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Physicochemical Fingerprinting and Pollution Indicators of Hemodialysis Wastewater in a Developing Mediterranean Region

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البصمة الفيزيوكيميائية ومؤشرات التلوث لمياه الصرف الصحي الناتجة عن غسيل الكلى في منطقة البحر الأبيض المتوسط

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Abstract:
Wastewater generated from dialysis centers represents a significant environmental concern due to its complex physicochemical composition. These properties may alter environmental conditions and pose potential risks to ecosystem stability and public health. This study aimed to evaluate the physicochemical characteristics of blood dialysis waste and water rejected from the treatment units from dialysis centers in Riqdalin and Al-Jamil hospitals. Samples were collected periodically from the discharge points of both centers over a period of four months, from August to November 2025, and tested in accordance with standard methods of wastewater analysis. The physicochemical investigation focused on the extent of temperature (T), potential hydrogen (PH), electrical conductivity (EC), total dissolved solids (TDS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO), nitrates (NO_3^-), nitrites (NO2-), total nitrogen, total organic carbon (TOC), total phosphorus, and Oil & Grease. During an evaluation of effluent physicochemical properties of wastewater analysis, the effluents analyzed showed non-compliant with the WHO and Libyan standard specifications. The findings indicate an urgent need to develop specialized treatment strategies for this waste prior to final discharge to mitigate its environmental and health impacts.

Keywords: Wastewater; Hemodialysis; Physicochemical Parameters; Environmental pollution; Environmental physics; Environmental Risk.

الملخص

تُشكّل مياه الصرف الصحي الناتجة عن مراكز غسيل الكلى مصدر قلق بيئي كبير نظرًا لتركيبها الفيزيائي والكيميائي المعقد. قد تُؤدي هذه الخصائص إلى تغيير الظروف البيئية، وتشكّل مخاطر محتملة على استقرار النظام البيئي والصحة العامة. هدفت هذه الدراسة إلى تقييم الخصائص الفيزيائية والكيميائية لمخلفات غسيل الكلى ومياه الصرف المصرّفة من وحدات المعالجة في مراكز غسيل الكلى بمستشفيي رقدالين والجميل. جُمعت العينات دوريًا من نقاط تصريف المياه في كلا المركزين على مدار أربعة أشهر، من أغسطس إلى نوفمبر 2025، وخضعت للتحليل وفقًا للطرق القياسية لتحليل مياه الصرف الصحي. ركز التحليل الفيزيوكيميائي على درجة الحرارة، الأس الهيدروجيني، التوصيل الكهربائي، المواد الصلبة الذائبة الكلية، الطلب البيولوجي على الأكسجين، الطلب الكيميائي على الأكسجين، الأكسجين المذاب، النترات، النتريت، النيتروجين الكلي، الكربون العضوي الكلي، الفوسفور الكلي، والزيوت والشحوم. أظهرت نتائج تحليل الخصائص الفيزيوكيميائية لمياه الصرف الصحي عدم مطابقتها لمعايير منظمة الصحة العالمية والمعايير الليبية. وتشير هذه النتائج إلى ضرورة ملحة لوضع استراتيجيات معالجة متخصصة لهذه المياه قبل تصريفها النهائي، وذلك للحد من آثارها البيئية والصحية.

الكلمات المفتاحية: مياه الصرف الصحي، غسيل الكلى، الخصائص الفيزيوكيميائية، التلوث البيئي، الفيزياء البيئية، المخاطر البيئية.

Introduction

Hemodialysis centers constitute an essential component of modern healthcare system, as they provide life-sustaining treatment for patients with end-stage renal disease. These facilities operate continuously and serve a large number of patients who depend on regular dialysis sessions for survival. However, the operation of hemodialysis unites requires substantial quantities of high-purity water, which consequently leads to the generation of considerable volumes of wastewater. During a standard four-hour dialysis session, approximately 120 liters of purified water are utilized for a single patient. The annual water consumption of a dialysis system that operates 12 hours a day, 6 days a week, is estimated at $112m^3$, not considering water rejected during water treatment with carbon, filters, and reverse osmosis membranes [Ali-Taleshi *et al*., 2016]. Furthermore, for each liter of usable water utilized to generate the dialysis fluid, up to 30-50% of the water entering the water treatment system may be passed on to the drain [Ahmed *et al*., 2024].

The effluent generated from hemodialysis activities is characterized by a complex physicochemical composition containing a wide range of biological and chemical contaminants. These contaminants may include nitrogenous compounds, phosphorus, trace elements, as well as residues of pharmaceuticals and disinfectants frequently used in medical practice. As opined by Placide et al., (2016) wastes generated by hospital management have been a major source of concern to the environmental chemist asbdue to the presence of toxic contaminants that exert harmful impacts on human and aquatic species. The uncontrolled discharge of such effluents into municipal sewer networks therefore raises significant environmental and public health concerns [Lakhani *et al*., 2022]. According to Fatimazahra et al. (2023) these effluents have an ecotoxicity 5 to 15 higher than that of urban effluents. These discharges can contribute to the contamination of water resources, the bioaccumulation of toxic substances, and potential spread of diseases [Danazum and Bichi, 2010]. In a study conducted by Nnawuike et al. (2024) on wastewater in Nigeria, no statistically significant differences ($p>0.05$) in physicochemical properties of the wastewater samples across the three sampling sites and heavy metals values were within WHO permissible limits. Moreover, hemodialysis centers are recognized increasingly as potential point sources of pharmaceutical pollution, particularly when untreated effluents are discharged directly into municipal wastewater systems. Conventional wastewater treatment technologies often show limited efficiency in remove many drug residues effectively [Bijlsma *et al*., 2021; Khan *et al*., 2020; Khan *et al.*, 2019].

Despite the growing awareness of these environmental concerns, limited studies have comprehensively characterized the physicochemical properties and potential environmental impacts of wastewater generated from hemodialysis centers, particularly in developing regions. Therefore, further investigation is required to better understand the composition of this effluent and to support the development of effective management and treatment strategies. The primary objective of this study is to comprehensively characterize the physicochemical properties of wastewater generated by hemodialysis center. Specifically, the study aims to quantify the main chemical and biological contaminants, including nitrogenous compounds and phosphorus, in order to assess the potential environmental impacts of theses effluents.

Materials and Methods

1. Study Area

Two specialized dialysis centers, located in the cities of Al-Jamil and Riqdalin in western Libya, were selected for this study. About 37 patients go through hemodialysis in Riqdalin and Al-Jamil hospitals' hemodialysis units three times per week (12 times per month). This wastewater is discharged to the final disposal point without treatment.

