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Biofilm Formation and Antibiotic Resistance in MDR Uropathogens: Evaluating Meropenem's Inhibitory Potential

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KEYWORDS

Biofilm, Multidrug resistance, *E. coli*, *K. pneumoniae*, Meropenem, Urinary tract infections

ABSTRACT:

Multidrug-resistant (MDR) urinary tract infections (UTIs), predominantly caused by *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*), pose a serious public health challenge due to their ability to form biofilms that protect them from antimicrobial agents. This study aimed to assess the prevalence of biofilm-forming MDR uropathogens and evaluate the inhibitory effects of meropenem on biofilm production. A total of 63 MDR urinary isolates were collected and identified using the VITEK system. Biofilm-forming ability was assessed using the Tissue Culture Plate method, with classification based on optical density readings. Antibiotic susceptibility testing was conducted using standard protocols. The effect of meropenem on biofilm inhibition was tested at concentrations ranging from 0.25 µg/mL to 1.0 µg/mL. *E. coli* (76%) was the most prevalent isolate, followed by *K. pneumoniae* (24%). Strong biofilm formation was observed in 46% of the isolates, with *K. pneumoniae* showing slightly higher biofilm-forming ability than *E. coli*. Strong biofilm producers demonstrated significantly greater resistance to ampicillin, ciprofloxacin, and gentamicin ($p < 0.05$). Meropenem showed a dose-dependent inhibitory effect on biofilm formation, with concentrations ≥ 0.75 µg/mL significantly reducing biofilm biomass, especially at 1.0 µg/mL. The study confirms a strong correlation between biofilm formation and antibiotic resistance in MDR uropathogens. Meropenem effectively inhibited biofilm formation at higher concentrations, suggesting its potential utility in managing biofilm-associated UTIs. These findings highlight the need for novel therapeutic approaches that combine antimicrobial and anti-biofilm strategies to combat persistent MDR infections.

1. Introduction

Urinary tract infections (UTIs) are among the most prevalent infections globally, affecting millions of people each year. The majority of UTIs are caused by *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*), which are responsible for a significant proportion of both uncomplicated and complicated urinary infections (1). In recent years, there has been an alarming rise in the prevalence of multidrug-resistant (MDR) strains of these pathogens, complicating treatment options and leading to increased morbidity and mortality (2). MDR pathogens are resistant to multiple classes of antibiotics, making treatment more challenging and often resulting in prolonged hospital stays, higher healthcare costs, and an increased risk of complications (1,3).

A major factor contributing to the persistence and chronicity of these infections is the ability of uropathogens, such as *E. coli* and *K. pneumoniae*, to form biofilms (1,4). Biofilms are complex, three-dimensional structures formed by bacterial cells embedded in a self-produced extracellular matrix. These biofilms are highly resistant to both host immune responses and antimicrobial agents, making infections difficult to eradicate (5). Biofilm formation is particularly problematic in UTIs, as it allows bacteria to evade antibiotic treatment, leading to recurrent and chronic infections. This is especially true for patients with indwelling urinary catheters or those with underlying conditions such as diabetes or immunocompromised states, who are more susceptible to biofilm-associated infections.

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Despite the growing recognition of the role of biofilm in UTI pathogenesis, there remains a critical gap in understanding the most effective ways to treat biofilm-associated infections caused by MDR pathogens (3,5,6). Traditional antibiotics, including beta-lactams, fluoroquinolones, and aminoglycosides, are often ineffective against biofilm-forming organisms due to the protective matrix and altered bacterial metabolism within the biofilm (4). As a result, there is an urgent need for novel therapeutic strategies that can target biofilm formation or enhance the activity of existing antibiotics against biofilm-forming uropathogens (6,7).

Meropenem, a broad-spectrum carbapenem antibiotic, is typically reserved for severe or resistant infections rather than used as a first-line agent for UTIs (1,3). However, it remains a key therapeutic option in the treatment of MDR Gram-negative infections, including those caused by *E. coli* and *K. pneumoniae* (4,8). The significance of using Meropenem in this study lies in its potential dual role—not only as a potent antimicrobial agent but also as a biofilm-inhibitory compound, a function that remains underexplored in clinical isolates from urinary tract infections. This is particularly important given the lack of effective treatment options once biofilms are established in MDR cases (1,5,9). While the biofilm-forming capability of these organisms is not novel in itself, this study offers a new perspective by correlating biofilm strength with antibiotic resistance profiles and evaluating Meropenem's concentration-dependent effect on biofilm inhibition. Unlike many earlier studies that tested antibiotics in planktonic conditions, this study specifically examines biofilm modulation by Meropenem using a quantitative Tissue Culture Plate (TCP) method, which simulates biofilm growth more realistically in vitro (9).

