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OCCURRENCE OF BACTERIAL CONTAMINANTS IN POULTRY MEALS AND THEIR ANTIBIOTIC RESISTANCE PATTERN

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ABSTRACT: By products and wastes of livestock and poultry processing units are used to make different meals for poultry feed as a source of good quality protein. Microbiological quality of poultry meals and antibiotic resistance pattern of isolates against commonly used poultry antibiotics was determined. Meal samples (n=40) from four feed processing plants (n=10, each) were processed for aerobic plate, total coliform and total yeast/mold count and *Salmonella* detection by standard microbiological techniques. Bacterial isolates were identified by morphology and biochemical testing. Antibiotic resistance pattern of isolates against 24 commonly used antibiotics was determined by disk diffusion method. Higher aerobic counts (10^7- 10^{13} CFU/g) were observed in all poultry meals. Coliforms (10^2-10^5 CFU/g) and total yeast/mold counts (10^4-10^6 CFU/g) were detected in 25 and 27.5 percent meals, respectively. Bacterial isolates (n=134) including *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Pseudomonas aeroguinosa*, *Staphylococcus epidermidis* and *Escherichia coli* were identified. Higher antibiotic resistance was observed in *B. subtilis* and *S. aurues*. It was concluded that poultry meals were contaminated by resistant bacteria and may be source of antibiotic resistance transfer to commensal bacteria of poultry.

Keywords: Poultry meals, microbial load, bacterial contaminants and antibiotic resistance.

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INTRODUCTION

Poultry is the second largest industry in Pakistan after textile industry (Anonymus, 2014). Its consumption rate is very high as compared to other animal protein sources, as it is cheaper compared to red meat (Maqsood, 2012). To fulfill increasing demand of poultry, quality of poultry production must be improved. Many factors affect poultry production. One factor is feeding process (Grepay, 2009).

Poultry feeds are food materials designed to contain all necessary feed ingredients for proper growth, meat and egg production in birds (Obi and Ozugbo, 2007). The major component of poultry feed is protein which is the key component of eggs and meat. Protein sources in poultry feeds are of plant, marine and animal origins. Plant proteins may lack some of essential amino acids, thus are incomplete proteins while proteins of animal origin are better growth promoter but their safety is a concern (Alali *et al*., 2011). Animal protein sources include products of animal origin. Livestock processing wastes and by products are used by rendering process to make different meals like poultry, fish, meat and bone, feather and poultry by products meal (Laban *et al*., 2014). Among all protein based meals, poultry meals and poultry by products meal are of superior quality and provide higher protein contents than plant, marine and meat based meals (Samli *et al*., 2006).

Chemicals as well as microbes can contaminate feed. Feeds of animal origin including meals have been implicated as one of the source of microorganisms to animals and poultry (Uwaezuoke and Ogbulie, 2008). Poultry meals are added in feed as a source of protein, which are clean tissues of slaughtered poultry including bone converted to poultry meals by the process of rendering (Anonymus, 2014). Cooking step in rendering process inactivates bacteria, viruses, protozoa, and parasites (Meeker and Hamilton, 2006). It reduces the number of microorganisms in meals (Hayes, 2014), converting them into rendered safe materials almost free from pathogens, but still meals get contaminated. Such contamination of meals is attributed to post processing contamination (Laban *et al*., 2014) by dust, air movements, contaminated processing equipment (Hofacre *et al*., 2001) or possible recontamination from raw material during air drying either by direct contact or indirectly by insects and rodents (Elsir, 2015).

Poultry meals are used as feed and as protein component of feed. Bacterial and fungal contamination of meals adversely affect poultry industry, thus ultimately human health. Therefore, there is a dire need of the time to determine various quality parameters of poultry meals to curtail the losses in poultry industry. The aim of present study was to determine microbial load in commercially available poultry meals and to evaluate antibiotic resistance pattern of common bacterial isolates from poultry meals.

MATERIALS AND METHODS

Poultry meal samples (n=40) were collected from four feed processing plants (n=10, each) in sterile labeled polythene bags and processed according to procedure described by Arotupin *et al*., (2007).

