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ISOLATION, IDENTIFICATION AND ANTIBIOGRAM STUDY OF BACTERIA ASSOCIATED WITH QUAIL FECES IN SYLHET SADAR, BANGLADESH

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Abstract

The current research was conducted for isolation, identification of isolates, and antibiogram study of quail feces isolated from different quail farms and hatchery in Sylhet, Bangladesh. The data used for this study were randomly collected from three different farms and hatchery in the Sylhet district. A total of 90 samples (30 samples from each farm) were settled for bacterial isolation. In this study, A series of culture and biochemical tests were carried out for the isolation and identification of isolated isolates. The antibiotic sensitivity tests of E. coli, Salmonella spp., and Pseudomonas spp. were determined using the Kirby Bauer discdiffusion method. Out of 90 samples, E. coli was found 74 (82.22%), and the highest number of 26(86.67%) was observed in shibganj Bazar Sylhet. The second most pathogen of Salmonella spp. was recorded in Hashem quail and poultry breeders with the prevalence of 23(76.67%), whereas the lowest number of Pseudomonas spp. was obtained in sarkar quail and poultry hatchery 16.67%, respectively. The antibiotic susceptibility test exposed that E. coli were 100% sensitive to Colistin sulfate and Neomycin, whereas cloxacillin showed 100% resistance. Salmonella spp. and Pseudomonas spp. were shown 100% resistant to Amoxicillin, Ampicillin, and cloxacillin. We isolates the three most pathogenic isolates from different farm and hatchery, and these bacteria create a severe public health risk.

1 Introduction

Bangladesh is an agriculture-based country. About 80% of people of the country are dependent on agricultureeither directly or indirectly. Livestock is one of the most important parts of agriculture.The livestock sector isthe integral sector of the agro-based economy (Modak et al., 2012). But the quail is a very newbird that is rearing on a commerciallevel. Quail farming in

Bangladesh is growing gradually because of its high rate, pre-sexual maturity, pleasantmeat, high egg production rate, and less floor space for maintenance. In Bangladesh, poultry, eggs, and meataccount for around 38% of total animal protein(Khatun et al., 2020). Quail maybe two sources of income in addition to chicken andducks for its immense potentiality for meat and egg production. At present, quail production has commerciallystarted in small ranges in Dhaka, BAU poultry farm, Mymensingh, Manikgonj, TMSS NGO in Sylhet, Barisal, andanotherdistrict in Bangladesh (Banglapedia). However, the rise of quail manufacturing is being seriously wastedbysomemanagementfactors,includingvariousfatalcontagious,noncontagious,andbloodsucking sicknesses (Barnes 1987). Colibacillosis, Salmonellosis, ulcerative enteritis, chicken cholera, quail bronchitis, Newcastle disease, andquail pox are the most prevalent infectious diseases associated with quail production. A disease outbreak canresult in high flock mortality as high as 50% to 90%, negatively affecting a grower's economic bottom line (DeJongDozieret al., 2010). Inpoultry, *E. coli* infections can cause egg peritonitis, omphalitis, coligranuloma, enlarged head syndrome, cellulitis,and colisepticaemia, as well as bird death. This disease is an extensive, severe form of infection characterized bythe existence of*E.coli* inblood andtheconsequence oforgans like theliver, spleen,andheart (Barnes et al., 2003).Therespiratorytract and air sacs are the most prevalent sites of infection, and it is usually due to a mycoplasma orvirusinfection. Affected birds show signs of perihepatitis, pericarditis, and air sacculus. Reduced food consumption,listlessness, ruffled three feathers, laborious quick breathing, and a peculiar"snicking" are all symptoms ofcolisepticemia (Mdegela et al., 2000).

Salmonellosis is another crucial complication that causes severe financial loss due to fatality and decreased eggproduction (Khan et al., 2005). Salmonellae may be involved in a variety ofpathogenic processes inhumans and animals,including poultry. Affected birds appear to be depressed and can show signs of scour. Post-mortem lesionsinclude acute dehydration, septicemia, acute enteritis and classical caecal cores of white caseous materials (Johnston, A. 2007).The mortality rate can be reasonably high, reaching 15% in rare cases. *Pseudomonas* spp. showed resistanceagainst most of the antibiotics, and it is clinically significant. Some species of *Pseudomona*s are opportunisticpathogenic for humans and animals and poultry like *P. aeruginosa*; they also play an essential role in spoilage offoods, dairy products, meat, poultry, and eggs. The clinical cases have accomplished the characterizationofmicroorganismslike E. coli, *Salmonella* spp., and *Pseudomonas* spp. in quail birds. For the proper treatment and control of any disease,theisolation,identification,andcharacterizationofnormalbacterialfloraareessentialbecauseth eywill behelpfulforthe selectionofantibioticsand vaccines.

