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Multiple antibiotic resistance of *Escherichia coli* isolates from newborn diarrhetic calves and their virulence factors

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ABSTRACT

Diarrhea in calves is a major worldwide problem threat calves industry and caused by bacterial ,viral or parasitic pathogens resulted in great economic losses. *Escherichia coli* is one of the major causes of calves diarrhea. This study has looked into the prevalence of *E. coli*, its virulence factors, antibiotic resistance genes, and antibiotic resistance pattern. Aseptically, 60 fecal samples were collected from calves that had diarrhea at the Kafr El-Sheikh Governorate, Egypt. The incidence of *E.coli* were 42%. Eight (O) serogroups were identified by serogrouping of the isolated strains of *E. coli*: O26 (11 strains), O128 (9 strains), O111 (7 strains), O17 (4 strains), O84, O91, O55 (3 strains, each), and O159 (2 strains). The *E. coli* isolates' antimicrobial susceptibility to ten antimicrobial agents were tested and revealed that they were 76.19% sensitive to Gentamicin and 50% sensitive to Ciprofloxacin, but they were 100% resistant to Amoxicillin and Cefotaxim, Sulfamethoxazole-trimethoprim and Tetracyclin (92.85%,each), then Florfenicol and Amikacin (71.42%,each). Besides, multidrug resistance was determined in 92.85% of *E.coli* isolates. Eight *E.coli* serogroups were found to have the virulence genes stx1, stx2, sta and eaeA : O128 and O55 carried stx1, O26, O17and O84 carried stx2, 1 strain of each O26, O17, and O159 carried sta while all 7 serogroups except O128 showed positive result for eaeA gene. Furthermore, the antibiotic resistance genes; bla_{TEM},TetA(A), sul1 and floR were present in all *E.coli* serogroups. In conclusion, Both shiga toxin-producing *E. coli* (STEC) and enterotoxigenic *E. coli* (ETEC) are commonly the cause of diarrhea in calves. Moreover, *E.coli* isolates has a multidrug resistance appearance.

INTRODUCTION

Newborn calves constitute a substantial source of animal output for breeding or meat production worldwide **(Africa Union, 2008)**. Many dairy and beef herds suffer significant financial losses as a result of neonatal diarrhea **(Sharma et al. 2017)**. In Egypt and around the world, diarrhea is a significant issue in the production of livestock **(Farid et al. 2001)**.

The most common bacterial pathogen implicated in diarrhea in young calves is *E. coli* **(Cho and Yoon 2014)**. Depending on the dis-

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ease they produce and their virulence characteristics, pathogenic *E. coli* are classified as either intestinal or extraintestinal **(Omerovic et al. 2017)**. Diarrheal *E. coli* (DEC) are the prevalent term for intestinal infections caused by *E. coli* strains. There are several forms of intestinal pathotypes, including enteropathogenic *Escherichia coli*, enterotoxigenic *Escherichia coli*, enterohemorragic *Escherichia coli*, enteroaggregative *Escherichia coli*, enteroinvasive *Escherichia coli*, diffusely adherent *Escherichia coli*, and shiga toxin-producing *Escherichia coli* **(Cartlon et al. 2010)**. Based on pathological and molecular criteria, the most common pathotypes of *Escherichia coli* linked to neonatal colibacillosis are shiga-toxigenic *Escherichia coli* (STEC), enterotoxigenic *Escherichia coli* (ETEC), and enterohemorrhagic *Escherichia coli* (EHEC) as mentioned by **Aref et al. (2018)**.

