Received: 03 September 2023, Accepted: 24 November 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 9, 1901-1906

Virulotyping and Antibiograms of Pathogenic *Escherichia coli* Isolated from Calves Suffering from Diarrhea

Mona El refaey*, Rasha Elkenany, Gamal Younis

Department of Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Mansoura University, 35516 Mansoura, Egypt.

***Correspondence**
Corresponding author: Mona El refaey
E-mail address: elrefaeym17@gmail.com

Abstract

Escherichia coli is an important cause of diarrhea in calves and has negative economic effects on the livestock industry worldwide due to high mortality and reduced growth rate. The present study was aimed to investigate the prevalence of *E. coli* in 120 fecal samples from diarrheic mixed-sex neonatal calves, molecular detection of virulence genes, and antimicrobial resistance testing. The high occurrence of *E. coli* (90/120; 75%) was detected in diarrheic calves. The molecular detection of virulence genes (*iron*, *pap*C, *ast*A, *iut*A, *omp*T, *hyl* and *iuc*) showed that the high occurrence of *ast*A (78.8%), *iuc*D (66.6%), and *pap*C (64.4%) genes in examined strains, while only 19 strains devoid of virulence genes. Furthermore, antimicrobial susceptibility testing of *E. coli* isolates showed absolute resistance to ampicillin (100%), followed by streptomycin (95.5%), tetracycline, sulfonamides, gentamycin and chloramphenicol (77.7% each). In the other hand, strains revealed high rates of susceptibility to ciprofloxacin (80%) and enrofloxacin (77.8%). The multidrug-resistant (MDR) *E. coli* strains were determined as 77.7% (70/90) and the most identified antimicrobial resistance patterns in 31 strains was C, S, CN, N, TET, AM, SXT. Also, the identified antibiotypes had MAR index values ranged from 0.1 to 0.9. The results of current study indicate the importance of routine monitoring of *E. coli* isolated from diarrhetic calves to reduce the transmission to humans and animals as well as to select the most appropriate antibiotics. The antibiograms in our study emphasizes the risks associated with the random use of antibiotics.

KEYWORDS
Antibiograms, Pathogenic *E. coli*, Diarrheic calves, Virulotyping

INTRODUCTION

Since it is associated with high mortality and considerable economic losses, *Escherichia coli* induced calf diarrhea is one of the most economically significant problems. This sickness may be fatal in complicated untreated calves because it causes severe mucoid diarrhea, dehydration, and rapid weight loss (Galal *et al*., 2013; Cho and Yoon, 2014), A member of the Enterobacteriaceae family, *E. coli* is a Gram negative, facultatively anaerobic bacterium. *It* has a rod-shaped, flagellated, and non-sporulating morphology. Based on the virulence of the bacteria and the clinical signs that the host experiences, there are six main categories of *E. coli* strains: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohaemorrhagic *E. coli* (EHEC), Shiga toxin-producing *E. coli* (STEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (Xia *et al*., 2010). The frequency of colibacillosis varies from 5.4 to 100%, and it is roughly predicted that 20% of calves dying from the disease might result in a 40% reduction in net profit. The pathogenicity of each species, which varies progressively with virulence factors, provides the basis for the diagnosis. Rate of virulence factor change considered as a warning about the endemic instance of the infection with a subsequent recommendation for exclusion from the afflicted area (Badouei *et al*., 2010). *E. coli* pathogenicity genes, such as the *iut*A, *iuc*D, *iron*, *pap*C, *hyl*F, *omp*T, and *ast*A genes, have been related to diarrhea in both humans and animals (Fröhlicher *et al*., 2008; Huehn *et al*., 2010). In contrast to *E. coli* isolated from cows, *omp*T, *hly*F, *iut*A, and *iss* were more frequently identified in *E. coli* isolated from calves (Mihailovskaya *et al*., 2022). The ability of bacteria to connect to host cells is a requirement for bacterial infections to populate the body. The term "tissue tropism" refers to the precise interaction in this phenomenon between the target receptors and the surface of a specific tissue (Klemm *et al*., 2010).

The process of specific adhesion heavily depends on a variety of surface properties. The three main groups of adhesins are fmbriae, afmbrial adhesins (Afa), and outer membrane proteins (OMPs) (Lindberg *et al*., 2008). By bringing the bacteria into close contact with the host cell wall, surface adhesin expression improves the pathogenicity of *E. coli*. Most of the genes that control whether fmbriae are present on the surface of bacterial cells are encoded on chromosomes or, less frequently, in plasmid DNA. Different bacterial adhesins have been modified to occupy a particular niche. Among isolates from UTI patients, S fmbrial adhesins (sfa), F1C ("pseudotype I") fmbriae (foc), coding P like pili, *pap*C, and Iha (iha) are the adhesins that are most frequently found (Hagan and Mobley, 2007).