The primary aim of this study is to evaluate the correlation between biofilm formation and antimicrobial resistance in MDR urinary isolates of *E. coli* and *K. pneumoniae*, with a specific focus on the biofilm-inhibitory potential of Meropenem (6). By exploring whether stronger biofilm formers are more resistant to commonly used antibiotics and whether Meropenem can attenuate biofilm biomass, this research seeks to inform more effective treatment strategies for persistent and recurrent UTIs caused by MDR pathogens.

The novelty of this study lies in its integrative approach—simultaneously examining resistance patterns, biofilm-forming ability, and the impact of a high-priority antibiotic (Meropenem) on biofilm production. The findings could help guide clinical decision-making in challenging cases of biofilm-associated UTIs, especially when first-line antibiotics fail (10).

Addressing the research gap related to biofilm formation and antibiotic susceptibility is crucial for improving the management of biofilm-related UTIs. With biofilms being a significant contributor to the chronicity and recurrence of these infections, understanding how to effectively disrupt biofilm formation could lead to more targeted and successful treatment strategies. This study aims to contribute valuable data that could pave the way for the development of more effective therapies, ultimately improving patient outcomes and reducing the burden of chronic and MDR-related UTIs.

Methodology

Study Design

This research was a cross-sectional study conducted over a six-month period, in the Clinical Microbiology Laboratory at Saveetha Medical College and Hospital, Chennai, India. The study aimed to evaluate the effects of Meropenem on biofilm formation in multidrug-resistant (MDR) urinary tract pathogens, particularly *Escherichia coli* and *Klebsiella pneumoniae*.

Ethical clearance: Ethical clearance for the study was obtained from the Institutional Ethical Committee of Saveetha Medical College and Hospital (Approval No: IHEC-II/0667/24).

Study Participants

The study included all MDR uropathogenic bacterial isolates obtained from urine samples of patients attending both outpatient and inpatient departments of Saveetha Medical College and Hospital. The participants were not directly involved in the research process but were part of routine clinical care for which urine samples were sent to the laboratory for microbiological testing. A total of 63 isolates from urine samples were included in the study, which were identified as MDR organisms. The study focused on *E. coli* and *K. pneumoniae*, which are

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the most prevalent pathogens responsible for urinary tract infections.

Ethical Considerations

Ethical approval for the study was obtained prior to sample collection. All bacterial isolates were obtained from routine clinical samples sent to the hospital's microbiology lab for diagnostic purposes. Patient confidentiality was strictly maintained, and no personal identifying information was used in the study. The study adhered to the principles outlined in the Declaration of Helsinki, and all research activities were conducted in accordance with ethical guidelines for medical research. As the study involved only microbiological isolates and not direct patient interaction, informed consent was not required.

Inclusion Criteria

The inclusion criteria were applied to select the study participants: (i) Only multidrug-resistant organisms were included in the study. MDR isolates are defined as those resistant to at least one antibiotic from three or more different antimicrobial classes. Resistance was determined using the VITEK 2 system, which provided a comprehensive antibiotic susceptibility profile for each isolate. (ii) Isolates were exclusively from urine samples obtained from patients in the outpatient or inpatient departments of the hospital. (iii) The isolates had to be identified as clinically significant pathogens (e.g., *E. coli* and *K. pneumoniae*) involved in urinary tract infections.

Exclusion Criteria

The exclusion criteria were: (i) Non-MDR Isolates: Isolates that were not resistant to at least three classes of antibiotics were excluded from the study, (ii) Isolates from Other Clinical Specimens: Only urinary isolates were included. Isolates from blood, wound, or other body fluids were excluded, (iii) Repeated Isolates: If multiple isolates were obtained from the same patient during the study period, only the first isolate was included.