Microbiological analysis: Suspension of meal samples (20%) was prepared in normal saline and was ten folds serially diluted (Arotupin *et al*., 2007). Aliquots of each dilution were spreaded on Nutrient agar (Himedia Labs, Mumbai, India) for aerobic plate count (APC) and MacConkey agar (Himedia Labs, Mumbai, India) for total coliform count (TCC) as described by Lateef and Kana (2014). Sabouraud dextrose agar (Himedia Labs, Mumbai, India) was used for total yeast/mold count (TYMC) (Krnjaja and Trenkovski, 2011). One mL from suspension was directly spread plated on Salmonella Shigella agar (Himedia Labs, Mumbai, India) for *Salmonella* detection (Maqsood, 2012). Plates were incubated at 37˚C for 24h for bacterial growth and at room temperature for 3-5 days for total yeast/mold count. After incubation, counts in colony forming units (CFUs) were converted to $\log_{10}$ values and means of $\log_{10}$ values were calculated. Counts were statistically compared using one way ANOVA followed by DMR test using SPSS version 16.0.

Identification of microorganisms: Bacterial colonies having different morphology were identified on the basis of their colony characteristics and microscopic features according to instructions given in Bergey's manual (2009). Selected isolates were further characterized through their biochemical profile.

Antibiotic resistance pattern: Antibiotic resistance pattern of bacterial isolates was determined on Mueller hinton agar (Himedia Labs, Mumbai, India) by Kirby-Bauer disk diffusion method (Bauer *et al.*, 1966), using antibiotic disks including mupirocin (Mu, 5µg), ticaricillin/clavulanic acid (TC, 75/10µg), oxacillin (Ox, 1µg), aziocillin (Az, 75µg), piperacillin/tazobactem (TZP, 110µg), spectinomycin (SE, 100µg), co-trimazine (Cm, 25µg), triple sulphas (S3, 300µg), cinoxacin (CIN, 100µg), cefuroxime (CXM, 30µg), cephradine (CE, 30µg), bacitracin (B, 10units), amikacin (AK, 30µg), streptomycin (S, 10µg), ciprofloxacin (CIP, 5µg), tetracycline (TE, 30µg), ceftriaxone (CRO, 30µg), kanamycin (K, 30µg), gentamicin (CN, 10µg), novobiocin (NV, 5µg), cloxacillin (Cx, 30µg), chloramphenicol (C, 25µg), norfloxacin (NOR, 10µg) and combination of amoxicillin and clavulanic acid (AMC, 30µg). Zones of inhibition were measured and compared to the criteria of clinical laboratory standards institute (CLSI, 2014). Isolates were declared sensitive or resistant on the basis of zone of inhibition. Data for resistance was converted to percentage antibiotic resistance.

RESULTS AND DISCUSSION

Microbial load of poultry meal samples was determined. In this study, all the samples were positive for aerobic plate count and total yeast/mold count, ten samples were positive for total coliform count and *Salmonella* was not found in any of the tested meal sample. Counts in CFU/g are shown in Table-1. Relatively high bacterial contamination was observed in meals, where the number of aerobic bacteria reached up to 10^{13} CFU/g. Mean aerobic plate count was 1.12×10^{11} CFU/g. Counts in all of the tested samples were higher than standard permissible limits of 3.0×10^{6} CFU/g (ISO 7218:2007). In order to determine microbiological quality of poultry meals, samples were collected from different feed processing plants. Higher aerobic plate counts were obtained in poultry meals and this corroborates to the findings of Kukier *et al*. (2013). Kukier reported higher bacterial contamination in animal meals. However, Kinley *et al*., (2010); Awachat *et al*., (2011) and Elsir (2015) demonstrated that meals after rigorous thermal processing found to have a lower bacterial count up to 10^{2} CFU/g.

