

Molecular confirmatory diagnosis of some *E. coli* serogroups isolated from feces and milk samples of buffaloes and cattle and from its milk products with special reference to antibiotic resistant genes

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Abstract

A total of 60 fecal and milk samples (30 samples of each) from cattle and buffaloes, were collected from different localities at Kalyobia governorate and also 60 samples of kareish cheese and ice-cream (30 samples of each) were collected from retail supermarkets located in Kalyobia governorate, all samples were examined bacteriologically, biochemically and serologically for detection of *E. coli*.

E. coli was detected in 12 (40%) of the examined fecal samples and the revealed serogroups were O157 (3.3%), O26 (16.6%), O15 (13.3%) and O111(6.7%). *E. coli* was detected in 10 (33.3%) of the examined milk samples and the serogroups were O18 (13.3%), O26 (10%) and O 44 (10%). *E. coli* was detected in 9(30%) of the examined samples of kareish cheese and the obtained serogroups were O44(10%), O15(10%), O157(6.7%) and O119 (3.3%), where *E. coli* was detected in the examined samples of ice-cream 7 (23.3%) and the recovered serogroups were O44(6.7%), O18(6.7%), O15(3.3%) and O86 (3.3%). The antibiogram pattern revealed that all serogroups were sensitive to enrofloxacin, spectinomycin and ciprofloxacin. Moreover all serogroups were resistant to amoxycillin, oxytetracyclin, tobramycin and ampicillin, while variable results were recorded with neomycin, colistin sulphate, gentamycin and cefadroxil. The isolated serogroups were confirmed by molecular technique by polymerase chain reaction (PCR) for detection of the *TEcol* gene (Which generated specific amplicons of 212 base pair and to detect the distribution of antibiotic-resistant genes in *Escherichia coli* isolates.

Key words: *E. coli*, milk, milk products, antibiotic sensitivity, PCR -antibiotic resistance genes.

Introduction:

Escherichia coli are normal inhabitant of the intestinal tract of the animals. However, particular *E. coli* strains such as enterotoxigenic *E. coli* are the causative agents of septicemic colli bacillosis and diarrhea (Gyles, 1994). The role of *E. coli* as pathogen is well known and many *E. coli* isolates have been associated with a wide variety of diseases. Caprioli *et al.*, (2005) stated that enterohemorrhagic *E. coli* (EHEC) serotype O157 which is Shiga toxin (Stx) – producing *E.coli* (STEC) had been reported in water buffaloes and cattle which considered to be the most important source of

human infections with EHEC O₁₅₇. Shiga-toxin-producing *Escherichia coli* (STEC), also called vero-toxin-producing *E. coli* (VTEC) is the most important recently emerged group of food-borne pathogens. These bacteria can cause severe disease in humans, such as hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). Coli bacillosis is the major cause of morbidity and mortality and is responsible for significant economic losses world wide (Schouler *et al.*, 2012). *E. coli* is considered to be opportunistic pathogen and originate from cow's fecally contaminated environment and infect the udder via teat canal. Cattle, espe-

cially young animals, have been implicated as a principal reservoir of STEC, undercooked ground beef and raw milk being the major vehicles of food-borne outbreaks. Human and bovine STEC elaborate two potent phage-encoded cytotoxins, called Shiga-toxins (Stx1 and Stx2) or verotoxins (VT1 and VT2) (**Paton and Paton, 1998**).

