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Isolation, Identification and Characterization of Poultry Associated Bacterial Pathogens

S. M. Shakira Begum [a++*], Amthul Azeez [b#] and K. Kavitha [c†]

[a] Department of Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai, Tamil Nadu, India.
[b] Postgraduate and Research Department of Zoology, Justice Basheer Ahmed Sayeed College for Women, Chennai, Tamil Nadu, India.
[c] Department of Microbiology, Madras Christian College, Chennai, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author SMSB designed the study, analysis of samples, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AA and KK managed the analyses of the study, supervised sample analysis, planning of the experiment, and interpretation of the data. All authors read and approved the final manuscript.

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ABSTRACT

In order to meet consumer demand, the global poultry business is constantly expanding; hence it is crucial to ensure the bio-safety of the poultry farms. In poultry farms, the main sources of bacterial development, increased antibiotic resistance, and environmental dispersion may be poultry feed,

[++] Research Scholar, Postgraduate and Research Department of Zoology, and Assistant Professor;
[#] Principal, Head and Associate Professor;
[†] Associate Professor;
**Corresponding author: Email: shaki.mb@gmail.com;*

litter, and water. The focus of this research is on the identification and characterization of bacterial pathogens found in commercial poultry farms, drinking water samples, feed samples, and litter samples. The 100 samples were gathered from chicken farms in different districts of Tamil Nadu and transported to the lab for additional examination. Serial dilution was used to analyse the samples, which were then examined for colony morphology, microscopic features, and the biochemical traits of the bacteria *Escherichia coli*, *Pseudomonas* spp., *Proteus* spp., *Salmonella* spp., *Shigella* spp., *Bacillus* spp., *staphylococcus* spp., and *Streptococcus* spp. The disc diffusion method was used to evaluate bacterial isolates against various antibiotic drugs. The results of this research indicate that the main sources of bacterial infections are chicken feed, drinking water samples and poultry litter. *Salmonella*, *Pseudomonas*, and *E. coli* prevalence rates were 94%, 80%, and 75%, respectively. Chloramphenicol and Tetracycline both had overall antibiotic resistance rates of 65.65% and 59.23%, respectively. Public health is alarmingly threatened by the presence of these harmful microorganisms in poultry habitats. To reduce the chance of transmitting the bacterial diseases linked to poultry, strict hygiene procedures must be put in place. The use of antibiotics in chicken for promoting growth and illness prevention needs to be properly regulated and executed.

Keywords: Antibiotic; antimicrobial resistance; bacterial pathogens; disc diffusion; poultry feed.

1. INTRODUCTION

Humans are at risk of contracting food-borne illnesses as a result of contaminated poultry products caused by bacterial infections. Food-related illnesses caused by germs like *Salmonella* spp. and *E. coli* are spread through poultry meat [1]. The elements that affect the contagious transmission of bacterial infections must be taken into consideration in order to prevent them from emerging from poultry habitats. Poultry feed (starters, growers, finishers, and layers) and drinking water from poultry farms are contaminated during processing [2], storage while adding the raw material ingredients, and ultimately during exposure of the product to the environmental microbes [3,4].

The primary worry of industrialised nations is the safety of drinking water and chicken feed in poultry farms. All birds' basic needs are food and water, and contaminated feed results in significant economic loss [5]. The abundance of different bacteria in chicken diets depends on a number of variables, including the nutrient makeup of the feed, pH, oxygen tension, and water activity. According to Aisha Ali [6] and [7], microbial proliferation can reduce feed quality by replacing the nutrients in the feed, and damage done during feed processing or the creation of toxins have a detrimental influence on animal health [8]. The safe use of poultry products increases the length of time that must pass before microbiological contaminants are assessed and exposed, which causes the customer undue harm [9]. According to Osei et al. [10], the material used to create poultry beds, feather waste, manure, and leftover feed are the main sources of litter utilised by poultry. Bacterial infections can spread through leftover poultry. The expanding population and the commerce in animals are under increasing health and ecological hazard [11]. Numerous illnesses in poultry are caused by bacterial infections such *Escherichia coli, Staphylococcus,* and *Bacillus* species. Although research is being done to repurpose chicken waste material for agricultural and feed additives for other animals [12], efficient poultry waste disposal practises are not currently in practise in developing countries [13,14].

