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Antimicrobial Susceptibility Trends in Uropathogens: A 6-Year Retrospective Study from Kerala, India

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ABSTRACT

Introduction: Antimicrobial resistance (AMR) among uropathogens is a critical global public health challenge, potentially exacerbated by the COVID-19 pandemic. This study evaluates the pandemic's impact on antimicrobial susceptibility trends among uropathogens at a tertiary care center in Kerala over six years (2018–2023). **Methods:** A retrospective analysis of 4,461 uropathogen isolates was conducted using data from laboratory records. Data were analyzed using IBM SPSS Statistics version 20, with Chi-square tests for associations. **Results:** *Escherichia coli* was the predominant pathogen (57.4%), followed by *Klebsiella pneumoniae* (14.3%) and *Enterococcus* spp. (6.0%). Other common isolates included *Pseudomonas aeruginosa* (4.2%) and *Acinetobacter baumannii* (3.3%), and fungi (6.8%). *E. coli* susceptibility to ampicillin increased significantly from 3.4% in 2018 to 13.9% in 2023 ($P< 0.001$), though overall resistance remained high; while susceptibility to cefoperazone-sulbactam and piperacillin-tazobactam declined ($P< 0.001$). *K. pneumoniae* susceptibility to cefoperazone-sulbactam decreased from 88.7% in 2018 to 73.0% in 2023 ($P< 0.001$). *Enterococcus* spp. susceptibility to nitrofurantoin declined from 91.0% in 2018 to 67.4% in 2023 ($P = 0.017$). *A. baumannii* showed increased ceftazidime susceptibility from 18.4% in 2018 to 56.0% in 2022, followed by a decline to 27.8% in 2023 ($P = 0.015$). *P. aeruginosa* exhibited increased susceptibility to gentamicin (57.6% to 77.2%; $P = 0.012$), ceftazidime (61.6% to 77.2%; $P = 0.043$), and fluoroquinolones (53.9% to 68.1%; $P = 0.019$) from 2018 to 2023. **Conclusion:** This study highlights dynamic shifts in antimicrobial susceptibility patterns among uropathogens, underscoring the need for continuous surveillance to guide empirical therapy and infection control strategies. Further research is warranted to explore contributing factors, including pandemic-related practices.

INTRODUCTION

Urinary tract infections (UTIs) represent a significant global health burden, exacerbated by the increasing prevalence of antimicrobial resistance (AMR) among uropathogens [1]. The rise of AMR in both community and healthcare-acquired UTIs threatens the efficacy of current treatment strategies, leading to increased morbidity, mortality, and healthcare costs. The predominant bacterial uropathogens and their antimicrobial susceptibility profiles exhibit geographic and temporal variability, posing a critical challenge to effective UTI management [2, 3].

Among uropathogens, *Escherichia coli*, a member of the *Enterobacterales* order (formerly *Enterobacteriaceae* family), is the most frequently isolated species, followed by *Klebsiella pneumoniae* and *Enterococcus* spp. [4, 5]. The emergence of the COVID-19 pandemic raised concerns about its potential impact on AMR trends [6]. The pandemic influenced AMR dynamics variably; while some studies reported reduced antimicrobial prescriptions due to fewer doctor consultations, others noted increased antimicrobial use in several countries, potentially contributing to the spread of resistance among bacterial pathogens [7, 8]. Public health measures, such as masking and social distancing, significantly reduced the transmission of respiratory pathogens, potentially decreasing antimicrobial use for these infections [9].

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The global impact of the COVID-19 pandemic on AMR patterns has been diverse and geographically varied [10–12]. Public health interventions aimed at curbing the spread of the epidemic also led to a decline in numerous respiratory infections, thereby decreasing antimicrobial use within the community [13]. These findings underscore the need for ongoing surveillance and coordinated efforts among healthcare providers, policymakers, and the public to address AMR. This six-year retrospective study (2018–2023) analyzes uropathogen prevalence and antimicrobial resistance trends at a tertiary care center in Kerala, encompassing pre-pandemic and pandemic periods. By characterizing the most prevalent uropathogens and their evolving resistance patterns, this study aims to provide locally relevant data to optimize antimicrobial prescribing practices for UTIs and inform regional antimicrobial stewardship efforts.

