

Article

Prevalence and antimicrobial susceptibility patterns of *Vibrio cholerae* in dairy excreta

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Abstract: *Vibrio cholerae* is a major etiological agent of human diarrhoea and has become epidemic across the world in the recent past. A cross-sectional study was conducted to assess the prevalence of *V. cholerae* from dairy excreta along with antimicrobial resistant status of the isolates. A total 50 samples were collected from 50 different household manure pit located at Bangladesh Agriculture University (BAU) surrounding area, Mymensingh. Alkaline peptone water was used for enrichment of the samples followed by inoculation onto thiosulfate citrate bile salt sucrose (TCBS) agar media for the isolation of *Vibrio* spp., which were further confirmed via *Vibrio* genus specific molecular assay. Biochemical tests were performed to identify *V. cholerae* from the isolates of *Vibrio* spp. Out of 50 samples 17 (34%) were confirmed as *Vibrio* spp. as they produced characteristic yellow colonies on TCBS agar and had found to possess recombinase A gene that confirmed the identity of *Vibrio* spp. From this 17 *Vibrio* isolates, 6 (12% in total from 50 samples) were identified as *V. cholerae* based on different biochemical tests. All the isolates fermented glucose, maltose, sucrose and mannitol with the production of only acid. The isolates were positive in oxidase, gelatinase, methyl-red (MR) and indole test, but negative in case of voges-proskauer (VP) test. In antimicrobial susceptibility testing, *V. cholerae* isolates showed 100% sensitivity to gentamycin, chloramphenicol and tetracycline with moderate sensitivity to ciprofloxacin and co-trimoxazole. A high level of resistance was observed to ampicillin (100%) followed by moderate resistance to erythromycin and imipenem. In the present study about 33.33% (n = 2) of 6 isolated *V. cholerae* were found to be multidrug resistant (MDR) as they demonstrated resistant against 3 antimicrobial agents. The findings of this study substantiate the presence of MDR *V. cholerae* in the dairy excreta, which indicates the role of domestic animals to serve as a reservoir that might pose a health risk to human. Hygienic management of animal waste is needed to reduce the burden of human illness.

Keywords: *Vibrio cholerae*; diarrhoea; dairy excreta; multidrug resistant; antimicrobial susceptibility

1. Introduction

Globally diarrhoeal diseases including cholera, are considered as a leading cause of death of children under 5 years of age as well as the morbidity in adults (Bryce *et al.*, 2005; Shrestha *et al.*, 2015) of which 10% contribute from the low and middle-income countries, including Bangladesh (WHO, 2021). *Vibrio cholerae* is the causative agent of cholera. Being a genetically versatile bacterial species, *Vibrio cholerae* has more than two hundred serogroups on the basis of the somatic O antigens (Vu *et al.*, 2018) among which O1 and O139 are two

major virulent strains that have the potential to cause an epidemic (Mehrabadi *et al.*, 2012). Classical and El Tor, two biotypes of *V. cholerae* O1 are documented as the causative agents of the sixth and the seventh pandemics respectively (Ramamurthy and Nair, 2010). *V. cholerae* O1 is still the prime cause of many outbreak in Asian countries from where it is frequently isolated (Shrestha *et al.*, 2015). Moreover, multiple antibiotic resistant (MAR) *V. cholerae* O1 El Tor biotype triggered the seventh cholera pandemic that expanded rapidly from Indonesia to Bangladesh, India, Iraq, and Iran (Safa *et al.*, 2010).

Approximately 1.3 billion people of the world are at risk of cholera where Bangladesh occupies a larger portion (Islam *et al.*, 2019). A recent update reveals that around 66 million people of Bangladesh are living with the risk of cholera where the estimated incidence is about 1.64 per thousand people (Ali *et al.*, 2015). In Bangladesh, about 109,000 cases of cholera are estimated yearly, where the case fatality rate is 3% (Ali *et al.*, 2015). Insufficient sanitation, poor access to pure drinking water and poor socio-economic status are considered as the major predisposing factors of cholera outbreaks. Infection of cholera happens mainly through fecal-oral route especially ingestion of faecally contaminated foods or water (Shrestha *et al.*, 2015). In the small intestine, *V. cholerae* secretes enterotoxin known as cholera toxin, which results in excessive release of water and electrolytes into the gut lumen causing massive fluid loss, diarrhea and vomiting (Maheshwari *et al.*, 2011).

