Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Emergence and Antimicrobial Resistance Patterns of ESBL-Producing *Escherichia coli* Isolated from Clinical and Subclinical Mastitis Cases in Egyptian Dairy Cattle

Amany M. Mohamed[*1], Mohamed K. Elbayoumy[1], Ahmad M. Allam[1], Hala A. A. Abou-Zeina[1], Marwa B. Salman[2] and Asmaa M. Elfiky[3]

[1]*Department of Parasitology and Animal Diseases, Veterinary Research Institute, National Research Centre, 33 Bohouth St., Dokki, Giza, Egypt.*

[2]*Department of Zoonotic Diseases, National Research Centre, 33 Bohouth St., Dokki, Giza, Egypt.*

[3]*Department of Environmental and Occupational Medicine, Environment and Climate Change Research Institute, National Research Centre, 33 Bohouth St., Dokki, Giza, Egypt.*

Abstract

THE emergence and global spread of ESBL-producing *Enterobacteriaceae* is a growing public health concern. ESBL-producing *E. coli* ESBL-PE poses a significant health risk, particularly with the emergence of new variants carrying bla_{SHV}, bla_{TEM} and bla_{CTX-M} genes. This study looked at antibiotic susceptibility, genotyping, and gene sequencing in order to discover ESBL-PE in milk from cows with subclinical and clinical mastitis in three Egyptian governorates. 186 milk samples were analyzed in this study in order to isolate *E. coli* and identify which ones developed ESBL Antibiotic resistance of ESBL- PE was assessed on Mueller-Hinton agar using 10 different commercial antibiotic disks. Resistance genes were genotyped using (PCR).The obtained sequences were evaluated using Chromas pro1.7 and the maximum-likelihood phylogenetic trees were constructed using MEGA11. *E. coli* was found in (n. 16/57) 28.07% and (n. 27/46) 58.69 %, while (ESBL- PE) were found in (13/57) 22.8% and (7/46) 15.2% with clinical mastitis and subclinical mastitis respectively. ESBL- PE showed greater resistance to ampicillin, amoxicillin and streptomycin at percentage of 91.67% while more sensitive to azithromycin , norfloxacin , and gentamicin by 83.33%. Results indicated that 8.3%, 16.7% and 8.3% of ESBL- PE were susceptible to ciprofloxacin, tetracycline, and amoxicillin. The bla_{TEM} gene was recorded in GenBank as PQ45739. This study detected that ESBL- PE, harboring bla_{SHV} and bla_{TEM} antibiotic resistance genes, were identified in mastitis milk. Consistent monitoring of ESBL- PE and proactive measures are crucial to avoiding the future lay out of resistance genes.

Keywords: Extended-Spectrum β-Lactamases; Antibiotic resistance; Polymerase chain reaction; Genotyping; Sequencing, Mastitis.

Introduction

Mastitis is a common significant cause, considers dangerous infectious disease in dairy manufacture that impacts welfare of the animal, and poses a risk to human health. Diverse bacteria, notably Escherichia coli (*E. coli*) [1], often cause mastitis. Worldwide, mastitis in dairy cattle remains a significant problem. The Mastitis clinical signs marked by inflammation of the udder, which subsequently affects the functions of mammary cells. As a result, it creates warning to cow's health. In addition, it increases disease treatment cost, moreover mastitis cause shortage in production of milk and losses in dairy farmers [2]. *E. coli* is a main reservoir of resistance genes that can lead to treatment failure [3].

Antibiotics are essential tools for combating microbial infections and lowering mortality from infectious diseases. Antimicrobial-resistant (AMR) bacteria have become more common in recent decades, which present an important threat. Research indicates that animals may serve as both vectors and

*Corresponding authors: Amany M. Mohamed, E-mail: amanymmm2010@gmail.com, Tel.: +2 01008775878
(Received 16 August 2025, accepted 28 October 2025)
DOI: 10.21608/ejvs.2025.414660.3050

reservoirs for resistance genes. Plasmids facilitate horizontal transfer of resistance genes to gram-negative bacteria, particularly strains of Enterobacterales. One possible factor contributing to human antimicrobial resistance is the increased antibiotic use in cattle. .The presence of antimicrobial-resistant bacteria and their genes in animals is crucial [4].

AMR poses a significant health challenge, causing an estimated 23,000 deaths annually and costing the U.S. nearly $55 billion each year in societal cost [5]. Globally, at least 700,000-person die annually attributed to AMR. By 2050, this figure could reach up to 10 million according to the WHO organization [6].

AMR in *E. coli* is a serious issue that affects animal and human health. *E. coli* is a common bacterium, which can infect different body systems, including gastrointestinal tract, urinary tract, respiratory tract, and bloodstream. *E coli* acquired, during past years, resistance genes through generation's transfer, where it acts as donor and recipient of these genes [3]. ESBL-PE strains can horizontally transfer resistance genes to both similar and different bacterial type, contributing to the spread of AMR in healthcare settings, communities, and livestock. Thus, rise of ESBL-PE in livestock presents a human health risk, highlighting the interconnectedness of animal, human and environmental health [7].