MATERIAL AND METHODS

Study design and setting. This retrospective study analyzed antimicrobial susceptibility data for uropathogens isolated from urine cultures at the Microbiology Laboratory, District Hospital, Palakkad, Kerala, from January 2018 to December 2023. Data included midstream and catheter-aspirated urine samples from patients of all ages and sexes.

Inclusion and exclusion criteria. All culture-positive urine samples were included in the analysis. To avoid overrepresentation, only the first positive urine culture per patient within a 14-day period was retained, unless there was a change in antimicrobial susceptibility pattern or a clinician suspected a recurrent infection. Samples with mixed growth (more than two isolates per sample) were excluded, and a properly collected repeat sample was requested.

Laboratory procedures. Urine samples underwent wet mount microscopy and were cultured on blood agar and MacConkey agar, incubated overnight at 37°C. Growth was considered significant when there were $\geq 10^5$ colony-forming units/mL of urine. Bacterial isolates were identified using standard biochemical tests, including catalase, oxidase, indole, urease, citrate, triple sugar iron, and mannitol motility tests for Gram-negative bacteria. For Gram-positive bacteria, catalase, tube coagulase, and aesculin hydrolysis tests were performed. Antimicrobial susceptibility testing was conducted via the Kirby-Bauer disk diffusion method, following Clinical and Laboratory Standards Institute (CLSI) guidelines (as per CLSI document M100 for each year).

Antibiotics tested. The following antibiotics were tested, selected as per CLSI guidelines based on the isolate.

- β-lactams: Ampicillin, Piperacillin, Amoxicillin-clavulanate, Cephalexin, Ceftriaxone, Ceftazidime, Cefoperazone-sulbactam, Piperacillin-tazobactam, Imipenem, Meropenem, Cloxacillin
- Aminoglycosides: Gentamicin, Amikacin
- Fluoroquinolones: Ciprofloxacin, Norfloxacin
- Others: Nitrofurantoin, Co-trimoxazole, Vancomycin, Tetracycline

Quality control. Monthly quality control was performed using reference strains: *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, and *E. faecalis* ATCC 29212.

Data collection and analysis. Demographic data (age, sex), bacterial isolates, and antibiograms were entered into Microsoft Excel 2016 and validated for accuracy through data entry validation, integrity checks, and cross-checking results. Statistical analysis was conducted using IBM SPSS Statistics v20. Categorical variables were summarized as frequencies and percentages, and temporal trends were analyzed using the Pearson Chi-square test ($P< 0.05$ considered statistically significant).

Ethical considerations. Ethical clearance (IEC/GMCPKD/3/2024/114) was obtained from the Institutional Ethics Committee of Government Medical College, Palakkad. Informed consent was waived due to the retrospective design and use of anonymized data.

RESULTS

Sample characteristics and culture positivity. From 2018 to 2023, 20,635 urine samples were received. The annual distribution was as follows: 3,647 (2018), 4,002 (2019), 2,423 (2020), 2,343 (2021), 4,322 (2022), and 3,898 (2023). The culture positivity rate was 21.6%, yielding 4,461 isolates from 4,375 patients (86 had polymicrobial infections). The female-to-male ratio was 1.59:1. The most frequently affected age group was 61–70 years (20.3%), as shown in Figure 1.

Distribution of uropathogens. Gram-negative bacilli constituted 84.7% (n = 3,779), Gram-positive 8.5% (n=380), and fungi 6.8% (n=302) of isolates. *E. coli* was the most prevalent uropathogen (57.4%, n = 2,559), followed by *K. pneumoniae* (14.3%, n = 638) and *Enterococcus* spp. (6.0%, n = 267). Among the isolates, 6.8% were fungi, which included *Candida albicans* and non-*Candida albicans* (63 and 239 isolates, respectively). The distribution and yearly trends are depicted in Figures 2–4.

