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Isolation, identification and antibiogram of bacterial flora from rectum of horses

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Abstract

In the present study, we isolated, identified and characterized bacteria in rectal swabs from apparently healthy horses around the campus of the Bangladesh Agricultural University (BAU). The study also focused on the determining the prevalence and investigation of drug sensitivity and resistant pattern of the isolates. For this purpose, 20 rectal swab samples were collected from Veterinary Teaching Hospital, BAU; D. C. park (Joinul Abedin Park), Mymensingh and Torar mor, Mymensingh. Most of these samples were found positive for *E. coli, Staphylococcus spp*. and *Salmonella spp.* Based on the origin of the samples, the highest prevalence of *E. coli, Staphylococcus aureus and Salmonella spp*. was found in BAU campus and Torar mor (100%), BAU campus and Torar mor (100%) and Joinul Abedin Park (80%) respectively. The overall prevalence of *E. coli, S. Aureus* and *Salmonella spp.* was 93%, 86.67% and 24.44% respectively. For the confirmation of identification and characterization of these bacteria staining, cultural, biochemical studies and Polymerase Chain Reaction (PCR) were accomplished. The antibiogram profiles exhibited that *E. coli, S. Aureus* and *Salmonella spp*. were highly sensitive to Erythromycin, Gentamycin and Ciprofloxacin respectively

Keywords: Isolate; Characterize; Bacterial flora; Prevalence; Polymerase Chain Reaction (PCR)

1. Introduction

Enterococci are commonly found in the gastrointestinal tract of healthy humans and animals [1]. They are Gram-positive facultative anaerobic bacteria, spherical shaped, which occur singly, in pairs or short chains and fit within the general definition of lactic acid bacteria[2]. This intestinal bacterium can be easily disseminated in different ecosystems. For this reason, fecal *E. coli* is considered to be an important indicator for the selective pressure exerted by the use of antimicrobials on intestinal populations of bacteria. The emergence of multi resistant *E. coli* has been previously reported in humans and in different animal species, increasing the public health concern [3]. Foals are mostly vulnerable to *E. coli* infection. Symptoms include diarrhea, weakness, a rise in temperature, lack of appetite and abdominal pain. In older animals there is a tendency of infection to localize itself in the joint of survivors. Lesions include enlarged, hemorrhagic spleens and the accumulation of synovial fluid and sometimes pus in affected joints[4]. *E. coli* can be grown easily and inexpensively in a laboratory. *E. coli* in horse can be isolated, identified and characterized using cultural, morphological, biochemical, serological and molecular techniques. [5], [6].*Staphylococci* also have a characteristic glistening, opaque and yellow to white appearance on blood agar. Patterns of α or β hemolysis may also be visible.

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Further identification of staphylococcal isolates is available using commercial test kits. *Staphylococcus* may also be identified by phage typing or by 16S ribosomal DNA typing. Salmonellosis is a disease condition caused by a wide variety of *Salmonella* spp. various hosts including horse which act as a causal agent of serious problem of oneself with public health significance throughout the world [7]. Antimicrobial resistance is a public health issue of growing concern. The use of antimicrobials can lead to the development of antimicrobial resistance in bacterial species[8].Patterns of microbial diversity in the G.I. tract have important implications for human and environmental health. Accordingly, characterization of the equine intestinal microbe is critical, since a good understanding of the 'normal' intestinal microbial is needed for interpretation of 'abnormal'. Most investigations of the equine microbes have typically involved bacterial culture of feces or intestinal contents. It is well established that the enteric bacteria are responsible as the predominant agent in bacterial abortion of mares and that they are one of the significant secondary invaders in equine infectious anemia. In other diseases of the horse, these bacteria seem to play a role as the causative agent. Though the necessity of studying the enteric bacterial flora in the intestinal tract of healthy horses has been recognized for a long time, no real study has been made for the lacking of a suitable method for classification of the enteric bacteria. In Bangladesh, the diagnosis of abdominal disorder and diarrhea in horse is being done only by symptoms. But for the confirmatory diagnosis- it needs proper isolation, identification, characterization and their antibiogram detection [9].To the best of our knowledge only one work has been carried out in Bangladesh around the Bangladesh Agricultural University by [6]. However, the present study reports on the enteric bacterial flora of the intestinal tract of apparently healthy horses in and around BAU campus. Therefore, the objectives of this study were: to investigate the prevalence of various bacteria from the GI tract of apparently healthy horse, to isolate and identify bacterial agent present in the GI tract of apparently healthy horses, and to determine the antibiogram of the isolated bacteria.

