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ANTIBIOTIC RESISTANCE PROFILE OF *ESCHERICHIA COLI* ISOLATED FROM RETAIL POULTRY OUTLETS OF SATARA DISTRICT, MAHARASHTRA

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ABSTRACT

In the present study, intestinal contents of 80 broiler birds were sampled from retail poultry outlet of Satara from Sept to Dec 2020. A total of 30 *Escherichia coli* (*E. coli)* isolates were identified based on characteristic colonies on selective agar and further confirmed by PCR. These isolates were tested for their antibiotic sensitivity against 8 different antibiotics using the Kirby-Bauer disc diffusion method. It showed that *E. coli* isolates were susceptible to Cephoxitin (90%), Cefoperazone (90%), Tobramycin (87%), Piperacillin/Tazobactam (87%), and Norfloxacin (87%) whereas, high sensitivity to Augmentin (93.3%), Imipenem (90%), and Nalidixic acid (66.7%) was also observed. Further, multidrug resistance was observed in 20 percent of the isolates.

Keywords: *E. coli*, Chicken, Antibiotic, Retail

INTRODUCTION

Escherichia coli is an important pathogen affecting poultry either as a primary pathogen or secondary bacterial infection, resulting in economic losses to the poultry industry. It is manifested by yolk sac infection, omphalitis, respiratory tract infection, septicaemia, polyserositis, enteritis, cellulitis, and salpingitis (Kabir, 2010). The control of avian colibacillosis relies mainly on the use of antibiotics. Moreover, antibiotics are also used as growth promoters in the poultry industry (Van Boeckel *et al.,* 2015). Such events can give rise to multi–drug resistant bacteria and create problems in the management of intestinal and extra–intestinal infections caused by *E. coli* (Gupta *et al.,* 2001). Multi–drug resistant *E. coli* can be transmitted to humans via food of animal origin (chicken, beef, mutton, milk etc.) or direct contact with infected animals and can act as a potential source for transportation of resistance genes to

human pathogens (Oliveira *et al.,* 2020, Vega Sánchez *et al.,* 2020, Reddy *et al.,* 2021). At slaughterhouses and retail shops, the spillage of gut contents often contaminates the poultry carcasses and can transmit the multi-drug resistant *E. coli* to consumers (Turtura *et al.,* 1990, Reddy *et al.,* 2021). The present study was designed to investigate the antimicrobial resistance patterns in *E. coli* isolated from intestinal samples obtained from retail poultry outlets of Satara.

MATERIALS AND METHODS

Sampling and Bacterial Isolation

Intestinal contents collected from retail poultry outlets of Satara district, Maharashtra, were suspended in 10 ml of nutrient broth and incubated at 37 ºC for 24 h. Overnight growth was sub cultured onto the MacConkey Agar (Hi-Media, India) plates and incubated at 37 ºC overnight. The lactose fermenting pink colonies obtained were inoculated on Eosin methylene blue (EMB) agar plates (Hi-Media, India) and incubated at 37 ºC for overnight. Colonies producing green metallic sheen were picked from EMB plates and confirmed by specific PCR.

Antibiotic Sensitivity test

The antibiotics resistance pattern of 30 *E. coli* isolates against eight different antibiotics *viz.,* Tobramycin (TOB) (10 mcg), Imipenem (IPM) (10 mcg), Augmentin (AMC) (30 mcg), Cephoxitin (CX) (30 mcg), Piperacillin/Tazobactam (PIT) (100/10 mcg), Cefoperazone (CPZ) (75 mcg), Nalidixic acid (NA) (30 mcg), norfloxacin (NX) (10 mcg) (HiMedia, India) was performed using Kirby- Bauer disc diffusion method on Mueller-Hinton agar (Bauer *et al.,* 1966) and CLSI (Clinical and Laboratory Standards Institute), 2018 guidelines. Isolates showing resistance to 3 or more antimicrobials were classified as MDR (Multi–Drug Resistant) isolates.

Isolation of genomic DNA and PCR based confirmation of *E. coli*

DNA extractions from *E. coli* isolates were carried out as per the method described previously (Chen and Kuo, 1993). For PCR primers targeting 16s rRNA(ECO-1Foward GACCTCGGTTTAGTTCACAGA and ECO1 Reverse -CACACGCTGACGCT GACCA) were used as previously described by Islam *et al.*(2016). The amplified products were subjected to gel electrophoresis (1% agarose, HiMedia, India) and stained with ethidium bromide.

