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Virulence and Antimicrobial Resistance Genes of *E. Coli* Isolated from Diarrheic Sheep in The North-West Coast of Egypt

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Abstract

The pathogenic Escherichia coli, which causes sheep diarrhea, represents a great concern and great economic losses, especially in desert areas. The aim of this study was to identify some virulence genes and investigate the antibiotic resistance genes isolated from diarrheic sheep collected from north-west coast, Egypt ,A total of 115 (69, 7%) strains of *E. coli* were isolated , 28 strains were virulent . PCR used for identifying virulence genes revealed *iss, sxt1 ,sxt2 , crl, fimH*, *TraT* and *pic* genes, with detection rates 96,4% (27/28), 67,8% (19¥28) , 78,6% (22¥28), 64% (18¥28), 60,7% (17¥28),57% (16¥28) , and ,28.5% (8¥28) in sequence The results of the antibiotic susceptibility tests indicated that the isolates were sensitive to NIT (97%), NOR (92%), and had high resistance to CTX (92%), E (89%), AMX (85%), followed by GM(82%) ,TE (80%) , TM (71%), NA (35,7%), OFX (32%), CIP (28.6%) and DOX (21%) . Detection rates of the resistance genes *blaTEM, floR, sul1, aada1, tetA, mcr1,* and *dfrA*, were 64.3%, 28,6%,25%,78,6%,14,3 %,17,8% and 21,4%, respectively. However, qnrA,and aadB were not detected, and no relationship was observed between drug resistance genes and genotypic profile.

Keywords: *E. coli*, diarrheic sheep, virulence gene, antibiotic resistance, Egypt

1. Introduction

Sheep diarrhea is consider to be from the high pervasive economic concerns in the veterinary industry. As sheep are the economic lifeline for beduen in the desert, it is significantly important to address aspects such as rapid identification, description, and try to curing of the main causal microorganism (Aiello &Moses 2016).

Agents of this disease *E. coli* which produce Diarrhea have antigens for colonization or bacteria can adhesions this make it enable to colonize the small intestine (Chakraborty et al., 2001) , Several researchers have documented that attachment , toxin production, biofilm formation, and iron uptake systems, which include a decrease in the amount of iron available by reducing intestinal iron absorption cosider to be the most virulence ability of E. coli (Croxen et al., 2013).

Studies have also confermed that adherence to epithelial cells is go between adhesions of fimbrial or afimbrial , afa/draBC, fimH, focG and pap cluster encoded the outer membrane proteins (Le Bouguenec,2005, Searle et al., 2015,Welch,2016 and Sarowska etal., 2019), Other researchers have termed these organisms as “enteroaggregative, verotoxin-producing E. coli.” STEC produces a toxin (Shiga toxin) characterized by the presence of stx1 or stx2 genes or their variants. (James et al., 2004,Bako, et al 2017), (Ferreira et al., 2015; Castro et al., 2017; Cundon et al., 2018)., Enteropathogenic fabricate a unique histopathology which called attaching and effacing (A/E) present on intestinal cells , EPEC has been classify into two subtypes, typical (tEPEC) and atypical (aEPEC), depend on the presence or absence of the EPEC plasmid adherence factor and bfpB gene

(Schmidt,2010), aEPEC secretes the pic protein , a factor that is implicated in mucinase activity, serum resistance, and hemagglutination. , Pic may be has multifunctional protein implicated in enteric pathogenesis. (Abreu, et al., 2016),In addition to this, fimbriae primarily genes encoding adhesion are from virulence factors in UPEC which help the colonization and invasion of epithelial cells and thin flexible aggregation protein filaments are called curli encoded by the genes crl and csgA (Pal & Singh 2007).

Adhesion raise the expression of bacterial toxins, iron gain, and elopement from defense mechanisms of the host (Blum, et al., 1994). The most prevalent adhesion genes are iha and fimH (Paniagua et al., 2017), traT gene used to overcome the serum bactericidal (Miajlovic and Smith, 2014). Quaternary ammonium compounds are generally used to control microorganisms in clinical and industrial environments which are cationic surface-active detergents. Besides their use for disinfection of hard surfaces (Ioannou et al., 2017), *qac* genes are mostly accompanied by antimicrobial resistance phenotypes (Zhang et al., 2016).