Extensive antibiotic use in livestock farming contributes to the emergence and spread of AMR in *E. coli*, which can select for resistant strains in the animal gut and contaminate the environment and food product. the transportation of *E. coli* via the fecal-oral route among humans, animals, and environmental sources, which can facilitate the exchange of resistance genes through plasmids or mutations, and the lack of adequate surveillance and monitoring of antimicrobial resistance in *E. coli* isolates from different sources, which can hinder the detection and prevention of outbreaks [6]. The WHO classifies some of these antibiotics as critically important for human medicine, which means that their resistance poses a high risk to public health [8]. Antibiotic resistance, particularly in ESBL-producing bacteria, increases infection-related illness, death, and treatment expenses. The most predominant types of ESBLs are *SHV*, *TEM*, and *CTX*-M [9].

Antimicrobial resistance gene patterns among ESBL-producing *Enterobacteriaceae* vary significantly across countries and regions. In Egypt, previous studies have reported the presence of ESBL-producing *E. coli* in mastitis cases, with identifying $bla_{CTX\text{-}M}$ followed by bla_{TEM} and bla_{SHV} [10]. Similarly, [1] found bla_{TEM} to be the most prevalent, with bla_{SHV} detected in isolates. Research from Malaysia [11] reported a predominance of combined $bla_{CTX\text{-}M}$ and bla_{TEM} genotypes, highlighting regional variation in resistance gene distribution. With an emphasis on gene sequencing, genotyping, and antibiotic susceptibility, this study examined ESBL-PE isolated from animals with (clinical or subclinical) mastitis in three Egyptian Governorates.

Material and Methods

Geographical area of this study and duration of time

This study was conducted between April 2023 and September 2023. Samples were obtained from private farms in three different Egyptian Governorates (Giza, Kaluobia and EL Menofia) as showed in Fig (1). Samples were examined at bacteriological labs in National Research Center (NRC), Giza, Egypt.

Milk samples Collection

One hundred eighty six quarter milk samples were gathered from milking cows located in different three Governorates as mentioned before. The age range of the dairy cows was three to eight years old. All animals were clinically checked through udder palpation and ocular inspection in order to identify any anomalies in the udder and to diagnose clinical mastitis before milk samples from infected animals were collected. While subclinical mastitis was detected via using California mastitis test. A plastic vessel with four shallow wells was used for collecting approximately 2 ml of milk from each udder quarter; then equal amount of alkali reagent (Schalm reagent) was added. A gentle circular motion was applied to the mixtures in horizontal plane for 5 seconds and the different degrees of gel were recorded. After inspection the teats orifices were disinfected using 70% alcohol, and the initial three squirts were eliminated, and Approximately 25 mL of milk was collected from each infected quarter in sterile screw-capped bottles under strict sanitary settings. All the milk specimens were stored in icebox and transfer to the laboratory and saved at 4°C until examination within 3-4 h from collection of the samples [12].

Isolation and identification of E. coli

Samples of milk were incubated for a period of 18 to 24 hours at 37°C, followed by culturing a loopful from each on MacConkey and blood agar. The culture plates were thereafter incubated at 37°C for duration of 24 to 48 hours. Pink colonies on MacConkey agar (lactose-fermenting colonies) suspected as *E. coli* were picked up and streaked on Eosin Methylene Blue (EMB) plate. Gram staining was performed on suspected *E. coli* colonies for confirmation and biochemically validated by IMViC test (indole, methyl red, Voges-Proskauer, and citrate tests), urease and also by triple sugar iron (TSI). *I*solates of *E. coli* were transferred to 20% glycerol for future identification in accordance with [13].

Phenotypes identification of ESBL-PE

Isolates of *E. coli* were cultured on selective MacConkey agar containing 1 mg/L cefotaxime (CFX), followed by ESBL-PE growth for phenotypic ESBL producer screening [14].

Susceptibility Testing

Antibiotic sensitivity was evaluated with the Kirby-Bauer disk diffusion technique [15]. The antibiotic susceptibility of ESBL- PE isolates were done using Mueller-Hinton agar (Hi Media, Mumbai, India) against 10 various antibiotics disks commercially available[ciprofloxacin; CIP (5µg), amoxicillin; AMX (10ug), azithromycin; AZM (15ug), tetracycline; Te (10ug), ampicillin; AM(10ug), cefoxitin; Fox(30ug), streptomycin ;S(10ug), norfloxacin; NX(10ug), erythromycin; E(15 ug), gentamicin CN(10ug). Sensitivity was determined via measurement of inhibition zones and categorized as resistant or sensitive in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [16].

Extraction of isolates DNA

DNA was extracted from bacterial colonies utilizing a GF-1 Bacterial Deoxyribonucleic Acid (DNA) Extraction Kit (Vivantis Technologies, Malaysia) following the instructions manufacturers. In short, bacterial colonies were isolated from the media of the agar and then the bacterial suspension was mixed well with 20 mL of lysozyme. Proteinase K was added to the reconstituted pellets at 65°C with periodic shaking following centrifugation. The addition of 100% ethanol was used as a homogenization phase. Centrifugation was used to cleanse and dry the treated fluid after the spinning column step. For DNA elusion, 50 µL of elusion buffer was used. Samples of DNA were preserved at -20°C until PCR was conducted and the concentrations of genomic DNA were measured with a Nanodrop Microvolume Spectrophotometer (Quawell Q9000CM).

