Detection of Shiga-toxin producing *Escherichia coli* from fecal samples of some cattle farms from Egypt

Abstract

Objective:*Escherichia coli* that produce the Shiga toxin (STEC) has become one of the most significant foodborne pathogens linked to serious illnesses. Our study was aimed todetectthe existence, antimicrobial profiles, andvirulence repertoire of different STEC serotypes from fecal samples of dairy cattle.

Study design: We collected 19 fecal samples (12 diarrheic and 7 non-diarrheic) from dairy cattle. *E. coli* isolation and identification were performed. Shiga toxins genes were detected and the antimicrobial resistance was investigated.

Results:*E. coli* was detected at the rate of 13/19 (68.4%) from the fecal samples.The frequency of *Stx*2a represented 12/13 (92.3%), *Stx*2 represented 3/13 (23.14%), and *Stx*1d, *Stx*2f, *Stx*2g, and *eae* represented 2/13 (15.4%). While the virulence genes *Stx*1, *Stx*1c, *Stx*2c, *Stx*2d, *Stx*2e, and *ehx*A represented 0/13 (0%). The most effective antimicrobials were tulathithromycin 30 (53.84%) followed by ceftiofur 30 and marbofloxacin 5 (46.16%), amikacin (15.38%), and the least resistance was recorded for nalidixic acid and oxytetracycline (7.70%).The resistant antibiotics with percentages reached to 100% were amoxicillin/ clavulanic acid, ceftriaxone, ciprofloxacin, doxycycline, erythromycin, gentamicin, linezolid, nitrofurantoin, penicillin G, streptomycin, trimethoprim/sulfamethoxazole, and vancomycin followed by chloramphenicol, nalidixic acid, oxytetracycline, and tyrosine 30 (92.30%), amikacin and ampicillin (84.62%), ceftiofur 30 (23.07%), marbofloxacin 5 (15.38%) and tulathithromycin 30 (7.70%).

Conclusions: The findings demonstrated that *E. coli* that are resistant to numerous groups of antimicrobialsspread through feces and may pose a risk to human and animal health.

Keywords: Dairy cattle, Shiga-toxigenic*E.coli*, Virulence genes, Antimicrobial resistance,

1. Introduction

Escherichia coli (E. coli) is a common inhabitant of the human and animals' intestinal tract**[1]** andhas been selected as a guard organism in worldwide antimicrobial

resistance (AMR) surveillance investigations**[2,3]**.Although ruminants are the mainreservoir of STEC that contaminate environment andfoods of animal and plant origins, STEC isolates wereestablished in poultry, pet birds, wild birds andpigeons, dogs, and pigs **[4,5]**.*E.coli* is one of the most prevalent raw milk pollutants comes from a variety of sources, primarily animal feces.The constant presence of *E. coli* indicates fecal contamination, and the presence of additional enteric pathogens in raw milk puts consumers' health at risk**[6]**.Many dairy food products were contaminated with *E. coli* through the fecal-oral rout as a result of poor hygienic measures in farms and the failure of pasteurization process**[7]**.

STEC infectionsareincriminated in hemolytic uremic syndrome and hemorrhagic colitis in human **[8,9]** and numerous food-borne epidemics**[10]**.In addition,STEC are associated with dysentery in cattle. Furthermore, they produce twotypes of Shiga-toxins; *Stx*1 and *Stx*2, and certain types of STEC have the ability to produce the intimin. The *E. coli* strains, which possess the eaeA gene and do not produce *stx*1 and *stx*2 genes, were defined as Enteropathogenic *E. coli***[11,12]**. It is known that, *stx*1a, *stx*1c, and *stx*1d are the genetic variants of *stx*1, while the variants of *stx*2 are *stx*2a, *stx*2b, *stx*2c, *stx*2d, *stx*2e, *stx*2f, and *stx*2g **[13]**. A single STEC strain may harbor one or more Shiga toxin-encoding genes (*stx*) in their genome**[14,15]**.