Regarding the function of acquisition of virulent genes also their influence on pathogenicity, the gain of resistance genes plays a vital function in therapeutic failure and increase in mortality rate. It has been estimated that an failure, to control the monitoring of multidrug resistance, and pan drug resistance also misapply, and overuse of antibiotics for therapeutic purposes in humans will increase the mortality rate of millions of people by the year 2050. As growth promoters in livestock can lead to the emergence of resistance to several antimicrobial classes such as tetracyclines, sulfonamides, cephalosporins, macrolides, polymyxin, & penicillins, many studies have proven the presence of qac genes repeatedly in negative bacteria associated with genes encoding resistance to sulfonamides as well as aminoglycosides, sulfonamides, chloramphenicol and trimethoprim plus beta-lactam (Zhao et al., 2012). Therefore, this research conducted for investigate genes responsible for virulence in addition to the resistance of drug pattern in virulent *E. coli* in sheep and the relationship with molecular typing.

Therefore, this research conducted for investigating genes responsible for virulence in addition to the resistance of drug patterns in virulent *E. coli* in sheep and the relationship with molecular typing. For this purpose, fecal samples were collected from diarrheic sheep on the north-west coast of Egypt and tested for the presence of virulent E*. coli*. Moreover, the fecal samples were subjected to tests for determining virulence genes, genes responsible for drug resistance, and genotyping, which could be useful for preventing and medication of several diseases caused by pathogenic *E. coli*.

2. Materials and methods

2.1. Ethics statement:

All approvals were obtained from Bedouin sheep breeders and follow all international instructions and institutional guidelines for the use and care of animals.

2.2 Isolation and detection of bacteria

165 fecal samples were obtained from sheep with diarrhea from the governorates of Matrouh and Alexandria, Egypt. They were transferred in 1 ml LB medium (Beijing Aoboxing Co., Ltd, Beijing, China) and transferred to the bacterial laboratory of the Desert Research Center**.** After 24h of incubation then inoculated in HiCrome Coliform Agar (HCCA, Sigma) select a single colony. The final determination of the presence of *E. coli* was done by detection of PhoA gene. Specific for *E. coli* by using PCR.

2.3 Detection of pathogenicity of *E. coli*

QC for E. coli screening gene (E. coli ATCC 25922)

The pathogenicity of all isolates was confirmed using mice pathogenicity tests. Each *E. coli* strain was injected intraperitoneally into three mice, Then, they were observed for 24 h, after which the results were recorded and necropsy was performed for dead mice.

2.4 Virulence gene detection:

We scrutinized seven genes encoding virulence factors accompanied by aggregative adherence, dispersion, biofilm formation, and toxin production. Positive and or negative controls were represented by a field sample that was previously confirmed to be positive or negative by PCR for the related genes in the Animal health research institute ,We used primers that have been previously described in the literature (Table 1). The confirmatory identification of *E. coli* was done by PCR amplification of the *phoA* gene using species-specific primers (Hu *et al*., 2011), *iss* (Yaguchi *et al*., 2007), *crl* and *fimH* (Ghanbarpour and Salehi, 2010), *TraT* (Kaipainen *et al.*, 2002), stx1 (Osman *et al.,* 2012) ,stx2 (Van Giau *et al*., 2016) *,* and *pic* (Boisen *et al*., 2009). The dealing with primers was done according to the manufacturer's instructions.

Oligonucleotide primers

Metabion (Germany) were supplied the primers which used and are recorded, in Table (1):

Table 1. Molecular characterization of *E. coli*

Target gene	Primer sequences	Specificity of the PCR for	Amplified segment (bp)	Reference
E. coli phoA	CGATTCTGGAAATGGCAAAAG CGTGATCAGCGGTGACTATGAC	E. coli	720	Hu *et al.,*2011
E. coli iss	ATGTTATTTTCTGCCGCTCTG CTATTGTGAGCAATATACCC	EXPEC	266	Yaguchi*et al*., 2007
E. coli crl	TTTCGATTGTCTGGCTGTATG CTTCAGATTCAGCGTCGTC	UPEC	250	Ghanbarpour and Salehi, 2010
E. coli fimH	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	UPEC	508	(Yun, *et al.,* 2015)
E. coli Stx1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	**STEC**	614	(Osman *et al.,* 2012)
E. coli TraT	GATGGCTGAACCGTGGTTATG	ExPEC	307	Kaipainen*et al.*, 2002

	CACACGGGTCTGGTATTTATGC			
E. coli Stx2	stx2F cca tga caa cgg aca gca gtt stx2R cct gtc aac tga gca ctt tgc	**STEC**	780	Vo Van Giau *et al.*,2016
E. coli pic	ACTGGATCTTAAGGCTCAGGAT	aEPEC	572	Boisen*et al*., 2009
	GACTTAATGTCACTGTTCAGCG			

