

Gene Alteration in Carbapenem-Resistant Bacteria Isolated from Urine Samples

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ABSTRACT

Purpose: The prevalence of multidrug-resistant uropathogens has changed the course of treatment of urinary tract infections (UTIs) from easily treatable conditions into complex clinical challenges therapeutic approaches. Characterizing resistance mechanisms, particularly β -lactamase genes such as oxa, made it very difficult to treat the infection so need a either combination of drug having high beta lactam inhibitory power. The primary objective of the current study was to examine the antibiotic resistance profiles of UTI-associated bacteria and gens associated the multi-antibiotic resistance The study was conducted at District Headquarters Hospital, which is located in Angul, Odisha.

Methodology: Urine specimens were collected from 1000 affected individuals, and completed the bacterial isolation, characterization, and identification with conventional and advanced microbiological techniques. The Kirby-Bauer disk diffusion method was employed in order to evaluate antibiotic susceptibility.

Results: Out of 1000 samples, 46 (4.6%) were positive for bacterial growth. Staphylococcus aureus was the popular uropathogens, accounting for 37.0% of cases. Pseudomonas spp. was the second most common, accounting for 19.6% of cases, and E. coli was third, accounting for 10.6% of cases. Carbapenem resistance was observed in 14 isolates, with the OXA gene detected in 3 samples (21.43% of carbapenem-resistant isolates). Antibiotic susceptibility testing demonstrated elevated resistance rates to numerous frequently utilized medicines, including ampicillin (57.14%), amoxicillin (50%), and cotrimoxazole (35.71%). Ciprofloxacin and Cefepime and Ceftriaxone showed relatively low resistance rates of 7.14 % respectively.

Conclusion: The present study provides significant insights into the incidence of uropathogens and their antibiotic resistance profiles in Angul, Odisha, which can guide treatment strategies for UTIs in this region.

Keywords: UTI, Uropathogens, Carbapenemase resistance, Antibiotic susceptibility, Infectious diseases

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INTRODUCTION

A urinary tract infection (UTI) is a condition that has the potential to develop in any area of the urinary system. The kidneys, the ureters, the bladder, and the urethra are the four components of urinary system. The lower urinary system, which is made up of the bladder and the urethra, is the most common site of infection [1]. It is also defined as bacteria accompanied by urine abnormalities [2]. A UTI occurs when microorganisms, mostly bacteria originating from the gastrointestinal tract, enter the urethral opening and undergo rapid multiplication [3]. It is among the prevalent microbial illnesses in both males and females. Nevertheless, females exhibit a higher susceptibility to urinary tract infections than males. A urinary tract infection (UTI) may be asymptomatic. however, when symptoms an insatiable need for urine, a burning sensation that occurs

when the person urinates, and frequent urination with minimal output, and urine that is cloudy or discolored, such as red, bright pink, or cola-colored, signifying the presence of blood[4]. Additionally, the urine may have a strong odor, and women may experience pelvic pain, particularly in the center of the pelvis and around the pubic bone [4]. Despite these uncomfortable symptoms, UTIs remain commonly occurring contagious infections observed in medical care, predominantly in poorer nations where the mortality and economic burdens are substantial [2, 5]. Poor hygiene habits are risk factors for UTI. The etiological agents responsible for UTIs differ and depending on the geographical location, susceptibility and emergence of resistance [6]. UTI can be caused by number of bacterial pathogens including Escherichia coli, S. aureus, Staphylococcus saprophyticus, Proteus sp.,

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Pseudomonas aeruginosa, *Klebsiella pneumoniae*, and enterococci are often found pathogenic bacteria associated with UTI [7, 8]. The infection caused by these bacteria are treated by various antibiotics that include penicillin, beta-lactams, fluoroquinolones and carbapenems. The carbapenems is broad spectrum antibiotics and known as last report however amongst the many beta-lactams, carbapenems exhibit a wide range of effectiveness against microbial infections and indicate the highest level of potency compared to both Gram-negative and Gram-positive bacteria.