Human and animal excreta harbor various enteric pathogens including *V. cholerae* (Delahoy *et al.*, 2018) which transmit diseases predominantly in children and cause deaths due to diarrhea in the developing countries including Bangladesh (Uddin *et al.*, 2012). At present, access to improved sanitation is keeping human away from the risk of their own excreta but not from animal waste. In developing countries, animals have frequent contact with humans and that is why animal feces may impose substantial burden of diseases in humans (Delahoy *et al.*, 2018). Therefore, lack of attention on the hygienic management of animal waste may arise a dangerous situation.

A few studies conducted in Bangladesh to explore sparsely the burden of *V. cholerae* from animal excreta (Akond *et al.*, 2008; Uddin *et al.*, 2012; Islam *et al.*, 2013). However, the present study was performed to figure out the role of animals to act as a reservoir of *V. cholerae*. Therefore, this study was conducted to investigate: (1) the prevalence, (2) identification and (3) antimicrobial resistance status of *Vibrio cholerae* from fecal materials of cattle of Mymensingh sadar upazila. The outcome may assist physician to the proper antibiotic choice for the therapeutic purpose and simultaneously provide significance to public health decision-makers with the more effective management of animal waste to reduce the burden of zoonotic diseases in humans.

2. Materials and Methods

2.1. Ethical statement

This study doesn't require any ethical statement. Verbal consent was taken during sample collection from farm owners.

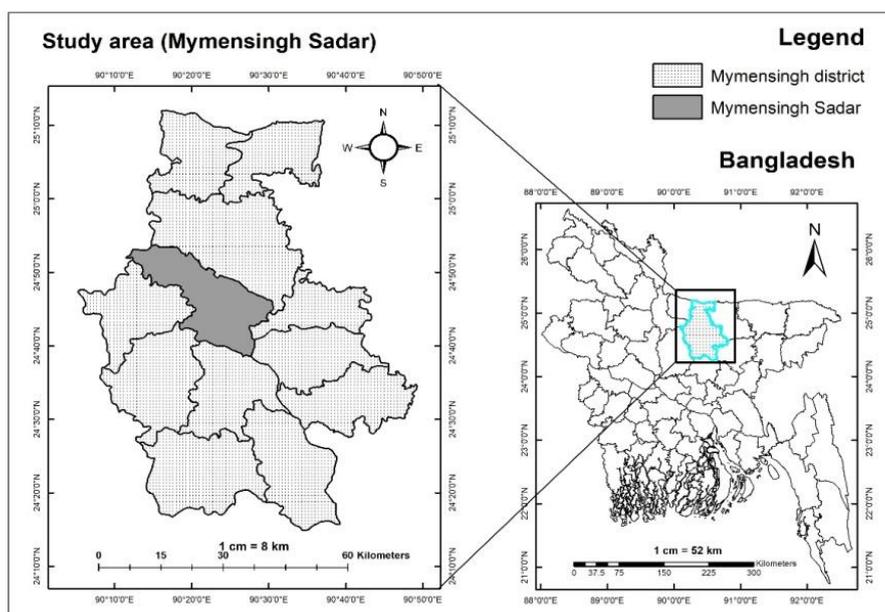


Figure 1. Study area map, created with ArcMap 10.7 software (ESRI, Redlands, California, USA) based on Geographical Information System (GIS).

2.2. Study area

The present study was carried out at the surrounding area of Bangladesh Agricultural University (BAU), Mymensingh Sadar Upazila (24.42° N, 90.25° E) of the Mymensingh district of Bangladesh (Figure 1) during the period of July 2019 to November 2019. In this area, people are mostly live on agriculture or animal rearing. In addition, different indigenous and cross breed of cattle are frequently available in this area.