One of the greatest problems that affect the human and animal health allover the world is the antimicrobial resistance**[16,17]**. It is encoded genetically as a result of different causes including the unnecessary low dosage of antibiotics, feed additives, and preventive therapy in dairy animals **[18,19]**. Continuous shedding of the antibacterial resistant bacteria onto dairy farms and the surrounding environment occurred through feces, milk, or urine **[20,21,22]**.Moreover, it maytransmit to humans through milk and meat consumption or manure land application as soil amendment for vegetables and crops for human consumption**[23,24,25,26]**.Antimicrobial resistance and virulence types in *E. coli* have been investigated on fecal or manure samples of dairy cattle in a variety of countries, including Egypt **[1,27]**, Canada **[28]**, USA **[29]**, and Switzerland **[30]**. Therefore, this study was performed to determine the prevalence of *E. coli* in fecal samplings from dairy cattlein Egypt and determine theirvirulence factors and antibiotic sensitivity.

2. Materials and methods

2.1. Samplings

A total of 19 fecal samples were collected from Egyptian dairy cattle. Twelve samples were from diarrheic feces and seven samples were from non-diarrheic feces.Sterile plastic bags were used for the fecal samplescollection. The samples werelabeled and kept on ice and transported to the Faculty of Veterinary Medicine, Sadat City University for examination in the laboratory of the Department ofBacteriology, Mycology, and Immunology.

2.2. *E. coli* isolation and identification

The collected samples were pre-enriched innutrient broth and incubated for 24 hrs at 37°C.After the appearance of confirmed growth, aloopful was streaked onto Mac Conkey agarmedium plates (MAC) and incubated at 37°C for 24 h. The pink Lactose fermentingcolonies were picked up with a sterile looptransferred onto Eosin Methylene Blue (EMB)and incubated for 24 h at 37°C. Discretecolonies with metallic green sheen typical of *E. coli* were inoculated into the semisolid medium bystabbing technique, incubated for 24 h at 37°C,and kept at 4°C. The suspected colonies were used for furthermorphological,biochemical, and molecularcharacterization. The isolated *E. coli* strainsupon specific media were confirmed at thespecies level using different biochemical tests such ascytochrome oxidase, indole test, triple sugar ironagar, and urease **[31,32]**. The *Staphylococcus aureus* ATCC 29737 was used as a negativecontrol and *E. coli* (O157:H7, *stx*1, *stx*2, *eae*A,*hly*A) ATCC 35150 was used as a positivecontrol.

2.3. Characterization of virulence genes harbored by *E. coli*serotypes

The preserved *E. coli* serotypes on the semisolidmedium were inoculated on MAC agar mediumplates. After overnight incubation at 37°C,a fewselectedcolonies were picked with a steriletoothpickfor DNA extraction using the QI AampMiniprep kit andperformed according to themanufacturer's instructions. All the *E. coli*serotypes obtained were examined for thepresence of virulence genes using primerstargeting *stx*1, *stx*2, *eae*A, and *hly*A.

2.4. Antimicrobial susceptibility test

The confirmed serotypes were cultivated on Mueller-Hinton (Oxoid) broth for 18 hrs at 37°C until the bacterial density was adjusted to $1.5x10^8$/1 ml (using McFarland tube 0.5). Then, 1 ml from each tube was spread on Mueller Hinton agar (Oxoid)

plates.The disk diffusion technique was used to determinesusceptibility to 21 antimicrobials in accordance withthe Clinical and Laboratory Standards Institute**[33]**guidelines. The following antimicrobials were used:amikacin, amoxicillin/clavulanic acid, ampicillin, ceftiofur 30, ceftriaxone, chloramphenicol, ciprofloxacin, doxycycline, erythromycin, gentamycin, linezolid, marbofloxacin 5, Nalidixic acid, Nitrofurantoin, oxytetracycline, penicillin, streptomycin, trimethoprim/sulfamethoxazole, tulathithromycin 30, tyrosine 30, and Vancomycin.