Carbapenems are considered to be a final option for antibiotic treatment in the case of illnesses that are caused by Gram-negative bacteria that are resistant to multiple drugs. The increasing number of Enterobacteriaceae that are resistant to carbapenem (CRE) is a significant risk to the public health of the entire globe[9] [10–12]. The synthesis of carbapenem-degrading enzymes, particularly OXA-type β -lactamases (oxacillinases), represents a key resistance mechanism against carbapenem antibiotics[13]. These enzymes arise due to gene-level alterations and horizontal transfer of blaOXA determinants, which play a critical role in the molecular evolution of carbapenem-resistant uropathogens. In addition to carbapenemase production, other genetic alterations also contribute significantly to carbapenem resistance. For instance, mutations or loss of the oprD porin gene have been strongly associated with reduced carbapenem susceptibility in *Pseudomonas aeruginosa* and have been reported in a high proportion of clinical isolates. The variability of this gene, which includes both substitutions and deletions of amino acids, enables the classification of isolates into genetic groups, which is indicative of the multifactorial nature of carbapenem resistance[14]. Among these, the blaOXA-48 gene and its variants are particularly about due to their ability to confer high levels of resistance while remaining difficult to detect using routine diagnostic tests[15].

The blaOXA genes are frequently situated on mobile genetic elements, enabling their horizontal movement across bacterial species and across different geographical areas [16]. In India, instances of OXA-mediated carbapenem resistance have been documented in many hospital environments[17]. However, information from rural or semi-urban regions, including Angul, Odisha, is limited. The change in predominant uropathogens, including the rising significance of *Staphylococcus aureus* and *Pseudomonas* spp., necessitates region-specific monitoring to customize successful empirical treatments.

Therefore, the current study objective is to identify uropathogens isolated from urine samples of UTI patients in Angul, Odisha, and then evaluate their antibiotic susceptibility patterns. The experiments of OXA genes in carbapenem-resistant isolates conducted using polymerase chain reaction (PCR) techniques. This work aims to enhance local antimicrobial resistance survey and it causes.

MATERIALS AND METHODS

Study area

This experiment was accomplished during (2022 –2024) years at the District Public Health Laboratory (DPHL) of the District Headquarters Hospital in Angul, Odisha, India. The study focused on identifying uropathogens in patients exhibiting urinary tract infection (UTI) symptoms, antibiotic sensitivity test, and detection of OXA genes in carbapenem resistant isolates.

Ethical Considerations

Ethical approval was obtained from the Independent Ethics Committee for Clinical Research, India (permission No: IECRI/23-24/03, dated 03.10.2023). Permission was taken before sample collection. Institutional approvals were taken.

Inclusion and Exclusion Criteria

The conditions for inclusion in this study comprised patients aged between 15 and 60 years with clinical symptoms of urinary tract infection (UTI), including dysuria, frequency, or urgency. Pregnant women were also included. Patients who were either beyond the age of sixty or under the age of fifteen were included in the exclusion criteria, as were patients who refused to grant their consent or were already receiving antibiotic treatment.

Sample Collection and Study Design

The midstream clean-catch urine specimens (10–20 mL) have been collected in sterile containers from both inpatients and outpatients from the various departments of hospital. Samples were accurately tagged with patient IDs and readily transferred to the laboratory for analysis within a 2-hour timeframe post-collection.

Culturing, Isolation, and Identification

The urine samples are cultured on CLED agar using semi-quantitative culture procedures with a standardized calibrated loop. Culture plates were incubated at temperature of 37°C overnight. Moreover, morphologic and microscopic colony identification was done, and CLED agar plates are shown in Figure 1. Biochemical tests were performed to identify the species of bacteria. Growth $\geq 10^5$ CFU/mL was considered significant. Isolates were subjected to a panel of standard biochemical tests for precise identification. Following Cheesbrough's criteria, isolates were identified by colony morphology, gram staining, and routine biochemical assays, including catalase, oxidase, indole, citrate, and urease tests [14]. Antibiotic sensitivity testing was conducted using the disc-diffusion approach of Kirby-Bauer on Mueller-Hinton agar medium.

