University of Thi-Qar Journal of Science (UTJsci)
E-ISSN: 2709-0256, P-ISSN: 1991-8690, Vol.12, No. 1, June. 2025

Clinical and Biochemical Insights into Biofilm-Forming Bacteria Isolated from Dialysis Patients

Noor S. Kadhem*[1a] and Ahmed Khassaf Atya[1b]

[1] *Department of Biology, College of Science, University of Thi-Qar, Thi-Qar, Iraq.*

[b]E-mail: ahmedkhassafatia@sci.utq.edu.iq
[a*]Corresponding author: noor.kadhim@utq.edu.iq

Received:2025-03-06, Revised: 2025-04-01, Accepted: 2025-04-11, Published: 2025-06-24

***Abstract*— Bacterial biofilms are difficult to eradicate and cause a significant number of complications in the management of dialysis patients resulting in more frequent infections and worse morbidity. The purpose of the present research paper is to study the characteristics of biofilm producing bacteria sampled from dialysis patients and their relationships with clinical and biochemical features. Urine cultures from 121 participants were used and yielded a total of 23 bacterial isolates. From the 23 samples, *E. coli* (n=15) had the highest frequency of moderate biofilm formation with 46.67% of the isolates belonged to this category while the rest of the isolates were equally distributed between the weak and strong biofilm producers (26.67% each). *Enterobacter cloacae* complex (n=3) formed moderate biofilms at a higher frequency (66.67%). On the other hand, *Klebsiella pneumoniae* (n=5) showed relatively weak biofilm formation, 60% had weak biofilm formation while 40% had moderate biofilm formation. We then analysed the antimicrobial resistance profiles of the isolates to determine the relationship between biofilm formation and resistance pattern. All the weak biofilm producers were multidrug-resistant while the resistance pattern of the moderate biofilm producers was also rather homogeneous; 72.7% of the isolates were multidrug-resistant and 27.3% were extensively drug-resistant. However, the resistance pattern of the strong biofilm producers was rather different, with only 20% of the isolates being multidrug-resistant and 80% being extensively drug-resistant. Duration and frequency of the dialysis sessions were observed to be important determinants of biofilm formation. These findings provide valuable information on the clinical and biochemical characteristics of biofilm forming bacteria in dialysis patients which may be helpful in the development of new strategies for the prevention and management of bacterial infections in such vulnerable patients.**

***Keywords*— Biofilms, Dialysis Patients, MDR, XDR, Antibiotics Resistant, Iraq.**

I. INTRODUCTION

Patients on dialysis treatment are at a high risk for several comorbidities and complications leading to have higher propensity to get worse infections associated with the use of medical devices, more so those that involve biofilm formation. Research topics in the area of diagnosis, intervention and prevention of biofilm related infections in dialysis patients is one of the interesting topics. Biofilm-related infections in medical devices have increased in hospitals and are associated with increased morbidity and mortality, longer hospital stay, challenging in treatment, and higher costs [1]. Biofilms defined as attached microorganisms to a biotic or abiotic surface or to each other in a polymeric substance of extracellular matrix secreted by the microorganisms [1]. Furthermore, biofilm has been found to play a role in the onset of recurrent peritonitis, a typical problem in patients receiving peritoneal dialysis treatment. This puts the dialysis patients at a disadvantage since they have compromised immune systems and have long term indwelling catheters or devices which can provide point of entry for bacteria and formation of biofilms [2]. The formation of biofilms may cause recurrence of infections, antibiotic resistance and other complications. Infections are more common among patients on dialysis than in the general population [3-4]. For example, sepsis is more frequent among dialysis patients and death from sepsis is 50 times higher than that of the general population [5]. Infections are, therefore, still the most common cause of morbidity, hospitalization, and death among the uremic population, especially among those on dialysis treatment [4], [6].

Bacterial biofilms are generally more resistant to antibiotics than their free-living counterparts owing to three major mechanisms of resistance: shielding, starvation, and transformation [7]. As demonstrated by Murray *et al*. (2022), bacteria present in biofilms are more resistant to antimicrobials than their planktonic counterparts [8]. The subgroup of dialysis patients with recurrent peritonitis and catheter loss can be identified by comparing the antibiotic sensitivities of a biofilm culture and a routine microbiologic culture of the same peritoneal dialysis effluent. These findings indicate that biofilm formation is a major factor in the persistence and recurrence of infections in these patients. [9].

They have been shown that bacterial biofilms in dialysis membranes identified *Staphylococcus aureus*, *Pseudomonas aeroginosa* and *Acinetobacter baumannii* as the most frequent biofilm producing bacteria [10-11]. These bacteria are adapted to the conditions and are prone to form strong biofilms that are difficult to eradicate by the host defences and antimicrobial therapy. Other studies have pointed *Klebsiella pneumoniae*, *Enterococcus faecalis* and

https://doi.org/10.32792/utq/utjsci/v12i1.1359

Escherichia coli as the prevalent biofilm forming pathogens in the dialysis facilities [11-13]. For instance, carbapenem-resistant *A. baumannii* is a major driver of the antimicrobial resistance (AMR) burden in low and middle income countries, while third-generation cephalosporin resistant *E. coli* is more prevalent in high income countries [8], [14]. These variations in the patterns of resistance have significant implications for the initial choice of antibiotic therapy and infection control measures in dialysis centres. It has been found that two class IIb bacteriocins, enterocins DD28 and DD93 from *Enterococcus faecalis*, inhibited effectively methicillin-resistant *Staphylococcus aureus* (MRSA) biofilm formation on stainless steel and glass surfaces. When these bacteriocins were combined with erythromycin or kanamycin the inhibitory effect was better suggesting their possibilities as agents in preventing or disrupting MRSA biofilms in medical and industrial setups [15].

However, despite the clinical significance of this issue, there is a lack of a comprehensive research examining the epidemiology and defining the characteristics of major bacterial infections that commonly afflict dialysis patient populations. Hence, this work aims to provide deeper clinical and biochemical insights into the biofilm-forming bacteria isolated from dialysis patients in Nasiriyah City/ Iraq, an area of crucial importance given the disproportionate burden of infectious complications in this vulnerable patient group.

II. Materials and Methods

A. Study Design, Population, and Data Collection

To examine the epidemiology and characteristics of biofilm forming bacteria isolated from a cohort of dialysis patients, this research used both clinical data collection and laboratory-based investigations. The cross-sectional study was carried out at the Al Hussein Teaching Hospital Dialysis Unit in Nasiriyah City/ Iraq. The Dialysis Unit offers both outpatient and inpatient dialysis services thus able us to meet a wide range of patient needs. The study sample comprised of 121 patients who were on hemodialysis at the unit between September 2024 and December 2024. The study also incorporated collecting clinical and demographic data from the hospital's electronic medical records system. This included the age, gender, comorbidities, dialysis modality and duration, frequency of dialysis sessions and current symptoms of the patient.

B. Urine Sample Collection and Transport

1. Collection Methodology

Patients with normal voiding function were asked to provide midstream clean catch urine (MSU) in accordance with the standard guidelines to prevent contamination [16]. The urine samples were collected in pre-labelled, sterile, screw-capped urine containers to prevent external contamination.