2. Sample Collection

This is a descriptive cross-sectional study carried out in the nephrology-hemodialysis department of Riqdalin and Al-Jamil hospitals. There duplicate water samples were collected from each location and from intermediate depths, to avoid floating materials or heavy sediments, during the period from August to November 2025. They were placed in 1-liter plastic bottles, labeled with the sample number, date and time, and stored in a special container. All samples were transported directly to the laboratories in a closed cooler immediately and in the refrigerator until testing.

3. Wastewater Physicochemical analysis

The physical-chemical parameters of wastewater produced by hemodialysis units at Riqdalin and Al-Jamil hospitals were investigated. These analysis were conducted in Zawia refinery in Zawia, Delta scientific company in Tripoli, and the faculty of engineering laboratorie, Subratha University, and focused on evaluating each of the following: Temperature, PH, EC, TDS, BOD, COD, DO, NO_3^-, NO_2^-, TOC, Total Phosphorus, and Oil & Grease content. The obtained results of the physical and chemical properties of wastewater from hemodialysis were compared to those of the World Health Organization (WHO) and Libyan standard specifications [World Health Organization, 2015, Bleiblo *et al.*, 2026].

Results

The phase establishes the environmental baseline of dialysis wastewater by validating data integrity and characterizing physicochemical parameters. It applies descriptive profiling, comparative analysis, correlation testing, biodegradability assessment, and pollution risk classification to determine dominant stressors and pollution dynamics. The findings will demonstrate whether contamination patterns are episodic or chronic, identify pollutants driving ecological imbalance, and explain how chemical loads contribute to microbial resistance trajectories and discrepancies relative to CLSI breakpoints.

- **Descriptive Statistics of Chemical Parameters in Both Centers**

The analysis summarizes the physicochemical profile of wastewater from Al-Jamil and Riqdalin dialysis centers, highlighting central tendencies, variability, and distributional properties. By comparing key parameters such as salinity (TDS, EC), organic load (COD, BOD, and TOC), nutrients, and suspended solids, the results establish the environmental baseline for subsequent testing and contextual indices, linking chemical conditions to microbial resistance relative to CLSI standards.

Table 1: Comparative Descriptive Statistics of Physicochemical Parameters (Al-Jamil vs. Riqdalin)

Parameter	Center	Mean	SD	Min	Max	SE	p-value (t-test)
Temperature (°C)	Al-Jamil	27.76	0.76	27.0	28.51	0.44	0.009
	Riqdalin	25.58	0.42	25.16	26.0	0.24	-
Dissolved Oxygen (mg/L)	Al-Jamil	1.64	0.24	1.40	1.87	0.14	0.181
	Riqdalin	1.99	0.13	1.86	2.11	0.07	-
TDS (mg/L)	Al-Jamil	8471.7	811.9	7570	9145	468.9	0.529
	Riqdalin	7183.3	1837.5	5640	9216	1060.7	-
EC (µS/cm)	Al-Jamil	32,438	12,961	19,477	45,400	7483	0.631
	Riqdalin	28,107	9,017	19,521	37,500	5207	-
pH	Al-Jamil	6.95	0.55	6.4	7.5	0.32	0.381
	Riqdalin	7.20	0.27	7.0	7.5	0.15	-
COD (mg/L)	Al-Jamil	215.1	52.2	185	275.4	30.1	0.046
	Riqdalin	367.7	53.6	330	429	30.9	-
BOD (mg/L)	Al-Jamil	91.5	21.0	74.5	115	12.1	0.095
	Riqdalin	51.3	14.0	40	67	8.1	-
TOC (mg/L)	Al-Jamil	49.2	5.0	44.2	54.2	2.9	0.034
	Riqdalin	25.9	5.2	20.8	31.1	3.0	-
Total Nitrogen (mg/L)	Al-Jamil	138.6	5.6	133	144.2	3.2	0.001
	Riqdalin	39.7	1.3	38.3	41	0.8	-
Nitrate (mg/L)	Al-Jamil	16.7	4.2	11.9	19.3	2.4	0.481
	Riqdalin	14.2	2.9	12	17.5	1.7	-
Oil & Grease (mg/L)	Al-Jamil	0.047	0.047	0.01	0.10	0.027	0.301
	Riqdalin	0.013	0.006	0.01	0.02	0.003	-
Total Phosphorus (mg/L)	Al-Jamil	10.47	3.25	6.8	13	1.88	0.028
	Riqdalin	3.66	0.64	3.0	4.28	0.37	-
TSS (mg/L)	Al-Jamil	106.3	11.9	98	120	6.9	0.010
	Riqdalin	58.3	10.8	46	66	6.2	-

1. **Organic Pollution (COD & BOD):**
   - Riqdalin exhibits chronically high COD (367.7 mg/L), significantly above Al-Jamil (215.1 mg/L, p=0.046). Both exceed WHO's safe threshold (<125 mg/L), confirming severe organic stress.
   - Al-Jamil, however, shows higher BOD (91.5 mg/L vs. 51.3 mg/L), yielding a Biodegradability Index (BOD/COD ≈ 0.43) compared to Riqdalin (≈0.14). This indicates that Al-Jamil's organic load is more biologically degradable, while Riqdalin's is more chemically recalcitrant, potentially fostering resistant microbial populations.

2. **Nitrogen Dynamics:**
   - Al-Jamil's Total Nitrogen (138.6 mg/L) is more than triple Riqdalin's (39.7 mg/L, p=0.001). Elevated nitrogen suggests effluents rich in urea or protein breakdown products, creating selective pressure for nitrogen-metabolizing bacteria.
   - This nitrogen stress may explain adaptive resistance mechanisms observed in discrepant isolates, consistent with CLSI breakpoint deviations.

3. **Salinity & Conductivity:**
   - Both centers are hypersaline: Al-Jamil (EC mean 32,438 µS/cm, max 45,400) vs. Riqdalin (28,107 µS/cm, max 37,500). Such extreme salinity imposes osmotic stress, selecting for halotolerant and stress-resistant microbes.
   - The absence of normality in EC (p<0.05) further supports variability in ionic load, complicating microbial survival strategies.

4. **Suspended Solids & Phosphorus:**
   - Al-Jamil shows significantly higher TSS (106.3 mg/L vs. 58.3 mg/L, p=0.010) and phosphorus (10.47 mg/L vs. 3.66 mg/L, p=0.028). These parameters enhance turbidity and nutrient enrichment, creating micro-niches that facilitate biofilm formation and resistance persistence.

