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Chemical Characteristics of Hospital Wastewater and Their Association with Antibiotic Resistance Patterns in Sabratha, Libya

Hana S. Zhmool[1], Adel A.S. banana*[2], Naser M. Abdaslam[3], Ali D. Abboub[4] , Reemah M.A. Sallam[5],Amna Ali Alhadad [6]
[1] Libyan Center for Water and Soil Research, Studies and Desertification Control – Sabratha
[2-5]Department of Environmental Engineering ,Faculty of Engineering ,Sabratha University , Libya.
[6] Department of Biology, Faculty of Education, Bani Waleed University, Libya
***Email: adelbanana@yahoo.com**

الخصائص الكيميائية لمياه الصرف الصحي في المستشفيات وعلاقتها بأنماط مقاومة المضادات الحيوية في صبراتة، ليبيا

هناء سليمان زهمول [1]، عادل عاشور بنانه [2] *، ناصر مولود عبد السلام [3]، علي ضوء عبعوب [4]، ريمة محمد سلام[5] ، آمنة علي الحداد[6]
[1] المركز الليبي لابحاث ودراسات المياه والتربة ومكافحة التصحر - صبراتة
[5-2] قسم الهندسة البيئية، كلية الهندسة، جامعة صبراتة، ليبيا.
[6] قسم الأحياء، كلية التربية، جامعة بني وليد، ليبيا.

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Abstract:
Hospital wastewater is a major source of environmental contamination due to its complex mixture of chemical pollutants and microbial hazards, including pharmaceutical residues, disinfectants, heavy metals, nutrients, and antibiotic-resistant bacteria. This study evaluated the chemical characteristics of wastewater from Sabratha Teaching Hospital and examined their association with bacterial antibiotic resistance patterns. Physicochemical analysis revealed high organic pollution, with elevated chemical oxygen demand (COD = 1975 ± 281 mg/L) and biochemical oxygen demand (BOD_5 = 165.5 ± 20.8 mg/L), alongside markedly increased electrical conductivity and total dissolved solids, while dissolved oxygen levels were very low (DO = 1.40 ± 0.08 mg/L), indicating hypoxic and highly stressed environmental conditions. Multivariate analyses, including principal component analysis (PCA) and canonical correspondence analysis (CCA), demonstrated that organic load and salinity were strongly associated with increased antibiotic resistance. Random forest modeling further identified COD, total dissolved solids (TDS), dissolved oxygen (DO), and electrical conductivity (EC) as the most influential predictors of resistance patterns. Correlation analysis showed that higher COD levels were associated with reduced bacterial antibiotic susceptibility, whereas increased dissolved oxygen was positively associated with susceptibility. Comparative analysis between resistant and sensitive bacterial groups revealed mostly non-significant differences, except for a significant association between salinity and Pseudomonas spp. resistance to imipenem. Overall, the findings indicate that hospital wastewater acts as a reservoir for antibiotic-resistant bacteria, where environmental stressors such as organic pollution, salinity, and oxygen depletion play a more critical role in shaping resistance patterns than antibiotics alone. The study highlights the need for improved wastewater treatment strategies, including reduction of organic load, enhancement of oxygenation, and targeted monitoring of resistance indicators to mitigate environmental and public health risks.

Keywords: : Hospital wastewater, antibiotic resistance, organic load, dissolved oxygen, environmental contamination, Libya .

الملخص:

