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Distribution of *TET, AAC* and *CTX-M* Genes among Antibiotic Resistant *Escherichia coli* Isolated From Poultry under Various Farming System of A and N Islands

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ABSTRACT

Background: Transmission of antibiotic resistance from animal food chain to human through animal food-borne pathogens have led to increased public concern. Wider surveillance on prevalence of antibiotic resistance in *E. coli* will provide information on evolution of resistance in various geographical locations. The purpose of this study was to investigate the presence of antimicrobial resistance of *E. coli* isolates from poultry under various farming system in A and N Islands and resistance genes of *tet*, *ctx-M* and *aac* encoding the isolates.

Methods: Isolates were obtained from cloacal swabs in poultry under various farming systems and tested against major antimicrobial derivatives to study multi drug resistance. The presence of genes associated with resistance to tetracycline (*tet A*), ESBL (*CTX*-M) and Gentamycin (*aac*(3)-IV) were determined by PCR.

Result: A total of 126 cloacal samples were analysed out of which 31.38% of the *E.coli* isolates from poultry under various farming systems were producing extended spectrum beta-lactamases and were multiple antimicrobial resistant. Poultry birds of commercial farms showed higher resistance levels (37.5%) than organised farms (24.76%) and desi birds (31.88%). Results indicate a high level of multi-drug resistance is emerging even in desi birds. It is suggested that an antimicrobial resistance surveillance program is needed in A and N Islands in order to detect bacterial resistance among rural poultry production as the 80 percentage of total poultry population belong to desi birds.

Key words: *E. coli*, ESBL, Farming systems, MDR, PCR, Poultry.

INTRODUCTION

Escherichia coli (E. coli) is normally considered to be one of the commonest commensal in human and animals and some causes severe illness such as gastroenteritis, cystitis, meningitis, colibacillosis etc (Yassin *et al*., 2017). The poultry industry suffers huge economic losses due to *E. coli* pathogen. Colibacillosis is one of the common poultry diseases showing variety of symptoms such as colisepticemia, coligranuloma, chronic respiratory disease, coliform cellulitis, swollen head syndrome, coliform peritonitis, coliform salpingitis, coliform osteomyelitis/ synovitis, coliform panophthalmitis and coliform omphalitis or yolk sac infection (Barnes *et al.,* 2008).

Antibiotics are sometimes used in poultry feed at sub optimum level as growth promoters and are mostly used to control and treat the infection in broilers (Lipsitch and Samore, 2002). Extensive usage of antibiotics to control colibacillosis in poultry production is considered to play the vital role in the development of antimicrobial resistance (Rahimi, 2013) in *E. coli*. Antibiotic resistance in animal food chain and its possible transmission to human through animal food-borne pathogens have led to increased public concern and scientific interest regarding the administration of therapeutic and sub therapeutic antimicrobials to animals (Piddock, 1996; Blanco *et al*., 1997; Mitsuda *et al*., 1998). *E. coli* surveillance data show that multi drug resistance in *E. coli* is consistently highest for antimicrobial agents that have been in use for longest time in veterinary medicine. Extended-spectrum β-lactamases (ESBLs) are plasmid-encoded enzymes found in Gram-negative bacteria especially in Enterobacteriaceae conferring resistance to first, second and third generation cephalosporins while they are inhibited by clavulanic acid. ESBL-producing enterobacteriaceae have emerged as pathogens in both poultry and humans. Many ESBL-producers are additionally mult-iresistant to non-β-lactam antibiotics, including fluoroquinolones, aminoglycosides, trimethoprim,

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tetracyclines, sulfonamides and chloramphenicols. Extended-spectrum-beta-lactamases (ESBLs) producing *E. coli* are widely spread and are of zoonotic importance as its interchange between humans and livestock is very likely leading to treatment failure. The CTX-M-type-lactamases confer resistance to extended-spectrum cephalosporins (Bonnet, 2004). Development of multidrug resistance Enterobacteriaceae organisms has a global impact on increased incidence of infections. The detection rate of beta lactamase, MDR (multi drug resistance) and pathogenic gram negative bacteria in these islands is still a pitiable state. *Escherichia coli* strains possessing CTX-M-type ESBL genes have been extensively detected in human since many decades. Similarly, aminoglycosides and tetracycline derivatives are commonly used in livestock and poultry. Therefore the present study was designed to ascertain the present scenario of multidrug resistance in *E. coli* from poultry under various farming system.

