Publisher homepage: www.universepg.com, ISSN: 2663-6913 (Online) & 2663-6905 (Print)

https://doi.org/10.34104/ajpab.023.056064

American Journal of Pure and Applied Biosciences

Journal homepage: www.universepg.com/journal/ajpab

Detection of Zoonotic Potential of *Salmonella* and *Escherichia coli* Isolated from Ostriches and Determine Their Antibiogram Study

Md. Raisul Azam[1], Md. Aoulad Hosen[1], Likhon Kumar Shil[1], Nirban Kumar Das[1], Nazmi Ara Rumi[1]*, and Md. Khaled Hossain[1]

[1]Department of Microbiology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh.

*Correspondence: rumi_dvm@yahoo.com (Dr. Nazmi Ara Rumi, Associate Professor, Department of Microbiology, Faculty of Veterinary & Animal Science, Hajee Mohammad Danesh Science & Technology University, Dinajpur-5200, Bangladesh).

ABSTRACT

The present research was conducted for molecular characterization of important zoonotic bacteria isolated from different samples in ostrich and also determined their antimicrobial activity. For this current research, 32 samples were randomly collected from 8 ostriches at different ages, of which 8 were oropharyngeal, 8 were cloacal swabs, 8 were environmental sand samples, and 8 were feces samples. In addition, the bacteria were isolated and identified by using standard microbiological methods, including cultural, biochemical and molecular techniques. 16S rRNA gene was used to detect *Escherichia coli* and *Salmonella* spp. molecularly. The Kirby-Bauer disc diffusion method was used to determine the antibiotic sensitivity test. Out of 32 samples, *E. coli* 8 (53.33%) and *Salmonella* spp. 7 (46.67%) were identified in young ostrich, while in adult ostrich, *E. coli* 2 (40%) and *Salmonella* spp. 3 (60%) were detected. According to our study, *E. coli* was the most predominant isolate found in cloacal swabs and ostrich feces. *Escherichia coli* were most sensitive to Amoxicillin and Azithromycin (100%), followed by Kanamycin, Chloramphenicol and Gentamicin (75%), while 100% resistant to Piperacillin, Bacitracin, Tetracycline, Cloxacillin, Novobiocin, Cefixime. *Salmonella* spp. was 100% sensitive to Azithromycin and also 100% resistant to Tetracycline, Piperacillin, Bacitracin, Chloramphenicol and Methicillin. Our research concluded that *E. coli* and *Salmonella* spp. are multi-drug resistant bacteria, and appropriate antibiotics should be used in ostrich farms to protect the multi-drug resistant bacteria. We suggest farm owners increase public awareness about zoonotic diseases and those working on ostrich farms.

Keywords: Antibiotic, Zoonotic bacteria, Multidrug-resistant, Zoonotic potential, and 16S rRNA.

INTRODUCTION:

Ostriches (*Struthio camelus*) have fascinated humanity for centuries. Ostrich farming has grown globally, offering an inexpensive trade of feathers, meat, prized skin, and eggs, making them essential replacement animals in the multiple nations (Cooper *et al.,* 2008). Additionally, ostriches are exhibited in the zoos and estates. There were numerous ostriches in the Sahara desert, which served as an area for hunting. Palestine, Iran, and the Arabian Desert have ostriches. In the late 19th century, Africa sent many ostriches to Australia, New Zealand, Europe, and North and South America (Boum, 2015). Ostrich farming is now an obscure part of South African agriculture, but it once dominated

certain regions' economies. Egypt's ostrich industry continues to expand, as are its farms (Cooper *et al.,* 2008). Egyptian ostrich farms produced 7.27 eggs/ hen/month, compared to South Africa's 5.99 (Youssef and Afifi, 2017; Rahman *et al*., 2019).