2. Material and methods

2.1. Study area and population

The research work was conducted in the department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh 2202 the period from January 2018 to November 2018. Samples were cottected from horses from teaching veterinary hospital, BAU; D. C. park (Joinul Abedin Park), Mymensingh and Torar mor, Mymensingh. Horses brought to the hospital for treatment purposes were selected for sampling

2.2. Collection of samples

In total, 20 samples comprising rectal swabs from TVH (n = 6), D.C park (n = 10), and Torar mor (n = 4) were collected from Horses. Horse were weakness and some skin problem were sampled during the study period. The samples were collected using sterile cotton and immediately transferred to Stuart's transport medium (Oxoid, Basingstoke, UK) and stored at 80 °C in the laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Bangladesh, for further use [10] .

2.3. Bacteriological investigation

Escherichia coli, Salmonella spp and Staphylococcus spp. were isolated and identified from the collected samples based on their cultural properties, biochemical tests, including pigment production, and hemolytic activities [11],[10].

2.3.1. Escherichia coli

The collected rectal swab samples were enriched overnight in buffered peptone water (BPW) (Oxoid Ltd., Basingstoke, UK). A loopful of the enriched broth was inoculated onto MacConkey agar (Oxoid Ltd, Basingstoke, UK) and incubated for 24 h at 37°C. The suspected large pink color colonies were subculture onto eosin-methylene blue agar (Oxoid Ltd, Basingstoke, UK) and incubated for 24 h at 37 °C. Colonies which produced a typical metallic sheen were subculture onto the blood agar and further confirmed by the Gram stain properties and biochemical tests.

2.3.2. Staphylococcus spp.

The collected rectal swabs were enriched overnight in BPW at 37 °C. One loopful of the enriched broth was directly streaked onto the Mannitol Salt Agar (MSA) (Oxoid Ltd., Basingstoke, UK) and incubated for 24 h at 37 °C. The susceptive positive colonies were identified based on the colony characteristics on the MSA. The suspected bright yellow positive colonies were then subculture onto the blood agar and incubated at 37 °C for 24 h to detect characteristics appearance on the blood agar and the hemolytic properties of the organism [5]. Then, the organism was confirmed by the Gram stain properties and several biochemical tests.

2.3.3. Salmonella Spp.

The collected rectal swabs were inoculated in screw cap test tube containing buffered Peptone Water (BPW) (primary enrichment media) and incubated for 24 hours at 37 °C. After primary enrichment sample from buffer peptone was picked up and streaked on BGA (Merck, PH: 6.9±0.2), XLD and Blood agar Agar (Oxoid Ltd., PH: 7.3±0.2). The agar plates then were incubated at 37 °C for 24 hours. After development of characteristic colony Then, the organism was confirmed by the Gram stain properties and several biochemical tests (TSI stab, Oxoid Ltd., PH: 7.2±0.2) to confirm Salmonella.

2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for all positive isolates was carried out using the disk diffusion technique suggested by the Clinical Laboratory and Standards Institute[17]. The ATCC25922 was used for superiority control during the disk diffusion technique. A total of 12 antimicrobials of different groups were used at the given disk content: penicillin (6 µg), ampicillin (10 µg), ephradine (30 µg), ceftriaxone (30 µg), erythromycin (15 µg), azithromycin (15 µg), gentamycin (30 µg), oxytetracycline (30 µg), doxycycline (5 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), and sulfamethoxazole/trimethoprim (25 µg). These antimicrobials were selected based on the history of commonly used antimicrobials at the TVH. The interpretation of the test results was made according to the CLSI guidelines [17].

2.5. Statistical analysis

Laboratory data obtained were entered into MS Excel 2010 spreadsheets and were analyzed using the R package [19].

