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Original Article

Poultry Feeds as Carriers of Antibiotic Resistant *Enterobacteriaceae*

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ABSTRACT

Antibiotic resistance in microorganisms, caused due to uncontrolled use of antibiotics has become an emerging problem in public health care. This study was undertaken to examine the incidence of antibiotic resistant bacteria in three varieties of poultry feeds (starter, finisher and layer) collected from 20 locations. Initially bacteria were isolated using selective media (EMB agar, MacConkey agar and *Salmonella-Shigella* agar) and identified biochemically. Twenty five Enterobacter belonging to 8 genera (*Klebsiella*, *Shigella*, *Salmonella*, *Pseudomonas*, *Enterobacter, Yersinia, Proteus* and *Escherichia*) were isolated from feed samples. Among these *Klebsiella* was the most dominant genus and *Salmonella* was highly distributed among the three feed types. The distribution of bacteria in the three feed types were in the order, layer> finisher> starter. The layer feed showed 11.11 % incidence of *Salmonella* spp. The susceptibilities of the 25 isolates towards 15 antibiotics were examined. Except one, all the other isolates showed multiple antibiotic resistances. A 100 % resistance was observed against ampicillin, whereas, all isolates were sensitive to amikacin. *Klebsiella* spp. showed resistance to 12 antibiotics. The layer feed harboured highly resistant isolates that were resistant to 13 antibiotics. The prevalence of antibiotic resistant strains in poultry feeds strengthens the need for effective control measures during feed processing.

Keywords: Antibiogram, multiple drug resistance, *Salmonella, Klebsiella*, layer feed.

1. INTRODUCTION

Poultry feeds are the food items supplied for farm poultry, including chickens, ducks, geese and other domestic birds. These food materials are used for raising poultry birds. As these foods contain all the nutritional material required for growth, egg and meat production in birds they are described as complete feeds[1]. Depending on the functions they perform in the birds, various brands of poultry feeds are available, namely starters, growers, finishers, layers etc. 'Starters' are provided for the first six weeks of baby chick lives. It consists of 22-24 % protein for meat birds (called broiler

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starter) and 20 % protein for laying breeds. Growers are given until the chick is 14 weeks old, and 14 weeks onwards, pullet developer or 'finishers' are given. Laying hens at maturity (around 22 weeks of age) require a 16-18 % protein level. Hence they are provided with 'layer' rations[2].

The quality of poultry feed is of utmost consideration to public as it affects the quality of the animal and wholesomeness of meat consumed by humans. But often these feeds may serve as carriers of pathogenic microorganisms. Contamination in feed can arise from ingredients of plant and animal sources, unhygienic manufacturing practices and improper storage. Poultry feeds with bacterial contamination not only reduce the quality of feed but also contribute to human food borne illness through the feed-poultry-food-human chain[3,4]. Thus microbiological safety regulations are required for poultry feeds to escape microbial contamination of the product[1].

Enterobacteriaceae is a large family of gram negative bacteria, consisting of 53 genera and above 170 named species, of which 26 genera are known to be associated with human infections. In poultry feed the most frequently observed bacteria are *Escherichia coli* and *Salmonella* species[1,5]. *Aspergillus flavus* and *Aspergillus parasiticus* are the filamentous species found in poultry feeds[6]. Contamination of animal feed has been thought to be the cause of various diseases such as salmonellosis, diarrhea, bacillary dysentery, colibacillosis, staphylococcosis, listeriosis and erysipelas usually seen in farm animals[7]. But, the potential and more deadly hazard associated with animal feed is the antibiotic resistance acquired by the contaminating organism.

