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Study Of Antibiotic Resistance In Bacteria Isolated From Poultry Fecal Sample.

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ABSTRACT

Antibiotic-resistant bacteria in gut of the poultry birds are the result of excessive antibiotic use. Gut flora of the animals is showing resistant to several antibiotics and contaminating the environment through the excrement of fowl. The present study was aimed to isolate, characterize, and assess the antibiotic resistance profiles of bacteria obtained from poultry fecal samples. 14 bacteria were isolated from poultry fecal sample using Nutrient agar, EMB and Cetrimide agar. The antibiotic resistance pattern of 14 isolated bacteria against a panel of 8 poultry therapeutically relevant antibiotics like Ciprofloxacin, Cefixime, Cotrimoxazole, Gentamicin, Cefotaxime, Chloramphenicol, Amoxicillin and Nitrofurantoin revealed that 6 bacterial isolates were found to be resistant to 5 or more antibiotics studied and thus were found to be multidrug resistant bacteria. Thus, 92% isolates were resistant to chloramphenicol, 85% were resistant to Amoxicillin and Nitrofurantoin, 71 % were resistant to Cotrimoxazole and 42% were resistant to Ciprofloxacin and Cefixime. Morphological and biochemical characterization revealed that they may belong to *E. coli*, *Pseudomonas* sp. and *Alcaligens* sp. Thus, present study suggests for the responsible use of antibiotics in poultry farming and consideration of alternative infection control measures to minimize the emergence and spread of antibiotic-resistant microorganisms and antibiotic-resistant genes.

Keywords: Antibiotic resistance, Antibiotic resistant bacteria, Poultry farm, poultry feces, *E. coli.*

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INTRODUCTION

Poultry farming is most prominent and fast-growing industry in animal husbandry. Almost half of the meat that is being consumed comes from poultry. Along with meat, the total production of egg in India is 142.77 billion nos. which also comes from poultry [1]. Despite of their advantages, there are many difficulties facing the poultry business, especially about enteric diseases. They lead to lower weight gain, higher death rates, and higher drug costs. Enteric illnesses can be caused by a variety of pathogens, such as bacteria, viruses, and parasites, either alone (mono-causal) or in combination with other microorganisms [2]. *Salmonella* species, *Escherichia coli*, and *Klebsiella* are among the enteric bacteria in the *Enterobacteriaceae* family that present serious health hazards in chicken production [3]. Additionally, chickens may behave as asymptomatic carriers, excreting these harmful bacteria into the environment through their feces [4]. Effective disease prevention programs are necessary in poultry farm management, particularly during the introduction of new flocks [5].

With increasing population, demand of meat and other livestock products is also increasing. To satisfy the demand, antibiotics are used to elevate the health and motivate growth of the animal [6][7]. Animals do not, however, completely metabolize antibiotics, and they are released into the environment along with excrement. The amount of waste produced in the livestock industry is decreased by using animal dung as fertilizer. However, microbes with antibiotic resistance genes (ARGs) are frequently found in manure. Then, the microbiome of manure applied to the soil may help propagate antibiotic resistance in the environment, including bacteria that live in the soil and are autochthonous [6].

Antibiotic resistant genes are transferred through conjugation with the help of plasmid [8]. The widespread utilization of antibiotics in poultry husbandry, for purposes including prophylaxis, infection treatment, and growth enhancement, raises substantial public health concerns. As a result of this practice, the prevalence of antibiotic-resistant bacterial strains within the food chain has increased over time [9]. Millions of deaths worldwide are attributed to antibiotic resistance each year, making it a major global health concern [10]. This worry is made worse by the wide spread of plasmid-mediated resistance genes among bacterial species in the gastrointestinal tract. The ability of these resistant plasmids to spread horizontally between unrelated bacteria further boosts resistance to several medications. Drug-resistant strains of *Escherichia coli* in the gut serve as reservoirs of resistance genes, making it challenging to treat extra intestinal infections in both people and animals. Even at trace amounts, antibiotics and their metabolites in soil can bioaccumulate and endanger the health of people and other living things. Because antibiotic residues may affect human endocrine function, metabolism, and development, their presence in the environment has sparked worries around the world [11].

Addressing the misuse of antibiotics in chicken farming is essential to halting the emergence of antibiotic resistance and safeguarding public health [12]. Thus, the present study aimed for studying the antibiotic resistance pattern of bacteria isolated from poultry fecal sample.

METHODOLOGY

Collection of Sample

Chicken fecal sample was collected in sterile container from Poultry farm located in Chandaj, District, Solapur, Maharashtra, India and properly sealed and transported to the laboratory.

