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Threats and Confronts regarding Poultry: Antimicrobial Multidrug Resistance of Pathogenic *Escherichia Coli* isolated from Layers in Ajmer Region of Rajasthan.

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ABSTRACT

Antimicrobial's efficiency is critically compromised by the emergence and spread of antimicrobial resistance, yet they are valuable therapeutics. Investigations were carried to test and analyze the antimicrobial susceptibility of *E. coli* isolates from Ajmer poultry farm. 24 tissue samples containing 6 liver, 12 small intestine, and 6 large intestine samples were processed for isolation and identification and characterization of pathogenic *E. coli* from 12 fowls of 4 selected poultry farms of Ajmer suspected for colibacillosis and subjected to detailed bacteriological and biochemical examination. 24 *E. coli* isolates were isolated following standard procedures. *E. coli* isolates found to be highly resistant to some of the classical drugs like tetracycline, amoxy clav, carbenicillin, cotrimoxazole and cefopodoxime and found to be highly sensitive (100%) to the remaining drugs, which is disclosed by antibiogram study. The most ineffective antibiotic drug was found to be tetracycline for all the *E. coli* isolates, as all these microbes were found 100% resistant to it. Multiple resistance to at least 2 to 4 antibiotics were observed in all the 24 isolates. 50% of the isolates were resistant to at least 4 antibiotics and 75% to at least 3 antibiotics and 25% to at least 2 antibiotics. Results from this study revealed the high prevalence rate of multidrug resistant *E. coli* isolates. It may suggest that the high resistance of *E. coli* to antibiotics constitutes a threat not only to poultry industry of Ajmer but also possesses a serious threat to public and animal health with adverse economic implications.

Keywords: Antimicrobial, Therapeutics, Multidrug resistance, Antimicrobial susceptibility, Colibacillosis, Antibiogram.

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INTRODUCTION

Over the past decades, the poultry sector's growth and trends towards intensification and concentration have given rise to a number of environmental concerns. A direct consequence of these structural changes (industrialization, geographical concentration and intensification) in poultry production is that far more waste than can be managed by land disposal is produced, resulting in environmental problems. This intensification has lead to increased susceptibility to various disease conditions in the birds. This paper analyzes the environmental impacts arising from intensive poultry production, evaluating such impacts across the food chain and all environmental media.

Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis[1]. This has increased resistance to commonly used antimicrobials both in the public health and veterinary sectors. Substantial data show elevated antibiotic resistance in bacteria associated with animals fed with antimicrobials and their food products. This resistance spreads to other animals and humans – directly by contact and indirectly via the food chain, water, air, and manured and sludge-fertilized soils. The intense use and misuse of antibiotics are undoubtedly the major forces associated with the high numbers of resistant pathogenic and commensal bacteria worldwide. Both the volume and the way antibiotics are applied contribute to the selection of resistant strains.

It is well documented that the contamination of food with pathogens is a major public health concern worldwide[2]. Because of the relatively high frequency of contamination of poultry with pathogenic bacteria, raw poultry and products are reported to be responsible for a significant number of cases of human food poisoning[3]. In the absence of hygienic conditions, birds may be highly exposed to the wide range of bacterial pathogens such as *Listeria monocytogenes, Campylobacter*, and other enteric bacteria[4]. Among these, the poultry industries are most vulnerable to attack by *Escherichia coli* that increased mortality of poultry chickens. *E. coli* is one of the common microbial flora of gastrointestinal tract of poultry and human being including other animals but may become pathogenic to both[5, 6]. Major species of *E. coli* encounter in the lower portion of the intestine of human, warm blooded animals and birds, where they are mostly responsible for gastroenteritis[7]. In view of the significance of *E. coli* infection in poultry, this study has been undertaken to isolate and to study their antibiotic drug resistance pattern.

MATERIAL AND METHODS

Sampling site and procedure:

The study was conducted in the 4 selected poultry farms of Ajmer region and 12 layers with possible colibacillosis collected in different farms were autopsied. The study was performed following approval from Institutional Animal Ethical Committee (IAEC) of Maharishi Dayanand Saraswati University, Ajmer. Ethical norms were strictly followed during all experimental procedures.

