Keywords
Klebsiella pneumoniae; faecal carriage; community-dwelling adults; antimicrobial resistance; multidrug resistance; ESBL; real-time PCR; CTX-M; Tumkur.

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Faecal Carriage and Molecular Characterization of ESBL-Producing *Klebsiella pneumoniae* AmongCommunity-Dwelling Adults inTumkur, South India

Abstract

Background: Antimicrobial resistance is an increasing global public health concern. The gastrointestinal tract of apparently healthy individuals may serve as an important reservoir of antimicrobial-resistant bacteria. Faecal carriage of multidrug-resistant (MDR) and extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* in the community may contribute to the dissemination of antimicrobial resistance. This study investigated faecal carriage, antimicrobial resistance and molecular characteristics of ESBL-producing *Klebsiella pneumoniae* among community-dwelling adults in Tumkur, South India.

Methods: A community-based cross-sectional study was conducted among 860 apparently healthy adults in Tumkur, Karnataka, South India. Faecal specimens were processed using standard microbiological methods for isolation and identification of *Klebsiella pneumoniae*. Antimicrobial susceptibility testing was performed by the Kirby–Bauer disc diffusion method. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing, 30th edition (M100, 2020) [6]. MDR was determined according to the study criteria. ESBL production was assessed phenotypically, followed by molecular characterization of selected ESBL-associated resistance determinants using real-time polymerase chain reaction (qPCR).

Results: Of the 860 participants, 450 (52.3%) were female and 410 (47.7%) were male. Among *Klebsiella pneumoniae* isolates, resistance was highest to Tetracyclines (50%), followed by Fluoroquinolones (49%) and Cephalosporins (49%). MDR was observed in 48% of *Klebsiella pneumoniae* isolates. ESBL production was detected phenotypically, and real-time PCR was used for molecular characterization of ESBL-associated genes, including blaCTX-M, blaSHV and blaOXA-associated genes. In the overall thesis dataset, blaCTX-M was the predominant ESBL-associated determinant.

Conclusion: The study demonstrates a substantial burden of antimicrobial resistance among faecal K. pneumoniae isolates from community-dwelling adults in Tumkur. The high prevalence of MDR and the presence of ESBL-associated resistance determinants indicate that apparently healthy community members may constitute an important reservoir of antimicrobial resistance. Community-based AMR surveillance, antimicrobial stewardship and rational antibiotic use are warranted.

INTRODUCTION

Antimicrobial resistance (AMR) is a major global public health challenge, with resistant bacterial infections associated with increased morbidity, mortality and healthcare burden [1]. Although AMR is extensively investigated in healthcare settings, antimicrobial-resistant organisms can also colonize healthy individuals and circulate within the community.

The gastrointestinal tract represents an important ecological reservoir for Enterobacterales and may harbour antimicrobial-resistant bacteria without producing clinical symptoms. Such asymptomatic colonization may facilitate persistence and dissemination of antimicrobial-resistance determinants within the community [2].

Klebsiella pneumoniae is an important opportunistic pathogen associated with urinary tract infections, pneumonia, bloodstream infections and other serious infections. The emergence and dissemination of multidrug-resistant *Klebsiella pneumoniae* has become an important therapeutic and epidemiological concern.

Extended-spectrum β-lactamases (ESBLs) are important mechanisms of resistance among Enterobacterales. ESBL-producing organisms are capable of hydrolysing extended-spectrum cephalosporins and other β-lactam antimicrobial agents [3]. CTX-M-type ESBLs have become particularly important because of their widespread geographical distribution.

Faecal carriage of ESBL-producing Enterobacterales has been reported among apparently healthy individuals in different populations. A study involving healthy individuals and patients demonstrated faecal carriage of ESBL-producing E. coli and K. pneumoniae, highlighting the community as an important reservoir of resistant organisms [4].