Cooper *et al.* (2009) reported that ostrich eggs are highly nutritious. Additionally, ostrich eggs may contain different pathogenic bacteria. A previous study investigated that 19.3% of several bacterial isolates were found in ostrich eggs (Youssef *et al.,* 2017). In Bangladesh, many ostrich farm owners breed ostrich on their farms for a high quantity of meat production. During the handling of foods, farm owners attach ostrich and ostrich eggs and meat, which could be a potential risk of much zoonotic disease transmission. Recently, ostrich farming and ostrich exhibition have been increased, and as a result, people are more conscious about the zoonotic disease risk associated with this bird, its products and by-products (Youssef *et al*., 2017). Adult ostriches have a high level of resistance to several diseases. Nevertheless, young birds, especially when being moved from their nests to the farm area, are more susceptible to the dangers posed by parasites and bacteria such as hemolytic *E. coli*, *Campylobacter* spp., and the *Salmonella* spp. (Cooper, 2005). *Escherichia coli* is a part of the intestinal microbiota of poultry, including ostriches, yet pathogenic strains cause colibacillosis, and poultry deaths often start with it (Scerbova *et al.,* 2016). *Salmonella* is found in clinically healthy ostrich and diseased ostriches (de Freitas Neto *et al.,* 2009). Enterotoxigenic *E. coli* strains cause watery diarrhoea in animals and birds worldwide (Marzouk *et al.,* 2004). *Escherichia coli* in ostrich products may impede meat and other product trade. As a widespread human food-borne infection, it threatens public health (Foley *et al.,* 2008; Smith *et al.,* 2008). Ostriches are widely farmed, although little is known about infections in their eggs. Bacterial infections inhibit extensively ostrich breeding. In ostriches, *E. coli, Salmonella,* and *Pseudomonas* infections are most important (Wieliczko *et al.,* 2000).

Antimicrobial resistance in microorganisms from animals, including food-producing animals, pets, fish, and wild animals, has generated interest significantly (Schwarz *et al.,* 2010). Only a few specific studies have been conducted on the antimicrobial resistance of organisms isolated from ostriches in Bangladesh. Therefore, this research aimed to: isolate and identify the zoonotic potential of *Salmonella* and *E. coli* strains from ostriches, molecular characterization of isolated strains by PCR, DNA sequencing and phylogenetic tree analysis and to determine antimicrobial resistance.

MATERIALS AND METHODS:

Selection of study site and period

This study was complemented at the bacteriology research laboratory of the Department of Microbiology at Hajee Mohammad Danesh Science and Technology University [HSTU] Bangladesh, during the period from July 2019 to December 2019. The research was carried out in Hajee Mohammad Danesh Science and Technology University [HSTU] ostrich farms in Dinajpur district.

Sample collection and processing

A total of 32 samples including oropharyngeal swabs (Adult=2, Young=6), cloacal swabs (Adult=2, Young =6), environmental sand (Adult=2, Young=6) and feces (Adult=2, Young=6) were collected from ostrich farm and transferred with ice-containing bags in the bacteriology laboratory for microbiological analysis **(Fig. 1)**.

Isolation and identification of bacteria

Samples were suspended in a sterile saline solution. The suspension was inoculated into nutrient agar and nutrient broth for the primary isolation of bacteria (Parvez *et al.,* 2016). For sub-culturing of the suspected bacteria, we have used different bacteriological agar media like MacConkey agar, Eosin Methylene Blue agar, Mannitol Salt Agar, Cetrimide agar and Salmonella-Shigella agar. All bacterial culture Petri dishes were incubated at 37˚C overnight for the confirmation of bacterial growth. Pure culturing of bacteria was then done by following the methods described earlier (Kundu *et al.,* 2021). All bacteriological and fungal agar media were derived from Hi-Media Laboratories Private Ltd. India. Primary identification of bacteria was made by using gram-staining methods, which showed morphological and staining characteristics under microscopy (Merchant and Packer, 1967). Using standard methods, bacteria were identified by different biochemical tests, inclu-

ding catalase, oxidase, indole, MR-VP, Simon citrate, and motility urease (Cheesbrough, 2003).

DNA extraction of *E. coli* and *Salmonella* spp. and phylogenic tree analysis

E. coli and *Salmonella* spp. were identified by biochemical tests. DNA was extracted from *E. coli* and *Salmonella* with a robotic DNA extractor (Maxwell-16, source: Promega-USA) as per manufacturer instructions. Genomic DNA purity and concentration of *E. coli* and *Salmonella* were measured with a Nano-drop spectrophotometer (ND-200, source: Thermo Scientific-USA). The final PCR band was found in agar gel electrophoresis and visualized and photo-graphed by a UV transilluminator. The PCR primer marks gene and PCR cycling procedure are demonstrated in **Table 1**. By applying the neighbor-joining method of 1000 replicates, a phylogenic tree was measured with the MEGA6 program (Tamura *et al.,* 2013).

Table 1: PCR primers and DNA extraction techniques.

