JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

# PREVALENCE, ANTIMICROBIAL RESISTANCE AND SUBSTANTIAL VIRULENCE-ASSOCIATED GENES OF *ESCHERICHIA COLI* ISOLATED FROM COLIBACILLOSIS IN NEONATAL CALVES IN EGYPT

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**doi: 10.15414/jmbfs.2020.9.6.1145-1150**

ARTICLE INFO

Received 13. 11. 2019
Revised 4. 2. 2020
Accepted 5. 2. 2020
Published 1. 6. 2020

Regular article

OPEN ACCESS

**ABSTRACT**

Pathogenic *Escherichia coli* (*E. coli*) consider the most common bacterial agent causes calf diarrhea, particularly in newborn calves. Therefore, this study highlights prevalence, serotyping, antibiogram pattern of *E. coli* recovered from diarrheic calves in Egypt. In addition to the investigation of six virulence encoding genes (*iss*, *fim*H, *tsh*, iutA, *stx*2 and *eae*A) using polymerase chain reaction (PCR). One hundred and twenty calves were examined for *E. coli* existence. A total of 16 (40%) *E. coli* strains were isolated from 40 diarrheic calves samples. Seven *E. coli* serotypes (O26, O157, O78, O125, O146, O44, and O18) were recovered, and O26 and O157 were the most common. The tested strains exhibit high susceptibility to ciprofloxacin, enrofloxacin and polymixin and amoxicillin/clavulanic acid, while a high resistance to streptomycin, gentamycin, tetracycline, sulfamethoxazole/trimethoprim, chloramphenicol, and neomycin was recorded. The *iss* and *fim*H genes were the most frequently identified virulence genes in all tested strains (100%), followed by *eae*A (31.25%) and *stx*2 (6.25%), whereas *tsh* and *iut*A were not detected at all. Our findings emphasize the existence of multi-antimicrobial resistance strain as well as virulence genes, which is crucial for developing novel methods to control of colibacillosis in calves.

**Keywords:** *Escherichia coli*, calf diarrhea, serotyping, antimicrobial profile, virulence genes

## INTRODUCTION

Calf diarrhea represents a prevalent financial dilemma in the animal industry worldwide (**Shahrani et al., 2014**). Diarrhea is a common cause for high morbidity and case fatality rates, particularly in newborn calves. Various microorganisms, such as protozoa (*Cryptosporidium parvum*), viruses (coronavirus and rotavirus) and bacteria can cause diarrhea (**Izzo et al., 2011**). Although various bacterial types have been recovered from calves with diarrhea, *E. coli* remains an important noteworthy pathogenic bacterium in diarrheic calves and its prevalence varies according to geographic area (**García et al., 2000**).
In general, this bacterium lives naturally in both human and animal intestines. However, certain strains can become pathogenic by producing virulence factors and can, therefore, cause enteric infections (**Picco et al., 2015**). Many researchers recorded that among ruminant, cattle may implicate as the primary barrage for of enterovirulent *E. coli* that can transfer into humans through the food series (**Pradel et al., 2001**). Accordingly, *E. coli* is considered a leading cause of some clinical syndrome, such as septicemia, diarrhea, pneumonia, meningitis, and panophthalmitis especially in newborn calves and death in complicated dehydrated cases (**Shahrani et al., 2014**).
Based on their virulence factors, pathogenic *E. coli* strains can be divided into six types: (i) enteropathogenic *E. coli* (EPEC); (ii) enterotoxigenic *E. coli* (ETEC); (iii) enterohemorrhagic *E. coli* (EHEC), also known as shiga toxin-producing *E. coli* (STEC); (iv) enteroaggregative *E. coli* (EAEC); (v) diffusely adherent *E. coli* (DAEC); and (vi) enteroinvasive *E. coli* (EIEC) (**Kaper et al., 2004**). All these pathotypes are mainly associated with diarrheagenic strains. A number of probable virulence of *E. coli* strains are potential based on the presence of substantial factors, such as adheins, fimbriae, invasins, toxins, capsules, siderophores and hemolysins which play a critical role to avoid or suppress defenses and inflammatory response of the host (**Bekal et al., 2003 and Croxen and Finlay, 2010**)
Among the ETEC and EPEC pathotypes, molecular analysis has revealed a considerable divergence in virulence genotypes (**Beutin et al., 2005**). The pathogenicity of *E. coli* based mainly on adhesion and binding to the receptor of the mucosa of the small intestine using of fimbrial factors and exotoxins (**Kaper et al., 2004**) including F5 (K99), F41 and heat-stable enterotoxin (**Acres, 1985**). Intimin (*eae*) gene and heat-stable enterotoxins were considered the most familiar virulence genotypes linked with EPEC *E. coli* (**Mainil, 2013**). Moreover, several virulence genes (*stx*1, *stx*2, *eae, ehly*, *hlyA, lt, st, cnf*1, *cnf*2, *cdt*III and *f*17c*)* are involved and predominated as virulence properties *of E. coli* strains particular of calves origin (**Shahrani et al., 2014**).
The use of antimicrobial agents is usually an effective tool applied for the prevention and treatment of neonatal calf diarrhea (**Berge et al., 2009**). However, this method may lead to the evolution of resistant bacterial strains to several antimicrobial drugs (**Hammerum and Heuer, 2009**). Furthermore, in recent years, much international surveillance recorded multidrug-resistant of *E. coli* strains of animals or human origin (**Woodford et al., 2011**). In animals, rapid evolution of antimicrobial resistance has been recently reported within E. *coli* isolates recovered from neonatal calves, especially those received growth promoters antibiotics in milk and milk substitutes (**Pereira et al., 2011 and Tadesse et al., 2012).**
The significant economic losses due to diarrhea in the dairy farm as well as in small-holder in Egypt encourage this work to investigate the prevalence, antimicrobial pattern, and distribution of the *iss*, *fim*H, *tsh*, *iut*A, *stx*2 and *eae*A virulence genes between, *E. coli* isolates recovered from diarrheic calves.