2.6. DNA extraction

The conventional crude boiling method was used to extract the genomic DNA of E. coli isolates [18].In brief, 2–3 fresh colonies were taken in a sterile 1.5-ml micro centrifuge tube containing 200-µl sterile Milli-Q water and vortexed thoroughly. After heating the micro centrifuge tube at 99°C for 10 min, it was rapidly frozen at −20 °C and centrifuged at 13,000 rpm for 5 min. Finally, the supernatant (100 µl) was collected and used as the DNA template in polymerase chain reaction (PCR), followed by storing at −20 °C for further use.

2.7. Statistical analysis

Laboratory data obtained were entered into MS Excel 2010 spreadsheets and were analyzed using the R package [19]. The heatmap of antimicrobial susceptibility testing phenotype was generated by using Graphpad Prism 7.0.

3. Results

The present study was conducted for the isolation, identification and characterization of bacteria in the rectal swab of healthy horses in and around BAU campus. In addition, determination of antibiotic sensitivity profile of the isolated bacteria was also carried out.

3.1. Bacterial flora isolated from rectal swab of apparently healthy horses

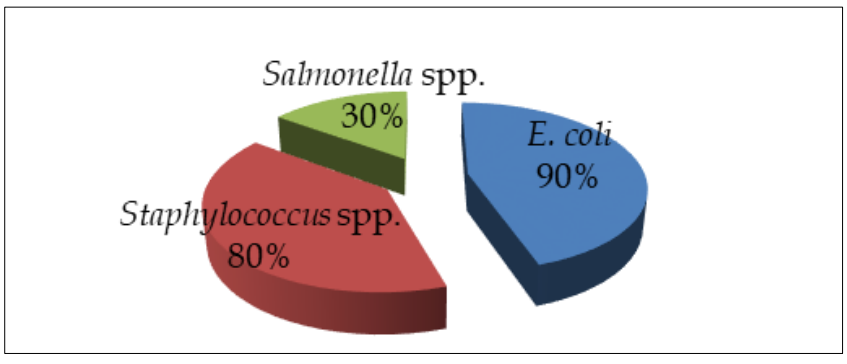

Figure 1 Percentage of isolated bacteria from rectal swab of apparently healthy horses in and around BAU campus

3.2. Prevalence of the isolated bacteria

3.2.1. Prevalence of E. coli

Table 1 Prevalence of *E. coli* in apparently healthy horse

Sources of sample	No of sample tested	No. of isolates of E. coli	Prevalence
BAU campus	6	6	100%
D.C. Park	10	8	80%
Torar mor	4	4	100%
Overall	20	18	90%

3.2.2. Prevalence of S. aureus

Table 2 Prevalence of *Staphylococcus* spp. in the rectal swab of apparently healthy horses

Sources of sample	No of sample tested	No. of isolates of Staphylococcus spp.	Prevalence (%)
BAU campus	6	6	100%
D.C Park	10	6	60%
Torar mor	4	4	100%
Overall	20	16	80%

3.3. Identification of *E. coli* by cultural examination findings

3.3.1. Culture on Nutrient agar, EMB agar & Mac Conkey agar

On nutrient agar the *E. coli* produce smooth, circular, and white to grayish white colony. The isolated *E. coli* produced smooth, circular colonies with metallic sheen on EMB agar and on M'c Conkey agar, the isolated *E. coli* produced large, bright pink colored colony.

3.3.2. Culture on Blood agar, Xylose-Lysine-Deoxycholate (XLD) Agar and identification of E. coli by microscopic examination through Gram's staining.

On Blood agar media the isolated organism produced small colorless colony with discoloration due to haemolysis. The organism produced large, flat, yellow colored colonies on XLD agar plates. In Gram's staining under microscope, the organism revealed Gram-negative, Pink colour, small rod shaped arranged as single or paired.

Table 3 Results of cultural, morphological and motility characteristics of *E. coli*

Sources of isolates	Cultural characteristics on					Staining characteristics	Motility
	Nutrient agar	EMB agar	Blood agar	XLD agar	Mac Conkey agar		
BAU campus	Smooth, circular, White to grayish white colony	Smooth, Large, circular, blue-black colonies with green metallic sheen	Colonies without hemolysis	Partial to complete; Large, flat, yellow colonies	Smooth, bright pink colored colony	Pink, short rod, Gram- negative, single or pair or in short chain	+
D.C. park							
Torar mor							

Legends: EMB = Eosin Methylene Blue, XLD= Xylose-Lysine-Deoxycholate agar.