Milk is an excellent medium for the growth of numerous microbes which produce consequential spoilage of the milk and various milk products or infections in consumers (**Oliver *et al*, 2005**). Because of the specific production, it is impossible to avoid contamination of milk with microorganisms therefore the microbial content of milk is a major feature in determining its quality (**Torkar and Teger, 2008**). The existence of food borne pathogens in raw milk may increase the threat of transmission of food borne pathogens and ingestion of various harmful toxins (**Srinu *et al*, 2012**). Huge numbers of microbes can get access to milk and various milk products including *E. coli* which is an indicator of fecal contamination constituting a public health hazard. The most important causes of food borne diseases are Shiga toxin-producing *E. coli* (STEC) among the other seropatho types of *E. coli* (**Kaufmann *et al*, 2006**). Culture alone can be insensitive, especially for the detection of small numbers of *E. coli* O157:H7, and it is unable to detect non-O157 verotoxin-producing *E. coli* (**Novicki *et al*, 2000** and **Tarr, 1995**). **Eraqi, (2010)** reported that the results of antimicrobial sensitivity test revealed that the main bacterial pathogens isolated from clinical mastitis *E. coli* were highly sensitive to nalidixic acid, sulbactam + Ampicillin, cefoperazone, flucloxacillin, ampicillin, amoxicillin+clavulonic acid and ofloxacin.

PCR has become a very rapid and reliable tool for the molecular biology-based diagnosis of a variety of infectious diseases (**Fredricks and Relman, 1999**). **Ahmed *et al*, (2004)** stated that polymerase chain reaction (PCR) proved to be rapid, accurate, sensitive and specific method for the detection of *E. coli* O₁₅₇:H₇ in

regard to conventional, biochemical and serological methods. PCR has been applied for the detection of microorganisms from microbial cultures and tissues and directly from clinical samples. **Amin *et al*, (2011)** reported that PCR assay can be used as a rapid, sensitive and specific routinely diagnostic tool to detect the presence of *Escherichia coli* in milk samples. **Gillespie and Oliver (2005)** reported that the application of molecular diagnostic technique such as Polymerase Chain Reaction assay (PCR) is based on unique signature sequences in the nucleic acid of pathogen and subject to less variability providing rapid, sensitive and specific diagnosis of pathogens. Multiplex PCR was developed for detection of virulence genes of *E. coli* (**Kimata *et al*, 2005**). PCR is a rapid, specific and sensitive assay for detection of *E. coli*. Fecal specimens are among the most complex specimens for direct PCR testing due to the presence of inherent PCR inhibitors that are often coextracted along with bacterial DNA (**Brian *et al*, 1992**). Potential PCR inhibitors found in stool specimens include heme, bilirubins, bile salts, and complex carbohydrates (**Demeke and Adams, 1992**). Few studies have evaluated or compared simple DNA extraction methods that would facilitate and improve the sensitivity of PCR detection of enteric pathogens from fecal specimens (**Caeiro *et al*, 1999**). Conventional methods for the detection and identification of *Escherichia coli* O157:H7 include growth of non-sorbitol fermenting *E. coli* colonies on Sorbitol MacConkey agar culture (SMAC), followed by serological confirmation with O157- and H7-specific antisera (**Karmali, 1989**). Recently, molecular techniques, especially polymerase chain reaction (PCR), have been widely used to study antimicrobial resistance genes due to the excessive use of antibiotics. The increasing number of multiple-antibiotic resistant pathogens has become a serious threat to human health (**Centers for Disease Control and Prevention [CDC], 2013; Review on Antimicrobial Resistance [RAR], 2014; World Health Organization WHO, 2014**). The resistance of

fecal bacteria from human and animal sources released into the environment impart antibiotic resistance genes to the non-resistant indigenous microorganisms (**Aminov, 2011; Tacão et al., 2014**). The subsequent transfer of antibiotic resistance genes from the indigenous microorganisms to human-associated bacteria may take place (**Devirgiliis et al., 2011**). Simultaneously, positive selective pressure for antibiotic resistance genes in the environment may be stimulated by the presence of antibiotics or other contaminants (**Gao et al., 2015**). Antibiotic resistance surveillance data show that *E. coli* has high resistance for older generation human and veterinary antibiotics including ampicillin, streptomycin, and tetracycline and the increasing resistance to newer antibiotics such as fluoroquinolones and cephalosporins (**Tadesse et al., 2012**). The gene encoding resistance to tetracycline class antibiotics, *tet(M)*, which is predominately found on transposons within enterococci has also been found on plasmids within *E. coli* (**Jurado-Rabadán et al., 2014**).