PCR condition and electrophoresis

PCR involved an initial 5-minute denaturation at 95°C, followed by 30 cycles of 30 seconds at 95°C for denaturation, 30 seconds of annealing at each primer's optimal temperature, and a 30-second extension at 72°C, with a final 10-minute extension.. Amplification products were confirmed using1.5% agarose gel stained with Red Safe (Intron, China) and analyzed using an ultraviolet transilluminator. The primer sequences used for identifying ESBL *E. coli* are provided [17] and recorded in table (1). Primers were provided by GeneDireX®, Taiwan. DNA molecular weight marker (100bp DNA Ladder H3 RTU, GeneDireX®, Taiwan) was utilized for estimation the products size. Control positive samples were included and kindly provided from positive isolates previously recorded in GenBank with the following accession numbers MW345819.1 and MW345820.1 for the *E. coli* bla_{TEM} gene and MW295407 for the *E. coli* bla_{SHV} [1].

Analysis of sequencing and phylogenetic

Purified PCR products were sequenced (Macrogen, Korea), assembled using Chromas Pro 1.7 (Technelysium, Australia), and compared to GenBank using the Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Maximum-likelihood phylogenetic trees constructed with MEGA11 version 11.0.13 [18] using 500 bootstrap replications.

Results

E. coli incidence in clinical and subclinical mastitis tested milk samples

A total of 186 of quarter milk samples were gathered from cows in particular dairy farms in the Governorates of Giza, Kaluobia, and EL Menofia, which accounted for 74, 67, and 45 milk samples, respectively .Collected milk samples showed 83 samples of normal milk in incidence of (44.62%), the rest of the samples were infected 57 samples from clinical mastitis cases (30.64%), 46 samples from subclinical mastitis cases (24.73%). Bacteria isolated from collected milk samples during the routine bacteriological protocols of our lab were 43 sample *E. coli* (41.75%), 32 samples *Staphylococcus aureus* (31.07%), 18 samples *Streptococcus agalactia (*17.48%) and 10 samples *Pseudomonas aeruginosa* (9.71%). Forty-three samples of *E. coli* (41.75%), thirty-two strains of *Staphylococcus aureus* (31.07%), 18 strains of *Streptococcus agalactia* (17.48%), and ten strains of *Pseudomonas aeruginosa* (9.71%) were isolated from milk samples. *E. coli* detected in 28.07% of clinical and 58.69% of subclinical mastitis cases (Table 2).

Phenotypic identification

Following phenotypic identification, ESBL-PE was found in 19.4% of the 103 analyzed mastitis milk samples, while non-ESBL-PE was found in 22.33%. According to table (3), non-ESBL-PE was 5.26% from clinical mastitis milk and 43.48% from subclinical mastitis milk, whereas ESBL-P E was 22.8% from clinical mastitis milk and 15.2% from subclinical mastitis milk samples.

Sensitivity test by disk diffusion

Table (4) illustrates the susceptibility of 12 ESBL-PE isolates to 10 antimicrobial agents. ESBL-PE isolates exhibited higher resistance to ampicillin (AM), amoxicillin (AMX) and streptomycin (S) at percentage of 91.67% while it was more sensitive to azithromycin (AZM), norfloxacin (NX), gentamicin (CN) at percentage of 83.33% Results indicated that 8.3%, 16.7% and 8.3% of ESBL-PE were sensitive also to ciprofloxacin, tetracycline, and amoxicillin, respectively.

Results of PCR

The evaluated strains tested positive for ESBL. Table 5 displays the spread of resistance genes bla_{CTX-M}, bla_{CMY-2}-group, bla_{TEM} and bla_{SHV} in ESBL-PE isolates. The blaC$_{MY-2}$-group and bla_{CTX} genes were not detected (Table 6). The PCR products of the isolated bla_{TEM} (H240408-016_F21) was sequenced and was subjected to nucleotide BLAST. The isolate showed 100% homology towards *E. coli* HJMH7 TEM-1 gene for beta-lactamase TEM-1 (Table 6), and the isolate was- phylogenetically closely related to *E. coli* (Figure 3). The phylogenetic tree presented was generated using the MEGA 11 software, based on beta-lactamase gene sequences obtained from our isolates and aligned with closely related sequences retrieved from GenBank. The tree was constructed using the Maximum Likelihood method, with bootstrap analysis performed using 500 replicates to evaluate the robustness of the branching.

Accession number

These sequence data have been submitted to the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) under accession number PQ45739.1

Discussion

Mastitis in dairy cows is regarded as a prevalent infection and a significant issue for the dairy industry and producers. Although other bacteria also contribute to mastitis, *E. coli* is the most prevalent and harmful environmental pathogen [1]. Because of their direct connections to the food chain, producing dairy cows have been shown in recent research to serve as reservoirs and carriers for the transmission of ESBL when animal products are consumed raw or unprocessed milk utilized in industry [19]. Clinical mastitis accounts for 33%-38% of healthcare costs in dairy herds and is the primary reason for antibiotic administration in dairy cattle during lactation, representing one-third of all antibiotics administered to cows [20]. This study found that the *E. coli* isolation rates for clinical and subclinical mastitis were 28.07% and 58.69%, respectively. Although these results are almost in line with earlier research that found 31% of cases of clinical mastitis and 40% of cases of subclinical mastitis in Egypt at 2011 [20]. The greater percentages, however, were found by [1], who reported that the infection rates of *E. coli* isolated from two kinds of mastitis were 80.5% and 85.7%, respectively.