Autopsy:

Organs showing characteristics lesions related with colibacillosis were inspected and samples were collected randomly for further analyses.

Sample collection:

The tissue samples of 24 samples of 12 poultry birds were collected in sterile containers based on clinical findings and pathogonomonic lesions observed during detailed post mortem examination of infected layers of the selected poultry farm. Samples like liver and intestine, small and large were collected aseptically from fresh carcasses exhibiting perihepatitis, enteritis, air sacculitis, yolk sac infection and pericarditis and processed for bacteriological isolation and identification.

Isolation and Identification of *E. coli:*

Briefly the collected tissue samples were inoculated into the MacConkey agar plates and incubated at 37°C for 24 hours. After incubation, the MacConkey agar plates were examined for bacterial growth. Discrete colonies of lactose fermenting (pink) were identified and selected. After 24 hours of inoculation, selected colonies were plated and purified on MacConkey agar (Himedia) and incubated at 37°C for 24 hours and gram stained for microscopy. The *E. coli* organisms were identified based on their morphology, cultural, biochemical and sugar fermentation characters as per the method described by Edwards and Ewing and Cruickshank[8, 9]. Various biochemical tests which were performed include catalase test, oxidase test, indole test, Methyl Red (MR) test, TSI agar test, H_2S production test and citrate utilization test. Other test like haemolysis on blood agar and motility test were also carried out.

Antimicrobial Susceptibility Testing:

The antibiotic sensitivity tests of the *E. coli* isolates were performed by employing the Bauer- Kirby disc diffusion method using antibiotic discs (Himedia) as per the method of Bauer[10]. The identified isolates were tested for susceptibility to 24 antibiotics, which were laid on solid medium of Mueller Hilton agar. The plates were incubated for 24 hours at 37°C and inhibition zones measured. Based on zones of inhibition, isolates were classified as either sensitive (S) or resistant (R) or intermediate (Int). Isolates resistant to three or more antibiotics were classified as MDR (Multi Drug Resistant) strains.

RESULTS AND DISCUSSION

In the present study, *E. coli* has been isolated from dead and diseased bird collected from the selected poultry farms which were submitted for necropsy. From 12 suspected birds, all were found to be positive for *E. coli* infection as they show characteristic lactose fermenting colonies on MacConkey agar and their biochemical and sugar fermentation characters were in accordance Edwards and Ewing[8].

The sensitivity and resistance pattern of these isolates for various antibiotics are presented in Table-1 and Figure 1. The results of antibiotic sensitivity test of the 24 isolates of *E. coli* showed that the isolates were highly sensitive to amikacin, aztreonam, cefepime, cefixime, cefoperazone-sulbactam, cefotaxime, ceftazidime, ceftriaxone, cefazolin, chloramphenicol, ciprofloxacin, cefoxitin, imepenem, netilmicin, ofloxacin and tobramycin (100%), gentamicin and piperacillin (75%) and resistance was observed against tetracycline (100%), amoxy clav, carbenicillin and cotromoxazole (50%), cefopodoxime and piperacillin (25%) (Table-1 and Figure 1).

