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Savita Sahu
Department of Veterinary
Pathology, Dau Shri Vasudev
Chandrakar Kamdhenu
Vishwavidyalaya, Anjora,
Durg, Chhattisgarh, India

Vivek Kumar
Department of Veterinary
Pathology, Dau Shri Vasudev
Chandrakar Kamdhenu
Vishwavidyalaya, Anjora,
Durg, Chhattisgarh, India

Dhananjay Kumar Jolhe
Department of Veterinary
Pathology, Dau Shri Vasudev
Chandrakar Kamdhenu
Vishwavidyalaya, Anjora,
Durg, Chhattisgarh, India

Ratan Chandra Ghosh
Department of Veterinary
Pathology, Dau Shri Vasudev
Chandrakar Kamdhenu
Vishwavidyalaya, Anjora,
Durg, Chhattisgarh, India

Kamlesh Kumar
MVSc, Department of
Veterinary Microbiology,
College of Veterinary and
Animal Sciences, Anjora, Durg,
Chhattisgarh, India

Corresponding Author:
Vivek Kumar
Department of Veterinary
Pathology, Dau Shri Vasudev
Chandrakar Kamdhenu
Vishwavidyalaya, Anjora,
Durg, Chhattisgarh, India

Biochemical characterization and antibiotic susceptibility pattern of *Escherichia coli* causing infections in chickens of Chhattisgarh plains

Savita Sahu, Vivek Kumar, Dhananjay Kumar Jolhe, Ratan Chandra Ghosh and Kamlesh Kumar

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Abstract

The present research work was carried out to elucidate the prevalence, pathology and biochemical characterization antibiotic susceptibility pattern of *Escherichia coli* causing avian *Colibacillosis* in Durg, Rajnandgaon, Raipur and Surajpur districts of Chhattisgarh. The duration of research work was 9 months from November 2021 to July 2022 in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Anjora, Durg, (C.G.). Detection of *E. coli* was done by cultural isolation, staining properties, biochemical tests, biofilm formation. The positive isolates were further subjected for antibiogram profile of *E. coli* isolates. Swab samples were collected from various organs of birds with suspected *Colibacillosis* across 12 commercial farms. Following inoculation in nutrient broth, isolates were cultured on MacConkey (MCA) and EMB agar. The resulting pink colonies on MCA and green metallic sheen on EMB tentatively identified the isolates as *E. coli*. Gram staining confirmed all 32 isolates as Gram-negative bacilli. Biochemical characterization showed all isolates were positive for indole, Methyl red, and catalase, but negative for Voges-Proskauer and citrate tests. The isolates fermented glucose, lactose, maltose, galactose, mannitol, sucrose, and dextrose with acid and gas production, but did not ferment inositol or adonitol. Seventeen (70.83%) *E. coli* isolates were positive for biofilm formation on Congo red agar. Antibiogram results showed that the PCR-positive isolates were most sensitive to chloramphenicol (94.11%), amikacin (88.23%), and streptomycin (76.47%). Conversely, the highest resistance was observed against levofloxacin (100%), trimethoprim (94.11%), and amoxiclav (70.58%). Intermediate resistance was primarily noted for azithromycin (and cefixime ((47.05%).

Keywords: Antibiogram, Biochemical tests, Biofilm, *Escherichia coli*, EMB, MacConkey

Introduction

Huge economic losses occurs as a result of mortality and decreased productivity of affected chickens, mainly during peak egg production Pathogenic *E. coli* strains including intestinal pathogenic *E. coli* (InPEC) and extra-intestinal pathogenic *E. coli* (ExPEC) depending on the location of the infection (Panth, 2019) [23]. APEC belongs to the ExPEC group is a major pathogen responsible for morbidity and mortality in chickens and causing high economic losses in poultry industries (Dhama *et al.*, 2013; Mellata, 2013 and Tawab *et al.*, 2014) [7, 18, 32]. APEC strains can affect all age groups of chickens, most commonly found in young chicks of up to 3 weeks of age. Many conventional bacteriological techniques, serological tests and molecular techniques are helpful for the identification of pathogenic *E. coli* and for the detection of certain virulence genes. Pathogenic *E. coli* possess one or more virulence properties that may help in establishment of the infection. Virulence properties of *E. coli* can be detected by Congo red binding and haemolytic activity. The association between the binding of Congo red dye and the pathogenicity of *E. coli* was confirmed by Berkhoff and Vinal, (1986) [5]. Conventional culture technique is commonly used for isolation of *E. coli*. This includes non-selective pre-enrichment (nutrient agar) followed by selective enrichment (MacConkey agar) and plating on selective agar (EMB). *E. coli* produce pink lactose fermenting colonies on MacConkey agar (MCA) after 24hrs. of incubation at 37°C, then performing subculture on EMB agar produce green metallic sheen. Suspected colonies are confirmed by various biochemical tests.

