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Colibacillosis in lambs and kids in Egypt: Prevalence, serogroups, antibiogram profile, virulence genes distribution and antimicrobial resistance genes

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Abstract

Background: Small ruminants have a socioeconomic impact on Egypt's production of meat, milk, and wool. Hence, every effort should be taken to prevent infections.

Aim: To elucidate the prevalence and serogrouping of *Escherichia coli* (*E. coli*) strains from diarrheic lambs and kids, determine their antibiotic susceptibility and associated risk factors affecting the occurrence of the disease, and establish the most common virulence genes marker and major antimicrobial resistance genes.

Methods: A total of 150 diarrheic animals (95 lambs and 55 kids) at different ages and seasons were subjected to clinical examination. Rectal swabs were collected from 150 diarrheic animals for isolation and biochemical identification of *E. coli*.

Results: The bacteriological examination revealed that 62/95 lambs and 26/55 kids with percentages of 65% and 47%, respectively, showed infection with *E. coli.* Serotyping of 88 isolates of *E. coli* revealed the strains belonging to O2(8), O55(17), O84(5), O17(4), O6(8), O91(17), O26(9), O103(5), O126(5), O124(6), and O159(4). A total of 21 isolates were examined by multiplex polymerase chain reaction assay for detection of virulence and resistance genes. All examined isolates possessed a combination between intimin gene and heat-stable toxin (100%), the serine protease (pic) gene on 8/21 isolates of O55, O2, O6 (38%), and α-hemolysin gene on 8/21 isolates of O26, O91(38%) while adherent invasive gene (invA) gene on 3/21 isolates of O124, O159 (14%) which divided diarrheagenic *E. coli* into four types assigned to be atypical enteropathogenic *E. coli* (48%), atypical enterohemorrhagic *E. coli* 35%), atypical enterotoxigenic *E. coli* (6%), and atypical enteroinvasive *E. coli* (11%). On the other hand, the results of antimicrobial susceptibility testing revealed high resistance to ampicillin, erythromycin, and tetracycline (100%) and amoxicillin/clavulanic acid (92%) but were highly sensitive to gentamicin, imipenem, norfloxacin, ciprofloxacin, chloramphenicol, and amikacin (100%). Concerning to ß lactams antibiotic resistance genes of examined isolates had blaSHV (100%) and blaCTX-M (43%). For tetracycline, we detected the tetA in all examined isolates.

Conclusion: The wide spread of atypical *E. coli* strains among diarrheic lambs and kids with marked resistance to several antibiotics of interest and the detection of major resistance genes assess the potential risk of this pathogen to animal and public health.

Keywords: *Escherichia coli*, Lambs, Kids, Antibiogram profile, Virulence genes.

Introduction

In Egypt, small ruminant products are the second source in human diet consumption after bovine, it is a popular home-raised species in small-scale farmers or village flocks (Abd-Allah *et al.*, 2019). Moreover, it could be a reservoir of many microorganisms (mo) that represent an economic and public health risk (Thomas *et al.*, 2020). Neonatal diarrhea is one of the health problems leading to economic losses and mortalities in small ruminants' flocks (Reidy *et al.*, 2006). Colibacillosis is caused by pathogenic *Escherichia coli*, represented as one of the infectious agents causing neonatal diarrhea in lambs and kids that possesses a few concentrations of circulating immunoglobulins (Constable *et al.*, 2017). Taxonomically, *E. coli* is categorized in the Enterobacteriaceae family. Six pathogenic strains of *E. coli* as enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli*, Shiga toxin-producing *E. coli* (STEC), diffusely adherents *E. coli*, enteroinvasive *E. coli* (EIEC), and enterotoxigenic *E. coli* (ETEC) are producing septicemia and diarrhea in human, animals and avian (Wani *et al.*, 2013; Bashahun and Amina, 2017). Heat stable and heat labile toxins, are produced by ETEC and are encoded by the genes est and elt,

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respectively (Weiglmeier *et al*., 2010). Moreover, the outer membrane protein of pathogenic *E. coli* strains (EHEC and EPEC) known as intimin (eae) that encoded by intimin gene (eaeA) (Omerovic *et al.*, 2017).