MATERIALS AND METHODS

Study design

A total of 126 cloacal samples were collected from organised farm, commercial farm and free range *desi* birds and from meat samples of slaughter house of different parts of Andaman and Nicobar islands during the period from October 2018-July 2019. Study was carried out at ICAR-Central island Agricultural Research Institute, Port Blair. The swab was inoculated in nutrient broth and incubated at 37°C overnight. The broth culture was streaked on next day to Eosine Methylene Blue (EMB) plates and incubated at 37°C overnight. *E. coli* isolates were identified based on colonial morphology on EMB and biochemical tests as per standard method (Pezzlo, 1992; Reisner *et al.,* 1999).

Disc method for phenotypic screening of antibacterial sensitivity

Antibacterial sensitivity tests were performed on all the isolates of *E. coli* by the (Bauer *et al.,* 1966) single-disk diffusion method in accordance with National Committee for Clinical Laboratory Standards (NCCLS, 2002). A total of 13 antibiotic discs such as amoxyclav (30µg), tetracycline (30µg), cephalexin (30µg), erythromycin (5µg), cloaxacillin (200µg), co-trimaxazole (25µg), clindamycin (2µg), ciprofloxacin (5µg), gentamycin (10µg), trimethoprim (1.25µg), sulphamethoxazole (50µg), ampicillin (25µg) and Chloramphenicol (30 µg) were assayed. The diameter of the zone of inhibition was measured. The antimicrobial susceptibility data was expressed as percentage of the isolates. Multi drug resistance was defined as resistance exhibited to three or more antimicrobials (Tricia *et al.,* 2006).

DNA extraction and PCR amplification of antibiotic resistance genes

The bacterial cultures were grown into Nutrient agar (Himedia, India) at 37°C for overnight. Genomic DNA was extracted from *E. coli* using a Genomic DNA purification kit according to the manufacturer's instructions (GCC, India). The purified DNA was checked and run in agarose gel electrophoresis (1.5%) and kept at -20°C for further use. The genes associated with resistance to most common antibiotics in the field were used. The presence of antimicrobial resistance *viz.* tetracycline (*tet A*), ESBL gene (*CTX*-M) and gentamicin [*aac* (3)-IV] were determined by PCR and the set of primers used for each gene is shown in Table 1. PCR was done by the method described by Fode-Vaughan *et al.* (2003). The product was then electophoresed in 1.5 % agarose gel and visualized using gel documentation system (Labmate Asia Pvt Ltd). A 100 bp DNA ladder was used as the standard to determine the size of the product. Except annealing temperatures for the genes, the PCR running conditions were 95°C for 5 min; 35 cycles of 95°C for 60s, 72°C for 60s; 72°C for 5 min. The PCR Taq2X Master mix was used in this study.

Statistical analysis

The prevalence of *E. coli* was quantified and was compared among the different source of origins. Similarly the pattern of antimicrobial resistance was also quantified and compared. Data were analysed as per the Snedeccor and Cochran (1994).

RESULTS AND DISCUSSION

A total of 63 *E. coli* were isolated from the 126 claocal swabs from different poultry under various farming system. Results shows that the prevalence of *E. coli* was highest amongst the birds of organised farm (57.14%) followed by backyard poultry (54.76%) and slaughter house (38.09%) respectively. The magnitude of prevalence of *E. coli* amongst the organised farm chicken (38.1%) was found to be more than the backyard (36.5%) and slaughter house (25.4%) sources. The phenotypic antibiotic sensitivity of all the *E. coli* isolates from different origins is depicted in Table 2. The overall antibiotic resistance to all the antibiotics were found to be highest among the isolates from the organised farm (33.86%) followed by backyard poultry (32.86%) and slaughter house (32.39%) respectively. No significant difference was obtained compared to the origin of the isolates; however the average percentage of resistance was found to be 33.04%. The lowest antibiotic resistance was found with the amoxyclav (7.75%) and the highest resistance was observed against cloxacillin (80.99%) (Table 3).

The recovery of *E. coli* isolates from the various farming system and the meat samples shows that the prevalence of *E. coli* was more among the chicken of organised farms. Similar types of results were also obtained by McPeake *et al*., 2005 and Samanta *et al*., 2015 from faeces of apparently healthy and consumable chickens. *E. coli* is a common commensal found in the GI tract of poultry and in the environment, some are pathogenic and some are of zoonotic importance. The presence of high number of *E. coli* from the organised farm, backyard birds and chicken meat samples were also reported by Messele *et al*., 2017. They

Table 1: *Escherichia coli* antimicrobial resistant genes and primer sequences used for PCR identification.