The use of antibiotics in animal feed at low doses for extended periods of time may contribute to microbial resistance. Due to the risk of resistance genes propagating to the environment, it is possible for resistant bacteria to enter sewage systems through chicken waste [15,16]. As a result, it was suggested that the current research work isolate, characterise, and identify the isolated pathogenic bacteria linked to poultry as a source of the potential contamination in poultry farms [17].

2. MATERIALS AND METHODS

2.1 Sample Collections

Six chicken farms in the Tamil Nadu state of India's Kancheepuram, Namakkal, Salem, Coimbatore, Cuddalore, and Thiruvallur districts provided samples of poultry litter, poultry water, and commercial feed. Litter samples from chicken farms were chosen at random, and feed and water samples were gathered directly from

the chicken farms' feeding operations. In total, 100 samples were acquired from each farm, including at least 6 litter, 6 feed and 6 water samples. The samples were taken in a sterile bag and container, and they were clearly labelled.

2.2 Analysis of Samples

The Mathan et al. [18] protocol was followed for the isolation and subsequent identification of the bacterial pathogens. 1 g of poultry litter, 1 g of chicken feed, and 1 ml of water sample were inoculated into a 100 ml nutritional broth and then incubated for an additional 24 hours at 37°C. All of the samples that were obtained underwent a total bacterial count on nutrient agar plates before being serially diluted at concentrations of 1 in 10. After 24 hours of incubation, the colony formation is expressed in CFU/mg using a colony counter [19].

2.3 Characteristics of Isolated Bacteria

2.3.1 Gram's staining

All bacterial strains had their cell walls stained in order to observe the morphology of their cell walls [20].

2.3.2 Colony morphology

After 24 hours of incubation, the colonial morphology of a bacterial culture including its size, shape, colour, and texture was inoculated into different media [20,21].

2.3.3 Biochemical tests

Biochemical tests such as the catalase test, oxidase test, citrate utilisation test, Methyl red test, Indole test, Voges-proskauer test, triple sugar iron test, and carbohydrate fermentation test were carried out using isolated bacterial strains that displayed distinctive colony morphology [22,23].

2.4 Testing for Antimicrobial Susceptibility

To assess the susceptibility of isolated bacterial strains to the chosen antibiotics, the disc diffusion method (Kirby-Bauer) was used [24,25]. On freshly made, sterile Mueller-Hinton agar plates, the 24-hour nutrient broth culture of bacterial isolates was compared with MacFarland standard (0.5M) turbidity aseptically swabbed. The chosen antibiotic discs were then put on Mueller-Hinton agar and incubated for 24 hours at 37 degrees Celsius. Cefotaxime (30 mg), ampicillin (10 mg), chloramphenicol (30 mg), vancomycin (5 mg), tetracycline (30 mg), erythromycin (15 mg), Nitrofurantoin (300 mg), and penicillin G (10 mg) were the antimicrobials selected for the current study. Following incubation, the published CLSI was used to record the zone of inhibition values derived from the observations.

2.5 Statistical Analysis

The DMRT (Duncan's Multiple Range Test) was used in the statistical analysis with the SPSS (Statistical Package for the Social Sciences) (Version 20.0) level (5%) package to calculate the significant difference in resource allocation across various management groups. Values are presented as significance (P 0.05) [26].

3. RESULTS AND DISCUSSION

The goal of the current research was to identify potential sources for the transmission of dangerous bacteria to the environment, including the existence of bacterial pathogens as pollutants in poultry feed, litter, and water from poultry feeding operations. Using varied and selective culture media, 6 samples from each farm were evaluated. There were 6 different poultry farms involved.

Table 1. Represent the total plate count of bacterial isolates for the obtained samples (commercial Feed, poultry litter and water)

S. No	CFU X Dilution factor
1	$3.9x10^{9}$
2	$3.5x10^{9}$
3	$5.5x10^{8}$
4	$2.5x10^{8}$
5	$3.2x10^{10}$
6	$1.5x10^{8}$
7	$3.5x10^{9}$
8	$4.9x10^{8}$
9	$3.2x10^{9}$
10	$3.8x10^{9}$

3.1 Bacterial Isolates' Characterization

The collected bacterial isolates were subjected to various culture medium and biochemical characteristics (Table 2), which were chosen based on colony morphology, growth, and number of biochemical tests.