E. coli strains with increased antibiotic resistance have become widespread worldwide in recent decades (de Been *et al*., 2014). The primary cause of the widespread antimicrobial resistance in agricultural animals is thought to be the overuse of antibiotics in illness prevention, disease treatment, and animal growth promotion. The consumption of these animals' milk or

ISSN: 2090-6277/2090-6269/

meat products can pass antimicrobial resistance genes to human. The majority of the resistant germs that contaminate other foods and the environment come from humans and animals)Adzitey *et al*, 2021). According to several studies, using antibiotics can reduce animal mortality to 10% from 75% in untreated animals (Wray and Davies, 2000). Although its use is only justified in bacterial infections that progress toward a systemic disease, veterinary surgeons nevertheless frequently utilize antimicrobials to treat neonatal calf diarrhea recurrence, Restrictions on the use of antimicrobials in veterinary practice have been put in place because the indiscriminate use of antibiotics for treating diarrheal calves raises the potential for the development of resistance. The virulence and genetic structure of *E. coli* isolated from calves in Egypt's large production system have been the subject of several research (Awadallah *et al*., 2016). However, there is little data from calves raised in Egypt using an intensive production strategy. The prevalence and antibiotic resistance profile of *E. coli* in humans, food animals, and the environment are also unknown, and there is no routine monitoring of antimicrobial resistance. Determining the virulence and antibiotic resistance characteristics of *E. coli* strains linked to diarrhea in calves at Egyptian dairy farms was the aim of this study.

MATERIALS AND METHODS

Ethical approval

This study was ethically approved by Research Ethics Committee of the Faculty of Veterinary Medicine, Mansoura University, Egypt (Code No: M/30).

Sampling and clinical examination

A total of 120 fecal samples were taken from neonatal mixed-sex calves aged under one month old in September 2020 from farm I (n=50) located in Kafr-Saad, Damietta province and in September 2021 from farm II (n=70) located in El-Dakahlia province at calving season . The fecal samples were collected after the onset of diarrhea on calves and before beginning of the medical plan. The fecal samples were obtained, stored in ice packs in sterile bags, and then transported right away to the lab for bacteriological investigation.

Clinical observation and examination of ill calves revealed widespread watery and mucoid diarrhea along with varying degrees of dehydration indicated by sunk eyes, decreased skin elasticity, cold extremities, and immobility. All affected calves displayed depression, tachycardia, tachypnea, and rough coats. Some animals showed systemic responses.

Isolation and identification of E. coli

The fecal samples were added to MacConkey's broth (Oxoid, UK), where they were then cultured for 24 hours at 37°C, after being subcultured into eosin methylene blue (EMB) (Oxoid, India), the enriched broth was incubated at 37°C for 24 hours.

Biochemical tests (oxidase, catalase, urease, indole, methyl Red, Voges-Praskauer, and citrate utilization tests) were used to identify the hypothesized typical *E. coli* colonies that had the appearance of bright metallic green sheen colonies in accordance with Koneman *et al*. (1983).

DNA extraction and PCR conditions

Using appropriate primers (Metabion, Germany) and PCR conditions, all isolates were submitted to confirmatory polymerase chain reaction (PCR) identification of pathogenic *E. coli* (16S rRNA gene) and certain virulence genes (*iron*, *pap*C, *ast*A, *iut*A, *omp*T, *hyl*, and *iuc*) (Table 1). According to Shah *et al*. (2009), the lysate boiling technique was employed to extract the bacterial DNA. The PCR reactions were carried out in 50 µl PCR reaction with a final volume of 50 µl of PCR water, 5 µl of DNA template, 1 µl of each primer (0.5 M), and 25 µl of 2x multiplex master mix (Takara). The electrophoresis of amplified DNA products on a 1.5% agarose gel was followed by ethidium bromide staining and UV detection.

Antimicrobial susceptibility test

In accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2019), disk diffusion was used to evaluate the antimicrobial susceptibility of all *E. coli* isolates against 11 antimicrobial discs (2020).The tested antimicrobial discs (Oxoid, UK) contained the following medications: tetracycline (TET; 30), chloramphenicol (C; 30), amoxicillin/clavulanic acid (AMC; 30), streptomycin (S; 10), enrofloxacin (ENR; 5), sulfamethoxazole/trimethoprim (SXT; 25), gentamicin (CN; 10µ),

Table 1. Primer sequences, target genes and amplified segments with PCR condition.

Gene	Primer Sequence	Primary denaturation	Secondary denaturation	Annealing	Extension	Final extension	Number of cycles	Amplicon size (bp)	Reference
16S rRNA	F: GACCTCGGTTTAGTTCACAGA. R: CACACGCTGACGCTGACCA	95°C for 5 minutes	94°C for 1 minute	58 for 1 minute	72°C for 1 minute	72°C for 7 minutes	35	585	Van Bost *et al* (2003)
*iut*A	F: GGCTGGACATCATGGGAACTG R: CGTCGGGAACGGGTAGAATCG	94°C for 4 minutes	94°C for 30 seconds	60 for 1 minute				302	Ewers *et al*. (2005)
*pap*C	F: TGATATCACGCAGTCAGTAGC R: CCGGCCATATTCACATAA							501	Chapman *et al* (2006)
*iuc*D	F: ACAAAAAGTTCTATCGCTTCC R: CCTGATCCAGATGATGCTC							714	
*omp*T	F: TCATCCCGGAAGCCTCCCTCACTACTAT R: TAGCGTTTGCTGCACTGGCTTCTGATAC							496	
iron	F: AATCCGGCAAAGAGACGAACCGCCT R: GTTCGGGCAACCCCTGCTTTGACTTT							553	
hyl	F:GGCCACAGTCGTTTAGGGTGCTTACC R:GGCGGTTTAGGCATTCCGATACTCAG							450	Piva *et al*. (2003).
*ast*A	F:TGCCATCAACACAGTATATCC R:TCAGGTCGCGAGTGACGGC							116	Chapman *et al*. (2006)

