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ORIGINAL ARTICLE

Investigation of antibiotic resistance pattern of *Staphylococcus aureus* in clinical samples of animals and humans from selective areas of Bangladesh

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Abstract

Background: *Staphylococcus aureus* (*S. aureus*) is a potential pathogen responsible for producing various infectious diseases. Determination of the prevalence of *S. aureus* infections both in animals and humans and elucidation of their antibiotic resistance pattern is crucial. The objective of this study was to find out the prevalence of Staphylococcal diseases in animals and humans, and their resistance pattern to commonly used antibiotics.

Methods: A total of 100 animal and 100 human clinical samples were analyzed by traditional method. The *S. aureus* was identified by their cultural characteristics, gram's staining, and catalase and coagulase tests. Antibiotic resistance pattern of the isolates was determined by disc diffusion method using various types of antibiotics.

Results: The prevalence of *S. aureus* in animals and humans were 54% and 40%, respectively. *S. aureus* isolates of animal origin were highly resistant against penicillin (64.81%) and oxytetracycline (42.59%), and the lowest resistance was against oxacillin (7.40 %). *S. aureus* isolates from human were also showed a higher percentage of resistance against penicillin (87.5%) then oxacillin, cloxacillin, amoxicillin (37.5% each), and lowest resistance was observed against fusidic acid (5%).

Conclusion: The findings of this study will certainly help veterinary clinicians or physicians to select appropriate antibiotics like oxacillin and fusidic acid for the treatment of different types of staphylococcal infections.

Key words: Penicillin, Oxytetracycline, Oxacillin, Clinicians, Veterinarian, Antibiogram

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Introduction

S. aureus is the most common cause of various types of infections known as staphylococcal infections. This organism was found commonly as a commensal on the human skin such as on the scalp, armpits, groins, nose (~25% humans), throat and may also be found less commonly in the colon and urine (Rahman et al., 2005; Islam et al., 2007; Gurung et al., 2020). They can also be found as commensals on domestic animals such as cattle, horses, dogs, cats, and other animals. Sir Alexander Ogston, a renowned surgeon, discovered *S. aureus* in the pus from surgical abscesses in Aberdeen, Scotland in 1880. *S. aureus* is a Gram-positive coccus which produces grape-like clusters under the microscope. On blood agar plate, the organism looks like round, largened, golden-yellow colonies with characteristic hemolysis (β-types).

The organism is responsible for causing both minor infections to major infections (life-threatening) in both humans and animals. The minor infections include pimples, impetigo, furuncles/boils, folliculitis, carbuncles (a collection of furuncles), cellulitis, and abscesses (Nwachukwu et al., 2009; Gurung et al., 2020). The life-threatening diseases include endocarditis, meningitis, pneumonia, toxic shock syndrome, septicemia, and septic arthritis, osteomyelitis etc. (Islam et al., 2019; Islam et al., 2020), and in chickens, it causes bumble foot. *S. aureus* secretes several toxins, depending on the strain. The organism causes a severe disease named staphylococcal scalded skin syndrome in infants. *S. aureus* is known as one of the crucial causal agents of mastitis in dairy animals.

The antibiotic resistant *S. aureus* has been unknown when penicillin first introduced in 1943 for the treatment of infectious diseases. (Islam et al., 2008; Chambers and DeLeo, 2009). Shortly after introducing antibiotics (e.g., by 1950) about 40% hospital isolates of *S. aureus* were resistant against penicillin and this had risen to 80% by 1960 (Rahman et al., 2005). At present, *S. aureus* has been found resistant to many commonly used antibiotics. Nowadays, due to the penicillinase (a form of β-lactamase), a very low percentage of *S. aureus* isolates were sensitive to penicillin globally (Islam et al., 2011). Then methicillin, flucloxacillin, oxacillin, and other β-lactamase-resistant penicillin were developed for the treatment of penicillin-resistant *S. aureus* which was used as first-line antibiotic agents (Ahmed et al., 2019). At present, there have been reports of resistance of *S. aureus* against β- lactamase-resistant penicillin (Chambers and DeLeo, 2009).

