

Original Research Article

<https://doi.org/10.20546/ijcmas.2023.1206.005>

Bacterial Load in Broiler Bedding Material and Antibiotic Effect on Broiler Farm

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ABSTRACT

Keywords

Broiler bedding
Material, antibiotic
sensitivity, bacterial
load, pathogen

Article Info

Received:

04 May 2023

Accepted:

05 June 2023

Available Online:

10 June 2023

In poultry farms, the mixture of bedding material, chicken excrement and feather seems to play an important role in pathogen development which may contribute spreading the potential risk of zoonosis disease through food chain and air. Which hinders the weight gain of broiler and increases their mortality rate. The aim of this study is to observe the bacterial load in the bedding material of the broiler farm, and the analyze the causes of uncontrolled mortality rate in which antibiotic sensitivity test is to be done by disc diffusion method and to reduce the mortality rate by concluding the antibiotic therapy. In the present study an effort was made to isolate, identify and characterize bacteria from the broiler bedding of an inhabitant of farmer's farm, Jaipur, Rajasthan, India, so as to correlate the role of these organisms as reservoirs in transmission of bacterial pathogens.

Introduction

Thousands of birds congested in an enclosed, warm and dusty environment is ideal for disease transmission. Poultry facilities cause stench by attracting flies, rodents and other pests which can cause nuisances and infectious diseases in the local area, it may represent a serious local problem, especially when large number of poultry birds kept in confinement area.

Poultry litter is a mixture of feces, wasted feed bedding material and feather hence its widespread use as manure worldwide because it is a rich organic

fertilizer. However its poultry litter can be contaminated with various types of pathogen including virus, bacteria, parasites and fungi.

Bacteria such as gram positive and gram negative or both are survive for months in water, soil and feed and risk of microbial contamination in poultry litter or bedding material, and this type of microbial contamination are reduce the poultry production and reduce weight gain in broiler birds and it promotes the mortality of poultry birds and harms the economy of poultry farm, and evidence suggest that poultry bedding air the main source of inside and outside air and soil pollution.

This study aimed to observe microbial contamination in bedding material of broiler poultry farm and systematic review the use of antimicrobial drugs in suspected diseased broiler farm and MDR (maximum drug resistance) in infected broilers. In addition to being essential for the treatment and prophylaxis of human infection, antibiotics are also widely applied in the food producing animal, which can serve as a reservoir of antibiotic resistant bacteria determinants that may be transferred to human (Marshall and Levy, 2011) subsequently the effectiveness of antibiotics in human decreases, resulting in treatment failure (Mellata, 2013).

In recent decade, broiler have grown in relevance as a meat source, about which the data of worldwide production of broiler meat is given in the figure below, (Statista.com, Global chicken meat production 2012-2022; Shahbandeh, 2022)

A chicken waste management system in which the several birds are left under a single roof and bring about mainly the droppings, along with a bedding material. These together form a litter and finally form compost. Birds are reared for 42 days at which they gain a marketable weight about 1.5-2.5 kg. The bedding material which is used is called as the poultry litter or broiler litter which is chiefly a mixture of poultry excreta, spilled food, feathers and the bedding material.

The poultry litter is mainly used for raising Broilers, Turkeys, Quails, and other birds. The common bedding materials include wood shavings, sawdust, peanut hulls, shredded sugarcane, straw, etc. The main function of the bedding material is to quickly absorb the faeces of birds and make themselves dry quickly.

Species wise meat contribution in India (source; basic animal husbandry and fisheries statistics, 2014). However 87% of poultry production is broiler production (FAO, 2010). In India broiler market 8% to 10% per annum, and Maharashtra is a largest producer of poultry meat in India.

Materials and Methods

Materials and sample collection

The data for this study were collected bedding material. Samples collected in different shade of poultry farm in sterile clean compartment and quantity of sample is 15 gm in each compartment, for detection of microbial contamination in bedding material, and post mortem sample collected in the transport media phosphate buffer saline for detection of antibiotic sensitivity in suspected broiler farm. It was been taken in these sample.

