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Original Article

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Antimicrobial Susceptibility Pattern and ESBL Prevalence in Urine Isolates from Hospitalized Patients in a Tertiary Care Hospital, Lucknow

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ABSTRACT

Background: Urinary tract infections (UTIs) are a common cause of morbidity among hospitalized patients. The emergence of extended-spectrum β-lactamase (ESBL)-producing bacteria poses significant challenges in treatment due to their resistance to commonly used antibiotics. This study was conducted to determine the prevalence of uropathogens among inpatients at IIMSR, Lucknow, India, and to evaluate their antimicrobial susceptibility profiles, with special emphasis on ESBL production.

Methods: This cross-sectional, observational study was conducted over 6 months from November 2023 to April 2024 at the Department of Microbiology, IIMSR, Lucknow, India. Urine samples from IPD with suspected UTIs were collected and processed for bacterial culture and sensitivity testing. Identification was done by using standard microbiological methods. AST was performed using the Kirby-Bauer disc diffusion method, as per CLSI guidelines. ESBL production was detected using the combined disc method.

Results: A total of 730 urine samples were analysed; 215 samples were culture-positive. The most isolated bacteria were *E. coli* (39%) and *Enterococcus* sp. (38%). Overall, 43% of the isolates were found to be ESBL producers. ESBL-producing strains showed high resistance to β-lactam/β-lactamase inhibitor and Cephalosporins. Carbapenems and aminoglycosides remained the most effective antibiotics.

Conclusion: This study highlights a significant prevalence of ESBL-producing bacteria among UTI patients in the IPD of IIMSR, Lucknow. The high resistance rates to commonly used antibiotics necessitate regular monitoring of antimicrobial sensitivity patterns and the implementation of stringent infection control measures to manage and prevent the spread of resistant strains.

Key-words: Antimicrobial Sensitivity, Colony-forming units (CFU), ESBL, Inpatient, Urinary tract infection (UTI)

INTRODUCTION

Urinary tract infection (UTI) is a common clinical condition encountered in both hospital-acquired and community-acquired settings, significantly contributing to patient morbidity. [1] It is defined microbiologically as the presence of ≥10^5 colony-forming units (CFU) per ml in a midstream urine sample, indicating active infection. [2] Gram-negative bacteria, including *E. coli, Proteus* sp., *Klebsiella* sp., *P. aeruginosa*, and *Acinetobacter,* primarily cause the infection. Gram-positive organisms such as *Enterococcus* sp., *S. saprophyticus*, and *S. agalactiae* also play a role. Opportunistic fungal pathogens, especially *Candida* sp., have gained clinical relevance in catheterized and immunocompromised patients due to the complexity of management and risk of systemic dissemination.[3] UTIs present with symptoms such as dysuria, increased frequency and urgency of urination, fever, and abdominal pain. If not promptly treated, they can lead to complications including renal scarring, hypertension, and kidney failure. [4]

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The increasing resistance to antibiotics, particularly due to the emergence of extended-spectrum beta-lactamase (ESBL)-producing organisms, has complicated the treatment landscape. ESBLs are plasmid-mediated enzymes that inactivate penicillins and third-generation cephalosporins, while carbapenems generally retain efficacy. [5] Their detection relies on the inhibitory effect of agents like clavulanic acid, sulbactam, and tazobactam. [6] Given the dynamic nature of antimicrobial resistance, ongoing local surveillance is essential to guide empirical therapy and prevent the spread of multidrug-resistant strains. [7] The present study was undertaken to assess the spectrum of uropathogens, their antimicrobial susceptibility profiles, and the prevalence of ESBL production in urine isolates from inpatients at the tertiary care hospital, Lucknow.

MATERIALS AND METHODS

Research design- This cross-sectional, observational study was conducted in the Department of Microbiology at IIMSR, Lucknow, over six months, from November 2023 to April 2024.

Specimen collection- Urine samples were collected from hospitalized patients with clinically suspected UTIs. Midstream clean-catch specimens were obtained in sterile, wide-mouthed containers, while catheterized samples were collected aseptically from the catheter port using sterile syringes after disinfection. Informed consent was obtained before collection. Samples were promptly transported to the microbiology laboratory and processed within 2 hours; if delayed, they were stored at 4–8°C to prevent bacterial overgrowth.