Milk samples from clinical mastitis cases had an ESBL-PE detection rate of 22.8%, whereas subclinical cases had an isolation rate of 15.2% [21], registered that ESBL-PE occurrence was 38.2% in subclinical cases and 39.3% in clinical cases. In this study we found that 19.4% of mastitis milk samples contained ESBL-PE, a result nearly comparable with the 23.53% reported in China [22]. In contrast [11] found that 66.7% samples of milk contained ESBL-PE. In Turkey [23] the percentage of ESBL-PE rose from 33.2% in year 2008 to 48.83% in year 2013.

The global rise in ESBL prevalence is driven by the plasmid-mediated transfer of resistance genes between bacterial strains and species. ESBL genes like bla_{TEM} and bla_{SHV} are plasmid-mediated β-lactamase mutants, whereas others, such as bla_{CTX-M}, result from environmental bacteria. [24].

Antibiotic resistance in mastitis-causing bacteria varies significantly by region, underscoring the need for antibiotic susceptibility testing and regular monitoring. In Gram-negative bacteria, β-lactam resistance mainly results from β-lactamases that hydrolyze the lactam ring, inactivating the antibiotic. [20].

According to antibiotic sensitivity testing, ampicillin, streptomycin, and amoxicillin were the most resistant antibiotics among the 12 isolates, accounting for 91.67% of the total. This incidence of amoxicillin is nearly the same as that recorded by [1] who found also that amoxicillin was extremely resistant at a rate of 100%. Similarly, [25] observed that tetracyline had a 81% incidence of resistance to ESBL-positive isolates, with an 83.33% resistance rate. Additionally, cefoxitin and erythromycin had high resistance rates of 83.33% and 75%, respectively.

This study found that azithromycin, gentamicin and norfloxacin were the most sensitive antibiotics to ESBL-positive isolates, with an incidence of 83.33% and a sensitivity to tetracycline in a percentage of 16.67%, which is the same as that found by [22].

Conclusion

This study highlights the public health relevance of multidrug-resistant ESBL-producing *E. coli* isolated from bovine mastitis cases in Egypt an area with limited molecular surveillance. The identification of bla_{TEM} and bla_{SHV} genes, with 100% identity to human-associated *E. coli* strains, raises concern over potential zoonotic transmission through dairy products. The research also aims to determine the most effective antibiotics for treating both clinical and subclinical mastitis in these governorates as gentamicin, azithromycin, and norfloxacin. By linking animal infections with antimicrobial resistance threats to humans, this research supports the One Health approach and emphasizes the need for integrated monitoring and control strategies to protect both animal and public health.

Acknowledgments

Special thanks to National Research Centre veterinary institute for permitting us to conduct this research.

Funding statement

This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The Ethical Committee approved this study for Medical Research at the National Research Centre of Egypt (approval number 16229) in compliance with local laws and regulations.

TABLE 1. Primer sequences for identifying ESBL *E. coli* (17)

Sequence	Gene	Annealing temp. °C	Amplicon size
F: ATGTGCAGYACCAGTAARGTKATGGC R:TGGGTRAARTARGTSACCAGAAYCAGCGG	bla_{CTX}	60	593 bp
F: GCACTTAGCCACCTATACGGCAG R: GCTTTTCAAGAATGCGCCAGG	bla_{CMY-2}	60	758 bp
F: TGAGTATTCAACATTTCCGTGT R: TTACCAATGCTTAATCAGTGA	bla_{TEM}	53	861 bp
F: CAAAACGCCGGGTTATTC R: TTAGCGTTGCCAGTGCT	bla_{SHV}	53	937 bp

ESBL=Extended spectrum β lactamase, *E. coli*=*Escherichia coli*

TABLE 2. Incidence of *E. coli* in milk from mastitis cases

Bacteria	Clinical mastitis milk samples		Subclinical mastitis milk samples		Total milk samples	
	No (Total number of clinical mastitis samples)	%	No (Total number of subclinical mastitis samples)	%	No (Total number of mastitis samples)	%
isolates of *E. coli*	16 (57)	28.07	27 (46)	58.69	43 (103)	41.74
Negative	41 (57)	71.92	19 (46)	41.30	60 (103)	58.25

TABLE 3. rate of phenotypically identified ESBL-PE

E. coli isolates	Clinical mastitis milk samples		Subclinical mastitis milk samples		Total milk samples	
	Total number	%	Total number	%	Total number	%
ESBL *E. coli*	13 (57)	22.8	7 (46)	15.2	20 (103)	19.4
Non ESBL *E. coli*	3 (57)	5.26	20 (46)	43.48	23 (103)	22.33

TABLE 4. show antibiotic susceptibility of ESBL-PE

Antibiotic agent	*ESBL E. coli* Sensitive		ESBL *E. coli* intermediate		ESBL *E. coli* Resistant	
	No.	%	No.	%	No.	%
Ciprofloxacin (CIP)	1	8.33	7	58.33	4	33.33
Amoxicillin (AMX)	1	8.33	0	0	11	91.67
Azithromycin AZM)	10	83.33	0	0	2	16.66
Tetracycline (TE)	2	16.67	0	0	10	83.33
Ampicillin (AM)	0	0	1	8.33	11	91.67
Cefoxitin (Fox)	1	8.33	1	8.33	10	83.33
Streptomycin (S)	0	0	1	8.33	11	91.67
Norfloxacin (NX)	10	83.33	1	8.33	1	8.33
Erythromycin (E)	0	0	3	25.0	9	75.0
Gentamicin (CN)	10	83.33	2	0	2	16.67