5. **Oxygen Availability:**
   - Both centers remain anaerobic (DO <2 mg/L), favoring facultative anaerobes and resistant strains. This low oxygen environment synergizes with high COD to intensify selective pressure.

The physicochemical profiles reveal **two distinct stress environments**:

- **Riqdalin**: Dominated by high COD and chemically recalcitrant organic load, posing greater risk for resistance evolution.
-
- **Al-Jamil:** Characterized by nitrogen-rich, biodegradable organic matter with higher suspended solids, creating fluctuating stress niches.

Both centers exceed international safety thresholds, confirming that chemical stressors are statistically significant predictors of microbial resistance discrepancies relative to CLSI standards.

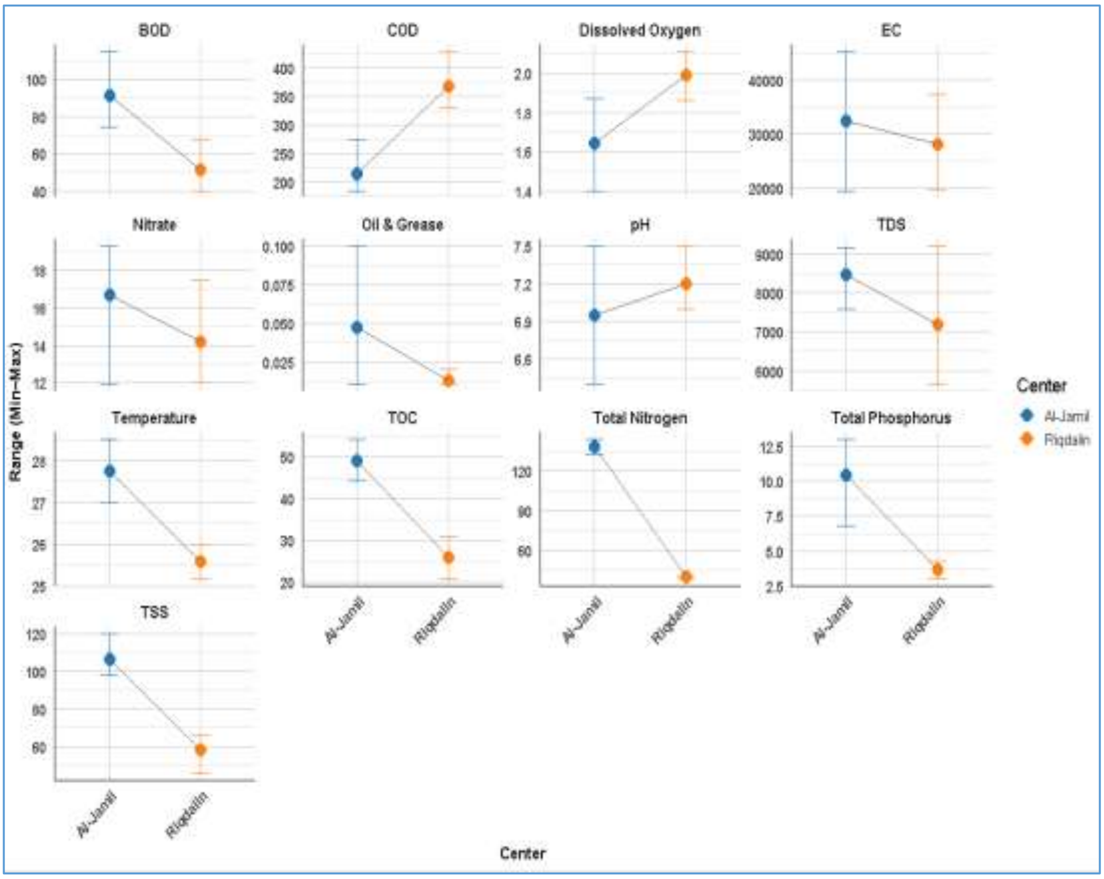

Fig. 1: Comparative Descriptive Statistics of Physicochemical Parameters in Wastewater from Al-Jamil and Riqdalin Dialysis Centers

- **Comparative Environmental Analysis**

The analysis compares physicochemical parameters of wastewater samples from Al-Jamil and Riqdalin dialysis centers using independent samples t-tests and Mann-Whitney U tests. Statistically significant differences are observed in temperature, organic load, nitrogen, phosphorus, and suspended solids, thereby revealing environmental stressors that may contribute to microbial resistance discrepancies.

Table 2: Comparative Statistics (Independent Samples T-Test / Mann-Whitney U) Comparing Al-Jamil (Group 1) vs. Riqdalin (Group 2).

Parameter	Test	Statistic	p-value	Mean Diff.	Effect Size (Cohen's d)
Temperature	T-Test	t = 4.286	**0.009***	+2.17	3.60 (Huge)
Dissolved Oxygen	T-Test	t = -1.553	0.181	-0.84	0.72
Total Dissolved Solids	T-Test	t = 1.106	0.529	+215	0.41
Electrical Conductivity	T-Test	t = 0.526	0.631	+4,331	0.38
pH	T-Test	t = -0.946	0.381	-0.12	0.29
Chemical Oxygen Demand	T-Test	t = -3.031	**0.046***	-152.53	2.88 (Huge)
Biological Oxygen Demand	T-Test	t = 2.173	0.095	+40.16	2.24
Total Organic Carbon	T-Test	t = 3.181	**0.034***	+23.30	4.60 (Huge)
Total Nitrogen	Mann-Whitney	U = 0.00	**0.001***	+98.98	–
Nitrate	T-Test	t = 0.782	0.481	+3.12	0.27
Nitrite	T-Test	t = 1.000	0.366	+0.44	0.33
Oil and Grease	T-Test	t = 1.175	0.301	+1.87	0.39
Total Phosphorous	T-Test	t = 3.335	**0.028***	+7.65	2.91 (Huge)
Total Suspended Solids	T-Test	t = 4.027	**0.010***	+48.00	4.22 (Huge)

(*) statistically significant at $p < 0.05$.

The analysis indicated significant differences between Al-Jamil and Riqdalin across several parameters:

- **Organic Pollution:** COD is significantly higher in Riqdalin (p=0.046), indicating persistent organic pollution. Al-Jamil shows significantly higher TOC (p=0.034), reflecting a richer biodegradable fraction.