Immunogenicity of New castledisease vaccine, fowl choleravaccine, and invivo growthand kinetics ofvirulentNewcastlediseasevirusinsomequailpopulationofBangladeshwereperformed (Ara et al., 2009).Nostudyhasbeenorganized yet on the prevalence of bacterial disease of quail concerning age, sex, verity location, and season.

Totreat bacterial disease, farmers are indiscriminately using a different types of antibiotics resulting in antibioticresistance. For this reason, the priority is to isolate and molecular characterization of the causal agent of thediseasesandtakeadequatecontrolmeasuresagainstthatdisease.Sothecurrentresearchisplannedtocharacterize the bacterial pathogen in the quail birds of Bangladesh by using selective antibiotics to control theeffectiveness of isolates. The research objective was to isolate and identify the bacterial isolates from Quail fecalsampleandtheir antibiogram profile atSylhetSadar.

2 Materialsandmethods

2.1 Studyareaselection

The current investigation was carried out in Sylhet to extract and identify bacteria from quail feces, as well as toconduct an antibiogram study on bacteria from different quail farms and hatcheries. The research was conductedduring the period of 2014-2015 in the Department of Microbiology and Immunology, Faculty of Veterinary andAnimalScience,SylhetAgricultural University (SAU).

2.2 Samplecollection

A total of 90 feces samples of quail were randomly collected from three different farms and hatchery, includingSarkarQuailandPoultryHatchery,KashemQuailandBreederHatchery,andShibganjBazarinSylhet,Bangladesh.30 sampleswerecollectedfromeachfarmandhatchery.

2.3 Isolationandidentificationofbacteria

After collection,sampleswereTransferredintothelaboratoryandprimarilyculturedonnutrientagar andnutrient broth media. Next, different culture media such as MacConkey agar, EMB agar, SS agar,BA, BGA, and MSA(Hi media, India) were used for subculture and pure culture for pure colony isolation of suspected isolates. Atthat point, all cultured plates were incubated at 37°C for 24 hours with the aseptic condition. In this study, purebacterial cultures were acquired based on Bergey's manual of determinative bacteriology (1994). Bacterial pure cultures were stored at -80°C with 20% glycerol for further use. A group biochemical test such as indole, MR,VP, oxidase, catalase, motility test, simons citrate, and five sugar fermentation tests was used by followingstandard protocols (Cheesbrough M. 2003).

2.4 Antibiogramstudyofisolatedbacteria

Eight commerciallyavailable antibioticswereused forantibiotic susceptibilitytestsin thisstudy.AccordingtotheguidelinesandrecommendationsofCLSIassesstheantibioticsensitivityofallisolatesusingMueller-Hintonagar(Hi-Media,India)platebydiskdiffusionmethod (CLSI. 2013).Theisolatesresistant tothreeor moreantibioticswereclassifiedasmultidrug-resistant(MDR)strains.Ciprofloxacin(5µg),Amoxicillin(10µg),Ampicillin(10 µg),Erythromycin(15µg),Colistinsulphate(25µg),Neomycin(30µg),Chloramphenicol(30µg), Cloxaciline (5 µg) (Hi media, India)were used for antibiogram study. The zone of inhibition is measured bytheguideline of CLSI (CLSI. 2009).

3 Results

3.1 Prevalenceofisolatedbacteriafromdifferentquailfarmsandhatchery

In this research, we collected a total of 90 samples from three different quail and poultry hatcheries. 30 sampleswere randomly collected from each farm. Out of 30 Sarkar quail and poultry hatcherysamples, *E. coli* werepositivefor20(76.67%),*Salmonella*spp.20(66.67%),*Pseudomonas*spp.5(16.67%).InKashemQuailand BreederHatchery,25(83.33%)*E. coli*,23(76.67%)*Salmonella* spp.,and6(20%)*Pseudomonas* spp.wereidentified. In shibgang Bazar, 26 (86.67%) *E. coli*, 22(73.33%) *Salmonella* spp., and 7(23.33%) *Pseudomonas* spp.were isolated in this study. The highest percentage (86.67%) of *Escherichia coli* was found in Shibganj Bazar, thehighest percentage (76.67%) of *Salmonella* spp. was found in Kashem Quail and Breeder Hatchery, and thehighest percentage (23.33%) of *Pseudomonas* spp. was found in Shibganj Bazar. The prevalence results are shown inTable1.