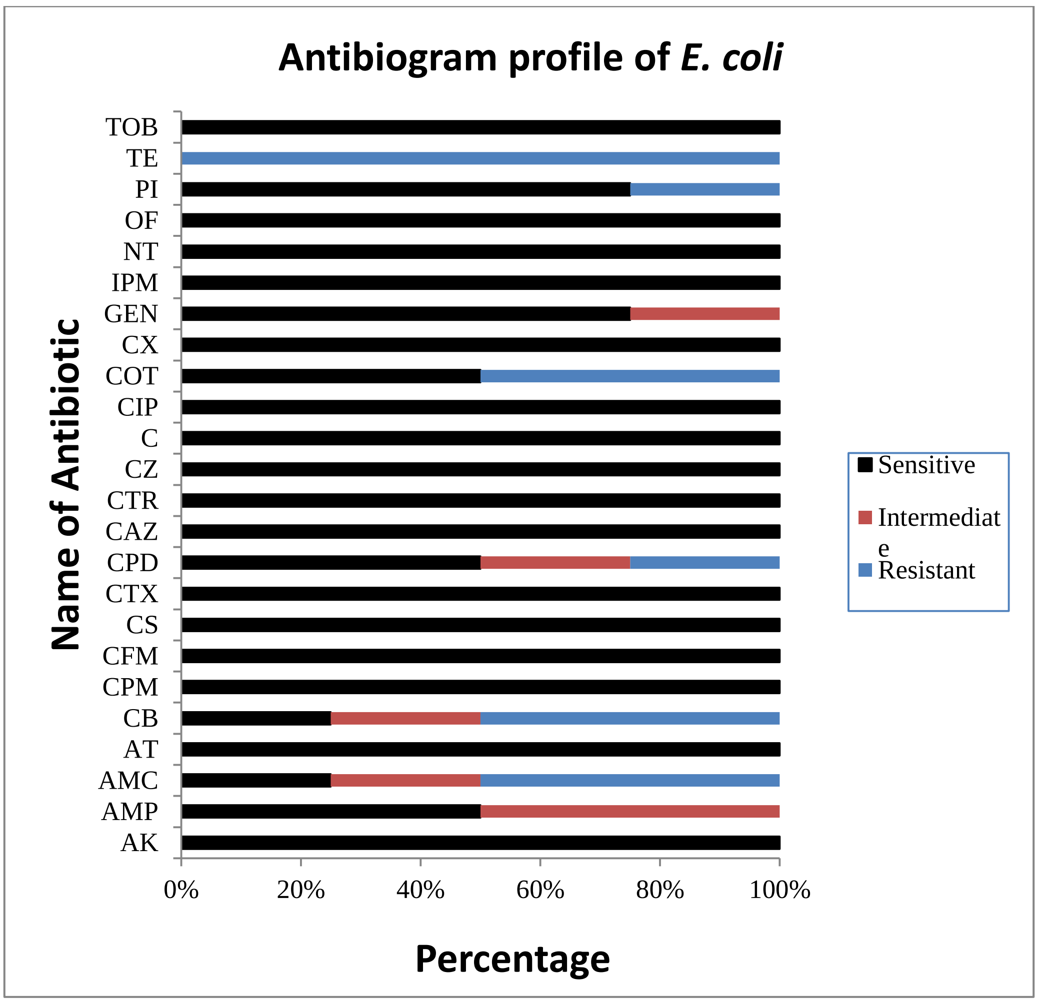

Figure 1 Graphical representation of Antibiogram profiling by poultry *E. coli.*

Table 1: Antibiotic susceptibility pattern of various antibiotics on 24 isolates of *E. coli* (samples: – 6 from liver, 12 from small intestine & 6 from large intestine of 12 diseased birds).

Sl. No.	Antibiotic tested	Percents of Samples or Total Number of Isolates (percentage)		
		Sensitive Isolates or High Sensitivity	Intermediate Isolates (drug not effective) or	Resistant Isolates or Resistant
1.	Amikacin (Ak)	*24(100)*	*0*	*0*
2.	Ampicillin (Amp)	12 (50)	12 (50)	0
3.	Amoxy clav (Amc)	6 (25)	6 (25)	12 (50)
4.	Aztreonam (At)	24 (100)	0	0
5.	Carbenicillin (Cb)	6 (25)	6 (25)	12 (50)
6.	Cefepime (Cpm)	24 (100)	0	0
7.	Cefixime (Cfm or Cfx)	24 (100)	0	0
8.	Cefoperazone-Sulbactam (Cs)	24 (100)	0	0
9.	Cefotaxime (Ctx)	24 (100)	0	0
10.	Cefopodoxime (Cpd)	12 (50)	6 (25)	6 (25)
11.	Ceftazidime (Caz)	24 (100)	0	0
12.	Ceftriaxone (Ctr)	24 (100)	0	0
13.	Cefazolin (Cz)	24 (100)	0	0
14.	Chloramphenicol (C)	24 (100)	0	0
15.	Ciprofloxacin (Cip)	24 (100)	0	0
16.	Cotrimoxazole (Cot)	12 (50)	0	12 (50)
17.	Cefoxitin (Cx)	24 (100)	0	0
18.	Gentamicin (Gen)	18 (75)	6 (25)	0
19.	Imipenem (Ipm)	24 (100)	0	0
20.	Netilmicin (Net or Nt)	24 (100)	0	0
21.	Ofloxacin (Of)	24 (100)	0	0
22.	Piperacillin (Pi)	18 (75)	0	6 (25)
23.	Tetracycline (Te)	0	0	24 (100)
24.	Tobramycin (Tob)	24 (100)	0	0