3.4. Identification of *E. coli* by biochemical tests

The organisms were checked and confirm their purity using selected media (EMB agar). Then they were grown in NB for biochemical test. For identification, a series of biochemical tests selective for *E. coli* were performed with the culture.

3.5. Results of sugar fermentation

All five basic sugars i.e., dextrose, maltose, lactose, sucrose and mannitol were fermented by *E coli* with acid and gas production indicated by the color change from reddish to orange yellow color and the gas production was manifested by the appearance of gas bubbles in the inverted Durham's tubes.

Table 4 Sugar fermentation test of *E. coli*

Name of the isolates	Fermentation characteristics	Name of the basic sugars				
		Dextrose	Maltose	Lactose	Sucrose	Mannitol
E. coli	Acid production	+	+	+	+	+
	Gas production	+	+	+	+	+

Legends: + = Positive

3.6. Results of other biochemical tests

All the *E. coli* isolates were MR positive, VP test negative and Indole test positive.

Table 5 Other Biochemical characteristics of *E. coli*

Name of the isolates	MR test	VP test	Indole test
E. coli	+	-	+

Legends: + = Positive; - = Negative

3.7. Identification of *E. coli* by PCR

Out of nine isolates, three isolates of *E. coli* were run for PCR confirmation and they showed positive result with molecular detection which is shown in Fig. 2

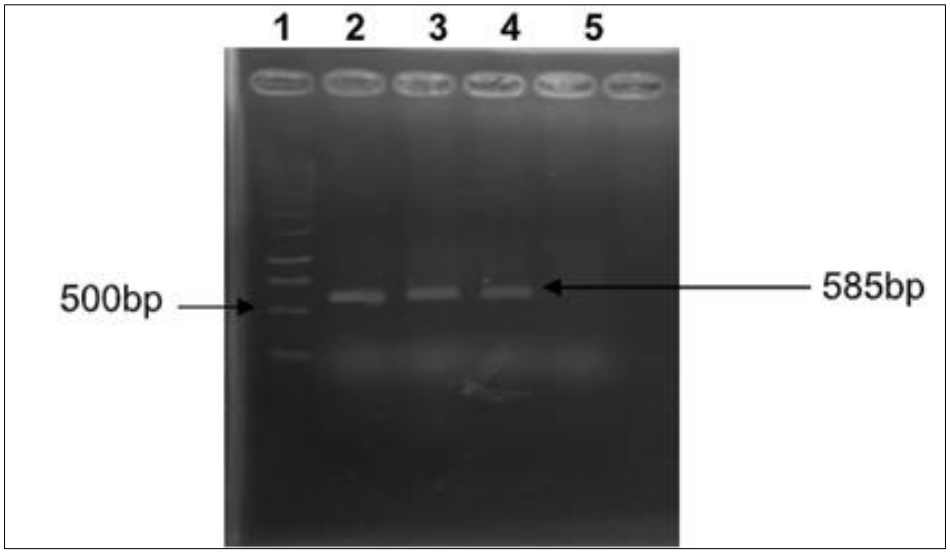

Figure 2 Detection of E. coli by PCR using EC16S rRNA primer. Lane-1, DNA marker, lane 2-4 E. coli isolates, Lane-5 negative control

3.8. Antibiotic sensitivity profile

A total of 2 isolated *E. coli* were analyzed for the antibiogram susceptibility. The overall susceptibility pattern of *E. coli* isolates presented in Table. The table data showed that the *E. coli* isolates were significantly 100% resistant to Ampicillin and Ciprofloxacin, 66.7% resistant to Amoxicillin and Gentamycin, 66.7% sensitive to Erythromycin.

Table 6 Antibiotic sensitivity pattern of *Escherichia coli*

Name of the bacteria	No of samp-les	Name of the antibiotics				
		Ampicillin (AMP)	**Amoxicillin (AMX)**	**Ciprofloxacin (CIP)**	**Gentamycin (GEN)**	**Erythromycin (E)**
E. coli	1	R	R	R	I	R
	2	R	R	R	R	S
	3	R	I	R	R	S

Legend: R= Resistant, I= Intermediate sensitive, S= Sensitive

3.9. Identification of *Staphylococcus* spp. by cultural examination findings

3.9.1. Culture on Nutrient broth

In nutrient broth, the growth of *Staphylococcus* spp. was characterized by diffused turbidity.

