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CHARACTERIZATION OF ANTIMICROBIAL RESISTANT *ESCHERICHIA COLI* ISOLATED FROM MASTITIC COWS AND BUFFALOES

By

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ABSTRACT

Mastitis is one of the most costly production diseases in the dairy industry that is caused by a wide array of microorganisms. *E. coli* is a common cause of bovine mastitis even in well-managed herds and therefore, *E. coli* was targeted in this study. Out of 200 milk samples from cows and buffaloes, 76 resulted in isolation of *E. coli* (38%). Of the recovered isolates, 71were obtained from 160 mastitic cows' milk samples and 5 were from 40 mastitic buffaloes' at Giza governorate of Egypt. *E. coli* isolates were isolated, identified biochemically and confirmed at the molecular level. According to serotyping, the detected *E. coli* serotypes of *E. coli* from cows' milk were O148, O125, O166, O158, O146, O126, O27, O26, O111, O1, O18, O119, O86a, O114, O78, O169, O25, O168, and O157 while serotypes detected from buffaloes' milk were O55, O125, O86a and O124 from buffalo mastitic milk. Antimicrobial susceptibility test was carried out on the isolates using the disk diffusion method to investigate the potential multi drug resistance (MDR) among the isolates. All isolates were resistant to one or more antibiotics. The recovered *E. coli* strains were also screened by polymerase chain reaction to detect certain antibiotic resistance genes (namely; *bla Tem, bla CTX-M, qnr, and Kan genes*) as well as virulence genes (*stx2* And *eae*A).

Key words:

Mastitis, *E. coli*, Serotypes, MDR, PCR, Antimicrobial Resistance Genes, Virulence Genes.

INTRODUCTION

Bovine mastitis is one of the most prevalent, expensive, and devastating infectious diseases in the dairy industry, this is attributed to severe drop in milk Production, increased veterinary expenses due to excessive use of medications, increased culling rate and decreased reproductive efficiency in high producing animals **(Dhakal *et al.*, 2002; Friedman *et al.*, 2004; Singh *et al.*, 2004)**. This poses a potential risk to public health if inadequately treated milk is consumed.

E. coli is a major environmental pathogen causing bovine mastitis **(Timofte *et al.*, 2014).** *E. coli* causes inflammation of the mammary gland in dairy animals around parturition and during lactation with striking local and sometimes systemic clinical symptoms **(El-Khodery *et al.*, 2008; Quesnell *et al.*, 2012).** The sources of *E. coli* infection include bedding materials, soil, manure and other organic matter in the environment of cows **(Hogan and Smith, 2003).** *E.coli* is classified into pathogenic and nonpathogenic types. Pathogenic *E.coli* can be classified into different types based on the pathogenic mechanism and virulence factors into enteropathogenic *E.coli*(EPEC), enterotoxigenic *E. coli* (ETEC), Shiga toxin-producing *E. coli* (STEC or VTEC), and enterohemorrhagic *E. coli* (EHEC) **(Fitzhenry *et al.*, 2002; Blum and leitner, 2013).** Shiga-like toxin producing *E. coli* (STEC) strains play important role in mastitis **(Güler *et al.*, 2007; Kobori *et al.*, 2004).** *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; Amira *et al.*, 2013).** The most common *E. coli* serotypes isolated from mastitic cows and buffaloes produced verotoxin, which is consistent with the hypothesis that verotoxin plays a major role in the pathogenesis of mastitis caused by *E.coli.* The pathogenicity of mastitis probably results from the production of verotoxin or Shiga-liketoxin which efficiency inhibits protein synthesis in mammalian cell free system **(Dalia *et al.*, 2007).** In dairy animals, mastitis is one of the most important reasons for the frequent and persistent use of antimicrobials.Unfortunately, *E.coli* strains resistant to almost all antimicrobial agents in bovine mastitis have been increasingly reported **(Eisenberger *et al.* 2018).** To successfully control infectious diseases of bovines and to prevent the possible hazard linked with bacterial resistance as well as therapeutic failure, it is essential to evaluate antibiotic resistance of pathogenic bacteria **(Authier *et al.*, 2006).** The improper and extensive use of antibiotics in veterinary practices is the main cause of the emerging bacterial resistance **(Sorum *et al.*, 2001)**. Therefore, a commensal bacteria that poses antimicrobial resistance determinants constitute a main resource of resistant genes for highly pathogenic bacteria **(Moyaert *et al.*, 2006).** The associated resistance is due to the fact that the strains of the bacteria become carriers of the factors of resistance to diverse classes of antimicrobials **(Shafiq *et al.*, 2021).** The prominent found multidrug resistance (MDR) was in ESBL type include *blaCTX-M, blaTEM* group b-lactamases found in *E. coli* causing mastitis **(Ali *et al.*, 2017).** Several