## MATERIALS AND METHODS

### Sample collection and Study area

The current study was performed from October 2018 to April 2019, through which cattle parturition rate is high. One-hundred and twenty calves under one-month-old were monitored clinically for the incidence of diarrhea cases from small-holder in Menoufiya Province, Egypt. The clinical examination was done as described by **Jackson and Cockcroft (2002)**, including clinical signs, systemic reaction, dehydration, pulse and respiratory rates. Forty fecal samples were aseptically collected from diarrheic calves using sterilized bacteriological swabs and directly transferred in the icebox to the lab for bacteriological examination.

### Bacteriological examination of *E. coli* strains

The collected fecal swab samples were inoculated into tryptose soy broth (mTSB- Difco La Jolla, CA, USA) and incubated at 37°C for 12 hours and then sub-cultured on a selective culture medium (MacConkey agar, MAC; Difco) at 37°C for one day. Pink color colonies were then picked up and cultured in eosin methylene blue (EMB; Difco) medium. Metallic green colonies were considered *E. coli*. Confirmation of all isolates were done through morphological characters, Gram staining and standard biochemical tests, including indole, citrate utilization, Voges-Proskauer, methyl red, Triple Sugar Iron (TSI), and urease tests as described by (**Cowan and Steels, 1985**) and biofilm activity on congo red medium according to **(Nivedith et al., 2012).**

**_E. coli_ isolates Serotyping**

Serotyping using the slide agglutination test for *E. coli* isolates was done as described by **(Edwards and Ewing, 1972**).

**Antibiogram profile**

The antimicrobial susceptibility test of *E. coli* strains was performed using a Kirby-Bauer disk diffusion assay according to (**CLSI, 2002**). Bacterial suspension was prepared according to (**Tenover, 2009**). *E. coli* isolates were then examined *in vitro* for their susceptibility to 10 antimicrobial discs (Oxoid): tetracycline: TET, (30µ); chloramphenicol C (30µ); amoxicillin/clavulanic acid AMC (30µ); streptomycin S (10µ); enrofloxacin ENR (5µ); sulfamethoxazole/trimethoprim SXT (25µ); gentamicin CN (10µ); neomycin N (30µ); ciprofloxacin CIP (5µ); and polymixin PB (300IU). The inhibition zone was measured to survey the resistance or the susceptibility according to the interpretation criteria established by CLSI standard.