Aumental use of antibiotics resulted in an increase in antimicrobial resistance (AMR) in both human health problems and veterinary medicine which become more prevalent worldwide (Blanco Crivelli *et al*., 2021). Many complex mechanisms are associated with the emergence of plasmid-mediated AMR of mo as metallo ß lactams and extended-spectrum beta-lactamses (ESBLs), active efflux pumps and plasmid-mediated quinolone resistance genes (Bhattacharyya *et al.*, 2022). Assessment and diversity of gene resistance among bacterial isolates are essential to diagnose and understand the epidemiology of AMR spread among humans and animals (Boerlin *et al.*, 2005).

Abundant use of a wide variety of antibiotics such as aminoglycoside, penicillin, streptomycin, and sulfonamide without control from the authorities especially in developing countries leading to elevate the pathogenicity of m.o and constitute a major health concern to human and animal health (Wang *et al.*, 2016; El-Adawy *et al*., 2018). Antibiotic resistance genes have been widely identified using polymerase chain reaction (PCR) (Momtaz *et al.*, 2012). The wide spread of tetracycline resistance among m.o is due to the localization of tet genes on plasmids, transposons, and integrons (Roberts, 2003). TetA, blaCTX, and blaSHV genes were predominant in antibiotic-resistant *E. coli* mainly leading to antimicrobial treatment failure (Gozi *et al.*, 2019).

This study was performed to determine the prevalence rate and its associated risk factors that provoke diarrhea in lambs and kids, determine the actual incriminated virulence genes, detect the actual level of resistance of antimicrobial agents against *E. coli* isolated from diarrheic lambs and kids, and evaluate the distribution of major resistance genes in these isolates to detect the level of resistance in the study region.

Material and Methods

Animals and E. coli strains identification

A total of 150 diarrheic rectal swab samples were collected from 95 lambs and 55 kids aged from 1 day up to 4 months during the period from October 2021 to December 2022 from Al-Sharkia and Al-Ismailia Governorates. The data on prospective risk factors was related to a questionnaire of owners and direct observations of diarrheic lambs and kids. The questionnaire incorporated season, age, sex, breed, and other hygienic conditions were performed on each animal. Briefly, rectal sterile cotton swabs were collected from diarrheic animals in sterile MacConkey broth (Oxoid Ltd., Basingstoke, UK) in an ice box to the Infectious Diseases Laboratory, Faculty of Veterinary Medicine, Zagazig University, Egypt, for bacteriological examination.

Rectal swabs were incubated aerobically at 37°C/24 hour to improve the probability of isolation. According to Quinn *et al.* (2011), a loopful of MacConkey broth was added to MacConkey's agar and cultured for 37°C/24 hour to isolate *E. coli* strains, then a streak of lactose fermenter (pink) colonies were over Eosin Methylene Blue agar. *Escherichia coli* strains were identified using different specific biochemical tests according to Collee *et al.* (1996).

Escherichia coli isolates serogroups

Escherichia coli serogroups were identified serologically using slide agglutination test according to Kok *et al.* (1996), by standard polyvalent and monovalent *E. coli* antisera**.**

Antimicrobial sensitivity test (AST)

AST was applied by disc diffusion method on *E. coli* isolates on Mueller–Hinton agar plates following 0.5 McFarland standards as per Clinical and Laboratory Standards Institute (CLSI, 2020) against thirteen antimicrobial agents (Oxoid, Basingstoke, UK)**:** amoxicillin/clavulanic acid (AMC), amikacin (AK), norfloxacin (NOR), erythromycin (E), ciprofloxacin (CIP), gentamicin (CN), ceftazidime (CAZ), cefotaxime (CTX), trimethoprim-sulfamethoxazole (STX), chloramphenicol (C), ampicillin (AMP), imipenem (IMP), and tetracyclin (TE). The studied antimicrobial drugs characterized the *E. coli* isolates as being either susceptible, intermediate, or resistant.

Detection of virulence and resistant genes by PCR technique

Following the instructions on the QIAamp DNA Mini kit (Qiagen, Germany, GmbH), DNA was extracted from pure *E. coli* colonies. In a nutshell, 200 µl of the sample suspension was treated with 10 µl of proteinase K and 200 µl of lysis buffer at 56°C/10 minutes. After incubation, 200 µl of 100% ethanol was added, and nucleic acid was eluted using 100 µl of the kit's provided elution buffer.

