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Characterization of Zoonotic Bacteria from Captive Crocodiles in Bangladesh

Ariful Islam[1], Md. Bakhtiar Lijon[1*], Md. Mahadee Hasan[1], Abu Syem Muhammad Arif[2], Mahbubul Pratik Siddique[1], Muhammad Tofazzal Hossain[1] , Md. Ariful Islam[1]

[1]*Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh*

[2]*Reptiles Farm Limited, Mymensingh, Bangladesh*

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Abstract

An attempt was undertaken to characterize the bacterial flora of saltwater crocodiles reared on a commercial farm at Valuka, Mymensingh, Bangladesh. Swab samples (n=60) comprised of the oral swab (n=20), nasal swab (n=20) and cloacal swab (n=20) were collected from apparently healthy farm-raised crocodiles. Samples were cultured into various selective media to isolate bacteria. Characterization of the isolated bacteria was performed by studying the cultural, staining, biochemical properties followed by polymerase chain reaction. The antimicrobial susceptibility patterns of bacteria were investigated against eight commonly used antibiotics by the disc diffusion method. A total of 135 bacterial isolates belonged to 5 genera such as *Escherichia* (22%), *Salmonella* (22%), *Staphylococcus* (21%), *Bacillus* (16%) and *Vibrio* (19%) were identified. A genus-specific PCR targeting *16s rRNA* gene successfully identified *Escherichia coli, Salmonella* spp. and *Staphylococcus* spp. In PCR assay, 11 of 30 (36.67%) *E. coli* isolates were found positive for *stx1* and *stx2* genes and 4 of 30 (13.33%) isolates were found positive for and *hly* gene. Five isolates of *Staphylococcus* spp. were found positive with *nuc* gene, indicating these were pathogenic strains of *Staphylococcus aureus*. Multidrug resistance profile was observed in *E. coli, Salmonella* spp. and *Vibrio* spp. The results of this study indicate that captive crocodiles in the farm harbor multidrug-resistant pathogenic bacteria, which may produce infection in crocodiles and might cause health problems if transmitted to a human.

* **Corresponding Author:** Md. Bakhtiar Lijon ✉ lijonmicro2014@gmail.com

Introduction

Crocodiles are usually hunted and farmed for their valuable skins, meat, oil and claws. Crocodile farming is a unique and lucrative activity in many parts of the world. However, the first commercial crocodile farms were established to meet the growing demand for skins in the early 1960s (Tosun, 2013). Crocodile skins are used to make the most luxurious lather items, where crocodile meat and eggs are delicious food products in many countries. Bones are used to making perfume as well as for decorative purposes. Teeth are used to make ornaments and claws are used to making keyrings (Lane and Ruppert, 1997). In Bangladesh, crocodile farming is a very new concept. The geophysical and climatic condition of this country is suitable for crocodile farming as it is the historical living place of saltwater crocodiles. Reptiles Farm Limited, located at Valuka, Mymensingh, is the first and only commercial crocodile farm in Bangladesh. This is, in fact, the only commercial crocodile farm in South East Asia, with the aim to earn foreign currencies through the export of crocodiles and different crocodile products (Husain *et al.*, 2012). There has been much development in crocodile farming worldwide over the past few years, and with this has come to an interest in the major diseases associated with farmed crocodiles.

The bite of crocodile can cause severe injuries to all living animals (Erickson *et al.*, 2003). Besides physical damage, it can develop wound infections caused by bacteria or fungi (Caldicott *et al.*, 2005). Studies revealed the presence of many bacteria in the oral cavity of wild and captive crocodilians (Charruau *et al.*, 2012). Although several antibiotics protect from wound infections, in some cases, patients may die from sepsis (Caldicott *et al.*, 2005).

Crocodiles have significant resistance and healing capacity to injuries and illness, but bacterial infections may lead them to death to some extent (Garcia *et al.*, 2008). Like other reptiles and amphibians, wild crocodiles are found to harbor *E. coli*, *Salmonella* spp., *Pseudomonas* spp., *Aeromonas* spp., *Staphylococcus* spp. *Vibrio* spp. and *Bacillus* spp. etc. (Charruau *et al.*, 2012). All these bacteria are not pathogenic; instead, most of them are commensal. Occasionally some bacterial diseases are caused by primary pathogens significantly when the immune system is compromised (Chinnaduria and DeVoe, 2009). Many potentially pathogenic bacteria, especially enterobacteria, are harbored by clinically normal crocodiles, and these bacteria are probably the most frequent cause of disease. Consumption of products (meat and egg) of both farmed and wild reptile is considered responsible for biological risks including bacterial infections caused by *Salmonella* spp., *Vibrio* spp. etc. (Magnino *et al.*, 2009). Exporting meat, eggs, skin, bones, claws and teeth is the primary purpose of commercial crocodile farming. Contamination of these products with bacteria during processing and packaging can hamper the whole export process. These kinds of obstacles can turn the entire investment into a significant economic loss. Characterizing bacterial flora of captive farm crocodiles is essential for understanding bacterial infection in these species and the health hazard of people associated with crocodile rearing and its product processing.

Characterizations of bacterial flora of farm-raised crocodiles in Bangladesh remain to be studied. However, no study has been conducted so far on the characterization of bacterial flora from captive crocodiles on the farm in Bangladesh.

The study's objective was to isolate and identify the bacterial flora from oral, nasal and cloacal cavities of captive crocodiles in Bangladesh and to characterize them through staining, cultural, biochemical features and polymerase chain reaction (PCR) and antibiotic sensitivity test.

Material and methods

Study area

The research work was conducted at the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh. The samples were collected from Reptiles Farm Ltd. (RFL); Valuka, Mymensingh, Bangladesh.

Collection and transportation of samples

To collect the oral swabs, a piece of wood was used to keep the mouth of the crocodile open. A tight rubber band was used to avoid biting. Then sterile cotton buds were introduced inside its mouth cavity and the swab was collected. For nasal swabs, sterile cotton buds were introduced into the nasal cavity of the crocodile. The cloacal swab was also collected by using sterile cotton buds. Aseptic measures were undertaken during the collection of samples. After collection, the swabs were transferred into cryovials containing 0.1% peptone water. Immediately after transferring in cryovials, all samples were brought to the Bacteriology Laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh, through cool chain maintaining.