According to Ewers *et al.*, (2005), a variety of virulence factors have been implicated in promoting these extraintestinal diseases in avian species, including adhesins (F1-, P-, AC/I-, and F17-fimbriae, curli fibers and fimbrial adhesins), hemolysins (hemolysin E and *tsh*), antibactericidal factors (outer membrane protein A, iss, lipopolysaccharide, K1-capsule) and toxins (heat stable toxin, cyto-verotoxin, flagella toxin, and vat toxin). All these virulence genes that contribute to the pathogenesis of *Colibacillosis* in chickens were also reported by (Johnson *et al.*, 2008; Zenda *et al.*, 2013 and Ibrahim *et al.*, 2019) [12]. Keeping the above facts into consideration, the present study was undertaken with the following objectives:

- Isolation of *Escherichia coli* on pre-enrichment and selective enrichment media.
- Determination of biochemical characteristics and biofilm formation phenomenon of *E. coli*

Materials and Methods

Gram's staining of *E. coli*

Single pink lactose fermenting colony was taken aseptically from MCA plate using a sterile loop and made smear on cleaned glass slide and then stained by Gram's method of staining (Himedia, K001-1KT). Following staining, slide was examined under microscope to determine the size and shape of the organism (Merchant and Packer, 1967).

3.6.4. Motility test

The motility test was performed according to the method described by (Cowan, 1985) [6] to differentiate the motile bacteria from the non-motile one by hanging drop method. A drop of 24 hrs. old pure broth culture of *E. coli* was taken on the clean glass slide and observed the movement of bacteria under microscope.

Biochemical tests

Biochemical tests such as catalase test, indole production, methyl red (MR), Voges-Proskauer (VP), citrate utilization, oxidase test and carbohydrate fermentation test were carried out as per the standard procedures described by Khaton *et al.*, (2008) and Levy *et al.*, (2020) [14, 11].

Catalase test

A loop full of pure colony from each isolate was picked up and transferred on a glass slide. One-two drops of 3% Hydrogen Peroxide (H₂O₂) were placed over culture to observe effervescence of bubbles for confirmation of catalase.

Indole production test

Peptone broth (supplemented with NAD) tubes were prepared freshly and inoculated with each isolate and incubated at 37°C for 48 hrs. After incubation, 0.5 ml Kovac's reagent was added into tubes and observed for appearance of red colour ring at the junction of medium and reagent to confirm Indole production.

Methyl Red test

Methyl red-Voges-Proskauer (MR-VP) broth was prepared in a tube and inoculated freshly pure culture and incubate at 37°C for 48 hrs. following incubation, 5 drop of a (0.05%) methyl red solution was added and observed the colour

change in the tubes. Development of red or pink colour was interpreted as positive test.

Voges-Proskauer test

MR-VP broth was prepared in a tube and inoculated freshly pure single colony of the each isolate of *E. coli* and incubated at 37°C for 48 hrs. After incubation, 0.6ml of VP reagent-1 and 0.2 ml of VP reagent-2 was added for each ml of culture, the ingredients were mixed thoroughly and allowed to stand. The appearance of pink colour indicated to positive test.

Citrate utilization test

Simmons citrate agar HiMedia, (M099S) slant was prepared in 10 ml tube by autoclaving at 15 lbs and 121°C. Small amount of 24 hrs old pure culture of *E. coli* inoculated into the slant by means of streaking in a zig-zag pattern. The tubes were incubated at 37°C for 48 hrs. Following incubation, colour change at the surface of slant from green to blue was interpreted as positive

Carbohydrate fermentation test

Carbohydrate fermentation tests was performed to detect the ability of the organism to ferment different sugars. Test was carried out by inoculating loop full fresh pure culture of *E. coli* into the 5ml Andrade peptone water (HiMedia, M885) then single carbohydrate disc was added in each tube and inverted Durham tube was placed inside the each tube and incubate at 37°C for 72 hrs. After incubation, colour changed and gas production was observed. Acid production was indicated by the colour change from red to yellow of the medium and the gas production was noted by the appearance of gas bubbles in the inverted Durham's tube (Edwards and Ewing, 1972 and Tonu *et al.*, 2011) [33].

Biofilm formation of *E. coli*

A simple qualitative approach for detecting biofilm production using Congo red agar (CRA) media, described by Freeman *et al.*, (1989) was followed. Brain heart infusion agar 37 g/l, sucrose 50 g/l, and Congo red indicator 0.8 g/l were used to make CRA media. The first Congo red stain was made as a concentrated aqueous solution and autoclaved separately from the other medium constituents (121°C for 15 min.). Then, at 55°C, it was put to autoclaved brain heart infusion agar with sucrose. CRA plates were inoculated with test organisms and aerobically incubated for 24 hours at 37°C. Biofilm was identified by black colonies with a dry crystalline appearance (Katongole *et al.*, 2020) [13].

Antibiogram of *E. coli* isolates

Antibiogram of *E. coli* isolates was studied by disc diffusion method and interpretation was made as per Clinical and Laboratory Standards Institute (CLSI, 2019). Twelve numbers of antibiotic discs (Himedia laboratories, Mumbai) are used as enlisted in Table. 4. The overnight grown broth culture was spread on Muller Hinton Agar Base (HiMedia, M173) and the antibiotic discs were impregnated and incubated at 37°C for 24-48 hrs in BOD incubator (Sahoo *et al.*, 2012) [25]. After incubation, result was interpreted as per CLSI guidelines as given in the Table. 1.

