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Research Article

PREVALENCE OF ANTIBIOTIC RESISTANCE GENES IN INDIAN DUCKS: A COMPARATIVE STUDY BETWEEN DESHI AND KHAKI CAMPBELL DUCKS

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ABSTRACT: The ESBL and ACBL producing genes are responsible for bacterial resistance to a number of antibiotics. This study was undertaken to detect the distribution pattern of ESBL and ACBL producing genes among healthy Deshi and Khaki Campbell ducks that have no apparent history of antibiotic intake. For this purpose, 110 and 92 cloacal swab samples were collected from Deshi and Khaki Campbell ducks respectively. Initially samples were screened for detection of *Escherichia coli, Salmonella* spp. and *Klebsiella pneumoniae* on the basis of their cultural, biochemical and morphological properties followed by PCR confirmation. Distribution pattern of three important ESBL producing genes *viz.* bla_{CTX-M}, bla_{TEM}, bla_{SHV} and one ACBL producing gene bla_{AmpC} were studied thoroughly among two different segments of ducks. Significantly higher occurrence of antibiotic resistance genes was found in free range Deshi ducks compared to that of Khaki Campbell ducks. This study indicates that promotion of rearing Khaki Campbell ducks among farmers is not only beneficial for higher production but also facilitates better management of the problem associated with antibiotic resistance.

Key words: Antibiotic resistance, ESBL, ACBL, Deshi ducks, Khaki Campbell ducks.

INTRODUCTION

According to the 20th livestock census, India has 31.20 million duck population out of which 12.45 million *i.e.* more than 1/3rd of the total population belongs to West Bengal only. Rearing Deshi (Indigenous) ducks in backyards is a very popular and profitable practice throughout the State. On the other hand, the Animal Resources Development Department of Government of West Bengal is making constant efforts for popularizing the practice of rearing Khaki Campbell ducks among the small and marginal farmers with attractive schemes including distribution of inputs and all kind of technical supports. Ducks commonly share both land and water bodies with man, animals and other birds. Owing to common access to land and water bodies, they are often exposed to different microbial organisms having antibiotic resistance properties. Exposed birds may harbour those organisms in their enteric tract with or without any clinical symptoms and shed those organisms in the environment. Thus, they may act as potential carriers to transmit those organisms to the human, animals and birds (Banerjee *et al.* 2019, Banerjee *et al.* 2020). There are distinct differences between rearing process of Deshi and Khaki Campbell ducks. Deshi ducks are generally reared in backyard semi-intensive system whereas Khaki Campbell is reared in more or less confined environment or as an important component of composite farming system *e.g.* fish cum duck farm. The present study was undertaken to evaluate whether the differences in rearing system can incite significant differences in the pattern of harbouring various antibiotic resistance bacteria among these two groups of ducks. To evaluate this comparative distribution pattern, three important members of *Enterobacteriaceae* family *viz. Escherichia coli*, *Salmonella* spp. and *Klebsiella pneumonia* were considered in the present study.

MATERIALS AND METHODS

Sampling

In this study, total 202 (n=202, single sample/duck) cloacal swab samples were collected aseptically from both Deshi ducks (110 samples) and Khaki Campbell ducks (92 samples) from different districts of West Bengal (Fig. 1, Table 1), India on a random basis. All these ducks

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were apparently healthy during the collection, without any known history of antibiotic intake. All collected swab samples were transported to laboratory in sterile peptone water (HiMedia, India) at 4°C for further processing.

Culture and isolation

Escherichia coli, *Salmonella* spp. and *Klebsiella* spp. were isolated from the collected samples by standard bacteriological techniques (OIE 2018). For isolation of *E. coli* and *Salmonella* spp., the collected swab samples were inoculated into MacConkey's agar (HiMedia) and Selenite broth (HiMedia), respectively and incubated at 37°C for overnight. The rose-pink colonies from MacConkey's agar were picked and transferred into EMB agar (HiMedia) followed by incubation at 37°C. Samples showing brick red colour in Selenite broth enrichment medium were streaked into brilliant green agar (HiMedia) and incubated overnight at 37°C. The colonies with metallic sheen in EMB agar and the convex, pale, red, translucent colonies from BGA were streaked into nutrient agar (HiMedia) slants for further morphological and biochemical confirmation of *E. coli* and *Salmonella* spp., respectively. Similarly, for isolation of *Klebsiella* spp., the collected samples were inoculated into HiChrome Klebsiella selective agar (HiMedia) and incubated at 37°C. The characteristic purple-magenta-coloured colonies were selected for further confirmation. The biochemically confirmed *E. coli*, *Salmonella* spp. and *Klebsiella* spp. isolates were subjected to confirmatory PCR (Wang *et al*. 1996, Brisse and Verhoef 2001, Salehi *et al*. 2005). All the confirmed *Klebsiella* spp. isolates were further tested for the identification of *Klebsiella pneumoniae* by specific PCR (Liu *et al*. 2008). (Fig. 2-5)