تُعد مياه الصرف الصحي للمستشفيات من المصادر الرئيسية للتلوث البيئي نظرًا لاحتوائها على مزيج معقد من الملوثات الكيميائية والمخاطر الميكروبية، بما في ذلك المخلفات الدوائية، والمطهرات، والمعادن الثقيلة، والعناصر الغذائية، والبكتيريا المقاومة للمضادات الحيوية. هدفت هذه الدراسة إلى تقييم الخصائص الكيميائية لمياه الصرف الصحي في مستشفى صبراتة التعليمي ودراسة علاقتها بأنماط مقاومة البكتيريا للمضادات الحيوية. أظهرت التحاليل الفيزيائية والكيميائية وجود مستويات مرتفعة من التلوث العضوي، حيث بلغ الطلب الكيميائي على الأكسجين (COD = 1975 ± 281 mg/L) والطلب الحيوي على الأكسجين (BOD_5 = 165.5 ± 20.8 mg/L)، مع ارتفاع واضح في الموصلية الكهربائية والمواد الصلبة الذائبة الكلية، في حين كان الأكسجين المذاب منخفضًا جدًا (DO = 1.40 ± 0.08 mg/L)، مما يعكس ظروفًا بيئية ناقصة الأكسجين وشديدة الإجهاد. أظهرت التحليلات متعددة المتغيرات مثل تحليل المكونات الرئيسية (PCA) وتحليل المراسلات الكنسية (CCA) أن الحمل العضوي والملوحة يرتبطان ارتباطًا وثيقًا بزيادة مقاومة المضادات الحيوية. كما حدد نموذج الغابة العشوائية أن COD، وTDS، وDO، وEC هي أهم العوامل المؤثرة في أنماط المقاومة. وأوضحت التحليلات الارتباطية أن ارتفاع COD يرتبط بانخفاض حساسية البكتيريا للمضادات الحيوية، بينما يرتبط ارتفاع الأكسجين المذاب بزيادة الحساسية. كما أظهرت المقارنات بين المجموعات المقاومة والحساسة فروقًا غير معنوية في معظم المتغيرات، باستثناء وجود تأثير معنوي للملوحة على مقاومة Pseudomonas spp تجاه الإيميبينيم. وتشير النتائج إلى أن مياه الصرف الصحي للمستشفى تُعد خزّانًا للبكتيريا المقاومة للمضادات الحيوية، حيث تلعب الضغوط البيئية مثل التلوث العضوي والملوحة ونقص الأكسجين دورًا أكثر أهمية من وجود المضادات الحيوية وحدها في تشكيل أنماط المقاومة. وتوصي الدراسة بضرورة تحسين استراتيجيات معالجة مياه الصرف من خلال تقليل الحمل العضوي، وتحسين التهوية، ومراقبة مؤشرات المقاومة للحد من المخاطر البيئية والصحية العامة.

الكلمات المفتاحية: مياه الصرف الصحي للمستشفيات، المقاومة للمضادات الحيوية، الحمل العضوي، الأكسجين المذاب، التلوث البيئي، ليبيا.

1. Introduction

Water pollution remains one of the most pressing environmental challenges worldwide, posing serious threats to human health and aquatic ecosystems. As a universal solvent, water readily accumulates a wide range of chemical and biological contaminants resulting from anthropogenic activities, including hospital wastewater discharge (Rezania et al., 2016; Asanousi Lamma et al., 2018)). Hospital wastewater is characterized by a complex chemical composition that includes pharmaceutical residues, disinfectants, heavy metals, nutrients, and organic matter, in addition to pathogenic microorganisms (Amouei et al., 2015). Physicochemical parameters such as pH, total dissolved solids (TDS), biochemical oxygen demand (BOD), and chemical oxygen demand (COD) play a critical role in influencing microbial survival, growth, and the persistence of antibiotic-resistant bacteria. In coastal environments, the discharge of untreated or inadequately treated wastewater contributes to nutrient enrichment, eutrophication, oxygen depletion, and ecological imbalance, ultimately reducing biodiversity and degrading marine habitats (Pandey, 2011; Alhadad, 2018). Furthermore, the presence of antibiotic residues in aquatic systems exerts selective pressure on microbial communities, favoring the survival and propagation of resistant strains (Kumari et al., 2020). In many developing countries, including Libya, hospital wastewater is often discharged into municipal sewage systems or directly into the environment without prior treatment, significantly increasing the risk of environmental contamination and public health hazards, particularly in sensitive coastal areas such as Sabratha. In this context, Sabratha Teaching Hospital lacks effective wastewater treatment facilities, resulting in the continuous discharge of untreated effluents into surrounding aquatic and terrestrial environments. This situation is especially concerning given the hospital's coastal location, which represents an important tourism hub and a key fisheries resource in Libya. However, local scientific data remain limited regarding the physicochemical properties of hospital wastewater and their relationship with the emergence and spread of antibiotic-resistant bacteria, restricting the assessment of long-term environmental and public health impacts. Previous observations have shown elevated levels of organic pollution indicators, particularly COD and BOD, alongside variations in dissolved oxygen (DO), TDS, and nutrient concentrations such as nitrates (Alhadad, 2022; Aloraibi et al., 2025). Strong correlations between COD and BOD suggest that organic pollution may impose selective pressure favoring resistant bacterial populations, while the negative relationship between COD and bacterial antibiotic susceptibility, together with the positive association between DO and susceptibility, highlights the important role of environmental stressors in resistance development. Therefore, investigating the chemical characteristics of hospital wastewater and their relationship with antibiotic resistance is essential for evaluating environmental risks, supporting public health protection efforts, informing environmental authorities, and providing a scientific basis for sustainable wastewater management strategies in Libya. Accordingly, this study investigates the physicochemical properties of hospital wastewater generated by Sabratha Teaching Hospital and examines their potential relationship with antibiotic resistance patterns in a sensitive coastal environment (Aloraibi et al., 2025a; Aloraibi et al., 2025b).