Resistance against antibiotic agents in bacteria was developed by bacterial gene mutations, gene rearrangement, or by the acquisition of resistant genes from other antibiotic-resistant bacteria by horizontal transfer (Dutta et al., 2013; Foster, 2017). Penicillin resistant *S. aureus* were developed by the production of penicillinase, the enzyme which breakdowns the β-lactam ring of the penicillin antibiotic (Lowy, 2003; Islam et al., 2011). Methicillin-resistant bacteria were developed by the acquisition of the *mecA* gene (Chambers and DeLeo, 2009; Dutta et al., 2013). The *mecA* gene encodes for an alternate penicillin-binding protein (PBP-2') which has a lower binding to β-lactam antibiotics (Islam et al., 2011). Thus, methicillin resistance in bacteria also develops resistance to all other β-lactam antibiotics (Ayukekbong et al., 2017). If the bacteria acquire the *vanA* gene then it becomes resistant against glycopeptides. The *vanA* gene codes for an enzyme which is responsible for producing alternative peptidoglycan to which vancomycin is not able to bind and ultimately treatment failure occurs (Dutta et al., 2013; Foster, 2017).

Infectious diseases caused by *S. aureus* and the susceptibility of *S. aureus* to commonly used antibiotics are a major concern worldwide among human and veterinary medicine for effective therapy. But a scarcity of surveillance and accuracy of the source of samples, methods of bacterial identification, methods of antibiotic susceptibility testing, guidelines for the interpretation of antibiotic susceptibility etc. of different studies have existed in many countries

of the world (WHO, 2014; Chambers and DeLeo, 2009). The present study was undertaken to find out the prevalence of staphylococcal diseases in animals and humans, and their resistance pattern to commonly used antibiotics. The findings of this study are crucial to search for antibiotics effective for clinical *S. aureus* treatment.

Materials and methods

Sample collection and preparation

Samples comprising wound swabs, pus, and various exudates were collected aseptically from different animals and humans. After cleaning the wounds with normal saline and removing the debris, swabs have taken using sterile swab sticks by applying moderate rubbing. Pus samples were also collected by immersing the swab sticks into the pus. From each patient a single type and a single sample was taken. After collection, the swabs, the swab sticks were put into sterile test tubes containing 10 ml nutrient broth medium and shifted to the laboratory for incubation.

The liquid samples such as the mastitic milk was also collected by strict aseptic precautions from the field. The teats were cleaned by normal saline just prior to collection of samples. Then milk was kept into a screw-capped vial and kept in an ice flask immediately. The samples were then kept in 4ºC temperature for few days if required. The samples from the refrigerator were carried to the laboratory by using ice flask. In the laboratory, the samples were inoculated into the Nutrient broth media. The animal origin clinical samples were collected from Bangladesh Agricultural University, Mymensingh and Milk Vita, Sirajganj, Bangladesh and human clinical samples were collected from Mymensingh Medical College Hospital (MMCH), Mymensingh, Bangladesh from January to June 2007.

Isolation and identification of *S. aureus*

Isolation and identification

Nutrient broth media of various types of clinical specimens were incubated in an incubator. When bacteria were grown, there was found the development of turbidity and sometimes formation of sediment after 24 hours. The positive clinical samples then subsequently re-cultured into the Nutrient agar and Blood agar media. On Nutrient agar media, 2-4 mm circular, golden yellow pigment producing, and convex shining surface colonies were detected as *S. aureus*. On Blood agar media, yellow to cream or sometimes white colonies with 1-2 mm in diameter showing zone of β hemolysis after overnight incubation was suspected as *S. aureus*. All the media were incubated for 24 hours at 37ºC in the incubator.

Gram's staining method

Gram's staining method was applied as described previously (Tenover and Hirschmann, 1990). After staining, the slide was examined under a microscope.

Subculturing on Mannitol Salt agar media

On Mannitol Salt Agar media, *S. aureus* was cultured and bacteria produce large colonies surrounded by yellow zones which are characteristic for *S. aureus*.

Biochemical tests

The biochemical test was applied as described previously (Cheesbrough, 2000). Both catalase and coagulase tests were used for confirmation of *S. aureus*.