Culture and Identification- Urine specimens were inoculated onto Cysteine Lactose Electrolyte Deficient (CLED) agar using a calibrated loop (0.001 mL) for semi-quantitative culture. The plates were incubated aerobically at 37°C for 18–24 hours. A growth of $\geq 10^5$ colony-forming units (CFU)/mL was considered significant for bacteriuria. Isolates were identified based on colony morphology, Gram staining, and standard biochemical tests, including indole, citrate utilization, urease, motility, triple sugar iron (TSI), Nitrate, Oxidative-Fermentative, Catalase, Coagulase, Bile esculin and oxidase tests, depending on the suspected organism.

Antibiotic Susceptibility Testing- Antimicrobial susceptibility testing of all bacterial isolates was performed using the Kirby-Bauer disk diffusion method**,** following the Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines**.** Bacterial suspensions were prepared by emulsifying 3–5 well-isolated colonies in nutrient broth and adjusting to match the 0.5 McFarland turbidity standard. The suspension was evenly inoculated onto Mueller-Hinton agar plates using a sterile cotton swab. Antibiotic discs were placed aseptically on the inoculated plates, which were then incubated at 37°C for 16–18 hours. After incubation, the diameters of the zones of inhibition were measured in millimetres, and results were interpreted as Sensitive, Intermediate, or Resistant according to CLSI 2023 criteria.[8] Quality control for susceptibility testing was ensured using standard strains *E. coli* ATCC 25922 and *S. aureus* ATCC 25923.

Antibiotics Tested- The antibiotic susceptibility of Gram-negative isolates was tested using the following discs: Ampicillin/ Sulbactam (10/10 µg), Piperacillin/ Tazobactam (100/10 µg), Cefazolin (30 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Cefepime (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Amikacin (30 µg), Aztreonam (30 µg), Tetracycline (30 µg), Cotrimoxazole (25 µg), Nitrofurantoin (300 µg), Norfloxacin (30 µg), Fosfomycin (200 µg), Meropenem (10 µg), Doripenem (10 µg), and Imipenem (10 µg).

For Gram-positive isolates, the antibiotics tested included Penicillin (10 units), Ampicillin (10 µg), Linezolid (30 µg), Vancomycin (30 µg), Teicoplanin (30 µg), High-level Gentamicin (120 µg), High-level Streptomycin (120 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Norfloxacin (30 µg), Tetracycline (30 µg), and Nitrofurantoin (300 µg).

Detection of Extended-Spectrum β-Lactamase (ESBL)- Isolates with reduced susceptibility to third-generation cephalosporins (ceftazidime, cefotaxime, or ceftriaxone) were screened for ESBL production.

Phenotypic confirmation was performed using the Combined Disk Test (CDT), following CLSI 2023 guidelines. [8] A 0.5 McFarland standard bacterial suspension was inoculated onto Mueller-Hinton agar. Discs of ceftazidime (30 µg) and ceftazidime-clavulanic

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acid (30/10 µg) was placed 25 mm apart. Plates were incubated at 37°C for 16–18 hours.

Interpretation- An increase of ≥5 mm in the zone diameter around the ceftazidime-clavulanic acid disc compared to ceftazidime alone was considered positive for ESBL production.

Inclusion criteria- All urine samples collected from hospitalized inpatients (IPD) with clinically suspected urinary tract infections (UTIs) were submitted to the Microbiology Laboratory of the Integral Institute of Medical Sciences and Research (IIMSR), Lucknow, for bacteriological analysis.

Exclusion criteria

- Urine samples collected from outpatients (OPD).
- Duplicate urine samples collected from the same patient during the study period.
- Patients or their guardians who declined to provide informed consent.

Statistical analysis- Data were entered in Microsoft Excel and analyzed using SPSS version 20.0. Descriptive statistics were used to calculate frequencies and percentages, while associations between ESBL production and antibiotic resistance were evaluated using the Chi-square test or Fisher's exact test, as appropriate. A p-value of <0.05 was considered statistically significant.

