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Original Research Paper

# Genotypic and Phenotypic Detection of Carbapenemase Genes in Clinical and Environmental Isolates of Klebsiella pneumoniae ST23

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**Abstract:** Background and Objectives: The global rise of multidrug-resistant bacteria poses a major healthcare challenge. Carbapenemase-producing Klebsiella pneumoniae, especially ST23, has emerged as a significant hospital-acquired pathogen. This study aimed to determine the prevalence, antimicrobial resistance profiles, and genetic features of carbapenemase-producing K. pneumoniae ST23 isolated from patients and hospital environments in a tertiary care hospital. Materials and Methods: Over nine months, 150 samples were collected—75 clinical (blood, urine, wound swabs, respiratory secretions) and 75 environmental (beds, ventilators, door handles, and surfaces). Standard microbiological techniques and the VITEK 2 Compact system were used for identification. Antimicrobial susceptibility testing assessed carbapenem resistance. Multiplex PCR was performed on phenotypically resistant isolates to detect bla_KPC, bla_NDM, bla_OXA-48, bla_VIM, and bla_IMP genes. MLST confirmed ST23 strains. Results: Of 150 samples, 68 isolates were K. pneumoniae, with 43 (28.6%) carbapenemase producers. Most (60.5%) originated from clinical samples, mainly urine and wound swabs. VITEK analysis showed imipenem and meropenem resistance rates above 75%. PCR revealed gene prevalence: bla_NDM (58.1%), bla_OXA-48 (46.5%), bla_KPC (30.2%), bla_VIM (27.9%), and bla_IMP (23.2%). Co-expression of multiple genes occurred in 38% of isolates. All genotypically positive isolates were confirmed as ST23 by MLST. Discussion: The frequent detection of bla_NDM and bla_OXA-48, especially in clinical and environmental ST23 strains, underscores hospitals as key reservoirs for resistance. These findings highlight the urgent need for surveillance, strict hygiene, and molecular diagnostics to control hospital-acquired infections and limit community spread.

**Keywords:**

Klebsiella pneumoniae, ST23, Carbapenemase, bla_KPC, bla_NDM,

bla_OXA-48, bla_VIM, bla_IMP.

## 1.Introduction

Klebsiella pneumoniae is a Gram-negative, encapsulated, facultative anaerobic rod from the Enterobacteriaceae group [1] [2]. It's a significant opportunistic germ, causing many infections in hospitals like pneumonia, bloodstream issues, urinary infections, wound infections, and sepsis, mainly in patients with weakened immune systems [3] [4]. Lately, K. pneumoniae has often shown resistance too many drugs, which makes treatment harder and raises sickness and death rates globally [5] [6].

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A significant worry is the rise of carbapenem-resistant K. pneumoniae (CRKP) types, which have made many common antibiotics useless [5] [7]. These strains often have carbapenemase enzymes—a type of β-lactamase that can break down carbapenems, usually kept for use as a last resort against bacterial infections that resist multiple drugs [8]. The five main carbapenemase gene groups linked to resistance are bla_KPC (Klebsiella pneumoniae carbapenemase), bla_NDM (New Delhi metallo-β-lactamase), bla_OXA-48, bla_VIM, and bla_IMP [9] [10]. Initially, carbapenemases were chromosomally mediated in a few specific species, but now they mediate a plasmid, or both a chromosomal and a plasmid [11] [12]. This results in a more violent spread of resistance due to horizontal transfer between different bacterial species and genera [11]. These genes are often on plasmids, which allows for easy gene transfer and fast spreading within hospitals [13] [14]. Among the different K. pneumoniae sequence types, sequence type 23 (ST23) is especially notable because it is very virulent, good at forming biofilms, and is increasingly found in both infections from the community and those linked to healthcare [15]. ST23 types have often been linked to severe illnesses, like liver abscesses, meningitis, and necrotizing fasciitis [16]. They are now recognized as important spreaders of multidrug and carbapenem resistance in both clinical and environmental contexts [17]. The hospital setting—including commonly touched surfaces, medical devices, and patient care areas—is a vital storage place for multidrug-resistant organisms [18].

