Veterinary Integrative Sciences 2022; 20(2): 431-442 DOI; 10.12982/VIS.2022.032

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

Molecular detection of *Vibrio cholerae* and *Vibrio parahaemolyticus* from healthy broilers and backyard chickens for the first time in Bangladesh- A preliminary study

Soudiya Akter, Farah Zereen, Md. Saiful Islam, Md. Abdus Sobur, Md. Ismail Hossen, Mahbubul Pratik Siddique, Muhammad Tofazzal Hossain, and Md. Tanvir Rahman*

Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Abstract

Many of the *Vibrio* spp. are major public health concerns globally. *Vibrio cholerae* and *Vibrio parahaemolyticus* are the etiology of pandemic and epidemic diarrhea and foodborne illness, respectively. Poultry has the potential to harbor pathogenic *Vibrio* spp., which can have a detrimental impact on public health if they are transmitted to humans. We, therefore, screened 54 cloacal swab samples from healthy chickens (broiler=27, backyard= 27) to detect *V. cholerae* and *V. parahaemolyticus*. *Vibrio* spp. were isolated and identified by culturing, biochemical tests, PCR, and antibiogram profiles were determined by disk diffusion method. By PCR, 29.63% (16/54; 95% CI: 19.14-42.83%) samples were positive for *Vibrio* spp., where backyard chickens had a significantly higher ($p< 0.05$) occurrence (44.44%; 27.59-62.69%) than broilers (14.82%; 95% CI: 5.92-32.48%). *V. parahaemolyticus* was found in 22.22% (6/27; 95% CI: 10.61-40.76%) of backyard chicken samples, which was significantly dominant ($p< 0.05$) than in broilers (0/27, 0%, 95% CI: 0.00-12.46%). In addition, *V. cholerae* was positive in 7.41% (2/27; 95% CI: 1.32-23.37%) of broiler, and 14.82% (4/27; 95% CI: 5.92-32.48%) of backyard chicken samples. The *toxR* gene was found in all *V. cholerae* isolates, suggesting the presence of other virulence genes, whereas no isolates of *V. parahaemolyticus* contained the *tdh* gene. Isolated *Vibrio* spp. had high to moderate resistance to tetracycline, azithromycin, erythromycin, and streptomycin. The occurrence of antibiotic-resistant *V. cholerae* and *V. parahaemolyticus* in broiler and backyard chickens is of public health concern because of the possibility of food chain contamination.

Keywords: Antibiotic resistance, Backyard chickens, Broilers, Pathogenic *Vibrio* spp., Public health, *toxR*.

Corresponding author: Md. Tanvir Rahman, Professor, Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. Email. tanvirahman@bau.edu.bd. Tel. + 880 1913323307, Fax. +88-09161510.

Funding;This research was partially supported by Bangladesh Agricultural University Research System (BAURES) project (Project No. 2019/8/BAU).

Article history; *received manuscript: 17 February 2022, revised manuscript: 16 April 2022, accepted manuscript: 21 April 2022, published online: 26 April 2022*

Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

Vibrio species can be found in a wide range of settings including aquatic, estuarine, and marine habitats (Hossain et al., 2013a). *Vibrio* spp. are vastly associated with human infections transmitted by the natural waterborne and seafood microbiome (Baker-Austin et al., 2018). *Vibrio* spp. infections in humans are usually caused by direct or indirect contact with contaminated water or by consuming contaminated seafood without sufficient preparation (Altekruse et al., 2000). Several *Vibrio* species have zoonotic characteristics, causing diseases and death in fish, shellfish, and domestic marine life (Rahman et al., 2020).

To date, a total of 136 *Vibrio* species have been identified, of which *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio alginolyticus* are of major concern for being pathogenic to humans and animals (Grimes, 2020; LPSN, 2021). In humans, pathogenic *Vibrio* spp. can develop two types of infections such as cholera and non-cholera type infections. *V. cholerae* is linked to the development of cholera infection, while *V. parahaemolyticus* and V. vulnificus assist to develop non-cholera infections (Baker-Austin et al., 2017).

V. cholerae is the etiology of cholera, a food- and water-borne pandemic and epidemic diarrheal disease that effects a wide range of hosts and species (Ismail et al., 2021). It is regarded as the most subversive agent since it kills a large number of people every year all over the world, especially low- and middle-income countries are affected mostly (Lutz et al., 2013). Each year, due to cholera, an estimated 2.9 million cases and 95,000 deaths worldwide (CDC, 2021) and more than 100,000 cases with about 3000 deaths occur in Bangladesh (Parvin et al., 2021). Along with sporadic cases of diarrhea, *V. cholerae* can cause otitis, bacteremia, and urinary tract infection in humans (Hackbusch et al. 2020). *V. cholerae* is primarily transmitted through the fecal-oral route which involves taking contaminated water. Bacteria can also be transmitted through food, or from person to person through close contact (Baker-Austin et al., 2018).

V. parahaemolyticus, a common marine and human pathogen, is generally found in raw seafood. Humans may develop acute gastroenteritis, septicemia, and wounds after consuming infected shellfish raw without proper preparation (Siddique et al., 2021). Contaminated water is also responsible for *V. parahaemolyticus* infections, although there is no evidence of person-to-person or fecal-oral transmission (Baker-Austin et al., 2010). In addition, *V. parahaemolyticus* can develop symptoms in humans such as fever, diarrhea, chills, nausea, abdominal cramps, and headaches (Honda and Iida, 1993).

