

ISOLATION AND CHARACTERIZATION OF MULTIDRUG RESISTANT BETA-LACTAMASE PRODUCING *SALMONELLA ENTERICA* FROM WILD MIGRATORY BIRDS

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Abstract. A multidrug resistant, an enteric pathogen, *Salmonella enterica* is the most frequent cause of food poisoning. They are gram negative, aerobic or facultative anaerobic bacteria belonging to the family *Enterobacteriaceae*. Migratory birds serve as key factor, may disseminate *Salmonella* to the susceptible people through shared environment, fecal shedding and by mean of direct contact. The aim of present study was to investigate the occurrence of beta-lactamase genes (CTX-M and TEM) within antibiotic resistant strains of *Salmonella enterica* isolated from migratory birds. Present study incorporated isolation of 60 samples of *Salmonella enterica* from saliva, intestinal fluid and blood of different migratory birds. They are catalase positive, oxidase negative and H₂S gas producer. The antibiotic resistance of all isolated strains (29) was tested against 10 antibiotics by Kirby-Bauer disc diffusion method. The antibiotics that were used include ciprofloxacin (5 µg), ceftriaxone (30 µg), meropenam (10 µg), aztreonam (30 µg), penicillin (10 µg), erythromycin (15 µg), streptomycin (10 µg), gentamycin (10 µg), vancomycin (10 µg) and imipenem (10 µg). These strains indicated more resistance towards penicillin (93%), streptomycin (100%), erythromycin (93%), aztreonam (62%) and vancomycin (90%) and low resistance towards ciprofloxacin (21%), ceftriaxone (54%) and imipenem (45%). For the detection of CTX-M and TEM gene in ciprofloxacin and ceftriaxone resistant strains, DNA was obtained through chemical method. These strains were further checked for the presence of CTX-M and TEM gene by polymerase chain reaction (PCR). PCR results demonstrated that all the strains contain TEM gene but CTX-M gene was not identified in any of these strains. PCR amplified product was sequenced, followed by BLAST, which confirmed the presence of TEM gene giving resistance to beta-lactam antibiotics.

Keywords: *Salmonella enterica*, TEM gene, antimicrobial resistance, CTX-M gene, polymerase chain reaction (PCR), BLAST

Abbreviations: SS agar: *Salmonella Sheila* agar, DNA: Deoxyribonucleic acid, STE buffer: Sodium Chloride-Tries-EDTA, TE buffer: Tries-EDTA, PCR: Polymerase Chain Reaction, CIP: Ciprofloxacin, CRO: Ceftriaxone, MEM: Meropenam, ATM: Aztreonam, S: Streptomycin, E: Erythromycin, P: Penicillin, CN: Gentamicin, VA: Vancomycin, IPM: Imipenem, BLAST: Basic Local Alignment Search Tool

Introduction

The bacterial genus *Salmonella* which is an enteric pathogen causes a wide spectrum of disease (Acheson and Hohmann, 2001). *Salmonella enterica* is mostly responsible for food poisoning (Herikstad et al., 2002). *Salmonella* is an exceptionally assorted family,

which contain two species that are *S. bongori* and *S. enterica* (Tindall et al., 2005). *S. bongori* infects mostly ectotherms and unusually humans while *S. enterica* incorporates around 2500 serovars (Brenner et al., 2000). It is subdivided into 6 subspecies *enterica*, *arizonae*, *diarizonae*, *salamae*, *indica* and *houtenae* dependent on biochemistry and genomics (Malorny et al., 2011). The normal characteristic environment for subspecies *enterica* is endotherms while for the subspecies *salamae*, *arizonae*, *diari zonae*, *houtenae*, *indica* and *S. bongori* is ectotherms and environment. Migratory birds may serve as vehicle for the transmission of various extrinsic microorganisms (Maeda et al., 2001). *Salmonella* have been isolated from wildlife including exotic birds (Refsum et al., 2002). Gourmelon et al. (2010) have also observed that aquatic system gets polluted by fecal material of these exotic species can lead to human infection. During annual migration, migratory birds move from one place to another in search of suitable weather and food and play an important role in causing infections in humans and animals (Foti et al., 2011). They can easily be transmitted through contaminated food and water, improper hygienic conditions and improper disposal of sewage. Antimicrobial resistance in *Salmonella* takes place due to horizontal and vertical transference of antimicrobial gene, inappropriate self-medication which leads to resistance in microorganisms against chemical, therapeutic agents (White et al., 2002). The excessive use of antimicrobials has brought about a development of antibiotic resistance in humans, animals and environment (Berendonk et al., 2015; Radhouani et al., 2014). Majority of ceftriaxone and ciprofloxacin resistance was because of the action of β -lactamase gene. The horizontal gene transfer plays an important part in expanding ceftriaxone and ciprofloxacin resistance.

The main objective of present study was to isolate different strains of *Salmonella* from oral, intestinal and blood samples of migratory birds and to investigate the occurrence of beta-lactamase genes (CTX-M and TEM) which give antibiotic resistance against beta-lactam antibiotics (ciprofloxacin and ceftriaxone).

Material and methods

Study design

This study involved biochemical identification of *Salmonella enterica* from blood, nasal and intestinal fluid of migratory birds and molecular characterization and PCR detection of β -lactamase (CTX-M and TEM) genes.

