A contribution on Coliforms causing mastitis in cows with reference to serotypes and virulence factors of E. coli isolates

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ABSTRACT

Escherichia coli (*E. coli*) **is the predominant coliform species causing intramammary infections. Where in the present study,** *E. coli* **isolates were 18 strains (17.82%) followed by** *Enterobacter aerogenes* **3 strains (2.97%) and** *Klebsiella pneumoniae* **one strain (0.99%) from 101 clinical mastitic milk samples of cows. Eighteen** *E. coli* **isolates were serotyped to nine different serogroups; O111:H4 (3), O127:H6 (3), O26 (2), O126 (2), O119:H6 (1), O114:H21 (1), O55:H7 (1), O44:H18 (1), O124 (1) and (3) untyped. Virulence tests were performed on the 18 isolated** *E. coli*, **it was found that 15 isolates (83.3%) were serum resistant, 13 isolates (72.2%) had Congo Red binding activity, 6 isolates (33.3%) were invasive and one isolate (5.6%) had haemolytic activity. PCR was applied to detect the presence of Shiga like toxin producing** *E. coli* (*stx*1 **and** *stx2* **genes) on the nine different strains (one strain for each serogroup), where** *stx*1 **and** *stx*2 **were found in 8 (88.9%) and 4 (44.4%) of the nine examined strains, respectively. While** *stx*1 **and** *stx*2 **genes were found together in 3 strains (33.3%). Conclusions:** *E. coli* **isolates usually posses one or more virulence factors that may help in establishment at the infection site and subsequently causing clinical bovine mastitis.**

Keywords: coliforms, *E. coli,* **serotypes, virulence factors,** *stx*1 **and** *stx* 2.

INTRODUCTION

Gram-negative bacteria that commonly cause bovine mastitis are classified as environmental pathogens. Genera classified as coliform are *E. coli*, *Klebsiella,* and *Enterobacter.* Other Gram-negative bacteria frequently isolated from intramammary infections include species of *Serratia, Pseudomonas,* and *Proteus* (**Koneman** *et al.,* **1983).** The point sources of coliform bacteria that cause infections include bedding materials, soil, manure and other organic matter in the environment of cows (**Hogan and Smith, 2003).**

E. coli is the predominant coliform species reported as causing intramammary infections; it causes inflammation of mammary gland in dairy animals around parturition and during lactation with striking local and sometimes systemic clinical symptoms. It is well known that bacterial, animal and environmental factors are interpedently and influence mastitis susceptibility (**Burvenich** *et al.,* **2003; Lehtolainen, 2004 and Quesnell** *et al.,* **2012).**

Virulence factors of bacterial strains can give it chance for colonization, multiplication and survival in udder in the face of host defense mechanism (**Fernandes** *et al.,* **2011).** Several virulence factors have been detected in pathogenic *E. coli*. These include toxins, adhesions, invasions, capsule production and the ability to resist serum complement, and iron scavenging. Only isolates with successful combinations of virulence factors will be capable of causing disease (**Kaper** *et al.,* **2004).**

Serogrouping of *E. coli* was carried out to give an idea about the most predominant serogroups associated with clinical mastitic cases. *E. coli* recovered from mastitis cases belonged to different serogroups and varied greatly in O groups and may not be attributed to epizootic strains (**Moussa** *et al.*, **2006 and Amira** *et al.*, **2013).**

Shiga-like toxin producing *E. coli* (STEC) also known as verotoxin producing *E. coli*. The most *E. coli* serotypes isolated from mastitic cows and buffaloes produced verotoxin and this result consistent with the hypothesis that verotoxin play a major role in the

pathogenesis of mastitis caused by *E. coli*. The pathogenicity of this disease probably results from the production of verotoxin or Shiga-like toxin which efficiency inhibits protein synthesis in mammalian cell free system (**Dalia and Amany, 2007).**

Congo Red Agar test has been used to differentiate invasive and noninvasive *E. coli* in poultry, this simple test was used to detect enteroinvasive *E. coli* of bovine origin (**Sharma *et al*., 2006).** Bactericidal activity of serum against Gram negative bacteria was mainly mediated by antibody and complements components, moreover complement activity responsible for bactericidal activity of serum by generating chemostatic factors and directly killing susceptible Gram negative bacteria (**Taylor, 1983).** The ability of *E. coli* organisms to survive in serum and grow in spite of the bactericidal activity of complement has recognized as an important attribution which contributes to its pathogenicity and suggests the possibility of multiplication in blood stream (**John *et al*., 1989).** Haemolysin production has been associated with pathogenicity of *E. coli* strains. Hemolytic activity could be used a phenotypic marker or virulence factor of *E. coli* serotype (**Gad El-Said *et al*., 2005).**

Consequently, the objective of this study was to describe the bacteriological findings of coliform mastitis in cows. Detect the serotypes and virulence factors, mainly serum resistance, Congo Red binding activity, invasiveness and haemolytic activities of *E. coli* strains isolated from clinical mastitic cow's milk samples, in addition PCR assay was used to detect of the genes encoding Shiga toxin 1 and 2 (*stx*1 & *stx*2).