Table 1: Antibiotics used to study antibiogram of *E. coli* isolates

S. No.	Antibiotics used	Antibiotics symbol and concentration /disc	Interpretation as per CLSI 2019		
			Sensitive	Intermediate	Resistant
1.	Amoxiclav	AMC (30µg)	≥18	14-17	≤13
2.	Amikacin	AK (30µg)	≥17	15-16	≤14
3.	Cefixime	CFM (5µg)	≥19	-	≤15
4.	Cefotaxime	CTX (30µg)	≥26	23-25	≤22
5.	Chloramphenicol	C (30µg)	≥18	13-17	≤12
6.	Streptomycin	S (10µg)	≥15	12-14	≤11
7.	Tetracycline	TE (30µg)	≥15	12-14	≤11
8.	Gentamicin	GEN (10µg)	≥15	13-14	≤12
9.	Ciprofloxacin	CIP (5µg)	≥21	16-20	≤15
10.	Trimethoprim	TR (5µg)	≥16	11-15	≤10
11.	Levofloxacin	LE (5µg)	≥17	14-16	≤13
12.	Azithromycin	AZM (15µg)	≥18	14-17	≤13

Results and Discussion

Isolation of *E. coli*

In the present study, swab samples were collected from various organs, including the liver, heart, intestine, oviduct, and yolk sac, for the isolation of *E. coli* from 12 commercial broiler and layer farms exhibiting gross lesions suggestive of *Colibacillosis*. Based on cultural, morphological, and biochemical characteristics, a total of 32 (68.08%) *E. coli* isolates were recovered from 47 positive cases of *Colibacillosis*. All 32 isolates manifested as pink-colored, lactose-fermenting colonies, 5–8 mm in diameter, on MacConkey agar (MCA) (Fig.1). Following 24 hours of incubation at 37°C, 24 of these isolates produced a characteristic green metallic sheen on Eosin Methylene Blue (EMB) agar (Fig.2). These findings align with those reported by previous authors (Tonu *et al.*, 2011; Sahoo *et al.*, 2012; Sarkar *et al.*, 2013; Srinivasan *et al.*, 2014; Matin *et al.*, 2017; Ahmad *et al.*, 2022; Koutsianos *et al.*, 2022) [33, 25, 26, 30, 17, 1, 16]. The *E. coli* isolation rate observed in this study (68.08%) is comparable to the isolation rates reported by Kilic *et al.* (2007) [15], Ramasamy *et al.* (2008) [24], and Sarkar *et al.* (2013) [26], who documented rates of 48%, 51.9%, and 67.3%, respectively.

Gram's staining and Motility test

All 32 isolates showed Gram-negative, pink coloured, rod-shaped, short chain in Gram's stain (Fig.3). previous workers described similar staining characteristics of *E. coli* on Gram's stain (Khaton *et al.*, 2008; Omer *et al.*, 2010; Tonu *et al.*, 2011 and Amine *et al.*, 2017) [14, 21, 33]. All the *E. coli* isolates were found to be motile with hanging

drop's preparation. The results of the motility test are akin to the findings of (Khaton *et al.*, 2008 and Dey *et al.*, 2013) [14].

Biochemical Characterization of *E. coli* isolates

All the isolates that have morphological characters of *E. coli* were subjected to biochemical test for identification. All the 32 isolates showed biochemical reactions pertaining to *E. coli*. The isolates were found to be positive for indole production, Methyl red (MR), catalase test and negative for Voges-Proskauer (VP) and citrate utilization test (Fig.4). All the 32 isolates revealed similar IMViC pattern (+ + - -). These findings in our study were in agreement with earlier reports by (Khaton *et al.*, 2008; Tonu *et al.*, 2011; Nasrin *et al.*, 2012; Olarinmoye *et al.*, 2013; Sarkar *et al.*, 2013; Singh *et al.*, 2018; Panchal *et al.*, 2020 and Choudhary *et al.*, 2020) [14, 33, 26, 19, 20, 29, 22].

All the isolates fermented 7 sugars such as glucose, lactose, maltose, galactose, mannitol, sucrose and dextrose with the production of acid and gas. Acid production was indicated by the color change from reddish to yellow and gas production was noted by the presence of gas bubbles in the inverted Durham's tubes and all the isolates were failed to ferment inositol and adonitol (Fig.5; Table 2) within 24-48 hrs. of incubation that supported the findings of others workers (Edwards and Ewing, 1972; Nasrin *et al.*, 2012; Sahoo *et al.*, 2012; Dey *et al.*, 2013; Dadheech *et al.*, 2016 and Boro *et al.*, 2018). Singh *et al.*, (2018) [8, 19, 29] reported all this isolates fermented glucose, lactose and mannitol but, only 3% of isolates fermenting adonitol.

