Singh et al | Article 11039
J Pure Appl Microbiol. 2026;20(3):2248-2260. doi: 10.22207/JPAM.20.3.15
Received: 10 October 2025 | Accepted: 27 April 2026
Published Online: 01 August 2026

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Antimicrobial Resistance Patterns of *Klebsiella pneumoniae* in Human–Camel–Environment Interfaces in Rajasthan, India

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Citation: Singh Y, Joseph B, Nayakvadi S, et al. Antimicrobial Resistance Patterns of *Klebsiella pneumoniae* in Human–Camel–Environment Interfaces in Rajasthan, India. *J Pure Appl Microbiol.* 2026;20(3):2248-2260. doi: 10.22207/JPAM.20.3.15

Abstract

***Klebsiella pneumoniae*, a critical pathogen with significant implications for global antimicrobial resistance (AMR), is a major threat to public health, particularly in regions with intensive human–animal–environment interfaces. This study investigated the AMR patterns of *K. pneumoniae* isolates from human–camel–environment interactions in Rajasthan, India, a region where camels are economically significant. A total of 138 samples were collected from camel populations, animal handlers, and their surrounding environments in the districts of Ajmer and Jaipur. Thirty-three isolates were confirmed to be *Klebsiella pneumoniae* by microbiological and molecular methods, as well as by using BD Phoenix M50. Antibiotic resistance was assessed using the Kirby-Bauer disc diffusion and BD Phoenix M50 AST methods. The study revealed that 66.66% of the isolates were multidrug-resistant (MDR), with the most pronounced resistance noted against ciprofloxacin (72.72%), as the organism exhibits intrinsic resistance to narrow-spectrum penicillins. Conversely, sulfafurazole, tetracycline, and trimethoprim–sulfamethoxazole exhibited 100% sensitivity. A significant proportion of the isolates (30.30%) showed a multiple antibiotic resistance (MAR) index greater than 0.2, indicating a high risk of spreading resistance. Thirty-three percent of the isolates exhibited phenotypic evidence of extended-spectrum beta-lactamase (ESBL) production, with the predominant ESBL gene identified as bla_{TEM}. Notably, one isolate demonstrated phenotypic colistin resistance, representing the first such report in a camel ecosystem in India. However, *mcr-1*, which is commonly associated with colistin resistance, was not detected in the present study. The genetic mechanism underlying colistin resistance remains unclear. Additionally, none of the isolates possessed carbapenem resistance genes. These findings underscore the critical need for robust and comprehensive AMR surveillance in camel ecosystems to protect public health, food safety, and the livelihoods of camel-dependent communities.**

Keywords: Camel ecosystem, Colistin resistance, ESBL, MDR, One Health Approach

INTRODUCTION

Antimicrobial resistance (AMR) is an increasing global health concern, responsible for an estimated 4.95 million deaths worldwide.[1] The rapid emergence and dissemination of AMR endanger human and animal health, thereby necessitating a One Health approach to understand its transmission dynamics. According to the World Health Organization (WHO), there is a strong need to track antimicrobial use in humans, animals, and the environment to address this crisis effectively.[2] The movement of antimicrobial-resistant bacteria and resistance genes among different ecological niches underscores the interconnected nature of AMR development and spread.[3]

A major contributor to the global AMR crisis is the group of ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species). Among these, *K. pneumoniae* is a Gram-negative opportunistic pathogen known for its extensive resistance gene repertoire. It exhibits high adaptability to diverse environments and plays a significant role in the transfer of antimicrobial resistance genes such as bla_{KPC}, bla_{OXA-48}, and bla_{NDM-1}, facilitating the spread of resistance among clinically relevant pathogens.[4] Although *K. pneumoniae* is primarily a human pathogen, it is also a commensal organism in animals, including livestock and wildlife, where it contributes to AMR transmission dynamics.

Despite the increasing global focus on AMR, limited research has been conducted on the prevalence of AMR in camels, particularly in regions where these animals play crucial socioeconomic and cultural roles. In Rajasthan, India, camels have a vital socioeconomic role, especially among nomadic pastoral communities, and are recognized as the state animal. However, the wandering nature of nomads also poses a significant risk for the spread of diseases and resistant pathogens. Despite their importance, research on camels in Rajasthan remains sparse, particularly in the context of AMR. The misuse and overuse of antibiotics in veterinary practice, exposure to contaminated environments, and

Table 1. Summary of sample collection from different sources in Ajmer and Jaipur districts, Rajasthan

District	Source	Sample type	Number of samples
Ajmer	Camels	Nasal swabs	8
		Rectal swabs	9
Jaipur	Camels	Nasal swabs	29
		Rectal swabs	30
	Environment	Soil samples	25
		Manger's soil samples	3
		Water samples	3
	Camel Handler's	Hand swabs	31
Total			138

close human–camel interactions contribute to the spread of resistant pathogens. These resistant bacteria can be transferred between camels, humans, and the environment, thereby affecting public health, food safety, and environmental health.