Tableno.1:Prevalenceofisolatedbacteriafromdifferentquailfarmsandhatchery

SL.NO	**Farm/Hatchery**	**No. of case**	***E. coli***		***Salmonella* spp.**		***Pseudomonas* spp.**	
1	Sarkar Quail and Poultry Hatchery	30	No. of positive case	%	No. of positive case	%	No. of positive case	%
			23	76.67	20	66.67	5	16.67

2	Kashem Quail and Breeder Hatchery	30	25	83.33	23	76.67	6	20
3	Shibganj Bazar	30	26	86.67	22	73.33	7	23.33
Total		**90**	**74**	82.22				

In this study, different media wereused for cultural and biochemical tests. *E. coli* gives large pink color colonies on MacConkey agar and Showsmetallic sheen colonies on EMB agar. In the biochemical test, *E. coli* gives positive results for the Indole test, MRtest, VP test. *Salmonella* spp. shows in black center colonies on SS agar and give positive biochemical test for MR,Citrate, and catalase test. *Pseudomonas* spp. express pink yellow colonies in MacConkey agar and sticky circularcolonies on EMB agar. Biochemical test positive for catalase and motility test. All bacterial culturaland biochemicaltestresultsareshowninFigures 1,2 and 3.

Figure 1: *E.coli* on MacConkey agar with pink colony (left) and EMB agar with mettalic sheen colony (right)

Figure 2:*Salmonella*spp.onEMBagarwithcolorlesscolony(left)andonSSagarwithblackcentercolony(right)

Figure 3: *Pseudomonas* spp. on EMB agar with blue color sticky colony (left) and hemolytic colony on Blood agar (right)

3.2 Antibiogram profile of the isolated bacteria

Commercially available eight antibiotics were used for antibiotic susceptibility patterns of isolated *E.coli*, *Salmonella* spp., and *Pseudomonas* spp. We selected each of five isolates for this antibiogram study. Three sensitivity profiles, such as resistant (R), intermediate (I), and sensitive (S), were observed in all isolates tested against antibiotics. *E. coli* were 100% sensitive to Colistin sulfate and Neomycin followed by ciprofloxacin, chloramphenicol, and amoxicillin 60%, respectively. In case of *Salmonella* spp. Colistin sulphate and ciprofloxacin were 100% sensitive. *Pseudomonas* spp. were 100% sensitive to ciprofloxacin and Colistin sulphate, whereas Amoxicillin, Ampicillin, and Cloxaciline were 100% resistant. The summary of antibiogram profiles is presented in Table 2 and figure 5, 6 and 7. The Clinical and Laboratory Standard Institute evaluated antibiotic sensitivity tests according to the disc diffusion method (CLSI. 2013).

Table 2: Antibiogram profiles of *Ecoli*, *Salmonella* spp. and *Pseudomonas* spp.

Antimicrobial agents	**Symbol**	**Disc concentration (µg/ml)**	***Ecoli* (n=5)**			***Salmonella* spp. (n=5)**			***Pesudomonas* spp. (*n=5*)**		
			R	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**
Ciprofloxacin	CLIP	5µg	1	1	3	0	0	5	0	0	5
Amoxicillin	AML	10µg	5	0	0	5	0	0	5	0	0
Ampicillin	AMP	10µg	4	0	1	5	0	0	5	0	0
Erythromycin	E	15µg	3	2	0	3	0	2	4	1	0
Colistinsulphate	CT	25µg	0	0	5	0	0	5	0	0	5

Neomycin	N	30µg	0	0	5	0	2	3	0	2	3
Chloramphenicol	C	30µg	1	2	3	0	3	2	4	1	0
Cloxaciline	OB	5µg	5	0	0	5	0	0	5	0	0

R=Resistant;I=Intermediate;S=Sensitive.