**Molecular characterization of _E. coli_ serovars virulence genes**

Freshly grown typical *E. coli*-like colonies were collected and the DNA extraction was done according to the manufacturer's guidelines using QIAamp DNA Mini Kit DNA extraction kit (Qiagen, GmbH, Germany). All *E. coli* strains were screened for six virulence genes (*iss*, *fim*H, *tsh*, *iut*A, *stx*2 and *eae*A) by PCR. Several PCR protocols were used to detect the target genes of *E. coli* isolates. Lists of primers used, the PCR conditions, and the amplified products are listed in Tables 1. Amplification of DNA was carried out a total reaction of 25 µl including 12.5 µl of PCR Master Mix, 1 µl of each primer of 20 pmol concentration, 6 µl of DNA template and 4.5 µl of purified water. The primers (Metabion, Steinkirchen, Germany) used for detecting the virulence genes of *E. coli* (*fim*H and *tsh*) (**Colom et al., 2003**), *eae*A (**Archambault et al., 2006**), *iss* and *iut*A (**Ghanbarpour and Salehi, 2010**) and for *stx*2 gene (**Bisi-Johnson et al., 2011**).

The reactions were performed in an Applied Biosystems 2720 thermal cycler (Applied Biosystems, Foster, CA). Five microliter aliquots of all PCR products and a 100 bp DNA ladder were loaded in the wells, electrophoresed through 1.5% agarose gels, stained with ethidium bromide and photographed under UV transillumite.

**Table 1** Target genes, primer sequences, amplicon sizes and cycling conditions of virulence genes of *E. coli* isolates

| Target gene | Primer sequences Virulence genes | Amplified amplicon (bp) | Primary denaturation | Secondary denaturation | Annealing | Extension | Final extension |
|---|---|---|---|---|---|---|---|
| *Iss* | ATGTTATTTTCTGCCGCTCTG<br>CTATTGTGAGCAATATACCC | 266 | 94˚C<br>5 min. | 94˚C<br>30 sec. | 54˚C<br>30 sec. | 72˚C<br>30 sec. | 72˚C<br>7 min. |
| *Stx2* | CCATGACAACGGACAGCAGTT<br>CCTGTCAACTGAGCAGCACTTTG | 779 | 94˚C<br>5 min. | 94˚C<br>30 sec. | 58˚C<br>45 sec. | 72˚C<br>45 sec. | 72˚C<br>10 min. |
| *fim*H | TGCAGAACGGATAAGCCGTGG<br>GCAGTCACCTGCCCTCCGGTA | 508 | 94˚C<br>5 min. | 94˚C<br>30 sec. | 50˚C<br>45 sec. | 72˚C<br>45 sec. | 72˚C<br>10 min. |
| *Tsh* | GGT GGT GCA CTG GAG TGG<br>AGT CCA GCG TGA TAG TGG | 620 | 94˚C<br>5 min. | 94˚C<br>30 sec. | 54˚C<br>30 sec. | 72˚C<br>30 sec. | 72˚C<br>10 min. |
| *iut*A | GGCTGGACATGGGAACTGG<br>CGTCGGGAACGGGTAGAATCG | 300 | 94˚C<br>5 min. | 94˚C<br>30 sec. | 63˚C<br>45 sec. | 72˚C<br>30 sec. | 72˚C<br>7 min. |
| *eae*A | ATG CTT AGT GCT GGT TTA GG<br>GCC TTC ATC ATT TCG CTT TC | 248 | 94˚C<br>5 min. | 94˚C<br>30 sec. | 51˚C<br>30 sec. | 72˚C<br>30 sec. | 72˚C<br>7 min. |

All genes were amplified for 35 cycles.

**RESULTS AND DISCUSSION**

**Prevalence and clinical examination of diarrheic calves**

Calf diarrhea is considered a major acute disease in neonatal calves causing high mortality and significant economic losses. This syndrome caused by multiple etiology (e.g. bacteria, viruses, protozoa) led to severe mucoid diarrhea, dehydration and rapid weight loss and may end with death in complicated non-treated calves (**Galal et al., 2013 and Cho and Yoon, 2014**). *E. coli* is the primary bacterial etiological agent responsible for calf diarrhea (**Osman et al., 2012**).