Previously antibiotics were incorporated into animal feed formulations at low levels to prevent minor microbial diseases and enhance the growth of birds[8]. But the increasing exposure to antibiotics has resulted in development of antibiotic resistant strains. Continuous exposure to antibiotics causes mutations in bacteria that make them resistant to the antibiotic, giving a survival advantage to mutated bacteria. In the presence of antibiotic this positive mutation is transferred via horizontal gene transfer, resulting in proliferation of resistant trait[9]. These resistant strains can be transmitted to humans through the feed-poultry-food-human chain causing serious problems to humans. Penicillin, tetracycline, aminoglycosides, streptomycin, ampicillin, norfloxacin, gentamycin, kanamycin, vancomycin, riphampicin, cefixime, norfloxacin, amoxicillin and augmentin are the most commonly used antibiotics in poultry feed. Avilamycin, avoparcin, flavomycin, monensin, olaquindox, carbadox and salinomycin are some of the antibiotics approved for growth[10]. The present study aims at isolating and identifying bacteria belonging to *Enterobacteriaceae* from different poultry feeds and to determine the antibiotic susceptibility of the isolated bacteria. The percentage of antibiotic resistance based on the organism and the feed type were also examined.

2. MATERIALS AND METHODS

Sample Collection

A total of 60 samples of poultry feed belonging to three varieties, namely starter, finisher, and layer were collected from 20 different sites. The samples were obtained in sterile containers. These samples were then subjected to various bacteriological and biochemical examinations in the laboratory.

Isolation of microorganisms

Sterile Brain Heart Infusion broth was prepared for pre-enrichment of samples and 5 ml of the broth was transferred into sterile test tubes[11, 12]. Then 0.55 g of sample was added to these tubes. They were incubated at 37 ^{o}C for 24 hours. From the pre-enrichment broth, 0.1 ml of culture was transferred to 10 ml sterile Rappaport-Vassiliadis medium[11] and kept for incubation at 42 ^{o}C for 18-24 hours. After incubation 0.1 ml enriched broth culture was plated on sterile EMB agar, MacConkey agar and SS agar (HiMedia) plates. The plates were then incubated at 37 ^{o}C for 24 hours. Pure cultures of isolated colonies were maintained on nutrient agar slants.

Identification of Microorganisms

The isolated organisms were subjected to different morphological and biochemical tests. The isolates were identified by comparing the results with Bergey's manual of determinative bacteriology [13].

Antibiotic susceptibility test

Pure cultures of all the isolated organisms were prepared in nutrient broth and standardised using 0.5 McFarland standard. The antibiotic susceptibilities of all the 25 isolates were examined against 15 different antibiotic discs (HiMedia) using Kirby-Bauer disc diffusion method, according to the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2012)[14]. Firstly, the organisms were swabbed on sterile Mueller–Hinton agar (MHA, HiMedia) plates and kept undisturbed for 5 min for drying. Later the discs were placed with the help of sterile forceps and incubated at 37 ^{o}C for 24 hours. The tested antibiotics included: Amoxicillin (25 µg) [AM], Amoxiclav (30 µg) [AMC], Ampicillin (2 µg) [A], Amikacin (30 µg) [AK], Chloramphenicol (25 µg) [C], Cephalothin (30 µg) [CEP], Ciprofloxacin (5 µg) [CIP], Erythromycin (15 µg) [E], Kanamycin (30 µg) [K], Neomycin (10 µg) [N], Nitrofurantoin (300 µg) [NIT], Penicillin G (10 units) [P], Streptomycin (25 µg) [S], Tetracycline (30 µg) [TE] and Gentamycin (30 µg) [GEN]. The zone diameters were measured in millimetres (mm) and results were compared with the published data for zone interpretation in *Enterobacteriaceae* from CLSI document M100-S23 (M02-A11)[14] and additionally from data published by Fall (2011)[15] for Amoxicillin. Based on the results the organisms were classified as sensitive, intermediate or resistant.

3. RESULTS AND DISCUSSIONS

Isolation of microorganism

Twenty five bacteria belonging to *Enterobacteriaceae* were isolated from 60 samples belonging to three varieties of poultry feeds (starter, finisher and layer). Most of the bacteria were obtained from layer feed type followed by finisher and starter. Similar observation for layer preference were also made earlier by Chowdhury *et al.* (2011)[1]. Atere *et al.* (2015)[16] also observed highest number of bacteria in layer sample of SBM brand of poultry feed.