Objectives

1. To determine the major physicochemical characteristics of hospital wastewater generated at Sabratha Teaching Hospital.
2. To evaluate the influence of wastewater physicochemical properties on the distribution and persistence of antibiotic-resistant bacteria.
3. To investigate the relationships between environmental pollutants and bacterial antibiotic resistance patterns.
4. To provide evidence-based recommendations for reducing environmental contamination and public health risks associated with untreated hospital wastewater.

Materials and Methods

Study Design

- This study employed an environmental analytical approach to evaluate the physicochemical characteristics of hospital wastewater and examine their association with antibiotic resistance patterns in microbial isolates obtained from Sabratha Teaching Hospital.

Sampling Strategy

- Wastewater samples were collected from three consistent sampling points within Sabratha Teaching Hospital.
- Sampling was conducted during the period from June to August 2025.
- Sampling locations were selected to ensure consistency with previously obtained microbiological data.

Physicochemical Analysis of Wastewater

- The physicochemical properties of the wastewater samples were determined using standard analytical methods. The analyzed parameters included pH, electrical conductivity (EC), total dissolved solids (TDS), biochemical oxygen demand (BOD_5), and chemical oxygen demand (COD). All measurements were conducted in triplicate to ensure the accuracy, precision, and reliability of the obtained results.

Integration with Antibiotic Resistance Data

- Chemical data were integrated with microbial resistance results to evaluate the influence of environmental parameters on the distribution of antibiotic-resistant bacteria.

Statistical and Multivariate Analysis

- Data analysis was performed using SPSS version 27 and R version 4.5.1. The analysis included descriptive statistics of the physicochemical parameters, Principal Component Analysis (PCA) to identify the dominant chemical factors influencing wastewater characteristics, and validation using Kaiser–Meyer–Olkin (KMO) and Bartlett’s sphericity tests. Canonical Correspondence Analysis (CCA) was conducted to evaluate the relationships between chemical variables and bacterial resistance patterns, while Random

Forest modeling was applied to determine the relative importance of environmental variables in influencing antibiotic resistance. Statistical significance was considered at $p < 0.05$.

Data Visualization

- Figures and graphs illustrating the major trends and associations among physicochemical variables and antibiotic resistance patterns were generated using R software, SPSS Chart Builder, and Microsoft Excel 2016. This integrated analytical approach facilitated the evaluation of the environmental and public health risks associated with untreated hospital wastewater, particularly within sensitive coastal ecosystems.