Enterotoxigenic *Escherichia coli* is a prevalent pathotype linked to calves' infectious diarrhea. Enterotoxigenic *Escherichia coli* is linked to watery diarrhea, and the newborn calves show a high affinity for it **(Foster and Smith 2009)**. Fimbriae and enterotoxins are the two main virulent components **(Eid et al. 2019)**. Enterotoxigenic *Escherichia coli* is adept at producing two main enterotoxin kinds in both humans and animals: heat-stable (STa and STb) and heat-labile (LT) as reported by **Olaogun et al. (2016)**. In young calves, STEC is linked to dysentery. stx1 and stx2, two powerful forms of shiga toxins, are produced by them, and some of their strains are also capable of producing intimin. The strains of *E. coli* that possess the eaeA gene but lack the stx1 and stx2 genes are known as enteropathogenic strains **(Algammal et al. 2016, Hashish et al. 2016).**

In animal medicine, antibiotic medication is frequently used to treat and prevent a variety of bacterial infections, including calf diarrhea **(Umpierrez et al. 2017)**. However, Antibiotic misuse is associated with the development of antimicrobial resistance in bacterial pathogens **(Hammerum and Heuer 2009)**. The emergence of antibiotic-resistant bacteria is a growing concern in veterinary medicine. These resistant organisms are dangerous to animals and may even be harmful to people **(Pomba et al.2017)**.

Pathogenic *E. coli* is usually associated with antimicrobial resistance, which may be caused by the widespread misuse of antibiotics. *E. coli* frequently exhibits multidrug resistance (MDR), which is mostly linked to a number of genes **(Algammal et al. 2020, Yamamoto et al. 2013)**.

The purpose of the current study is to determine the prevalence of *E. coli* linked to calf diarrhea. focusing on virulence genes. Also its antibiogram and antibiotic resistance genes are screened.

MATERIAL AND METHODS

Sample collection

A total of 60 fecal samples were randomly collected using sterile rectal swabs from diarrheic calves (3 months of age) during November 2023 to May 2024 at different private farms of Kafr El Sheikh governorates. The samples were sent immediately to Animal Health Research Institute lab in an icebox for bacteriological examination .

Isolation and Identification of *E.coli*

Inoculating the swabs was done in nutrient broth (Oxoid) then incubated at 37°C for 24 hour aerobically followed by a 24-hour subculture on to MacConkey agar plates (Oxoid) and incubated as above, lactose-fermenting colonies were further cultivated onto eosin methylene blue (EMB) agar plates (Oxoid) and incubated at 37°C aerobically for the entire night. Colonies with metallic green shine were regarded as *E. coli*. Examinations of morphology, culture, and biochemical characters were conducted in accordance with **Murray et al. (2003)**. The slide agglutination test and rapid diagnostic *E. coli* antisera kits (DENKA SEIKEN Co., Japan) were used to serotype the isolates according to **Kok et al. (1996).**

Antimicrobial susceptibility test of *E.coli* isolates

Sensitivity of *E. coli* isolates to antibiotic was tested using ten different antimicrobial agents.; Amoxicillin (AMX, 25 ug), Cefotaxim

(CTX, 30ug), Tetracyclin (TE, 30ug), Sulfamethoxazole-Trimethoprim (SXT, 25ug), Florfenicol (FFC, 30ug), Amikacin (AK, 30ug), Doxycycline (DO, 30ug), Streptomycin (S, 10ug), Ciprofloxacin (CIP, 5ug) and Gentamicin (CN, 10ug) (Oxoid).using disc diffusion method as the description provided by **CLSI, (2018)**, the inhibitory zone's diameter was measured in millimeters and categorized as sensitive, moderate, and resistant.

Detection of virulence genes and antibiotic resistance genes of *E.coli* isolates using PCR (Uniplex Polymerase Chain Reaction)

Out of 42 *E. coli* isolates, eight (one from each serogroup) were subjected to PCR in order to identify four genes related to virulence and four genes related to antibiotic resistance. The QIAamp DNA mini Kit (Qiagen, Germany, GmbH) was used to extract DAN from samples according to the manufacturers recommendations. Table 1 displays the list of primers utilized in this case. These primers came from Metabion in Germany. The primers were utilized in a 25 –ul reaction containing 12.5 ul of EmeraldAmp Max PCR Master Mix (Takara, Japan). The reaction was performed in an Applied biosystem 2720 thermal cycler. Then the PCR products were separated by electrophoresis on 1.5% agarose gel in 1×TBE buffer using gradients of 5V/cm. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions

Target gene	Primers sequences	Amplified segment	Amplification (35 cycles)					Reference
			Primary denaturation	Secondary denaturation	Annealing	Extension	Final extension	
Stx1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	614 bp	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Dipineto et al., 2006
Stx2	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	779 bp	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	
eaeA	ATGCTTAGTGCTGGTTTAGG GCCTTCATCATTTCGCTTTC	248 bp	94˚C 5 min.	94˚C 30 sec.	51˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	Bisi Johnson et al., 2011
STa	GAAACAACATGACGGGAGGT GCACAGGCAGGATTACAACA	229 bp	94˚C 5 min.	94˚C 30 sec.	57˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	Lee et al., 2008
Sul1	CGGCGTGGGCTACCTGAACG GCCGATCGCGTGAAGTTCCG	433 bp	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.	72˚C 7 min.	Ibekwe et al., 2011
blaTEM	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516 bp	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Colom et al., 2003
floR	TTTGGWCCGCTMTCRGAC SGAGAARAAGACGAAGAAG	494 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Doublet et al., 2003
TetA(A)	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	570 bp	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Randall et al. 2004

RESULTS

Prevalence of *E.coli* isolated from diarrhetic calves.

Out of 60 fecal samples from diarrhetic calves examined bacteriologically; 42 samples (70%) were positive for *E. coli* isolation based on morphological characteristics and biochemical analysis and 18 (30%) were negative for *E.coli* isolation table 2

Table 2. Prevalence of *E.coli* in diarrhetic calves

Number of samples examined	+ve for *E.coli*		-ve for *E.coli*	
60	No	%	No	%
	42	70	18	30

* % calculated according to the No. of samples examined (60)

Serotyping identification of *E.coli* isolates

Forty two *E. coli* isolates were subjected to serological analysis, which revealed eight distinct serogroups, including O26 (eleven strains), O128 (nine strains), O111 (Seven strains), O17(four strains), O84, O91 and O55 (three strains, each) and O159 (two strains) table 3

Table 3. Serotyping of *E.coli* isolates (n=42)

Serogroups of *E.coli*	Total no. of strains	Percentage (%)
O26	11	26.19
O128	9	21.42
O111	7	16.66
O17	4	9.52
O84	3	7.14
O91	3	7.14
O55	3	7.14
O159	2	4.76

Antimicrobial susceptibility of *E.coli* isolates

The strains of isolated *E. coli* were examined for antibiotic susceptibility, and demonstrated resistance to cefotaxime and amoxicillin (100% each). sulfamethoxazole- trimethoprim and tetracyclin (92.85% each), then florfenicol and amikacin (71.42% each). However, gentamicin and ciprofloxacin showed 76.19 % and 50% of susceptibility, respective-

Table 4. Antimicrobial susceptibility of *E.coli* isolates (n=42)

Antimicrobial drugs	*E.coli* isolates					
	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Amoxicillin (AMX)	-	-	-	-	42	100
Cefotaxim (CTX)	-	-	-	-	42	100
Tetracyclin (TE)	-	-	3	7.14	39	92.85
Sulfamethoxazole- Trimethoprim (SXT)	3	7.14	-	-	39	92.85
Florfenicol (FFC)	8	19.04	4	9.52	30	71.42
Amikacin (AK)	12	28.57	-	-	30	71.42
Doxycycline (DO)	4	9.52	11	26.19	27	64.28
Streptomycin (S)	6	14.28	15	35.71	21	50
Ciprofloxacin (CIP)	21	50	14	33.33	7	16.66
Gentamicin (CN)	32	76.19	6	14.28	4	9.52

* % calculated according to the No. of tested *E.coli* isolates (42)

