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Genotypic Characterization of *Escherichia coli* Pathotypes Based on Antibiotic Resistance Genes

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ABSTRACT

Diarrhea represents a major health challenge in young calves and is a leading cause of mortality, arising from infectious and non-infectious factors. The present study aimed to investigate antibiotic-resistant, multi-virulent diarrheagenic *Escherichia coli* (*E. coli*) strains isolated from human and animal samples. The current study included 23 *E. coli* strains obtained from calves across four different farms in Egypt, as well as nine *E. coli* isolates derived from 18 stool samples from workers. These samples were transferred to the microbiological laboratory for bacteriological and molecular analysis utilizing polymerase chain reaction (PCR). Molecular analysis of the antibiotic resistance gene was performed using PCR, followed by antibiotic sensitivity testing against ciprofloxacin, enrofloxacin, norfloxacin, ampicillin, cephalosporin, and colistin. Among 23 *E. coli* isolates obtained from calves, the resistance genes, including *qnrS* (21.7%), *gyrA* (34.7%), and *blaTEM* (13%), were identified. Conversely, *qnrA*, *blaOXA-1*, and *blaSHV* were not detected. Only one isolate exhibited colistin resistance, which was uncommon. Human *E. coli* isolates demonstrated moderate prevalence of *qnrA* (55.5%), *qnrS* (33.3%), *gyrA* (33.3%), and *blaTEM* (22.2%), while *blaOXA* and *blaSHV* were not detected, and a colistin resistance isolate (*mcr-1*) was rare. The antibiotic sensitivity test of *E. coli* isolates from calves exhibited that 43% were resistant to ciprofloxacin and 35% to enrofloxacin. Meanwhile, 22% of isolates demonstrated resistance to norfloxacin. The β-lactam group revealed 13% resistance to ampicillin and cephalosporin. Only 4% of all isolates exhibited resistance to colistin. Antibiotic sensitivity test of *E. coli* isolates from humans revealed significant resistance patterns. Among the quinolones, 78% demonstrated resistance to ciprofloxacin, 56% to enrofloxacin, and 45% to norfloxacin. In contrast, only 22% of isolates were resistant to ampicillin and cephalosporin, while resistance to colistin was detected in just 11% of isolates. The present study confirmed that the misuse of different antibiotics is a significant factor in the development of antimicrobial resistance genes, especially in *E. coli* strains isolated from both humans and infected calves.

Keywords: Antibiotic sensitivity test, *Escherichia coli*, Multidrug resistance, PCR

INTRODUCTION

Diarrhea causes substantial morbidity and mortality in developing nations. Given that several distinct phenotypic enteric bacteria have been identified as causative agents of severe gastroenteritis (Davis and Brown, 2016). *Escherichia coli* (*E. coli*) is the main cause of diarrhea and can be categorized as either commensal or pathogenic (Davis and Brown 2016). The pathogenic group of *E. coli* comprises two pathotypes, including diarrheagenic *E. coli* (DEC) and extraintestinal pathogenic *E. coli* (ExPEC), each responsible for distinct diseases in humans and animals (Kunert et al., 2015). Neonatal meningitis and uropathogenic *E. coli* are the two pathovars of ExPEC (Bae et al., 2006). Furthermore, regarding the mechanisms underlying diarrhea, six pathovars of DEC have been identified, including diffusely adherent *E. coli*, enteropathogenic *E. coli*, enteroaggregative *E. coli*, enterohaemorrhagic *E. coli*, and entero-invasive *E. coli* (Croxen and Finlay, 2010). *Escherichia coli* has been linked to foodborne outbreaks in humans, resulting in thrombotic thrombocytopenic purpura (TTP) in adults, hemolytic uremic syndrome (HUS), and bloody diarrhea in children (Bae et al., 2006). There are two major public health crises involving *E. coli* infections. Firstly, the prevalent pathogenic *E. coli* strains in environmental waters, such as those used for irrigation and drinking, cause severe infections in humans and animals (Koczura et al., 2011). Secondly, the presence of multi-virulent and multidrug-resistant (MDR) *E. coli* strains that exhibited resistance to at least three antimicrobial medicines from different classes (Hamelin et al., 2007).