Double disc diffusion test

PCR confirmed *E. coli*, *Salmonella* spp. and *K. pneumoniae* isolates were subjected to double disc diffusion test with cefotaxime (30 µg, HiMedia) and ceftazidime (30 µg, HiMedia) with or without clavulanate (10 µg, HiMedia) (CLSI 2014). The cefoxitin-cloxacillin double disc synergy (CC-DDS) was performed with all the bacterial isolates for phenotypic confirmation of AmpC production (Tan *et al*. 2009).

Detection of beta-lactamase (bla_{CTX-M}, bla_{TEM}, bla_{SHV}) and chromosomal bla_{AmpC}

All the phenotypically beta-lactamase producing *E. coli*, *Salmonella* spp. and *K. pneumoniae* isolates including controls were subjected to PCR for detection of bla_{CTX-M}, bla_{TEM}, bla_{SHV} and bla_{AmpC} (Cao *et al*. 2002, Féria *et al*. 2002, Weill *et al*. 2004). The positive controls were provided by Central Agricultural University, Aizawl and non-ESBL producing strain *E. coli* ATCC 25922 (HiMedia) was used as negative control (Fig. 6-9). The selected PCR products were sequenced from commercially available sources (Xcelris Genomics, Ahmedabad, India). The sequence homology searches were conducted using the BLAST algorithm (Altschul *et al.* 1990).

Antibiotic sensitivity

All the beta-lactamase producing isolates were tested for their phenotypical resistance against ampicillin (10 µg), ampicillin/ cloxacillin (10/10 µg), amoxycillin/ clavulanic acid (20/10 µg), cefotaxime (30 µg), cefotaxime/clavulanic acid (30/10 µg), ceftazidime (30 µg), ceftazidime/clavulanic acid (30/10 µg), ceftriaxone (30 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5µg), tetracycline (30 µg), co-trimoxazole (25 µg) and chloramphenicol (30 µg) (Hi Media) (CLSI 2014).

Fig. 1. Places of Sample Collection.

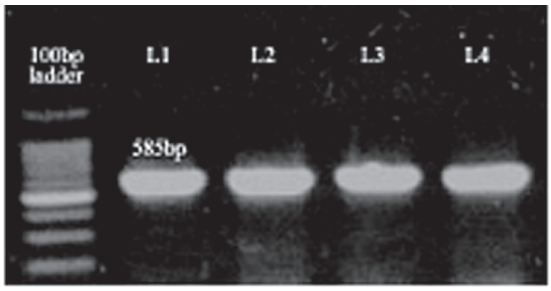

Fig. 2. Agarose gel electrophoresis showing the amplified product of *Escherichia coli* (16S rRNA).

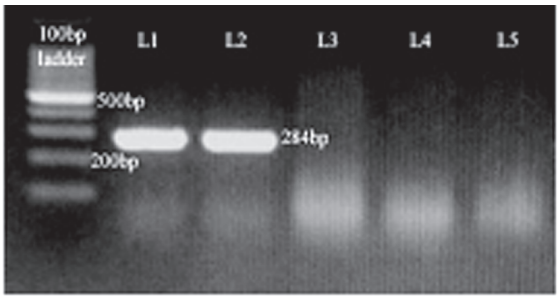

Fig. 3. Agarose gel electrophoresis showing the amplified product of *Salmonella* spp. (*invA*).

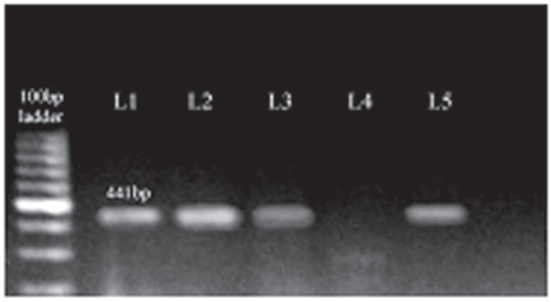

Fig. 4. Agarose gel electrophoresis showing the amplified product of *Klebsiella* spp.