2.5. Ethical Approval

The study was reviewed and approved by the Animal Use Ethics Committee of the Faculty of Veterinary Medicine, Sadat City University.

2.6. Statistical analysis

3. Results

3.1. Prevalence of *E. coli* from dairy cattle fecal samples

A total of nineteen fecal samples from dairy cattle (12 diarrheic and 7 non-diarrheic) were exposed toisolation and biochemical identificationprocedures using specific media. Nine samples (75%) of the diarrheic samples were positive for *E. coli* while four samplesof the non-diarrheic samples (54.14%) were positive. So, the total positive number and percentage for the isolation of *E. coli* was 13 (68.4%) as shown in Table 1.

Table 1: Results of isolation of *E. coli*

Samples	No. of collected samples	No. and percentage of isolates
Diarrheic Feces	12	9 (75%)
Non-diarrheic Feces	7	4 (57.14%)
Total Samples	19	13 (68.4%)

3.2. Percentage of virulence genes among the obtained isolates

The obtained results in Table 2 regarding the detection of virulence genes showed that *Stx*2a represented 12/13 (92.3%),*Stx*2 represented 3/13 (23.14%), and *Stx*1d,*Stx*2f, *Stx*2g, and *eae*represented2/13 (15.4%). While the virulence genes *Stx*1, *Stx*1c, *Stx*2c, *Stx*2d, *Stx*2e, and *ehx*A represented 0/13 (0%).

Table 2: Results of virulence genes of *E.coli*

Isolate code	Source	Virulence factors											
		***Stx*1**	***Stx*1d**	***Stx*1c**	***Stx*2**	***Stx*2a**	***Stx*2c**	***Stx*2d**	***Stx*2e**	***Stx*2f**	***Stx*2g**	***eae***	***ehx*A**
1	Diarrheic feces	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2	Diarrheic feces	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
3	Diarrheic feces	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
4	Diarrheic feces	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
5	Diarrheic feces	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
6	Diarrheic feces	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
7	Diarrheic feces	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
8	Diarrheic feces	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
9	Diarrheic feces	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve
10	Non-diarrheic feces	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
11	Non-diarrheic feces	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
12	Non-diarrheic feces	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
13	Non-diarrheic feces	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve
Total		0/13 (0.0 %)	2/13 (15.4%)	0/13 (0.0%)	3/13 (23.1%)	12/13 (92.3%)	0/13 (0.0%)	0/13 (0.0%)	0/13 (0.0%)	2/13 (15.4 %)	2/13 (15.4%)	2/13 (15.4 %)	0/13 (0.0%)

3.3. Antimicrobial susceptibility pattern of *E. coli* isolates

The positive *E. coli* samples were tested for antibiotic resistance and were applied antibiotics disks,amikacin,amoxicillin/clavulanic acid, ampicillin,ceftiofur 30, ceftriaxone,chloramphenicol,ciprofloxacin, doxycycline, erythromycin, gentamycin, linezolid, marbofloxacin 5,Nalidixic acid, Nitrofurantoin,oxytetracycline,penicillin,streptomycin,trimethoprim/sulfamethoxazole , tulathithromycin 30, tyrosine 30, and Vancomycin.The resistant antibiotics with

percentages reached to 100% were amoxicillin/ clavulanic acid, ceftriaxone, ciprofloxacin, doxycycline, erythromycin, gentamicin, linezolid,nitrofurantoin, penicillin G, streptomycin,trimethoprim/sulfamethoxazole, and vancomycin followed by chloramphenicol,nalidixic acid, oxytetracycline, and tyrosine 30 (92.30%), amikacin and ampicillin (84.62%), ceftiofur 30 (23.07%), marbofloxacin 5 (15.38%) and tulathithromycin 30 (7.70%).Antibiotics that revealedhigh antimicrobial activities with good effect fortulathithromycin 30 (53.84%) followed by ceftiofur 30 and marbofloxacin 5 (46.16%), amikacin (15.38%), and the least sensitive was recorded for nalidixic acid and oxytetracycline (7.70%), (Table 3).