Contamination with coliforms in meal samples was detected 25% of tested samples. Coliform counts were found in range of 10^{2}-10^{5} CFU/g, with a mean count of 1.17×10^{1} CFU/g. Permissible limit of 300 CFU/g for Enterobacteriaceae (Kukier *et al*., 2013) was exceeded in 22.5% of tested meal samples, 2.5% samples showed counts within standard limit, while remaining 75% showed no counts. Coliforms were found in 1/4 of poultry meal samples with counts higher than standard permissible limits, while remaining meal samples showed no coliform counts. Such variation in coliform count have also been reported by Uwaezuoke and Ogbulie (2008), Radziejewska *et al*. (2013) who reported contamination with Enterobacteriaceae in feeds varied. Such difference in coliform counts of meal samples collected from different feed plants could be attributed to improper unhygienic conditions for handling and storage.

Mycological examination of poultry meals revealed that counts of 72.5 percent samples didn't exceed maximum permissible limit (2.0×10^{5} CFU/g), whereas, 27.5 percent samples showed counts slightly higher than standard value. Counts were found within the range of 10^4-10^6 CFU/g, with a mean total yeast/mold count of $9.33x10^4$ CFU/g. Generally, feeds of animal origin show lowest mold contamination as a result of processing and storage (Čabarkapa *et al*., 2009). My findings are found in coherence, as almost 73 percent of meal samples under study conformed to standard permissible limits. Similar trend was also recorded by Krnjaja and Trenkovski (2011), Kukier *et al*., (2012, 2013).

All of the meal samples were found negative for *Salmonella* that conformed to official European Union (EU) standards for feed. *Salmonella* was not found in any of tested meal samples. Similar findings were recorded by Čabarkapa *et al*. (2009) where none of the poultry feed sample contained *Salmonella* and Awachat *et al*. (2011) who reported absence of *Salmonella* in poultry by products meal. Good microbiological principles like hazard analysis critical control points, has been employed by animal protein producer industry to reduce incidence of *Salmonella* recontamination in rendering plants since 1995 (Hofacre *et al*., 2001). It can account for no recovery of *Salmonella* in poultry meals in present study.

Out of poultry meal samples (n=40), 134 bacterial isolates of five genera were isolated and identified. The isolates included *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Pseudomonas aeroguinosa*, *Staphylococcus epidermidis* and *E. coli* accounting for 38.80, 26.86, 16.41, 8.95, 6.71 and 2.23 percent occurrence, respectively. The type of bacterial isolates reported are similar to those previously reported by Nkang *et al*., (2010); Aliyu *et al*., (2012) and Lateef and Kana (2014). Occurrence of similar bacterial isolates in meals with the exception of presence of *Klebsiella spp.*, *Streptococcus spp.* and *Corynebacterium spp.* was reported in another study (Elsir, 2015). The presence of *E. coli* in poultry meals is a hygiene indicator. In present study, its presence in only two samples suggested recent fecal contamination. Animal feed containing animal tissues and by-products is a major concern, as spore forming bacteria likely will be present even after processing (Gilchrist *et al*., 2007). Rendering process ensures complete removal of microorganisms, but still presence of *Bacillus spp.* in meal samples may suggest that it was a spore forming bacteria whose spores were not destroyed in thermal processing, but competing microflora was killed, so spores germinated and resulted in increase in such a high number of occurrence of *Bacillus spp.* in meals. The *S. aureus*, *M. luteus*, *P. aeroguinosa* and *S. epidermidis* are non-spore formers that can easily be killed at high processing temperatures in rendering process. Their presence in meal samples that have low water activity suggested recent contamination from environment. The *S. aureus* is the normal flora of nose and skin and its presence in poultry meals pretended improper handling practices.