Multiplex PCR technique

DNA extract of *E. coli* isolates was exposed to multiplex PCR to recognize eaeA, heat-stable toxin (STa), serine protease (pic), α-hemolysin (hlyA), invE, Stx2, and (heat-labile toxin) LT virulence genes and blaSHV, blaCTX-M, and tetA resistant genes with specific oligonucleotide primers (Metabion, Germany) (Table 1). A 50 µl reaction containing 25 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer, 13 µl of water, and 8 µl of DNA template was used for the final PCR amplification. Initial denaturation took place during the amplification process at 94°C for 5 minutes. The annealing temperature was 58°C for (Stx2), 57°C for (Sta and LT), 64°C for (hlyA), 59°C for (invE), 58°C for (pic), 51°C for (eaeA), 54°C for (blaSHV), 54°C for (blaCTX-M), and 50°C for (TetA) and extension at 72°C for 40 seconds.

At room temperature, the PCR products were separated on a 1% agarose gel (Applichem, Germany, GmbH). Before gel analysis, each gel slot was filled with 40 µl

Table 1. Oligonucleotide primers sequence of virulence and resistance genes *E. coli* strain.

References	Length of amplified product (bp)	Primer sequence (5'-3')	Target gene
Virulence genes			
Bisi-Johnson *et al*. (2011)	248	ATG CTT AGT GCT GGT TTA GG GCC TTC ATC ATT TCG CTT TC	eaeA
Lee *et al.* (2008)	229	GAAACAACATGACGGGAGGT GCACAGGCAGGATTACAACA	STa
Boisen *et al*. (2009)	572	ACTGGATCTTAAGGCTCAGGAT GACTTAATGTCACTGTTCAGCG	pic
Wang *et al.* (2002)	569	AGCTGCAAGTGCGGGTCTG TACGGGTTATGCCTGCAAGTTCAC	hlyA
da Cruz *et al.* (2014)	766	CGATAGATGGCGAGAAATTATATCCCG CGATCAAGAATCCCTAACAGAAGAATCA	invE
Dipineto *et al.* (2006)	779	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	Stx2
Lee *et al.* (2008)	605	GGTTTCTGCGTTAGGTGGAA GGGACTTCGACCTGAAATGT	LT
Resistance genes			
Colom *et al*. (2003)	392	AGGATTGACTGCCTTTTTG AGGATTGACTGCCTTTTTG	blaSHV
Archambault *et al*. (2006)	593	ATG TGC AGY ACC AGT AAR GTK ATG GC TGG GTR AAR TAR GTS ACC AGA AYC AGC GG	blaCTX-M
Randall *et al.* (2004)	576	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	TetA(A)

of the duplex PCR products and 20 µl of the uniplex PCR products. Thermo's Fermentas 100 bp ladder was used to calculate the sizes of the fragments. A gel documentation system (Alpha Innotech, Biometra) was used to visualize the gel, and computer software was used to analyze the data.

Statistical analysis

Using SPSS version 25 (SPSS Inc., Chicago, IL), the data were examined. One-sample chi-square and binomial tests were run, with $p \leq 0.05$ considered statistically significant.

Ethical approval

The Zagazig University Institutional Animal Care and Use Committee (ZU-IACUC) reviewed and authorized this study with permission N. (ZU-IACUC/2/F/116/2022).

Results

The colibacillosis-infected lambs and kids displayed a variety of clinical signs, including mild to profuse watery white diarrhea, minor dehydration, tachycardia, pale mucous membranes, severe weakness, a little rise in rectal temperature, and elevated respiratory rate. The bacteriological and biochemical identification of 150 rectal swabs of diarrheic lambs and kids (95 lambs and 55 kids aged from 1 day up to 4 months) illustrated a percentage of 65% (62/95) and 47% (26/55) *E. coli* infection in lambs and kids, respectively. The statistical analysis revealed a significant difference ($p = 0.004$) among lambs and kids (Table 2).