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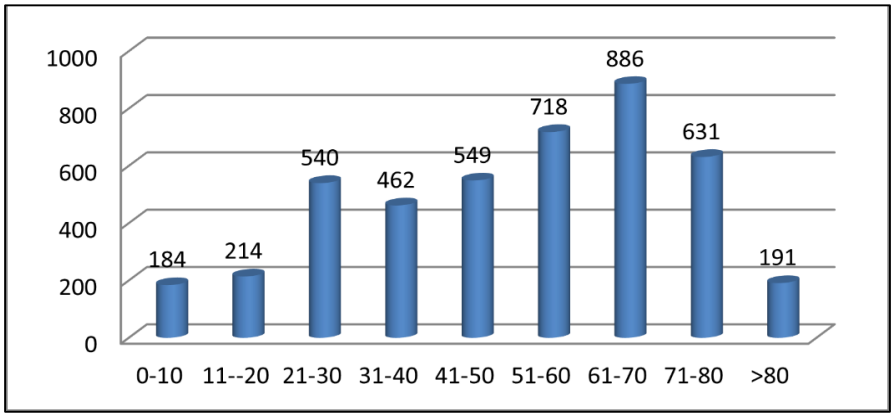

Fig. 1. Age group distribution of patients (X axis: Age group, Y axis: Number of patients)

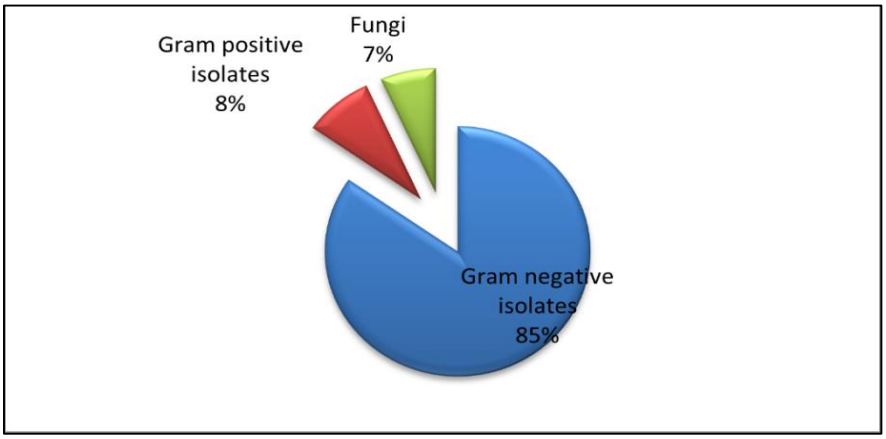

Fig. 2. Distribution of Gram-positive, Gram-negative, and fungal isolates

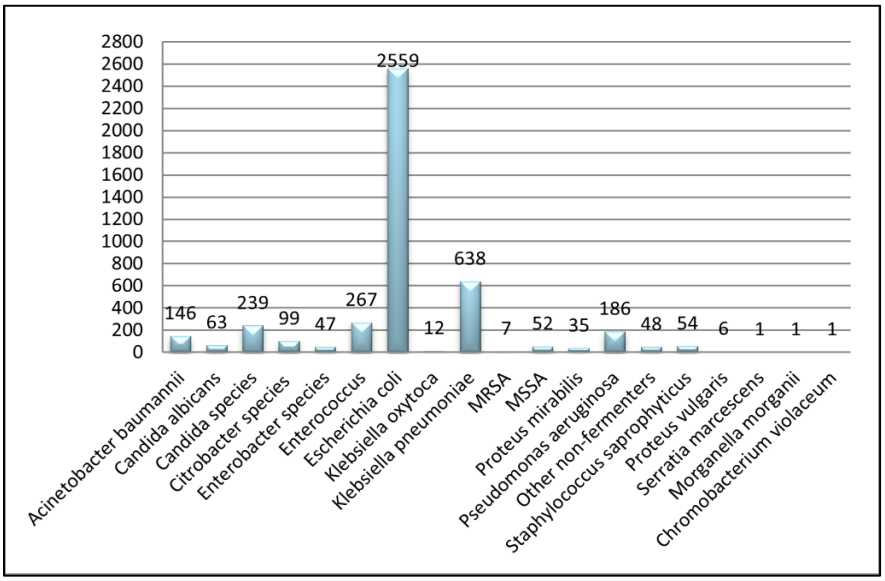

Fig. 3. Distribution of isolates (X axis: Isolates, Y axis: Number of isolates)