Poultry rearing and farming have become popular in Bangladesh as a source of protein and economical supports (Sabuj et al., 2019). As a result, people come into close contact with poultry and poultry products during raising poultry, purchasing live poultry and poultry eggs, and processing poultry meat (Akond et al., 2008). Poultry and poultry products have been reported as carriers and reservoirs for both pathogenic and non-pathogenic microbes, therefore they have the potential to be major pathways for pathogen transmission (Shanker et al., 1982). Furthermore, in some developing nations, poultry feces and excreta are commonly utilized for fish feed and to replace chemical fertilizers to

increase land fertility. The use of poultry feces and excreta in fish feed, as well as the replacement of chemical fertilizers, puts animal and human health at risk of disease exposure.

Antimicrobial resistance (AMR) is a serious public health issue of the twenty-first century that affects all aspects of one health (Islam et al., 2021a, Roy et al., 2021). AMR has also become a major threat to economic progress (Ievy et al., 2020). If nothing is done, millions of people would die, the global economy will suffer catastrophic losses, and livestock output will plummet as a result of AMR (Islam et al., 2021b, Sobur et al., 2021). Moreover, the impact of AMR on low- and middle-income countries like Bangladesh will be devastating (Hossain et al., 2021). Antibiotic overuse and misuse in livestock and humans without proper prescription results in selective pressure and, eventually, antibiotic resistance (Tawyabur et al., 2020, Urmi et al., 2021). Antibiotic-resistant pathogens have now the adverse effects on humans, animals, and the environment by circulating extensively within the environment (Hossain et al., 2020). Antibiotic-resistant bacteria, such as *Vibrio* ssp., may be carried out and spread by poultry. It is possible that poultry has the ability to spread antibiotic resistant and pathogenic *Vibrio* spp. to humans, animals, and the environment and thereby contaminates all the components of the one-health.

Several studies have previously detected *Vibrio* spp. in poultry in Bangladesh (Akond et al., 2008; Uddin et al., 2012), but to the best of our knowledge, no data on the molecular detection of *V. cholerae* and *V. parahaemolyticus* in poultry has been published in Bangladesh. We, therefore, screened cloacal swabs of healthy broiler and backyard chickens in Bangladesh to detect *V. cholerae* and *V. parahaemolyticus* as well as their antibiogram profiles.

MATERIALS AND METHODS

Ethical statement

The Institutional Ethical Committee approved the methodologies and related protocols used in this study [(AWEEC/BAU/2022(19)]. Samples were collected by expert veterinarians and microbiologists.

Study area

This study was carried out in Mymensingh Sadar (24.7851° N, 90.3560° E) and Gauripur Upazila (24.7546° N, 90.5678° E) of Mymensingh district in Bangladesh (Figure 1).

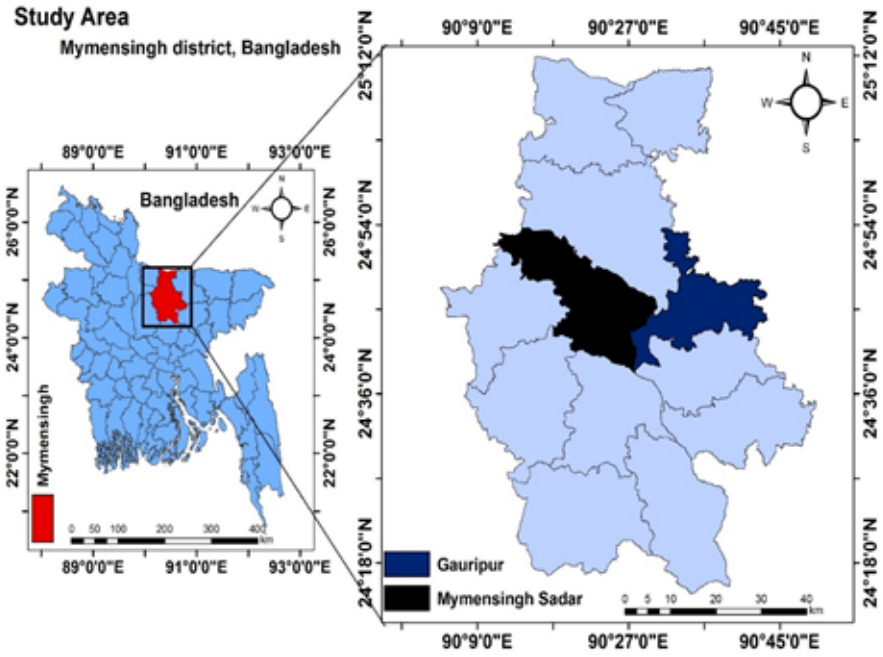

Figure 1 Map of sample collection sites, created with ArcMap software (version 10.7; ESRI, Redlands, CA, USA).

Sampling and sample processing

A total of 54 cloacal swab samples (27 from broilers and 27 from backyard chickens) were collected aseptically. Each sample was collected using sterile cotton buds and was dipped directly into a sterile screw-caped test tubes containing alkaline peptone water. After giving a particular tag number, samples were directly transported to the laboratory maintaining room temperature and incubated aerobically overnight at 37°C.

