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Molecular, Antibiogram Characterization and Assessment Biocidal Potency of Some Essential Oils in Combating the Virulent Pathogenic *Escherichia coli* from Different Sources

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Abstract

Escherichia coli (*E. coli*) is a food-borne bacterium responsible for several clinical infections in animals and humans. This study aimed to isolate *E. coli* from feces and milk of animals in a dairy farm along with urine samples gathered from workers managing these animals. Serological, molecular characterization and evaluation of the antimicrobial efficacy of routinely used antibiotics were assessed. Finally, finding a way to overcome high resistance of *E. coli* by using thyme, peppermint, capsaicin, orange, green tea, tea tree and onion oils. Samples (n=610) were collected from feces, milk and urine. *E. coli* was significantly found in 44.87, 22.2, and 28.4%, respectively at X2=348.467, P<0.001. Serologically, O86 was the prominent serotype and O55 was found in milk and feces. Also, O44, O128 and O111 serotypes were denoted in milk. While O91 and O78 were noticed in urine only. Genetically, quorum-related gene (*LuxS*) was amplified in all isolates. Pathotype (*ChuA*, *YjaA*, *TspE4C2*) and virulent (*Iss*, *IutA*, *Tsh*) gene markers were observed in nearly all traits. Furthermore, a considerable multidrug resistance at P<0.001 was found in human and animal strains. It was obvious that tea tree oil was significantly showed antimicrobial efficiency whereas, thyme oil was ineffective in inhibiting development of *E. coli*. It was concluded that the existence of virulent and quorum determinants in *E. coli* in animals and humans is a noteworthy prospect for the public health concern of cow's milk and feces. Furthermore, the inhibitory and biocidal elements of essential oils are a realistic technique for eliminating resistant *E. coli*.

KEYWORDS
E. coli, Prevalence, Pathotypes, Virulence, Quorum, Resistance, Tea tree, Essential oils

INTRODUCTION

Escherichia coli (*E. coli*) is a Gram negative non-sporulating flagellated, facultative anaerobic bacilli in the family *Enterobacteriaceae*. This species is extensively dispersed and inhabits the large intestine as a part of the common microflora of both warm-blooded animals, humans and birds (Rojas-Lopez *et al*., 2018). However, few pathogenic strains of *E. coli* can cause intestinal and extraintestinal disorders in healthy and immune-compromised persons (Kaper *et al*., 2004).

In calves, *E. coli* causes septicemia and diarrhea, it is the leading cause of death and associated with severe morbidity and resulting in huge economic losses that affect the livestock industry and productivity worldwide (Yadegari *et al*., 2019). In Egypt, calf scouring is a major reason for neonatal mortality, accounting for around 27.4-55.0% of the total deaths in young animals (El-Seedy *et al*., 2016). Furthermore, *E. coli* is also one of the most common occasions of mastitis in cattle and the bacterium can readily be spread to customers through consumption of contaminated milk and dairy products (Keba *et al*., 2020).

E. coli infection is one of the emerging food-borne zoonotic bacteria in man, causing multiple clinical symptoms such as watery or bloody diarrhea. In the developing world, pathogenic *E. coli* is the most prevalent reason for acute watery diarrhea in children and adults, causing approximately 400 million diarrheal episodes and 380,000 fatalities in children under five years old annually (Qadri *et al*. 2005). Besides, potentially fatal syndromes such as thrombotic thrombocytopenic purpura, hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and acute renal failure (Costanzo *et al*., 2020) are prevalent. Also, *E. coli* is usually related to the community and hospital-acquired clinically significant blood stream infections (Loque *et al*., 2012).

Based on the virulence and pathogenicity features, *E. coli* are divided into two major categories and six pathotypes for instance intestinal forms including, attaching, and effacing *E. coli* (AEEC), Enterotoxigenic (ETEC), enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC) or shiga toxin producing *E. coli* (STEC), diffusely adherent *E. coli* (DAEC), enteroinvasive *E. coli* (EIEC) (Donnenberg, 2013; Ori *et al*., 2019). Whereas the extra-intestinal types are uropathogenic *E. coli* (UPEC), meningitis-associated *E. coli* (MNEC), avian pathogenic *E. coli* (APEC) and necrotoxigenic *E. coli* (NTEC) (Nojoomi and Ghasemian, 2019).