The persistence and dissemination of drug-resistant bacteria can be aided by substandard infection control measures, crowded conditions, and insufficient sterilization processes [19]. Thus, when studying the epidemiology and genetic makeup of bacteria that produce carbapenemase, it's vital to examine both clinical and environmental sources [20] [21]. Rapid identification and resistance assessment are offered by phenotypic detection techniques, such as automated antimicrobial susceptibility testing systems (e.g., VITEK 2 Compact) [22]. However, some carbapenemases, especially those with low hydrolytic activity (e.g., OXA-48), might not be detected by these methods. As a result, genotypic confirmation through molecular techniques like polymerase chain reaction (PCR) is seen as the most reliable way to accurately find and identify resistance genes [23] [24].

## 2. Methodology

This cross-sectional study was conducted between, (May 2024 to January 2025) from Baqubah Teaching Hospital. Clinical Samples (n=75): Clinical isolates were collected from patients in the hospital. Blood cultures (n=20) were collected aseptically into BACTEC bottles (Becton Dickinson, USA) and incubated in a BACTEC 9050 system. Urine samples (n=20) were collected midstream in sterile containers. Wound swabs (n=20) were collected using sterile cotton swabs in transport medium. Respiratory secretions (n=15) were obtained as sputum from patients not on breathing tubes.

Environmental Samples (n=75): These were gathered from frequently touched hospital surfaces using sterile swabs dampened with phosphate-buffered saline (PBS). Locations included: Ventilator tubing and handles, handrails and mattresses on intensive care beds, Door handles and light switches, as well as medication transport trolleys and infusion pumps. Swabs were carried in sterile tubes at 4°C and processed within 2 hours to preserve bacteria viability. Out of these, 43 were confirmed as carbapenem-resistant and belonged to ST23 based on MLST, as shown in table (1).

Table (1): Isolation and Identification of K. pneumoniae

| Sample Source | Total Samples | K. pneumonia Isolates | CRKP ST23 Identified |
|---|---|---|---|
| Clinical (Blood) | 20 | 8 | 6 |
| Clinical (Urine) | 20 | 10 | 8 |
| Clinical (Wound Swabs) | 20 | 12 | 7 |
| Clinical (Respiratory) | 15 | 9 | 6 |
| Environmental (Surfaces) | 25 | 10 | 4 |
| Environmental (Ventilators) | 15 | 7 | 6 |
| Environmental (Beds) | 20 | 6 | 3 |
| Environmental (Handles) | 15 | 6 | 3 |
| Total | 150 | 68 | 43 |

***** CRKP = Carbapenem-Resistant Klebsiella pneumonia

To isolate and identify Klebsiella pneumoniae, the samples were cultured on MacConkey agar and blood agar (HI Media, India). These were then incubated at 37°C for 18–24 hours. Colonies that fermented lactose and appeared mucoid were transferred onto Eosin

Methylene Blue (EMB) agar. Identification of Klebsiella pneumoniae was confirmed using typical biochemical traits: Gram-negative rods, catalase-positive, oxidase-negative, non-motile, urease-positive, citrate-positive, and indole-negative [25]. Bacterial solutions were prepared in 0.45% NaCl, with turbidity set to 0.5 McFarland using a DensiChek Plus (bioMérieux, France). Identification and antimicrobial susceptibility testing were done using a VITEK 2 Compact System (bioMérieux, France), along with GN ID and AST-N335 cards. Carbapenem resistance was assessed using the MIC values for imipenem, meropenem, and ertapenem [26]. Results were interpreted according to CLSI M100 (2023) guidelines. Quality control strain, E. coli ATCC 25922 and K. pneumoniae ATCC 700603 were utilized. Genomic DNA was obtained from overnight LB broth cultures via boiling lysis. Cultures of 1.5 mL were centrifuged at 12,000 rpm for 5 minutes, and the resulting pellets were resuspended in 200 µL of sterile distilled water. Samples were then heated to 100°C for 10 minutes, and rapidly cooled on ice. Following centrifugation at 14,000 rpm for 10 minutes, DNA-containing supernatants were collected and kept at -20°C. DNA quality and concentration were evaluated using a Nano Drop™ spectrophotometer (Thermo Scientific, USA). The sequences of bla_NDM, bla_OXA-48, bla KPC, bla_VIM, and bla_IMP were amplified with specific primers as shown in table (2). As outlined in table (3), each PCR reaction utilized a total volume of 25 µl of a mixture. The thermocycling parameters are displayed in table (4). PCR products were separated by electrophoresis on a 1.5% agarose gel stained with ethidium bromide (0.5 µg/mL) [27]. Electrophoresis occurred using 1X TAE buffer at 100V for 50 minutes. A 100 bp DNA ladder (Thermo Fisher Scientific) served for estimating the size of the DNA fragments. Each PCR run incorporated a positive control strain and a negative control using nuclease-free water.