Table 2: Phenotypic screening of *Escherichia coli* isolates from chickens affected with *Colibacillosis*

S.No.	<i>E. coli</i> isolates	Gram negative rods	Cultural characteristics		Biochemical properties									Biofilm formation on CRA	
			Pink lactose fermenting colonies on MCA	Green metallic sheen on EMB	IMViC Pattern	Sugar fermentation									
						Glucose	Lactose	Maltose	Galactose	Mannitol	Sucrose	Inositol	Adonitol		
1	DRG1	+	+	+	++--	+	+	+	+	+	+	-	-	+	
2	DRG2	+	+	+	++--	+	+	+	+	+	+	-	-	+	
3	DRG3	+	+	+	++--	+	+	+	+	+	+	-	-	-	
4	DRG4	+	+	+	++--	+	+	+	+	+	+	-	-	+	
5	DRG5	+	+	-	++--	+	+	+	+	+	+	-	-	NT	
6	DRG6	+	+	+	++--	+	+	+	+	+	+	-	-	+	
7	DRG7	+	+	+	++--	+	+	+	+	+	+	-	-	+	
8	DRG8	+	+	+	++--	+	+	+	+	+	+	-	-	+	
9	DRG9	+	+	+	++--	+	+	+	+	+	+	-	-	-	
10	DRG10	+	+	+	++--	+	+	+	+	+	+	=	-	-	
11	DRG11	+	+	-	++--	+	+	+	+	+	+	-	-	NT	
12	DRG12	+	+	+	++--	+	+	+	+	+	+	-	-	+	
13	DRG13	+	+	+	++--	+	+	+	+	+	+	-	-	+	
14	DRG14	+	+	+	++--	+	+	+	+	+	+	-	-	+	

15	DRG15	+	+	+	++--	+	+	+	+	+	+	-	-	-
16	RAI 1	+	+	+	++--	+	+	+	+	+	+	-	-	+
17	RAI 2	+	+	+	++--	+	+	+	+	+	+	-	-	+
18	RAI 3	+	+	-	++--	+	+	+	+	+	+	-	-	NT
19	RAI 4	+	+	-	++--	+	+	+	+	+	+	-	-	NT
20	RAI 5	+	+	+	++--	+	+	+	+	+	+	-	-	+
21	RAI 6	+	+	+	++--	+	+	+	+	+	+	-	-	+
22	RAI7	+	+	+	++--	+	+	+	+	+	+	-	-	-
23	RAI8	+	+	-	++--	+	+	+	+	+	+	-	-	NT
24	RJN 1	+	+	-	++--	+	+	+	+	+	+	-	-	NT
25	RJN 2	+	+	+	++--	+	+	+	+	+	+	-	-	+
26	RJN 3	+	+	-	++--	+	+	+	+	+	+	-	-	-
27	RJN4	+	+	+	++--	+	+	+	+	+	+	-	-	+
28	RJN5	+	+	+	++--	+	+	+	+	+	+	-	-	+
29	SJP1	+	+	+	++--	+	+	+	+	+	+	-	-	+
30	SJP2	+	+	-	++--	+	+	+	+	+	+	-	-	NT
31	SJP3	+	+	-	++--	+	+	+	+	+	+	-	-	NT
32	SJP4	+	+	+	++--	+	+	+	+	+	+	-	-	-
Total		32	32	24	32	32	32	32	32	32	32	32	32	17

DRG - isolates of Durg, RAI- isolates of Raipur, RJN- isolates of Rajnandgaon, SJP- isolates of Surajpur, NT-Not tested



Fig 1: Showing pinkish coloured lactose fermenting colonies of *E. coli* on MacConkey agar



Fig 3: Gram's staining revealed pink coloured Gram-negative short bacilli



Fig 2: Exhibiting characteristic green metallic sheen colonies of *E. coli* on EMB agar

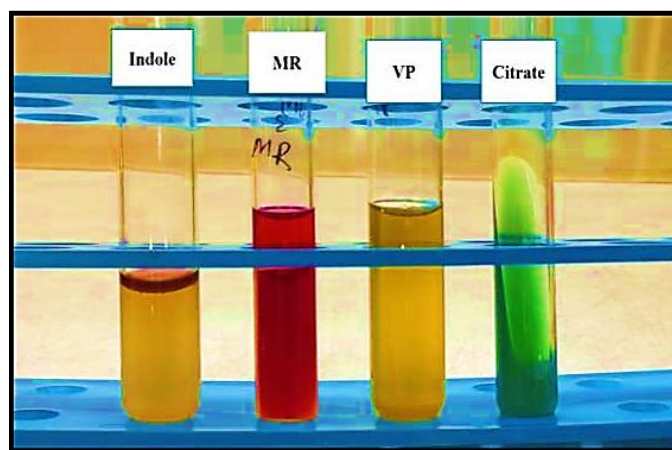


Fig 4: Showing result of IMViC test for *E. coli* (Indole & MR +ve and VP & citrate -ve)

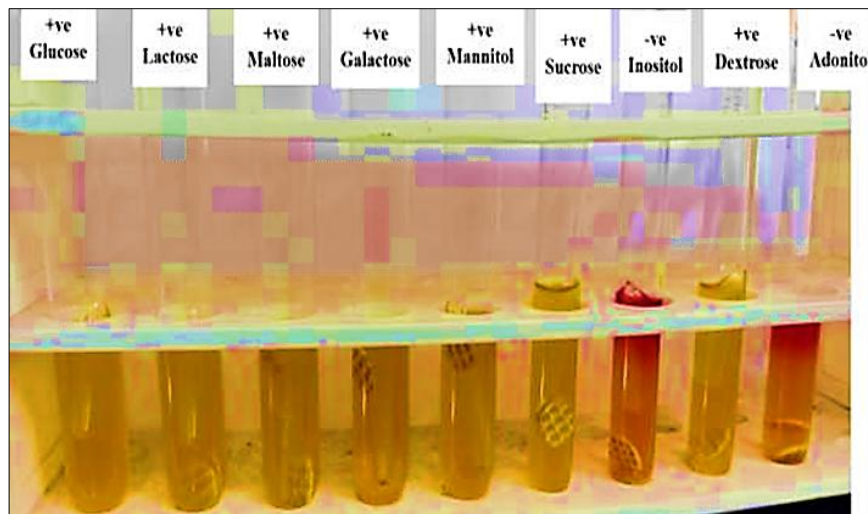


Fig 5: Showing result of carbohydrate fermentation of *E. coli* isolates with production of acid and gas