Table 3: Results of antimicrobial susceptibility pattern of *E. coli* isolates

No.		Amikacin	Amoxicillin/ clavulanic acid	Ampicillin	Ceftiofur 30	Ceftriaxone	Chloramphenicol	Ciprofloxacin	Doxycycline	Erythromycin	Gentamicin	Linezolid	Marbofloxacin 5	Nalidixic acid	Nitrofurantoin	Oxytetracycline	Penicillin G	Streptomycin	Trimethoprim/sulfamethoxazole	Tulathithromycin 30	Tylosin 30	Vancomycin
1	DF*	R	R	R	S	R	R	R	R	R	R	R	I	R	R	R	R	R	R	S	R	R
2	DF	R	R	I	I	R	R	R	R	R	R	R	S	R	R	R	R	R	R	I	R	R
3	DF	R	R	R	I	R	R	R	R	R	R	R	I	R	R	R	R	R	R	S	R	R
4	DF	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	S	R	R
5	DF	R	R	R	S	R	R	R	R	R	R	R	S	R	R	R	R	R	R	I	R	R
6	DF	S	R	R	S	R	R	R	R	R	R	R	S	R	R	R	R	R	R	S	R	R
7	DF	R	R	R	I	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R
8	DF	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
9	DF	R	R	R	S	R	R	R	R	R	R	R	I	S	R	R	R	R	R	I	R	R
10	NDF**	R	R	R	S	R	R	R	R	R	R	R	I	R	R	R	R	R	R	S	R	R
11	NDF	S	R	R	S	R	R	R	R	R	R	R	I	R	R	R	R	R	R	I	R	R
12	NDF	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	R	R	R	S	R	R
13	NDF	R	R	R	I	R	R	R	R	R	R	R	S	R	R	R	R	R	R	I	R	R
S		2/13 15.38%	0/13 0%	0/13 0%	6/13 46.16%	0/13 0%	0/0 0%	0/13 0%	0/13 0%	0/13 0%	0/13 0%	0/13 0%	6/13 46.16%	1/13 7.70%	0/13 0%	1/13 7.70%	0/13 0%	0/13 0%	0/13 0%	7/13 53.84%	0/13 0%	0/13 0%
R		11/13 84.62%	13/13 100%	11/13 84.62%	3/13 23.07%	13/13 100%	12/13 92.30%	13/13 100%	13/13 100%	13/13 100%	13/13 100%	13/13 100%	2/13 15.38%	12/13 92.30%	13/13 100%	12/13 92.30%	13/13 100%	13/13 100%	13/13 100%	1/13 7.70%	12/13 92.30%	13/13 100%
I		0/13 0%	0/13 0%	2/13 15.38%	4/13 30.77%	0/13 0%	1/13 70.70%	0/13 0%	0/13 0%	0/13 0%	0/13 0%	0/13 0%	5/13 38.46%	0/13 0%	0/13 0%	0/13 0%	0/13 0%	0/13 0%	0/13 0%	5/13 38.46%	1/13 70.70%	0/13 0%

***DF: Diarrheic feces**

****NDF: Non-diarrheic feces**

4. Discussions

In this study,68.4%(13/19) of fecal samples(diarrheic and non-diarrheic)from cattlewere positivefor *E. coli*.These results are nearly similar to the study that performed in Egypt (63.6%) **[34]**. Furthermore, our findings are lower than what was reported in diarrheic calves in India (85.04%) **[35]**. However, a lower prevalence of *E. coli* was reported in previous investigations in Switzerland**[36]**, Korea**[37]**, Egypt**[38]**, Argentina**[39]**, and Ethiopia **[40],**with percentages of (5.5%), (22%), (28.8%), (30.1%), and (36.8%), respectively. These differences in the prevalence of *E. coli* may be attributed to different causes including, geography, hygienic measures for animals and the surrounding environment, health conditions of cattle and mixing of different ages of animals together **[34,38,39,41]**.Other researchers have similarly reported the isolation of *E. coli*from diarrheic calves in Egypt **[27,42,43]**.

