Alexandria Journal of Veterinary Sciences 2015, 47: 90-96
ISSN 1110-2047, www.alexjvs.com
DOI: 10.5455/ajvs.202843

Molecular Characterization of *Escherichia Coli* Isolated from Buffalo Calves in El-Behera Governorate

Mahmoud Abdulgayeid[1], Hazem Shahin[2], Seham Foad[1], Madiha S. Ibrahim[3*]
[1]Department of Microbiology, Animal Health Institute, Damanhour Branch, Egypt
[2]Department of Pharmacology, Faculty of Veterinary Medicine, Damanhour University, Egypt
[3]Department of Microbiology, Faculty of Veterinary Medicine, Damanhour University, Egypt

Key words

E. coli,

Diarrhea, Buffalo calves, *tsh* gene, Egypt

ABSTRACT:

The present study aimed to detect diarrheagenic *Escherichia* co*li* in buffalo calves with or without diarrhea, its virulence factors and antibiotic resistant pattern(s) as well as antibiotic resistance genes. Rectal swabs were collected from193 buffalo calves at different ages in El-Behera Governorate (110 and 83 samples from diarrheagenic and non-diarrheagenic calves, respectively). Ninety-five (49.2%) *E. coli* positive samples were detected from calves with or without diarrhea by culturing and biochemical tests with higher incidence within the first 2 weeks of age. *E. coli* samples were subjected to antimicrobial disc diffusion susceptibility test by using 11 different antibiotic discs, which are the most commonly used in field treatment of calf diarrhea. High sensitivities (100%) to individual antibiotics as Amoxycillin and clavulonate, Lincospectin and Cefotaxime was detected while only two showed high resistances as oxytetracycline and Sulfamethoxazole trimethobrim with 91.6% and 50.5% resistance, respectively. PCR for identification of *E. coli* by detection of *phoA* gene revealed that 100% of the samples were *E.coli* positive. PCR for detection of *E. coli* virulence genes*; eaeA* and *tsh* as well as the antibiotic resistance genes; *tetA(A)* and *Sul1* was performed. The *eae(A)*, *tsh*, *tetA(A)* and *Sul1* genes were found in 20%, 100%, 100% and 60% of *E.coli* samples, respectively. These results collectively indicate that buffalo calves can harbor enteropathogenic *E. coli* causing diarrhea at different ages. To our knowledge, this is the first report of detecting the *tsh* gene in *E.coli* isolated from diarrheagenic buffalo calves. This may raise concerns regarding the real distribution of *tsh*-positive *E. coli* in buffaloes, especially calves. Further, the *tsh* gene is mainly or exclusively detected in the Avian pathogenic *E. coli* (APEC) raising concerns on the role of buffaloes as a reservoir for pathogenic *E. coli.* Together, this can pose therapeutic challenge(s) to farm animals as well as zoonotic potential to contact human.

***Corresponding Author :** Madiha Salah Ibrahim; madihasalah@yahoo.com

1. INTRODUCTION

Buffaloes are widely raised in Egypt as an important source of high quality meat and milk, however, buffaloes can be also a reservoir of pathogenic *Escherichia coli* (*E. coli*) (Borriello *et al*., 2012; Malik *et al*., 2012; Osman *et al*., 2013; Anwarullah *et al*., 2014; Beraldo *et al*., 2014). *E. coli* normally inhabits the intestinal tract of man and animal with a potential to produce from mild to severe pathological conditions (Croxen and Finlay, 2010; Borriello *et al*., 2012; Osman *et al*., 2013). Several studies have addressed the high distribution of *E. coli* in calf diarrhea in different countries (Nguyen *et al*., 2011; Malik *et al*., 2012; Anwarullah *et al*., 2014; Shahrani *et al*., 2014). Diarrheagenic *E. coli* includes *Enteropathogenic E. coli* (EPEC), *Enterohemorrhagic E. coli* (EHEC), *Enterotoxigenic E. coli* (ETEC), *Enetroaggregative E. coli* (EAEC), *Enteroinvasive E. coli* and *Diffusely adherent E. coli* (DAEC) (Kaper *et al*., 2004).

