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Bacterial load determination of poultry feed with seasonality effect in Karachi, Pakistan.

Shajeela Iram[1,2], Tabinda Khawaja[2], Mehmood ul Hassan[3], Muhammad Jawad Khan[4], & Tanveer Abbas[2]

[1]Department of Microbiology, Baqai College of Veterinary Sciences, Baqai Medical University, Karachi-Pakistan.
[2]Department of Microbiology, University of Karachi, Karachi-Pakistan.
[3]Department of Pharmacology, Faculty of Veterinary and Animal Sciences, Lasbela University of Agriculture, Water and Marine Sciences, Uthal Balochistan-Pakistan.
[4]Livestock Dairy Development Board, Islamabad, Karachi-Pakistan.

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Corresponding Author Email: shajeelazhar@gmail.com
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Abstract

Background: The escalating rate of food-borne diseases via poultry feeds necessitates the characterization of the pathogens to reduce the health risk factor for humans and animals. The poultry feed microbial analysis help to control food-borne illness. In addition, the knowledge about seasonal effects on microbial growth helps the manufacturers to take precautionary measures in alarming months to maintain the standard quality of poultry feed.

Methodology: This study was designed to identify and enumerate bacteria and their seasonal variation. Total feed samples (n=204) were evaluated through traditional culture techniques, microscopic inspection, and biochemical properties. However, bacterial load was determined by using the total viable count.

Results: As a result, five genera, including Salmonella enterica (39.05%), Escherichia coli (22.48%), Bacillus subtilis (18.34%), Staphylococcus aureus (11.24%), and Streptococcus sp (8.87%), were isolated. The proportion of occurrence of the bacterial load was lowest in December-February (64.4%) and highest in June-August (96.2%). Whereas in September-November was (87.6%) and March-May (77.5%). On the whole, the total percentage of positive samples was 82.8%. Statistical analysis revealed that (9.9×10-8 cfu/g) was the highest viable bacterial count recorded from June to August. The presence of food-borne pathogens, especially S.enterica and E.coli, is bothersome. Moreover, June to August is considered the most troubling month due to the elevated level of contamination.

Conclusion: To evade microbial contamination, the microbiological security rules must be followed throughout the process of formulation and storage period, especially in sensitive hot and humid months of June to August. Also, standard inspection should be taken to control the dissemination of food-borne illness.

Keywords

Food-Borne Illness, Bacterial Contamination, Total Viable Count, Seasonal Variation.

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Introduction

The most vibrant and effervescent agriculture segment in Pakistan is the poultry sector, with a major contribution (1.3%) to the national GDP. The poultry sector works as a bridge to cover the gap between supply and demand for protein. In developing countries, the poultry sector plays a key part in economic development, but poultry diseases are a big concern[1]. Poultry feed is the major cause of the dissemination of various food-borne diseases and is the most critical constraint for the intensification of the poultry industry[2]. Hence the hygiene and safety of feed are of prime importance. Poultry feed contaminated with animal feces or other ecological niches such as soil, dust, and insects are the main vectors from which poultry feed acquire diverse microflora during harvesting, mixing, and storage. Therefore, all the feed ingredients, either chemical or nutritional, constitute the matrix of feed and the source of transmission of pathogens. The most common food-borne illnesses are salmonellosis and colibacillosis. However, other illnesses caused by poultry feed are staphylococcosis, pasteurellosis, streptococcosis, and mycoplasmosis, which rarely produce any severe infections[3]. Food-borne pathogens in feed, for instance, Salmonella, E.coli, Clostridium perfringens, Listeria monocytogenes, and Staphylococcus aureus, are measured as food protection hazards[4].

Salmonellosis is the most destructive disease of poultry because of its high morbidity and mortality ratio. It also has zoonotic importance through poultry meat and eggs transmitted to humans as food[5]. In humans, it may cause ulcers, vociferous diarrhea, and its toxins may also lead to septic shock[6]. Colibacillosis caused by virulent opportunistic strains of E.coli due to unhygienic measures by introducing contaminated feed and water in the flock is responsible for enormous monetary losses[7]. The pathogenic strain of avian E.coli shared some genes to Shiga toxigenic E.coli, a causative agent for fatal diseases in humans such as hemolytic uremic syndrome and hemorrhagic colitis[8].

Environmental factors, particularly season, play a fundamental part in propagating infections[9]. Percentage of humidity affects the frequency of pathogens and is steadfastly comparative to its expansion. Due to climatic changes, chances of horizontal spread in poultry sheds are also increased[10].

This study was intended to characterize the pathogens of poultry feed that are transferred to human beings through food series and cause severe food-borne illness. However, the bacterial load of feed concerning seasonal variation was also determined.

