A study on Extended Spectrum β-lactamase Producing (ESBL)and Fluoroquinolone-resistant*Escherichia coli* in the Japanese quails (*Coturnix japonica*)from Puducherry, India

ABSTRACT

Antimicrobial resistance (AMR) is a growing public health threat worldwide, and members of the Enterobacteriaceae family are among the clinically important bacteria rapidly developing resistance to available antibacterial agents. The most important mechanism of resistance to third-generation cephalosporins among members of the Enterobacteriaceae is the production of extended-spectrum β-lactamase (ESBL) enzymes. Quails also represent a source of animal protein deficiency in developing countries including India. This study aimed to detect the presence of extended-spectrum β-lactamases (ESBLs) and fluoroquinolone-resistant *Escherichia coli* from fecal samples of quails in Puducherry. A total of 36 fecal samples of quails from six different retail outlets were collected. *E. coli* could be isolated from 27 fecal samples and confirmed as *E. coli* by polymerase chain reaction by targeting the *uspA* gene. The isolates were preliminarily screened for ESBL production by indicator antimicrobial disc and phenotypic ESBL production was confirmed by combination disc method.The isolates were subjected to genotypic detection of ESBL (*bla* CTX-M, *bla*TEM, and *bla* SHV) by PCR. Out of 27 isolates, 16 isolates were positive for the presence of ESBL-producing genes, in which,9 harbored*bla*CTX-M, 2 harbored*bla*TEM, and 5 harboured *bla*SHV genes.Similarly, out of 27 isolates 13 isolates were found positive for fluoroquinolone resistance in which 13 (48.18%) isolates harbored the *qnr*S gene, and 11 (40.74%) isolates harbored the *qnr*B gene. Antimicrobial susceptibility test showed that the isolates exhibited a high level of resistance against Cefpodoxime (90%), Amoxycillin/clavulanic acid (70%), Ceftriaxone (60%), Cefotaxime (55%), Ceftazidime (45%), Ciprofloxacin (40%) and Aztreonam (30%).

Keywords: AMR, ESBL, Fluoroquinolone resistant, *E. coli*, *uspA*

1. INTRODUCTION

Over the past five decades, there has been a significant increase in poultry meat production, which now constitutes nearly one-third of global meat production (1,2).The rearing of birds other than chickens as quails has been developed in recent years worldwide to supply human food requirements (3).Quail farming is increasing globally daily as it requires low-rearing investment and has fast financial returns. In addition, quails differ from other poultry species in that they use less feed, overgrow, reach sexual maturity early, have short generation intervals, have short incubation periods, have highly nutritious meat, and are less susceptible to viral diseases. (4,5,6).Common bacteria in the poultry gut include *Escherichia coli*, which causes various diseases such as omphalitis, cellulitis,

swollen head syndrome, coli granuloma, and colibacillosis (7). The primary cause of the emergence, persistence, and dissemination of resistant bacteria is the overuse of antibiotics in intensive animal agriculture, particularly in raising pigs and poultry (8). Swine, cattle, poultry, and turkey have all been found to harbor ESBL-producing bacteria (9). The emergence of plasmid-mediated quinolone resistance was first reported in strains of *Klebsiella pneumoniae* in the United States in 1998. In the following years, several distantly related plasmids-mediatedQnr determinants were described in Enterobacteriaceae (*qnr*A, *qnr*B, *qnr*C, *qnr*D, and *qnr*S). It is becoming more common to find ESBLs and additional plasmid-mediated antibiotic-resistance genes such as *qnr* (10). Humans may acquire multidrug-resistant bacteria through zoonotic transmission from broiler farms and agricultural settings, or indirectly by food-borne diseases (11).

Puducherry, a state with intensive quail farming, lacks data regarding antibiotic use and antibiotic resistance in poultry farms. This study was therefore done to determine the antibiotic-resistant*E. coli*, study their antibiotic susceptibility pattern, and identify the resistance genes in quails in Puducherry.