Toxins, adhesins, plasmids, iron acquisition factors, polysaccharide capsules and mobile genetic elements are among the

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virulence factors (VF) linked to the pathogenicity of distinct *E. coli* subtypes, allowing bacteria colonization. Non-pathogenic strains of *E. coli* could gain additional virulence Issues from accessory DNA, found on plasmid and/or the chromosome (El-Shaer *et al*., 2018; Sobhy *et al*., 2020). In addition, quorum sensing (QS) is a chemical signaling system that Gram-negative and Gram-positive bacteria use to regulate motility, biofilm creation, enzyme synthesis and expression of virulence traits (Abisado *et al*., 2018).

Globally, antimicrobial resistance (AMR) is a severe public health Issue in both animals and humans since the resistant bacteria can be transferred to man through the food chain and environmental contamination of food and water (Bryce *et al*., 2016). Animals with antibiotic-resistant *E. coli* could serve as crucial reservoirs for colonization and infection in humans (Banjari *et al*., 2020). AMR has emerged as one of the most serious public health concern of the twenty-first century responsible for killing approximately 10 million persons every year by 2050 (O'Neill, 2016).

Many techniques have recently been implemented to combat multidrug-resistant bacterial infections (Edwards-Jones, 2013). The use of essential or volatile oils (EOs) containing high concentrations of bioactive chemicals is one of the results concentrating on identifying safe, eco-friendly, innovative, and effective antibacterial agents (Gadisa *et al*., 2019; Trifan *et al*., 2020). These compounds have substantial medicinal and pharmacological potential as well as antibacterial capabilities, which have already been demonstrated for Gram-positive and Gram-negative bacteria found in various animal species as well as in humans (Zago *et al*., 2009).

The goal of this work was to characterize *E. coli* isolated from animal and human sources and compare the pathogenic recovered *E. coli* strains based on phenotypic and genotypic-virulence markers using molecular assays. Moreover, analyze the antibiotic sensitivity profile and evaluate the biocidal effect of some essential oils in combating the MDR-virulent-quorum potent traits of *E. coli*.

MATERIALS AND METHODS

Study location and design

This study was performed in a private dairy farm in Beni-Suef locality (coordinates 29° 04′ N-31° 05′E), Egypt in the period between January and December 2021. The farm contained 250 lactating dairy cows grouped according to their milk production into 10 groups (n=25) and kept in a partially covered yards with an earthen floor. Cows were milked twice a day in an abreast parlor provided with seven milking units and sanitary measures were generally fair to moderate on the farm. Samples gathered during the study were authorized by International Animal Care and Use Committee (IACUC) and Institutional Review Board (IRB) of Beni-Suef University. All collected samples were cultured for isolation of *E. coli*. Serological and molecular assays were used for strain characterization then verified for their sensitivity against 13 antibiotics by the disc diffusion method and the isolates showed multi-drug resistance to more than two categories of antibiotic were subjected to evaluate the antimicrobial effect of various EOs using well agar diffusion approaches.

Sample collection

Fecal (n=410) and milk (n=90) samples were collected aseptically according to the ethical standard approval of Institutional Animal Care and Use Committee (IACUC), Ref. No: IORG 238-022), Beni-Suef University. All samples were obtained under extensive aseptic restrictions. The animal samples were taken after getting an oral permIssion from the farm manager. The samples were stored on ice and sent to the lab for further bacteriological examination. Moreover, ten urine samples were taken from the workers in the farm under investigation as well as 100 urine samples were gathered from outpatients' individuals attending Beni-Suef University Hospital. Urine samples were collected from these individuals after taking an oral consent from them and in accordance with Institutional Review Board (IRB), Ref. No: IORG 238-022), Beni-Suef University.

Isolation and identification of E. coli

All the collected samples (n=610) were pre–enriched on tryptic soy broth (Oxoid, Basingstoke, UK) for 18–24 h at 37°C then a loopful from each tube was cultivated on the surface of Eosin Methylene blue agar (EMB, Oxoid, Basingstoke, UK) and incubated aerobically for 24 h at 37°C. Typical colonies showed the characteristic blue-black color with a green metallic sheen (Ojo *et al*., 2010). The suspected isolates samples were picked, stored on Tryptic Soya Agar (TSA) slopes and kept at 4°C for further assessments.

Biochemical identification of the isolated E. coli

All colonies showed the characteristic features of *E. coli* on EMB surface were selected and identified biochemically in accordance with schemes described by Quinn *et al*. (2011).