neomycin (N; 30µ); ciprofloxacin (CIP; 5µ), and polymyxin (PB; 300IU). According to the zone diameter interpretation guidelines advised by CLSI (2020), the strains were classified as susceptible, intermediate, or resistant. The strains were referred to as multi drug resistant (MDR) isolates if they were resistant to three or more antibiotic classes (Lee *et al*., 2009). Using the formula provided by (Singh *et al*. ,2010) the multiple antibiotic resistances (MARs) index for each resistant pattern is determined. MAR index = Number of resistant antibiotics/Total number of antibiotics tested (isolates classified as intermediate based on inhibition zone were considered as sensitive for MAR index).

Statistical analysis

The description of antibacterial patterns was done using the statistical program SPSS 20. The statistical program SPSS version 20 was used to compare the rates of antibiotic resistance between *E. coli* with virulence genes and *E. coli* without virulence genes using Pearson's Chi-squared (x 2) tests. For comparisons, P0.05 was regarded as statistically significant (de Verdier *et al*., 2012).

RESULTS

Prevalence of pathogenic E. coli

Out of 120 examined diarrheic calves' samples, 90 (75%) were identified as pathogenic *E. coli* based on their morphology, biochemical and molecular characteristics. The bacteria were visible under a microscope as rod-shaped, motile, Gram-negative, non-sporulated organisms of a medium size. On EMB agar, the bacteria flourished and produced colonies with a distinctive metallic sheen. All isolates tested positive for the biochemical assay's catalase, indole, and methyl-red. The tests for cytochrome oxidase, Voges-Proskauer, citrate utilization, and urease were all negative at the same time. The 16S rRNA gene was present in all isolates, according to the molecular analysis. In terms of where the farms were, bacteriological testing revealed that farm I had a 60% (30/50) prevalence of *E. coli* in diarrheic calves while farm II had an 85.7% (60/70) prevalence.

Virulotyping in E. coli strains

Multiplex PCR of virulence genes in all *E. coli* strains showed that *E. coli* isolates had various virulence genes (Figure 1). The results revealed the high occurrence of *ast*A, *iuc*D, and *pap*C genes in examined strains. The *ast*A gene was detected in 78.8% (71/90) of *E. coli* strains, followed by *iuc*D gene 66.6% (60/90), *pap*C gene 64.4% (58/90), *iron* 56.6% (51/90), *hly*F 55.5% (50/90), *iut*A and *omp*T (50%; 45/90 each). Forty-five strains carried all examined virulence genes, while only 19 strains devoid of virulence genes.

Two strains had *ast*A and *iuc*D genes. Six strains contain *ast*A, *iuc*D, *pap*C, *iron*, *hly*F gene, seven strains had *ast*A, *iuc*D, and *pap*C gene, and ten strains had *ast*A gene.