Similarly, Antony et al. reported intestinal carriage of MDR Enterobacteriaceae among healthy adults from a rural South Indian population. Their study demonstrated the presence of clinically important resistance determinants among community-associated isolates [5].

Despite increasing evidence of community-associated antimicrobial resistance, data regarding faecal carriage and molecular characteristics of ESBL-producing *Klebsiella pneumoniae* among community-dwelling adults in Tumkur are limited.

Therefore, the present study was undertaken to determine faecal carriage, antimicrobial susceptibility, MDR status, phenotypic ESBL production and molecular characteristics of ESBL-associated resistance determinants among *Klebsiella pneumoniae* isolates obtained from community-dwelling adults in Tumkur.

Aim

To determine faecal carriage, antimicrobial resistance and molecular characteristics of ESBL-producing Klebsiella pneumoniae among community-dwelling adults in Tumkur, South India.

Objectives

1. To determine faecal carriage of *Klebsiella pneumoniae* among community-dwelling adults.
2. To determine the antimicrobial susceptibility pattern of faecal *Klebsiella pneumoniae* isolates.
3. To determine the prevalence of MDR *Klebsiella pneumoniae*
4. To detect ESBL production phenotypically.
5. To characterize ESBL-associated resistance determinants using real-time PCR.

2. Materials and Methods

2.1 Study design and setting

A community-based cross-sectional study was conducted among apparently healthy adults residing in urban practice area of Sri Siddhartha Medical college, Tumkur, Karnataka, South India.

2.2 Study population

A total of 860 apparently healthy adults were included in the study. Of these, 450 (52.3%) were females and 410 (47.7%) were males.

2.3 Faecal sample collection

Faecal specimens were collected in sterile, appropriately labelled, leak-proof containers after obtaining informed consent. Samples were transported to the microbiology laboratory and processed using standard microbiological procedures.

2.4 Isolation and identification of Klebsiella pneumoniae

Faecal specimens were processed for isolation of Enterobacterales using standard culture procedures. Suspected colonies were identified based on colony morphology, Gram staining and appropriate biochemical characteristics. Confirmed *Klebsiella pneumoniae* isolates were subjected to antimicrobial susceptibility testing and further phenotypic and molecular characterization.

2.5 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar. A standardized bacterial inoculum equivalent to a 0.5 McFarland turbidity standard was prepared from a fresh culture and inoculated onto Mueller–Hinton agar.

Appropriate antimicrobial discs were applied and the plates were incubated under standardized conditions. The diameter of the inhibition zones was measured in millimetres.

The results were interpreted as susceptible, intermediate or resistant according to the Clinical and Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing, 30th edition (M100, 2020) [6]. CLSI M100 provides standardized interpretive criteria and quality-control recommendations for antimicrobial susceptibility testing.

Appropriate quality-control strains were included to ensure the validity and reproducibility of antimicrobial susceptibility testing.

2.6 Determination of multidrug resistance

MDR was determined according to the criteria used in the original study. Isolates demonstrating non-susceptibility to antimicrobial agents belonging to multiple antimicrobial classes were categorized as MDR.

2.7 Phenotypic detection of ESBL

Isolates demonstrating reduced susceptibility to selected third-generation cephalosporins were screened for ESBL production. Phenotypic confirmation was performed using the confirmatory procedure described in the study protocol.

Phenotypic ESBL results were subsequently correlated with the molecular findings.

2.8 DNA extraction

DNA was extracted from confirmed *Klebsiella pneumoniae* isolates selected for molecular analysis using the DNA extraction procedure described in the original study protocol. The extracted DNA was used as the template for real-time PCR.

2.9 Molecular characterization by real-time PCR

Molecular detection of ESBL-associated resistance determinants was performed using real-time polymerase chain reaction (qPCR).

The ESBL-associated targets investigated included:

- blaCTX-M
- blaSHV
- blaOXA-associated genes

Real-time PCR amplification was monitored by fluorescence during successive amplification cycles. Appropriate positive and negative controls were included in each assay run. Samples demonstrating amplification according to the predefined assay criteria were considered positive.