2.3. Collection of samples

Samples were collected from 50 different household manure pit located in Bangladesh Agriculture University (BAU) surrounding area. Sample was collected from different location of a manure pit to make a pooled sample from each household cattle farm. Samples were taken with the help of sterile cotton buds and dipped into sterile normal saline. Collected samples were transported immediately to the bacteriology laboratory of the Department of Microbiology and Hygiene, BAU, Mymensingh, for bacterial isolation and identification.

2.4. Isolation of *Vibrio* spp.

Isolation of *Vibrio* spp. was performed following the procedure described by Shrestha *et al.* (2015) with some modifications adopted from Uddin *et al.* (2012). Accordingly, samples were enriched in alkaline peptone water (APW) (HiMedia, India) at 37 °C for 8 h. Enriched samples were then subjected to 10 fold serial dilution up to 10⁻⁴. From 10⁻², 10⁻³ and 10⁻⁴ dilutions, 0.1 ml samples were inoculated onto thiosulfate citrate bile salt sucrose (TCBS) agar (HiMedia, India) and incubated at 37 °C for 24 h. After incubation, sucrose fermenting yellow colored colonies on TCBS agar were further streaked onto the same agar and incubated at 37 °C for 24 h. Characteristic yellow colored colonies were selected and stained to observe the morphology of the bacteria.

2.5. Molecular detection of *Vibrio* spp.

Template DNA for the identification of *Vibrio* spp. was carried out by boiling method following the procedure described by Sarker *et al.* (2020). PCR was performed as per the method described previously by Kim *et al.* (2015) with some modifications. Amplification of recombinase A gene was performed to identify *Vibrio* genus with a DNA thermal cycler (Thermo cycler, ASTEC, Japan) using the published primers. The list of primers is shown in Table 1. PCR reactions were carried out with 20 µl volume that included 10 µl master-mix (Promega, USA), 1 µl forward primer (Macrogen Inc., Korea), 1 µl reverse primer (Macrogen Inc., Korea), 2 µl DNA template and 6 µl deionized water. The thermocycling conditions consisted of an initial denaturation at 94 °C for 5 min, followed by 25 cycles of 30 s denaturation at 94 °C, 30 s annealing at 60 °C and 30 s extension at 72 °C. Thereafter, a final extension of 10 min at 72 °C ended up the PCR reaction. PCR products were taken for gel electrophoresis with 1.5% agarose gel at 100 volts for 45 min. Then the gel was submerged in ethidium bromide having final concentration of 4 mg/ml for 15 min maintaining a dark condition followed by washing with distilled water for 5 min. Afterwards, the DNA was visualized under UV transilluminator (Biometry, Germany).

Table 1. Primers used for the molecular detection of *Vibrio* genus.

Primers	Sequence (5'-3') ^a	Amplicon size (bp)	Reference
VG C2694352 F46	GTC ARA TTG AAA ARC ART TYG GTA AAG G	689	Kim <i>et al.</i> , 2015
VG C2694352 R734	ACY TTR ATR CGN GTT TCR TTR CC		

^aMixed base: R = A + G; Y = C + T; N = A + C + G + T

2.6. Identification of *Vibrio cholerae*

Suspected *Vibrio* isolates were screened for the presence of cytochrome oxidase and gelatinase activity by subculturing onto gelatin agar media. Isolates, found to harbor cytochrome oxidase and showed gelatinase activity were selected to perform further confirmation by other biochemical tests like motility test, sugar fermentation test, triple sugar iron (TSI) agar test, indole test, urease test, Voges-Proskauer (VP) test and salt tolerance test according to 'Microbiological Laboratory Manual' (Cappuccino and Sherman, 1996).