Antimicrobial Agent	Resistance Gene	Sequence	Product Size	Annealing temperature (°C)	References
Tetracycline	*tet A*	5'-GTAATTCTGAGCACTGTCGC-3 5'-CTGCCTGGACAACATTGCTT-3	500 bp	57	Miranda *et al.* 2003
Gentamicin	aac(3)-IV	5'-AGTTGACCCAGGGCTGTCGC-3 5'-GTGTGCTGCTGGTCCACAGC-3	300 bp	63	Brau, *et al.* 1984
ESBL gene	CTX-M1	5'- CCATGGTTAAAAAATCACTGCG-3' 5'-GGGTRAARTARGTSACCAGAAYSAGCGG-3	836 bp	66	Moodle *et al.,* 2009

Table 2: Antimicrobial susceptibility/resistance of *E. coli* from different poultry origin.

Antibiotics		Organised farm (n= 24)	Backyard (n= 23)	Slaughter house (n= 16)
Amoxyclav (30µg)	S	21(85.63%)	20(87.5%)	6(35.61%)
	I	3(12.15%)	3(12.5%)	9(55.16%)
	R	1(2.22%)	3(12.5%)	1(9.23%)
Tetracycline (30µg)	S	7(29.17%)	7(31.74%)	2(12.50%)
	I	11(45.83%)	3(11.74%)	7(43.75%)
	R	6(25.0%)	13(56.52%)	7(43.75%)
Cephalexin (30µg)	S	5(20.83%)	13(56.45%)	5(29.92%)
	I	6(25.0%)	8(34.35%)	2(13.63%)
	R	13(54.17%)	2(9.2%)	9(56.45%)
Erythromycin (5µg)	S	5(20.83%)	16(69.57%)	3(18.75%)
	I	8(33.33%)	3(12.43%)	5(31.25%)
	R	11(45.83%)	9(39.13%)	8(50.0%)
Cloaxacillin (200µg)	S	1(4.16%)	2(8.33%)	3(18.75%)
	I	1(4.16%)	2(8.33%)	3(19.63%)
	R	22(91.66%)	19(83.33%)	10(61.62%)
Co-trimaxazole (25µg)	S	12(50.0%)	8(34.78%)	3(21.74%)
	I	2(8.33%)	3(13.04%)	1(4.35%)
	R	10(41.66%)	12(52.17%)	7(43.48%)
Clindamycin (2µg)	S	15(62.5%)	4(17.4%)	2(12.5%)
	I	5(20.83%)	6(26.1%)	5(31.25%)
	R	4(16.66%)	0(0%)	9(56.25%)
Ciprofloaxcin (5µg)	S	5(20.83%)	6(27.39%)	2(12.50%)
	I	6(25.0%)	2(7.39%)	5(31.25%)
	R	13(54.17%)	15(65.22%)	9(56.25%)
Chloramphenicol	S	12(50.0%)	7(30.43%)	2(12.50%)
	I	5(20.50%)	10(43.48%)	7(43.75%)
	R	7(29.50%)	6(26.09%)	6(37.50%)
Gentamycin (10µg)	S	15(62.50%)	12(52.17%)	3(18.75%)
	I	3(12.50%)	1(4.35%)	2(12.50%)
	R	6(25.0%)	10(43.48%)	11(68.75%)
Ampicillin (25µg)	S	15(63.0%)	11(47.83%)	5(31.25%
	I	5(22.50%)	5(21.74%)	2(12.50%)
	R	8(35.0%)	8(34.78%)	9(56.25%)
Trimethoprim (1.25µg)	S	9(37.50%)	13(56.52%)	4(26.09%)
	I	8(33.33%)	5(21.74%)	5(30.43%)
	R	7(29.17%)	5(21.74%)	7(43.48%)
Sulphamethoxazole (50µg)	S	14(56.52%)	11(47.83%)	10(62.50%)
	I	4(17.39%)	5(21.74%)	5(31.25%)
	R	6(26.09%)	7(30.43%)	1(6.25%)

Table 3: Percentage of bacterial showing resistant to different antibiotics.