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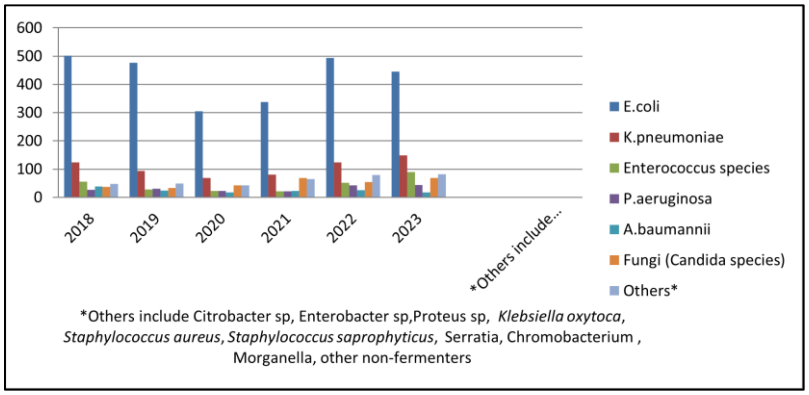

Fig. 4. Distribution of isolates by year (X axis: Isolates in each year, Y axis: Number of isolates)

Antimicrobial susceptibility patterns

A) *E. coli and K. pneumoniae*

Among *E. coli* isolates, susceptibility to ampicillin was low (8.0%, n = 205), while susceptibility to nitrofurantoin was high (90.5%, n = 2,317). All *K. pneumoniae* isolates (n=638) were resistant to ampicillin, with only 29% (n = 182) susceptible to nitrofurantoin. *K. pneumoniae* showed higher susceptibility to co-trimoxazole (57% *vs.* 51%, $P< 0.05$), norfloxacin (61% *vs.* 41.6%, $P< 0.001$), cephalexin (45% *vs.* 28%, $P< 0.001$), and ceftriaxone (53% *vs.* 33%, $P< 0.001$) compared to *E. coli*. Conversely, *E. coli* exhibited greater susceptibility to amikacin (92% *vs.* 82%), cefoperazone-sulbactam (91% *vs.* 84%), piperacillin-tazobactam (91% *vs.* 84%), imipenem (97% *vs.* 94%), and meropenem (97% *vs.* 95%) compared to *K. pneumoniae* (Figure 5).

B) *Enterococcus spp.*

All *Enterococcus* spp. isolates (n = 267) were susceptible to vancomycin. High susceptibility was observed for nitrofurantoin (79.0%, n = 211), amoxicillin-clavulanate (71.2%, n = 190), and ampicillin (64.4%, n = 172). Figure 6 shows the percentage of sensitive strains of *Enterococcus* spp.

C) *P. aeruginosa and A. baumannii*

P. aeruginosa (n = 186) showed higher susceptibility to ceftazidime (74.7%, n = 139) compared to *A. baumannii* (35.0%, n = 51). Susceptibility to cefoperazone-sulbactam was high for both (96.0% for *A. baumannii* vs. 95% for *P. aeruginosa*). Susceptibility to piperacillin-tazobactam was 93.2% (n = 137/147) for *A. baumannii* and 94.6% (n = 176/186) for *P. aeruginosa*. Figure 7 shows the percentage susceptibility of *P.aeruginosa* and *A. baumannii*.

Temporal trends in antimicrobial susceptibility

A) *E. coli*

Susceptibility to ampicillin increased significantly ($P< 0.001$) from 3.4% in 2018 to 13.9% in 2023. Gentamicin susceptibility peaked at 75.0% in 2021 ($P< 0.001$). Similar trends were observed for co-trimoxazole, ciprofloxacin, norfloxacin, amoxicillin-clavulanate, cephalexin, and ceftriaxone ($P< 0.001$ for all). Susceptibility to cefoperazone-sulbactam and piperacillin-tazobactam decreased ($P< 0.001$) from 91.0% in 2018 to 87.0% in 2023. Imipenem and meropenem susceptibility remained high, reaching 100% in 2021 (Table 1, Figure 5).