Ruminants are a known reservoir of potentially pathogenic *E. coli* that can represent a significant threat to public health (De Verdier *et al*., 2012; Malik *et al.*, 2012; Shahrani *et al.*, 2014; Beraldo *et al*., 2014; Bok *et al*., 2015); however, information regarding the *E. coli* distribution in buffaloes in Egypt is scarce. Thus it is crucial to monitor the prevalence of different *E. coli* pathotypes in buffaloes in Egypt through the detection of virulence determinants and antimicrobial resistance. Therefore, here we aimed to study the prevalence of diarrheagenic *E. coli* in buffalo calves in EL-Behera Governorate. Rectal swabs were collected from diarrheagenic and non-diarrheagenic calves from one day old up to six months. Bacterial culture and identification as well as antimicrobial susceptibility testing was carried out. Further, *E. coli* positive isolates were subjected to PCR for detection of *phoA* gene, virulence genes; *eaeA* and *tsh* genes, and antibiotic resistance genes; *tetA(A)* and *Sul1* genes.

2- MATERIALS AND METHODS

2.1. Animal samples and bacterial cultures:

A total of 193 rectal swabs were collected from buffalo calves at ages from one day up to six months from different localities of El-Behera Governorate (110 were diarrheagenic and 83 were non-diarrheagenic buffalo calves). Rectal swabs were cultured into nutrient broth for 18 h and then inoculated onto MacConkey agar and Eosine methyelene blue (EMB) agar and incubated aerobically at 37°C for 24 h and then examined for bacterial growth. Colonies from each sample were subjected to gram staining and biochemical testing as catalase test, urease test, IMViC and triple sugar iorn agar (TSI) (MacFaddin, 1985).

2.2. Antimicrobial susceptibility testing:

Samples were inoculated on MacConky agar and Eosine methyelene blue (EMB) agar and three colonies per sample were collected and then cultured onto nutrient agar for Antimicrobial susceptibility testing, which was carried out according to the Clinical laboratory standards Inistitute **(CLSI, 2012)**. The following antibiotic discs were used: Amoxycilline+clavulanic acid (AMC), 20/10 µg; Florfenicole (FFC), 30 µg; Norfloxacin (NOR), 10 µg; Gentamycin (CN), 10 µg; Marbocyl (MAR), 10 µg; spectinomycine (SH), 10 µg; Oxytetracycline (OT) 30 µg; Sulfatrimethoprime (SXT), 25 µg; Lincospectine (LSP), 100 µg; Cefotaxime (CTX) 30 µg and Cholistin sulphate (CT), 10 µg.

2.3. Detection of *E. coli phoA* gene, virulence factors and antibiotic resistance genes by Polymerase Chain Reaction (PCR):

From pure cultures, DNA was extracted by phenol-chloroform method according to Sambrook *et al*., (1989). PCR (Emerald Amp GT PCR master mix (Takara) Code No. RR310A kit) was conducted by adding 12.5 µl of Emerald Amp GT PCR master mix (2x premix), 4.5 µl PCR grade water, 1 µl forward primer (20 pmol), 1 µl reverse primer (20 pmol) and 6 µl template DNA to a total volume of 25 µl. Primers used for the detection of the different genes are listed in **Table (1)**

3. RESULTS

3.1. Detection of *E. coli* in rectal swabs collected from buffalo calves

A total of 193 rectal swabs were collected from buffalo calves (110 diarrheagenic and 83 non-diarrheagenic buffalo calves). Samples were cultured for isolation of *E. coli* and subjected to biochemical tests. Gram negative rods were detected by Gram staining and the isolates produced characteristic black metallic sheen colonies on EMB agar and pink colonies on MacConky agar. *E. coli* was isolated from 73 out of 110 (66%) diarrheagenic calves and from 22 out of 83 (26.5%) non-diarrheagenic calves with a total percentage of 49.2% (95 out of 193) as shown in Table (2). *E. coli* isolation showed higher incidence in younger calves of both diarrheagenic and non-diarrheagenic calves specially during the first 2 weeks as shown in Table (2).

Table (1): Oligonucleotide primers sequences used for PCR.

