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Studies on Antimicrobial Resistance in *E. coli* Isolates of Poultry Farms in and around Meerut

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ABSTRACT

The increase in antimicrobial-resistant (AMR) bacteria is an emerging public health problem. The indiscriminate use of antibiotics in domestic animals particularly in poultry sector is considered to be major contributor to the modern-day crisis of antimicrobial resistance (AMR). E. coli, being commensal bacteria, its antimicrobial resistance is a potent public health hazard. However, a little is understood about acquisition of drug resistance, once it leaves its host. To know the status of AMR E. coli, the current study was conducted in and around Meerut during January to April 2020. A total of fifty faecal swabs were collected from different poultry flocks. E. coli were isolated and identified based on cultural, morphological and biochemical tests. The overall prevalence of E. coli was found 54.00%. Antimicrobial susceptibility test by disc diffusion method against different antibacterial drugs revealed 100 % resistance to Cefotaxime, followed by Enrofloxacin (89%), Doxycycline hydrochloride (85%), Tetracycline (70%), Ciprofloxacin (52%), Chloramphenicol and Ampicillin/Sulbactam (44%). On the other hand, the highest susceptibility was recorded against Gentamicin (100%) followed by Streptomycin (96%), Ampicillin/Sulbactam (41%), Ofloxacin and Chloramphenicol (33 %) as well as some isolates show nominal sensitivity against the ciprofloxacin (15%). The higher prevalence may attribute to poor management practices. Higher drug resistance against antibacterial might be due to indiscriminate use of these drugs. It is therefore, firmly endorse to diminish the unethical utilization of antibiotics to limit the spread of resistance strain of microbes.

Keywords: *E. coli, Antibacterial, Resistance, Antibiogram*

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INTRODUCTION

The poultry industry is one of the wide ranging and fast moving developing agro-based industries in the world. It is one of the boom sectors of Indian agriculture in these days, with annual growth rates of 5.57 % and 11.44 % in egg and broiler production, respectively. The sector is involving in giving employment direct or indirect to 6.5 million individuals. In today scenario India stands third largest egg producer in the world (after China and the United States of America), and the nineteenth largest broiler producer [1]. This is often related to an expanding demand for poultry meat and egg products. Poultry flocks are often raised under intensive conditions using large volume of antimicrobials to avoid and to manage infection, in addition to for growth promotion. Antimicrobial use has multiplied the life expectancy of humans and greatly improved the productiveness of animals. However, these benefits were compromised by the development of antimicrobial resistance (AMR)[2].

The emergence of antimicrobial resistance amongst meat borne pathogens and commensals has add up a problem in meat safety that poses a serious public health risk. A large range of antimicrobials is used to raise poultry in most of the countries [3,4] mostly via the oral route, not only for the purpose to prevent and to treat disease but also to enhance growth and productivity [5]. The broad-based use of antimicrobials in animal farming is possibly to step up the development of AMR in pathogens, as well as in commensal organisms. Moreover, AMR in poultry pathogens is in all likelihood to cause to economic losses, derived from the expenditure on ineffective antimicrobials, in addition to the burden of untreated poultry disease. Establishment of AMR bacteria in the intestinal tract of young chicks may cause

perseverance of these bacteria within the intestine throughout the existence of the chicken [6]. *Escherichia coli* is one of the fundamental causes of economic losses in the poultry industry globally [7]. It is a common inhabitant of the intestinal microflora of poultry at abundances of as much as 10^6 colony-forming units per gram of faeces. In healthy chickens, 10 to 15% of faecal coliforms can also belong to probably pathogenic serogroups, carrying virulence and antimicrobial resistance genes [7]. In addition to resistance phenotypes data, information is also required for the AMR pattern of faecal *E. coli*. Therefore, the objective of this study was to assess the prevalence of AMR patterns of faecal *E. coli* isolates of chickens from in and around Meerut and to determine differences in AMR pattern among broiler and layer poultry species.

MATERIALS AND METHODS

Samples collection:

A total of 50 cloacal swab samples were collected aseptically from broiler and layer poultry in and around Meerut during the period of January to April 2020. Immediately after collection swabs were placed into a sterile container containing buffered peptone water (BPW) (Himedia, India). The samples were carried into ice box to Department of Immunology & Defence Mechanism, College of Biotechnology, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut-250110 for further processing.

