ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 4, pp: (254-259), Month: October - December 2018, Available at: www.researchpublish.com

Microbiological quality of commercially available poultry feeds from Chittor District

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Abstract: **This study was carried out to determine the microorganisms contaminating poultry feeds from Chittor District, Andhra Pradesh. A total of 10 feed samples of different feed types (Quails, Broilers, Layers, Sussex) were collected from sree venkateswara poultry farm. Samples were cultured in Nutrient Broth for isolation of other bacteria and then sub-cultured on Blood and MacConkey's Agars. A total of 3 isolates were obtained, some of the feed samples showed no growth. The isolates were identified according to their microscopic, cultural and biochemical properties. Gram negative bacteria included *Escherichia coli, Klebsiella pneumonia* and *Salmonella enteritidis.* The highest percentage of occurrence was obtained with *Escherichia coli* (35.6%). The fungi isolated were *Aspergillus niger, Aspergillus flavus,, Rhizopus sps, Mucor sps.* The highest bacterial and fungal count was obtained in Broiler feed 5.4 x 10^6 cfu/g and 8.1 x 10^4 cfu/g respectively for bacteria and fungi, while the least count was recorded in Sussex with the range 2.19 x 10^6 cfu/g (bacteria) and 5.7 x 10^4 cfu/g (fungi).**

Keywords: **Poultry feed, Microbial quality, Bacteria and Fungi.**

I. INTRODUCTION

The world consumption of poultry products: meat and eggs is remarkably increasing with increase in number of people and this is because of good quality and effective price. The wide spread of human consumption of poultry meat and eggs necessitates the control of microbial contamination. The safety of poultry products raises the importance of efforts that should be exerted towards evaluation and detection of microbial hazard, which represents a great risk to the consumer.

Poultry feed is considered as one of the important sources of contamination of poultry products [6]. The safety and quality of poultry feeds are currently of major concern in developed counties, that safety of feed is a fundamental requirement for all birds. Unsafe feed may lead to great economic losses.

Poultry feeds are formulated in order to meet the complex nutrient requirements of birds. Due to the simple digestive tract of birds and the intestinal flora making little contribution towards food digestion, it is necessary that poultry feed is complete and easily digestible [14]. Materials for formulation of feeds are sourced from different origin both animals and plants and are mostly agrowastes. However, most of these feed additives have been implicated amongst the principle sources of microorganisms of public health concern. Presence of microorganisms in the feed might be attributed to the normal flora of the feed, cross contamination. Accordingly, several poultry diseases with various pathological manifestations and of different origins: viral (*Avian influenza, Newcastle disease*), Bacterial (*Salmonellosis* and *infectious coryza*) and fungal origin (*Aspergillosis*, *Candidiasis* and *Histoplasmosis*) and also consumption of mycotoxins can cause immunosuppression[1].

The world feed manufacture and stock industries have faced severe food safety issues throughout the last two decades such as the outbreak of bovine encephalopathy (BSE) and Belgium dioxin crisis, which occurred in 1999 due to contaminated fat supplied to stock feed manufacture [6]. These incidents showed the importance of feed safety in ensuring the safety of human food. One of the major areas of concern in the bacterial contamination of poultry feed come from the

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stock feed, raw materials and farms [13]. There has been an increased focus on food as source of bacterial contamination of livestock production units and there are standard measures that every feed factory or industry should follow and produce high quality, efficiency and pathogen-free feed. Industry must accept greater share of responsibility for the quality and safety of poultry feed production [5].

II. MATERIALS AND METHODS

Collection of samples: Total of 10 samples of poultry feeds of Quails, Broilers, Layers, Sussex were collected from sree venkateswara poultry farm in Jowkupalle Village, Ramakuppam Mandal, Chittoor District, Ramakuppam, Andhra Pradesh.

Method For collection of samples: sterile plastic bags were used. A sample size of about 50 g was taken for each feed (Fig 1). The samples were immediately brought to the laboratory and processed.

Fig 1: Poultry feed collected from sree venkateswara poultry farm

(i)Q-Quails(ii)B-Broilers(iii)L-Layers(iv)S-Sussex.

Feed sample processing and isolation of microorganisms: Samples were processed according to Ogbulie et al. 1999[11]. One gram of each sample was homogenized in 9ml of sterile physiological saline, serial dilution carried out up to 10^{-7}dilution. One ml of the solution was inoculated onto already prepared and solidified MacConkey agar, Nutrient agar and potato dextrose agar (PDA) by spread plate method and incubated for 24 hours at 37ºC, PDA plates were incubated at 25ºC for 3-7 days. Using the viable plate count method colony forming units (cfu) was calculated.