2. Sample Transport and Storage

Urine samples were transported to the microbiology laboratory at the Al-Hussein Teaching Hospital within 1 hour of collection using an ice-packed container at 4°C to maintain bacterial viability. If immediate processing was not possible, samples were refrigerated at 4°C for up to 24 hours [17].

C. Bacterial Isolation and Culture Conditions

The urine samples received inoculation onto culture media which combined selective with differential and enriched types to achieve maximum bacterial recovery and initial identification. The following culture media were used:

1. Blood Agar (Himedia, India) functions as a non-selective enriched medium which supports both fastidious and non-fastidious organisms while hemolysis patterns serve for differentiation between β-hemolysis, α-hemolysis and γ-hemolysis.
2. MacConkey Agar (Himedia, India) functions as a selective medium that separates Gram-negative enteric bacteria into lactose-fermenting (pink) and non-fermenting (colorless) colonies through neutral red pH indicator reactions.
3. Mannitol Salt Agar (Himedia, India) selects *Staphylococcus* species through its 7.5% NaCl concentration which blocks non-halotolerant bacteria from growing. The mannitol fermentation reaction resulted in yellow colonies because the phenol red indicator turned acidic.

A sterile loop transferred urine samples onto agar plates for inoculation. The culture plates underwent 24–48 hours of incubation at 37°C while researchers checked for colony growth during the first 24 hours.

D. Bacterial Identification

Bacterial identification was performed using the VITEK 2 Compact system (bioMérieux, France). Pure bacterial colonies (18–24 hours old) were suspended in 0.45% sterile saline to a 0.5 McFarland standard and loaded into the system with the appropriate identification (ID) card. The system analysed 64 biochemical reactions over 6–8 hours, providing species-level identification based on fluorescence and turbidity changes.

E. Antimicrobial susceptibility testing

The antimicrobial susceptibility of all the bacterial isolates was checked using the VITEK 2 Compact system which identified as well as gave the resistance profiles of the isolates. This wide range antimicrobial susceptibility profiling assessed the susceptibility patterns of the isolates to a wide range of antibiotics, including β-lactams, carbapenems, aminoglycosides, fluoroquinolones, and polymyxins. The isolates were categorized as susceptible, multidrug-resistant (MDR) or extensively drug-resistant (XDR) according to the guidelines of the Clinical and Laboratory Standards Institute and the European Committee on Antimicrobial Susceptibility Testing.

F. Biofilm formation assay

Biofilm formation was assessed using the Crystal Violet Microtiter Plate Assay, as previously described [18]. Briefly, 200 µL of the bacterial suspension was inoculated into 96-well flat-bottom polystyrene microtiter plates in triplicate. Negative control wells containing sterile broth were included in each assay. Plates were incubated statically at 37°C for 24 hours to allow biofilm formation. Following incubation, non-adherent cells were carefully removed, and wells were washed three times with phosphate-buffered saline (PBS, pH

7.2) to eliminate planktonic bacteria. Adherent biofilms were then stained with 0.1% crystal violet (CV) solution (200 µL per well) for 15 minutes at room temperature. Excess stain was removed by washing with PBS, and the plate was air-dried. To quantify biofilm biomass, bound crystal violet was solubilized by adding 200 µL of 30% acetic acid per well and incubating for 10 minutes at room temperature. The optical density (OD) was measured at 570 nm using a microplate reader (BioTek, United States). The degree of biofilm formation was classified as weak, moderate, or strong based on predetermined cutoff values.

G. Biochemical Analysis

Patients underwent aseptic blood collection of 5 mL venous blood samples through vacutainer gel tubes. The blood samples rested at room temperature for thirty minutes before undergoing 3500 rpm centrifugation for ten minutes for serum separation. A total of 13 biochemical markers were measured, categorized as follows: The liver function tests included Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), Alanine Transaminase (ALT) and Albumin (ALB). The renal function tests included Creatinine (Cr) and Urea. The iron metabolism markers included Unsaturated Iron Binding Capacity (UIBC), Iron, Hemoglobin (HB), and Total Serum Bilirubin (TSB). The mineral metabolism markers consisted of Calcium (Ca), Phosphate (PO_4) and Total Protein (TP). The Gesan Chem-200 platform (Gesan Production SRL, Italy) was used to analyse all biochemical parameters through manufacturer-defined protocols to guarantee precise and reproducible assay results. The laboratory followed standard clinical chemistry guidelines to establish quality control procedures.

H. Data analysis

As for descriptive statistics, they were applied for the characterization of the study population as well as the prevalence of biofilm forming bacteria. Bivariate analyses were used to determine the correlation between biofilm formation, antimicrobial resistance and patient clinical characteristics. All the statistical analyses were done using GraphPad Prism (version 10) and all the p values were considered significant if they were < 0.05.

I. Ethical Considerations

The research protocol was reviewed and approved by the Institutional Review Board at Al-Hussein Teaching Hospital (Approval No: [252/2024]). All participants gave informed consent before they were enrolled in the study.

III. Results

A. Demographic Characteristics

A total of 121 patients were recruited in the study with the age of the patients ranging from 8 to 90 years with an approximate mean age of 55 years. Age had a range of IQR=47−66 which indicated that the patients were mainly of middle or elderly age. The gender distribution was also quite comprehensive with 64 males and 57 females. An overlayed histogram was used to look at the age distribution by gender (Figure 1). This result showed that the age distribution of male and female patients was similar. But there was a bit of a pattern where males seemed to account for a relatively greater proportion of the patients in the 50−60 years age range than females.

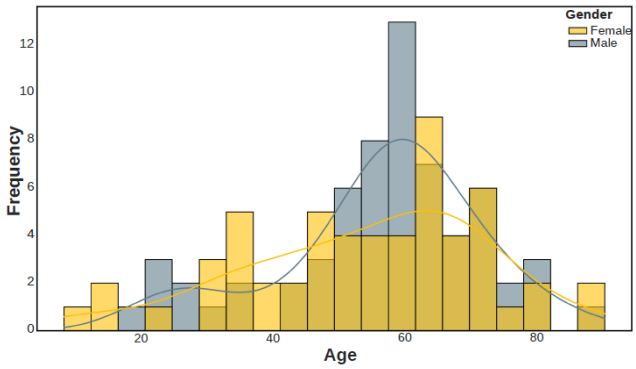

Fig. 1: Age distribution of study participants by gender. A histogram displaying the frequency of male (grey) and female (yellow) patients across different age groups. KDE line indicates the kernel density estimate smoothing curve for each gender.

B. Bacterial Isolates and Antimicrobial Resistance

Urine cultures from the 121 study participants yielded a total of 23 bacterial isolates. The 23 culture-positive samples revealed *Escherichia coli* as the main pathogen at 65.2% followed by *Klebsiella pneumoniae* at 21.7% and *Enterobacter cloacae* complex at 13.1%. The antimicrobial susceptibility testing showed significant antimicrobial resistance levels. *E. coli* showed a 93.3% resistance rate to beta-lactams, but all isolates remained susceptible to carbapenems. *K. pneumoniae* showed 100% resistance to amoxicillin/clavulanic acid as well as cefazolin and ciprofloxacin and trimethoprim/sulfamethoxazole. The *E. cloacae* isolate showed complete resistance to beta-lactams but demonstrated 100% susceptibility to fosfomycin. Furthermore, we evaluated how many bacterial isolates demonstrated MDR and XDR resistant patterns. The analysis revealed MDR strains in 60% of *E. coli* and 100% of *K. pneumoniae* while XDR strains appeared in 40% of *E. coli* and 33.3% of *E. cloacae*.