Media reagents and chemicals:

The media and chemicals used in the study were obtained from Hi-media, Mumbai (India) and prepared in the laboratory as per the standard procedures[8].

Isolation and Identification of *E. coli:*

On the day of collection and arrival in the laboratory, faecal swabs have been streaked directly on MacConkey agar (Himedia, India) and incubated aerobically at 37⁰C for 24 hrs. Lactose-fermenting colonies have been picked and re-streaked on eosin methylene blue (EMB) agar (Himedia, India) and incubated for 24 h at 37⁰C. The green metallic sheen colonies have been taken into consideration to be *E. coli*. These colonies were subjected to biochemical tests. *E. coli* isolates were preserved in Brain heart infusion agar (BHI) slants (Himedia, India) and stored with 15% glycerol at -20°C until used[9].

Antibacterial susceptibility testing:

Antimicrobial susceptibility testing of *E. coli* isolates was achieve on Muller-Hinton agar plates (Himedia, India) using the Kirby-Bauer disc diffusion method to assess the antibiotic susceptibility of all the *E. coli* isolates as described by Bauer[10]. The antimicrobial agents (Himedia, India) tested included Ampicillin/Sulbactam (10/10mcg), Cefotaxime (5 mcg), Chloramphenicol (10 mcg), Ciprofloxacin (5 mcg), Doxycycline Hydrochloride (30 mcg), Enrofloxacin (5 mcg), Gentamicin (10 mcg), Ofloxacin (5 mcg), Streptomycin (10 mcg) and Tetracycline (30 mcg) were used for antibiotic susceptibility test. The interpretation was made as per the zone size interpretation chart provided by the manufacturer of discs. Zones of inhibition around each disc were measured and recorded as Sensitive, Intermediate and Resistant.

RESULTS

Isolation and identification of *E. coli:*

A total of 50 poultry faecal samples from four different places were processed for the presence of clinically important *E. coli*. 27 samples were found positive for *E. coli* isolates. The results of gross colony morphology of *E. coli* on EMB agar, MacConkey agar and Grams staining and motility test are summarized (Table 1). On biochemical characterization all 27 isolates fermented the five basic sugars producing acid and gas. All the isolates were Methyl Red (MR) positive, Voges-Proskauer (VP) negative and Indole positive. The prevalence of *E. coli* in the faecal sample was 54.0% (Table 2 and Fig.1). Bhattacharjee *et. al*.[11]communicate 40.82% prevalence of *E. coli* in chicken from Bangladesh but Nazir[11]stated the overall prevalence was 62.5% from chicken, which is closed to the present findings.

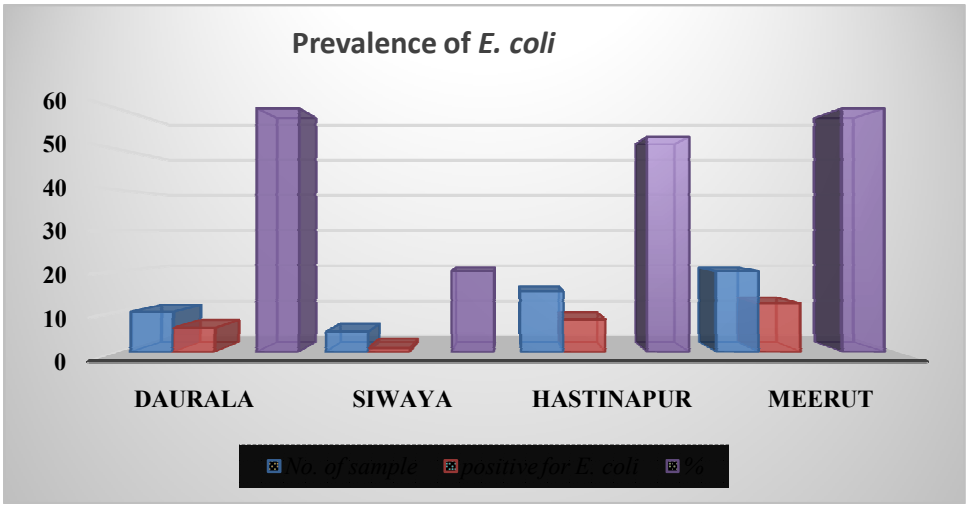
Table 1: identifying characteristics of *E. coli*