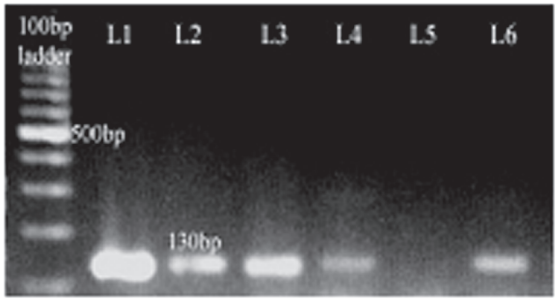

Fig. 5. Agarose gel electrophoresis showing Amplified product of *K. pneumoniae.*

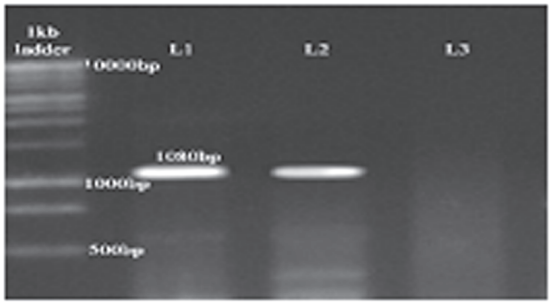

Fig. 6. Agarose gel electrophoresis showing amplified product of bla_{TEM} specific gene.

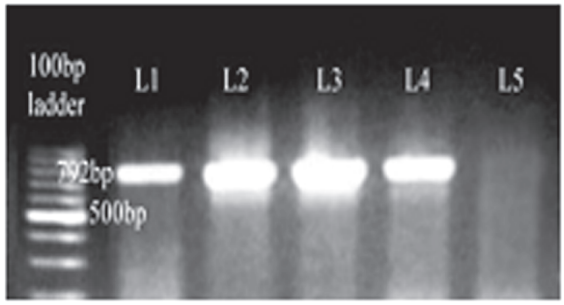

Fig. 7. Agarose gel electrophoresis showing amplified product of bla_{SHV} specific gene.

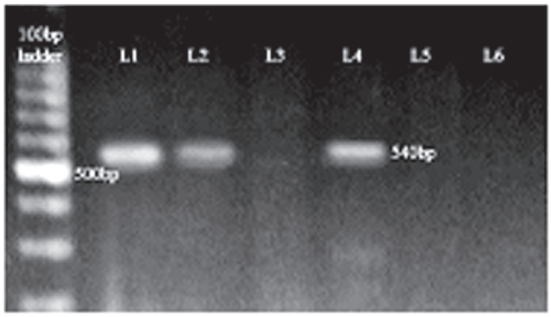

Fig. 8. Agarose gel electrophoresis showing amplified product of bla_{CTX-M} specific gene.

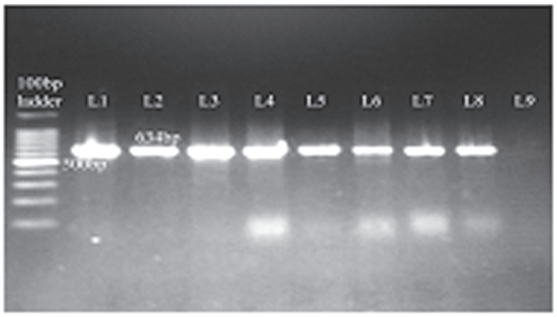

Fig. 9. Agarose gel electrophoresis showing amplified product of bla_{AmpC} specific gene.

RESULTS AND DISCUSSION

Out of 202 cloacal swab samples, 109 (53.96%), 13 (6.44%) and 30 (14.85%) isolates were confirmed positive for *E. coli*, *Salmonella* spp. and *K. pneumoniae* respectively. Among them 88 *E. coli* (88/109, 79.81%), 09 *Salmonella* (09/13, 69.23%) and 15 *K. pneumoniae* (15/30, 50.00%) were phenotypically detected as beta-lactamase producers. Significantly higher occurrence of beta-lactamase producing isolates ($p<0.05$) were observed in Deshi ducks than the Khaki Campbell ducks (Table 2).

Among the 88 phenotypically beta-lactamase producing *E. coli* isolates; 19 (19/109, 17.43%), 06 (06/109, 05.05%) and 15 (15/109, 13.76%) isolates were detected positive for bla_{TEM}, bla_{SHV} and bla_{CTX-M} respectively.