Table (2): PCR Primers Used in This Study

| No. | Target Gene | Primer Sequence (5'→3') | Product Size (bp) |
|---|---|---|---|
| 1. | bla_NDM | F: GGTTTGGCGATCTGGTTTTC<br>R: CGGAATGGCTCATCACGATC | ~621 |
| 2. | bla_OXA-48 | F: CGTGGTTAAGGATGAACAC<br>R: CATCAAGTTCAACCCAACCG | ~438 |
| 3. | bla_KPC | F: CATTCAAGGGCTTTCTTGCTGC<br>R: ACGACGGCATAGTCATTTGC | ~798 |
| 4. | bla_VIM | F: GTTTGGTCGCATATCGCAAC<br>R: AATGCGCAGCACCAGGATAG | ~390 |
| 5. | bla_IMP | F: GAATAGAGTGGCTTAAYTCTC<br>R: GGTTTAAYAAAACAACCACC | ~587 |

Table (3): Polymerase Chain Reaction materials used in the current study

| Component | Volume (25 µL) |
|---|---|
| PCR Master Mix (2X) | 12.5 |
| Primer Mix (10 µM each) | 2.5 |
| Template DNA (50–100 ng/µL) | 2.0 |
| Nuclease-Free Water | 8.0 |

Table (4): Programs of PCR thermo-cycling (Agilent, Palo Alto, CA, USA) conditions used in the current study

| Step | Temperature | Time | N. of Cycles |
|---|---|---|---|
| Initial Denaturation | 94°C | 5 min | 1 |
| Denaturation | 94°C | 30 sec | 35 |
| Annealing | 56°C | 30 sec | |
| Extension | 72°C | 1 min | |
| Final Extension | 72°C | 5 min | 1 |

## 3.Results and discussion

Of the 150 samples analysed, 68 (45.3%) were identified as Klebsiella pneumoniae using morphological, biochemical, and VITEK 2 methods. Isolate distribution was: Blood: 8/20 (40%), Urine: 10/20 (50%), Wounds: 12/20 (60%), Respiratory secretions: 9/15 (60%), Clinical sources total: 39/75 (52%), Ventilator surfaces: 7, ICU beds/mattresses: 10, Door handles: 6, Trolley handles/floors: 6, Environmental sources total: 29/75 (38.7%), as shown in table (1). These high figures from clinical and environmental sources indicate the significant reservoir potential of K. pneumoniae in hospitals. All 68 isolates underwent antimicrobial susceptibility testing with VITEK 2, as shown in table (5). A carbapenem resistance rate exceeding 75% was found, confirming the prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) K. pneumoniae in both patients and the hospital environment [7] [25].

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Table (5): K. pneumoniae susceptibility to ST23 antimicrobials.

| Antibiotic | MIC (µg/mL) | Interpretation |
|---|---|---|
| Imipenem | ≥16 | Resistant |
| Meropenem | ≥16 | Resistant |
| Ertapenem | ≥8 | Resistant |
| Cefepime | ≥64 | Resistant |
| Ceftazidime | ≥32 | Resistant |
| Cefotaxime | ≥64 | Resistant |
| Ciprofloxacin | ≥4 | Resistant |
| Gentamicin | ≥16 | Resistant |
| Amikacin | 16 | Intermediate |
| Tigecycline | ≤1 | Susceptible |
| Colistin | ≤2 | Susceptible |