Sample collection

Oral, intestinal and blood samples were collected from different seasonal avian species at watery sites of Hazara division (Tarbela Lake, Chakai, Siran Valley, and Khanpur). During period from September 2018 till February 2019, migratory birds were captured for study by licensed hunters from different watery sites of Hazara division. A sterile culturette was used to collect samples. A total of 60 samples were collected by rubbing swabs to oral, intestine and blood of different migratory birds. 47 blood samples were obtained through sterile syringes while 6 swabs from saliva and 7 swabs from intestinal fluid were obtained. After sampling, the culturettes were placed in their plastic sheath having nutrient broth and kept in refrigerator at 4 °C. Then the samples were enriched by adding peptone water and incubated at 37 °C for 24 h. After incubation, the pre-enriched samples were examined for the presence of *Salmonella*.

Isolation and identification

The samples were inoculated on sterile petri plates containing *Salmonella Shigella* agar and incubated at 37 °C for 24 h. The isolated colonies were picked up and again streaked on SS agar medium to obtain a pure culture. Purified bacterial colonies were exposed to cultural, colony morphology and biochemical identification by using catalase, oxidase and citrate test.

Antibiotic sensitivity

Antibiotic Sensitivity of 29 positive isolates of *Salmonella enterica* to 10 antibiotics was checked on Muller Hinton agar by Kirby-Bauer disc diffusion method. A fresh colony of *Salmonella* was picked up from the culture plate and spread over the entire petri plate containing Muller Hinton agar by using sterile cotton swab. Ten antibiotics with different concentrations were used which included ciprofloxacin, ceftriaxone, penicillin, erythromycin, streptomycin, vancomycin, gentamicin, imipenem, meropenam and aztreonam. The antimicrobial disks were placed on the surface of agar with the help of forceps and plates were incubated overnight at a temperature of 37 °C. After incubation, the zone diameters were measured to nearest millimeter and classified as sensitive (S), intermediate (I) and resistant (R) according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Table 1).

Table 1. Concentration (μg) and breakpoints (mm) of antibiotics

Antibiotics	Disc code	Potency (μg)	Zone diameter breakpoint (mm)	
			S \geq	R <
Ciprofloxacin	CIP	5	22	19
Ceftriaxone	CRO	30	23	20
Penicillin	P	10	14	15
Erythromycin	E	15	21	15
Streptomycin	S	10	15	11
Vancomycin	VA	10	17	14
Gentamicin	CN	10	17	14
Imipenem	IPM	10	22	16
Meropenam	MEM	10	22	16
Aztreonam	ATM	30	24	21

DNA extraction

DNA was extracted by chemical method (Ausubel et al., 1994). The 1 ml growth culture of bacterial suspension was incubated overnight and centrifuged at 8000 g for 2 min. Supernatant was removed. 400 μl STE buffer was added twice and cells were centrifuged at 8000 g for 2 min. Supernatant was discarded again and 200 μl TE buffer was added to the pellets. Then, 100 μl Tris-saturated phenol was added and tubes were vortexed for 1 min. Samples were centrifuged at 13,000 g for 5 min at 4 °C so that the aqueous phase is separated from organic phase. Then 40 μl TE buffer was added to 160 μl upper aqueous phase and mixed with 100 μl chloroform and centrifuged at 13,000 g for 5 min at 4 °C. 150 μl upper aqueous phase was taken into clean eppendorf tube and that contained the required purified DNA.

Polymerase chain reaction (PCR)

PCR was performed for DNA amplification on thermocycler (Multigene Optimax, USA). Two sets of primer pairs specific for TEM and CTX-M gene were used (Table 2). The reactions were performed in final volume of 25 µL that involved 3 µL of DNA template, 1 µL of forward primer, 1 µL of reverse primer, 10 µL of 5X FIREPol® master mix (FIREPol® DNA Polymerase, 5X reaction buffer B (0.4 M Tris-HCl, 0.1 M (NH₄)₂SO₄, 0.1% w/v Tween 20), 7.5 mM MgCl₂, 1 mM dNTPs of each dATP, dGTP, dCTP, dTTP) (Solis BioDyne) and 10 µL of distilled water. Amplification was carried out using optimum conditions as follows: 1 cycle of 5 min for initial denaturation at 94 °C, 35 cycles of 45 s for final denaturation at 94 °C, 45 s for annealing at 54 °C, 1 min for initial extension at 72 °C and 1 cycle of 10 min for final extension at 72 °C in case of CTX-M gene. TEM gene was amplified using the same thermocycler with the exception of annealing at 52 °C for 45 s.

The amplification products were interpreted by gel electrophoresis. The samples were loaded in 1.2% gel stained with ethidium bromide (2 µg/mL) with the help of micro pipette and run at 90 V for 45 min. A 100-bp ladder (Solis BioDyne) was used as molecular weight marker. Gel was placed in Cleaver Scientific UV transilluminator (CSLUVTL312) to visualize the DNA bands under UV light for the presence of target gene.