Ethical Approval- The study received ethical clearance from the Institutional Research Committee and Institutional Ethics Committee of IIMSR, Lucknow (Approval No. IEC/IIMSR/2023/21, dated 17th October 2023).

RESULTS

During the study period, a total of 730 urine samples from suspected UTI cases were processed. Significant bacterial growth was detected in 215 samples, corresponding to a culture positivity rate of 29.4%. Most positive cases originated from the Medicine (35%), Emergency (22%), and Paediatrics (21.3%) departments. Females comprised 67% of cases, with the highest prevalence observed in the 15–30 years' age group (35%), followed by patients aged <15 years (23%), 31–45 years (20%), 46–60 years (14%), and >60 years (8%).

Among the 215 culture-positive isolates, Gram-negative bacteria were slightly predominant, accounting for 48% (n = 103), followed by Gram-positive bacteria at 46% (n = 98) and *Candida* sp. at 6.5% (n = 14). *E. coli* was the most frequently isolated pathogen (39%), followed by *Enterococcus* sp. (37%) and *Klebsiella* sp. (4%). Other isolates included *Staphylococcus* sp. (2%) and, less frequently, *Pseudomonas*, *Acinetobacter*, *Citrobacter*, and *Proteus* sp., each representing 1–2% of total isolates (Fig. 1).

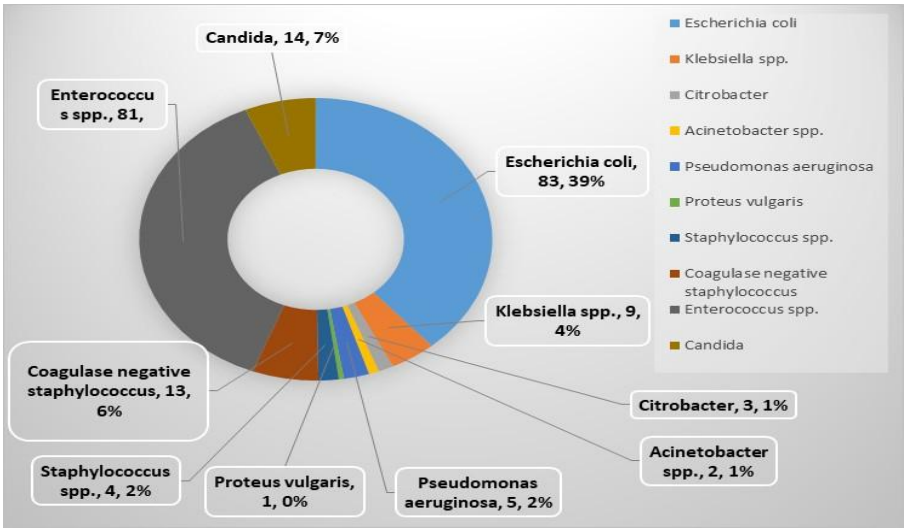

Figure 1: Microbiological profile of uropathogens.

Of the 83 *E. coli* isolates, 43 (51.8%) were confirmed as extended-spectrum β-lactamase (ESBL) producers. Among *Klebsiella* sp. (n = 9), 2 isolates (22.2%) were ESBL-positive. The overall prevalence of ESBL-producing uropathogens was 43%. (Fig. 2)

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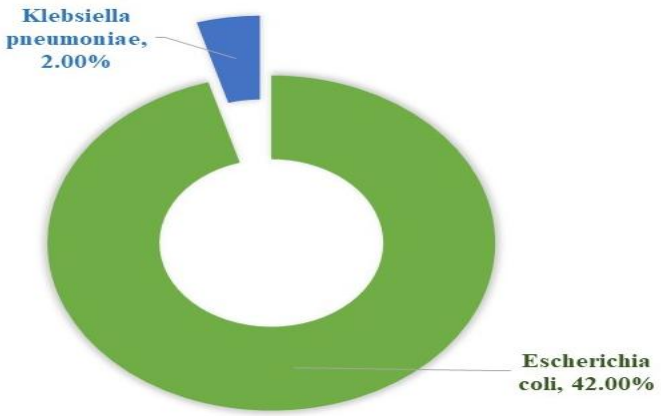

Fig. 2: Prevalence of ESBL-producing uropathogens.

