



Detection and molecular characterisation of *Klebsiella* spp in human, animal and environmental interface: A one health approach

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Abstract

The *gyrA* gene in *Klebsiella* spp. is a 441 base pair long gene coding for the DNA gyrase A subunit of the organism. The present study was undertaken for the isolation, identification, and molecular confirmation of *Klebsiella* spp. from the environmental, animal and human samples by targeting the *gyrA* gene. A total of 175 samples were collected from the human patients, water and soil of the patients' domestic environment and faecal samples of the domestic animals reared in the patients' households. All the samples were subjected to the isolation and identification of *Klebsiella* spp. by conventional cultural methods and biochemical characterisation. The current study identified the occurrence of *Klebsiella* spp. in 94.34, 20.00, 14.00 and 36.36 per cent of samples collected from human, soil, water and animal sources, respectively. The recovered isolates were subjected to PCR-based amplification of the *gyrA* gene and all the presumptively identified isolates were found to harbour the *gyrA* gene.

Keywords: *Klebsiella* spp., *gyrA* gene, PCR

Klebsiella spp. is a capsulated, gram-negative, non-motile bacterium of the Enterobacteriaceae family. The source of *Klebsiella* spp. causing infections in man can be varied ranging from soil, water, domestic environment and also the livestock and companion animals sharing the same environment as that of the humans. *Klebsiella pneumoniae* is a clinically significant member of the genus *Klebsiella*, reportedly accounting for approximately 86 per cent of

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human infections caused by *Klebsiella* (Fatima *et al.*, 2021). The WHO had classified ESBL-producing, carbapenem-resistant *Klebsiella pneumoniae* as one of the 'priority status' pathogens in 2017 (Guerra *et al.*, 2022). The gene *gyrA* is the one which codes for the DNA gyrase A subunit of *Klebsiella spp.* It has been demonstrated that DNA gyrase is necessary for bacterial growth thus making it a housekeeping gene (Dimri and Das., 1990).

A total of 53 phenotypically confirmed drug-resistant *Klebsiella spp.* isolates were collected from the Department of Microbiology, Dr Moopens Medical College (DMMC) Meppadi, Wayanad. The environmental samples like water and soil (50 each) and faecal samples (22 Nos.) from the domestic animals were collected from the households of confirmed patients. All the collected samples were subjected to isolation and identification of *Klebsiella spp.*

The enriched samples were streaked onto MacConkey agar (MCA) and incubated at 37°C for 24 h. Characteristic large, pink mucoid colonies were selected and streaked onto the selective agar base, m-Kleb agar. Three to five representative colonies that showed a typical deep blue to bluish-green colour suggestive of *Klebsiella spp.* were selected and later confirmed by biochemical assay. The presumptively identified colonies of *Klebsiella spp.* were subjected to PCR targeting the *gyrA* gene (Fatima *et al.*, 2021).

Out of the 175 samples analysed, 75 (42.86%) isolates were found positive for *Klebsiella spp.* by conventional culture techniques. Of this, a total of 50 out of the 53 isolates recovered from human samples were phenotypically confirmed as *Klebsiella*

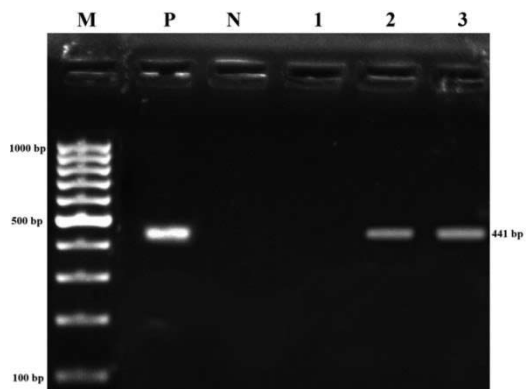


Fig. 1. Identification of *gyrA* gene, Lane M-Marker, Lane N, 1: Negative samples; Lane P: Positive control; Lane 2, 3: Positive samples

spp. Among the 50 soils and water samples collected from the selected patients' domestic environment, 20.00 and 14.00 per cent samples were found to harbour the organism, respectively (Table 1). Iwu *et al.* (2020) reported a similar rate of recovery (20.00 %) of *Klebsiella spp.* from the agricultural soil samples and Samanta *et al.* (2018) reported a similar rate (16.60 %) of recovery in water samples collected from the pig farms of West Bengal, India. In the case of 22 samples collected from the animals, 36.36 per cent of samples were found to carry the organism of interest. The study conducted by Hamza *et al.* (2016) reported an occurrence rate of 35.00 per cent of *Klebsiella spp.* in diseased broiler chicken samples collected from Egypt.

The presumptively identified isolates from all the sources were subjected to molecular confirmation using PCR targeting the *gyrA* gene. Fatima *et al.* (2021) and Aly *et al.* (2014) used the *gyrA* gene for the species-level identification of *Klebsiella spp.* in isolates recovered from human samples. Out of the 75

Table 1. Occurrence of *Klebsiella spp.* in human, animal and environmental samples

Type of samples	No. of samples	Recovered isolates	
		No.	Percentage
Human	53	50	94.34
Soil	50	10	20.00
Water	50	7	14.00
Animal	22	8	36.36
Total	175	75	42.86

isolates recovered from different sources all the isolates were found to harbour the *gyrA* gene (Fig. 1) demonstrating the reliability of the gene for the confirmation of the species-level identification of *Klebsiella spp.*

Summary

The current study demonstrated that *Klebsiella spp.*, which is a major cause of respiratory tract infection in humans is present in varied niches such as soil, water and animals and *gyrA* gene is one of the most reliable housekeeping genes for the identification of *Klebsiella spp.*

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Conflict of interest

The authors declare that they have no conflict of interest

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