2.2 susceptibility test of antimicrobial

Due to procedures of the standard operational, the susceptibility tests for antimicrobial were done by using Mueller-Hinton agar (Oxoid, Hampshire, England) using disk diffusion method of Kirby Bauer. They were: nitrofurantoin (NIT ,300 µg), norflaxocin (NOR, 10 µg), Cefotaxime (CTX,5 µ g), erythromycin (E ,15 µ g), amoxicillin (AMX ,10 µ g), gentamicin (GM, 10 µ g), tetracycline (TE ,30 µ g), Tobramycin (TM, 10 µ g), Nalidixic (NA, ,30 µ g), Ofloxacin (OFX , 5 µg), ciprofloxacin (CIP ,5µg), Doxycycline (DOX,30 µg), These antibiotics belong to the classes of quinolone , Cephalosporins , macrolide , β-lactams, aminoglycoside ,tetracycline, and fluoroquinolones (Davies et al., 2010).

2.6. Antibiotic resistance gene detection

10 resistance genes for drug were detected by using PCR, including beta-lactam :blaTEM (Colom *et al*., 2003),: quinolones:qnrA (Robicsek *et al*., 2006), gentamycin:aadB (Frana *et al*., 2001), florphenicol:floR (Doublet *et al*., 2003), sulfonamides:sul1 (Ibekwe*et al*., 2011), streptomycin:aada1 and tetracycline:tetA(A) (Randall *et al*. 2004), trimethoprim:dfrA (Grape *et al*., 2007), colistin:mcr1 (Newton-Foot *et al*., 2017), and quaternary ammonium compounds:qacED1 (Chuanchuen *et al*., 2007).

Oligonucleotide primers

The primers used for detecting antibiotic resistance gene were supplied by Metabion (Germany) and are listed in Table 2.

Table 2. Primer sequences, antibiotic resistance genes

Target gene	Primer sequences	Amplified segment (bp)	Reference
E. coli blaTEM	ATCAGCAATAAACCAGC	516	**Colom et al., 2003**
	CCCCGAAGAACGTTTTC		
E. coli qnrA	ATTTCTCACGCCAGGATTTG	351	**Robicsek et al., 2006**
	GATCGGCAAAGGTTAGGTCA		
E. coli aadB	GAGCGAAATCTGCCGCTCTGG	319	**Franaet al., 2001**
	CTGTTACAACGGACTGGCCGC		
E. coli floR	TTTGGWCCGCTMTCRGAC	494	**Doublet et al., 2003**
	SGAGAARAAGACGAAGAAG		
E. coli Sul1	CGGCGTGGGCTACCTGAACG	433	**Ibekweet al., 2011**
	GCCGATCGCGTGAAGTTCCG		
E. coli aadA1	TATCAGAGGTAGTTGGCGTCAT	484	**Randall et al. 2004**
	GTTCCATAGCGTTAAGGTTTCATT		
E. coli tetA(A)	GGTTCACTCGAACGACGTCA	576	
	CTGTCCGACAAGTTGCATGA		
E. coli dfrA	TGGTAGCTATATCGAAGAATGGAGT	425	**Grape et al., 2007**
	TATGTTAGAGGCGAAGTCTTGGGTA		
E. coli Mcr1	CGGT CAGTCCGTTTGTTC	308	**Newton-Foot et al. 2017**
	CTTGGTCGGTCTGTAGGG		
E. coli qacED1	TAA GCC CTA CACAAA TTG GGA GAT AT	362	**Chuanchuen et al., 2007**
	GCC TCC GCA GCG ACT TCCACG		

1. RESULTS

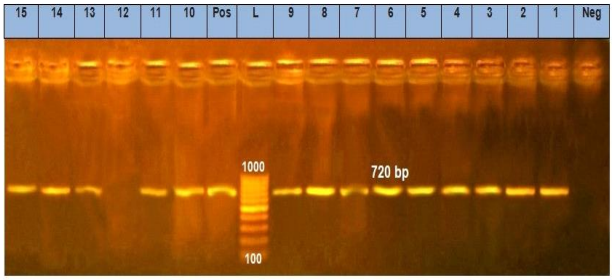
40. isolation and pathogenicity results for *E. coli*

115 (69.7%) isolates of *E. coli* were confirmed by using PCR, the results of the pathological susceptibility test showed no change in the control mice. As for the mice subjected to the experiment, they showed different symptoms, namely, loss of appetite and lethargy, and after injection of E. 28 (24.3%) caused the death of the mice. The post-mortem examination of the dead mice showed pulmonary congestion and the presence of yellow secretions that filled the abdominal cavity, accompanied by severe congestion in all internal organs, necrosis, and enlarged spleen.