Isolation of *Vibrio* spp.

Vibrio spp. were isolated by streaking one loopful broth culture on Thiosulfate-citrate-bile salts sucrose (TCBS) agar plates (HiMedia, India). Yellowish or green colonies on TCBS agar plates were primarily selected as *Vibrio* spp. after overnight culture at 37°C. Before selecting, colonies were purified by applying multiple cultures on TCBS agar plates. Then, pure colonies of suspected isolates were subjected to Gram's staining technique and biochemical tests (Sugar fermentation test, Methyl red test, Voges-Proskauer test, Indole test, catalase test, oxidase test, and motility test) for initial screening (Wang and Leung, 2000).

PCR detection and differentiation of Vibrio cholerae and Vibrio parahaemolyticus

Vibrio spp. isolated by conventional methods were confirmed by polymerase chain reaction (PCR) with groEL primers specific for *Vibrio* genus. These *Vibrio* spp. were finally confirmed as *V. cholerae* and *V. parahaemolyticus* at the species level by PCR with new sets of primers that are specific for *V. cholerae* and *V. parahaemolyticus* (Table 1). Potential pathogenic *Vibrio* spp. were detected by PCR targeting *toxR* and *tdh* genes (Table 1).

Table 1 Primers with their annealing temperature and amplicon size

Isolates	Targeted gene	Primer sequence (5'-3')	Amplicon size (bp)	Annealing temperature (◦C)	References
Vibrio spp.	*groEL*	F: TCCARAACATGGGCGCACAA R: ACGTTTTGYTCTTCGTTGTCRC	1117	69	Hossain et al., 2014
V. cholerae	*groEL*	F: GATCTTGACTGGCGGTGTTGTG R: GTCACCCACCAGAGAAGAGAGT	418	69	Hossain et al., 2013b
V. parahaemolyticus	*groEL*	F: AGGTCAGGCTAAGCGCGTAAGC R: GTCACCGTATTCACCCGTCGCT	510	69	Hossain et al., 2012
Potential pathogenic *V. cholerae*	*toxR*	F: GAAGCTGCTCATGACATC R: AAGATCAGGGTGGTTATTC	275	55	Neogi et al., 2010
Pathogenic *V. parahaemolyticus*	*tdh*	F: TATCCATGTTGGCTGCATTCA AAAC R: TCTTCACCAACAAAGTTAGCTACA	382	69	Hossain et al., 2013c

Bacterial genomic DNA for PCR was extracted by boiling method as previously described by Hossain et al., (2012). Each PCR reactions were carried out in a final volume of 25 µL. The content of each reaction mixture included 12.5 µL of 2X master mixture (Promega, Madison, WI, USA), 1 µL of each desired reverse and forward primer, 5 µL of DNA template (50 ng/ µL), and 5.5 µL of deionized water. After completion, the amplified products were assessed using 1.5% agarose gel electrophoresis, stained by 0.5 ng/mL ethidium bromide, and visualized under ultraviolet transillumination (Biometra, Germany). A 100 bp DNA ladder (Promega, Madison, WI, USA) was used to compare the amplicon size.

Antibiotic susceptibility testing

All *Vibrio* isolates were subjected to antibiotic susceptibility test (AST) using the disk diffusion method (Bauer et al., 1966). Six commonly used antibiotics, namely gentamicin (10 µg), tetracycline (30 µg), streptomycin (10 µg), erythromycin (15 µg), azithromycin (15 µg), and ciprofloxacin (5 µg) were used. For AST, bacteria were grown on TCBS agar plates for 18-24 hours and 2-3 colonies were suspended with 0.85% sterile normal saline solution to adjust to concentration equal to 0.5 McFarland standard unit. Using sterile cotton buds, the inoculum was then spread on Mueller-Hinton agar plates and dispensed with preselected antibiotics, followed by incubating for 24 hours at 37°C. The results were illustrated by following the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2018).

Statistical analysis

Data obtained from the current study were initially taken into Microsoft Excel-2010 (Los Angeles, CA, USA), and subsequently transferred into the GraphPad Prism 8.4.2 (GraphPad Software, Inc.) and the Statistical Package for the Social Sciences (SPSS) software (IBM SPSS- version 25.0, USA) to analyze them. By GraphPad Prism, the binomial 95% confidence intervals (CI) were calculated following the Wilson/Brown Hybrid method as previously described (Brown et al., 2001). In addition, a Fisher's exact test was performed to check the feasible variations in the occurrence rate of any of two the variables. Statistically significant variations were deemed at less than 0.05 of p- value.

RESULTS

Occurrence of *Vibrio* spp.

Out of 54 samples, 22 (40.74%; 95% CI: 28.68-54.03%) were found positive for *Vibrio* spp. based on their cultural characteristics and biochemical properties. Among them, seven (25.93%, 95% CI: 13.17-44.68%) out of 27 broiler samples and 15 (55.56%, 95% CI: 37.31-72.41%) out of 27 backyard chicken samples were found to be positive for *Vibrio* spp. By PCR targeting groEL gene, overall, 16 (29.63%; 95% CI: 19.14-42.83%) samples were positive for *Vibrio* spp., of which, the occurrence rate of *Vibrio* spp. was significantly dominant (p = 0.035) in backyard chickens (44.44%; 27.59-62.69%) compared to broiler chickens (14.82%; 95% CI: 5.92-32.48%) (Table 2).