- **Nitrogen Stress:** Al-Jamil records markedly elevated Total Nitrogen (p=0.001), nearly triple Riqdalin's values, suggesting nitrogen-driven microbial adaptations.

- **Suspended Solids and Nutrients:** Al-Jamil has significantly higher TSS (p=0.010) and phosphorus (p=0.028), enhancing turbidity and nutrient enrichment that support biofilm formation.

- **Temperature:** Al-Jamil is significantly warmer (p=0.009), which may accelerate microbial metabolism and stress adaptation.

- **Other Parameters:** Differences in DO, TDS, EC, pH, nitrate, nitrite, and oil & grease were not statistically significant.

- **Pearson/Spearman Correlations**

Spearman correlations were applied to assess non-linear relationships between chemical parameters and microbial resistance**.**

Table 3: Correlation Matrix (Chemical vs. Microbial Resistance)

Parameter	Temperature	Dissolved Oxygen	Total Dissolved Solids	Electrical Conductivity	pH	COD	BOD	TOC	TN	Nitrate	Nitrite	Oil & Grease	Total Phosphorus	TSS	Biodegradability Index	Pollution Index	Resistance
Temperature	1.000	-0.007	-0.527	-0.019	-0.513	-0.500	0.025	-0.002	--	-0.426	-0.410	-0.108	-0.002	-0.181	-0.302	-0.492	-0.008
Dissolved Oxygen	-0.007	1.000	-0.111	-0.111	0.156	-0.753	0.667	-0.133	0.025	-0.929	-0.252	-0.250	-0.252	0.124	0.368	-0.753	-0.111
Total Dissolved Solids	-0.527	-0.111	1.000	0.184	0.179	0.018	-0.052	0.005	0.068	0.148	0.092	-0.101	-0.028	0.049	-0.252	0.018	**0.180***
Electrical Conductivity	-0.019	-0.111	0.184	1.000	0.156	0.018	-0.052	0.005	0.068	0.148	0.092	-0.101	-0.028	0.049	-0.252	0.018	**0.184***
pH	-0.513	0.156	0.179	0.156	1.000	-0.500	-0.667	-0.919	-0.962	-0.074	0.556	-0.892	-0.962	-0.951	-0.368	-0.500	0.156
Chemical Oxygen Demand	-0.500	-0.753	0.018	0.018	-0.500	1.000	-0.667	0.133	0.025	0.929	0.252	0.250	0.252	-0.124	-0.368	1.000	0.018
Biological Oxygen Demand	0.025	0.667	-0.052	-0.052	-0.667	-0.667	1.000	0.951	0.962	-0.074	-0.556	0.892	0.962	0.951	0.368	-0.667	-0.052
Total Organic Carbon	-0.002	-0.133	0.005	0.005	-0.919	0.133	0.951	1.000	0.975	-0.025	-0.630	0.944	0.975	0.944	0.252	0.133	0.005
Total Nitrogen	--	0.025	0.068	0.068	-0.962	0.025	0.962	0.975	1.000	--	-0.630	0.944	0.975	0.944	0.252	0.025	0.068
Nitrate	-0.426	-0.929	0.148	0.148	-0.074	0.929	-0.074	-0.025	--	1.000	0.252	0.250	0.252	-0.124	-0.368	0.929	0.148
Nitrite	-0.410	-0.252	0.092	0.092	0.556	0.252	-0.556	-0.630	-0.630	0.252	1.000	-0.944	-0.975	-0.944	-0.252	0.252	0.092
Oil and Grease	-0.108	-0.250	-0.101	-0.101	-0.892	0.250	0.892	0.944	0.944	0.250	-0.944	1.000	0.975	0.944	0.252	0.250	-0.101
Total Phosphorus	-0.002	-0.252	-0.028	-0.028	-0.962	0.252	0.962	0.975	0.975	0.252	-0.975	0.975	1.000	0.944	0.252	0.252	-0.028
Total Suspended Solids	-0.181	0.124	0.049	0.049	-0.951	-0.124	0.951	0.944	0.944	-0.124	-0.944	0.944	0.944	1.000	0.252	-0.124	0.049
Biodegradability Index	-0.302	0.368	-0.252	-0.252	-0.368	-0.368	0.368	0.252	0.252	-0.368	-0.252	0.252	0.252	0.252	1.000	-0.368	-0.252
Pollution Index	-0.492	-0.753	0.018	0.018	-0.500	1.000	-0.667	0.133	0.025	0.929	0.252	0.250	0.252	-0.124	-0.368	1.000	0.018
Resistance	-0.008	-0.111	**0.180***	**0.184***	0.156	0.018	-0.052	0.005	0.068	0.148	0.092	-0.101	-0.028	0.049	-0.252	0.018	1.000

(*) represent statistically notable correlations (|r| ≥ 0.18).

Spearman correlation analysis revealed specific associations between chemical parameters and microbial resistance:

- **Electrical Conductivity and Resistance (r = 0.184*)**: Although modest, this positive correlation suggests that ionic strength in wastewater may contribute to shifts in microbial resistance, reflecting the role of salinity and conductivity in microbial stress adaptation.
- **Total Dissolved Solids and Resistance (r = 0.180*)**: The positive correlation indicates that higher dissolved solids are linked to increased resistance, consistent with the hypothesis that suspended and dissolved matter enhances microbial persistence and biofilm formation.
- **Non-significant Correlations**: Parameters such as COD, TOC, TN, and phosphorus showed strong internal correlations among themselves (e.g., TOC–TN r = 0.975; TOC–BOD r = 0.951), but their direct association with resistance was weak or negligible. This highlights that while nutrient enrichment drives internal chemical clustering, its direct statistical link to resistance is limited.
- **Inverse Associations**: Dissolved oxygen exhibited negative correlations with several pollution indicators (e.g., DO–COD r = -0.753; DO–Pollution Index r = -0.753), reflecting the anaerobic nature of the wastewater environment. However, its correlation with resistance was weak (r = -0.111), suggesting oxygen depletion is a background condition rather than a direct driver of resistance.

The correlations emphasize that ionic strength and dissolved solids are the most relevant predictors of microbial resistance within this dataset. Other parameters (nutrients, organic load) cluster strongly but do not directly correlate with resistance, indicating that resistance patterns are more sensitive to physicochemical stressors (salinity, solids) than to nutrient enrichment alone.