Total five *Salmonella* spp. (05/13, 38.46%) isolates were found to be positive for bla_{CTX-M}. In case of *K.*

Table 1. Details of sample collection.

Place of collection	Zone	Sector	Sample	Source	*E.coli*	*Salmonella*	*Klebsiella*
North 24 Parganas	New Alluvial	Organized	52 samples from Khaki Campbell duck	Cloacal swab	21	05	00
Hooghly (Rajbalhat, Jangipara)	Old Alluvial	Unorganized	14 samples from Desi duck	Cloacal swab	08	00	11
Nadia (Kalyani)	New Alluvial	Organized	40 sample from Khaki Campbell duck	Cloacal Swab	27	03	14
Burdwan (Uchalan, Raina-1)	Old Alluvial	Unorganized	19 samples from Desi duck	Cloacal Swab	12	03	06
Kolkata (Belgachia)	New Alluvial	Unorganized	02 samples from Desi duck	Cloacal Swab	02	02	00
Howrah (Bargachia, Jagatballavpur)	New Alluvial	Unorganized	33 samples from Desi duck	Cloacal Swab	25	00	06
Nadia (Bamanpukur, Nabadwip)	New Alluvial	Unorganized	32 samples from Desi duck	Cloacal Swab	08	00	04
Bankura (Panchbaga, Bankura-1)	Red Laterite	Unorganized	10 samples from Desi duck	Cloacal Swab	06	00	03
Unorganized (Desi duck)			110		61	08	30
Organized (Khaki Campbell)			92		48	05	14
TOTAL			202		109	13	44

Table 2. Distribution of beta-lactamase producing *Escherichia coli, Salmonella* spp. and *Klebsiella pneumoniae* from Khaki Campbell and Deshi ducks in West Bengal, India.

Source of samples	Beta-lactamase producing *E. coli*	Beta-lactamase producing *Salmonella* spp.	Beta-lactamase producing *Klebsiella pneumoniae*	Total number and percentage of ESBL producing bacteria
Khaki Campbell duck Sample (92) *E. coli i*solates (48) *Salmonella* spp. isolates (8) *Klebsiella pneumoniae* isolates (14)	32* (32/48, 66.67%)	05[a] (5/8, 62.5%)	08 (8/12, 66.67%)	45[b] (45/68, 66.17%)
Deshi Ducks sample (110) *E. coli i*solates (61) *Salmonella* spp. isolates (4) *Klebsiella pneumoniae* isolates (8)	56* (56/61, 91.8%)	04[a] (4/5, 80%)	08 (8/18, 57.14%)	68[b] (68/84, 80.95%)

***** Differs significantly ($p < 0.05$), [a] Differs significantly ($p < 0.05$), [b] Differs significantly ($p < 0.05$).

Table 3. Antibiotic sensitivity test of ESBL producing *Escherichia coli.*

Sl. No.	Antimicrobial agent (concentration)	Sensitive (%) n=47	Intermediate (%) n=47	Resistance (%) n=47
01	Ampicillin (10µg)	05 (10.64)	02 (04.26)	40 (85.11)
02	Ampicillin/ Cloxacillin (10/10µg)	08 (17.02)	00	39 (82.97)
03	Amoxicillin + Clavulanic acid (20/10µg)	06 (12.76)	08 (17.02)	33 (70.21)
04	Cefotaxime 30µg	01 (02.12)	03 (06.38)	43 (91.48)
05	Cefotaxime + Clavulanic acid (30/10µg)	15 (31.91)	28 (59.57)	04 (08.51)
06	Ceftazidime (30µg)	02 (04.25)	15 (31.91)	30 (63.83)
07	Ceftazidime + Clavulanic acid (30/10µg)	12 (25.53)	15 (31.91)	20 (42.55)
08	Ceftriaxone (30µg)	04 (08.51)	09 (19.15)	34 (72.34)
09	Gentamicin (10µg)	23 (48.94)	20 (42.55)	04 (08.51)
10	Amikacin (30µg)	39 (82.98)	06 (12.77)	02 (04.26)
11	Ciprofloxacin (5µg)	27 (57.45)	05 (10.64)	15 (31.91)
12	Tetracycline (30µg)	29 (61.70)	07 (14.89)	11 (23.40)
13	Co-Trimoxazole (25µg)	15 (31.91)	07 (14.89)	25 (53.19)
14	Chloramphenicol (30µg)	44 (93.62)	02 (04.26)	01 (02.12)

pneumoniae, 10 (10/30, 33.33%), 03 (03/30, 13.33%) and 04 (04/30, 13.33%) isolates were detected positive for bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ respectively.