Antibiotics	Resistance %
Amoxyclav (30µg)	7.75
Clindamycin (2µg)	20.63
Sulphamethoxazole (50µg)	22.64
Trimethoprim (1.25µg)	30.09
Chloramphenicol	30.29
Cephalexin (30µg)	38.33
Ampicillin (25µg)	40.32
Tetracycline (30µg)	41.27
Gentamycin (10µg)	42.86
Erythromycin (5µg)	44.44
Co-trimaxazole (25µg)	45.96
Ciprofloaxcin (5µg)	58.73
Cloaxacillin (200µg)	80.99

reported the prevalence of *E. coli* by 21.6 % from the chicken meat samples. Commensal *E. coli* are generally present in the GI tract of the chicken, human and in the environment. There is high possibility that during the processing of the chicken meat in the slaughter house/retail shop the meats are contaminated with the contaminated water, utensils, environment *etc*.

In the present study it was found that the isolates have resistance to almost all tested antimicrobial agentsat various rates. The mean of 31.38% of the *E. coli i*solates from poultry under various farming systems were producing extended spectrum beta lactamases and were multiple antimicrobial resistant. The antibiotics used for the disc diffusion test are commonly found to be used in the treatment of colibacillosis and are closely related to those used in human medicine. Poultry birds of commercial farms showed higher resistance levels (37.5%) than organised farms (24.76%) and desi birds (31.88%). Percentage of resistance rates in commercial farms were 56.25%, 43.75%, 25, 6.25%, 37.5% for aminoglycosides and penicillin, phenicols, tetracyclines, quinolones and macrolides derivatives respectively. While the respective percentage of resistance rates in desi and commercial birds were 26.09, 52.17, 21.74, 47.83, 17.39, 26.09 and 25, 29.17, 23,56, 8.33, 20.83, 41.66. In the present study the highest antibiotic resistance was found against cloxacilin and ciprofloxacin which is also very alarming as these antibiotics are third generation antibiotics. As expected the antibiotic resistance was reported against the old antibiotics *viz.* amoxicillin, tetracycline, chloramphenical *etc*. The high level of antibiotic resistance might be due to the widespread and indiscriminate usage of antibiotics in the treatment of poultry diseases. Report of high level of antibiotics resistance against the older as well as the newer generation antibiotics has also been reported by many workers (Hawkey *et al*., 2009; Tadesse *et al*., 2012; Maryam *et al*., 2014).

The total 63 isolates, 3 (4.8%) isolates showed resistant to one antibiotic, 9 (14.3%) resistant to two antibiotics, 14 (2.2%) resistant to three antibiotics, 19 (30.15%) resistant to four antibiotic and 18 (28.57%) showed resistant to more than four antibiotics (multiple drug resistant). The percentage of multidrug resistant bacteria (> 4 antibiotics) was found to be very high. Four bacterial isolates showed resistant to 7 antibitoics. The antibiotic resistance and multiple drug resistance pattern is shown in Table 4.The most common antibiotics found to be multiple resistant were cloxacillin (80.1%), ciprofoloxacin (58.73%), co-trimoxazole (45.96%), erythromycin (44.44%), gentamicin (42.86%), tetracycline (41.27%), ampicilin (40.32%) etc. Similar to our study, Yohanes *et al*., 2017 also reported the highest percentage of antibiotic resistance in ampicilin, tetracycline, erythromycin which indicated that the use of these antibiotics are not safe due to development of antibiotic resistance. They also reported the multidrug resistance of *E. coli* isolated from poultry and found that 39.7% of isolates harbored resistance gene responsible to three or more drugs. In the present study the percentage of multidrug resistance with more than 3 or more antibiotic was 58 %. The occurrence of multidrug resistance in the present study has been found to be very high. The prevalence of cross resistance to several antibiotics has been reported. In a study it was found that cross resistance of tetracycline to fluoroquinolones and cepharlosproins in *E. coli* from broiler has been reported. Velhner and Milanov (2015) suggested that this is probably the consequence of the fact that the resistance determinants are often found on mobile genetic elements.