Antibiogram study

Antibiogram study for the isolated *S. aureus* was performed by disc diffusion method (Kirby-Bauer technique) (Bauer et al., 1966) as per the recommendation of National Committee for Clinical Laboratory Standards (NCCLS, 1997). Various types of antibiotics were tested, and the tests were performed on Mueller-Hinton agar plate. In brief, suspension of *S. aureus* was prepared to have equivalent turbidity to 0.5 McFarland standards. Then the suspension of *S. aureus* was taken out by a cotton swab squeezing

on the wall of the test tube. Then the surface of the Mueller-Hinton agar plate was lightly and uniformly inoculated by cotton swab (Islam et al., 2008). The inoculated plates were incubated at 35ºC for 24 hours. After incubation, the zone of inhibition of specific antimicrobials on agar plates was measured (NCCLS, 1997). Then the results were interpreted as sensitive (S) or resistant (R) according to the reference zone of inhibition of specific antibiotics. The known strain e.g., ATCC 25923 of *S. aureus* has used as a control for maintaining the quality.

Results

The cultural characteristics, staining characteristics, and biochemical characteristics of *S. aureus* shown in Fig. 1. The antibiotic susceptibility pattern of isolated *S. aureus* shown in Fig. 2. A total of 100 animal and 100 human clinical samples were examined. Majority of the animal's origin specimens were collected from cattle 87 (87%) followed by goats 10 (10%) and then buffalo 3 (3%). Most of the specimens were mastitic milk 62 (62%) followed by pus 21 (21%), wound swab 17 (17%) (Table 1). All the culture-positive specimens were catalase positive.

The overall prevalence of *S. aureus* in animal samples was 54%. However, the prevalence of *S. aureus* in mastitic milk, pus and wound swab were 39%, 8% and 7% respectively (Table 1).

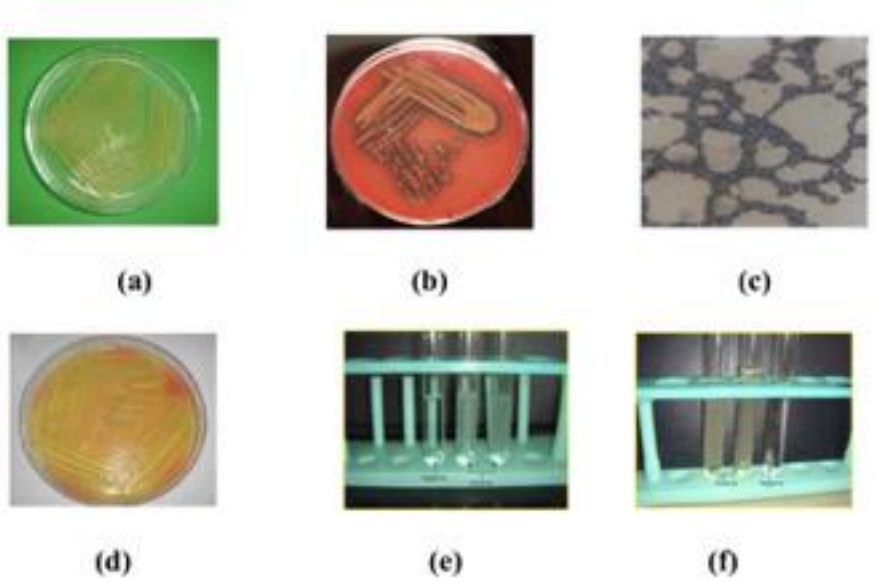
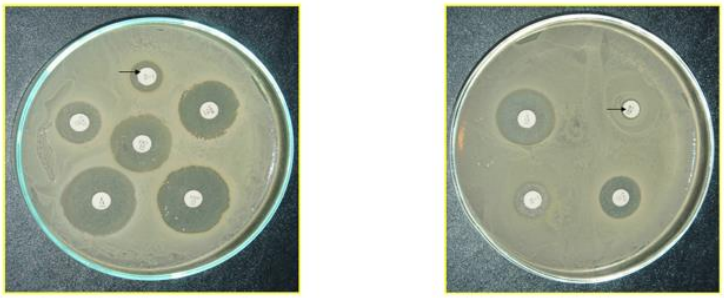


Fig. 1 (a). Nutrient agar media showing growth of *S. aureus*. Fig. 1 (b). Blood agar media showing growth of *S. aureus* with hemolysis. Fig. 1 (c). Gram-positive spherical cells of *S. aureus* arranged in grape-like clusters. Fig. 1 (d). Mannitol Salt Agar media showing yellowish colour after growth of *S. aureus*. Fig. 1 (e). Catalase test showing gas bubbles as an indication of positive reaction for *S. aureus* in two of three test tubes. Fig. 1 (f). Coagulase test showing positive coagulase reaction in two of three test tubes (conversion of plasma into a soft and/or stiff gel).