Given this background, the human–camel–environment interface in Rajasthan is a critical area for AMR research. Through the adoption of a One Health approach that integrates human, animal, and environmental health, highly effective strategies can be developed to monitor and combat the spread of AMR. This is essential for safeguarding not only public health but also the sustainable livelihoods of those dependent on camels and the health of the broader ecosystem.

MATERIALS AND METHODS

Sample collection

Study samples were randomly sourced from two districts in Rajasthan, namely, Ajmer and Jaipur, during a 1-year period from December 2023 to December 2024, as detailed in Table 1. Samples were collected from camels (nasal and rectal swabs), the surrounding environment (soil, manger soil, and water), and camel handlers (hand swabs). All the samples were collected under sterile conditions and processed accordingly.

Sample processing and isolation

Each sample was inoculated into nutrient broth and incubated overnight for further analysis. The following day, samples were streaked onto MacConkey agar for preliminary isolation. *Klebsiella pneumoniae* isolates were subcultured on Simmons citrate inositol agar (SCIA). The isolates were identified using biochemical tests. Primary tests included Gram staining and microscopy using immersion oil, whereas motility was assessed using a motility test medium and catalase and oxidase tests. Secondary biochemical tests were performed for further characterization.

Tentative *Klebsiella* spp. isolates were confirmed using molecular techniques targeting the 16S-23S rDNA internal transcribed spacer (ITS) region.

Molecular characterization of *K. pneumoniae*

Genomic DNA was extracted using a HipurA bacterial genomic DNA kit and the snap-chilling method. The 16S-23S rDNA ITS region of the isolates was amplified using species-specific primers.[5] The PCR mixture consisted of 12.5 µL of 2X PCR Master Mix (Thermo Scientific, Waltham, MA, USA), 1 µL each of forward and reverse primers at 10 pM, and 2 µL of template DNA. Amplification was performed in a Nexus Gradient Thermal Cycler (Eppendorf, Hamburg, Germany) under the following conditions: initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 45 °C for 1 min (annealing), and 72 °C for 1 min (extension), with a final extension at 72 °C for 10 min. The amplified product (approximately 130 bp) was verified by electrophoresis on a 1.5% agarose gel.

Testing of antibiotic susceptibility

Disc diffusion method

Antibiotic susceptibility of the isolates was determined using the Kirby-Bauer disc diffusion method with the following antibiotics:

piperacillin/tazobactam, penicillin G, cefepime, cefoxitin, ciprofloxacin, gentamicin, amikacin, imipenem, tetracycline, ceftazidime, cefotaxime, cefixime, tobramycin, meropenem, sulfafurazole, trimethoprim, levofloxacin, ofloxacin, norfloxacin, doxycycline, and minocycline (HiMedia, Mumbai, India). Antibiotic discs were carefully placed on Mueller–Hinton agar (MHA) plates uniformly inoculated with test cultures. Plates were incubated at 37 °C for 16-18 hrs. Following incubation, the zones of inhibition were measured, and isolates were classified as susceptible, intermediate, or resistant according to the CLSI (2024) guidelines.[4]

BD Phoenix™ M50 automated system

Susceptibility and minimum inhibitory concentration (MIC) of antimicrobial agents were assessed using the BD Phoenix™ M50 automated system (Becton Dickinson, Franklin Lakes, NJ, USA), which determines MIC based on broth microdilution and automated reading.

Escherichia coli ATCC 25922 was used as a quality control strain for both methods used for antibiotic susceptibility testing.

Phenotypic resistance testing

ESBL production

The double-disc diffusion test (DDST) was used to detect ESBL production. The test was performed using third-generation cephalosporins (ceftazidime and cefotaxime) alone and in combination with clavulanic acid. An increase of ≥5 mm in the inhibition zone was interpreted as indicative of ESBL production.

Detection of carbapenemase production

A modified Hodge test (MHT) was used to detect carbapenemase production. A positive result was identified by the appearance of a cloverleaf shaped indentation around the meropenem disc placed on the MHA plate after overnight incubation.