Concerning with the obtained findings of virulence genes, the *Stx*2a represented 12/13 (92.3%).The high *Stx*2 percentage that is associated with clinical signs was concluded in the studies of **[44]** and **[27]** who stated that the *Stx*2 gene was more prevalent than *Stx*1 and that both were associated with *eae* gene in STEC strains. In contrast to our finding, the prevalence virulence was*Stx*1 and *eae* genes **[45]**. Moreover, the previous study performed by Elsayed et al.,**[46]** reported that both the *stx*1 and*stx*2 genes were highly prevalent. In our study the *eae* genes were noticed in 15.4% of *E. coli* isolates, which is in accordance with Ishii et al.,**[47]**but is in contrast to**[48]** and **[49]**.

Regarding the results of antimicrobial resistance against E. coli, the isolated strains exhibited the maximum resistance (100%) againstamoxicillin/ clavulanic acid, ceftriaxone, ciprofloxacin, doxycycline, erythromycin, gentamicin, linezolid, nitrofurantoin, penicillin G, streptomycin, trimethoprim/sulfamethoxazole, and vancomycin followed by chloramphenicol, nalidixic acid, oxytetracycline, and tyrosine 30 (92.30%), amikacin and ampicillin (84.62%), ceftiofur 30 (23.07%), marbofloxacin 5 (15.38%) and tulathithromycin 30 (7.70%). In a previous study performed in Egypt **[50]** concluded that the most prevalent resistance antimicrobials were ampicillin (71.4%), amoxicillin (64.3%),trimethoprim/sulfamethoxazole(50%), and gentamicin (42.8%). Moreover, in Iranian investigation there was amaximum resistance of *E. coli* to lincomycin, penicillin, streptomycin,sulfamethoxazole, and tetracyclinefromdiarrheic calves **[51]**. Furthermore, inSwitzerland in 2022 data reported the antimicrobial pattern of resistance as ampicillin (48%),

tetracycline(45%), and chloramphenicol (27%) **[30]**.Notably, the present study reported that the *E. coli* isolates were sensitive to tulathithromycin 30, ceftiofur 30, marbofloxacin 5, amikacin, nalidixic acid, and oxytetracycline with the percentages of(53.84%), (46.16%),(46.16%),(15.38%),(7.70%), and(7.70%) respectively.However, Elsayed et al., **[46]**established that STEC serotypes were highlysensitive to chloramphenicol and doxycyclinemore than that of ampicillin andamoxycillin/clavulinic acid.Overall, our data of antimicrobial resistance in the study indicated the misuse of the veterinary drugs in the cattle farms and also the usage of the Egyptian paramedical people of these drugs.

5- Conclusion

E. coli continues to be one of the major causes of severeeconomic losses all over the world. The Shiga toxin gene; *Stx*2a,is the most prevalent virulence genes associated with STEC followed by *Stx*2, *Stx*1d, *Stx*2f, *Stx*2g, and *eae*.The majority of the isolated *E. coli* have resistance pattern to amoxicillin/ clavulanic acid, ceftriaxone, ciprofloxacin, doxycycline, erythromycin, gentamicin, linezolid, nitrofurantoin, penicillin G, streptomycin, trimethoprim/sulfamethoxazole,vancomycin, chloramphenicol, nalidixic acid, oxytetracycline, and tyrosine 30, amikacin, and ampicillin.Moreover, tulathithromycin 30, ceftiofur 30, and marbofloxacin 5are the most effective antimicrobial agents against the isolated *E. coli*. Our findings should inform veterinarians about the possible prognosis of *E. coli* infections in dairy cattle thereby reducing the unselective usage ofantibiotics.

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