat production or their feathers. Poultry comes from the French word poul, which was derived from Latin word pullus meaning small animals. Poultry farms (PFs) have appeared successful and wide spread business-industry in Nigeria, which often remains contaminated with various hazardous microorganisms when standard hygiene practices are compromised. Poultry remains the largest domestic animal stock in the world in terms of the number of animals. In Nigeria, poultry droppings are extensively used as manure for crop cultivation. The application of poultry droppings to land provides nutrients for the crop's growth as well as organic matter for soil conditioning but this can pose danger to public health especially when the crops are eaten raw [1].

The industry has expanded extensively in commercial levels as well as in household traditional levels in Nigeria. More than three million people are employed directly in poultry sector, which provides the largest supply of meat and eggs [2], so as to meet up the major protein sources for entire population of the country. Poultry is a fast growing source of meat in the world today, representing a quarter of all the meat produced [3]. Meat and poultry products are some of the sources for transmitting food borne pathogens to humans with 40% of the clinical cases attributed to the consumption of egg and other poultry products [4-6].

The incidence of food borne diseases in humans has

increased considerably worldwide in the last few years. Poultry products have been repeatedly implicated in food borne infections. Poultry can harbour different food borne pathogens. Many reports in recent years have shown that Salmonella and Campylobacter spp are the most common causes of human food borne bacterial diseases linked to poultry [7]. Food borne infections and intoxications have been estimated to cause about one billion cases of acute diarrhoea annually in children under the age of 5 Years in Africa, Asia, Latin America and other developing countries. [8, 9].

Poultry feeds are infected during processing, by handling, mixing of ingredients and exposing the raw materials and finished products to the atmospheric microorganisms. Therefore, high rate of poultry disease and death occur as a result of consumption of contaminated feeds.

The most common vehicles for transmission of food-borne salmonellosis are meat, meat products, eggs, and egg products that are contaminated as a direct result of animal infection or faecal contamination during processing [6]. The problem of non-typhoidal Salmonella in Africa is very serious, but has generally been overshadowed by the 'big three': malaria, human immunodeficiency virus (HIV) and tuberculosis [10]. Prominent bacterial species in the poultry farms include Escherichia, Enterococcus, Proteus, Clostridium, Salmonella, Providencia and Lactobacillus that have been shown to be of critical importance in tropical countries [11] and elsewhere in the world [12].

2. Materials and Methods

2.1. Materials

Autoclave, Incubator, weighing balance, Bunsen burner, Wire loop, Hot air Oven, Cotton wool, Spatula, Aluminium foil, Microscope, Beakers, Conical flask, Slides, Cover slip, Petri dishes, Reagent bottles, Test tube and Calibrated cylinder.

2.2. Media and Reagents

Methyl red, Kovac' reagent, Potassium hydroxide (KOH), Hydrogen peroxide (H_2O_2), Oxidases reagent and Peptone water. Selenite Feaces (SF) broth) and Bismuth sulphite agar

2.3. Sterilization of Materials

The glassware and the wire loops were properly washed, air dried, wrapped with Aluminium foil paper and sterilized in hot air oven at 180°C for 2 hours.

2.4. Collection of Samples

Thirty (30) samples (10 samples of feed, droppings and water) each were collected from each of the five (5) poultry farms. All samples were collected under aseptic conditions. Sterile sampling materials and disposable gloves were used. Various samples were collected in sterile polythene bags and immediately transported to the Laboratory of the Department of Microbiology, Kaduna State University, Kaduna for laboratory analyses.

2.5. Preparation of Media Used

The media were aseptically prepared as when necessary according to the manufacturer's instructions on the labels of the media and autoclaved at 121°C for 15 minutes.

2.6. Isolation of Bacteria from Poultry Feed and Droppings

Five grams of poultry droppings and feed and 5mls of water were pre-enriched in 45mls of a selective enrichment broth (selenite Feaces (SF) broth), incubated at 37°C for 24 hours and sub cultured unto plates of Bismuth sulphite agar. The cultured plates were incubated at 37°C for 24 hours. The bacterial isolates were then identified following standard microbiological procedures based on morphology and biochemical characteristics as described by [13].

2.7. Biochemical Tests

2.7.1. Indole Test

Five ml of peptone water was incubated for 24 hours at 37°C in test tubes. The isolates were grown into the peptone water and allowed to stay for 24 hours. After, 5 drops of Kovac's reagent were added separately on each test tube and swirled gently for 5 minutes. Positive reactions were indicated by the development of a red colour in the reagent layer above while in the negative reaction (result) the indole reagent retained its yellow colour.