Procedure to calculate the bacterial load in bedding material

Some quantity of the collected sample was mixed with distilled water and the resulting bacterial suspension was saved in the laminar flow, after this, preparation of ten-fold dilutions of a bacterial suspension before conducting a viable count to find number of bacteria/ml in the original sample. The sample should be thoroughly mixed before sampling and a separate pipette should be used for each transfer step.

In this process, samples were collected from different bedding material from different sections of the broiler farm. After this, the collected sample was applied on the plate count media agar and the bacterial colony was grown by incubating for 37 °C for 24 hours, after this the bacterial colony was counted by the electric counter and 10⁹-9cfu/ml gram positive and gram negative both found bacterial load in bedding material of broiler farm. After this, antibiotic sensitivity test (ABST) was used to find out the reason for the rate of increase in mortality after antibiotic therapy in broiler farm and to observe the effectiveness of antibiotics, in this process sample of visceral organs of 9 broiler (29th day age) were collected during post mortem which include liver tissue, pericardial fluid, intestinal content, tracheal swab and straw color abdominal fluid. These sample of bacterial multiplication with the help of nutrient agar and after the multiplication process antibiotic sensitivity test are performed with

the help of Kirby-Bauer disc diffusion method, in which zone of inhibition was not obtained and this is the conclusion that both type of bacteria (gram positive and gram negative) are multiple drug resistant (MDR)

Experimental Protocol

The complete experimental protocol was divided into three parts: In our research protocol we analyzed twenty bedding samples (five samples from each farmer's farm sites and isolated seventy different bacterial colonies, on the basis of their specific characteristic features.

From different broiler bedding material samples, 25 different bacterial colonies were isolated from farm sites-1 matter, 15 were isolated from farm sites-2, 15 were isolated from farm sites-3 and 15 bacterial colonies were isolated from farm sites-4 (Table: 3.1). These isolated bacterial colonies were purified for further investigations.

These bacterial colonies were isolated from total bacterial population counted at 10^9 serial dilutions from broiler bedding material collected from different areas (Table- 3.2 & 3.3)

Studies clearly revealed that maximum types of bacterial colonies were observed from sites-1-4 sample whereas equal numbers of bacterial colonies were found in sample (Fig.). Seasonal variations such as summer, winter and rainy seasons also influenced the bacterial populations in broiler bedding material. As per our observations, maximum numbers of bacterial populations were found in rainy season in bedding material Site-1 (Table:2).

The quantitative analysis of the bacterial populations present in broiler bedding material indicated that the highest bacterial load (average of three seasons) was present in Sites-1 sample which registered 60.36×10^9 cfu/ml as compared to 50.23×10^9 cfu/ml, 46.3×10^9 cfu/ml and 32×10^9 cfu/ml in Sites-2 sample, Sites-3 sample and Sites-4 sample respectively (Table: 3.3).

Study Site

Survey was made to locate the sites of broiler bedding from different areas of Jaipur city. Their broiler bedding material were collected and further cultured in desired culture medium (enriched, selective and differential).

Collection of Broiler bedding material

Broiler bedding material samples were collected from known species of Broiler bedding. In systematic screening program for isolation of bacteria, broiler bedding materials were collected from different locations. Samples of fecal matter were collected from site 1-4 area of Jaipur (Northern Rajasthan). These all type of broiler bedding material were collected in different seasons, summer season i.e (March to June), rainy season (July to September) and winter season (October to February). The temperature of various season ranges from 45°C in Summer to 7°C in winter. (Fig.52, Plate)

The samples of fecal matter of Broiler bedding material were collected under sterile conditions. In order to avoid the contamination with environmental bacterial flora these were placed immediately inside sterile plastic containers and were later processed in laboratory for subsequent culture. Some portion of faeces was also preserved in laboratory at 4°C for further utilization.

Results and Discussion

70 colonies obtain in the 10^{-6} dilution. So the colony forming unit pr ml is; $\text{CFU/ML} = 126 \times 10^6$ or 1.26×10^8 . Based on the findings, the bacterial load in the bedding material samples of broiler farm was found to be approximate 10^9

Procedure of antibiotic sensitivity test

ABST is a laboratory test, in which determine how effective antibiotic therapy is against a bacterial infection, this test will control the use of antibiotics in clinical practice. The Kirby-Bauer disc diffusion

method is a flexible and relatively inexpensive technique that is commonly used.