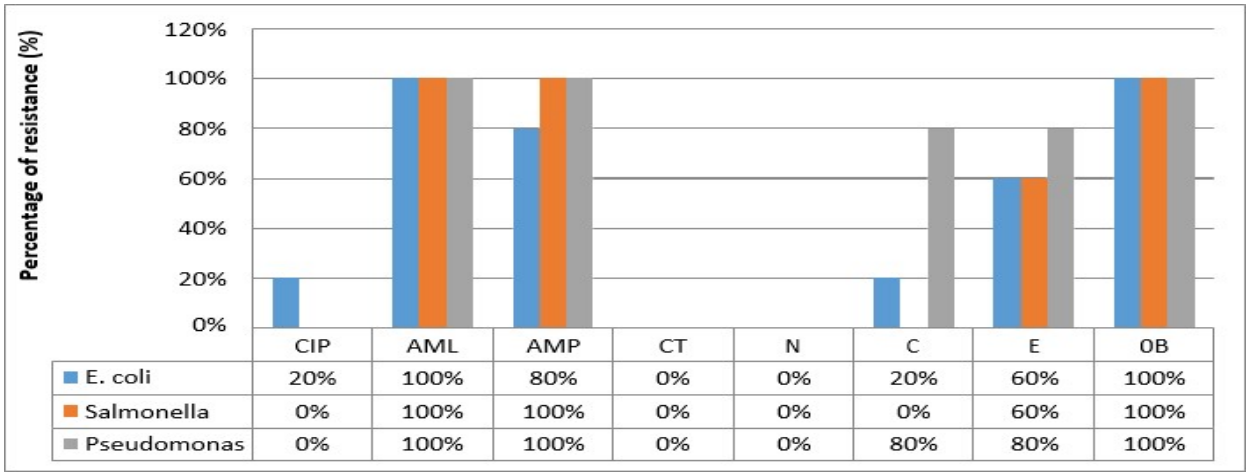


Figure5: Graphicalpresentationofresistancetoantibioticsin*Ecoli;Salmonella*spp.and *Pseudomonas*spp.

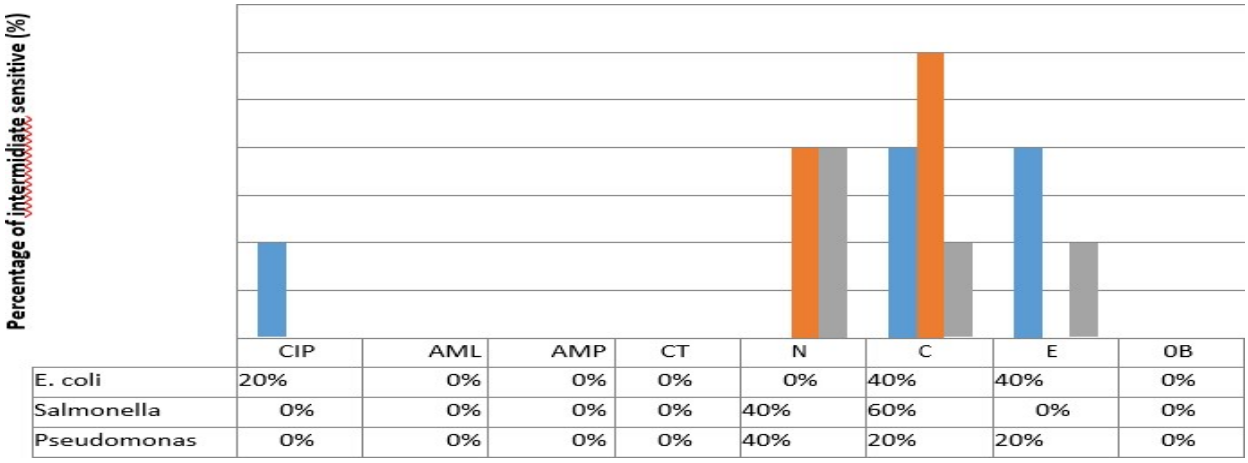
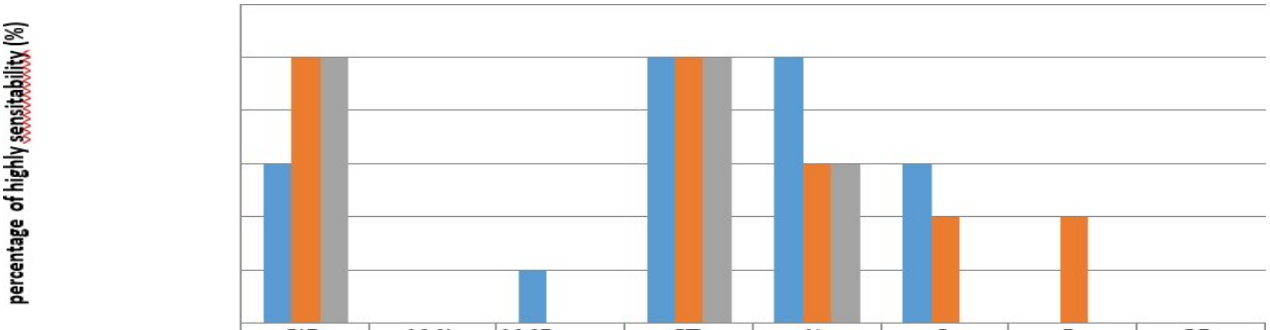


