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## Isolation and antibacterial susceptibility of *Escherichia coli* from poultry

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**Abstract**

*Escherichia coli* is one of the leading cause of morbidity and mortality of poultry throughout the world, leading to remarkable economic burden. Human cases of antibiotic resistance has been recently linked to drug resistant animal source bacteria, which have drawn more attention towards poultry industry in particular. The present study was conducted on broiler population to isolate and identify the bacteria from clinical cases, followed by antibiotic study. A total of 24 isolates out of 68 liver and blood samples were successfully detected. Maximum resistance was found against enrofloxacin (86.3%), oxytetracycline (75%) and norfloxacin (66.7%). Whereas sensitivity was found maximum against chloramphenicol (95.8%) and streptomycin (87.5%) followed by amoxicillin, amikacin and cephalexin, making them available for use in treatment and control of the disease.

**Keywords:** *Escherichia coli*, antibiotic, poultry

**Introduction**

Poultry sector in India and particularly Haryana and Punjab states, is mostly organized sector which work as industry, generating employment and revenue for farmers and government. Broilers are reared by farmer for one to two months period and its selling generate income within short period, which results in encouragement for setting up new units. Risk of bacterial and viral infection remains high due to intensive farming, resulting in use of antibiotics for treatment and promotion of growth<sup>[1, 2]</sup>. *Escherichia coli* (*E. coli*) is Gram negative bacteria of Enterobacteriaceae family, causes colibacillosis disease in poultry, which is affect multiple organs resulting in pericarditis, air-sacculitis, peritonitis, yolk sac infection or osteomyelitis. As per one report<sup>[3]</sup>, this bacterial infection leads to huge losses to poultry industry due to high morbidity and mortality. In India, 8.88% mortality in broilers was reported from 1987 to 1997 from some parts of Haryana<sup>[4]</sup>. Animal were reported as source of human infection of drug-resistant *E. coli*<sup>[5]</sup>. Antibiotic residue has been detected in 40% of 70 chicken meat samples tested in New Delhi, where enrofloxacin, ciprofloxacin, doxycycline and oxytetracycline were most abundant<sup>[6]</sup>. High prevalence of extended-spectrum  $\beta$ -lactamase (ESBL) producing *Enterobacteriaceae* in poultry production have been reported from many parts of the world including India<sup>[7, 1, 8]</sup>.

**Material and methods**

**Sampling and isolation of *E. coli*:** The present study was conducted on bird showing symptoms of colibacillosis presented at the time of postmortem. The heart blood and liver samples were collected from 68 broiler bird of all age group from Hisar, Jind and Sonipat district of Haryana. The culture and antibiotic sensitivity work was performed at Department of Biotechnology, DCR University of Science and Technology, Murthal, Sonipat. Isolation and identification of bacteria was done as per protocol of<sup>[9]</sup>. Briefly, liver tissue and heart blood were inoculated on Mac Conkey's agar (Hi-Media) and incubated for 24h at 37 °C aerobically. Identification of isolates was performed by cultural morphology and biochemical characteristics as per standard method<sup>[10]</sup>. Various biochemical reactions performed on pink colonies suspected for *E. coli* include indole production, Voges-Proskauer test, methyl red test and citrate test.

**Antibiotic sensitivity assay:** All the isolates were tested for sensitivity as per disc diffusion method<sup>[11]</sup>, against 16 antibiotics namely Co-trimethoxazole (25  $\mu$ g), Oxytetracycline (30  $\mu$ g), kanamycin (30  $\mu$ g), Cefotaxime (30  $\mu$ g), Nitrofurantoin (300  $\mu$ g), Ampicillin (10  $\mu$ g), Amoxicillin (10  $\mu$ g), Nalidixic Acid (30  $\mu$ g), Norfloxacin (30  $\mu$ g), Enrofloxacin (05  $\mu$ g), Chloramphenicol (30  $\mu$ g), Ciprofloxacin (05  $\mu$ g), Amikacin (30  $\mu$ g), Streptomycin (10  $\mu$ g), Cefalexin (30  $\mu$ g) and Gentamycin (10  $\mu$ g). Resistance and sensitivity was determined by comparing zone size with chart supplied (Hi-Media).