virulence factors have been detected in pathogenic *E. coli*. These include toxins, adhesins, invasions and capsule production. Only isolates with successful combinations of virulence factors will be capable of causing disease depending on host defense mechanism **(Fernandes *et al.*, 2011).** In addition, various virulence genes that are important for the pathogenicity in *E. coli* from mastitic cows include genes encoding aerobactin, autoagglutinating adhesion proteins,verotoxins, intimin, and hemagglutinin **(Zhang *et al.* 2018; Obaidat *et al.*, 2018; Tark *et al.*, 2017; Tavakoli *et al.*, 2017; Zhao *et al*., 2018).** Hence, understanding of the resistance and virulence factors of *E. coli* isolates will assist in the treatment and management of bovine mastitis. In this study, the key objective was to determine the accurate information about antimicrobial resistance and virulence determinants and the unconditional association of resistance genes and virulence genes in *E. coli* recovered from mastitic animals**.**

MATERIAL AND METHODS

Collection of the samples:

Two hundred milk samples were collected from mastitic dairy animals (160 cows 40 buffaloes) located in different farms at Giza governorate during the period from December, 2019 till January, 2022). The samples were collected in sterile 50 ml falcon tubes, labeled and transported while cold, to the laboratory **(Quinn *et al.*, 2002)**.

Isolation and identification of *E. coli*:

Upon arrival to the laboratory, milk samples were centrifuged and the sediment were streaked onto the surface of MacConkey's agar plates and incubated aerobically at 37°C for 24 h. Lactose fermenting (Pink colored) colonies were sub-cultured on eosin methylene blue agar (EMB) agar plates. Colonies showing characteristic green metallic sheen on EMB agar were further confirmed biochemically and serologically. Biochemical identification of *E. coli* was based on indole production from tryptaphane, methyl red test, Voges-Proskauer test, citrate utilization test, catales test, sugar fermentation tests, oxidase test, behavior in triple sugar iron agar slopes and urea hydrolysis test **(Quinn *et al.*, 2004)**.

Serotyping of *E. coli* isolates:

Confirmed *E. coli* isolates were serotyped using polyvalent and monovalent *E. coli* antisera sets. This was done in the Serology Unit of at the Animal Health Research Institute (AHRI, Dokki, Giza) according to the method of **Kok *et al.* (1996).**

Briefly, *E. coli* isolates were cultured overnight in Luria-Bertani broth at 37°C. The isolates were serotyped employing the slide agglutination assay using 46"O" antigens **(Schroeder *et al.*, 2002).**

Antimicrobial susceptibility test of *E. coli* serotypes:

E. coli isolates were tested for susceptibility to different antimicrobials using the disc diffusion method **(CLSI, 2018)**. The tested drugs were amoxicillin-clavulanic acid (30µg), ciprofloxacin (5µg), cefotaxime (30µg), ceftazidime (30µg), amikacin (30µg), cefepime (30µg), tobramycin (10µg), imipenem (30µg), meropenem (10µg), norofloxacin (10µg), ampicillin (10µg) and kanamycin (10µg).