Table 1. Antimicrobial susceptibility trends in *E. coli* (2018–2023)

Antibiotic	2018	2019	2020	2021	2022	2023	*P*-value
Ampicillin	17/501 (3.4%)	13/477 (2.7%)	16/305 (5.2%)	23/338 (6.8%)	74/493 (15.0%)	62/445 (13.9%)	<0.001
Norfloxacin	178/501 (35.5%)	173/477 (36.3%)	113/305 (37.0%)	153/338 (45.3%)	246/493 (49.9%)	202/445 (45.4%)	<0.001
Amoxicillin-clavulanate	154/501 (30.7%)	153/477 (32.1%)	116/305 (38.0%)	133/338 (39.3%)	225/493 (45.6%)	208/445 (46.7%)	<0.001
Cephalexin	122/501 (24.4%)	89/477 (18.7%)	81/305 (26.6%)	99/338 (29.3%)	190/493 (38.5%)	129/445 (29.0%)	<0.001
Ceftriaxone	146/501 (29.1%)	141/477 (29.6%)	88/305 (28.9%)	106/338 (31.4%)	209/493 (42.4%)	155/445 (34.8%)	<0.001
Co-trimoxazole	213/501 (42.5%)	219/477 (45.9%)	160/305 (52.5%)	181/338 (53.6%)	288/493 (58.4%)	249/445 (56.0%)	<0.001
Imipenem	462/501 (92.2%)	468/477 (98.1%)	302/305 (99.0%)	338/338 (100%)	491/493 (99.6%)	425/445 (95.5%)	<0.001
Meropenem	462/501 (92.2%)	468/477 (98.1%)	302/305 (99.0%)	338/338 (100%)	491/493 (99.6%)	425/445 (95.5%)	<0.001

Note: Values represent number of sensitive strains/total isolates, with percentage in parentheses

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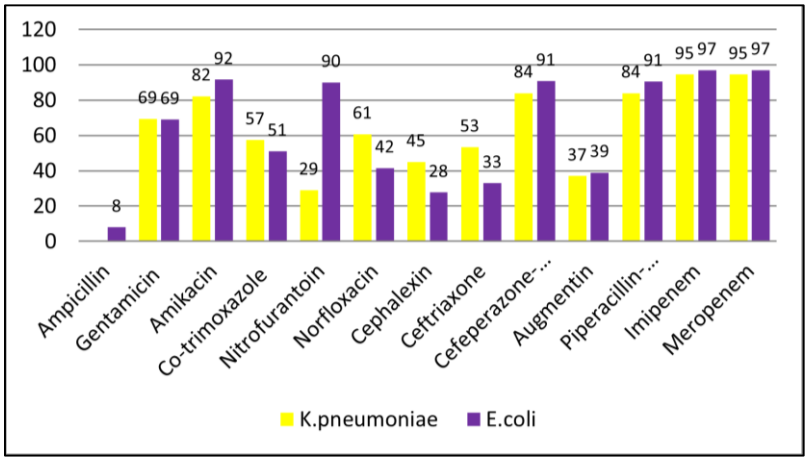

Fig. 5. Percentage of sensitive strains of *E. coli* and *K. pneumoniae* (X axis : Antibiotics, Y axis: Percentage of sensitive strains)

B) *K. pneumoniae*

Nitrofurantoin susceptibility increased (P< 0.001), peaking at 55.9% in 2020. Susceptibility to cefoperazone-sulbactam (73% in 2023), piperacillin-tazobactam (73% in 2023), imipenem (88% in 2023), and meropenem (88% in 2023) declined after 2021 (all P< 0.05) (Figure 5).

C) *Enterococcus spp.*

Nitrofurantoin susceptibility decreased (P = 0.017) from 91.0% in 2018 to 67.4% in 2023 (Figure 8).

D) *A. baumannii*

Ceftazidime susceptibility increased (P = 0.015), peaking at 56.0% in 2022, then declining to 27.8% in 2023. Piperacillin-tazobactam susceptibility decreased (P = 0.011) from 100% in 2020 to 77.8% in 2023. Imipenem and meropenem susceptibility was lowest in 2022 (Figure 9).

E) *P. aeruginosa*

Piperacillin susceptibility decreased (P = 0.001), reaching 23.8% in 2021. Gentamicin susceptibility increased (P = 0.012), peaking at 95.5% in 2020, with similar trends for norfloxacin and ceftazidime (Figure 10).