2.7.2. Methyl Red Test

The isolates were grown in 5 ml of MR both (glucose – phosphate peptone water) and incubated for 24 hours at 37°C. Thereafter, 3 drops of methyl red were added into each test tube. A reddish colour on the addition of indicator signified a positive result while a yellowish colour denoted negative result.

2.7.3. Voges – Proskauer's Test

Isolates were grown in 5 ml of Peptone water and glucose, respectively. This was incubated for 24 hours at 37°C then 5 drops of potassium hydroxide (KOH) was added. The tubes were shaken at intervals to ensure maximum aeration after 5 minutes. The development of red colour within 30 s and 60 s indicated a Voges-Proskauer positive test. But no red colour was seen which showed a VP negative result.

2.7.4. Oxidase Test

A piece of filter paper was wetted with a few drops of 1% oxidase reagent solution. A bit of the isolate was obtained with a sterile wire loop and smeared on the wetted portion of the filter paper. The development of an intense purple colour within 30 seconds indicated a negative test.

2.7.5. Triple Sugar Iron Agar Test (TSI)

In this test, 10 ml of peptone water was introduced into each of 3 sterile test tubes. 1 g of carbohydrate such as glucose, lactose and sucrose were added into each of the test tubes, respectively and labelled accordingly. They were stirred to dissolve completely over a Bunsen burner. After

which 3 drops of methyl red were added into each of the test-tubes which served as an indicator and a base medium. The tubes were then plugged with cotton wool and sterilized at 115°C for 15 minutes. They were then incubated at 37°C for 24 hours. A change in coloration of the medium after 24 hours from purple to yellow indicated acid production due to the fermentation of the sugar by the organism while retention of the purple colour indicated a negative reaction. Gas production was shown by the present of gas bubbles on the surface of the medium and the result was noted and recorded.

3. Results

In this study, Salmonella, E. coli and P. mirabilis were identified in the different feed and dropping samples. The bacteria were identified by determining the colony morphology in the media used. The distribution of the isolates according to sample type is shown in Table 1. The occurrence of the organisms in poultry feed samples was 2.67% while that of the dropping was 1.33%. However higher occurrences of 8.3% was observed in Ado Ekiti [14]. This might be due to different environmental condition, managemental condition, and mixed infection with other microbes.

Biochemical characteristics of bacteria isolated from feed and droppings of poultry farms are shown in Table 2. The bacteria include the following: Salmonella, E. coli and P. mirabilis. All the isolates fermented the sugars (glucose, lactose and sucrose) producing acid and gas.

Table 1. *Distribution of Isolates According to Sample Types.*

Sample Type	Number of Samples Collected	Number of Positive samples (%)	No of Isolates
Feed	50	4(2.67)	4
Water	50	0(0.00)	0
Droppings	50	2(1.33)	2
Total	150	6(4.00)	6

Table 2. *Biochemical characteristics of Bacteria Isolated from Feeds and Droppings of Poultry Farms.*

S	Isolate Code	TSI				Oxidase	Urease	Indole	MR	VP	Citrate	Probable Organism
		Slope	Butt	Gas	H_2S							
1	KS1	R	Y	+	+	–	+	+	+	–	+	Salmonella species
2	KS2	R	Y	+	+	–	+	–	+	+	+	Proteus mirabilis
3	KS3	R	Y	+	+	–	+	+	+	–	+	Salmonella species
4	GG4	R	Y	+	+	–	+	+	+	–	+	Salmonella species
5	GG5	Y	Y	+	+	–	+	+	+	–	–	Escherichia coli
6	GG6	R	Y	+	+	–	+	+	+	–	+	Salmonella species

Y =Yellow, R = Red, (+) = Positive, (–) = Negative, MR = Methyl red, VP = Voges Proskauer, KS = Kamazou, GG =Gonin-gora.

4. Discussion

Animal feeds have been listed as one of the sources of microbes of farmed animals and poultry [15]. This study revealed that three bacterial were isolated in the feed and dropping samples analysed, thus the bacterial recovered may indicate a potential hazard to the animals. The occurrence of bacterial species of public health concern may indicate obvious health hazard in terms of direct consumption of bacteriological contaminated feed or their toxins by farmed animal [16]. Animal feeds are rich source of nutrient for microbial growth especially when the environmental conditions are favourable. Monitoring of microbial contamination of animal production environment is an important first step in determining how such contaminants pass through the food chain. In a similar research carried out by Uwaezuoke and Ogbulie, (2008) the presence of Pseudomonas, Escherichia coli, Staphylococcus aureus and Salmonella was reported which is in line with what was observed in this research.