MATERIALS AND METHODS

I- Bacterial isolates and phenotypic identification

Thirty-two isolates of Gram negative bacilli isolated from 101 clinical mastitis cow's milk samples were used in the present study. A part of single typical lactose fermenting colony was inoculated onto Eosin methyline blue media and Sorbitol MacConkey agar plates (Diffco). The inoculated plates were incubated aerobically at 37°C for 24 hours. Biochemical conformation of the strains was performed by conventional IMVIC (Indole, Methyl red, Voges proskauer, and citrate utilization tests), urease test, motility, triple sugar iron agar (TSI) inoculation and sorbitol, raffinose and cellobiose fermentation, according to **Quinn *et al*. (2004).**

II- Serological identification of *E. coli*

The isolates were serologically identified according to **Kok *et al*. (1996)** by using rapid diagnostic *E. coli* antisera sets (**DENKA SEIKEN Co., Japan**) were used for serotyping of *E. coli* isolates. They include 8 vials of polyvalent in addition to the 43 vials of monovalent antisera and 5 H-sera.

III- Virulence factors for eighteen *E. coli* isolates

A)- Haemolytic activity (haemolysin) (**Beutin *et al*., 1989**): *E. coli* isolates were inoculated onto blood agar containing 5% sheep blood for detection of enterohaemolysis after 6 hours of incubation at 37 °C.

B)- Congo Red (CR) binding test (**Panigrahy and Yushen, 1990**): The medium used for CR dye binding was tryptose agar with 0.2% galactose and 0.03% CR dye. *E. coli* isolates were streaked onto CR agar plates and incubated at 37 °C for 24 hours. The plates were further incubated at room temperature for additional 48 hours. The colonies were examined at 18, 24, 48 and 72 hours of incubation. The *E. coli* that produced red colonies between 18 and72 hours of incubation were recorded as Congo Red positive and the ones that produced grayish-white colonies and remained so throughout the incubation period were recorded as Congo Red negative.

C)- Serum inactivation assay (**Timmis., 1979**): *E. coli* isolates were inoculated at 0.1%(v/v) inoculums level into glucose phosphate broth containing bromothymol blue and 2% human serum and incubated at 37 °C for 24 hours. The serum resistance was observed by change in colour from green to yellow.

D)- Invasiveness test (Sereny test), (**Sereny, 1955**): *E. coli* isolates were grown overnight in nutrient broth. The growth was centrifuged at 5000 rpm for 15 min at 4 °C and the bacterial pellet was collected. The concentration was adjusted with 0.9% normal saline solution to 5×10^5 cfu/ mL. A volume of approximately 50-microlitre suspension was in-

oculated at the conjunctival sac of guinea pig. Reaction was observed for 96 hours and development of keratoconjunctivitis was recorded as a positive reaction.

IV- Polymerase Chain Reaction (PCR) for detection of Shiga-like toxin (stx1 & stx2 genes): PCR was performed on nine different *E. coli* strains (one strain of *E. coli* for each serotype).

1. Materials used for PCR:

1.1. Reagents used for agrarose gel electrophoresis:

1.1.1. Agarose powder, Biotechnology grade (Bioshop[R], Canda inc. lot No: OE16323).

It prepared in concentration 2% in 1× TAE buffer.

1.1.2. Tris acetate EDTA (TAE) electrophoresis buffer (50×liquid concentration) (Bioshop[R], Canda inc. lot No: 9E11854).

The solution diluted to 1× by adding 1 ml stock solution to 49 ml bidistilled water to be used in the preparation of the gel or as a running buffer.

1.1.3. Ethidium bromide solution (stock solution) biotechnology grade (Bioshop ® Canda Inc, Lot No: 0A14667):

The stock solution was diluted by 25µl /200ml bidistilled water and stored covered at 4ºC. It was used for staining of PCR products that electrophoreses on agarose gel to be visualized by UV light.

1.2. Gel loading buffer (6×stock solution)(Fermentas, lot No: ooo56239).

The components were dissolved in sterile bidistilled water and stored covered with aluminum foil at room temperature.

1.3. DNA ladder (molecular marker): 185 & 160 bp (Fermentas, lot No: 00052518).

1.4. 5X Taq master (Fermentas):

Containing polymerase enzyme, Magnesium chloride (Mg Cl_2), Deoxy nucleotide triphosphate (dNTP) and PCR grade water.

1.5. Shiga toxin (*stx*1 & *stx*2) primer sequences of *E. coli* used for PCR identification system **(Fagan *et al.,* 1999):**

It had the amplicon length of 185 bp at which any *E. coli* producing *stx*1 could be identified. The sequence of this primer as follow Table (1):

PRIMER NAME	TARGET GENE	OLIGONUCLEOTIDE SEQUENCE (5′ → 3′)
1Slt224	*stx*1	ATG TCA GAG GGA TAG ATC CA
1Slt385	*stx*1	TAT AGC TAC TGT CAC CAG ACA AT

However, the sequence of the primer used for identification of *E. coli* producing *stx*2 at amplicon length of 160 bp is shown in the following Table (2):

PRIMER NAME	TARGET GENE	OLIGONUCLEOTIDE SEQUENCE (5′ → 3′)
2Slt537	*stx*2	AGT TCT GCG TTT TGT CAC TGT C
2Slt678b	*stx*2	CGG AAG CAC ATT GCT GAT T

2. DNA preparation from bacterial culture **(McKillip and Drake, 2000):**

An overnight bacterial culture (200µl) was mixed with 800µl of distilled water and boiled for 10 min. The resulting solution was centrifuged and the supernatant was used as the DNA template.