2.7. Antimicrobial susceptibility testing

All the isolates of *V. cholerae* were subjected to antibiotic susceptibility against eight antibacterial drugs of eight different antibiotic classess by modified Kirby-Bauer method (Bauer *et al.*, 1996) on Mueller-Hinton Agar (HiMedia, India) using antibiotic discs (Oxoid, England) including ampicillin (10 µg), chloramphenicol (30 µg), co-trimoxazole (25 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamycin (10 µg), imipenem (10 µg) and tetracycline (30 µg). The zones of growth inhibition connected with the zone diameter for individual

antimicrobial agents were then interpreted as susceptible, intermediate or resistant categories according to the interpretation table supplied by the manufacturer (Oxoid limited, England). All interpretations were confirmed by the completion of all disk diffusion tests for three times. In the present study, isolates showing resistant to three or more classes of antibiotics were referred to as Multidrug Resistant (MDR) bacteria as described earlier (Sweeney *et al.*, 2018).

3. Results

3.1. Prevalence of *Vibrio cholerae*

A total of 50 different dairy excreta samples from 50 dairy farms of Bangladesh Agricultural University (BAU) surrounding area, Mymensingh were screened to isolate and identify *Vibrio cholerae*. The study estimated an overall prevalence of *V. cholerae* as 12% (6 positive out of 50 samples) including all assessment methods though culture, biochemical tests and finally molecular detection (Table 2). Out of 50 samples, a total of 28 (56%) isolates showed positive growth on thiosulfate citrate bile salt (TCBS) agar plates (HiMedia, India) and produced small (2-3 mm), raised, circular, yellow (21 out of 28 culture positive case) as well as green, round, 2-3 mm diameter colonies (7 out of 28 culture positive case). However, out of 28 culture positive isolates, 17 (60.71%) were confirmed as *Vibrio* spp. through polymerase chain reaction (PCR) targeting recombinase A gene of *Vibrio*. Furthermore, 6 (35.29%) out of 17 positive *Vibrio* spp. were confirmed as *Vibrio cholerae* based on different biochemical tests and remaining 11 (64.70%) were suspected as other *Vibrio* spp. (Figure 2).

Table 2. Frequency distribution of the *Vibrio* isolates from the 50 samples.

Sample type	No. (%) of sample tested	No. (%) of isolates showed positive cultural characteristics	No. (%) of isolates confirmed as <i>Vibrio</i> spp. through PCR	No. (%) of isolates confirmed as <i>Vibrio cholerae</i> based on biochemical tests	No. (%) of <i>Vibrio</i> spp. other than <i>Vibrio cholerae</i>
Dairy excreta	50 (100)	28 (56)	17 (34)	6 (12)	11 (22)

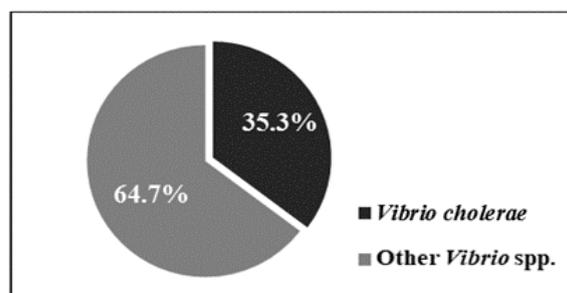


Figure 2. Percentage of *Vibrio cholerae* and other *Vibrio* spp. from 17 positive *Vibrio* isolates.

3.2. Molecular identification of *Vibrio* spp. by PCR

DNA extracted from all culture positive samples were screened through PCR assay for specific identification of *Vibrio* genus. Amplification of recombinase A gene of *Vibrio* spp. from the isolated genomic DNA produced 689 bp (Figure 3) that confirmed the identity of *Vibrio* genus.