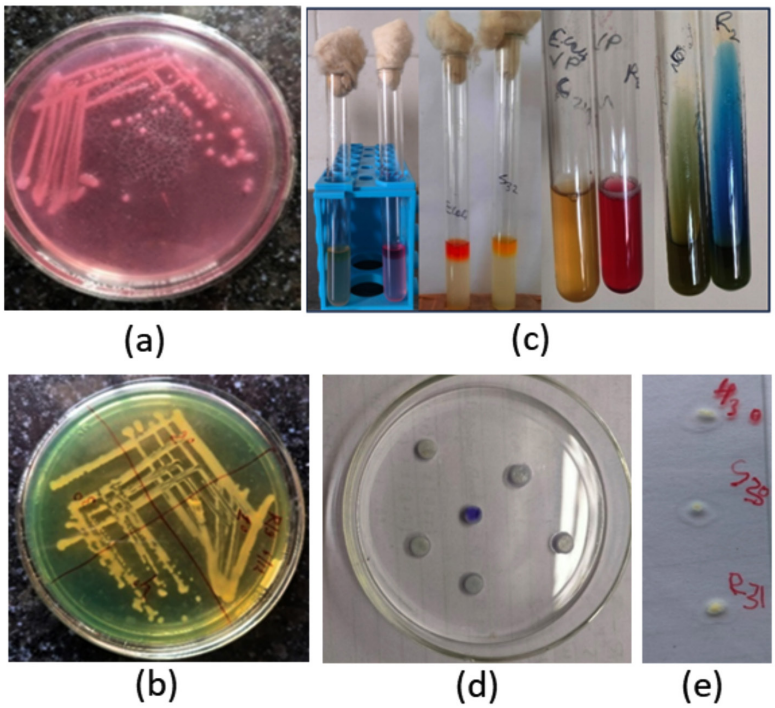


Figure 1. Cultural and biochemical characterization of *Klesbsiella pneumoniae* isolates. (a) McConkey Agar: pink lactose fermenting mucoid colonies. (b) Simmons Citrate Inositol Agar (SCIA): Yellow mucoid colonies. (c) IMViC reactions: Indole (-), Methyl red (-), Voges-Proskauer (+), Citrate (+) (- - + +). (d) Oxidase Test: Negative. (e) Catalase Test: Positive

Table 2. PCR conditions and primers used for characterization of producing *K. pneumoniae*

Gene	Forward primer sequence (5' to 3')	PCR conditions	Amplicon Size	Ref.
16S- 23S rDNA ITS[5]	F: ATT TGA AGA GGT TGC AAA CGA T R: TTC ACT CTG AAG TTT TCT TGT GTT C	Initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 1 min, 45°C for 1 min, and 72°C for 1 min; final extension at 72°C for 10 min; hold at 4°C.	130 bp	5
bla_{TEM}	F: GCG GAA CCC CTA TTT G R: ACC AAT GCT TAA TCA GTG AG	Initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 1 min, 42°C for 1 min, and 72°C for 1 min; final extension at 72°C for 10 min; hold at 4°C.	964 bp	6
bla_{CTX-M}	F: ATG TGC AGY ACC AGT AAR GTK ATG GC R: TGG GTR AAR TAR GTS ACC AGA AYS AGC GG	Initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 1 min, 54°C for 1 min, and 72°C for 1 min; final extension at 72°C for 10 min; hold at 4°C.	593 bp	7
bla_{SHV}	F: AGC CGC TTG AGC AAA TTA AAC R: ATC CCG CAG ATA AAT CAC CAC	95°Cx5 m / 95°Cx1 m-54°Cx1m -72°Cx1 m (35Cycles) / 72°Cx10 m-4°C Initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 1 min, 54°C for 1 min, and 72°C for 1 min; final extension at 72°C for 10 min; hold at 4°C.	713 bp	8
bla_{KPC}	F: CGT CTA GTT CTG CTG TCT TG R: CTT GTC ATC CTT GTT AGG CG	Initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 1 min, 54°C for 1 min, and 72°C for 1 min; final extension at 72°C for 10 min; hold at 4°C.	498 bp	9
bla_{NDM}	F: GGT TTG GCG ATC TGG TTT TC R: CGG AAT GGC TCA TCA CGA TC	Initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 1 min, 54°C for 1 min, and 72°C for 1 min; final extension at 72°C for 10 min; hold at 4°C.	621 bp	10
bla_{VIM}	F: GAT GGT GTT TGG TCG CAT A R: CGA ATG CGC AGC ACC AG	Initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 1 min, 52°C for 1 min, and 72°C for 1 min; final extension at 72°C for 10 min; hold at 4°C.	390 bp	10
bla_{IMP}	F: GGA ATA GAG TGG CTT AAY TCT C R: GGT TTA AYA AAA CAA CCA CC	95°Cx5 m / 95°Cx1 m-52°Cx1m -72°Cx1 m (35Cycles) / 72°Cx10 m-4°C Initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 1 min, 52°C for 1 min, and 72°C for 1 min; final extension at 72°C	232 bp	10
qnrA1 to *qnrA6*	F: AGA GGA TTT CTC ACG CCA GG R: TGC CAG GCA CAG ATC TTG AC	Initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 1 min, 54°C for 1 min, and 72°C for 1 min; final extension at 72°C	580 bp	11
qnrB1 to *qnrB6*	F: GGM ATH GAA ATT CGC CAC TG R: TTT GCY GYY CGC CAG TCG AA (M= A/C, H= A/C, Y=C/T)	95°Cx5 m / 95°Cx1 m-54°Cx1m -72°Cx1 m (35Cycles) / 72°Cx10 m-4°C Initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 1 min, 54°C for 1 min, and 72°C for 1 min; final extension at 72°C	264 bp	11
qnrS1 to *qnrS2*	F: GCA AGT TCA TTG AAC AGG GT R: TCT AAA CCG TCG AGT TCG GCG	Initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 1 min, 54°C for 1 min, and 72°C for 1 min; final extension at 72°C	428 bp	11
gyrA	F: AAA TCT GCC CGT GTC GTT GGT R: GCC ATA CCT ACG GCG ATA CC	Initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 1 min, 50°C for 1 min, and 72°C for 1 min; final extension at 72°C	344 bp	12
parC	F: CTG AAT GCC AGC GCC AAA TT R: GCG AAC GAT TTC GGA TCG TC	Initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 1 min, 49°C for 1 min, and 72°C for 1 min; final extension at 72°C	168 bp	12
mcr1	F: AGT CCG TTT GTT CTT GTG GC R: AGA TCC TTG GTC TCG GCT TG	Initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 1 min, 53°C for 1 min, and 72°C for 1 min; final extension at 72°C	320 bp	13

