Original Article

Antibiotic Resistance and Biofilm Formation in Uropathogenic Bacteria: A Study of Clinical Isolates from Hilla City

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Abstract

Background: Urinary tract infections (UTIs) are among the most common bacterial infections worldwide, often leading to significant morbidity if not promptly and properly treated. The growing incidence of antibiotic-resistant bacteria and their ability to form biofilms complicates the clinical management of these infections. **Objectives:** This study aimed to isolate and identify the bacterial pathogens causing UTIs, assess their antibiotic resistance profiles, and evaluate their biofilm formation capabilities. **Materials and Methods:** A total of 100 urine samples were collected from patients presenting with UTI symptoms at Al-Hashimiya General Hospital in the Babylon Governorate from November 15, 2023 to March 15, 2024. Bacterial isolates were obtained through standard microbiological techniques, including culture on selective media and subsequent identification via biochemical assays and the VITEK 2 system. Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method, while the extent of biofilm formation was quantified using the microtiter plate assay. **Results:** Out of the 100 samples, 47% yielded pathogenic bacteria. Gram-negative bacteria comprised 79% of these isolates, whereas Gram-positive bacteria accounted for 21%. The predominant pathogens identified were Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis, and Staphylococcus aureus. High rates of multidrug resistance were observed: 83% in E. coli, 84% in K. pneumoniae, 75% in P. mirabilis, and 70% in S. aureus. Furthermore, a considerable proportion of the isolates exhibited moderate to strong biofilm formation, which likely contributed to their resistance patterns. **Conclusions:** The study highlights the challenge posed by antibiotic-resistant, biofilm-forming UTI pathogens and underscores the need for robust antimicrobial stewardship and the exploration of alternative treatment strategies.

Keyword: antibiotic resistance, MDR, biofilm formation, Gram-negative, Gram-positive, UTIs

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial illnesses in the world. They affect millions of people every year and can cause serious health problems if they are not treated or controlled properly. UTIs usually cause inflammation in the urothelial tissues because uropathogens, which are usually good bacteria that live in the gut flora, get into the body. These infections, which include cystitis and pyelonephritis, are hard to treat because they keep coming back and are linked to bad health effects, especially in people who are already weak [1]. Alexander Fleming discovered penicillin in 1928, which was the first antibiotic. This was a huge step forward in medical history. These drugs have changed the way infectious diseases are treated, making illnesses that used to be deadly much easier to deal with [2]. Rising antimicrobial resistance (AMR), on the other hand, means that bacteria are developing ways to avoid drugs, which could undo this progress. Increasing multidrug-resistant infections are leading to treatment failures, prolonged hospital

stays, and higher mortality rates [3]. Formation of biofilms is one of the main reasons why bacterial germs stay alive in UTIs. Biofilms are organized groups of bacteria cells surrounded by an extracellular polymeric substance (EPS) matrix. This makes them more resistant to drugs and immune reactions. This ability lets bacteria live in harsh conditions, which can lead to repeated attacks and make treatment plans more difficult [4]. AMR is made easier for bacteria to get by things like horizontal gene transfer (plasmids and bacteriophages), genetic recombination, and random changes [5]. These things make it easier for resistance genes to spread quickly across bacterial populations. AMR is spreading faster around the world because antibiotics are used too much and in the wrong places in both medicine and farming. Infectious diseases are still thought to be the second most common cause of death in the world. Drug-resistant germs are a major threat to public health, especially in low- and middle-income countries [6]. AMR also has a huge impact on the economy. Higher healthcare costs and missed output make things even harder for healthcare systems that are already under a lot of stress [7]. Because MDR bacteria are becoming more common and biofilm formation makes things more complicated, we need new ways to fight these problems right away. To stop the spread of AMR and make sure that future treatments work, it is very important to study other treatments like anti-biofilm agents, phage therapy, and new antibiotic chemicals. Furthermore, strict rules on the use of antibiotics along with public health efforts to educate people about AMR are needed to lessen its effects and protect health around the world [8]. This study aims to identify uropathogenic bacteria in Hillah City, assess their antibiotic resistance and biofilm formation capabilities, and elucidate how these factors contribute to treatment challenges. Ultimately, it seeks to inform strategies for improved therapeutic management and control the spread of resistant strains.