Isolation and identification of bacteria

Samples were inoculated into the Nutrient broth and incubated for 24 hours at 37°C for better nourishment of the desirable organisms. Isolation of bacteria is carried out based on the cultural characteristics of different selective media. For this one loopful of an enrichment culture of oral, nasal and cloacal swabs was separately streaked in duplicate onto Mannitol salt agar (MSA, Hi-media, India), Thiosulfate Citrate Bile Salts Sucrose agar (TCBS, Hi-media, India), Salmonella-Shigella (SS) agar (Hi-media,India), Eosin Methyline Blue agar (EMB, Hi-media, India), Blood agar(BA, Hi-media, India) respectively and incubated for 24 hours at 37 °C. Single colony grown onto selective media were further sub-cultured onto the particular media until pure bacterial cultures were obtained. To identify the bacteria Gram's staining method (Ashikuzzaman *et al.*, 2015), motility test (Lijon *et al.*, 2015), sugar fermentation test (Hemraj *et al.*, 2013) and biochemical tests such as Catalase (Hemraj *et al.*, 2013), Indole (Hemraj *et al.*, 2013), MR-VP (Hemraj *et al.*, 2013) and coagulase tests (Islam *et al.*, 2016) were performed.

Molecular detection of bacteria by PCR

Polymerase chain reaction (PCR) method was used to identify *E. coli* by amplifying 585bp, 606 bp, 374 bp, 889 bp and 497 bp fragments of *16srRNA*gene , *stx1* and *stx2* gene, hly gene and *rfb*0157 gene, respectively (Schippa *et al.*, 2010; Heuvelink *et al.*, 1995; Wieler *et al.*, 1996; Paton and Paton, 1998). For detection of *Salmonella* spp. 469 bp and 274 bp fragments of *16srRNA* gene and *typh* gene were amplified respectively by following PCR method (Cohen *et al.*, 1993). Polymerase chain reaction (PCR) assay was also performed to identify *Staphylococcus* spp. by amplifying 241 bp and 279 bp fragments of *16s rRNA* gene and *nuc* gene, respectively (Stuhlmeier and Stuhlmeier, 2003; Kalorey *et al.*, 2007).

Antibiotic sensitivity test

Antibiotic sensitivity test of isolated *E. coli* (n=30), *Salmonella* spp. (n=30), *Bacillus* spp. (n=10), *Staphylococcus* spp. (n=10) and 10 *Vibrio* spp. (n=10) were performed against eight commercially available antibiotic discs such as Ampicillin, Azithromycin, Cephalexin, Ciprofloxacin, Chloramphenicol, Gentamicin, Penicillin and Vancomycin (Hi-media, India). The disc diffusion method was used to detect antibiotic susceptibility of the isolated bacteria according to the guidelines of Cockerill and Clinical and Laboratory Standard Institute (CLSI), 2012.

Results

A total of 135 bacterial isolates belonged to five genera of bacteria, i.e., *Escherichia, Salmonella, Staphylococcus, Bacillus* and *Vibrio* were isolated from 30 swab samples of the crocodile in this study.

Table 1. Summary of isolation of bacteria from oral, nasal and cloacal swab of crocodiles.

Samples (n)	Bacterial genera					Total
	E. coli (n)	*Salmonella* spp. (n)	*Staphylococcus* spp. (n)	*Bacillus* spp. (n)	*Vibrio* spp. (n)	
Oral swab (10)	10	10	9	7	8	44
Nasal swab (10)	10	10	10	9	8	47
Cloacal swab (10)	10	10	9	8	9	46
Total	30	30	28	22	25	135

A total of 44 isolates were recovered from oral swab, 47 isolates were recovered from nasal swab and 46 isolates were recovered from a cloacal swab of crocodiles. The number of each isolated bacteria with total isolates from each type of sample is presented in Table 1.

Isolation and identification of bacteria

The cultural characteristics of *E. coli*, *Salmonella* spp., *Staphylococcus* spp., *Bacillus* spp. and *Vibrio* spp. were found a green metallic sheen colony on EMB agar, translucent black smooth colony SS agar, small whitish or yellowish colony on MS agar, thick grayish-white or cream color colony on BA and yellow colony on TCBS agar respectively.

Bacteria were identified on the basis of colony morphology, Gran's staining, Motility test, Sugar fermentation reaction and several biochemical tests. Morphological and Gram's staining characteristics of bacteria isolated from nasal, oral and cloacal swab samples elucidate in Table 2.

Table 2. Morphological and Gram's staining characteristics of the bacterial isolates recovered from oral, nasal and cloacal swab samples.

Morphological and staining characteristics			Bacteria identified
Shape	Arrangements	Gram's staining reaction	
Short plump rods	Single, paired or in short chain	(-) ve	*E. coli*
Very short plump rods	Single	(-) ve	*Salmonella* spp.
Coccid in shape	Arranged in grapes like cluster	(+) ve	*Staphylococcus* spp.
Rod with square ends	Arranged in chains	(+) ve	*Bacillus* spp.
Curved rod or comma shaped	Single	(-) ve	*Vibrio* spp.

Legend: (+) ve =Positive; (-) ve=Negative.

All the isolates of *E. coli, Salmonella* spp., *Bacillus* spp. and *Vibrio* spp. were shown motile feature but *Staphylococcus* spp. were shown non-motile feature when examined by hanging drop method. Results of Sugar fermentation reaction using five basic sugars (Dextrose, Maltose, Lactose, Sucrose and Mannitol), Catalase, Coagulase, MR-VP and Indole production tests are listed in Table 3. *E. coli, Salmonella* spp., *Bacillus* spp. were found Catalase negative, but *Vibrio* spp. and *Staphylococcus* spp. were found Catalase positive (Table 3). *Staphylococcus* spp.

Isolates from swab samples of crocodiles were found Coagulase positive (Table 3).

Table 3. Biochemical characteristics of the bacterial isolates recovered from crocodiles.

Sugar fermentation reaction profiles					Catalase test	Coagulase test	MR test	VP test	Indole test	Interpretation
DX	ML	L	S	MN						
AG	AG	AG	AG	AG	-	ND	+	-	+	*E. coli*
A	A	-	-	A	-	ND	+	-	-	*Salmonella* spp.
A	A	A	A	A	+	+	+	+	-	*Staphylococcus* spp.
AG	A	A	AG	AG	-	ND	+	-	+	*Bacillus* spp.
A	A	-	A	A	+	ND	+	-	+	*Vibrio* spp.

Legend: DX= Dextrose; ML = Maltose, L= Lactose, S= Sucrose, MN = Mannitol; A = Acid, AG = Acid and Gas; MR= Methyl Red; VP = Voges-Proskauer; '+' = Positive, '-'= Negative; ND = Not Done.

Molecular detection of bacteria

Molecular detection of *E. coli, Salmonella* spp. *and Staphylococcus* spp. was performed by PCR method. The results of PCR are described in Table 4 and Figs. 3, 4, 5, 6, 7, 8 and 9.

Prevalence of bacteria

The prevalence of *E. coli* and *Salmonella* spp. were 100% in all the samples. *Staphylococcus* spp. had a 100% prevalence in a nasal swab. The prevalence of *E. coli, Salmonella* spp., *Staphylococcus* spp., *Bacillus* spp. *and Vibrio* spp. in oral, nasal and cloacal swab of crocodiles is presented in Fig. 1. Among the isolated bacteria, the *E. coli* was 22%, *Salmonella* spp. was 22%, *Staphylococcus* spp. was 21%, *Bacillus* spp. was 16% and *Vibrio* spp. was 19% (Fig. 2).