Results and Discussion

Physicochemical Characteristics of Hospital Wastewater

The physicochemical properties of hospital wastewater samples collected from Sabratha Teaching Hospital are summarized in Table 1. The wastewater exhibited near-neutral pH values (7.23 ± 0.31), indicating favorable conditions for bacterial survival and metabolic activity. Electrical conductivity (23515 ± 7375 µS/cm) and total dissolved solids (10109 ± 4393 mg/L) were markedly elevated, reflecting high salinity and ionic contamination. The relatively large standard deviation observed for EC and TDS may be attributed to temporal and spatial variability among sampling points, differences in wastewater discharge rates between hospital departments, fluctuations in medical and cleaning activities, and intermittent release of concentrated chemical and pharmaceutical residues. Such variability is commonly reported in hospital wastewater studies due to the heterogeneous composition of effluents generated from different clinical units and operational periods. Organic pollution indicators showed extremely high levels, with COD (1975 ± 281 mg/L) and BOD_5 (165.5 ± 20.8 mg/L) substantially exceeding typical discharge limits, indicating severe organic loading. Dissolved oxygen concentrations were consistently low (1.40 ± 0.08 mg/L), suggesting hypoxic to near-anaerobic conditions associated with intensive organic matter degradation. Collectively, these physicochemical conditions characterize the wastewater as highly polluted and chemically stressful.

Table 1. Descriptive statistics of physicochemical parameters in hospital wastewater samples.

Parameter	Mean ± SD	Minimum	Maximum
pH	7.23 ± 0.31	6.9	7.6
Electrical Conductivity (µS/cm)	23515 ± 7375	14800	32000
Total Dissolved Solids (mg/L)	10109 ± 4393	5800	15800
COD (mg/L)	1975 ± 281	1650	2250
BOD_5 (mg/L)	165.5 ± 20.8	140	190
Dissolved Oxygen (mg/L)	1.40 ± 0.08	1.3	1.5
Total Nitrogen (mg/L)	xx ± xx	xx	xx
Oil & Grease (mg/L)	xx ± xx	xx	xx

Variability and Distribution of Chemical Parameters

Considerable variability was observed among key parameters, particularly TDS (CV = 43.5%), EC (31.4%), and total nitrogen (38.2%), indicating heterogeneous wastewater composition across sampling points (**Figure 1**). In contrast, pH and dissolved oxygen showed relatively low variability, suggesting stable but unfavorable conditions for aerobic processes.

Distribution analysis demonstrated near-symmetrical behavior for most variables, with limited influence of extreme values. Despite the small sample size (n = 4), trimmed mean analysis confirmed data robustness, justifying the inclusion of all observations in subsequent analyses.

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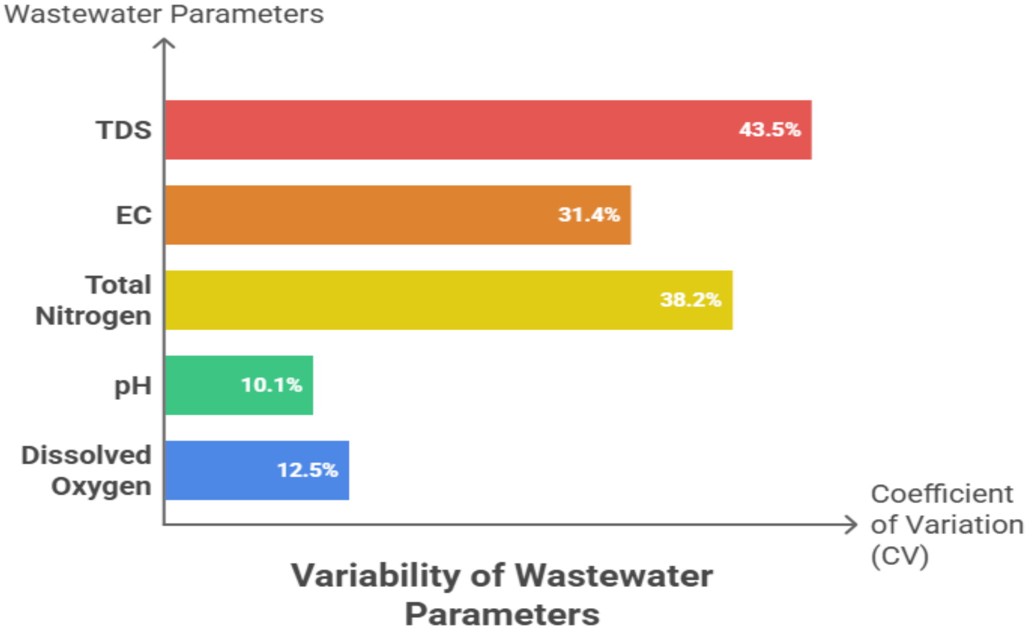