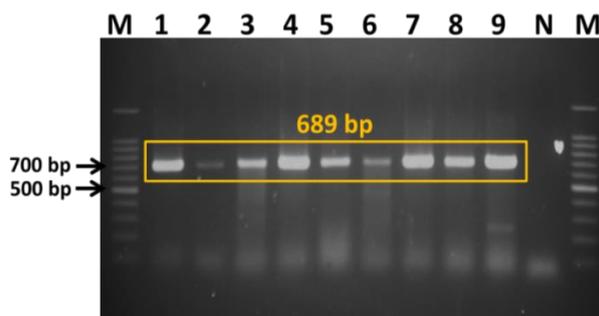


Figure 3. Recombinase A gene based PCR of *Vibrio* genus showing positive band at 689 bp. Here lane M: 100 bp DNA ladder; lane N: Negative control; Lane 1-9: Positive sample of *Vibrio* spp.

3.3. Biochemical characterization of *Vibrio cholerae*

All the isolates, confirmed via PCR were further identified as *Vibrio cholerae* based on the morphological and biochemical characteristics. Microscopically, *V. cholerae* appeared as gram negative, curved rod, motile organism. All the *V. cholerae* isolates were found positive in catalase, oxidase, gelatinase, MR and indole test. However, negative reactions were noticed in VP, Urease and H₂S production test. They exhibited an acid (yellow) slant and acid (yellow) butt without the production of gas and H₂S on TSI agar slant. *V. cholerae* fermented glucose, sucrose, galactose, maltose, mannitol without gas production but did not ferment lactose. Biochemical characteristics of *V. cholerae* were highlighted in Table 3.

Table 3. Results of biochemical characteristic of isolated *Vibrio cholerae*.

Characteristics	Results
Gram stain	-
Shape	Curved rod
Motility	+
Oxidase	+
Catalase	+
Gelatinase test	+
Triple Sugar Iron Agar (TSI)	+
Methyl-red test	+
Voges-Proskaur	-
Indole	+
Urease	-
H ₂ S production	-
Arginine dehydrolase	-
Lysine and ornithine decarboxylase tests	+
Sugar fermentation test	
Glucose	+
Lactose	-
D-galactose	+
Sucrose	+
Maltose	+
Mannitol	+
Growth in nutrient broth	
+ 0% NaCl	+
+ 2% NaCl	+
+ 4% NaCl	+
+ 6% NaCl	+
+ 8% NaCl	-

3.4. Antibiogram profiles of isolated *Vibrio cholerae*

Antibiotic sensitivity tests revealed that all *V. cholerae* isolates (n = 6) were resistant to ampicillin (100%). Additionally, *V. cholerae* isolates were resistant to imipenem (83.33%) and erythromycin (33.33%). Conversely, all the 6 isolates were found 100% sensitive to chloramphenicol, gentamicin and tetracycline whereas, 83.33% to ciprofloxacin. The overall antibiotic resistance profile of the isolated *V. cholerae* is presented in Figure 4.