Table 4. Summary of PCR result of *E. coli* with *16s rRNA*, *stx1*, *stx2*, *hly* and *O175* genes, *Staphylococcus* spp. with *16s rRNA* and *nuc* genes and *Salmonella* spp. with *16s rRNA* and *typh* genes.

Samples (n)	Number of PCR positive samples amplified								
	E. coli					*Staphylococcus* spp.		*Salmonella* spp.	
	16s rRNA gene	*stx1* gene	*stx2* gene	*hly* gene	*O157* gene	16s rRNA gene	*Nuc* gene	16s rRNA gene	*Typh* gene
Oral swab (10)	10	3	3	1	0	9	1	10	0
Nasal swab (10)	10	2	3	1	0	10	3	10	0
Cloacal swab (10)	10	6	5	2	0	9	1	10	0

Legend: RNA= Ribonucleic Acid; *stx1*& *stx2* = Shiga toxigenic genes; *hly* = hemolysin gene.

Table 5. Summary of antibiotic sensitivity test.

Antibiotics	Antibiogram profile				
	E. coli	*Salmonella* spp.	*Staphylococcus* spp.	*Bacillus* spp.	*Vibrio* spp.
Ciprofloxacin	R	R	I	I	R
Azithromycin	R	R	R	I	R
Vancomycin	R	R	S	S	R
Cephalexin	R	R	S	I	I
Gentamicin	S	S	S	S	S
Ampicillin	R	R	R	R	R
Penicillin	R	R	R	R	R
Chloramphenicol	I	S	S	S	I

Legend: S = Sensitive; I = Intermediate sensitive; R= Resistant.

Antibiogram

Each genus was subjected to an antibiotic sensitivity test against the eight most commonly used antibiotics. Antibiotic sensitivity test results are summaries in Table 5 and Fig. 10. The zone diameters were compared with the information supplement document on antimicrobial susceptibility testing of Cockerill and CLSI, 2012.

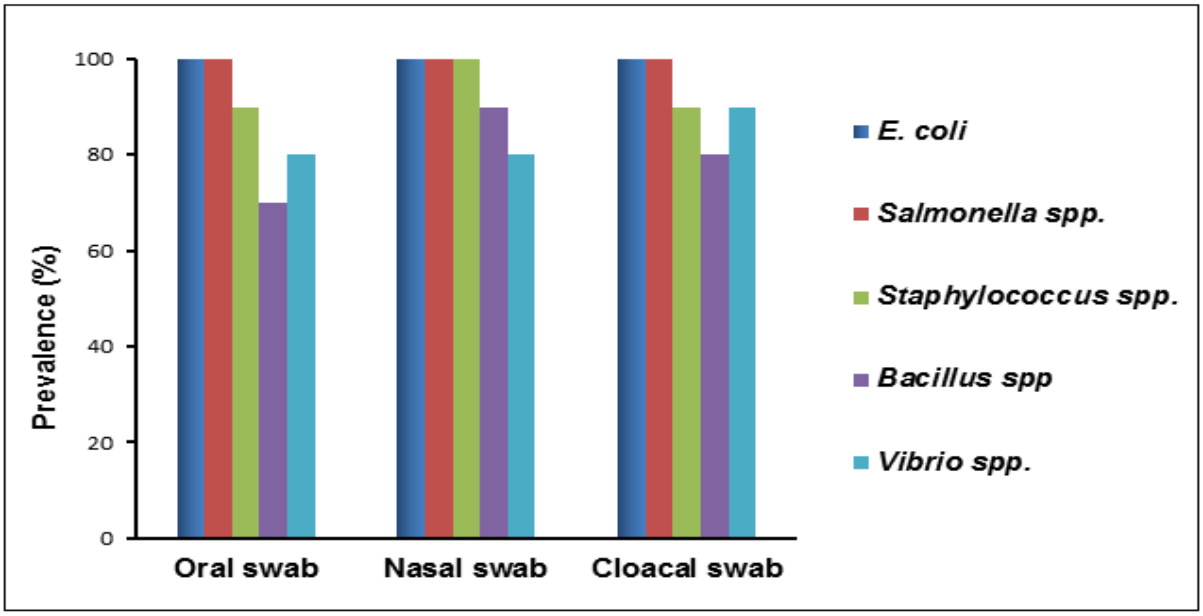

Fig. 1. Prevalence of bacteria in oral, nasal and cloacal swabs of crocodiles.

The results of antibiotic sensitivity of *Staphylococcus* spp. revealed that all the isolates were sensitive to Vancomycin, Cephalexin, Gentamicin, Chloramphenicol and intermediately sensitive to Ciprofloxacin and resistant to Azithromycin, Ampicillin and Penicillin, respectively. Most *Bacillus* spp. revealed sensitivity to Vancomycin, Gentamicin, Chloramphenicol and intermediately sensitive to Ciprofloxacin, Azithromycin and Cephalexin and resistance to Ampicillin, Penicillin. *E. coli* revealed

that they were resistant to Ciprofloxacin, Azithromycin, Vancomycin, Cephalexin, Ampicillin, Penicillin, Chloramphenicol and only sensitive to Gentamicin. Most of the isolates of *Salmonella* spp. were found to be sensitive to Gentamicin and Chloramphenicol and resistant to Ciprofloxacin, Azithromycin, Vancomycin, Cephalexin, Ampicillin and Penicillin, respectively. *Vibrio* spp. was found to be sensitive to Gentamicin only and intermediately sensitive to Chloramphenicol. *Vibrio* spp. was also shown the resistant properties against Ciprofloxacin, Azithromycin, Vancomycin, Cephalexin, Ampicillin and Penicillin.