Gene	Sequence	Amplified product	Reference
phoA	CGATTCTGGAAATGGCAAAAG CGTGATCAGCGGTGACTATGAC	720 bp	**Hu *et al*., 2011**
eaeA	ATG CTT AGT GCT GGT TTA GG GCC TTC ATC ATT TCG CTT TC	248 bp	**Bisi-Johnson et al., 2011**
tsh	GGT GGT GCA CTG GAG TGG AGT CCA GCG TGA TAG TGG	620 bp	**Delicato *et al*., 2003**
tetA(A)	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	576 bp	**Randall *et al*. 2004**
Sul1	CGG CGT GGG CTA CCT GAA CG GCC GAT CGC GTG AAG TTC CG	433 bp	**Ibekwe *et al*., 2011**

3. RESULTS

3.1. Detection of *E. coli* in rectal swabs collected from buffalo calves

A total of 193 rectal swabs were collected from buffalo calves (110 diarrheagenic and 83 non-diarrheagenic buffalo calves). Samples were cultured for isolation of *E. coli* and subjected to biochemical tests. Gram negative rods were detected by Gram staining and the isolates produced characteristic black metallic sheen colonies on EMB agar and pink colonies on MacConky agar. *E. coli* was isolated from 73 out of 110 (66%) diarrheagenic calves and from 22 out of 83 (26.5%) non-diarrheagenic calves with a total percentage of 49.2% (95 out of 193) as shown in **Table (2)**. *E. coli* isolation showed higher incidence in younger calves of both diarrheagenic and non-diarrheagenic calves specially during the first 2 weeks as shown in **Table (2)**.

3.2. Antimicrobial susceptibility

Antimicrobial susceptibility was observed in *E. coli* isolates from all samples. As shown in **Table (3)**, the highest percentage of resistance was to the oxytetracycline and Sulfamethoxazole trimethobrim with 91.6% and 50.5%, respectively. While all isolates were sensitive to Amoxycillin and clavulonate, Lincospectin and Cefotaxime with 100% sensitivities, followed by spectinomycin (98.9%), Norfloxacin (95.7%), Gentamycin (91.5%), Marbocyl (89.5%), Florfenicole (85.3%) and Cholestin sulphate (73.6%). Multiple antimicrobial resistance was observed in 66% (63/95) of the *E. coli* isolates.

3.3. Detection of the *E. coli phoA* gene, virulence factors and antimicrobial resistance genes by PCR:

Some of *E. coli* positive samples, as shown in **Table (4)** and **Figure (1)** were subjected to PCR for the detection of *E. coli phoA* (alkaline phosphatase) gene, virulence factors as *eaeA* (intimin) and *tsh* (temperature-sensitive hemagglutinin) genes as well as the antimicrobial resistance genes; *tetA(A)* and *Sul1* genes. All isolates were positive for the *phoA* gene, the *tsh* virulence gene and the *tetA(A)* gene detection. Only one isolate was positive for the intimin gene detection.

Table (2): Prevalence of *E. coli* among diarrheagenic and non-diarrheagenic buffaloes calves in correlation to age.

Age	Diarrheagenic Calves			Non-Diarrheagenic Calves		
	No.	*E. coli* +ve	%*	No.	*E. coli* +ve	%*
0day -1week	35	34	46.5	25	10	45.5
1week-2weeks	25	19	26	15	6	27.3
2weeks-3weeks	20	11	15	10	2	9
3weeks-4weeks	10	4	5.5	10	2	9
1-2months	10	3	4	10	1	4.5
2-6months	10	2	3	13	1	4.5
Total	110	73	66.4	83	22	26.5

*The percentage is calculated from the positive number in relation to the total number of positive *E. coli* isolates in each group; diarrheagenic and non-diarrheagenic.

Table (3): Antibiotic sensitivity of *E. coli* isolates

Antibiotics	Sensitive	Resistant
Norfloxacin 10	84[a] (95.7%)[b]	11[a] (4.3%)[b]
Amoxycillin & clavulonate	95 (100%)	-
Lincospectin	95 (100%)	-
Cholestin sulphate	70 (73.6%)	25 (26.4%)
Florfenicole 30	81 (85.3%)	14 (14.7%)
Marbocyl	85 (89.5%)	10 (10.5%)
Cefotaxime	95 (100%)	-
Oxytetracycline	8 (8.5%)	87 (91.5%)
Sulfamethoxazoletrimethobrim	47 (49.5%)	48 (50.5%)
Gentamycin	87 (91.5%)	8 (8.5%)
Spectinomycine	94 (98.9%)	1 (1.1%)

a; number of positive isolates, b; percentage of positive isolates.

Table (4): Detection of *E.coli phoA* gene, virulence factors and antimicrobial resistance genes.