All the bacterial isolates were confirmed by PCR to know the prevalence of antibiotic resistance genes. Out of the total isolates, CTX-M and aac(3)-IV genes were identified in 42.8% and 45.2% of isolates of organised farm. Presences of these genes were recorded in 16.6% and 19% of isolates of slaughter house; whereas, 38% and 28% of isolates of desi birds from backyard possessed these genes. None of E. coli isolates were found with tet genes. The tetracycline resistant gene (tetA) was not found in any of the isolates. The ESBL resistant gene (CTX-M1) was found to be present in 42.8% of the bacterial isolates from organised farm followed by desi birds (38.1%) and slaughter house (16.6%) respectively. The gentamicin resistant gene (aac(3)-IV) was found be present in 45.2% of the bacteria from organised farm followed by 28.57% from backyard poultry and 19.04 % from slaughter house isolates. The aac (3)-IV gene and CTX-M1 gene were present in almost 60.93% and 65.07% of the *E. coli* isolates from all the sources. Overall, 6.25 % of the total bacteria does not possess any antibiotic resistance gene. However, 62.5 % and 31.25% of the total bacteria possess one and two antibiotic resistance genes respectively (Table 5). During the last decade the drug resistance in *E. coli* has been increased dramatically worldwide. The increase in the resistance has been mainly due to the prevalence of extended spectrum β-lactamases. In the present study, the isolates were screened for the presence of tetA representing tetracycline, CTX-M1 representing β-lactamases and aac(3)-

Table 4: Antibiotic resistance pattern of *E. coli* isolates from different origin.

Number of antibiotic classes (No. isolates %)	Antibiotics	Number of isolates	%
One (n=3, 4.76%)	cip	1	1.6
	G	1	1.6
	amp	1	1.6
Two (n=9, 14.26%)	cli*G	1	1.6
	co-tri*G	1	1.6
	clox*co-tri	2	3.1
	co-tri*clin	1	1.6
	amp*G	1	1.6
	amp*G	2	3.1
	cip*clin	1	1.6
Three (n=14, 22.22%)	amx*cep*clox	1	1.6
	cep*clox*co-tri	1	1.6
	E*clox*G	1	1.6
	clin*cip*G	1	1.6
	clin*clox*co-tri	3	4.7
	clox*co-tri*C	1	1.6
	cip*clin*tet	1	1.6
	amox*cep*tet	1	1.6
	clin*clox*amp	1	1.6
	amox*cep*clin	1	1.6
	cip*clin*tet	1	1.6
	clin*clox*co-tri	1	1.6
Four (n=19, 30.16%)	E*Cl*S*t	3	4.7
	Amx*E*clo*S	1	1.6
	amox*tet*cep*E	1	1.6
	amx*cep*clox*co-tri	1	1.6
	amx*cep*clox*cip	1	1.6
	clox*co-tri*t*S	1	1.6
	amx*clox*co-tri*S	2	3.1
	clox*co-tri*E*G	1	1.6
	cep*co-tri*E*G	1	1.6
	co-tri*E*C*G	1	1.6
	amox*cep*tet*amp	2	3.1
	clox*co-tri*E*C	2	3.1
	cip*clin*tet*amp	1	1.6
	clox*co-tri*G	1	1.6
Five (n=5, 7.94%)	Amx*cep*E*co-tri*cip	1	1.6
	clox*co-tri*tet*amp*G	1	1.6
	amox*cep*clox*tet*amp	1	1.6
	amox*cep*co-tri*E*G	1	1.6
	clin*clox*co-tri*C*G	1	1.6
Six (n=9, 14.29%)	amx*tet*cep*E*clox*G	1	1.6
	amx*clox*E*amp*C*G	1	1.6
	clin*clox*E*amp*C*G	1	1.6
	cep*co-tri*E*tet*amp*C	1	1.6
	clin*co-tri*E*amp*C*G	1	1.6
	clin*clox*tet*amp*C*G	1	1.6
	amox*clin*co-tri*E*tet*amp	1	1.6
	amox*clin*clox*co-tri*tet*amp	1	1.6
	cip*clin*clox*tet*amp*G	1	1.6
> Seven (n=4, 6.35%)	cep*E**co.tri*cip*t*S*Amx	1	1.6
	amx*clin*clox*co-tri*amp*C*G	1	1.6
	amox*cep*clox*clin*E*tet*amp	1	1.6
	amox*cep*clox*co-tri*E*tet*amp	1	1.6

Table 5: Distribution of antibiotic resistance genes in *E.coli* isolates (n=63) by PCR.