Table 2 Occurrence of *Vibrio* spp., *V. cholerae*, *V. parahaemolyticus*

Poultry	*Vibrio* spp. (P, %, 95% CI)	*V. cholerae* (P, %, 95% CI)	*V. parahaemolyticus* (P, %, 95% CI)	Positive *V. cholerae* for toxR gene (P, %, 95% CI)	*Positive V. parahaemolyticus for tdh gene* (P, %, 95% CI)
Broiler (n=27)	4, 14.82, 5.92-32.48%	2, 7.41, 1.32-23.37%	0, 0, 0.00-12.46%	2, 7.41, 1.32-23.37%	NA
Backyard (n=27)	12, 44.44, 27.59-62.69%	4, 14.82, 5.92-32.48%	6, 22.22, 10.61-40.76%	4, 14.82, 5.92-32.48%	0, 0, 0.00-12.46%
p-value	0.035	0.669	0.023	0.669	NA

Here, a p-value less than 0.05 was deemed as statistically significant; P = Positive, CI = Confidence interval; V. = *Vibrio*, NA= Not applied

Occurrence of *V. cholerae* and *V. parahaemolyticus*

In broiler samples, 7.41% (2/27; 95% CI: 1.32-23.37%) samples exhibited positive for *V. cholerae*, whereas 14.82% (4/27; 95% CI: 5.92-32.48%) backyard chickens' samples were positive for *V. cholerae*. However, there was no significant variation in the occurrence rate of *V. cholerae* in samples of broiler and backyard chickens (p > 0.05) (Table 2).

For *V. parahaemolyticus*, significantly (p = 0.023) higher occurrence rate was observed in the samples of backyard chickens (6/27; 22.22%, 95% CI: 10.61-40.76%) compared to samples of broiler chickens (0/27, 0%, 95% CI: 0.00-12.46%) (Table 2).

Occurrence of *toxR* and *tdh* genes

No *V. parahaemolyticus* isolate was found to contain the *tdh* gene. Whereas six (12.96%; 95% CI: 6.42-24.42%) samples harbored potentially pathogenic *V. cholerae* as revealed by the detection of *toxR* gene, of them, two (7.41%, 95% CI: 1.32-23.37%) and four (14.82%, 95% CI: 5.92-32.48%) were found to be positive in broiler and backyard chickens respectively. No statistically significant was found in the occurrence of *toxR* gene in *V. cholerae* isolates (p > 0.05) (Table 2).

Antibiotic susceptibility testing of the isolated *Vibrio* spp.

Antibiotic sensitivity test showed different degrees of resistance of isolated *Vibrio* spp. to azithromycin (backyard, 41.67% vs broiler, 50%), erythromycin (66.67% vs 50%), streptomycin (16.67% vs 50%), and tetracycline (50% vs 50%). In addition, ciprofloxacin and gentamicin were highly sensitive to isolated *Vibrio* spp. The overall antibiogram profiles of isolated *Vibrio* spp. are showed in Figure 2.

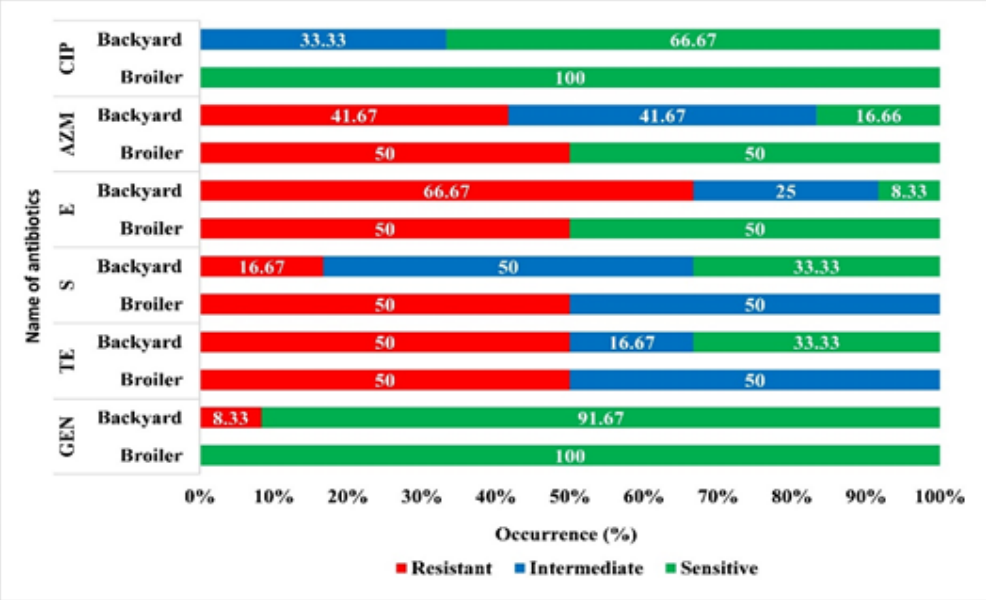

Figure 2 Antibiogram profiles of *Vibrio* spp. isolated from broiler and backyard chickens, GEN= Gentamicin, TE= Tetracycline, S= Streptomycin, E= Erythromycin, AZM= Azithromycin, CIP= Ciprofloxacin.