Materials and Methods

Sample collection

One hundred urine samples were collected from patients with symptoms of urinary tract infections from Al-Hashimiya General Hospital in the Babylon Governorate whose ages were 15-60 years from 15 November 2023 to 15 Match 2024. Patients were selected based on the results of a general urine examination. Samples were collected in sterile urine tubes and cultured on MacConkey and blood agar using the spreading method, and then incubated at 37°C for 24 hours to isolate the bacteria.

Culture Media

The culture media was prepared (Brain Heart Infusion Broth, MacConkey Agar, Muller-Hinton Agar, Nutrient Agar, Blood Agar, EMB agar) according to the manufacturer's instructions (Himedia (India), which were attached to the package of each medium. The autoclave was sterilized to the medium at 121°C and 15 lb/inch pressure for 15 minutes. Then, the culture media was poured into sterile Petri dishes (China) and tubes (China) and incubated at 37°C for 22 hours to ensure that they were not contaminated. Then, they were stored in the refrigerator at 4°C until use. Blood agar base medium was prepared according to the manufacturer's instructions, sterilized in an autoclave, and cooled to 45-50°C. After that, 5% blood was added to it, mixed gently, then poured into sterile Petri dishes and left to solidify. EMB agar medium was used as a selective medium for the diagnosis of *E. coli*.

Isolation and identification of bacteria

Urine samples cultured on nutrient agar for 24 hours at 37°C were subcultured on MacConkey and blood agar. Bacterial isolates were identified using Gram staining, microscopy, and the VITEK 2 system (BioMérieux, France), with colony morphology and Gram reaction examined [9-11].

Antibiotic susceptibility testing

The antibiotic susceptibility of bacteria strains was performed based on the Kirby–Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [12]. The antimicrobial susceptibility assays about 17 antibiotics (Bioanalyze / Turkey) were performed using commercially available antibiotics including Ampicillin (10µg) AMP , Amoxicillin-clavulanate (20/10µg) AMC , Ceftazidime (30 µg) CAZ , Cefotaxime (30 µg) CTX, Ceftriaxone (30 µg) CRO , Meropenem (10 µg) MEM , Amikacin (30 µg) AMK, Streptomycin (*300*µg) STR , Azithromycin (15µg) , Doxycycline (30 µg) DOX , Ciprofloxacin (5 µg) CIP, Levofloxacin (5 µg) LVX , Trimethoprim-sulfamethoxazole (1.25/ 23.75 µg) SXT , Gatifloxacin (5µg), Chloramphenicol (30µg) CHL, Nitrofurantoin (300µg) NIT , Trimethoprim (5µg) TMP, Aztreonam (30µg) ATM . Suspension of each bacteria isolate was spread by sterile glass rods on the surface of Mueller Hinton agar (Oxoid, UK). Then antibiotic discs (Bioanalyze/ Turkey) were placed onto the surface of the inoculated Mueller Hinton agar plate. The plate was then incubated at 35◦C for 18 h. Antimicrobial susceptibility was determined by measuring the diameter of the inhibition zone according to CLSI 2023.

Phenotypic detection of Biofilm Formation microtiter plate method (MTP)

The methodology adhered to the protocols established by [13]. The mean absorbance values from the replicate wells were determined, and the degree of biofilm formation was calculated using the following equation: Biofilm degree = Mean OD 630 of tested bacteria - Mean OD630 of control. The obtained results were evaluated in accordance with Table 1. The modified TCP method was regarded as the gold standard [14].

Table 1: Classification based on optical density (OD) values [15].

Mean OD value	Adherence	Biofilm Formation
< 0.120	Non	Non/ Weak
-0.240	Moderate	Moderate
>0.240	Strong	Strong

Statistical analysis

The data was entered and analyzed using Excel version 21 and SPSS version 20.