The analysis of the biofilm formation dataset was helpful in understanding the frequency of biofilm production as well as the relationships among the optical density (OD) measurements. The histogram of biofilm average was fairly normal with most of the values being clustered around the mean of about 0.56, since there were no extreme outliers in the range of biofilm production observed in samples (Figure 2). The pair plots of biofilm triplicates and biofilm average also gave a clear picture of the relationship between the separate OD measurements and their total averages (Figure 3).

From the 23 samples, the frequency of biofilm forming bacteria was categorized as weak, moderate or strong and it was observed that moderate biofilm formation was the most common overall. When the data was further broken down by bacterial group, certain tendencies were seen (Figure 4A). Out of the three bacterial types, *E. coli* (n=15) had the highest frequency of moderate biofilm formation and 46.67% of the isolates belonged to this category while the rest of the isolates were equally distributed between the weak and strong biofilm producers (26.67% each). The three strains of *Enterobacter cloacae* complex analysed formed moderate biofilms at a higher frequency (66.67%), while one-third of the isolates formed moderate biofilms, and none was categorized as weak. On the other hand, *Klebsiella*

pneumoniae (n=5) showed relatively weak biofilm formation, 60% had weak biofilm formation while 40% had moderate biofilm formation. This suggests that biofilm forming abilities are different in the various bacteria. The moderate biofilm formation by *E. coli* and *E. cloacae* complex seems to go hand in hand with conditions that require moderate biofilm formation for pathogenicity. On the other hand, the prevalence of weak biofilm formation in *Klebsiella pneumoniae* may be associated with different mechanisms of adhesion or biofilm maturation. The finding from the stratified analysis has significant implications for the development of appropriate antimicrobial therapies and the study of biofilm-related resistance in different bacterial pathogens.

We then determined the antimicrobial resistance profiles of the isolates using the VITEK 2 Compact system to determine the relationship between biofilm formation and resistance. This analysis helped in gaining an important understanding of how the resistance is developed by the biofilms. We observed a distinct gradient in the resistance patterns as a function of biofilm formation. A multidrug-resistant (MDR) phenotype was seen to be consistent with low biofilm formation; all the weak biofilm producers were MDR (Figure 4B). The resistance pattern of the moderate biofilm producers was also rather homogeneous; 72.7% of the isolates were MDR and 27.3% were XDR. However, the resistance pattern of the strong biofilm producers was rather different, with only 20% of the isolates being MDR and 80% being XDR (Figure 4B). These differences were statistically substantiated by chi-square test of independence which gave chi-square value of approximately $\chi 2=8.92$ (df = 2) with p-value of p=0.0116, which means that the association between biofilm formation and resistance patterns is probably not a chance association.

In summary, these results have potential clinical implications in relation to antimicrobial resistance and biofilm formation. They seem to suggest that increased biofilm formation could be an integral role in boosting stronger resistance mechanisms by bacterial isolates. Therefore, incorporating biofilm assessment into routine resistance profiling may help in improving the current understanding of the development of resistance, and in consequence, inform the development of new and better therapeutic strategies.

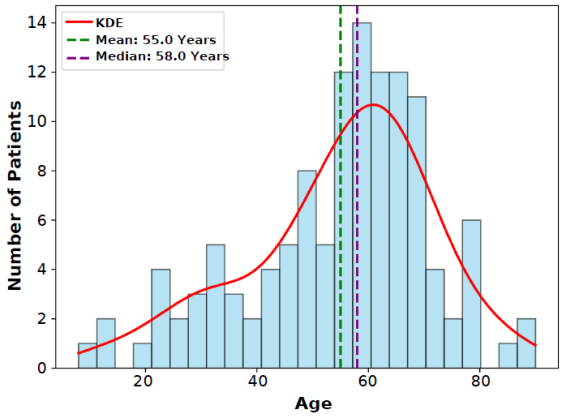

Fig. 2: Histogram of average biofilm optical density. The histogram illustrates the distribution of the average biofilm optical density values measured for the 23 bacterial isolates. KDE line indicates the kernel density estimate smoothing curve. Dished red lines represent the mean, while the dished green lines represent the median.

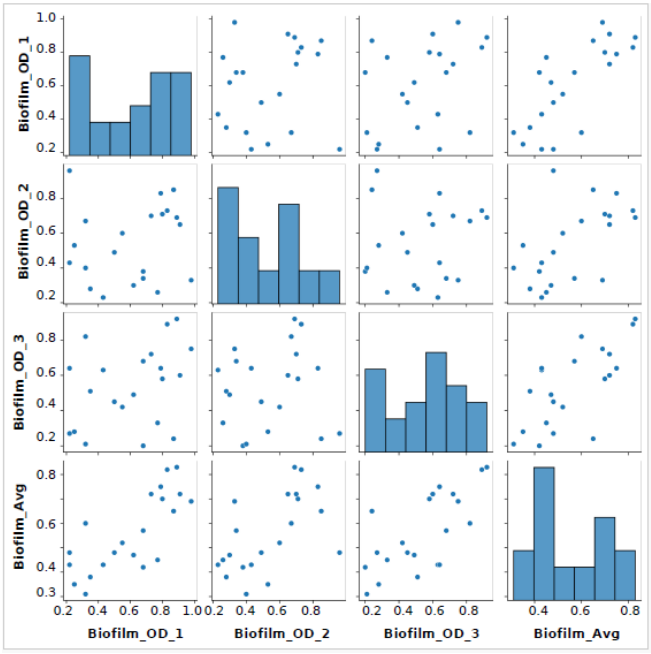

Fig. 3: Pair plots of biofilm optical density measurements. The panel shows the pairwise scatter plots of the three biofilm optical density replicates and their average (avg). The distribution of each individual measure and the average are presented along the diagonal.

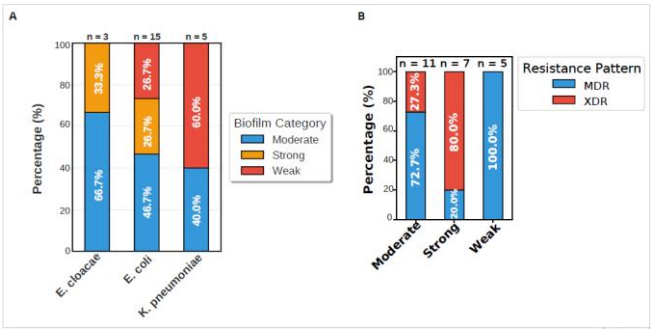

Fig. 4: Biofilm formation by bacterial species. **A**. Stacked bar chart showing the distribution of biofilm production intensity across the three bacterial species identified. **B**. Stacked bar chart illustrating the relationship between biofilm formation intensity and antimicrobial resistance patterns.