Biofilm formation

For this test pure colony of the *E. coli* isolates were picked and streaked on CRA plates and incubated at 37°C for 24 hours aerobically. Out of 24 EMB positive *E. coli* isolates, 17 isolates produced dark coloured colonies with dry crystalline consistency on Congo red agar plate were considered positive biofilm formation (Fig.6) whereas, red colonies negative biofilm forming *E. coli* (Fig.7). These

findings were in accordance with the findings of Katongole *et al.*, (2020) ^[13] who showed the dark crystalline colonies of pathogenic *E. coli* on Congo red agar.

In contrast to the present findings observed by Berkhoff and Vinal (1986) ^[5], Ahmed *et al.*, (2009) and Seifi *et al.*, (2015) ^[27] who reported that biofilm positive *E. coli* isolates produce grayish white colonies on CRA.



Fig 6: Biofilm formation of *E. coli* isolate showing dark crystalline colonies on Congo red agar



Fig 7: Non biofilm forming *E. coli* isolates showing red colonies on Congo red agar

Antibiogram of APEC isolates

In the present study, antibiotic sensitivity pattern of all the PCR positive *E. coli* isolates were determined using disc diffusion method. The *E. coli* isolates were tested against 12 commonly used antibiotic discs viz. chloramphenicol, amikacin, streptomycin, tetracycline, gentamicin, ciprofloxacin, cefixime, cefotaxime, amoxycillin+clavulanic acid (amoxycylav), trimethoprim, levofloxacin and Azithromycin (Fig.8). The antibiotic susceptibility of each isolates was shown in the form of percentage of sensitivity, intermediate and resistant (Table 3 and Graph 1).

E. coli isolates were highly sensitive to antibiotic chloramphenicol (94.11%) followed by amikacin (88.23%), streptomycin (76.47%), gentamicin (58.82% and ciprofloxacin (41.17%), whereas highest resistant was observed to antibiotic levofloxacin (100%) followed

trimethoprim (94.11%), amoxiclav (70.58%), cefotaxime (52.94%), tetracycline (47.05%), azithromycin (41.17%) and cefixime (35.29%). Intermediate resistance was observed to antibiotics azithromycin, cefixime (47.17%) followed by tetracycline, cefotaxime (35.29%), gentamicin (29.41%), amoxyclav (23.52%) and trimethoprim (5.88%).

The highest susceptibility of *E. coli* to chloramphenicol was also reported by previous authors Akond *et al.*, (2009) [2], Sahoo *et al.*, (2012) [25] and Shecho *et al.*, (2017) [28]. In contrast, Omer *et al.*, (2010) [21] detected almost all the *E. coli* isolates resistant to chloramphenicol isolated from chicken affected with *Colibacillosis*. Similarly, sensitivity to amikacin (88.23%) observed during the present study was also well supported by Subedi *et al.*, (2018) [31] and Choudhari *et al.*, (2020) [4] with sensitivity percentage of *E. coli* isolates observed were (84%) and (83.33%) respectively. During the present study highest resistance (100%) to *E. coli* isolates was observed against levofloxacin. Whereas Younis *et al.*, (2017) and Shrivastav *et al.*, (2018) observed 50% resistance of APEC isolates towards levofloxacin during their study. Halfaoui *et al.*, (2017) reported higher resistant to trimethoprim which was in agreement with the present study.

In the present study the APEC isolates clearly demonstrated higher resistance to most of the antibiotics (Graph 2) studied might be due to increased use of antibiotics as feed

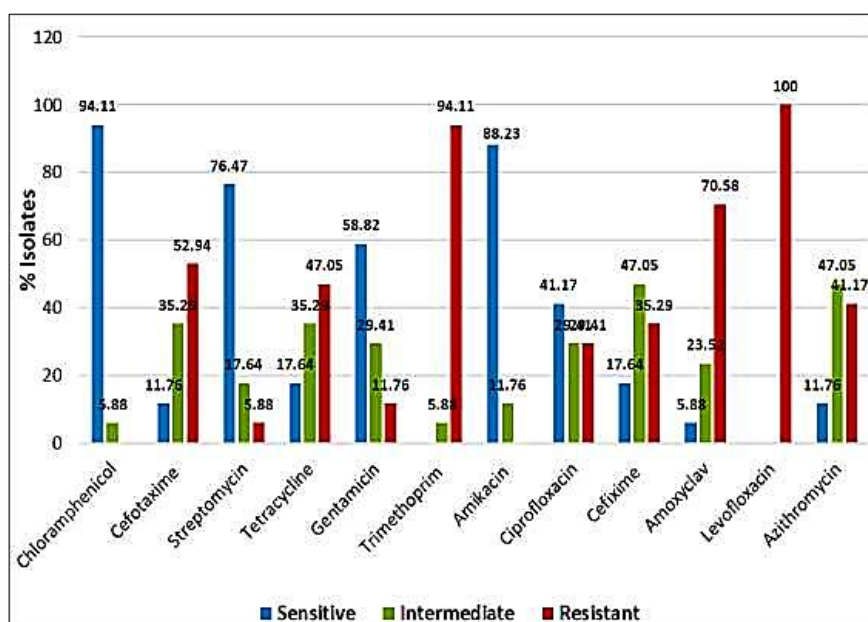
additives, growth promotion and therapeutic purpose for the prevention of diseases in poultry. For this reason, these antibiotics are ineffective or less effective against pathogenic *E. coli* strains in the present time (Omer *et al.*, 2010) [21].