The presence of virulence genes and antimicrobial resistance was demonstrated to have a strong negative correlation (Bendary et al., 2022). The high prevalence of multiple virulence genes among MDR *E. coli* isolates from human,

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animal, and environmental sources indicated a significant pathogenic potential, making these infections difficult to treat due to their multidrug resistance (James et al., 2025). Antimicrobial resistance among *E. coli* strains has become a growing concern worldwide. In 2017, Iranian hospitals documented an unexpected rise in resistance to commonly used antibiotics such as ampicillin and ceftazidime, underscoring the growing difficulty of managing nosocomial *E. coli* infections (Eshrati et al., 2025). The number of antibiotic resistance genes (ARGs) in *E. coli* has gradually increased over recent decades, influenced by factors such as global antibiotic use, the spread of mobile genetic elements, and various climatic conditions (Wang et al., 2026). Gamal et al. (2019) analyzed different human and animal samples to identify specific pathogenic genes. In this context, the present study aimed to assess antibiotic-resistant and MDR strains of highly virulent diarrheagenic *E. coli*, which pose significant health challenges to both humans and animals.

MATERIAL AND METHODS

Ethical approval

Ethical approval was not required for the present study according to institutional and journal guidelines, as it relied solely on previously isolated *E. coli* DNA samples and did not involve any animal experimentation or handling.

Strains

In the current investigation, nine *E. coli* isolates from humans and 23 *E. coli* strains from calves were utilized. The *E. coli* isolates used in the present experiment were sourced from a prior study by Gamal et al. (2019), who collected 43 fecal samples from diarrheic calves across four farms in New Valley, Egypt, and 18 stool samples from workers in 2019. These samples were transferred to the microbiological laboratory for bacteriological and molecular examination. Polymerase chain reaction (PCR) was used to genotype all 23 calves and 9 human samples as *E. coli* (Gamal et al., 2019).

Extraction of DNA

Genomic DNA was isolated from both human and animal *E. coli* cultures utilizing the EasyPURE Bacterial Genomic DNA Kit (Cat. No. EE161-01), following the manufacturer's provided protocol. To collect bacterial cells, one mL of each overnight culture was centrifuged at 12,000 × g for one minute. The cell pellet was subjected to enzymatic lysis using the provided lysis buffer and Proteinase K at 55°C for 15 minutes. The DNA binding was performed using the kit's binding buffer, then the mixture was applied to a silica column and centrifuged at 12,000 × g for 30 seconds. The column was subsequently washed according to the manufacturer's protocol, and DNA was eluted in 50 µL of elution buffer. The concentration and purity of the extracted DNA were determined using a spectrophotometer.

Polymerase chain reaction

A reaction mixture was prepared in 0.5 mL microfuge tubes containing 5 µL of genomic DNA, 12.5 µL of Dream Taq Green Master Mix (2x Easy Taq PCR Supermix [AS111]; Thermo Fisher Scientific, China), 1 µL of each primer (50 pmole/µl; Table 1), and 5.5 µL of deionized water. Then, amplification was performed under the following conditions. The thermal cycling profile for the *qnrA*, qnrS, and *gyrA* gene primers consisted of an initial denaturation step of 95°C for five minutes. This was followed by 35 cycles, each comprising 45 seconds at 94°C (denaturation), 45 seconds at 51°C (annealing), and 45 seconds at 72°C (extension). A final extension was performed at 72°C for seven minutes. For the *blaOXA-1*, *blaSHV*, and *blaTEM* genes (Colom et al., 2003), the protocol involved an initial denaturation at 94°C for five minutes, followed by 35 cycles. Each cycle consisted of 30 seconds at 94°C, 30 seconds at 54°C, and 1 minute at 72°C. A final extension was performed at 72°C for 10 minutes. For the *mcr1* gene primer (Rebelo et al., 2018), the protocol started with a 15-minute denaturation at 94°C. This was followed by 25 cycles, each comprising 30 seconds at 94°C, 90 seconds at 58°C, and one minute at 72°C. The final step was a 10-minute extension at 72°C. The PCR products were analyzed on a 1% agarose gel prepared in 1× TAE buffer and stained with ethidium bromide (0.5 µg/mL). A 100-base pair DNA ladder was included as a molecular size marker. Electrophoresis was performed at 100 V and 80 mA for 30 minutes. After separation, DNA bands were visualized and photographed under UV transillumination.

Antibiotic sensitivity test

In compliance with the guidelines supplied by the Clinical and Laboratory Standards Institute (CLSI, 2019), the *in vitro* susceptibility patterns of all tested *E. coli* isolates from both animal and human origin were determined using Mueller-Hinton agar and standard antimicrobial discs using the Kirby-Bauer disc diffusion method.