Ethical approval

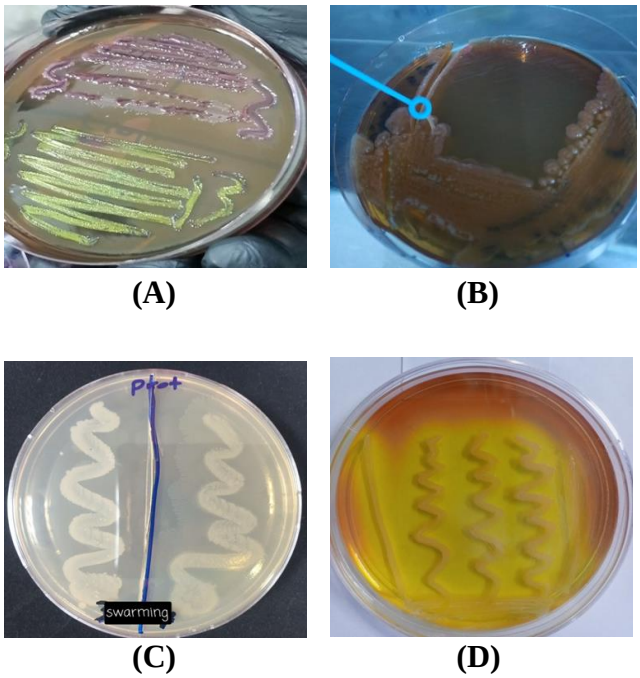
This study was conducted with the approval of the College of Biotechnology, Al-Qasim Green University, Iraq. Approval was also obtained from the Training and Development Department, Babil Health Directorate, Iraqi Ministry of Health, No.1767 Dated 15/11/2023.

Results

Morphological Examination

Urine samples were cultured on nutrient agar at 37°C for 24 hours, followed by subculture of bacterial growth on nutrient and MacConkey agar at 37°C for 24 hours. The isolates were grown on selective and differential media often used for bacterial identification, Blood Agar and MacConkey Agar. While MacConkey Agar separated bacteria depending on lactose fermentation, Blood Agar was used to track

hemolysis patterns—a vital diagnostic tool. Whereas *P. mirabilis* grew as non-lactose fermenters (colorless colonies), *E. coli* and *Klebsiella pneumoniae* showed growth with lactose fermentation (pink colonies). Gram-positive *S. aureus* did not grow on MacConkey Agar but showed hemolysis patterns on Blood Agar. The bacteria grow to form colonies with a metallic green sheen when grown on EMP agar as shown in (Figure 1A). On the other hand, *K. pneumoniae* showed a mucoid appearance when grown on blood agar without any hemolysis, so the surrounding area remained unchanged in color (Figure 1B). Whereas *P. mirabilis* appeared as crowded bacteria on the agar, with colonies widely spread on the surface of the medium, forming a wave-like gradient pattern (Figure 1C), This phenomenon is a characteristic feature of this genus. *S. aureus* showed beta-hemolysis when grown on blood agar, with a clear zone surrounding it due to hemolysis of red blood cells (Figure 1D) and did not grow on MacConkey agar.



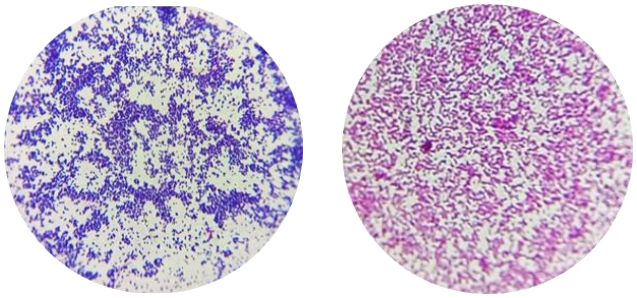
(A) **(B)**

(C) **(D)**

Figure 1: the morphological diagnosis of some bacteria. A. The green metallic sheen color of *E. coli* appears on EMB medium. *(B)K. pneumoniae* appears on blood agar as large, elevated, mucoid colonies. (C)The swarming of *P. mirabilis* appears. (D) *S. aureus* appears as beta-hemolytic erythrocytes on blood agar.

Microscopic detection

Following Gram staining, microscopic observation revealed Gram-positive bacteria as dark purple/blue and Gram-negative bacteria as pink/red, as shown in (Figure 2).