Identification of the isolates

All the isolates were identified using routine microbiological tests. Based on the results, these 25 organisms could be grouped into 8 genera. The genus *Klebsiella* was the most dominant with 36 % followed by *Shigella* (20 %), *Salmonella* (16 %), *Pseudomonas* (8 %) and 4 % each of *E.coli, Enterobacter, Yersinia, Proteus* and *Escherichia*. Dominance of *Klebsiella* spp. in poultry feeds was reported by Cox N. *et al.* (1983)[5] and Ezekiel *et al.* (2011)[17].

Salmonella spp. was the most distributed in all the feed types. This is of concern to public health as salmonellosis is the most common food-borne disease and is transmitted mainly by poultry products. In the study, it was noted that the layer feed which harboured the highest number of organism showed 11.11 % prevalence of *Salmonella* spp. Alshawabkeh (2010)[18] reported an overall 2.33 % incidence of *Salmonella* spp. in feed samples with 2.4 % particularly in layer feed. Veldman, A., *et al.* (1995)[19] observed 21 % *Salmonella* contamination in mash feeds used for layer-breeders and 1.4 % in pelleted feeds. A higher percentage of contamination (29.16 %) was reported by Islam *et al.* (2014)[20]. Chowdhury *et al.* (2011)[1] found that the largest number of *Salmonella* spp. were present in the layer feed samples. Detection of these organisms in this study agrees to the fact that bacteria are part of the enteric flora of the poultry feeds.

Antibiotic susceptibility of different *Enterobacteriaceae*

All the isolated organisms were tested for their susceptibility to selected 15 antibiotics. The zones formed were measured and the values obtained were compared with the interpretation of zone diameters of antibiotics and finally the antibiogram was prepared. From the antibiogram a high incidence of resistance against individual antibiotics was confirmed. A great variation was observed in the resistance patterns of organisms and samples from different locality and varieties. The findings of the present study were in consonance with other studies that have also confirmed the high incidence of antibiotic resistance among bacteria isolated from poultry feeds[16, 17].

From the study, it was observed that all the isolates were resistant to one or the other antibiotic except in the case of amikacin. None of the organisms were resistant to amikacin; whereas, a 100 % resistance was shown on ampicillin by all the organisms. This was followed by penicillin (96 %), amoxiclav (88 %,), amoxicillin (80 %), cephalothin (64 %), erythromycin (60 %), tetracycline (44 %), nitrofurantoin (40 %), ciprofloxacin and kanamycin (16 %) and streptomycin (8 %). Only 4 % of the organisms showed resistance towards the chloramphenicol, neomycin and gentamycin. The high resistance to ampicillin and penicillin could be due to frequent usage which gives a chance for resistance development. On the other hand, sensitivity to amikacin may be because of least usage as it is the newest of aminoglycosides.

Multiple antibiotic resistance (MAR) or resistance towards two or more antibiotics by single organism were seen during the analysis. All 25 isolates, excluding one, showed resistance to at least 3 drugs and to a maximum of 10 antibiotics. An isolate belonging to genus *Shigella*, was an exception as it showed resistance only towards ampicillin. Isolates belonging to genus *Klebsiella* showed the highest resistance towards multiple antibiotics (12 antibiotics), whereas *E. coli* showed the least resistance among the multidrug resistant strains (4 antibiotics). Low level of antibiotic resistance of *E. coli* was previously reported by Bywater *et al.* in 2004[21].

The resistance percentage of the dominant isolates- *Klebsiella, Salmonella* and *Shigella* against all 15 antibiotics were depicted in Figure 1. *Klebsiella* spp. and *Salmonella* spp. showed 100 % resistance to ampicillin, amoxiclav and penicillin; whereas in the case of *Shigella*, 100 % resistance was observed only against ampicillin. All three isolates were sensitive to amikacin. Other than this antibiotic, *Klebsiella* was sensitive to only two antibiotics (chloramphenicol and ciprofloxacin), *Salmonella* to five antibiotics *(*kanamycin, streptomycin, neomycin, chloramphenicol and gentamycin) and *Shigella* was sensitive to gentamycin and ciprofloxacin.

