International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(12): 333-335
www.biochemjournal.com
Received: 13-09-2025
Accepted: 16-10-2025

Peyi Mosing
Division of Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Bhand Akshata Chandrakant
Division of Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Shantishree Das
Department of Veterinary Surgery & Radiology, College of Veterinary Science, Khanapara, Assam, India

Siddharth Chaudhary
Division of Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Corresponding Author:
Peyi Mosing
Division of Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Detection of the *uidA* Gene in enterotoxigenic *Escherichia coli* associated with acute diarrhoea in neonatal piglets

Peyi Mosing, Bhand Akshata Chandrakant, Shantishree Das and Siddharth Chaudhary

DOI: https://www.doi.org/10.33545/26174693.2025.v9.i12e.6499

Abstract
Neonatal diarrhoea poses a major health and economic challenge in pig production, with *Escherichia coli* being one of the principal bacterial agents. Three twenty-four-day-old piglets with acute watery diarrhoea were investigated to determine the etiological agent using conventional and molecular diagnostic approaches. Bacteriological culture on MacConkey and EMB agar yielded lactose-fermenting colonies with characteristic metallic sheen and biochemical profiling was consistent with *E. coli*. Molecular confirmation was achieved through PCR amplification of the species-specific *uidA* gene, which encodes β-glucuronidase and produced the expected 200 bp amplicon. Screening for major enterotoxigenic genes (LT1 & STa) was negative, indicating the involvement of a non-toxigenic or atypical strain. Antimicrobial susceptibility testing guided therapeutic intervention, and the piglet responded rapidly to enrofloxacin and supportive care. The findings underscore the diagnostic value of uidA-based PCR for accurate identification of *E. coli*, particularly in diarrhoeal cases where classical virulence genes are absent. Incorporation of such molecular markers into routine diagnostic workflows can enhance the precision and timeliness of pathogen detection in neonatal piglet enteric infections.

Keywords: *Escherichia coli*, uidA, neonatal diarrhea, piglet, PCR, β-glucuronidase

Introduction
Neonatal diarrhoea remains one of the leading health challenges in pig production, with enterotoxigenic *Escherichia coli* (ETEC) constituting a major pathogen responsible for acute morbidity and mortality (Liu *et al.*, 2015). Although diagnostic emphasis typically lies on detecting toxin and adhesin genes, molecular markers such as *uidA*, which encodes β-glucuronidase, have emerged as highly reliable indicators for *E. coli* identification. The *uidA* gene is conserved across *E. coli* strains and provides enhanced sensitivity, particularly in cases where isolates lack traditional virulence factors or present atypical biochemical characteristics (Garcias *et al.*, 2024) [1]. The present case describes the clinical and laboratory investigation of a neonatal piglet with acute diarrhoea in which *E. coli* was confirmed through *uidA* gene amplification.

Case Study
Three crossbred piglets of around twenty-four-day-old age (less than a month) were attended at farmers' doorstep at Byrnihat, under Kamrup district of Assam, India with a history of sudden onset diarrhoea. The animals exhibited profuse yellowish watery faeces, dehydration, lethargy, sunken eyes, mild hypothermia and an inability to suckle. The farm caretaker reported that no antimicrobial treatment had been administered prior to presentation. The clinical picture was consistent with acute neonatal diarrhoea, and further diagnostic evaluation was undertaken.

Materials and Methods
Sample Collection
Fresh rectal swabs and diarrhoeic faecal samples were collected aseptically from the affected piglets in Cary-Blair transport medium (Himedia, India) and immediately transported to the laboratory for microbiological examination.

Bacteriological and Biochemical Examination

Primary isolation on MacConkey agar yielded lactose-fermenting smooth pink colonies (Fig.1)., which were further subcultured onto eosin methylene blue (EMB) agar. The colonies produced a characteristic metallic sheen, strongly suggestive of *E. coli*. Further, Gram staining revealed Gram-negative, pink-coloured, short rod-shaped bacilli consistent with suggestive morphological characteristics of *E. coli* isolates (Fig.2). Biochemical profiling of the isolate revealed a typical *E. coli* pattern: positive reactions for indole and methyl red tests, and negative results for Voges-Proskauer and citrate utilisation tests. These findings supported a presumptive identification of *E. coli*.