Out of 88 ESBL producing *E. coli*; 32 were detected from organized sector and rest 56 from unorganized sector. 66.67% (32 out of 48) and 91.8% (56 out of 61) sample collected from Khaki Campbell and Deshi ducks respectively were found to be positive for ESBL production. So, much higher prevalence of ESBL producing *E. coli* was found in Deshi ducks compared to

Table 4. Antibiotic sensitivity test of ESBL producing *Salmonella* spp.

Sl. No.	Antimicrobial agent (concentration)	Sensitive (%) n=9	Intermediate (%) n=9	Resistance (%) n=9
01	Ampicillin (10µg)	01 (11.11)	01 (11.11)	07 (77.78)
02	Ampicillin/ Cloxacillin (10/10µg)	02 (22.22)	00	07 (77.78)
03	Amoxicillin + Clavulanic acid (20/10µg)	03 (33.33)	01 (11.11)	05 (55.56)
04	Cefotaxime 30µg	02 (22.22)	02 (22.22)	05 (55.56)
05	Cefotaxime + Clavulanic acid (30/10µg)	05 (55.56)	00	04 (44.44)
06	Ceftazidime (30µg)	04 (44.44)	00	05 (55.56)
07	Ceftazidime+ Clavulanic acid (30/10µg)	04 (44.44)	02 (22.22)	03 (33.33)
08	Ceftriaxone (30µg)	03 (33.33)	00	06 (66.67)
09	Gentamicin (10µg)	06 (66.67)	01 (11.11)	02 (22.22)
10	Amikacin (30µg)	09 (100.0)	00	00
11	Ciprofloxacin (5µg)	05 (55.56)	03 (33.33)	01 (11.11)
12	Tetracycline (30µg)	02 (22.22)	05 (55.56)	02 (22.22)
13	Co-Trimoxazole (25µg)	01 (11.11)	04 (44.44)	04 (44.44)
14	Chloramphenicol (30µg)	08 (88.89)	01 (11.11)	00

Table 5. Antibiotic sensitivity test of ESBL producing *Klebsiella pneumoniae.*

Sl. No.	Antimicrobial agent (concentration)	Sensitive (%) n=15	Intermediate (%) n=15	Resistance (%) n=15
01	Ampicillin (10µg)	00	03 (20.00)	12 (80.00)
02	Ampicillin/ Cloxacillin (10/10µg)	00	05 (33.33)	10 (66.67)
03	Amoxicillin + Clavulanic acid (20/10µg)	03 (20.00)	03 (20.00)	09 (60.00)
04	Cefotaxime 30µg	03 (20.00)	04 (26.67)	08 (53.33)
05	Cefotaxime + Clavulanic acid (30/10µg)	07 (46.67)	01 (06.67)	07 (46.67)
06	Ceftazidime (30µg)	03 (20.00)	05 (33.33)	07 (46.67)
07	Ceftazidime+ Clavulanic acid (30/10µg)	06 (40.00)	03 (20.00)	06 (40.00)
08	Ceftriaxone (30µg)	02 (13.33)	04 (26.67)	09 (60.00)
09	Gentamicin (10µg)	05 (33.33)	06 (40.00)	04 (26.67)
10	Amikacin (30µg)	10 (66.67)	03 (20.00)	02 (13.33)
11	Ciprofloxacin (5µg)	07 (46.67)	03 (20.00)	05 (33.33)
12	Tetracycline (30µg)	03 (20.00)	02 (13.33)	10 (66.67)
13	Co-Trimoxazole (25µg)	00	06 (40.00)	09 (60.00)
14	Chloramphenicol (30µg)	10 (66.67)	00	05 (33.33)

Khaki Campbell ducks.

In case of *Salmonella* spp. 05 out of 08 (62.50%) ESBL producing isolates were obtained from Khaki Campbell ducks and 04 out of 05 (80%) was detected from Deshi ducks. So, higher prevalence of ESBL producing *Salmonella* spp. was found in Khaki Campbell ducks compared to Deshi ducks.

However, in case of *Klebsiella pneumoniae* slightly higher prevalence of ESBL producing isolates were detected in Khaki Campbell ducks compared to that of Deshi ducks.