So this study is aimed to isolate pathogenic *E. coli* from feces, milk and milk products, investigation of their antibiotic sensitivity pattern and detection of antibiotic resistant genes using Polymerase chain reaction.

Materials and Methods

Collection of samples:

A total of 60 fecal and milk samples (30 samples of each) were collected from different localities of cattle and buffaloes at Kalyobia governorate and also 60 samples of kareish cheese and ice-cream (30 samples of each) were collected from retail supermarkets located in Kalyobia governorate were collected under aseptic precautions. The samples were sent as soon as possible to the lab. Fecal samples were collected as aliquots of 5 grams of rectal feces from 30 diarrheic feverish buffalo and cattle calves aged from 1-4 months and were transferred to the laboratory in an ice box to be cultured immediately. The milk samples from mastitic buffalos and cattle were col-

lected in sterilized sample bottle and other milk products samples were collected in sterilized polyethylene bags.

Isolation and identification of *E. coli*:

fecal samples were inoculated into peptone water and incubated at 37°C for 24h, then cultivated onto Sorbitol MacConkey agar media and MacConkey agar media and incubated for 24h at 37°C.

Milk samples were incubated aerobically at 37°C for 18-24 hours, then 10ml of milk samples were transferred into sterile small centrifuge tubes. The tubes were centrifuged at 3000 RPM for 20 minutes. The cream and supernatant fluid were discarded. The sediment was streaked onto Sorbitol MacConkey agar, blood agar, MacConkey's agar and EMB agar.

Twenty five grams of kareish cheese and ice cream were separately homogenized with 225ml of modified Tryptone soy broth supplemented by novobiocin (20mg/l) for 2 min. using sterile homogenizer (Universal Laboratory, Poland) and incubated at 37°C for 24 hours. The inoculated plates were incubated aerobically at 37°C for 24 hours and examined for bacterial growth, suspected colonies appearing on different media were picked up and subcultured for further confirmation via colony characteristics, morphology, Gram's reaction and haemolysis patterns. Then, confirmed the isolates by various biochemical tests such as IMVC test, Urease test were performed according to **Quinn et al., (2002)**.

Serological identification:

Antisera of *Escherichia coli* were used for serological identification of somatic antigen "O" using slide agglutination test according to **Koneman et al., (1997)**. The *E. coli* immune-O sera (Polyvalent sera, 8 vials Monovalent sera, 43 vials) and K99 were obtained from Denka Seiken Co. LTD, Tokyo, Japan.

Antibiogram pattern: Antibiogram was applied on the different "O" serogroups of *E. coli* using in vitro disc diffusion technique according to **Quinn et al., (2002)** using Mueller Hinton agar plates. The following antimicrobial discs 'Oxoid' were used: Enrofloxacin (10mg),

neomycin (30mg), amoxicillin (25mg), oxytetracycline(30mg), cefadroxil (30mg), colistin-sulphate (50mg), gentamycin (10mg), tobramycin (10mg), ampicillin (10mg), spectinomycin (100mg) and ciprofloxacin(5mg). The results were interpreted according to **CLSI, (2009)**.

Primer sets for the patho types and virulence genes of the isolated *E. coli serogroups* according to **Paradis *et al.*, (2005)**.

Target gene	Primer	Nucleotide Sequence (5' - 3')	Amplicon size (bp)	Reference
TEcol553	F	(5'-TGGGAGCGAAAATCCTG-3')	212 bp	Paradis <i>et al.</i> , 2005
TE col 754	R	(5'-CAGTACAGGTAGACTTCTG-3')		

F: Forward.