Mark gene	Primer sequence (5′-3′)	Seg. (bp)	Pre Heat	Amplification- 35 cycles			Final extention	Ref.
				De-naturation	Annealing	Extension		
16s rRNA	Forward primer 27F (5'AGAGTTTGATCCTEGGCTCAG3')	1465	95°C for 2 min	95°C for 30 sec	52° C for 30 sec	72°C for 50 sec	72°C for 5 min	Tsen *et al.* 1998,
	Reverse primer 1492 R (5'-TACCTTGTTACGACTT3')							
	Forward- Primer S139 (F): (5'GTGAAATTATCGCCA CGTT CGGG CAA 3')	284						Rahn *et al.* 1992
	Reverse Primer S141 (R): (5' TCAT CGCA CCGTCAAAGGAACC 3')							

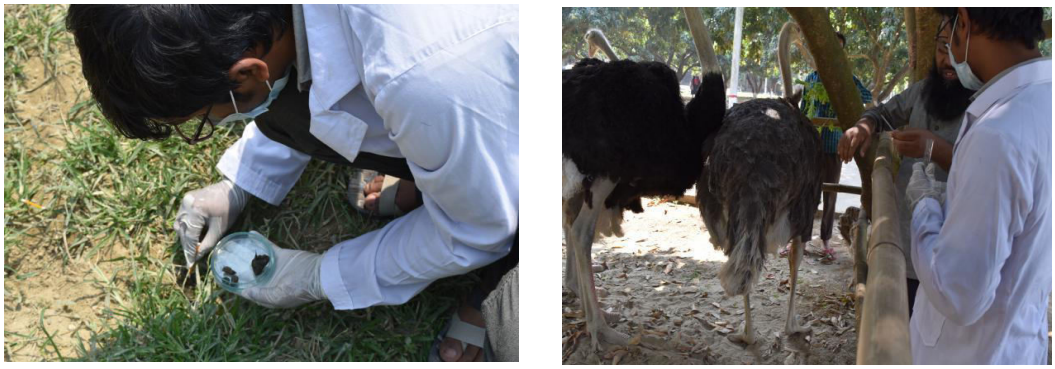

Fig. 1: Collection of samples from faces (left) and cloacal swab (right)

Antibiotic sensitivity tests

According to Clinical and Laboratory Standards Institute (CLSI), agar disc diffusion techniques were used to determine the antibiotic sensitivity patterns of isolates on Muller-Hinton agar plates (CLSI, 2014). A total of 15 commercially available antibiotics, including Kanamycin (30 µg), Amoxicillin (30 µg), Piperacillin (10 µg), Bacitracin (10 µg), Tetracyclin (15 µg), Erythromycin (15 µg), Azithromycin (15 µg), Cloxacillin (5 µg), Chloramphenicol (30 µg), Gentamicin (10 µg), Novobiocin (30 µg), Methicillin (5 µg), Cefixime (5 µg), Vancomycin (30 µg), and Cefradin (25 µg) were used. All antibiotic discs were purchased from (Oxoid Limited, UK). After biochemical identification, colonies of the pure isolates were spread on Muller-Hinton agar, and selected antibiotic discs were placed using sterile forceps. Finally, incubate the plates at 37°C for 24 hours, and then observe and the measure the zone of inhibition on a millimeter scale according to company guidelines.

RESULTS:

Prevalence of isolates

Among thirty-two (32) samples, *E. coli* 8 (53.33%) and *Salmonella* spp. 7 (46.67%) were isolated from young ostrich, and in adult ostrich, *E. coli* 2 (40%) and

Salmonella spp. 3 (60%) were identified. In young ostrich, *E. coli* 3 (75%) and *Salmonella* spp. 1 (25%) from oropharyngeal swab, and *E. coli* 5(45.45%) and *Salmonella* spp. 6 (54.55%) from feces were found **(Table 2 and Fig. 2)**.

Table 2: Prevalence of *E. coli* and *Salmonella* spp. from young ostrichs.

Bacterial isolates	Oropharyngeal swab/n	Cloacal swab /n	Cloacal swab (%)	Environmental sand/n	Feces/n	Feces (%)	Percentage (%)
Escherichia coli	0	3	75%	0	5	45.45%	8(53.33)
Salmonella spp.	0	1	25%	0	6	54.55%	7(46.67)
Total isolates		4			11		15 (100)

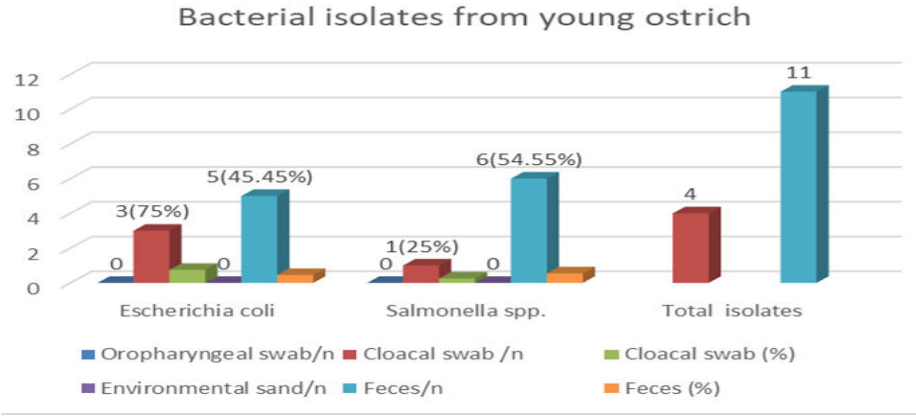

Fig. 2: Prevalence of bacterial isolates from young ostrich.