Table 1. Resistance gene primers used in the present study

Genes	Primer sequence	Amplicon size	Reference
qnrA	F 5-ATTTCTCACGCCAGGATTTG-3 R 5-GATCGGCAAAGGTTAGGTCA-3	516	Hammad et al. (2009)
qnrS	F 5-ACGACATTCGTCAACTGCAA-3 R 5-TAAATTGGCACCCTGTAGGC-3	417	Hammad et al. (2009)
gyrA	F 5-AAATCTGCCCGTGTCGTTGGT-3 R 5-GCCATACCTACGGCGATACC-3	344	Eguale et al. (2017)
blaOXA-1	F 5-TCAACTTTCAAGATCGCA-3 R5-GTGTGTTTAGAATGGTGA-3	609	Colom et al. (2003)
blaSHV	F 5-AGGATTGACTGCCTTTTTG-3 R 5-ATTTGCTGATTTCGCTCG-3	392	Colom et al. (2003)
blaTEM	F 5-ATCAGCAATAAACCAGC-3 R 5-CCCCGAAGAACGTTTTC-3	516	Colom et al. (2003)
mcr-1	F 5-AGTCCGTTTGTTCTTGTGGC-3 R 5-AGATCCTTGGTCTCGGCTTG-3	320	Rebelo et al. (2018)

RESULTS

The present results indicated that the *qnrA* resistance gene was not detected in any of the 23 *E. coli* isolates from calves (Table 2). However, the *qnrS* resistance gene was detected in 5 of the 23 isolates, producing a 417 bp PCR product (Figure 2A and 1A). The prevalence rates for the *qnrA*, *qnrS*, and *gyrA* resistance genes in the 23 *E. coli* isolates from calves were 0%, 21.7%, and 34.7%, respectively. Specifically, the *gyrA* gene was detected in eight isolates via PCR, producing a 344 bp product (Figure 3A). Analysis of the β-lactam resistance genes revealed that all 23 calf isolates were negative for *blaOXA-1* and *blaSHV* (Figure 4A). However, *blaTEM* was detected in three isolates, producing a 516 bp PCR product (Figure 5A). The prevalence rates for *blaOXA-1* and *blaSHV* were 0%, while the prevalence for *blaTEM* was 13%. Regarding colistin resistance, only 1 of 23 calf isolates tested positive for the *mcr-1* gene, yielding a prevalence of 4.3%. This positive result yielded a 320 bp PCR product (Figure 6A).

Analysis of nine *E. coli* isolates from human sources revealed the presence of resistance genes. Specifically, three isolates were positive for *qnrS*, yielding a 417 bp PCR product (Figure 1 B), and five isolates were positive for *qnrA*, yielding a 516 bp PCR product (Table 3; Figure 2B). The *gyrA* resistance gene was detected in three human isolates, yielding a 344 bp PCR product (Figure 3B). The prevalence of *qnrA*, *qnrS*, and *gyrA* resistance genes among the human isolates was 55.5%, 33.3%, and 33.3%, respectively. In the β-lactamase gene analysis, all nine human *E. coli* isolates yielded negative results for the *blaOXA* and *blaSHV* genes (Figure 4B). In contrast, the *blaTEM* gene was found in two of the nine human strains, producing a 516 bp PCR product (Figure 5B). The prevalence rates for *blaOXA*, *blaSHV*, and *blaTEM* were 0%, 0%, and 22.2%, respectively. For colistin resistance genes, the *mcr-1* gene was detected in one out of the nine human *E. coli* isolates, yielding a 320 bp PCR product. The prevalence rate for *mcr-1* among human strains was 11.1%.

The antibiotic sensitivity test results for *E. coli* isolates from calves against quinolones, β-lactams, and colistin are presented in Table 4. Susceptibility testing for quinolones in 23 *E. coli* calf isolates indicated that resistance to ciprofloxacin was observed in 10 isolates (43%). Resistance to enrofloxacin was detected in eight isolates (35%), and resistance to norfloxacin was found in five isolates (22%). The remaining isolates demonstrated different degrees of sensitivity to these antibiotics. Resistance to ampicillin and cephalosporin antibiotics (β-lactam group) was identified in 3 of 23 calf isolates (13%). Colistin resistance was found in only one calf isolate (4%), while the other 23 isolates demonstrated sensitivity to colistin.