R: Reverse

DNA amplification:

The samples were done in Bio Rad Thermal cycler (T100-England) with the following thermal profile: initial denaturation at 95°C for 3 min. then denaturation at 95°C for 30 sec., annealing at 58°C for 30 sec., extension for 1 min.at 72°C for 35 cycles and after the last cycle, the mixture was incubated for final extension at 72°C for 10 min .

Detection of PCR products: amplified prod-

Polymerase chain reaction (PCR):

Extraction of genomic DNA: Genomic DNA was extracted by using Gene JET Genomic DNA Purification Kit (**Fermentas#k0721**). **Oligonucleotide primers for *E. coli serogroups (TEcolgene)*.**

ucts were detected in 1.5% agarose gel electrophoresis pre-stained with ethidium bromide, at 80 V for 1 hour.

Antibiotic Resistance Gene Detection:

Plasmid DNA Extraction: Plasmid DNA was extracted by using **EZ-10 Spin Column Plasmid DNA Mini Preps Kit.(Bio Basic IMC Canada)**.

Escherichia coli antimicrobial resistant genes and primer sequences used for PCR identification:

Antimicrobial agent	Resistance gene	Sequence	Size (bp)	Annealing temperature (°C)	References
Beta-lactams	<i>BlaSHV</i>	(F) TCGCCTGTGTATTATCTCCC (R)CGCAGATAAAATCACCACAATG	768	52	Van <i>et al.</i> (2008)
Quinolones	<i>qnrA</i>	(F)GGGTATGGATATTATTGATAAAG (R) CTAATCCGGCAGCACTATTTA	670	50	Mammeri <i>et al.</i> (2005)
Trimethoprim	<i>dfrA1</i>	(F) GGAGTGCCAAAGGTGAACAGC (R)GAGGCGAAGTCTTGGGTAAAAAC	367	45	Toro <i>et al.</i> (2005)

Plasmid DNA amplification: PCR reactions were performed in a total volume of 25 µl, including 1.5mM MgCl₂, 50mM KCl, 10mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 200 µm of each dNTP (Fermentas), 1 µm primers, 1 IU of Taq DNA polymerase (Fermentas), and 5 µl (40–260 ng/µl) of DNA. Amplification reactions were carried out using Bio Rad Thermal cycler (T100-England) with the following thermal profile: initial denaturation at 95°C for 3

min., followed by 35 cycles each consisting of denaturation at 94°C for 1 min, annealing at ~55°C for 90 s and extension at 72°C for 1 min, followed by a final extension step of 10 min at 72°C.

Amplified samples were analyzed by electrophoresis in 1.5% agarose gel and stained by ethidium bromide. A molecular weight marker (100 bp DNA ladder, Fermentas) was used as a size standard.

Results

Table (1). Prevalence of *E. coli* in the examined fecal and milk samples from mastitic cattle and buffaloes (n=30).

Samples	NO. of the examined samples	No. +ve samples	% of +ve samples	<i>E.coli</i> Serogroups		
				Sero- groups	No.	%
Feces	30	12	40%	O157	1	3.3%
				O 26	5	16.6%
				O15	4	13.3%
				O111	2	6.7%
Milk	30	10	33.3%	O 18	4	13.3%
				O26	3	10%
				O44	3	10%

Table (2). Prevalence of *E. coli* in the examined samples of milk products. (n=30).

Milk products Samples	NO. of the examined samples	No. + ve samples	% of + ve samples	<i>E. coli</i> Serogroups		
				Sero-groups	No.	%
Kareish cheese	30	9	30%	O44	3	10%
				O15	3	10%
				O157	2	6.7%
				O119	1	3.3%
Ice- cream	30	7	23.3%	O44	2	6.7%
				O18	2	6.7%
				O15	1	3.3%
				O86	1	3.3%

Table (3). In vitro sensitivity test of the recovered *E. coli* pathogenic serogroups