RESULTS AND DISCUSSION

Out of 80 samples, 37.5 per cent (n = 30) were contaminated with *E. coli*. DNA extracted from them was used in the

PCR assay. The PCR primers targeting 16S rRNA gene of *E. coli* amplified 585 bp fragments (Fig. 1) of DNA confirmed the identity. Isolated *E. coli* were analyzed for the antibiotic susceptibility (Table 1).

Results of antimicrobial susceptibility test showed that most of the isolates of *E. coli* were sensitive to CX, CPZ, TOB, PIT and NX and resistant to IPM and AMC. For NA, majority of isolates (43.3 per cent) of this study had intermediate sensitivity. High sensitivity (100 per cent) of *E. Coli* isolates to CPZ has also been reported previously from Rajasthan, India (Dadheech *et al.,* 2014) and from Czech Republic (94 per cent) (Kolář *et al.,* 2002). On the contrary, a resistance of 56.6 per cent has been reported in poultry *E. coli* from Haryana (Kumar and Gupta, 2019). Similar to CPZ, high sensitivity to CX (100 per cent) has been reported from Rajasthan, India (Dadheech *et al.,* 2014); from Australia (99.5 per cent) (Abraham *et al.,* 2019); from Czech Republic (94per cent) (Kolář *et al.,* 2002); from Tanzania (93.51 per cent) (Hamisi *et al.,* 2014). On the other hand high resistance of *E. coli* isolates to CX (90.4 per cent) has been reported previously from Egypt (Younis *et al.,* 2017). Similar to CPZ, high sensitivity of *E. coli* isolates (100 per cent) to TOB has been reported previously from Rajasthan, India (Dadheech *et al.,* 2014); from Bhubaneswar, India (88.84 per cent) (Senapati *et al.,* 2020); from Czech Republic (94 per cent) (Kolář *et al.,* 2002). On the other hand high resistance of *E. coli* isolates (71.42 per cent) to TOB has been reported from Rajasthan, India (Sharma *et al.,* 2017). Similar to CPZ, high sensitivity of *E. coli* isolates (100 per cent) to PIT has been reported from Czech Republic (Kolář *et al.,* 2002). On the other hand high resistance of *E. coli* isolates (85.2

Table 1: Antibiotic susceptibility pattern of various antibiotics in 30 isolates of *E. coli*

		Number of isolates (Percentage)		
Sl. No.	Antimicrobial tested	Sensitive	Intermediate	Resistant
1	Tobramycin	26 (86.7)	4 (13.3)	0 (0.0)
2	Imipenem	3 (10)	7 (23.3)	20 (66.7)
3	Augmentin	2 (6.7)	5 (16.7)	23 (76.7)
4	Cephoxitin	27 (90)	3 (10)	0 (0.0)
5	Piperacillin/Tazobactam	26 (86.7)	4 (13.3)	0 (0.0)
6	Cefoperazone	27 (90)	3 (10)	0 (0.0)
7	Nalidixic acid	10 (33.3)	13 (43.3)	7 (23.3)
8	Norfloxacin	26 (86.7)	1 (3.3)	3 (10)

Table 2: Multiple antimicrobial resistance patterns observed in this study

Sl. No.	Resistance pattern	Number of isolates (Percentage)
1	IPM, AMC, NX	2 (6.7)
2	IPM, AMC, NA	5 (16.7)
3	IPM, AMC, NX, NA	1 (3.3)

per cent) to PIT has been reported from Rajasthan, India (Senthamils elvan, 2020). With regards to NX, high sensitivity of *E. coli* (100 per cent) has been reported from Ethopia (Sarba *et al.,* 2019); Bangladesh (86 per cent) (Kmetova, 2009); from Bhubaneswar (83.04 per cent) (Senapati *et al.,* 2020); Bangalore, India (75.39 per cent) (Sharada *et al.,* 2009); from Egypt (63.1 per cent) (Younis *et al.,* 2017). On the other hand high resistance of *E. coli* isolates to NX has been reported from Haryana, India (78.3 per cent) (Kumar and Gupta, 2019); and Anand, India (83.33 per cent) (Choudhari *et al.,* 2020). With regards to NA, 83 per cent , 76.79 per cent isolates were reported sensitive from Egypt (Sarba *et al.,* 2019) and Bhubaneswar (Senapati *et al.,* 2020) whereas 81.2 per cent, 85.3 per cent, 100 percent isolates were reported resistant from China (Yassin *et al.,* 2017), Jamaica (Miles *et al.,* 2006) and Nigeria (Awogbemi *et al.,* 2018), respectivley. Similar to findings of this study 34.6 per cent *E. coli* isolates were reported sensitive to NX from Tamil Nadu, India (Senthamils elvan, 2020). Contrary to results of this study, 100 per cent sensitivity of *E. coli* isolates was reported from Rajasthan, India (Dadheech *et al.,* 2014), Jordan (Ibrahim *et al.,* 2019) and 94.2 per cent sensitivity was reported from Bhubaneswar (Senapati *et al.,* 2020). With regards to Augmentin, similar to findings of this study high resistance against this antibiotic has been reported from Nigeria (Dadheech *et al.,* 2014).

