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Isolation of *Escherichia coli* from Clinical Mastitis of Dairy Cattle in Diyala Governorate

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Abstract:

Background: Mastitis is worldwide distributed disease that affects farm animals especially cattle and cause economic losses such as decreased milk production.

Aims: The current study aimed to determine the percentage of separated *Ecoli* infection in dairy cattle in Diyala governorate. The study conducted between 20July to 20 October, 2024. **:** Isolation of *Escherichia coli* by using MacConkey and EMB agers then the isolates were confirmed by PCR method depending on the 16s ribosomal gene

R**esults**: The results demonstrated that there were 20 samples in the clinically-diagnosed mastitis milk revealed positive infections with *Escherichia coli* (about 40% of the total samples that collected), while there were 30 samples of clinically-dignosed mastitis showed negative results (about 60% of total collected samples). *Escherichia coli* isolates were shown positive results for 16s ribosomal gene

Conclusions: *Escherichia coli* is a common cause of clinical mastitis in the cattle with high prevalence in comparison with other causative agents.

Keyword: *Escherichia coli*, mastitis, cattle

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Introduction:

Escherichia coli (*E. coli*) are gram negative bacteria that are present as normal habitat organism or as pathogenic bacteria which contribute to wide range of disease of human and animals. About 91% of human and animals have different *E coli* strains in their intestinal tract. *E. coli* strains are outnumbered by anaerobic or facultative bacteria; they constitute the predominant aerobic microorganisms in the human and animal intestinal tracts. Additionally, *E coli* strains were the first bacterial species that localized in the intestinal tract of new born (Secher et al., 2016).

Bovine mastitis is the disease with the high negative effects on dairy farms that affect health of the herd productivity causing economic losses in all around the world because it leads to reduction in milk yield, quality and quantity of milk, and increase in the therapeutic costs (Down et al., 2017). The mastitis is either: clinical form, which is characterized by abnormal udder consistency such as painful when touched, hardness, altered milk contents (e.g., pus, blood, necrotic tissue or debris and abnormal milk color), systemic signs such as an elevation of body temperature, anorexia, loss of body condition and finally death in sever untreated; or subclinical mastitis, which is characterized by normal udder and milk appearance but there is an elevation in somatic cell count within the milk (Adkins and Middleton, 2018). Mastitis is one of a most dangerous health issue that affected dairy animals, it was worldwide distributed problem that affect dairy cattle and impaired the farm economy and dairy industries especially countries that depend on the dairy products (Kumar et al., 2013). The Bactria and viruses are the most causative bacteria of mastitis, manifested by inflammation of the glandular tissue of the udder (McDougall, 2002). These defects lead to several disorders such as animals health problems, minimal input and reduced milk quality (Hiitio et al., 2017). One of the most causative agent of clinical mastitis is *E coli* that is environmental pathogen and present in animal houses (Vangroenweghe et al., 2020); and thus, it was called mammary pathogenic *E coli*. Due to their antigenic variation among the *E coli* strains there were eight phylogenetic groups (b, b 1, a ,c ,d ,e , f and g) most *E coli* is represented within the phylogenic group (A and B) (Zhang et al., 2018). Additionally, *E. coli* is responsible for the most bacterial pathogenicity that causes clinical mastitis and merits (Mohammed, 2024).

Recently, polymerase chain reaction (PCR) method that have rapid, sensitive, specific and relatively relyable results is used for the detection and identification of *E coli* in samples like milk (Haftu et al., 2012). This method is used for identification of *E coli* and other bacteria depending on the 16s

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ribosomal gene that acts as vital gene for several bacterial species (Lindstedt et al., 2018). This study aimed to determine the presence of *Ecoli* infection in dairy cattle in the Diyala governorate.