Sample	Genes				
	E. coli	**virulence**	**antimicrobial resistance**		
	phoA	***tsh***	***eaeA***	***tetA(A)***	***Sul1***
1	+	+	-	+	+
2	+	+	-	+	-
3	+	+	+	+	+
4	+	+	-	+	-
5	+	+	-	+	+

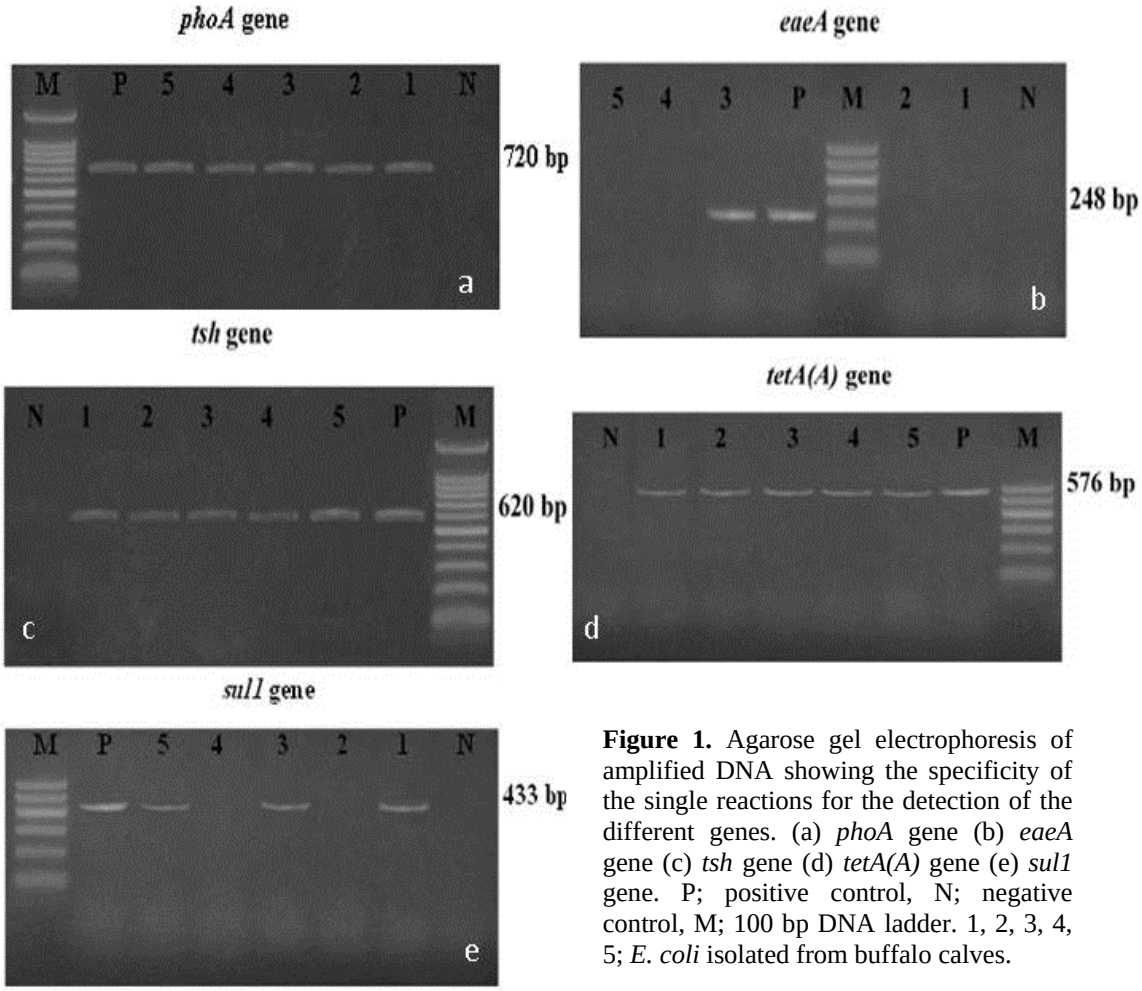

Figure 1. Agarose gel electrophoresis of amplified DNA showing the specificity of the single reactions for the detection of the different genes. (a) *phoA* gene (b) *eaeA* gene (c) *tsh* gene (d) *tetA(A)* gene (e) *sul1* gene. P; positive control, N; negative control, M; 100 bp DNA ladder. 1, 2, 3, 4, 5; *E. coli* isolated from buffalo calves.