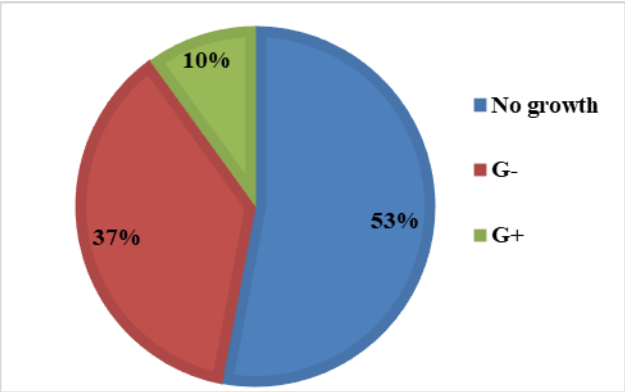


(A) **(B)**

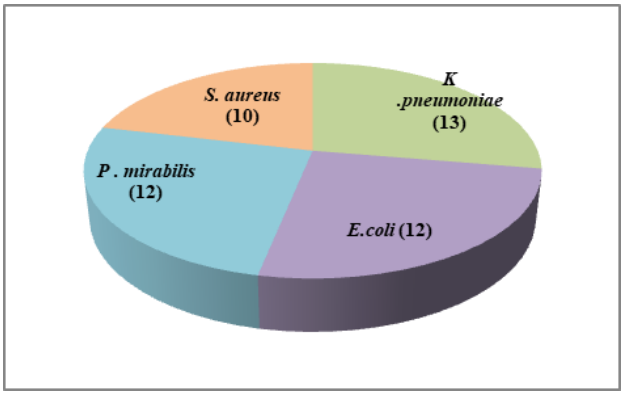
Figure 2: The color of the bacteria under the microscope after staining with Gram stain, where (A) shows the shape of *S. aureus* in blue and (B) shows the shape of *E. coli* in pink.

Identification of uropathogenic bacterial isolates from UTI patients

Of the 100 urine samples collected from persons suspected of having UTI, 47 (47%) contained bacteria. These bacterial isolates were classified as 37 (79%) Gram-negative and 10 (21%) Gram-positive. In addition, 53 (53%) of the samples showed no growth. Among the Gram-negative bacteria, *K. pneumoniae* was the most prevalent, with 13 (35%), followed by E. coli and P. mirabilis 12 (32 %) each. Among the Gram-positive bacteria, all isolates were *Staphylococcus* 10 (21%). As shown in the figure 3 below**:**



(A)



(B)

Figure 3 A, B: the number of positive and negative bacterial isolates taken from clinical specimens of patients with urinary tract infection.

Biochemical test for bacteria isolates

Biochemical and culture-based tests identified four bacteria: E. coli, S. aureus, K. pneumoniae, and P. mirabilis. Table 2 summarizes the findings: E. coli was indole-positive, produced pink colonies on MacConkey Agar (lactose fermentation), and was motile but non-hemolytic on Blood Agar. *K. pneumoniae* was urease-positive, produced pink colonies on MacConkey Agar (lactose fermentation), and was non-motile and gamma-hemolytic on Blood Agar. *P. mirabilis* was indole and urease positive, exhibited swarming motility on solid media, and produced colorless colonies on MacConkey Agar (no lactose fermentation) and characteristic swarming patterns on Blood Agar. *S. aureus* was coagulase-positive, displayed beta-hemolysis on Blood Agar, and did not grow on MacConkey Agar.

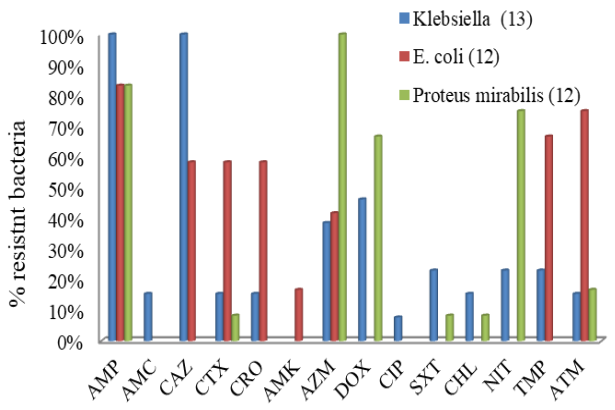
Table 2: Biochemical and Cultural Characteristics of *E. coli, S. aureus, K. pneumoniae, and P. mirabilis.*

Bacteria	Indole Test	Coagulase Test	Catalase Test	Urease Test	MacConkey Agar	Blood Agar	Motility Test
E. coli	+	-	+	-	Pink Colonies	Gamma Hemolysis	Motile
S. aureus	-	+	+	-	No Growth	Beta Hemolysis	Non motile
K. pneumoniae	-	-	+	+	Pink Colonies	Gamma Hemolysis	Non motile
P. mirabilis	+	-	+	+	Colorless Colonies	Swarming	Highly Motile