2. MATERIAL AND METHODS

2.1 Collection and Processing of Samples

A total of six retail outlets were chosen for sample collection and from each retail outlet six cloacal swabs were collected using a sterile swab aseptically from healthy quails randomly in and around Puducherry. The collected 36 cloacal swabs were transported to the Department of Veterinary Microbiology Laboratory, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry for further processing.

2.2 Isolation and Identification of *E. coli*

Each collected cloacal swab was inoculated into a Luria broth and subjected to incubation overnight at 37°C. A loopful of the culture was streaked onto MacConkey agar and incubated. The pink colonies that were lactose fermenting were subjected to Gram staining. Bergey's Manual of Systematic Bacteriology (1984) was used to identify the gram-negative bacteria as *E. coli* up to the species level based on their biochemical tests and culturalcharacteristics.

PCR was employed for genotypic confirmation of the *E. coli* isolates, utilizing primers targeting the *uspA* gene (12). PCR amplification was conducted using an automated thermal cycler (Eppendorf Mastercycler, Germany), following the PCR reaction conditions outlined in the protocol (12). The PCR products were analyzed by agarose gel electrophoresis with the expected product size is 884 bp for the *uspA*gene. Following PCR confirmation of *E. coli* isolates, the resulting PCR products underwent analysis through agarose gel electrophoresis. Subsequently, all confirmed *E. coli* isolates were subjected to further detection of ESBL through both phenotypic and genotypic methods, along with genotypic detection of fluoroquinolone resistance.

2.3 Phenotypic detection of ESBL production

The preliminary phenotypic screening of ESBL production was carried out by using five indicator antimicrobial discs: ceftriaxone (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefpodoxime (10 µg) and aztreonam (30 µg) discs. Among these five indicator discs isolatesshow resistance to at least one antibiotic was considered as positive for ESBL production. Further ESBL production was confirmed by following the combination disc method using cefotaxime and cefotaxime/ clavulanic acid, in the plate of Mueller-Hinton agar at a distance of 25 mm apart as per the recommendation of CLSI guidelines (2019). Plates were incubated for 16-18 hoursat 37°C.The isolates were confirmed as ESBL producers if the inhibition zone diameter around the combination disc was ≥ 5 mm when compared to the disc containing respective cephalosporin alone (13).

2.4 Genotypic Detection Antimicrobial Resistance Genes

Following identification as *E. coli*, all isolates underwent PCR analysis using primers targeting *bla* TEM, *bla* SHV, and *bla* CTX-M genes for ESBL confirmation and also confirmation of fluoroquinolone-resistant genes such as *qnr*S, and *qnr*Bby PCR. Template DNA preparation from *E. coli* strains followed the method described by Zhang *et al.* (14).

Table 1: Primers details used for PCR in this study and their amplicon size

Target	Primer sequence (5'-3')	Size (bp)	Annealing Temperature	References
uspA	CTGGAAGAGGCTAGCCTGGACGAG AAAATCGGCACCGGTGGAGCGATC	844	60°C	Chen *et al.,* 1998 (12)
bla TEM	ATGAGTATTCAACATTTCCG CTGACAGTTACCAATGCTTA	867	56°C	Rasheed *et al.,* 2003 (15)
bla SHV	AGGATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG	393	56°C	Colom *et at.,* 2003 (16)
bla CTX-M	GAAGGTCATCAAGAAGGTGCG GCATTGCCACGCTTTTCATAG	560	59°C	Sharma *et al.,* 2013 (17)
*qnr*S	GCAAGTTCATTGAACAGGGT TCTAAACCGTCGAGTTCGGCG	428	52°C	Cattoir *et al.,* 2007 (18)
*qnr*B	GGMATHGAAATTCGCCACTG TTTGCYGYYCGCCAGTCGAA	264	50°C	

2.5 Antibiotic susceptibility testing

Antimicrobial susceptibility test was done on Mueller-Hinton agar (HiMedia, Mumbai) plate as per the recommendation of Clinical Laboratory Standard Institute (2019) using the following commercially (HiMedia, Mumbai) available antimicrobial discs (HiMedia) used in this study were Amoxycillin/clavulanic acid (30 µg), Cefpodoxime (30 µg), Cefotaxime (30 µg), Aztreonam (30 µg), Ceftazidime (30 µg), Enrofloxacin (30 µg), and Ceftriaxone (30 µg).