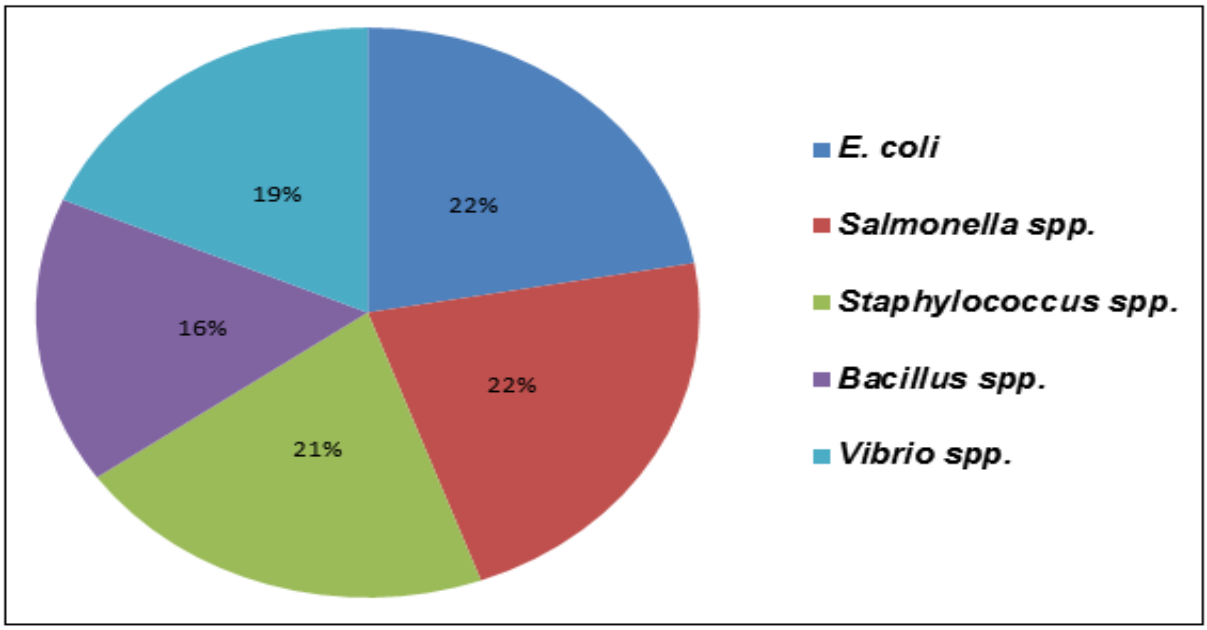

Fig. 2. Distribution of bacterial population in oral, nasal and cloacal swabs of crocodiles.

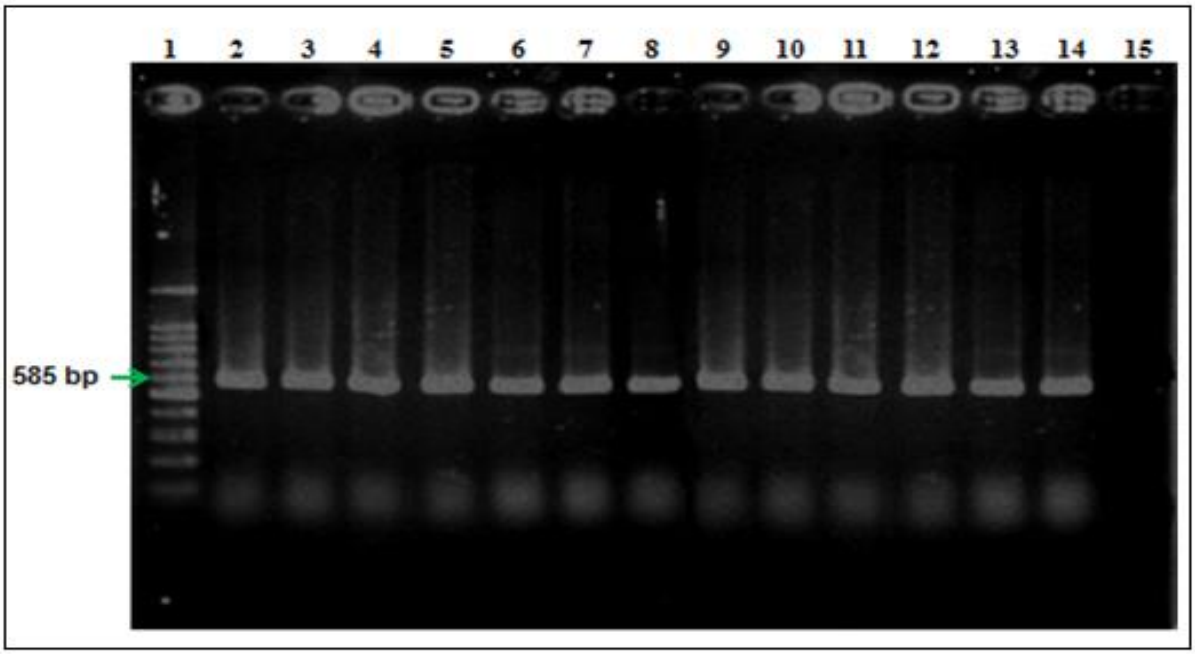

Fig. 3. PCR assay to amplify 16S rRNA of *E. coli* isolates recovered from crocodiles. Lane 1: 100bp size DNA marker; lane 2, 3, 4 and 5: representative *E. coli* isolates from oral swab; lane 6, 7, 8 and 9: representative *E. coli* isolates from nasal swab; lane 10, 11, 12 and 13: representative *E. coli* isolates from cloacal swab; lane 14: Positive control and lane 15: Negative control without DNA.

Discussion

The bacterial flora of crocodiles can be detrimental to crocodiles as well as human health. Bacterial infections are the most common cause of death in farmed crocodiles (Huchzermeyer, 1997). The human may get bacterial infections not only from the consumption of crocodile meat and eggs but also from various activities at the crocodile farm and crocodile bites (Caldicott *et al.*, 2005; Magnion *et al.*, 2009).

Bacteria can also be an important obstacle in the international trade of crocodile products in consideration to public health hazard risk (Huchzermeyer, 1997).