1.1.* PCR with species-specific primers for virulence genes of *E. coli

The results demonstrated seven virulence genes, including *iss, crl, fimH, TraT, pic, Stx1, and Stx2,* with the detection rates of 96.4% (27/28), 64% (18/28), 60.7% (17/28), 57% (16/28), 28.5% (8/28), 67.8% (19/28), and 78.6% (22/28), respectively. All samples of *E. coli* carrying the phoA gene yielded positive bands and were amplified at the size of approximately 720 bp. The molecular detection of the virulent genes iss, crl, fimH, TraT, pic, Stx1, and Stx2 in the samples yielded positive bands and were amplified at the size of approximately 266, 250, 508, 307, 572, 510, and 780 bp, respectively, as shown in Figs. 1–7). There were 15 strains carrying the iss, crl, fimH, TraT, Stx1, and Stx2 genes; 12 strains carrying the iss, crl, fimH, TraT, Stx2, and pic genes.

5 strains carrying the iss, crl, and pic genes; and 3 strains carrying the fimH and pic genes. The strains carrying the iss and TraT genes are considered to be ExpEC; those containing the crl and fimH genes were classified as UPEC; the pic-carrying strain was considered to be EPEC; and strains carrying the Stx1 **and Stx2 genes were classified as STEC(shgatoxigenic).**



(Fig 1) *E. coli*, all samples were positive at 720 bp except 12

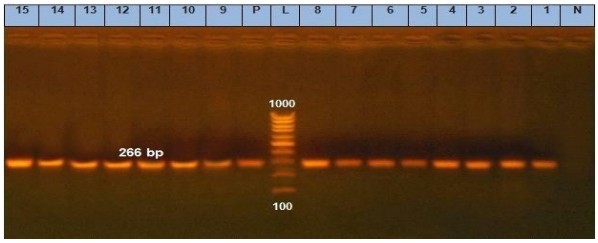


Figure 2. *iss gene*, all samples were positive at 266 bp

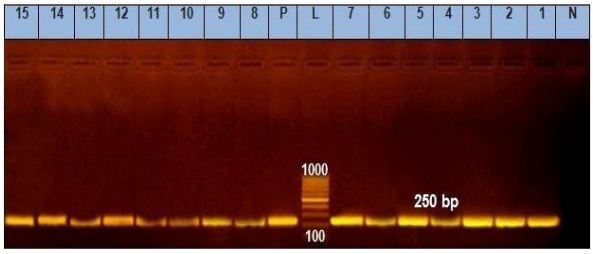


Figure 3. *crl gene*, all samples were positive at 250 bp

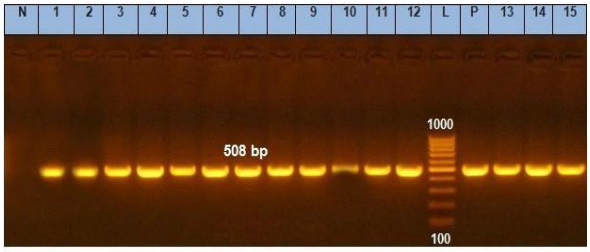


Figure 4. *fimH* gene, all samples were positive at 508 bp

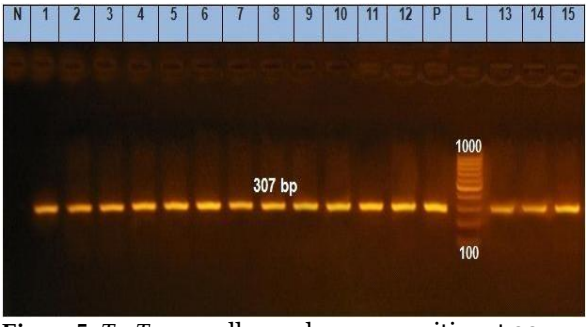


Figure 5. *TraT* gene, all samples were positive at 307

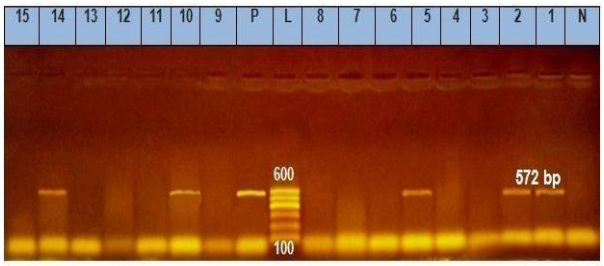


Figure 6. *pic* gene, samples were positive at 572 bp, except lanes3,4,6,7,8,9,11,12,13, and 15 represents the negative samples