As depicted in Table 3, all lambs and kids were diarrheic at the time of sample collection with the high risk recorded in winter (58% lambs, 56% kids) and spring (21% lambs, 26% kids) seasons. The highest prevalence rate was demonstrated significantly in lambs and kids at age 1 day-1 week (58% and 55%, respectively). A significant difference among diarrheic male and female lambs ($p = 0.003$) as male lambs (63%) were more susceptible than females (37%), in contrast, no statistical significance among male and female kids ($p = 0.59$) was recorded. lambs and kids breeds had a role in the prevalence of diarrhea, Osseimi lambs breed (74%)were more vulnerable than the Barki lambs breed (26%) and the Baladi kids breed (73%) were more vulnerable than the Zaraibi breed (27%) ($p = 0.001$). Moreover, diarrheic lambs were more prevalent

Table 2. The prevalence of *E. coli* between lambs and kids using chi-square test.

Species	No. of animals	Bacteriological examination				Chi-square test (*p*-value*)
		Negative		Positive		
		No.	%	No.	%	
Lambs	95	33	35	62	65	0.004
Goat-kids	55	29	53	26	47	
Total	150	62	41	88	59	

*The *p*-value of chi square test.is ≥ 0.05, is not statistically significant differences, and vice versa.

Table 3. Risk factors associated with colibacillosis in lambs and kids using one sample binomial test.

Risk factors		No. of diarrheic animals (%)		*p*-value *	
		Lambs (95)	Kids (55)	Lambs *p*-value *	Kids *p*-value *
Season	Winter	55 (58%)	31 (56%)	0.004	0.005
	Spring	20 (21%)	14 (26%)		
	Summer	15 (16%)	6 (11%)		
	Autumn	5 (5%)	4 (7%)		
Age	1 day–1 week	55 (58%)	30 (55%)	0.005	0.004
	1–8 weeks	25 (26%)	15 (27%)		
	8–16 weeks	15 (16%)	10 (18%)		
Sex	Male	60 (63%)	30 (55%)	0.003	0.59
	Female	35 (37%)	25 (45%)		
Breed	Osseimi	70 (74%)	-	0.001	0.001
	Barki	25 (26%)	-		
	Baladi	-	40 (73%)		
	Zaraibi	-	15 (27%)		
Locality	Al-Sharkia	65 (68%)	35 (64%)	0.001	0.06
	Al-Ismailia	30 (32%)	20 (36%)		
Housing	Open flock	65 (68%)	30 (55%)	0.001	0.59
	Closed flock	30 (32%)	25 (45%)		
Hygienic status	Good	25 (26%)	10 (18%)	0.001	0.001
	Bad	70 (74%)	45 (82%)		
Source of drinking water	Pond	30 (32%)	15 (27%)	0.003	0.003
	Tube-well	5 (5%)	5 (9%)		
	Municipality	60 (63%)	35 (64%)		

*The *p*-value of the one-sample binomial test. If the computed *p*-value is greater than the significance level alpha = 0.05, There are no statistically significant differences, and vice versa.

in Al-sharkia (68%) than Al-Ismailia (32%) while there is not statistical difference between Al-sharkia (64%) and Al-Ismailia (36%) localities in the prevalence of diarrhea in kids ($p = 0.06$). Furthermore, the prevalence of diarrheic lambs housed in open flocks (68%) was higher than in closed flocks (32%) ($p = 0.001$), while no statistical significance between open housed (55%) and closed housed (45%) concerning diarrheic kids flock ($p = 0.59$). A significant association ($p = 0.001$) in a hygienic condition, poor hygienic status showed a

higher diarrheic rate of 74% in lambs and 82% in kids than good status. Source water supplied for animals had an effect on the prevalence rate of diarrhea among lambs and kids as in this study, pond and municipality water were highly significant ($p = 0.003$).

Serogrouping of *E. coli* isolates revealed 11 different *E. coli* serogroups, belonged to O2(8), O55(17), O84(5), O17(4), and O6(8) represented as atypical enteropathogenic *E. coli* (48%), O91(17), O26(9), and O103(5) represented as atypical enterohemorrhagic *E. coli* (35%), O126(5) represented as atypical enterotoxigenic *E. coli* (6%), and O124(6) and O159(4) represented as atypical enteroinvasive *E. coli* (11%). The highest prevalent serotypes were O55 and O91 (19.3%) and the lowest ones were O17 and O159 (4.5%). A significant difference between O55 and O91 in comparison with both O17 and O159 ($p = 0.0006$) is illustrated in Table 4.