In Nigeria wastes from commercial poultry are not properly disposed and most rural farmers use these wastes as manure, which are often kept at the backyards before moving them to farms. These poultry wastes may serve as source of enteric organisms that habour novel factors for birds that feed on such wastes as reported by Okoli, (2006). The result revealed the presence of Salmonella species, E. coli and P. mirabilis from the samples analyzed (feed and droppings). Table 1 shows the distribution of isolates according to sample types, droppings (1.33%), and feed (2.67 %). There was no significant association ($p = 0.125$, $p > 0.05$) between sample types. Table 2 shows the biochemical characteristics of bacteria isolated from feed and droppings of poultry. The organisms were: E. coli 1 (16.67%), Salmonella 4 (66.66%) and Proteus mirabilis 1 (16.67%). All the organisms isolated from this study are important members of the coliform group. The overall member of enteric bacteria in this study is lower than that of Park et al (2010) [17] who recorded 38.6% of Proteus in poultry droppings in poultry farms in Bangladesh.

The presence of Escherichia coli, Proteus mirabilis and Salmonella species may suggest faecal as well as environmental contamination. Some of these organisms are well known pathogens of birds and farmed animal. E. coli for example was reportedly implicated in disease conditions such as colibacillosis which occurs in forms such as enteric and septicaemic colibacillosis whereas Salmonella is capable of producing acute and chronic infections in all or most types of birds and animals

The presence of Salmonella in the feed is also of public

health importance, this is because, in general the transmission of Salmonella spp through the environment has been shown to be cyclic, and poultry feeds had been reportedly viewed as important links for contamination in poultry [18] Although little is known about the relative significance of different sources of contamination of poultry feeds, it may depend partially upon the contamination levels of individual feed ingredients used in mixing the feed [19, 20]. It is important to state that one of the features observed during the study was that poultry feed and droppings shared Salmonella, E. coli and P. mirabilis. These organisms have been reported to have a host range and can infect both humans and animals. The finding in this study is lower compared to the report of Brenner et al (2010). They reported an incidence rate of 17% from different sample feeds in Nigeria. The lower rate in this study could be attributed to increased concentration of antibiotics in the feed, number of samples collected as well as proper storage of feed Supplies of contaminated feeds, the presence of rodents in the farms are some of the factors that could be responsible for the spread of Salmonella species in poultry farm [21].

However, in this study, there were no isolates from water. Musa et al., (2014), however, obtained a 9% prevalence from water samples in a study on isolation and antibiogram of Salmonella species from water and feed in selected poultry farms in Zaria. The result reported absence of enteric bacterial contamination from water samples, could be that the source of water to the farms visited are treated/purified.

5. Conclusion

This study shows that these enteric bacteria Proteus mirabilis, Salmonella and E. coli are the predominant enteric bacteria present in poultry feeds and droppings in the farms visited. Their presence is usually of economic and public health significance. They lead to great economic loss and hence, the need for concerted efforts to control them.

In the light of the potential risk associated with the type of organisms present in poultry feeds sold around Kaduna metropolis, the microbial contaminations of the feeds should be reduced to the barest minimal. Production and storage must be appropriate, bearing in mind the risks contaminations pose on the safety of the animals and public health. Chemical amendment, heat treatment, irradiation and careful sourcing of materials are proven methods of reducing bacterial loads in feed ingredient.

Conclusively, the present results provide evidence that poultry droppings can serve as an environmental reservoir of multiple enteric bacteria and hence as potential route for the entry of zoonotic pathogens into human population. This have very important implications for human health, as infections are difficult to treat and often requires expensive antibiotics and long term therapy. This can substantially increase the cost of treatment and even mortality. Therefore, how to take effective measures to prevent and control infectious diseases from chickens is the most important risk and it could also be said that poultry farms should be periodically checked for the presence of pathogens and biosecurity plan to the farms should be taken accordingly.

References

[1] Food and Agriculture Organization (2013). Climate Change: Implications for Food Safety. p: 13.

[2] Nandi, S.P., Sultana, M., Hossain, M.A. (2013). Prevalence and Characterization of Multidrug Resistant Zoonotic Enterobacter spp. in Poultry of Bangladesh. Foodborne Pathogens and Disease 10:420-427.

[3] Adeleke, E. O. and Omafuvbe B. O., (2011). Antibiotic Resistance of Aerobic Mesophilic Bacteria Isolated from Poultry Feaces. Research Journal of Microbiology, 6: 356-356.