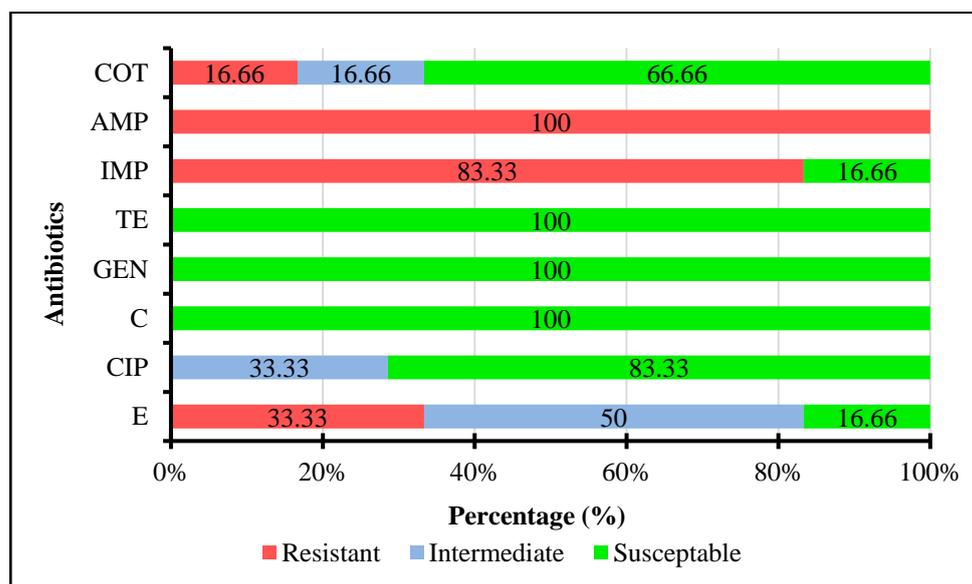


Figure 4. Antibiogram profiles of *V. cholerae* isolated from dairy excreta. COT = Co-Trimoxazole; AMP = Ampicillin; IMP = Imipenem; TE = Tetracycline; GEN = Gentamicin; C = Chloramphenicol; CIP = Ciprofloxacin; E = Erythromycin.

3.5. Distribution of MDR *Vibrio cholerae*

In the present study, we had found 2 (33.33%) out of 6 (100%) *V. cholerae* isolates exhibiting resistance against at least three antibiotic agents (Table 4). Each of the two isolates was found resistant against three antibiotics of three different classes. Two isolates demonstrated two different antimicrobial resistance pattern, E-IMP-AMP and IMP-AMP-COT respectively.

Table 4. Multidrug resistance (MDR) profiles of *V. cholerae* (n = 6) isolated from dairy excreta.

Pattern no.	Antibiotic resistance patterns	No. of antibiotics (classes)	No. (%) of MDR isolates	Overall no. (%) of MDR isolates
1	E- IMP- AMP	3 (3)	1 (16.66)	2 (33.33)
2	IMP- AMP-COT	3 (3)	1 (16.66)	

4. Discussion

Contaminated food and water are to be observed as the potential sources for cholera transmission. Cholera infection in human often progress with diarrhea where contaminated water from the liquid stool of diarrheic patient further continue the transmission cycle (Uddin *et al.*, 2012). It has been evidenced that other than the human stool, animal and poultry excreta can also act as the reservoir of cholera infection (Uddin *et al.*, 2012; Akond *et al.*, 2008; Sanyal *et al.*, 1974).

In the present study, we evaluated prevalence, molecular and biochemical confirmation with antimicrobial resistant pattern of *Vibrio cholerae* isolated from 50 dairy excreta samples that had been collected from individual cattle farm of BAU surrounding area. The occurrence of *V. cholerae* was found 12% from the fecal sample of cattle. This finding is comparable with the finding of some other similar study. Uddin *et al.* (2012) found *V. cholerae* to be present in cow dung, poultry and human excreta and the concentration of *V. cholerae* was found to be higher in human excreta. According to Akond *et al.* (2008) *V. cholerae* was isolated as 16% and 24% from each 50 samples of cloacal swab and intestinal fluid of poultry respectively. Out of 786 samples from cow, Sanyal *et al.* (1974) recorded 37 (4.71%) positive case for *V. cholerae*. However, *V. cholerae* recovered from some other natural sources has been documented also like 12% from human stool in Bangladesh (Zereen *et al.*, 2019), 6.3% from retail seafood (Vu *et al.*, 2018), 0.7% from shrimp samples (Ahmed *et al.*, 2018), 4.88% from human diarrheic sample (Shrestha *et al.*, 2015) and 30.4% from seafood of Nigeria (Adebayo-Tayo *et al.*, 2011). Variations in the occurrence of *V. cholerae* may be the different sample types and geographical locations. We could not compare our study finding of the occurrence of *V. cholerae* in great extent due to lack of reference data from animal sources. *Vibrio* spp. can be found naturally in an aquatic environment, more precisely the evidence of these organisms in raw seafood is very common (Adebayo-Tayo *et al.*, 2011). However, Sanyal *et*

al. (1974) had clearly documented that *V. cholerae* can exist in nonhuman reservoirs such as household cows, goats, dogs, and chickens. Moreover, these reservoirs may play role in the transmission cycle of the infection in humans.