The 88 *E. coli* isolates were tested against 13 different antimicrobial agents, and exhibited high resistance to ampicillin, erythromycin and tetracycline (100%), amoxicillin/clavulanic acid (92%), sulfamethoxazole/trimethoprim (75%), and ceftazidime (50%) but were sensitive to gentamicin, imipenem, norfloxacin, ciprofloxacin, chloramphenicol, amikacin (100%), and cefotaxime (58%) as showed in Table 5.

Out of 88 *E. coli* isolates, 21 isolates represented for detection of virulence genes using multiplex PCR. In this study, eaeA and STa (100%) were the most prevalent virulence genes represented, the pic gene for EPEC represented on 8/21 isolates of O55, O2, O6 (38%), and hlyA gene for EHEC also represented on 8/21 isolates of O26, O91(38%) while invA gene for EIEC represented only on 3/21 isolates of O124, O159 (14%) but Stx2 and LT did not detect.

Upon virulence gene characterization, *E. coli* isolates were alienated into typical ETEC (10%) and atypical combinations of ETEC/EHEC (38%), ETEC/EPEC (38%), and ETEC/EIEC (14%). All O55, O2, and O6 serotypes possessed the pic that represented an additional factor with eaeA and STa leading to the *E. coli* as aEPEC [A-typical EPEC].

Molecular diagnosis of *E. coli* AMR genes exhibited: blaSHV, blaCTX-M for (amoxicillin/clavulanic acid, cefotaxime, ampicillin, and ceftazidime) and tetA (for tetracycline). For amoxicillin/clavulanic acid, ampicillin, cefotaxime, and ceftazidime; blaSHV 21/21 (100%) showed a higher frequency than blaCTX-M 9/21 (43%). About 12/21 (57%) had a single gene, either blaCTX-M or blaSHV while 9/21 (43%) had both genes. For tetracycline, we detected the tetA in 21/21 (100%) of the isolates.

Discussion

Neonatal diarrhea is a worldwide infectious disease causing financial losses in the livestock industry mainly in the first few weeks of life (Croxen *et al.*, 2013). The same clinical signs of colibacillosis observed during this investigation were mentioned by Hassan *et al.* (2014) and Constable *et al.* (2017).

A variation in the prevalence of colibacillosis among lambs and kids, as lambs 65% were more susceptible to infection by *E. coli* than kids 47%, might be due to the difference in number of examined animals. The prevalence of colibacillosis in lambs was 65% which exceeded the level mentioned by Ahmed *et al*. (2010) in Nigeria (36.84%). The current study's high incidence could be related to improper preventative and control methods, untaken colostrum on the first days of lamb life, and unclean sheep housing. The prevalence

Table 4. Serovars distribution of *E. coli* isolated from diarrheic lambs and kids.

Strain	Serovars	No. of isolates	% of isolates	Total %
EPEC	O2:H6	8 [ab]	9.1	48
	O55:H7	17 [a]	19.3	
	O84:H4	5 [ab]	5.7	
	O17:H18	4 [b]	4.5	
	O6:H4	8 [ab]	9.1	
EHEC	O91:H21	17 [a]	19.3	35
	O26:H11	9 [ab]	10.2	
	O103:H2	5 [ab]	5.7	
ETEC	O126:H21	5 [ab]	5.7	6
EIEC	O124	6 [ab]	6.9	11
	O159	4 [b]	4.5	
		88		100
Kruskal–Wallis test (*p*-value)*		0.0006		

*Kruskal–Wallis test is used for comparing two or more independent samples of equal or different sample sizes. Means carrying different superscripts (a and b) are significant at $p < 0.05$.

Table 5. Antimicrobial sensitivity testing of *E. coli* isolated from diarrheic lambs and kids.

Antimicrobial class	Antimicrobial agents	No of *E. coli* isolates (%)					
		R	%	I	%	S	%
Aminoglycosides	Amikacin (AK)	0	-	0	-	88	100
Beta-lactams inhibitor (penam)	Amoxicillin/clavulanic acid (AMC)	81	92	0	-	7	8
Penicillins	Ampicillin (AMP)	88	100	0	-	0	-
Cephalosporins (third generation)	Cefotaxime (CTX)	15	17	22	25	51	58
Cephalosporins (third generation)	Ceftazidime (CAZ)	44	50	29	33	15	17
Fluoroquinolones	Ciprofloxacin (CIP)	0	-	0	-	88	100
Phenicols	Chloramphenicol (C)	0	-	0	-	88	100
Macrolides	Erythromycin (E)	88	100	0	-	0	-
Aminoglycosides	Gentamicin (CN)	0	-	0	-	88	100
Carbapenem	Imipenem (IPM)	0	-	0	-	88	100
Fluoroquinolones	Norfloxacin (NOR)	0	-	0	-	88	100
Sulfonamides	Sulfamethoxazole/Trimethoprim (SXT)	66	75	0	-	22	25
Tetracyclines	Tetracycline (TE)	88	100	0	-	0	-