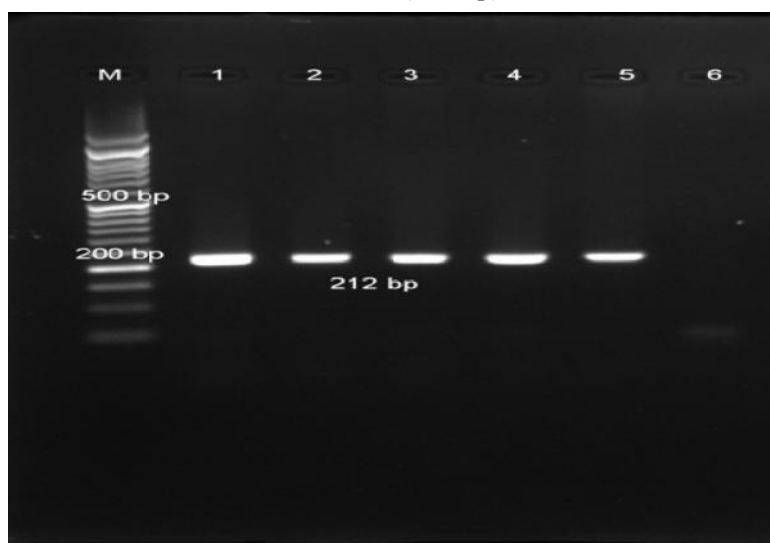
Chemotherapeutic agent	Conc. mg	O ₁₅₇	O ₁₅	O ₁₁₉	O ₁₈	O ₁₁₁	O ₈₆	O ₄₄	O ₂₆
Enrofloxacin	10	S	S	S	S	S	S	S	S
Neomycin	30	S	S	R	S	R	R	R	S
Amoxycillin	25	R	R	R	R	R	R	R	R
Oxytetracyclin	30	R	R	R	R	R	R	R	R
Cefadroxil	30	M	S	R	R	M	R	S	R
Colistinsulphate	50	S	R	R	R	R	R	S	R
Gentamycin	10	R	S	R	R	S	S	R	R
Tobramycin	10	R	R	R	R	R	R	R	R
Ampicillin	10	R	R	R	R	R	R	R	R
Spectinomycin	100	S	S	S	S	S	S	S	S
ciprofloxacin	5	S	S	S	S	S	S	S	S

N.B: S: Sensitive

M: Intermediate

R: Resistant

Figure (1). Agarose gel electrophoresis 1.5% showing PCR products of *T Ecol* gene of *E. coli* serogroups (212 bp).



Lane M: Molecular marker (50bp DNA ladder, Thermo).

Lane (1): DNA sample extracted from bacterial liquid culture of *E.coli* O15.

Lane (2): DNA sample extracted from bacterial liquid culture of *E.coli* O18.

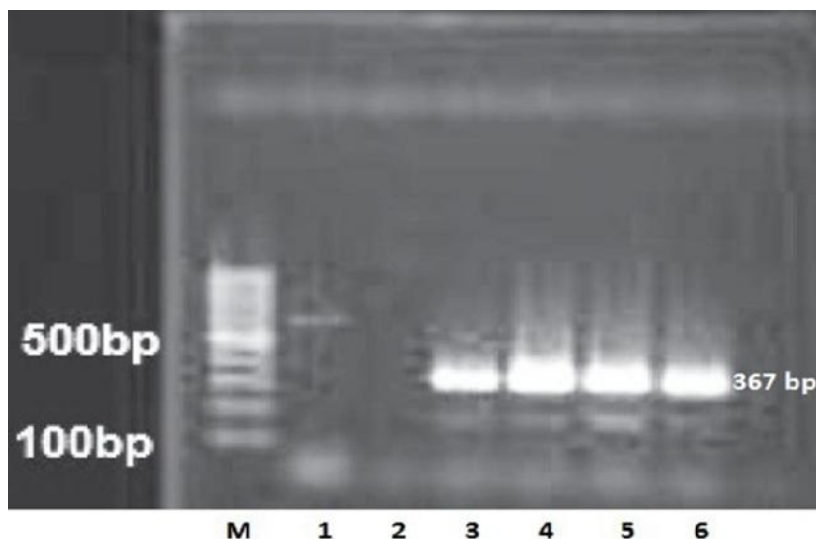
Lane (3): DNA sample extracted from bacterial liquid culture of *E.coli* O26.