Methodology

Seventeen poultry farms situated in different areas of Karachi were recruited for this study. A total of 204poultry feed samples were assembled hygienically and shifted to the microbiology laboratory, University of Karachi, during the year 2015-2016.

As per the recommendation of ISO, serial dilution from 10-1 to 10-8 was arranged by mixing poultry feed samples (one gram) in normal saline water (9ml). Using the spread plate method, each dilution (1ml) was plated onto nutrient agar. After an incubation period of twenty-four hours, bacterial colony count was made and recorded (cfu/ml)[11].

Bacterial load determination was planned month-wise. The year was arranged quarterly from December to February, March to May, June to August, and September to November. For bacteriological evaluation, selective media: Tryptone Soya agar, Salmonella-Shigella agar, Blood agar, MacConkey agar, Mannitol Salt agar were used. The feed sample (1ml) was mixed into normal saline (9 ml) and incubated for 24 hours. Each medium was cultured with 0.1ml of inoculums and incubated for 24 hours. Colony recognition was made by colony characters[12]. Gram staining was used to examine and differentiate between gram-positive and gram-negative bacteria under microscope[13].

For bacterial identification, biochemical tests (Catalase test, Carbohydrate fermentation test, Voges Proskauer test, Citrate utilization, Urease test, Indole test, Methyl red, Oxidase test, Motility test, Hydrogen sulfide production) were

performed as given by Barrow et al.[14] with slight amendments.

The statistical connection between the seasonal variation and bacterial contamination was determined by ANOVA (one-way analysis of variance), chi-square, frequency, and percentage using SPSS (version 22.0).

Results

For bacterial load determination, 204 samples were evaluated all over the year. Considerable total viable count difference of bacteria with the p-value ($p<0.01$) was observed, whereas $F=26.887$ was analyzed in different seasons by ANOVA (Table 1 and 2).

Table 1: Total viable bacterial count with seasonal variation.

Seasons	Total viable bacterial count (Log10 CFUs/g)			95% Confidence Interval for Mean		
	Positive Samples	Minimum	Maximum	Mean±SEM	Lower Bound	Upper Bound
Dec-Feb	29	1.10	7.3	3.78±0.45	2.860	4.704
Mar-May	31	1.75	8.2	4.57±0.42	3.712	5.442
Jun-Aug	52	2.71	9.9	8.09±0.21	7.662	8.517
Sep-Nov	57	2.09	9.1	6.36±0.32	5.723	7.014

Table 2: ANOVA for CFUs log10/g levels in poultry feeds seasonally.

Groups	Df	Mean Square	F	p-value
Between Groups	3	146.089	26.887	0.000
Within Groups	165	5.433		

Significant variation ($p < 0.000$) of microbial incidence among seasons and the highest bacterial load (87.6%) was recorded in the summer season (Table 3).

Table 3: Seasonal bacterial distribution of feed samples.

Seasons	Total samples	Positive samples%	Chi-square	p-value
Sep-Nov	65	57(87.6)	19.472	0.000
Dec-Feb	45	29(64.4)		
Mar-May	40	31(77.5)		
Jun-Aug	54	52(96.2)		
Total	204	169(82.8)		

Based on bacterial morphological characters, E.coli, Salmonella enterica, Staphylococcus aureus, Bacillus subtilis, and Streptococcus were identified (Table 4 & Figure 1). Bacterial isolates were identified microbiologically using the gram staining method (Figure 2).