Isolation of bacteria from poultry fecal sample

1 gm fecal sample was weighed and added in the test tube containing 9 ml sterile saline to make the suspension and vortexed. The serial dilutions from 10^{-1} to 10^{-7} were performed. An amount of 100 µl each from 10^{-5}, 10^{-6} and 10^{-7} dilutions was spread on sterile Nutrient Agar (NA), Eosin Methylene Blue Agar (EMB), and Cetrimide Agar (CA). The plates were incubated at 37°C for 24 hours. Subsequently, growth was observed, and isolated colonies were streaked onto sterile Nutrient Agar plate.

Antibiogram of the bacterial isolates

To study the antibiotic resistance of bacterial isolates from poultry fecal samples, different antibiotics viz. Ciprofloxacin, Cefixime, Cotrimoxazole, Amoxicillin, Nitrofurantoin, Gentamicin, Cefotaxime, Chloramphenicol of 20 µg/ml concentration were used. The antibiotic resistance was observed by spot inoculation method on sterile Muller Hinton Agar (MHA). Overnight grown cultures of bacterial isolates were spotted on the MHA plates containing respective antibiotic at concentration of 20 µg/ml. The plates were incubated at 37°C for 18-24 hours. After incubation, presence of growth or no growth of different bacterial cultures on plates with different antibiotics was checked and noted.

Characterization and Identification of multi drug resistant isolates

The selected bacteria showing resistance to the antibiotics were characterized based on morphological and biochemical characters and the identified using Bergey's Manual of Determinative Bacteriology. Gram staining, motility, oxidase test, catalase test, sugar fermentation test, Nitrate reduction test, and IMVIC were performed for characterization of isolates [13].

RESULTS

Isolation of bacteria from poultry fecal sample

100µl of 10^{-5} to 10^{-7} dilutions of poultry fecal sample was spread on sterile Nutrient Agar and Selective Media like Cetrimide Agar and EMB Agar and then plates were incubated at 37°C for 24 hours. After 24 hrs, well isolated colonies having different morphology were picked and streaked on respective plates for purification and further studies. On the basis of different colony morphology on NA plates, 10 bacterial isolates namely N1, N2, N3, N4, N5, N6, N7, N8, N9, N10, were obtained. Two bacterial isolates viz. E1, E2 were obtained from EMB agar plates and two isolates viz. C1 and C2 were obtained from cetrimide agar plates. Thus, in total 14 bacterial isolates were obtained from poultry fecal sample. Streak plate method was used for purification of cultures and representative bacterial cultures viz. N1, E1, N5 and N8 were shown in Figure 1.

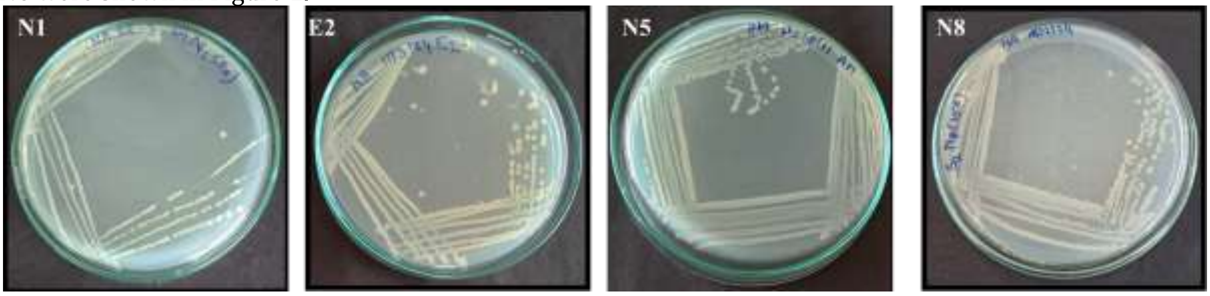

Figure 1: Pure cultures of representative bacterial isolates of fecal sample by Streak plate method

Study of antibiotic resistance of isolated bacteria by using different antibiotics

Antibiotic resistance pattern of isolated bacteria from poultry fecal sample was studied by spot inoculation method. Figure 2 and Table 1 describe the results of antibiotic resistance pattern of 14 bacterial isolates. As shown in Table 1, seven bacterial isolates were found to be resistant to the Ciprofloxacin, 7 isolates were resistant to Cefixime, 11 isolates were resistant to Cotrimoxazole while only 2 isolates were found to be resistant to Gentamicin and Cefotaxime. 12 isolates were found to be resistant to Amoxicillin and Nitrofurantoin. From Table 1, it was observed that isolate N1 was resistant to all eight antibiotics studied. Thus, isolate N1 can be called as multidrug resistant bacteria isolated from poultry fecal sample. Isolate N3, N5, N8, C1 and C2 were found to be resistant to five different antibiotics out of eight antibiotics studied. Isolates E2 were also found to be resistant to seven different antibiotics studied. Thus, 7 bacterial isolates were found to be multi drug resistant which were isolated from poultry fecal sample. In our study, out of 14 bacterial isolates 92% bacterial isolates were resistant to chloramphenicol, 85% were resistant to Amoxicillin and Nitrofurantoin. while 71 % were resistant to Cotrimoxazole and 42% were resistant to Ciprofloxacin and Cefixime.