Antibiotic sensitivity test results of human *E. coli* isolates to quinolones, β-lactams, and colistin are presented in Table 5. For quinolones, seven of nine human *E. coli* isolates (78%) were resistant to ciprofloxacin, while two isolates (22%) were sensitive (Table 5). The current findings indicate that five of the nine human *E. coli* isolates (56%) were resistant to enrofloxacin, whereas the remaining isolates showed varying degrees of sensitivity. Additional findings indicated that four of the nine human *E. coli* strains (45%) were resistant to norfloxacin, and the remaining isolates (5/9) were sensitive to norfloxacin. Resistance to ampicillin and cephalosporin antibiotics (β-lactams) was observed in two of nine human *E. coli* isolates (22%). Colistin resistance was noted in only one human *E. coli* isolate (11%).

Table 2. PCR results of *Escherichia coli* isolated from calves in New Valley, Egypt

Calves-tested isolates		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Target resistance genes																								
Quinolones	*qnrA*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	qnrS	-	-	+	+	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
	gyrA	+	+	+	-	-	-	+	-	-	-	+	-	+	-	-	-	+	+	-	-	-	-	-
β- lactam	*blaOXA-1*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	blaSHV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	blaTEM	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Colistin	*Mcr-1*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-

+: Means presence of gene, -: Means absence of genes

Table 3. PCR results of *Escherichia coli* isolated from humans in New Valley, Egypt

Human-tested isolates		5E	6E	9E	15E	51E	54E	55E	65E	67E
Resistance genes										
Quinolone group	*qnRA*	-	+	-	+	-	+	+	+	-
	qnrS	-	-	+	-	-	-	+	-	+
	gyrA	-	-	+	+	-	-	-	-	+
β-lactam group	*blaOXA-1*	-	-	-	-	-	-	-	-	-
	blaSHV	-	-	-	-	-	-	-	-	-
	blaTEM	+	-	-	-	+	-	-	-	-
Colistin	*mcr-1*	-	+	-	-	-	-	-	-	-

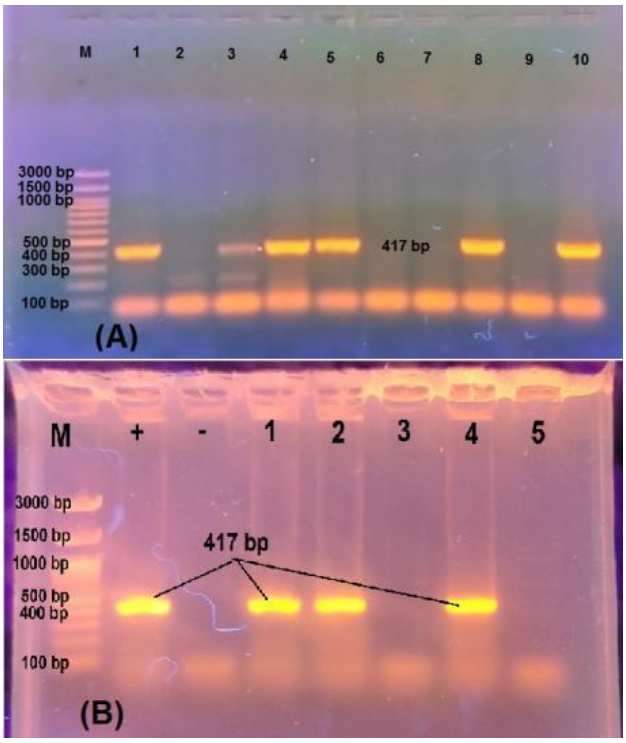

Figure 1. The *qnrS* gene of *Escherichia coli* isolated from different sources in Egypt. **A**: Calves, **B**: Humans