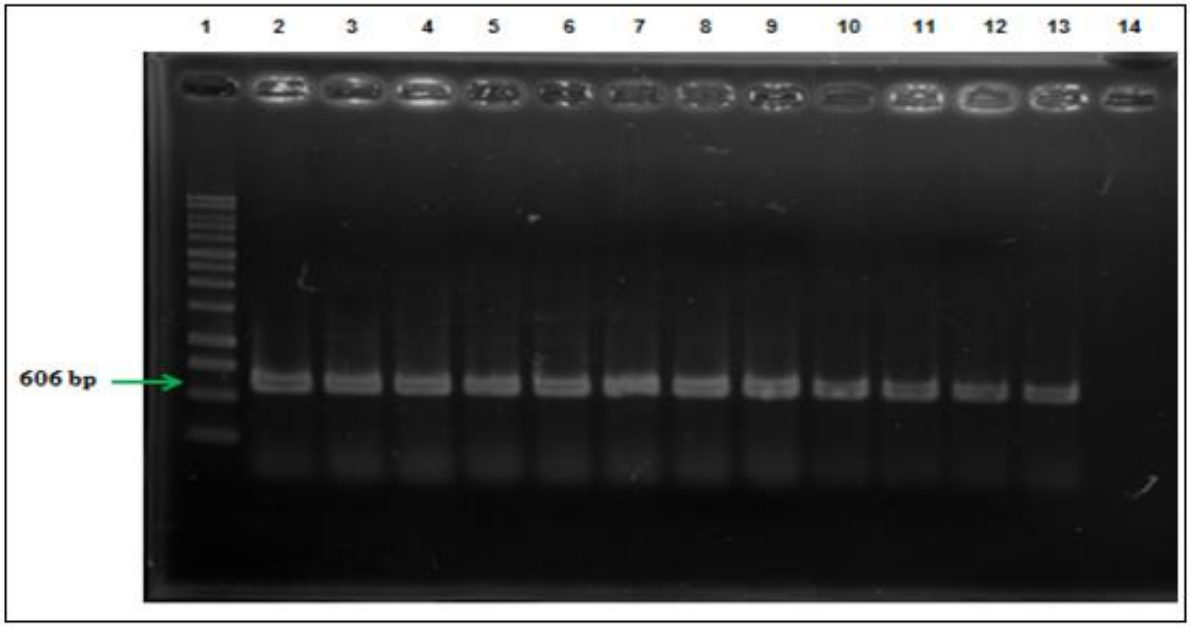

Fig. 4. PCR assay to amplify *stx1* gene of *E. coli* isolates recovered from crocodiles. Lane 1: 1kb size DNA marker; lane 2, 3 and 4: *E. coli* isolates from oral swab; lane 5 and 6: *E. coli* isolates from nasal swab; lane 7, 8, 9, 10, 11 and 12: *E. coli* isolates from cloacal swab; lane 13: Positive control and lane 14: Negative control without DNA.

In the current study, five genera of bacteria were isolated from swab samples of crocodiles. Among the total number of isolated bacteria, the distribution of *E. coli* and *Salmonella* spp. were the highest (22%) and *Bacillus* spp. was the lowest (16%). *Staphylococcus* spp. and *Vibrio* spp. had 21% and 19% distribution, respectively. A study conducted in the Mexican Caribbean by Charruau *et al.* (2012) recorded 47 bacterial species of 28 genera from a cloacal and oral swab of crocodiles.

In addition to these five genera, Charruau *et al.* (2012) isolated *Aeromonas, Citrobacter, Corynebacterium, Enterococcus, Streptococcus, Klebsiella, Moraxella, Proteus, Shigella, Pseudomonas, Pasteurella, Serratia and Fusobacterium*. Charruau *et al.* (2012) also reported the most common bacteria in oral and cloacal samples were *Salmonella* spp. and *Vibrio* spp., *E. coli, Arcanobacterium pyogenes and Aeromonas hydrophila*.

Though crocodiles are considered to have resistance to bacterial infection, many pathogenic bacteria are harbored by clinically healthy crocodiles and thought to be the most frequent cause of disease and death of crocodiles (Garcia *et al.*, 2008). A study conducted by Sultana *et al.* (2012) and reported *E. coli* septicemia as the leading cause of death of farmed crocodiles in Bangladesh.

Crocodile meat contaminated with bacteria is also reported to cause human infection. In the Amazonas crocodiles, meat consumption has been reported to have a condition by *Bacillus cereus* (Johnston *et al.*, 2010).

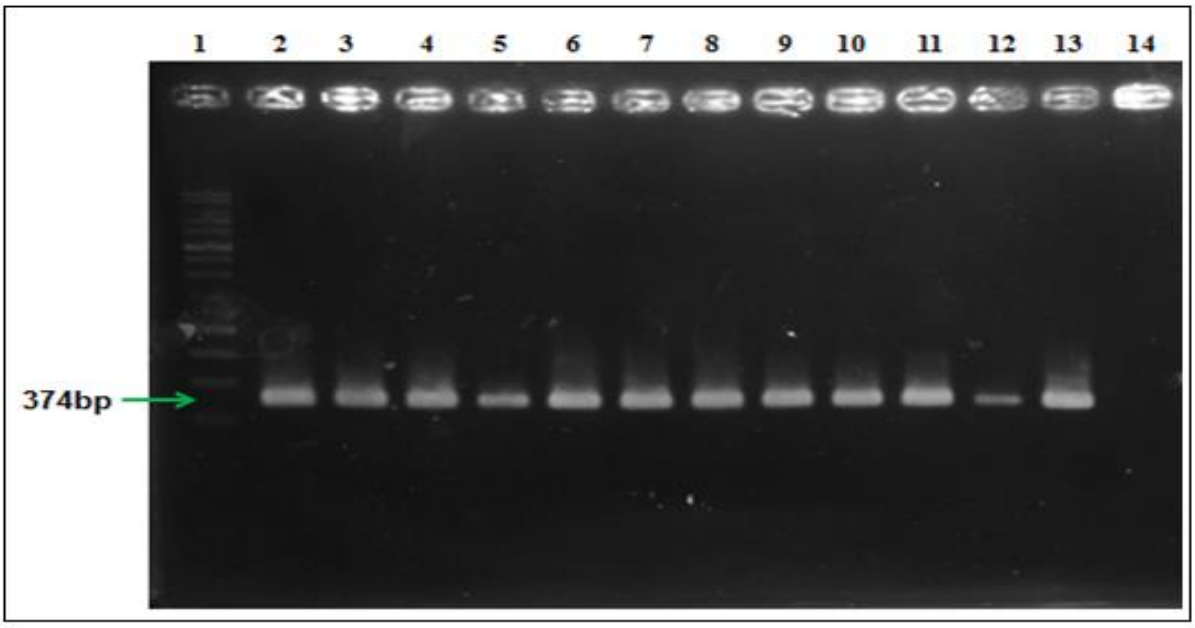

Fig. 5. PCR assay to amplify *stx2* gene of *E. coli* isolates recovered from crocodiles. Lane 1: 1kb size DNA marker; lane 2, 3 and 4: *E. coli* isolates from oral swab; lane 5, 6 and 7: *E. coli* isolates from nasal swab; lane 8, 9, 10, 11 and 12: *E. coli* isolates from cloacal swab; lane 13: Positive control and lane 14: Negative control without DNA.