3. RESULTS AND DISCUSSION

In this study, among 36 cloacal swabs examined, a total of 27 (75%)*E. coli* isolates were obtained, using cultural and biochemical characterization followed by genotypic confirmation of PCR using *usp*A gene specific for *E. coli* (844 bp) (*Fig.*1).The higher occurrence rate of *E. coli* is about 65%, and a lower rate of *E. coli* 3.5 % reported from quail cloacal samples (3,19).

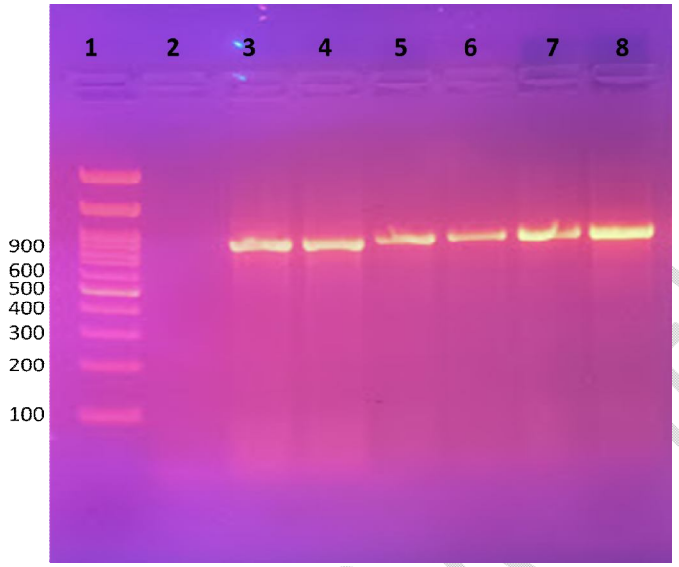

Fig 1. Agarose gel electrophoresis showing the results of polymerase chain reaction amplified product of 844 bp for the *usp*A gene in *E. coli* isolates. Lane 1: 100 bp ladder; Lane 2: Negative control; Lane 3: Positive control; Lane 4,5,6,7,8: Field isolates for *usp*A gene in *E. coli*

In this study, 20 (74.07%) isolates were found preliminarily positive for ESBL production by indicator antimicrobial agents. Out of 20, phenotypically 17 (62.9%) isolates were confirmed as ESBL producers by combination disc method (*Fig.*2).

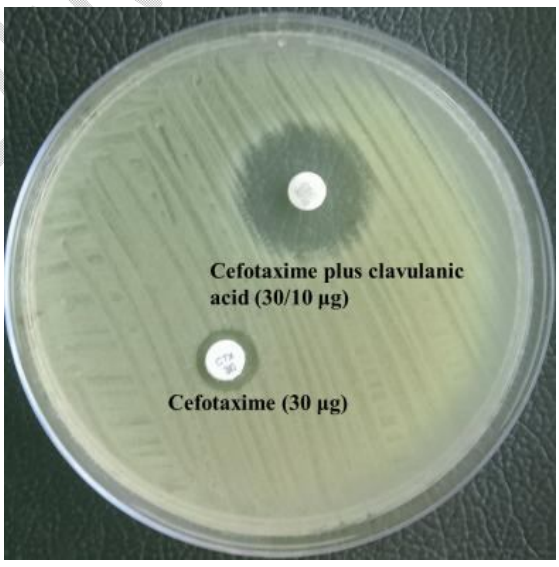

Fig 2. Phenotypic confirmation of ESBL production of *E. coli* isolates

In Kerala, 6/22 (27.27%) isolates were phenotypically positive for ESBL production in quailswerereported (20). The genotypic method confirms the presence of genes associated with ESBL production in *E. coli* isolates. In this study, out of 27 isolates, 16 (59.25%) isolates were found positive for ESBL-producing genes in which 9 (33.33%) isolates harbored CTX-M (*Fig.* 3), 2 (7.4%)isolates harbored*bla* TEM (*Fig.* 4) and 5 (18.51%) isolates harbored*bla* SHV gene (*Fig.* 5).