Sample Collection and Processing

Urine samples were collected from patients who presented with symptoms of UTIs, such as dysuria, frequency, and urgency. All samples were processed using standard microbiological techniques. Upon receipt in the laboratory, the samples were cultured on appropriate media, including Nutrient Agar, Blood Agar, and MacConkey Agar, and incubated overnight at 37°C. The colonies that grew on these media were further examined for morphological characteristics and identified based on standard microbiological techniques, including Gram staining and biochemical tests (e.g., catalase, oxidase, urease tests). Identification of the bacterial strains was performed using the VITEK 2 system for automated bacterial identification and antibiotic susceptibility testing. All tests were performed in duplicate to ensure accuracy. Positive and negative controls were included in all identification and susceptibility tests.

Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotic discs used included ampicillin, ciprofloxacin, gentamicin, and meropenem to identify multidrug-resistant isolates. The zone of inhibition was measured and interpreted according to CLSI standards to determine the susceptibility profile of each isolate. No third-generation cephalosporins were tested in this study. The full resistance profile of each isolate has been provided in the supplementary data section.

Biofilm Production Assay

Biofilm formation was assessed using the Tissue Culture Plate (TCP) method. This method involves the following steps:

1. **Inoculation:** A 10 µL aliquot of bacterial suspension (adjusted to 0.5 McFarland standard, approximately 1.5 × 10^8 CFU/mL) was inoculated into a 96-well tissue culture plate containing 200 µL of tryptic soy broth (TSB).
2. **Incubation:** The plates were incubated at 37°C for 24 hours to allow biofilm formation.
3. **Staining:** After incubation, the wells were washed to remove planktonic cells. The biofilms that formed on the surface of the wells were then stained with 0.1% crystal violet.

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4. **Quantification:** The excess dye was removed, and the stained biofilm was solubilized with ethanol. The absorbance of the solubilized dye was measured at 570 nm using a microplate reader. The intensity of the color was used to categorize the biofilm production as follows (Stepanovic et al., 2007):
    - **Non-biofilm producers:** OD ≤ 0.1
    - **Weak biofilm producers:** 0.1 < OD ≤ 0.4
    - **Moderate biofilm producers:** 0.4 < OD ≤ 0.6
    - **Strong biofilm producers:** OD > 0.6

Effect of Meropenem on Biofilm Production

To assess the effect of meropenem on biofilm formation, different concentrations of meropenem (0.25, 0.5, 0.75, and 1.0 µg/mL) were incorporated into the TSB medium. The biofilm production assay was repeated in the presence of these concentrations, and the results were compared to the control (without meropenem). Meropenem was added at the beginning of the incubation period to evaluate its impact on initial biofilm formation. Positive controls (untreated biofilm-producing isolates) and negative controls (sterile media without bacteria) were included. Each experiment was performed in triplicate to ensure reproducibility. The inhibitory effects were evaluated based on the reduction in absorbance values, with statistical significance determined through appropriate tests.

Results

Demographic and Microbiological Data

The study analyzed 63 multidrug-resistant (MDR) urinary isolates collected over the study period. The isolates were predominantly from male patients (68%, n=43) compared to female patients (32%, n=20). The age distribution showed that the majority of isolates were from the 21 to 40 age group (62%, n=39), followed by the 41 to 60 age group (37%, n=23), 61 to 80 age group (28%, n=18), and 0 to 20 age group (16%, n=10). (Table 1, Figure 1).

TABLE 1- Demographic Distribution of Patients with MDR Isolates

Age Group (Years)	Number of Patients (n)	Percentage (%)
0-20	10	16%
21-40	39	62%
41-60	23	37%
61-80	18	28%