Table 2. Primers sequence of CTX-M and TEM gene

Primers	Target gene	Length	Sequence (5'-3')	References
TEM F_5 TEM R_5	TEM	20 20	5' TTGGGTGCACGAGTGGGTTA 3' 5' TAATTGTTGCCGGGAAGCTA 3'	Gangoue-Pieboji et al., 2005
CTX-M F_5 CTX-M R_5	CTX-M	20 22	5' ACCGCCGATAATTCGCAGAT 3' 5' GATATCGTTGGTGGTGCCATAA 3'	Kaftandzieva et al., 2011

Statistical analysis

The Vassar Stats.net was used for *Chi Square* test (χ^2) to estimate significance level between sources of *S. enterica* isolates.

Genome sequencing and BLAST

Identification of resistant gene was confirmed by genome sequencing. The DNA of isolated strains was sequenced by Sanger sequencing method. The nucleotide sequence was analyzed and compared with sequences available on databases by using Basic Local Alignment Search Tool (BLAST).

Results

For the isolation of *Salmonella enterica*, *Salmonella Shigella* agar was used. Out of 60 samples 29 samples were positive for different serovars of *Salmonella enterica* and 31 samples were negative. Total isolation frequency of *Salmonella enterica* was found to be 29/60 (48%) (Fig. 1).

Out of these 29 samples, 22 (30%) samples were positive from blood, 3 (27%) from intestinal fluid and 4 (43%) from saliva (Fig. 2).

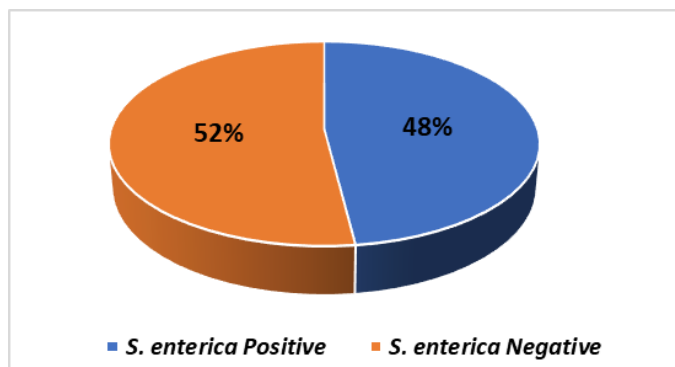


Figure 1. Isolation frequency of *Salmonella enterica* from migratory birds

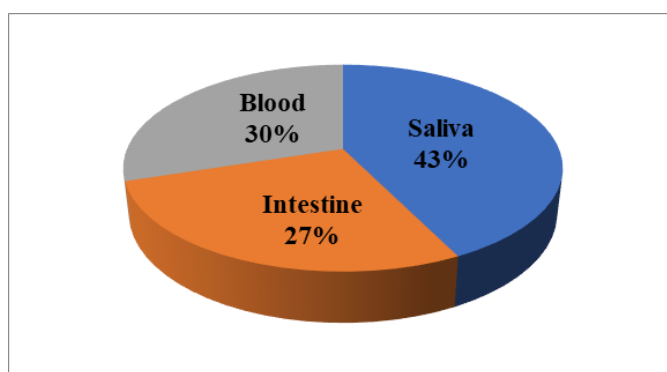


Figure 2. Percentage comparison of migratory birds positive for *S. enterica*

The chi square test (χ) revealed a non-significant difference between sources of *S. enterica* for all antibiotics tested except meropenem where a significant difference was observed ($p < 0.3$) (Table 3). The pink or yellow with black centered, flat and irregular/smooth colonies were observed on SS agar. The isolated colonies were stained with Gram's stain and appeared as rod shaped and pink in color under microscope (100X) (Fig. 3).

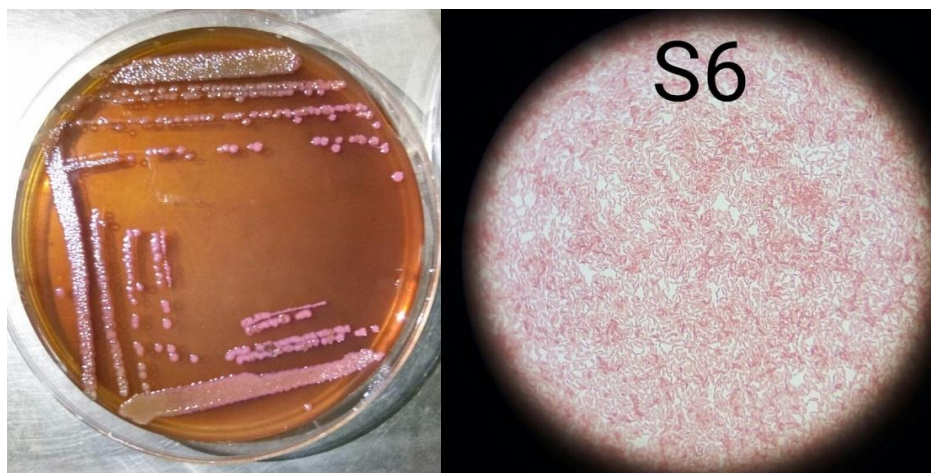


Figure 3. Cultural and microscopic (100X) identification of *S. enterica* isolated from migratory birds

All the positive isolates were catalase positive and oxidase negative (Fig. 4).