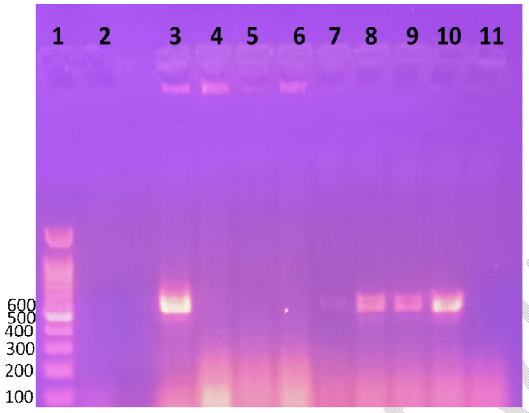

Fig 3. Agarose gel electrophoresis showing the results of polymerase chain reaction amplified product of 540 bp for the CTX-M gene in *E. coli* isolates. Lane 1: 100 bp ladder; Lane 4 to 11: Field isolates for CTX-M gene in *E. coli*; Lane 2: Negative control; Lane 3: Positive control

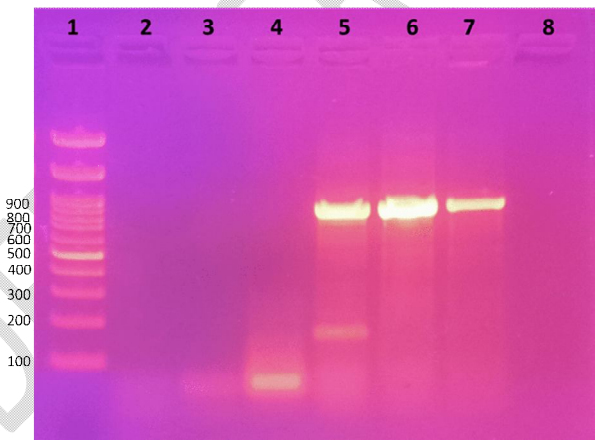

Fig 4. Agarose gel electrophoresis showing the results of polymerase chain reaction amplified product of 867 bp for the TEM gene in *E. coli* isolates. Lane 1: 100 bp ladder; Lane 2&3,4,5,6: Field isolates for TEM gene in *E. coli*; Lane 7: Positive control; Lane 8: Negative control

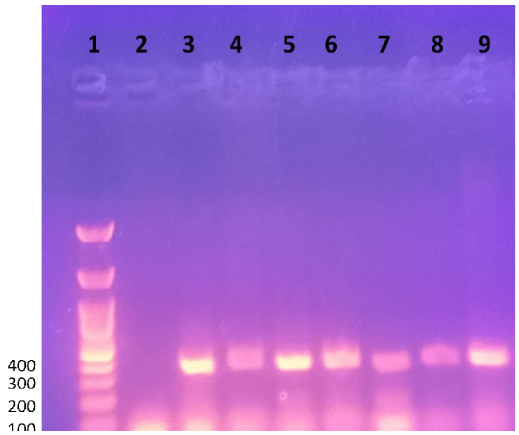

Fig 5. Agarose gel electrophoresis showing the results of polymerase chain reaction amplified product of 393 bp for the SHV gene in *E. coli* isolates. Lane 1: 100 bp ladder; Lane 4,5,6,7,8,9: Field isolates for SHV gene in *E. coli*; Lane 3: Positive control; Lane 2: Negative control

Two isolates (7.4%)harbored both *bla* TEM and *bla* SHV.20 isolates (90.9%) harbored at least one ESBL gene, 10 isolates (45.45%) harbored*bla*CTX-M gene, and 15 isolates (68.18%) harbored*bla*TEM gene in quails reported in India (20).In this study, out of 27 isolates 13 isolates were found positive for fluoroquinolone resistance in which 13 (48.18%) isolates harboredthe *qnr*S gene (*Fig.* 6), and 11 (40.74%) isolates harboredthe *qnr*Bgene (*Fig.* 7).