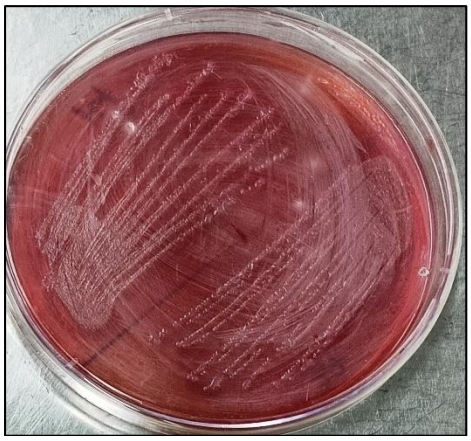

Fig 1: Culture plate shows lactose fermenting pink colony of *E. coli* isolates on MacConkey agar

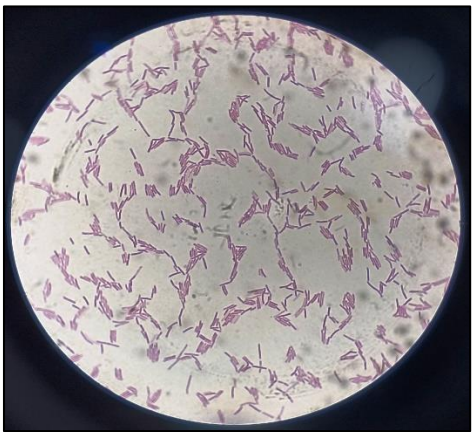

Fig 2: Gram staining revealed Gram-negative, pink-coloured, short rod-shaped bacilli consistent with suggestive morphological characteristics of *E. coli* isolates

DNA extraction and Molecular Confirmation

Genomic DNA was extracted from overnight bacterial cultures using a Genomic DNA by the snap chill or boiling lysis method (Johura *et al.*, 2017) [4]. Briefly, a loopful of overnight culture was suspended in 200 µL sterile distilled water, boiled at 95 °C for 10 min, and centrifuged at 10,000 rpm for 5 min, after which the supernatant containing crude DNA was stored at −20 °C. Molecular confirmation of *E. coli* was performed by PCR targeting the species-specific *uidA* gene encoding β-glucuronidase. PCR was carried out in a 25 µL reaction containing 12.5 µL Master Mix, 1 µL each of uidA-specific primers, 2 µL template DNA and nuclease-free water. The cycling protocol consisted of initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 7 min. Amplified products were electrophoresed on 1.5% agarose and visualized under UV illumination. Molecular confirmation was carried out by PCR targeting the *uidA* gene using published primers (Forward: 5'-TGGTAATTACCGACGAAAACGGC-3'; Reverse: 5'-ACGCGTGGTTACAGTCTTGCG-3'), yielding an expected amplicon size of 200 bp (Fig.3). PCR amplification produced a distinct band corresponding to this size, confirming the presence of the *uidA* gene and thereby validating the identity of the isolate as *E. coli*. Further screening of virulence-associated genes showed no amplification for *stx1*, *stx2* suggesting that the isolate represented a non-toxin-producing or atypical strain of *E. coli*.

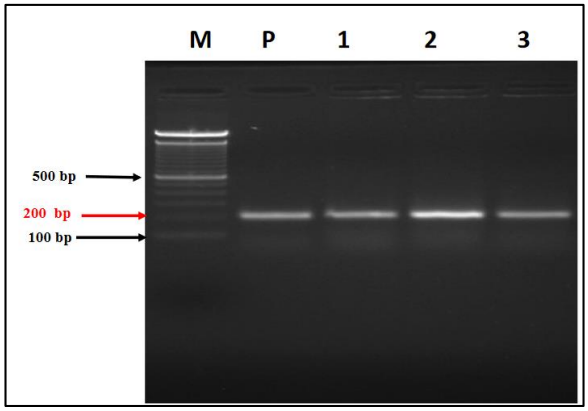

Fig 3: Agarose gel electrophoresis showing PCR amplification of the *uidA* gene (200 bp) for molecular confirmation of *E. coli* isolates.

