



Molecular Detection and Antimicrobial Resistance Profiling of *Klebsiella oxytoca* in Female Patients with Urinary Tract Infections Using *pehX* Gene PCR in Najaf, Iraq

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Abstract

Klebsiella oxytoca is a Gram-negative opportunistic pathogen, increasingly associated with urinary tract infections (UTIs), especially in healthcare-associated infections. This study was designed to investigate the prevalence of *K. oxytoca* in female UTI patients, the antimicrobial resistance pattern of the isolated strains and their clinical relevance.

During May 2024 – December 2025, 192 female patients with symptomatic UTIs were recruited from Al-Zahra Teaching Hospital in Najaf, Iraq. Standard microbiological and biochemical methods and polymerase chain reaction (PCR) targeting the species-specific *pehX* gene were used to identify bacterial isolates.

Thirty-six isolates (18.75%) were found to be *K. oxytoca*, and all produced the 344-bp PCR amplicon. The antimicrobial susceptibility test showed high resistance to ceftriaxone and ciprofloxacin and low resistance to amikacin. The isolates were most susceptible to imipenem.

The results highlight the significance of using molecular diagnostic methods for correct identification of *K. oxytoca* and the growing problem of antimicrobial resistance of clinical isolates. To manage patients optimally and enhance infection control practices, the continuous epidemiological surveillance and implementation of targeted antimicrobial strategies are essential. Molecular identification of *Klebsiella oxytoca* can be reliably achieved using the *pehX* gene.

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Introduction

Klebsiella Bacteria and Associated Infections

The *Klebsiella* genus consists of Gram-negative non-motile, facultatively anaerobic bacteria in the family *Enterobacteriaceae*. They are usually found in short chains, in pairs or singly as short rods, measuring 0.3–1 µm in width and 0.6–6 µm in length. They grow best at 37°C, and are characterized by the utilization of citrate and the production of urease, lysine decarboxylase and lactose fermenting; they are negative for ornithine decarboxylase. Their biochemistry is oxidase-negative, catalase-positive, methyl red-negative and Voges-Proskauer positive. Of particular interest, *Klebsiella oxytoca* is also indole (INDO) positive which differentiates it from other species.^[1]

One of the defining characteristics of *Klebsiella* species is the large polysaccharide capsule, which gives colonies a characteristic mucoid appearance, easily seen on MacConkey agar. After incubation, colonies may have a sticky consistency that looks like string and is characteristic of a high capsule content. This structure is not only important for laboratory identification, but also for immune evasion and is crucial to virulence.^[2,3]

Klebsiella species are part of the transient flora of the gastrointestinal tract and nasopharynx of humans and are found in large numbers in various environments and host-related circumstances. Although they are commensal organisms, they are well known opportunistic pathogens and can cause a wide range of infections such as pneumonia, septicemia, urinary tract infection (UTI)

and soft tissue infections. The capsule and other virulence factors are believed to contribute to their pathogenicity, due to their anti-phagocytic properties^[4,5,6]

Klebsiella spp. *Klebsiella oxytoca* and *Klebsiella pneumoniae* are commonly associated with urinary tract infections, particularly in healthcare facilities, particularly in patients with catheters and surgical patients^[7]. These infections constitute a significant proportion of both hospital-acquired urinary tract infections (HAUTIs) and catheter-associated urinary tract infections (CAUTIs). These bacteria produce urease which may help to alkalize urine and can lead to the development of renal stones. Severe infections may result in septicemia and multi-organ involvement^[8,9].

***Klebsiella oxytoca* and Urinary Tract Infections**

UTIs are one of the most prevalent bacterial infections in the world, with more than 150 million people developing one each year^[10]. While *Klebsiella pneumoniae* is still the most common species, *Klebsiella oxytoca* has been steadily becoming a clinically relevant organism in community and hospital settings.

K. oxytoca is a Gram-negative bacterium which usually inhabits the gastrointestinal and respiratory tracts of between 2-10% of people. It has the potential to become pathogenic from a commensal organism under the right conditions, especially if the person is immunocompromised or has an impaired epithelial barrier. It is a multi-factorial pathogen, with adhesion molecules, biofilm development and evasion of the immune system being all involved in its virulence^[11,12]

UTIs result from a sequence of events that includes the ascent of uropathogens, adhesion to uroepithelial cells, and colonization of the urinary tract. Aside from these classical uropathogenic mechanisms, there is recent molecular evidence that microbial dysbiosis is a key player in female reproductive health^[13] followed by adhesion to uroepithelial cells and then colonization of the urinary tract. Formation of bio-film is a key factor in chronic infections, especially indwelling urinary catheters. Host proteins like fibrinogen that are deposited on catheter surfaces help bind bacteria to the catheter surface and contribute to the formation of bacterial biofilm, which may result in resistance to microbial killing and host immune responses.^[14]

The host defense mechanisms, such as responses from macrophages and neutrophils, help to put an end to infection, but over-activation of inflammation can lead to tissue damage. From a clinical perspective, UTIs are divided into uncomplicated, complicated, and recurrent UTIs, depending on the clinical pattern of the disease, and the presence of underlying risk factors^[15,16].