a b

Fig. 2 (a). Arrow indicator antibiotic sensitivity disc in Mueller Hinton agar media showed Oxacillin sensitive *S. aureus*. Fig. 2 (b). Arrow indicator antibiotic sensitivity disc in Mueller Hinton agar media showed Oxytetracycline resistant *S. aureus*

Table 1. Detection of *S. aureus* from various clinical specimens of different type of animals

Specimen type	Species of animal			Total	
	Cattle	Goat	Buffalo	Number of Specimens	Detection of *S. aureus* (%)
Mastitic Milk	62	-	-	62	39 (62.9)
Pus	11	10	-	21	8 (38.1)
Wound swab	14	-	3	17	7 (41.2)
Total	87	10	3	100	54 (54.0)

Table 2. Detection of *S. aureus* from different clinical specimens of human

Specimen type	Number of specimens	Detection of *S. aureus* (%)
Swab from surgical wound	50	17 (34.0)
Exudate from burn ulcer	14	4 (28.6)
Swab from infected ear	11	8 (72.7)
Skin infection pus	19	9 (47.4)
Exudate from diabetic ulcer	5	1 (20.0)
Swab from vaginal infection	1	1 (100.0)
Total	100	40 (40.0%)

Table 3. Antibiogram study for *S. aureus* isolated from different domestic ruminants by disc diffusion method (n = 54)

Name of Antibiotics	Sensitive	Resistant
Penicillin	19 (35.19%)	35 (64.81%)
Oxacillin	50 (92.60%)	4 (7.40%)
Ampicillin	40 (74.07%)	14 (25.93%)
Amoxycillin	34 (62.96%)	20 (37.04%)
Sulphamethoxazole (Trimethoprim)	34 (62.96%)	20 (37.04%)
Oxytetracycline	31 (57.41%)	23 (42.59%)
Gentamicin	40 (74.07%)	14 (25.93%)
Streptomycin	41 (75.93%)	13 (24.07%)
Tobramycin	47 (87.04%)	7 (12.96%)
Erythromycin	47 (87.04%)	7 (12.96%)
Ceftriaxone	47 (87.04%)	7 (12.96%)
Cephradine	48 (88.89%)	6 (11.11%)

Table 4. Antibiogram study for *S. aureus* isolated from different clinical specimens of human by disc diffusion method (n = 40)

Name of Antibiotics	Sensitive	Resistant
Penicillin	5 (12.5%)	35 (87.5%)
Oxacillin	25 (62.5%)	15 (37.5%)
Amoxycillin	25 (62.5%)	15 (37.5%)
Cloxacillin	25 (62.5%)	15 (37.5%)
Cephradine	30 (75%)	10 (25%)
Gentamicin	32 (80%)	8 (20%)
Ceftriaxone	32 (80%)	8 (20%)
Vancomycin	33 (82.5%)	7 (17.5%)
Ciprofloxacin	34 (85%)	6 (15%)
Erythromycin	34 (85%)	6 (15%)
Cefuroxin	34 (85%)	6 (15%)
Doxycycline	35 (87.5%)	5 (12.5%)
Rifampicin	37 (92.5%)	3 (7.5%)
Fusidic acid	38 (95%)	2 (5%)

The human clinical samples were collected from hospital admitted patients. The distribution of human samples was presented in Table 2. All the culture-positive specimens were catalase positive. The overall prevalence of *S. aureus* in human specimens was 40%. However, the prevalence of *S. aureus* in different human specimens varied from 20-100% (Table 2).

Antibiotic resistance pattern of animal origin *S. aureus* isolates was presented in Table 3 (n = 54). The highest resistance was found against penicillin (64.81%). Antibiotic resistance pattern of human origin *S. aureus* was shown in Table 4. Most isolates of human samples were resistant to penicillin (87.5%).