Materials and Methods:

Clinical mastitis was diagnosed in caws using their milk at the milking times by observing any anomalies, including flakes, blood, pus, or color changes. The initial milk streams were visually examined in a black-backed strip cup. This study involved 50 milk samples. The teat ends were then cleaned with cotton pads soaked in 70% alcohol, and 15 mL of milk was gathered into a sterile plastic vial following the application of the premilking hygiene procedures (stripping, predipping, and drying of teats with paper towels) were completed. Samples were then transported into the lab for microbiological culturing while being stored in ice boxs. Milk samples were collected from cows in the Diyala Governorate between 20 July 2024 and 20 September 2024. The Scientific Ethical Committee of the College of Veterinary Medicine, University of Diyala, Iraq, has approved this study (Approval no: Vet Medicine (205), August 2024, O and W.

Isolation of *E. coli*

Fifty milk samples were transferred into nutrition broth using an inoculation loop and incubated under aerobic conditions for 18 hours. The resultant growth was cultured on MacConkey agar and Eosin Methylene Blue agar and incubated under aerobic conditions for onother18 hours.

DNA template preparation by boiling method.

DNA template was prepared by boiling method as described by (Ali et al., 2018). Briefly, 5 isolated colonies of overnight growth bacteria were suspended thoroughly in 2 ml distilled water and boiled in a water bath, for 10 min. After centrifugation, the supernatant was used as template DNA for the PCR.

PCR method for identification of *E.coli* isolates.

The PCR amplification procedure for the molecular detecting of local isolates of *E coli* was performed as in the following:

Final volume for PCR mixture was 25 µl (12.5 of master mix 2x, 5 µl template DNA), 1 µl primer (the oligonucleotide primers which in lyophilization status were dissolved and diluted first in free nuclease D.S.D.W to obtains 100 picomol/µl), then this stock was diluted in free nuclease D.D.W to obtain nearly 10 picomol/µl. This technique was applied on all primers in this study, as listed in table (2). The specifications primers of all genes for each forward and reverse primer provided from Alpha DNA Company, USA (Table 1). Finally, 5.5 µl nuclease free water was used in uniplex PCR

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eppendorf tubes with amount changed in multiplex PCR and mixed briefly via vortex, then been placed in thermocycler polymerase chain reaction. Annealing gradients were set, where appropriate, and gradually increased from 52°C to 62°C. The program used for each PCR mixture was illustrated in the table (2).

Table 1: Primer oligonucleotide sequences used in identification of *E.coli*

Gene	Sequences (5'-3')	Product size/bp	Reference
16S rRNA	F: 5′-GTGCCAGCAGCCGCGCTAA-3′	850	(Maikalu et al., 2023).
	R: 5′- AGACCCGGGAACGTATTCAC-3′		

Table 2: Amplification program of PCR .

Amplified gene	Initial denaturation	No. of cycle	Denaturation	Annealing	Elongation	Final extension
16S rRNA	94°C/ 5min	40	94°C/ 45 sec	50°C/45 sec	72°C/45 sec	72°C/7min

Results:

E coli bacteria were isolated from milk of mastitis-diseased cows (Figure 1). Out of 50 milk samples, 20 samples (40%) were yielded positive infection with *E.coli* on MacConkey and Eosin Methylene Blue agars, whereas in the other 30 samples (60%), no positive results were detected (see Table 3).

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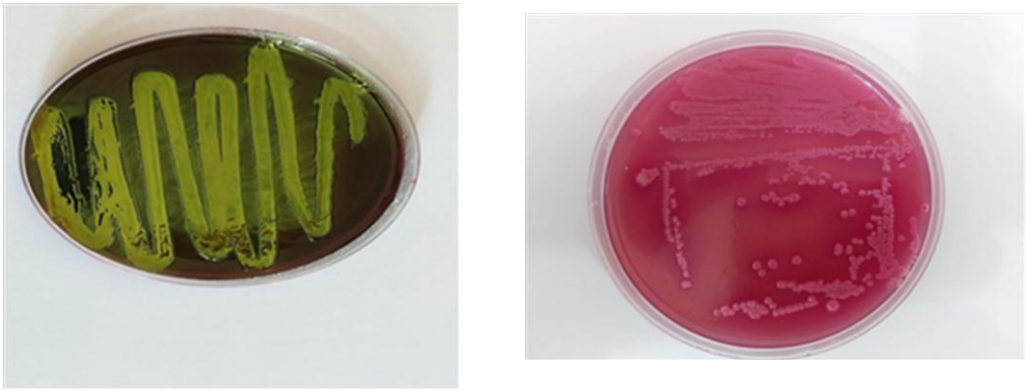
Table 3: the presence of *E coli* in the milk of mastitis-diseased cows