Detection of antibiotic resistance genes and virulence genes in *E. coli* strains by multiplex PCR:

DNA extraction:

Bacterial DNA was extracted using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) following the manufacturer instructions. Briefly, 200 µl of the bacterial broth suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

PCR amplification:

Primers were obtained from Metabion International AG **(**Germany**).** Primers' information are listed in (Table 1). Amplifications were performed in 25-µl reaction volumes containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20-picomole concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler. The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using electric gradients of 5V/cm. For gel analysis, 15 µl of the products was

loaded in each gel slot. A gelpilot 100 bp ladder (Qiagen, gm bh, Germany) and gene ruler 100 bp ladder (Fermentas, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table (1): Oligonucleotide primers information.

Gene	Primer sequence (5'-3')	Length of amplified product	Reference
QnrA	**ATTTCTCACGCCAGGATTTG**	**516 bp**	**Robicsek *et al*., 2006**
	GATCGGCAAAGGTTAGGTCA		
Kan	**GTGTTTATGGCTCTCTTGGTC**	**621 bp**	**Frana *et al*., 2001**
	CCGTGTCGTTCTGTCCACTCC		
BlaTEM	**ATCAGCAATAAACCAGC**	**516 bp**	**Colom *et al*., 2003**
	CCCCGAAGAACGTTTTC		
Stx2	**CCATGACAACGGACAGCAGTT**	**779 bp**	**Dipineto *et al*., 2006**
	CCTGTCAACTGAGCAGCACTTTG		
EaeA	**ATGCTTAGTGCTGGTTTAGG**	**248 bp**	**Bisi-Johnson *et al*., 2011**
	GCCTTCATCATTTCGCTTTC		
BlaCTX-M	**ATGTGCAGYACCAGTAARGTKATG GC**	**593 bp**	**Archambault *et al*., 2006**
	TGGGTRAARTARGTSACCAGAAYCAGCGG		

RESULTS

E. coli colonies were pink on MacConkey's agar and green metallic sheen on EMB agar. Microscopically, the bacterial cells were Gram -ve rods. Biochemically, all *E. coli* isolates were lactose fermenters, indole and methyl red, and catalase positive. Meanwhile, they were negative for oxidase, urea hydrolysis, citrate utilization and Voges- Proskauer tests and did not produce H2S in TSI slopes butt.

***E. coli* incidence in milk samples of mastitic animals:**

As depicted in (Table 2) the overall incidence of *E. coli* in milk samples of dairy cows and buffaloes was 38% (76 of 200). Concerning incidences in milk samples of cows and buffaloes, they were 44.4% in cows' samples (71 of 160 samples) and 12.5% in buffaloes' samples (5 of 40).

Table (2): Incidence of *E. coli* in mastitis milk samples.

Animal	Number of milk samples	*E. coli* containing samples
Cows	**160**	**71 (44.4%)**
Buffaloes	**40**	**5 (12.5%)**
Total milk samples	**200**	**76 (38%)**

Serotypes of *E. coli* isolates recovered from milk samples:

Of the *E. coli* isolates recovered in this study, 42 different serotypes were identified. Of the isolates recovered from cows' samples the serotypes O148, O125, O166, O158, O146, O126, O27, O26, O111, O1, O18, O119, O86a, O114, O78, O169, O25, O168, O157 were identified. On the other hand, serotypes identified among buffaloes-originated isolates were O55, O125, O86a and O124 (Table 3).

Antimicrobial susceptibility of *E. coli* serotypes:

Using the disk diffusion method, all tested *E. coli* isolates were resistant to one or more of antibacterial therapeutics **(CLSI, 2018)**. (Table 4) illustrates the results of the assay using 12 different antibacterial agents.