Quinolone resistance testing

MIC testing for ciprofloxacin resistance was performed using the Ezy MIC™ strip (HiMedia, Mumbai, India) on MHA plates. The MIC values were determined by observing the intersection of the inhibition ellipse with a scale printed on the strip.

Colistin resistance

Colistin agar test: MHA plates were

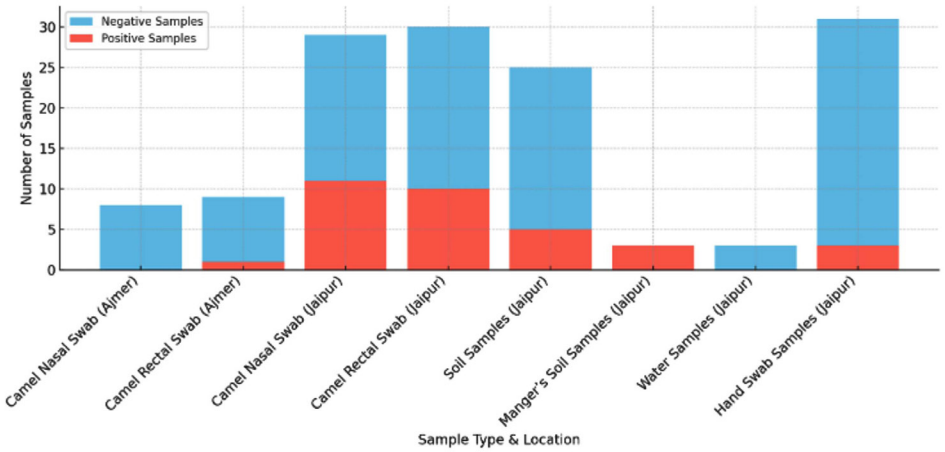


Figure 2. Stacked bar chart visualizing *Klebsiella pneumoniae* positivity across different sample types and locations. Red (salmon) bars indicate positive samples, whereas blue (skyblue) bars indicate negative samples