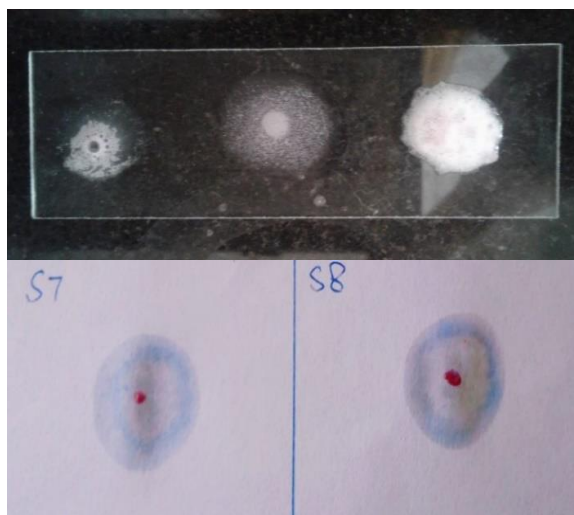


Figure 4. Biochemical identification of *Salmonella enterica* isolated from migratory birds

The *Salmonella enterica* serotypes were confirmed by citrate test. *Salmonella enterica* is citrate positive except *S. typhimurium* (Fig. 5).



Figure 5. Citrate test for the detection of *Salmonella enterica*

Antibiotic sensitivity of 29 isolates of *Salmonella enterica* obtained from saliva (n = 4), intestine (n = 3) and blood (n = 22) was checked against the ten antibiotics. In this study, *Salmonella enterica* showed resistance towards most antibiotics. The isolated strains showed more resistance against penicillin (93%), streptomycin (100%), erythromycin (93%), vancomycin (90%), aztreonam (59%) and meropenam (59%). They showed low resistance against ciprofloxacin (21%), ceftriaxone (52%), imipenem (45%) and gentamycin (55%). 25% (1/4) resistance was observed against ciprofloxacin and ceftriaxone in saliva samples, 33% (1/3) resistance against ciprofloxacin and 100% (3/3) resistance against ceftriaxone was noticed in intestinal fluid and 18% (4/22) resistance against ciprofloxacin and 50% (11/22) resistance against ceftriaxone were recognized in blood samples of migratory birds (Figs. 6, 7 and 8).

Statistical analysis was performed by chi-square test (Vassarstats.net) with P values for the comparison of difference between resistance rates of *S. enterica* to different antibiotics among the three sample sources (blood, intestine, saliva) (Table 3). The P values showed that there were no significant differences in antibiotic prescription patterns between blood, intestine and saliva sample except meropenam (0.03) (P value < 0.05 = significantly different).

Table 3. Chi-square test for comparison of *S. enterica* resistance rate (%) to various antibiotics among different sources

Antibiotics	Blood	Intestine	Saliva	X ²	P value
Ciprofloxacin	4/22 (18%)	1/3 (33%)	1/4 (25%)	0.42	0.81
Ceftriaxone	11/22 (50%)	3/3 (100%)	1/4 (25%)	3.97	0.13
Meropenam	10/22 (45%)	3/3 (100%)	4/4 (100%)	6.51	0.03
Aztreonam	11/22 (50%)	3/3 (100%)	3/4 (75%)	3.23	0.19
Penicillin	20/22 (91%)	3/3 (100%)	4/4 (100%)	0.68	0.71
Erythromycin	20/22 (91%)	3/3 (100%)	4/4 (100%)	0.68	0.71
Streptomycin	22/22 (100%)	3/3 (100%)	4/4 (100%)	0.00	1.00
Gentamycin	10/22 (45%)	2/3 (67%)	4/4 (100%)	4.25	0.11
Vancomycin	19/22 (86%)	3/3 (100%)	4/4 (100%)	1.06	0.58
Imipenem	8/22 (36%)	2/3 (67%)	3/4 (75%)	2.69	0.26

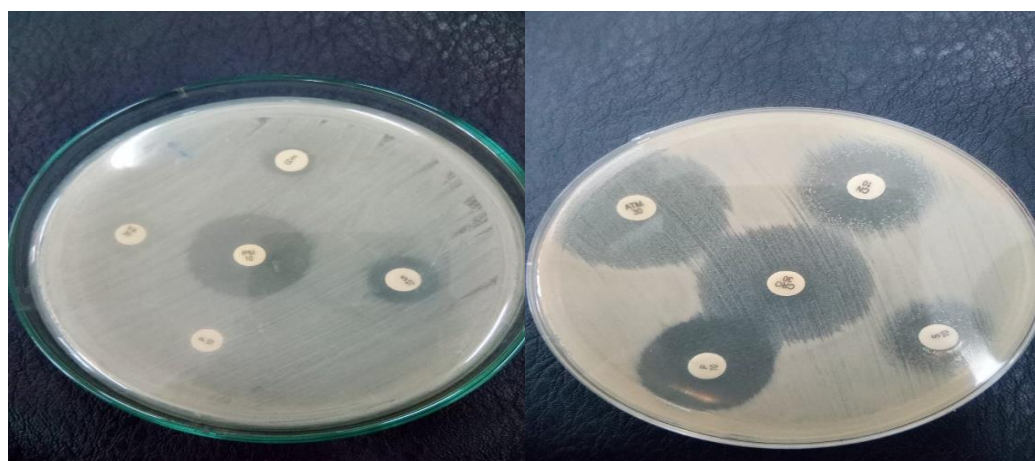


Figure 6. Antibiotic sensitivity of different antibiotics against *S. enterica* isolates