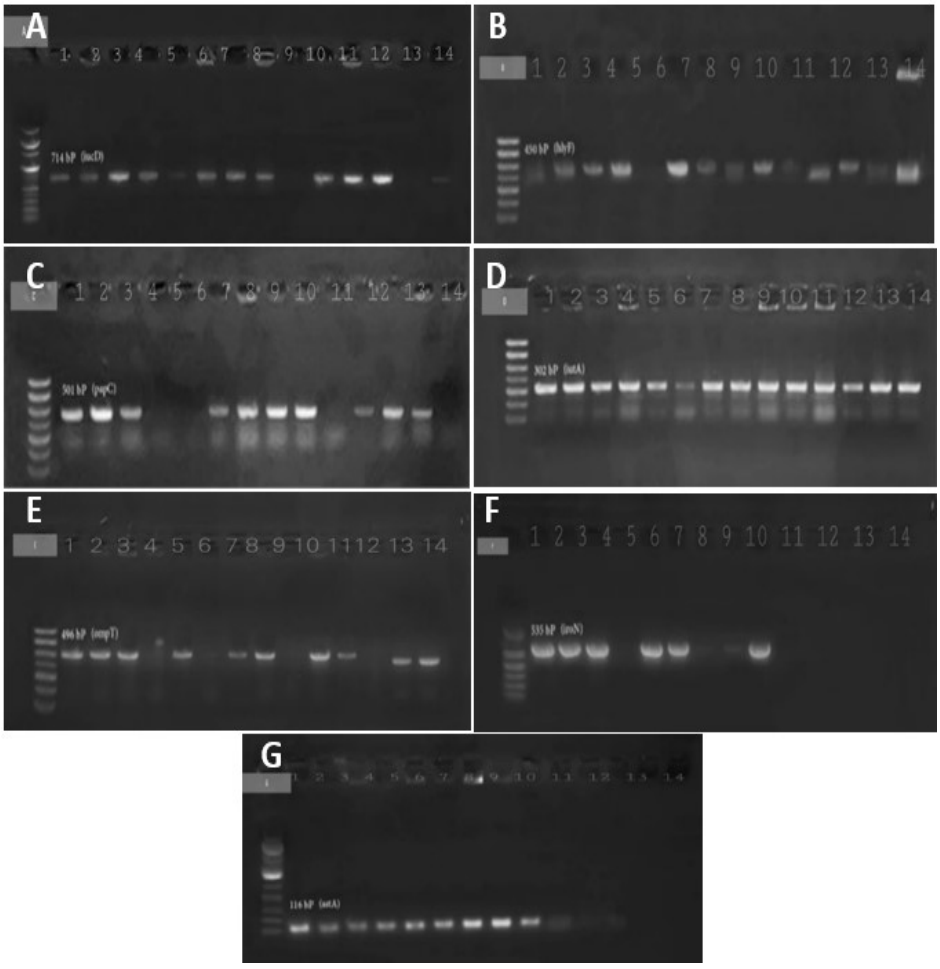

Figure 1. Agarose gel electrophoresis showing amplified PCR-products of various virulence genes of E.-coli strains. A: Amplification of *iuc*D-gene at 714 bp, lane 1,2,3,4,6,7,8,10,11,12: positive samples; B: Amplification of *hly*F gene at 450 bp,-lane-2,3,4,6,9.14:-positive samples; C: Amplification of E.-coli *pap*C gene at 501 bp, lane1-3,6-9,11-13: positive samples; D: Amplification of *iut*A gene at 302bp, lane1-5,7-14: positive samples; E: Amplification of *omp*T gene at 496 bp, lane1-3,5,7,8,10,11,13,14: positive samples; F: Amplification of *iron* gene at 535 bp,-lane1-3,5,6,9: positive samples; G: Amplification of *ast*A gene at 116 bp, lane 1-9: positive samples.

Antibiogram results

The results of examination for all *E. coli* strains (n=90) against eleven antimicrobial agents from 7 different classes by disc diffusion method showed complete resistance to ampicillin (100%) (Table 2). Also, the strains showed high rates of resistance to streptomycin (95.5%), followed by tetracycline, sulfonamides, gentamycin and chloramphenicol (77.7% each). In the other hand, strains revealed high rates of susceptibility to ciprofloxacin (80%) and enrofloxacin (77.8%). Noticeable differences in the number of *E. coli* isolates resistant to each antibiotic were found (Figure 2). Four strains were resistant only to one antibiotic (4.4%), 16 strains were resistant to two antibiotics (17.7%), 18 strains (20%) were resistant to six antibiotics,31strains (34.4%) were resistant to seven antibiotics, 10 strains (11.11%) were resistant to eight antibiotics, 11 strains (12.22%) were resistant to nine antibiotics. The multidrug-resistant (MDR) *E. coli* strains were determined as 77.7% (70/90) and the most identified antimicrobial resistance patterns in 31 strains was C, S, CN, N, TET, AM, SXT (Table 3). Additionally, anti-biotypes identified in this study had MAR index values ranged from 0.1 to 0.9 (Table 3).

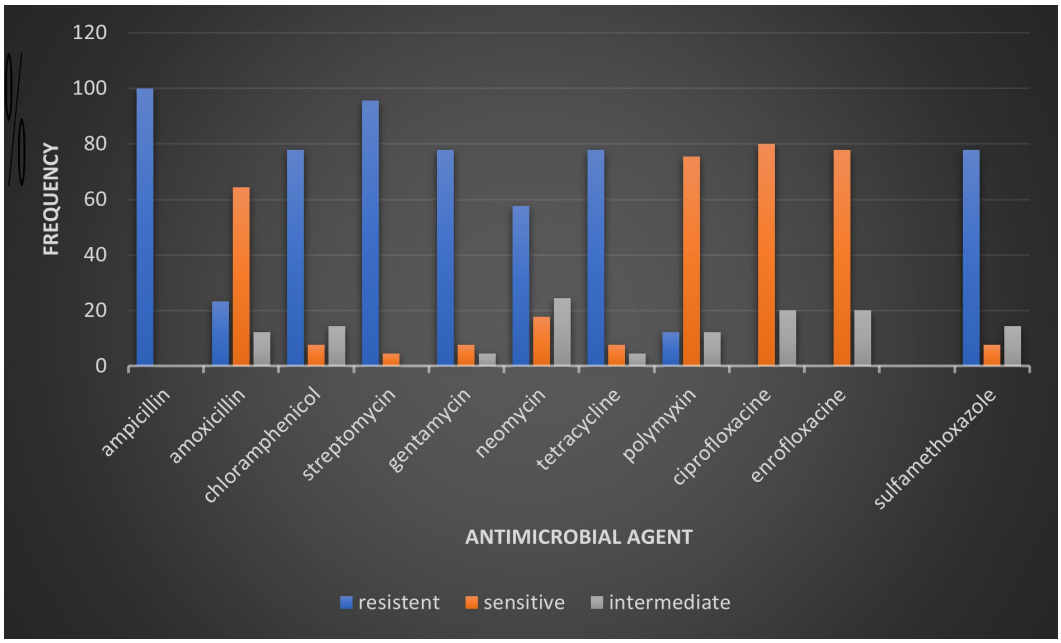

Fig. 2. Antimicrobial sensitivity test results of *E. coli* strains (n=90) isolated from diarrheic calves against various antimicrobial agents.