3. DNA amplification reaction of *E. coli* **(Pass *et al.,* 2000):**

Multiplex PCR was used for demonstration of suspected *E. coli* isolates. The bacterial genomic DNA samples were amplified by PCR in a reaction mixture (25µl) containing 13.25 sterile distilled H_2O, 2.5µl 10 x

buffer, 0.63µl 10mMNTPs, 1µl 25Mm Mgcl$_2$, 1.25 µl primer F(20pmol/ml) , 1.25 µl primer R(20 pmol/ml) and fill up to 25 µl PCR grade water. The PCR protocol consisted of the following steps: An initial denaturation (2 min at 95°C) for 30 cycles, primer denaturation (1 min at 95°C) 1 cycle, primer annealing (1 min at 57°C), primer extension (2 min at 72°C) and a final elongation (5 min at 72°C). The PCR products were electrophoresed in 2.5% agarose gel and stained with ethidium bromide.

RESULTS

Table (3): Prevalence of coliforms isolated from 101 clinical mastitis cow's milk samples.

E. coli		Enterobacter aerogenes		Klebsiella pneumoniae		Total of coliforms	
No	%	No.	%	No.	%	No.	%
18	17.82	3	2.97	1	0.99	22	21.78

Table (4): Relationship between different serogroups and phenotypic virulence factors of *E. coli* isolated from clinical mastitic cow's milk samples.

Serogroups	No.	Serum resistance	Congo Red binding	Invasiveness activity	Haemolytic activity
		No. of +ve (%)	No. of +ve (%)	No. of +ve (%)	No. of +ve (%)
O111:H4	3	3	3	0	0
O26	2	2	1	0	1
O127:H6	3	3	3	2	0
O126	2	2	2	0	0
O119:H6	1	1	0	0	0
O114:H21	1	1	1	1	0
O55:H7	1	1	1	1	0
O44:H18	1	1	1	1	0
O124	1	1	1	1	0
Untyped	3	0	0	0	0
Total	18	15 (83.3%)	13 (72.2%)	6 (33.3%)	1 (5.6%)

Table (5): *Stx*1 and *stx*2 genes profile of different nine *E. coli* strains isolated from clinical mastitic cow's milk samples.
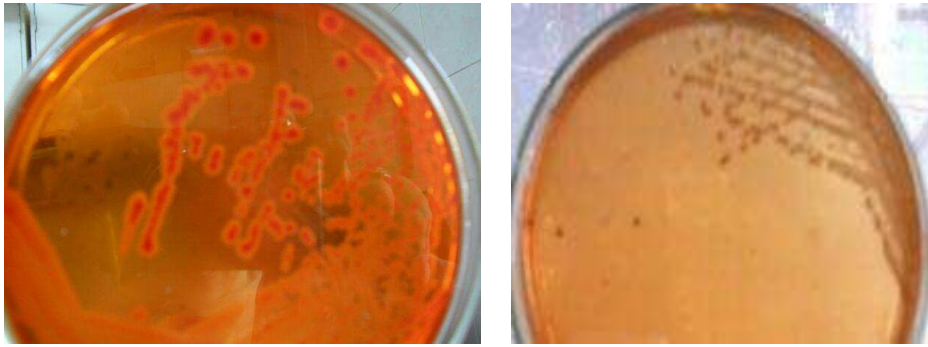
| Virulence genes	Positive serogroups	Number of +ve serogroup (%)
*stx*1	O26, O44:H18, O55:H7, O111:H4, O114:H21, O119:H6, O126 and O127:H7	8 (88.9%)
*stx*2	O26, O111:H4, O126 and O124	4 (44.4%)
*stx*1and *stx*2	O26, O111:H4 and O126	3 (33.3%)

Fig. (1): Serum resistance test.

**A): control negative, (green colour).**

B): positive serum resistance *E. coli* isolate, (yellow colour).

Fig. (2): Congo Red binding test. Left: Congo Red positive *E. coli* isolate (red colonies). Right: Congo Red negative *E. coli* isolate (grayish-white colonies).
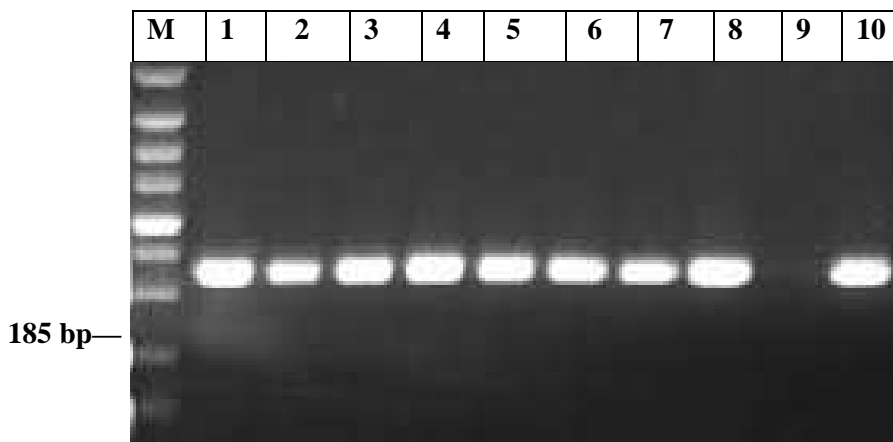
185 bp—