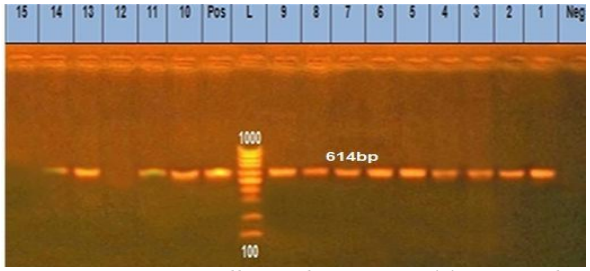


Figure 7. *stx1* gene all samples were positive at 614 bp

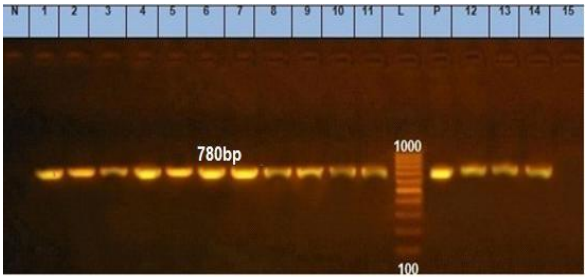


Figure 8. *stx2* gene, all samples were positive at 780 bp except lane15

Antibiotic susceptibility test results: -

The results of the antibiotic susceptibility tests indicated that the isolates were sensitive to NIT (97%), NOR (92%), and had high resistance to CTX (92%), E (89%), AMX (85%), followed by GM(82%) ,TE (80%) , TM (71%), NA (35,7%), OFX (32%), CIP (28.6%) and DOX(21%) ,isolates strains exhibited multidrug resistance, against 12 as shown in table 3.

Type of resistance	Antimicrobial spectrum	Numberof strains
2	**CTX, E**	**6**
3	**E, TE, AMX**	**5**
3	**NA, E, GM**	**5**
4	**E, TE, CTX, OFX**	**3**
4	**E, TE, GM, CTX**	**2**
4	**NA, E, TE, CTX**	**2**
5	**AMX,E,TE,GM, TX**	**1**
5	**AMX, NA, E, TE, CIP**	**1**
6	**NA, E, TE, CIP, CTX, OFX**	**1**
6	**AMX, E, TE, NA, CTX, DOX**	**1**
6	**AMX, NA, E, TE, OFX, CTX**	**1**

Drug resistance gene test results: -

The detection rate of blaTEM was 64.3% (18/28). Among the beta-lactam resistance genes, the disclosure rate of florphenicol resistance genes: floR was 28.6% (8/28). The detection rate of sulfonamides resistance gene: sul1 was 25% (7/28). The detection rate streptomycin aada1 gene was % (3/28), The detection rates of tetracycline resistance genes were 78.6% (22/28). The detection ratesof colistin: mcr1 was 14.3% (4/28). The detection rate of dfrA:trimethoprim was 17.8% (5/28). The detection rate of qacED1: QACs was 21.4% (6/28), and those aminoglycosides resistance gene gentamycin:aadB and quinolones resistance gene :qnrA were not detected.. (Figs. 9-18).

Antibiotic resistance genes for E.col by using PCR

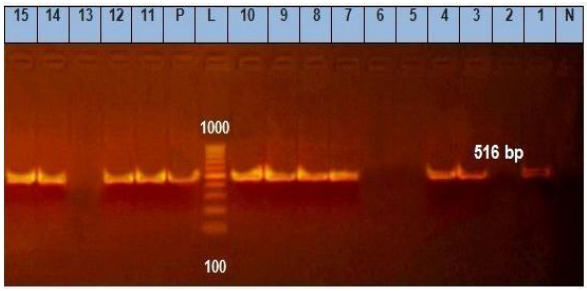


Figure 9. *blaTEM* gene *lanes,* 1,3,4,7,8,9,10,11,12,14 and 15 positive at 516 bp,5,6and 13 represent negative samples