Antimicrobial Susceptibility pattern- Overall, antimicrobial susceptibility testing revealed distinct resistance profiles between Gram-negative and Gram-positive isolates. Among Gram-negative bacteria, tigecycline demonstrated the highest activity (72%), followed closely by nitrofurantoin (70%), amikacin (69%), and imipenem (68%). In contrast, resistance was highest to cefazolin (99.1%) and ciprofloxacin (90%). Of particular concern, all *Proteus* sp. isolates showed complete resistance to ampicillin/sulbactam, tetracycline, and cefazolin (Table 1).

Table 1: Antimicrobial susceptibility pattern of Gram negative uropathogens to various antibiotics.

Antimicrobial Agents	***E. coli***	***Proteus* sp.**	***Pseudomonas* sp.**	***Klebsiella* sp.**	***Citrobacter* sp.**	***Acinetobacter* sp.**	**Total (%)**
	83 (%)	**1 (%)**	**5 (%)**	**9 (%)**	**3 (%)**	**2 (%)**	
Ampicillin/ Salbactam	18(22%)	0 (0%)	3 (60%)	2 (22%)	2 (67%)	NA	25 (24%)
Piperacillin/ Tazobactam	47(57%)	1(100%)	2 (40%)	5 (56%)	2 (67%)	1 (50%)	58 (56%)
Tetracycline	15(18%)	0 (0%)	NA	2 (22%)	NA	1 (50%)	18 (18%)
Gentamicin	51(61%)	1(100%)	4(80%)	5 (56%)	1 (33%)	1 (50%)	63(61%)
Amikacin	62(75%)	1(100%)	3 (60%)	4 (44.4%)	1 (33.3%)	NA	71(69%)
Tobramycin	42(51%)	1(100%)	4 (80%)	3 (33%)	2 (67%)	2 (100%)	54(52%)
Cefazolin	1 (1%)	0 (0%)	NA	NA	NA	NA	1 (0.9%)
Ceftriaxone	9 (11%)	1(100%)	1 (20%)	2 (22%)	NA	1 (50%)	14(14%)
Cefepime	15(18%)	1(100%)	4 (80%)	2 (22.2%)	1 (33.3%)	NA	23(22.3%)
Cotrimoxazole	19(23%)	NA	1 (20%)	3 (33%)	1 (33%)	2 (100%)	26(25%)
Aztreonam	13(16%)	1(100%)	3 (60%)	3 (33%)	2 (67%)	1 (50%)	23(22%)
Ciprofloxacin	5 (6%)	NA	3 (60%)	2 (22%)	NA	NA	10(10%)
Tigecycline	63(76%)	1(100%)	1 (20%)	4 (44%)	3 (100%)	2 (100%)	74(72%)

Impipenem	59(71%)	1(100%)	3 (60%)	5 (56%)	1 (33%)	1 (50%)	70(68%)
Doripenem	57(69%)	1(100%)	3 (60%)	5 (56%)	2 (67%)	1 (50%)	69(67%)
Meropenem	51(61%)	1(100%)	3 (60%)	4 (44.4%)	1 (33%)	1 (50%)	61(59%)
Norfloxacin	12(14%)	1(100%)	NA	2 (22%)	1 (33%)	1 (50%)	17(16%)
Nitrofurantoin	62(75%)	1(100%)	1 (20%)	5 (56%)	1 (33%)	2 (100%)	72(70%)

Gram-positive isolates exhibited greater overall susceptibility, with linezolid (96%), vancomycin (95%), teicoplanin (88%), and nitrofurantoin (84%) being the most effective agents. However, resistance to fluoroquinolones was notably high, with ciprofloxacin and levofloxacin resistance rates of 96% and 95%, respectively (Table 2). These findings highlight the sustained efficacy of certain last-line agents such as tigecycline and linezolid, while underscoring the alarming resistance to commonly used first-line antibiotics, particularly fluoroquinolones and cephalosporins.