TABLE 5. Prevalence of various resistence genes in isolates of ESBL-PE using PCR

Type of virulence gene	Examined Positive isolates	
	No. (total examined isolates)	%
bla_{TEM}	10 (12)	83.3
bla_{SHV}	11 (12)	91.6
bla_{CTX}	0	0
bla_{CMY}	0	0

TABLE 6. Results of the identification of isolate (PQ45739.1)

Description	Max Score	Total Score	Query Cover	E value	Per. ident	Accession no.
***Escherichia coli* BAGh-N1 *bla*TEM gene for beta-lactamase TEM-57, partial sequence**	110	110	19%	5.00E-19	94.44	LC712755.1
***Escherichia coli* HJMH7 TEM-1 gene for beta-lactamase TEM-1, partial sequence**	99	99	14%	1.00E-15	100	LC759075.1
Shigella sonnei blaTEM-1 gene for extended-spectrum beta-lactamase, partial cds, strain: *S. sonnei*-w44	97.1	97.1	14%	4.00E-15	100	LC310937.1
***Escherichia coli* strain RIGLD-2D9-CRO-F1 TEM-1 (tem-1) gene, partial cds**	97.1	97.1	14%	4.00E-15	100	KP634897.1
***Klebsiella pneumoniae* strain SJ12 broad-spectrum beta-lactamase (*bla*TEM-1) gene, partial cds**	95.3	95.3	13%	1.00E-14	100	MF345939.1
***Shigella sonnei bla*TEM-1 gene for extended-spectrum beta-lactamase, partial cds, strain: *S. sonnei*-w81**	95.3	95.3	13%	1.00E-14	100	LC310939.1
***Klebsiella pneumoniae* strain RK11 extended spectrum beta-lactamase (*bla*TEM) gene, *bla*TEM-116 allele, complete cds**	95.3	95.3	15%	1.00E-14	98.18	KY496567.1
***Klebsiella pneumoniae* strain Co-CRKP7 TEM family class A beta-lactamase (*bla*TEM) gene, partial cds**	95.3	95.3	13%	1.00E-14	100	OQ428237.1
***Francisella philomiragia* strain 14IUHPL001 CO2-regulated beta lactamase TEM-1 (tem1) gene, partial cds**	95.3	95.3	13%	1.00E-14	100	KT781076.1
***Escherichia coli* strain RIGLD-2A5-F2 TEM-1 (tem-1) gene, partial cds**	95.3	95.3	13%	1.00E-14	100	KP634895.1
***Salmonella enterica* subsp. enterica serovar Typhi strain BKQT8S plasmid unnamed, complete sequence**	93.5	93.5	13%	5.00E-14	100	CP160060.1
***Salmonella enterica* subsp. enterica serovar Typhi strain BKQU3X plasmid unnamed, complete sequence**	93.5	93.5	13%	5.00E-14	100	CP160062.1

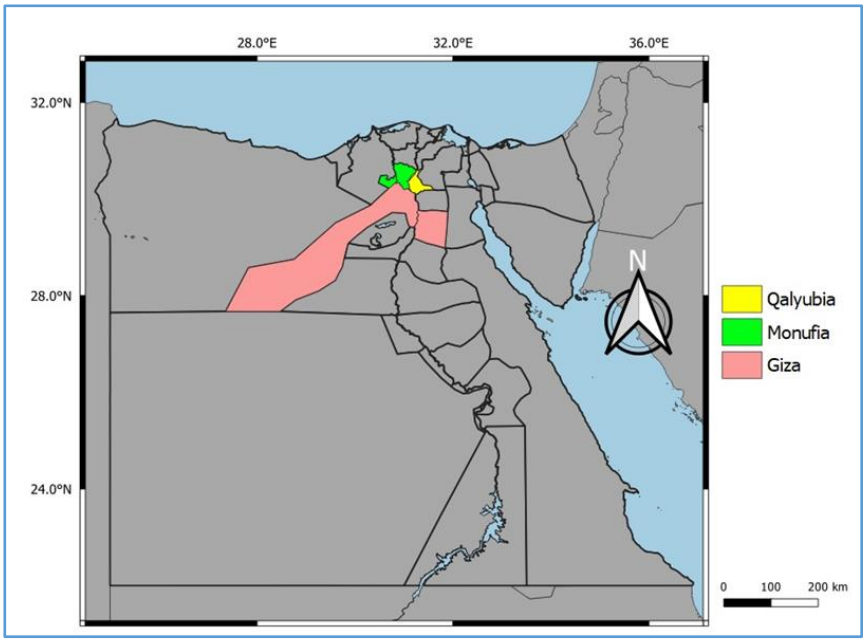

Fig. 1. Map of Egypt displaying Governorates from which milk samples were collected (Giza, Kaluobia and EL Menofia)