Table 3: Antibiotic susceptibility profile of APEC isolates

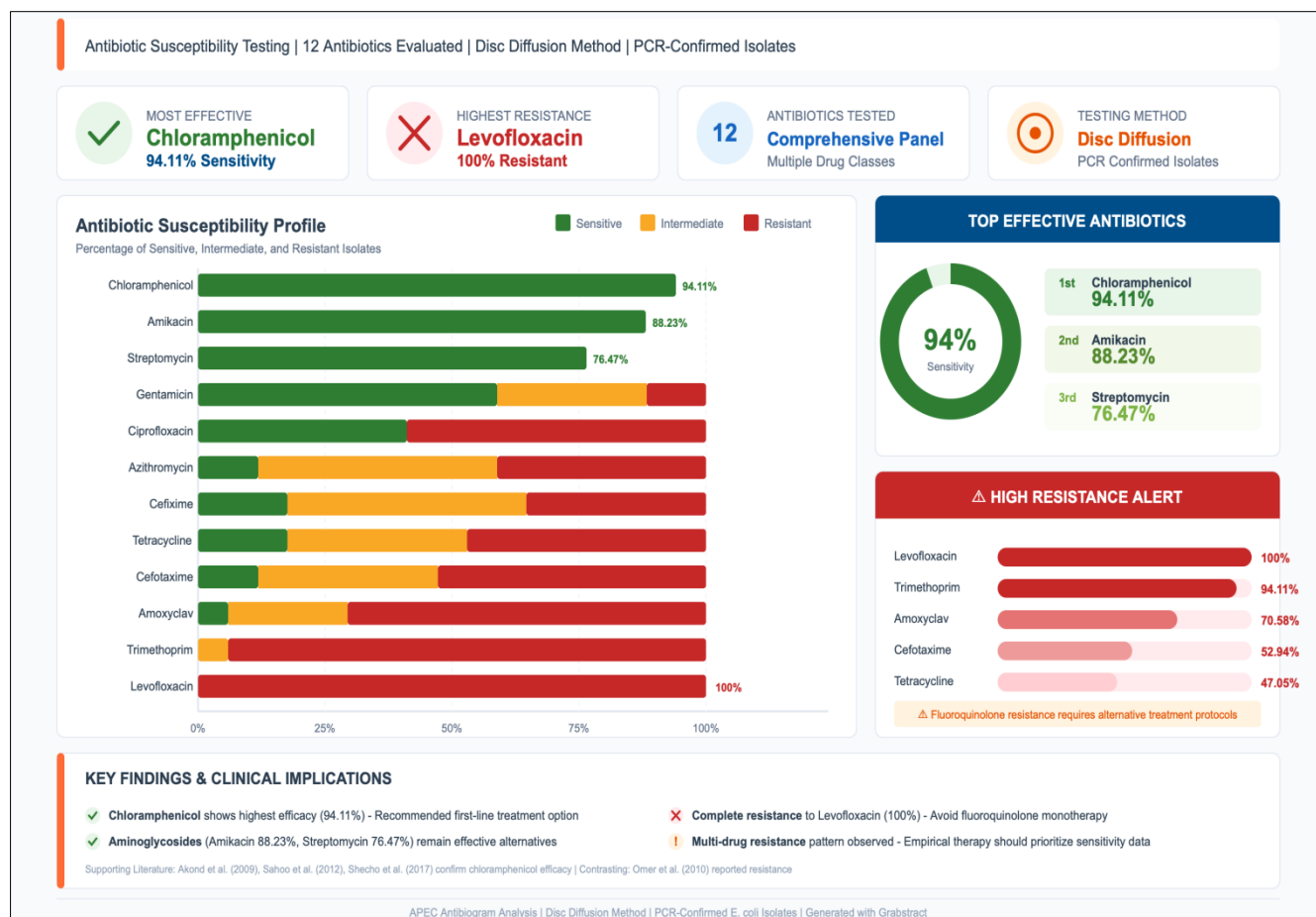
S.No.	Antibiotics	Susceptibility of <i>E. coli</i> isolates		
		Sensitive(S)	Intermediate(I)	Resistant(R)
1.	Chloramphenicol (C)	16 (94.11%)	1 (5.88%)	0
2.	Cefotaxime (CTX)	2 (11.7%)	6 (35.29%)	9 (52.94%)
3.	Streptomycin (S)	13 (76.47%)	3 (17.64%)	1 (5.8%)
4.	Tetracycline (TE)	3 (17.64%)	6 (35.29%)	8 (47.05%)
5.	Gentamicin (GEN)	10 (58.82%)	5 (29.41%)	2 (11.76%)
6.	Trimethoprim (TR)	0	1 (5.88%)	16 (94.11%)
7.	Amikacin (AK)	15 (88.23%)	2 (11.76%)	0
8.	Ciprofloxacin (CIP)	7 (41.17%)	5 (29.41%)	5 (29.41%)
9.	Cefixime (CFM)	3 (17.64%)	8 (47.05%)	6 (35.29%)
10.	Amoxyclav (AMC)	1 (5.88%)	4 (23.52%)	12 (70.58%)
11.	Levofloxacin (LE)	0	0	17 (100%)
12.	Azithromycin (AZM)	2 (11.76%)	8 (47.05%)	7 (41.17%)