Fig. (3): Agarose gel electrophoresis of PCR amplification products using Shiga toxin 1 (*stx*1) primers of *E. coli* (1Slt224 and 1Slt385). Lane M: 185 bp ladder as molecular DNA marker. Lane 1: Control positive for *stx*1 producing *E. coli.* Lane 2 (*E. coli* O26), Lane 3 (*E. coli* O44), Lane 4 (*E. coli* O55), Lane 5 (*E. coli* O111), Lane 6 (*E. coli* O114), Lane 7 (*E. coli* O119), Lane 8 (*E. coli* O126) and Lane 10 (*E. coli* O127): Positive *E. coli* for *stx*1 production. Lane 9 (*E. coli* O124): Negative *E. coli* for *stx*1 production.
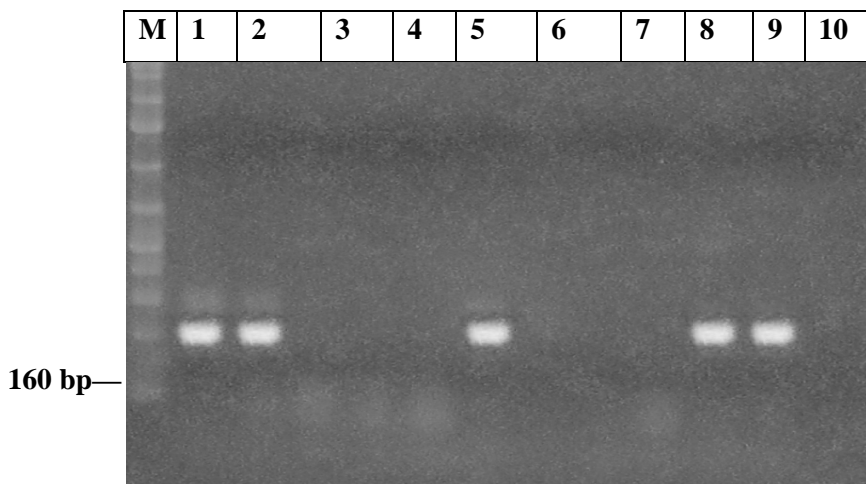
160 bp—

Fig. (4): Agarose gel electrophoresis of PCR amplification products using Shiga toxin 2 (*stx*2) primers of *E. coli* (2Slt537 and 2Slt678b).Lane M: 160 bp ladder as molecular DNA marker. Lane 1: Control positive for *stx*2 producing *E. coli.* Lane 2 (*E. coli* O26), Lane 5 (*E. coli* O111), Lane 8 (*E. coli* O126) and Lane 9 (*E. coli* O127): Positive *E. coli* for *stx*2 production. Lane 3 (*E .coli* O44), Lane 4 (*E. coli* O55), Lane 6 (*E. coli* O114), Lane 7 (*E. coli* O119), Lane 10 (*E. coli* O124): Negative *E. coli* for *stx*2 production.

DISCUSSION

Gram-negative bacteria are the main etiological agents of most of severe clinical cases of mastitis. Many studies have implicated coliforms as most common Gram-negative bacteria pathogens isolated from cases of bovine mastitis (**Oliver, 1988; El-Khodery and Osman, 2008**). In this study, out of 101 milk samples of clinically mastitic cows, coliforms were detected in twenty two milk samples with an incidence of 21.78% (Table 3). The higher incidence of coliforms (80.36%) was detected by **El-Khodery and Osman (2008)** in buffaloes. Dairy cattle with acute coliforms mastitis, caused primarily by *E. coli* (**Wenz et al., 2006; Blum and Leitner, 2013**). *E. coli* is the most important coliforms that has received more attention due to its high incidence relatively to other mastitis pathogens (**Amira et al., 2013**). The frequently isolated coliforms, in the present study, were *E. coli* followed by *Enterobacter aerogenes* and *Klebsiella pneumoniae* (17.82, 2.97 and o.99%, respectively), as shown in Table (3). *E. coli* was the most common coliforms species identified in milk samples from cows with mild to moderate clinical mastitis, followed by *Klebsiella spp.* and *Enterobacter spp.* (**Schukken et al., 2011**). This result of *E. coli* in close agreement with previous findings; 15.9, and 18.47% by **Ericsson et al. (2009) and Kalmus et al. (2011)** from cases of clinical mastitis, respectively. Variable incidences of *E. coli* among clinically mastitic cows were recorded: 34.7% (**Bradley and Green, 2001**); 32% (**Longo et al., 2001**); 21.05% (**El-Mahrouki et al., 2006**); 39.9% (**Dalia and Amany, 2007**); 34.48% (**Rangel and Marin, 2009**) and 26.7% (**Breen et al., 2009**). The higher prevalence of isolation *Klebsiella pneumoniae* was recorded previously as: 6.57% **El-Mahrouki et al. (2006)**; 35.6% **El-Khodery and Osman (2008)** and 4.2% **Ericsson et al. (2009)**. The higher prevalence of *Klebsiella pneumoniae* may be attributed to the nature of environment which encourages the multiplication of such organism, where the use of rice straw as a bedding that was contaminated and harbored coliforms organisms particularly *Klebsiella spp.* (**El-Khodery and Osman, 2008**).