So far authors' best knowledge it is the first-time reported study on occurrence of beta-lactamase producing *Salmonella* spp. and *Klebsiella pneumoniae* from ducks in India.

Out of 109 *E. coli* isolates 87 (79.82%), out of 13 *Salmonella* spp. isolates 06 (46.15%) and out of 30 *Klebsiella pneumoniae* isolates 13 (43.33%) were detected positive for bla_{AmpC} gene with PCR amplified product size of 634bp. In present study, bla_{AmpC} was found to be the most prevalent gene compared to other ESBL genes detected in all three types of bacteria.

In further analysis 31 out of 48 (64.58%) and 56 (91.80%) out of 61 *E. coli* isolates were found to be positive for bla_{AmpC} gene from Khaki Campbell and Deshi ducks respectively. So, higher prevalence of bla_{AmpC} genes was detected in Deshi ducks.

02 (25%) out of 08 and 04 (80%) out of 05 *Salmonella* spp. isolates were found to be positive for bla_{AmpC} gene from Khaki Campbell and Deshi ducks respectively. So, much higher prevalence of bla_{AmpC} gene was detected from Deshi ducks.

In case of *Klebsiella pneumoniae* similar distribution trend of bla_{AmpC} gene was also detected. 05 (41.67%) out of 12 and 08 (44.44%) out of 18 *K. pneumoniae* were found to be positive for bla_{AmpC} gene from Khaki Campbell and Deshi ducks respectively.

In present study overall higher prevalence of ESBL producing isolates was found in Deshi ducks compared to Khaki Campbell ducks. But no history of antibiotic use was recorded in Deshi ducks. This may be due to the fact that the Deshi ducks are reared and maintained in backyard or semi-intensive system and their habitat *i.e.* water bodies is often contaminated with antibiotic residue from sewage system, antibiotics used in aquaculture and agriculture. The birds also have ample chances of coming in close contact with antibiotic resistant bacteria as they commonly share land and water bodies with other animals and human beings, often exposed to indiscriminate antibiotic therapy (Banerjee *et al.* 2019). Earlier Samanta *et al.* (2015) from West Bengal reported higher prevalence of ESBL producing *E. coli* from indigenous pigs compared to the pigs maintained in organized farms. They also pointed out contaminated environment as source of ESBL producing *E. coli* for indigenous pigs. However, Singer *et al.* (2006) demonstrated that the use of antibiotic and emergence of resistant bacteria is not always correlated.

In this study, ESBL producing *Escherichia coli*, *Salmonella* spp. and *Klebsiella pneumoniae* were also detected from Khaki Campbell ducks. Particularly in case of *Klebsiella pneumoniae* the prevalence was slightly higher in Khaki Campbell ducks compared to Deshi ducks. This may be due to the common practice of duck farmers feeding antibiotic supplements to their Khaki Campbell ducks as growth promoter and disease preventer, as they sometimes consider this practice as an important part of management of Khaki Campbell ducks.

Majority of the isolates in this study possessed bla_{CTX-M}, although the ducks did not receive any antibiotics especially the higher generation cephalosporins. Contaminated environment might be an additional source, as the CTX-M type of beta-lactamase can better survive in the environment due to possession of certain plasmids associated with survival benefits (Schaufler *et al.* 2016).

Isolates exhibited phenotypical resistance against ampicillin, ampicillin/cloxacillin and ceftriaxone in antibiotic sensitivity test (Table 3-5). Similar kind of resistance against cefotaxime, ampicillin and susceptibility to chloramphenicol was observed in beta-lactamase producing *E. coli* in ducks (Ma *et al.* 2012).

CONCLUSION

From the present study we can conclude that ducks may act as a potential reservoir of several beta-lactamase producing bacteria coming under *Enterobactriaceae* family and may disseminate those organisms to cause major public health hazards. However, considering importance of duck rearing in Indian economy, particularly rural economy we will have to continue it. Needless to say, that ducks are serving as one of the most important sources of protein for their meat and eggs in our state as well as our country. In this situation promotion of rearing Khaki Campbell Ducks instead of Deshi ducks may be a good alternative. The present study revealed that Khaki Campbell ducks are safer in terms of handling and rearing compared to Deshi ducks, as they harbour lesser number of antibiotic resistance genes. Our State Government is constantly motivating the farmers to rear Khaki Campbell ducks as it produces more eggs than

Deshi ducks. Now this practice should be encouraged further to manage the problem of antibiotic resistance in a better way.

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