In our present work, a total of 120 samples were collected from under one month old calves that monitored clinically for the incidence of diarrhea cases from small-holder in Menoufiya Province, Egypt and examined for the incidence of *E. coli*. The clinical observation and examination of diseased calves revealed profuse watery and mucoid diarrhea with a variable degree of dehydration that was detected by a decrease in skin elasticity, sunken eyes, cold extremities and inability to stand. All affected calves were depressed and had a rapid pulse and respiratory rates and rough coats. Systemic reactions were observed in some of the affected animals.

Of 120 examined calves samples, forty (33.3%) were diarrheic, sixteen (40%) of the examined diarrheic calves were positive for *E. coli*. Our result, nearly similar to those of other studies in India reported by (**Malik et al., 2013 and Shekhar et al., 2017**) who isolated *E. coli* from diarrheic calves samples with a prevalence rate of 37.61% and 41.6%, respectively. In Egypt (**Galal et al., 2013**) reported an *E. coli* prevalence rate of 28.57%. On contrary, this result was lower than that reported by (**Osman et al., 2013; Anwarullah et al., 2014 and Majueeb et al., 2014**) with a prevalence rate of 63.6%, 72.8%, and 50%, respectively. While, (**Duse et al., 2015 and El-Seedy et al., 2016**) reported a lower prevalence rate with 14.6% and 18.1%, respectively. The variations in the results might attribute to the difference in the number of collected samples, geographical regions, stress factors, hygienic and management systems.

**Serogrouping of _E. coli_ isolates**

Serogrouping of sixteen *E. coli* isolates in our study revealed that O26 and O157 were the most common identified *E. coli* serogroups with prevalence rate 25% of each type. Other *E. coli* serogroups (O78, O125, and O146) were identified with a prevalence rate of 12.5% for each and finally *E. coli* serogroups (O44 and O18) with a prevalence rate of 6.25% for each. Our results are in agreement with (**Shahrani et al., 2014**) who conducted that *E. coli* O26 and O157 were the most *E. coli* serogroups isolated from diarrheic calves. Other studies reported by (**El-Seedy et al., 2016**) revealed that O26 and O103 were the most prevalent (17.7% of each) *E. coli* serogroups isolated from neonatal diarrheic calves. However,

(**Badouei et al., 2010**) recorded that O26 is the most prevalent *E. coli* serotypes with (18.4%) as well as O157:H7, O111, and O26 were also serotyped from diarrheic and non-diarrheic calves. While, (**Saridakis et al., 1997**) reported that O26, O114 and O119 were the most identified *E. coli* strains obtained from diarrheic calves. In addition to (**Mora et al., 2011**) who typing 52% of bovine *E. coli* strains serovars as belonged to O4, O20, O22, O26, O77, O105, O113, O157, and O171 serovars. The variation in the dominate serogroups of *E. coli* may explain the difference in geographic areas and numbers of the collected samples.

**Antimicrobial susceptibility pattern of *E. coli* serotypes from diarrheic calves**

Concerning antimicrobial susceptibility testing in this study, tested *E. coli* strains showed sensitivity against ciprofloxacin, enrofloxacin, polymixin and amoxicillin/clavulanic acid with percentage 81.25%, 75%, 75% and 62.5%, respectively. Meanwhile, streptomycin showed complete resistance (100%); followed by gentamycin, tetracycline, sulfamethoxazole/trimethoprim and chloramphenicol (81.25% for each) and neomycin-(56.25%) were noticed (Table 2). Similar findings were reported by (**Khachatryan et al., 2006**) who showed that *E. coli* exhibit phenotypic resistance to streptomycin, sulfonamide and tetracycline. Other studies have supported our result recorded by (**Pereira et al., 2011 and Shahrani et al., 2014**) they showed that all isolates of *E. coli* exhibited high susceptibility to ciprofloxacin and cefepime and resistance to tetracycline, streptomycin, and sulfamethoxazole-trimethoprim. However, (**Srivani et al., 2017**) reported high antimicrobial resistance of STEC strains isolated from diarrheic calves to tetracycline (63.21%) and susceptible to chloramphenicol, gentamycin (96.33%) and imipenem (99.06%) in addition to 69.81% of these strains expresses multidrug resistance. Furthermore, (**Hang et al., 2019**) found that tetracycline was detected as the most resistant antibiotic by *E. coli* strains followed by sulfamethoxazole, ampicillin, trimethoprim, and ciprofloxacin.