The percentages of resistance in the three feed types were represented in Figure 2. The data reveals that percentage resistance in 'starter' feed was high. About 100 % resistance was seen towards amoxicillin, ampicillin, erythromycin, amoxiclav and penicillin; whereas 50 % towards ciprofloxacin, tetracycline, nitrofurantoin and cephalothin. In the case of 'layer' feed samples 100 % resistance was towards ampicillin and penicillin; 89 % towards amoxiclav; 78 % amoxicillin; least percentage towards rest of the antibiotics. While considering 'finisher', 100 % of resistance was shown towards ampicillin; 80 % to amoxicillin, amoxiclav and penicillin; 60 % to tetracycline and cephalothin; 40 % resistance to kanamycin, erythromycin, ciprofloxacin and nitrofurantoin, and 20 % to streptomycin and chloramphenicol. From the graph it could be concluded that starter shows a high resistance percentage towards antibiotics even though isolated organisms were less. The layer feed samples showed resistance towards a broad range of antibiotics [13] although the resistance towards individual antibiotics was low.

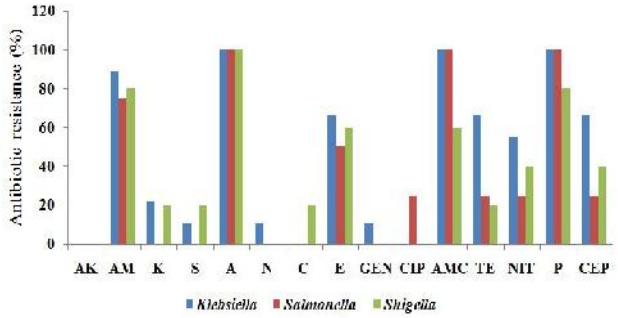

Fig 1: Resistance pattern of the most dominant isolates
(Amikacin [AK], Amoxicillin [AM], Kanamycin [K], Streptomycin [S], Ampicillin [A], Neomycin [N], Chloramphenicol [C], Erythromycin [E], Gentamycin [GEN], Ciprofloxacin [CIP], Amoxiclav [AMC], Tetracycline [TE], Nitrofurantoin [NIT], Penicillin G [P] and Cephalothin [CEP])

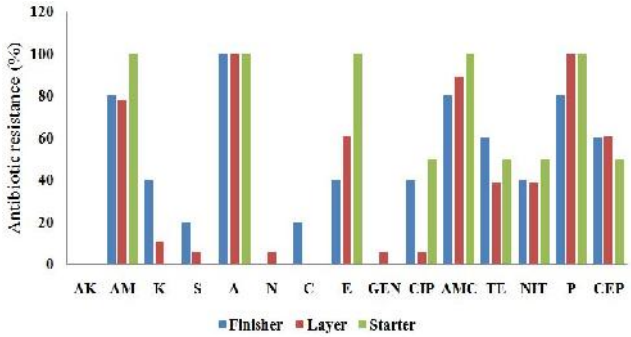

Fig 2: Resistance pattern of feed samples
(Amikacin [AK], Amoxicillin [AM], Kanamycin [K], Streptomycin [S], Ampicillin [A], Neomycin [N], Chloramphenicol [C], Erythromycin [E], Gentamycin [GEN], Ciprofloxacin [CIP], Amoxiclav [AMC], Tetracycline [TE], Nitrofurantoin [NIT], Penicillin G [P] and Cephalothin [CEP])

4. CONCLUSION

This study reveals the high incidence of antibiotic resistant *Enterobacteriaceae* in poultry feeds. Among the three feed types, layer feed harboured the most number of bacteria. Among the 25 bacteria identified *Klebsiella* was most dominant genus followed by *Salmonella* and *Shigella.* The high prevalence of *Salmonella* in feed samples raises concern regarding the safety of feed given to poultry. Contamination of feed may have occurred during the time of production or processing or even packaging. Whatever the stage may be, this will lead to great pathogenic infections both in birds as well as humans. All the isolates except one showed resistance to multiple antibiotics. This is a serious hazard, not only to the birds, but also the poultry workers and consumers. Such resistance may be acquired during the careless or over usage of antibiotics. This calls for urgent intercession by the authorities to take proper safety measures in feed production processes. Much more studies are required in this field to create an awareness regarding the risks associated with contamination of poultry feed and to develop strategies for quality control in poultry industry.

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