Microbial Culture Preservation

Bacterial isolates were maintained in tryptic soy broth supplemented with 20% glycerol. Microorganisms were cultured in 1 ml of sterile TSB overnight, after which an equivalent volume of sterile 20% glycerol was added under

aseptic conditions. The mixture was thoroughly agitated and subsequently frozen at -20°C for future molecular studies.



Figure 1. Representative culture plates of uropathogens grown on CLED agar showing species-specific colony morphology and chromogenic differentiation used for preliminary identification prior to biochemical tests.

Antibiotic susceptibility test

In accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) for the year 2023, the modified Kirby–Bauer disc diffusion method was used to conduct antibiotic susceptibility testing on Mueller-Hinton Agar (HiMedia, India)[18]. The following antibiotics were evaluated in the study: β -lactams (Amoxicillin, Amoxyclov), Ampicillin, cephalosporins (Cefuroxime, Ceftriaxone, Cefixime), carbapenems (Meropenem, Imipenem, Ertapenem), fluoroquinolones (Ciprofloxacin, Norfloxacin). Other antibiotics assessed included Cotrimoxazole, Gentamycin, Amikacin, Linezolid, and Piperacillin-Tazobactam. Inhibition zone measurements were sorted as sensitized, indeterminate, or resistant based on Clinical and Laboratory Standards Institute (CLSI) criteria.

Screening for Carbapenemase Production

Carbapenemase production was screened using the Kirby-Bauer disc diffusion method. Mueller-Hinton agar was used to cultivate *Escherichia coli* ATCC 25922, and a lawn culture was then developed. Bacterial strains exhibiting resistance to meropenem, ertapenem, and imipenem discs were chosen. If the inhibition zone was less than 19 mm for meropenem and imipenem, the isolates went confirmation tests for carbapenemase production. Incubation conditions included temperatures of $35\text{--}37^{\circ}\text{C}$ overnight.

Plasmid DNA Extraction

The urine samples were streaked on cled agar plates and kept them for incubation aerobically at $35\text{--}37^{\circ}\text{C}$ for 72 hours.

After the incubation process, single (each) colonies picked from each petri plates of carbapenem resistance isolates respectively (for 10 plates), and mixed in $100\mu\text{L}$ 1XPBS (Phosphate buffered saline is a buffer solution commonly used in biological research. The buffer helps to maintain a constant pH, osmolarity and ion concentrations of the solution, and stored in

-20°C . Plasmid DNA was isolated through the alkaline lysis technique. Following the completion of the rinsing of the DNA pellet using 70% ethanol, the pellet was subsequently re-suspended in 50 microliters of TE buffer. A Nano-drop spectrophotometer was used to determine the concentration and purity of the sample.

PCR Amplification of OXA Gene

The PCR reaction was formulated with a mastermix of dNTPs, Taq polymerase, buffer, and primers specific for the OXA gene. With a reaction volume of $25\mu\text{L}$, PCR amplification was carried out. The reaction volume consisted of $12.5\mu\text{L}$ of PCR Direct Buffer, $5\mu\text{L}$ of DNA template, $0.5\mu\text{L}$ of polymerase mix, $5\mu\text{L}$ of nuclease-free water, and $1\mu\text{L}$ each of forward and reverse primers. The

first step in the polymerase chain reaction (PCR) process is denaturation, which involves heating the sample to 95 degrees Celsius for five minutes. This is followed by thirty cycles of denaturation, during which the sample is heated to 98 degrees Celsius for forty-five seconds, annealing, during which the sample is heated to 68 degrees Celsius for thirty seconds, and extension, during which the sample is heated to 72 degrees Celsius for thirty seconds. The process is completed with a final extension step, during which the sample is heated to 72 degrees Celsius for ten minutes.

Agarose Gel Electrophoresis

PCR products were separated on a 1% agarose gel produced in 1X TAE buffer and stained with 2 microlitre ethidium bromide. A 100 bp molecular weight DNA marker was used for fragment size analysis. Electrophoresis proceeded at 70 volts for 90 minutes, and DNA bands were detected using

120 min ZelDoc System (Ibriht1500). A 438 bp band was considered positive for the OXA gene.