Discussion

The prevalence of *S. aureus* in human and animal samples were determined in this study. Moreover, the antibiogram of the isolates were also studied. Oxacillin and fusidic acid for the treatment of

animal and human diseases due to *S. aureus* can be recommended based on their antibiogram profile. The diseases caused by *S. aureus* in the animal population are quite high (54%). A similar finding is also reported in a recent study (Anueyiagu et al., 2020). The most likely reason for the higher prevalence might be due to the commensal nature of *S. aureus*. They can produce fatal infections to animals during stress conditions (Rahman et al., 2005; Chambers and DeLeo, 2009; Islam et al., 2011). Among all the animal specimens, the prevalence of staphylococcal mastitis was 62.90%. Several authors also reported similar prevalence of staphylococcal mastitis (Plozza et al., 2011; Abebe et al., 2016). The higher prevalence of staphylococcal mastitis in the animal population may be due to substandard hygiene, high density of milking cows, lack and/or no teat dipping after milking (Abebe et al., 2016). It has been revealed that milk from the cows affected by mastitis have a potential public health significance. So, measures should be taken to prevent mastitis caused by *S. aureus*.

Half of the human clinical specimens were wound swabs which indicates that wound is a common affection among clinical patients admitted in the hospital. In favorable conditions, *S. aureus* might produce abscess on the wound and ultimately there might be septicemia and other fatal infections (Rahman et al., 2005; Nwachukwu et al., 2009). Previously, swabs from wound were also reported as major specimens in the bacteriological laboratory by other authors (Pandey et al., 2012; Gurung et al., 2020). The higher proportion of the positive culture of wound specimen might be due to the higher number of wound infection in clinical patients in hospitals. Because of the poor hygiene in the hospital, unhygienic water supply for hand washing (Aiken et al., 2014), prolonged hospitalization and overcrowded patients, and lack of expert staffs favor the spread of *S. aureus* in hospital (Aiken et al., 2014; Ahmed et al., 2019). Besides, it is well known that wound infection usually caused by multiple-resistant *S. aureus* (multi-drug resistant *S. aureus*). So, to reduce the morbidity of surgery patients with wounds, the clinician should dispatch the wound specimen to the laboratory for culture, and sensitivity to select effective antibiotic.

The culture positivity of human samples was 70% which is similar to a previous study (Anupurba et al., 2003). The higher number of culture-positive samples were due to the lack of hygiene in the hospitals. Thus, for the prevention of nosocomial infections, the standards of hygiene in the hospitals should be improved (Chambers and DeLeo, 2009; Aiken et al., 2014). The overall prevalence of *S. aureus* in human specimens (40%) is similar with the findings of previous studies (Shibabaw et al., 2014; Suzuki et al., 2020) and are considered as quite high. The majority of *S. aureus* (42.5%) were from surgical wound swab. The higher proportion of *S. aureus* from the wound was also reported previously (Nwachukwu et al., 2009; Pandey et al., 2012; Roy et al., 2017). The prevalence of *S. aureus* infection in clinical patients in hospital was high because *S. aureus* remains as commensals on the skin (Rahman et al., 2005; Chambers and DeLeo, 2009; Gurung et al., 2020). From skin, *S. aureus* spread to the infection site if the wound surface of the patient remains exposed.

Antimicrobial resistance of the pathogenic bacteria is of great concern both in human and veterinary medicine worldwide and is a significant problem in the treatment of human and animal diseases caused by pathogenic *S. aureus* (Chambers and DeLeo, 2009; Dutta et al., 2013; Ahmed et al., 2019). *S. aureus* has become resistant to many commonly used antibiotics through the acquisition of antibiotic resistance genes (Foster, 2017). A major factor is the acquisition of resistance genes from other resistant bacteria (e.g., the spread of plasmid-encoded multidrug transporters) and self-acquisition of antibiotic-resistant genes (e.g., overexpression of endogenous multidrug transporters) due to irrational antibiotic prescribing by physicians and surgeons, a habit of self-medication among patients in developing countries, and indiscriminate use and huge consumption of antibiotics in animal husbandry,

and agriculture farming globally (Lowy, 2003; Foster, 2017). Nowadays, we have to combat these insidious enemies by finding effective therapeutic agents (Dutta et al., 2013).