Serological examination of the isolated E. coli

The biochemical suspected isolated were and serologically identified using eight polyvalent antisera and 43 monovalent antisera (Ewing and Edwards, 1972) in the Animal Health Research Institute, Egypt. Briefly, a homogenous suspension of each isolate was prepared using a drop of physiological saline on a slide. A drop of the specific *E. coli* polyvalent antisera was added to the suspension and thoroughly mixed. Positive agglutination could be easily seen with the naked eye within one minute. A delayed or partial agglutination was considered negative. The same way was carried out as described above using the subsequent monovalent sera for the positive polyvalent one.

Molecular identification of virulence and pathotype gene of E. coli

Amplification of *E. coli* specific virulence, pathotype and quorum genes was performed in the Biotechnology Unit at the Animal Health Research Institute, Egypt, data on the gene sequences were illustrated in Table 1. Molecular characterization was individually operated for each one primer pair using 40 pmol of each primer, 1 U of Platinum Taq DNA polymerase, $MgCl_2$ (Invitrogen), dNTPs and 5 µL of target DNA in a total volume of 25 µL for each reaction.

The thermocycling parameters were summarized as follows; an initial denaturation cycle at 94°C for 5 minutes then 30 cycles of the subsequent program: 94°C for 30 sec, the annealing temperature was 55°C for 45 sec for each primer. The final extension stage was 72°C for 7 min. The PCR reaction runs performed in an Applied biosystem 2720 thermal cycler. The secondary PCR products were separated by electrophoresis in 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm for 30 min and finally the gel photographed visualized through UV transilluminator (Alpha Innotech, Biometra) and analyzed through DigiDoc-It Imaging System

Table 1. Gene sequences of the targeted virulence, pathotype and quorum-related determinants, amplified products specific for *E. coli* in the current study.

Target Genes	Sequences	Amplified products	References
ChuA	GAC GAA CCA ACG GTC AGG AT TGC CGC CAG TAC CAA AGA CA	279 bp	Jeong *et al*. (2012)
YjaA	TGA AGT GTC AGG AGA YGC TG ATG RAG AAT GCG TTC CTC AAC	211 bp	
TspE4C2	GAG TAA TGT CGG GGC ATT CA CGC GYC AAC AAA GTA TTR CG	162 bp	
Iss	ATGTTATTTTCTGCCGCTCTG CTATTGTGAGCAATATACCC	266 bp	Yaguchi *et al*. (2007)
IutA	GGCTGGACATGGGAACTGG CGTCGGGAACGGGTAGAATCG	300 bp	
Tsh	GGT GGT GCA CTG GAG TGG AGT CCA GCG TGA TAG TGG	620 bp	Delicato *et al*. (2003)
LuxS	ATGCCGTTGTTAGATAGCTTCA GATGTGCAGTTCCTGCAACTTC	513 bp	Wang *et al*. (2016)
Stx1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	614 bp	Dipineto *et al*. (2006)
Stx2	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	779 bp	

software.

Antimicrobial susceptibility testing of the isolated E. coli

Randomly selected identified *E. coli* isolates (n=50) were selected for susceptibility to 13 frequently used antibiotics in both veterinary and human practice, which included: Amoxicillin clavulanic acid (25 µg), Doxycycline (30), Gentamicin (10 µg), Ciprofloxacin (5), Lincomycin (300 µg), Fosfomycin (10 µg), Colistin (25 µg), Clotrimazole (100 µg), Spectinomycin (10 µg), Norfloxacin (15 µg), Erythromycin (30 µg), Amaksin (5 µg) and Enrofloxacin (5 µg) using disc diffusion procedure. The bacterial sensitivity was performed on Mueller Hinton agar (MHA, Oxoid, Basingstoke, UK), the agar plates were inoculated aseptically with bacterial suspension at final concentration of 1x108 CFU/mL equivalent to McFarland 0.5 that was assessed by BD PhoenixSpec, Nephelometer Becton Dickinson and Company, Sparks, Maryland, USA). The zone of inhibition was screened and interpreted (CLSI, 2018).

Evaluation of biocidal activity of essential oils on the isolated E. coli

The biocidal activity of eight pure essential oils, purchased from Sigma Aldrich, including Thyme (*Thymus vulgaris*), Clove (Syzygium aromaticum), Peppermint (Mentha x piperita), Capsaicin (*Capsicum annum*), Orange (*Citrus sinensis* fruit), Green tea (*Camellia sinensis*), tea tree (*Melaleuca alternifolia*) and Onion oil (*Allium cepa*), were tested on 17 MDR randomly selected *E. coli* isolates and was analyzed using agar well diffusion technique. Briefly, over-night grown isolates on Tryptone soya agar (TSA) then the cultivated colonies were suspended in physiological saline (NaCl, 0.9%) at McFarland reaction of 1.5×10^8 CFU/mL. TSA was prepared and autoclaved at 121ºC for 15 min and allowed for cooling to 55ºC. The tested oils were mixed with polyethylene glycol and tween 80 at an equal volume, then mixed with TSA according to the tested concentration. The oil-agar medium was poured into sterile petri dishes and allowed to be solidified. The bacterial suspensions were inoculated at 6 mm well diameter on TSA surface. The plates were then incubated at 37˚C for 24-48h. After incubation, the diameter of the growth inhibition zones was measured and evaluated (Jeff-Agboola *et al*., 2012).