Polymerase Chain Reaction (PCR) in Bacterial Identification

Traditional biochemical methods can be useful but cannot be used to distinguish between closely related species like *K. pneumoniae* and *K. oxytoca*. The molecular diagnostic tests including polymerase chain reaction (PCR) have greater sensitivity and specificity in the identification of bacteria.

Polygalacturonase (*pehX*) gene is a species-specific marker for *K. oxytoca*. This gene can be easily and rapidly identified by the presence of a characteristic 344-bp fragment generated by amplification. This molecular method is superior to the traditional and automated identification systems which could lead to ambiguous or overlapping results.^[17,18]

Antimicrobial Resistance

AMR has become a critical public health issue and has occurred in *K. oxytoca*. This pathogen shows many resistance mechanisms, including the production of extended-spectrum β -lactamases (ESBLs) which greatly hinder susceptibility to β -lactam antibiotics like cephalosporins. In addition, this fluoroquinolone resistance and other frequently used antimicrobials are problematic in therapy.^[19,20]

It is important to routinely test the antimicrobial susceptibility pattern, because the emergence of multidrug-resistant strains is becoming more common in the world and this requires proper treatment choices to be made and better treatment outcomes achieved. The combination of molecular diagnosis with resistance profiling offers an integrated way of managing infections and conducting epidemiological surveillance^[21].

Patients and Methods

Sample Collection

One hundred ninety-two female patients with symptomatic urinary tract infections (UTIs) were recruited in Al-Zahra Teaching Hospital, Najaf, Iraq, from May 2024 to December 2025. Of those, 36 (18.75%) were molecularly confirmed as *Klebsiella oxytoca* by the detection of the *pehX* gene that results in a 344-bp PCR amplicon. The identification of the initial strains was done by conventional microbiological and biochemical techniques, and confirmed by VITEK 2 system. Midstream urine samples were obtained in sterile containers and collected aseptically with the help of trained microbiology lab staff.

Bacterial Identification

Morphological Identification

Initial classification was done using the colony morphology on MacConkey agar. Colonies were large, mucoid and lactose fermenting which suggested the presence of enteric gram-negative bacteria.

Microscopic Examination

The isolated colonies were cultured into bacterial smears which were heat fixed on clean glass slides and stained using Gram staining procedure. The cells were examined under the microscope for their morphology, arrangement and Gram reaction.

Biochemical Characterization

Biochemical identification was performed by using the following standard tests:

Indole Test: The peptone water cultures were incubated at 37°C for 24 hours and then the Kovac's reagent was added. A

red ring meant that there was a positive result.

Voges – Proskauer Test: MR-VP broth was incubated at 37°C for 24 hours, and the addition of reagent caused a red color to develop, which was considered a positive reaction.

Citrate Utilization Test: Simmons' citrate agar was inoculated and incubated at 37°C for 24 hours, a change in color from green to blue was considered to be positive.

The Kligler's Iron Agar Test used 24 hours of incubation at 37°C with the presence of yellow coloration showing carbohydrate fermentation and gas production was seen as bubbles and cracks.

Oxidase Test: Colonies were tested with oxidase reagent, purple colour appeared in 2-10 seconds was considered as positive.

Bacterial colonies when treated with hydrogen peroxide, produced bubbles instantly which showed catalase positivity.

Molecular Identification

DNA Extraction

Two methods were used to extract genomic DNA from overnight cultures of bacteria. A boiling method was first used: Two colonies were suspended in 1 mL of distilled water, boiled for 10 minutes followed by centrifugation at 1000 rpm for 5 minutes. The supernatant was then used for the DNA template and 5 µL of the supernatant was used. Optimal DNA quality was obtained by further extraction of the DNA using a commercial Bioneer kit following manufacturer's protocol.

Polymerase Chain Reaction (PCR)

Species-specific primers for *pehX* gene of *K. oxytoca* were used for PCR amplification. The sequences of the primers were 5'-GAT ACG GAG TAT GCC TTT ACG GTG-3' (forward) and 5'-TAG CCT TTA TCA AGC GGA TAC TGG-3' (reverse).