(a): S.enterica

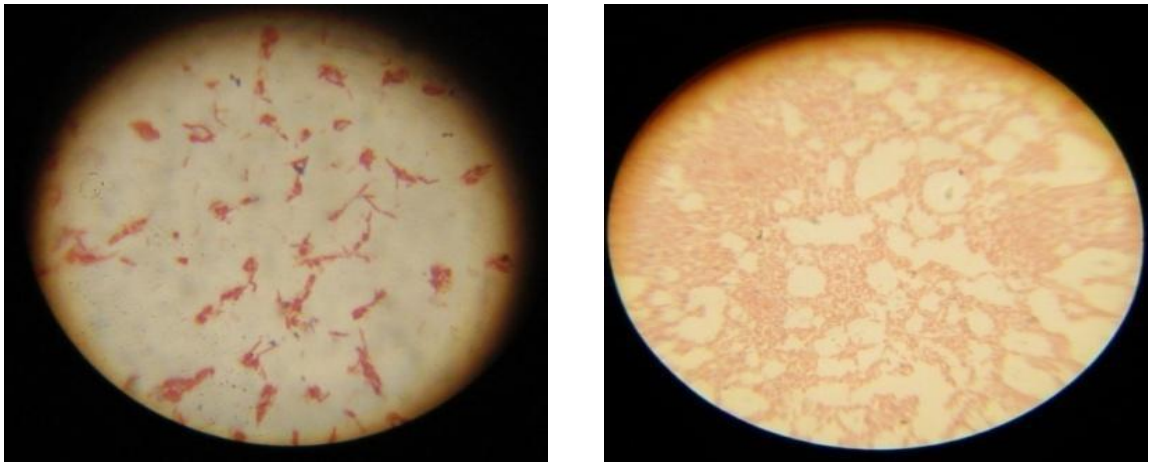
(b): E.coli

(c): B.subtilis

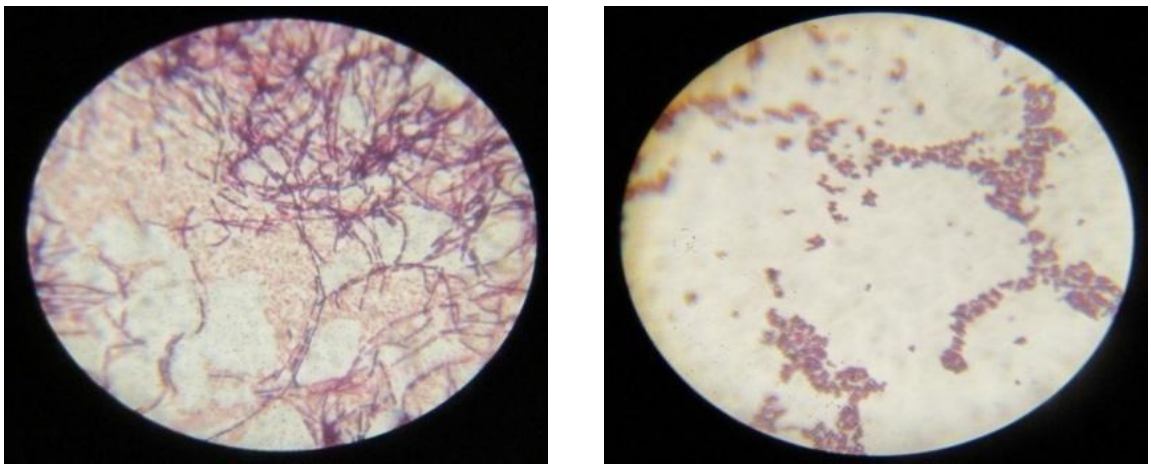
(d): S.aureus

(e): Streptococcus spp

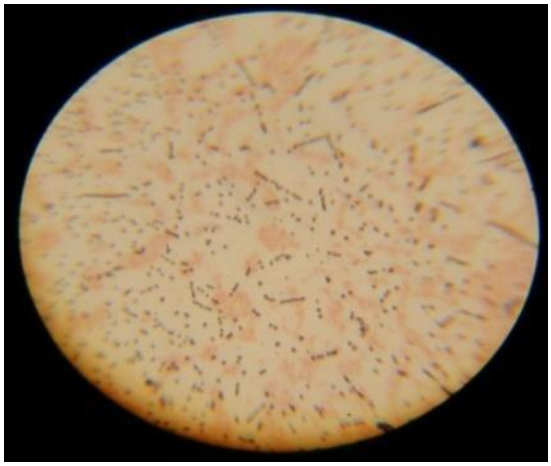
Figure 1: Cultural characteristics of bacterial isolates



(a): S.enterica **(b): E.coli**



(c) B.subtilis **(d): S.aureus**



(e) Streptococcus spp

Figure 2: Microscopic characteristics of bacterial isolates.

Table 4: Cultural and microscopic characteristics of bacterial isolates.

Isolates	Media	Macroscopic characteristics	Microscopic characteristics
Salmonella enterica	Salmonella-Shigella agar	Transparent and flat colonies with a dark core.	Gram-negative, short rod
E.coli	MacConkey agar	Pink colonies with surrounding darker pink area	Gram-negative, short rod
Staphylococcus aureus	Mannitol Salt agar	Tiny, golden-yellow colonies with yellow zones	Gram-positive cocci, cluster shape
Bacillus subtilis	Tryptone Soya agar	Smooth, big, asymmetrical colonies with lobate margins	G+, Rectangular shaped cells with square-cut ends with elliptic central spore give the shape of bamboo rods.
Streptococcus spp	Blood agar	Large zones of beta-hemolysis	Gram-positive, spherical cocci occur in the chain

Alleged colonies via conventional culture techniques and microscopic inspection were further confirmed by biochemical properties on the presence (+) or absence of bases criteria (Table 5)

Table 5: Biochemical Characters of bacteria.