Statistical analysis

The collected data were reported using the Microsoft Excel spreadsheet and prepared for statistical analysis. The prevalence of *E. coli* isolated from different examined samples and biocidal activity of tested essential oils against *E. coli* isolates were calculated using nonparametric tests (Chi-Square Test) using statistical package for social sciences (SPSS, Inc., version 22.0, Chicago, IL, USA).

RESULTS

The obtained results shown in Table 2, revealed the total number of positive samples for *E. coli* were 235/610 (20.4%), recovered from different sources. The pathogen was mainly prominent in feces (44.87%) followed by urine (28.4%) and milk (22.2%) ($X2= 348.467$ at $P<0.001$).

As illustrated in Table 3, *E. coli* serogroups were analyzed in a total of 16 samples including milk, feces and urine. *E. coli* was recovered with different percentages and biotypes. Serotype O86:K6 (EPEC, EAEC) isolated from all the examined samples, along with the serotype O55:K59 (EPEC, EHEC) reported in feces, urine. Furthermore, O126:H71 and O119:K69 (EPEC, EHEC) reported in calf feces only even as O78:K80 (EAEC, ETEC), O91(EPEC, EHEC) recorded in urine only. also, serotypes O44:K74 (EPEC, EAEC, EHEC), O128:K67 (EPEC, ETEC, EHEC) and O111:K58 (EPEC, EHEC, EAEC) recovered just from milk. Serotype O157:H7 failed detection in all studied samples.

Also, regarding the virulence, pathotype and quorum-related genes were analyzed using the conventional PCR in the serologically identified strains (Table 3). In milk, fecal and urine samples, virulence markers (*Iss* Fig. 1a, *IutA* Fig. 1b and *Tsh* Fig. 1c) found in O86:K6, O55:K59, O44:K74, O128:K67, O111:K58, and O91 whereas only *Iss* and *IutA* genes harbored in O126:H71, O119:K69 and O78:K80 from feces and urine. Concerning the pathotypes re-

lated genes, *ChuA* (Fig.2a), *YjaA* (Fig. 2b) and *TspE4C2* (Fig. 2c), identified in O86:K6, O55:K59, O126:H71, O91, O119:K69 and O78:K80 in samples of different sources but *ChuA* and *TspE4C2* genes found in O44:K74 and O111:K58 from milk. Moreover, only milk harbored O128:K67 yielded *YjaA* and Tsp4c2 genes. It was surprising that quorum sensing (QS) marker amplified *LuxS* gene in all serotypes (Fig. 3a). Unfortunately, both *Stx2* and *Stx2*-related genes were not identified in any serogroups (Fig. 3b).

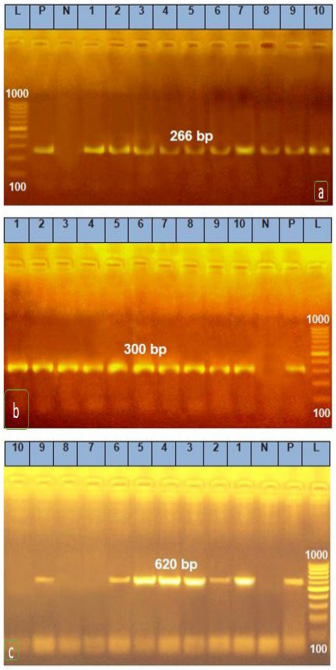

Fig. 1. Agarose gel electrophoresis for PCR products of virulent *E. coli* by *Iss* gene (a), amplified 266 bp, *IutA* amplified 300bp (b) and *Tsh* amplified 620 bp (c). Lane (L): 100 bp Ladder ''Marker'', Lane): 1-10), the examined samples, Lane Pos: Positive control, Lane Neg: Negative control.