Antibacterial susceptibility testing

Gram-negative isolates mostly showed a high resistance to ampicillin (100 and 83.3%), *K. pneumoniae* (100%), and *E. coli* (58.3%) resistant to ceftazidime. whereas *P. mirabilis* was resistant to each of Azithromycin (100%), Nitrofurantoin (75%), and Doxycycline (66.7%). The results showed that most *E. coli* isolates are resistant to several antibiotics in varying proportions figure (4, A). *S. aureus* isolates showed a high resistance rate to penicillin (90%), gatifloxacin (60%), and rifampin (60%). As shown in figure (4, B). In the current study, the antimicrobial sensitivity pattern in urine samples showed that *E. coli* isolates were highly resistant to ampicillin was 10 (83.30%) isolates, followed by 9 (75%) isolates was resistance to aztreonam, 8 (66.70%) isolates resistance to trimethoprim, and resistance to ceftriaxone was 7 (58.3 %). While the *E. coli* isolates was highly sensitive to nitrofurantoin 10(83.3%) isolates. *K. pneumonia* showed 100% resistance to penicillin and ceftazidime. *P. mirabilis* isolates demonstrated a high level of resistance to azithromycin (12 isolates, 100%), penicillin (10 isolates, 83%),

and nitrofurantoin (9 isolates, 75%), while they were sensitive to meropenem 12(100%), As shown in figure (4, A).



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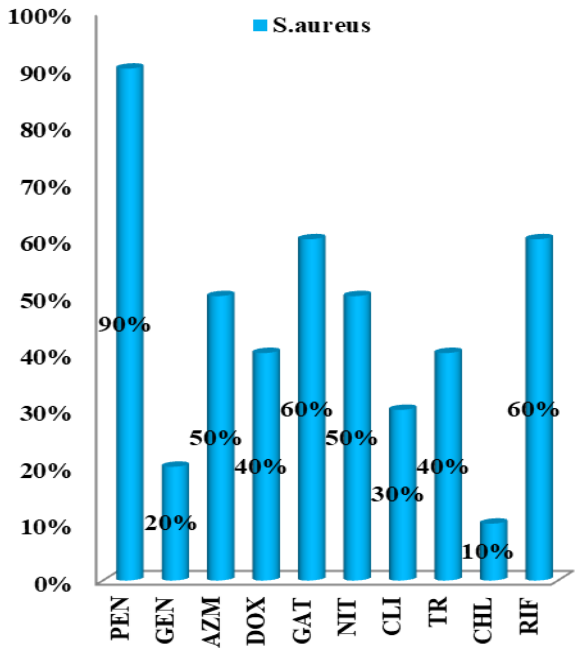

(B)

Figure 4 A and B: Ratio of uropathogenic Gram-negative and Gram-positive resistance among prevalent antibiotics.

Multi drug resistance (MDR) rate among uropathogenic bacteria

Most of Gram-negative and Gram-positive bacterial isolates showed multidrug resistance (MDR), indicating resistance to at least one antibiotic from three or more classes, according to the antimicrobial susceptibility testing guidelines developed by the Clinical and Laboratory Sciences Institute (CLSI), as shown in table (2). Antibiotic resistance data presented the prevalence of multidrug-resistant (MDR), non-MDR and extensively drug-resistant (XDR) strains, which was clearly defined for each bacterial species. *E. coli* shows a significant burden of resistance, with 83% of the strains (10 out of 12) identified as MDR and a smaller fraction, (17%), as non-MDR (2 out of 12), indicating a high level of resistance to multiple antibiotics. *K. pneumonia* also demonstrates considerable resistance, with 85% of its strains (11 out of 13) being MDR and 15% Non-MDR (2 out of 13), suggesting pervasive resistance within this species. *P. mirabilis* presents a slightly lower but still substantial MDR rate of 75% (9 out of 12) and a non-MDR rate of 25% (3 out of 12), reflecting varied resistance patterns that may impact treatment strategies. Due to its 70% MDR rate (7 out of 10) and 20% XDR strains (2 out of 10), S. aureus infections are difficult to treat. This succinct assessment of resistance rates emphasizes the need for focused antimicrobial stewardship and innovative treatment ways to control and combat antibiotic resistance.

Table 2: Distribution of MDR, Non-MDR, and XDR Cases among Bacterial Species

Bacteria Species	MDR (%)	Non-MDR (%)	XDR (%)
E. coli	83.3	16.7	0.0
K. pneumonia	84.6	15.4	0.0
P. mirabilis	75.0	25.0	0.0
S. aureus	70.0	10.0	20.0

Biofilm formation for bacteria isolate

Thirty isolates were randomly chosen from a total of 47 bacterial isolates to perform the biofilm experiment. The chosen isolates were 8

isolates of *K. pneumoniae*, 6 isolates each of *E. coli and S. aureus*, and 10 isolates of *P. mirabilis*.