Bacteria	IT	VT	MT	CU	CFT			HPT	UT	CT	OT	MT
					G	L	S					
Salmonella enteica	-	-	+	+	+	-	-	+	-	-	-	+
E.coli spp	+	-	+	-	+	+	+	-	-	-	+	+
Bacillus subtilis	-	-	-	-	-	-	-	-	-	+	+	+
Staphylococcus aureus	-	+	+	+	+	+	+	-	+	-	+	-
Streptococcus spp	-	-	-	-	+	+	+	-	-	-	-	-

G-Glucose; L-lactose; S-Sucrose; IT-Indole Test; VT-Vp Test; MT-MR Test; CU-Citrate Utilization; CFT-Carbohydrates fermentation Test; HPT-H2S production test; UT-Urease Test; CT-Catalase Test; OT-Oxidase Test; MT-Motility Test

Overall, for bacterial load, positive poultry feed samples were 82.8%, and negative were17.1%. Whereas bacterial seasonal frequency percentages are given in (Table 6). Which showed Salmonella enterica with the highest occurrence rate, followed by E.coli, Bacillus subtilis.

Table 6: Rate of occurrence of bacterial isolates.

Isolates	Dec-Feb	Mar-May	Jun-Aug	Sep-Nov	n	%
Salmonella enteric	10	14	22	20	66	39.05
E.coli	8	5	12	13	38	22.48
Bacillus subtilis	4	5	13	9	31	18.34
Staphylococcus aureus	3	4	3	9	19	11.24
Streptococcus spp	4	3	2	6	15	8.87
Total	29	31	52	57	169	82.8

Discussion

This study was planned to assess the quality of poultry feed to minimize the damage caused by food-borne illness to the poultry business and community health. By utilizing unhealthy poultry feed, numerous food-borne diseases have been found associated with it[15].

Salmonella enterica (39.05%), E.coli (22.48%), Bacillus subtilis (18.34%), Staphylococcus aureus (11.24%) and Staphylococcus spp (8.87%) were isolated by culture conventional method. These are classified by microscopic inspection and biochemical characters. These findings support Matthew et al.[16] and D'Mello rsearch[17], which stated the widespread of feed contaminants with the frequency of occurrence of Salmonella spp (31.1%), Escherichia coli (11.1%), Bacillus spp (11.1%), Lactobacillus spp (11.1%) and Staphylococcus spp (35.6%) respectively. A high incidence of food-borne pathogens was recorded in this study. As these pathogens have vital significance concerning food quality so this is an alarming scenario[18]. A similar study was conducted by Islam et al.[19]; they isolated Salmonella enterica 29.16% and E.coli 37.50% from poultry feed. During the last 25 years, the incidence of food-borne illness has been amplified due to which one-fourth population of the world is at momentous health threat[20,21]. Furthermore, climatic changes, storage, handling techniques, and shipping technologies affect the extent and variety of microbes.

In a recent research study, Bacillus subtilis may ascribe to probiotics for chick's growth enhancement[22]. Likewise, Pedroso et al.,[23] found Bacillus subtilis (probiotics) as a weight promoter in the broiler, improved the eggshell quality and facilitated feed conversion ratio in the layer. The prevalence of total coliforms recorded in feed, such as staphylococcus aureus, may be due to mishandling of the product[24]. However, these are opportunistic bacteria, but viral infection or immunosuppression can lead to fatal diseases in poultry like septicemia and osteomyelitis[25]. On the other hand, pathotypes of S.aureus may cause communal health problems by infecting the by-products of poultry[26,27]. Streptococcus may be attributed to normal flora, but it may lead to lethal diseases (endocarditis, peritonitis, salpingitis, septicemia, etc.) depending on species category and complimentary pre-disposing factors[28].

Microbial contamination prevailed throughout the year in the feed. However, the highest total viable count recorded was (8.09 ± 0.21) ×108 cfu/g from June to August due to high humidity and temperature. Likewise, Yunus et al.[29] and Anjum[30] indicated the highest bacterial contagion from April to September. Nasrin et al.[31] also documented the highest total viable count (6.5 ± 1.87)×105 cfu/gm in poultry feed due to environmental changes.

Conclusion

The prevalence of high levels of food-borne pathogens in poultry feed is a distressing condition. For standard quality and purity maintenance of feed, effective precautions and regular inspection by skilled manufacturers and health authorities are needed, especially during the alarming months, to avoid the public health hazard.

Conflicts of Interest

The authors have declared that no competing interests exist.

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