Lane (4): DNA sample extracted from bacterial liquid culture of *E.coli* O44.

Lane (5): DNA sample extracted from bacterial liquid culture of *E.coli* O157.

Lane (6): Control negative.

Figure (2). Agarose gel electrophoresis 1.5% showing multiplex PCR products of antibiotic resistance genes of *E. coli* serogroups showing a positive band at 367 bp for trimethoprim.



Lane M: Molecular marker (100 bp DNA ladder, Fermentas)

Lane (1): Control negative

Lane (2): Plasmid DNA sample extracted from bacterial liquid culture of *E.coli* O15.

Lane (3): Plasmid DNA sample extracted from bacterial liquid culture of *E.coli* O18.

Lane (4): Plasmid DNA sample extracted from bacterial liquid culture of *E.coli* O26.

Lane (5): Plasmid DNA sample extracted from bacterial liquid culture of *E.coli* O44.

Lane (6): Plasmid DNA sample extracted from bacterial liquid culture of *E.coli* O157.

Discussion

In the present study a total of 60 fecal and milk samples (30 samples of each) from cattle and buffaloes and some milk product samples (kareish cheese and ice-cream, 30 samples of each) were collected from retail supermarkets located in Kalyobia governorate were examined bacteriologically, biochemically and serologically for detection of *E. coli*. Antibio gram was applied on the different "O" sero groups of *E. coli* using in vitro disc diffusion technique according to **Quinn et al., (2002)** using Mueller Hinton agar plates. The isolated sero groups were confirmed by detection of the *TEcol* gene which is the target *E. coli* specific PCR primers (**Paradis et al., 2005**). Then detection of the distribution of antibiotic resistant genes in *Escherichia coli* isolates by multiplex PCR.

Prevalence of *E. coli* in milk and fecal samples:

E. coli recovered from mastitis cases belonged to different serogroups and varied greatly in O-groups (**Fuse et al., 1993**). Therefore increasing attention is being given to the role played by livestock in the epidemiology of this organism and to study of genetic diversity among groups of *E. coli* strains rather than biochemical, serological identification and antibiotic sensitivity.

Lurthus Retha et al., (2006) examined 30 milk samples from mastitic cows and found that the most predominant isolates were *E. coli*. The results of present study revealed that *E. coli* was isolated from milk samples of mastitic cattle and buffaloes (33.3 %) as shown in table (1). Nearly similar prevalence rate was recorded by **Eraqi, (2010)** (31.49%) and **Bradley and Green, (2001)** (34.7 %). Higher results (57%) obtained by **Wadhwa et al., (1996)** who related these result to higher contamination of hind quarters with urine and feces, also **Bradely and Green, (2001)** mentioned the prevalence of *E. coli* as a major pathogen in clinical mastitis and may be primarily attributed to its endotoxins. Also **Aziz, (2002)** re-

ported that *E. coli* was predominant isolate (37.7%). By contrast a low incidence rate of clinical mastitis was recorded by **Workineh et al., (2002)** (21.5 %). The relatively high incidence of *E. coli* isolated from mastitic cow in the present study may be attributed to heavy contamination of bedding and poor hygienic measures with consequent under infection from fecal contamination on theses beddings **Guerin Fauble et al., (2003)**.

E. coli was detected in 12 (40%) of the examined fecal samples and the revealed serogroups were O157 (3.3%), O26 (16.6%), O15 (13.3%) and O111(6.7%) as shown in table (1). The most important bacterial enteropathogens are enterotoxigenic *E. coli* causing diarrhea in calves, the disease is usually referred to as colibacillosis and include either enteric or systemic colibacillosis (**Warry et al., 1993**).