The reaction mixture (20 µL total volume) consisted of 10 µL of ready master mix, 2 µL of each primer, 5 µL of DNA template, and nuclease-free water to complete the final volume. The thermal cycling conditions used were an initial denaturation step at 95°C for 5 min followed by 35 cycles of denaturation (94°C for 30 s), annealing (55°C for 30 s), and extension (72°C for 30 s); a final extension step (72°C, 7 min) was performed.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was carried out by the Kirby Bauer disk diffusion technique on MHA following the guidelines of the Clinical and Laboratory Standards Institute (CLSI). A standardized bacterial suspension of 0.5

McFarland turbidity was prepared and the bacteria were then evenly spread on the surface of the agar.

The antibiotics tested were Imipenem (10 µg), Ciprofloxacin (5 µg), Ceftriaxone (30 µg) and Amikacin (30 µg). Aerobic incubation for 18-24 hours at 37°C was used to incubate plates. The zone of inhibition was measured in millimeters and the results were graded sensitive or resistant based on the CLSI guidelines. The number of susceptible and resistant isolates were expressed as percentages.

Inclusion and Exclusion Criteria

Patients included had a diagnosis of bacterial UTI involving any segment of the urinary tract (kidneys, ureters, bladder, and urethra). Patients with non-bacterial urinary conditions and within 30 days of antibiotic use were excluded.

Ethical Considerations

The study protocol was approved by the Institutional Ethics Committee for Human Studies - Al-Zahra Teaching Hospital - Najaf, Iraq. All subjects gave informed written consent before sample was taken.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) software, version 25.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Data were summarized using descriptive statistics which were presented in frequency and percentages. The Chi-square (χ^2) test was used for the comparative analysis of the antimicrobial resistance pattern of the tested antibiotics. The Fisher's exact test was used for cells with expected frequencies less than 5. P-value < 0.05 was regarded as statistically significant.

Results

Cultural Characteristics of *Klebsiella oxytoca* Isolates

The 36 isolates recovered from 192 clinical samples of urine were cultured and examined culturally and typical growth characteristics were observed on various culture media which were consistent with *Klebsiella oxytoca*.

MacConkey Agar

K. oxytoca isolates grew on MacConkey agar with large, smooth, circular colonies that were mucoid in appearance. The colonies were pink to red because they were able to ferment lactose and produce acidic fermentation products that caused the pH of the medium to change. The mucoid texture is due to the presence of a good polysaccharide capsule, which is an important virulence factor in the protection and persistence of the bacteria.



Fig 1: *Klebsiella oxytoca* bacteria on MacConkey medium

Isolates were gram stained for microscopic examination and *Klebsiella oxytoca* was found to be a Gram-negative bacterium, which were pink to red in colour due to the decolorization step and counterstaining. The cells were rod-shaped (bacillary) and were seen mainly as single cells, but in some cases as pairs and short chains.

When seen under capsule specific stains like India ink or negative stain, a conspicuous polysaccharide capsule was

seen, as a clear halo around the bacterial cells. It is a capsular structure that is a significant virulence factor and a factor in the resistance of bacteria to phagocytosis and survival in the host.

The isolates were also non-motile, characteristic of *Klebsiella* species of the Enterobacteriaceae family, as *Klebsiella* species lack flagella.

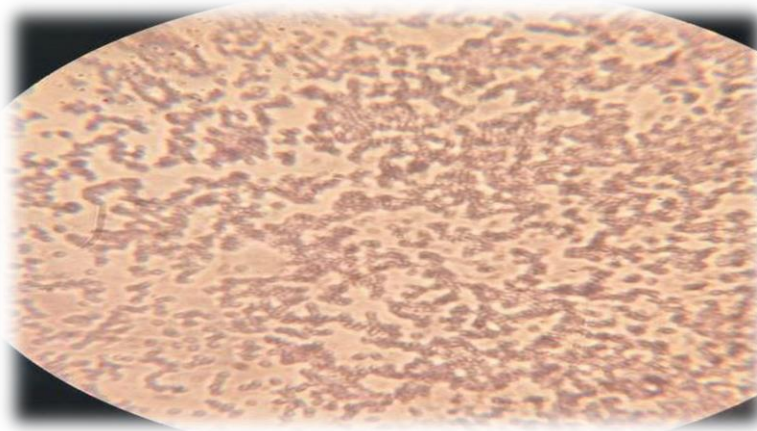


Fig 2: *Klebsiella oxytoca* bacteria under the microscope.