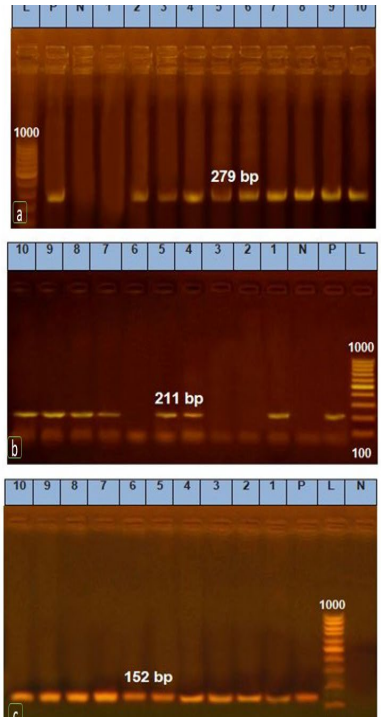

Fig. 2. Agarose gel electrophoresis for PCR products of pathotypic *E. coli* using *ChuA* gene (a), amplified 279bp, *YjaA* amplified 211bp (b) and *TspE4C2* amplified 162 bp (c). Lane (L): 100 bp Ladder ''Marker'', Lane): 1-10), the examined samples, Lane Pos: Positive control, Lane Neg: Negative control.

The obtained results of antibiotic susceptibility pattern (Table 4) revealed that the collected *E. coli* bacteria were remarkably resistant to two or more of the tested antibiotics, a phenomenon known as MDR. Similarly, Doxycycline and Lincomycin were entirely resistant in both human and animal samples (100.0%) at P<0.001. Complete resistance to Gentamicin, Amoxicillin Clavulanic Acid, Colistin, and Clotrimazole (100.0%) was also observed in isolates from feces and urine. Amikacin and Norfloxacin showed complete resistance in milk and urine (100.0%) at P<0.001, respectively. Additionally, all samples showed a higher

Table 2. Distribution of *E. coli* isolated from animal and human samples in the current study.

Species	Sample type	No. examined	No. positive	%
Cow	Feces	410	184	44.8
	Milk	90	20	22.2
Humans	Urine	110	31	28.4
Total		610	235	20.4

X2= 348.467, P value <0.001

Table 3. Distribution of the virulent, pathotypic and quorum sensing pattern of *E. coli* serotypes in the examined animal and human samples.

Serotypes	Isolates tested (*n*=16)	Percentage (%)	Samples origin	Virulence genes detected	Biotype/pathotype
O86:K61	4	25	Feces, Urine, Milk	*ChuA, YjaA TspE4C2, Iss, IutA, Tsh, LuxS*	EPEC, EAEC
O55:K59	2	12.5	Feces, Urine	*ChuA, YjaA, TspE4C2, Iss, IutA, Tsh, LuxS*	EPEC, EHEC
O44:K74	2	12.5	Milk	*ChuA, TspE4C2, Iss, IutA, Tsh, LuxS*	EPEC, EAEC, EHEC
O126:H71	2	12.5	Feces	*ChuA, YjaA, TspE4C2, Iss, IutA, LuxS*	EPEC, EHEC
O128:K67	1	6.25	Milk	*YjaA, Tsp4c2, Iss, IutA, Tsh, LuxS*	EPEC, ETEC, EHEC
O91:K-	1	6.25	Urine	*ChuA, YjaA, TspE4C2, Iss, IutA, Tsh, LuxS*	EPEC, EHEC
O119:K69	2	12.5	Feces	*ChuA, YjaA, TspE4C2, Iss, IutA, LuxS*	EPEC, EHEC
O111:K58	1	6.25	Milk	*ChuA, TspE4C2, Iss, IutA, Tsh, LuxS*	EPEC, EHEC, EAEC
O78:K80	1	6.25	Urine	*ChuA, YjaA, TspE4C2, Iss, IutA,*	EAEC, ETEC
O157:H7	0	0	-	-	-

Table 4. Antimicrobial susceptibility profile of the isolated *E. coli* from different sources.