In adult ostrich, *E. coli* 1(50%) and *Salmonella* spp. 1 (50%) from cloacal swab, *E. coli* zero and *Salmonella* spp. 1 (100%) from oropharyngeal swab and *E. coli* 1(50%) and *Salmonella* spp. 1 (50%) from oropharyngeal swab samples were found, respectively **(Table 3 and Fig. 3)**.

Table 3: Prevalence of *E. coli* and *Salmonella* spp. from adult ostrichs.

Bacterial isolates	Oropharyngeal swab/n	Cloacal swab/n	Cloacal swab (%)	Environmental sand/n	Environmental sand (%)	Feces /n	Feces (%)	Percentage (%)
Escherichia coli	0	1	50%	0	0	1	50%	2(40%)
Salmonella spp.	0	1	50%	1	100%	1	50%	3(60%)
Total number of isolates		2		1		2		5(100%)

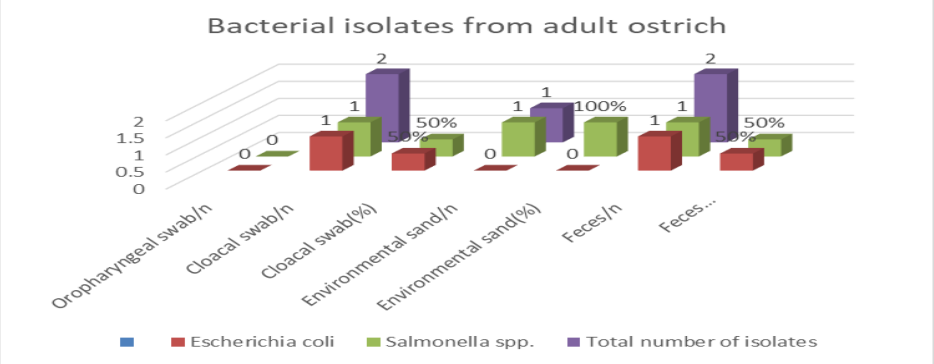

Fig. 3: Prevalence of bacterial isolates from adult ostrich.

Isolation and Identification of Bacteria

In this research, a total of 20 isolates were isolated and identified by cultural and biochemical tests. *E. coli* produces metallic sheen (greenish black) colonies on EMB agar **(Fig. 4A)**, and *Salmonella* spp. produce white colony on Brilliant Green agar **(Fig. 4B)**. *E. coli* demonstrated positive results for MR-VP, TSI, SC and SF in young ostrich, while negative results showed in TSI and OXI in adult ostrich below **(Fig. 5** and **Table 4).** *Salmonella spp.* gives positive results for MR-VP, SC and SF in young ostrich, whereas negative results showed in TSI and OXI in adults **(Table 4)**.

Table 4: Result of biochemical test for the representative isolates of *E. coli* and *Salmonella* spp.

Type	Sample No	MR	VP	Indole	MIU	TSI	SC	LF	OXI	CT
Young	2	+	+	+	+	+	+	+	-	+
	3	+	+	+	+	-	+	+	-	+
	4	+	+	+	+	-	+	+	-	+
	5	+	+	+	+	+	+	+	-	+
	6	+	+	-	+	+	+	+	-	+
Adult	2	+	+	+	+	-	+	+	-	+

Note: + = positive, - = negative, MR=Methyl Red, VP=Voges-Proskaur, MIU=Motility Indole Urease, TSI=Tripple Sugar Iron, SC=Simmons Citrate, LF=Lactose Fermented, OXI=Oxidase, CT=Catalase