All the culture positive isolates were confirmed as *Vibrio* spp. through molecular assay. 17 isolates out of 50 samples were confirmed as *Vibrio* spp. though a genus specific PCR assay. Recombinase A gene was targeted for the identification of *vibrio* genus, which was documented by Kim *et al.* (2015). Kim *et al.* (2015) described a newly developed multiplex PCR for the detection of *Vibrio* genus along with five pathogenic *Vibrio* species where he evaluated 117 strains and all the isolates were identified to be *Vibrio* species. Unfortunately the present study did not adopt the entire multiplex PCR method due to some limitations, rather we have followed the molecular identification method of *Vibrio* genus and found the similar type of result.

In the present study, 6 (12%) isolates of 17 *Vibrio* spp. were confirmed to be *Vibrio cholerae* based on cultural, morphological and biochemical characteristics. Due to the lack of molecular identification facilities, biochemical methods were solely performed to identify *V. cholerae*. Morphologically the isolated colonies showed small (2-3 mm), circular, and yellow color flattened colonies on thiosulfate citrate bile salts sucrose (TCBS) agar plates (HiMedia, India), previously described by Zereen *et al.* (2019) and Uddin *et al.* (2012). Microscopically isolated *V. cholerae* was observed as motile organism (Zereen *et al.*, 2019). Dua *et al.* (2017) described four traits to identify *V. cholerae* (fermentation of sucrose, non-requirement of external NaCl for the growth, absence of esculin and presence of oxidase), which are also reflected in the present study. Our results of sugar fermentation test match with the findings of Udoh and Itah (2012) who had found glucose, sucrose, mannitol, D-mannose, D-galactose to be fermented by *V. cholerae* whereas no fermentation was observed for lactose. Uddin *et al.* (2012) exhibited an acid (yellow) slant and acid (yellow) butt without the production of gas and H₂S on TSI agar slant, which are also in agreement with the present study.

Antibiotic resistance is considered as a major public health problem. The irrational and misuse of antimicrobial agents contributed to the development and spread of antibiotic-resistant pathogens in animals and humans (Simonsen *et al.*, 2004). In response to this malpractice, WHO has stated AMR (antimicrobial resistance) as a serious public health concern across the world (Ventola, 2015). All the isolated *V. cholerae* strains in this study were found resistant to ampicillin, suggesting this drug is inefficient in the treatment of cholera. Das *et al.* (2011) and Shrestha *et al.* (2015) also reported 100% resistance to the above antibiotic. Harris *et al.* (2012) and Kansakar *et al.* (2011) sparsely documented macrolide resistance in their studies. However, a slightly high level erythromycin resistance (33.33% resistance with 50% intermediate) was observed in our study which is in line with the finding of Akond *et al.* (2008) and Shrestha *et al.* (2015). Our study identified imipenem resistant (83.33%) *V. cholerae*, which may cause a threat to public health as carbapenem group of antibiotic is mostly applied in the serious infectious cases of human (Islam *et al.*, 2021). This result contrast with the finding of Uddin *et al.* (2012). Resistance to imipenem may have revealed due to the transmission of bacteria from human sources, as carbapenems are not authorized for use in livestock (Poirel *et al.*, 2014). Further studies at the molecular level are required to follow up on these findings. Additionally, all isolated strains showed 100% sensitivity to chloramphenicol, gentamicin and tetracycline whereas, 83.33% to ciprofloxacin which may be effective therapeutic agents for the treatment of cholera. This finding support the result of previously conducted studies (Shrestha *et al.*, 2015; Akond *et al.*, 2008). However, Akond *et al.* (2008) and Shah *et al.* (2012) reported 100% and 81.8% resistance to tetracycline respectively, Uddin *et al.* (2012) reported 100% resistance to tetracyclin and chloramphenicol, Garg *et al.* (2000) reported high-level resistance to chloramphenicol in India. These findings contrasted with our findings.

