Microbiological Analysis of Poultry Feeds Along with the Demonstration of the Antibiotic Susceptibility of the Isolates and the Antibacterial Activity of the Feeds

Chanda Rani Roy1, Tasnia Ahmed1, Md. Aftab uddin1*

1Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka 1217, Bangladesh.

Poultry farms and poultry product processing industries are increasing worldwide to fill up the expanding demand for protein of the escalating population. To get good growth and more eggs from the domestic birds in the poultry farms, nutritious food supplements are commonly known as the poultry feed provided. These feeds also serve as sources of bacterial, fungal and viral contamination which in turn can cause diseases in the birds and ultimately can infect the consumers if the poultry is not processed and cooked properly. In this study, in order to determine the pathogenic bacterial load five different poultry feed samples sold in local markets of Dhaka city were analyzed. All samples harbored total viable bacteria up to 5.0×10⁶ cfu/gm and total fungal count up to 4.5×10⁵ cfu/gm. While Escherichia coli was absent in all samples; Klebsiella spp. and Aeromonas spp. were found to be present in only one sample (1.4×10⁶ cfu/gm in sample 4 and 2.9×10⁵ cfu/gm in sample 5 respectively). Staphylococcus aureus and Shigella spp. were found to be prevalent in all the samples. Pseudomonas spp. and Vibrio spp. were present in 3 and 4 samples, respectively. Aeromonas spp., Vibrio spp., Salmonella spp. and Staphylococcus aureus showed 100% drug resistance towards ER, NA, NVB, KAN antibiotics. The finding of the study emphasis on the prevention of contamination through a sound maintenance of quality during poultry feeds preparation, storage and maintenance. Diseased birds and their excreta must be destroyed during poultry farming. Usage of excess antibiotics must be regulated as suggested from the data of the current study that shows high resistance of the bacterial isolates from the food. Finally, the consumers should process and cook the poultry items properly to save themselves from further food hazards.

Key words: Poultry feed, contamination, pathogenic bacteria, resistance, health hazards.

Introduction

Poultry products are very popular among the people worldwide providing a source of high protein containing food. Any type of avian species used for consumption as protein source by providing meat and eggs is called poultry. The birds may be chicken, duck, turkey, pigeons, quail etc 1,2. Poultry feeds are nutritious elements given to these domestic birds to make them grow properly and provide more meat and eggs 3,4,5. Poultry feeds are prepared using bone meal, cassava waste, grains, cereals, soya beans and vegetables like tridax, water leaf, amaranthus spp. etc 1,6. The feeds which are given to the domestic birds can be a source of microorganisms to them which includes bacteria, fungi as well as their secreted toxins. Escherichia spp., Enterobacter spp., Salmonella spp., Listeria spp., Streptococcus spp., Clostridia spp., etc have been isolated so far from poultry feeds and many of them showed multi-drug resistance 7-19. The growth of molds deteriorates the quality of the feed and production of toxins make the feed toxic, carcinogenic and often teratogenic resulting in different types of diseases in the domestic birds 20-29. Diseases like cholera, colibacillosis, listeriosis, staphylococcosis, amoebic dysentery, bacillary dysentery, salmonellosis, avian influenza, newcastle disease, infectious coryza are most common among those diseases which can transmit to the consumers as well 9,11,30,31. Human diseases like Salmonella paratyphoid fever, traveller’s diarrhea etc. can occur due to the consumption of poultry birds which contracted infections from poultry feeds 5. The poultry feeds can be contaminated during preparation, by using contaminated raw materials, by improper handling, from poor storage conditions, due to high humidity, by exposure to environmental microorganisms and so on 32. Contamination can be reduced by applying organic acids, organic salts, exposure to heat (>80°C) etc.

Poultry farming is quite popular in Bangladesh because of its ease of handling as well as lower investment cost which is manageable for the middle class societies. However, the microbiological concerns for feeds are not properly addressed in Bangladesh which may impose several disease complications. Such a concern for public health drove the current study to (1) isolate and enumerate bacteria from the poultry feeds along with the further determination of the antibiotic susceptibility of the
bacterial isolates and (2) to detect the antibacterial activity of the feeds if present.

Materials and methods

Study area and sampling

Five types of poultry feed samples were collected from different areas of Dhaka city between the time frame of January, 2017 to April, 2017. Samples were collected in sterile glass bottles following the aseptic technique. After collection of the samples, they were transported to the laboratory and kept at room temperature until the laboratory procedures started.