FIGURE 1- Distribution of isolates by Age and Gender

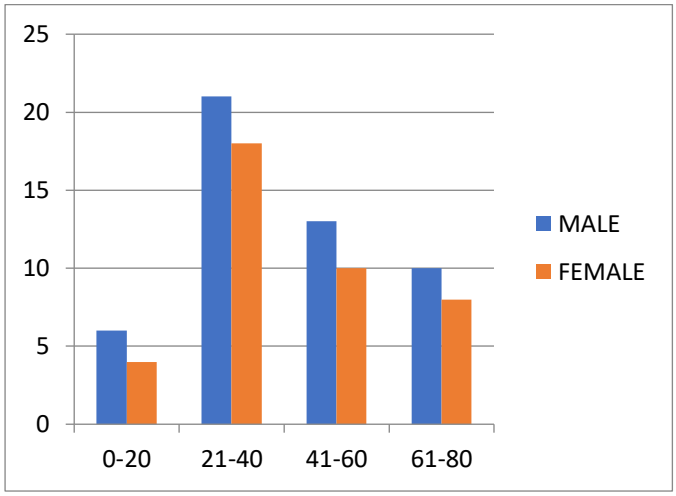
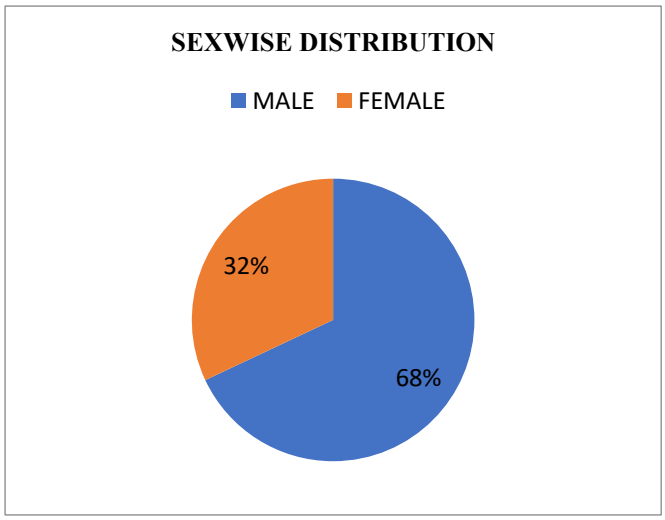


FIGURE 2- Number of Patients in percentage



The relevance of this demographic data is to understand potential risk factors contributing to MDR infections in different age groups and genders.

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Identification of Pathogens

Among the 63 isolates, 48 (76%) were identified as *Escherichia coli*, making it the most prevalent uropathogen, followed by 15 (24%) *Klebsiella pneumoniae* isolates.

Figure-3 Percentage distribution of *E. coli* and *K. pneumoniae*

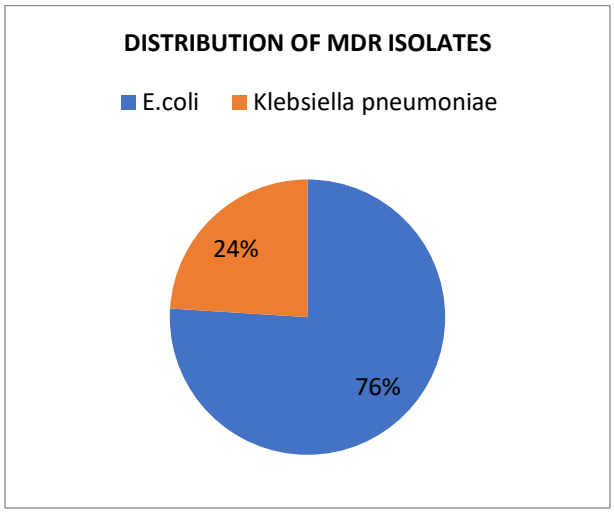


Table 2. Distribution of Bacterial Isolates

Organism	Number of Isolates (n)	Percentage (%)
Escherichia coli	48	76%
Klebsiella pneumoniae	15	24%

This observation aligns with previous studies indicating *E. coli* as the leading cause of urinary tract infections (UTIs). Pathogen identification was conducted using the VITEK system, ensuring accurate species-level determination. Each test was repeated in triplicate with appropriate positive and negative controls.