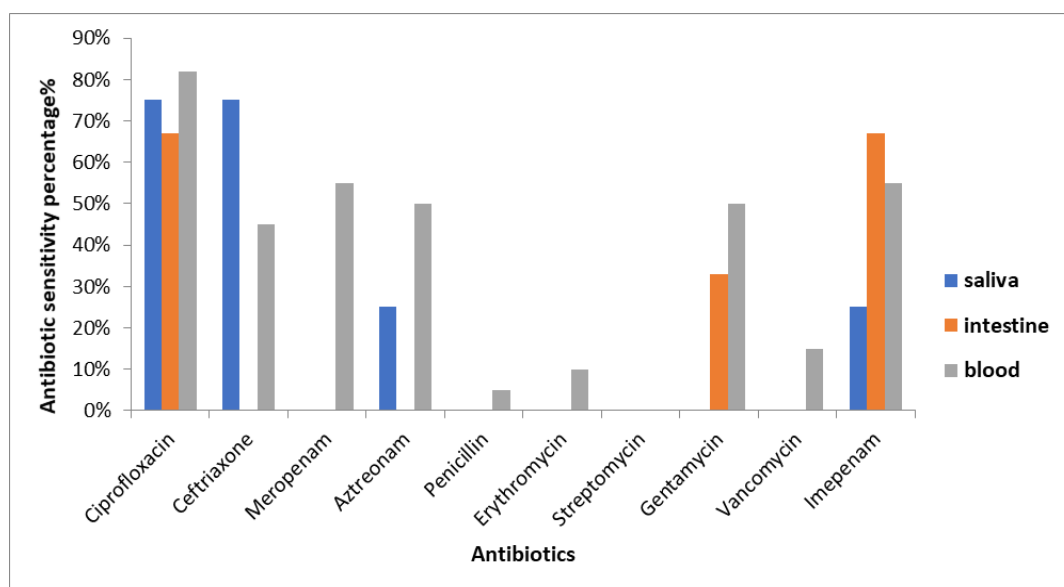


Figure 7. Antibiotic sensitivity pattern of isolates obtained from migratory birds

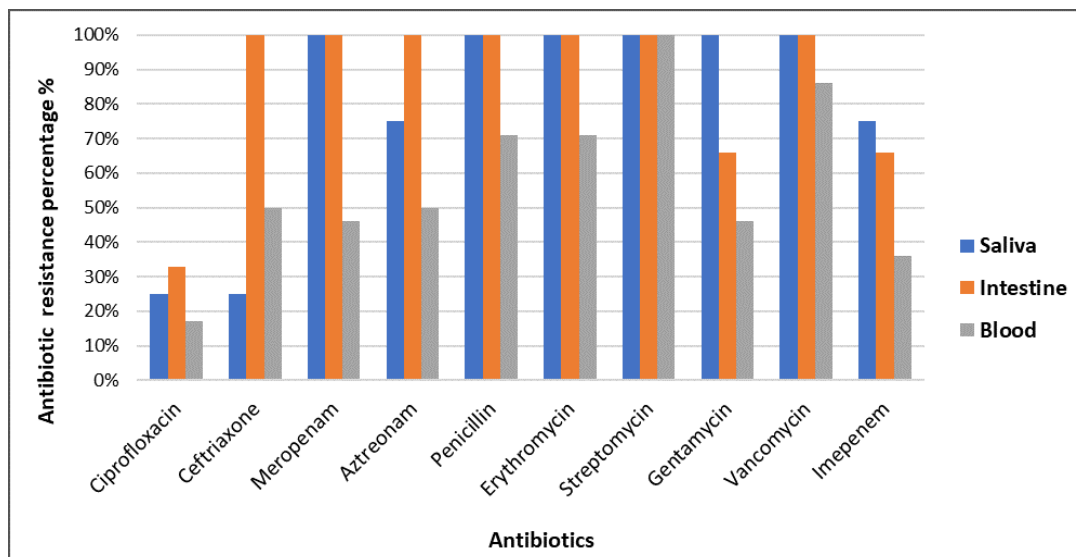


Figure 8. Antibiotic resistance pattern of isolates obtained from migratory birds

The extracted DNA of isolated strains was visualized by using gel electrophoresis (Fig. 9).