Serogrouping of *E. coli* was carried out to give an idea about the most important and predominant srogroups associated with clinical mastitic cases. In the present study, the obtained results showed that isolated *E. coli* belonged to nine different serovars O111:H4 (3), O127:H6 (3), O26 (2), O126 (2), O119:H6 (1), O114:H21 (1), O55:H7 (1), O44:H18 (1), O124 (1) and three untyped (Table 4). These results cleared that among the serotyped strains of *E. coli* there was no predominant serogroup. This result agreed with **Moussa et al. (2006) Wenz et al. (2006); El-Mahrouki et al. (2006); Fernandes et al. (2011) and Amira et al. (2013).** They recorded that *E. coli* strains recovered from mastitis cases belonged to different serogroups and varied greatly in O groups and may not be attributed to epizootic strains. This indicates that *E. coli* mastitis is not caused by a limited number of specific pathogenic strains, but seems to be associated with environmental fecal contamination and be multifactorial (**Rangel and Marin, 2009**).

Differentiation of pathogenic strains from normal flora strains depends on the identification of virulence characteristics. Virulence factors associated with strains of *E. coli* include adhesions, toxins, cell wall, capsule production, and serum resistance (**Gyles, 1993**). In the present study virulence factors were discussed because it thought to play an important role in pathogenicity of *E. coli*. Serum resistance was the most common virulence factor detected in this study, where 15 strains [O111:H4 (3), O127:H6 (3), O26 (2), O126 (2), O119:H6 (1), O114:H21 (1), O55:H7 (1), O44:H18 (1), O124 (1)], (83.3%) out of 18 *E. coli* strains were serum resistant as shown in Table (4) and Fig. (1), and this agrees with other researchers **DaRocha et al. (2002); Kaipainen et al. (2002); Zaki et al. (2004; Fernandes et al. (2011) and Amira et al. (2013),** (88.9, 95, 87.5, 96.2, and 79.37%, respectively). Moreover **Zaki et al. (2004) and Amira et al. (2013**) suggest that serum resistance is the only characteristic that could be related to virulence in *E. coli* strains isolated from bovine intramammary infections.

Congo red agar test could be used as a detective of virulence for *E. coli* strains and distinguished between virulent and avirulent *E. coli* strains (**El-Mahrouki et al., 2006 and**

Sharma *et al.,* 2006). The addition of an iron source or galactose to CR agar medium enhances absorption of CR dye by some isolates of *E. coli* **(Panigrahy and Yushen 1990).** In present investigation Congo Red binding ability showed that 13 strains[O111:H4 (3), O127:H6 (3), O26 (1), O126 (2), O114:H21 (1), O55:H7 (1), O44:H18 (1), O124 (1)], (72.2%) out of 18 *E. coli* strains could be bind actively Congo Red dye, Table (4) and Fig. 2. Congo Red binding activity of isolated *E. coli* strains was detected by many authors **Berkhoff and Vinal (1986); Panigrahy and Yushen (1990); Zaki *et al.* (2004); El-Mahrouki *et al.* (2006); Sharma *et al.* (2006); Dalia and Amany (2007) and Amira *et al.* (2013)** with an incidences of 50, 61.9, 33, 46, 47.42, 48.71 and 38.1%, respectively. Many studies were conducted to determine the correlation between Congo Red binding ability and virulence for *E. coli* strains and many of these studies emphasized that there is strong correlation between expression of Congo Red binding phenotype and virulence of *E. coli* **(Salman, 1999). Sharma *et al.* (2006)** reported that CRA test was 100% specific, and this test can be used for primary screening of non-invasive *E. coli* from potentially invasive *E. coli.* On the other side, some authors found that Congo Red binding activity did not correlate well with pathogenicity **(Zaki *et al.,* 2004).**

The term invasive *E. coli* referred to those strains of *E. coli* which able to induce keratoconjunctivitis in eyes of Guinea pigs **(Sereny, 1955).** In the present study 6 isolates [O126:H6 (2), O114H21 (1), O55:H7 (1), O44:H18 (1) and O124 (1)], (33.3%) of 18 *E. coli* strains were had ability to induce keratoconjunctivitis in eyes of Guinea pigs, (Table 4). *E. coli* strains were found to be invasive with an incidence 70, 58.69, 40.5% among *E. coli* strains recovered from clinical mastitis by **Zaki *et al.* (2004); Sharma *et al.* (2006) and Amira *et al.* (2013).** This result is in contrast to that found by **Mosherf (2004)** who found no invasive *E. coli* strains isolated from cases of mastitis.

In the present study one isolate [O26], (5.6%) out of 18 *E. coli* strains had haemolytic activity (Table 4). **Zaki *et al.* (2004) and Amira *et al.* (2013)** found that 12.5% and 13.5% of isolated *E. coli* strains were haemo-lytic, respectively. While **Gad El-Said (2005)** recorded that 81.25% of *E. coli* serotyped showed haemolytic activity.