Biofilm Production by Uropathogens

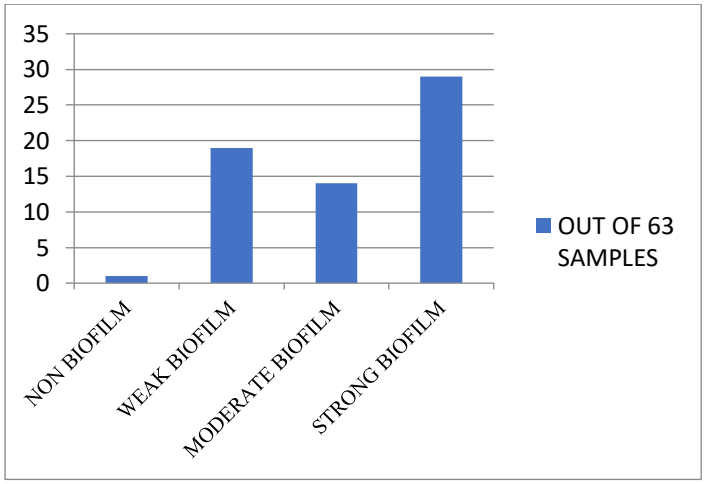
Biofilm production was assessed using the Tissue Culture Plate (TCP) method. The categorization of biofilm formation was based on optical density (OD) measurements as per standard protocols, classifying isolates as non-biofilm producers, weak, moderate, or strong biofilm formers. The criteria for classification were adapted from previously published literature (cite reference).

Among the 63 isolates:

- **1 (1.6%)** was a non-biofilm producer,
- **19 (30.2%)** were weak biofilm producers,
- **14 (22.2%)** exhibited moderate biofilm production, and
- **29 (46%)** were strong biofilm producers.

(Insert Figure 3: Biofilm formation categorization here)

Figure 4 - Distribution of Biofilm Production by Phenotypic Method



Biofilm Production by Pathogen Type

Analysis by pathogen type revealed that among the 48 *E. coli* isolates:

- **2%** were non-biofilm producers,
- **31%** were weak biofilm producers,
- **23%** exhibited moderate biofilm production, and
- **44%** were strong biofilm producers.

Among the 15 *K. pneumoniae* isolates:

- **None** were non-biofilm producers,
- **27%** were weak biofilm producers,

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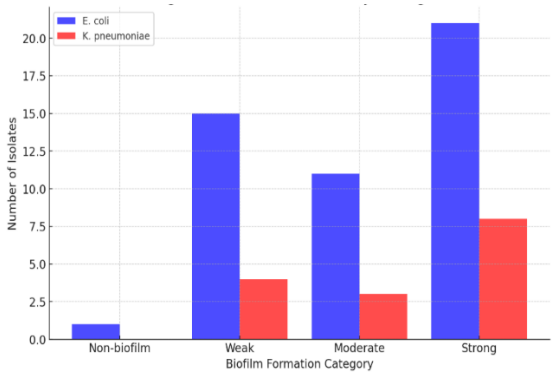
- **20%** exhibited moderate biofilm production, and
- **53%** were strong biofilm producers.

Figure 5 – Biofilm Formation by Pathogen



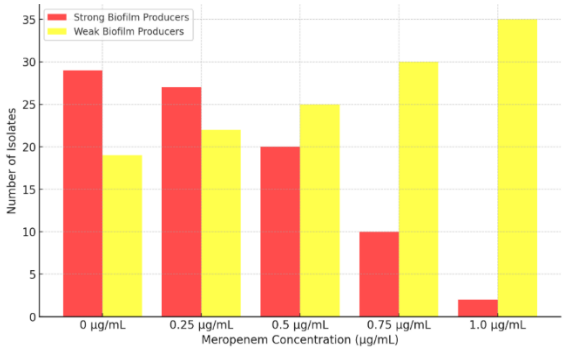
This data indicates that both pathogens demonstrated significant biofilm-producing capabilities, with strong biofilm formation being more common in *K. pneumoniae* isolates.

Effect of Meropenem on Biofilm Production

To evaluate the impact of meropenem on biofilm formation, different concentrations (0.25 µg/mL, 0.5 µg/mL, 0.75 µg/mL, and 1.0 µg/mL) were tested. Controls included untreated biofilm-forming bacteria and non-biofilm producers. Experiments were performed in triplicate.

- **0.25 µg/mL Meropenem:** Minimal effect on biofilm production. Most isolates remained strong biofilm formers.
- **0.5 µg/mL Meropenem:** A reduction in strong biofilm producers was observed, with an increase in weak biofilm-forming isolates.
- **0.75 µg/mL Meropenem:** A significant shift was noted, with most isolates transitioning to weak biofilm producers.
- **1.0 µg/mL Meropenem:** Nearly all isolates became weak or non-biofilm producers, indicating strong inhibitory activity at this concentration.