Sample	No. examined	Antibiotic used (Conc./ ug)																											
		Doxycycline (30)		Gentamicin (10)		Amoxicillin Clavulanic acid (30)		Ciprofloxacin (5)		Lincomycin (300)		Fosfomycin (10)		Colistin (25)		Clotrimazole (100)		Spectinomycin (10)		Norfloxacin (15)		Erythromycin (30)		Amikacin (5)		Enrofloxacin (5)			
		R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S		
Feces	50	50	-	50	-	50	-	10	40	50	-	42	8	50	-	50	-	42	8	40	10	39	11	41	9	32	18		
		100	0	100	0	100	0	20	80	100	0	84	6	100	0	100	0	84	6	80	20	78	12	82	8	64	16		
Milk	15	15	-	12	3	11	4	5	10	15	-	9	6	14	1	13	2	10	5	8	7	14	1	15	-	13	2		
		100	0	80	20	73	27	33.3	66.7	100	0	60	40	93.3	6.7	86.6	13.4	66.7	33.3	53.3	46.7	93.3	6.7	100	0	86.7	13.3		
Urine	15	15	-	15	-	15	-	6	9	15	-	10	5	15	-	15	-	11	4	15	-	12	3	15	-	7	8		
		100	0	100	0	100	0	40	60	100	0	66.7	33.3	100	0	100	0	73.3	26.7	100	0	80	20	100	0	46.7	53.3		
p-value		0.025		0.001		0.001		0		0.025		0.001		0.001		0.001		0.001		0.001		0.001		0.001		0.001			

Table 5. *In-vitro* evaluation of the biocidal effect of the tested essential oils on *E. coli* isolates in the current study.

Essential oils used	Concentration of essential oils used/ no. of isolates tested *(n.=17)*							
	0.50%				1.00%			
	R	%	S	%	R	%	S	%
Thyme	17	100	-	0	17	100	-	0
Peppermint	13	76.4	4	23.5	-	0	17	100
Capsaicin	17	100	-	0.-	2	11.7	15	88.2
Orange	17	100	-	0	7	41.1	10	58.8
Green tea	14	82.3	3	17.6	12	70.5	5	29.4
Tea tree	-	0	17	100	-	0	17	100
Onion	17	100	-	0	9	52.9	8	47
P-value	0.001				0.001			

degree of resistance to Fosfomycin, Spectinomycin, Erythromycin and Enrofloxacin. On the other hand, strains recovered from fecal, milk and urine samples were highly sensitive primarily to Ciprofloxacin (80.0, 66.7 and 60.0%, respectively) at P<0.001. Variable degrees of resistance were noted against the other used antibiotics.

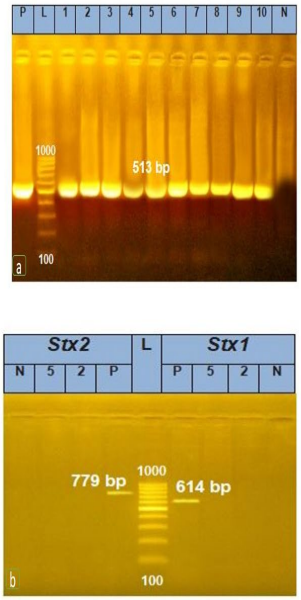

Fig. 3. Agarose gel electrophoresis for PCR products of *E. coli* by quorum markers using *lux* S gene (a), amplified 513bp, *Stx2* and *Stx2* 614 bp and 779 bp, respectively. Lane (L): 100 bp Ladder ''Marker'', Lane: (1-10), the examined samples, Lane Pos: Positive control, Lane Neg: Negative control.

The data showed in Table 5, tested the biocidal effect of some essential oils including thyme, peppermint, capsaicin, orange, green tea, tea tree and onion at concentration of 0.5% and 1.0% done against 17 randomly selecting multi-drug resistant, virulent and quorum sensing determinants from the well-identified *E. coli* of animal and human sources. It was revealed that at a concentration of 0.5 %, thyme, capsaicin, orange, and onion EOs failed to prevent bacterial development, however peppermint (23.5%) and green tea (17.6%) had a least effect on *E. coli* multiplication. At a concentration of 1.0%, peppermint demonstrated the most prevalent bioactive effect (100.0%), followed by capsaicin, orange, onion, and green tea, which showed rates of 88.2, 58.8, 47.0, and 29.4%, respectively at P<0.001. Thyme oil, on the other hand, failed to show any reduction of bacterial growth (0.0%) at both con-

centrations tested. Moreover, tea essential oil was found to have the maximum biocontrol efficacy against *E. coli* at the two concentrations applied (P<0.001).