Table (3): Serotypes of *E. coli* isolates recovered from cows and buffaloes' milk samples**.**

***E. coli* Positive samples**	**Serotype**	**Number of isolates**	**% (out the isolates)**
Cow's milk (42)	**O148**	**5**	**11,9**
	O125	**6**	**14.3**
	O166	**1**	**2.4**
	O158	**3**	**7,1**
	O146	**1**	**2.4**
	O126	**3**	**7.1**
	O27	**3**	**7.1**
	O26	**2**	**4.8**
	O111	**1**	**2.3**
	O1	**1**	**2.4**
	O18	**1**	**2.4**
	O119	**2**	**4.8**
	O55	**3**	**7.1**
	O86a	**1**	**2.4**
	O114	**1**	**2.4**
	O78	**1**	**2.4**
	O169	**1**	**2.4**
	O25	**2**	**4.8**
	O168	**1**	**2.4**
	O157	**1**	**2.4**
	Untypable	**2**	**4.8**
Buffaloes' milk (5)	**0125**	**1**	**20**
	055	**1**	**20**
	086a	**2**	**40**
	0124	**1**	**20**

Table (4): Antimicrobial susceptibility test results of *E. coli* strains identified in this study against 12 different agents R: resistantS: susceptible I: intermediate susceptible**.**

Serotype	NOR 10mg	AK 30mg	AMP 10mg	TOB 10mg	AMC 30mg	IBM 10mg	OFX 10mg	FEB 30mg	CAZ 30mg	MRP 10mg	K 30mg	CTX 30mg
O25	R	S	R	S	R	S	S	R	R	S	R	R
O157	R	S	R	S	R	s	R	I	R	S	R	R
O169	R	S	R	S	R	S	S	R	I	S	S	R
O158	R	S	R	S	R	S	R	I	I	S	R	I
O168	R	S	R	R	R	S	R	R	R	S	S	R
O25	R	S	R	`S	R	S	R	R	R	S	R	R
O55	R	S	R	S	R	S	R	R	R	S	R	R
O78	R	S	R	S	R	S	R	R	R	S	R	R
O114	S	R	R	I	R	S	R	I	I	I	S	I
O124	S	I	R	R	R	S	I	R	R	S	S	R
O111	S	S	R	S	R	S	S	R	R	S	S	R
O26	I	S	I	S	R	S	S	S	I	S	S	R
O26*	I	S	I	S	R	S	I	R	R	S	S	R
O1	S	S	S	R	R	I	I	R	R	S	I	R
O18	I	S	I	R	R	I	I	R	R	S	S	R
O68	S	R	S	R	R	S	I	R	R	S	I	R
O27	R	S	R	S	R	S	R	I	R	S	S	I
O125	R	R	R	R	R	S	R	R	R	R	I	R
Total	55%	17%	72%	33%	100%	0%	50%	72%	78%	5%	33%	83%

Multiplex PCR amplification on DNA of *E. coli* serotypes to detect antimicrobial resistance and virulence genes:

PCR amplification of DNA extracted from different serotypes of *E. coli* recovered in this study resulted in the detection of intimin (*eae*A) gene in O55, O124, O157, O158 and O25 serotypes while the *stx*2 gene was not detected in any of them.

The antibiotic resistance gene *Bla*TEM was detected in the O55, O86a, O125, O157, O111, O26, O158, O25 and O168 serotypes. The *Bla*CTX-M gene was detected in the serotype O125 and the *qnr*A gene was detected in the serotypes O55, O124, O86a, O125, O157, O111, O26, O158, O25 and O168 while the *Kan* gene was not detected in any of the tested serotypes (Table 5) and Fig. (1, 2, 3, 4, 5 and 6).

Table (5): PCR amplifications of six virulence and antibiotic resistance genes in ten *E. coli* serogroups.

***E. coli* serogroups**	**The targeted gene**					
	***eae*A**	***Stx*2**	***bla*TEM**	***Bla*CTX-M**	***qnr*A**	***Kan***
O55	+	-	+	-	+	-
O124	+	-	-	-	+	-
O86a	-	-	+	-	+	-
O125	-	-	+	+	+	-
O157	+	-	+	-	+	-
O111	-	-	+	-	+	-
O26	-	-	+	-	+	-
O158	+	-	+	-	+	-
O25	+	-	+	-	+	-
O168	-	-	+	-	+	-