Both gram positive and negative bacterial colony was obtained on the nutrient agar with the help of Plate inoculation technique from the collected broiler post mortem sample. With a sterile inoculating loop, a sample of the specimen (the inoculum) is spread over a small area at the edge of the plate. The loop is sterilized by flaming before inoculation of each area, after inoculation on the each plate the loop should be sterilized by flaming on completion of inoculation to ensure that no pathogens survive on the loop. After the completion of the inoculation of each sample, the plates are placed in an incubator at 37°C temperature for 24 hours.

Antibiogram Test

The 12 hour bacterial culture was spread on Mueller Hinton agar (MHA) using a sterile swab. The plates were dried and then the antibiotic disc were placed by means of a sterile forceps and incubated at 37°C overnight. Clear zone around the antibiotic disc indicated that the organism is sensitive. The diameter of each zone of inhibition was measured, recorded and interpreted according to the zone size interpretative charts (CLSI Approved Standard M173).

This antibiotic disc are used in the ABST test, After placing the antibiotic disc, the plate is placed in an incubator at 37°C temperature for 24 hours and after the time is over, the plates is taken out and the diameter of the zone of inhibition is measured in mm with the help of a scale. Both gram positive and gram negative bacteria were cultured for antibiotic sensitivity test from broiler post mortem samples. In which we found that the cultured both type bacteria show the condition of MDR (multiple drug resistance) along with the antibacterial drugs. In this test not found the zone of inhibition.

The study shows that poor standardization of poultry housing means poor air quality and poor management of bedding material, this study show

that higher microbial load in bedding material impact on the broiler production and increase mortality rate in the poultry farm. The poultry litter used in the present study contained 10^9 aerobic bacteria per gm of material as determined by CFU on plate count agar. The level of total bacteria in our samples were similar than those detect by Jingrang *et al.*, (2002) and higher detected by Martin and Mccann (Microbiological survey of Georgia poultry litter. J. Appl. Poult. Res. 7:90-98.) in which total bacterial counts range between 1,200 and 8.4×10^7 .

Also antibiotic sensitivity test result were found MDR (multiple drug resistant) both gram positive and gram negative bacteria, which indicates antibiotics being used in poultry farm are not effective on bacteria and effect on economy, as unspecified treatment or growth promoter. Misuse of antibiotics can increase generation of antibiotic resistance gene in bacteria that infect poultry farm and can lead to higher cost of treating infection farms due to lake of antibiotic that can be used to treat multidrug- resistant strains. Can be done for bacteria, as well as it can affect human health.

The present study recommended that antibiotics not be used as growth promoter, that infected farm be tested for antibiotic susceptibility before any antibiotic are given as treatment, and that antibiotics use to protect human health keep in mind the timing of withdrawals. We should seek other ways to treat microbial infection such as the use of natural products.

Antibiogram Test

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Table.1

Chicken meat production worldwide from 2012 to 2022(in 1,000 metric tons)		Consumption volume of poultry meat in India from 2013 to 2022 (in 1,000 metric tons)	
Year	Production in thousand metric tone	Year	Consumption in thousand metric tone
2022	100,984	2022	4,243.74
2021	100,547	2021	4,207.2
2020	99,264	2020	3,985.7
2019	97,3290	2019	3,757.3
2018	92,767	2018	3,780.3
2017	93,665	2017	3,550.6
2016	92,262	2016	3,438.04
2015	91,552	2015	3,283.7
2014	86,786	2014	3,264.96
2013	84,469	2013	3,873.85
2012	83,267	Source; Consumption of Poultry Meat In India 2013 -2022 ; Published By A. Minhas , Nov 2, 2022	

Table.2

Sample characters :- (age of the broilers is 29 th day)	
Nasal swab	Liver tissue
Tracheal swab	Intestinal swab
Pericardial fluid	Intestinal content
Straw color glutinous fluid in abdominal cavity	

Table.3 Number of Isolated Bacterial Colonies from Different broiler bedding material

