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 (stx1 and stx2 genes) on the nine different strains (one strain for each serogroup), where stx1 and stx2 were found in 8 (88.9%) and 4 (44.4%) of the nine examined strains, respectively. While stx1 and stx2 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, stx1 and stx2.

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 interdependently 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 vero- toxin 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 complement 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, invasive ness 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 (stx1 & stx2).

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 methylene 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 in oculted 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 and 72 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 5×10³ 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 agarose gel electrophoresis:

1.1.1. Agarose powder, Biotechnology grade (Bioshop®, 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®, 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.

1.2. Gel loading buffer (6×stock solution)(Fermentas, lot No: 00056239).

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 Cl₂), Deoxy nucleotide triphosphate (dNTP) and PCR grade water.

1.5. Shiga toxin (stx1 & stx2) 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 stx1 could be identified. The sequence of this primer as follow Table (1):

<table>
<thead>
<tr>
<th>PRIMER NAME</th>
<th>TARGET GENE</th>
<th>OLIGONUCLEOTIDE SEQUENCE (5’ → 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1Slt224</td>
<td>stx1</td>
<td>ATG TCA GAG GGA TAG ATC CA</td>
</tr>
<tr>
<td>1Slt385</td>
<td>stx1</td>
<td>TAT AGC TAC TGT CAC CAG ACA AT</td>
</tr>
</tbody>
</table>

However, the sequence of the primer used for identification of E. coli producing stx2 at amplicon length of 160 bp is shown in the following Table (2):

<table>
<thead>
<tr>
<th>PRIMER NAME</th>
<th>TARGET GENE</th>
<th>OLIGONUCLEOTIDE SEQUENCE (5’ → 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2Slt537</td>
<td>stx2</td>
<td>AGT TCT GCG TTT TGT CAC TGT C</td>
</tr>
<tr>
<td>2Slt678b</td>
<td>stx2</td>
<td>CGG AAG CAC ATT GCT GAT T</td>
</tr>
</tbody>
</table>

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 H₂O, 2.5µl 10 x...
buffer, 0.63µl 10mM NTPs, 1µl 25Mm MgCl₂, 1.25 µl primer F(20 pmol/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.

<table>
<thead>
<tr>
<th>E. coli</th>
<th>Enterobacter aerogenes</th>
<th>Klebsiella pneumoniae</th>
<th>Total of coliforms</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>18</td>
<td>17.82</td>
<td>3</td>
<td>2.97</td>
</tr>
</tbody>
</table>

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

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

Table (5): Stx1 and stx2 genes profile of different nine E. coli strains isolated from clinical mastitic cow’s milk samples.

<table>
<thead>
<tr>
<th>Virulence genes</th>
<th>Positive serogroups</th>
<th>Number of +ve serogroup (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>stx1</td>
<td>O26, O44:H18, O55:H7, O111:H4, O114:H21, O119:H6, O126 and O127:H7</td>
<td>8 (88.9%)</td>
</tr>
<tr>
<td>stx2</td>
<td>O26, O111:H4, O126 and O124</td>
<td>4 (44.4%)</td>
</tr>
<tr>
<td>stx1 and stx2</td>
<td>O26, O111:H4 and O126</td>
<td>3 (33.3%)</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>M</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
</table>

**Fig. (3):** Agarose gel electrophoresis of PCR amplification products using Shiga toxin 1 (stx1) primers of *E. coli* (1Slt224 and 1Slt385). Lane M: 185 bp ladder as molecular DNA marker. Lane 1: Control positive for stx1 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 stx1 production. Lane 9 (*E. coli* O124): Negative *E. coli* for stx1 production.

<table>
<thead>
<tr>
<th>M</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
</table>

**Fig. (4):** Agarose gel electrophoresis of PCR amplification products using Shiga toxin 2 (stx2) primers of *E. coli* (2Slt537 and 2Slt678b). Lane M: 160 bp ladder as molecular DNA marker. Lane 1: Control positive for stx2 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 stx2 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 stx2 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 0.99%, 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 serogroups 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
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), O114:H21 (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 haemolytic, 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 stx1 and stx2 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 stx1 and stx2 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 stx1 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 stx1 gene; 12 (60%) stx2 gene and non of both], Rangel and Marin (2009). Stx1 and stx2 genes were detected by Lira et al. (2004); Dalia and Amany (2007); Galal, et al. (2013) in bovine mastitic milk. While stx2 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 stx1 and stx2 genes (Murinda et al., 2004; Wenz et al., 2006; Guler and Gunduz, 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.
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المختص العربي

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

سيد محمد سيد

باحث أول - معهد بحوث صحة الحيوان (المعمل الإقليمي بأسيوط)

يعتبر الميكروب القولوني الأكثر شيوعاً في مجموعة ميكروبات الكوليفورم المسببة لالتهاب الضرع في الأبقار. حيث تم عزل 18 عرة (17.88%) من الميكروب القولوني ليها الأنيتيروباكتير أروجيانيز 3 عرات (2.97%) ثم ميكروب الكيليسيليا نيموني عرة واحدة (0.99%) من 11 عينة لابار اصابة بالتهاب الضرع الإكلينيكي. تم أجراء الخصوص السيرولوجي لعترات الميكروب القولوني حيث تبين أنها تتنتمي إلى تسبع O111:H4(3), O127:H6(3), O26(2), O126 (2), O119:H6 (1), O114:H21 (1) مجموعات هي (1) O44:H18 (1) and O124 (1) وثلاث عرات لم يتم تصنيفها. بإجراء اختبارات الضراوة لعترات الميكروب القولوني المعزولة (18 عرة)، وجد 15 عرة (82.32%) مقابلة للمصل 12 عرة (72.02%) لديها القدرة على الارتباط بصبغة الكونغرو الأحمر و6 عرات (33.32%) لديها القدرة على أحداث التهاب ملتحمة العين في حِنازير غينيا وعترة واحدة (5.91%) لديها المقدرة على تحليل الدم، وباستخدام تفاعل البلمرة المتسلسل على تسبع عرات مختلفة للميكروب القولوني (عرة واحدة لكل مجموعة)، وجد 8 عرات (80.9%) تحتوي على الجين stx1 و4 عرات (44.44%) تحتوي على الجين stx2. كما أسفرت النتائج عن ثلاث عرات (33.33%) تحتوي على الجينين ومعاً. الخلاصـة: عترات الميكروب القولوني المعزولة لديها واحد أو أكثر من عوامل الضراوة التي تمكنها من إحداث التهاب الضرع الإكلينيكي.