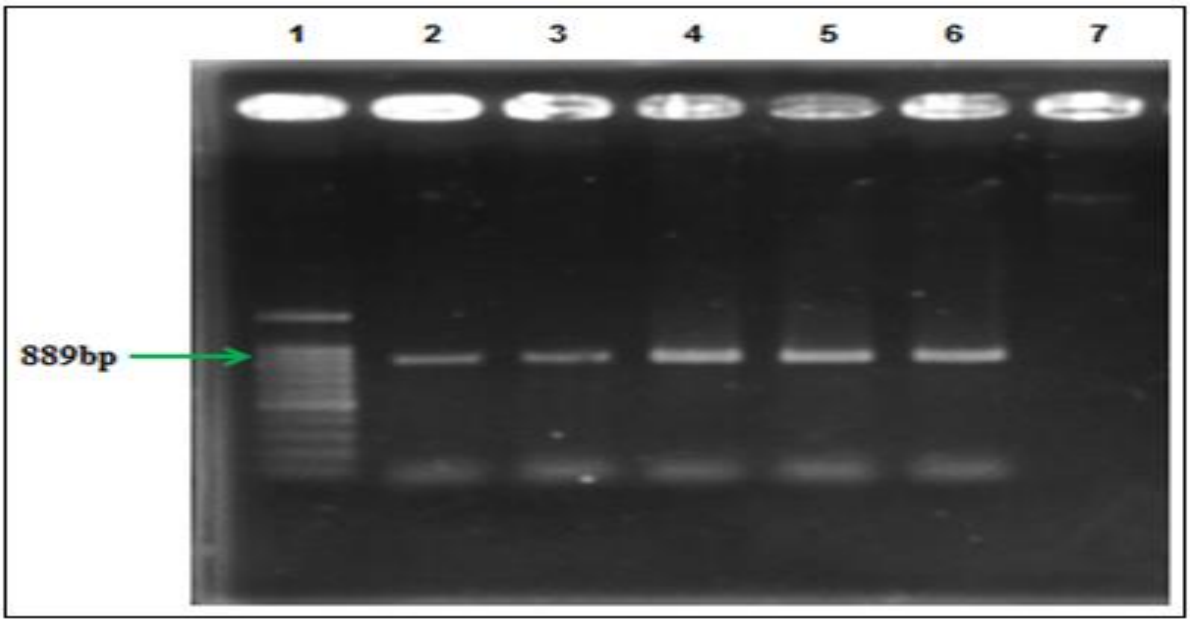

Fig. 6. PCR assay to amplify *hly* gene of *E. coli* isolates recovered from crocodiles. Lane 1: 100bp size DNA marker; lane 2: *E. coli* isolates from oral swab; lane 3: *E. coli* isolates from nasal swab; lane 4 and 5: *E. coli* isolates from cloacal swab; lane 6: Positive control and lane 7: Negative control without DNA.

A man found in South Africa was suffering from salmonellosis after consumption of crocodile meat infected by *Salmonella enterica diarizonae* (Narayana *et al.*, 2008). Consumable products for export, especially meat and eggs, can be contaminated during processing which may hamper international trade due to public health concerns. For isolation of *E. coli, Salmonella* spp., *Staphylococcus* spp., *Vibrio* spp. and *Bacillus* spp. various selective media were used. In this study, *E. coli* were found to produce a greenish black colony with a metallic sheen in EMB agar media. Sultana *et al.*, (2012) reported similar colony characteristics on EMB agar media. Morphologically, *E. coli* were Gram-negative, rod

shape, and motile bacteria stay in a paired or single arrangement; similar morphological characteristics of *E. coli* were described by Sultana *et al.* (2012).

The identified bacteria were re-confirmed by sugar fermentation and other biochemical tests, which found similar to the findings of Sultana *et al.* (2012). *Salmonella* spp. on SS agar was observed to produced translucent black, smooth, small round colonies, which were similar to findings of Faruq *et al.* (2016). Morphologically, *Salmonella* was Gram-negative very short plump rods that appeared in a single or paired arrangement under the microscope and were motile. The morphological and biochemical properties of *Salmonella* spp. were found similar with Hasan *et al.* (2011).

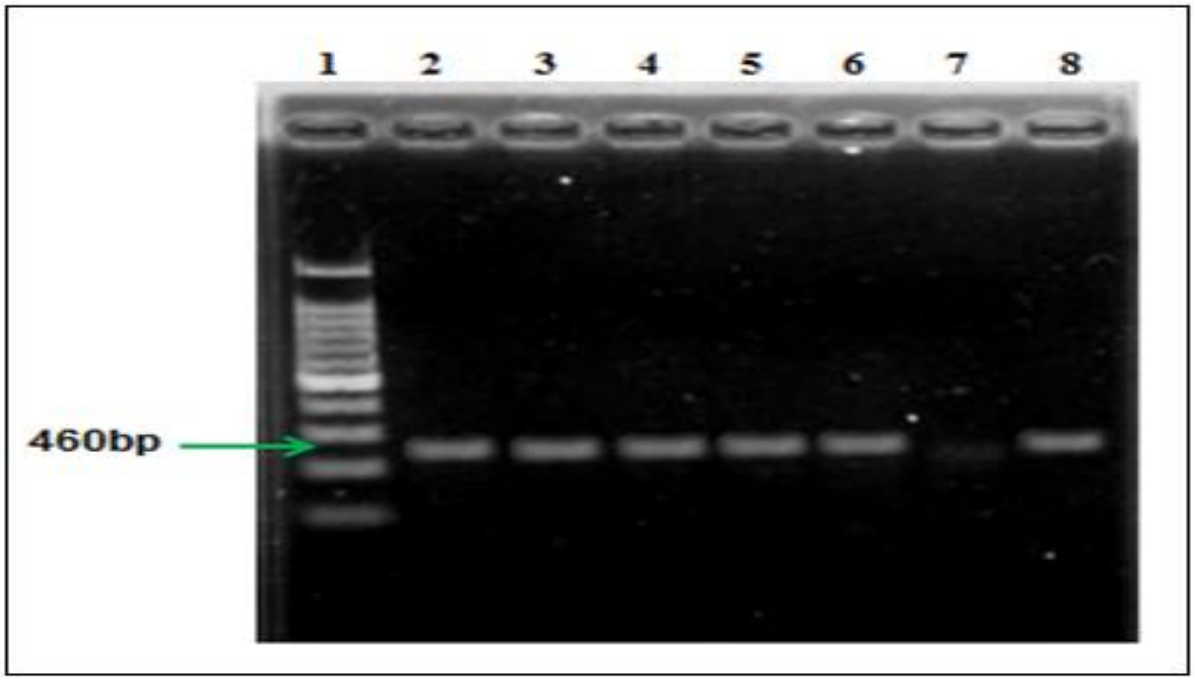

Fig. 7. PCR assay to amplify 16s rRNA gene of Staphylococcus spp. isolates recovered from crocodiles. Lane 1: 100bp size DNA marker; lane 2 and 3: representative Staphylococcus spp. isolates from oral swab; lane 4 and 5: representative Staphylococcus spp. isolates from nasal swab; lane 6: representative Staphylococcus spp. isolates from cloacal swab; lane 7: Negative control without DNA and lane 8: Positive control.

In this research work, *Staphylococcus* spp. culturally produced yellowish colony on MS agar, morphologically they appeared as Gram-positive coccoid shape in a grape-like arrangement under the microscope. Islam *et al.* (2016) reported similar cultural and morphological characteristics of *Staphylococcus* spp. in their study. *Staphylococcus* spp. was found to ferment five essential sugars with no gas production. Similar sugar fermentation test results were described by Hasan *et al.* (2011) and Rall *et al.* (2008). Catalase and Coagulase tests results of *Staphylococcus* spp. were found positive with bubble formation and coagulation of plasma, respectively. These findings were supported by the result of Islam *et al.* (2016).