After incubation period, discrete colonies were picked and sub-cultured on Nutrient Agar and incubated at 37° C for 24 h. Sub-culturing on Nutrient Agar was repeated for pure isolation. Smears were made before and after purification from each type of colony. The smears were dried into air, fixed by heating and stained by Gram's method .The stained smears were examined under microscope for bacterial cell morphology, arrangement and staining reaction.

Morphological Identification of bacterial and fungal isolates: Bacterial isolates were identified based on the cultural characters, morphological, Gram staining properties.

Fungal isolates were identified based on their morphological characteristics on PDA and Lactophenol cotton blue stain identification and compared with criteria in Barnett et al., 1979[3].Biochemical characterization of bacterial and fungal isolates: biochemical tests (indole test, methyl red test, voges- proskauer test, citrate utilization test, catalase test, coagulase test) and compared with criteria in bergey's manual of determinative bacteriology (1994). For biochemical characterization Himedia (KB001 and KB002) ready kit was used to carry out the above mentioned tests including sugar fermentation test(glucose, adonitol, arabinose, lactose, sarbitol, mannitol ,rhamnose and sucrose). Based the standard chart provided in the kit bacteria could be identified up to genus and species level.

III. RESULTS

Isolation of microorganisms: Out of 10 poultry feed samples, 3 isolates were obtained. The mean bacterial and fungal counts of the feed samples produced in sree venkateswara poultry farm with varying range are represented in Table 1, the highest mean bacterial count and fungal count was obtained in Broiler (5.4×10^6 cfu/g) and (8.1×10^4 cfu/g) respectively while the least was obtained in sussex (2.19×10^6 cfu/g) and (5.7×10^4 cfu/g) respectively.

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Table 1: Mean total bacteria count and fungi count of poultry feed samples.

Poultry Feed	Bacteria (Cfu/G)	Fungi (Cfu/G)
Quails	2.9×10^6	7.4×10^4
Broilers	5.4×10^6	8.1×10^4
Layers	4.2×10^6	7.1×10^4
Sussex	2.19×10^6	5.7×10^4

Morphological Identification of bacterial and fungal isolates:

Bacterial isolates were identified based on their Gram staining and cultural characters (Fig 2 and 3). Fungal isolates were identified based on their morphological characteristics,colour of the colony and spores on PDA and Lacto phenol cotton blue staining was used for microscopic confirmation of fungi(Fig 4 and 5).

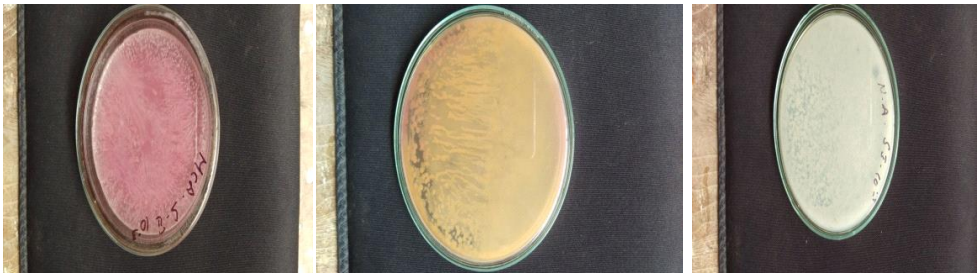

Fig 2: Colony formation on Mac conkey agar and Nutrient agar medium

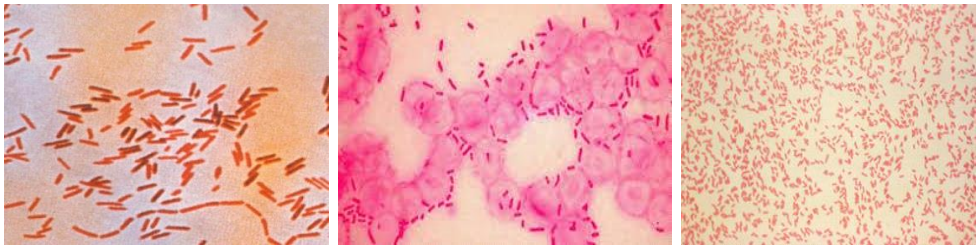

Fig 3: Phase contrast microscopic view of gram stained smears picked from Mac conkey and nutrient agar medium plates. (i)Klebsiella (ii)E.coli (iii) Salmonella

