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A Regional Epidemiological Study on the Prevalence, Species Diversity, and Multidrug Resistance Patterns of Urobacteria Isolated from Urinary Tract Infections in the Nashik District, Maharashtra, India

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A B S T R A C T

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide, with increasing concern due to multidrug resistance (MDR) among uropathogens. This study investigated the diversity and antibiotic resistance profiles of urobacteria isolated from urine samples collected between November 2022 and April 2023 across 70 hospitals in the Nashik district, Maharashtra, India. Of the 541 samples analysed, 482 (89.09%) showed bacterial growth, while 59 (10.90%) exhibited fungal presence. A total of 36 bacterial species were identified, including 24 (66.66%) Gram-negative and 12 (33.33%) Gram-positive strains. *Escherichia coli* was the predominant pathogen (36.3%), followed by *Enterococcus faecalis* (9.5%), *Klebsiella pneumoniae* (7.8%), and multidrug-resistant (MDRO) *Pseudomonas aeruginosa* (6.2%). MDR analysis revealed that 37.5% of *E. coli* isolates were resistant to three antibiotic classes, while *K. pneumoniae* MDRO strains showed extensive resistance, with 69.69% resistant to six classes. *Enterococcus faecalis* VRE isolates displayed high-level resistance to five and eight antibiotic classes, and *Providencia rettgeri* showed 75% resistance to seven classes. The study emphasized the alarming spread of MDR in both dominant and rare uropathogens and highlights the need for region-specific antimicrobial stewardship and continuous surveillance to mitigate further escalation of resistance.

Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide, affecting individuals across all age groups and healthcare settings. They are a major cause of morbidity, recurrent hospitalization, and economic burden on healthcare systems, particularly in developing countries (M. S. Kumar & A. P. Das, 2016). The emergence of antibiotic-resistant uropathogens has become a critical public health challenge, limiting therapeutic options and increasing the risk of treatment failure. In India, the problem is

particularly alarming due to widespread self-medication, antibiotic misuse, and inadequate surveillance mechanisms that contribute to the rapid spread of multidrug-resistant (MDR) bacterial strains.

Over the past decade, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* have been reported as predominant UTI pathogens. Conventional antibiotic therapies, including β-lactams, fluoroquinolones, and aminoglycosides, have been extensively used for their treatment.

However, increasing resistance to these commonly prescribed antibiotics has significantly reduced their clinical efficacy (Torres-Sangiao *et al.,* 2022).

Automated systems such as VITEK 2 have improved bacterial identification and susceptibility testing, providing rapid results that aid in antibiotic selection (N. Shetty *et al.,* 1998). Despite these advancements, antibiotic resistance remains a dynamic and region-specific issue influenced by local prescribing patterns, healthcare practices, and microbial ecology.

Several surveillance studies have attempted to map antibiotic resistance trends across India, yet most are restricted to tertiary hospitals or limited populations, overlooking regional variations. Furthermore, data on the prevalence and resistance patterns of urinary pathogens in semi-urban and district-level populations such as Nashik are scarce (Odsbu *et al.,* 2018).

This information gap hampers the formulation of targeted antibiotic policies and rational therapy guidelines at the local level. Furthermore, in developing countries like India, the antibiotic susceptibility pattern study is farm more crucial due to prevalent and unprescribed antibiotic use (Porter *et al.,* 2021).

To address these limitations, the present study investigates the antibiotic resistance patterns of urobacteria isolated from urine samples collected across multiple hospitals in the Nashik district, Maharashtra, India. Using the VITEK 2 Compact system, this research provides a comprehensive analysis of bacterial diversity, frequency distribution, and multidrug resistance (MDR) profiles. The study aims to generate region-specific data that can contribute to the development of evidence-based antimicrobial stewardship strategies and help mitigate the growing threat of antimicrobial resistance in urinary tract infections.

Materials and Methods

Collection of samples

The present study was conducted from November 2022 to April 2023. Ethical approval for this study was obtained from the Regional Research Ethics Committee, Nashik. The urine samples were collected from male and female patients with positive urinary tract infections for the population aged 4-90 years. In total 541 urine samples were collected from ---- laboratory that handles the patient samples from approximately 70 different hospitals from the Nashik region, Maharashtra. Around 4 to 5 mL of Clean Catch Midstream urine samples were collected in sterile vials of ---ml and immediately transported to laboratory. Guidelines for proper sample collection were provided to all the patients.