Recorded morphological characteristics and sugar fermentation tests result of *Bacillus* spp. coincided with the findings of Hasan *et al.* (2011). The colony characteristics of *Vibrio* spp. on TCBS media was found yellow, in Gram's staining bacteria exhibited curved shaped Gram-negative short rods, fermented five basic sugars and produced acid only and gave a negative result to VP test, which is similar to the findings of Uddin *et al.*, (2012).

PCR assay was used for molecular detection of bacteria. Different primers targeting 16s rRNA genes were used for specific identification of the bacteria at the genus level by PCR. In this study, *Escherichia, Salmonella* and *Staphylococcus* genera were

identified by amplification of 16s rRNA genes. Seidavi *et al.* (2010), Cohen *et al.* (1993) and Stuhlmeier and Stuhlmeier (2003) also used 16s rRNA target primers to detect *Escherichia, Salmonella* and Staphylococcus genus, respectively.

In the case of *E. coli*, the presence of *stx1* and *stx2* genes indicates Shiga toxigenic strains and *hly* gene indicates entero-hemorrhagic *E. coli* strains. In this study, PCR assays were performed to detect *stx1, stx2* and *hly* genes in *E. coli*. Results of PCR assays showed that 11 of 30 (36.66%), 11 of 30 (36.66%) and 4 of 30 (13.34%) *E. coli* isolates harbored *stx1, stx2* and *hly* genes, respectively. Heuvelink *et al.* (1995) and Wieler *et al.* (1996) reported similar results in their study.

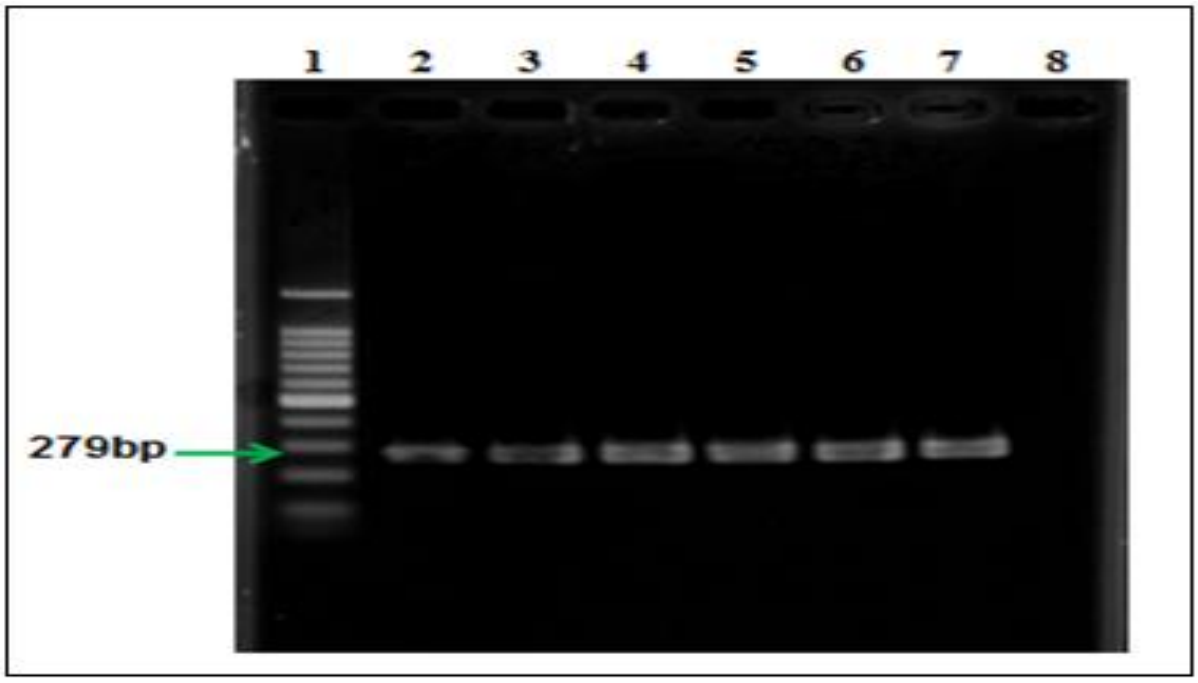

Fig. 8. PCR assay to amplify nuc gene of Staphylococcus spp. isolates recovered from crocodiles. Lane 1: 100bp size DNA marker; lane 2: Staphylococcus spp. isolates from oral swab; lane 3, 4 and 5: Staphylococcus spp. isolates from nasal swab; lane 6: Staphylococcus spp. isolates from cloacal swab; lane 7: Positive control and lane 8: Negative control without DNA.

The result signifies the presence of Shiga toxigenic and entero-hemorrhagic strains of *E. coli* in crocodiles. In this study, PCR assay did not amplify *rfb O157* gene from *E. coli* which is in disagreement with the findings of Paton & Paton (1998). Presence of thermo nuclease gene (*nuc*) in the genome of *Staphylococcus* spp. indicates pathogenic strains of *Staphylococcus aureus*.

In this study, 5 of 30 (16.67%) of the *Staphylococcus* spp. were found to carry *nuc* gene in their genome, indicating pathogenic strains of *Staphylococcus aureus*. Our data coincided with that recorded by Kalorey *et al.* (2007). The *in-vitro* antibiotic sensitivity tests of bacterial isolates to 8 commonly used antibiotics were studied. A slight variation was noticed in the results of the sensitivity of isolates against the antibiotics used. Among the isolated bacteria *E. coli, Salmonella* spp. and *Vibrio* spp. showed multidrug resistance properties. *E. coli was* found to be resistant to 7 of the used antibiotics and were sensitive only to gentamicin. Isolated *Vibrio* spp. and *Salmonella* spp. were found to be resistant to 6 used antibiotics and sensitive to 2 antibiotics. Several researchers reported this type of multidrug resistance of these bacteria (Hasan *et al.*, 2011, Uddin *et al.*, 2012, Magiorakos *et al.*, 2012). The antibiogram profile of *Bacillus spp.* and *Staphylococcus* spp. of this study are similar to the findings of Andrews and Wise (2002) and Islam *et al.* (2016), respectively.