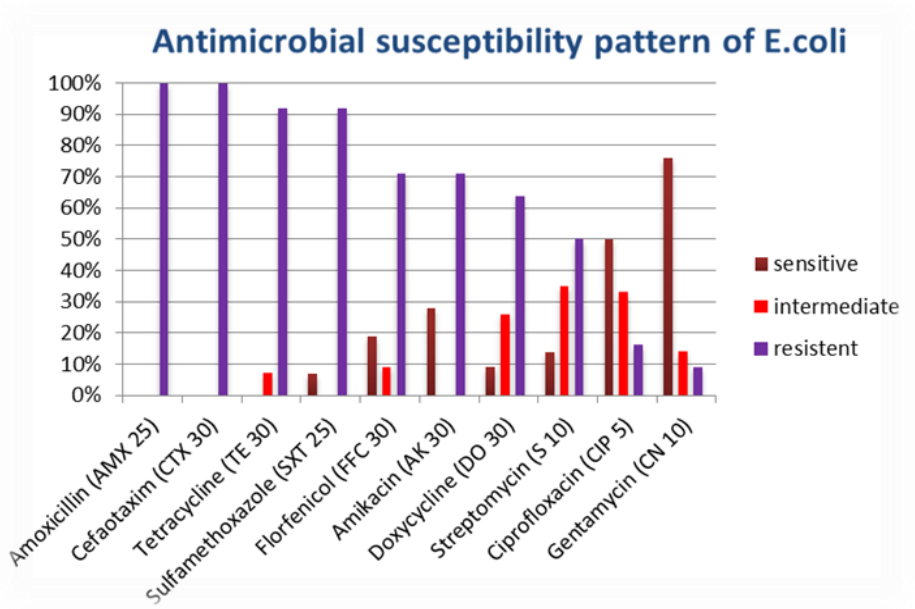

Figure 1 . Antimicrobial susceptibility pattern of *E.coli* isolates

Table 5. The distribution of the multidrug resistance pattern among the tested *E.coli* isolates (n= 42)

No. of isolates	% of isolates	Antibiotic resistant pattern	Multidrug resistance isolates (MDR)
			%
14	33.33	AMX,CTX,TE,SXT,FFC,AK,DO,S	(39 out of 42)
9	21.42	AMX,CTX,TE,SXT	(92.85%)
6	14.28	AMX,CTX,TE,SXT,FFC,AK,DO	
4	9.52	AMX,CTX,TE,SXT,FFC,AK,DO,S,CIP,CN	
3	7.14	AMX,CTX,TE,SXT,FFC,AK,DO,S,CIP	
3	7.14	AMX,CTX,TE,SXT,FFC,AK	

MDR: Multidrug resistance to at least three different antimicrobial classes

Detection of virulence and antibiotic resistance genes in *E.coli* isolates (No=8)

Regarding to virulence genes; the highest detected gene was eaeA which was present in an incidence of (87.5%) followed by stx2 and sta (37.5% ,each) while the lowest incidence was stx1 (25%). Furthermore All isolates (100%) had the antibiotic resistance genes bla_{TEM}, floR, TetA (A), and sul1 table 6.

Table 6. Prevalence of virulence genes & antibiotic resistance genes in 8 different serogroups of *E.coli* isolated from diarrhetic calves.

Isolates	No. of isolates	Serogroups	Virulance genes								Antibiotic resistance genes							
			stx1		stx2		eaeA		sta		bla_{TEM}		floR		TetA(A)		sul1	
E.coli	8	O26	-		+		+		+		+		+		+		+	
		O128	+		-		-		-		+		+		+		+	
		O111	-		-		+		-		+		+		+		+	
		O17	-		+		+		+		+		+		+		+	
		O91	-		-		+		-		+		+		+		+	
		O55	+		-		+		-		+		+		+		+	
		O84	-		+		+		-		+		+		+		+	
		O159	-		-		+		+		+		+		+		+	
		Total	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
			2	25	3	37.5	7	87.5	3	37.5	8	100	8	100	8	100	8	100

*% calculated according to the No. of tested isolates (8) .