Statistical Analysis

Statistical methods were employed to encapsulate demographic data and resistance behaviors. Chi-square tests were used to evaluate the association between pathogen type and carbapenem resistance. The Wilson score method was used to construct confidence intervals (95%). A p-value of less than 0.05 was considered to be statistically significant. Analyses were conducted utilizing Microsoft Excel.

RESULTS

Identification of Uropathogens

One of the most often diagnosed illnesses affecting the human urinary tract is known as a urinary tract infection. Treatments are often administered informally, with the selection criteria for antimicrobials based upon the most probable infection and it shows resistance profile within the vicinity [19]. Out of 1000 urine samples processed, 46 (4.6%) yielded significant bacterial growth listed in Table 1. Based on the gram staining and biochemical characterization as shown in Figure 2. Among these, the most common uropathogen was *Staphylococcus aureus* (37.0%), followed by *Pseudomonas* spp. (19.6%), and *E. coli* (10.9%) and so on. The following distribution of isolated organisms is shown in Table 2. The bacteria isolated from the sample are *E. coli* observed in 5 samples, *Klebsiella* in 3 samples, *P. vulgaris* in 2 samples, streptococcus in 2 samples, pseudomonas in 9 samples, staphylococcus aureus in 17 samples, *Acinetobacter Baumannii* in 3 samples, and coagulase-negative staphylococci (CONS) in 5 samples. Overall, it was observed that *Staphylococcus aureus* is the most common and prevalent urinary pathogen, accounting for 17 (37.0%) out of 46 positive samples, followed by *Pseudomonas* spp. (19.6%) and *E. coli* (10.6%). Similarly, prior research has demonstrated a significant association between the increased prevalence of *S. aureus* and participation in receptive anal intercourse, as well as the presence of HIV infections[20, 21]. The distribution of Uropathogens shown in a Figure 3 graph.



Figure 2. Biochemical identification of uropathogens using methyl red test, urease test, Citrate utilization test.

Table 1. Distribution of Urine Culture Outcomes in Study Participants.

Culture Result	Number	Percentage (%)
Positive	46	4.6 %
Negative	954	95.4 %
Total	1000	100%

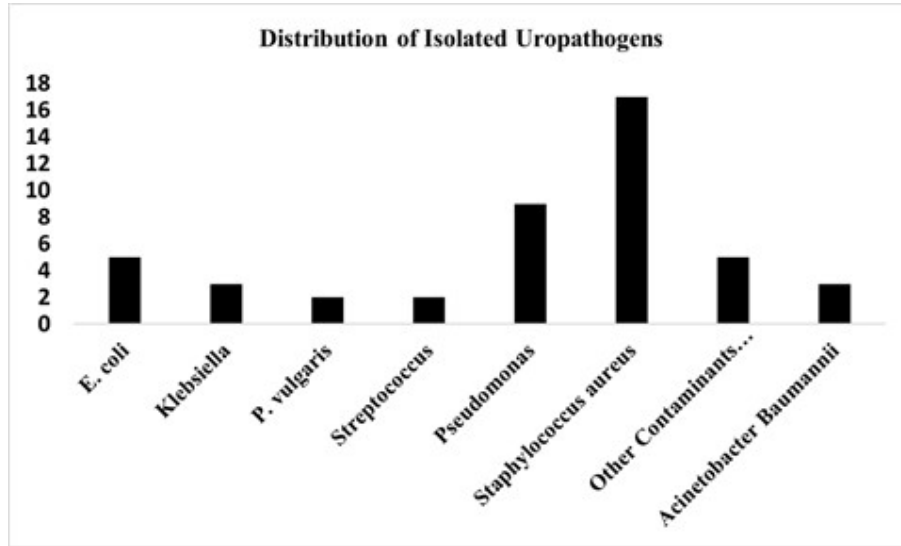


Figure 3. Distribution of Uropathogens in UTI-positive Samples.

Table 2. Distribution of Isolated Organisms from Urinary Tract Infection (UTI).

Organism	No of samples	Percentage (%)
E. coli	05	10.9 %
Klebsiella	03	6.5 %
P. vulgaris	02	4.3 %
Streptococcus	02	4.3 %
Pseudomonas aeruginosa	09	19.6 %
Staphylococcus aureus	17	37.0 %
Coagulase-negative staphylococci	05	10.9 %
Acinetobacter Baumannii	03	6.5 %
Total	46	100

Furthermore, the organism divided as gram negative and gram-positive category as shown in Table 3. In addition, it was observed that the in the total of samples males are 11

and females are 35. This suggested the infection mostly occurred in the females.