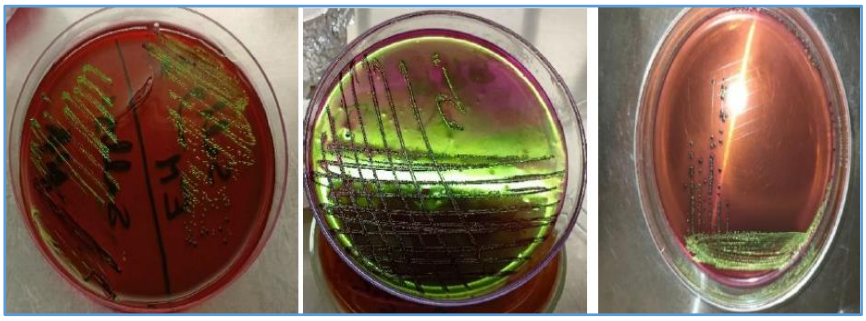

Fig. 2. Show appearance of *E.coli* on eosin methylene blue (EMB) plate

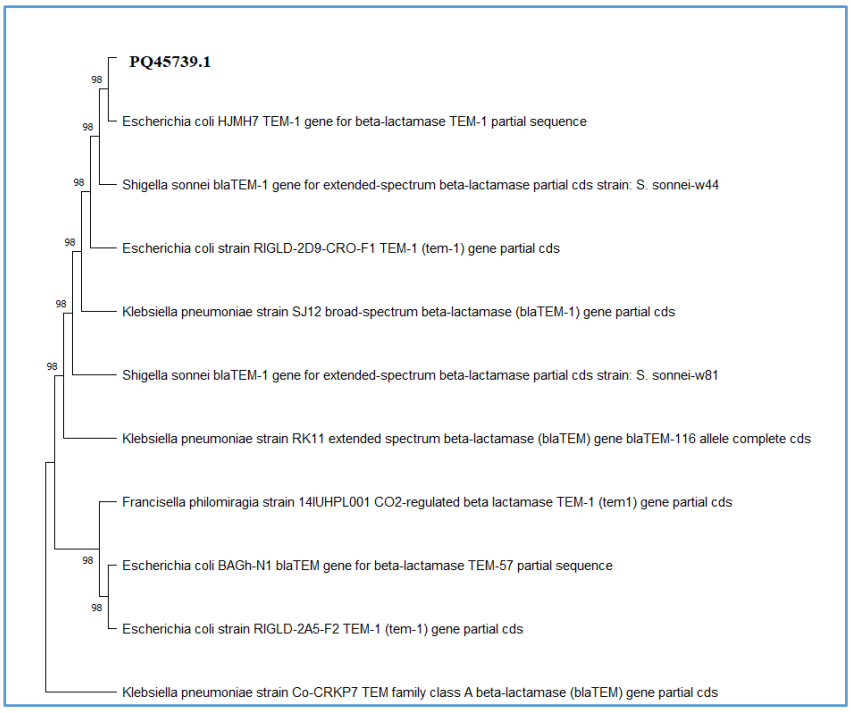

Fig. 3. Phylogenetic tree with the *E. coli* isolate using the MEGA 11 program by The maximum-likelihood method

References

1. Poirel, L., Madec, J-Y., Lupo, A., Schink, A.K., Kieffer ,N., Nordmann, P. and Schwarz, S. Antimicrobial Resistance in *Escherichia coli*., *Microbiology Spectrum*, **6**(4) (2018). https://doi.org/10.1128/microbiolspec.ARBA-0026-2017

2. Tseng, C-H., Liu, C-W. and Liu, P-Y. Extended-Spectrum β-Lactamases (ESBL) Producing Bacteria in Animals, *Antibiotics* **12**, 661 (2023). https://doi.org/10.3390/antibiotics12040661

3. El-Mohandes, S.S., Eid, R.H., Allam, A.M., Abou-Zeina ,H.A.A. and Elbayoumy, M.K. Phenotyping and genotyping studies on extended-spectrum β-lactamase-producing Escherichia coli isolates from mastitic cows on dairy farms in Egypt, *Veterinar World*, **15**, 890–897 (2022). https://doi.org/10.14202/vetworld.2022.890-897

4. Roussel, P., Porcherie, A., Répérant-Ferter, M., Cunha, P., Gitton, C., Rainard, P. and Germon, P. *Escherichia coli* mastitis strains: In vitro phenotypes and severity of infection in vivo. *PLoS One*, **12**(7), e0178285 (2017). https://doi.org/10.1371/journal.pone.0178285

5. Lee, S., Mir, R.A., Park, S.H., Kim, D., Kim, H.Y. Boughton, R.K., Morris, J.G .Jr. and Jeong, K.C., Prevalence of extended-spectrum β-lactamases in the local farm environment and livestock: challenges to mitigate antimicrobial resistance, *Critical Reviews in Microbiology*, **46**(1), 1–14 (2020). https://doi.org/10.1080/1040841X.2020.1715339

6. Mancuso, G., Midiri, A., Gerace, E. and Biondo, C., Bacterial Antibiotic Resistance: The Most Critical Pathogens, *Pathogensn*, **10**(10), 1310 (2021). https://doi.org/10.3390/pathogens10101310

7. Nery Garcia, B.L., Dantas, S.T.A., da Silva Barbosa, K., Mendes Mitsunaga, T., Butters, A., Camargo, C.H. and Nobrega, D.B**.** Extended-Spectrum Beta-Lactamase-Producing Escherichia coli and Other Antimicrobial-Resistant Gram-Negative Pathogens Isolated from Bovine Mastitis: A One Health Perspective, *Antibiotics* (Basel, Switzerland), **13**(5), 391 (2024). https://doi.org/10.3390/antibiotics13050391

8. Gharavi, M.J., Zarei, J., Roshani-Asl, P., Yazdanyar, Z., Sharif, M. and Rashidi, N. Comprehensive study of antimicrobial susceptibility pattern and extended spectrum beta-lactamase (ESBL) prevalence in bacteria isolated from urine samples, *Scientific Reports*, **11**, 578 (2021). https://doi.org/10.1038/s41598-020-79791-0

9. Liebana, E., Carattoli, A., Coque, T.M., Hasman, H., Magiorakos, A.P., Mevius, D., Peixe, L., Poirel, L., Schuepbach-Regula, G., Torneke, K., Torren-Edo, J., Torres, C. and Threlfall, J. Public health risks of enterobacterial isolates producing extended-spectrum β-lactamases or AmpC β-lactamases in food and food-producing animals: an EU perspective of epidemiology, analytical methods, risk factors, and control options, *Clinical Infectious Diseases*, **56**(7), 1030–1037 (2013). doi: 10.1093/cid/cis1043.