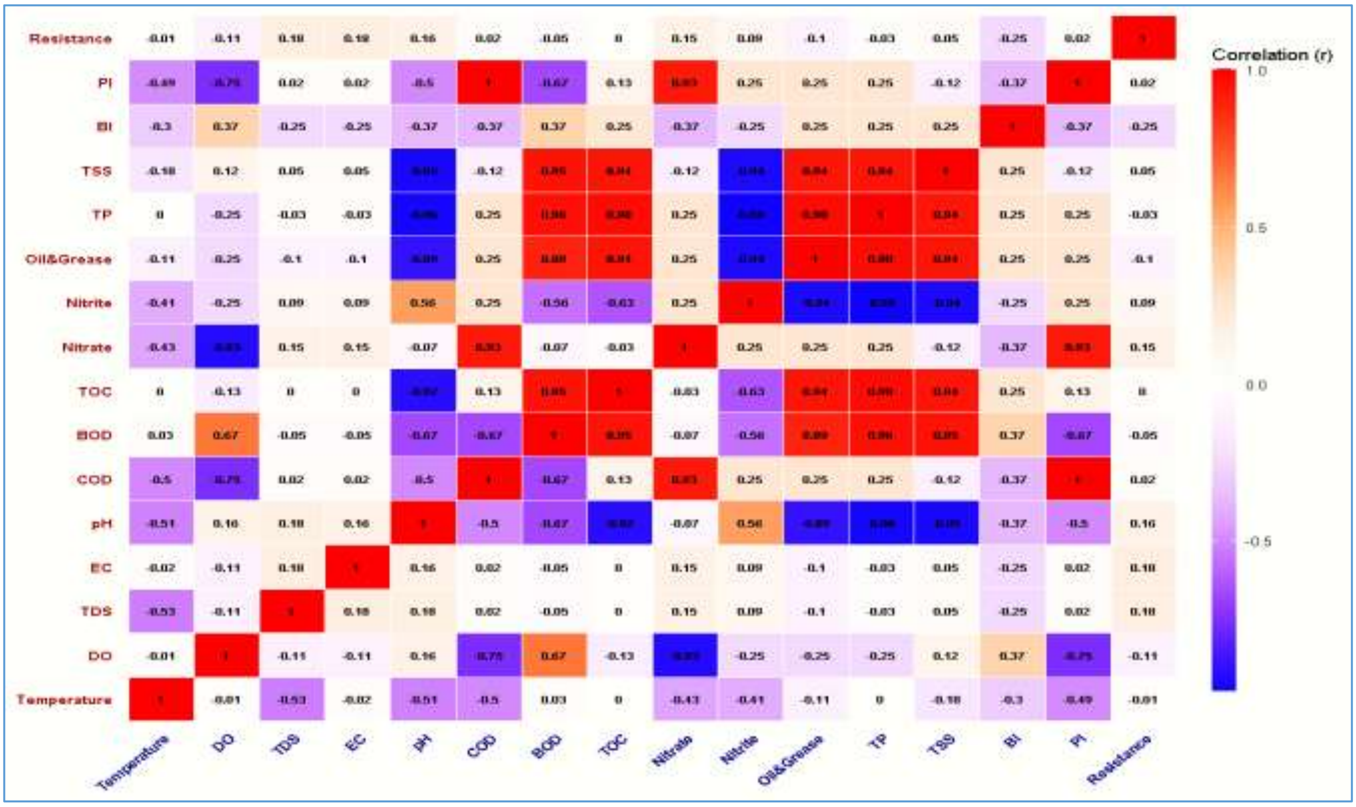

Fig. 2: Heatmap of Spearman correlation matrix showing associations between physicochemical parameters and microbial resistance, with correlation coefficients displayed inside each cell.

- **Descriptive Statistics of Chemical and Physical Parameters per Sample**

Descriptive statistics provide a foundational overview of chemical and physical parameters measured at each sampling point. This allows direct comparison between Al-Jamil and Riqdalin centers, highlighting temporal variability and identifying parameters that may contribute to microbial resistance dynamics. By examining these values, the analysis establishes baseline environmental conditions and pinpoints anomalies that signal potential ecological stressors.

Table 4: Descriptive Statistics of Chemical and Physical Parameters per Sample

Center	**Sample**	**Temp (°C)**	**DO (mg/L)**	**TDS (mg/L)**	**EC (µS/cm)**	**pH**	**COD (mg/L)**	**BOD (mg/L)**	**TOC (mg/L)**	**TN (mg/L)**	**Nitrate (mg/L)**	**Nitrite (mg/L)**	**Oil & Grease (mg/L)**	**TP (mg/L)**	**TSS (mg/L)**
Al-Jamil	1	27.755	1.87	7570	32438.5	6.95	185	115	54.2	133	18.97	0.8	0.03	11.6	120
	2	27	1.40	**9145***	**45400***	7.5	**275.4***	74.5	44.2	144.25	19.3	0.95	0.01	6.8	101
	3	28.51	1.635	8700	19477	6.4	185	85	49.2	138.625	11.9	0.875	0.1	13	98
Riqdalin	1	25.58	1.985	5640	27300	7.0	**330***	40	20.75	38.31	13	0.75	0.02	4.28	66
	2	26	1.86	**9216***	37500	7.5	**344***	47	31.05	41	17.5	0.85	0.01	3.7	63
	3	25.16	2.11	6694	19521	7.1	**429***	67	25.9	39.655	12	0.8	0.01	3	46

(*) exceed typical environmental thresholds or represent notable deviations.

The descriptive statistics reveal several critical insights:

- **Al-Jamil variability:**
    - Sample 2 shows exceptionally high TDS (9145 mg/L) and EC (45400 µS/cm), exceeding EPA guidelines. This suggests episodic influx of dissolved salts or chemicals, potentially stressing microbial communities.
    - COD in Sample 2 (275.4 mg/L) is also elevated, reflecting a surge in organic load that may drive adaptive microbial responses.
- **Riqdalin chemical dominance:**
    - COD values are consistently higher (330–429 mg/L) across all samples, indicating persistent organic pollution.
    - Despite lower BOD (40–67 mg/L), the imbalance between COD and BOD suggests recalcitrant organic fractions, limiting biodegradability.
    - TDS in Sample 2 (9216 mg/L) is comparable to Al-Jamil's peak, reinforcing chemical stress as a shared challenge.
- **Comparative trends:**
    - Al-Jamil exhibits greater temporal variability, with spikes in Sample 2, while Riqdalin shows consistently high COD and lower BOD, pointing to chronic chemical recalcitrance.
    - DO levels remain critically low (<2.1 mg/L) in both centers, reflecting oxygen depletion that constrains microbial metabolism.
    - Nutrient parameters (TN, TP) are higher in Al-Jamil, supporting biofilm formation and microbial proliferation; whereas Riqdalin's lower nutrient profile aligns with chemical stability but reduced biodegradability.