Source (n=50)	Motility	Colony Characteristics		Morphology	Staining Properties
		EMB agar	MacConkey Agar		
Faecal Swabs	+	Green metallic sheen	Bright pink colonies	Short rod, single, pair	Gram Negative

Table 2: Number of samples and cultural prevalence of *e. Coli* from different places in and around Meerut

Place	No. of sample	Positive for *E. coli*	Prevalence (%)
Daurala	10	6	60
Siwaya	05	1	20
Hastinapur	15	8	53
Meerut	20	12	60
Total	**50**	**27**	**54**

Fig. 1: Graphical representation of prevalence pattern of *E. coli* from different places in and around Meerut



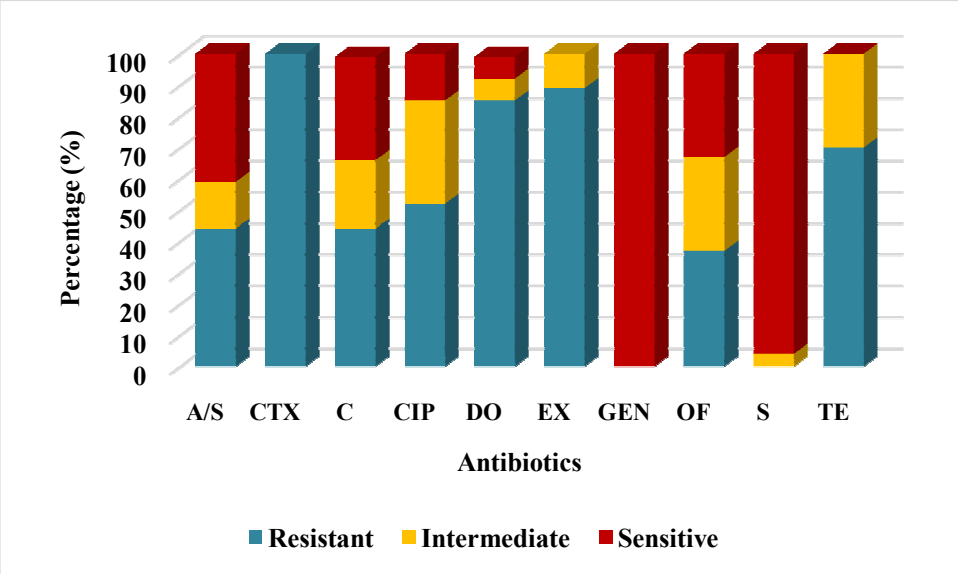
Antibiogram of *E. coli:*

Antimicrobial resistance and sensitivity patterns of the tested *E. coli* isolates against ten selected antimicrobial agents were determined using the agar disc diffusion method; the results are summarized in Table 3. All the isolates were resistant to Cefotaxime (100%) followed by Enrofloxacin (89%), Doxycycline hydrochloride (85%), Tetracycline (70%), Ciprofloxacin (52%), Chloramphenicol and Ampicillin/Sulbactam (44%). On the other hand, the highest responsiveness rates were recorded against Gentamicin (100%) followed by Streptomycin (96%), Ampicillin/Sulbactam (41%), Ofloxacin and Chloramphenicol (33%) as well as some isolates show nominal sensitivity against the ciprofloxacin (15%) (Fig. 2).

Table 3: Antimicrobial susceptibility patterns among the investigated *e. Coli* isolates

Antimicrobial Agents	*E. coli* isolates (n=27)					
	Resistant		Intermediate		Sensitive	
	n	%	n	%	n	%
Aminoglycosides						
Gentamicin (GEN)	0	0	0	0	27	100
Streptomycin (S)	0	0	1	4	26	96
Tetracycline						
Tetracycline (TE)	19	70	8	30	0	0
Doxycycline Hydrochloride (DO)	23	85	4	15	0	0
Fluroquinolones						
Ofloxacin (OF)	10	37	8	30	9	33
Enrofloxacin (Ex)	24	89	3	11	0	0
Ciprofloxacin (CIP)	14	52	9	33	4	15
Macrolides						
Chloramphenicol ©	12	44	6	22	9	33
Cephalosporin						
Cefotaxime (CTX)	27	100	0	0	0	0
Other						
Ampicillin/Sulbactam (A/S)	12	44	4	15	11	41

Fig. 2: Antibiogram profile of *E. coli* isolates against different antibiotics. A/S=Ampicillin/Sulbactam, CTX=Cefotaxime, C=Chloramphenicol, CIP=Ciprofloxacin, DO=Doxycycline Hydrochloride, EX= Enrofloxacin, GEN=Gentamycin, OF= Ofloxacin, S=Streptomycin and TE=Tetracycline.