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## Results and Discussion

Out of 68 samples tested, *E. coli* was isolated from 24 samples based on cultural and biochemical characteristics. A total of 35.29% sample were successfully detected for *E. coli* from the cases suspected for colibacillosis (Table1). Out of 30 samples tested from Hisar district, 40% were successfully detected for presence of *E. coli*. Whereas from Jind and Sonipat district, 50% and 11.1% samples were detected for the bacteria out of 20 and 18 samples tested respectively. This is suggestive of systemic infection in these bird, resulting in high level of detection from organs. The similar finding of systemic nature of colibacillosis infection has been reported from Haryana <sup>[12]</sup>, where 86% isolation was performed from heart and liver samples of broilers, whereas detection from spleen and intestine were low.

**Table 1:** Detail of *E. coli* isolated from broiler population suspected for colibacillosis

S. No.	District	Samples tested	<i>E. coli</i> isolated	% positive
1	Hisar	30	12	40
2	Jind	20	10	50
3	Sonipat	18	2	11.1
	Total	68	24	35.29%

At the time of post-mortem, fibrinous layer was found on liver and heart, in most of cases. In some cases abdominal cavity also found covered with fibrinous exudate. Air sac were found cloudy with fibrinous mass. Similar observations have been reported by some investigators from different areas <sup>[13, 12]</sup>.

Most of broiler population in Haryana is concentrated in Hisar and Jind districts whereas layer population is mostly in Ambala, Karnal, Panipat and Yamunanagar districts. The present study was limited to broiler population hence sampling was done from Hisar, Jind and Sonipat district which was found justified as more cases were found from former two while detection from Sonipat district was less comparatively. The detection was not fully achieved from all suspected cases, which might be because of possible administration of antibiotic to the diseased bird.

Antibiotic sensitivity assay result showed various in resistance to different antibiotics (Table2). Out of 24 *E. coli*

isolates tested, 20 (83.3%) were found resistant to Enrofloxacin, followed by 19 (79.2%) to Oxytetracycline, 18(75%) Norfloxacin, 16(66.7%) to Cotrimethoxazole and 16 (66.7%) to Nitrofurantoin. Maximum sensitivity was found against Chloramphenicol (95.8%) followed by Streptomycin (87.5%), Amoxicillin (83.3%), Amikacin (83.3%), Cefalexin (83.3%), Cefotaxime (79.2%), Gentamycin (75%) and Ampicillin (70.8%). Sensitivity to ciprofloxacin (66.7%) and kanamycin (62.5%) was found fairly good followed by low sensitivity of Nalidixic acid (41.7%). The more resistance to Enrofloxacin and Norfloxacin showed resistance to quinolones and fluoroquinolone (Fig.1), which is in agreement with report <sup>[14]</sup> from Haryana state. Another excessively used antibiotic oxytetracycline was found highly resistant in the present study, which was also in agreement with above mentioned observation <sup>[14]</sup>. From other parts of the country (Telangana, Karnataka and Andhra Pradesh), resistance rate (100%) was found towards tetracycline and quinolone class of antibiotics <sup>[8]</sup>, which was also in agreement with the present study. This shows that almost similar types of antibiotics are in maximum use or primarily used in most different parts of the country, which may be either as feed additive or in treatment of the various bacterial diseases. Whereas from Eastern part of the country, maximum resistance was found against cefuroxime followed by penicillin and ampicillin <sup>[15]</sup>, which was found different from the present study. However, resistance to co-trimethoxazole (73.1%) was found high which was also observed in the present study. From adjoining state Punjab, maximum resistance to nalidixic acid, tetracycline, ampicillin and co-trimethoxazole has been reported <sup>[1]</sup>. The situation in both the state was found almost consistence in resistance to sulfonamide and tetracycline class of antibiotics, whereas the present study found moderate resistance to nalidixic acid. Study from another state of northern India, geographically adjacent to Haryana, recorded maximum resistance to piperacillin, tetracycline and co-trimethoxazole <sup>[16]</sup>. This was partially in agreement with the present study, in *E. coli* resistance to tetracycline and sulfonamide. Whereas, the disagreement was found in resistance towards Cefotaxime, which was found highly sensitive in the present study. This suggests that use of this drug in Haryana is very limited for treatment in poultry.