Isolation of bacteria from urine sample

Isolation of bacterial species was performed by spread plate technique. The urine samples were diluted by 10^5 fold with sterile water and 100 ml of the diluted sample was spread onto chromogenic nutrient agar plates M. Kumar and A. Das (2016). Medium was prepared by dissolving 56.8 g/L HiCrome UTI agar medium (Himedia, India). The bacteria were incubated at 37^0 C for 24 h. The VITEK 2 compact system also identified the bacteria as the cards contain 29 different biochemical assays.

A measured volume of urine was inoculated onto nutrient agar medium (Merck, Germany) using the calibrated loop method for colony count analysis. A growth of $\geq 10^4$ CFU/mL of a single potential pathogen, or of each of two potential pathogens, was interpreted as indicative of a positive urinary tract infection (UTI). Samples yielding 10^2–10^4 CFU/mL were re-cultured for confirmation, while those with $<10^2$ CFU/mL were considered negative for UTI [10].

Identification of bacteria

Gram-staining was performed and the bacteria were inoculated on the cards separately on the basis of their Gram positive and Gram negative nature accordingly (N Shetty *et al.,* 1998). Incubation times varied from two to 15 hours depending on the growth rate of the organism. The Vitek programmed computer determined whether each well is positive or negative by measuring light

attenuation with an optical scanner. After the completion of the incubation period, the reactions were analyzed automatically, and the identification was printed.

Antimicrobial susceptibility test

After identification, the samples were processed, and antimicrobial susceptibility test was run similarly on cards which contain dilutions of antimicrobials to determine the breakpoint minimum inhibitory concentration (MIC) against the organisms. The MIC cutover values differentiating sensitive, moderate, and resistant status for an organism against appropriate antimicrobials are programmed into the system. Briefly, from the positive samples, a 0.5 McFarland turbidity suspension inoculum was prepared and susceptibility to different antibiotics by the VITEK 2 System (bioMerieux) was tested. There are separate cards for Gram positive and Gram-negative organisms. The antibiotics tested were Amphotericin B, Ampicillin, Amoxyclav, Amikacin, Aztreonam, Chloramphenicol, Caspofungin, Ceftazidime, Cefalotin, Cefixime, Ciprofloxacin, Clindamycin, Ceftriaxone, Colistin, Ceftazolone/tazobactum, Cefotaxime, Cefuroxime, Cefuroxime axetil, Ceftazidime/avibactum, Ceftizoxime, Daptomycin, Doxicycline, Doripenem, Erythromycin, Ertapenem, Flucytosine, Cefpime, Fluconazole, Fosfomycin, Cefoxitin, Nitrofurantoin, Gentamicin, Gentamicin, Inducible Clindamycin Resistance Imipenem, Levofloxacin, Linezolid, Micafungin, Meropenem, Minocycline, Moxifloxacin, Nalidixic acid, Netilmycin, Norfloxacin, Ofloxacin, Oxacillin, Cefoxitin Screen, Benzylpenicillin, Polymyxin B, Rifampicin, Ampicillin/sulbactum, Cefoperazone/sulbactum, Co-trimoxazole TCC-Other-Expertized, Tetracycline, Teicoplanin, Tigecycline, Ticarcillin, Tobramycin, Piperacillin/tazobactum, Vacomycin, Voriconazole as well as the ESBL test (N-388 AST card, bioMerieux). (Torres-Sangiao *et al.,* 2022). The MDR were considered as if a microbe showed resistance in three or more than three major classes. The classification of major classes is given in Table 2.

Statistical analysis

Statistical analysis was performed using the GraphPad Prism program (GraphPad Software, San Diego, CA, USA). To analyze paired elements, we used the paired *t*-test. Normality (Kolmogorov-Smirnov) and homogeneity of variance (Bartlett's) tests were applied to all variables. When under normal distribution and homogeneous variance, parametric tests (ANOVA with post-hoc Tukey's multiple comparison) were applied, and the results are expressed as the mean ± SEM. Otherwise, we used non-parametric tests (Kruskal-Wallis test with Dunn's multiple comparison), and the results are expressed as median, maximum, and minimum values. Differences were considered significant when $p \leq 0.05$ (Rodrigues *et al.,* 2016)

Results and Discussion

From the current results it was found that out of 541 urine samples, 482 (89.09 %) samples had bacterial occurrence and remaining 59 (10.90 %) samples had fungal occurrence.

The majority of positive samples were from female patients exhibiting 271 (56.22 %) and 211 samples (43.77 %) were from male patients.