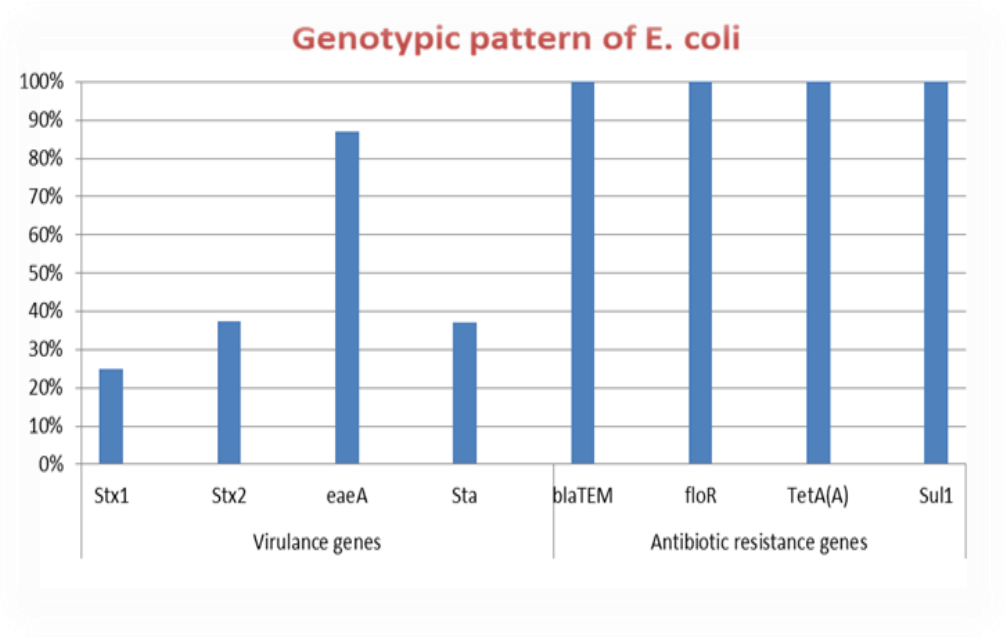

Figure 2. Genotypic pattern; prevalence of virulence and antibiotic resistance genes in *E.coli* isolates

Detection of virulence genes in 8 different serogroups of *E.coli* isolated from diarrhetic calves by PCR.