In the present study, three multidrug resistance phenotypes were observed Table 2. The patterns IPM, AMC, NX and IPM, AMC, NA was observed in 6.7 per cent and 16.7 per cent isolates, respectively. One isolate (3.3 per cent) was resistant to IPM, AMC, NX, and NA. In total, 20 per cent of the isolates from this study showed multidrug resistance. To conclude, results of the present study revealed that *E. coli* isolates from retail poultry outlets of Satara were susceptible to Cephoxitin, Cefoperazone, Tobramycin, Piperacillin/Tazobactam, and Norfloxacin whereas they were resistant to Augmentin, Imipenem, and Nalidixic acid. Multidrug resistance was also observed in 20 per cent of the isolates. These findings will be helpful in choosing antibiotics for treatment of *E. coli* infections of poultry in Satara region.

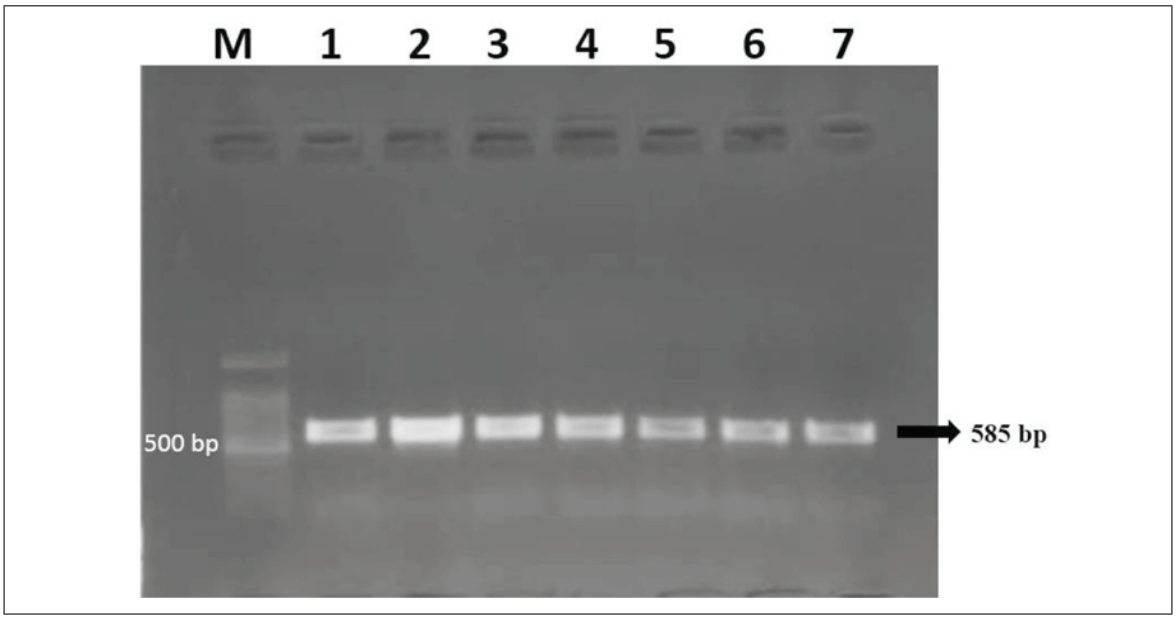

Fig. 1: PCR amplification of 16S rRNA gene of *E. coli.* Lane M- DNA ladder, Lanes 1 to 7 – *E. coli* samples

CONCLUSION

In the present study, *E. coli* were isolated and identified based on characteristic colonies on selective agar and further confirmed by PCR. It was concluded that *E. coli* isolates were susceptible to Cephoxitin (90%), Cefoperazone (90%), Tobramycin (87%), Piperacillin/ Tazobactam (87%), and Norfloxacin (87%). The isolates were resistant to Augmentin (93.3%), Imipenem (90%), and Nalidixic acid (66.7%). Further, multidrug resistance was observed in 20 per cent of the isolates.

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CONFLICT OF INTEREST

None declared

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