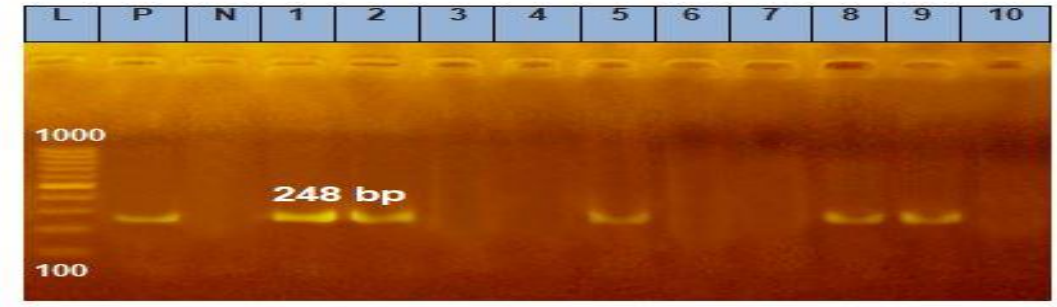

Fig. (1): Results of PCR for amplification of *eaeA* gene of *E. coli* serogroups. The positive result is indicated by a product of 248 pb.

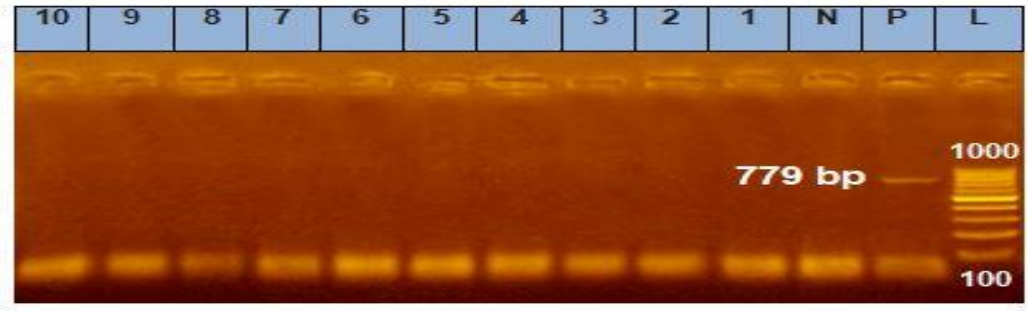

Fig. (2): Results of PCR for amplification of *stx2* gene of *E. coli* serogroups. The positive result is indicated by a product of 779 bp.

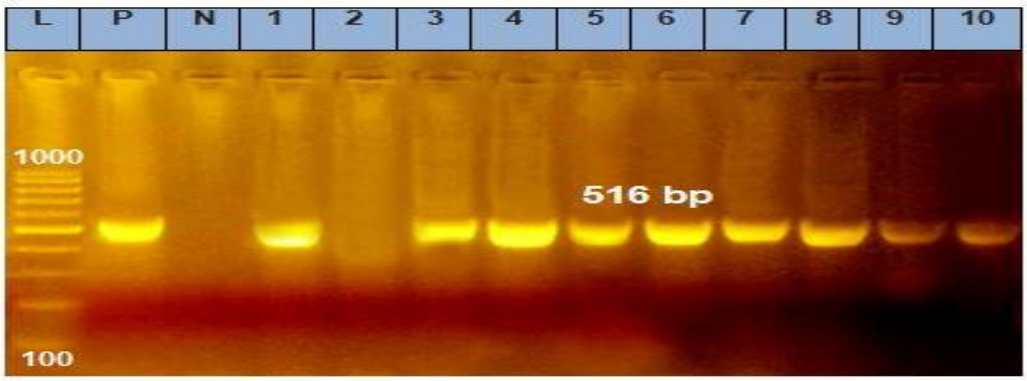

Fig. (3): Results of PCR for amplification of *bla TEM* gene of *E. coli* serogroups. The positive result is indicated by a product of 516 bp.