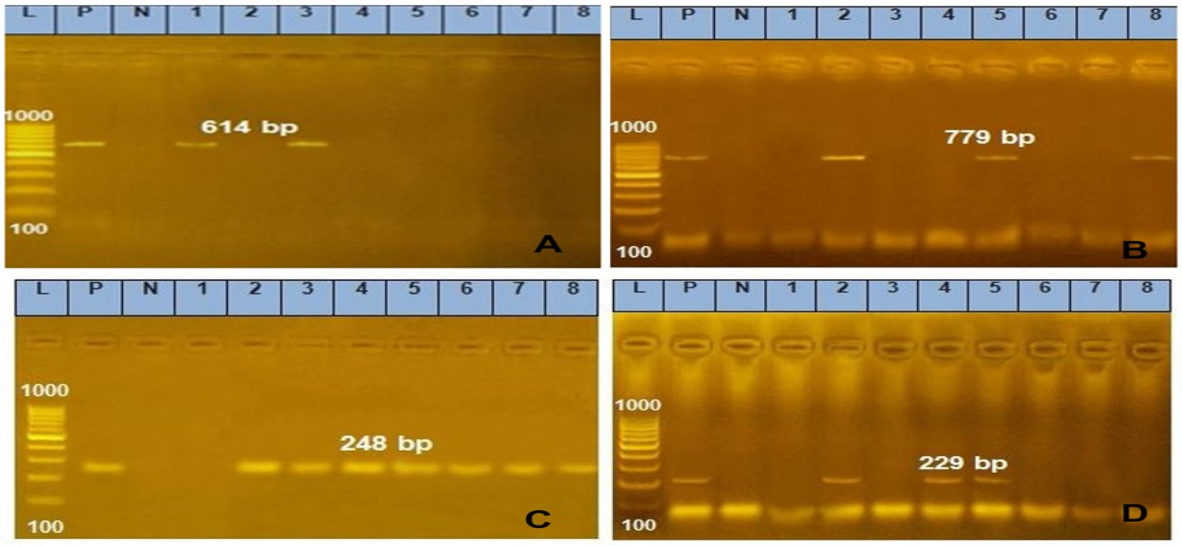

Photo1.(A) Agarose gel electrophoresis of PCR amplification products of stx1 virulence gene of *E.coli* (614bp): L:100-1000bp DNA ladder; P:Positive control; N:Negative control; Lanes:1,3 positive *E.coli* strains for stx1 gene.
(B) Agarose gel electrophoresis of PCR amplification products of stx2 virulence gene of *E.coli* (779bp): L:100-1000 bp DNA ladder; p:positive control; N:Negative control; Lanes: 2,5,8 positive *E.coli* strains for stx2 gene. (C) Agarose gel electrophoresis of PCR amplification products of eaeA virulence gene of *E.coli* (248bp): L:100-1000 bp DNA ladder; P:Positive control; N:Negative control; lanes 2-8 positive *E.coli* strains for eaeA gene.(D)Agarose gel electrophoresis of PCR amplification products of sta virulence gene of *E.coli* (229 bp): L:100-1000bp DNA ladder; P:Positive control; N:Negative control; lanes 2,4,5 positive *E.coli* strains for sta gene.

Detection of antibiotic resistance genes in 8 different serogroups of *E.coli* isolated from diarrheic calves by PCR

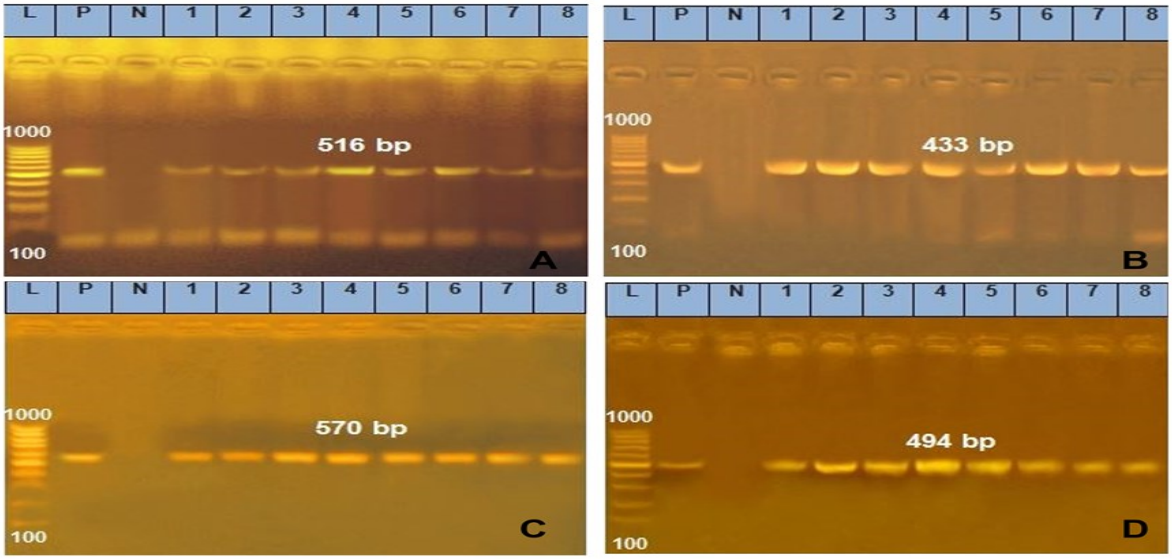

Photo2.(A)PCR amplification products of bla_{TEM} antibiotic resistance gene of *E.coli* (516bp):L:100-1000 bp DNA ladder; P:Positive control; N:Ngative control; lanes 1-8 positive *E.coli* strains for bla_{TEM} gene.
(B)PCR amplification products of sul1 antibiotic resistance gene of *E.coli* (433bp) :L:100-1000 bp DNA ladder; P:Positive control; N:Negative control; lanes 1-8 positive *E.coli* strains for sul1 gene. (C)PCR amplification products of TetA(A) antibiotic resistance gene (570bp): L:100-1000bp DNA ladder; P:Positive control; N:Negative control; lanes 1-8 positive *E.coli* strains for TetA(A) gene. (D)PCR amplification products of floR antibiotic resistance gene (494bp): L:100-1000bp DNA ladder; P:Positive control; N:Negative control; lanes 1-8 positive *E.coli* strains for floR gene.