Milk sample	*E.coli*	Percentage %
Positive	20	40 %
Negative	30	60 %
Total	50	100 %



Figure(1): The *E coli* colonies on the MacConkey agar. Pink colonies, lactose fermenter appear on the EMB ager and the presence of characteristic shiny green metallic colonies**.**

Molecular identification of *E. coli*

E. coli isolates from milk of cows infected with mastitis was confirmed by PCR method by 16S rRNA as shown in Figure 2.

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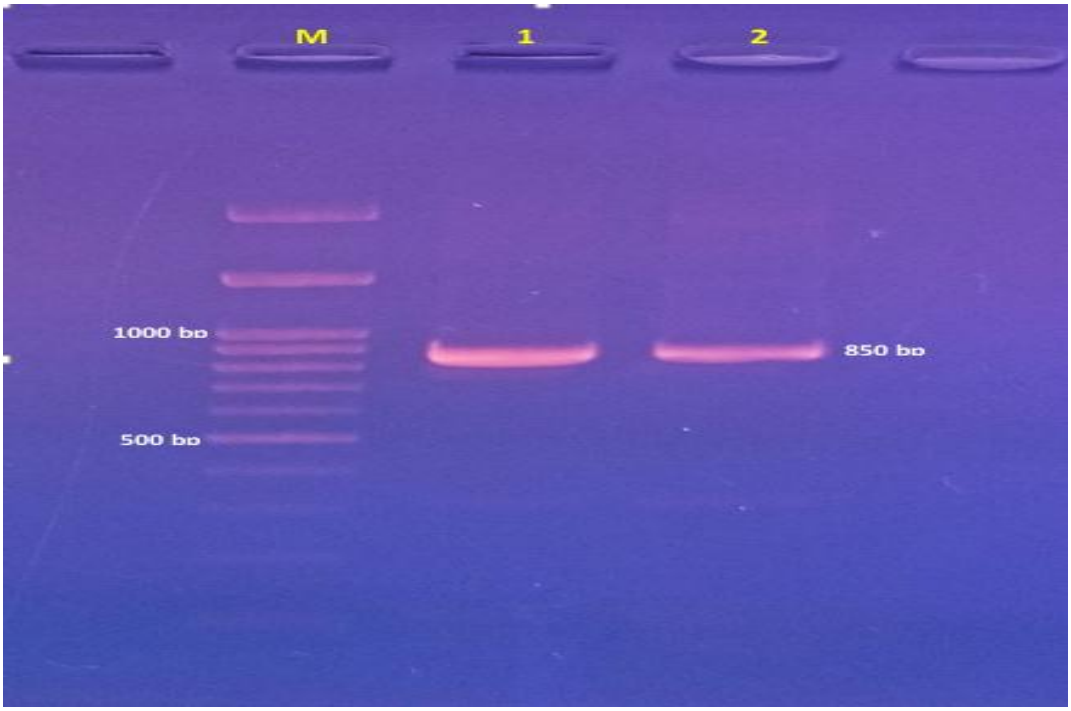

Figure (2): Agarose gel electrophoresis of *E. coli* (1.5% agarose,7v/cm^2 for 60 min) for 16S gene (850 bp amplicon), lane M represents M100bp DNA Ladder, lanes 1and 2 represent *E. coli* isolates band.