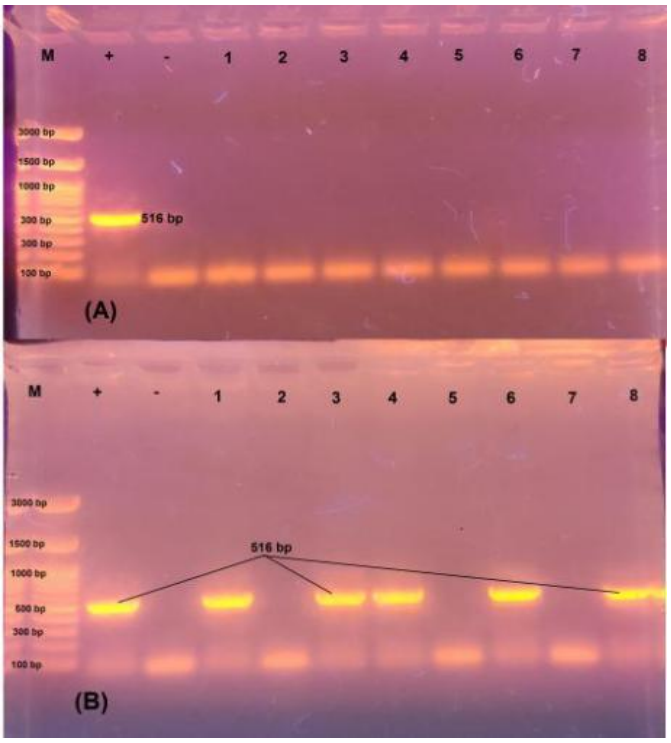

Figure 2. The *qnr A* gene of *Escherichia coli* isolated from differen sources in Egypt. **A**: Calves, **B**: Humans

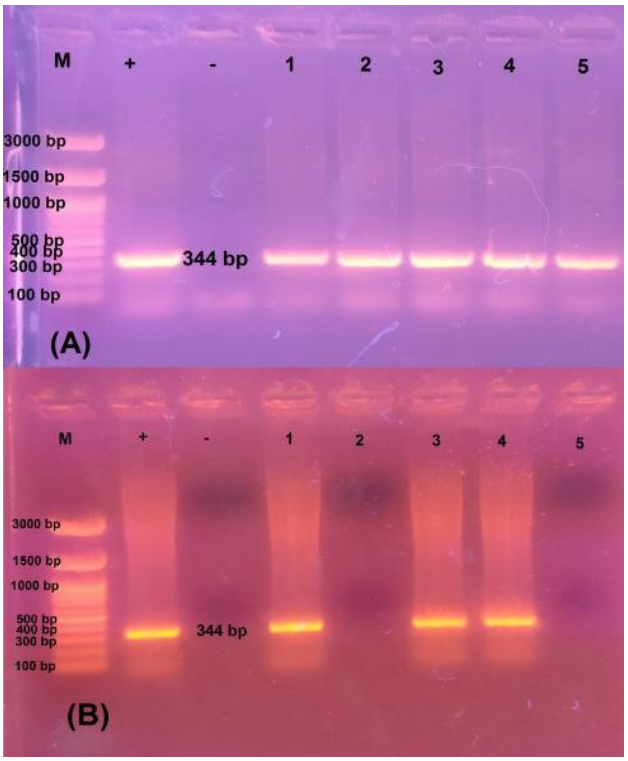

Figure 3. The *gyrA* gene of *Escherichia coli* isolated from different sources in Egypt. **A**: Calves, **B**: Humans

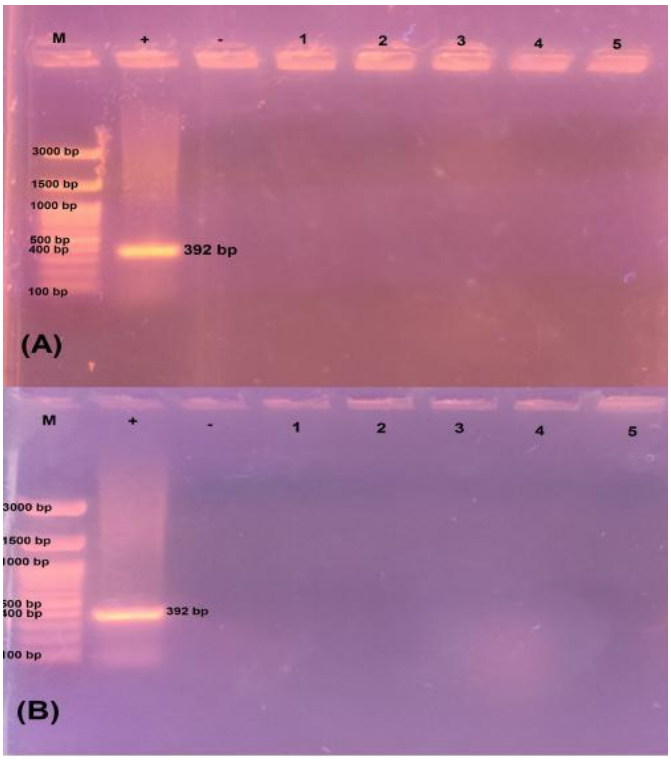

Figure 4. The *blaSHV* gene of *Escherichia coli* isolated from different sources in Egypt. **A**: Calves, **B**: Humans