**Table 2** Antibiogram of different *E. coli* isolates

| Antimicrobial class | Antimicrobial agents | No of *E. coli* isolates (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | **R** | % | I | % | S | % |
| Chloramphenicol | Chloramphenicol (C) | 13 | 81.25 | 2 | 12.5 | 1 | 6.25 |
| Aminoglycosides | Streptomycin (S) | 16 | 100 | 0 | - | 0 | - |
| Aminoglycosides | Gentamycin (CN) | 13 | 81.25 | 3 | 18.75 | 0 | - |
| Aminoglycosides | Neomycin (N) | 9 | 56.25 | 4 | 25 | 3 | 18.75 |
| Tetracycline | Tetracycline (TET) | 13 | 81.25 | 1 | 6.25 | 2 | 12.5 |
| Beta –lactams | Amoxicillin/Clavulanic acid (AMC) | 4 | 25 | 2 | 12.5 | 10 | 62.5 |
| Sulfonamides | Sulfamethoxazole/trimethoprim (SXT) | 13 | 81.25 | 1 | 6.25 | 2 | 12.5 |
| Fluoroquinolones | Ciprofloxacin (CIP) | 0 | - | 3 | 18.75 | 13 | 81.25 |
| Fluoroquinolones | Enrofloxacin (ENR) | 1 | 6.25 | 3 | 18.75 | 12 | 75 |
| Polymixins | Polymixin B (PB) | 2 | 12.5 | 2 | 12.5 | 12 | 75 |

**R**=Resistance **I**=Intermediate **S**=Sensitive

A comparative study in Egypt was carried out (**El-Seedy et al., 2016**) demonstrated that *E. coli* isolates had high susceptibility to marbofloxacin, spectinomycin, and neomycin. Otherwise, (**Ortman and Svensson, 2004**) discussed that trimethoprim/sulfa and enrofloxacin were considered an effective treatment for diarrhea in young calves.

The high resistances of E. coli isolates to tetracycline in our study were previously supported by many authors (**Schroeder et al., 2002; Rigobelo et al., 2006; Alberto et al., 2011 and Hang et al., 2019**). However, higher resistance to streptomycin, gentamycin, tetracycline, chloramphenicol, sulfamethoxazole/trimethoprim and neomycin in our study was in contrary to other reports by (**Wani et al., 2013; Rehman et al., 2014 and Srivani et al., 2017**), that reported STEC isolates from diarrheic calves showed high susceptibility to gentamycin and chloramphenicol.

The high incidence of resistant or multi-resistant *E. coli* isolates in the present work may be attributed to that the wide and haphazard using of antimicrobials in animals for treatment, prevention, and control of infectious diseases, and as growth promoters for potential livestock production (**Marshall and Levy, 2011**). In Egypt, the random using of these traditional antibiotics without veterinarian prescription and the overuse or misuse of antimicrobials in the veterinary field by dairy farmers, lead to emerging of their resistance genes which are of public concern as it could be passed on to humans (**WHO, 2014**).

**Molecular detection of *E. coli* virulence genes**

Regarding *E. coli* strains, their pathogenicity mainly relies on the existence of powerful toxins and certain virulence determinants, of these shiga toxins (*ST*) and intimin (*eae*A) that facilitate the adhesion and colonization of *E. coli* strains in the host cells (**Oswald et al., 2000**). Intimin (*eae*A gene) is an essential genetic element for attaching/effacing (A/E) lesion formation. Moreover, numerous fimbriae genes are proposed to have critical roles in the adhesion, attachment, and colonization of *E. coli* (**Mainil, 2013**). Shiga toxins are responsible for binding to the glycolipids on (Gb3) sites on the cell surface, resulting in the stoppage of protein synthesis and causing cell death (**Kaper et al., 2004**).