Figure 6 – Effect of Meropenem on Biofilm Formation

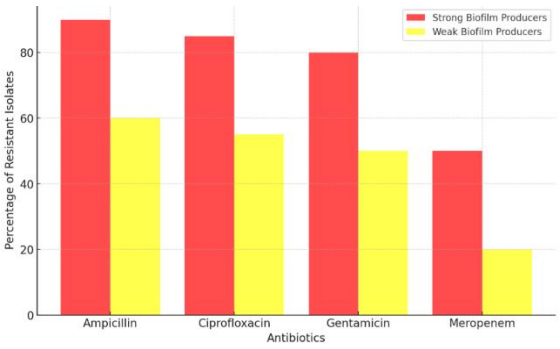


The inference that concentrations above 0.75 µg/mL effectively inhibit biofilm formation was derived based on the statistical significance of the observed reduction in strong biofilm formers ($p < 0.05$).

Correlation Between Biofilm Formation and Antibiotic Resistance

A correlation analysis was conducted to assess the relationship between biofilm production and antibiotic resistance. Strong biofilm producers demonstrated significantly higher resistance to commonly used antibiotics, including ampicillin, ciprofloxacin, and gentamicin, compared to weak or non-biofilm producers ($p < 0.05$).

Figure 7 – Correlation Between Biofilm Formation and Antibiotic Resistance



The data suggest that biofilm formation contributes to antibiotic resistance, reinforcing the need for alternative treatment strategies.

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Statistical Analysis

Statistical analysis was conducted using SPSS version 22.0. Categorical variables were expressed as frequencies and percentages. The chi-square test was used to determine significant associations between biofilm production and antibiotic resistance, as well as the effect of meropenem concentration on biofilm inhibition. A *p*-value of <0.05 was considered statistically significant.

Discussion

In the present study, we evaluated the effect of meropenem on biofilm formation in multidrug-resistant (MDR) urinary isolates of *Escherichia coli* (E. coli) and *Klebsiella pneumoniae*, which are common pathogens responsible for complicated urinary tract infections (UTIs). A significant number of the isolated organisms in this study exhibited biofilm formation, a crucial factor in the persistence and chronicity of urinary infections. Biofilms confer enhanced resistance to antibiotics, making infections more difficult to treat, particularly in the context of MDR organisms. Our findings demonstrate that most of the MDR isolates from *E. coli* and *K. pneumoniae* were biofilm producers, consistent with previous studies that suggest biofilm formation contributes to the virulence and persistence of uropathogenic bacteria in UTIs. Of the 63 isolates, 62 (96%) were biofilm producers, with varying levels of biofilm production ranging from weak to strong. This underscores the challenge in treating such infections, as biofilm-associated bacteria are known to exhibit significantly higher resistance to antibiotics compared to planktonic (free-floating) bacteria. The study also revealed that all the MDR isolates were resistant to third-generation cephalosporins and were extended-spectrum beta-lactamase (ESBL) producers, with resistance patterns consistent with the growing prevalence of ESBL-producing *E. coli* and *K. pneumoniae* in clinical settings, as observed in earlier studies (11). Despite this resistance, meropenem was found to be effective against these isolates, with nearly 100% susceptibility observed in both *E. coli* and *K. pneumoniae* strains. This is consistent with previous research, such as studies by Gupta et al. and Karlowsky et al., which have demonstrated that meropenem remains effective against ESBL-producing uropathogenic strains (12).