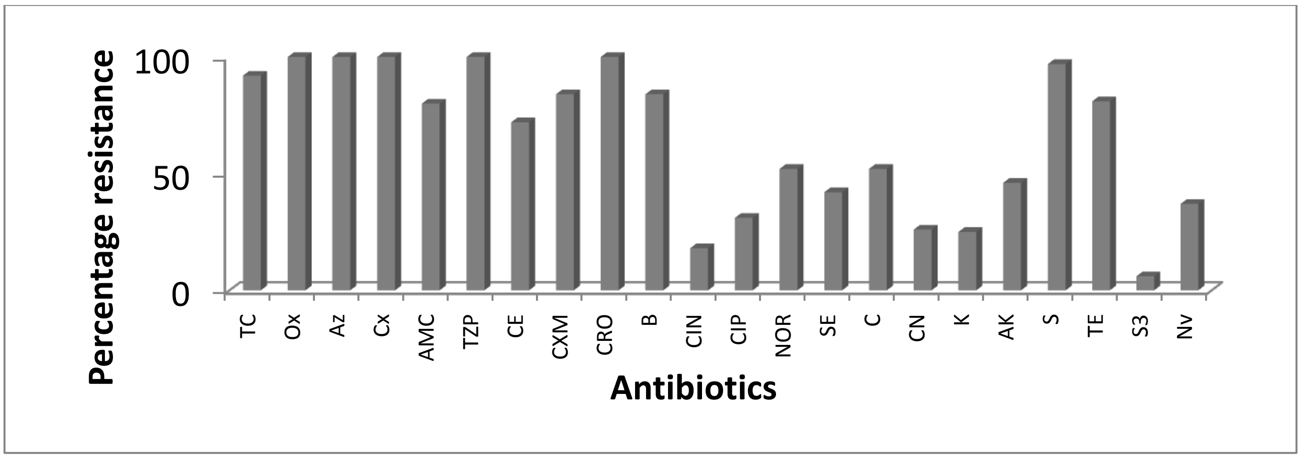
In present study, four different feed processing plants were selected for collection of poultry meal samples. Aerobic plate count and coliform count of location A differed significantly ($P\leq 0.05$) from other three locations (B, C and D), whereas, counts of location B, C and D differed non-significantly ($P\geq 0.05$). Similarly, total yeast/mold count of meal samples from location B differed significantly ($P\leq 0.05$) from counts of location A, C and D. Microbial loads as well as type of microorganisms can be used to determine quality of meals as well as source of contamination (Nkang *et al*., 2010).

Antibiotic resistance pattern of bacterial isolates was determined against 24 antibiotics. All of 134 isolates were found resistant to oxacillin, aziocillin, ceftriaxone, piperacillin/tazobactem, cloxacillin and mupirocin (Fig-1). Highest resistance was observed to penicillins and penicillin group derivatives. Least resistance was shown to amino glycosides (kanamycin, gentamicin and amikacin) and sulfonamides (combination of sulphadimidine, sulphadiazine and sulphathiazole). The *S. aureus* isolates were oxacillin, aziocillin, piperacillin/tazobactem resistant, while, moderate resistance was observed against ticaricillin and clavulanic acid, amoxicillin/clavulanic acid, gentamicin and cefuroxime (Fig-2). Multi drug resistant *E. coli* was isolated from poultry meals that were resistant to gentamicin, cefuroxime and penicillin derivatives (Fig-3).

A large number of poultry meal samples contained bacteria that were resistant to oxacillin, aziocillin, ceftriaxone, streptomycin, bacitracin, cefuroxime, tetracycline and amoxicillin/clavulanic acid, whereas few samples were contaminated with bacteria resistant to ciprofloxacin, kanamycin and sulfa drugs. Similar observations were recorded by Hofacre *et al*. (2001), who reported similar resistance trend in bacteria isolated from poultry meals. *B. subtilis* and *S. aureus* were most commonly isolated antibiotic resistant bacteria in poultry meal samples. *E. coli* showed resistance to all test antibiotics except spectinomycin, gentamicin, amikacin, kanamycin, cephradine and amoxicillin/clavulanic acid for which it showed low resistance. *E. coli* was found to be 100 percent resistant to tetracycline and streptomycin, that were against the findings of (da Costa *et al.*, 2007) who reported 41.4 and 17.0 percent resistance, respectively. Presence of tetracycline and streptomycin resistant *E. coli* has also been reported by Hofacre *et al*., (2001).