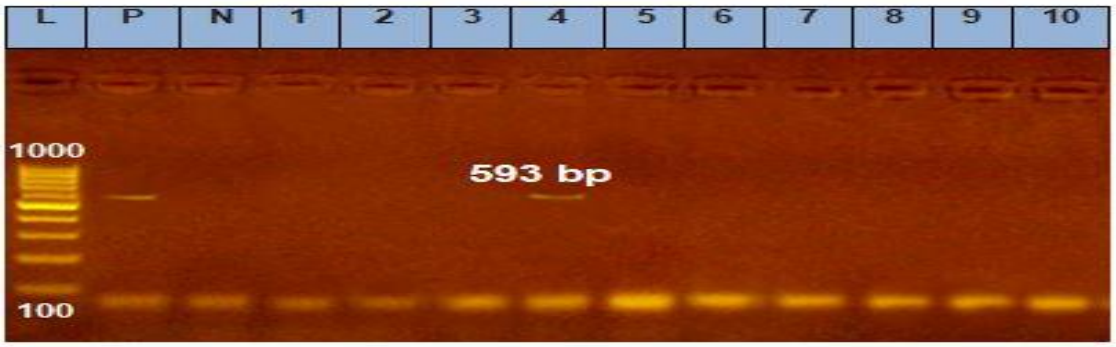

Fig. (4): Results of PCR for amplification of *bla CTX-M* gene of *E. coli* serogroups. The positive result is indicated by a product of 593 bp.

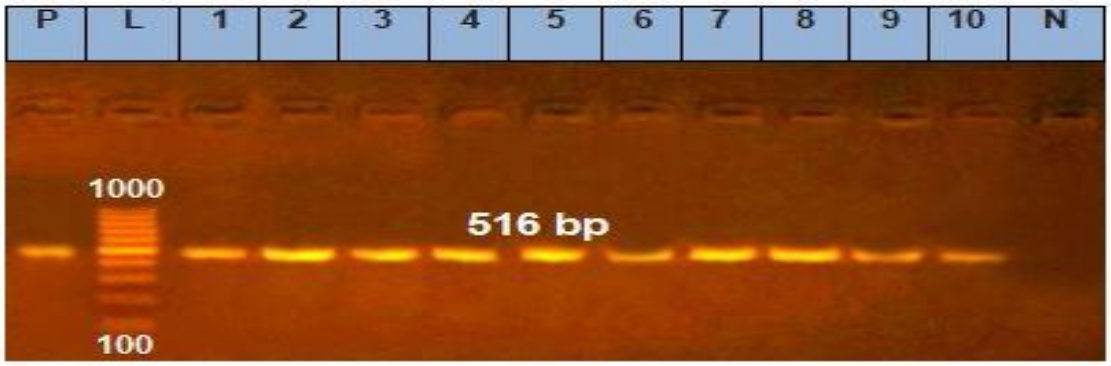

Fig. (5): Results of PCR for amplification of *qnrA* gene of *E. coli* serogroups. The positive result is indicated by a product of 516 bp.

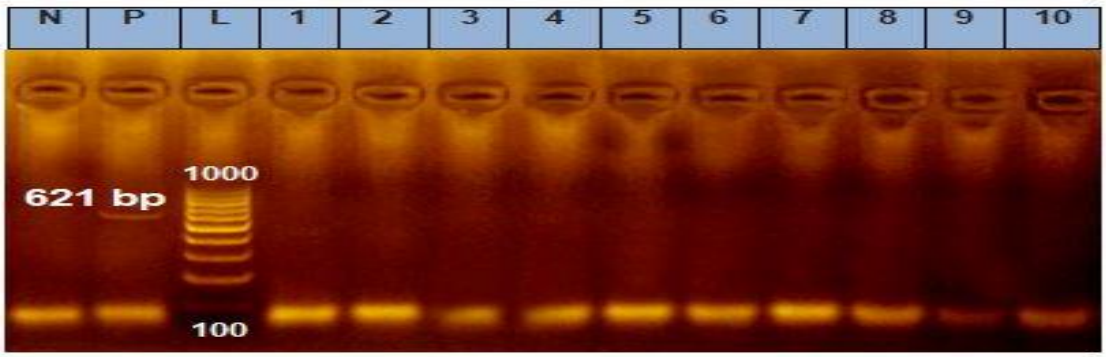

Fig. (6): Results of PCR for amplification of *Kan* gene of *E. coli* serogroups. A product of 621 bp indicates the positive result.