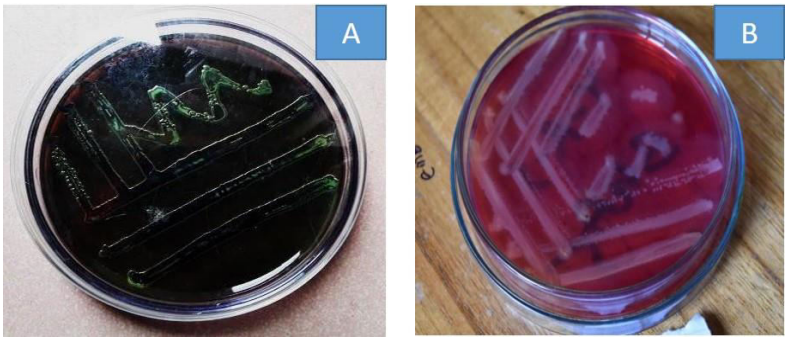

Fig. 4: *E. coli* produce metallic sheen (greenish black) colony on EMB agar (A), *Salmonella* spp. on Brilliant Green Agar (B).

Table 5: Antibiotic sensitivity tests results of *E. coli* and *Salmonella* spp.

Antimicrobial agent with disc cons. (µg)	*E. coli* (4)		*Salmonella* spp. (4)	
	%S	%R	%S	%R
Kanamycin (30)	75%	25%	25%	75%
Amoxicillin (30)	100%	0%	0%	100%
Piperacillin (10)	0%	100%	0%	100%
Bacitracin (10)	0%	100%	0%	100%
Tetracycline (15)	0%	100%	0%	100%
Erythromycin (15)	50%	50%	25%	75%
Azithromycin (15)	100%	0%	100%	0%
Cloxacillin (5)	0%	100%	0%	100%
Chloramphenicol (30)	75%	25%	0%	100%
Gentamicin (10)	75%	25%	75%	25%
Novobiocin (30)	0%	100%	25%	75%
Methicillin (5)	0%	100%	0%	100%
Cefixim (5)	0%	100%	0%	100%
Vancomycin (30)	25%	75%	25%	75%
Cefradin (25)	25%	75%	25%	75%

Legends: cons.: Concentration; S: Sensitivity; R: Resistant; %: Percentage

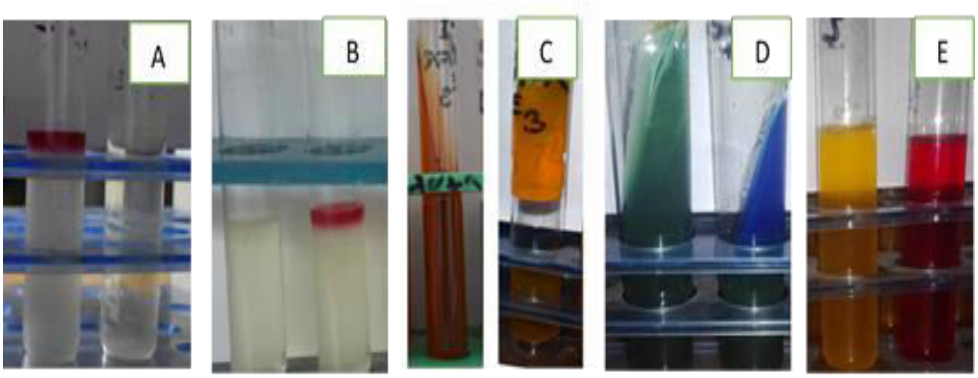

Fig. 5: *E. coli* show positive results in A: Methyl red, B: Voges-proskaeur, C: TSI, D: SC and E: SF.

Results of Antibiotic Sensitivity Test

A total of 15 commercially available antibiotics were used to determine the antibiotic sensitivity tests of this research. *Escherichia coli* were most sensitive to Amoxicillin and Azithromycin (100%), followed by Kanamycin, Chloramphenicol and Gentamicin (75%), while 100% resistant to Piperacillin, Bacitracin, Tetracycline, Cloxacillin, Novobiocin, Cefixime **(Fig. 6A)**. *Salmonella* spp. was 100% sensitive to Azithromycin and 100% resistant to Tetracycline, Piperacillin, Bacitracin, Chloramphenicol and Methicillin **(Fig. 6B)**.