Table 3. Pattern of Gram-Positive and Gram-Negative Bacterial Isolates.

Organism	Number	Percentage
Gram Positive	24	52.17
Gram Negative	22	47.82
Total	46	100

Initial Screening for Carbapenem Resistance

Among the 46 uropathogenic isolates, 14 (30.4%) exhibited resistance to carbapenem antibiotics (imipenem,

meropenem, Ertapenem) based on disc diffusion method, using CLSI breakpoints. The distribution of resistant isolates across different bacterial species depicted in Figure 4 and Table 4.

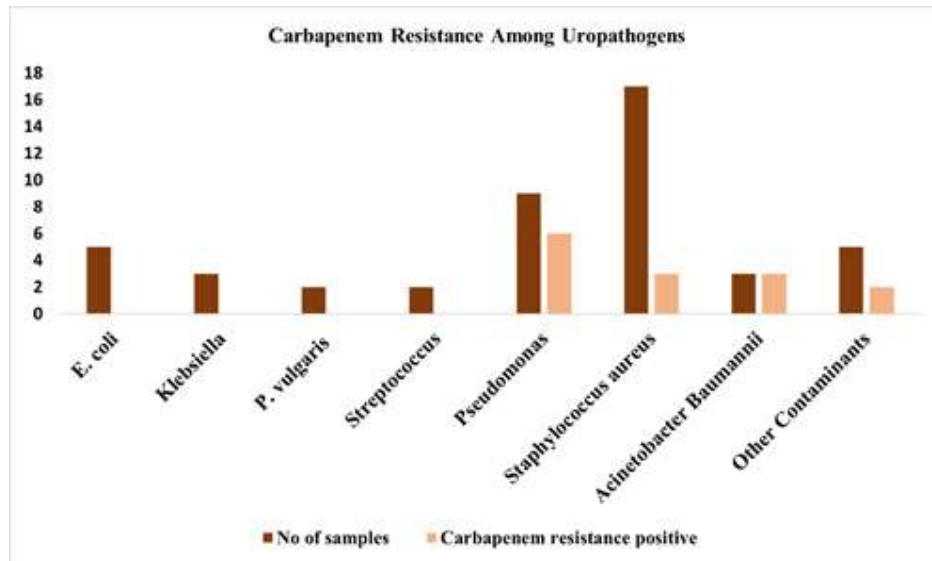


Figure 4. Distribution of carbapenem resistance among various uro-pathogens isolated from urine samples.

Table 4. Prevalence of Carbapenem Resistance among Isolated Uropathogens.

Organism	No of samples	Carbapenem resistance positive	Percentage (%)
E. coli	05	0	0
Klebsiella	03	0	0
P. vulgaris	02	0	0
Streptococcus	02	0	0
Pseudomonas	09	06	66.66 %
Staphylococcus aureus	17	03	17.64 %
Acinetobacter Baumannii	03	03	100%
Other Contaminants	05	02	40%
Total	46	14	30.43%

Molecular Detection of OXA Gene

DNA extraction was performed by the alkaline lysis method, and a spectrophotometer was used to quantify the eluted nucleic acid. 10 viable carbapenem-resistant isolates were subjected to PCR to detect the OXA gene. 3 (30%) isolates tested positive, corresponding to a frequency of 21.43% among all resistant cases. Three of the positive samples were *A. baumannii*. PCR was performed using OXA-specific primers, and the amplified product was determined on a 1% agarose gel and analyzed after running it for 120 min using the ZedDoc System (Ibriht1500). A distinct band at 438 bp indicated the presence of the blaOXA gene, as shown in Figure 5.

Inclusively, the study emphasizes that *Staphylococcus aureus* is the most common uropathogen in Angul, Odisha, with *Pseudomonas* spp. being the second most prevalent. The significant occurrence of these pathogens indicates the necessity for specific antibiotic treatments in this area.