DISCUSSION

Escherichia coli is the most prevalent and significant pathogenic bacteria that causes newborn diarrhea in animals, despite the fact that only some types of *E.coli* are pathogenic **(Muktar et al. 2015).** Similar to other research, our findings showed that *E. coli* was recovered from diarrhetic calves with an incidence of 70% table 2. **Yeshiwas and Fentahum (2017)** isolated *E.coli* as from diarrhetic calves70.6% and **El Bably et al. (2016)** as 68.3%, while the results were nearly similar to **Naena and Abou Zeid (2018)** who isolated *E.coli* in an incidence of 75%. But these results were lower than that reported by **Picco et al. (2015)** 85.04%, however, much lower prevalence was detected **by Khalifa et al. (2019)** 9%.

Overall, *E.coli* seems to be a significant causal agent, either by alone or in conjunction with other bacteria in calf diarrhea etiology. In our investigation, *E. coli* was identified with the highest frequently from calves that had diarrhea. The high isolation rate of *E. coli* in this investigation may have resulted from mixing of different ages, unsanitary and unfavorable environmental conditions, or inadequate colostrum quantity and/or quality. However Variations in geography, management techniques, health conditions, and calf age may all contribute to variations in *E. coli* prevalence **(Enany et al. 2019).**

Different serogroups of *E. coli* were found in diarrheal calves, as O26, O128, O111, O17, O159, O91, O55 and O84 table 3. Similar *E.coli* serogroups has been reported **by Al gammal et al. (2020) and El Bably et al. (2016)** who isolated O26, O128, O111, O91 serogroups and O26, O159 and O55 serogroups from diarrheic calves, respectively. While our results disagree with **Shaaban (2018)** who detected *E.coli* O18 and O158 serogroups from diarrheic calves .

Table 4 and Figure 1 display the antimicrobial susceptibility pattern of the *E. coli* strains that were identified from the diarrheal calves. The bacterial strains showed 100% resistance to cefotaxime and amoxicillin. **Ansari et al. (2014) and Abd-Elrahman et al. (2011)** showed similar findings, indicating higher resistance to amoxicillin (100%) and cefotaxime 87.18% and 60%, respectively. In contrast **Abdulgayeid et al. (2015)** found that the most susceptible antibiotics was cefotaxime (100%).

Also in the present study, the resistance was recorded against tetracyclin, sulfamethoxazole –trimethoprime and florfenicol 92.85%, 92.85% and 71.42% respectively. In accordance with our findings, both **Malik et al. (2013) and Ansari et al. (2014)** expressed 100% resistance. However, **Hossain et al. (2012)** declared 100% sensitivity to tetracycline. Furthermore our results agreement with **Shahrani et al. (2014)** who observed high resistance to sulfamethoxazole-trimethoprime as 90.31%. Moreover near agreement with **Cengiz and Adiguzel (2020) and Haydardedeoglu et al. (2023)** who demonstrated 70.22% and 75% resistance to florfenical, respectively but not **with Abdulgayeid et al. (2015)** who found that *E.coli* was sensitive to florfenical 85.3%. On the other hand the most susceptible antibiotic in our study were gentamycin and ciprofloxacin 76.19% and 50% respectively. According to similar results, *E. coli* isolates showed a moderate susceptibility 50% to ciprocin and high sensitivity 75% to gentamycin **by Naena and Abou Zeid (2018). Additionally, Sikrodia et al. (2024)** exhibit sensitivity to the antibiotics ciprofloxacin (32.81%) and gentamycin (68.75%), **whereas Hossain et al. (2012)** found gentamycin as 100% resistant.

In the present investigation, multidrug resistance *E.coli* (resistance to more than 3 drug clones) was observed in (39 isolates; 92.85%) table 5. The incidence of multiple resistance is less than the 4.9% recorded by **Li et al. (2018),** but it is comparable to the 93.82% and 98.73% reported by **Cengiz and Adiguzel (2020) and Algammal et al. (2020)** respectively.