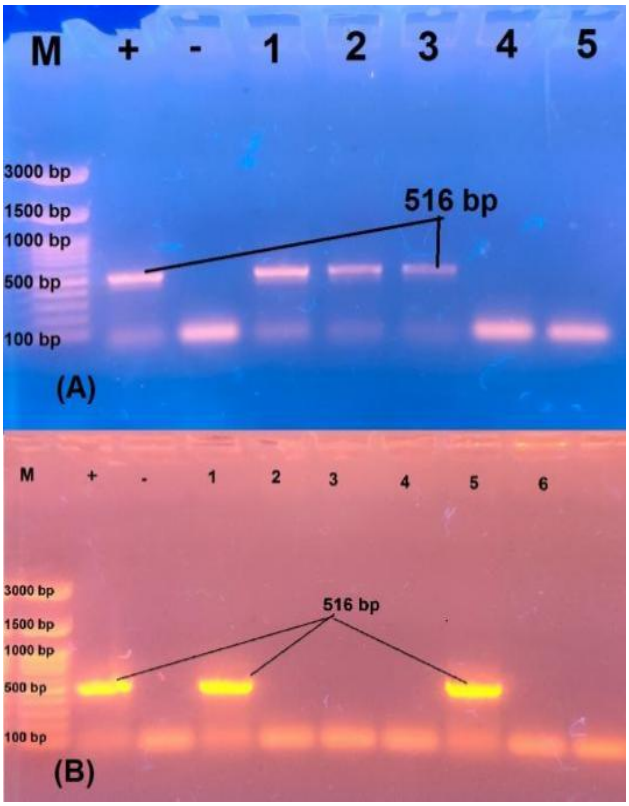

Figure 5. The *blaTEM* gene of *Escherichia coli was* isolated from different sources in Egypt. **A**: Calves, **B**: Humans

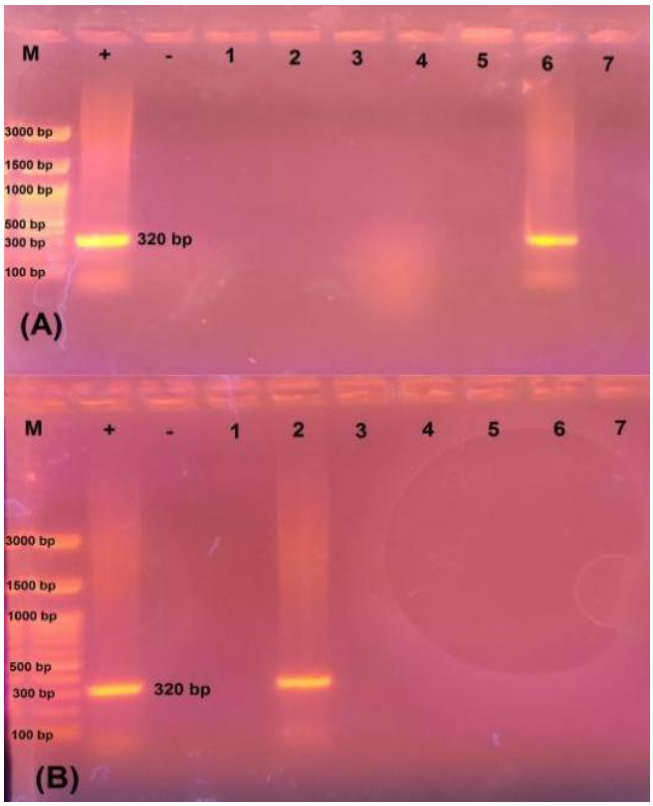

Figure 6. The *mcr-1* gene of *Escherichia coli* isolated from different sources in Egypt. **A**: Calves, **B**: Humans

Table 4. Antibiotic sensitivity test of *Escherichia coli* isolated from calves, New Valley, Egypt

Calves-tested isolates		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**
Antibiotic																								
Quino-Lons group	Ciprofloxacin	-	-	-	-	+++	+++	-	+++	+++	++	-	+++	-	++	-	++	-	-	+++	++	+++	++	++
	Enrofloxacin	-	-	-	+	++	++	-	++	+++	++	-	++	-	++	+	+++	-	-	++	+++	+++	+++	+++
	Norfloxacin	+	+	-	-	+++	+++	-	++	++	+++	+	++	-	+++	-	+++	+	+	++	+++	++	++	++
β-lactam group	Ampicillin	++	+	-	+++	-	+++	-	+	++	+	+++	+++	+	+	++	+++	+	+	+++	++	++	+	+
	Cephalosporin	+++	+	-	++	-	++	-	+++	+++	++	+	+	++	+++	+++	+	+	+++	+	+	+++	+++	++
Colistin		+	++	++	+	+	+++	+	++	+++	++	+	+	++	++	+++	++	+++	++	++	+++	-	++	++