DISCUSSION

Vibrio spp. are the etiological agents of life-threatening diarrheal and foodborne diseases. They are involved in epidemic and pandemic diseases, but their distribution patterns are unclear yet. Diseases caused by *Vibrio* spp. are frequently associated with unhygienic conditions that are common in Bangladesh. Poultry has the potential to acquire, carry, and spread pathogenic *Vibrio* spp. We, therefore, designed the present study to detect *V. cholerae* and *V. parahaemolyticus* from cloacal swabs of healthy broilers and backyard chickens in Bangladesh.

In this study, *Vibrio* spp. were detected in 29.63% of the samples analyzed, of which backyard chickens harbor a significantly higher occurrence than that of broilers. Backyard chickens are usually reared in villages with the free-ranging system (Islam et al., 2020), which easily allows them to come into contact with *Vibrio* spp. contaminated sources. During their vast mobility, they can easily pick up *Vibrio* spp. from contaminated aquatic environmental and other sources. Significant exponents of environmental findings of *Vibrio* spp. indicate that the aquatic environmental sources have the potential to be natural reservoirs of *Vibrio* spp., however, the theory is complex and unknown yet (Ismail et al., 2021). Broiler chickens can acquire *Vibrio* spp. from

contaminated supply water. Previously, Akond et al. (2008) and Uddin et al. (2012) isolated and identified *Vibrio* spp. from poultry samples in Bangladesh based on culture and biochemical properties, however, they didn't perform any molecular assays to confirm the isolates. Molecular approaches are the most effective in microbiological practices, such as the one used in our study, for detecting any bacteria targeting their specific gene (Jo et al., 2013).

Here, 7.41% broiler samples and 14.82% backyard chicken samples were found positive for *V. cholerae*; on the contrary, 22.22% of backyard chicken samples harbored *V. parahaemolyticus* which was significantly higher than broiler samples (0%). Backyard chickens are more likely to come into contact with water and foods contaminated with *V. cholerae* and *V. parahaemolyticus*. Previously, several studies detected *V. cholerae* and *V. parahaemolyticus* in household rectal swabs, drinking water, and household food items (Albert et al., 1997; Rafique et al., 2016; Li et al., 2020; Maje et al., 2020). Because backyard chickens roam freely in the household, they can readily pick up *V. cholerae* and *V. parahaemolyticus* from contaminated water and other household items. Broilers can come in contact with these organisms in the unhygienic conditions of the farms and water including feeds. In addition, aquatic water birds have the potential to transmit *Vibrio* spp. to other birds. Ismail et al. (2021) previously demonstrated the link between *V. cholerae* transmission from aquatic birds (ducks) to the broiler farms. Furthermore, free-ranging backyard chickens have the ability to come into contact with free-ranging waterfowls and can acquire *V. cholerae* and *V. parahaemolyticus* from them through fecal contamination of fecal sources in the environment.

Pathogenic *Vibrio* spp. are linked to a variety of human diseases, including those that are life-threatening (Hossain et al., 2013c). In this study, the regulator gene *toxR* carrying *V. cholera* was detected in 12.96% of poultry isolates, however, no pathogenic *V. parahaemolyticus* with the *tdh* gene was found. The *toxR* is a membrane-bound regulatory protein that plays a significant role in the development of virulence and persistence in *V. cholerae* (Zhang et al., 2018). It assists *V. cholerae* in surviving in unfavorable environments (Valeru et al., 2012). It also regulates cholera toxin, toxin-coregulated pili (TCP), and a number of other DNA-binding and transmembrane proteins that are important in cholera pathogenesis (Parsot and Mekalanos, 1990). Therefore, the detection of *toxR* gene carrying *V. cholerae* in broiler and backyard chickens raises serious human health concerns due to the possibility of transmission to humans via the food chain and close contact. The presence of them in cloacal swabs further suggests that poultry has the potential to contaminate the environment and infect humans.

Antimicrobial resistance is a worldwide problem (Talukder et al., 2021). Antibiotic resistance is increasing that exposes *Vibrio* spp. as a potentially deadly infection (Islam et al., 2021c). It is quite concerning that 50% of *Vibrio* spp., isolated from both broiler and backyard chickens were resistant to tetracycline, indicating a harmful influence on healthcare systems. Tetracycline is a first-choice antibiotic in the treatment of cholera disease (Chopra and Roberts, 2001). Therefore, the presence of tetracycline resistant *Vibrio* spp. limits the use of this antibiotic to treat cholera. With resistance against tetracycline, isolated *Vibrio* spp. were found to be resistant against azithromycin,

erythromycin, and streptomycin. The presence of these antibiotic resistant *Vibrio* spp. has the potential to transmit to humans via the food chain which shows a serious public health risk.

One of the major limitations of this study is a lack of adequate samples. However, this was a preliminary study to check only the presence of two important *Vibrio* species *V. cholerae* and *V. parahaemolyticus* from cloacal swabs of broilers and backyard chickens. In addition, during the present study, the COVID-19 outbreak took place in Bangladesh and everything was on lockdown for a long time, resulting in no field trip to have access to extra samples.