Shiga like toxin-producing *E. coli* (STEC) strains are considered to be the most important pathogens between recently emerged groups of food borne strains. This type of strain is a major cause of gastroenteritis and also can be responsible for hemorrhagic colitis (HC) or the hemolytic uremic syndrome (HUS), the major cause of acute renal failure in children **(Beutin *et al.,* 2004).** Domestic ruminants, especially cattle, sheep and goats, have suggested being the principal reservoirs of STEC strains that cause human infections **(Zschock *et al.* 2000).** In the present work, PCR analysis of nine *E. coli* strains (one strain for each serogroup) demonstrated the presence of *stx*1 and *stx*2 genes in eight *E. coli* serogroups [O26, O44:H18, O55:H7, O111:H4, O114:H21, O119:H6, O126 and O127:H7], (88.9%) and four *E. coli* serogroups [O26, O111:H4, O126 and O124], (44.4%) in the examined nine *E. coli* serogroups, respectively; also *stx*1 and *stx*2 genes were detected together in three *E. coli* isolates [O26, O111:H4 and O126], (33.3%) in the examined nine *E. coli* serogroups, Table (5) and Fig. 3 & 4. These results were agreed with **Momtaz *et al.* (2012)** who found that *stx*1 gene had the highest prevalence in *E. coli* isolated from bovine mastitic milk. Also twenty (8.6%) out of 231 *E. coli* strains were detected to harbor the Shiga-toxin genes [8 (40%) the *stx*1 gene; 12 (60%) *stx*2 gene and non of both], **Rangel and Marin (2009).** *Stx*1 and *stx*2 genes were detected by **Lira *et al.* (2004); Dalia and Amany (2007); Galal, *et al.* (2013)** in bovine mastitic milk. While *stx*2 gene only was detected by **Amira *et al.* (2013).** On the contrary, others had mentioned that *E. coli* strains isolated from cows with clinical mastitis are negative for both *stx*1 and *stx*2 genes **(Murinda *et al.,* 2004; Wenz *et al.,* 2006; Güler and Gündüz, 2007).**

Conclusions: The results obtained in the present study indicate that the pathogenic role of *E. coli* in bovine mastitis is not consequence of specific virulence factors. *E. coli* isolates usually posses one or more virulence factors that may help in establishment at the infection site and subsequently causing clinical bovine mastitis.

REFERENCES

Amira, El-Sayed Lamey; Ammar, A. M.; Zaki, E. R.; Norhan, K.; Badea S. M. and Refai, M. K. (2013): Virulence factors of *Escherichia coli* isolated from recurrent cases of clinical and subclinical mastitis in buffaloes. International J. of Microbiological Research, 4 (1): 86-94.

Berkhoff, H. A. and Vinal, C. A. (1986): Congo red medium to distinguish between invasive and noninvasive *Escherichia coli* pathogenic for poultry. Avian Diseases, 30(1): 117-121.

Beutin, L.; Krause, G.; Zimmermann, S.; Kaulfuss, S. and Gleier, K. (2004): Characterization of Shiga toxin-producing *Escherichia coli* strains isolated from human patients in Germany over a 3-year period. J. Clin. Microbiol. 42:1099-1108.

Beutin, L.; Montenegro, M. A.; Orskov, I.; Orskov, F.; Prada, J.; Zimmermann, S. and Stephan, R. (1989): Close association of verotoxin (Shiga like toxin) production with enterohaemolysin production in strains of *E. coli.* J. Clin. Microbiol. 27(11):2559-2564.

Blum, S. E. and Leitner, G. (2013): Genotyping and virulence factors assessment of bovine mastitis *Escherichia coli*. Vet. Microbiol., 163(3-4):305-312.

Bradley, A.J. and Green, M.J. (2001): Aetiology of clinical mastitis in six Somerest dairy herds. Vet. Rec., 148(22):683-686.

Breen, J. E.; Green, M. J. and Bradley, A. J. (2009): Quarter and cow risk factors associated with the occurrence of clinical mastitis in dairy cows in the United Kingdom. J Dairy Sci., 92(6): 2551–2561.

Burvenich, C.; Van Merris, V.; Mehrzad, J.; Diez-Fraile, A. and Duchateau, L. (2003): Severity of *E. coli* mastitis is mainly determined by cow factors. Vet. Res., 34(5):521- 564.

Dalia, M. Mohsen and Amany, N. Dapgh (2007): Detection of gene sequence of *E. coli* toxins isolated from mastitic buffalo and cows milk. Vet. Med. J. Giza, 55(2): 451-467.

DaRocha, A.C.; DaSilva, A.B.; DeBrito, A.B.; Moraes, H. L.; Pontes, A.P.; Ce, M.C.; DoNascimento, V.P. and Salle, C.T. (2002): Virulence factors of avian pathogenic *E. coli* isolated from broilers from the South of Brazil. Avian Dis., 46(3):749-753.

El-Khodery, S. A. and Osman, S. A. (2008): Acute coliform mastitis in buffaloes (*Bubalus bubalis*): Clinical findings and treatment outcomes. Trop. Animal Health and Production, 40(2): 93-99.

El-Mahrouki, A. M.; Nevine, M. Sobhy and Aggour, M. G. (2006): Detection of coliform mastitis in cattle with special reference to molecular characterization of enterotoxigenic *E. coli* using polymerase chain reaction (PCR). J. Egypt. Vet. Med. Ass., 66(1): 47-58.

Ericsson, U. H.; Lindberg, A.; Persson, W. K.; Ekman, T.; Artursson, K.;Nilsson-Ost M. and Bengtsson, B. (2009): Microbial aetiology of acute clinical mastitis and agent-specific risk factors. Vet. Microbiol., 137(1-2):90-97.

Fagan, P.; Hornitzky, M.; Bettelheim, K. and Djordjevic, S. (1999): Detection of Shiga-like toxin (*stx1* and *stx2*), intimin (*eaeA*), and enterohemorrhagic *E. coli* (EHEC) hemolysin (EHEC *hlyA*) genes in animal feces by multiplex PCR. Appl. Environ. Microbiol. 65:868-872.