Table 2. Antimicrobial sensitivity test results of *E. coli* isolates from diarrheic calves (n=90).

Antimicrobial agent	Antimicrobial class	Resistant	Intermediate	Sensitive
Chloramphenicol (C)	Chloramphenicol	70(77.7%)	13(14.4%)	7(7.7%)
Streptomycin(S)	Aminoglycosides	86(95.5%)	0(0%)	4(4.4%)
Gentamycin (CN)	Aminoglycosides	70(77.7%)	13(14.4%)	7(7.7%)
Neomycin (N)	Aminoglycosides	52(57.7%)	22(24.4%)	16(17.7%)
Tetracycline (TET)	Tetracycline	70(77.7%)	13(14.4%)	7(7.7%)
Amoxicillin (AMC)	Beta-lactams	21(23.33%)	11(12.2%)	58(64.4%)
Ampicillin (AM)	Beta-lactams	90(100%)	0(0%)	0(0%)
Sulfamethoxazole/trimethoprim (SXT)	Sulfonamides	70(77.7%)	13(14.4%)	7(7.7%)
Ciprofloxacin (CIP)	Fluoroquinolones	0(0)	18(20%)	72(80%)
Enrofloxacin (ENR)	Fluoroquinolones	0(0)	20(20%)	70(77.8%)
Polymyxin B(PB)	Polymyxins	11(12.22%)	11(12.2%)	68(75.5%)

Table 3. Antimicrobial resistance profiles of *E. coli* (n=90) isolated from diarrheic calves.

Pattern	Pattern of resistance	No. of isolates	No of resistant antibiotics	MARI
I	AM	4	1	0.1
II	C, S, CN, N, TET, AM, AMC, SXT	10	8	0.8
III	C, S, CN, N, TET, AM, AMC, SXT, PB	11	9	0.9
IV	S, AM	16	2	0.2
V	C, S, CN, TET, AM, SXT	18	6	0.6
VI	C, S, CN, N, TET, AM, SXT	31	7	0.7

C: Chloramphenicol; S: Streptomycin; AMC: Amoxicillin; CIP: Ciprofloxacin; SXT: Sulfamethoxazole/trimethoprim; AM: Ampicillin; TET: Tetracycline; ENR: Enrofloxacin; PB: Polymyxin; N: Neomycin; CN: Gentamycin.

DISCUSSION

One of the most prevalent bacterial infections linked to diarrhea in calves is *E. coli* (Osman *et al*., 2012; Gharieb *et al*., 2019). Thus, this study sought to identify the prevalence, pathogenicity, and antibiotic resistance profiles of *E. coli* strains that had recently been identified from diarrheal calves in Egyptian dairy farms.

According to the current study, diarrheal calves had a high (75%) prevalence of *E. coli*. This outcome is in line with the earlier Egyptian study that discovered a high incidence of *E. coli* (66%) in diarrheal calves (Abdulgayeid *et al*., 2015). Also, *E. coli* was found (Tarabees *et al*., 2020) in significant concentrations (95% of diarrheal calves in Egypt). In fecal samples, 88.5% of the time, *E. coli* was present (Begum *et al*., 2014). In contrast, a prior study found that the prevalence of *E. coli* was lower (40%) in samples from Egyptian calves (Mousa *et al*., 2020). However, Luna *et al*. (2009) reported that only 18.9% of the tested samples in Austria were positive for *E. coli*. The differences in the results may be attributed to the different geographic regions, stress factors, farm sizes, meteorological circumstances, hygiene and management methods, as well as differences in the number of samples gathered.

Inadequate management techniques and risk factors like overcrowding and malnutrition, which were thought to be the main causes of immunosuppression, may be to blame for the greater frequency of *E. coli* in early age groups, particularly in areas where these animals are not raised under intense farming settings. Additionally, *E. coli*, a commensal bacterium, causes diarrhea in calves, especially those that receive little to no maternal antibodies through colostrum (Malik *et al*., 2012), which is common in areas where milk is mostly used for commercial purposes.

According to numerous early studies, *E. coli* strains still have virulence factors that help with tissue invasion and colonization, which contribute to the development of calf colibacillosis (Delicato *et al*., 2003; Zaho *et al*. 2005). Seven virulence genes were screened for in the current investigation using multiplex PCR on retrieved *E. coli* isolates (n=90), and the results showed that the recovered *E. coli* strains possess between 2 and 7 virulence genes, which are assumed to be connected to the pathogenesis of pathogenic *E. coli*. According to our analysis of virulence genes, the *ast*A gene (78.8%), *iuc*D gene (66.6%), and *pap*C gene (64.4%) were the most frequently found virulence genes in *E. coli* strains, whereas other genes (*iron* 56.6%, *hly*F 55.5%, *iut*A, and *omp*T 50%) reported varying rates. The *pap*C gene is one of the most important virulence factors in *E. coli* (Pearce *et al*., 2006; Paixao *et al*., 2016). (Ana Umpiérrez *et al*., 2020) found the *iuc*D gene to be the most prevalent virulence gene in Uruguay (81.3%), which is consistent with our findings. Previously, the *hly*F gene was found in Zimbabwe and Egypt in percentages of 24.4% and 16.6%, respectively (Mbanga and Nyararai, 2015; Tarabees *et al*., 2020). Also, in earlier studies conducted in Russia and Egypt, the frequency of the *iut*A gene was found to be 44.9% and 50%, respectively (Mihailovskaya *et al*., 2022; Fathy *et al*., 2019). Furthermore, Mbanga and Nyararai (2015) concluded that 2.2% of Zimbabweans were infected with the *omp*T. On the other hand, no examined *E. coli* isolates had the *iut*A gene, according to Mousa *et al*. (2020). According to Ji Hyoung Ryu *et al*. (2020), finding *E. coli* strains, whether they had virulence factors or not, was not linked to diarrhea in pre-weaned calves.