+: Sensitive to antibiotic, -: Resistance to antibiotic

Table 5. Antibiotic sensitivity test of *Escherichia coli* isolated from humans in New Valley, Egypt

Human-tested isolates		**5E**	**6E**	**9E**	**15E**	**51E**	**54E**	**55E**	**65E**	**67E**
Antibiotic										
Quinolone group	Ciprofloxacin	++	-	-	-	++	-	-	-	-
	Enrofloxacin	++	-	-	-	+++	-	+	+	-
	Norfloxacin	+++	+	-	+	++	+	-	-	-
β-lactam group	Ampicillin	-	+++	+	+++	-	+	++	+++	++
	Cephalosporin	-	++	+	++	-	+	++	+++	+++
Colistin		+++	-	++	++	+	+++	++	+	+++

Sensitive to antibiotic, -: Resistance to antibiotic

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DISCUSSION

Escherichia coli strains in cattle can exist asymptomatically, primarily within the digestive tract. This environment facilitates the transfer of resistance traits from non-pathogenic to pathogenic strains. Increasing bacterial antibiotic resistance poses a major challenge for treating diarrheagenic *E. coli* infections, especially in low-income countries. These infections can pose a major hazard due to their widespread environmental distribution.

In the current study, resistance gene profiling of *E. coli* isolated from calves revealed prevalence rates of 21.7% and 34.7% for *qnr* and *gyrA*, respectively. In contrast to the present findings, Tabran et al. (2022) revealed that 4.76% (2/42) of the isolates tested negative for all resistance genes, and *tet(A)* and *tet(B)* were the main genes found in 64.2% (27/42) of the shiga toxin-producing *E. coli* (STEC) strains, while *sul1* and *aadA1* were present in 52.3% (22/42) of the pathogenic strains examined.

For the β-lactam group, no *blaOXA* or *blaSHV* genes were detected in the present study, whereas *blaTEM* was found in 13% of isolates. In contrast to the present results, Tabran et al. (2022) reported β-lactam resistance genes in 2.3% (1/42) of isolates for ^{bla}*SHV* and a higher prevalence of 23.8% (10/42) for *blaTEM*. For the colistin resistance group, the current results reported a prevalence rate of 4.3%. However, Zhang et al. (2019) reported notably higher prevalence rates of *E. coli* isolates in calves (57.14%), developing cows (75%), and milking cows (73.68%), which contrasted with the present findings. Additionally, Bai et al. (2016) collected 93 STEC isolates from fecal, small intestinal, and colonic contents of healthy pigs in Beijing, China. Of these 93 STEC isolates, 10 were resistant to colistin, and the *mcr-1* gene was detected in all 10 isolates.

Antibiotic sensitivity testing confirmed the PCR results for strains encoding the *qnr* and *gyr* genes, which exhibited resistance to ciprofloxacin, enrofloxacin, and norfloxacin. The current findings were inconsistent with those of Tabran et al. (2022), who reported that nalidixic acid had the highest resistance mechanism against pathogenic *E. coli*, followed by ciprofloxacin and tetracycline. In the β-lactam group, this study reported resistance rates of 13% for both ampicillin and cephalosporins, and 4% for colistin. In comparison, Tabran et al. (2022) observed a notable decrease in resistance levels within the cephalosporin class. Similar to Romania, 31% of intestinal samples from cattle exhibited resistance to cefotaxime and ceftazidime; whereas only 10% (1/3) of the isolates from France exhibited a similar resistance pattern.

The current findings on *E. coli* isolates from human samples demonstrated a high resistance to the *qnr* gene (88.8%), with 55.5% for *qnrA* and 33.3% for *qnrS*. Additionally, the *gyrA* gene was present in 33.3% of isolates. These findings were considerably higher than those reported by Tahou et al. (2017) in Côte d'Ivoire, who detected *qnr* genes in 31.2% of *E. coli* isolates, with a prevalence rate of 14.6% for *qnrB*, 9.9% for *qnrA*, and 2.7% for *qnrS*. Furthermore, in Niger, Moumouni et al. (2017) found that 44.4% of *E. coli* isolates carried *qnr* genes, with specific prevalence rates of 64.3% for *qnrS*, 26.2%for *qnrB*, and9.5%for *qnrA*. Further findings from Morocco indicated that 18.7% of *E. coli* isolates harbored *qnrB* (23%), *qnrA* (10%), and *qnrS* (3%; Bouchakour et al., 2010).