10. Ibrahim, E.S.F.H., El-Wahab, A.M.A., Khalil, S.A. and Torky, H.A. ,Prevalence of ESBL producing Enterobacteriacae isolated from bovine mastitis milk, *Alexandria Journal of Veterinary Sciences*, **58** (1), 102-108 (2018). DOI: 10.5455/ajvs.285225

11. Kamaruzzaman, E.A., Abdul Aziz, S., Bitrus, A.A., Zakaria, Z. and Hassan, L., Occurrence and Characteristics of Extended-Spectrum β-Lactamase-Producing Escherichia coli from Dairy Cattle, Milk, and Farm Environments in Peninsular Malaysia, *Pathogens* (Basel, Switzerland) , **9**(12), 1007 (2020). https://doi.org/10.3390/pathogens9121007

12. Amer,S., Galvez, F.L.A., Fukuda, Y., Tada, C., Jimenez, I.L., Valle, W. F.M. and Nakai, Y., Prevalence and etiology of mastitis in dairy cattle in El Oro Province, Ecuador, *Journal of Veterinary Medical Science*, **80**(6), 861–868 (2018). https://doi.org/10.1292/jvms.17-0504

13. Quinn, P.J., Markey, B.K., Leonard, F.C., FitzPatricket, E.S, Fanning, S., Hartiganal, P.J.**,** *Veterinary Microbiology and Microbial Disease* John Wiley & Sons **(**2011).

14. Wilson, G. and McCabe, D. The use of antibiotic-containing agars for the isolation of extended-spectrum beta-lactamase-producing organisms in intensive care units. Clinical microbiology and infection : *the official publication of the European Society of Clinical Microbiology and Infectious Diseases*, **13**(4), 451–453 (2007). https://doi.org/10.1111/j.1469-0691.2006.01667.x

15. Hudzicki, J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. American Society for Microbiology (2009).

16. CLSI 2020 Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 30th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA (2020).

17. Weiner, M., Rózańska, H., Kubajka, M., Szulowski, K., Krajewska, M., Wasiński, B.,Occurrence and characterisation of MRSA and extended-spectrum ß-lactamases producing *Escherichia coli* isolated from mastitic cows' milk, *Bulletin of the Veterinary Institute in Pulawy*, **59**,191–195 **(**2015). https://doi.org/10.1515/bvip-2015-0029

18. Kar, D., Bandyopadhyay, S., Bhattacharyya, D. and Samanta, I., Molecular and phylogenetic characterization of multidrug resistant extended spectrum beta-lactamase producing Escherichia coli isolated from poultry and cattle in Odisha, India, *Infection, Genetics and Evolution*, **9**, 82–90 (2015). https://doi.org/10.1016/j.meegid.2014.11.003

19. Liu, G., Ding, L., Han, B, Piepers, S. and Naqvi, S.A., Characteristics of *Escherichia coli* Isolated from Bovine Mastitis Exposed to Subminimum Inhibitory Concentrations of Cefalotin or Ceftazidime, *Biomed. Res. Int.*, 1628 eCollection (2018). https://doi.org/10.1155/2018/4301628

20. Ahmed, A.M. and Shimamoto, T. Molecular characterization of antimicrobial resistance in Gram-negative bacteria isolated from bovine mastitis in Egypt, *Microbiology and Immunology*, **55**, 318–327 (2011). https://doi.org/10.1111/j.1348-0421.2011.00323.x

21. Eisenberger, D., Carl, A., Balsliemke, J., Kämpf, P., Nickel, S., Schulze, G. and Valenza, G., Molecular Characterization of Extended-Spectrum β-Lactamase-Producing Escherichia coli Isolates from Milk Samples of Dairy Cows with Mastitis in Bavaria, Germany, *Microbial Drug Resistance*, **24**, 505–510 (2018). https://doi.org/10.1089/mdr.2017.0182

22. Ali,T., Ur Rahman, S., Zhang, L., Shahid, M., Zhang, S., Liu, G., Gao, J. and Han, B., ESBL-Producing Escherichia coli from Cows Suffering Mastitis in China Contain Clinical Class 1 Integrons with CTX-M Linked to ISCR1, *Frontiers in Microbiology*, **7**, 1931 (2016). https://doi.org/10.3389/fmicb.2016.01931

23. Tekiner, İ.H. and Özpınar, H., Occurrence and characteristics of extended spectrum beta-lactamases-producing Enterobacteriaceae from foods of animal origin, *Brazilian Journal of Microbiology*, **47**(2), 444–451 (2016). https://doi.org/10.1016/j.bjm.2015.11.034