Demographic Analysis

Among the 14 carbapenem-resistant samples, 4 positive samples are male and 10 positive samples are female. The age and gender distribution among the 14 samples are described in Table 5.

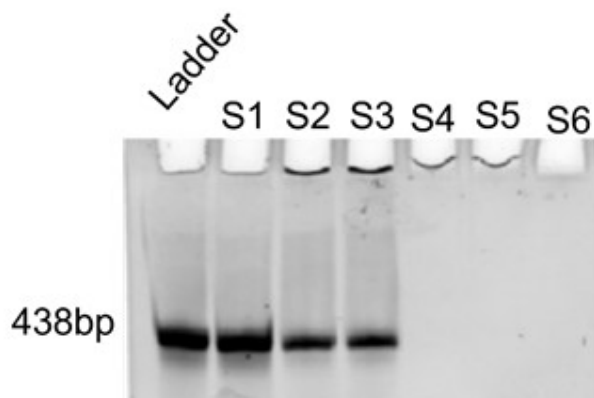


Figure 5. Representative gel image showing OXA gene PCR product.

Table 5. Demographic Profile of Carbapenem-Resistant Specimens by Age and Sex (n=14).

Age	Gender	No. of samples
15-20 years	Females	1
21-30 years	Male and Females	2 and 7 respectively
31-40 years	Females	1
41- 50 years	Females	1
51-60 years	Males	2

Departmental Distribution of Isolates (n=46)

The isolates were obtained from different departments as follows: Medicine 25 (52%), Urology 2 (8%), Surgery 9 (19%), Outpatient Department (OPD) 9 (19%), and Obstetrics and Gynecology 1 (2.17%) as shown in Figure 6. This distribution highlights the importance of targeted infection control measures in high-risk departments, particularly Obstetrics and Gynecology and Urology.

Antibiotic Sensitivity and Resistance (n=14)

Sensitivity and resistance to antibiotics were determined and are shown in Table 5. The high resistance observed in

Ampicillin exhibited a resistance rate of 57.14%, Amoxicillin-clavulanic acid (AMC) 50%, Ciprofloxacin (CIP) 7.14%, Cotrimoxazole (COT) 35.71%, Fosfomycin (FO) 14.28%, Norfloxacin (NX) 14.28%, Cefepime (CPM) 7.14%, Ceftriaxone (CTR) 7.14%, Ertapenem (ETP) 28.57%, Amikacin (AK) 0%. Nevertheless, Amikacin exhibited a notable level of sensitivity, with a resistance rate of 0%. This indicates that Amikacin has the potential to be an effective empirical therapy choice. Ciprofloxacin had a comparatively low resistance rate of 7.14% as compared to sensitivity, indicating that it is a suitable alternative for the care of urinary tract infections.

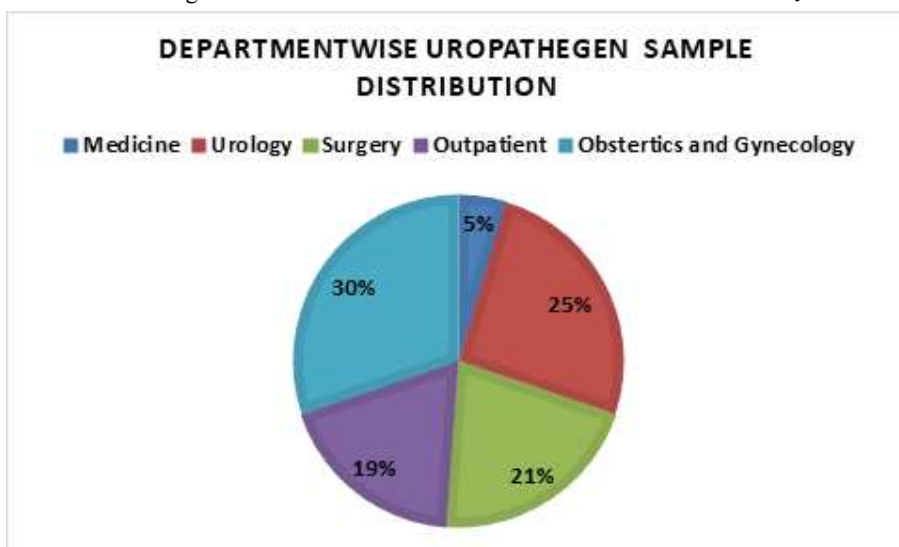


Figure 6. Department-wise Distribution of Uropathogenic Isolates.