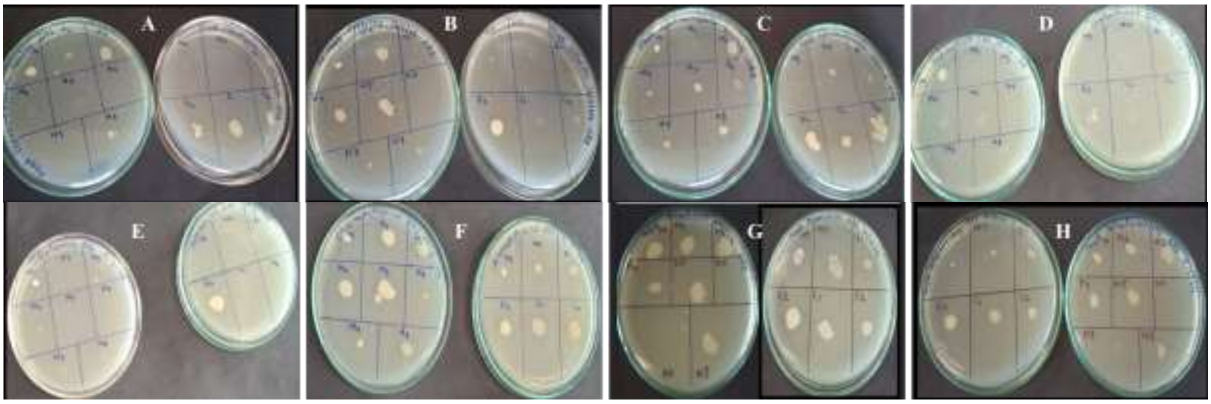

Figure 2: Antibiogram of the bacterial isolates. A: Isolates resistant to Ciprofloxacin; B: Isolates resistant to Cefixime; C: Isolates resistant to Cotrimoxazole; D: Isolates resistant to Gentamicin; E: Isolates resistant to Cefotaxime; F: Isolates resistant to Chloramphenicol; G: Isolates resistant to Amoxicillin; H: Isolates resistant to Nitrofurantoin.

Table 1: Antibiotic resistance pattern of bacterial isolates

Isolates	**Ciprofloxacin**	**Cefixime**	**Cotrimoxazole**	**Gentamicin**	**Cefotaxime**	**Chloramphenicol**	**Amoxicillin**	**Nitrofurantoin**
N1	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
N2	Sensitive	Resistant	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant
N3	Resistant	Sensitive	Resistant	Sensitive	Sensitive	Resistant	Resistant	Resistant
N4	Sensitive	Resistant	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant
N5	Sensitive	Resistant	Resistant	Sensitive	Sensitive	Resistant	Resistant	Resistant
N6	Sensitive	Sensitive	Resistant	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
N7	Sensitive	Resistant	Resistant	Sensitive	Sensitive	Resistant	Sensitive	Sensitive
N8	Resistant	Sensitive	Resistant	Sensitive	Sensitive	Resistant	Resistant	Resistant
N9	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant
N10	Sensitive	Sensitive	Resistant	Sensitive	Sensitive	Resistant	Resistant	Resistant
E1	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant
E2	Resistant	Resistant	Resistant	Sensitive	Resistant	Resistant	Resistant	Resistant
C1	Resistant	Sensitive	Resistant	Sensitive	Sensitive	Resistant	Resistant	Resistant
C2	Resistant	Sensitive	Resistant	Sensitive	Sensitive	Resistant	Resistant	Resistant

5 Bacterial isolates viz. N1, N3, N5, E1, and C1 showing muti drug resistance were selected for identification by morphological and biochemical characterization. Bacterial isolates N1, N3 and N5, E1 and C1 were found to be Gram negative short rods. All isolates except C1 was found to be motile. All 5 isolates were catalase and oxidase positive. The biochemical characterization of 5 isolates was depicted in Table 2.