Since recent decades, *E. coli* antibiotic resistance has raised alarm across the globe. All pathogenic *E. coli* strains in this investigation were resistant to three or more families of antibiotics, and many of the strains were also resistant to most first-line antibiotics used in veterinary medicine. Our isolates were remarkably resistant to ampicillin (100%), streptomycin (95.5%), tetracycline, sulfonamides, gentamycin and chloramphenicol (77.7% each), with prophylactic and therapeutic usages in calves with diarrhea, whereas strains revealed high rates of susceptibility to ciprofloxacin (80%) and enrofloxacin (77.8%). These findings were in line with those of Alberto Prieto *et al*. (2022), who found that a large proportion of ETEC strains in Spain were concurrently resistant to tetracyclines, trimethoprim, sulphonamides fluoroquinolones, and a variety of beta-lactam antibiotics in addition to ampicillin. On the other hand, *E. coli* isolates obtained from diarrhea were shown to be resistant to penicillin, streptomycin, tetracycline, lincomycin, and sulfamethoxazole in Iran and to amoxicillin, tetracycline, and cefotaxime in Bangladesh (Ansari *et al*., 2014). In addition, the current investigation found that MDR *E. coli* strains are quite prevalent (77.7%) (de Verdier *et al*., 2012; Lee *et al*., 2009). Additionally, a significant incidence of MDR *E. coli* isolates (49.6%) was reported by Khawaskar *et al*. (2022) in India. MDR *E. coli* strains were discovered in several reports, and it was shown that resistance to routinely used antibiotics such ampicillin, amoxicillin, clavulanic acid, oxytetracycline, and streptomycin in particular had developed (Manna *et al*., 2006; Wani *et al*., 2013; Adiguzel *et al*., 2018). Excessive use of antibiotics has led to the emergence of highly pathogenic MDR *E. coli* strains, which pose a serious threat to the health of cattle by causing numerous severe infections and large financial losses in the production of livestock. Additionally, the development of strains that are resistant to penicillins, cephalosporins, and carbapenems signals a public health emergency and emphasizes the challenging nature of treating infections brought on by these strains. The proper application of antimicrobial agents in the veterinary and health fields, as well as the regular use of antimicrobial susceptibility testing, are also advised.

CONCLUSION

The current investigation showed that virulent and MDR *E. coli* strains isolated from diarrheal calves are very common in Egyptian dairy farms. Additionally, the rise of MDR *E. coli* strains has been linked to the abuse of antibiotics in veterinary medicine, which could be dangerous for the public's health. Therefore, to reduce the monetary losses brought on by antibiotic-resistant strains in animals as well as the zoonotic danger, antimicrobial resistance must be continuously monitored in both human and veterinary medicine.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

Abdulgayeid, M., Shahin H., Foad, S., Ibrahim, M.S., 2015. Molecular Characterization of *Escherichia coli* Isolated from Buffalo Calves in El-Behera Governorate. Alexandria Journal of Veterinary Sciences 47, 90-96.

Adzitey, F., Huda, N., Shariff, A.H.M., 2021. Phenotypic antimicrobial susceptibility of *E. coli* from raw meats, ready-to-eat meats, and their related samples in one health context. Microorganisms 9, 1-11.

Adiguzel, M.C., Diren Sigirci, B., Celik, B., 2018. Phenotypic and genotypic examination of antimicrobial resistance in thermophilic Campylobacter species isolated from poultry in Turkey. J. Vet. Res. 62, 463-468.

Alberto, P., 2022. Antimicrobial Susceptibility of Enterotoxigenic *Escherichia coli* from Diarrhoeic Neonatal Calves in Spain, Animals 12, 264.

Ana, U., 2021. Virulence genes of *Escherichia coli* in diarrheic and healthy calves, Revista Argentina de Microbiología 53, 34-38

Ansari, A.R., Rahman, M.M., Islam, M.Z., 2014. Prevalence and antimicrobial resistance profile of *Escherichia coli* and *Salmonella* isolated from diarrheic calves. J. Animal Health Prod. 2, 12-15.

Awadallah, M.A, Ahmed, H.A., Merwad, A.M., Selim, M.A., 2016. Occurrence, genotyping, shiga toxin genes and associated risk factors of *E. coli* isolated from dairy farms, handlers and milk consumers. Vet. J. 217, 83–88.