Fig.8: Showing antibiotic susceptibility pattern of *E. coli* by disc diffusion method



Graph 1: Antibigram Profile of APEC isolates



Graph 2: Antibigram analysis of APEC isolates (Antibiotic susceptibility testing)

Conclusion

Based on cultural characteristics and morphological observation, a total of 24 *E. coli* isolates were successfully recovered and identified from EMB agar plates. The comprehensive antibiogram profiling of these *E. coli* isolates revealed that the highest level of sensitivity was exhibited toward chloramphenicol, which was followed in decreasing order of susceptibility by amikacin, streptomycin, gentamycin, and ciprofloxacin. Conversely, a significant patterns of antimicrobial resistance were observed, with the highest resistance rates recorded for levofloxacin, followed by trimethoprim, cefotaxime, cefixime, amoxycylav, tetracycline, and azithromycin. These findings strongly emphasized the urgent necessity for the judicious and evidence-based selection of antibiotics to ensure effective clinical treatment outcomes. Furthermore, the phenotypic evaluation of virulence factors showed that a majority of the *E. coli* isolates (70.83%) were identified as biofilm formers when tested on Congo Red Agar (CRA).

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Competing interests

The authors declare that there are no competing interests regarding this work.

Consent for publication

All authors have granted their formal consent for the publication of this manuscript.

References

- Ahmad T, Fiaz M, Sharif A, Nadeem M, Rehman SU, Rizwan M, *et al.* *In vitro* and *in vivo* evaluation of antimicrobials in *Escherichia coli* infection in broilers and evaluation of ciprofloxacin in induced *Colibacillosis*. *Pure Appl Biol.* 2022;11(3):744–754.
- Akond MA, Alam S, Hassan SMR, Shirin M. Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. *Int J Food Saf.* 2009;11:19–23.
- Amin U, Kamil SA, Shah SA, Dar TA, Mir MS, Ali R, *et al.* Serotyping and prevalence of avian pathogenic *Escherichia coli* infection in broilers in Kashmir. *Pharma Innov Int J.* 2017;6(10):336–338.
- Choudhari P, Ghodasara DJ, Kabariya DV, Bhandari BB, Momin SG. Isolation, antibiogram and molecular characterization of *E. coli* in broiler flocks. *Indian J Vet Sci Biotechnol.* 2020;16(4):35–39.
- Berkhoff HA, Vinal AC. Congo red medium to distinguish between invasive and non-invasive *Escherichia coli* pathogenic for poultry. *Avian Dis.* 1986;30(1):117–121.
- Cowan ST. Biochemical behavior of *E. coli*. *J Gen Microbiol.* 1985;8:391.
- Dhama K, Chakraborty S, Barathidasan R, Tiwari R, Rajagunalan S, Singh SD. *Escherichia coli*, an economically important avian pathogen, its disease manifestations, diagnosis and control and public health significance. *Res Opin Anim Vet Sci.* 2013;3(6):179–194.