Fernandes, JB. C.; Zanardo, L. G.; Galvão, N.N.; Carvalho, I. A.; Nero, L. A. and Moreira, M.A. S. (2011): *Escherichia coli* from clinical mastitis: serotypes and virulence factors. J. of Vet. Diagnostic Investigation, 23(6) 1146–1152.

Gad EL-Said, W. A.; El-Jakee, J. K.; Xandel, M. M. and Mona, A. El-Shabrawy (2005): Presence of E. coli O157:H7 in raw milk and meat samples. J. Egypt Vet. Med. Ass., 65(3): 341-350.

Galal, H. M.; Hakim, A. S. and Sohad, M. D. (2013): Phenotypic and virulence

genes screening of *Escherichia coli* strains isolated from different sources in delta Egypt. Life Science Journal, 10(2):352-361.

Güler, L. and Gündüz, K. (2007): Virulence properties of *Escherichia coli* isolated from clinical bovine mastitis. Turk. J. Vet. Anim. Sci., 31(5): 361-366.

Gyles, C. L. (1993): *Escherichia coli*. In: Gyles, C. L., Thoen, C.O. (Eds.). Pathogenesis of bacterial infections in animals. Iowa State University Press, Ames, Iowa, pp. 164-187.

Hogan, J. S and Smith, K. L. (2003): Coliform mastitis. Vet. Res., 34:507-519.

John, M.; Jacques, M. and Lariviere, S. (1989): Pathogenicity of *E.coli* O115:K V165 strains isolated from pigs with diarrohea. Am. J. Vet. Res., 50(1): 1029-1036.

Kaipainen, T.; Pohjanvitra, T.; Sphigel, N. Y.; Shwimmer, A.; Pyolara, S. and Pelkonen, S. (2002): Virulence factors of *E. coli* isolated from bovine clinical mastitis. Vet. Microbiol., 85(1):37-46.

Kalmus, P.; Aasmäe, B.; Kärssin, A.; Orro, T. and Kask, K. (2011): Udder pathogens and their resistance to antimicrobial agents in dairy cows in Estonia. Acta Vet Scand. 53: 4.

Kaper, J. B.; Nataro, J. P. and Mobley, H. L. (2004): Pathogenic E. coli. Nat. Rev. Microbiol., 2:123–140.

Kok, T.; Worswich, D. and Gowans, E. (1996): Some serological techniques for microbial and viral infections. In Practical Medical Microbiology (Collee, J.; Fraser, A.; Marmion, B. and Simmons, A., eds.) 14th ed., Edinburgh, Churchill Livingstone, UK.

Koneman, E. W.; Allen, S. D.; Dowell V. R. and Sommer H. M. (1983): The Enterobacteriaceae, in: Color Atlas and Textbook of Diagnostic Microbiology, J.B. Lippincott Company, New York, USA, pp. 57– 124.

Lehtolainen, T. (2004): *E. coli* mastitis: Bacterial factors and host response, Ph. D. thesis Department of clinical veterinary

sciences, Faculty of veterinary Medicine, Helsinki, University, Finland.

Lira, W. M.; Macedo, C. and Marin, J. M. (2004): The incidence of Shiga toxin-producing *Escherichia coli* in cattle with mastitis in Brazil. J.of Applied Microbiology, 9: 861–866.

Longo, F.; Salat, O. and Gool, V.F. (2001): Incidence of clinical mastitis in French dairy herds: epidemiological data and economic costs. Folia Veterinaria, 45: 45-46.

McKillip, J. and Drake, M. (2000): Molecular beacon polymerase chain reaction detection of *Escherichia coli* O157:H7 in milk. J. Food Protect. 63: 855-859.

Momtaz, H.; Dehkordi, F. S.; Taktaz, T.; Rezvani, A. and Yarali, S. (2012): Shiga toxin-producing *Escherichia coli* isolated from bovine mastitic milk: Serogroups, virulence factors, and antibiotic resistance properties. The Scientific World Journal, Article ID 618709, 9 pages.

Mosherf, B. E. (2004): Studies on microbial causes of mastitis in buffaloes, Ph. D. thesis (Microbiology), Fac. Vet. Med. Cairo Univ.

Moussa, I. M.; Mostafa, M. and Mohamed, K. F. (2006): Determination of phylogenetic relationships among *E. coli* isolates recovered from bovine fecal and milk samples. J. Egypt. Vet. Med. Ass., 66: 7-25.

Murinda, S.E.; Nquyen, L.T.; Landers, T.L.; Draughon, F.A.; Mathew, A.G.; Hogan, J.S.; Smith, K.L.; Hancock, D.D. and Oliver, S.P. (2004): Comparison of *Escherichia coli* isolates from humans, food and farm and companion animals for presence of Shiga toxin-producing *E. coli* virulence markers. Foodborne Pathogens and Disease, 1(3): 178-184.

Oliver, S.P. (1988): Frequency of isolation of environmental mastitis-causing pathogens and incidence of new intramammary infection during the non-lactating period. American Journal of Veterinary Research, 49(11): 1789-1793.

Panigrahy, B. and Yushen, L. (1990): Differentiation of pathogenic and non pathogenic *Escherichia coli* isolated from poultry. Avian Dis., 34: 941-943.

Pass, M., Odedra, R. and Batt, R. (2000): Multiplex PCR for identification of *Escherichia coli* virulence genes. J. Clin. Microbiol. 38:2001-2004.