24. Overdevest, I., Willemsen, I., Rijnsburger, M., Eustace, A., Xu, L., Hawkey, P., Heck, M., Savelkoul, P., Vandenbroucke-Grauls, C., van der Zwaluw, K., Huijsdens, X. and Kluytmans, J., Extended-spectrum β-lactamase genes of *Escherichia coli* in chicken meat and humans, The Netherlands, *Emerging Infectious Diseases*, **17**(7), 1216–1222 (2011). https://doi.org/10.3201/eid1707.110209

25. Abro, S.M., Sahito, J.K., Soomro, A.A., Mirani, A.H., Memon, M.Z. and Kalhoro, N.H. Detection of extended-spectrum beta-lactamase genes among *Escherichia coli* isolates of buffalo mastitis milk. *Ecological Genomics and Genetics*, **33**, 100297 (2024). https://doi.org/10.1016/j.egg.2024.100297

أنماط ظهور ومقاومة مضادات الميكروبات لبكتيريا الإشيريشيا كولاى المنتجة لمثبطات إنزيم بيتا لاكتاماز المعزولة من حالات التهاب الضرع السريرية وشبه السريرية في الأبقار الحلوب المصرية

أمانى محمد محمد محمد[1]، محمد كرم البيومي[1]، أحمد محمد علام[1]، هالة عبد الله أحمد أبو زينة[1]، مروة بدوي سلمان[2] و أسماء محمد أحمد الفقي[3]

[1] قسم الطفيليات وأمراض الحيوان، معهد البحوث البيطرية، المركز القومي للبحوث، الجيزة، مصر.

[2] قسم الأمراض المشتركة بين الإنسان والحيوان، معهد البحوث البيطرية، المركز القومي للبحوث، الجيزة، مصر.

[3] قسم الطب البيئي والمهني، معهد أبحاث البيئة وتغير المناخ، المركز القومي للبحوث، الجيزة، مصر.

الملخص

أن ظهور وانتشار البكتيريا المعوية المنتجة لـ ESBL على مستوى العالم يشكل مصدر قلق متزايد بشأن الصحة العامة، حيث تشكل خطرًا صحيًا كبيرًا خاصة مع ظهور متغيرات جديدة تحمل جينات مقاومه للمضادات الحيوية مثل bla_{SHV} و bla_{TEM}، bla_{CTX-M}. تناولت هذه الدراسة حساسية المضادات الحيوية، والنمط الجيني، وتسلسل الجينات من أجل اكتشاف البكتيريا المعوية المنتجة لـ ESBL في حليب الأبقار المصابة بالتهاب الضرع السريري وتحت السريري في ثلاث محافظات مصرية. تم تحليل عدد 186 عينة حليب في هذه الدراسة من أجل عزل "الإشيريشيا كولاى "، وتحديد أي منها قام بتطوير مقاومة ضد المضادات الحيوية التى تم تقييمها على أجار مولر هينتون باستخدام 10 انواع مختلفة من المضادات الحيوية المستخدمة فى علاج حالات التهاب الضرع عالمياً. تم تحديد النمط الجيني لجينات المقاومة باستخدام تفاعل البلمرة المتسلسل (PCR)، كما تم تقييم التسلسلات التي تم الحصول عليها باستخدام Chromas pro1.7 وتم بناء الأشجار التطورية ذات الاحتمالية القصوى باستخدام MEGA11. تم العثور على "الإشيريشيا كولاى " في (n. 16/57) بنسبة 28.07٪ و (n. 27/46) بنسبة 58.69٪، بينما تم العثور على "الاشيريشيا كولاى المنتجة ل "الطيف الموسع بيتا لاكتاماز"" في (57/13) بنسبة 22.8٪ و (46/7) بنسبة 15.2٪ مع التهاب الضرع السريري والتهاب الضرع تحت السريري على التوالي. أظهرت الاشيريشيا كولاى المنتجة ل "الطيف الموسع بيتا لاكتاماز" مقاومة أكبر للأمبيسيلين والأموكسيسيلين والستربتومايسين بنسبة 91.67٪ بينما كانت أكثر حساسية للأزيثروميسين والنورفلوكساسين والجنتاميسين بنسبة 83.33٪. أشارت النتائج إلى أن 8.3% و16.7% و8.3% من بكتيريا الاشيريشيا كولاى المنتجة ل "الطيف الموسع بيتا لاكتاماز" كانت حساسة للسيبروفلوكساسين وللتتراسيكلين والأموكسيسيلين. كشفت هذه الدراسة عن وجود بكتيريا الاشيريشيا كولاى المنتجة ل "الطيف الموسع بيتا لاكتاماز"، التي تحمل جينات مقاومة المضادات الحيوية bla_{SHV} و bla_{TEM} ، في حليب الأبقار المصابة بالتهاب الضرع السريري وتحت السريري و تم تسجيل جين و bla_{TEM} في بنك الجينات برقم PQ45739.1. يُعدّ الرصد المستمر لبكتيريا الاشيريشيا كولاى المنتجة ل "الطيف الموسع بيتا لاكتاماز" واتخاذ التدابير الاستباقية أمرًا بالغ الأهمية لتجنب ظهور جينات المقاومة مستقبلًا.

الكلمات الدالة: الاشيريشيا كولاى المنتجة لـ "الطيف الموسع بيتا لاكتاماز"، مقاومة المضادات الحيوية، تفاعل البلمرة المتسلسل، النمط الجيني، التسلسل، التهاب الضرع.