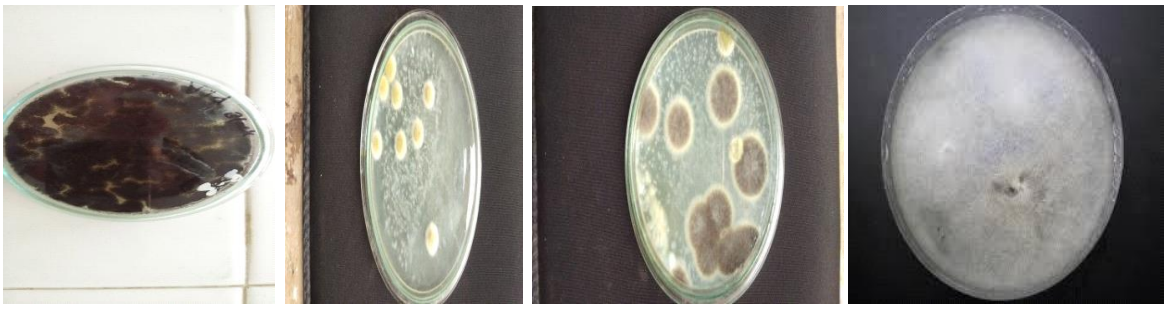

Fig 4: Fungal colonies obtained on PDA (i) *Aspergillus niger* (ii) *Aspergillus flavus* (iii) *Mucor sps.* (iv) *Rhizopus sps.*

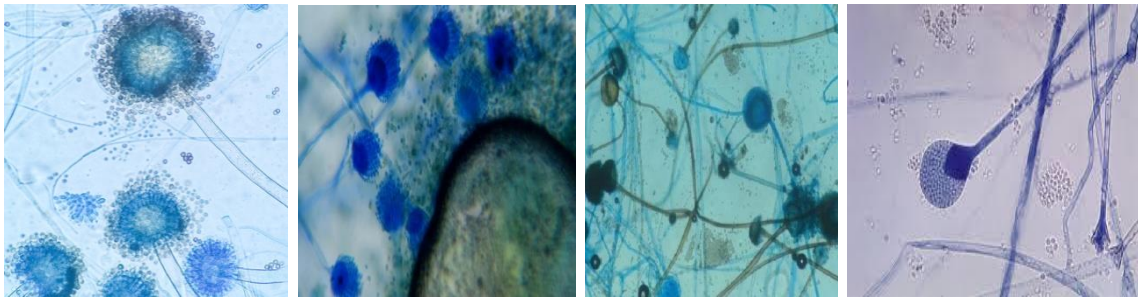

Fig 5: Microscopic view of fungi with Lactophenol cotton blue staining

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(i) ***Aspergillus niger*** **(ii)** ***Aspergillus flavus*** **(iii)** ***Mucor sps.*** **(iv)** ***Rhizopus sps.***

Biochemical test kit of himedia (KB001 ,KB002) used to confirm (indole test, methyl red test, voges- proskauer test, citrate utilization test, catalase test, coagulase test, sugar fermentation tests(glucose, adonitol, arabinose, lactose, sarbitol, mannitol ,rhamnose and sucrose. and compared with the standard chart for identification of bacterial isolates up to genus and species level(Fig 6 and Table 2).

Fig 6: Biochemical characterization of bacterial cultures using Himedia kits (KB001 and KB002)

(i) *Salmonella enteritidis* **(ii)** *Escherichia coli* **(iii)** *Klebsiella pneumoniae*

Table 2: Biochemical characterization of bacterial isolates

.No	Test	B1	B2	B3
1	Indole	-VE	V	-VE
2	Methylred	+VE	+VE	+VE
3	Vogesprouskar	-VE	-VE	-VE
4	Citrate Utilization	+VE	-VE	V
5	Glucose	+VE	+VE	+VE
6	Adonitol	-VE	-VE	+VE
7	Arabinose	+VE	V	+VE
8	Lactose	-VE	V	V
9	Sorbitol	+VE	V	V
10	Mannitol	+VE	+VE	+VE
11	Rhamnose	+VE	V	V
12	Sucrose	-VE	V	V

+VE – Positive , -VE- Negative, V- Slightly positive

The percentage occurrence of bacteria on the poultry feed samples from sree venkateswara poultry farm is represented in Table 3, which also presented the three genera of bacteria was isolated from the feed samples include- *Escherichia coli*, *Klebsiella pneumoniae, and Salmonella enteritidis* of which *Escherichia .coli* the highest frequency of occurrence (35.6%) while *Klebsiella pneumoniae, and Salmonella enteritidis* has the same frequency of occurrence (11.1%) which is the least.