The biofilm production ability of bacterial isolates was examined by using micro-titer plate method (MTP) (Fig5). The results of the current study showed that while moderate biofilm formation of *E. coli* and *S. aureus* isolates was (83.3%) for both as well as (80%), (75%) for *P. mirabilis* and *K. pneumonia* respectively.

Table 3: Biofilm formation (OD $_{630}$ nm) for bacteria isolate

Bacteria	Total Isolates	Weak Biofilm (%)	Moderate Biofilm (%)	Strong Biofilm (%)
E. coli	6	1 (16.7%)	5 (83.3%)	0 (0%)
S. aureus	6	1 (16.7%)	5 (83.3%)	0 (0%)
P. mirabilis	10	0 (0%)	8 (80%)	2 (20%)
K. pneumonia	8	0 (0%)	6 (75%)	2 (25%)

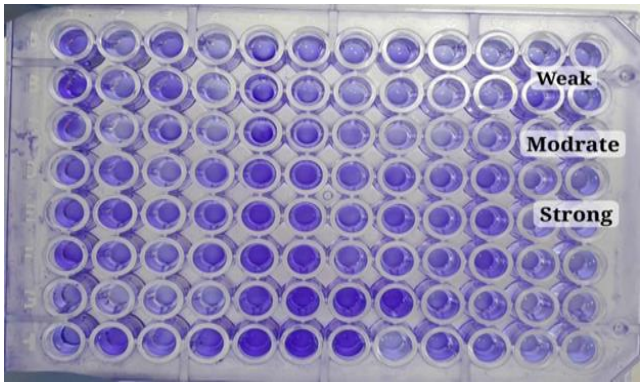

Figure 5: Biofilm production by isolates of *E. coli S. aureus, P. mirabilis and K. pneumoniae* using 96-well microtiter plate method

Discussion

Gram-negative isolates mostly showed a high resistance to ampicillin (100 and 83.3%), *K. pneumoniae* (100%), and *E. coli* (58.3%) resistant to ceftazidime. whereas *P. mirabilis* was resistant to each of Azithromycin (100%), Nitrofurantoin (75%), and Doxycycline (66.7%). E. coli isolates exhibited varying resistance to multiple antibiotics. S. aureus isolates showed high resistance to penicillin (90%), gatifloxacin (60%), and rifampin (60%)". *S. aureus* isolates showed a high resistance rate to penicillin (90%), gatifloxacin (60%), and rifampin (60%). As shown in figure (4, B).The results of current study was agreement with the study that presented by [16] in Iraq which showed that the antibiotics resistance for penicillin and ceftazidime was (100%). In another study, these results do not agree with the results of that study, as *K. pneumoniae* bacteria showed less resistance to the aforementioned antibiotics [17]. The 46% resistance of *K. pneumoniae* to doxycycline poses a major treatment challenge. This finding is consistent with a study by [18] in which the researchers found that *K. pneumoniae* isolates showed high rates of doxycycline resistance, reflecting the widespread presence of resistance genes such as tet(A) and tet(B). On the other hand, this result differs from a study that showed higher doxycycline resistance rates of 80% in *K. pneumoniae* isolates [19]. This difference is attributed to geographic variation in antibiotic use patterns, as excessive and unregulated use of doxycycline in some regions leads to selective pressure on bacteria, which promotes the emergence of resistant strains. The results of current study agreement to results of study that presented by [20] in Iraq that showed the *E. coli* isolates resistance revealed highly resistant to ampicillin (87.8%), and ceftriaxone (61.0%) . while sensitivity to nitrofurantoin was (88.9%). Another study was consistent with the current study, in which *E. coli* bacteria showed varying resistance to antibiotics, as the rates of resistance to ampicillin were (81%) and to ceftriaxone (62.3%), and it was highly susceptible to nitrofurantoin (89.3%) [21]. On the other hand, a