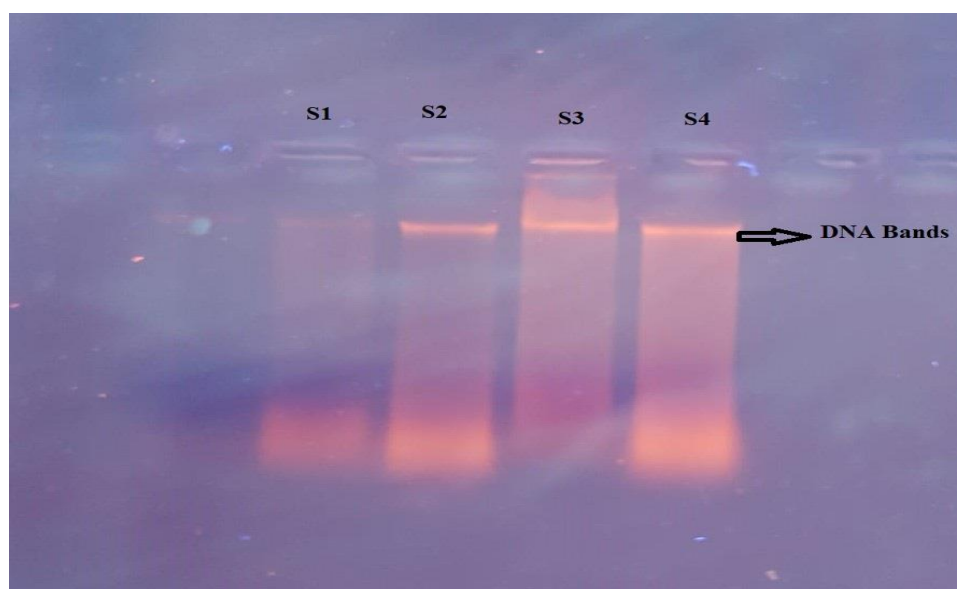


Figure 9. Gel electrophoresis of extracted DNA of isolated strains

Amplified PCR samples were analyzed by using gel electrophoresis. TEM gene was amplified in 100% strains of *S. enterica* tested for the presence of antibiotic resistant gene, yielding a strong band as dictated by correlation with size markers keep running on similar gel while no one isolates have CTX-M gene and its presence is 0% (Fig. 10).

Sample numbers S1, S2, S3, S4 and S5 showed a 508 bp fragment of TEM gene and L (Ladder) showed a 100 bp DNA size marker. Genome sequencing, followed by BLAST, confirmed the presence of TEM gene. The BLAST analysis showed highest similarity (88% identity) with *Klebsiella oxytoca*, which also harbors TEM gene (Fig. 11).

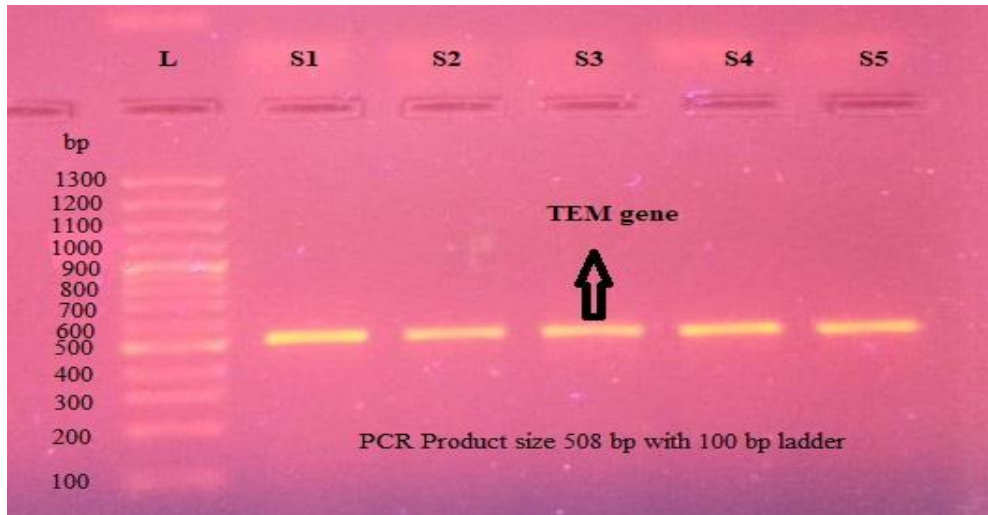


Figure 10. Amplification of TEM gene through polymerase chain reaction (PCR)

Klebsiella oxytoca extended-spectrum beta-lactamase (blaTEM1) gene, partial cds					
Sequence ID: KU991932.1 Length: 621 Number of Matches: 1					
Range 1: 121 to 535 GenBank Graphics ▼ Next Match ▲ P					
Score	Expect	Identities	Gaps	Strand	
510 bits(276)	2e-140	369/417(88%)	6/417(1%)	Plus/Plus	
Query 56	AGCACTGTT-ATGTTGTGCTATGTGTRTGCGGYATTATCAGTTATTGACGCC-GGCAAGA	113			
Sbjct 121	AGCACTTTTAAAGTTCGCTATGTG-GTGCGGTATTATCCCGTATTGACGCCGGGCAAGA	179			
Query 114	TCAACTCGGTCGCCGCGTACACTATTTCGCACAA-GACTGGGCTGATTATTGRCCAGCGTC	172			
Sbjct 180	GCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGTTGAGTACTCACCAGTAC	239			
Query 173	AGAAGAGCATCTTACGCATGGGGTGCCAGCMGGAGAATTATGCMGGGCTGCTGTAACCCA	232			
Sbjct 240	AGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAAGTCTGCCATAACC-A	298			
Query 233	CTCKTG-CACCCCAACGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAA	291			
Sbjct 299	TGAGTGATAAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAA	358			
Query 292	CCGCTTTTTTGCAACAACATGGGGGATCATGTAACCTCGCCTTGATCGTTGGGAACCGGAGC	351			
Sbjct 359	CCGCTTTTTTGCAACAACATGGGGGATCATGTAACCTCGCCTTGATCGTTGGGAACCGGAGC	418			
Query 352	TGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCCTGTAGCAATGGCAACAA	411			
Sbjct 419	TGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCCTGTAGCAATGGCAACAA	478			
Query 412	CGTTGCGCAAACTATTAACGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAA	468			
Sbjct 479	CGTTGCGCAAACTATTAACGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAA	535			