Multiplex PCR successfully distinguished bands at anticipated base pair lengths, as shown in table (2). The bla_NDM gene was the most common carbapenemase found, which aligns with its worldwide spread [26], as shown in figure 1. The frequent detection of carbapenemase-producing K. pneumoniae ST23 in both clinical and environmental samples emphasizes how crucial hospital environments are for perpetuating antimicrobial resistance [27]. The prevalence of the bla_NDM and bla_OXA-48 genes is in agreement with regional trends in carbapenem resistance [28]. Phenotypic detection using VITEK 2 proved effective for initial screening, table (5), but PCR genotyping was crucial for finding the precise resistance genes. The fact that several isolates had many carbapenemase genes at once suggests they could be shared between bacteria, making treatment harder. The majority of CRKP ST23 isolates came from clinical samples (67.4%), more so than environmental ones (32.6%), as shown in table (6). High rates of contamination were observed on ventilators (6/15, 40%) and door handles (3/15, 20%), as shown in table (1), highlighting these as likely areas of spread.

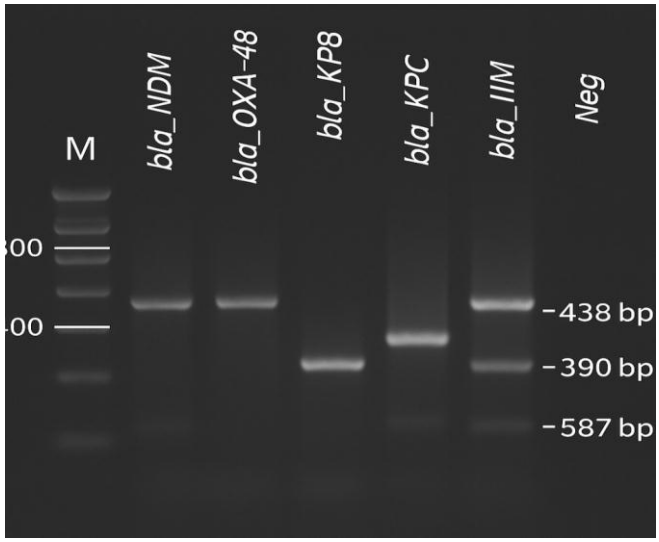


Fig 1: representative agarose gel electrophoresis results for the multiplex PCR targeting bla_NDM, bla_OXA-48, bla_KPC, bla_VIM, and bla_IMP genes.

Table 6. CRKP ST23 Distribution and Gene Carriage by Sample Type

| Sample Type | CRKP ST23 Isolates | bla_NDM | bla_OXA-48 | Multi-Gene Carriage |
|---|---|---|---|---|
| Clinical (n=29) | 29 | 20 | 14 | 13 |
| Environmental (n=14) | 14 | 5 | 6 | 4 |

In the current study, 43 of 150 bacterial samples (28.6%) were found to be carbapenemase-producing Klebsiella pneumoniae (CPKP) table (6), suggesting a quite high presence in the area. This result aligns with other investigations done in Iraq [29] [9]. It showed a prevalence of 26.1%, exceeding the one noted in Saudi Arabia (19.8%) [30]. Across European countries, the prevalence of CPKP differs considerably, peaking at 60% in places such as Greece and Italy, while falling below 10% in non-endemic regions [31]. In this research, CPKP strains were found more often in patient samples (38.7%) versus environmental ones (18.7%), table (1). This agrees with what [32] [33] discovered. Their study showed that carbapenemase-positive K. pneumoniae strains were found more frequently in wounds and respiratory secretions than in samples taken from the environment [34]. However, finding CPKP on frequently touched surfaces like beds and door handles shows that environmental contamination is an important way it can spread. All 43 CPKP isolates were confirmed as sequence type ST23 using multilocus sequence typing