**Table 2:** Antimicrobial susceptibility pattern of *E. coli* isolates (n=24)

Antibiotics applied	code	No. of Resistant isolate	% Resistant	No. of Sensitive isolates	% Sensitive
Cotrimethoxazole	Cot 25	16	66.7	8.0	33.3
Oxytetracycline	O 30	19	79.2	5.0	20.8
Kanamycin	K 30	9	37.5	15.0	62.5
Cefotaxime	Ctx 30	5	20.8	19.0	79.2
Nitrofurantoin	Nit 300	16	66.7	8.0	33.3
Ampicillin	Amp10	7	29.2	17.0	70.8
Amoxicillin	Amx10	4	16.7	20.0	83.3
Nalidixic acid	Na30	14	58.3	10.0	41.7
Norfloxacin	Nx 10	18	75.0	6.0	25.0
Enrofloxacin	Ex5	20	83.3	4.0	16.7
Chloramphenicol	C30	1	4.2	23.0	95.8
Ciprofloxacin	Cip5	8	33.3	16.0	66.7
Amikacin	Ak30	4	16.7	20.0	83.3
Streptomycin	S10	3	12.5	21.0	87.5
Cefalexin	Cn30	4	16.7	20.0	83.3
Gentamycin	Gen10	6	25.0	18.0	75.0

Maximum sensitivity was recorded against chloramphenicol and streptomycin in the present study, which suggest its less use in recent past and this may be event of re-emergence of

sensitivity. The same report regarding these two drugs have also been reported from another studies on *Salmonella* spp. <sup>[17, 18]</sup>. Maximum sensitivity of *E. coli* against chloramphenicol,

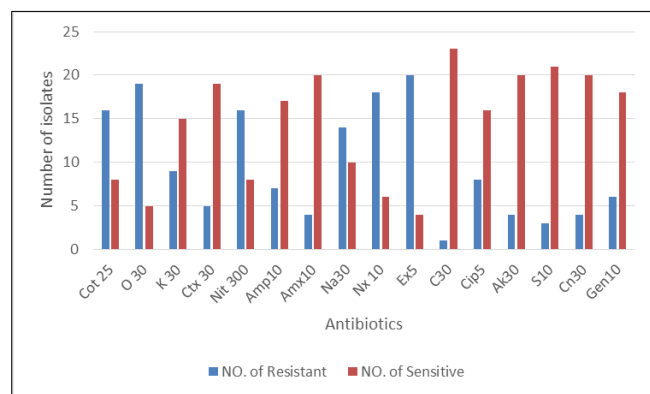
gentamycin and amikacin was also reported by [16], which was in agreement with the present study, making these drug preferred choice for treatment. *E. coli* resistance to gentamycin (73%) observed from previous study [19], was found in consistent with the present study. The bacteria has not developed resistance to this drug even after 20 years from the state, which reflect either this drug is being less used or full course in appropriate dose may be given. Similar higher sensitivity against chloramphenicol and gentamycin has been reported previously [1]. So, this make the present study constant with above study from Punjab.

### Conclusion

So, it can be concluded that *E. coli* is one of the leading pathogen affecting poultry either as primary cause or result of secondary infection. As the disease mostly spread through oro-faecal route, good hygienic condition as farm level may help in reduction of incidence in poultry, which in turn may reduce human risk of food borne infection. The best choice for isolation of samples may be liver and heart blood, as invasive infection is very common. The incidence of infection was found more in Jind followed by Hisar district of the state, which are also most populated broiler areas. The resistance to quinolone, tetracycline and sulfonamide is worrisome and again emphasize need for judicious use of these drugs. Re-emergence of *E. coli* sensitivity against chloramphenicol and streptomycin may prove beneficial making them available for treatment alongwith other third generation cephalosporins and amikacin. Further research involving sampling more area of the state and inclusion of layer, hatcheries and meat shops is required for adopting better control strategy.

Ethical Statement: All the animal carcasses were handled ethically. No live animal was harmed.

**Conflict of Interest:** There is no known conflict of interest to anyone regarding the present study.



**Fig.1.** Result of antibiogram pattern of *E. coli* isolates from broiler affected with colibacillosis

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