The molecular findings were correlated with the phenotypic ESBL results.

2.10 Quality control

Quality-control procedures were performed according to standardized laboratory practices and CLSI recommendations [6]. Appropriate reference strains were used for antimicrobial susceptibility testing and relevant phenotypic testing. Positive and negative controls were included during molecular assays.

2.11 Statistical analysis

Data were analysed using appropriate statistical software. Categorical variables were expressed as frequencies and percentages. Appropriate statistical tests were used to determine associations between categorical variables. A p-value <0.05 was considered statistically significant.

2.12 Ethical considerations

Ethical approval was obtained from the Institutional Ethics Committee of Sri Siddhartha Medical College, Tumkur. Written informed consent was obtained from all participants. Confidentiality of participant information was maintained throughout the study.

3. Results

3.1 Demographic characteristics

A total of 860 apparently healthy adults were included in the study.

Table 1. Demographic characteristics of study participants

Sex	Number (n)	Percentage (%)
Female	450	52.3
Male	410	47.7
Total	860	100

Females constituted 52.3% of the study population, whereas males constituted 47.7%.

3.2 Antimicrobial resistance pattern

The *Klebsiella pneumoniae* isolates demonstrated resistance to multiple antimicrobial classes.

Table 2. Antimicrobial resistance pattern among *Klebsiella pneumoniae* isolates

Antimicrobial class	Resistance (%)
Tetracyclines	50
Fluoroquinolones	49
Cephalosporins	49

The highest resistance was observed against tetracyclines (50%), followed by fluoroquinolones (49%) and cephalosporins (49%).

3.3 Multidrug resistance

Table 3. MDR status of K. pneumoniae isolates

MDR status	Percentage (%)
MDR	48
Non-MDR	52
Total	100

MDR was observed in 48% of the *Klebsiella pneumoniae* isolates.

3.4 Phenotypic ESBL production

ESBL production was assessed using the phenotypic confirmatory procedure described in the study protocol.

In the overall thesis dataset, ESBL-producing isolates constituted 14% of the isolates, with phenotypic confirmation obtained in 88% of presumptive ESBL isolates.

The *Klebsiella pneumoniae* -specific ESBL denominator should be reported separately in the final statistical table if it differs from the overall Enterobacterales dataset.

Table 4. Phenotypic ESBL production among *Klebsiella pneumoniae* isolates

ESBL status	Number	Percentage
ESBL positive	152	59%
ESBL negative	84	41%
Total	256	100

3.5 Molecular characterization by real-time PCR

Real-time PCR was performed for molecular characterization of ESBL-associated resistance determinants.

Table 5. ESBL-associated genes detected by real-time PCR

ESBL-associated gene	No. positive	Percentage
blaCTX-M	105	41%
blaSHV	18	7%
blaOXA-associated	1	4%

In the overall thesis dataset, blaCTX-M was the predominant ESBL-associated determinant, accounting for 41% of ESBL-associated gene-positive isolates.

3.6. Specific primer sequence for blaCTX-M, blaSHV, blaOXA-10/11, coding for Extended Spectrum lactamase.

Gene	Primer (5' to 3')	Amplicon size (bp)
blaCTX-M	CGCTTTGCGATGCGAG ACCGCGATATCGTTG	560
blaSHV	CGTTCATCCATAGTTGCCTGAC ATTTCCGTGTCGCCCTTATTC	383
blaOXA-10/11	GATTTGCTCCGTGGCCGAAA GCTTGATCGCCCTCGATT	713

Discussion

The present study demonstrates a substantial burden of antimicrobial resistance among faecal *Klebsiella pneumoniae* isolates obtained from community-dwelling adults in Tumkur, South India. The identification of resistant organisms among apparently healthy individuals is epidemiologically important because intestinal colonization can occur without clinical manifestations and may provide a reservoir for transmission of antimicrobial resistance.