DISCUSSION

Results shown in Table 2, revealed that the total number of positive samples for *E. coli* isolated from different sources were 235/610 (20.4%). *E. coli* was prominent in feces (44.87%) followed by urine (28.4%) and milk (22.2%). Much higher isolation rates were reported by Ali and Abdelgadir (2011) who recovered *E. coli* at a rate of 63.0%, also Dawit (2012) denoted greater results than our findings (64.0%) in feces whereas, 55.0 and 54.67% detections of *E. coli* were noticed in milk by El nahas *et al*. (2015) and Abd El-Tawab *et al*., (2020), respectively in Egypt. Besides, Azab *et al*. (2021) clarified higher infection rates of *E. coli* in urine (55.36%). Conversely to our finding Uhde *et al*. (2008), Ojo *et al*. (2010) and Galal *et al*. (2013) showed lower detection for *E. coli* in feces (5.5, 15.5 and 13.3%, respectively) and Dibua *et al*. (2014) 8.0% in urine samples. Nearly records were hypothesized by Thaker *et al*. (2012) 38.0% and Gebregiorgis and Tessema (2016) 36.8% in feces, Hassuna *et al*. (2020) 33.5% in urine and Elbagory *et al*. (2015) 25.0% in milk samples.

The higher isolation rates in this study (Table 2) was mainly associated with diarrhea, that was the main clinical signs in calves at the time of sample collection, also the small ages of the screened animals, most of which aged 5-30 days (Radostits *et al*., 2007), as well as management and environmental conditions of the farms such as deficient and/or inadequate-quality colostrum intake by the calves as declared by Charles *et al*. (2003). The poor hygiene in the farm unit often permits accumulation of pathogens in the young animal's environment, resulting in contaminated milk with *E. coli* during or after milking due to improper washing and disinfection of the udder or contact with contaminated milking pails and utensils, posing a public health risk of *E. coli* for young animals, workers and consumers.

Findings in the present study (Table 3), were matched to those reported by El-Jakee *et al*. (2012) who investigated O111 from milk and O119 from feces, Osman *et al*. (2012) in Egypt detected O86 in urine, Merwad *et al*. (2014) in Egypt recovered O55, O86 and O119 from feces but O128 from milk, El nahas *et al*. (2015) found O111 in milk. Also, O26 and O128 were determined in milk by Abdel Maaboud (2014) in Egypt and O111 and O128 were identified in milk by Momtaz *et al*. (2012) in Iran. In contrast Ojo *et al*. (2010) from Nigeria recovered O26, O111 and O128 from feces and Amraei *et al*. (2016) identified O142 and O25 in urine in Iran.

The existence of various virulence, pathotypes and quorum markers in samples of different origins (humans and animals) are ideal targets for determining the potential pathogenicity of *E. coli* isolate pathotypes (Frydendahl, 2002) as the commensal *E. coli* seldom have virulence genes (Boerlin *et al*., 2005), that revealed a prospective of the public health concern of *E. coli* found in cow's milk and feces. As result of the great impact associated with the intestinal infection including EPEC, which can cause diarrhea in children and animals. In addition to, HC and HUS in humans caused by EHEC strains. The EAEC traits are related to persistent diarrhea in man (Beutin, 1999) and the EIEC, are involved in invasive intestinal infections, watery diarrhea, and dysentery in man and animals (Martin *et al*., 1997). Reporting of many pathotypes and biotypes from human samples (workers or out-patients), make attention for the uropathogenic strains responsible (UPEC) for sepsis, meningitis, diarrhea and urinary tract infections (UTIs) (Sarantuya *et al*., 2004). Concerning, EHEC strains (non-O157) are regularly exhibited in the isolated serotypes with absence of O157 in all the samples studied, which could be attributed to its existence in small numbers among very high levels of other competing bacteria (Siriken *et al*., 2006). It also refers to the fact that milk contains immune (primarily IgA) and non-immune factors (lactoferrin and various free secretory components) that could prevent STEC from adhering to and multiplying on certain cell substrates as well as glycolipids and triglycerides that bind to and inactivate Stx (Sprong *et al*., 2002). Also, QS marker could be produced by both Gram-negative and Gram-positive bacteria, is the communication tool among bacteria, it regulates cellular aggregation-dependent aspects including control motility, antibiotic production, attack the host cells, biofilm formation, production of enzymes, as well as other virulence traits (Wei and Zhao, 2018). Commonly, *E. coli* could generate Acyl-Homoserine Lactones (AHLs), which can be detected by QS receptors. Based on this, the targeting of QS was shown as a promising approach to diminish the bacterial virulence (Khayyat *et al*., 2021; Saqr *et al*., 2021).