Table 2: Antimicrobial Susceptibility pattern of gram positive uropathogens to various antibiotics

Antimicrobial Agents	***Enterococcus* sp. 81(%)**	***Staphylococcus* sp. 4 (%)**	***Coagulase-negative Staphylococcus* 13 (%)**	**Total (%)**
Penicillin	50 (62%)	1 (25%)	3 (23%)	54 (55%)
Ampicillin	50 (62%)	NA	NA	50 (51%)
Linezolid	79 (98%)	3 (75%)	12 (92%)	94 (96%)
Vancomycin	77(95%)	4 (100%)	12 (92.3%)	93 (95%)
Teicoplanin	75 (93%)	3 (75%)	8 (62%)	86 (88%)
Ciprofloxacin	2 (2%)	NA	2 (15.3%)	4 (4%)
Levofloxacin	3 (3.7%)	NA	2 (15.3%)	5 (5.1%)
Tetracycline	3 (3.7%)	2 (50%)	10 (77%)	15 (15%)
Doxycycline	3 (3.7%)	1 (25%)	5 (38%)	9 (9%)
Norfloxacin	4 (5%)	NA	1 (8%)	5 (5%)
Nitrofurantoin	66 (81%)	4 (100%)	12 (92%)	82 (84%)

Table 3 summarises the antimicrobial susceptibility of ESBL-producing and non-ESBL-producing *E. coli*. Non-ESBL producers consistently demonstrated higher susceptibility rates across most antibiotic classes. Significant differences ($p<0.05$) were observed for cefazolin, ceftriaxone, aztreonam, norfloxacin, and nitrofurantoin, with non-ESBL strains showing significantly higher sensitivity.

Among ESBL producers, the highest susceptibility was noted for imipenem (79%), doripenem (74.4%), amikacin (77%), tigecycline (74.4%), and nitrofurantoin (72%), with the lowest for ceftriaxone (2.3%) and fluoroquinolones (≤7%). Both groups retained good activity against carbapenems, tigecycline, nitrofurantoin, and aminoglycosides, although susceptibility rates were slightly lower in ESBL producers.

Table 3: Comparative Antimicrobial susceptibility pattern of ESBL and non-ESBL producing *E. coli*.

Antimicrobial Agents	**ESBL producing *E. coli* 43(%)**	**Non-ESBL producing *E. coli* 40 (%)**	**p-value**
Ampicillin/Sulbactam	7 (16.2%)	11 (27.5%)	0.23
Piperacillin/Tazobactam	26 (60.4%)	26 (65%)	0.68
Tetracycline	7 (16.2%)	10 (25%)	0.32

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Gentamicin	29 (67.4%)	28(70%)	0.80
Amikacin	33 (77%)	32 (80%)	0.72
Tobramycin	22 (51.1%)	23 (57%)	0.59
Cefazolin	0%	8(20%)	0.004*
Ceftriaxone	1 (2.3%)	8 (20%)	0.016*
Ceftazidime	4 (9.3%)	9 (23%)	0.10
Cefepime	6 (14%)	9 (23%)	0.29
Cotrimoxazole	10 (23.2%)	11 (27.5%)	0.65
Aztreonam	2 (5%)	11 (27.5%)	0.008*
Ciprofloxacin	5 (12%)	7 (17.5%)	0.48
Tigecycline	32 (74.4%)	31 (78%)	0.70
Imipenem	34 (79%)	35 (87.5%)	0.31
Doripenem	32 (74.4%)	34 (85%)	0.23
Meropenem	30 (70%)	35 (87.5%)	0.05
Norfloxacin	3 (7%)	10 (25%)	0.02*
Nitrofurantoin	31(72%)	36 (90%)	0.03*