of colibacillosis in kids was 47% which was lower than mentioned by Islam *et al*. (2016) in Bangladesh (52%) but higher than the recorded in Turkey (36.4%) by Türkyılmaz *et al.* (2014), in Saudia Arabia (30.8%) by Shabana *et al.* (2017), and in Rajasthan (31.43%) by Sharma *et al.* (2020). This dissimilarity might have been due to hygienic measures, geographic location, virulence, and strain of *E. coli*.

Colibacillosis was more common in the winter season followed by the spring season. Similar to Sonawane *et al.* (2012) but inconsistent with Islam *et al.* (2016) demonstrated that the diarrhea prevalence did not show any seasonal variation and Abdou *et al.* (2021) stated that diarrhea was more common in the dry season than the wet one. In this scenario, small ruminants aged from 1 day to 1 week were more likely to have a greater prevalence of infection, which matched the finding of Sharma *et al.* (2020) and Aklilu *et al.* (2013) reported that diarrhea was common in lambs and goat-kids of age less than 1 month. On the contrary, Shabana *et al.* (2017) noticed that sheeps and goats with age up to 12 months were highly susceptible to diarrhea.

The prevalence of diarrhea in lambs was more common in open flocks (68%) than closed ones (32%) might be due to exposure to extreme cold conditions. This disagreed with Nasr *et al.* (2014) stated that diarrhea was higher in the closed system (83.70%) than in the open system (46.80%) and Abdou *et al.* (2021) noticed that diarrhea occurred in both open and closed flocks equally.

The hygienic state of the area where lambs and kids were raised was shown to be significantly correlated, and low hygienic status exhibited a higher prevalence than good hygienic status. As detected by Islam *et al.* (2016) stated that poor hygiene has a main role in *E. coli* spread. Water sources for animal drinking from a pond and municipality had a high *E. coli* prevalence. Environmental contamination of water sources may be the main accusatory factor (Rashid *et al.*, 2015).

The 88 *E. coli* isolates were belonging to O2, O55, O84, O17, O6, O91, O26, O103, O126, O124, and O159 serotypes. The highest prevalent serotypes were O55 and O91 (19.3%) and the lowest ones were O17 and O159 (4.5%). This was in line with Ruchi and Kataria (2012) found that O55 was the most frequently isolated serogroup from diarrheic lambs and kids. In addition, Nasr *et al.* (2014) isolated the serogroups O55, O78, O125, O101, and O22 from diarrheic lambs. Hence, the analysis of our results proved that small ruminants in Egypt could be a potential source of infection in humans.

Antimicrobial drugs are frequently misused in Egypt because their usage for feed efficiency and growth promotion is not properly restricted. Farmers typically administer excessive doses of antibiotics to sick animals based exclusively on their own experience and without a veterinarian's prescription, supervision, or lab diagnosis, this creates selective pressure on the growth and distribution of infections that are resistant to antibiotics, including those that affect humans and animals (El-Twab *et al.*, 2016).

The antimicrobials with high resistance were erythromycin, ampicillin, and tetracycline (100%), followed by AMC (92%), SXT (75%), CAZ (50%),

and CTX (17%) with (100%) sensitivity to amikacin, ciprofloxacin, chloramphenicol, gentamicin, imipenem, and norfloxacin which considered specific drugs for treatment of *E. coli* infection in lambs and kids. These findings were in line with Croxen *et al.* (2013) who mentioned that *E. coli* isolates were resistant to ampicillin and tetracycline and sensitive to chloramphenicol, gentamicin, and norfloxacin. While disagreed with Imre *et al.* (2022) who reported that *E. coli* isolates were less resistant to ceftazidime (3.6%, 1/28).