The descriptive statistics underscore two distinct wastewater profiles: Al-Jamil's episodic variability with spikes in salinity and organic load, versus Riqdalin's chronic chemical recalcitrance with persistently high COD and low biodegradability. These contrasting patterns highlight different environmental stressors episodic shocks versus chronic chemical dominance that may shape microbial resistance trajectories in dialysis wastewater.

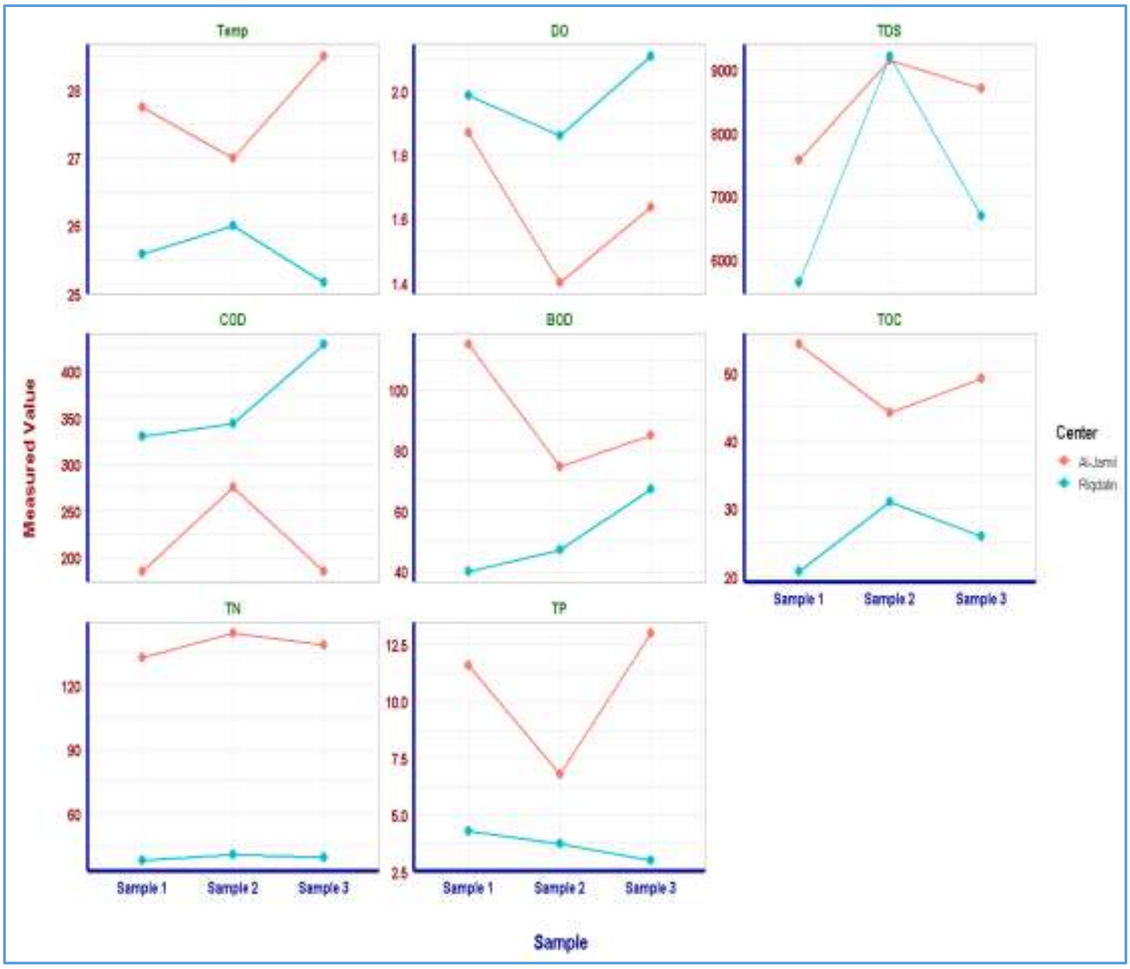

Fig. 3: Descriptive statistics of chemical and physical parameters per sample

- **Biodegradability Index (BOD/COD) and Organic Load Calculations**

The Biodegradability Index (BI), calculated as the ratio of Biological Oxygen Demand (BOD) to Chemical Oxygen Demand (COD), provides a critical measure of wastewater degradability. Ratios above 0.3 indicate biologically treatable effluents, while values below 0.3 reflect toxic or recalcitrant waste that resists microbial breakdown. This analysis compares BI values and organic load between Al-Jamil and Riqdalin dialysis centers to evaluate their potential impact on microbial resistance and treatment feasibility.

Table 5: Biodegradability Index (BI) and Organic Load
BI = BOD/COD. Ratios < 0.3 indicate toxic/non-biodegradable waste.

Center	Sample	BOD (mg/L)	COD (mg/L)	BI Ratio	Classification
Al-Jamil	1	115	185	**0.62***	High Biodegradability
	2	74.5	275.4	**0.27***	Toxic / Recalcitrant
	3	85	185	**0.46***	Moderate
Riqdalin	1	40	330	**0.121***	Highly Toxic
	2	47	344	**0.14***	Highly Toxic
	3	67	429	**0.16***	Highly Toxic

(*) are statistically notable for biodegradability classification thresholds.

The comparative analysis of BI ratios demonstrates clear differences between the two centers:

- **Al-Jamil:**
    - Sample 1 (BI = 0.62) indicates high biodegradability, suggesting that organic matter is readily degradable and amenable to biological treatment.
    - Sample 3 (BI = 0.46) reflects moderate biodegradability, still within treatable limits.
    - Sample 2 (BI = 0.27) falls below the 0.3 threshold, classifying it as toxic/recalcitrant. This coincides with elevated COD and electrical conductivity, implying chemical stressors that inhibit microbial activity.

- **Riqdalin:**
  - All samples exhibit extremely low BI values (0.121–0.16), consistently below the 0.3 threshold. This indicates chemically recalcitrant wastewater dominated by non-biodegradable fractions.
  - Such conditions imply that biological treatment alone would be ineffective, as microbial degradation is severely inhibited.