Table 2: Biochemical characterization of selected multidrug resistant bacterial isolates

Isolates	Indole	Methyl red	Citrate utilization	Sugar fermentation					
				D-Xylose	D-Glucose	D-Fructose	D-Mannose	L-Arabinose	Maltose
N1	-	+	-	+	+	+	+	+	-
N3	-	-	-	+	+	+	+	+	-
N5	+	+	+	+	+	+	+	+	-
E1	+	+	-	+	-	-	+	+	+
C1	-	-	+	+	-	-	+	-	+

Based on morphological and biochemical characterization, and by referring the Bergey's Manual of Determinative Bacteriology, bacterial isolates N1, N3 and N5 may belong to *Alcaligens* sp. Isolate E1 may belong to *E. coli* while isolate C1 may belong to *Pseudomonas* sp. Thus, these results showed that poultry feces collected from Poultry farm located in Chandaj, Solapur District is the source of multi drug resistant bacteria and thus suggests the responsible use of antibiotics to chickens during their growth. Proper disposal and management of chicken feces will help to prevent the spread of these multi resistant bacteria to humans and the environment.

DISCUSSION

The present study aimed at antibiotic resistance of the bacteria isolated from poultry fecal sample. Such study is essential for studying the microbiota of poultry, their antibiotic resistance pattern and its impact on poultry health and food safety.

A similar study provided the information about different pathogenic bacteria like *E. coli*, *Salmonella* and *S. aureus* found in chicken feces in three different regions of Mansehra District of Pakistan. The study also highlighted the importance of monitoring and controlling the spread of pathogenic bacteria in chicken feces, as they can pose a significant public health risk. Proper disposal and management of chicken feces can help prevent the spread of these bacteria to humans and the environment [2]. Another study reported the presence of multidrug resistant *E. coli* from poultry in Bangladesh. 66–100% of *E. coli* bacteria isolated from Bangladeshi poultry showed resistance to tetracycline, penicillin, erythromycin, and chloramphenicol [14]. Thus, results of present study agree with the earlier reports [2] [14]

A study on fecal samples from two broiler farms and one live bird market in Qatar reported isolation of 90 commensal *E. coli* from Out of 90 isolates, 81 (90%) had at least one antibiotic resistance, 14 (15.5%) had colistin resistance, 2 (2.2%) produced extended-spectrum b-lactamases, and 2 (2.2%) had multidrug resistance to four antibiotic classes, according to the results [15]. In our study, out of 14 bacterial isolates 92% bacterial isolates were resistant to chloramphenicol, 85% were resistant to Amoxicillin and Nitrofurantoin. while 71 % were resistant to Cotrimoxazole and 42% were resistant to Ciprofloxacin and Cefixime.

Langata et al. [16] gathered fresh chicken droppings from 150 randomly chosen chicken families in Nairobi. Antimicrobial susceptibility testing was done on *Salmonella* and *Escherichia coli* after they were isolated. Another study by Thuan et al. [3] collected 3,055 samples from year 2017 to 2020 from Mekong Delta households and the broiler farms and were able to isolate *Salmonella* and the isolates were tested for antibiotic resistance to 14 different antibiotics. In contrast, current study could not find *Salmonella* in any sample.

According to similar research by Abbassi et al. [17], multi antimicrobial-resistant *E. coli* was isolated from chickens, thus supporting the findings of present work.

CONCLUSION

Culture based analysis of poultry fecal sample collected from poultry farm of Chandaj, District, Solapur revealed valuable insights into the microbial composition, antibiotic resistance profiles, and the presence of potentially pathogenic bacterial species. 14 bacteria were isolated from poultry fecal sample using different media like Nutrient agar, EMB and Cetrimide agar. All cultures were screened for antibiotic resistance using 8 different antibiotics like Ciprofloxacin, Cefixime, Cotrimoxazole, Gentamicin, Cefotaxime, Chloramphenicol, Amoxicillin and Nitrofurantoin. Out of 14, 6 bacterial isolates viz. N1, N3, N5, N8, E1, and C1were found to be resistant to 5 or more antibiotics studied and thus were called as multidrug resistant bacteria. In our study, out of 14 bacterial isolates 92% bacterial isolates were resistant to chloramphenicol, 85% were resistant to Amoxicillin and Nitrofurantoin. while 71 % were resistant to Cotrimoxazole and 42% were resistant to ciprofloxacin and Cefixime. The morphological and biochemical characterization revealed that they may belong to *E. coli*, *Pseudomonas* sp. and *Alcaligens* sp. Thus, the present study suggests the responsible use of antibiotics in poultry farming, emphasizing the importance of veterinary oversight, adherence to dosage regimens of antibiotics, and consideration of alternative infection control measures to minimize the emergence and spread of antibiotic resistance bacteria and antibiotic resistance genes to the surrounding environment. This study also shed light on the necessity of such studies to address public health concerns, understand environmental impacts, and guide antibiotic stewardship practices in poultry farming.

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