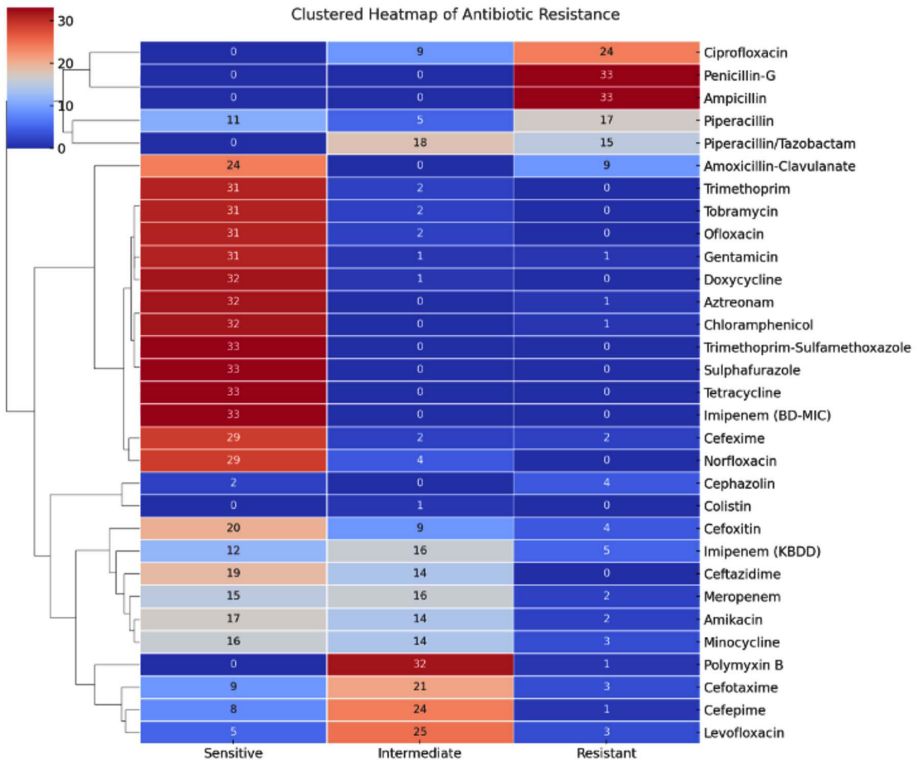


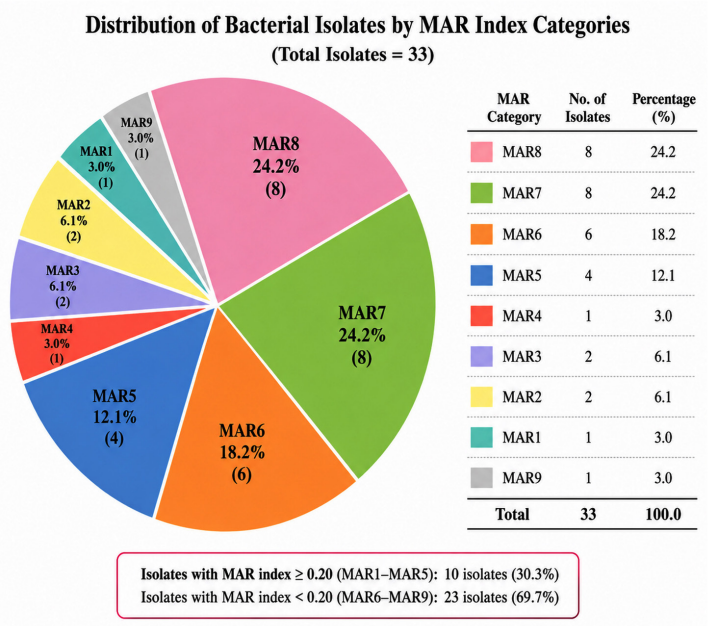
Figure 3. Clustered heat map of antibiotic resistance pattern of *Klebsiella pneumoniae* isolates

supplemented with 0.25-4 µg/mL colistin. After 24 hrs of incubation at 37 °C, growth on the plate was indicative of colistin resistance.

Polymyxin B MIC testing: Polymyxin B Ezy MIC™ strips were used, and the MIC was assessed following 16 hrs of incubation at 37 °C.

Detection of genes Associated with Antibiotic Resistance Genes (ARGs)

The presence of specific ARGs was assessed by PCR. Genes for beta-lactamase (bla_{SHV}, bla_{TEM}, bla_{CTX-M}), carbapenem resistance (bla_{VIM}, bla_{IMP}, bla_{KPC}, bla_{NDM}), quinolone resistance (*qnrA*, *qnrB*, *qnrS*), and colistin resistance (*mcr-1*) were



MAR Category	No. of Isolates	Percentage (%)
MAR8	8	24.2
MAR7	8	24.2
MAR6	6	18.2
MAR5	4	12.1
MAR4	1	3.0
MAR3	2	6.1
MAR2	2	6.1
MAR1	1	3.0
MAR9	1	3.0
Total	**33**	**100.0**

Figure 4. Distribution of 33 bacterial isolates among nine Multiple Antibiotic Resistance (MAR) index categories. MAR7 and MAR8 were the most prevalent categories, each comprising 24.2% of the isolates, followed by MAR6 (18.2%) and MAR5 (12.1%). Isolates with MAR Indices ≥ 0.20 (MAR1-MAR5) represented 30.3% of the total isolates, indicating a substantial proportion of multidrug-resistant strains

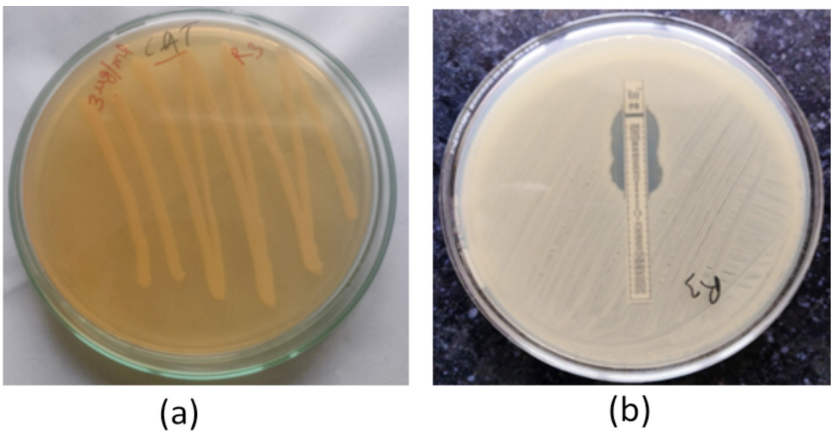


Figure 5. Colistin resistance by colistin agar test. (a) Isolate growth on 3 µg/mL colistin agar indicating resistance. (b) Colistin resistance by polymyxin-B MIC testing. Polymyxin isolate RCKR3 shows a MIC of 6 mcg/mL

amplified using specific primers. The PCR mixture consisted of 12.5 µL of 2X PCR Master Mix, 1 µL each of forward and reverse primers, and 2 µL of DNA template. Details of the primers and thermal cycling conditions are presented in Table 2.