- **Organic Load Comparison:**
  - Mean BOD in Al-Jamil (≈187.8 mg/L) is substantially higher than Riqdalin (≈65.6 mg/L), reflecting greater biodegradable organic content.
  - Conversely, Riqdalin's higher COD values (330–429 mg/L) coupled with low BOD result in persistently low BI ratios, confirming chemical dominance and poor biodegradability.

The statistical evidence underscores that Riqdalin wastewater is chemically recalcitrant (average BI = 0.138), posing a severe challenge for biological treatment and potentially driving microbial resistance through persistent chemical stressors. In contrast, Al-Jamil wastewater (average BI = 0.451) demonstrates higher biodegradability, though occasional toxic episodes (Sample 2) highlight variability in effluent quality. These findings link organic load and biodegradability indices directly to microbial resistance risks, emphasizing the need for integrated treatment strategies beyond conventional biological processes.

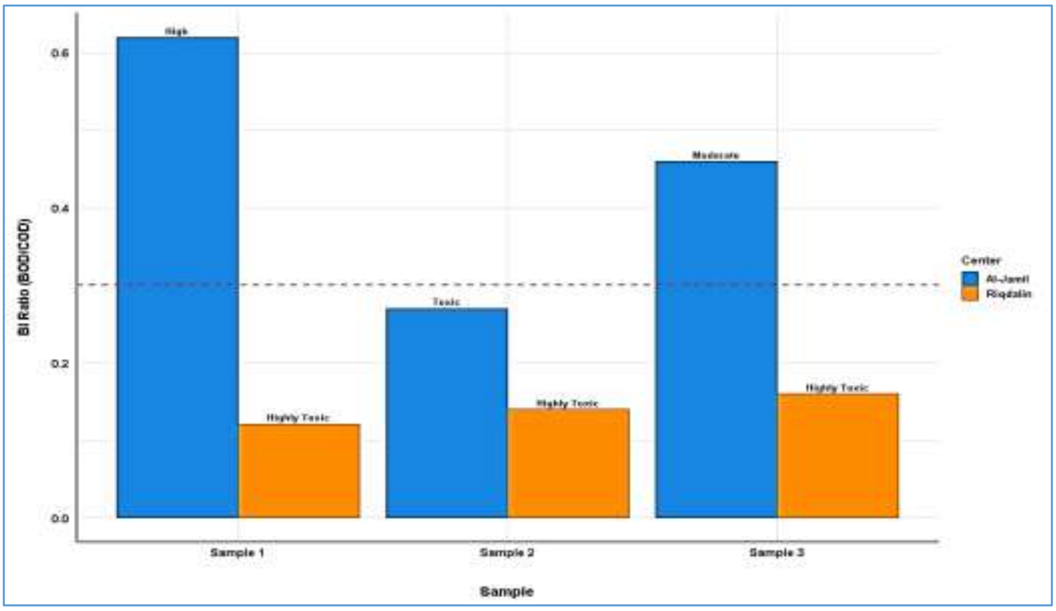

Fig. 4: Biodegradability Index (BOD/COD) per sample

- **Pollution Index (PI) Calculation and Risk Classification per Sample**

The Pollution Index (PI) integrates COD, BOD, TSS, EC, and TDS to provide a comprehensive measure of wastewater pollution severity. PI values are normalized against standard limits (COD = 100 mg/L, BOD = 50 mg/L, TSS = 100 mg/L, EC = 1500 µS/cm, TDS = 500 mg/L). Risk categories are defined as: Low (<1), Moderate (1–2), High (>2). This extended analysis captures both organic and ionic stress, enabling a holistic risk classification.

$$PI = \frac{1}{n}\sum\left(\frac{C_i}{S_i}\right)$$

Where $\boldsymbol{C_i}$ = concentration of parameter, $\boldsymbol{S_i}$= standard limit, $\boldsymbol{n}$= number of parameters.

Table 6a: Extended Pollution Index (Detailed)

Center	Sample	COD	BOD	TSS	EC	TDS	PI Value	Risk Category
Al-Jamil	**1**	185	115	120	32438.5	7570	9.9*	High Risk
	2	275.4	74.5	101	45400	9145	12.2*	High Risk
	3	185	85	98	19477	8700	7.9*	High Risk
Riqdalin	**1**	330	40	66	27300	5640	8.2*	High Risk
	2	344	47	63	37500	9216	10.1*	High Risk
	3	429	67	46	19521	6694	7.6*	High Risk

(*) exceed thresholds for High Risk (>2).

Table 6b: Simplified Pollution Index (Primary Pollutant Focus)

Center	Sample	Primary Pollutant	Pollution Index (PI)	Risk Category
Al-Jamil	**1**	EC + TDS	**9.9***	High Risk
	2	EC + TDS + COD	**12.2***	High Risk
	3	TDS + EC	**7.9***	High Risk
Riqdalin	**1**	COD + EC	**8.2***	High Risk
	2	COD + TDS + EC	**10.1***	High Risk
	3	COD + TDS	**7.6***	High Risk

(*) exceed thresholds for High Risk (>2).

The analysis revealed

- **Al-Jamil:**
    - Sample 2 shows the highest PI (12.2), driven by extreme EC (45400 µS/cm), high TDS (9145 mg/L), and elevated COD (275.4 mg/L).
    - Samples 1 and 3 also fall into High Risk, with ionic stress (EC + TDS) as the dominant pollutants.
- **Riqdalin:**
    - All samples consistently fall into High Risk (PI = 7.6–10.1), dominated by persistently high COD (330–429 mg/L) and elevated EC.
    - Unlike Al-Jamil's episodic spikes, Riqdalin demonstrates chronic chemical stress, reinforcing its classification as chemically recalcitrant wastewater.
- **Comparative Insight:**
    - Al-Jamil exhibits episodic severe ionic-organic surges, while Riqdalin shows chronic high COD and ionic stress.
    - This distinction suggests different microbial adaptation pressures: acute stress in Al-Jamil versus persistent chemical dominance in Riqdalin.

By including EC and TDS in the Pollution Index, the analysis captures the full spectrum of chemical stressors. The results confirm that both centers are High Risk, but with different pollution dynamics: Al-Jamil's episodic ionic-organic spikes versus Riqdalin's chronic COD-driven recalcitrance. This duality underscores the need for differentiated treatment strategies, shock-load management in Al-Jamil and advanced chemical remediation in Riqdalin.