Presented in Table 4 is the distribution of fungi in the poultry feeds, showing four isolated genera of which include *Rhizopus sps.,* has the highest distribution frequency (37.5%) while *Mucor sps.*, has the least distribution frequency (14.6%).

Table 3: Frequency of occurrence of bacteria in poultry feed samples.

Isolates	No of Samples Positive				Frequency	Frequency (%)
	Q	B	L	S		
Klebsiella pneumoniae	1	2	2	0	5	11.1
Escherichia coli	5	4	3	4	16	35.6
Salmonella enteritidis	2	0	2	1	5	11.1

Q-Quails,B-Broilers,L-Layers,S-Sussex.

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Table 4: Percentage occurrence of fungi in poultry feed samples.

Isolates	No of Samples Positive				Frequency	Frequency (%)
	Q	B	L	S		
Aspergillus niger	6	2	3	2	10	20.8
Rhizopus sps.	3	3	5	4	18	37.5
Mucor sps	0	4	3	0	7	14.6
Aspergillus flavus	0	6	3	4	13	27.1

IV. DISCUSSION

Most times poultry birds get infected through consumption of contaminated feeds, making the quality and safety of poultry feeds important part of poultry farming. All samples analyzed in this study showed the presence of microorganisms, which is an indication that poultry feeds serve as good growth medium for microorganisms owing to the nutritional quality. The result of this study may suggest that both bacteria and fungi might be implicated in health problems on the farm.

Most of the microorganisms isolated in this study have been associated with diseases of the poultry farm. Salmonellosis is caused by bacterium of the genera *Salmonella*, this infection is common in two weeks old chicks and ducklings, *Salmonella* gastroenteritis of human have been associated with consumption of infected birds, hence the infection of birds with *Salmonella* has been attributed to contaminated feeds [12].Isolation of *E. Coli* is a coliform is an indication of feacal material contamination which can be associated with poor hygiene.

D Mello (2006) described about the presence of microorganisms in poultry feeds vary due to climatic conditions, harvesting of raw materials, feed formulation process, storage and transport technologies employed[7]. *Bacillus sps.* and *Staphylococcus aureus* have been implicated by the studies of Dhand *et al.,* 1998[8] in the poultry farming microbial disease outbreak and reported about the beneficial effect of lactic acid bacteria on poultry feed.

The isolation of fungi genera (*Aspergillus*, *Mucor* and *Rhizopus*) which could be mycotoxigenic from the poultry feeds can be linked to cereal raw materials used in feed formulation, mycotoxins are economically important toxins which are immunosuppressive and can result to low poultry production. *Aspergillus sps.* can also cause aspergillosis in birds the presence of *Aspergillus sps.* in food should be of a concern [9]. *Rhizopus sps* and *Mucor sps* were the predominant fungi in several studies which might cause deterioration of the feeds ingredients making less nutrients available for the birds. Also some species of *Rhizopus* are mycotoxigenic. The least fungal count was obtained in Sussex value 5.7 x 10^4cfu/g, which exceeds the safety quality indicator of total fungi count less than or equal to 1x10^5 cfu/g [10]. The bacterial count was highest in broiler feed 5.4 x 10^6 cfu/g and least in Sussex at 2.19 x 10^4 cfu/g, The reported high microbial count in broilers feed is in line with the findings of Arotupin *et al.,* 2007 [2]and maybe depicts the level at which the ingredients used in the feeds production is contaminated, more so the use of agro wastes such as fish waste, cassava flour, bone meal, millet, lysine, maize, wheat offal, oyster shell, fish meal, groundnut cake, palm kernel cake, soya bean cake, brewery waste.

V. CONCLUSION

In the current study bacteria isolated are pathogenic and most fungi isolated are potentially toxigenic , making it a necessity to establish a quality control measures to be adopted during feed formulation, storage, use and equally educating local poultry farmers on how to apply this strategy and equally adopting the practice of incorporation of probiotics into feed. This will enhance poultry farm production and reduce the incidence of human diseases associated with poultry product consumption.

ACKNOWLEDGEMENTS

Authors would like to thank DST CURIE and Department of Applied Microbiology for the support.

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