Sample collection and sample preparation:

Samples were collected early in the morning and transported and stored. Then it was subjected to microbiological analysis. Ten gm of each sample was transferred to 90 ml normal saline for homogenization and then serial dilution was done up to 10⁻⁵. For isolation of VBNC bacteria for example Vibrio spp., Salmonella spp. & Shigella spp., 1 ml homogenized sample was inoculated into 9 ml APW (alkaline peptone water) and 9 ml SCB (selenite cysteine broth) respectively for all the samples. The inoculated APW and SCB tubes were vortex mixed properly and incubated at 37°C for 4 to 6 hours for enrichment. After enrichment the tubes were subjected to serial dilution.

Total Viable Bacterial Count (TVBC) and Total Fungal Count (TFC)

0.1 ml diluted sample from 10⁻³ and 10⁻⁵ dilution of the homogenized samples of poultry feed were spread onto the nutrient agar (NA) plates and sabouraud dextrose agar (SDA) plates for TVBC and total fungal load respectively. NA plates were kept in the 37°C incubator for 24 hours and SDA plates were kept in the 25°C incubator for 48 hours.

Determination of Pseudomonas spp., Staphylococcus spp., Escherichia coli & Klebsiella spp.

0.1 ml sample from 10⁻³ and 10⁻⁵ dilution was spread over Pseudomonas Agar, MSA (Mannitol Salt Agar), MAC (MacConkey Agar) plates and incubated overnight at 37°C to isolate Pseudomonas spp., Staphylococcus spp., Klebsiella spp. and Escherichia coli respectively.

Determination of VBNC bacteria

The enriched SCB and APW tubes were subjected to serial dilution up to 10⁻³ in normal saline. 0.1 ml of sample from 10⁻² and 10⁻³ dilutions were inoculated onto Thiosulfate Citrate Bile Salt Sucrose (TCBS) agar plates and Salmonella-Shigella (SS) agar plates for the detection of Vibrio spp., Aeromonas spp., Salmonella spp., and Shigella spp. All plates were incubated at 37°C for 24 hours.

Antibiotic susceptibility of the isolates found in poultry feeds

Bacterial susceptibility to antibiotics was determined by using the standardized agar disc-diffusion method known as the Kirby Bauer method. Antibiotics used in this study included Gentamycin (10/μg), Erythromycin (15/μg), Ceftriaxone (30/μg), Rifampin (5/μg), Kanamycin (30/μg), Novobiocin (30/μg), Nalidixic Acid (30/μg), Amoxicillin (30/μg), Ciprofloxacin (5/μg). A suspension of the Salmonella spp., Enterobacter spp., Pseudomonas spp., Klebsiella spp., Escherichia coli, Proteus spp., Vibrio spp., Staphylococcus aureus were prepared. A sterile cotton swab was dipped into the prepared bacterial suspension and used to make lawn of the bacterial suspension evenly over the entire surface of a Mueller-Hinton agar plate. Antibiotic disc were placed aseptically over the surface of the inoculated plates using sterile forceps. The plates were then incubated at 37°C for 8 hours. After incubation the plates were examined & the diameters of the zones of inhibition were measured in mm.

Antibacterial activity of the poultry feeds against clinical isolates

The antimicrobial activity of the homogenized poultry feed samples were determined by agar well diffusion method. Suspensions of clinical isolates (Staphylococcus spp., Pseudomonas spp., Klebsiella spp., Vibrio spp., Escherichia coli) were used to make lawn directly on the Muller-Hinton agar (MHA) media separately. Each of the five samples were then introduced (volume of 100/μl) separately in the specified hole made in the MHA agar plates with sterile cork borer. After incubation period of 24 hours, the presence of clear zone around the sample solution indicated the presence of antibacterial activity. This indirectly indicated the presence of antibiotics in poultry feeds tested.