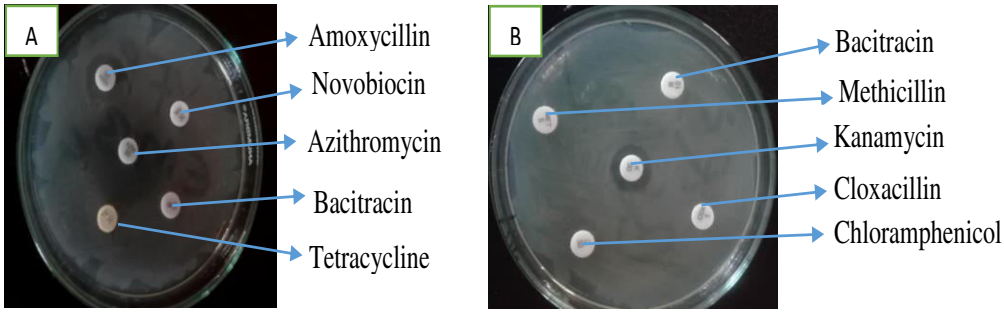

Fig. 6: Antibiotic sensitivity test for *E. coli* (A) and *Salmonella* spp. (B)

Results of PCR, gene sequencing and phylogenic tree analysis

16S rRNA gene region of *E. coli* was amplified with the universal primers, Forward primer 27F (5'-AGAGTTTGATCCTEGGCTCAG3') and Reverse primer 1492 R (5'-TACCTTGTTACGACTT3'), and found at 1465 bp. For *Salmonella* spp. DNA was amplified with specific S139- F and S141- R primers, and a 284 bp band was found, which are shown in **Fig. 7A** and the phylogenic tree in **Fig. 7B.** Whole genome sequencing was experimented by the National Institute of Biotechnology at Savar, Dhaka, Bangladesh.

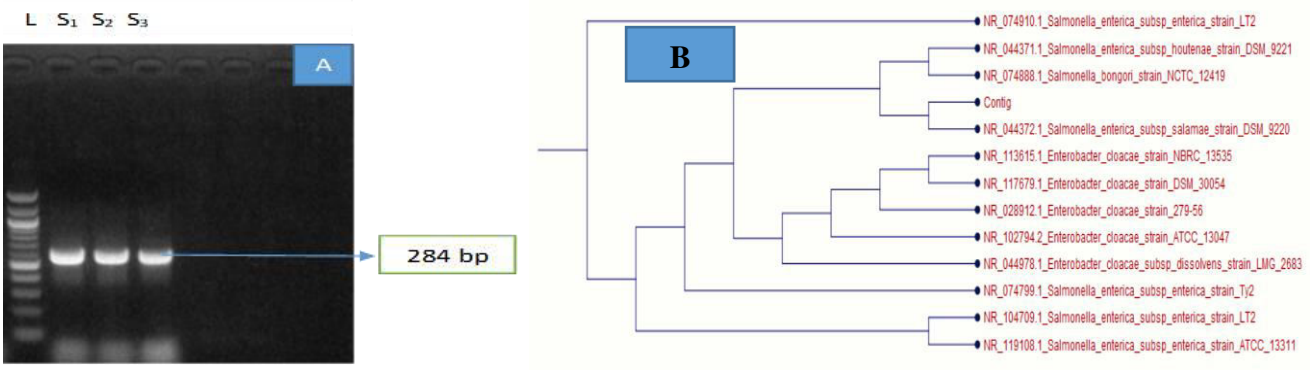

Fig. 7: 284 bp of *Salmonella enterica*; L=500kb ladder; S_1, S_2, S_3: sample (A); Phylogenic tree analysis of *Salmonella enterica* (B).

DISCUSSION:

In Bangladesh, very few ostrich farms are available. Different food items such as vegetables, green leaves, poultry feeds, rice, wheat and water are needed to feed ostrich. All these foods are related to microbial contamination during handling. Our research mainly focused on identifying microorganisms directly or indirectly related to the ostrich, the farm environment and persons working on ostrich farms. Different zoonotic bacteria cause different diseases and transmit to human through animals. This represents the latest research in Bangladesh to assess the microbial community in the oropharyngeal swabs, cloacal swabs, feces and environmental sand samples from ostrich farms, along with determining their antibiogram study. A previous study reported that there was very little information about the zoonotic potential of *Salmonella* spp. and *E. coli* strains (Youssef AI *et al., 2017).* Earlier researchers reported that ostriches are potential reservoirs of *Salmonella* spp. and *E. coli* as well as transmit important zoonotic bacteria from animals to humans (Jahan *et al*., 2017). In our study, most prevalence *E. coli* (53.33%) were identified from different ages, which was lower than the observation of Asmaa *et al*. (2016), who found 58.4% *E. coli* in Ostrich Farms in Egypt. In our study, the overall cultural prevalence of *Salmonella* spp. from different age's ostrich was (46.67%), which was higher than the value (20.8%) observed by Asmaa *et al*. (2016).