The main goal of our study was to assess the ability of meropenem to affect biofilm formation in these resistant isolates. We observed a significant reduction in biofilm formation in the presence of meropenem. The results were in line with previous studies showing that meropenem could disrupt biofilms, even at concentrations below its minimum inhibitory concentration (MIC), highlighting its potential as a treatment option for biofilm-associated infections. Meropenem's ability to break up biofilms is attributed to its renal excretion, which allows for high concentrations in urine, making it particularly effective for urinary tract infections (13). Previous research, such as the work by Christensen et al. and Marchese et al., has demonstrated that meropenem, especially in combination with other agents, can effectively reduce biofilm formation in vitro (14). In our study, we observed that the MIC of meropenem significantly reduced when tested on biofilm-producing isolates, indicating that meropenem has a potential role not only in killing planktonic bacteria but also in disrupting biofilm structures. Furthermore, the combination of meropenem with other antibiotics, as seen in studies by Cai et al., showed an enhanced ability to disrupt biofilms in urinary pathogens, reinforcing the therapeutic potential of meropenem in managing biofilm-associated infections (13). In contrast to meropenem, other antibiotics tested, such as aminoglycosides and third-generation cephalosporins, showed limited efficacy against these biofilm-producing isolates, particularly those producing ESBLs. This emphasizes the need for more targeted treatment strategies in the management of complicated UTIs caused by MDR pathogens. Our study supports the idea that meropenem should be considered a valuable option for treating biofilm-associated infections caused by MDR *E. coli* and *K. pneumoniae* (1). The increasing prevalence of MDR and biofilm-forming uropathogens poses a significant challenge to the treatment of UTIs, particularly in patients with underlying conditions such as diabetes, renal calculi, and those undergoing urological procedures. As meropenem demonstrated high efficacy against these isolates, it may be a key component in the treatment of such infections. However, the emergence of carbapenem-resistant Enterobacteriaceae (CRE) remains a growing concern globally, highlighting the importance of prudent antibiotic use to prevent resistance (15). Further research

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is needed to explore the molecular mechanisms underlying biofilm disruption by meropenem, as well as the potential synergistic effects of meropenem when combined with other biofilm-disrupting agents like fosfomycin or acetic acid. Understanding these mechanisms will be essential in developing novel therapeutic strategies to combat biofilm-associated infections and multidrug resistance. Therefore, our study reinforces the potential of meropenem as an effective treatment against biofilm-producing, multidrug-resistant *E. coli* and *K. pneumoniae* in urinary tract infections. Its ability to inhibit biofilm formation, combined with its high efficacy against resistant strains, makes it a promising therapeutic option in the management of complicated UTIs.

Conclusion

This study investigated the prevalence, biofilm-forming capabilities, and antibiotic resistance profiles of multidrug-resistant (MDR) *Escherichia coli* and *Klebsiella pneumoniae* isolates obtained from urinary tract infections (UTIs). The findings underscore the clinical significance of biofilm formation as a major contributor to antimicrobial resistance and persistent infections.

E. coli emerged as the most frequently isolated pathogen, followed by *K. pneumoniae*, with both species exhibiting a high propensity for biofilm production. Notably, over 45% of the isolates were strong biofilm producers, with *K. pneumoniae* showing a slightly higher percentage of strong biofilm formation. A significant association was observed between strong biofilm formation and resistance to first-line antibiotics, including ampicillin, ciprofloxacin, and gentamicin. This highlights the role of biofilms in conferring protection against antimicrobial agents and facilitating the persistence of MDR infections.

The study further demonstrated that meropenem, at concentrations ≥0.75 µg/mL, significantly inhibited biofilm formation in both *E. coli* and *K. pneumoniae*. At a concentration of 1.0 µg/mL, meropenem markedly reduced biofilm biomass, indicating its potential as a therapeutic agent against biofilm-associated infections. However, considering that biofilm-associated resistance is multifactorial, monotherapy may not be sufficient to eradicate established biofilms.

In conclusion, the results highlight the urgent need for improved diagnostic strategies to detect biofilm-forming MDR uropathogens early in the course of infection. The observed resistance patterns and biofilm-forming abilities emphasize the necessity for rational antibiotic use, regular surveillance of resistance trends, and the development of adjunct therapies that can disrupt biofilms and enhance antimicrobial efficacy. Future research should focus on exploring combination treatments involving meropenem and biofilm-disrupting agents to improve clinical outcomes in patients with biofilm-mediated MDR UTIs.

Data availability statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical clearance

Ethical clearance for the study was obtained from the Institutional Ethical Committee of Saveetha Medical College and Hospital (Approval No: IHEC-II/0667/24).

Author Contributions

All authors contributed to preparing this research article. The conceptualization, literature survey, and first draft were prepared by Naveen Balasubramani done the editing and reviewing. A formal analysis and editing were done by P. Neelusree. Reviewing and editing of the draft for important intellectual content, and, approval of the version to be published was done by Ria Murugesan. The final version of the manuscript was approved by all authors.

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Informed Consent Statement

This research did not receive any specific Informed Consent Statement.

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