DISCUSSION

Antimicrobial resistance in chickens is a recurrent problem in India and other developing countries due to the uncertain utilization of antibiotics agents as feed component and prophylactic treatment of infectious diseases. Antimicrobial agents have emerged a significant and prevalent public health risks mainly while there are few or not to be had alternative effective antimicrobial agents for the treatment of infections caused by microorganism. In spite of the fact that most strains of *E. coli* are innocuous and normally found in the gut of peoples and warm-blooded animals. In the current study, we explored the prevalence and the determinants of antimicrobial resistance among *E. coli* isolates from faecal samples in and around Meerut. The overall prevalence of *E. coli* from faecal samples was 54.00%. In step with Hossain *et al.*[13] mentioned the overall prevalence of *E. coli* was 60% from apparently healthy broilers and layers while Jakaria *et al.*[14]revealed a higher prevalence of 78.86% in cloacal sample of chicken. Among the isolates of *E. coli* have been resistant in varying degrees to commonly used antimicrobial agents, such as Cefotaxime followed by Enrofloxacin, Doxycycline hydrochloride, Tetracycline, Ciprofloxacin, Chloramphenicol and Ampicillin/Sulbactam. Amin *et al.*[15]reported *E. coli* resistance to third generation cephalosporin asnd tetracycline, while 12 strains were resistant to fluoroquinolone and sulphamethoxazole, 10 strains were to aminoglycosides and 3 strains were to nitrofurantoin. Additionally, Hossain *et al.* [13]also confirmed high level of sensitivity of *E. coli* isolates to chloramphenicol, ciprofloxacin, kanamycin and cephalexin. However, 32.26% *E. coli* from layer were found resistant to Ciprofloxacin and 25.81% to Ampicillin. This expanding pattern of resistance was recorded in both broiler and layer isolates which is supporting to our study. One of the finding to our outcomes was supported by the study of Al-Ghamdi *et al.*[16]who mentioned a very high resistance level of *E. coli* (99.1%) to tetracycline in Saudi Arabia. However, Prescott and Baggot[17]suggested good activity of erythromycin against some gram-negative bacteria. About 91.43% broiler isolates were moderately sensitive to cephalexin, 77.74% to ciprofloxacin and 85.71% to kanamycin while 54.28% isolates were highly sensitive to chloramphenicol. In keeping with Miles *et al.*[18] and Kang *et al.*[19]there is strong evidence that the usage of antimicrobial agents can prompt to the emergence and dissemination of resistant *E. coli*, which would able to be passed onto individuals by means of food or through direct contact with animals. Antimicrobial sources are additionally utilized as feed additives, poultry feed etc. which likewise bring about progressive increase of resistance to *E. coli.* Advancement of resistance may be because of the vigorous utilization of antibiotics for control of diseases in poultry. This higher prevalence may be because of poor management practices at poultry farms as *E. coli* is opportunistic pathogen. *E. coli* infection may happen as secondary infection when birds are immunosuppressed because of different diseases or environmental stress. Poor managemental practices and general hygienic conditions add to higher infection of *E. coli*[20]. Antibiotics agents are utilized broadly in poultry and animals without any prescription which brought in development of resistance strain of *E. coli* and thus antibiotic affectivity decreased with passage of time. Results of the present study

are in accordance with the findings of Catry *et al.*[21]who reported that resistance developed with extensive use of antibiotics. *E. coli* is one of the most significant factors of making financial losses resulting from diseases in commercial poultry farms and inflicting mortality as well as condemning the carcasses in slaughterhouses. Antibiotics are the drugs used for stopping economic losses because by *E. coli* and increasing the production efficiency. However, increasing utilization of these drugs prompts dispersing them into manure and other poultry wastes and transferring them to peoples by their remains in carcasses and can be the beginning of bacterial resistances. Because of vivacious utilization of antibiotics for control of diseases and in feed additives, resistance to the regular antibiotics in the microbes developed. It is strongly recommended to diminish the unethical utilization of antibiotics to limit the development of resistance strain of microbes within the future.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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