Figure 11. Sequence analysis of TEM gene showing similarity with Gene bank database sequence. Vertical lines represent identical nucleotides

Discussion

The *S. enterica* was found higher in oral cavity (43%) followed by blood (30%) and intestinal fluid (27%). The different isolation frequency of *Salmonella* was due to reason that it was found in oral cavity by eating contaminated food and its presence in blood indicates bacteremia. Experimental studies have demonstrated that gastrointestinal carriage of *Salmonella* take place in wild birds after disease; hence it is conceivable that healthy birds could be persisting carriers. Samad (2011) declared that *Salmonella* was transmitted through birds which obtain this infection from their surrounding environment. Present data was identical to the findings of Samad that

infected birds can play a main role in the transmission of diseases in humans and animals. In current study, among the ten antibiotics used a high rate of antibiotic resistance was observed against penicillin (93%), streptomycin (100%), erythromycin (93%) and vancomycin (90%). Thung et al. (2018) have also discovered comparable outcomes, who found higher prevalence of *Salmonella* in retail beef meat from different retail markets of Selangor, Malaysia. Antibiotic susceptibility was checked against 15 antibiotics. They also found that all the isolates showed resistance towards penicillin, erythromycin and vancomycin but sensitivity was observed for tetracycline, gentamicin and amoxicillin/clavulanic acid.

TEM gene was present in all positive strains (100%) tested for the presence of antibiotic resistant gene but CTX-M was absent in these strains and its presence is (0%). The presence of TEM gene was further confirmed by genome sequencing which showed 88% similarity with database sequence of *Klebsiella oxytoca* by using BLAST. This indicates that *S. enterica* might have acquired TEM gene from *K. oxytoca*. Threfall et al. (2002) reported *Salmonella* from India and different locations of Asia. They presumed that one of the primary factors of failure of treating *Salmonella* is because of resistance to ciprofloxacin. Majority of ceftriaxone and ciprofloxacin resistance was the result of activity of β -lactamase gene which includes CTX-M and TEM gene (Elumalai et al., 2014). Beta-lactamases producing *Enterobacteriaceae* have progressively developed because of widespread utilization of cephalosporin and represent a major challenge in disease control (Chong et al., 2018). The horizontal gene transfer through wild birds plays an important role in expanding ciprofloxacin and ceftriaxone resistance.

CTX-M gene was not amplified in any *S. enterica* isolate. This is due to the reason that the universal CTX-M primer used in current study was unable to recognize all positive strains even though showing positive results of resistance to beta lactam drugs. The primers for one group of CTX-M gene were unable to recognize the other groups of CTX-M gene, which results in no amplification of DNA. Pitout et al. (2004) also demonstrated a high level of specificity for the group specific primers.

Wild migratory birds have been recommended as reservoir of beta-lactamase producing pathogenic bacteria in various studies around the world (Bonnedahl et al., 2015; Atterby et al., 2016). Mohsin et al. (2017) indicated beta-lactamase producing *E. coli* in migratory birds along the Indus migration in Pakistan. He revealed that all ESBL-producing *E. coli* have CTX-M gene as the most prevailing genotype. Study carried out in Korea by Kang et al. (2015) declared that *Salmonella enterica* subsp. were the infectious agent mostly found in wild birds and brings about mortality in certain species of birds.

Conclusion

The increase in antimicrobial resistance has turned into a serious issue around the world. It has also been found that migratory birds are playing a main role in the transmission of multidrug resistant pathogens from one place to another. It presents potential risk around the world since these species can easily be utilized as expected and unexpected agents of serious foodborne disease. To avoid the occurrence of antibiotic resistance and MDR *Salmonella*, it is basic to keep up the continuous checking of antimicrobial resistance and pursue an objective remedy of antimicrobials dependent on local antimicrobial pattern.

