SAR Journal of Pathology and Microbiology

Abbreviated Key Title: *SAR J Pathol Microbiol*
Home page: https://sarpublication.com/journal/sarjpm/home
DOI: https://doi.org/10.36346/sarjpm.2025.v06i02.003

OPEN ACCESS

ISSN 2707-7756 (P)
ISSN 2709-6890 (O)

Original Research Article

Isolation and Identification of *Escherichia Coli* that Cause Diarrhea in Neonatal Calves

Noor Ali Abdulzahra[1], Rawaa Adday Ali[1*]

[1]Microbiology Department, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq

***Corresponding Author:** Rawaa Adday Ali
Microbiology Department, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq

Article History: | Received: 02.03.2025 | Accepted: 08.04.2025 | Published: 10.04.2025 |

Abstract: *Escherichia coli* (*E. coli*) is a major bacterial pathogen responsible for neonatal diarrhea in calves, leading to significant economic losses in the livestock industry due to decreased productivity and increased veterinary costs. This study aims to isolate and identify *E.coli* from intestinal infections in calves using microbiological and biochemical tests and vitik. A total of 75 rectal swab samples were collected from diarrheic calves, different ages (2 days to 6 months) as well as different sex (male or female) from several farms in the Babylon district between October 2024 and February 2025. Out of the 75 samples, 70 (93.3%) showed positive bacterial growth, while 5 (6.66%) were negative bacterial growth, the positive growth include Gram Negative bacteria 50(71.4%) isolates were identified as *Escherichia coli* 30 (60%),*Salmonella typhimurium* 8 (16%), *Klebsiella* spp. 6 (12%) , *Mycoplasma bovin* 4 (8%), *Haemophilis somnus* 2 (4%) ,while the remaining Gram positive bacteria 20(28.5%) which revealed 8 (32 %) isolates of *Staphylococcus spp.* 9 (45%), *Enterococcus spp.* 6 (30%), 3 (15%)*Listeria monocytogenes*, 2 (10%)*Clostridium spp*.

Keywords: Bacteria, *E.coli*, neonatal, calves, diarrhea.

INTRODUCTION

Diarrhea is a common health issue in neonatal calves and is a leading cause of high mortality rates in this age group, resulting in significant economic losses in the livestock industry. Among the primary causes of this condition are microbial agents, including viruses and bacteria, with *Escherichia coli* being one of the main bacterial pathogens responsible for neonatal diarrhea (Aditya *et al*., 2023). Pathogenic strains of *Escherichia coli*, such as enterotoxigenic *E. coli* (ETEC), cause damage to the intestinal lining, leading to impaired absorption of fluids and nutrients, which results in severe diarrhea (Rathor *et al*., 2021). In addition to *Escherichia coli*, other bacteria such as *Salmonella* and *Klebsiella* can also contribute to diarrhea in neonatal calves (Algamma *et al*., 2020). Early detection and identification of these bacteria through techniques like microbiological culturing and biochemical tests are essential for developing effective prevention and treatment strategies to manage this condition, which significantly impacts calf health and farm productivity (Bakry *et al*., 2020). The virulence factors of *Escherichia coli* (*E. coli*) play a critical role in causing neonatal diarrhea in calves. Key virulence factors include enterotoxins, such as heat-labile toxin (LT) and heat-stable toxin (ST), produced by enterotoxigenic *E. coli* (ETEC), which damage the intestinal epithelium and disrupt fluid absorption. Additionally, adhesins such as fimbriae help the bacteria adhere to the intestinal lining, further contributing to the pathogenicity of *E. coli* in neonatal calves (Alegbeleye, O.O *et al*.,2020).

METHODOLOGY

Sample Collection:

Between October 2024 and January 2025, collected 75 rectal swabs from calves suffering from diarrhea from differant farms in the Babylon Governorate. The rectal swab was taken from the calves in aseptic way. Each swab was carefully collected using sterile equipment to avoid contamination. After collection, the samples were immediately placed in an ice box to preserve their viability during transport. The samples were then delivered to the laboratory, where they were cultured to identify potential pathogens.

A Laboratory-Prepared Media:

All the agricultural media were used in this study, according to the instructions of their manufacturers, after preparing the types of culture media such as(Eosin Methylene blue, Blood agar, MacConkey agar, Brain heart broth, Nutrient agar and S.S agar) sterilized at 121 °C for 15 min. and incubated at 37 ℃ for 24 hours, the pH was adjusted to 7

Laboratory Diagnosis of Isolates:

After obtaining the bacterial isolates, the diagnosis was done by microscopical examination, and stained with Gram-stain to observe the specific shape and type of reaction and identification of bacteria by using: biochemical tests and VITEK2 system (bioMerieux, Paris, France) according to the manufacturer's instructions to diagnose the bacteria isolated.