There was a high rate of multidrug resistance (resistance to three or more unrelated antimicrobials) among the isolates. A total of 100% isolates were resistant to at least two antibiotics and 75% were resistant to at least three antibiotics. 50% of isolates were resistant to at least four antibiotics (Table-1 and Figure 1). However, the trend of resistance of isolates for tetracycline were more elevated followed by amoxy clav, carbenicillin and cotrimoxazole than cefopodoxime and piperacillin.

E. coli isolates showed variable percentages of sensitivity and resistance to the different antibiotics. It was observed that most of the antibiotics used were found to be cent percent effective or 100% sensitive contrary to the research of Sharada[11]. High levels of resistance were found against tetracycline (100%), amoxy clav, carbenicillin and cotrimoxazole (50%), cefopodoxime and piperacillin (25%). *E. coli* isolates are resistant to these drugs because of regular usage in poultry industry for control of pathogenic avian colibacillosis. *E. coli* isolates of this study were highly sensitive (100%) to amikacin, aztreonam, cefepime, cefixime, cefoperazone, cefotaxime, ceftazidime, ceftriaxone, cefazolin, chloramphenicol, ciprofloxacin, cefoxitin, imipenem, netilmicin, ofloxacin and tobramycin, which was followed by gentamicin and piperacillin (75%). Moderate sensitivity to antibiotics such as ampicillin, cefopodoxime and cotrimoxazole (50%), amoxy clav and carbenicillin (25%) was exhibited by the isolates. However the results of this study are in variance with the findings of other workers, indicating that antibiotic pattern varies with different isolates, time and development of multiple drug resistance among different *E. coli* isolates related to transmissible R factor/ plasmid[12]. The transmission of resistance plasmids of *E. coli* from poultry to human have also been reported[13].

Isolates of *E. coli* from the poultry farms were found frequently resistant to two or more antibiotics, especially if they have been widely used in poultry industry over a long period (e.g., tetracyclines)[14, 15]. Antibiotics once effective at controlling *E. coli* infections are now ineffective due to the bacterium's acquired resistance to these compounds.

This high level of resistance to antimicrobial drugs among pathogenic isolates has been reported which is in accordance with the reports of Kaul, Negeleka *et al.*, Lambie and Islam[16-19]. The high level of resistance of *E. coli* isolates to antimicrobial drugs, as observed in this study, may reflect an extensive use of these drugs in local poultry farming, Such practices, especially without prior antibiotic sensitivity testing of bacterial isolates, may lead to the development of a pool antibiotic resistant genes and thus to the selection of increasing numbers of *E. coli* resistant clones, many with potential negative impact on layers and broilers industry. The study revealed that all the *E. coli* isolates were found sensitive to amikacin, aztreonam, cefepime, cefixime, cefoperazone, cefotaxime, ceftazidime, ceftriaxone, cefazolin, chloramphenicol, ciprofloxacin, cefoxitin, imipenem, netilmicin, ofloxacin and tobramycin. Hence, on the basis of above data, the antibiotic of choice for *E. coli* infection treatment in layers appeared to be a combination of these antibiotic drugs.