The choice of drugs, practiced globally over the years for controlling *V. cholerae* serogroup O1 infection are mostly tetracyclines, fluoroquinolones and macrolides (Leibovici-Weissman *et al.*, 2014). Additionally, according to WHO- erythromycin, trimethoprim-sulphamethoxazole, and chloramphenicol have also been found effective in case of treating the infectious cholera agents (Faruque *et al.*, 2007). However, resistance to all these commonly used drugs has been reported as a result of chromosomal mutations or by gaining mobile genetic elements like plasmids, transposons etc. (Ceccarelli *et al.*, 2016). In Bangladesh, although tetracycline and doxycycline have long been considered as the antibiotics of choice for cholera (Faruque *et al.*, 2007), clinicians are also prescribing other WHO recommended drugs. So, this might be the cause that the present study showed some isolated *V. cholerae* strain resistant to erythromycin and co-trimoxazole. But, it's a message of hope that our study evidenced the effectiveness of tetracycline, ciprofloxacin and chloramphenicol in treating the infectious case with *V. cholerae*.

It is a matter of concern that out of 6 isolated *V. cholerae*, 2 (2/6, 33.33%) were found to be multidrug resistance (MDR) in the present study. Shrestha *et al.* (2015) and Roy *et al.* (2012) reported 100% MDR *V. cholerae* from human stool samples, which contrasted with the present study. Whereas, Garbern *et al.* (2021) documented

28.1% MDR *V. cholerae* from same type of samples in Bangladesh which nearly matched with the present study. However, No document was retrieved about MDR *V. cholerae* from animal samples.

MDR cholera epidemics have already been reported previously from Bangladesh (Faruque *et al.*, 2006). Moreover, epidemics of MDR cholera (both classical and El Tor biotypes) have also been reported worldwide (Mandal *et al.*, 2011). Therefore, as a potential source of cholera, human can play a vital role in the transmission of *V. cholerae* in livestock, since cattle, buffalo, goat, sheep and poultry are habited in close contact of human in Bangladesh. Although Delahoy *et al.* (2018) documented a limited or no transmission of cholera in human by animal feces, the risk can not be ignored as pathogenic cholera strain are being recovered from the animal feces (Uddin *et al.*, 2012; Akond *et al.*, 2008). For additional survival advantages newly emerged cholera strains are being evolved to replace the prevailing epidemic strains through genetic changes (Faruque *et al.*, 2006) like spontaneous mutation or horizontal transfer of resistance genes (Shrestha *et al.*, 2015).

This present study is the first approach to highlight the role of animals as a reservoir of cholera infection which is supported by the isolation and identification of *V. cholerae* from the animal waste. The findings of the present study may assist the clinician to select appropriate antibiotics for serious cholera cases and simultaneously provide significance to public health decision-makers with the more effective management of animal waste to reduce the burden of zoonotic diseases in humans.

The main limitation of this study was the time and fund constrain. As a result, molecular detection of *V. cholerae* was not attained, rather identification was solely dependent on biochemical tests. Further studies are badly needed to characterize these isolates through molecular assay to find out whether they are virulent for humans or have the potential to cause an epidemic.

5. Conclusions

Vibrio cholerae is one of the major agents associated with diarrhoea outbreaks in Bangladesh. Present study isolated MDR *V. cholerae* from cattle feces, evidencing the possibility of animals being served as the reservoir of cholera that might pose substantial risk to human health if these MDR bacteria enters into the food chain. Inadequate management of animal feces from human domestic environments can happen fecal-oral transmission of zoonotic pathogens including *V. cholerae* due to the poor sanitation practice. Therefore, composting of animal and poultry feces can be a better managemental strategy which will lower the risk of spreading potential pathogens and reduce environmental pollutions through hygienic waste disposal.

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Conflict of interest

None to declare.

Authors' Contribution

Mohammad Arif and S. M. Lutful Kabir designed the experiment. Nilofa Eashmen, Mohammad Arif and Basana Sarker collected the samples and conducted an experiment. Mohammad Arif drafted the first version of the manuscript. S. M. Lutful Kabir and Mir Rowshan Akter critically reviewed the article and finally approved for publication.

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