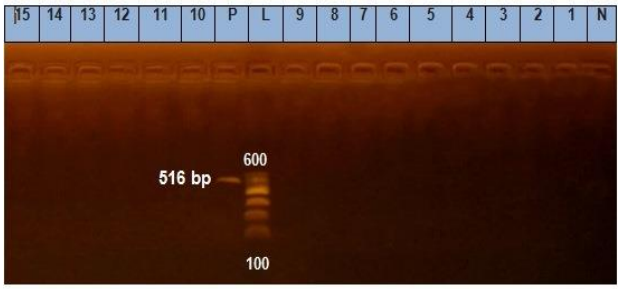


Figure 10. PCR amplification of qnrA: *gene* all sample are negative at 516bp

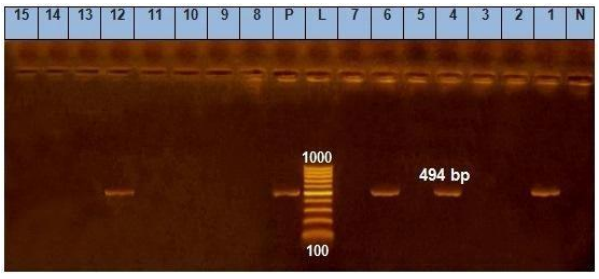


Figure 11. floR: *gene* lanes, 1, 4,6, and 12 positive at 494bp;2,3,5,7,8,9,10,11,13,14 and 15 represent negative

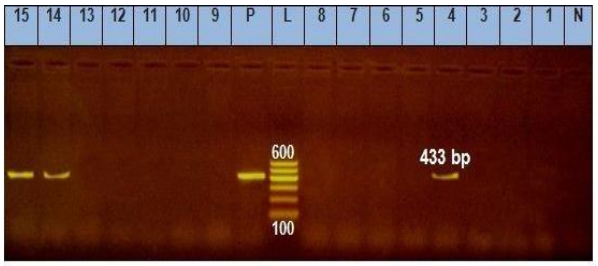


Figure 12. sul1 *gene* DNA, 4, 14 and 15 positive at 433 bp,1,2,3,5,6,7,8,9,10,11,12, and 13 represent negative

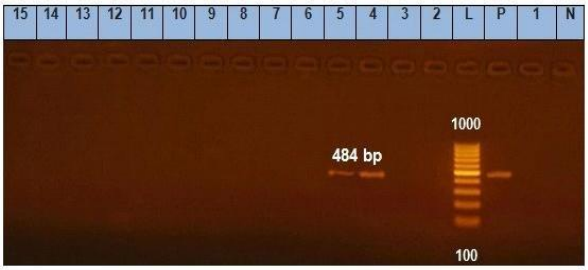


Figure 13. aada1 *gene* lanes, 4and 5 positive at 484 bp,1,2,3, 6,7,8,9,10,11,12,13,14 and 15 represent negative

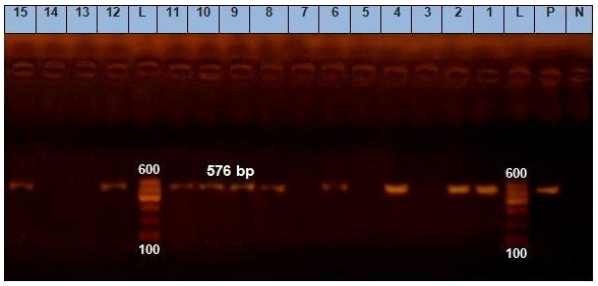


Figure 14. lanes, 4 and 5 positive at 576 bp; 1,2,4, 6, 8,9,10,11,12, and 15,5,7, and 13 represent negative

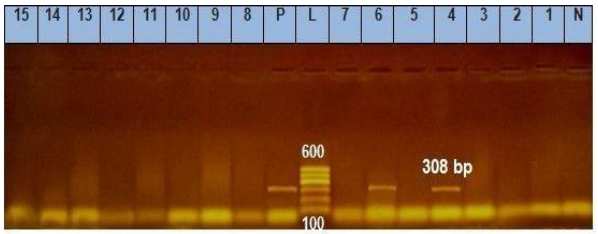


Figure 15. mcr1 *gene* lanes, 4 and 6 positive at 308 bp, 1,2,3,5,7,8,9,10,11,12,13,14 and 15 represent negative

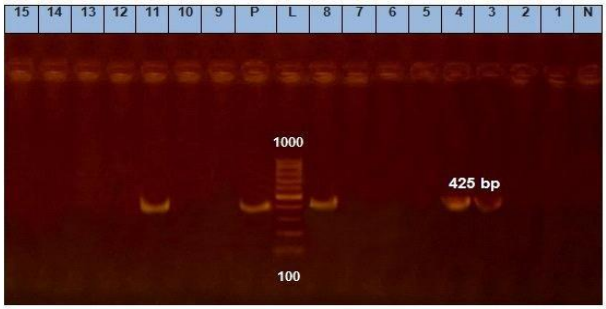


Figure 16. dfrA *gene* lanes, 3,4 and 11 positive at 425 bp, 1,2, 5, 6,7,8,9,10, 12,13,14 and 15 represent negative