S. No.	Habitat	No. of Fecal Samples Examined	Types of isolated Bacterial Colonies
1	Sites-1	5	25
2	Sites-2	5	15
3	Sites-3	5	15
4	Sites-4	5	15

Table.4 Seasonal variation in bacterial population

S. No	Sites of broiler bedding material Collection	Seasonal Variation In Bacterial Population In One gmbroiler bedding material (Cfu/MI)		
		Summer	Winter	Rainy
1	Sites-1	45.6x10 ⁹	42.30x10 ⁹	93.3x10 ⁹
2	Sites-2	35.7x10 ⁹	31.70x10 ⁹	83.2x10 ⁹
3	Sites-3	32.6x10 ⁹	27.80x10 ⁹	78.6x10 ⁹
4	Sites-4	22.1x10 ⁹	20.30x10 ⁹	53.5x10 ⁹

Table.5 Quantitative Analysis of Bacterial Population Fecal Sample from Different Sites

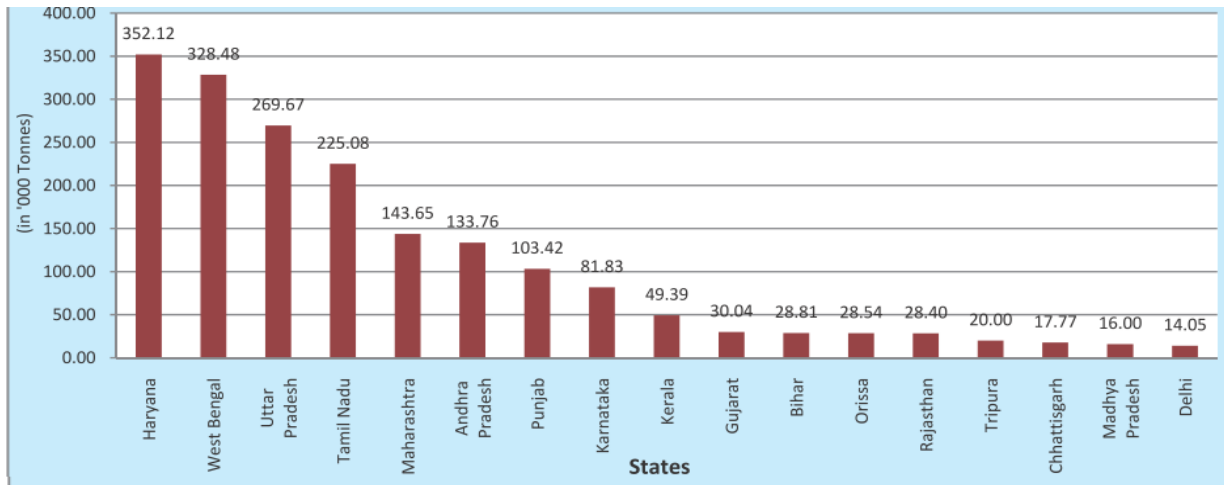
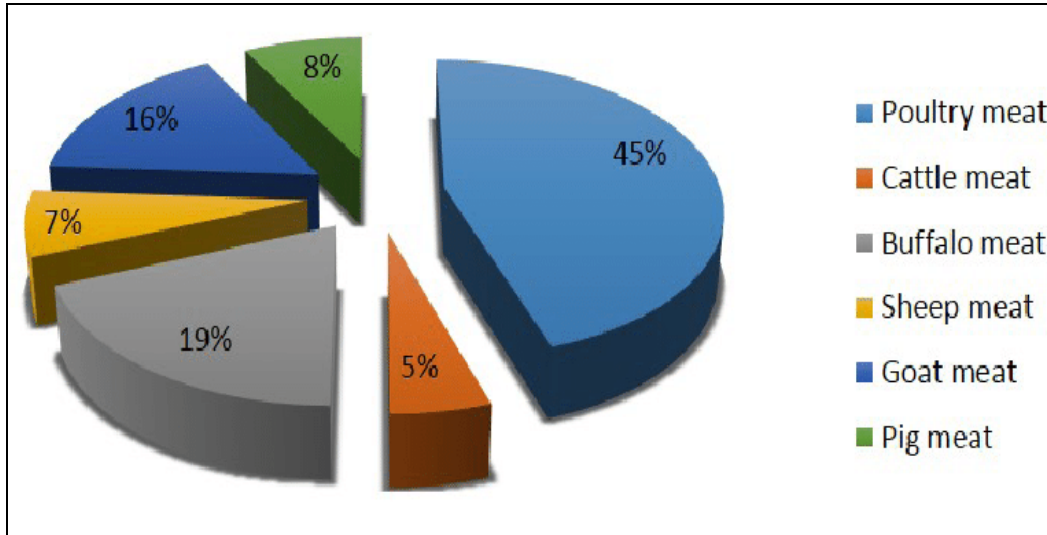
S. No	Sites Of broiler bedding material Collection	Total Average Bacterial population (cfu/ml)
1	Sites-1	60.36×10 ⁹
2	Sites-2	50.23×10 ⁹
3	Sites-3	46.3×10 ⁹
4	Sites-4	32×10 ⁹