One of the biggest issues facing the veterinary and medical fields is high and variable antibiotic resistance, particularly in situations when humans and animals are in close contact under such rearing systems. This makes it pos-

sible for resistance strains to spread between species and between animals, and even between animals and humans. This can have detrimental implications on the management of pathogenic *E. coli* and the treatment of diseases caused by *E. coli* in various hosts.

Table 6, figure 2 and photo1 illustrate the results of this study's virulence gene screening, which revealed that the *E. coli* isolates tested positive for eaeA (87.5%) followed by stx2 and sta (37.5% each). The lowest incidences were stx1 (25%) that were agreed with **Coura et al. (2015)** who found that stx2, stx1, eaeA, and sta were the most prevalent virulence profiles of *E. coli* strains. On the other hand our results in harmony with **Borriello et al. (2012) and Jia et al. (2022)** who detected eaeA gene in a high prevalence 100% and 76%, respectively. Slightly higher results were recorded where stx2 and sta genes were detected with a percentage of 39.7% and 40.98% **Sobhy et al. (2020) and Coskun and Sahin (2023),** respectively. However near finding was reported by **Naena and Abou Zeid (2018)** who stated that stx1 was habord in *E.coli* isolates with a percentage of 16.6%.While exception have been reported by **Osman et al. (2012)** who detected that eaeA gene as 5.9% and **Mohammadi et al. (2013)** who stated that every *E.coli* isolate tested was negative for eaeA. In contrast *E.coli* isolates excusively contained stx1 and stx2 **(Osman et al. 2012).** While **Umpierrez et al. (2021)** note that only 5.36% of *E.coli* isolates had the sta gene. Seasons, farm management techniques, farm size and animal population, hygienic conditions and detection techniques could all affect the incidence of virulence genes. According to **Belardo et al. (2014)** the intimin gene is mostly associated with the EPEC pathotype, and strains that are eaeA positive are thought to be more virulent to humans than those that are eaeA negative. STEC is defined as strains that carry eaeA and have either stx1 or stx2 variants **(Ishii et al. 2007).**

stx2 is linked to severe infections and has a potent cytotoxic effect on endothelial cells **(Bertin et al. 2001 and Caprioii et al. 2005).** The high frequency of stx2 is linked to clinical symptoms that support the findings of **Wani et al. (2003)** in that the stx2 gene was more common in *E.coli* isolates from diarrhetic calves than the stx1 gene and that the eaeA gene in STEC was linked to both.

Four distinct antibiotic resistance genes, shown in table 6, figure 2 and photo2 were detected in the isolates in order to compare the phenotypic and genotypic resistance. TetA(A), sul1, floR, and bla_{TEM} gene detection rates were 100% each. The results of **Abdeen et al. (2019), Abdulgayeid et al. (2015), and Elsayed et al. (2020)** are all in strong accord with the current study, which discovered that 100% of *E. coli* isolates had the bla_{TEM}, TetA, and sul1 genes, respectively. However, lower percentages of the bla_{TEM}, TetA, sul1, and floR genes were reported by **Jia et al. (2022),** at 29%, 19%, 0%, and 24%, respectively.

Geographical differences, the complexity of the healthcare facilities involved, and the use of antibiotics and antibiotic stewardship procedures may all play a role *E. coli* genes detected.

CONCLUSION

In conclusion, *E.coli* was highly prevalent in the diarrhetic calves which constitute a common bacterial cause of diarrhea in calves resulting in severe economic losses and discovered that both O26 and O128 were the most common *E. coli* serogroups in diarrhetic calves. Furthermore, *E.coli* strains demonstrated greater resistance to variety of antibiotic .

However, susceptibility was observed to gentamicin and ciprofloxacin which are used in veterinary medicine for treatment . As well as, it was discovered that 92.85% of all isolates were multidrug resistant, highlighting the severity of *E. coli* infection in calves and its implications for public health. We also found a strong correlation between antimicrobial resistance genes and antibiogram profiles, which may help to understand the seriousness of the problem facing veterinarians. Hence, it is crucial to implement efficient control measures to reduce the spread of *E. coli* that is resistant to antibiotics.

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