[4] Behravesh, C.B., Brinson, D., Hopkins, B.A., Gomez, T.M. (2014). Backyard Poultry Flocks and Salmonellosis: A Recurring, yet Preventable Public Health Challenge. Clinical of Infectious Diseases, 58;1432-1438.

[5] Brenner, F. W., O'Hara, C. M., Miller, J. M. (2010). Classification, Identification, and Clinical Significance of Proteus, Providencia and Morganella. Clinical Microbiology Review 13: 534-546.

[6] Hossain, M.A., Islam, M.M., Islam, A.F., Iji, P.A. (2011). Constraints to use all-vegetable Feed Ingredients and Strategies to Improve such Diets for Poultry Birds: A review. Research Publication Journal. 6(1):120-125.

[7] Hafez, H.M. (2005). Governmental Regulations and Concept behind Eradication and Control of some Important Poultry Diseases. World's Poultry Science, 61: 569-582.

[8] Gillespie, B.E, Mathew, A.G, Draughon, F.A, Jayarao B. M., Oliver, S.P. (2012). Detection of Salmonella Enteric Somatic Groups. Journal of Food Protection; 66:2367-2370.

[9] Mulata, G., Beyene, G., Zeynudin, A. (2014). Prevalence of Shigella, Salmonella, and Campylobacter species and their Susceptibility Patterns among Under Five Children with Diarrhea. Ethiopian Journal of Health Science, 24; 101-108.

[10] Musa, I.W., Mansur, M.S., Saidu, L., Mohammed, B., Kaltungo, B.Y., Lawan, M.K. (2014). Isolation and Antibiogram of Salmonella Species from Waste and Poultry Feed in Selected Commercial Farms in Zaria, Nigeria. Times Journal of Agriculture and Veterinary Science 2(2):75-80.

[11] Chowdhuri, A., Iqbal, A., Giasuddin, M., Bhuiyan, A.A. (2011). Study on Isolation and Identification of Salmonella and Escherichia coli from different Poultry Feeds of Savar Region of Dhaka, Bangladesh. Journal of Scientific Research. 3(2):403-411.

[12] Mohanta, M.K., Islam, M.S., Saha, A.K., Khatun, S., Khatun, Z. (2016). Antibiogram Profiles of Bacterial Isolates from Poultry Feeds. Imperial Journal of Interdisciplinary Research 2(5):41-46.

[13] Chessbrough, M. (2006). District Laboratory Practice in Tropical Countries, Part 2. 1st Edition., Cambridge University Press, Cambridge ISBN-10: 113944929X, pp: 135-140, 188-189, 440.

[14] David, O. M. and Ogunlade, J. T. (2013) Qualities of Poultry Feeds Produced by Local Small-scale Feed Mills in Ekiti State, Nigeria: A Public Health and Feed Safety Study. Animal Vetenary. Science, 3(9), 297-302.

[15] Uwaezuoke, J. C. and Ogbulie, J. N. (2008) Microbiological Quality of Commercially Available Poultry Feeds sold in Parts of Eastern Nigeria. Journal of Applied Science and Environmental Management 12(1) 113 – 117.

[16] Okoli, I.C. (2006). Antimicrobial Resistance Profiles of E. coli Isolated from Free Range Chickens in Urban and Rural Environments of Imo State, Nigeria. Online Journal of Health and Allied Science. 5(1), 1-6.

[17] Park, S.D., Uh, Y., Lee, G., Lim, K., Kim, J.B. (2010). Prevalence and Resistance Patterns of Extended-spectrum and AmpC Beta-lactamase in Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, and Salmonella serovar Stanley in a Korean tertiary hospital. APMIS118:801-808.

[18] Maciorowski, K. G., Herrera, P., Kundinger, M. M. and Ricke, S. C. (2006). Animal Feed Production and Contamination by Foodborne Salmonella. Journal fur Verbraucherschutz und Lebensmittelsicherheit, 1: 197-209.

[19] Good Manufacturing Practices (GMP). (2008). GMP Certification Scheme Animal Feed. Sector 2006, Appendix 1: Product Standards; Regulations on Product Standards in the Animal Feed Sector. Good Manufacturing Practices, 14: 1-39.

[20] Maciorowski, K. G., Jones, T. F., Pillai, S. D. and Ricke, S. C. (2004) Incidence, Source and Control of Food-borne Salmonella spp. in Poultry Feeds. World's Poultry Journal 60(5): 446-457.

[21] Noohi, N., Ebrahimipour, G., Rohani, M., Talebi, M., Pourshafie, M.R. (2014). Phenotypic Characteristics and Probiotics Potential of Lactobacillus Spp. Isolated from Poultry. Journal of Microbiology 7(9): e17824.