The current study revealed that the molecular characterization of *E. coli* and *Salmonella enterica* that had been done by PCR amplification and phylogenic tree analysis. To determine the genetic diversity and evolutionary relationship between *Salmonella* spp. and other similar species, we applied phylogenic tree analysis with BLAST search tools. Overall on BLAST analysis, *Salmonella_ enterica* subsp_ salamae_strain_ DSM_9220 were identified in our sample and it was closely linked with *Salmonella_ enterica_* subsp_ houtenae_strain_DSM_9221 and *Salmonella_ enterica* _subsp_ entarica_strain_Ty2. The present study reported that *E. coli* can resist strong antibiotics such as Amoxicillin and Azithromycin (100%), and Chloramphenicol, Kanamycin and Gentamicin (75%), which are often used to treat pathogenic bacteria. A previous study by Jahan *et al.* (2017) showed that amoxicillin and azithromycin have strong antimicrobial activity against *E. coli*. And the result is similar to our current research. A previous study by Yadav *et al.* (2017) revealed that the rate of *Salmonella* spp. resistant to amoxicillin, ampicillin, tetracycline, erythromycin, and gentamycin was the highest (100%), followed by colistin sulfate (83.33%), pefloxacin (38.88%) enrofloxacin (38.88%), gentamycin (11.1%) and Ceftriaxone (0%). The present study reported that *Salmonella* spp. could be inhibited with strong antibiotics such as azithromycin (100%), followed by gentamycin (75%), which are often used to treat pathogenic bacteria, whereas they were more resistant to amoxicillin, piperacillin, bacitracin, tetracycline, cloxacillin (100%). Our study provides a similar result to (Janan *et al.,* 2017).

The obtained results of our study mentioned that *E. coli* and *Salmonella* are the most predominant pathogens, which are mainly found in ostrich feces. Spreads of zoonotic bacteria and causes of diseases in animals and humans are common in ostrich farms due to a lack of proper management. The persons directly or indirectly involved in an ostrich farm as well as an immune-compromised person and pregnant women, have a high potential risk of the developing diseases caused by zoonotic bacteria.

CONCLUSION:

In the present study, the most prevalent bacteria were found to be *E. coli* and *Salmonella* spp., which were isolated from the most significant sources, such as cloacal swabs and ostrich faces, and were responsible for the transmission of zoonotic pathogens from animals to humans. Due to wide use of antibiotics without proper prescription microorganisms exhibit their resistance character in ostrich farm in Bangladesh as well as other countries in the world. Usually, hygiene levels in ostrich farms determine the presence of these microorganisms, and contamination may result from domestic ostrich sanitation and handling. Thus, ostrich farms need proper antimicrobials and biosecurity. Ostrich farming is a developing sector in Bangladesh. Therefore, this study will benefit investors, prescribers, and the ostrich owners. Additionally, Bangladeshi ostrich farms must utilize antibiotics rationally to prevent the multi-drug-resistant micro-

organisms. Finally, the precautions must be taken to prevent the spread of zoonotic diseases among the ostrich farming workers.

Ethical approval

The ethical committee of Hajee Mohammad Danesh Science and Technology University in Basherhat, Dinajpur, Bangladesh [HSTU] approved the research's methodology [approval number: HSTU/IRT/94].

ACKNOWLEDGEMENT:

The Microbiology department at Hajee Mohammad Danesh Science and Technology University [HSTU] in Basherhat, Dinajpur, Bangladesh, supported laboratory facilities (bacteriological analysis) for this research. The IRT project gave financial support during research conduct.

CONFLICTS OF INTEREST:

The authors have no conflict of interest.

REFERENCES:

1) Asmaa, M.A, Shimaa, A.E., & Elshater M.A.H. (2016). Prevalence of *Escherichia coli* and Salmonella Species in Ostrich Farms in Egypt. *IOSR J Env Sci,* **10**(4), 06-11. https://doi.org/10.9790/2402-1004020611
2) Boum, A., & Bonine, M. (2015). The elegant plume: ostrich feathers, African commercial networks, and European capitalism. *The J. North African Studies*, **20**(1), 5-26. https://doi.org/10.1080/13629387.2014.983733
3) Cheesbrough, M. (2003). Laboratory manual for tropical countries. Volume II. Microbiology. *Trop Health Tech, ELBS, London, UK*, 214-20.
4) Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests; 9th ed.; Document M2-A9; *Clinical and Laboratory Standards Institute* (CLSI): Wayne, PA, USA; 2014.
5) Cooper, R. G. (2005). Bacterial, fungal and parasitic infections in the ostrich (*Struthio camelus var. domesticus*). *Anim Sci J*, **76**(2), 97-106. https://doi.org/10.1111/j.1740-0929.2005.00243.x
6) Cooper, R. G., Mahrose, K. M., & Marai, I. F. M. (2008). Ostrich (*Struthio camelus*) production in Egypt. *Trop Anim Health Prod*, **40**, 349-355. https://doi.org/10.1007/s11250-007-9108-z
7) de Freitas Neto, O. C., Lages, S. & Berchieri Junior, A. (2009). Search for *Salmonella* spp. in ostrich productive chain of Brazilian southeast region. *Trop Anim Health Prod*, **41**, 1607-1614. https://doi.org/10.1007/s11250-009-9354-3
8) Foley, S. L., Lynne, A. M., & Nayak, R. (2008). Salmonella challenges: prevalence in swine and poultry and potential pathogenicity of such isolates. *J Anim Sci*, **86**(suppl_14), E149-E162. https://doi.org/10.2527/jas.2007-0464
9) Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. *American Society for Microbiol*, **15**, 55-63.
10) Jahan, I., Rumi, N. A., Akter, S., & Miah, A. G. (2017). Microbial assessment of different samples of ostrich (*Struthio camelus*) and determination of antimicrobial susceptibility profiles of the isolated bacteria. *Asian J Med Biol Res*, **3**(4), 437-445. https://doi.org/10.3329/ajmbr.v3i4.35334
11) Kundu, T., Rumi, N. A., & Halder, J. (2021). Isolation of multidrug-resistant *Escherichia coli* from turkeys in Dinajpur, Bangladesh, and their antibiogram profile. *J. Adv Vet Anim Res*, **8**(1), 64. https://doi.org/10.5455/javar.2021.h486
12) Marzouk, A., Gray, A. I., & Deans, S. G. (2004). Transformed root cultures of Solanum dulcamara and production of secondary metabolites. In *Poisonous plants and related toxins* (pp. 167-174). Wallingford UK: CABI Publishing. https://doi.org/10.1079/9780851996141.0167
13) Merchant, I.A., & Packer, R.A. (1967). *Vet Bacteriol Virol*. (No. QR49 M4).
14) Parvez, M. A. K., Mahmud, S. A., & Rahman, S. R. (2016). Isolation of multidrug resistant pathogennic bacteria from common flies in Dhaka. *Bangladesh J. Entomol*, **13**(4), 141-147.
15) Rahman MA, Haque A, Uddin ME, and Ahmed R. (2019). Isolation, identification, and antibiotic sensitivity pattern of Salmonella spp from locally isolated egg samples. *Am. J. Pure Appl. Sci*., **1**(1), 1-11. https://doi.org/10.34104/ajpab.019.019111
16) Rahn, K., McEwen, S. A., & Gyles, C. L. (1992). Amplification of an invA gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. *Mol Cell Probes*, **6**(4), 271-279. https://doi.org/10.1016/0890-8508(92)90002-F

17) Scerbova, J., & Lauková, A. (2016). *Escherichia coli* strains from ostriches and their sensitivity to antimicrobial substances. *Polish J Vet Sci*, **19**(2). https://doi.org/10.1515/pjvs-2016-0052

18) Schwarz, S., Silley, P., Johnson, A. P., & Gaastra, W. (2010). Assessing the antimicrobial susceptibility of bacteria obtained from animals. *J Antimicrob Chemother*, **65**(4), 601-604. https://doi.org/10.1093/jac/dkq037

19) Tamura, K., Stecher, G., & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol*, **30**(12), 2725-2729. https://doi.org/10.1093/molbev/mst197

20) Tsen, H. Y., Lin, C. K., & Chi, W. R. (1998). Development and use of 16S rRNA gene targeted PCR primers for the identification of *Escherichia coli* cells in water. *J. Applied Microbiol*, **85**(3), 554-560. https://doi.org/10.1046/j.1365-2672.1998.853535.x

21) Wieliczko, A., & Kuczkowski, M. (2000). Selected issues of infectious diseases in ostrich (*Struthio camelus*). *Medycyna Weterynaryjna*, **56** (1), 23-28.

22) Youssef, A. I., & Afifi, R. A. (2017). Zoonotic potential of *Salmonella* and *Escherichia coli* isolated from ostrich eggs of a flock in a recreational park. *Human Vet Med*, **9**(3), 71-75.

Citation: Azam MR, Hosen MA, Shil LK, Das NK, Rumi NA, and Hossain MK. (2023). Detection of zoonotic potential of *Salmonella* and *Escherichia coli* isolated from ostriches and determines their antibiogram study. *Am. J. Pure Appl. Sci.*, **5**(4), 56-64. https://doi.org/10.34104/ajpab.023.056064