C. The Relationship between Dialysis-Related Factors and Biofilm Formation

To further investigate the potential clinical relevance of biofilm-forming bacteria in the dialysis population, we examined various dialysis-related factors and their associations with biofilm formation. First, we investigated the relationship between biofilm formation and dialysis duration. The distribution of dialysis duration (in months) was significantly different according to biofilm categories (Figure 5A). The median longest dialysis duration was seen in the Strong biofilm category with more spread of the values than in the Weak and Moderate categories. This could mean that the stronger biofilm formation is, the longer the dialysis duration. The opposite was observed in the Weak biofilm category which had the shortest median dialysis duration and a more restricted range. To establish whether there were any significant differences in dialysis duration as a function of biofilm categories, a one-way analysis of variance (ANOVA) was conducted. Descriptive statistics for the distribution of the dialysis duration (in months) across biofilm categories

are presented in Table 1. The Moderate group had a mean dialysis duration of 22.00 months (SD = 14.63), the Strong group had a mean of 32.00 months (SD = 20.70), and the Weak group had a mean of 16.43 months (SD = 17.69). The one-way ANOVA revealed no statistically significant differences in dialysis duration between the biofilm categories ($F(2, 20) = 1.24$, $p = 0.3101$). The effect size ($\eta^2 = 0.1105$) indicated a medium effect.

Next, we evaluated the relationship between biofilm formation and the number of dialysis sessions. The majority of the patients in all categories received dialysis sessions three times a week. However, the Weak biofilm category had a slightly increased proportion of patients on two times a week dialysis as compared to the other categories. On the other hand, the Moderate and Strong biofilm categories had more patients on three times a week dialysis than the other categories, which suggests that there may be a relationship between the increased dialysis frequency and the level of biofilm formation (Figure 5B). These findings showed that biofilm formation may be associated with dialysis-related parameters, including the duration of the procedure and the frequency of sessions. It seems that those patients with longer dialysis times and more frequent dialysis sessions are at risk of developing more potent biofilms that may lead to chronic infections and treatment failures.

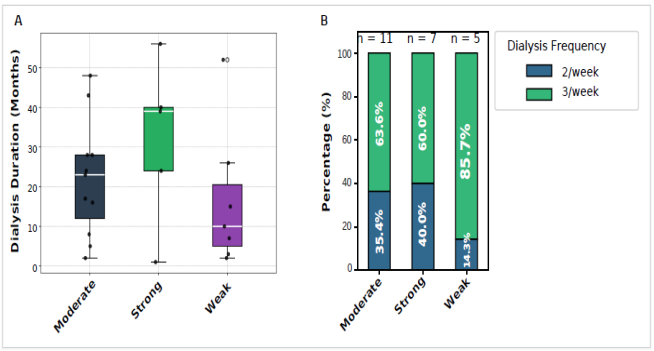

Fig. 5: Clinical Outcomes and Biofilm Association. **A**. Boxplots showing the distributions of dialysis duration across biofilm formation categories. **B**. Stacked bar chart illustrating the frequency of dialysis sessions per week in relation to biofilm formation intensity.

D. Comorbidities, Symptoms and Biofilm Dynamics

To further understand the clinical significance of biofilm forming bacteria in dialysis patients, we assessed the association between patient comorbidities, symptomatology and biofilm formation. For comorbidities (Figure 6A), moderate biofilm formation was highly correlated with diabetes mellitus (DM) which was observed in 36.36% of cases. This may suggest that DM might have a significant role in the manifestation of moderate biofilm formation. On the other hand, the weakest biofilm formation was observed in the cases with diabetes mellitus and hypertension (DM+HTN) which was seen in 57.14% of the cases. However, the strongest biofilm formation was observed in diabetes mellitus, hypertension and cardiovascular disease (DM+HTN+CVD) where 40.0% of the cases were observed. These findings showed how the comorbidity clusters may impact the strength of biofilm formation. The comparison of the clinical symptoms revealed clearly distinct tendencies (Figure 6B). The great majority of patients with moderate biofilm formation had several symptoms (36.36%) and flank pain (27.27%), which means that this group had a more complicated symptom profile. The symptoms of fever (40.0%) and several symptoms (40.0%) were most severe in patients with strong biofilm formation, with dysuria and oedema being also quite typical (20.0%). Pains in the chest and dysuria were most frequent among patients with weak biofilm formation (28.57%), and other focal symptoms including flank pain, fever, and oedema were seen in 14.29% of patients. These findings showed that symptoms were different in their severities and regionalization depending on the level of biofilm formation and that strong biofilm formation was associated with more numerous and widespread symptoms than that of weak biofilm formation.

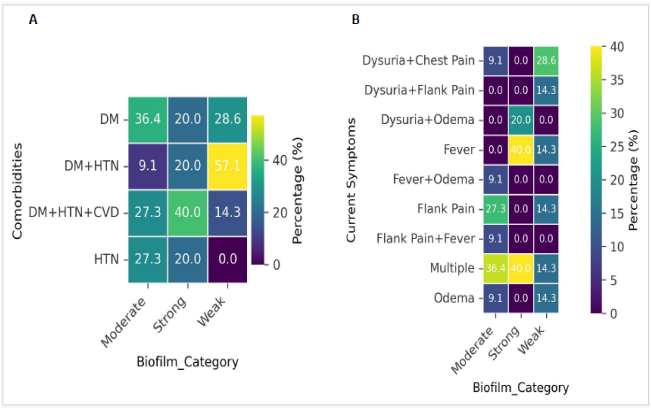

Figure 6: Comorbidities, Symptoms and Biofilm Association. **A**. A heatmap showing the relationship between comorbidities and biofilm categories. **B**. A heatmap depicting the association between clinical symptoms and biofilm categories. DM (Diabetes Mellitus), HTN (Hypertension), CVD (Cardiovascular disease).

E. Biochemical Markers Profiling

Biofilm strength was also analysed in association with biochemical markers from the biofilms to find out if there was any relationship between the metabolic parameters of the host and the bacterial biofilm formation. Since biofilms are a major clinical problem in dialysis patients and resistant to antimicrobial agents and host immune response, it is important to know the biochemical environment which can either favor or go against the formation of biofilms. We have assumed that some biochemical markers can be significantly different between patients with different levels of biofilm (Weak, Moderate and Strong) to show some metabolic factors that may be involved in the pathogenesis of biofilm or to predict the complications associated with biofilm.

A one-way analysis of variance (ANOVA) was used to determine the differences in the levels of various biochemical markers among the category of biofilm groups. Thirteen biochemical markers were assessed including those of liver function (Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), Alanine Transaminase (ALT), Albumin (ALB)), renal function (Creatinine (Cr), Urea), iron metabolism (Unsaturated Iron Binding Capacity (UIBC), Iron, Hemoglobin (HB), Total Serum Bilirubin (TSB)), and mineral metabolism (Calcium (Ca), Phosphate (PO4), Total Protein (TP)).

The results indicated a statistically significant difference in Alkaline Phosphatase (ALP) levels among the three biofilm categories ($F = 5.98$, $p = 0.0097$), suggesting that

increased biofilm formation is associated with alterations in ALP levels. In contrast, other evaluated biochemical markers, such as Creatinine, Urea, and markers of iron and mineral metabolism, did not show statistically significant differences (all $p > 0.05$). Notably, although Iron levels approached significance ($F = 1.80$, $p = 0.19$), the overall findings point toward a specific association between biofilm strength and Alkaline Phosphatase levels.