RESULTS

Samples (n = 138) were collected from camels, their handlers, and the surrounding environment. Of these, 34 isolates initially exhibited pink mucoid colonies on MacConkey agar and yellow, dome-shaped mucoid colonies on SCIA. Microscopy after Gram staining indicated small, pink, Gram-negative rods. All the isolates were non-motile. Further, all the isolates tested positive for catalase and negative for oxidase activity, followed by an IMViC pattern is --++. On triple sugar iron (TSI) testing, all isolates displayed yellow butt, yellow slant, and gas production without H_2S production. The results of the primary and biochemical characterizations are displayed in Figure 1. Based on these preliminary findings and the amplification of the 16S-23S rDNA ITS region, 33 isolates were confirmed to be *K. pneumoniae* at the molecular level.

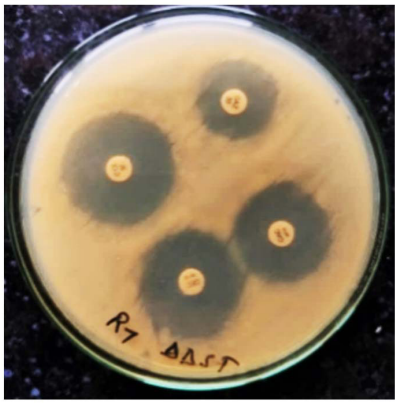


Figure 6. ESBL production was assessed phenotypically by the double disc diffusion method

Occurrence and distribution of *K. pneumoniae*

The overall occurrence of *Klebsiella pneumoniae* infection in the camel ecosystem was 23.9% (33/138). Among the camels, 22 isolates were recovered from 76 samples (28.95%), including 11 of the 37 nasal swabs (29.73%) and 11 of the 39 rectal swabs (28.20%). From the surrounding environment, eight isolates were obtained from 31 samples (25.80%), with five out of 25 soil samples (20%) and three out of three manger soil samples (100%) testing positive, while none of the three water samples (0%) yielded isolates. In contrast, three isolates were recovered from 31 samples (9.68%) collected from camel handlers. The stacked bar chart in Figure 2 shows *K. pneumoniae* positivity across different sample types and locations.

Antibiotic susceptibility profile of *K. pneumoniae* isolates

All isolates (100%) exhibited resistance to penicillin and ampicillin, which is attributable to the intrinsic resistance of *K. pneumoniae* resulting from constitutive production of a chromosomal

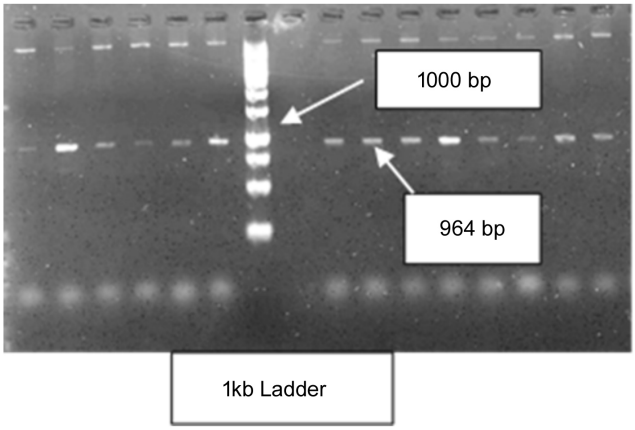


Figure 7. Amplification of the bla_{TEM} gene of *Klebsiella pneumoniae* by PCR

class A β-lactamase. Antibiotic sensitivity pattern of *Klebsiella pneumoniae* isolates against different classes of antibiotics by Kirby-Bauer Disc diffusion method and BD phoenix M50 method is mentioned in the supplementary Table S1 and S2, respectively. High resistance against ciprofloxacin (CIP5) (72.72%) and piperacillin (PIP) (51.51%) was observed. A clustered heatmap of the antibiotic resistance patterns of the isolates is shown in Figure 3. Antibiotic resistance profile of *Klebsiella pneumoniae* isolated from Camels, their handlers and surrounding environment by Kirby-Bauer disc diffusion method/MIC/ BD Phoenix M50 is mentioned in the supplementary Table S3.