CONCLUSION

To the best of our knowledge, this is the first study in Bangladesh to describe the molecular detection of antibiotic resistant *V. cholera* with their *toxR* gene, and *V. parahaemolyticus* from broiler and backyard chickens. Antibiotic resistant *Vibrio* spp. found in broiler and backyard chickens have the potential to transfer to humans via food chain supply and affect human health. The implementation of effective surveillance combined with a strong one-health approach is pivotal to minimize the harmful effects of *V. cholerae* and *V. parahaemolyticus*. Furthermore, adopting proper management practices, compatible hygiene norms, biosecurity in the sphere of poultry production, and implementation a training programs to farm owners about infectious diseases could aid in breaking the cycle of human-poultry transmission.

ACKNOWLEDGEMENTS

We thank the poultry owners for giving access us for sampling. We are sincerely grateful to the Ministry of Science and Technology, Government of the People's Republic of Bangladesh, for providing National Science and Technology (NST) fellowship to SA.

AUTHOR CONTRIBUTIONS

Conceptualization- MTR; Methodology- MTH and MTR; Software- MSI; Validation- MTR; Formal analysis- MSI and MAS; Investigation- SA, FZ, and MIH; Data curation- SA and MSI; Writing—original draft preparation- MSI, SA, and MTR; Writing—review and editing- MSI, MAS, MPS, MTH, and MTR; Visualization, MSI and SA; Supervision, MTH and MTR; Project administration, MTR; Critical revisions and writing, MTR. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interest.

REFERENCES

Akond, M.A., Alam, S., Hasan, S.M.R., Uddin, S.N., Shirin, M., 2008. Antibiotic resistance of *Vibrio cholerae* from poultry sources of Dhaka, Bangladesh. Advan. Biol. Res. 2(3-4), 60-67.

Albert, M.J., Neira, M., Motarjemi, Y., 1997. The role of food in the epidemiology of cholera. World Health Stat. Q. 50(1-2), 111-118.

Altekruse, S.F., Bishop, R.D., Baldy, L.M., Thompson, S.G., Wilson, S.A., Ray, B.J., Griffin, P.M., 2000. *Vibrio* gastroenteritis in the US Gulf of Mexico region: the role of raw oysters. Epidemiol. Infect. 124(3), 489-495.

Baker-Austin, C., Oliver, J.D., Alam, M., Ali, A., Waldor, M.K., Qadri, F., Martinez-Urtaza, J., 2018. *Vibrio* spp. infections. Nat. Rev. Dis. Primers 4(1), 1-19.

Baker-Austin, C., Stockley, L., Rangdale, R., Martinez-Urtaza, J., 2010. Environmental occurrence and clinical impact of *Vibrio vulnificus* and *Vibrio parahaemolyticus*: a European perspective. Environ. Microbiol. Rep. 2(1), 7-18.

Baker-Austin, C., Trinanes, J., Gonzalez-Escalona, N., Martinez-Urtaza, J., 2017. Non-cholera *Vibrios*: the microbial barometer of climate change. Trends Microbiol. 25(1), 76-84.

Bauer, A.T., 1966. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. 45(4), 149-158.

Brown, L.D., Cai, T.T., DasGupta, A., 2001. Interval estimation for a binomial proportion. Stat. Sci. 16(2), 101-133.

CDC, 2021. Cholera - *Vibrio cholerae* infection. Available online: https://www.cdc.gov/cholera/general/index.html (Accessed on January 1, 2022).

Chopra, I., Roberts, M., 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol. Mol. Biol. Rev. 65(2), 232-260.

Clinical and Laboratory Standards Institute (CLSI), 2018. Performance standards for antimicrobial susceptibility testing: twenty-eight informational supplement M100-S28. CLSI, Wayne, PA, USA.

Grimes, D.J., 2020. The *Vibrios*: scavengers, symbionts, and pathogens from the sea. Microb. Ecol. 80(3), 501-506.

Hackbusch, S., Wichels, A., Gimenez, L., Döpke, H., Gerdts, G., 2020. Potentially human pathogenic *Vibrio* spp. in a coastal transect: occurrence and multiple virulence factors. Sci. Total Environ., 707, 136113.

Honda, T., Iida, T., 1993. The pathogenicity of *Vibrio parahaemolyticus* and the role of the thermostable direct haemolysin and related haemolysins. Rev. Med. Microbiol. 4(2), 106-113.

Hossain, M., Attia, Y., Ballah, F.M., Islam, M., Sobur, M., Ievy, S., Rahman, A., Nishiyama, A., Hassan, J., Rahman, M., 2021. Zoonotic significance and antimicrobial resistance in Salmonella in poultry in Bangladesh for the period of 2011–2021. Zoonotic Dis. 1(1), 3-24.

Hossain, M.J., Islam, M.S., Sobur, M.A., Zaman, S.B., Nahar, A., Rahman, M., Rahman, M.T., 2020. Exploring poultry farm environment for antibiotic resistant *Escherichia coli*, *Salmonella* spp., and *Staphylococcus* spp. having public health significance. J. Bangladesh Agril. Univ. 18(3), 615-622.

Hossain, M.T., Kim, E.Y., Kim, Y.R., Kim, D.G., Kong, I.S., 2012. Application of groEL gene for the species-specific detection of *Vibrio parahaemolyticus* by PCR. Lett. Appl. Microbiol., 54(1), 67-72.