Table 2. Morphological identification of bacterial isolates

Morphological characterization of isolated bacteria	Gram staining	Shape	organism
Green pigment-producing colonies	Gram –ve	Rod	*Pseudomonas* spp
Pink, convex with smooth-edged colonies	Gram –ve	Rod	*Escherichia coli*
Colonies with irregular margins, flat and white colour	Gram +ve	Rod	*Bacillus* spp
Colonies were white and swampy	Gram –ve	Rod	*Proteus* spp
Black, convex colonies with Smooth edges	Gram –ve	Rod	*Shigella* spp
Creamy, round, convex smooth Edged colonies	Gram –ve	Rod	*Salmonella spp,*
Shiny, spherical colonies on blood agar	Gram +ve	Cocci in chains	*Streptococcus* spp
Creamy and convex with Smooth edged colonies	Gram +ve	Cocci in clusters	*Staphylococcus* spp

Table 3. Bacterial pathogens distribution as sample wise

Sample	Bacterial pathogens
Poultry feed	*Pseudomonas* spp, *E. coli*, *Salmonella* spp, *staphylococcus* spp, *Vibrio* spp, *Shigella* spp and *Proteus* spp.
Poultry litter	*Salmonella* spp, *Pseudomanas* spp, *E.coli, Streptococcus* spp, *Bacillus substilis* and *Shigella* spp
Water sample	*Pseudomonas* spp, *Shigella* spp. *Salmonella* spp and *Vibrio* spp

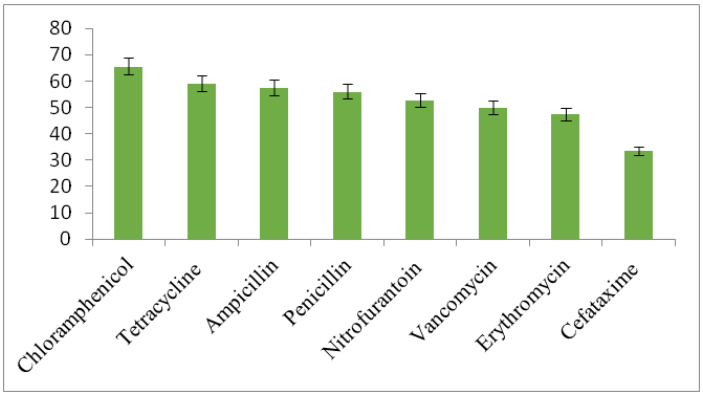

Fig. 1. Antibiotic resistance among the bacteria isolated from poultry

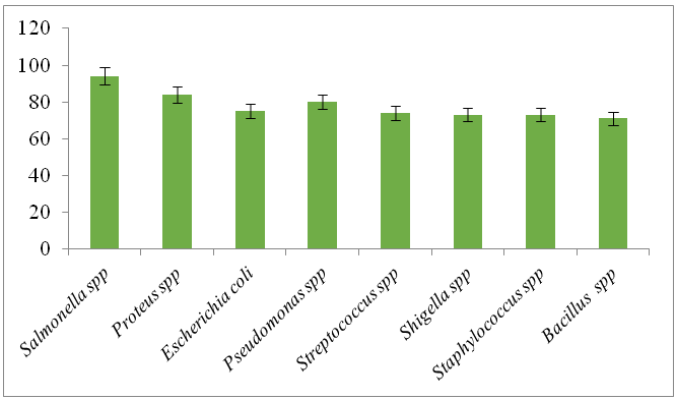

Fig. 2. Prevalence of antibacterial resistance among bacterial isolates

3.2 Identification of Bacterial Isolates

The isolated bacteria were identified by the routine biochemical test and distribution of bacterial isolates according to sample is given in Table 3. The overall prevalence of *Salmonella, Pseudomonas* and *E. coli* were recorded at 94%, 80%, and 75%, respectively.