Multidrug-resistance

Of the isolates, 22 (66.67%) were MDR and exhibited resistance to three or more classes of antibiotics, as summarized in Figure 4. The multiple antibiotic resistance (MAR) index was calculated for each isolate. Detection and distribution of multiple antibiotic resistance (MAR) Index of the isolates are presented in the supplementary Table S4. The MAR index reflects the potential risk of each isolate acquiring or transmitting drug resistance to other bacterial pathogens. MAR typing of the isolates was conducted based on the MAR index, which resulted in nine MAR categories. In this study, ten isolates (RCKR2, RCKN12, RCKR13, RCKR15, RCKR18, RCKN24, RCKM26, RCKS27, RCKH30, and RCKS30) exhibited MAR indices exceeding 0.2%. All these isolates were from camels (six isolates: RCKR2, RCKN12, RCKR13, RCKR15, RCKR18, and RCKN24), humans (one isolate: RCKH30), or the environment (three isolates: RCKM26, RCKS27, and RCKS30), indicating the potential risk of camel rearers picking up MDR *K. pneumoniae* infection if they do not follow proper sanitary precautions.

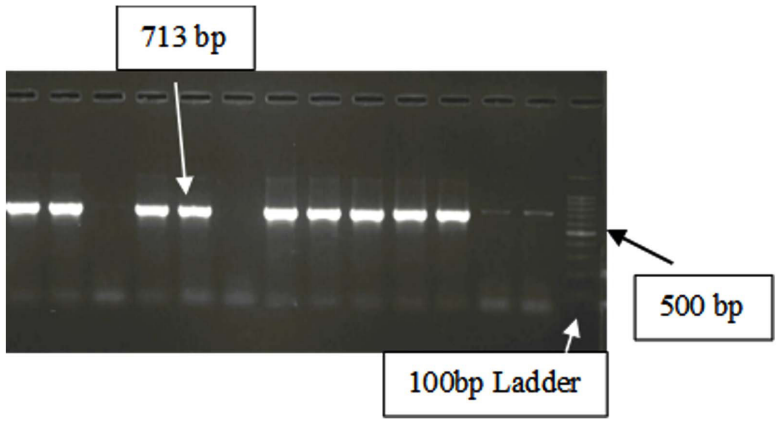


Figure 8. PCR amplification of the bla_{SHV} gene of *Klebsiella pneumoniae*

Figure 9. Phenotypic detection of quinolone resistance by ciprofloxacin MIC testing

Carbapenem resistance

Although five isolates were resistant to imipenem (IPM10) upon disc diffusion, all isolates were confirmed to be susceptible to carbapenems via BD Phoenix M50.

Colistin resistance

One isolate (RCKR3) was phenotypically resistant to colistin based on the colistin agar test and BD Phoenix M50 MIC testing (MIC >2 µg/mL) (Figure 5).

ESBL detection in *K. pneumoniae*

Phenotypic ESBL production was confirmed in 11 isolates (33.33%) using the double-disc diffusion test (Figure 6). Genotypic screening identified ESBL-associated genes, of which bla_{TEM} was the most prevalent gene, detected in 31/33 isolates (93.9%) (Figure 7); bla_{SHV} was detected in 22/33 isolates (66.66%) (Figure 8), and bla_{CTX-M} was absent in all isolates.

Detection of carbapenem and quinolone resistance genes

Carbapenem resistance genes (bla_{VIM}, bla_{IMP}, bla_{KPC}, bla_{NDM}) were not detected in any isolate. None of the isolates harbored plasmid-mediated quinolone resistance genes (*qnrA*, *qnrB*, *qnrS*). Given the high resistance to ciprofloxacin (84.84%) (Figure 9), mutations in *gyrA* may be responsible for this resistance.

Colistin resistance and *mcr-1* gene screening

Only one isolate (RCKR3) was colistin resistant. Screening for the *mcr-1* gene by PCR was negative, indicating that resistance could be mediated by other *mcr-1-10* variants or chromosomal mutations. Further whole-genome sequencing of this isolate is required to identify the exact mechanism underlying the colistin resistance.

DISCUSSION

The present study provides key insights into the prevalence, antimicrobial resistance, and genetic determinants of *K. pneumoniae* at the camel–human–environment interface, emphasizing its significance within the One Health framework. The findings indicate that *K. pneumoniae* is not only prevalent in camels but also in their associated environment and handlers, with an overall isolation rate of 23.9%. These results are consistent with earlier observations on *K. pneumoniae* from camels and other livestock, highlighting its ubiquitous nature and potential role in cross-contamination and zoonotic transmission.

Comparative prevalence and epidemiological significance

The isolation rate recorded in the current investigation resembles prior findings in camels, with a reported prevalence of 26.71% in healthy camels in Egypt,[14] of which the isolation rate from nasal and lung swabs from camels in Gharbia was 7.31%.[15] Similarly, another One Health study reported *K. pneumoniae* isolation rates of 11.6% in animals, 8.4% in humans, and 7.0% from environmental sources in Punjab, Pakistan.[16] The detection of *K. pneumoniae* across multiple reservoirs shows its remarkable adaptability in diverse ecological contexts.

Multidrug-resistance and ESBL production in Camel ecosystem

One of the most significant findings was the predominance of ESBL-producing *K. pneumoniae* in apparently healthy camels (31.8%). This is particularly concerning, as previous studies have reported no ESBL producers in healthy camels (0.0%) and 31.9% in diseased camels.[17] The increasing prevalence of ESBL-producing strains in healthy animals suggests an increasing trend of AMR, possibly owing to environmental contamination, unregulated antibiotic use, or horizontal gene transfer.