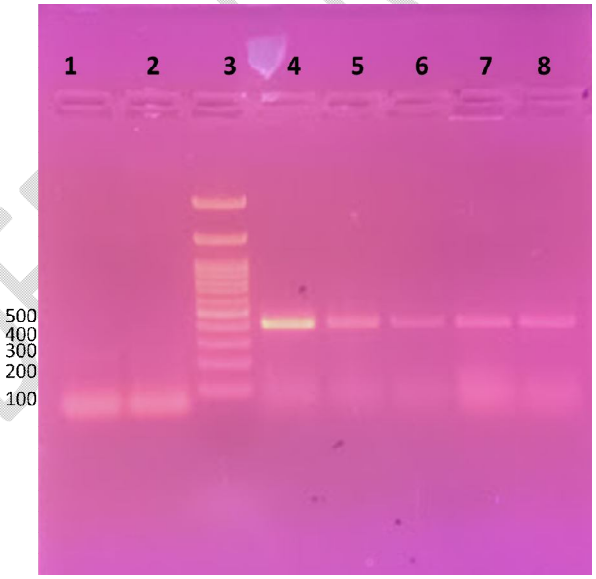

Fig 6. Agarose gel electrophoresis showing the results of polymerase chain reaction amplified product of 428 bp for the qnrS gene in *E. coli* isolates. Lane 3: 100 bp ladder; Lane 1,5,6,7,8,9,10: Field isolates for *qnr*S gene in *E. coli*; Lane 2: Negative control; Lane 4: Positive control

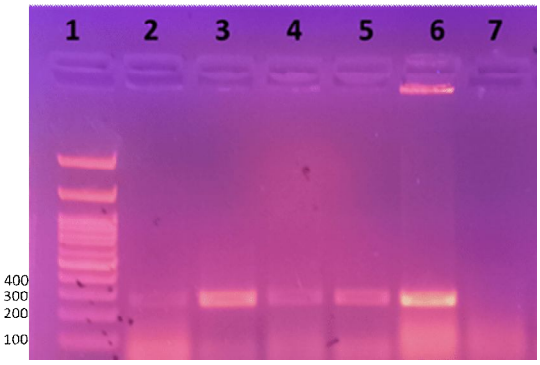

Fig 7. Agarose gel electrophoresis showing the results of polymerase chain reaction amplified product of 264 bp for the *qnr*B gene in *E. coli* isolates. Lane 1: 100 bp ladder; Lane 2&3,4,5: Field isolates for *qnr*B gene in *E. coli*; Lane 6: Positive control; Lane 7: Negative control

No study has reported the presence of fluoroquinolone resistance genes in Japanese quails in India. In poultry farms of Southern Karnataka, a study was conducted and found results that the *qnr*Bgene 38%was commonly detected followed by the *qnr*Sgene 27% and *qnr*A gene 21.5% in *Escherichia coli* in collected fecal samples. From North India, the prevalence of *qnr*S, and *qnr*B were 37. 50% and 5.83% respectively in from the cloacal swabs of commercial chickens reported (23,24,25). This is the first study conducted on Japanese quails of Puducherry and detected the presence of ESBL-producing and fluoroquinolone-resistant *E. coli* in fecal samples of quails in Puducherry. Antimicrobial susceptibility test showed that the majority of the isolates exhibited a high level of resistance against Amoxicillin/clavulanic acid (100%), Cefpodoxime (100%), Cefotaxime (80%), Aztreonam (70%), Ceftazidime (70%), Enrofloxacin (70%) and Ceftriaxone (40%).

4. CONCLUSION

The present study's findings indicate that Japanese quails may serve as a reservoir for ESBL and fluoroquinolone resistance*E. coli.*Similarly, the presence of ESBL-producingand fluoroquinolone-resistant*E. coli* in quails was a major concern indicating infection at the farm level. The present study showed the increased occurrence of ESBL-producing *E. coli* in quails in the Puducherry region and their resistant antibiogram pattern among quails.

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