REFERENCES

- [1] Acheson, D., Hohmann, E. L. (2001): Nontyphoidal salmonellosis. – *Clinical Infectious Diseases* 32(2): 263-269.
- [2] Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., Struhl, K. (1994): *Current Protocols in Molecular Biology*. – John Wiley & Sons, Inc., Chichester.
- [3] Atterby, C., Ramey, A. M., Hall, G. G., Järhult, J., Börjesson, S., Bonnedahl, J. (2016): Increased prevalence of antibiotic-resistant *E. coli* in gulls sampled in Southcentral Alaska is associated with urban environments. – *Infection Ecology & Epidemiology* 6(1): 32334.
- [4] Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Bürgmann, H., Sørum, H., Norström, M., Pons, M. N., Kreuzinger, N. (2015): Tackling antibiotic resistance: the environmental framework. – *Nature Reviews Microbiology* 13(5): 310.
- [5] Bonnedahl, J., Stedt, J., Waldenström, J., Svensson, L., Drobni, M., Olsen, B. (2015): Comparison of extended-spectrum β -lactamase (ESBL) CTX-M genotypes in Franklin Gulls from Canada and Chile. – *PLoS One* 10(10): e0141315.
- [6] Brenner, F. W., Villar, R. G., Angulo, F. J., Tauxe, R., Swaminathan, B. (2000): *Salmonella* nomenclature. – *Journal of Clinical Microbiology* 38(7): 2465-2467.
- [7] Chong, Y., Shimoda, S., Shimono, N. (2018): Current epidemiology, genetic evolution and clinical impact of extended-spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*. – *Infection, Genetics and Evolution* 61: 185-188
- [8] Elumalai, S., Muthu, G., Selvam, R. E. M., Ramesh, S. (2014): Detection of TEM-, SHV- and CTX-M-type β -lactamase production among clinical isolates of *Salmonella* species. – *Journal of Medical Microbiology* 63(7): 962-967.
- [9] Foti, M., Rinaldo, D., Guercio, A., Giacobello, C., Aleo, A., De Leo, F., Fisichella, V., Mammina, C. (2011): Pathogenic microorganisms carried by migratory birds passing through the territory of the island of Ustica, Sicily (Italy). – *Avian Pathology* 40(4): 405-409.
- [10] Gangoué-Piéboji, J., Bedenic, B., Koulla-Shiro, S., Randegger, C., Adiogo, D., Ngassam, P., Ndumbe, P., Hächler, H. (2005): Extended-spectrum- β -lactamase-producing Enterobacteriaceae in Yaounde, Cameroon. – *Journal of Clinical Microbiology* 43(7): 3273-3277.
- [11] Gourmelon, M., Caprais, M. P., Mieszkin, S., Marti, R., Wery, N., Jardé, E., Derrien, M., Jadas-Hécart, A., Communal, P. Y., Jaffrezic, A., Pourcher, A. M. (2010): Development of microbial and chemical MST tools to identify the origin of the faecal pollution in bathing and shellfish harvesting waters in France. – *Water Research* 44(16): 4812-4824.
- [12] Herikstad, H., Motarjemi, Y., Tauxe, R. V. (2002): *Salmonella* surveillance: a global survey of public health serotyping. – *Epidemiology & Infection* 129(1): 1-8
- [13] Kaftandzieva, A., Trajkovska-Dokic, E., Panovski, N. (2011): Prevalence and molecular characterization of extended spectrum beta-lactamases (ESBLs) producing *Escherichia coli* and *Klebsiella pneumoniae*. – *Prilozi* 32(2): 129-41
- [14] Kang, M. S., Jeong, O. M., Kim, H. R., Jang, I., Lee, H. S., Kwon, Y. K. (2015): Arthritis in an egret (*Egretta intermedia*) caused by *Salmonella* Typhimurium and its potential risk to poultry health. – *Journal of Wildlife Diseases* 51(2): 534-537.
- [15] Maeda, Y., Tohya, Y., Nakagami, Y., Yamashita, M., Sugimura, T. (2001): An occurrence of *Salmonella* infection in cranes at the Izumi Plains, Japan. – *Journal of Veterinary Medical Science* 63(8): 943-944.
- [16] Malorny, B., Hauser, E., Dieckmann, R. (2011): *New Approaches in Subspecies-Level Salmonella Classification*. – *Salmonella - From Genome to Function*. Caister Academic Press, Norfolk, pp.1-23

- [17] Mohsin, M., Raza, S., Schaufler, K., Roschanski, N., Sarwar, F., Semmler, T., Schierack, P., Guenther, S. (2017): High prevalence of CTX-M-15-Type ESBL-producing *E. coli* from migratory avian species in Pakistan. – *Frontiers in Microbiology* 8: 2476.
- [18] Pitout, J. D., Hossain, A., Hanson, N. D. (2004): Phenotypic and molecular detection of CTX-M- β -lactamases produced by *Escherichia coli* and *Klebsiella* spp. – *Journal of Clinical Microbiology* 42(12): 5715-5721.
- [19] Radhouani, H., Silva, N., Poeta, P., Torres, C., Correia, S., Igrejas, G. (2014): Potential impact of antimicrobial resistance in wildlife, environment and human health. – *Frontiers in Microbiology* 5: 23.
- [20] Refsum, T., Handeland, K., Baggesen, D. L., Holstad, G., Kapperud, G. (2002): *Salmonellae* in avian wildlife in Norway from 1969 to 2000. – *Appl. Environ. Microbiol.* 68(11): 5595-5599.
- [21] Samad, M. A. (2011): Public health threat caused by zoonotic diseases in Bangladesh. – *Bangladesh Journal of Veterinary Medicine* 9(2): 95-120.
- [22] Threlfall, E. J. (2002): Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food-and water-borne infections. – *FEMS Microbiology Reviews* 26(2): 141-148.
- [23] Thung, T. Y., Radu, S., Mahyudin, N. A., Rukayadi, Y., Zakaria, Z., Mazlan, N., Tan, B. H., Lee, E., Yeoh, S. L., Chin, Y. Z., Tan, C. W. (2018): Prevalence, virulence genes and antimicrobial resistance profiles of *Salmonella* serovars from retail beef in Selangor, Malaysia. – *Frontiers in Microbiology* 8.
- [24] Tindall, B. J., Grimont, P. A. D., Garrity, G. M., Euzeby, J. P. (2005): Nomenclature and taxonomy of the genus *Salmonella*. – *International Journal of Systematic and Evolutionary Microbiology* 55(1): 521-524.
- [25] White, D. G., Zhao, S., Simjee, S., Wagner, D. D., McDermott, P. F. (2002): Antimicrobial resistance of foodborne pathogens. – *Microbes and Infection* 4(4): 405-412.