In our study, the screening of six virulence genes in *E. coli* isolates (*iss*, *fim*H, *eae*A, *stx*2, *tsh* and *iut*A) was carried out using specific primers sets in PCR assay. The results revealed that *iss* and *fim*H were the most frequently detected genes in all tested isolates (Fig. 1A-B), followed by the intimin (*eae*A) gene in 31.25% (Fig. 1C). Our results related to the study reported by (**Kwon et al., 2008**) who explaining the vital role of fimbrial genes such as F5 (K99) in *E. coli* colonization in the mucosa of the small intestine of calves and by studying the frequency of the *iss* gene he reported that *iss* gene ranged from 80 to 100%. Other fimbrial genes (F41 and F17) were identified in ETEC in neonatal calves (**Mellata et al., 2003**). Another study reported by (**Lynne et al., 2007**) clarified the function of the *iss* and *bor* genes in the pathogenicity of *E. coli* strains and reported that these genes believed to prevent the effect of inhibitory mediators generated by the host complement and resist the process of phagocytosis, and therefore, enhance the pathogenic influence of *E. coli* strains on the target host.

Likewise, the incidence of the *stx*2 gene in our investigation was low as existed in only one isolate (6.25%) (Fig. 1D). Our results are in agreement with (**Wilson et al., 1992**), who revealed a lower incidence of the *stx*2 gene (3.5%) in *E. coli* strains of young calves in Canada. On contrary, in Egypt (**Abotalp et al., 2017**) recorded a high prevalence (43.75%) of the *stx*2 gene. Interestingly, neither the *tsh* nor the *iut*A gene was detected in any tested samples in the current investigation, which attributed to variation in the results obtained by the researchers. In accordance with the previous study, (**Saidenberg et al., 2013**) illustrated that the frequency of the *tsh* gene in *E. coli* avian strains was 85.3%. Another investigation in Egypt by (**Abdulgayeid et al., 2015**) revealed that the prevalence of the *tsh* gene in *E. coli* of diarrheic calves was 100%. This difference requires further investigation regarding the role and prevalence of this gene in animal species and the possibility of interspecies transmission and the environmental risk factor.

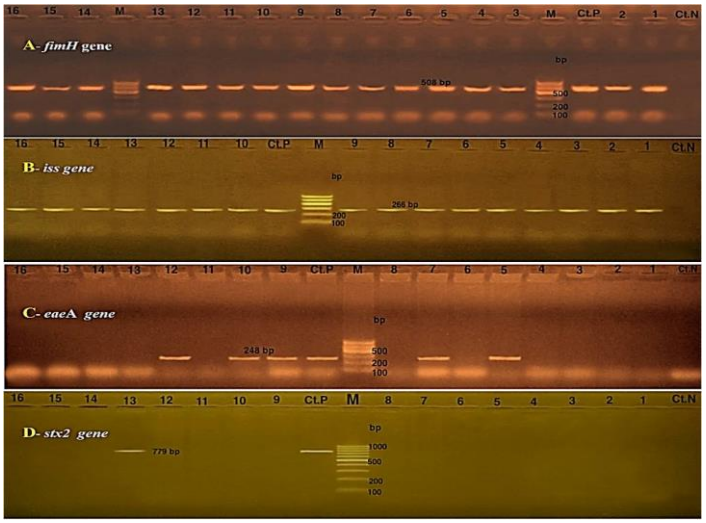


**Figure 1** Agarose gel electrophoresis for PCR products for the detection of virulence genes in 16 *E. coli* strains; **(A)** All *E. coli* strains (1-16) were positive for the *fim*H gene (508 bp). **(B)** All *E. coli* strains (1-16) were positive for the *iss* gene (266 bp). **(C)** Lanes 5, 7, 9, 10 and 12 were positive for the *eae*A gene (248

bp), while lanes 1, 2, 3, 4, 6, 8, 11, 13, 14, 15 and 16 were negative. **(D)** Only one strain (Lane 13) was positive for the *stx2* gene (779 bp), whereas 15 strains (lanes 1-12 and 14-16) were negative for this gene. Lane M: 100 bp DNA marker; lane Ct.N: Negative control; and lane Ct.P: Positive control.