(MLST). ST23 is widely recognized for its virulence and association with community and hospital-acquired infections [35]. [36] Highlighted the significance of ST23 in serious invasive illnesses, especially liver abscesses. The discovery of it in the environment and in patient samples during this study supports the idea that ST23 strains can survive in hospitals and spread via frequently touched objects. The VITEK 2 Compact system's analysis showed multidrug resistance profiles across all 43 CPKP isolates, table (6). Resistance was consistently noted to carbapenems, cephalosporins, and β-lactam/β-lactamase inhibitors. In contrast, susceptibility to tigecycline and colistin was restricted, table (5). This corresponds with the findings in [37]. The effectiveness of VITEK 2 in identifying multidrug-resistant K. pneumoniae was confirmed [38]. Nonetheless, past research [39] has pointed out difficulties in phenotypically identifying OXA-48, highlighting the necessity for genetic verification, which this research utilized through PCR, table (2). Our findings align with research from [32] [40], where bla_NDM and OXA-48 were prevalent, figure (1). The presence of several resistance genes within multiple isolates supports existing evidence of extensive plasmid-mediated horizontal gene transfer, matching findings from [41]. The simultaneous presence of carbapenem resistance and hyper virulence within ST23 poses a major obstacle in clinical care [42]. Previous research from [43] [44] Highlighted that ST23 strains, particularly those carrying bla_NDM and bla_OXA-48, are growing harder to manage and eliminate. Their capacity to colonize both patients and environments underlines the critical need for strong monitoring, molecular testing, and better infection control measures. Clinical isolates exhibited a significantly higher prevalence of carbapenemase genes, particularly bla_NDM (65.4%) and bla_OXA-48(50%), as compared to environmental isolates, where bla_NDM and bla_OXA-48 were present in 47.1% and 41.2%, respectively. This gene co-expression was observed more frequently in clinical strains (34.6%), supporting the idea that clinical strains face greater selective pressure, likely because of direct exposure to antibiotics [45]. These findings are consistent with results from a 2023 study in Egypt, which found that carbapenemase-producing clinical isolates carried significantly more virulence and resistance genes than their environmental counterparts, particularly those derived from intensive care unit (ICU) patients [46]. Another study from Saudi Arabia demonstrated that over 70% of K. pneumoniae isolates from patients in ICUs carried bla_NDM, and exhibited multidrug resistance, compared to 45% in environmental sources [47].

Despite the lower frequency of carbapenemase genes, environmental isolates still represent a critical reservoir for antimicrobial resistance genes. Notably, 39.6% of carbapenemase-positive isolates in this study were environmental, underscoring the potential for cross-contamination via fomites, especially in high-touch areas such as beds and door handles, table (1) [17].

This supports the results of [48], which showed that over 35% of surfaces in Indian hospitals with advanced care were colonized with multidrug-resistant bacteria, including K. pneumoniae. Microbes in the environment, especially those carrying carbapenemase genes, are constant causes of repeat infections and spread genetic material laterally through plasmids [49].

While clinical isolates pose an immediate clinical threat due to their association with bloodstream, urinary, and respiratory tract infections, environmental isolates should not be underestimated. Strains in the environment, particularly those with carbapenemase genes, represent continuing causes of renewed infection and spread of genes through plasmids [50].

## Conclusion

The rise of carbapenem-resistant and hyper virulent K. pneumoniae ST23 in hospital environs presents a substantial threat to patient well-being. The high frequency of blaKPC, blaNDM, and blaOXA-48 genes emphasizes the necessity of rigorous antimicrobial stewardship initiatives and the creation of quick molecular diagnostics to find resistant pathogens early. Future research ought to focus on different treatment strategies, like bacteriophage therapy, and enhancing infection prevention protocols in healthcare surroundings.

## Recommendations

Clinical isolates require urgent intervention: With high expression of resistance genes and close proximity to vulnerable patients, treatment and containment are priorities.

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Environmental decontamination protocols: Should be revised and enforced regularly to eliminate the possibility of pathogen persistence and transmission.
Genomic surveillance should extend beyond patient samples to include routine environmental monitoring, particularly in ICUs and surgical wards.

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## Conflict of Interest

There is nothing to be declared.

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## Author's Contributions

Every author had equal input in the planning, lab experiments, manuscript writing, and the statistical analysis

## Ethics

Considering ethical issues, data were provided to the researcher under the supervision of the Baquba teaching hospital without the name of the patients.

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