Table.6 Zone diameter interpretive standards chart for the determination of antibiotic sensitivity and resistance status by the Disk Diffusion method)

Name of antibiotics (dose)	Abbreviation	Sensitive (S)	Moderately sensitive (MS)	Resistant zone of inhabitation
Amikacin	Ak	≥ 17	15-16	14
Amoxicillin	AMX	≥ 25	19-25	22
Ampicillin	Amp	≥ 17	14-46	13
Ceftriaxone	CTR	≥ 25	20-22	19
Vancomycine	VA	≥ 20	14-20	13
Ciprofloxacin	CIP	≥ 21	16-20	15
Enrofloxacin	EX	≥ 24	18-22	20
Erythromycin	E	≥ 23	14-22	13
Gentamicin	GEN	≥ 15	13-14	12
Nalidaxiac acid	NA	≥ 19	14-18	13
Ofloxacin	OF	≥ 18	15-17	14
Oxytetracycline	O	≥ 28	22-28	22
Penicillin G	P	≥ 29	22-28	28
Roxithromicin	RO	≥ 30	22-30	28

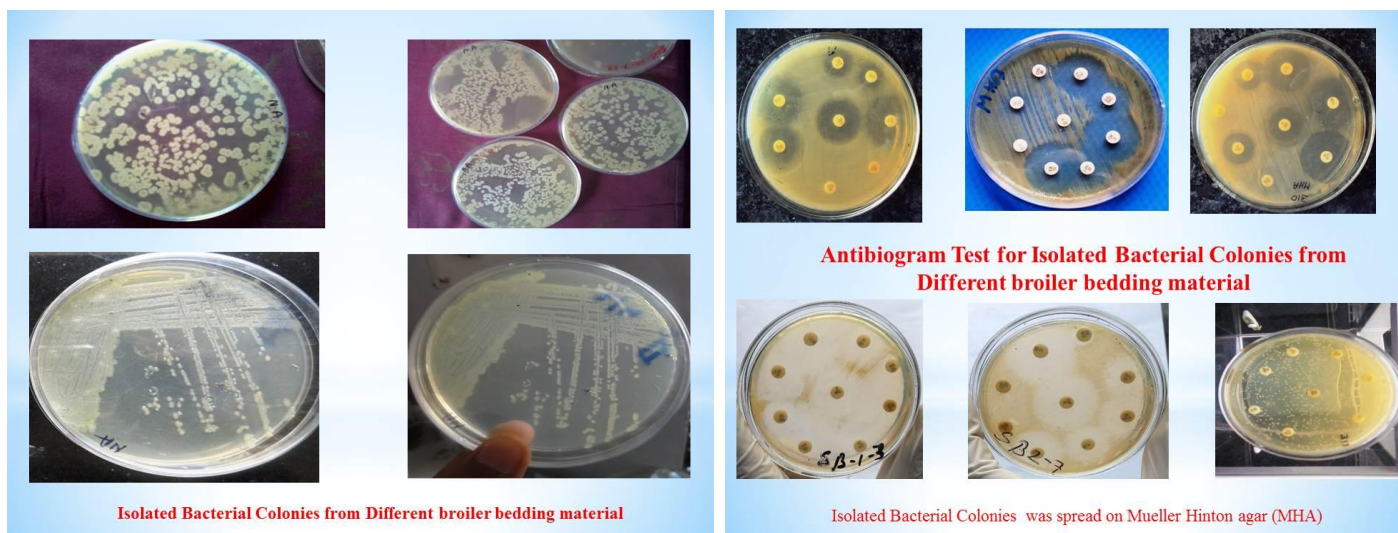
Abbreviation	Antibiotic	Disclod (microgram)	Abbreviation	Antibiotic	Disclod (microgram)
CFM	Cefixime	10	NX	Norfloacin	10
CF	Cefalotin	30	TE	Tetracycline	30
AN	Amikacin	30	P	Penicillin g	10
ENX	Enrofloxacin	5	AMX	Amoxicillin	30
OT	Oxytetracycline	30 iu	LEV	levofloxacin	5
AMP	Ampicillin	10	S	Streptomycin	10
OF	Ofloxacin	5	C	Chlormphenicol	30
VA	Vancomycine	30	GEN	Gentamicine	10
Dx	Doxycycline	30	N	Neomycin	30
Cxm	Cefuroxime	30	Azm	Azithromycine	30