DISCUSSION

Mastitis in lactating cows remains a serious problem and the environmental pathogens are flourishing in some governorates of Egypt through the practice of teat dipping **(Hamouda *et al.*, 2014; Günther *et al.*, 2016; Allam *et al.*, 2017; Hakim *et al.*, 2017).** The pathogenic *E. coli* induces 90% of these infections **(Wang *et al.*, 2008).** In Egypt, coliform is a major inducer of *E. coli* mastitis which is the most important coliform microorganism that has received more attention due to its high incidence relatively to other mastitis pathogens **(Abdel-Fattah *et al.*, 2008)**. The present study recorded that total incidence rate of *E. coli* from mastitic buffaloes and cows was 38% (76 out of 200 milk samples). The incidence was 44.4% among cow samples and 12.5% in buffaloes. These results are consistent to some extend with that obtained by **Elias *et al*. (1991) and Lamey *et al*. (2013).** A higher incidence (90%) was obtained by **Allam *et al.* (2018) and Shafiq *et al.* (2021)** while a lower incidence (13.3%) was reported by others **(Elbably *et al.*, 2013; Lan *et al.*, 2020; Ahmed *et al.*, 2021)**. Of the 76 *E. coli* isolates recovered in this study, 47 serogroups were detected (42 of cow samples and 5 of buffalo samples). The identified serogroups among cows' isolates are O148 (12%), O125 (14.3%), O166 (2.4%), O158 (7.1), O146 (2.4%), O126 (7.1%), O27 (7.1%), O26 (4.8%), O111 (2.4%), O1 (2.4%), O18 (2.4%), O119 (4.8%), O119 (7.1%), O86a (2.4%), O114 (2.4%), O78 (2.4%), O169 (2.4%), O25 (4.8%), O168 (2.4%), O157 (2.4%) and Untyped (4.8%). Serogroups identified amon buffaloes' isolates are O125 (20%), O55 (20%), O86a (40%) and 0124 (20%). Our recorded seropgroups match those reported by **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, varied greatly in O groups, and could not be considered epizootic strains. In addition, nearly similar results were obtained by **Allam *et al.* (2018) and Gamal *et al.* (2018).** Most of the O serogroups have not been detected in cows. Approximately 77.4% of *E. coli* isolates belonged to four different O serogroups (O26, O86, O111, and O127) in Egypt **(Osman *et al.*, 2012).** 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 multifactorial **(Rangel and Marin, 2009).** Antimicrobial resistance in Gram-negative bacteria is rising globally, especially for *E. coli*. More troubling are the increasing reports of MDR pathogenic *E. coli* from food-producing animals, raising worries about animals and public health **(Seiffert *et al.* 2013).** Antimicrobials are commonly used in the control and/or prevention of mastitis. Unfortunately, the misuse of antimicrobials contributes to the emergence of bacterial strains with antimicrobial resistance **(Metzger *et al.*, 2013).** In this study, a high percentage of strains were resistant to amoxicillin-clavulanic acid (100%), followed by cefotaxime (84%), ceftazidime (78%), ampicillin and cefepime (72%), Ciprofloxacin (50%), norofloxacin (55.5%), kanamycin and tobramycin (33%), meropenem (5%), imipenem (0%) as shown in (Table 4). The resistance rate towards ampicillin was higher in our study than that reported by **Zhang *et al.* (2018) and Lan *et al.* (2020)** where the rates were 25.3% and 41.3%, respectively. In contrast, **Shafiq *et al.*, (2021)** found higher rates of *E. coli* resistance against cefotaxime (96%), cefepime (94.76%), ampicillin (93.84%) and ceftazidime (98.15%). **Nüesch-Inderbinen *et al.* (2019)** recorded resistance rates of *E. coli* against ampicillin (22 %), kanamycin (3.7 %) and amoxicillin-clavulanic acid (2.4 %). **Barbour *et al*. (2015)** reported that the resistance rates of *E. coli* isolates to 9 antimicrobials as follows: amoxicillin-clavulanic acid (100%), ceftazidime (100%), norfloxacin (100%), cefotaxime (97%), tobramycin (88%), kanamycin (77%), ciprofloxacin (65%), amikacin (65%) and Cefepime (53%). Broad-spectrum antibiotics, especially aminoglycosides, beta-lactams and quinolones are frequently used in the clinical treatment of mastitis. The continuous use of antibiotics may cause MDR in dairy herds**.** Therefore, it is important to control antibiotic use to prevent the risk of MDR **(Chajęcka *et al.*, 2016; Zurfluh *et al.*, 2014).** PCR based methods, as multiplex PCR is very useful as it allows