Results and discussion

In Bangladesh, poultry farms are very common which are responsible for commercially providing poultry feeds in the retail and super shops. Both small scale domestic poultry farms and commercial large poultry farms are available in our country. The proper growth and health status of the poultry birds depends on the quality of the supplied poultry feed. Very often low quality, locally produced feeds are provided to the birds of small farms. Such poultry products can cause diseases in human if not processed and cooked properly. The pathogenic bacteria from the poultry products can infect human body causing various acute and chronic diseases. Salmonella spp. can undergo chronic stage in human patients who might become carrier of salmonellosis towards other susceptible individual. Till now, 82 serotypes of Salmonella spp. have been reported of which 45 isolates were isolated from poultry feeds. Enterobacteriaceae group is one of the major contaminants of poultry feed. Poultry droppings from infected birds must be cleaned and disposed properly to reduce the transmission from droppings to other feeds, to human during handling, to fishes when they are used as fish feed.
viable bacterial count was within the range of $5.0 \times 10^4$ cfu/gm to $5.0 \times 10^6$ cfu/gm. Highest total fungal count ($4.5 \times 10^5$ cfu/gm) was observed in sample 04 and the lowest count ($2.0 \times 10^3$ cfu/gm) was found in sample 05. *Escherichia coli* was absent in all the samples. *Aeromonas* spp. and *Klebsiella* spp. were found in only one sample (sample 5 and sample 4 respectively). *Staphylococcus aureus* ($2.7 \times 10^4$ cfu/gm to $5.0 \times 10^3$ cfu/gm) and *Shigella* spp. ($3.2 \times 10^4$ cfu/gm to $1.1 \times 10^6$ cfu/gm) were the most abundant pathogenic bacteria found to be present in all the samples. *Pseudomonas* spp. was present in sample 01, 03 and 04. *Vibrio* spp. was the second most abundant pathogen in the poultry feeds. Four samples out of five harbored *Vibrio* spp. within the range of $1.8 \times 10^5$ cfu/gm in sample 5 to $2.4 \times 10^5$ cfu/gm in sample 4.

In case of locally made feeds, the quality must be maintained by physical and chemical treatments to lessen the bacterial load. Storage conditions, packaging, handling should also be maintained properly. Cross contaminations must be prevented. The consumption of uncooked eggs and undercooked meat of poultry should be discouraged strongly. If not maintained properly the food products can not only be contaminated but also the contamination level will increase and finally find its way into the consumers causing illness. In a data provided by CDC it had been found that 29444 illnesses cases were confirmed among consumers causing illness. In this study, Rifampin and Erythromycin are 90% resistant against *Klebsiella* spp. whereas Ciprofloxacin and Ceftriaxone were the most sensitive. *Pseudomonas* spp. was 100% susceptible to Ceftriaxone. *Staphylococcus aureus* was completely resistant towards Kanamycin, Novobiocin and Erythromycin. *Salmonella* spp. and *Shigella* spp. both showed resistance towards Ceftriaxone. Novobiocin was susceptible for maximum isolates except *Vibrio* spp. which showed 100% resistance. And finally *Aeromonas* spp. was completely resistant towards Erythromycin. The resistant isolates which will cause disease in the domestic birds, could be transferred into the human body if consumed undercooked. Then the infection which might arise could not be treated using the antibiotics towards which they have become resistant. Choice of susceptible drugs is decreasing very fast. This may result in the death from simple diseases which will fail to resume for unavailability of appropriate susceptible drugs.

Homogenized poultry feed samples were subjected to detect antibacterial activity towards clinical bacterial isolates. The isolates included *Pseudomonas* spp., *Staphylococcus* spp., *Vibrio* spp., *Escherichia* spp. and *Klebsiella* spp. Antibacterial activity against these bacteria have been found to be negative.

### Table 1: Number of different bacteria isolated from poultry feed.

| Sample | TVBC     | Total Fungal count | E. coli | *Klebsiella* spp. | *Pseudomonas* spp. | *Staphylococcus* aureus | *Vibrio* spp. | *Salmonella* spp. | *Shigella* spp. | *Aeromonas* spp. |
|--------|----------|--------------------|---------|-------------------|---------------------|------------------------|--------------|-------------------|----------------|-----------------|
| S1     | $5.0 \times 10^6$ | $2.0 \times 10^4$ | 0       | 0                 | $2.0 \times 10^4$ | $1.0 \times 10^4$      | 0            | 0                 | $5.0 \times 10^4$ | 0               |
| S2     | $2.5 \times 10^6$ | $1.2 \times 10^4$ | 0       | 0                 | 0                   | $2.7 \times 10^5$      | $2.0 \times 10^4$ | 0                 | $8.8 \times 10^4$ | 0               |
| S3     | $5.0 \times 10^4$ | $1.3 \times 10^4$ | 0       | 0                 | $1.6 \times 10^4$  | $1.8 \times 10^5$      | 0            | 0                 | $1.1 \times 10^6$ | 0               |
| S4     | $2.1 \times 10^6$ | $4.5 \times 10^5$ | 0       | $1.4 \times 10^6$ | $2.5 \times 10^6$  | $8.8 \times 10^4$      | $2.4 \times 10^5$ | $7.0 \times 10^3$ | $3.2 \times 10^4$ | 0               |
| S5     | $4.0 \times 10^5$ | $2.0 \times 10^3$ | 0       | 0                 | 0                   | $5.0 \times 10^5$      | $1.8 \times 10^5$ | 0                 | $2.3 \times 10^5$ | $2.9 \times 10^5$ |
Table 2: Antibiotic susceptibility of the isolates