study conducted in Pakistan showed that *E. coli* was resistant to the antibiotics penicillin, aztreonam, and ceftriaxone at rates of (100%), (44.8%), and (43.3%) respectively, which is different from the current study [22]. In another study conducted in North Eastern, Nigeria, E. coli showed resistance to penicillin and ceftriaxone (64% and 48%) respectively and sensitivity to nitrofurantoin (78%), which is not compatible with the results of the current study [23]. This may be due to incorrect or irrational use of antibiotics or due to the exchange of resistance genes between bacteria, so bacteria show varying resistance to antibiotics [24]. *P. mirabilis* isolates exhibited high resistance to azithromycin (100%), penicillin (83%), and nitrofurantoin (75%), but were universally sensitive to meropenem (100%). The results of the current study are relatively consistent with the results of the study which was presented in Iraq, which showed that resistant Proteus mirabilis isolates showed high resistance to azithromycin (97.5%) and penicillin, nitrofurantoin was (67.5%) and (100%) respectively were highly sensitive to meropenem (95%) [25]. It differs from the study conducted in Jordan, which showed a different level of antibiotic resistance for both azithromycin, penicillin and nitrofurantoin, which is (22%) (50%) and (25%) respectively [26]. The reason may be due to a difference in the sample population, incorrect use of antibiotics, or due to the transmission of Genes between bacteria [5]. Most of the *S. aurous* isolates showed high resistance to penicillin 9 (90%), gatifloxacin 6(60%), rifampicin 6(60%), and nitrofurantoin 5 (50%), and high sensitivity to chlorofenicol 7(70%) and gentamicin 6(60%). These results are relatively consistent with a study conducted in Iraq, which showed resistance to penicillin (86.7%), while it was highly sensitive to chlorophenol (67.9%) [27]. Another study conducted in India showed that the bacteria were resistant to penicillin at 54.8% and to catifloxacin at 60%, which is almost consistent with the current study [28]. This study was not consistent with a study in Nigeria, which showed resistance to penicillin, Chloramphenicol and nitrofurantoin (72%), (80.4%) and (32%) respectively [29]. In this study, the antibiotic resistance of *S. aureus* was evaluated against ten antibiotics, the most prominent of which were: penicillin, rifampicin, gatifloxacin, and chloramphenicol. Many hospital- and community-acquired infections are caused by *S. aureus*. Penicillin resistance was the most common, perhaps due to its widespread usage, which has resulted to resistant bacterial strains. Rifampicin and gatifloxacin showed reduced resistance, demonstrating they still work against bacteria's resistance mechanisms. Chloramph-enicol has a 30% resistance rate and 70% sensitivity rate among the strains examined, demonstrating its therapeutic efficacy. This report emphasizes the necessity for antibiotic stewardship and antibiotic resistance studies. In this investigation, 83.33% of *E. coli* samples were MDR. Compared to other investigations, these *E. coli* isolates were highly drug-resistant. As in the current study, *E. coli* was 81% resistant to various medications in Pakistan [22]. Similar to the current study, *K. pneumonia* exhibited a 90% multidrug resistance rate in Iraq [30]. Another Ghanaian research found *P. mirabili*s had 84% multidrug resistance. It resembles the current study [31]. Another Iraqi research found 62% S. aureus resistance, which matches the present data [32]. Iqbal et al. [33] reported a low drug resistance rate for both *E. coli* and *K. pneumoniae* strains, which was 7.5 and 24.3%, respectively, but the drug resistance rate for both strains was 92.06 and 75.7%, respectively, which