Fig.1



Graph shows the percentage achievement of meat production against the target set by the various states. The states like Bihar, Rajasthan, Karnataka, Punjab, Tripura, Orissa, Uttar Pradesh, Andhra Pradesh (includes Telangana), Madhya Pradesh, Lakshadweep, Haryana, Maharashtra, Puducherry, and Sikkim have achieved the target for production of meat during 2013-14.

Fig.2 Plate showing single isolated colony & ABST Test against bacterial colony



References

- Al-Zenki, S., Al-Nasser, A., Al-Safar, A., Alomirah, H., Al-Haddad, A., Hendriksen, R.S., Aarestrup, F.M. Prevalence and Antibiotic Resistance of Salmonella Isolated from a Poultry Farm and Processing Plant Environment in the State of Kuwait. *Foodborne Pathog. Dis.* 2007, 4, 367–373.
- Brooks, J.P. McLaughlin, M.R. Adeli, A. Miles, D.M. Cultivation and qPCR Detection of Pathogenic and Antibiotic-Resistant Bacterial Establishment in Naive Broiler Houses. *J. Environ. Qual.* 2016, 45, 958–966.
- Bryan Markey, Finola Leonard, Marie A. Ann Cullinane, Dores Maguire. *Clinical veterinary microbiology*, second edition, 2013 Elsevier Ltd. ISBN 9780723432371
- Jingrang Lu, Susan Sanchez, and Margie D. Lee. 2022. Evaluation of broiler litter with reference to microbial composition as assessed by using 16s rRNA and functional gene markers, PMID 12571010, 25. <https://doi.org/10.1128/AEM.69.2.901-908.2003>
- Marshall Bonnie, Stuart B Levy 2011 Food animals and antimicrobials: impacts on human health. *Clin.Microbiol.Rev.* 2011 Oct;24(4):718-33. <https://doi.org/10.1128/CMR.00002-11>
- Martin and Mccann Microbiological survey of Georgia poultry litter. *J. Appl. Poult. Res.* 7:90-98. <https://doi.org/10.1093/japr/7.1.90>
- Mellata M. 2013. Human and avian extra intestinal pathogenic Escherichia coli: infections, zoonotic risks, and antibiotic resistance trends. *Foodborne Pathog Dis* 10:916–932.
- Shahbandeh, 2022 Livestock and Poultry: 2022 World Markets and Trade Foreign Agricultural Service/USDA 18 April 2023 Global Market Analysis United States Department of Agriculture Foreign Agricultural Service April 11, 2023.
- Statista.com, Global chicken meat production 2012-2022 ; published by M. Shahbandeh, Apr13, 2022

How to cite this article:

Harshil Kuldeep, Hariom Kumawat, Sandeep Kumar and Sushil Kumar Barolia. 2023. Bacterial Load in Broiler Bedding Material and Antibiotic Effect on Broiler Farm. *Int.J.Curr.Microbiol.App.Sci.* 12(06): 38-45. doi: <https://doi.org/10.20546/ijcmas.2023.1206.005>