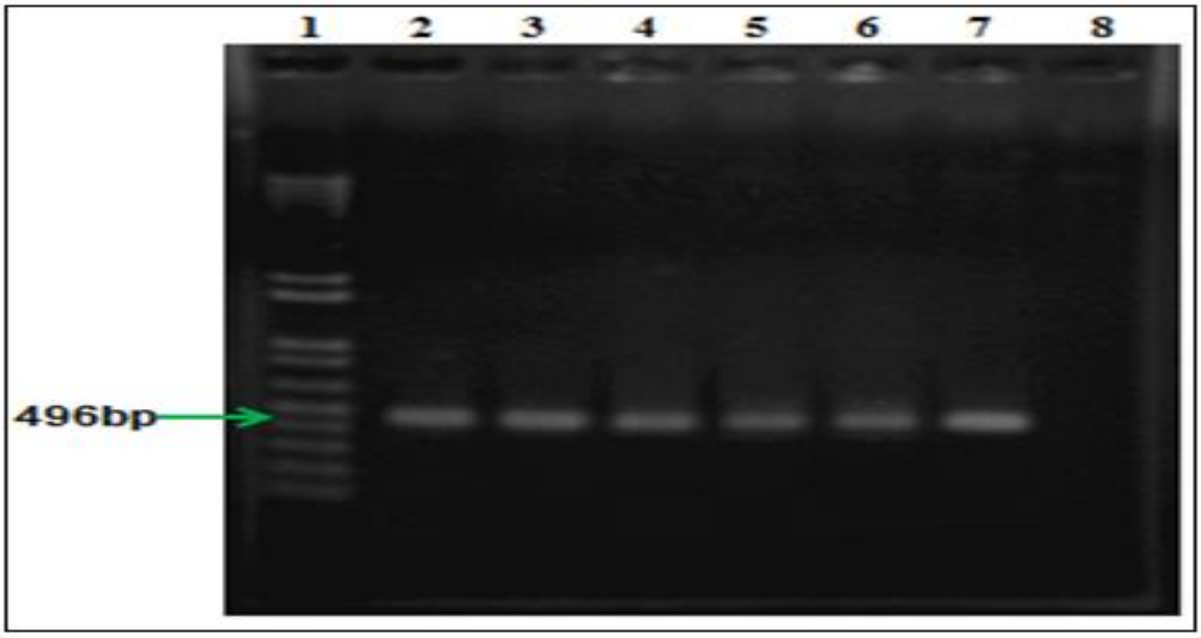

Fig. 9. PCR assay to amplify 16s rRNA gene of *Salmonella* spp. isolates recovered from swab samples of crocodiles. Lane 1: 100bp size DNA marker; lane 2: *Salmonella* spp. isolates from oral swab; lane 3 and 4: *Salmonella* spp. isolates from nasal swab; lane 5 and 6: *Salmonella* spp. isolates from cloacal swab; lane 7: Positive control and lane 8: Negative control without DNA.

The results of this research work suggest that crocodiles harbor multidrug-resistant and pathogenic bacteria, which might play a crucial role in the development of crocodile disease. The isolated bacterial flora may also cause serious health hazards to the workers and hamper the export of crocodile products. Contamination of crocodile meat principally occurs during slaughter and dressing procedures (Kalorey *et al.*, 2007). Results of this study indicate the need to implement biosecurity and control measures of bacteria, safety measures for workers, and proper hygiene practice during processing of crocodile products for export to ensure safe production and safeguard public health.

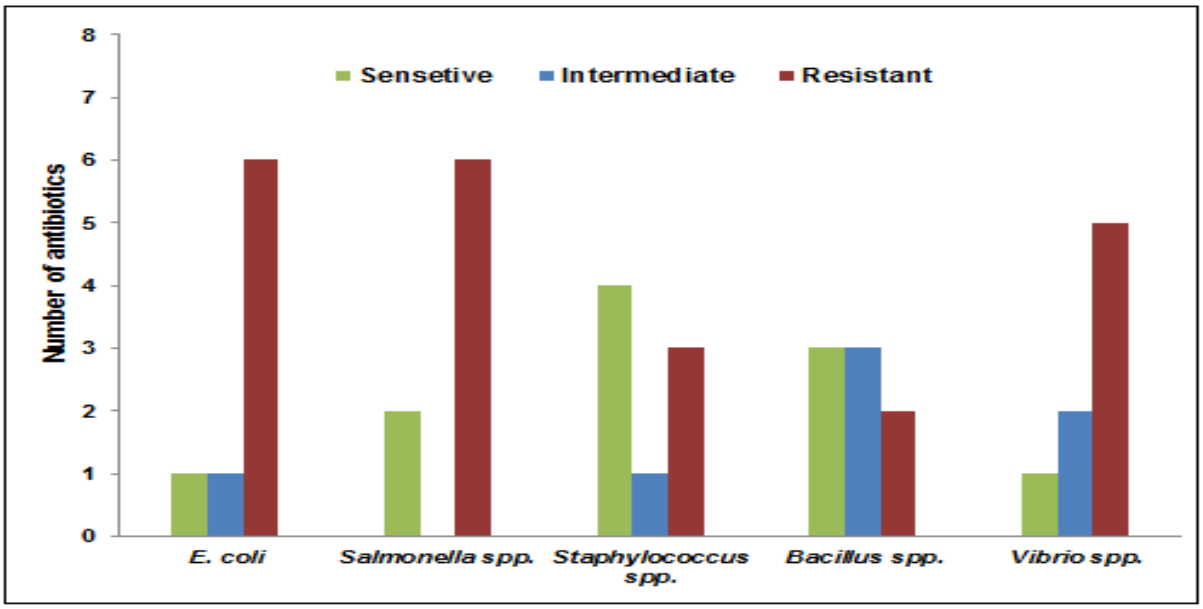

Fig. 10. Summary of antibiogram profile of *E. coli*, *Salmonella* spp. *Staphylococcus* spp., *Bacillus* spp. and *Vibrio* spp. against 8 antibiotics.

Conclusion

Crocodiles harbor hazardous and highly pathogenic strains of some bacteria, which may cause disease at the time of stress and results in significant economic loss due to morbidity and mortality. Bacterial genera isolated in this study may cause public health hazards that implicate people's health risk who come in contact with the crocodiles. Food-borne pathogenic bacteria might also contaminate crocodile's meat during slaughtering and processing if proper hygienic measures are not undertaken, which can result in loss of their acceptance for export in the international market. Multidrug-resistant bacteria found in crocodiles can make it hard for treatment if there is an infection.

Authors' contributions

Mahbubul Pratik Siddique designed the experiment, negotiated with the Reptiles Farm, and was directly involved in sample collection. Ariful Islam and Md. Mahadee Hasan collected the samples and conducted the majority of the research works. Abu Syem Muhammad Arif, as Farm Manager, took the whole responsibilities at the Farm level and helped in sample collection. Md. Ariful Islam and Muhammad Tofazzal Hossain worked as research supervisor and research co-supervisor, respectively. Md. Bakhtiar Lijon wrote the manuscript and helped in various molecular research and analyses. All the authors read the manuscript carefully and approved the final version for submission to the journal.

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Competing Interest

The authors have no competing interests.

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