Note: *Significant differences (p<0.05) are marked with an asterisk (*).*

DISCUSSION

The increasing prevalence of multidrug-resistant (MDR) organisms has emerged as a major global health threat, with a particularly severe impact in developing countries where antimicrobial misuse is more common. Urinary tract infection (UTI) is one of the most frequent nosocomial infections, affecting patients across all age groups, and is increasingly complicated by the emergence of extended-spectrum β-lactamase (ESBL) producers. In the present study involving inpatients, the culture positivity rate was 29.4%, which is comparable to the prevalence reported by Kumar *et al*. [9] and Singh *et al*. [10] Females accounted for 67% of UTI cases, consistent with previous studies attributing this higher prevalence to anatomical and behavioral factors such as a shorter urethra, proximity to the anus, and sexual activity. [11,12]

The highest incidence was noted in the 15–30-year age group (35.3%), similar to the findings of Dubey *et al*. [13], indicating that age is an important risk factor in inpatient UTIs. Gram-negative bacilli were the predominant pathogens, with *E. coli* (39%) being the most common isolate, followed by *Enterococcus* sp. (37%) and *Klebsiella* sp. (4%). This pattern is consistent with other hospital-based studies. [14, 15] Knowledge of such local pathogen profiles is vital for clinicians to initiate appropriate empirical therapy and reduce complications such as pyelonephritis and urosepsis.

In terms of antimicrobial susceptibility, tigecycline (72%), nitrofurantoin (70%), and amikacin (69%) were the most effective agents against Gram-negative isolates, while linezolid (96%), vancomycin (95%), and nitrofurantoin (84%) were highly active against Gram-positive organisms. Nitrofurantoin retained good overall sensitivity against both Gram-negative and Gram-positive pathogens, making it a valuable option for empirical therapy in stable hospitalized patients while awaiting culture results. Similar findings on nitrofurantoin's effectiveness have been reported in multiple Indian studies. [16–18]

ESBL production was detected in 43% of isolates, predominantly *E. coli* and *Klebsiella* sp. This falls within the national range of 21.8–64.8% reported in earlier studies [15,19,20] and agrees with Behera *et al.* [21] ESBL producers showed high resistance to ceftriaxone (98%), ceftazidime (95%), ceftazidime–clavulanic acid (93%), and ciprofloxacin (88%), but retained relatively higher susceptibility to carbapenems (70–79%), nitrofurantoin (72%), and amikacin (77%). Similar trends have been documented by Chandrashekhar *et al.* and Umadevi *et al.* [22,23]

Notably, nitrofurantoin maintained substantial activity against both ESBL and non-ESBL isolates, consistent with findings by Asha *et al.* [24]. The persistence of nitrofurantoin's efficacy, coupled with its affordability

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and oral availability, supports its role as a first-line empirical treatment in this setting.

The high proportion of ESBL-producing isolates among inpatients highlights the urgent need for stringent infection control measures, rational antimicrobial prescribing, and routine resistance surveillance. Minimizing the use of third-generation cephalosporins can help reduce selection pressure for resistant strains. [25] Regularly updated hospital antibiograms are vital for guiding empirical therapy and improving patient outcomes. [17,18] In light of the high prevalence of ESBL production and fluoroquinolone resistance, cephalosporins and fluoroquinolones should be avoided for empiric treatment, with preference given to agents retaining good activity, such as nitrofurantoin, aminoglycosides, and carbapenems for severe infections.

CONCLUSIONS

This study reveals a high burden of antimicrobial resistance among uropathogens in hospitalized patients at an IIMSR in Lucknow. *E. coli* was the most common isolate, with half producing ESBL, indicating widespread multidrug resistance. ESBL producers showed significantly lower susceptibility to β-lactams, cephalosporins, fluoroquinolones, and aztreonam, while both ESBL and non-ESBL strains remained highly sensitive to carbapenems, tigecycline, nitrofurantoin, and aminoglycosides. Gram-negative pathogens exhibited alarming resistance to fluoroquinolones and first-generation cephalosporins, whereas Gram-positive isolates retained good susceptibility to glycopeptides and linezolid. These findings highlight the need for ongoing resistance monitoring and early ESBL detection to guide empirical therapy and protect last-line drugs.

Future research should focus on rapid diagnostics, genomic surveillance, and antibiograms to guide timely treatment. Additionally, predictive tools and national AMR programs can improve outcomes, reduce costs, and prevent outbreaks by enhancing infection control, definitive therapy, and the careful use of carbapenems.

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