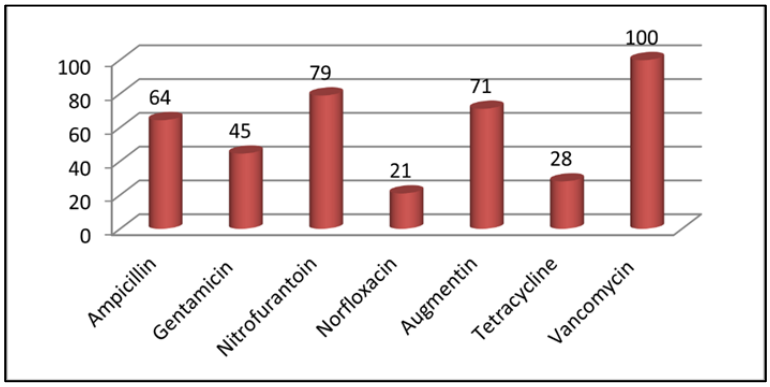

Fig. 6. Percentage of sensitive strains of *Enterococcus* spp.(X axis: Antibiotics, Y axis: Percentage of sensitive strains)

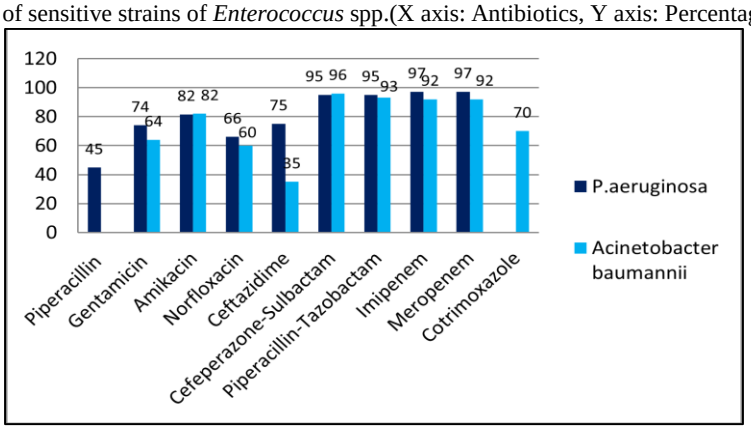

Fig. 7. Percentage of sensitive strains of *P. aeruginosa* and *A. baumannii* (X axis: Antibiotics, Y axis: percentage of sensitive strains)

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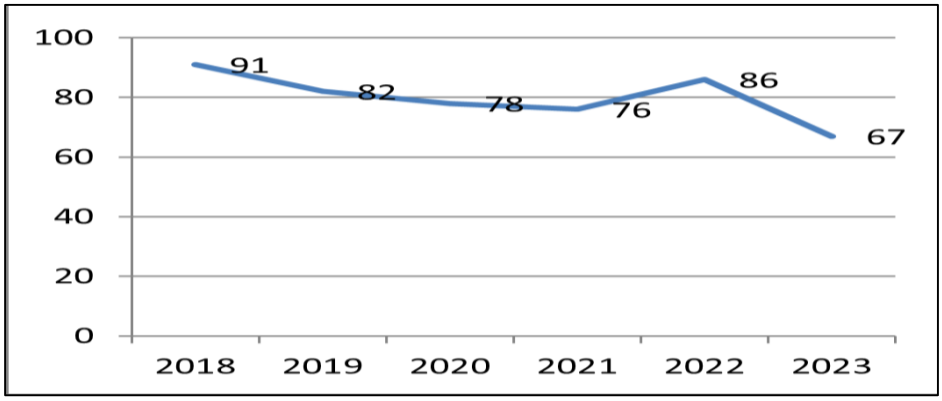

Fig. 8. Trend in nitrofurantoin-sensitive strains of *Enterococcus* spp. over the years (X axis: Years, Y axis: percentage of sensitive strains)