Discussion

Bovine mastitis is a disease that affects the udder of cattle and characterized by abnormal milk contents such as the present of blood, clots, pus, and changes the chemical and physical properties of milk. Mastitis occurs as either clinical or subclinical form. The causative agent may be bacterial, viral or fungal. High prevalence of mastitis effect farms that leads to economic losses by decrease the milk yield, decrease herd productivity and increase mortality rate of cattle that are failed to recover (Orsi et al., 2023). Several bacterial species have the ability to cause mastitis include either G + or G – bacteria such as *E coli*, *Streptococcus* and other bacteria (Naji et al., 2019). *E coli* have high prevalence among the bacterial species that cause clinical mastitis (Haftu et al., 2012). The results in this study shown that the percentage of clinical mastitis caused by *E coli* was too high as shown in table (3), this explained the prevalence of causative agent. The results of this study agrees with Haftu et al (2012) and Neamah et al (2022). They demonstrate that the *Ecoli* have the ability to cause clinical mastitis in high percentages. A recent study by Zuhier Moqkbel & Walaa Najm (2024) revealed that *E coli* can cause clinical mastitis and this agrees with the results of the current study.

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Other studies depend on the PCR method for confirmation of the diagnosis of several causative agents because the PCR technique have a rapid, sensitive and specific results compared with other traditional diagnostic methods (Clermont,et al2013, 2019). Many studies were focused on the 16s ribosomal gene for confirm the diagnosis of *E coli* (Neamah et al., 2022; Orsi et al., 2023). In this study, *E. coli* infection in bacterial isolates from milk of mastitis-diseased cows were confirmed by PCR method by 16S rRNA as shown in Figure (2). The current study confirmed that the *E coli* is one of the most prevalence bacterial causative agent of mastitis of dairy cattle in Diyala Governorate.

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References

Adkins, P. R. F., and J. R. Middleton. 2018. Methods for diagnosing mastitis. Vet. Clin. North Am. Food Ani Ruegg, P. L. 2017. A 100-year review: Mastitis detection, manage ment, and prevention. J. Dairy Sci. 100:10381–10397. https://doi .org/10.3168/jds.2017-13023.m. Pract. 34:479–491. https://doi.org/10.1016/j.cvfa.2018.07.003.

Ali MR, Khudhair AM (2018) Detection of colony adhesion factors and genetic background of adhesion genes among multidrug-resistant uropathogenic Escherichia coli Isolated in Iraq. J Pure Appl Microbiol 12:2017–2025, http://dx.doi.org/10.22207/JPAM.12.4.38

Clermont, O., J. K. Christenson, E. Denamur, and D. M. Gordon. 2013. The Clermont Escherichia coli phylo-typing method revisit ed: Improvement of specificity and detection of new phylo-groups. Environ. Microbiol. Rep. 5:58–65. https://doi.org/10.1111/1758-2229.12019.

Down, P. M., A. J. Bradley, J. E. Breen, and M. J. Green. 2017. Factors affecting the cost-effectiveness of on-farm culture prior to the treatment of clinical mastitis in dairy cows. Prev. Vet. Med. 145:91–99. https://doi.org/10.1016/j.prevetmed.2017.07.006.

Haftu R, Taddele H, Gugsa G, Kalayou S. Prevalence, bacterial causes, and antimicrobial susceptibility profile of mastitis isolates from cows in large-scale dairy farms of Northern Ethiopia. Trop Anim Health Prod. 2012;44(7):1765-1771. DOI: 10.1007/s11250-012-0135 z.

P-ISSN: 2410-8863
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https://djvs.uodiyala.edu.iq
Pages: 76-84

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https://doi.org/10.71375/djvs.2025.03207

Hiitio H, Vakkamaki J, Simojoki H, Auto T, Junnila J, Pelkonen S, Pyorala S. Prevalence of subclinical mastitis in Finnish dairy cows: changes during recent decades and impact of cow and herd factors. Acta Vet Scand. 2017;59(1):22**. DOI: 10.1186/s13028-017-0288-x**.