Prevalence of *E. coli* in milk products samples:

The Prevalence of *E. coli* in kareish cheese is 30% (table 2), similar result was recorded by **Fadel et al., (2009)** (29.2%) but higher prevalence in cheese was reported by **Paneto et al., (2007)** (96%). However, lower prevalence of *E. coli* in cottage cheese was reported by **Singh and Prakash (2008)** (12.9%) and 16.6 % in cheese was reported by **Farzan et al., (2012)**.

The Prevalence of *E. coli* in ice-cream was 23.3 % in the present work (table 2). Lower result was reported by **Farzan et al., (2012)** (16.6 %). In contrast, high prevalence of *E. coli* (58%) was recorded by **Amany et al., (2008)** and **Fadel et al., (2009)** (31.8%). In vitro determination of antimicrobial sensitivity test on the isolated microorganisms is important in order to guide the choice of the most appreciated drug required to produce therapeutic effect.

Concerning to the results of sensitivity test, results obtained in table (3) revealed that the antibiogram pattern of all detected serogroups were sensitive to enrofloxacin, spectinomycin

and ciprofloxacin. Moreover all serogroups were resistant to amoxycillin, oxytetracyclin, tobramycin and ampicillin, while variable results were recorded with neomycin, colistin sulphate, gentamycin and cefadroxil. This results nearly similar to that obtained by **Chigor *et al.*, (2010)** where they showed that pathogenic *E. coli* isolated from clinical sources were found to be resistant to oxytetracycline, cotrimoxazole, ampicillin and other used antibiotics, and added, multidrug resistance and plasmid were observed in clinical isolates. Nearly similar results obtained by **El-Shehedi, (2013)** where she showed that all tested *E. coli* serogroups detected from milk samples of mastitic cattle were resistant to neomycin, oxytetracycline, colistin sulphate, Tobramycin and ampicillin. The results recorded in Table (3) revealed that most strains of *E. coli* were highly sensitive to Enrofloxacin. These results simulate what was reported by **Jha *et al.*, (1994)** also **Schroder *et al.*, (2005)** mentioned that *E. coli* isolated from mastitic cows were sensitive to enrofloxacin. **Nagib, (2008)** showed that *E. coli* O₁₅₇:H₇ isolates were 100% resistant to ampicillin and vancomycin. **Sayed and Mohamed, (2008)** mentioned that *E. coli* isolated from dairy Friesian mastitic cows was highly sensitive to ciprofloxacin and gentamicin, but intermediate sensitive to flumequine, nalidixic acid and resistant to streptomycin and colistin sulphate.

The specificity of the oligonucleotide primers were carried out by testing of all isolated *E. coli* serogroups with PCR using the primer pairs targeting the *TEC* gene (specific for all members of *E. coli* species). In this study, the most predominant *E. coli* serogroups (O15, O18, O26, O44 and O157) detected by bacteriological examination were tested by PCR using the same primer pair after selective enrichment media on EMB agar. All the examined *E. coli* serogroups were positive for amplification of 212bp fragments of *TEC* gene as shown in (Fig. 1).

The results obtained in Fig. (2) showed that *E. coli* serogroups (O18, O26, O44 and O157)

harbour a resistant gene for trimethoprim (*dfrA1*) giving a positive band at 367 bp. but *E. coli* O15 is negative for the trimethoprim resistant gene (*dfrA1*) the examined *E. coli* serogroups (O15, O18, O26, O44 and O157) not have a resistant gene for trimethoprim (*dfrA1*), quinolones (*qnr*) and β -lactams (*blaSHV*). The presence of genes that confer resistance to some antimicrobial agents indicating that *E. coli* isolates originating from meat and milk could be a reservoir of antimicrobial resistance (**Momtaz *et al.*, 2012**).

Conclusion and recommendation

The isolated strains were confirmed by polymerase chain reaction (PCR) of the isolated *E. coli* serogroups for detection of *E. coli* specific gene (*TEC* gene).