Figure6: Graphicalpresentationofintermediatesensitivetoantibioticsin*E.coli;Salmonella*spp.and *Pseudomonas*spp.



	CIP	AML	AMP	CT	N	C	E	OB
E. coli	60%	0%	20%	100%	100%	60%	0%	0%
Salmonella	100%	0%	0%	100%	60%	40%	40%	0%
Pseudomonas	100%	0%	0%	100%	60%	0%	0%	0%

Figure7: Graphicalpresentationofsensitivitytoantibioticsin*E.coli,Salmonella*spp.,and*Pseudo*

*mon*spp.

4 Discussion

Bangladesh'seconomyisbasedonagriculture.Morethan70%offamiliesinBangladesh'sruralareasraise poultry. Poultry production, particularly in animal husbandry, serves a crucial role in producing protective food in humannutrition. Quail is one of the species of poultry. The current investigation was carried out to isolate and identifybacteria found in the feces of quail birds, and an antibioticsensitivity test was also performed. The researchwork was carried out at Sarkar Quail and Poultry Hatchery, Kashem Quail and Breeder Hatchery, and ShibganjBazar, Sylhet and in the laboratory of Microbiology and Immunology Department, Faculty of Veterinary andAnimalScience,Sylhet AgriculturalUniversity, Sylhet. Total 90 feces samples were collected from Sarkar Quail and Poultry Hatchery,Kashem Quail andBreederHatchery, and Shibganj Bazar (30 samples from each farm). The numbers of positive feces samples of Quail fromSarkar Quail and Poultry Hatchery, Kashem Quail and Breeder Hatchery and Shibganj Bazar for *E. coli* were 23,25, and 26 respectively, and their percentage were 76.67%, 83.33%, and 86.67%, respectively and the resultwere compared with earlier results (El-Dosoky et al., 1999; Burns et al., 2003).
Thenumbers of positive feces samples of Quail from Sarkar Quailand Poultry Hatchery, Kashem Quail and Breeder Hatchery and Shibganj Bazar for *Salmonella* spp. were 20, 23,and 22 respectively and their percentage were 66.67%, 76.67% and 73.33% respectively and the result werecompared with earlier results (El-Dosoky et al., 1999; Burns et al., 2003) . The numbers of positive feces samples of Quail from Sarkar Quail andPoultry Hatchery, Kashem Quail and Breeder Hatchery and Shibganj Bazar for*Pseudomonas* spp. were 5, 6 and 7respectively, and their percentage were 16.67%, 20.00%, and 23.33% respectively and the result was comparedwith earlier results (El-Dosoky et al., 1999; Burns et al., 2003). The colonies characteristic of*E. col*i on different culturemedia such as NA, MAC,EMB agar were identical to other findings (Husseina et al., 2008;