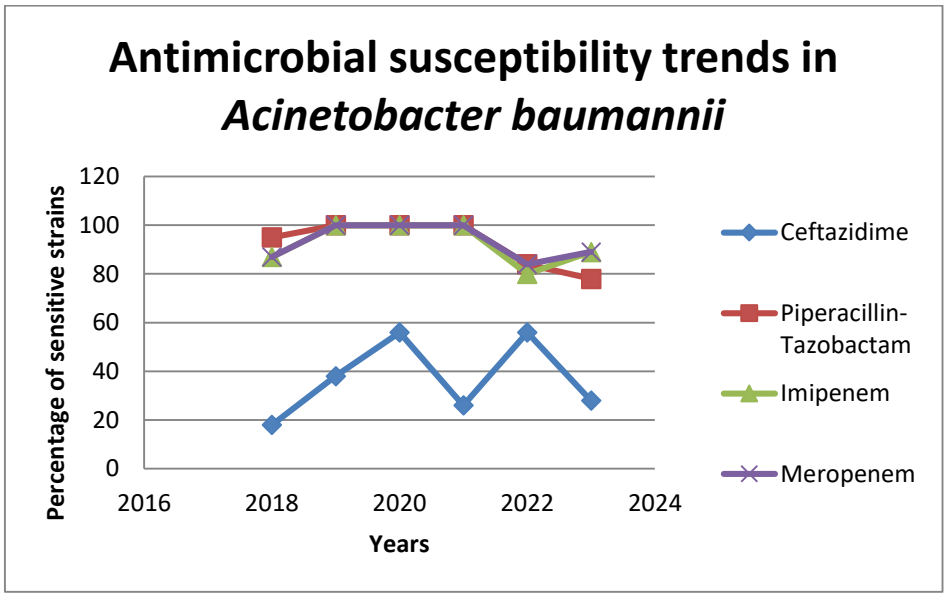

Fig. 9. Trends in antimicrobial susceptibility of *A. baumannii* (X axis: Years, Y axis: percentage of sensitive strains)

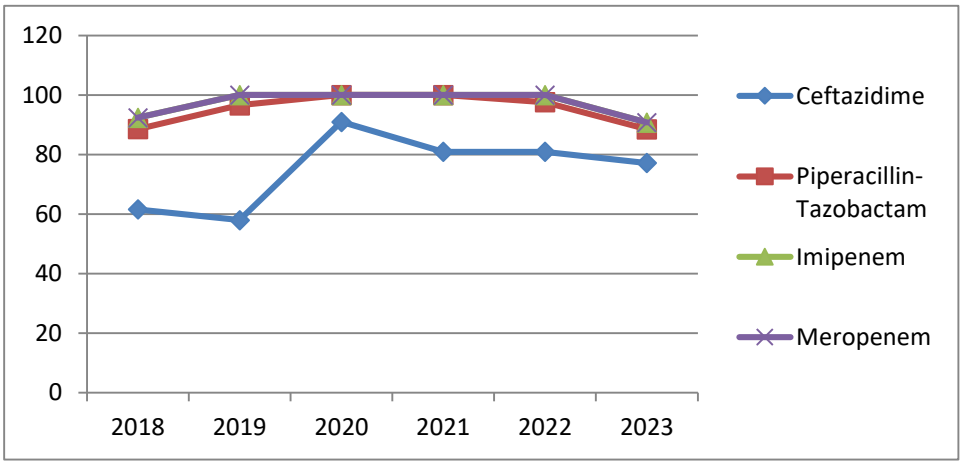

Fig. 10. Trends in antimicrobial susceptibility of *P.aeruginosa* (X axis: years and Y axis: percentage of sensitive strains)

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DISCUSSION

In our study, 21.6% of urine cultures were positive, lower than the 29.1% positivity rate reported in a 2023 study from North India [14]. This discrepancy may reflect differences in study populations, diagnostic criteria, or regional epidemiology. No significant shift in culture positivity rates was observed between pre-COVID (20.8%) and post-COVID (22.1%) periods ($P > 0.05$), suggesting the COVID-19 pandemic did not substantially alter the prevalence of positive urine cultures in our cohort.

In contrast, Yadigaroglu *et al*. (2022) [15] reported a significant increase in urine culture positivity during the pandemic (11.6% *vs*. 6.6%), possibly due to higher rates of catheter-associated UTIs or other pandemic-related factors. Consistent with studies from India [16, 17] and Nepal [18], *E. coli* and *K. pneumoniae* were the most frequently isolated uropathogens in our study, underscoring their persistent predominance in UTIs across diverse regions.

The distribution of uropathogens remained stable pre- and post-COVID-19. During 2018–2019, prevalence rates for *E. coli*, *K. pneumoniae*, and *Enterococcus* spp. were 62.5%, 13.9%, and 5.3%, respectively, compared to 54.6%, 14.5%, and 6.4% during 2020–2023. This stability aligns with findings from a meta-analysis by Langford *et al*. (2022) [11], which reported no significant changes in Gram-positive uropathogen incidence. However, Gandra *et al*. (2023) [19] observed higher culture positivity rates (29.3% *vs*. 18.8%) in an Indian community hospital during the pandemic, likely due to differences in study settings or infection control practices.