3.9.2. Culture on Nutrient Agar, Blood agar and Mannitol Salt Agar (MSA)

On nutrient agar media small, circular and smooth raised gray white or yellowish colonies were observed by *Staphylococcus* spp.; on blood agar media produced circular, small, smooth and raised colony colored as gray white or yellowish, hemolysis was seen on BA media; the production of yellowish colonies and some plates showed whitish colony on MSA.

Table 7 Results of cultural, morphological and motility characteristics of *Staphylococcus* spp

Sources of samples	Cultural characteristics on			Staining characteristics
	Nutrient agar	**Mannitol salt agar**	**Blood agar**	
BAU Campus,	Small, circular, smooth raised yellowish colonies.	Small, circular, yellowish colonies with fermentation.	Circular, smooth raised yellowish colonies.	Gram positive, violet colored, cocci shaped, arranged in grapes like cluster.
D.C. Park				
Torar mor				

3.10. Identification of *Staphylococcus* spp. by biochemical tests

3.10.1. Results of fermentation reaction with five basic sugars

All the tested isolates fermented dextrose, maltose, lactose, sucrose and mannitol and produced only acid. Acid production was indicated by the color change from reddish to yellowish. No gas production was noticed as there were no gas bubbles in the inverted Durham's tubes.

Table 8 Summary of the results of sugar fermentation test of *Staphylococcus* spp. isolated from rectal swab of horses

Name of the isolates	Fermentation characteristics	Name of sugars				
		Dextrose	**Maltose**	**Lactose**	**Sucrose**	**Mannitol**
Staphylococcus spp.	Acid production	+	+	+	+	+
	Gas production	_	_	_	_	_

Legend: + = Positive, - = Negative

3.10.2. Results of catalase test

Catalase test was performed to differentiate *Staphylococci* (catalase producer) from *Streptococci* (non-catalase producer). Hydrogen peroxide was breakdown into water and oxygen. Production of oxygen was indicated by the

bubble formation. All *Staphylococcus* isolates were catalase positive. Out of 8 *Staphylococcus* isolated sample all showed positive result to catalase test. The catalase test was done by slide method.

3.10.3. Results of coagulase test

All *Staphylococcus* isolates gave positive reaction in coagulase test indicated that they were pathogenic *S. aureaus*. On the other hand, no isolates were found to be coagulase negative. The positive result was confirmed by the formation of curd like clotting compare to negative control where there is no formation of curd like clotting.

3.11. Antibiotic sensitivity pattern of *S. aureus*

The antibiotic sensitivity profiles are also studied for the coagulase positive *S. aureus* isolates from the feces of horse. A total of 3 isolated *S. aureus* were analyzed for the antibiogram susceptibility. The overall susceptibility pattern of ***S. aureus*** isolates presented in Table. The table data showed that the *S. aureus* isolates were significantly 100% resistant to Ampicillin, 100% sensitive to Gentamycin, 66.67% resistant to Amoxicillin and Erythromycin, 33.3% sensitive to Ciprofloxacin.

Table 9 Antibiotic sensitivity pattern of *S. aureus*

Name of bacteria	No. of samples	Name of the antibiotics				
		Ampicillin (AMP)	Amoxicillin (AMX)	Ciprofloxacin (CIP)	Gentamycin (GEN)	Erythromycin (E)
S. aureus	1	R	R	I	S	R
	2	R	R	S	S	R
	3	R	I	I	S	I

Legend: R= Resistant, I= Intermediate sensitive, S= Sensitive

3.12. Identification of *Salmonella* spp. by cultural examination findings

3.12.1. Culture on Nutrient broth

The growth of the isolated organism in NB was characterized by diffused turbidity.

3.12.2. Culture on Nutrient Agar, Salmonella-Shigella (SS) Agar and Blood Agar & Xylose-Lysine-Deoxycholate (XLD) Agar

On nutrient agar the isolated organism produce circular, smooth, opaque and translucent colonies; On SS agar, the isolates produced opaque, translucent, black, smooth and round colonies with black center and produces well to excellent, opaque colonies with black centers in XLD agar.

3.13. Results of microscopic examination after Gram's staining

The microscopic examination of Gram's stained smears revealed Gram-negative, pink colored, small rod shaped appearance, arranged in single and paired.