| Isolates, Antibiotes             | Klebsiella spp (N = 8) | Pseudomonas aerogenes spp (N = 6) | Staphylococcus aureus (N = 5) | Salmonella spp (N = 3) | Shigella spp (N = 5) | Vibrio spp (N = 4) | Aeromonas spp (N = 4) |
|---------------------------------|------------------------|----------------------------------|------------------------------|------------------------|---------------------|-------------------|---------------------|
| RFM (5µg)                       | 90% (R) 10% (S)        | 70% (R) 30% (S)                  | 60% (R) 40% (S)              | 60% (R) 40% (S)        | 70% (R) 30% (S)    | 40% (R) 60% (S)    | 30% (R) 70% (S)     |
| CFT (30µg)                      | 10% (R) 90% (S)        | 0% (R) 100% (S)                  | 10% (R) 90% (S)              | 0% (R) 100% (S)        | 0% (R) 100% (S)    | 20% (R) 80% (S)    | 10% (R) 90% (S)     |
| KAN (30µg)                      | 60% (R) 40% (S)        | 10% (R) 90% (S)                  | 100% (R) 0% (S)              | 80% (R) 20% (S)        | 10% (R) 90% (S)    | 30% (R) 70% (S)    | 10% (R) 90% (S)     |
| NVB (30µg)                      | 40% (R) 60% (S)        | 30% (R) 70% (S)                  | 80% (R) 20% (S)              | 100% (R) 0% (S)        | 40% (R) 60% (S)    | 80% (R) 20% (S)    | 40% (R) 60% (S)     |
| NA (30µg)                       | 30% (R) 70% (S)        | 80% (R) 20% (S)                  | 100% (R) 0% (S)              | 60% (R) 40% (S)        | 10% (R) 90% (S)    | 0% (R) 10% (S)     | 10% (R) 90% (S)     |
| GEN (10µg)                      | 20% (R) 80% (S)        | 40% (R) 60% (S)                  | 20% (R) 80% (S)              | 20% (R) 80% (S)        | 40% (R) 60% (S)    | 10% (R) 90% (S)    | 40% (R) 60% (S)     |
| AMO (30µg)                      | 20% (R) 80% (S)        | 20% (R) 80% (S)                  | 0% (R) 100% (S)              | 40% (R) 60% (S)        | 30% (R) 70% (S)    | 45% (R) 55% (S)    | 30% (R) 70% (S)     |
| ER (15µg)                       | 90% (R) 10% (S)        | 70% (R) 30% (S)                  | 100% (R) 0% (S)              | 50% (R) 50% (S)        | 75% (R) 25% (S)    | 20% (R) 80% (S)    | 100% (R) 0% (S)     |
| CIP (5µg)                       | 10% (R) 90% (S)        | 10% (R) 90% (S)                  | 0% (R) 100% (S)              | 30% (R) 70% (S)        | 45% (R) 55% (S)    | 40% (R) 60% (S)    | 45% (R) 55% (S)     |

N.B. R = Resistant, S = Sensitive

RFM= Rifampin (5µg), GEN= Gentamycin (10µg), ER= Erythromycin (15µg), CFT= Ceftriaxone (30µg), KAN= Kanamycin (30µg), NVB= Novobiocin (30µg), NA= Nalidixic Acid (30µg), AMO= Amoxicillin (30µg), CIP= Ciprofloxacin (5µg).

Conclusion

Poultry products are good sources of proteins. This sector is expanding in Bangladesh to meet the protein demand of the growing population. But this protein source can be responsible for public health hazards if it harbors adequate amount of pathogenic bacteria. Pathogenic bacteria can be introduced from the ingredients, storage conditions, environmental facilities. Commercially available good quality feeds should be provided to the domestic birds to avoid diseases of domestic birds as well as their transmission to consumers.

Conflict of interest: None

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