Urobacterial pathogens

The 485 total samples that showed the presence of bacterial pathogens had a diversity of 36 bacterial species showing majority of 24 (66.66 %) species were gram negative and 12 (33.33 %) species were gram positive. The most common urinary bacteria isolated were *E. coli* (36.3 %) followed by *Enterococcus faecalis* (9.5 %), *Klebsiella pneumoniae* (7.8 %) and *Pseudomonas aeruginosa* MDRO (6.2 %) (Table 1).

Multiple drug resistance

The distribution of multidrug resistance varied notably among the isolates. E. coli showed resistance to three, four, and five antibiotic classes in 37.5%, 10.79%, and 2.84% of cases, respectively, while E. coli CRE isolates exhibited broader resistance, with 45.45% resistant to four classes and 13.63% each to five and six classes. Enterococcus faecalis showed lower resistance overall, whereas VRE isolates displayed higher multidrug resistance, with 33.3% resistant to both five and eight classes. Klebsiella pneumoniae demonstrated moderate resistance, with 15.78% of isolates resistant to four classes, while K. pneumoniae MDRO strains showed extensive resistance, with 69.69% resistant to six classes. Proteus mirabilis and Providencia rettgeri isolates exhibited resistance across multiple classes, with P. rettgeri showing up to 75% resistance to seven classes.

Table.1 Frequency distribution and MDR pattern of urobacteria isolated from Nashik region

Genus	Species	Total frequency (n)	Percentage (%)	Total male	Total female	No. of MDR (Female)	No. of MDR (Male)
Gram Negative bacteria							
Acinetobacter	*Acinetobacter baumannii*	1	0.2	0	1	0	0
	Acinetobacter baumannii complex	1	0.2	1	0	0	1
	Acinetobacter haemolyticus	1	0.2	0	1	1	0
	Acinetobacter lwoffii	1	0.2	0	1	0	0
Burkholderia	*Burkholderia cepacia*	4	0.8	1	3	2	0
Chryseobacterium	*Chryseobacterium indologenes*	1	0.2	1	0	0	1
Citrobacter	*Citrobacter koseri*	2	0.4	2	0	0	2
Enterobacter	*Enterobacter aerogenes*	1	0.2	0	1	0	0
	Enterobacter cloacae	4	0.8	1	3	2	1
	Enterobacter cloacae complex	5	1	4	1	0	2
Escherichia	*Escherichia coli*	176	36.3	66	108	55	33
	Escherichia coli CRE	22	4.5	11	11	11	11
Klebsiella	*Klebsiella oxytoca*	1	0.2	1	0	0	1
	Klebsiella pneumoniae ozaenae	1	0.2	0	1	1	0
	Klebsiella pneumonae pneumonae	38	7.8	14	24	10	4
	Klebsiella pneumonae	1	0.2	1	0	0	1
	Klebsiella pneumonae MDRO	33	6.8	21	12	21	12
Leclercia	*Leclercia adecarboxylata*	1	0.2	1	0	0	0
Morganella	*Morganella morganii*	2	0.4	1	1	1	1
Myroides	*Myroides spp*	1	0.2	0	1	1	0
Pantoea	*Pantoea agglomerans*	1	0.2	1	0	0	1
Proteus	*Proteus mirabilis*	12	2.5	12	2.5	3	7

Providencia	*Providencia rettgeri*	4	0.8	4	0.8	0	4
Pseudomonas	*Pseudomonas aeruginosa*	24	4.9	24	4.9	4	5
	Pseudomonas aeruginosa MDRO	30	6.2	30	6.2	11	19
	Pseudomonas fluorescens	1	0.2	1	0.2	1	0
	Pseudomonas luteola	2	0.4	2	0.4	0	2
	Pseudomonas oleovorans	1	0.2	1	0.2	0	1
	Pseudomonas putida	2	0.4	2	0.4	1	1
	Pseudomonas spp	3	0.6	3	0.6	0	1
Serratia	*Serratia marcescens*	2	0.4	2	0.4	0	2
Sphingomonas	*Sphingomonas paucimobilis*	1	0.2	1	0.2	1	0
Gram Positive Bacteria							
Enterococcus	*Enterococcus faecalis*	46	9.5	46	9.5	20	13
	Enterococcus faecalis VRE	9	1.9	9	1.9	6	2
	Enterococcus faecium	7	1.4	7	1.4	5	2
	Enterococcus faecium VRE	7	1.4	7	1.4	3	4
Staphylococcus	*Staphylococcus aureus*	1	0.2	1	0.2	1	0
	Staphylococcus epidermis (MRSA)	4	0.8	4	0.8	1	2
	Staphylococcus epidermidis	3	0.4	3	0.4	1	1
	Staphylococcus haemolyticus	13	2.7	13	2.7	8	4
	Staphylococcus hominis	1	0.2	1	0.2	0	0
	Staphylococcus saprophyticus	3	0.6	3	0.6	2	1
	Staphylococcus warneri	1	0.2	1	0.2	0	0
Streptococcus	*Streptococcus agalactiae*	4	0.8	4	0.8	0	0
	Streptococcus oralis	1	0.2	1	0.2	0	1
	Streptococcus mutans	1	0.2	1	0.2	0	1
	Streptococcus sanguinis	2	0.4	2	0.4	2	0
		485	100	212	273	193	161