Source of *E. coli*	*tet A*	aac(3)-IV	CTX-M1
Organised farm (n= 24)	0	19(45.23%)	18 (42.85%)
Backyard (n= 23)	0	12 (28.57%)	16 (38.1%)
Slaughter house (n= 16)	0	8 (19.04%)	7 (16.6%)
Total = 63	0	39(60.93%)	41 (65.07%)

IV representing aminoglycosidase. These three resistance genes were selected on the basis of the common use of the antibiotic classes in the poultry farming in the island region. Similar type of study was conducted by Samanta *et al*. (2015) with 272 *E. coli* strains from 360 backyard poultry from the four agro-climatic zones of West Bengal, India (Terai, New Alluvial, Coastal, Red Laterite soils) and reported that none of the *E. coli* isolates from the backyard poultry and farmed poultry in costal and red laterite soil were positive for any studied ESBL gene by PCR whereas 29.4 per cent of *E. coli* isolates from the farmed poultry in terai and new alluvial zones were found to produce the ESBL genes. The study (Garcia-Graells *et al.,* 2013) conducted on a subset of *E. coli* isolates from poultry recorded 82.45 per cent of them were positive for ESBL and also recorded that CTX-M (ESBL) is predominantly produced by the commensal *E. coli*. Further, Emmanuel *et al*. (2013) has compared the ESBL production by *E. coli* isolated from cloacal samples (74 per cent) with faecal swabs (67 per cent) from the same poultry. Moreover, different strains of *E. coli* in the same poultry have shown different resistance types (ESBL and AmpC genes). This could be involvement of other plasmid-mediated ESBLs such as OXA, CEP-1 and others (Jacoby and Sutton, 1985). Similar to our study Maryam *et al*., 2014 also reported the prevalence of 54.54% of gentamicin genes among the resistant *E. coli.* In the present study the presence of aac(3)-IV and CTX-M1 correlated with the presence of phenotypic antibiotic resistance however, there was no correlation with the presence of *tet* A gene and phenotypic correlation with the tetracycline antibiotic. Similar types of observation were also reported by Yohanes *et al*., 2017. They also reported lack of association between phenotypic erythromycin resistance and molecular detection of ere(A), streptomycin and aadA1, chloramphenicol and catA1 resistance gene. Reports suggest that the presence of resistance phenotype might not represent all the underlying resistance gene or absence of a resistance gene might not indicate the particular isolate is resistant or susceptible to an antimicrobial (Aarts *et al*., 2006).

The three derivatives of aminoglycosides, penicillin and phenicols are extremely used in commercial birds from day one onwards and hence might have resulted in highest MDR of isolates in commercial birds as compared to poultry from other farming systems. The overall second highest resistance rate reported in desi birds is due to resistance of isolates to tetracycline. Tetracycline is the broad spectrum and one of the oldest antibiotic commonly used in the field and hence largely given to the desi birds for various ailments.

This might have obviously attributed to highest resistance of isolates in desi birds. High prevalence of resistance rate of *E.coli* isolates recovered from poultry to tetracycline may be the consequence of mismanagement of tetracycline antibiotics by the farmers for the desi birds. Yassin *et al*., 2017 also reported that highest rates of resistance (>75%) were found with tetracycline, nalidixic acid, sulfamethoxazole, ampicillin, enrofloxacin and trimethoprim-sulfamethoxazole. The lowest MDR of isolates in the birds of organised farm indicates the controlled use of antibiotics for the poultry. There was lower rate of resistance level for gentamicin in the isolates of organised farm and desi birds. In a similar study, *E. coli* isolated from commercial chickens in Enugu State, Nigeria has been recorded as 30.6 per cent and 36.5 per cent ESBL producers for the antibiotics of ceftazidime and cefotaxime respectively with the disc method (Chah and Oboegbulem, 2007). Saberfar *et al.* (2008) also reported that resistance rate (12%) to gentamicin in *E. coli* isolated from colibacillosis in broiler is low. Reports also reveal that none of the *E.coli* isolates was found resistant to Gentamicin (Tricia *et al.,* 2006). As reported in the present study in commercial birds, resistance of *E. coli* isolates from broiler chickens to ampicillin, with 11-95 % range has been reported at various geographical locations (Apun *et al.,* 2008; Rahman *et al.,* 2008; Akond *et al.,* 2009).Resistance rate to ampiclllin in this study was higher than the average. In recent years, ampicillin has been widely used by commercial poultry farmers. Excessive and inappropriate treatment of diseases with ampicillin might have led to emergence of resistant *E. coli* strains. The resistance frequencies of the present study to chloramphenicol were relatively lesser to previous reports (Islam *et al.,* 2008; Saberfar *et al*., 2008; Salehi and Bonab, 2006).