Biochemical diagnosis

The results of biochemical tests are presented conducted for the identification of *Klebsiella oxytoca*. The biochemical

characteristics of the bacterial isolates were confirmed by the use of these tests which helped to differentiate the bacteria from other closely related species.

Table 1: Chemical properties

Test	Result
Indole Production	(+)
Methyl Red	(-)
Voges-Proskauer	(+)
Citrate Utilization	(+)
Urease Production	(+)
Oxidase	(-)

Antimicrobial Resistance

The observed resistance pattern revealed a high resistance rate to β -lactam, especially to Ceftriaxone which can be

related to the presence of ESBL-producing strains, an Imipenem is still the most effective antibiotic against *Klebsiella oxytoca* isolates.

Table 2: Antimicrobial Susceptibility Profile of *Klebsiella oxytoca* Isolates (n = 36 isolates)

Antibiotic	Sensitive n (%)	Resistant n (%)	Interpretation
Imipenem	34 (94.4%)	2 (5.6%)	Highly active against isolates
Ciprofloxacin	18 (50.0%)	18 (50.0%)	Moderate resistance observed
Ceftriaxone	10 (27.8%)	26 (72.2%)	High resistance (ESBL-associated)
Amikacin	26 (72.2%)	10 (27.8%)	Relatively effective

The antimicrobial susceptibility analysis revealed a statistically significant variation in antimicrobial susceptibility patterns among the tested antibiotics ($P < 0.001$). Thirty-six isolates of *Klebsiella oxytoca* were identified, with imipenem having the highest sensitivity rate of 94.4%, which reflects its high efficacy against *Klebsiella oxytoca* isolates. Ceftriaxone was the most resistant antibiotic (72.2%), indicating the possible presence of extended-spectrum β -lactamase (ESBL)-producing isolates. Ciprofloxacin showed moderate resistance (50%) and Amikacin – excellent sensitivity (72.2%). Overall, these results suggest that there is a wide range of antimicrobial

activity, and it is important to select an appropriate antibiotic, depending upon the susceptibility test.

Results of antimicrobial susceptibility testing demonstrated high resistance rates to ceftriaxone, whereas imipenem exhibited the highest sensitivity against *Klebsiella oxytoca* isolates.

Molecular Identification of *Klebsiella oxytoca*

To confirm the presence of *K. oxytoca*, a 344 bp fragment amplified by PCR using the *pehX* gene was observed on agarose gel electrophoresis (Figure 3).

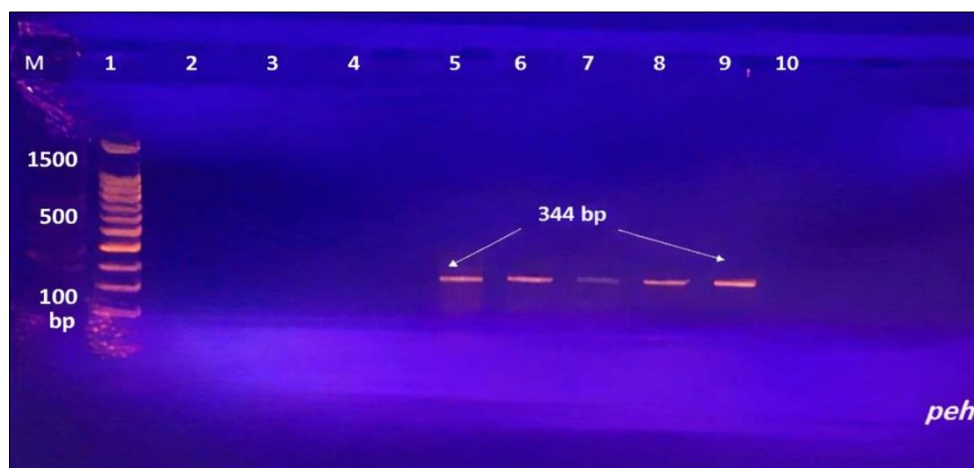


Fig 3: PCR product of the (*pehX*) gene for *Klebsiella oxytoca* by gel electrophoresis.

Discussion

Klebsiella oxytoca is a clinically important gram-negative Enterobacteriaceae species that is emerging as an important cause of urinary tract infections (UTIs), especially in health care settings (HAS), including patients with urinary catheters. In the current study, 36 isolates (18.75%) of *K. oxytoca* were isolated from 192 urine samples, supporting the significant role of *K. oxytoca* in UTI etiology in the investigated population.