4. DISCUSSION

Diarrhea in young calves is the main cause of economic losses through poor growth, morbidity and mortality **(**Constable 2004; Gaber 2004), and the role played by *E. coli* in producing diarrhea in calves has received a great attention by many researchers Shahrani *et al*., (2014). In our study, 193 rectal swabs were collected from buffalo calves from one day up to six months of age, with a total percentage of *E.coli* isolation of 49.2% (95/ 193) as shown in Table (1). This agrees with Paul *et al*., (2010), who showed that *E.coli* was isolated with an incidence of 50%. Meanwhile, other researchers isolated *E.coli* from calves with lower incidence as described by Anwarullah *et al*., (2014), who isolated *E.coli* with an incidence of 14.6%. On the other hand, higher incidence of *E.coli* was recorded by Majueeb *et al*., (2014) who isolated *E.coli* with an incidence of 72.8%.

The higher prevalence of *E.coli* isolation from diarrheagenic and non-diarrheagenic buffaloes calves was observed in young ages; one and two weeks. The higher prevalence of *E. coli* in these ages may be due to poor managemental practices and predisposing factors like overcrowding and

malnutrition, which are supposed to be a primary cause of immunosuppression. Especially, where these animals are not reared under intensive farming conditions. Further, *E. coli* is a commensal organism and is responsible for diarrhea in calves, particularly calves receiving less or no maternal antibodies through colostrum Malik *et al*., (2012) where milk is mainly used for commercial purposes.

Detection of the alkaline phosphastase gene (*phoA*) showed that the isolates were 100% positive for the *phoA* gene. This results agree with Chang *et al*., (1986) and Kong *et al*., (1999) who reported that *phoA* gene is a housekeeping gene present in all *E.coli* strains.

The detection of the *eaeA* (intimin) gene by PCR showed that 20% of the examined isolates were positive for the *eaeA* gene. This result agrees with Hala A.A., (2012) who detected the *eaeA* gene by 20% but disagree with Nguyen *et al*., (2011) who detected the *eaeA* gene by 9.8% and Mohammadi *et al*., (2013) who reported that all of their isolates were *eaeA*-negative. The intimin gene detection is mainly linked to the EPEC pathotype (Beraldo *et al*., 2014) and *eaeA*-positive strains are considered to be more virulent to human than the *eaeA*-negative ones. This indicates a possible participation of buffalo calves in the zoonotic transmission of pathogenic *E.coli*.

The *tsh* gene encodes a temperature-sensitive hemagglutinin of *E.coli*, first identified by Provence and Curtiss Provence and Curtiss (1994). Also the *tsh* protein was the first identified member of an expanding subclass of the *IgA* protease family of autotransporters present in *Shigella* spp. and numerous pathotypes of *E. coli* (Stathopoulos *et al*., 1999). In the present study result of PCR for the detection of the *tsh* gene showed that 100% of the tested isolates were positive. This nearly agrees with Janßen *et al*. (2001) and Saidenberg *et al*., (2013) who detected the *tsh* gene in 85.3% and 78.3%, respectively, but do not agree with Mohamed *et al*., (2014), Delicato *et al*., (2003) and Ewers *et al*., (2004), who detected a *tsh* positive frequency of 28%, 39.5% and 53.3%, respectively. However, those authors detected the *tsh* gene from the APEC isolated from poultry, where our *E. coli* isolates were isolated from fecal samples from buffalo calves. This could indicate a possibility of either the expression of the *tsh* gene is under-estimated in different animal species or a poultry-to-buffalo transmission of APEC and/or plasmid transfer. Different animal species are generally reared together in the country side and this allows the continuous interaction between the microbial environments of those species with its possible inter-species mixing requiring a more detailed study of the co-existence of such mixed microbial populations. Further, this may indicate a possible wider role of buffalo calves as reservoir for extraintestinal infections to human.

Antibiotics are widely used in the treatment and prevention of disease in the veterinary practice as well as growth enhancer in animals. To date, there are many reports regarding *E. coli* resistance in many countries and regions (Johns *et al*., 2012, Szmolka and Nagy, 2013).