Table 10 Results of cultural and morphological characteristics of *Salmonella* spp

Sources of sample	Cultural characteristics on			Staining Characteristics
	Nutrient agar	SS agar	XLD agar	
BAU Campus	Circular, smooth, opaque, and translucent colonies	Opaque, translucent, black and round colonies with black center	Colony with black centers	Gram-negative, pink colored, small rod shaped, arranged in single and paired.
D.C. Park				
Torar mor				

Legend: SS= Salmonella-Shigella, XLD= Xylose-Lysine-Deoxycholate

3.14. Results of biochemical tests of *Salmonella* isolates

3.14.1. Results of fermentation reaction with five basic sugars

All of the *Salmonella* spp. isolates fermented dextrose, maltose and mannitol and produce acid and gas but did not ferment lactose and sucrose.

Acid production was marked by the colour change from reddish to yellow and the gas production was noted by the presence of gas bubbles in the inverted Durham's tubes kept inside each of the test tubes containing sugar media.

Table 11 Biochemical characteristics of *Salmonella spp.* Isolates

Name of bacteria	No. of samples	Name of the antibiotic discs				
		Ampicillin (AMP)	Amoxicillin (AMX)	Ciprofloxacin (CIP)	Gentamycin (GEN)	Erythromycin (E)
Salmonella spp.	1	I	R	S	S	R
	2	R	R	S	S	R
	3	R	I	S	I	R

Legend: + = Positive; - = Negative

3.15. Results of other biochemical tests

All the *Salmonella* spp. isolates were methyl-red positive, VP test negative and Indole test negative.

Table 12 Other Biochemical characteristics of *Salmonella* spp.

Name of the isolates	Indole test	MR test	VP test
Salmonella spp.	-	+	-

Legend: + = Positive; - = Negative

3.16. Antibiotic sensitivity profile

The antibiotic sensitivity profiles are also studied for the *Salmonella* spp. isolates from the rectal swab of horse. A total of 3 *Salmonella* spp. isolated were analyzed for the antibiogram susceptibility. The overall susceptibility pattern of *Salmonella* spp. isolates presented in Table. The table data showed that the *Salmonella* spp. isolates were significantly 100% sensitive to Ciprofloxacin, 66.7% sensitive to Gentamycin, 100% resistant to Erythromycin, 66.67% resistant to Ampicillin and Amoxicillin.

Table 13 Antibiotic sensitivity pattern of *Salmonella* spp.

Name of the isolate	Fermentation characteristics	Name of sugars				
		Dextrose	Maltose	Lactose	Sucrose	Mannitol
Salmonella spp.	Acid production	+	+	_	_	+
	Gas production	+	+	_	_	+

Legend: R= Resistant, I= Intermediate sensitive, S= Sensitive

4. Discussion

In this study, three different types of bacteria e.g. *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. were isolated from rectal swab samples of healthy horses supported by [12],[13], [14], [15]

The prevalence of *E. coli, Staphylococcus* spp. *and Slamonella* spp. were 100%, 100% and 33.33%. At D.C. Park, out of 5 samples from horses the prevalence of *E. coli, Staphylococcus* spp. *and Slamonella* spp. were 80%, 60% and 40%. At

Torar mor, out of 2 samples from horses the prevalence of *E. coli, S. aureus* and *Slamonella* spp. were respectively 100%, 100% and 0%.

Several culture media were used simultaneously in this investigation to culture *E. coli*. In this study, colony characteristics of *E. coli* observed on NA, EMB, MC and BA agar were similar to the findings [11]. Differences in colony morphology in different cultural media by the isolates may be due to losing or acquiring some properties by the transfer of host or choice of host tissue observed by [10]. Specific media were used for the characterization of *Salmonella* which were previously suggested by [16],[17]. In this study the colony characteristics of *Salmonella* spp. observed in NA, XLD agar and SS agar were similar to the findings of [18].