Based on genotypic and phenotypic characteristics, most of the *E. coli* isolates (8/21, 38%) in this study were classified as aEPEC, which was in line with prior investigations (Vettorato *et al.*, 2009; Maluta *et al.*, 2014) that discovered few tEPEC strains in sheep and other animal species. Monitoring aEPEC in farm animals, such as sheep, became especially crucial since pathogenic strains can be transferred from animals to humans through food or direct contact with animals and their natural habitats (Brandal *et al.*, 2012; Otero *et al.*, 2013). In the actual result, observed that aEPEC was seriously found in lambs and kids. Similar findings in Spanish sheep and goats had been reported as well by Cortés *et al.* (2005).

ETEC is one of the most significant pathogens that causes diarrhea in young small ruminants (Pourtaghi and Sodagari, 2016). About 10% of the isolates in this investigation carried specific ETEC genes mainly STa. According to the most recent research from India and Turkey, lamb fecal isolates included 9% and 11.2% ETEC (Bandyopadhyay *et al.*, 2011; Türkyılmaz *et al.*, 2014), respectively. In this study, the absence of LT in the isolates is not surprising as LT is considered atypical in ruminant isolates (Türkyılmaz *et al.*, 2014).

Eight (38%) of the *E. coli* isolates in the current investigation showed mixed combinations of ETEC and EPEC. The current results supported those of other research that discovered mixed combinations of several *E. coli* pathotypes in calves (Sharma *et al.*, 2017; Aref *et al.*, 2018). Other unusual combinations were also found, including ETEC/EHEC (38%) and ETEC/EIEC (14%). The emergence of novel pathotypes that are more pathogenic and cause severe diarrhea in young small ruminants may result from these unusual combinations. These isolates were thought to pose a risk to Egypt's public health and were the cause of newborn diarrhea (Aref *et al.*, 2018). This happens when virulence genes are added or removed, altering the lineage and promoting the formation of novel variants with distinct characteristics and pathogenicity (Ahmed *et al.*, 2008). In this study, blaCTX-M and blaSHV AMR genes were responsible for developing the resistance against amoxicillin/clavulanic acid, ampicillin, cefotaxime, ceftazidime where blaSHV gene was predominant among ESBL genotypes and recorded in 21/21 (100%), blaCTX gene detected in 9/21 (43%). These results disagreed with (Abdallah *et al.*, 2022; Mahmood *et al.*, 2022) reported that the blaCTX gene was the most prevalent among *E. coli* isolates.

The most common tet genes discovered in enterobacteria were tetA and tetB (Van *et al.*, 2008). However, tetA markers are more prevalent than tetB markers (67%) compared to 31% (Karczmarczyk *et al.*, 2011). In this study, all isolates carried the tetracycline efflux gene tetA. These findings agreed with Gozi *et al.* (2019) noted that among the tested *E. coli* isolates, tetA was found in 54 (69.2%) of the samples.

Conclusion

This research proves the wide distribution of diarrheal *E. coli* pathotypes in lambs (65%) and kids (47%), with atypical EPEC (48%) being the most predominant pathotypes. The presence of eaeA and STa genes in all isolates created a typical virulent combination that threatened the public health hazard. Consequently, with the growing resistance of mostly used antibiotics [ampicillin, erythromycin, and tetracycline (100%), amoxicillin/clavulanic acid (92%), saulfamethoxazole/trimethoprim (75%) and ceftazidime (50%)], isolated *E. coli* carried the most important antibiotic-resistant genes, as all were revealed to be positive for the blaSHV and tetA genes (100%) while 43% were positive to blaCTX-M. Thus, there is a need to implement an accurate surveillance program and exact control measures for drug administration in the veterinary field to minimize the dissemination of these virulent, resistant, and atypical strains to humans.

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Author contributions

HHN, MIE, NZA, and EMF participated in the concept development, and study execution. While HHN, EBA, and AAS conducted data analysis and interpretation processes. The first draught of the manuscript was written by HHN and EMF. The manuscript revision and editing were done by HHN, EBA, and EMF. All of the study's data were completely accessible to the authors, who also accepted responsibility for the accuracy of the data analysis and final submission.

Conflict of interest

The authors declare that there is no conflict of interest.

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Data availability

The manuscript data are included within the manuscript.

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