In antibiotic resistance test, animal origin samples exhibited a higher resistance against penicillin which is similar with the findings of previous studies (Kurjogi and Kaliwal, 2011). The most likely reason for the higher resistance of penicillin among dairy cows might be due to the habit of indiscriminate use of antibiotics in livestock farming (Chambers and DeLeo, 2009; Mostafa and Kumar, 2012; Sutradhar et al., 2014; Biswas et al., 2014). These findings indicate that choice of penicillin for the treatment of staphylococcal disease in the animal are not a good option. This might cause failure to cure diseases of animals as well as there might be chances of transmission of antibiotic resistance bacterial genes to the human cycle through meat, milk, eggs, and other animal products (Ahmed et al., 2019). So judicious use of antibiotics for the treatment of staphylococcal diseases are suggested (Foster, 2017). Choice of alternate antibiotics, controlled use of antibiotics through strict regulations, educating the general public and health care persons (Chen et al., 2016) about hazards of antibiotics might reduce antibiotic-resistant microorganisms globally.

The antibiotic resistance pattern of *S. aureus* isolates from human samples are similar to that described previously (Naik and Teclu, 2009; Chen et al., 2016; Gurung et al., 2020). The probable reason behind this resistance phenomenon might be due to the acquisition of a plasmid and chromosome mediated resistance genes as reported by a previous study (Chamberland et al., 2001). Relatively higher resistance of *S. aureus* against penicillin was detected in human samples compared to animal samples which is similar to the findings of a previous study (Pandey et al., 2012). The most likely reason for this might be due to extensive use of penicillin for the therapy of human diseases caused by *S. aureus* (Lowy, 2003). Other factors might also be responsible for the development of penicillin resistance are the misuse of penicillin, poor quality drugs, undernutrition, compromised immunity, substandard health care facilities, inability to afford costly but more effective drugs (Chambers and DeLeo, 2009; Aiken et al., 2014; Ayukekbong et al., 2017). So relevant authorities should take appropriate measures to formulate and market other alternative antibiotics to prevent treatment failure caused by commonly used penicillin.

Besides penicillin, other tested antibiotics showed some form of resistance (multidrug-resistant) against *S. aureus* which is similar to the findings of previous studies (Erami et al., 2014; Neyra et al., 2014; Chen et al., 2016; Roy et al., 2017; Anueyiagu et al., 2020). The lowest percentage of animal origin *S. aureus* were resistant to oxacillin and the lowest percentage of human origin *S. aureus* were resistant to fusidic acid. The lowest resistance of animal origin *S. aureus* to oxacillin is due to the absence of oxacillin preparation in the local market for the therapy of animal diseases (Islam et al., 2011). The present findings indicate that except penicillin, all other tested antibiotics could be used for the treatment of animal and human diseases caused by *S. aureus*. But every nation should undertake and implement effective surveillance programs (antibiotic stewardship programs) following standardized methodologies to find out the development of new antimicrobials resistance (Sumon et al., 2018) to prevent the spread of antimicrobial-resistant *S. aureus* globally through humans, animals, animal products, and environment etc. (Sjölund et al., 2008; Dutta et al., 2013; Haque et al., 2020).

One of the main limitations of the present study is that the disc diffusion method cannot detect low levels of antibiotic resistance in microorganisms. Future studies should undertake to find out resistance of microbes against different antibiotics by using molecular detection techniques. But such methods are costly and requires highly experienced personnel.

Conclusion

The infections caused by *S. aureus* both in humans and animals could be considered as high. *S. aureus* isolates from both animals and humans exhibit a higher percentage of resistance against penicillin. So, care should be taken during use of penicillin for the therapy of infections caused by *S. aureus* in animals and humans. It is essential to find alternate antimicrobials for the treatment of diseases caused by *S. aureus* in both animals and humans to prevent the spread of staphylococcal resistance genes among animal health, human health, and environment. Novel technologies leading to rapid and accurate diagnosis of *S. aureus* infection and improved understanding of the pathogenesis and pathology of the *S. aureus* diseases are crucial. Along with the antibiotic approach, non-antibiotic approaches are essential for the prevention of the spread of multi-drug resistant *S. aureus* infections globally.

Conflict of Interest

None to declare

Ethical considerations

We did not use experimental animals or humans. Thus, approval was not required but verbal permission was taken from the Ethical Committee.

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None

Author contributions

M. A. Islam: sample collection, sample analysis, writing original draft manuscript M. S. Uddin: sample collection, sample analysis. M. J. Islam: sample collection, sample analysis M. U. Ahmed: supervision, critical evaluation of the manuscript, M. M. Alam: supervision, critical evaluation of the manuscript.

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