The results of our *E.coli* antibiotic resistance demonstrated in Table (3) showed high resistance to Oxytetracycline (91.5%) which was nearly in agreement with Shahrani, *et al*. (2014) who found 98.09% resistance of *E. coli* against tetracycline and Balasubramaniam *et a*l., (2014) who found the resistance of *E. coli* clinical strains against tetracycline from India in 88% but not with Nizza *et al*., (2010) who found *E.coli* resistance tetracycline of 34%. We found high resistance to Sulfamethoxazole-trimethoprime (SXT) (50.5%) among the *E.coli* isolates. High resistance to Sulfamethoxazole-trimethoprime (90.31%) was observed by Shahrani *et al*., (2014). This is quite important as Sulfamethoxazole-trimethoprime and tetracycline are commonly used in veterinary and human practices. Further, the detection of the SXT resistant gene in our isolates was 60% positive for the *sul1* gene. Close percentage was reported by Nelson *et al*., (2014) who detected the *sul1* gene in 73% of his samples. On the other hand, Hilbert D.W., (2011), Momtaz *et al*., (2013), Dehkordi *et al*., (2014) and Shahrani *et al*., (2014), detected the *sul1* gene in 39.5%, 82.78%, 18% and 90.31%, respectively.

The detection of *tetA(A*) gene; tetracycline resistant gene, was 100% positive in the tested isolates. This agrees with Daini and Adesemowo (2008); Hilbert D.W., (2011); Dehkordi *et al*., (2014) and Balasubramaniam *et al*., (2014), Who detected the *tetA(A*) gene by 88%, 84.2%, 76% and 88%, respectively. While do not agree with Nizza et al., (2010), Momtaz *et al*., (2012) and Momtaz *et al*., (2013), who detected *tetA(A*) gene by 34%, 52.63% and 51.63%, respectively.

High and variable antimicrobial resistance is one of the main problems facing the veterinary and the medical fields equally. Especially, where animal and man are in close and continuous contact under such rearing systems in the country side of El-Behera region and other regions as well. This opens the way for the transfer of the resistance

stains in between different animals as well as different species and further between animal and human with its negative effects on the control of pathogenic *E. coli* and treatment of *E. coli*-induced diseases in different hosts.

To our knowledge, this is the first report on the detection of the *tsh* gene in *E. coli* isolates derived from a species other than poultry, which may indicate a more wider distribution of the *tsh*-carriers than it is estimated. Further, this may indicate, together with the high antibiotic resistance to commonly used antibiotics, that buffaloes could be equal to poultry in the risk of zoonotic transfer of pathogenic *E. coli* species to human.

5. REFRENCES

Anwarullah, M., Khan, J.A., Khan, M. S., Ashraf, K., Avais, M. 2014. Prevalence of salmonella and *Escherichia coli* associated with diarrhea in buffalo and cow calves. Buffalo Bull. 33: 3.

Balasubramaniam, A., Arthanari, Eswaran, M., Suresh, P., Sukumar, K. 2014. Detection of tetracycline resistance determinant *tetA* gene and antimicrobial resistance pattern in *Escherichia coli* isolates recovered from healthy layer chickens, Vet. World 7(9): 635-638.

Beraldo, L.G., Borges, C., Maluta, R.P., Cardozo, M.V., Rigobelo, E.C., Avila, F. A. 2014.Detection of Shiga toxigenic (STEC) and enteropathogenic (EPEC) *Escherichia coli* in dairy buffalo. Vet. Microbiol. http://dx.doi.org/10.1016/j.vetmic.2014.01.023.

Bisi-Johnson, M.A, Obi, C.L., Vasaikar, S.D., Baba, K.A., Hattori, T. 2011.Molecular basis of virulence in clinical isolates of *Escherichia coli* and Salmonella species from a tertiary hospital in the Eastern Cape, South Africa. Gut Pathogens 2011, 3:9.

Borriello, G., Lucibelli, M. G., de Carlo, E., Auriemma C D., Cozza G., Ascione., Scognamiglio F., Iovane G., Galiero G. 2012. Characterization of Enterotoxigenic E. coli (ETEC), Shiga-toxin producing *E. coli* (STEC) and necrotoxigenic E. coli (NTEC) isolated from diarrhoeic Mediterranean water buffalo calves (Bubalus bubalis). Res. Vet. Sci. 93: 18-22.

Bok, E., Mazurek, J., Stosik, M., Wojciech M, Baldy-Chudzik K. 2015. Prevalence of Virulence Determinants and Antimicrobial Resistance among Commensal *Escherichia coli* Derived from Dairy and Beef Cattle. Int. J. Environ. Res. Public Health, 12 (1):970–985.

Chang, C. N, Kuang, W. J, Chen E Y. 1986. Nucleotide sequence of alkaline phosphatase gene of *Escherichia* coli. Gene 44 (1): 121 - 125.

Clinical and Laboratory Standerds Institute (CLSI). 2012. Performance standards for antimicrobial susceptibility testing; 23th informational supplement; no.M100-S23, 33 (1)

Constable P. D. 2004. Antimicrobial use in the treatment of calf diarrhea. J. Vet. Int. Med. 18: 8-17.