Boxplot visualizations also further confirmed these results by showing clearly distinct median differences for Alkaline Phosphatase across the biofilm categories, with the Strong biofilm group having a notably higher median ALP than the Moderate and Weak groups. These findings indicated that Alkaline Phosphatase may be a potential biochemical marker for biofilm-related pathophysiology in this patient population and thus warrant further investigation.

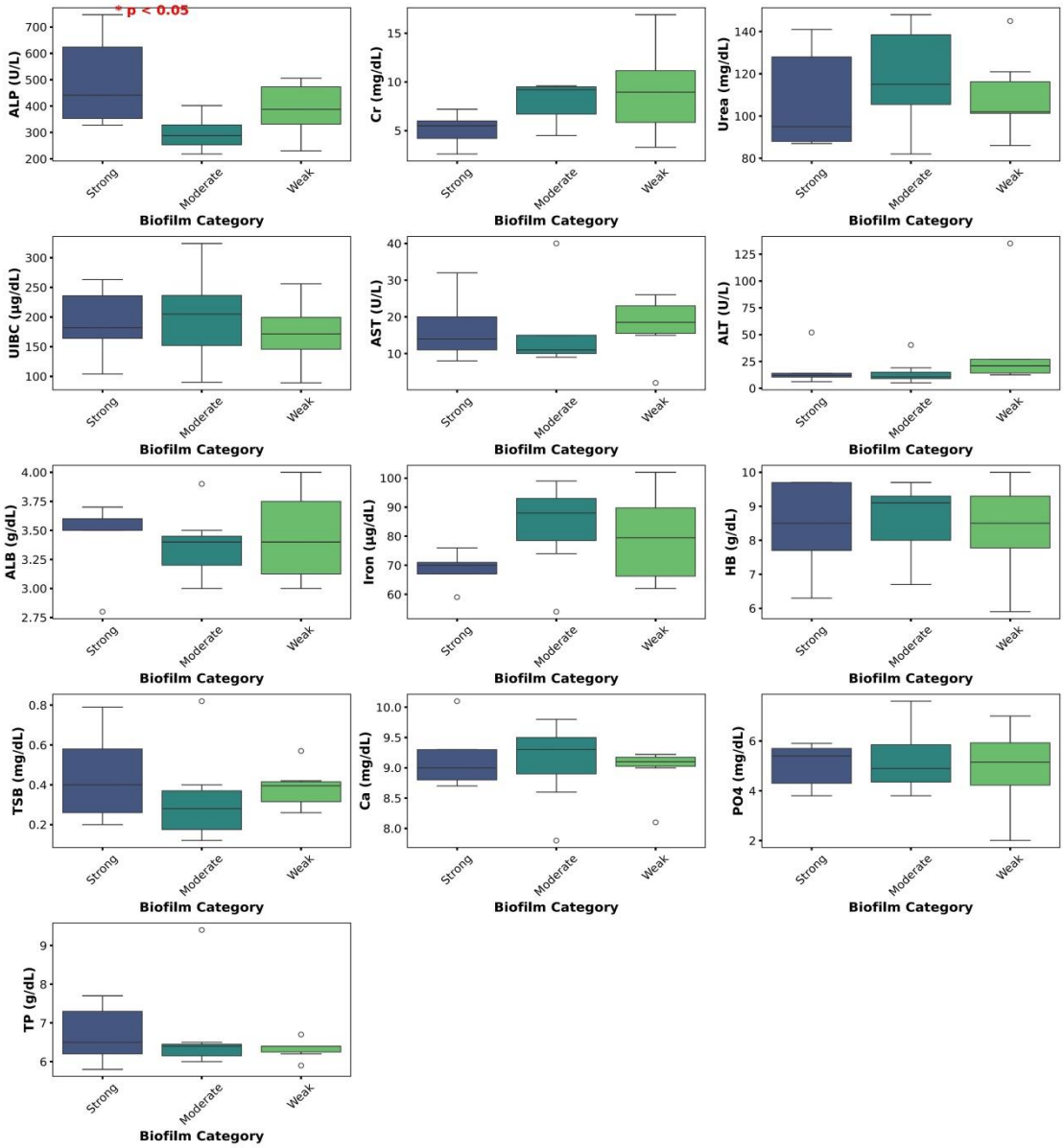

Fig. 7: Biochemical Markers and Biofilm. Boxplots demonstrating various biochemical levels across biofilm categories.

Pearson correlation analysis was conducted on biochemical markers (Figure 8). Overall correlation patterns revealed considerable variability in marker relationships, with a mean correlation coefficient of 0.019 (range: -0.457 to 0.528). When stratified by biofilm production, distinct correlation patterns emerged. Weak biofilm producers exhibited a mean correlation of 0.033 (range: -0.994 to 0.860), Moderate biofilm producers showed a mean correlation of 0.011 (range: -0.727 to 0.686), and Strong biofilm producers demonstrated a mean correlation of 0.006 (range: -0.972 to 0.938). These differences in correlation distributions suggest potential associations between biofilm production capacity and specific biochemical interrelationships in dialysis patients. In the Strong biofilm group, several marker pairs exhibited exceptionally strong positive correlations: ALB and Iron ($r = 0.938$), AST and ALT ($r = 0.934$), Urea and TSB ($r = 0.860$), Cr and Iron ($r = 0.859$), and Cr and ALB ($r = 0.844$). These robust correlations suggest that in patients with Strong biofilm production, these biochemical markers are tightly regulated in tandem, potentially reflecting altered metabolic processes specific to this subgroup.

Comparison of mean marker values across biofilm categories revealed notable differences. Strong biofilm producers exhibited markedly elevated ALP levels (498.8 U/L) compared to Moderate (296.5 U/L) and Weak (381.0 U/L) producers. Conversely, Weak biofilm producers showed higher Cr levels (8.24 mg/dL) compared to Moderate (6.74 mg/dL) and Strong (5.10 mg/dL) producers. ALT levels were notably higher in Weak biofilm producers (34.36 U/L) compared to Moderate (13.42 U/L) and Strong (18.88 U/L) producers. These differences suggest potential associations between biofilm production capacity and specific biochemical alterations in dialysis patients.

The largest differences in correlation patterns between Strong and Weak biofilm producers were observed for several marker pairs, including ALB-Ca (difference = 1.47), ALT-TSB (difference = 1.30), and ALP-ALB (difference = 1.25). Similarly, substantial correlation differences were found between Strong and Moderate biofilm producers for TSB-PO_4 (difference = 1.43), ALP-HB (difference = 1.28), and Cr-PO_4 (difference = 1.27). These marked differences in correlation patterns underscore the distinct biochemical interrelationships that characterize each biofilm category. A cluster analysis on these markers in the form of clustermaps gave a general picture of these complex interrelationships. Functionally related markers were also consistently placed together in the dendrograms, indicating that these statistical associations are biologically relevant. For example, liver function markers (AST, ALT) were found to cluster together irrespective of the biofilm category while markers associated with iron metabolism and nutritional status had different clustering patterns according to biofilm production capacity.