Lanes 1-3: Amplified products from representative *E. coli* isolates showing bands at 200 bp;
Lane1: Positive Control
Lane M: 100 bp DNA ladder used as molecular weight marker.

Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing was performed by the standard disc diffusion method against commonly used antibiotics based on their application in veterinary medicine. Results were interpreted as per the method of the Clinical and Laboratory Standards Institute guidelines (CLSI 2008).

Treatment Outcome

Therapeutic management included administration of oral electrolyte solutions to correct dehydration, parenteral enrofloxacin (used as per antibiogram assay) at a dosage of 5 mg/kg body weight, warm fluid therapy and probiotic supplementation to restore gut microbial balance. The piglet responded favourably to treatment, with substantial improvement observed within 48 hours. Diarrhoea gradually subsided, and the animal resumed normal feeding behaviour.

Results and Discussion

The *uidA gene*, which encodes β-glucuronidase, has long been recognised as a highly conserved and dependable molecular marker for the identification of *Escherichia coli*. Its diagnostic utility becomes particularly relevant in cases where toxin genes associated with ETEC are absent or where isolates display atypical biochemical characteristics

that complicate conventional identification (Jacobson, 2022) [3]. In this case, although no classical enterotoxigenic genes were detected, the clinical signs and isolation of *E. coli* confirmed via *uidA* PCR strongly indicated its role in the diarrhoeic episode.

Neonatal piglets are predisposed to gastrointestinal infections due to immature immune function and an underdeveloped gut microbiome (Mahato *et al.*, 2023) [6]. Even strains lacking classical virulence determinants may proliferate extensively under favourable intestinal conditions, leading to diarrhoea through mechanisms such as colonisation-induced malabsorption, disruption of normal flora or intestinal inflammation (Garcias *et al.*, 2024) [1]. The rapid clinical recovery following antimicrobial and supportive therapy further supports the pathogenic involvement of the identified *E. coli* strain.

This case emphasises the importance of incorporating molecular tools, particularly uidA-based PCR, into routine diagnostic protocols for neonatal diarrhoea. Such approaches enhance diagnostic accuracy and ensure timely therapeutic interventions, especially in situations where phenotypic methods may yield incomplete or misleading results.

Conclusion

The present case study demonstrates the diagnostic significance of *uidA* gene detection in confirming *Escherichia coli* infection in neonatal piglet diarrhoea. *uidA*-based molecular assays offer a rapid, sensitive and species-specific method for the identification of *E. coli*, proving especially valuable when conventional virulence markers are absent. Incorporation of such molecular tools in diagnostic workflows can greatly improve the accuracy and reliability of pathogen identification in enteric disease investigations.

Acknowledgements

Funding: No funding was received for conducting this study.

Conflict of Interest: The authors declare that they have no conflict of interest.

References

1. Garcias B, Martin M, Darwich L. Characterization of antimicrobial resistance in *Escherichia coli* isolated from diarrheic and healthy weaned pigs in Catalonia. Animals. 2024 Feb 1;14(3):487.
2. Liu D. Diarrhoeagenic *Escherichia coli*. In: Molecular Medical Microbiology. London: Academic Press; 2015. p. 1133-46.
3. Jacobson M. On the infectious causes of neonatal piglet diarrhoea—a review. Vet Sci. 2022 Aug 10;9(8):422.
4. Johura FT, Parveen R, Islam A, Sadique A, Rahim MN, Monira S, Khan AR, Ahsan S, Ohnishi M, Watanabe H, Chakraborty S. Occurrence of hybrid *Escherichia coli* strains carrying Shiga toxin and heat-stable toxin in livestock of Bangladesh. Front Public Health. 2017 Jan 9;4:287.
5. Clinical and Laboratory Standards Institute (CLSI). Methods for antimicrobial susceptibility testing of anaerobes bacteria. 9th ed. Wayne (PA): CLSI; 2018. (CLSI document M07-A8).
6. Mahato G, Dutta M, Sarma KK, Arif SA, Gogoi SM, Basumatary P, Das T. First report of isolation of enteropathogenic *Escherichia fergusonii* from the faeces of elephant calf (*Elephas maximus*). Explor Anim Med Res. 2023;13(2).