**Relationship between phenotypic resistance and virulence determinant genes among *E. coli* serotype**

The results in Table 3 showed a correlation between phenotypic resistance and virulence genes among different *E. coli* serotype, our finding emphasizes that the *E. coli* serotype which exhibits more phenotypic resistance carried more virulence genes. Moreover, *E. coli* O26 and O157 were the most *E. coli* serovars which exhibit multidrug resistance to more than five antimicrobial agents and carried three virulence determinant genes. Other serovars (O78, O146, O125, O18, and O44) observed in our result also displayed multidrug resistance to three or more antimicrobial agents. The proper association between drug resistance and virulence genes determinants is still poorly unclear to understand. However, several evidence pointed out a correlation between phenotypic–resistance and virulence determinant genes (**Orden et al., 2000; Rasko et al., 2008; Badri et al., 2009 and Bonyadian et al., 2014**) clarified that many of the *E. coli* strains showed multidrug resistance to several antimicrobial groups and thus enhanced more virulence to these strains, which may lead to complicate the treatment of some urinary tract and enteric infections in animals. Additionally, (**Srivani et al., 2017**) observed that 69.81% of shiga toxingenic *E. coli* serovars from diarrheic calves expressed multidrug resistance and serovars which carried *hly*A and *eae*A genes may possess a zoonotic threat and high prevalence of multidrug resistance. A similar finding in Brazil was conducted by (**Rigobelo et al., 2006**) who described that STEC isolates of diarrheic calves showed 100% multidrug resistance. Meanwhile, (**Kumar et al., 2013**) explained that the indiscriminate and widespread use of antimicrobial drugs act as the main reason of prevalent multi-drug resistance among bacteria and obstacle in the treatment of many bacterial diseases.

**Table 3** Relationship between antimicrobial resistance and virulence factor genes among *E. coli* serotype

| Isolates no. | Serotype | Phenotypic resistance | |
|---|---|---|---|
| 1 | O26 | S, TET, SXT, C, N, AMC | *fimH, iss, eaeA* |
| 2 | O26 | S ,TET, SXT, C, N, PB | *fimH, iss* |
| 3 | O26 | S , TET, SXT, C, N, AMC | *fimH, iss* |
| 4 | O26 | S , TET, SXT, C, N | *fimH, iss* |
| 5 | O157 | S , TET, SXT, C, N, ENR, CIP, AMC | *fimH, iss* |
| 6 | O157 | S , TET, SXT, C, N, AMC | *fimH, iss* |
| 7 | O157 | S , TET, SXT, C, N, PB, AMC | *fimH, iss* |
| 8 | O157 | S , TET, SXT, C, N, PB | *fimH, iss* |
| 9 | O78 | S ,TET, SXT, C, N | *fimH, iss* |
| 10 | O78 | S , TET, SXT, C | *fimH, iss* |
| 11 | O125 | S , TET, SXT, C | *fimH, iss* |
| 12 | O125 | S , TET, SXT, C, AMC | *fimH, iss* |
| 13 | O146 | S , TET, SXT, C | *fimH, iss* |
| 14 | O146 | S, ENR, PB | *fimH, iss* |
| 15 | O44 | S, CIP, ENR | *fimH, iss* |
| 16 | O18 | S, CIP, ENR | *fimH, iss* |

**Legend S**=Streptomycine **TET**=Tetracycline **SXT**=Sulfamethozol/Trimethoprim **C** = Chloramphenicol **N**=Neomycin **AMC**=Amoxicillin/Clavulanic acid **PB**=Polymixin B **ENR**=Enrofloxacin **CIP**=Ciprofloxacin

## CONCLUSSION

Given from the above-mentioned results, concluded the high importance of diarrhegenic *E. coli* which constitute a potential problem in neonatal calves as well as it acts as a source of infection dissemination in animal population. The problem is more dangerous with the existence of multi-antimicrobial resistant strains as well as *E. coli* associated virulence genes that could contribute to the capability of *E. coli* to cause diarrhea in calves. Furthermore, it is significant to provide valuable knowledge about the virulence ability of the circulating *E. coli* strains in Egypt and the ideal methods for prevention and control of such problems in the animal industry.

**Ethics statement:** This study was approved by the Ethics Committee and current legislation on research and ethical approval of the Faculty of Veterinary Medicine (Local ethical approval), Sohag and Sadat City Universities.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding**: This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

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