The prevalence in this study is similar with those previously reported such as 12.8% [21] and 10.83% [17] and is higher than some lower prevalence (1.1-2%) reported in other investigations [21,23]. These differences can be explained by differences in geographic distribution, patient demographics, clinical environments and diagnostic methods, and underscore the context-dependent epidemiology of *Klebsiella* infections.[24]

Klebsiella species are a challenging group of organisms to identify accurately because of the close phenotypic and biochemical similarity between *K. oxytoca* and *Klebsiella pneumoniae*. This has resulted in a large number of misclassifications, especially in the studies that are based only on the conventional method. For this purpose, molecular methods like the polymerase chain reaction (PCR) offer an important aid to species level identification.[25]

In the present study, PCR using *pehX* gene was used to successfully detect the presence of *K. oxytoca* by amplification of a characteristic 344-bp fragment. The results are in agreement with previous reports [17,24] that proved *pehX* as a reliable species-specific genetic marker. The PCR application not only improves the diagnostic value, but also minimizes the risk of misidentification, thus promoting epidemiological monitoring and clinical management.[26]

Importantly, the antimicrobial susceptibility results showed an alarming trend of resistance, especially to β -lactam

antibiotics like ceftriaxone. This resistance profile could potentially be associated with the production of extended-spectrum β -lactamases (ESBL) by *Klebsiella* species, which is a well-established mechanism. Past studies have shown that chronic bacterial infections can have a profound effect on antioxidant levels, and can play a role in oxidative stress-related tissues damage in infected patients [27].

The presence of such strains of resistance indicates the adaptive ability of *K. oxytoca* and its role in MDR infections. Beyond the classical uropathogenic pathways, there is recent molecular evidence that microbial dysbiosis is crucial for female reproductive health. Changes in the vagina's microbiota, including a decrease in protective *Lactobacillus* species and a buildup of opportunistic pathogens like *Klebsiella oxytoca* and *Escherichia coli*, have been strongly linked to problems with reproductive tract inflammation. This dysbiosis can cause damage to the epithelial integrity, reduce sperm motility, and contribute to ascending infections leading to poor fertility results.^[13]

The molecular identification combined with antimicrobial susceptibility testing is a powerful diagnostic tool, providing correct identification of the pathogen in addition to the encouragement of targeted therapy. It is crucial to implement a comprehensive approach to improve patient outcomes, minimize unnecessary antibiotic prescribing, and enhance infection control measures in healthcare facilities.

The overall results highlight the importance of *Klebsiella oxytoca* as a uropathogen and a possible factor involved in the inflammatory conditions of the reproductive tract, and thus, the necessity of combined diagnostic and therapeutic strategies.

Conclusion

This study provides valuable insights about the presence and molecular identification of *Klebsiella oxytoca* for urinary tract infection among female patients in Najaf, Iraq. UTI is an important cause of infection in this population, with 18.75% of all 192 samples analysed being confirmed as *K. oxytoca*.

Application of PCR-based *pehX* gene-based identification was highly sensitive and specific, successfully overcoming the weaknesses of conventional diagnostic techniques. These results support the need to include molecular assays as a standard clinical microbiology practice to increase diagnostic accuracy.

In addition, the patterns of antimicrobial resistance observed, especially for β -lactam, indicate a rising concern of multidrug resistance and possible involvement of ESBL-producing strains. Molecular characterization is thus crucial in guiding appropriate antimicrobial use and better infection control practices, in addition to surveillance.

In conclusion, the association of molecular diagnostics and antimicrobial susceptibility testing provides a comprehensive tool for the management of *K. oxytoca* infections and the impact of antimicrobial resistance in the hospital.

Limitations

Although this study has yielded some positive results, there are still some drawbacks to be noted. First, the study was

carried out in a single healthcare center in Najaf, Iraq, and results may not be generalizable to other areas or health care providers. Second, one might not be able to interpret the results of the relatively small sample size as representative of the larger population of UTI patients.

Furthermore, this study was mainly descriptive in nature and did not include sophisticated statistical comparisons of the various patterns of antimicrobial resistance, potentially compromising the detection of significant associations between the antimicrobial resistance patterns and the clinical variables. In addition, other significant resistance/virulence genes, such as those responsible for extended-spectrum β -lactamase (ESBL) production, were not examined; only the presence of the *pehX* gene was examined.

Lastly, patient-related factors such as antibiotic use, previous hospitalizations or catheterization were not studied in great detail and may have helped to illuminate the risk factors for *Klebsiella oxytoca* infection and antimicrobial resistance.

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