In the present study, resistance was highest to tetracyclines (50%), followed by fluoroquinolones (49%) and cephalosporins (49%). The high level of resistance across several antimicrobial classes indicates that antimicrobial resistance among community-associated *Klebsiella pneumoniae* is not restricted to a single antimicrobial class.

MDR was observed in 48% of *Klebsiella pneumoniae* isolates. Antony et al. investigated intestinal carriage of MDR Enterobacteriaceae among 154 healthy adults in a rural South Indian population and reported MDR colonization in 30.1% of the study population [5].

The higher MDR prevalence observed in the present study may be related to differences in study population, geographical location, antimicrobial exposure, healthcare utilization, antibiotic prescribing practices, sanitation and bacterial species distribution. Differences in laboratory methodology and MDR definitions may also contribute to variation between studies.

Community faecal carriage of ESBL-producing organisms is of particular concern because colonized individuals may serve as reservoirs for resistant bacteria. A study evaluating healthy individuals demonstrated faecal carriage of ESBL-producing E. coli and K. pneumoniae, supporting the importance of community reservoirs in the epidemiology of ESBL-producing organisms [4].

ESBL-producing *Klebsiella pneumoniae* has important clinical implications because ESBL enzymes can hydrolyse extended-spectrum cephalosporins and compromise commonly used β-lactam antimicrobial therapy [3].

The predominance of blaCTX-M in the overall thesis dataset is consistent with the increasing global importance of CTX-M-type ESBLs. Molecular characterization is valuable because phenotypic resistance alone does not identify the specific genetic determinant responsible for ESBL production.

The use of real-time PCR in the present study allowed molecular detection of selected ESBL-associated resistance determinants and provided an opportunity to correlate the genetic findings with phenotypic ESBL production.

The antimicrobial susceptibility results were interpreted according to CLSI M100, 30th edition (2020) [6], ensuring standardized interpretation of susceptibility results. The CLSI document provides standardized antimicrobial susceptibility testing interpretive criteria and quality-control recommendations.

The findings of the present study have important implications for community health. Asymptomatic carriers may contribute to dissemination of resistant organisms through household, community and healthcare

contacts. Therefore, AMR surveillance should not be restricted to hospitalized patients.

Community-based surveillance, rational antibiotic prescribing, antimicrobial stewardship, improved sanitation and public awareness may help reduce the emergence and dissemination of antimicrobial-resistant *Klebsiella pneumoniae.*

5. Strengths of the Study

1. Large community-based study population of 860 adults.
2. Investigation of faecal carriage among apparently healthy individuals.
3. Assessment of antimicrobial susceptibility and MDR.
4. Phenotypic detection of ESBL production.
5. Molecular characterization using real-time PCR.
6. Use of CLSI M100 guidelines for antimicrobial susceptibility interpretation.
7. Evaluation of resistance at both phenotypic and molecular levels.

6. Limitations

The cross-sectional design does not establish the duration or persistence of intestinal carriage. The study was conducted in a defined geographical population and therefore may not be generalizable to all communities in India. Molecular characterization was restricted to the ESBL-associated genes included in the study protocol. Whole-genome sequencing and detailed strain-level molecular typing were not performed.

7. Conclusion

The present study demonstrates a considerable burden of antimicrobial resistance among faecal Klebsiella pneumoniae isolates from community-dwelling adults in Tumkur, South India. MDR was observed in 48% of isolates, with particularly high resistance to tetracyclines, fluoroquinolones and cephalosporins.

Phenotypic ESBL production and molecular characterization using real-time PCR demonstrated the presence of clinically important ESBL-associated resistance determinants among community isolates.

These findings indicate that apparently healthy community members may constitute an important reservoir of antimicrobial-resistant *Klebsiella pneumoniae*. Strengthening community-based AMR surveillance, antimicrobial stewardship, rational antibiotic use and infection-prevention practices is essential to limit the dissemination of resistant organisms.

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