Statistical Analysis:

The statistical analysis was performed using SPSS 23. The statistical differences across distinct groups were determined using the Pearson chi-square test (Team R C, R Foundation for Statistical Computing).

RESULTS

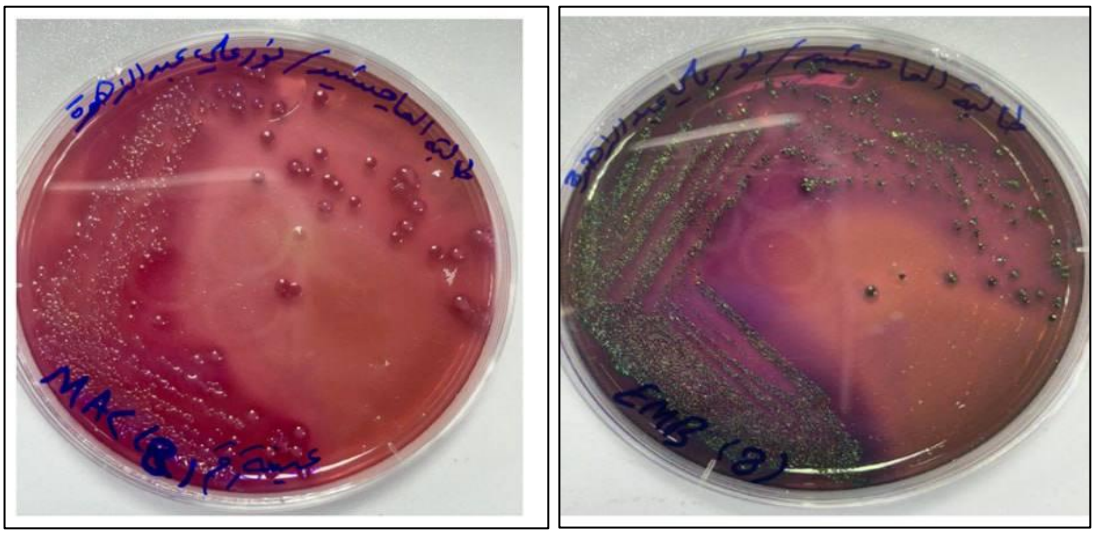
The results showed 70(93.3%) sample were positive bacterial growth, while 5(6.66%) were negative bacterial growth, table (1). The positive growth were include Gram Negative bacteria 50(71.4%) isolates were identified as *Escherichia coli* 30 (60%) Figure 1(A,B*)*, *Salmonella typhimurium* 8 (16%), *Klebsiella* spp. 6 (12%) , *Mycoplasma bovin* 4 (8%), *Haemophilis somnus* 2 (4%) ,while the remaining Gram positive bacteria 20(28.5%) which revealed isolates of *Staphylococcus* spp 9(45%), *Enterococcus* spp. 6 (30%), *Listeria monocytogenes* 3(15%), *Clostridium* spp 2(10%) Table (2).

Table 1: Isolate distribution based on bacterial growth, categorized as positive or negative

No. of culture	G-culture	G+culture
75	5	70
100%	6.66%	93.3%

Table 2: Distribution of bacterial isolates

Type of bacterial isolates	Total
Gram Negative bacteria 50(71.4%)	
E.coli	30 (60%)
Salmonella typhimurium	8 (16%)
Klebsiella spp	6 (12%)
Mycoplasma bovin	4 (8%)
Haemophilis somnus	2 (4%)
Gram positive bacteria 20(28.5%)	
Staphylococcus spp	9 (45%)
Enterococcus spp	6 (30%)
Listeria monocytogenes	3 (15%)
Clostridium spp	2 (10%)



A **B**

Figure 1: A) *E.coli* on MacConkey agar; B) *E.coli* on EMB at 37 °C for 24 hrs

DISCUSSION

The current study identifies *Escherichia coli* as the primary cause of neonatal diarrhea in calves, with 57.1% of bacterial isolates being *E. coli* (Chen *et al*., 2021). This is consistent with previous findings, which highlight *E. coli* as a leading pathogen due to its production of enterotoxins that disrupt fluid absorption in the intestines (Muhie OA *et al*., 2019). Other bacterial pathogens, such as *Salmonella* spp., *Klebsiella* spp., and *Staphylococcus* spp., were also detected, though less frequently (Batabyal *et al*., 2020). The high prevalence of *E. coli* underscores the importance of early intervention and proper management practices, including adequate colostrum intake, vaccination, and maintaining a clean environment to prevent infection (Alomari *et al*., 2021). the study emphasizes that calves' susceptibility to infection, especially during the first few days of life, is influenced by factors such as poor immune transfer and inadequate sanitation (Kim *et al*., 2021). Effective control strategies, including good nutrition and environmental management, are crucial for reducing the incidence of diarrhea and improving overall herd health (Tadesse *et al*., 2020). Early diagnosis and appropriate treatment can help mitigate the economic losses associated with neonatal diarrhea(Wu *et al*., 2021).