8. Edwards PR, Ewing WH. Identification of Enterobacteriaceae. 3rd ed. Minneapolis (MN): Burgess Publishing Co.; 1972.
9. Ewers C, Janssen T, Kiesling S, Philipp HC, Wielera LH. Rapid detection of virulence-associated genes in avian pathogenic *E. coli* by multiplex PCR. Avian Dis. 2005;49:269–273.
10. Halfaoui Z, Menoueri NM, Bendali LM. Serogrouping and antibiotic resistance of *E. coli* isolated from broiler chicken with *Colibacillosis* in centre of Algeria. Vet World. 2017;10(7):830–835.
11. Ievy S, Islam MS, Sobur MA, Talukder M, Rahman MB, Khan MR, et al. Molecular detection of avian pathogenic *Escherichia coli* (APEC) for the first time in layer farms in Bangladesh and their antibiotic resistance patterns. Microorganisms. 2020;8(7):1021.
12. Johnson TJ, Wannemuehler Y, Doetkott C, Johnson SJ, Rosenberger SC, Nolan LK. Identification of minimal predictors of avian pathogenic *Escherichia coli* virulence for use as a rapid diagnostic tool. J Clin Microbiol. 2008;46(12):3987–3996.
13. Katongole P, Nalubega F, Florence NC, Asiimwe B, Andia I. Biofilm formation, antimicrobial susceptibility and virulence genes of UPEC isolated from clinical isolates in Uganda. BMC Infect Dis. 2020;20(1):453.
14. Khaton R, Haider MG, Paul PK, Das PM, Hossain MM. *Colibacillosis* in commercial chickens in Bangladesh. Bangladesh Vet. 2008;25(1):17–24.
15. Kilic A, Ertas HB, Muz A, Ozbey G, Kalender H. Detection of the *eaeA* gene in *Escherichia coli* from chickens by polymerase chain reaction. Turk J Anim Sci. 2007;31(4):215–218.
16. Koutsianos D, Athanasiou LV, Mossialos D, Franzo G, Cecchinato M, Koutoulis KC. Investigation of serotype prevalence of *Escherichia coli* strains isolated from layer poultry in Greece and interactions with other infectious agents. Vet Sci. 2022;9(4):152.
17. Matin MA, Islam MA, Khatun MM. Prevalence of *Colibacillosis* in chickens in greater Mymensingh district of Bangladesh. Vet World. 2017;10(1):29–33.
18. Mellata M. Human and avian extraintestinal pathogenic *Escherichia coli*: infections, zoonotic risks, and antibiotic resistance trends. Foodborne Pathog Dis. 2013;10(11):916–932.
19. Nasrin S, Islam MA, Khatun M, Akhter L, Sultana S. Characterization of bacteria associated with omphalitis in chicks. Bangladesh Vet. 2012;29(2):63–68.
20. Olarinmoye AO, Oladele OO, Adediji AA, Ntiwunka UG, Tayo GO. Antibigrams of avian pathogenic *Escherichia coli* isolates from commercial layers with *Colibacillosis* in Southwest Nigeria. Malays J Microbiol. 2013;9(4):317–325.
21. Omer MM, Abusalab SM, Gumaa MM, Mulla SA, Omer EA, Jeddah IE, et al. Outbreak of *Colibacillosis* among broiler and layer flocks in intensive and semi-intensive poultry farms in Kassala state, eastern Sudan. Asian J Poult Sci. 2010;4(4):173–181.
22. Panchal PP, Patel JM, Vihol PD, Raval JK, Patel DR, Danger N, et al. Serotyping and prevalence of *Escherichia coli* infection in poultry of South Gujarat based on culture isolation and identification. Int J Curr Microbiol Appl Sci. 2020;9(10):3876–3883.
23. Panth Y. *Colibacillosis* in poultry: A review. J Agric Nat Resour. 2019;2(1):301–311.
24. Ramasamy GKM, Dorairajan N, Balasubramaniam GA, Dinakaran AM, Saravanabava K. Pathogenic bacteria related to respiratory diseases in poultry with reference to *Ornithobacterium rhinotracheale* isolated in India. Vet Arhiv. 2008;78(2):131–140.
25. Sahoo TK, Sahoo L, Sarangi LN, Panda SK, Panda HK. Prevalence, isolation, characterization and antibiogram study of pathogenic *Escherichia coli* from different poultry farms of Odisha. J Adv Vet Res. 2012;2(3):169–172.
26. Sarkar M, Roy JP, Batabyal K. Characterization and antibiogram of enteropathogenic *Escherichia coli* isolated from poultry. Explor Anim Med Res. 2013;3(2):65–168.
27. Seifi S, Khoshbakht R, Atabak AR. Antibiotic susceptibility, serotyping and pathogenicity evaluation of avian *E. coli* isolated from broilers in northern Iran. Bulg J Vet Med. 2015;18(2):173–179.
28. Shecho M, Thomas N, Kemal J, Muktar Y. Cloacal carriage and multidrug resistance *E. coli* O157 from poultry farms, eastern Ethiopia. J Vet Med. 2017;17:1–9.
29. Singh GK, Niyogi D, Tripathi KK, Joshi RK, Singh SV, Choudhary PK. Incidence of spontaneous *E. coli* infection in broiler chickens in Faizabad and Sultanpur districts of Uttar Pradesh. Int J Curr Microbiol Appl Sci. 2018;7(5):5175–5181.
30. Srinivasan P, Balasubramaniam GA, Murthy TR, Balachandran P. Bacteriological and pathological studies of egg peritonitis in commercial layer chicken in Namakkal area. Asian Pac J Trop Med. 2013;3(12):988–994.
31. Subedi M, Luitel H, Devkota B, Bhattarai RK, Phuyal S, Panthi P, et al. Antibiotic resistance pattern and virulence genes content in avian pathogenic *Escherichia coli* from broiler chickens in Chitwan, Nepal. BMC Vet Res. 2018;14(1):113.
32. Tawab AA, Maarouf AA, Samir AEA, Hofy FI, Mougy EAE. Detection of some virulence genes of avian pathogenic *E. coli* by polymerase chain reaction. Benha Vet Med J. 2014;26(2):159–176.
33. Tonu NS, Sufian MA, Sarker S, Kamal MM, Rahman MH, Hossain MM. Pathological study on *Colibacillosis* in chickens and detection of *Escherichia coli* by PCR. Bangladesh J Vet Med. 2011;9(1):17–25.
34. Zende RJ, Chavhan DM, Suryawanshi PR, Rai AK, Vaidya VM. PCR detection and serotyping of enterotoxigenic and shigatoxigenic *Escherichia coli* isolates obtained from chicken meat in Mumbai, India. Vet World. 2013;6(10):770–773.