the simultaneous detection of several specific genes of each bacterial strain targeted **(Touron *et al.*, 2005)**. In the current study, PCR analysis of some *E. coli* serogroups using *eaeA*, *stx2* primers demonstrated the presence of *eaeA* gene in five out of ten examined *E. coli* strains (50%) representing the serogroups O55, O124, O157, O158 and O25. These results are nearly similar with the reports of other authors **(Clements *et al.*, 2003; Moussa *et al.*, 2006; Lamey *et al.*, 2013)** who detected *eaeA* gene in three out of five examined *E. coli* strains.In other investigations, a lower incidence of *eaeA* genes was reported only in 1% of 100 *E. coli* strains **(Bean *et al.*, 2004**; **Wenz *et al.*, 2006**; **Güler *et al.*, 2007)**. In the present study, the *stx2* gene was not detected in any of the examined strains. Similar findings were reported by **Murinda *et al.* (2004)**, **Solomakos *et al.* (2009) and Lan *et al*. (2020)**. Meanwhile, other authors, detected the *stx2* gene in some *E. coli* strains isolated from cases of mastitis **(Cursons *et al.*, 2005; Kobori *et al*., 2004; Sabry *et al.*, 2006**; **Sayed *et al.*, 2014)**. The detection of *eae* gene in some *E. coli* strains in this study suggested that adhesion of *E. coli* to mammary epithelial cells have a role in pathogenesis of mastitis. The *eae* gene has been considered an enteropathogenic *E. coli* (EPEC) marker implicated in developing countries as the cause of infantile diarrhea **(Nataro *et al.*,1998).** It was suggested that strains of *E. coli* recovered from mastitis cases in dairy cows adhere selectively to part of the cells**(Gaastra *et al.*, 2001; Dogan *et al.*, 2006).** In our study, further genotypic characterization was carried out to detect the major ESBL-encoding genes (*blaTEM*, *blaCTX-M* and Kan genes) and quinolone resistance genes (*qnrA*). The qnr gene was the most prevalent (100%) followed by *blaTEM*, *blaCTX*-M and *Kan* genes that were detected in 90%, 10%, and 0% in the examined *E. coli* strains,respectly. On the other hand, **Shafiq *et al*. (2021)** reported multi-drug resistance in more than 85% of ESBL-producing *E. coli* isolates with the dominance of the *blaCTX-M* gene, followed by the *blaTEM* gene. **Memon *et al.* (2016)** reported the presence of *blaCTX-M* and *qnr* genes in 48% of *E. coli* isolates from mastitis. In addition, **Ahmed *et al*. (2021)** recorded the predominance of one or two types of ESBL(*blaCTX-M* and *blaTEM*) genes in mastitic *E. coli* isolates recovered from mastitic animals while the quinolone resistance gene (*qnrA*) was present in only a few isolates. **Effendi *et al*. (2019) and Ahmed *et al.* (2021)** mentioned that kanamycin resistance in *E. coli* can be connected with the presence of Kan gene.

CONCLUSION

E. coli strains recovered from milk samples of mastitic cows and buffaloes were highly resistance to most of the tested agents. Moreover, important resistance and virulence genes were detected. This is alarming and could be the main reason for the clinical mastitis treatment failure. More studies are necessary to appraise the role of *E. coli* in bovine mastitis infection to develop proper approach for treatment and control of coliform mastitis.

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