In the β-lactam group, the present findings indicated prevalence rates of 0% for *blaOXA*, 0% for ^{bla}*SHV*, and 22.2% for *blaTEM*. The present results contrast with the findings of Salah et al. (2019), who found that all *qnr*-positive *E. coli* and *Klebsiella* spp. strains were linked to the spectrum β-lactamase (ESBL) producers. Salah et al. (2019) identified CTX-M1 in 102 *qnr*-positive strains, *SHV* in 52, and *TEM* in 96. Furthermore, the characterization of *qnr*-positive isolates in Mexico revealed that the *SHV* ESBL-type (*SHV*-12, -5, -2a) was the most prevalent isolate, followed by CTX-M-15 (Silva-Sanchez et al., 2011). Notably, CTX-M-15 was the most predominant isolate among pediatric populations (Silva-Sanchez et al., 2013).

According to the present results, the prevalence of the *mcr-1* gene was 11% in the colistin group. Skov et al. (2016) reported the discovery of over 100 *Enterobacteriaceae* strains carrying *mcr* from human samples worldwide, mainly *E. coli* strains from patients who had recently visited Asian countries. Liu et al. (2016) reported the first detection of *mcr-1*, identifying 16 clinical *E. coli* strains that were *mcr-1*-positive.

Regarding the antibiotic sensitivity results of *E. coli* isolates from human samples, among strains encoding *qnr* and *gyr* genes in the present study, 78% were resistant to ciprofloxacin, 56% to enrofloxacin, and 45% to norfloxacin. In contrast to the present findings, Bouchakou et al. (2010) reported that among *E. coli* isolates carrying the *qnr* and *gyr* genes, resistance rates were 100% to ceftazidime, 71% to cefotaxime, 78% to ciprofloxacin, and 57% to nalidixic acid. In Mexico, the resistance rate for *qnr*-positive pediatric infections was 41.1% for nalidixic acid, 29.4% for ciprofloxacin, 82.3% for ceftazidim, and 100% for cefotaxim (Silva-Sanchez et al., 2013).

Based on the present results, the resistance rate for β-lactam antibiotics was 22% for ampicillin and cephalosporins. The current findings indicated a colistin resistance rate of 11%. However, Hassan et al. (2022) reported higher prevalence rates of *E. coli* isolates resistant to ampicillin (91%), cephalosporins (46-68%), nitrofurantoin (56%), gentamicin (26%), and amikacin (21%).

CONCLUSION

The present findings indicated a higher prevalence of resistance genes in humans than in calves, which was due to the smaller number of human samples included in this study. The presence of resistance genes, including *qnrS*, *gyrA*, *blaTEM*, and *mcr-1*, in humans and calves is of significant concern and requires further investigation. The current results indicated that antimicrobial resistance genes were identified through both PCR and antimicrobial susceptibility testing. Therefore, the high prevalence of resistance genes and phenotypic resistance, especially in human isolates, emphasized the urgent need for antimicrobial stewardship programs. The current findings highlighted the need to impose restrictions on the improper use of medications and antibiotics. Antibiotic use should be guided by antimicrobial susceptibility testing to reduce the spread of resistant strains and the horizontal transfer of resistance genes.

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DECLARATIONS

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Authors' contributions

Mohamed Saeed Diab, Yasser El-Naker, Nermin Awad, and Marwa Salah Eldin Diab contributed to sample collection. Fatma El Zahraa Gamal and Fatma Mohamed Gadallah have contributed to the processing and cultivation of the bacteria, as well as to the development of the PCR assay and the antibiotic sensitivity test. Selim Selim Salamawas was involved in molecular analysis, data analysis, drafting the article, and critically revising its intellectual content. Fatma Mohamed Gadallah participated in the study design and critically revised the manuscript. Selim Selim Salama and Fatma Mohamed Gadallah supervised and approved the final edition before submission. All authors read and approved the final edition of the manuscripts before publication in the present journal.

Availability of data and materials

All datasets used in the current study are available upon reasonable request from the corresponding author.

Competing interests

The authors declared no conflict of interest.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors. The authors confirmed that no AI tools were used in conducting and preparing the present study.

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