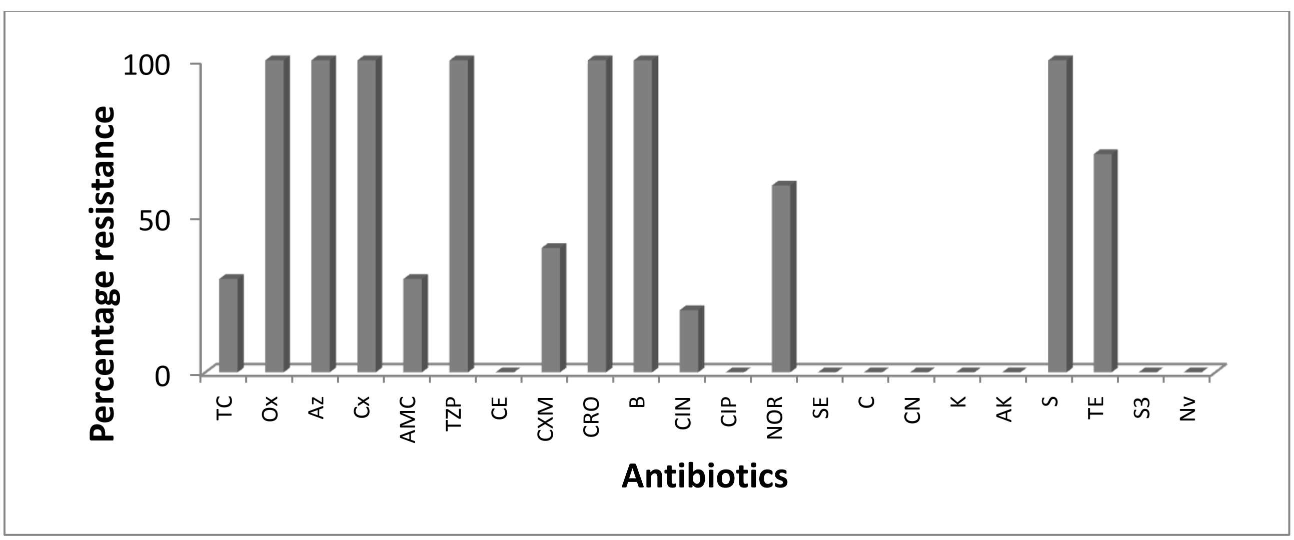
Table-1: Mean counts (CFU/g) of aerobic plate count, total coliform and total yeast/mold count

Poultry meal sources locations	No. of samples	Mean Counts (CFU/g) Aerobic plate	Total coliform	Total yeast/mold
A	10	2.51×10^{8}	1.99×10^{4}	5.75×10^{4}
B	10	2.51×10^{12}	Nil	3.98×10^{5}
C	10	3.98×10^{12}	Nil	3.98×10^{4}
D	10	2.50×10^{12}	Nil	5.01×10^{4}
Total	40	1.12×10^{11}	1.17×10^{1}	9.33×10^{4}