The predominant ESBL genotype detected in this study was bla_{TEM} (93.9%), followed by bla_{SHV} (66.66%), which is consistent with the findings of other studies from Rajasthan.[18,19] These results further support the notion that bla_{TEM} is the dominant genotype in this region. The high prevalence of bla_{TEM} (93.9%) may reflect either the clonal expansion of a successful ESBL-producing lineage or widespread dissemination mediated by mobile genetic elements. Given the recovery of isolates from multiple ecological niches within the camel–human environment interface, horizontal gene transfer under antimicrobial selection pressures appears plausible. However, molecular typing studies are required to delineate clonal relatedness and transmission dynamics. The absence of the globally dominant ESBL bla_{CTX-M} in our study contrasts with previous reports, which found a higher bla_{CTX-M} prevalence in *K. pneumoniae*

from bovines in Northeast India and West Bengal.[20,21] The absence or very low prevalence of bla_{CTX-M} in Rajasthan's camel ecosystem may be due to limited third-generation cephalosporin pressure and reduced spillover of hospital-origin strains. Due to the limited use of third-generation cephalosporins in camel ecosystems, there is insufficient selective pressure to drive bla_{CTX-M} expansion. This geographical variation in ESBL gene distribution warrants further genomic surveillance to track the development and dissemination of resistance determinants.

Resistance to quinolone and role of chromosomal mutations

This study identified a high ciprofloxacin resistance rate (84.84%). Since no plasmid-mediated quinolone resistance genes (*qnrA*, *qnrB*, *qnrS*) were found, chromosomal alterations, particularly in *gyrA* and *parC*, likely contributed to the quinolone resistance in these isolates. These findings are consistent with reports of 80% resistance to quinolones in *K. pneumoniae* isolates from poultry farms in coastal Karnataka.[22] In contrast, one study reported no ciprofloxacin-resistant isolates in healthy camels and only 8.5% resistance in diseased camels, highlighting the emerging resistance trend over time.[17]

Colistin resistance and implications for last-resort antibiotics

The detection of the colistin-resistant isolate RCKR3 is notable because colistin is a last-line antibiotic used against MDR Gram-negative infections. Although *mcr-1* was not detected, the resistance might be mediated by other *mcr* variants (*mcr-1* to *mcr-10*) or chromosomal mutations in *mgrB* or *pmr*. Reports on colistin-resistant *K. pneumoniae* in camels are extremely rare, and this study is the first to document colistin resistance in *K. pneumoniae* in camels in India. Resistance to colistin has been previously reported in pigs (Portugal),[23] turkey meat (Czech Republic),[24] and camels (Dubai),[25] where *E. coli* with *mcr-1* was detected and colistin-resistant *Enterobacter cloacae* (Tunisia)[26] was negative for *mcr-1*. Phenotypic colistin resistance in a camel-associated ecosystem, despite the absence of *mcr-1*, highlights the need for ongoing genomic surveillance to detect emerging resistance mechanisms, risk assessment studies to evaluate zoonotic transmission pathways, and investments in research on resistance in nontraditional livestock systems.

Public health and one health implications

These results have significant public health relevance, particularly in areas where camels are central to milk production, meat supply, and transportation. Our findings provide empirical evidence that camel ecosystems can serve as reservoirs of clinically significant resistant pathogens, particularly in regions where camels are a key source of milk, meat, and transportation.

Given the rapid evolution of AMR, integrating the One Health approach into future research is critical for understanding the transmission dynamics between animals, humans, and the environment.

CONCLUSION

This study provides the first comprehensive analysis of ESBL-producing, quinolone- and colistin-resistant *K. pneumoniae* isolates from a camel ecosystem in India. The high prevalence of MDR isolates across camels, handlers, and the environment underscores the need for continuous surveillance, strict antimicrobial stewardship, and interdisciplinary collaboration to mitigate the spread of AMR within the One Health Framework.

SUPPLEMENTARY INFORMATION

Supplementary information accompanies this article at https://doi.org/10.22207/JPAM.20.3.15

Additional file: Tables S1-S4.

ACKNOWLEDGMENTS

The authors are grateful to the Vice Chancellor of RAJUVAS, Bikaner, and the Dean of PGIVER, Jaipur, for their support through the provision of laboratory facilities and research consumables. The authors also sincerely acknowledge the Director of ICAR-NIVEDI for granting permission to use the institute's laboratory facilities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

YS, BJ, SN, SKS, SSS, TB, AF, RK and VKM conceptualized and designed the study. YS, RK and VKM performed material preparation. YS, BJ, SN and SKS designed the methodology. YS, AF, TB and SSS performed data collection and analysis. YS wrote the manuscript. All authors reviwed, revised and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and/or in the supplementary files.

ETHICS STATEMENT

Not applicable.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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