Badouei, A.M., Salehi, T.Z., Khorasgani, M. R., Tadjbakhsh, H., Brujeni, G.N., Nadalian, M.G., 2010. Virulencegene profiles and intimin subtypes of Shiga toxinproducing *E. coli* isolated from healthy and diarrheic calves. Vet. Rec. 167, 858–861.

Begum, S., Hazarika G.C., Rajkhowa, S., 2014. Prevalence of *E. coli* from pigs and cattle. Journal of Animal Health and Production JAHP. 2, 38 –39.

Chapman T.A., Wu X.-Y., Barchia I., Bettelheim K.A., Driesen S., Trott D.,

Wilson M., Chin J.J.-C., 2006. Comparison of virulence gene profiles of *Escherichia coli* strains isolated from healthy and diarrheic swine. Appl. Environ. Microbiol. 72, 4782-4795.

Cho, Y.I., Yoon, K.J., 2014. An overview of calf diarrhea-infectious etiology, diagnosis, and intervention. J. Vet. Sci. 15, 1-17.

CLSI (Clinical Laboratory Standards Institute), 2019, Performance standards for antimicrobial susceptibility testing, 29th edition; M100. CLSI, 29, 320.

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. Veterinary Microbiology 94, 97– 103.

de Verdier, K., Nyman, A, Greko, C., 2012. Antimicrobial resistance and virulence factors in *Escherichia coli* from Swedish dairy calves. Acta Vet. Scand. 54, 2.

de Been, M., Lanza, V.F., de Toro, M., Scharringa, J., Dohmen, W., Du, Y., Hu, J., Lei, Y., Li, N., ToomingKlunderud, A., Heederik, D.J., Fluit, A.C., Bonten, M.J., Willems, R.J., de la Cruz, F., van Schaik, W., 2014. Dissemination of cephalosporin resistance genes between *E. coli* strains from farm animals and humans by specific plasmid lineages. PLoS Genet. 10, e1004776.

Ewers, C., Janssen, T., Kiessling S., Philipp, H.C., Wieler, L.H., 2005. Rapid detection of virulence-associated genes in avian pathogenic *Escherichia coli* by multiplex polymerase chain reaction. Avian Diseases 49, 269–273.

Fathy, H., Eman, A., Alaa Eldin, H.M., Gamal, Y., 2019. Development of PCR and Multiplex-PCR for Detection of Some *Escherichia coli* Virulence Genes from Bovine Neonates Diarrhea. Journal of Current Veterinary Research 1, 11-16.

Fröhlicher, E., Krause G., Zweifel C., Beutin L., Stephan, R., 2008. Characterization of attaching and effacing *Escherichia coli*., AEEC. isolated from pigs and sheep. BMC Microbiol. 8, 144.

Galal, H.M., Hakim, A.S., Sohad, M.D., 2013. Phenotypic and virulence genes screening of *Escherichia coli* strains isolated from different sources in delta Egypt. Life Sci. J. 10, 352-361.

Gharieb, R., Elshaima, F., Ibrahim, E., 2019. Antibiogram, virulotyping and genetic diversity of *Escherichia coli* and *Salmonella* serovars isolated from diarrheic calves and calf handlers. Comparative Immunology, Microbiology and Infectious Diseases 67, 101367.

Hagan, E.C., Mobley, H.L.T., 2007. Uropathogenic *Escherichia coli* outer membrane antigens expressed during urinary tract infection. Infect Immun. 75, 3941–3949.

Huehn, S., La Ragione, R.M., Anjum, M., Saunders, M., Woodward, M.J., Bunge, C., Helmuth, R., Hauser, E., Guerra, B., Beutlich, J., 2010. Virulotyping and antimicrobial resistance typing of *Salmonella* enterica serovars relevant to human health in Europe. Foodborne Pathog. Dis. 7, 523–535.

Ji Hyoung, R., SuHee, K., Jinho, P., Kyoung Seong, C., 2020. Characterization of virulence genes in *Escherichia coli* strains isolated from pre-weaned calves in the Republic of Korea Acta Veterinaria Scandinavica 62, 45.

Khawaskar, D.P., Sinha, D.K., Lalrinzuala, M.V., Athira, V., Kumar, M., Chhakchhuak, L., Mohanapriya, K., Abhishek, I.S., Kumar, O.R.V., Chaudhuri, P., Singh, B.R., Thomas, P., 2022. Pathotyping and antimicrobial susceptibility testing of *Escherichia coli* isolates from neonatal calves. Veterinary Research Communications 46, 353–362.

Klemm, P., Hancock, V., Schembri, M.A., 2010. Fimbrial adhesins from extraintestinal *Escherichia coli*. Environ. Microbiol. Rep. 2, 628–640.

Koneman, E.W., Allen, S.D., Dowell, V.R., Janda, W.H., Sommers, H.M., 1983. Color Atlas and Textbook of Diagnostic Microbiology. 4th Ed., J. B. Lippincott Co., New York.

Lee, J.H., 2009. Antimicrobial resistance of *Escherichia coli* O26 and O111 isolates from cattle and their characteristics. Vet. Microbiol. 135, 401-405.