Kumar DRA, Chakraborty S, Verma AK, Tiwari R, Dhama K, Singh U, Kumar S. Trends in diagnosis and control of bovine mastitis: a review. Pakistan J Biol Sci PJBS. 2013;16(23):1653-1661. **DOI: 10.3923/pjbs.2013.1653.1661.**

Lindstedt, B. A., M. D. Finton, D. Porcellato, and L. T. Brandal. 2018. High frequency of hybrid Escherichia coli strains with com bined Intestinal Pathogenic Escherichia coli (IPEC) and Extraint estinal Pathogenic Escherichia coli (ExPEC) virulence factors iso lated from human faecal samples. BMC Infect. Dis. 18:544. **https: //doi.org/10.1186/s12879-018-3449.**

McDougall S. Bovine mastitis: epidemiology, treatment and control. New Zeeland Vet J. 2002;50(sup3):81-84. **DOI: 10.1080/00480169.2002.36274.**

Mishra, B. B. T. S.; Patel, B. B., & Tiwari, S. (2010). Colloidal nano carriers: a review on formulation technology, types and applications toward targeted drug delivery. Nanomedicine: Nanotechnology, biology and medicine; 6.24-9, **https://doi.org/10.1016/j.nano.2009.04.008**

Mohammed, S. N. (2024). Bacteriological Finding, Vaginal Discharges, and Endometrial Cytology for Endometritis Detection in Postpartum Buffaloes. *Diyala Journal for Veterinary Sciences*, *2*(3), 31-49.DOI: https://doi.org/10.71375/djvs.2024.02304

Morovat, T.; Bahram, F.; Mohammad, E.; Setareh, S., & Mohamad Mehdi, F .(2009).Distribution of different carbapenem resistant clones of Acinetobacterbaumannii in Tehran hospitals. The new microbiologica; 32(3):265.

Naji HA, Saleh WMM, Hanoon M, Imad I, Salim Y. Serotyping, virulence gene expression and phenotypic characterization of E. coli O157:H7 in colibacillosis affecting buffalo calves in Basra governorate. Iraqi J Vet Sci. 2019;33(2):445-451. **DOI: 10.33899/ijvs.2019.163198**.

Neamah, A., Fahed, K., Sadeq, J., & Alfatlawi, M. (2022). Molecular characterization and phylogenetic analysis of Escherichia coli isolated from milk of cattle affected by mastitis. **DOI: 10.33899/ijvs.2021.129934.1702**

Orsi, H., Guimarães, F. F., Leite, D. S., Guerra, S. T., Joaquim, S. F., Pantoja, J. C., Hernandes, R. T., Lucheis, S. B., Ribeiro, M. G., & Langoni, H. (2023). Characterization of mammary pathogenic Escherichia coli reveals the diversity of Escherichia coli isolates associated with bovine clinical mastitis in Brazil. *Journal of dairy science*, *106*(2), 1403-1413. https://doi.org/10.3168/jds.2022-22126

Secher, T., Brehin, C. & Oswald, E. (2016) Early settlers: which E. coli strains do you not want at birth? *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 311(1), G123-G129. **https://doi.org/10.1152/ajpgi.00091.2016**

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Pages: 76-84

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https://doi.org/10.71375/djvs.2025.03207

Vangroenweghe, F., L. Duchateau, and C. Burvenich. 2020. J- 5Esche richia coli vaccination does not influence severity of an Escherichia coli intramammary challenge in primiparous cows. J. Dairy Sci. 103:6692–6697. **https://doi.org/10.3168/jds.2019-17799**.

Zhang, D., Z. Zhang, C. Huang, X. Gao, Z. Wang, Y. Liu, C. Tian, W. Hong, S. Niu, and M. Liu. 2018. The phylogenetic group, an timicrobial susceptibility, and virulence genes of Escherichia coli from clinical bovine mastitis. J. Dairy Sci. 101:572–580. **https:// doi.org/10.3168/jds.2017-13159.**

Zuhier Moqkbel, O., & Walaa Najm, A. (2024). Modulation of Immune system by Oral Administration Pathogenic Escherichia coli Isolated from Cow Mastitis. *Diyala Journal for Veterinary Sciences*, *2*(4), 111-117.DOI: https://doi.org/10.71375/djvs.2024.02410