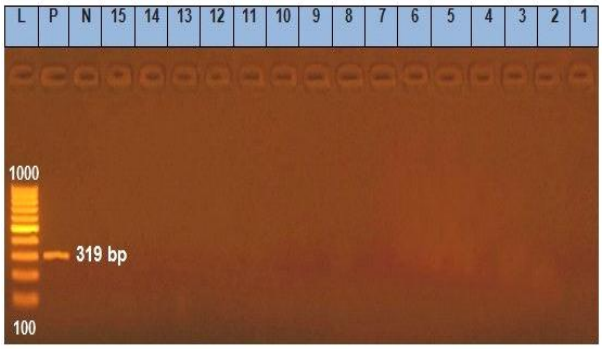


Figure 17. aadB: *gene* all samples represent negative at 319 bp

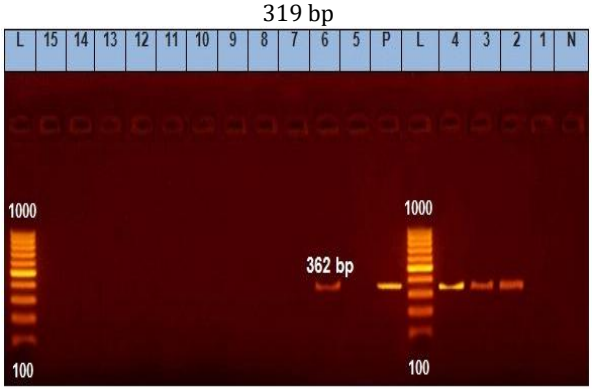


Figure 18. *qacED1* gene DNA from different samples; shown above the lanes, 2,3,4, and 6 positive at 362 bp; 1,5,7,8,9,19,11,12,13,14, and15 represent negative samples

Discussion

Sheep are the mainstay of the economy, especially in the desert. There has been an increase in the prevalence and severity of diarrhea in sheep in the north-west coast of Egypt, *E. coli* is the most renowned bacterial agent that causes severe casualties in sheep farms.

E. coli has disclosed a rate of (69.7%), identified in the fecal samples of diarrheic sheep. This finding is consistent with those of prior studies in Confirms that E coli is one of the most important microbes that cause diarrhea in sheep & lamb.

in this study we dealt with isolating 115 strains of pathogenic coli out of 165 samples (69.7%), of which 28 strains are pathogenic, at a rate of 24.3%, and this is approximately to what was stated. Aiello and Moses (2016) they isolated it from Iranian lambs by 71.66%, and Andrade *et al.* (2012) Isolated it by 59.26% of calves in Brazil, and this confirms that the percentage of E. coli isolation varies in different regions.

E. coli isolates were positive for at least one of the virulence genes. Similar results have been reported by Rigobelo et al. (2006) and Andrade et al. (2012). We got the highest percentage of the *iss* gene (96.4%), Next in order sxt1, sxt2, crl, fimH, TraT, and pic genes (67.8%, 78.6%, 64%, 60.7%, 50%, and 28.5%, respectively).

Regarding the high prevalence of the iss gene (266 bp fragment), only Yang, H. et al. (2011) found a similar result (97%), whereas other authors obtained a lower percentage, e.g., 82.7% reported by Ewers et al. (2004), 80.5% by Dissanayake et al. (2014), and 64.29% by Al-Arfaj et al. (2016).

We found that the stx2 gene had a higher presence than the stx1 gene,This result is in contrast to a report that demonstrated that STEC strains isolated from small ruminants harbor the stx1 gene more frequently (Mahanti et al., 2015) this corresponds to what has been reported by Bandyopadhyay et al. (2011), who also found a predominance of the stx2 gene in STEC strains isolated from small ruminants with diarrhea in India.

Regarding the TraT gene that showed a prevalence of 59%, a previous study (Montenegro et al., 1985) also isolated this gene at the rate of 23%–67%. The TraT gene was also Isolated patients with urinary tract infection at a rate of 47.1% (Derakhshandeh et al. 2015), where the authors mentioned that the prevalence of traT is related to the expression of the K1 capsule (Nojoomi && Ghasemian 2019).

from the most essential, achievements of the twentieth-century were Antibiotics, which is very important for killing or inhibit microorganisms, but now a day from the most serious problems is Antibiotic resistance in e.coli which isolated from fecal samples is which increases day by day, making it a major animals health problem So it is very important to detect the antibiotic resistance patterns in E. coli isolates for the achievement of accurate prescriptions. (Goncuoglu *et al.* 2010).