Table.2 Resistance of microbes according to MDR classes

Microbe	Total isolates	3 classes	4 classes	5 classes	6 classes	7 classes	8 classes	9 classes
Burkholderia cepacia	4	2	0	0	0	0	0	0
E coli	176	66	19	5	0	0	0	0
***E. coli* CRE**	22	6	10	3	3	0	0	0
Enterobacter cloacae	4	1	1	0	0	0	0	0
***Enterobacter cloacae* complex**	5	0	1	1	0	0	0	0
Enterococcus faecalis	46	12	15	3	6	0	0	0
***Enterococcus faecalis* VRE**	9	0	0	3	0	1	3	1
Klebsiella pneumonae	38	5	6	2	0	0	0	0
***Klebsiella pneumonae* MDRO**	33	0	2	8	23	0	0	0
Proteus mirabilis	12	2	2	3	1	2	0	0
Providencia rettgeri	4	0	0	0	1	3	0	0
Pseudomonas aeruginosa	24	8	1	0	0		0	0
***Pseudomonas aeruginosa* MDRO**	30	28	2	0	0	0	0	0
***Staphylococcus epidermis* MRSA**	4	0	2	0	0	0	0	0
Staphylococcus haemolyticus	13	3	4	3	1	0	0	0

Pseudomonas aeruginosa displayed limited resistance, but its MDRO variant showed high resistance, with 93.33% resistant to three antibiotic classes.

As observed in several studies, Escherichia coli was identified as the predominant pathogen associated with urinary tract infections, accounting for approximately 70.1% of all cases. Being a normal inhabitant of the intestinal microbiota, *E. coli* can readily colonize the urinary tract, most commonly leading to infections such as cystitis. In the present study it was found that the occurrence of bacteria other than *E. coli* like *P. aeruginosa*, the MDR strain of *P. aeruginosa*, *Staphylococcus* spp. *P. mirabilis, P. rettigeri*, the MDR strain of *K. pneumonae* were higher in males than in females. Similar results were obtained by (Silva *et al.,* 2022) who reported higher incidence of *P. aeruginosa* in males. Consistent with previous findings, Amna *et al.,* (2013) reported that non-*E. coli* pathogens are more commonly associated with urinary tract infections in men, which is largely attributed to the higher prevalence of catheter use and the consequent development of complicated UTIs in this group (Shackley *et al.,* 2017). Notably, Enterococcus and Pseudomonas species are frequently implicated in catheter-associated urinary tract infections (CAUTIs) (Cole *et al.,* 2014).

In the present study, the third most prevalent pathogen was found to be *K. pneumoniae* replacing *S. aureus* over the years. It was reported that ten years ago the prevalence rate of *K. pneumoniae* was 4.3 % of the UTI. However, it changed gradually over the years and in the current study it was reported to be 7.8 %. This can be related to the increase in the resistance of bacterium. In the study it was found that out of 33 isolates of *K. pneumoniae* MDRO strain, 23 isolates were resistant to at least 6 classes of antibiotics.

Even bacteria with low incidence may demonstrate antibiotic resistance likely via horizontal gene transfer (HGT). The similar resistance patterns observed across diverse species support a strong possibility of acquired resistance through HGT (Sun *et al.,* 2019).

In conclusion, the present study revealed a high prevalence of multidrug-resistant uropathogens in the Nashik district, with *E. coli* as the dominant species followed by Enterococcus and Klebsiella. The extensive resistance in *K. pneumoniae* MDRO and VRE isolates is concerning, indicating the spread of resistance genes across bacterial taxa. Even bacteria with low frequency demonstrated similar resistance profiles, suggesting horizontal gene transfer as a major mechanism. The study demonstrated the urgent need for strict antibiotic stewardship, infection control measures, and continuous regional monitoring to curb MDR proliferation.

Author Contributions

J. H. Maniyar: Investigation, formal analysis, writing—original draft. S. B. Mali: Validation, methodology, writing—reviewing. P. P. Dixit:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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