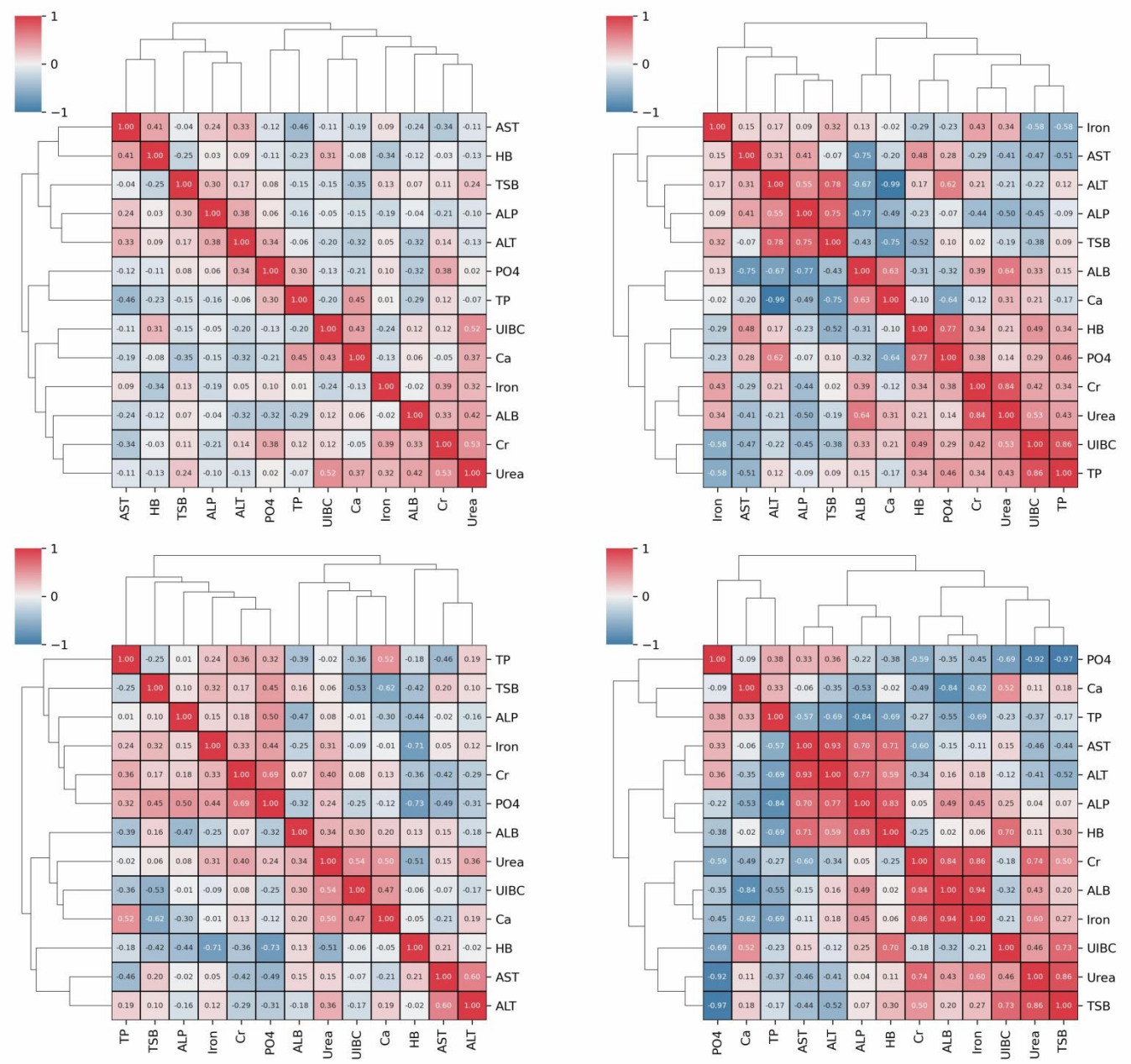

Fig. 8: Heatmap of Biochemical Marker Correlations. Clustered heatmap depicting the correlation matrix of biochemical markers, stratified by biofilm production capacity.

IV. DISCUSSION

A. Bacterial Isolates and Antimicrobial Resistance

The study provides important insights into the antimicrobial resistance patterns, clinical characteristics, and biochemical factors associated with biofilm formation by bacteria isolated from dialysis patients. The research findings showed that *E. coli* (65.2%) was the most common pathogen which confirms previous studies that have identified it as the primary uropathogen in dialysis patients [19], [20]. The high resistance rate of *E. coli* to beta-lactams (93.3%) and the 100% resistance of *K. pneumoniae* to amoxicillin/clavulanic acid, cefazolin, ciprofloxacin, and trimethoprim/sulfamethoxazole suggest alarming trends in antimicrobial resistance. The study results match worldwide studies which show rising antibiotic resistance in urinary tract infections affecting high-risk groups including dialysis patients [21]. The most important result was that all isolates had a MDR phenotype. One interesting finding isolates with strong biofilm formation were more resistant to many antimicrobial agents than the other two groups and classified as XDR. This finding broadly supports the work of other studies in this area linking biofilm formation with antimicrobial resistance [7], [22], [23]. The high MDR rates of 60% in *E. coli* 100% in *K. pneumoniae*, XDR strains at 40% in *E. coli* and 33.3% in *Enterobacter cloacae* complex demonstrated the necessity for new treatment approaches. All isolates showed complete susceptibility to carbapenems and fosfomycin but their uses need careful monitoring because they could lead to developing antibiotic resistance.

B. Biofilm Formation and Its Clinical Relevance

The results showed that the majority of patients had moderate biofilm production, 47.8%, while 30.4% and 21.7% were classified as weak and strong biofilm producers, respectively. Several reports have also shown that biofilm forming pathogens are prevalent in dialysis associated infections and the difficulty in managing these patients [7], [24]. The most obvious finding to emerge from the analysis is that these isolates were highly resistant to multiple antimicrobials commonly used in the hospital including third-generation cephalosporins and carbapenems, as these results provide further support for the increasing burden of antimicrobial resistance in healthcare facilities worldwide [8], [25]. The bacterial species demonstrated different levels of biofilm formation where *E. coli* and *E. cloacae* complex produced moderate biofilms more frequently than *K. pneumoniae* which tended to create weaker biofilms. The observed differences in biofilm formation may result from different adhesion and maturation processes which research has documented in biofilm-associated resistance studies [26]. The relationship between biofilm strength and resistance patterns proved to be highly significant. The majority of XDR strains (80%) were found among strong biofilm producers, but all weak biofilm producers remained in the MDR category. The documented phenomenon in chronic infections shows that bacterial resistance to antimicrobials increases when bacteria form biofilms [27], [28]. The statistical test ($\chi 2=8.92$, $p=0.0116$) confirms that biofilm development actively contributed to antimicrobial resistance rather than being an accidental outcome of bacterial expansion.

C. Dialysis-Related Factors and Biofilm Formation

The relationship between biofilm formation and dialysis-related parameters provides additional insights into potential risk factors. No statistically significant differences were observed in dialysis duration between biofilm categories ($p=0.3101$), but the trend of longer dialysis durations being associated with stronger biofilm formation suggests a possible cumulative effect of repeated exposure to indwelling catheters and microbial colonization. Patients with moderate and strong biofilm-forming bacteria had an increased frequency of dialysis sessions. This is consistent with previous studies that have shown that repeated vascular access and prolonged dialysis durations are associated with higher rates of catheter-associated infections [29].