Table 6. Antibiotic Susceptibility Profile of Uropathogenic Isolates (n=14).

Antibiotics	Sensitivity no., %	Resistance no., %
Ampicillin (AMP)	0	08(57.14%)

Amoxicillin-clavulanic acid (AMC)	0	07(50%)
Ciprofloxacin (CIP)	04(28.57%)	01(7.14%)
Amikacin (AK)	01(7.14%)	0
Cotrimoxazole (COT)	01(7.14%)	05(35.71%)
Fosfomycin (FO)	02(14.28%)	02(14.28%)
Norfloxacin (NX)	01(7.14%)	02(14.28%)
Cefepime (CPM)	01(7.14%)	01(7.14%)
Ceftriaxone (CTR)	02(14.28%)	01(7.14%)
Ertapenem (ETP)	0	04(28.57%)

Statistical Findings

The proportion of carbapenem resistance among pathogens varied significantly. *Staphylococcus aureus* exhibited a resistance proportion of 17.6% (95% CI: 3.9%–43.4%),

while *Pseudomonas* had the highest resistance proportion of 66.7% (95% CI: 35.4%–87.9%). The overall association between pathogen type and carbapenem resistance was statistically significant, as determined by a Chi-Square test ($\chi^2 = 19.22$, $p=0.0075$) as shown in Table 7.

Table 7. Statistical Findings of pathogens (n=46).

Pathogen	Total Samples	Carbapenem Resistance	Resistance Proportion	95% CI Lower	95% CI upper
<i>E. coli</i>	5	0	0.0	0.0	0.43
<i>Klebsiella</i>	3	0	0.0	0.0	0.56
<i>P. vulgaris</i>	2	0	0.0	0.0	0.65
<i>Streptococcus</i>	2	0	0.0	0.0	0.65
<i>Pseudomonas</i>	9	6	0.66	0.35	0.87
<i>Staphylococcus aureus</i>	17	3	0.17	0.06	0.41
Other Contaminants	5	2	0.4	0.11	0.76
<i>Acinetobacter Baumannii</i>	3	3	1.0	0.43	1.0

CONCLUSION

The present study emphasizes the detection of OXA gene in carbapenem resistance uropathogens in Angul, Odisha. *Staphylococcus aureus* emerged as the predominant pathogen, followed by *Pseudomonas* spp. and *E. coli*, Coagulase-negative staphylococci (CONS) which differs from the typical etiology of UTIs reported in many other regions. The high prevalence of carbapenem resistance (30.4% of positive samples) and the presence of the OXA gene in some isolates are concerning and needs careful monitoring. The antibiotic susceptibility results indicated increased resistance rates to multiple frequently used antibiotics, the necessity for prudent antibiotic application and ongoing monitoring of local resistance patterns. Amikacin and ciprofloxacin demonstrated relatively low resistance rates, their potential as effective empirical treatment options for UTIs in this area. These findings highlight the necessity of customized antibiotic therapies informed by regional susceptibility trends to successfully address UTIs and limit the increasing incidence of antibiotic resistance. The following studies should concentrate on identifying the divisor that bring to the increased incidence of *S. aureus* in urinary tract infections within this region, as well as examining the molecular mechanisms underlying antibiotic resistance in these isolates.

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Conflict of Interest

The author has no conflict of interest regarding this study.

Ethical Approval

The study titled "Detection of OXA genes in carbapenem resistance uropathogens isolated from urine samples at Angul, Orisaa" received ethical approval from the **Independent Ethics Committee (Clinical Research) India, Kolkata**, on **03.10.2023** (Approval No. **IECCRI/23-24/03**). The approval is valid until **02.10.2024**. The committee reviewed the research proposal through an expedited process and approved it with standard ethical recommendations, including written informed consent and institutional permissions.

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