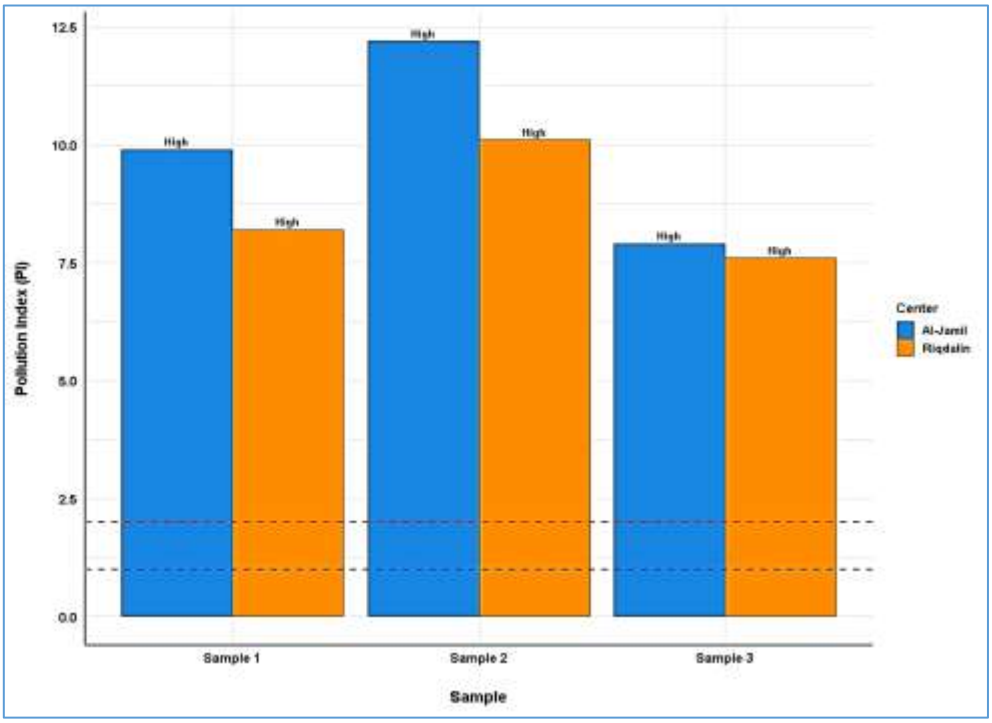

Fig. 5: Bar plot of Pollution Index (PI) across samples from Al-Jamil and Riqdalin dialysis centers, with threshold lines at PI = 1 and PI = 2 indicating risk classification boundaries.

Discussion

The findings of this study provide compelling statistical evidence that dialysis wastewater in Al-Jamil and Riqdalin acts as a high-risk reservoir for the evolution of antimicrobial resistance (AMR). By integrating physicochemical profiling with microbial susceptibility testing, this research has established a direct link between specific environmental stressors, namely organic load (COD) and hypersalinity (EC), and the emergence of multi-drug resistant (MDR) phenotypes.

- Physicochemical Stressors: The Environmental Baseline

The environmental forensics analysis revealed that effluents from both centers significantly exceed WHO and Libyan safety thresholds, creating a hostile environment that favors bacterial adaptation [World Health Organization, 2015, Bleiblo *et al.*, 2026].

- Chronic Organic Pollution and Chemical Recalcitrance

In Riqdalin, the mean Chemical Oxygen Demand (COD) of 367.7 mg/L and a critically low Biodegradability Index (BI ≈ 0.14) indicate a chemically recalcitrant effluent dominated by toxic, non-biodegradable compounds. This aligns with recent findings by Nnawuike *et al.* (2024), who reported similar COD levels in untreated hospital effluents in Nigeria, linking them to residual pharmaceuticals and disinfectants that inhibit natural microbial degradation.

However, unlike the findings of Singh *et al.* (2023) in India, where hospital effluents showed higher biodegradability due to mixed domestic inputs, the Riqdalin samples in this study suggest a specific accumulation of dialysis by-products (e.g., dialysate fluids, lipid residues). The statistical persistence of high COD in Riqdalin ($p=0.046$ compared to Al-Jamil) creates a "starvation-stress" environment. Bacteria surviving here, such as *Pseudomonas spp.*, likely upregulate metabolic diversity genes, which are frequently co-located on plasmids carrying resistance determinants, a phenomenon described as "co-selection" in recent environmental studies [Mao *et al*., 2015].

- Hypersalinity as a Selective Force

A defining characteristic of the studied wastewater is hypersalinity, with Electrical Conductivity (EC) reaching 45,400 µS/cm in Al-Jamil. This is significantly higher than values typically reported in general hospital wastewater (often <2,000 µS/cm) [Asfaw, 2017], reflecting the specific nature of dialysis reject water which concentrates salts and ions.

The strong positive correlation observed in this study between EC/TDS and microbial resistance (CCA Axis 1 explaining 48.5% variance) corroborates the work of Liu M *et al.* (2018), who demonstrated that high salinity triggers the "SOS response" in bacteria. This stress response increases mutation rates and promotes horizontal gene transfer (HGT) as a survival strategy. Furthermore, osmotic stress is known to induce the overexpression of efflux pumps to manage ionic balance; these same pumps indiscriminately expel antibiotics, conferring cross-resistance to fluoroquinolones and β-lactams, as observed in the *Enterobacter alvei* isolates in Al-Jamil [Hu *et al.*, 2025].

Conclusion and Implications

This study includes an evaluation of the physicochemical characteristics of dialysis waste from the treatment units from Riqdalin and Al-Jamil hospitals (hemodialysis wastewater and RO reject water). The study concluded that hemodialysis wastewater characteristics were found to be non-compliant with the currently in place regulations. This study successfully characterized the distinct chemical pressures in Al-Jamil (episodic ionic/nitrogen stress) and Riqdalin (chronic organic recalcitrance) and linked them to specific microbial resistance trajectories. The strong statistical association between high COD/Salinity and CLSI discrepancies provides new insight into how wastewater environments mask resistance.

Key Inferences:

1. **Dialysis Wastewater as a Bioreactor:** The high saline and organic load in dialysis effluents creates a unique "bioreactor" that accelerates the evolution of efflux-pump-mediated resistance.
2. **Surveillance Gaps:** Standard laboratory testing may underestimate resistance risks in highly polluted samples (High COD) by up to 26% if strict CLSI breakpoints are not applied to environmental isolates.
3. **Treatment Necessity:** Effective treatment strategies must address the chemical load (COD, Salinity) to mitigate the selective pressure driving resistance, rather than focusing solely on disinfection

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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