Significant changes in antimicrobial susceptibility were observed over the 6-year period. *E. coli* susceptibility to ampicillin increased ($P< 0.001$) from 3.4% in 2018 to 13.9% in 2023, possibly due to reduced ampicillin prescribing, as reported in a Polish study [8]. Similar significant increases in susceptibility were observed for amoxicillin–clavulanate, gentamicin, co-trimoxazole, cephalexin, ceftriaxone, and norfloxacin (all $P < 0.001$). Susceptibility to imipenem and meropenem remained high, reaching 100% in 2021 (Table 1). An Egyptian study [20] similarly reported a significant increase in E. coli susceptibility to ciprofloxacin ($P < 0.001$), whereas a Mexican study [21] documented persistent ampicillin resistance (>70%) along with a slight rise in imipenem resistance. A Romanian study [22] reported increased *E. coli* resistance to amoxicillin-clavulanate, ceftazidime, and nitrofurantoin post-COVID.

K. pneumoniae showed decreased susceptibility to cefoperazone-sulbactam (88.7% to 73.0%, $P< 0.001$), piperacillin-tazobactam, imipenem, and meropenem ($P< 0.05$), but increased nitrofurantoin susceptibility ($P< 0.001$). A Delhi study [17] reported reduced resistance in *Enterobacterales* post-COVID, while a Romanian study [22] noted increased *K. pneumoniae* resistance to fluoroquinolones and ceftazidime.

Enterococcus spp. showed decreased nitrofurantoin susceptibility ($P = 0.017$) from 91.0% in 2018 to 67.4% in 2023. A Mexican study [21] reported increased *E. faecalis* ampicillin resistance, while Mares *et al*. (2022) [22] noted increased *Enterococcus* susceptibility to ampicillin and nitrofurantoin but decreased vancomycin susceptibility.

A. baumannii ceftazidime susceptibility increased ($P = 0.015$), peaking at 56.0% in 2022, while piperacillin-tazobactam susceptibility decreased ($P = 0.011$). A Pakistan study [23] reported increased *A. baumannii* resistance to meropenem, imipenem, and piperacillin-tazobactam during the pandemic. Studies from Egypt [20] and India [24] noted increased multidrug-resistant (MDR) and extensively drug-resistant (XDR) *A. baumannii* post-COVID.

P. aeruginosa showed decreased piperacillin-tazobactam susceptibility ($P = 0.001$) but increased susceptibility to gentamicin, norfloxacin, and ceftazidime. A Delhi study [17] reported decreased gentamicin resistance, while a Chinese study [25] noted increased resistance to ceftazidime, ciprofloxacin, and gentamicin.

Increased resistance gene prevalence in hospital wastewater [26] and higher outpatient antibiotic use post-COVID vaccination [27] may have contributed to resistance trends. Studies also reported increased trimethoprim [3] and colistin [28] resistance in *Enterobacterales* and *Klebsiella* spp., respectively.

The retrospective design limited control over patient selection and sampling, potentially introducing selection bias. Exclusion of 122 patients with incomplete data on age, sex, specimen type, or antimicrobial use may affect generalizability. The single-center setting may not reflect broader regional trends.

In conclusion, the urine culture positivity rate was 21.6% and remained stable pre- and post-COVID-19. Significant changes in antimicrobial susceptibility were observed, including increased *E. coli* susceptibility to ampicillin, amoxicillin-clavulanate, gentamicin, co-trimoxazole, fluoroquinolones, cephalexin, and ceftriaxone post-COVID. In contrast, *E. coli* and *K. pneumoniae* exhibited decreased susceptibility to cefoperazone-sulbactam and piperacillin-tazobactam. *Enterococcus* spp. showed reduced nitrofurantoin susceptibility. *A. baumannii* had increased ceftazidime susceptibility but decreased piperacillin-tazobactam susceptibility. *P. aeruginosa* showed increased susceptibility to gentamicin, norfloxacin, and ceftazidime.

These findings provide a baseline for uropathogen susceptibility patterns in Kerala, guiding clinicians in selecting empirical antibiotic treatments for UTIs while awaiting susceptibility testing results. Continuous surveillance and further research into pandemic-related

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factors are essential for effective antimicrobial stewardship.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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