Quesnell, R. R.; Klaessig, S.; Watts, J. L. and Schukken, Y. H. (2012): Bovine intramammary *Escherichia coli* challenge infections in late gestation demonstrate a dominant anti-inflammatory immunological response. J. Dairy Sci., 95(1):117-126.

Quinn, P. J.; Carter, M. E.; Markey, B. and Carter, G. R. (2004): Clinical veterinary microbiology. 6th ed., Mosby, Edinburgh, London, New York, Philadelphia, St. Louis, Sydney, Toronto.

Rangel, P. and Marin, J. M. (2009): Analysis of *Escherichia coli* isolated from bovine mastitic milk. Pesq. Vet. Bras. 29(5):363-368.

Salman, A. M. (1999): Serological and bacteriological studies on *Escherichia coli* in chicken. Ph. D. Thesis (Microbiology), Fac. Vet. Med., Cairo Univ.

Schukken, Y.H,; Bennett, G.J.; Zurakowski, M.J.; Sharkey, H.L.; Rauch, B.J.; Thomas, M.J.; Ceglowski, B.; Saltman, R.L.; Belomestnykh, N. and Zadoks R.N. (2011): Randomized clinical trial to evaluate the efficacy of a 5-day ceftiofur hydrochloride intramammary treatment on non severe gram-negative clinical mastitis. J. Dairy Sci., 94(12):6203-6215.

Sereny, B. (1955): Experimental shigella keratoconjunctivitis. Acta Microbiol. Acad. Sci. Hung., 2: 293-296.

Sharma, K. K.; Soni, S. S. and Meharchandani, S. (2006): Congo red dye test as an indicator test for detection of invasive bovine *Escherichia coli* – short communication. Veterinarski Arhiv, 76(4):363-366.

Taylor, P. w. (1983): Bacteriocidal and bacteriolytic activity of serum against Gram negative bacteria. Microbial. Rev., 47: 46-83.

Timmis, K. N. (1979): Plasmid gene that specifies resistance to the bactericidal activity of serum. In Plasmids of medical, environmental and commercial importance. Edited by K. N. Timmis & Puhler, A. (Elsevier, North Holland Biomedical Press, New York, 145-153.

Wenz, J. R.; Barrington, G. M.; Garry, F. B.; Ellis, R. P. and Magnuson, R. J. (2006): *E. coli* isolates, serotypes, genotypes and virulence genes and clinical coliform mastitis severity. Journal of Dairy Science, 89(9): 3408-3412.

Zaki, E.R.; Riad, E. M. and Sobhy, N. M. (2004): Correlation between *E. coli* serotypes isolated from buffalo mastitic milk with different virulence patterns. J. Egypt Vet. Med. Assoc., 64: 53-63.

Zschock, M.; Hamann, H. P.; Kloppert, B. and Wolter, W. (2000): Shiga-toxin producing *E.coli* in faeces of healthy dairy cows, sheep and goats: prevalence and virulence properties. Lett. Appl. Microbiol. 31:203-208.

الملخـص العربـي

إسهام ميكروبات الكوليفورم في إحداث التهاب الضرع في الأبقار، مع الإشارة إلي التصنيف السيرولوجي وتحديد عوامل الضراوة لعترات الميكروب القولوني المعزولة

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يعتبر الميكروب القولوني الأكثر شيوعاً في مجموعة ميكروبات الكوليفورم المسببة لالتهاب الضرع في الأبقار. حيث تم عزل ١٨ عترة (١٧،٨٢%) من الميكروب القولوني يليها الأنتيروباكتر أيروجينز ٣ عترات (٢،٩٧%) ثم ميكروب الكليبيسيلا نيموني عترة واحدة (٠،٩٩%) من ١٠١ عينة لبن لأبقار إصابة بالتهاب الضرع الإكلينيكي. تم أجراء الفحص السيرلوجي لعترات الميكروب القولوني حيث تبين أنها تنتمي إلي تسع مجموعات هي O111:H4(3), O127:H6(3), O26(2), O126 (2), O119:H6 (1), O114:H21 (1), (1) O124 and (1) O44:H18 , (1) O55:H7 وثلاث عترات لم يتم تصنيفها. بإجراء اختبارات الضراوة لعترات الميكروب القولوني المعزولة (١٨ عترة), وجد ١٥ عترة (٨٣،٣%) مقاومة للمصل و١٣ عترة (٧٢،٢%) لديها القدرة علي الارتباط بصبغة الكونغو الأحمر و6 عترات (٣٣،٣%) لديها القدرة علي أحداث التهاب ملتحمة العين في خنازير غينيا وعترة واحدة (٥،٦%) لديها المقدرة علي تحليل الدم. وباستخدام تفاعل البلمرة المتسلسل علي تسع عترات مختلفة للميكروب القولوني (عترة واحدة لكل مجموعة), وجد ٨ عترات (٨٨،٩%) تحتوي علي الجين stx1 و٤ عترات (٤٤،٤%) تحتوي علي الجين stx2. كما أسفرت النتائج عن ثلاث عترات (٣٣،٣%) تحتوي علي الجينين stx1 and stx2 معا. الخلاصــــة: عترات الميكروب القولوني المعزولة لديها واحد أو أكثر من عوامل الضراوة التي تمكنها من إحداث التهاب الضرع الإكلينيكي.