PCR is a rapid, reliable specific method and allows the quick detection of pathogenic *E. coli* cells and the current study supports the finding that raw milk and various milk products can be regarded as critical source of pathogenic *E. coli*.

This explains the need for:

Control measures for hygienic practice particularly in milking, surveillance and legislation have to be improved in order to prevent fecal contamination of milk and dairy products.

Strict monitoring and surveillance for effective measures of hygiene and sanitary practice during production of milk and various milk products.

Application and implantation of hazard analysis and critical control point (HACCP) system as a microbiological control system.

Educational programs for all employees concerned with the production and handling of milk and milk products.

Avoid consuming of milk products labeled by unknown trade name or not carry expired date.

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التشخيص الجزيئي للتأكد من بعض عترات الميكروب القولوني المعزول من الجاموس والأبقار ومن بعض منتجات الألبان مع الإشارة للجينات المقاومة للمضادات الحيوية
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المخلص العربى

تم إجراء هذه الدراسة على ٦٠ عينة من البراز و اللبن من بعض تجمعات الأبقار و الجاموس (٣٠ عينة من كل نوع) وعلى ٦٠ عينة بعض منتجات الألبان مكونة من الجبن القريش والأيس كريم (٣٠ عينة من كل نوع) والتي تم جمعها من بعض السوبر ماركت بمحافظة القليوبية وتم إرسال العينات على وجه السرعة وتحت ظروف صحية إلى المعمل. وقد تم فحص العينات لمعرفة مدى تواجد الميكروب القولوني وذلك بالطرق البكتيريولوجية والبيوكيميائية والسيرولوجية. وقد تم عزل الميكروب القولوني من البراز ١٢ (٤٠%) وكانت العترات المعزولة هي O١٥٧ (٣.٣%) و O٢٦ (١٦.٦%) و O١٥ (١٣.٣%) و O١١١ (٦.٧%) وتم عزل الميكروب القولوني من عينات اللبن ١٠ (٣٣.٣%). وكانت العترات المعزولة هي O١٨٠٤٤, O٢٦, بنسبة ١٣.٣% و ١٠% و ١٠% على التوالي. وقد تم عزل الميكروب القولوني من بعض منتجات الألبان مثل الجبن القريش والأيس كريم بنسبة ٣٠% و ٢٣.٣% على التوالي وكانت العترات المعزولة من الجبن القريش هي O١٥, O٤٤O١٥٧, O١١٩ بنسبة ١٠% و ١٠% و ٦.٧% و ٣.٣% على التوالي. وكانت العترات المعزولة من الأيس كريم هي O١٥, O١٨٠٨٦, O٤٤ بنسبة ٦.٧% و ٦.٧% و ٣.٣% و ٣.٣% على التوالي. وقد تم إجراء اختبار الحساسية للمضادات الحيوية ووجد أن العترات المعزولة كانت مقاومة للامبسلين والتوبراميسين والأكسيتتراسيكلين والأموكسيسيلين وكانت حساسه للإنزيم فلوكلوكساسين وسيتينوفايسين وسيفروفلوكساسين في حين كانت نتائج متباينة مع النيوميسين والجنتاميسين والسيفادروكسيل وسلفات الكولستين. وقد تم استخدام اختبار تفاعل إنزيم البلمرة المتسلسل للكشف عن وجود الجين (*TEcolgene*) الذى يوجد في جميع عترات الميكروب القولوني وقد وجد أن جميع العترات المعزولة بالطرق التقليدية كانت إيجابية لوجود هذا الجين (٢١٢bp) وكذلك تم التعرف على الجينات المقاومة لبعض المضادات الحيوية باستخدام تفاعل إنزيم البلمرة المتسلسل. هذا وقد ناقش البحث مدى خطورة الميكروب القولوني على الصحة العامة للإنسان عن طريق تواجده في منتجات الألبان.