Croxen, M.A., Finlay, B.B., 2010. Molecular mechanisms of *Escherichia coli* pathogenicity. Nature Rev. Microbiol. 8: 26–38.

Daini, O. A., Adesemowo A. 2008. Antimicrobial susceptibility pattern and R plasmids of clinical strains of Escherichia coli. Aus. J. Basic Appli. Sci., 2: 397-400.

Dehkordi, F. S., Yazdani, F., Mozafari J., Valizadeh, Y. 2014. Virulence factors, serogroups and antimicrobial resistance properties of *Escherichia coli* strains in fermented dairy products. BMC, Res., Notes. 7. 217.

De Verdier, K., Nyman, A., Greko, C., Bengtsson, B. 2012. Antimicrobial resistance and virulence factors in *Escherichia coli* from Swedish dairy calves. Acta Vet. Scand. 54(1):2.

Delicato, E.R., de Brito, B.G., Gaziri, L.C.J., Vidotto, M.C. 2003.Virulence-associated genes in *Escherichia coli* isolates from poultry with colibacillosis. Vet. Microbiol. 94: 97–103.

Ewers, C., Janßen, T., Kießling, S., Philipp H., Wieler, L. H. 2004. Molecular epidemiology of avian pathogenic Escherichia coli (APEC) isolated from colisepticemia in poultry. Vet. Microbiol.104 (1-2):91–101.

Gaber, S.A. 2004. Polymerase chain reaction and DNA electrophoretic pattern of certain types of *Escherichia coli*. M.V.Sc Thesis, Fac. Vet. Med. Alexandria Univ.

Hala, A. A. 2012. Characterization of *E.coli* isolated from cattle and buffalo calves suffering from diarrhea. M.V.Sc Thesis, Fac. Vet. Med. Alexandria Univ.

Hilbert D W. 2011. Uropathogenic Escherichia coli: The Pre-Eminent Urinary Tract Infection Pathogen. Nova Science Publishers, Hauppauge (pp. 1-67).

Hu, Q., Tu J., Han, X., Zhu Y., Ding C., Yu S. 2011. Development of multiplex PCR assay for rapid detection of Riemerella anatipestifer, *Escherichia coli*, and *Salmonella enterica* simultaneously from ducks. J. Microbiol. Methods 87: 64–69.

Ibekwe, A. M, Murinda, S. E, Graves, A. K. 2011. Genetic Diversity and Antimicrobial Resistance of *Escherichia coli* from Human and Animal Sources Uncovers Multiple Resistances from Human Sources. PLoS ONE, Vol. 6, Issue 6, e20819.

JanBen, T., Schwarz, C., Preikschat P., Voss M., Philipp H C., Wieler L H. 2001. Virulence-associated genes in avian pathogenic Escherichia coli (APEC) isolated from internal organs of poultry having died from colibacillosis". Inter. J. Med. Microbiol., 291(5): 371-378.

Johns, I., Verheyen, K., Good, L., Rycroft, A.. 2012. Antimicrobial resistance in faecal *Escherichia coli* isolates from horses treated with anti-microbials: A longitudinal study in hospitalised and non-hospitalised horses. Vet. Microbiol.159: 381-389.

Kaper, J.B., Nataro, J.P., Mobley, H.L. 2004. Pathogenic *Escherichia coli*. Nat Rev Microbiol. 2: 123-140.

Kong, R. Y. C., So, C. L., Law, W.F., Wu, RSS. 1999. A sensitive and versatile multiplex PCR system for the rapid detection of enterotoxigenic (ETEC)

enterohemorrhagic (EHEC) and enteropathogenic (EPEC) strains of *Escherichia coli*. Marine Poll. Bulletin 38 (12): 1207 - 1215.

MacFaddin, J. F. 1985. Media for isolation, cultivation, identification, maintenance of medical bacteria, vol. 1., Williams & Wilkins, Baltimore, MD.

Majueeb, U., Rehman, M. R., Javeed, A. S., Mohd, A. B. 2014. Molecular epidemiology and antibiotic resistance pattern of Enteropathogenic *Escherichia coli* isolated from bovines and their handlers in Jammu, India. J. Adv. Vet. Anim. Res.1(4): 177-181.