Hossain, M.T., Kim, Y.R., Kim, E.Y., Lee, J.M., Kong, I.S., 2013a. Detection of *Vibrio* cholerae and *Vibrio vulnificus* by duplex PCR specific to the groEL gene. Fish Sci. 79(2), 335-340.

Hossain, M.T., Kim, E.Y., Kim, Y.R., Kim, D.G., Kong, I.S., 2013b. Development of a groEL gene–based species-specific multiplex polymerase chain reaction assay for simultaneous detection of *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio* vulnificus. J. Appl. Microbiol. 114(2), 448-456.

Hossain, M.T., Kim, Y.O., Kong, I.S., 2013c. Multiplex PCR for the detection and differentiation of *Vibrio parahaemolyticus* strains using the groEL, *tdh* and trh genes. Mol. Cell. Probes 27(5-6), 171-175.

Hossain, M.T., Kim, Y.R., Kong, I.S., 2014. PCR–restriction fragment length polymorphism analysis using groEL gene to differentiate pathogenic *Vibrio* species. Diagn. Microbiol. Infect. Dis. 78(1), 9-11.

Ievy, S., Islam, M., Sobur, M., Talukder, M., Rahman, M., Khan, M.F.R., Rahman, M.T., 2020. Molecular detection of avian pathogenic Escherichia coli (APEC) for the first time in layer farms in Bangladesh and their antibiotic resistance patterns. Microorganisms. 8(7), 1021.

Islam, M., Sobur, M., Rahman, S., Ballah, F.M., Ievy, S., Siddique, M.P., Rahman, M., Kafi, M., Rahman, M., 2021a. Detection of blaTEM, blaCTX-M, blaCMY, and blaSHV genes among extended-spectrum beta-lactamase-producing Escherichia coli isolated from Islam, M., Nayeem, M., Hasan, M., Sobur, M., Ievy, S., Rahman, S., Kafi, M., Ashour, H.M., Rahman, M., 2021b. Virulence determinants and multidrug resistance of *Escherichia coli* isolated from migratory birds. Antibiotics. 10(2), 190.

Islam, M.S., Paul, A., Talukder, M., Roy, K., Sobur, M.A., Ievy, S., Nayeem, M.M.H., Rahman, S., Nazir, K.N.H., Hossain, M.T., Rahman, M.T., 2021c. Migratory birds travelling to Bangladesh are potential carriers of multi-drug resistant *Enterococcus* spp., *Salmonella* spp., and *Vibrio* spp. Saudi J. Biol. Sci. 28(10), 5963-5970.

Islam, M.S., Sabuj, A.A.M., Haque, Z.F., Pondit, A., Hossain, M.G., Saha, S., 2020. Seroprevalence and risk factors of avian reovirus in backyard chickens in different areas of Mymensingh district in Bangladesh. J. Adv. Vet. Anim. Res. 7(3), 546-553.

Ismail, E.M., Kadry, M., Elshafiee, E.A., Ragab, E., Morsy, E.A., Rizk, O., Zaki, M.M., 2021. Ecoepidemiology and potential transmission of *Vibrio cholerae* among different environmental niches: an upcoming threat in Egypt. Pathogens. 10(2), 190.

Jo, G.A., Kwon, S.B., Kim, N.K., Hossain, M.T., Kim, Y.R., Kim, E.Y., Kong, I.S., 2013. Species-specific duplex PCR for detecting the important fish pathogens *Vibrio* anguillarum and Edwardsiella tarda. Fish. Aquat. Sci. 16(4), 273-277.

Li, Y., Xie, T., Pang, R., Wu, Q., Zhang, J., Lei, T., Xue, L., Wu, H., Wang, J., Ding, Y., Chen, M., 2020. Food-borne *Vibrio parahaemolyticus* in China: prevalence, antibiotic susceptibility, and genetic characterization. Front. Microbiol. 11, 1670.

LPSN, 2021. List of Prokaryotic names with Standing in Nomenclature. Available online: https://lpsn.dsmz.de/genus/*Vibrio* (Accessed on January 1, 2022).

Lutz, C., Erken, M., Noorian, P., Sun, S., McDougald, D., 2013. Environmental reservoirs and mechanisms of persistence of *Vibrio cholerae*. Front. Microbiol. 4, 375.

Maje, M.D., Kaptchouang Tchatchouang, C.D., Manganyi, M.C., Fri, J., Ateba, C.N., 2020. Characterization of *Vibrio* species from surface and drinking water sources and assessment of biocontrol potentials of their bacteriophages. Int. J. Microbiol. 2020, 8863370.

Neogi, S.B., Chowdhury, N., Asakura, M., Hinenoya, A., Haldar, S., Saidi, S.M., Kogure, K., Lara, R.J., Yamasaki, S., 2010. A highly sensitive and specific multiplex PCR assay for simultaneous detection of *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*. Lett. Appl. Microbiol., 51(3), 293-300.

Parsot, C., Mekalanos, J.J., 1990. Expression of *ToxR*, the transcriptional activator of the virulence factors in *Vibrio cholerae*, is modulated by the heat shock response. Proc. Natl. Acad. Sci. 87(24), 9898-9902.