CONCLUSION

Escherichia coli isolates were cultured and isolated from clinical samples, and the identification was performed by culture examination, biochemical tests and VITEK2 system.

Acknowledgments: The authors declare that no competing exist.

Conflict of Interest: None

REFERENCES

1. Aditya A, Tabashsum Z, Martinez ZA, *et al.*, Diarrheagenic *Escherichia coli* and their antibiotic resistance patterns in dairy farms and their microbial ecosystems. *Journal of Food Protection*. 2023;86(3):100051. doi: 10.1016/j.jfp.2023.100051 [DOI] [PubMed] [Google Scholar].
2. Rathor AS, Bhati T, Singh AP. In vitro antibacterial activity of extracts of Dalbergiasissoo and Aegle marmelos against Enterotoxigenic Escherichia coli from calves. J Phytopharmacol. 2021;10(5):289-293.
3. Algammal AM, El-Kholy AW, Riad EM, Mohamed HE, Elhaig, MM, Yousef SAA, *et al.*, Genes encoding the virulence and the antimicrobial resistance in enterotoxigenic and shiga-toxigenic E. coli isolated from diarrheic calves. Toxins. 2020;12(6):383.
4. Bakry NM, Awad WS, Ahmed SA. Virulence genes, antibiotic resistance and phylotyping of Escherichia coli O157 recovered from diarrheic calves. International Journal of Veterinary Science. 2021;10(1):1-7.
5. Alegbeleye, O.O.; Sant'Ana, A.S. Pathogen subtyping tools for risk assessment and management of produce-borne outbreaks. *Curr. Opin. Food Sci.* 2020, *32*, 83–89. [Google Scholar] [CrossRef].
6. Chen, H., Wang, C., Huasai, S., Chen, A. (2021). Effects of Dietary Forage-to-Concentrate Ratio on Nutrient Digestibility, Ruminal Fermentation and Rumen Bacterial Composition in Angus Cows. *Sci. Rep.* 11, 17023. doi: 10.1038/s41598-021-96580-5.
7. Muhie OA. Antibiotic Use and Resistance Pattern in Ethiopia: Systematic Review and Meta-Analysis Int J Microbiol. 2019. 2019: 2489063.
8. Batabyal K, Mahanti A, Dey S, Joardar SN, Samanta I, Isore DP, *et al.*, Prevalence of Extended-spectrum and AmpC Beta-lactamases Producing STEC in Bovine Diarrhoea Cases in West Bengal, India. International Journal of Current Microbiology and Applied Sciences. 2020;9(10):100-110.
9. Alomari, M. M. M., Dec, M., Nowaczek, A., Puchalski, A., Wernicki, A., Kowalski, C., *et al.*, (2021). Therapeutic and Prophylactic Effect of the Experimental Bacteriophage Treatment to Control Diarrhea Caused by E. Coli in Newborn Calves. *ACS Infect. Dis.* 7, 2093–2101. doi: 10.1021/acsinfecdis.1c00010.
10. Kim, E. T., Lee, S. J., Kim, T. Y., Lee, H., Atikur, R. M., Gu, B. H., *et al.*, (2021). Dynamic Changes in Fecal Microbial Communities of Neonatal Dairy Calves by Aging and Diarrhea. *Animals* 11 (4), 1113. doi: 10.3390/ani11041113.
11. Tadesse N. Prevalence and Multidrug Resistance Profiles of Escherichia coli in Dairy Farms. International Journal of Veterinary Science and Research. 2020;6(2):142-147.
12. Wu, D., Wang, C., Li, T., Wang, H., Hu, J. H., Peng, X. (2021). Study on the Effect and Mechanism of Recombinant Human Intestinal Trefoil Factor on Intestinal Mucosal Injury and Repair in Burned Mice. *Zhonghua Shao Shang Za Zhi Zhonghua Shaoshang Zazhi Chin. J. Burns* 37, 811–820. doi: 10.3760/cma.j.cn501120-20210412-00125.