D. Comorbidities, Symptoms, and Biofilm Dynamics

The comorbidity analysis revealed important associations between biofilm formation and diabetes mellitus. The highest biofilm formation level occurred in patients who had diabetes mellitus together with hypertension and cardiovascular disease (40.0%). The highest rate of moderate biofilm formation occurred among diabetic patients at 36.36%. The findings align with earlier research which demonstrated that elevated blood sugar levels enhance bacterial attachment and biofilm development by creating advanced glycosylation end-products which support microbial survival [30]. Strong biofilm producers presented with complex clinical symptoms that included fever at 40.0% and multiple symptoms at 40.0%. Biofilm-related infections in dialysis patients present as severe chronic conditions which are hard to treat because of their resistances to standard treatments [31].

E. Biochemical Markers and Biofilm Association

Among the biochemical markers analysed, Alkaline Phosphatase (ALP) levels were found to be significantly associated with biofilm strength ($p=0.0097$) and the strongest biofilm producers had the highest median ALP levels. The identification of Alkaline Phosphatase as a possible biochemical marker of increased bacterial biofilm is a very interesting finding as it suggests possible metabolic or signalling pathways that may be involved in the regulation of bacterial biofilm formation. Previous studies have linked elevated ALP levels with inflammatory conditions, tissue damage, and changes in bone and mineral metabolism that may predispose to biofilm growth [32], [33]. As an example, in this study, the relationship between ALP and biofilm strength was investigated and the results indicate that this marker can potentially affect or be affected by the biofilm related infection pathogenesis process in dialysis patients and therefore further studies are needed to understand the mechanisms of this relationship. The correlation patterns observed in this study suggest that biofilm production in dialysis patients is associated with distinct biochemical profiles and marker interrelationships. The strong positive correlations of liver function markers (AST, ALT) and of markers of nutritional status (ALB, Iron) in strong biofilm producers may be the sign of particular pathophysiological processes in this subgroup. These findings are in line with the emerging data indicating that biofilm formation can affect the metabolic processes in patients on dialysis [11], [29], [34]. These results agree with the possible role of correlation-based marker profiling in risk stratification and personalized care of dialysis patients. It is important to mention that through the identification of different

biochemical signatures related to various biofilm production capacities it may be possible for clinicians to predict complications and, therefore, tailor interventions accordingly [7], [35]. Therefore, there is a need for future prospective studies with larger cohorts to further examine their clinical significances.

V. CONCLUSION

The present study was designed to determine the clinical and biochemical characteristics of different levels of biofilm forming-bacteria from patients on dialysis. The data analysis emphasises the severe clinical complexity of biofilm related infections in this high-risk cohort population, with symptoms and comorbidities being closely linked to the level of biofilm maturity. Of particular interest is the strong relationship between the level of biofilm formation and antimicrobial resistance, with the most potent biofilm producing isolates having increased resistance to several antimicrobial agents. Furthermore, the identification of alkaline phosphatase as a putative biomarker of increased biofilm formation could be a potential step toward improving the recognition and management of such hard-to-treat infections.

Therefore, this research stresses the necessity of a more precise, patient-specific approach to the diagnosis and treatment of biofilm infections in dialysis patients when considering the results of this study together. This work also helps to further establish the basis for the targeted interventions and personalized care plans that are required to manage these critical clinical issues by demonstrating the complex interplay between biofilm formation, antimicrobial resistance, and the systemic metabolic and inflammatory disturbances of these conditions. In addition, the study recommends that more comprehensive research should be carried out to fully understand the mechanisms that are involved in the relationships that were observed to enable clinicians to better predict, prevent and control the complications of these devastating biofilm infections in this special group of patients.

ACKNOWLEDGMENT

The authors would like to thank the staff of Al-Hussein Teaching Hospital Dialysis Unit for their support and assistance during sample collection and data acquisition.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

REFERENCES

[1] L. T. Oliveira, L. G. Lopes, S. B. Ramos, C. H. G. Martins, M. C. Jamur, and R. H. Pires, 'Fungal biofilms in the hemodialysis environment', *Elsevier BV*, vol. 123, pp. 206–212, Jul. 2018, doi: 10.1016/j.micpath.2018.07.018.

[2] A. Zhao, J. Sun, and Y. Liu, 'Understanding bacterial biofilms: From definition to treatment strategies', *Frontiers Media*, vol. 13. Apr. 2023. doi: 10.3389/fcimb.2023.1137947.

[3] I. W. Apata *et al.*, 'Opportunities to Improve Antibiotic Prescribing in Outpatient Hemodialysis Facilities: A Report From the American Society of Nephrology and Centers for Disease Control and Prevention Antibiotic Stewardship White Paper Writing Group', *Elsevier BV*, vol. 77, no. 5, pp. 757–768, Oct. 2020, doi: 10.1053/j.ajkd.2020.08.011.

[4] H. Kanamori, D. J. Weber, J. E. Flythe, and W. A. Rutala, 'Waterborne Outbreaks in Hemodialysis Patients and Infection Prevention', *Oxford University Press*, vol. 9, no. 3, Feb. 2022, doi: 10.1093/ofid/ofac058.

[5] B. Wang, J. Xu, P. Fu, and L. Ma, 'MicroRNAs in septic acute kidney injury', *BioMed Central*, vol. 11, Jan. 2023, doi: 10.1093/burnst/tkad008.

[6] J. Himmelfarb, R. Vanholder, R. Mehrotra, and M. Tonelli, 'The current and future landscape of dialysis', *Nature Portfolio*, vol. 16, no. 10. pp. 573–585, Jul. 2020. doi: 10.1038/s41581-020-0315-4.

[7] J.-P. Cuevas *et al.*, 'Characterization of the Bacterial Biofilm Communities Present in Reverse-Osmosis Water Systems for Haemodialysis', *Multidisciplinary Digital Publishing Institute*, vol. 8, no. 9, pp. 1418–1418, Sep. 2020, doi: 10.3390/microorganisms8091418.

[8] C. J. L. Murray *et al.*, 'Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis', *Elsevier BV*, vol. 399, no. 10325. pp. 629–655, Jan. 2022. doi: 10.1016/s0140-6736(21)02724-0.

[9] J. Sampaio *et al.*, 'Deciphering the Contribution of Biofilm to the Pathogenesis of Peritoneal Dialysis Infections: Characterization and Microbial Behaviour on Dialysis Fluids', *Public Library of Science*, vol. 11, no. 6, Jun. 2016, doi: 10.1371/journal.pone.0157870.

[10] A. K. A. Atya, K. Drider-Hadiouche, A. Vachée, and D. Drider, 'Potentialization of β-lactams with colistin: in case of extended spectrum β-lactamase producing Escherichia coli strains isolated from children with urinary infections', *Elsevier BV*, vol. 167, no. 3, pp. 215–221, Dec. 2015, doi: 10.1016/j.resmic.2015.12.002.