Lindberg, S., Xia, Y., Sondén, B., Göransson, M., Hacker, J., Uhlin, B.E., 2008. Regulatory interactions among adhesin gene systems of uropathogenic *Escherichia coli*. Infect. Immun. 76, 771–780.

Luna, C.H., Klein, D., Lapan, G., RevillaFernandez, S., Haschek, B., SommerfeldStur,I., Moestl, K., and Baumgartner, W., 2009. Characterization of virulence factors in*E. coli* isolated from diarrheic and healthy calves in Austria shedding various enteropathogenic agents. Veterinarni Medicina, 54,1–11.

Manna, SK., Brahmane MP, Manna C., 2006. Occurrence, virulence characteristics and antimicrobial resistance of *Escherichia coli* O157 in slaughtered cattle and diarrheic calves in West Bengal, India. Lett. Appl. Microbiol. 43, 405-409.

Malik S, Verma AK, Kumar A., 2012. Incidence of calf diarrhea in cattle and buffalo calves in Uttar Pradesh. India. Asian J. Anim. Vet. Adv. 7, 1049–1054.

Mbanga, J., Nyararai, Y.O., 2015. Virulence gene profiles of avian pathogenic *Escherichia coli* isolated from chickens with colibacillosis in Bulawayo, Zimbabwe. The Onderstepoort Journal of Veterinary Research 82, 850.

Mihailovskaya, V.S., Remezovskaya, N.B., Zhdanova, I.N., Starčič Erjavec, M., Kuznetsova, M.V., 2022. Virulence potential of fecal *Escherichia coli*. strains isolated from healthy cows and calves on farms in Perm Krai. Vavilov Journal of Genetics and Breeding 26, 486-494.

Moulin-Schouleur, M., Répérant, M., Laurent, S., Brée, A., Mignon Grasteau, S., Germon, P., Rasschaert, D., Schouler, C., 2007. Extraintestinal pathogenic *Escherichia coli* strains of avian and human origin: link between phylogenetic relationships and common virulence patterns. J. Clin. Microbiol. 45, 3366-3376.

Mousa, W.S., 2020. Prevalence, antimicrobial resistance and substantial virulence-associated genes of *Escherichia coli* isolated from colibacillosis in neonatal calves in Egypt. J. Microbiol. Biotech. Food Sci. 9, 1145-1150.

Osman, K.M., Mustafa, A.M., Aly, M.A.K., Abd El-Hamed, G.S., 2012. Serotypes, virulence genes, and intimin types of shiga toxin-producing *Escherichia coli* and enteropathogenic *Escherichia coli* isolated from mastitic milk relevant to human health in Egypt. Vector Borne Zoonotic Dis. 12, 297-305.

Paixao, A.C., Ferreira, A. C., Fontes, M., Themudo, P., Albuquerque, T., Soares, M. C., Fevereiro, M., Martins, L., Corrˆea de, S.M.I., 2016. Detection of virulence-associated genes in pathogenic and commensal avia *Escherichia coli* isolates. Poultry Science Association Inc. pp.1646–1652.

Pearce, M.C., 2006. Prevalence and virulence factors of *Escherichia coli* serogroups O26, O103, O111 and O145 shed by cattle in Scotland. Appl. Environ. Microbiol. 72, 653–659

Piva, I.C., 2003. Virulence markers of enteroaggregative *Escherichia coli* isolated from children and adults with diarrhea in Brasília, Brazil. J. Clin. Microbiol. 41, 1827–1832.

Singh, S., Yadav, A., Bharti, P., 2010. Prevalence of *Salmonella* in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. Food Research International 43, 2027-2030.

Tarabees,R., Younis, G., El-Khetaby, H., 2020. Molecular Screening of Certain Virulence Encoding Genes Associated with *E. coli* Strains Isolated from Diarrheic Calves. Journal of Current Veterinary Research 2, 80-88.

Van Bost, S., Jacquemin, E., Oswald, E., Mainil, J., 2003. Multiplex PCRs for identification of necro toxigenic *Escherichia coli*. Journal of Clinical Microbiology 41, 4480–4482.

Wani, S.A., Hussain, I., Beg, S.A., 2013. Diarrhoeagenic *Escherichia coli* and *Salmonella*e in calves and lambs in Kashmir: absence, prevalence and antibiogram. Rev. Sci. Tech. 32, 833-840.

Wray, C., Davies, R.H., 2000. *Salmonella* Infections in Cattle. In: *Salmonella* in Domestic Animals. CABI BOOKS, pp. 169–190

Xia, X., Meng, J., McDermott, P.F., Ayers, S., Blickenstaff, K., Tran, T.T., Abbott, J., Zheng, J.,and Zhao, S., 2010. Presence and characterization of shiga toxin-producing *E. coli* and other potentially diarrheagenic *E. coli* strains in retail meats. Applied Environmental Microbiology 76, 1709-1717.

Zaho, S., Maurerb, J.J., Huberta, S., De Villenac, J.F., McDermotta, P.F., Mengc, J., Ayers, S., Englisha, L., White, D.G., 2005. Antimicrobial susceptibility and molecular characterization of avian pathogenic *Escherichia coli* isolates. Vet. Microbiol. 20, 215-224.