Nazir et al., 2005).The cultural andbiochemical test results of*E.coli* werereportedas(Cheesbrough M. 2003). *Salmonella* spp. colony characteristics on NA, SS agar, were equivalent to other authors (Khan et al., 2005;Mondal et al., 2008). According to (Jonget al., 2012)*E.coli* bacteria demonstrated full-sugar fermentation by producing acid and gas. *Pseudomonas* spp. isusually found in the urinary bladder, respiratory tract, wounds infection, and blood, but it can also be foundelsewhere in the body. In this experiment, the bacteria was found mainly in tracheal swabs supported by (Ogunleye, A. O. 2012).*Pseudomonas* spp. Found positive in catalase test, no acid and gas producing in sugar fermentation test andnegative in indole, methyl red, and VP test; the conditions described by (Ogunleye, A. O. 2012). On blood agar, a hemolytic reactionwas observed by (Ogunleye, A. O. 2012). Antibiogram study explained that *E. coli* were found 100% sensitive to Colistin sulphateandNeomycin;followed byCiprofloxacinandchloramphenicol60%, Ampicillin20%whereas100%resistanttoAmoxicillin and Cloxacillin and 80%, 60%, and 20% case of resistance found against Ampicillin; Erythromycinrespectively. The resultswererelatedtootherresearchers (Lee et al., 2005, Hossneara et al., 2007, Akond et al., 2009; Ozaki et al., 2011).

Incaseof*salmonella*spp.found100%sensitivetoCiprofloxacin,Colistinsulphate,whichpartlyagreesw ith (Khan et al., 2005),60%sensitivetoNeomycin;40%sensitivetoChloramphenicol,andErythromycinand0%sensiti vetoAmoxicillin,Ampicillin,Cloxacillin.Theorganismwasintermediatesensitiveto ChloramphenicolandNeomycin60%and40%,respectively.*Salmonella*spp.isresistanttoAmoxicillin, Ampicillin,Cloxacillin(100%).Elsethat60%casesofresistancewerefoundagainstErythromycin.*Pse udomonas*spp.wasfound100%sensitivetoCiprofloxacinandColistinsulfatefollowedby60%sensitive toNeomycinwhereas100%resistanttoAmoxicillin,Ampicillin,Cloxacillinfollowedby80%resistanc ewasfoundagainstChloramphenicolandErythromycin. Quailbirds and their egg's nutritional value is very high and are rich in protein and poultry meat.

Theemergenceofmultidrug-resistantbacteriaisconcerning.Thisresistancemayacquireaccessintheenvironmentandhumansandan imals,potentiallyresultingindifficultiesintreatingbacterialinfectionswithmedicines.Thereisminimalr esearchconductedinquailfecesinBangladesh,andit'sanoutstandingsignificanceifweconductgoodresearch aboutthisandfindoutnewresearchoutput.Ourresearchhasmanylimitationsandalackofproperfunding;we didnotcharacterizepathogenicisolatesbyPCRtechniques.Furtherresearchneedsforthe propercharacterization ofisolates.

5 Conclusion

Quail faces samples from three different quail farms and hatchery are the main reservoirs of isolated isolatessuch as *E.coli*, *Salmonell*a spp., and *Pseudomonas* spp. In this research, we identified multidrug resistance *E. coli*.Customer consumes quailbird meat,and it creates a public

health problem due to the antibiotic resistancecharacter of *E.coli*, *Salmonella* spp., and *Pseudomonas* spp. Because of antibiotic resistance, the treatment of quailbird disease is complex. Proper control measures should be taken urgently against antimicrobial resistance of bacteriasoon.

Listofabbreviations

MA= MacConkey Agar. NA= Nutrient Agar, EMB= Eosin Methylene Blue, BGA= Brilliant Green Agar, BA= BloodAgar, SS= Salmonella - Shigella, MR= Methyl Red, VP= Voges-Proskauer, MDR=Multidrug Resistance, SAU= SylhetAgriculturalUniversity.

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Conflictofinterests

Theauthorshavenoconflict ofinterest.

Author'scontribution

Conceptualization, methodology and supervision: Dr. A.T.M Mahbub-E-Elahi. Resources:Md. Abdullah Al-Amin, Md. Safwan Hussain. Data collection: Md. Hasibul Hasan and Harunur Rashid. Manuscriptdrafting: All authors contribute to prepare manuscript and finally manuscript written by Md. Aoulad Hosen.Review: manuscript reviewed by Dr. A.T.M Mahbub-E-Elahi. Plagiarism and grammarcheck: Md. Aoulad Hosen.Finalcorrection:Md.AouladHosen,Md.HasibulHasanfinallycorrectedthemanuscript.Beforefinal submissionandpublication,allauthorsreadthemanuscriptandapprove.

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