Malik, S., Verma, A.K., Kumar, A., Gupta, M. K., Sharma, S. D. 2012. Incidence of calf diarrhea in cattle and buffalo calves in Uttar Pradesh, India. Asian J. Anim. Vet. Adv. 7: 1049-1054.

Mohamed, M. A., Shehata, M. A., Rafeek, E. 2014 Virulence Genes Content and Antimicrobial Resistance in *Escherichia coli* from Broiler Chickens. Vet. Med. Int.

Mohammadi P, Abiri R, Rezaei M, Salmanzadeh-Ahrabi S. 2013.Isolation of Shiga toxin-producing *Escherichia coli* from raw milk in Kermanshah, Iran. Iran. J. Microbiol. 5 (3), 233–238.

Momtaz, H., Dehkordi, F. S., Hosseini, M. J., Sarshar, M., Heidari M. 2013. Serogroups, virulence genes and antibiotic resistance in Shiga toxin-producing *Escherichia coli* isolated from diarrheic and non-diarrheic pediatric patients in Iran. Gut Pathogens, 5: 39.

Momtaz H., Rahimi E. Moshkelani S. 2012. Molecular detection of antimicrobial resistance genes in *E. coli* isolated from slaughtered commercial chickens in Iran. Vet. Med., 57 (4): 193–197.

Nelson, J., Abdlelaziz, A., Elbanna, T., Nagiub, H., Abdel Ghani, S. 2014. Mechanisms of Resistance to Antibiotics in *Escherichia Coli* from Patients with Urinary Tract Infections in Egypt. J. Am. Sci. 10 (10): 235-239.

Nizza, S., Mallardo, K., Marullo, A., Iovane, V., De Martino, L., Pagnini, U. 2010. Antibiotic susceptibility of haemolytic *E. coli* strains isolated from diarrheic feces of buffalo calves. Italian J. Animal Sci. 9 (1), e26.

Nguyen, T. D., Vo, T.T., Vu-Khac H. 2011. Virulence factors in *Escherichia coli* isolated from calves with diarrhea in Vietnam. J. Vet. Sci, 12(2):159–164.

Osman, K.M., Mustafa, A.M., Elhariri, M., Abdelhamed, G S. 2013. The Distribution of Escherichia coli Serovars, Virulence Genes, Gene Association and Combinations and Virulence Genes Encoding Serotypes in Pathogenic *E. coli* Recovered from Diarrheic Calves, Sheep and Goat. Transbound Emerg Dis., 60: 69-78.

Paul, S.K., Khan, M.S.R., Rashid, M.A, Hassan, J., Mahmud, S.M.S. 2010. Isolation and characterization of *Escherichia coli* isolated from buffalo calves in some selected areas of Bangladesh. Bangl. J. Vet. Med. 8 (1): 23 – 26.

Provence, D. L., Curtiss R III. 1994. Isolation and characterization of a gene involved in hemagglutination by an avian pathogenic *Escherichia coli* strain. Infect. Immun. 62:1369–1380.

Randall, L.P., Cooles, S.W., Osborn, M.K., Piddock LJV, Woodward M.J. 2004. Antibiotic resistance genes, integrons and multiple antibiotic resistances in thirty-five serotypes of *Salmonella enterica* isolated from humans and animals in the UK. J. Antimicrobial Chemotherap. 53: 208–216.

Saidenberg, A. A. B., Allegretti, L., Astolfi-Ferreira C C S., Ferreira A J P., Almeida M A., Raso TF. 2013. Some virulence genes of Escherichia coli isolated from cloacal swabs of healthy Alagoas Curassows (Pauxi mitu) in Brazil. Pesquisa Vet. Brasi¬leira 33(4):523-527.

Sambrook J, Fritscgh EF, Mentiates. 1989. Molecular coloning. A laboratory manual. Vol., Cold spring Harbor Laboratotry press, New York.

Shahrani, M., Dehkordi, F. S, Momtaz H. 2014. Characterization of *Escherichia coli* virulence genes, pathotypes and antibiotic resistance properties in diarrheic calves in Iran. Biol. Res. 47: (1) 28.

Stathopoulos C, Provence D L, Curtiss R.III. 1999. Characterization of the avian pathogenic *Escherichia coli* hemagglutinin Tsh, a member of the immunoglobulin A protease-type family of autotransporters. Infect Immun. 67:772–781.

Szmolka, A., Nagy, B. 2013. Multidrug resistant commensal *Escherichia coli* in animals and its impact for public health. Front Microbiol. 4: 258-270.