All *E. coli* isolates fermented the five basic sugars producing acid and gas. Acid production was indicated by the colour change from reddish to yellow and the gas production was noted by the appearance of gas bubbles in the inverted Durham's tubes, which was supported by [19],[20]. The isolates also revealed positive reaction in MR test and Indole test but negative reaction in VP test found by[6]. Isolates of *Staphylococcus* spp. was revealed a complete fermentation of 5 basic sugars and production of acid which was supported by [20]. Coagulase test of *Staphylococcus* spp. was performed to determine whether the organism is pathogenic or not pathogenic. It was found that the isolated *Staphylococcus* spp. were coagulase positive *i.e.,* they were pathogenic. *Salmonella* spp. isolates were able to ferment three basic sugars (Dextrose, Maltose and Mannitol) by producing both acid and gas, however differentiation of *Salmonella* into species level was difficult based on their sugar fermentation pattern [21]. The present study showed similarities with the findings of other researchers [22].

The isolated *E. coli* bacteria showed 100% resistant to Ampicillin and Ciprofloxacillin, while the *S. aureus* and *Salmonella* spp. represented respectively 100% resistant to Ampicillin, Erythromycin and 100% resistant to Erythromycin.

PCR is a highly specific molecular technique for the confirm identification of microbial agents. In this study molecular detection of isolated *E. coli* was carried out using primers specific for *E. coli 16S rRNA* gene that amplified 585bp amplicon. Presence of specific sized amplicon in the PCR here confirmed the isolates as *E. coli.* This PCR protocol has also been used by others for the detection of *E. coli* [23],[24],[25]

5. Conclusion

The present study was undertaken during the period of July 2014 to December 2014 for the isolation and identification of the bacterial flora present in the rectal swab originated from apparently healthy horses reared in and Bangladesh Agricultural University (BAU) campus, Mymensingh using conventional and PCR based approach. In addition, antibiotic sensitivity profiles of the isolated bacteria against 5 commonly used antibiotics were also determined.

10 rectal swab samples were collected from the Veterinary Teaching Hospital, BAU; D.C Park (Joinul Abedin Park), Mymensingh and Torar mor, Mymensingh. The overall prevalence of *E. coli*, *S. Aureus* and *Salmonella* spp. were successively 93%, 86.67% and 24.44%.

The present study revealed that some of the isolated bacteria were resistant to more than one antibiotic (100% resistance of *E. coli* to Ampicillin, Ciprofloxacillin, 100% resistance *S. aureus* to Ampicillin, Erythromycin and 100% resistance of *Salmonella* spp. to Erythromycin) that might be a hazard for the personnel working with horse and also risk for the environment. The multidrug resistant gene might be transmitted to other bacteria and expressed as a new biohazard for humans. To successfully fight the increasing numbers of drug resistant and multi drug-resistant bacteria, knowledge of the updated information regarding current distribution of resistance pattern is required. The other vital aspect of controlling the spread of multi-drug resistant organisms is by providing sufficient hygienic measures and proper care of animal during infectious period of that animal. Present study suggested that Erythromycin, Gentamycin and Ciprofloxacin might be of first choice of treatment against *E. coli, S. aureus* and *Salmonella* spp. infection in horses. Sufficient efforts in using antimicrobial agents wisely and strict attention to infection may prevent the emergence of resistant organisms in a great extent.

Considering the findings of this research work, it may be concluded that:

- *E. coli, Staphylococcus aureus* and *Salmonella spp*. were isolated from the rectal swab of horses in and around BAU campus.
- *E. coli* were successfully isolated and confirmed by different biochemical test and PCR.
- Isolated bacteria showed resistant to one or more antibiotics.

- Erythromycin, Gentamycin and Ciprofloxacin could be the first choice of antibiotic for the treatment of *E. coli, S. aureus and Salmonella spp*. infection in horses in the study areas.

Possible future studies of this present work could be:

- Determination of virulence properties of the isolated bacteria.
- Identify the molecular basis of antibiotic resistance by PCR and sequencing of antibiotic resistant genes.

Compliance with ethical standards

Acknowledgments

The author would like to convey my heartiest gratitude to the respected supervisor, co-supervisor, reviewers and well-wishers for this piece of work. I like to thank National Science and Technology (NST) fellowship authority for their monetary support and would like announce that there is no conflict with any researcher for the presentation of the research.

Disclosure of conflict of interest

There was no conflict of interest. The co-authors are well informed and hence they are delighted to be the part of this work.

Statement of ethical approval

The corresponding author would like to disclose that during this study; the University Ethical Committee approved the research methodology. The review board members reviewed this piece of work as expert.

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Author's short biography

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