Resistance rate to tetracycline in desi birds of present study was lower than the similar reports (Rahimi, 2013; Tabatabaei and Nasirian, 2003). Plasmid mediated pathway with various genetic determinants leads to bacterial resistance to tetracycline. Hence, the susceptible bacterium acquires resistance (Tricia *et al.,* 2006). As reported in the present study, quinolone-resistant avian *E. coli* (QREC) isolates has been reported in various countries of the world (Saenz *et al.,* 2003; Yang *et al.,* 2004). This high prevalence of resistant *E.coli* to the quinolones in the world indicates the wide and inappropriate use of quinolones in commercial broilers. Evolution of resistance to quinolones can be very rapid during the period of treatment (Jacob, 2005). These reports reiterate the need of the hour to monitor quinolone resistant bacteria in chicken production. Recently, 14 *E. coli* isolates out of 32 isolates collected from poultry intestine have been reported as ESBL producers.

In our study, phenotypically similar isolates were positive for ESBL in poultry from various farming systems in particular desi birds. The prevalence of MDR was recorded from the cloacal swab of chicken as well as from the fresh meat samples from the slaughter house. It indicates that there is a potential possibility of *E. coli* entering food supply either from farm premises directly or during slaughter by contamination of intestinal contents. The high percentage of *E. coli* in the meat samples reported that poor hygienic practices is responsible for contamination of meat by indiscriminate exploitation of antimicrobial agents, causing MDR may be occurring in A & N islands. It has been reported that generally antibiotic resistance transmits to the human through food chain or water chain and sometimes by environmental contamination (Velhner *et al*., 2010). Inappropriate antimicrobial therapy and excessive use of antibiotics as feed additives for growth promotion and prophylaxis may be the probable causing factors for the transfer of resistance and cross resistance among different bacteria. Results show that cloxacillin, gentamicin, ampicillin and amoxyclav were the most common antibiotics showed resistant with disc as well as possessing the resistant genes. However, the tetracycline doesn't show any correlation with the presence of resistance genes. The occurrence of antibiotic resistance in *E. coli* may be due to number of factors such as use of antibiotic in the poultry, clonal spread of resistance among the commensal *E. coli and* transmission of resistance plasmids. In the present study it is observed that the highest antibiotic resistance was reported for cloxacilin, followed by ciprofloxacin, co-trimoxazole, erythromycin, gentamicin, tetracycline and ampicillin. Hence, continuous monitoring of isolates for resistance genes is essential in humans who are in close contact during farm activities and slaughter. Food supply chain should always be continuously monitored by molecular epidemiology and phylogenetic analysis to find out the probable source of origin for contamination and thereby emergence of antibiotic resistant isolates can be controlled in the cycle of the food chain. Similar to the present study (Amsaveni *et al*., 2018) also reported that identification of antibiotic resistance in *E. coli* in faeces of poultry origin indicates zoonotic and public health significance which emphasizes routine screening of poultry meat thereby appropriate strategies can be adopted to combat this resistance. In the present study the percentage of E. coli isolates from the meat samples was found to be 25.4% which doesn't indicate that the infection due to human through the meat samples is the only sole source responsible. However, there are other factors which are responsible *viz*. transportation from slaughter house to the human table, processing, cooking etc. However, the number of isolates recovered from the meat samples gives an indication about the possible transmission of the antibiotic resistance *E. coli* to the human food chain.

CONCLUSION

These comprehensive results indicate a high level of multi-drug resistance is emerging even in desi birds. The monitoring of antibiotic resistance in commensal *E. coli* isolated from randomly selected healthy poultry provides valuable information about the level of multidrug resistance in the *E. coli*. It is suggested that an antimicrobial resistance surveillance program is needed in A & N Islands in order to

detect bacterial resistance among rural poultry production as the 80 percentage of total poultry population belong to desi birds. This study shows that routine monitoring of ESBL producing *E. coli* is highly important as it enters into food chain from farm premises or during slaughter. These type of studies has public health significance in controlling the emergence and spread of ESBL producing *E. coli* isolates from poultry sector. It is also emphasises that there is need to improve the poultry farming practices with strict regulation of usage of antibiotics. The proper and strict measure will ensure the reduction in the likelihood of horizontal gene transfer of the resistant genes to other bacteria through food chain.

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