

A universal primer set for specific vis-à-vis simultaneous differentiation of *Escherichia coli* and *Klebsiella* species

Escherichia coli and *Klebsiella* species are major human and animal pathogens that produce broad-spectrum antibiotic resistance. Antibiotic resistance spreads rapidly, owing mostly to horizontal gene transfer across populations of competent bacteria, i.e. from faecal germs to environmental isolates or vice versa¹. The horizontal gene transfer approach is well recognized for producing extended spectrum beta-lactamases (ESBLs), which are resistance determinants and have clinical value in the family Enterobacteriaceae, particularly *Klebsiella pneumoniae* and *E. coli*^{2–4}.

Differentiation of Enterobacteriaceae species, such as *E. coli* and *Klebsiella* sp. is difficult in clinical laboratories because the bacteria are genetically and phenotypically related. To distinguish *E. coli* from *Klebsiella* species, a costly series of biochemical tests is required. In clinical settings, pathogen species detection is critical for selecting the proper antibiotic treatment as well as outbreak tracing and disease epidemiology. The duration and expense of the

microbiological procedures are also major considerations. Many microbiological methods are used for this purpose, with the gold standard being automated platforms that test bacteria in a complex set of biochemical reactions for various metabolic activities and compare the profile to an established database^{5,6}. However, these procedures take around 24 h after pathogen isolation to produce results and are not cost-effective. Label-free Raman spectroscopy was recently used to differentiate clinical *E. coli* and *Klebsiella* species⁷. However, this process necessitates the use of costly apparatus and knowledge.

Though PCR-based methodology has been developed for specific and sensitive detection of *E. coli* and *Klebsiella* species, it requires more than one set of primers specific for each pathogen⁸. The present study deals with a single universal set of novel primers that can differentiate *E. coli* and *Klebsiella* species in clinical and environmental samples. We designed a single set of novel universal primers (an Indian

Patent filed with application number 202311041898, dated 23 June 2023), which can easily differentiate *E. coli* and *Klebsiella* species without multiple sets of primers (Figure 1). A total of 55 samples (piggery farm wastewater, livestock slaughterhouse wastewater, drainage water, hospital wastewater, etc.) were tested using the developed universal primer set. The detection limit of the assay was found to be 10^{-3} ng/ μ l of the plasmid DNA. The developed assay is simple, specific and efficient for quick differentiation of *E. coli* and *Klebsiella* species.

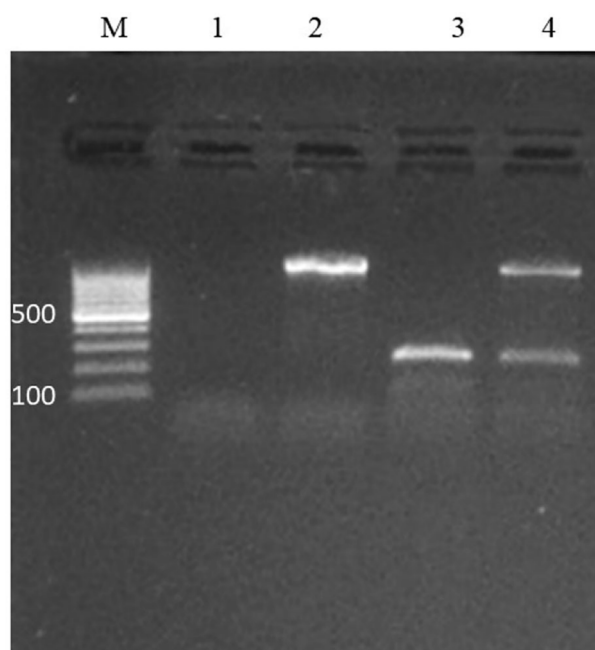


Figure 1. Universal primers for differentiating *Escherichia coli* and *Klebsiella* species. Lane 1, No template control; lane 2, *E. coli*; lane 3, *Klebsiella* species; lane 4, Mixed culture having *E. coli* and *Klebsiella* species; M, Molecular marker (base pairs).

1. Baquero, F., Martínez, J. L. and Cantón, R., *Curr. Opin. Biotechnol.*, 2008, **19**, 260–265.
2. Carattoli, A., *Int. J. Med. Microbiol.*, 2013, **303**, 298–304.
3. Jonas, D., Spitzmüller, B., Daschner, F. D., Verhoef, J. and Brisse, S., *Res. Microbiology*, 2004, **155**(1), 17–23.
4. Neog, N., Phukan, U., Puzari, M., Sharma, M. and Chetia, P., *Curr. Microbiol.*, 2021, **78**(4), 1115–1123.
5. Al Masoud, N., Muhamadali, H., Chisanga, M., Al Rabiah, H., Lima, C. A. and Goodacre, R., *Analyst*, 2021, **146**(3), 770–788.
6. Espagnon, I. *et al.*, *J. Biomed. Opt.*, 2014, **19**(2), 027004.
7. Nakar, A., Pistiki, A., Ryabchikov, O., Bocklitz, T., Rösch, P. and Popp, J., *J. Biophotonics*, 2022, **15**(7), e202200005.
8. Li, P., Zhang, D., Li, H., Pang, J., Guo, H. and Qiu, J., *Front. Vet. Sci.*, 2020, **7**, 588173.

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