3.3 The Trend of Bacterial Isolates' Antibiotic Resistance

Based on the susceptibility of the bacterial isolates, the chosen antibiotics showed a range of action, antibiotic resistance prevalence is also identified Chloramphenicol and Tetracycline both had overall antibiotic resistance rates of 65.65% and 59.23%, respectively (Figs. 1, 2).

Salmonella showed the highest antibacterial resistance among the tested bacterial isolates for the prevalence of antibiotic resistance. The presence of these resistant bacterial pathogens in commercial poultry feed, litter, and water is a concern for global health. To improve hygienic procedures in commercial poultry industries, the feed and water used in poultry farms must be stored in clean, closed bags to prevent the spread of bacterial pathogens to people and the environment. The appropriate removal of chicken waste, on the other hand, is a poultry worker's primary responsibility in order to reduce the risk of the spread of resistant bacteria to the general populace. The use of antibiotics by poultry for the prevention of sickness and the stimulation of growth is rigorously regulated in order to manage this scenario among the poultry sectors.

Gram-positive and Gram-negative bacteria were found in the environment, and the total viable counts (TVCs) of the isolates in the reproductive organs were much greater than those in the chicken feeds. Against eight antibiotics, the majority of the isolates (33.33%) were sensitive, hypersensitive (28.13%), resistant (25.0%), and intermediate (13.33%). Three bacteria, including *Sphingobacterium daejeonens*, *Bacillus cereus*, and *Bacillus* sp., were identified [27,28]. Faecal samples from 19 isolates, on the other hand, revealed 100% sensitivity to ciprofloxacin, gentamicin, and tetracycline, 53% resistance to erythromycin, and 47% resistance to streptomycin [29]. Recently, it was discovered that chicken flesh was contaminated with *Salmonella enterica* and *E. coli*, with the former species showing 100% resistance to augmentin and amoxicillin and 93% resistance to tetracycline [30].

Salmonella, *Lactobacillus*, and a number of lactic acid bacterium species' antibiotic sensitivity patterns were examined in broiler chicken faeces, poultry feed and 21 day old chicks, respectively [31]. Moreover, antimicrobial sensitivity profiles of seven strains of Bacillus species using 12 antibiotics were investigated, where all strains were resistant to bacitracin but were susceptible to gentamycin, neomycin, ormethoprim, triple sulfa and spectinomycin [32,33], while *Clostridium perfringens* from poultry sources was found resistant to gentamycin, streptomycin, kenamycin and tetramycin [34]. Due to the potential spread of multi-drug resistant (MDR) bacteria from birds to people, antibiogram profile investigations of bacterial isolates from various poultry sources have recently attracted a lot of attention [35]. In Bangalore, India, *E*. *coli* isolates from dead poultry birds showed maximum resistance to nitrofurazone (90.77%), followed by tetracycline (83.08%) and cotrimoxazole (76.92%), but the bacteria was extremely sensitive to ciprofloxacin and enrofloxacin (83.08%), chloramphenicol (81.54%), pefloxacin (76.92%), and norfloxacin (75.39%) [36,37].

4. CONCLUSIONS

Six different places the 5 samples isolated were isolated through gram staining confirmed that gram +ve and –ve bacteria, through colony morphology analysis, various biochemical tests confirmed organisms *Pseudomonas* spp, *Escherichia coli*, *Bacillus* spp, *Proteus* spp, *Shigella* spp, *Salmonella* spp, *Streptococcus* spp, *staphylococcus* spp. overall prevalence of *Salmonella, Pseudomonas* and *E. coli* were recorded at 94%, 80%, and 75%, respectively. Antibiotic resistance prevalence is also identified Chloramphenicol and Tetracycline both had overall antibiotic resistance rates of 65.65% and 59.23%, respectively. We recommended that poultry farms regularly checked for the presence of bacterial pathogens, microbial infections, and to examine regularly for bio safety, may be to reduce the spread of bacterial infection and resistance genes from poultry sources. The current study concluded that regular microbial checking very essential to avoid spreading bacterial diseases among the chicks, chickens, and humans.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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