is not consistent with the current study. Another study conducted in Iraq showed that the multi-resistance of *E. coli, K. pneumoniae, P. mirabilis, and S. aureus* bacteria was (28.59%, 14.28%, 1.58%, and 20.63%), respectively. This is completely different from the current study [34].The differences in rates of antibiotic resistance between Iraq and other countries can be attributed to several factors. Regulatory control over the use of antibiotics in Iraq is not as strict or effective as in some other countries, leading to cover prescription and misuse of antibiotics. This contributes to increasing the selective pressure that favors the emergence and spread of resistant bacterial strains. The differences in the sample population can significantly affect the variation in rates related to antibiotic resistance between different studies or countries. A multidisciplinary approach is necessary to address antibiotic resistance. These strategies should include promoting education and public awareness of the importance of responsible use and rationalization of antibiotics, developing strict protocols to control the prescription and distribution of antibiotics, as well as encouraging research and development in the field of discovering new antibiotics and alternative methods of treating infections. The results of the current study showed that moderate biofilm formation of *E. coli* and *S. aureus* isolates was (83.3%) for both as well as (80%), (75%) for *P. mirabilis* and *K. pneumonia* respectively. The results of the current study were consistent with the results of the study presented by [35], which showed that (50%), (25%) of *K. pneumonia* isolates had moderate and strong biofilm formation, respectively. The results of current study was agreement with results of study that presented by [36], where showed that (80%) , (14%) of *E.coli* isolates was moderate and weak biofilm formation respectively. Another study conducted in Iraq showed that *E. coli* formed biofilms at a rate of 72% and 18%, medium and weak, respectively. This is almost consistent with the current study [37]. The reason for the formation of biofilm in *E. coli* may be due to the presence of some factors that help in producing biofilm. It was mentioned [37] that *E. coli* consumes the *fim H*, *csg A*, and *ag 43* genes in 92% to 98% of clinical isolates, which help them in producing biofilm. Another study conducted in Iran showed that bacteria formed biofilm (25%) of medium and (56.25%) of weak biofilm reaction from isolates of medium and weak respectively, which is different from the current study. [38] The reason may be due to the difference in the sample community or the difference in the genes that make up the biofilm. The results in the current study showed that 80% and 20% of *P. mirabilis* isolates exhibited moderate and strong biofilm formation, respectively. When compared with previous studies, there is a high agreement with some results and differences with others, reflecting the potential influence of environmental and clinical factors, as well as differences in experimental methodologies. This study is in complete agreement with the results of a study on biofilm development in isolates associated with medical devices. Reflecting the above results, the study found that 80% of isolates showed moderate biofilm-forming potential and 20% were strong producers [39]. This important agreement suggests that isolates from similar clinical or environmental settings may exhibit consistent patterns of biofilm formation, particularly when associated with catheter- or medical device-associated diseases. Much research revealed different ranges of biofilm-forming capacity. In *P. mirabilis*, for example, 60% of isolates were strong producers, 24% were moderate, and 16% were weak based

on studies on virulence parameters. These data show a predominance of strong biofilm producers, which contrasts with the results of this investigation where moderate producers were somewhat common [40]. Comparatively, another study found 15% of isolates were strong producers, 22.5% were moderate, 12.5% were poor, and 50% did not form biofilm at all. Although the proportion of strong biofilm producers (15%) is somewhat near to the results of this study (20%), the proportion of moderate producers is noticeably lower than the 80% recorded here [41]. Variations in the source of isolates (e.g., clinical against environmental), methodological approaches (e.g., the use of crystal violet staining or other biofilm quantification techniques), or variations in the clinical conditions under which the isolates were obtained can help to explain these variances. The findings of this investigation revealed that whereas 16% of *S. aureus* isolates showed poor biofilm formation, 83% of them displayed moderate biofilm formation. Closely matching the 83% found in this investigation, a 2016 Dakheel et al. study found that 88% of *S. aureus* isolates displayed moderate biofilm development [42]. This consistency emphasizes the prevalence of moderate biofilm development in *S. aureus*, especially in clinical settings where environmental variables include nutrition availability and surface contacts affect biofilm dynamics [43]. Another study found that more than 72% of the isolates produced biofilms, divided into 54.64% weak, 14.43% moderate, and 3.09% strong biofilm producers. These results differ significantly from the results of this study [44]. By a multi-step process comprising first attachment to surfaces, accumulation of cells via intercellular adhesion, and maturation into a structured biofilm protected by an extracellular polymeric matrix, *S. aureus* develops biofilms. Genetic elements including the ica operon control this process, while environmental variables including surface type and nutrition availability shape it as well. By improving resistance to medicines and immunological defenses, biofilm development gives S. aureus a survival advantage and lets it survive in hostile conditions, especially in clinical settings like medical devices or chronic infections [45]. The noted variation in biofilm generation among bacterial strains emphasizes the need of developing focused therapy plans. This can entail creating anti-biofilm chemicals catered to the special traits of every bacterial species.

Conclusion

This work exposes high rates of antibiotic resistance and biofilm generation in bacterial isolates from UTI patients, therefore challenging accepted treatment guidelines. The results highlight how urgently creative ideas—including the creation of strong antibiotics, investigation of alternative medicines, and tight rules on antibiotic use—should be developed. Furthermore, crucial for lowering the usage of antibiotics and stopping the emergence of resistant strains are public awareness efforts.

Interest Conflicts

None**.**

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None.

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