[11] M. K. Dasgupta and M. Larabie, 'Biofilms in Peritoneal Dialysis', *SAGE Publishing*, vol. 21, pp. 213–217, Dec. 2001, doi: 10.1177/089686080102103s37.

[12] S. Berman, E. W. Johnson, C. H. Nakatsu, M. Alkan, R. Chen, and J. W. LeDuc, 'Burden of Infection in Patients with End-Stage Renal Disease Requiring Long-Term Dialysis', *Oxford University Press*, vol. 39, no. 12, pp. 1747–1753, Dec. 2004, doi: 10.1086/424516.

[13] R. N. Foley, 'Infectious Complications in Chronic Dialysis Patients', *SAGE Publishing*, vol. 28, pp. 167–171, Jun. 2008, doi: 10.1177/089686080802803s31.

[14] A. K. Atya, M. H. Alyasiri, R. Altamimy, and S. Ethaib, 'Assessment of Airborne Fungi in Indoor Environment for Biological Lab Rooms', *J Pure Appl Microbiol*, vol. 13, no. 4, pp. 2281–2286, Dec. 2019, doi: 10.22207/JPAM.13.4.42.

[15] A. K. A. Atya *et al.*, 'Anti-MRSA Activities of Enterocins DD28 and DD93 and Evidences on Their Role in the Inhibition of Biofilm Formation',

Frontiers Media, vol. 7, no. undefined, May 2016, doi: 10.3389/fmicb.2016.00817.

[16] T. M. Hooton *et al.*, 'Diagnosis, Prevention, and Treatment of Catheter-Associated Urinary Tract Infection in Adults: 2009 International Clinical Practice Guidelines from the Infectious Diseases Society of America', *Clinical Infectious Diseases*, vol. 50, no. 5, pp. 625–663, Mar. 2010, doi: 10.1086/650482.

[17] A. Tripathi, K. Sathua, V. Pachauri, and S. J. S. Flora, 'Collection, storage, and transportation of samples for offsite analysis', in *Handbook on Biological Warfare Preparedness*, Elsevier, 2020, pp. 133–149. doi: 10.1016/B978-0-12-812026-2.00007-4.

[18] G. A. O'Toole, 'Microtiter Dish Biofilm Formation Assay', *MyJOVE*, vol. undefined, no. 47, Jan. 2011, doi: 10.3791/2437.

[19] B. Foxman, 'The epidemiology of urinary tract infection', *Nat Rev Urol*, vol. 7, no. 12, pp. 653–660, Dec. 2010, doi: 10.1038/nrurol.2010.190.

[20] A. L. Flores-Mireles, J. N. Walker, M. Caparon, and S. J. Hultgren, 'Urinary tract infections: epidemiology, mechanisms of infection and treatment options', *Nat Rev Microbiol*, vol. 13, no. 5, pp. 269–284, May 2015, doi: 10.1038/nrmicro3432.

[21] E. Tacconelli *et al.*, 'Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis', *The Lancet Infectious Diseases*, vol. 18, no. 3, pp. 318–327, Mar. 2018, doi: 10.1016/S1473-3099(17)30753-3.

[22] S. Parikh, J. Patel, R. Patel, and R. Patel, 'Editorial: Novel approaches in the prevention of bacterial biofilm formation', *Frontiers Media*, vol. 13. Jul. 2023. doi: 10.3389/fcimb.2023.1212386.

[23] L. K. Vestby, T. Grønseth, R. Simm, and L. L. Nesse, 'Bacterial Biofilm and its Role in the Pathogenesis of Disease', *Multidisciplinary Digital Publishing Institute*, vol. 9, no. 2. pp. 59–59, Feb. 2020. doi: 10.3390/antibiotics9020059.

[24] M. K. Dasgupta, K. Kowalewaska-Grochowska, and J. W. Costerton, 'Biofilm and Peritonitis in Peritoneal Dialysis', *SAGE Publishing*, vol. 13, pp. 322–326, Jan. 1993, doi: 10.1177/089686089301302s79.

[25] M. Jihad and M. Salih, 'Microbial Detection and Antibiotic Susceptibility patterns of Clinical Isolates from Women With Urinary Tract Infection in AL-Nasiriyah City/Iraq', *UTJsci*, vol. 11, no. 1, pp. 45–54, Jun. 2024, doi: 10.32792/utq/utjsci/v11i1.1168.

[26] L. Hall-Stoodley, J. W. Costerton, and P. Stoodley, 'Bacterial biofilms: from the Natural environment to infectious diseases', *Nat Rev Microbiol*, vol. 2, no. 2, pp. 95–108, Feb. 2004, doi: 10.1038/nrmicro821.

[27] R. M. Donlan and J. W. Costerton, 'Biofilms: Survival Mechanisms of Clinically Relevant Microorganisms', *Clin Microbiol Rev*, vol. 15, no. 2, pp. 167–193, Apr. 2002, doi: 10.1128/CMR.15.2.167-193.2002.

[28] P. S. Stewart and J. William Costerton, 'Antibiotic resistance of bacteria in biofilms', *The Lancet*, vol. 358, no. 9276, pp. 135–138, Jul. 2001, doi: 10.1016/S0140-6736(01)05321-1.

[29] A. G. e Silva, M. Grosso, C. M. Takiya, and L. R. Andrade, 'Patient's cells colonize the biofilm of Tenckhoff catheters used in peritoneal dialysis', *Taylor & Francis*, vol. 27, no. 6, pp. 603–608, Jun. 2011, doi: 10.1080/08927014.2011.591488.

[30] J. Azeredo, P. García, and Z. Drulis-Kawa, 'Targeting biofilms using phages and their enzymes', *Current Opinion in Biotechnology*, vol. 68, pp. 251–261, Apr. 2021, doi: 10.1016/j.copbio.2021.02.002.

[31] D. Lebeaux, A. Chauhan, O. Rendueles, and C. Beloin, 'From in vitro to in vivo Models of Bacterial Biofilm-Related Infections', *Pathogens*, vol. 2, no. 2, pp. 288–356, May 2013, doi: 10.3390/pathogens2020288.

[32] G. Cannalire, S. Pilloni, S. Esposito, G. Biasucci, A. D. Franco, and M. E. Street, 'Alkaline phosphatase in clinical practice in childhood: Focus on rickets', *Frontiers Media*, vol. 14. Feb. 2023. doi: 10.3389/fendo.2023.1111445.

[33] X. Cheng and C. Zhao, 'The correlation between serum levels of alkaline phosphatase and bone mineral density in adults aged 20 to 59 years', *Wolters Kluwer*, vol. 102, no. 32, Aug. 2023, doi: 10.1097/md.0000000000034755.

[34] C. Liu, D. Sun, J. Zhu, J. Liu, and W. Liu, 'The Regulation of Bacterial Biofilm Formation by cAMP-CRP: A Mini-Review', *Frontiers Media*, vol. 11. May 2020. doi: 10.3389/fmicb.2020.00802.

[35] B. Branger *et al.*, 'Using Detachment-Promoting Agents for the Prevention of Chronic Peritoneal Dialysis-Associated Infections', *Wiley*, vol. 32, no. 12, pp. 918–924, Dec. 2008, doi: 10.1111/j.1525-1594.2008.00684.x.