In the present study 28 pathogenic strains *E. coli* isolated from sheep exhibited various resistance levels to most of the antibiotics. many strains were show resistance to six or two antibiotics, and few strains resistance to three, and four antibiotics. The results of the antibiotic susceptibility tests indicated that the isolates were sensitive to NIT (97%), NOR (92%), and had high resistance to CTX (92%), E (89%), AMX (85%), followed by GM(82%) ,TE (80%) , TM (71%), NA (35,7%), OFX (32%), CIP (28.6%) and DOX (21%) Elsayed et al. (2018) reported the sensitivity rates to chloramphenicol (73.4%) and doxycycline (73.4%).

The mechanism of action of group Quinolones inhibition of DNA replication, beta-lactam *antibiotics and* Cephalosporins (CTX) inhibiting and disrupt the synthesis of the peptidoglycan layer of bacterial cell walls, macrolide, aminoglycoside and tetracycline inhibition of bacterial protein biosynthesis.

It is evident that most *Escherichia coli* isolates are highly resistant to many antibiotics, This may be due to the prolonged administration of many different types of antibiotics to animals without testing for sensitivity to antibiotics, or due to the fact that taking the antibiotic leads to selective pressure ultimately raise the generation of resistant bacteria (Olorunmola et al., 2013)

And it was found that the resistance rate of coli to ampicillin was 71.4% and 63.4%, respectively, and that to sulfaprim was 50%, and the strain was highly sensitive to norfloxacin and trimethoprim-sulfamethoxazol ,(35.4%). this is what Hakim *et al.* (2017) and Huang et al. (2016) said, All these results were a strong indication that the pathogen *E. coli* differs in its results with regard to its resistance to antibiotics, and based on this, susceptibility testing for *E. coli* -related diseases must be performed first before the antibiotic is prescribed.

Sheep and goats do not pose a threat to public health in that they are only a reservoir for STEC, but that over time these bacteria have become resistant to many antibiotics (Bay et al., 2016). Therefore, specialists had to track antimicrobial resistance to STEC due to the possibility of horizontal transmission of resistance genes, Resistance from the notorious STEC to other pathogens is appearing to offer a new approach to treatment that will help develop effective control strategies to stop the spread of resistance (Islam et al., 2008, Elsayed et al., 2018).

The tested strains carry many resistance genes, and this

reinforces the concept, that these genes play an important part in encoding of the tested strains resistance, I found that ,tet (A) were mainly encoded resistance genes for tetracycline which give (78.6%),

Which is compatible with the results of Liao et al. (2019), the active efflux is still the primary mechanism underlying *E. coli* resistance to tetracyclines. The proportion of quinolone resistance gene qnrA was 35.7%, which was primarily related to the Drug resistance may be the result of DNA and topoisomerase IV mutation of amino acids in cells and mutations in gyrA and parC,It was found that sulI, which was isolated at a rate of 25%, which is used to encode the genes of resistance to sulfonamide, and this result was compatible with Mohsin et al. (2017).

And it has been discovered that the main cause of resistance to a new generation of beta-lactamase antibiotics is Long-acting plasmid-mediated beta-lactamases (ESBLs). The results show that the blaTEM gene had the highest rate of 64.3%, which is similar to the results of Ali et al. (2018).

The detection rates of the florphenicol resistance gene: floR, colistin:mcr1, trimethoprim:dfrA, and qacED1:quaternary ammonium compound genes were 28.6%, 14.3%, 17.8% and 21.4%, respectively. The aminoglycoside resistance gene aadB was not detected.

The detection rate of the florphenicols resistance gene: floR is 28,6%, colistin mcr114,3 %, trimethoprim dfrA 17,8% qacED1: quaternary ammonium compounds21,4%, In this study, aminoglycoside resistance gene is aadB was not detected

We found that the number of phenotypic resistance genes is less than the number of antibiotic resistance gene discoveries, and the reason may be related to the scale of expression of drug resistance genes as well as the quantity of enzyme which produced by bacteria.

Conclusions

This study confirms that sheep require special concern as they act as an important reservoir for *E. coli*. Most of the isolated strains carried several virulent genes that were confirmed to be multidrug-resistant to most of the antimicrobial agents that are critical for animal health thus, molecular detection and a pugnacious watching of the resistance of antimicrobial may be helpful for meliorative effective control strategies against E. coli and for the production of new antimicrobials responsible for lowering the ability for antimicrobial resistance.

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