Results shown in Table 4, were like those reported by Sobhy *et al*. (2020) in Egypt, who found extreme resistance to Tetracyclines for *E. coli* isolates from cattle feces. Mirsoleymani *et al*. (2014) confirmed that *E. coli* isolated from urine showed 100.0% resistance to Amikacin, and Mandal *et al*. (2018) reported the highest resistance rate of *E. coli* against Ciprofloxacin in urine. Tadesse *et al*. (2012) stated that MDR in *E. coli* increased from 7.2% in the 1950s to 63.6% in the 2000s, confirming the previous findings. Even so, recent assumptions have found increasing levels of resistance to many antibiotic classes, including β-lactams, fluoroquinolones, tetracyclines and aminoglycosides in pathogenic *E. coli* isolates from all over the world, including Egypt (Flament-Simon *et al*., 2020; Khairy *et al*., 2020; Masoud *et al*., 2021).

On the contrary, Aghamahdi *et al*. (2013) reported that Ciprofloxacin represents the least effective medicine in controlling the bacterial uro-pathogens also Malekzadegan *et al*. (2018) and Abdelraouf *et al*. (2020); advised using of Fosfomycin, and Colistin as the ultimate alternatives for illnesses initiated by MDR strains of *E. coli*. It was clear that nearly all human and animal samples share the consistent resistance pattern to the same medications, owing to the widespread use of human drugs for treatment of diseased animals resulting in resistance transfer to animal hosts (Wellington *et al*. 2013). Additionally, the elimination of these antibiotics and their metabolites in human sludge, from which resistance is then passed on to animals using slurry as a fertilizer or irrigation with wastewater (Heuer *et al*. 2006). Additionally, bacteria can develop resistance to various antibiotics categories beyond mutation and/or horizontal gene transfer. Mutation arises spontaneously limiting the drug permeability and decreasing their effectiveness (Levy and Marshall, 2004). while horizontal gene transfer play a crucial role in bacterial evolution and the propagation of antimicrobial resistance determinants (Da Silva and Mendonca 2012). Resistance to various types of antimicrobial drugs is on the rise, posing a severe health threat by reducing treatment efficacy and limiting treatment alternatives (Monroy-Perez *et al*. 2020).

Antimicrobial abuse in the animal sector, whether for prophylactic or therapeutic purposes, is significantly linked to the development of antimicrobial resistance in a variety of bacteria, including *E. coli*. In several nations, antibiotics have been prohibited (Kammon, 2017). As a result, veterinary medicine practices are constantly looking for better, safer alternatives such as essential oils or volatile oils extracted from various aromatic and medicinal plants are widely used in animal industry, comprise many nontoxic bioactive compounds that have been revealed to be that are safe as food additives used in food industries (Bhavaniramya *et al*. 2019). In addition, they have a promising potential as growth promoters and treatment without the adverse effects like that of antibiotics due to their antibacterial antiviral, antiseptic, antifungal, antioxidant, anti-parasitic and insecticidal activities (Saljoughian *et al*., 2018).

The obtained results in Table 5 were equivalent to those of Mumu and Hossain (2018); Sharifi *et al*. (2018) and Puvača *et al*. (2021) who stated that the bioactive effect of tea oil is owing to its richness in terpenoids including terpinen, D-limonene and 8-cineole, the chemical ingredients exhibited a broad spectrum antimicrobial potency also the tea tree oil's bactericidal efficacy is due to its ability to suppress respiration and promote bacterial

cytoplasmic permeability, thus disrupts the *E. coli* cell's growth lag phase (Mohsen *et al*., 2020) as well as the remarkable antibacterial effects through inhibition of QS (Šimunovic *et al*. 2020). In addition, limonene and α-terpinene are the main constituents in peppermint, capsaicin, orange and onion volatile oils (Abuhlega *et al*. 2018; Martinelli *et al*. 2017; Banjari *et al*. 2020, Amiri *et al*. 2020, respectively), demonstrating their optimal anti-Qs determinants as well as the potent antimicrobial efficiencies. Conversely to our observations many studies showed thyme as effective antibacterial effect (Hippenstiel *et al*. 2011; Bassolé and Juliani 2012). Although, according to Ruiz-Navajas *et al*. (2012) emphasized that thyme at concentration of 1.0% could not prevent *E. coli* development.

CONCLUSION

This study has showed that virulence and quorum markers are extremely distributed among distinctive *E. coli* traits of animal and human sources. Also, Antimicrobial resistance is on the rise, and the advent of MDR poses a severe health threat, limiting treatment choices for infections caused by pathogenic *E. coli* isolates. Furthermore, essential oils' bioactive constituents comprise a viable strategy to reduce MDR stains, necessitating potential applications in the food, pharmaceutical, and other industries.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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