The results showed in Table 1 and Figure 1 revealed that multiple drug resistance was observed in almost all the isolates of *E. coli* exhibiting simultaneous resistance to more than two antibiotics. These finding correlates well with the observation of Mary Jones, Mishra, Saha and Sahoo[20-23]. The resistance pattern showed that the *E. coli* isolates are sensitive to the modern antibiotics which were less used in the poultry farms and there is development of higher resistance to those antibiotics which are traditionally used as feed additives or chemotherapeutic agents which is in accordance with the findings of Sahoo[23]. The development of multiple antibiotics resistance may be as a result of transfer of R factor (borne on plasmids) and *E. coli* are noted to carry multiple plasmids which can carry any number of multiple resistant genes[24].

In *E. coli*, the high values of resistance observed against tetracycline (100%), amoxy clav, carbenicillin and cotrimoxazole (50%), cefopodoxime (25%) in contrast to the appreciable sensitivity values (100%) recorded towards the fluoroquinolones such as ciprofloxacin and ofloxacin, were in contradiction to the findings of Akond[25] who reported 82% resistance of *E. coli* from poultry and poultry environment in Bangladesh to fluoroquinolones. However, the report of Sharada[11] and Ezekiel[26] correlates with our data since they suggested a high sensitivity of *E. coli* from poultry to fluoroquinolones in Bangalore and Nigeria, respectively. The high resistance values in our study are similar to those observed in *E. coli* isolates from other sources against inexpensive, first-line broad-spectrum and readily available antibiotics by investigators[27-29].

Antimicrobial-resistant *Escherichia coli* can be transferred from poultry to humans through consumption of contaminated food and food products and thus present a public health risk. Again, concern about antibiotic resistance and its transmission to human pathogens is important because these resistant bacteria may colonize the human intestinal tract and may contribute resistance genes to human endogenous flora. The episomal transfer of resistance factor between the intestinal pathogens may lead to evolution of drug resistant bacterial strains in human being which is of public health importance. The emergence of antimicrobial resistant bacterial strains has been linked with the use of antimicrobial agents in animals. The continuous use of antimicrobials as therapeutic, prophylactic and growth promoter agents creates selective pressure that ultimately leads to the emergence of resistant bacterial strains[30, 31].

Blind antimicrobial therapy, excessive usage of antimicrobial agents for prophylaxis and inappropriate treatment may explain the high incidence of multiresistance of *Escherichia coli* in poultry rearing in Ajmer. Such practices, especially without prior antibiotic sensitivity testing of bacterial isolates may lead to the development of a pool of antibiotic-resistant genes and to the selection of increasing numbers of resistant *Escherichia coli* clones. *Escherichia coli* of avian origin could act as a possible source for the transfer of antibiotic resistances to other bacterial species including human pathogens[32].

CONCLUSION

In conclusion, the findings clearly demonstrates multiple antimicrobial resistant *E. coli* isolates, that are commonly present among diseased layer chickens in Ajmer. Resistance to existing antimicrobials is widespread and of concern to poultry veterinarians. The significant increase in the incidence of resistance against antibiotics in the *E. coli* strains isolated from layer chickens is probably due to increased use of antibiotics as feed additives for growth promotion and prevention of diseases, use of inappropriate antibiotics for treatment of diseases and resistance transfer among different bacteria. Thus, introduction of surveillance programs to monitor antimicrobial resistance in pathogenic bacteria is strongly needed in this region because in addition to animal health problems, transmission of resistant clones and resistance plasmids of *E. coli* from food animals (especially poultry) to humans can occur. Hence, special emphasis need to be given for judicious selection of antibiotics, preferably after antibiotic sensitivity testing and judicious use of such antibiotics at an optimum dose for sufficient duration to ensure effective treatment and control of various diseases caused by *E. coli* in poultry. In fine, our study reveals that, the poultry borne bacteria could be a good source as vectors in transmitting drug resistance. Attention is to be paid for personal hygiene in processing and handling of poultry and poultry products and the excess use or abuse of antibiotics should be reduced or stopped by the judicious application of antibiotics for the safety of public health in Ajmer region.

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