Parvin, I., Shahid, A.S.M.S.B., Das, S., Shahrin, L., Ackhter, M.M., Alam, T., Khan, S.H., Chisti, M.J., Clemens, J.D., Ahmed, T., Sack, D.A., 2021. *Vibrio cholerae* O139 persists in Dhaka, Bangladesh since 1993. PLOS Negl. Trop. Dis. 15(9), e0009721.

Rafique, R., Rashid, M.U., Monira, S., Rahman, Z., Mahmud, M.D., Mustafiz, M., Saif-Ur-Rahman, K.M., Johura, F.T., Islam, S., Parvin, T., Bhuyian, M., 2016. Transmission of infectious *Vibrio cholerae* through drinking water among the household contacts of cholera patients (CHoBI7 trial). Front. Microbiol. 7, 1635.

Rahman, M., Sobur, M., Islam, M., Ievy, S., Hossain, M., El Zowalaty, M.E., Rahman, A.M.M., Ashour, H.M., 2020. Zoonotic diseases: etiology, impact, and control. Microorganisms. 8(9), 1405.

Roy, K., Islam, M.S., Paul, A., Ievy, S., Talukder, M., Sobur, M.A., Ballah, F.M., Khan, M.S.R., Rahman, M.T., 2021. Molecular detection and antibiotyping of multi-drug resistant Enterococcus faecium from healthy broiler chickens in Bangladesh. Vet. Med. Sci. 8(1), 200-210.

Sabuj, A.A.M., Mahmud, T., Barua, N., Rahman, M.A., Islam, M.S., Bary, M.A., 2019. Passive surveillance of clinical poultry diseases in an Upazila Government Veterinary Hospital of Bangladesh. Afr. J. Microbiol. Res. 13(29), 632-639.

Shanker, S.I.V.A.R.A.J., Rosenfield, J.A., Davey, G.R., Sorrell, T.C., 1982. Campylobacter jejuni: incidence in processed broilers and biotype distribution in human and broiler isolates. Appl. Environ. Microbiol. 43(5), 1219-1220.

Siddique, A.B., Moniruzzaman, M., Ali, S., Dewan, M., Islam, M.R., Islam, M., Amin, M.B., Mondal, D., Parvez, A.K., Mahmud, Z.H., 2021. Characterization of pathogenic *Vibrio parahaemolyticus* isolated from fish aquaculture of the Southwest Coastal Area of Bangladesh. Front. Microbiol. 12, 266.

Sobur, M., Islam, M., Haque, Z.F., Orubu, E.S.F., Toniolo, A., Choudhury, M. and Rahman, M., 2021. Higher seasonal temperature enhances the occurrence of methicillin resistance of *Staphylococcus aureus* in house flies (Musca domestica) under hospital and environmental settings. Folia. Microbiol. 67(1), 109-119.

Talukder, M., Islam, M.S., Ievy, S., Sobur, M.A., Ballah, F.M., Najibullah, M., Rahman, M.B., Rahman, M.T., Khan, M.F.R., 2021. Detection of multidrug resistant Salmonella spp. from healthy and diseased broilers having potential public health significance. J. Adv. Biotechnol. Exp. Ther. 4(2), 248-255.

Tawyabur, M., Islam, M., Sobur, M., Hossain, M., Mahmud, M., Paul, S., Hossain, M.T., Ashour, H.M., Rahman, M., 2020. Isolation and characterization of multidrug-resistant Escherichia coli and Salmonella spp. from healthy and diseased Turkeys. Antibiotics, 9(11), 770.

Uddin, M.A., Ullah, M.W., Noor, R., 2012. Prevalence of *Vibrio cholerae* in human-, poultry-, animal excreta and compost samples. Stamford J. Microbiol. 2(1), 38-41.

Urmi, M.R., Ansari, W.K., Islam, M.S., Sobur, M.A., Rahman, M., Rahman, M.T., 2021. Antibiotic resistance patterns of Staphylococcus spp. isolated from fast foods sold in different restaurants of Mymensingh, Bangladesh. J. Adv. Vet. Anim. Res. 8(2), 274-281.

Valeru, S.P., Wai, S.N., Saeed, A., Sandström, G., Abd, H., 2012. *ToxR* of *Vibrio cholerae* affects biofilm, rugosity and survival with Acanthamoeba castellanii. BMC Res. Notes 5(1), 1-8.

Wang, X.H., Leung, K.Y., 2000. Biochemical characterization of different types of adherence of *Vibrio* species to fish epithelial cells. Microbiology. 146(4), 989-998.

Zhang, Y., Hu, L., Osei-Adjei, G., Zhang, Y., Yang, W., Yin, Z., Lu, R., Sheng, X., Yang, R., Huang, X., Zhou, D., 2018. Autoregulation of *ToxR* and its regulatory actions on major virulence gene loci in *Vibrio parahaemolyticus*. Front. Cell. Infect. Microbiol. 8, 291.

How to cite this article;

Soudiya Akter, Farah Zereen, Md. Saiful Islam, Md. Abdus Sobur, Md. Ismail Hossen, Mahbubul Pratik Siddique, Muhammad Tofazzal Hossain, and Md. Tanvir Rahman. Molecular detection of *Vibrio cholerae* and *Vibrio parahaemolyticus* from healthy broilers and backyard chickens for the first time in Bangladesh- A preliminary study. Veterinary Integrative Sciences. 2022; 20(2): 431- 442.