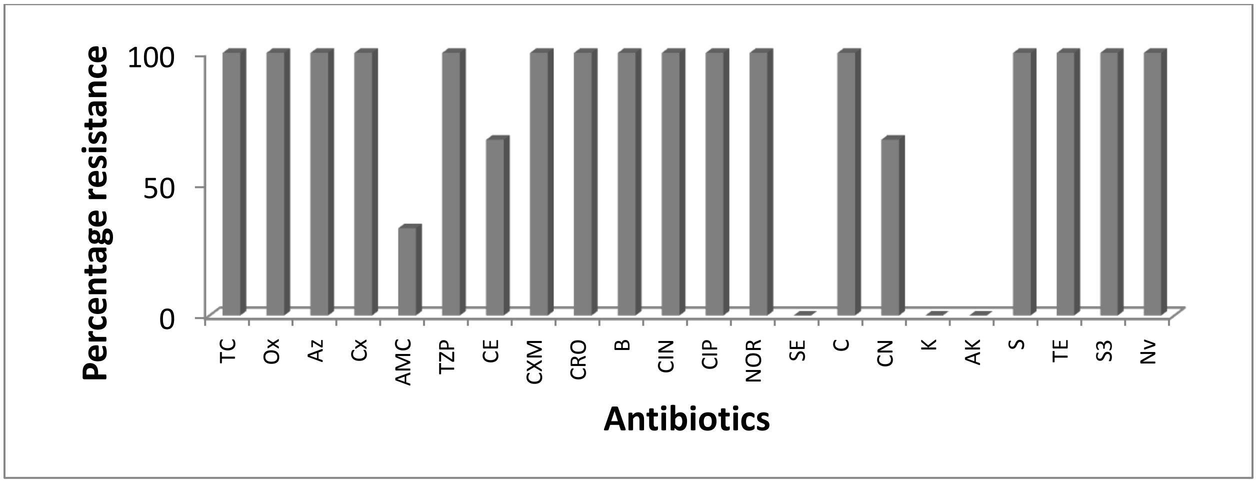
Mu: Mupirocin (5µg) , TC: Ticaricillin/Clavulanic acid (75/10µg), Ox: Oxacillin (1µg), Az: Aziocillin (75µg) , TZP: Piperacillin/Tazobactem (110µg) , SE: Spectinomycin (100µg), Cm: Co-trimazine (25µg), S3: Triple sulphas (300µg) , CIN: Cinoxacin (100µg), CXM: Cefuroxime (30µg), CE: Cephradine (30µg), B: Bacitracin (10units), AK: Amikacin (30µg), S: Streptomycin (10µg), CIP: Ciprofloxacin (5µg), TE: Tetracycline (30µg), CRO: Ceftriaxone (30µg), K: Kanamycin (30µg), CN: Gentamicin (10µg), NV: Novobiocin (5µg), Cx: Cloxacillin (30µg), C: Chloramphenicol (25µg), NOR: Norfloxacin (10µg) and AMC: Combination of Amoxicillin and Clavulanic acid (30µg)

Figure-1: Antibiotic resistance pattern of bacterial isolates from poultry meals



Mu: Mupirocin (5µg) , TC: Ticaricillin/Clavulanic acid (75/10µg), Ox: Oxacillin (1µg), Az: Aziocillin (75µg) , TZP: Piperacillin/Tazobactem (110µg) , SE: Spectinomycin (100µg), Cm: Co-trimazine (25µg), S3: Triple sulphas (300µg) , CIN: Cinoxacin (100µg), CXM: Cefuroxime (30µg), CE: Cephradine (30µg), B: Bacitracin (10units), AK: Amikacin (30µg), S: Streptomycin (10µg), CIP: Ciprofloxacin (5µg), TE: Tetracycline (30µg), CRO: Ceftriaxone (30µg), K: Kanamycin (30µg), CN: Gentamicin (10µg), NV: Novobiocin (5µg), Cx: Cloxacillin (30µg), C: Chloramphenicol (25µg), NOR: Norfloxacin (10µg) and AMC: Combination of Amoxicillin and Clavulanic acid (30µg)

Figure-2: Antibiotic resistance pattern of *S. aureus* isolated from poultry meals



Mu: Mupirocin (5µg) , TC: Ticaricillin/Clavulanic acid (75/10µg), Ox: Oxacillin (1µg), Az: Aziocillin (75µg) , TZP: Piperacillin/Tazobactem (110µg) , SE: Spectinomycin (100µg), Cm: Co-trimazine (25µg), S3: Triple sulphas (300µg) , CIN: Cinoxacin (100µg), CXM: Cefuroxime (30µg), CE: Cephradine (30µg), B: Bacitracin (10units), AK: Amikacin (30µg), S: Streptomycin (10µg), CIP: Ciprofloxacin (5µg), TE: Tetracycline (30µg), CRO: Ceftriaxone (30µg), K: Kanamycin (30µg), CN: Gentamicin (10µg), NV: Novobiocin (5µg), Cx: Cloxacillin (30µg), C: Chloramphenicol (25µg), NOR: Norfloxacin (10µg) and AMC: Combination of Amoxicillin and Clavulanic acid (30µg)

Figure-3: Antibiotic resistance pattern of *E. coli* isolated from poultry meals

Conclusion: Multi-drug resistant microbes in poultry meals can transfer antibiotic resistance through poultry feed to commensal bacteria of poultry.

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