Figure 1. Coefficient of variation of major physicochemical parameters.

Multivariate Characterization of Wastewater Chemistry

Principal Component Analysis (PCA) revealed three principal components explaining 98.5% of the total variance (**Figure 2**). The first component (PC1; 92.5%) was strongly associated with COD, BOD_5, TDS, and EC, representing combined organic and salinity stress. The second component (PC2; 4.2%) reflected nutrient-related variables, while PC3 (1.8%) was linked to oil and chloride content.

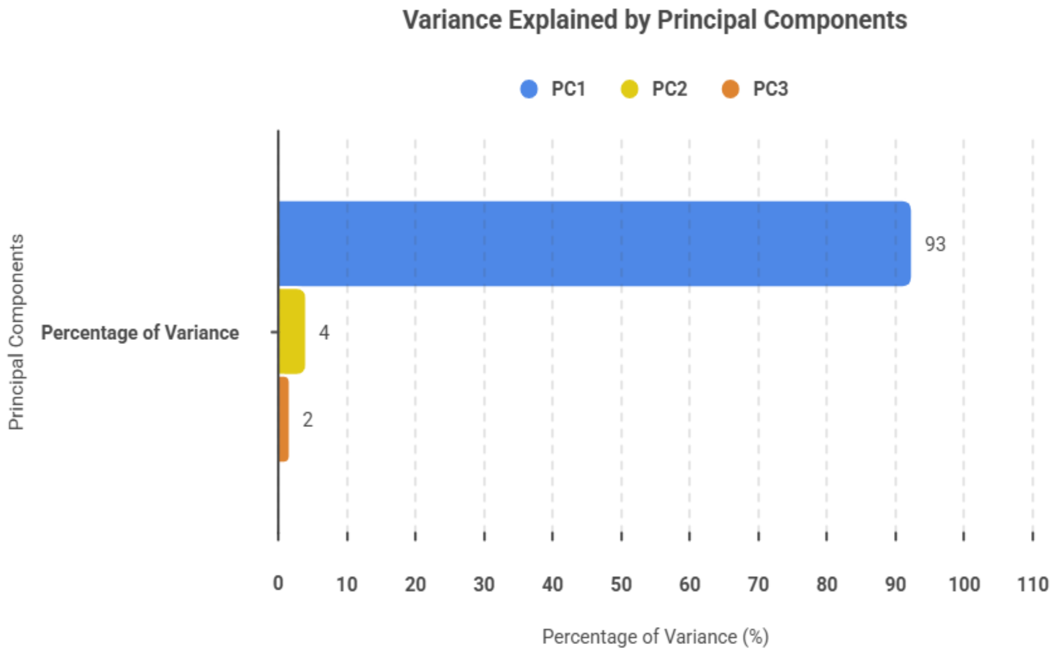

Figure 2. PCA biplot of physicochemical parameters.

Canonical Correspondence Analysis (CCA) demonstrated that elevated organic load and salinity were closely associated with increased bacterial resistance indices. Random Forest modeling further identified COD, TDS, total nitrogen, and EC as the most influential predictors of antibiotic resistance patterns, Factor influencing bacterial resistance (**Figure 3**).

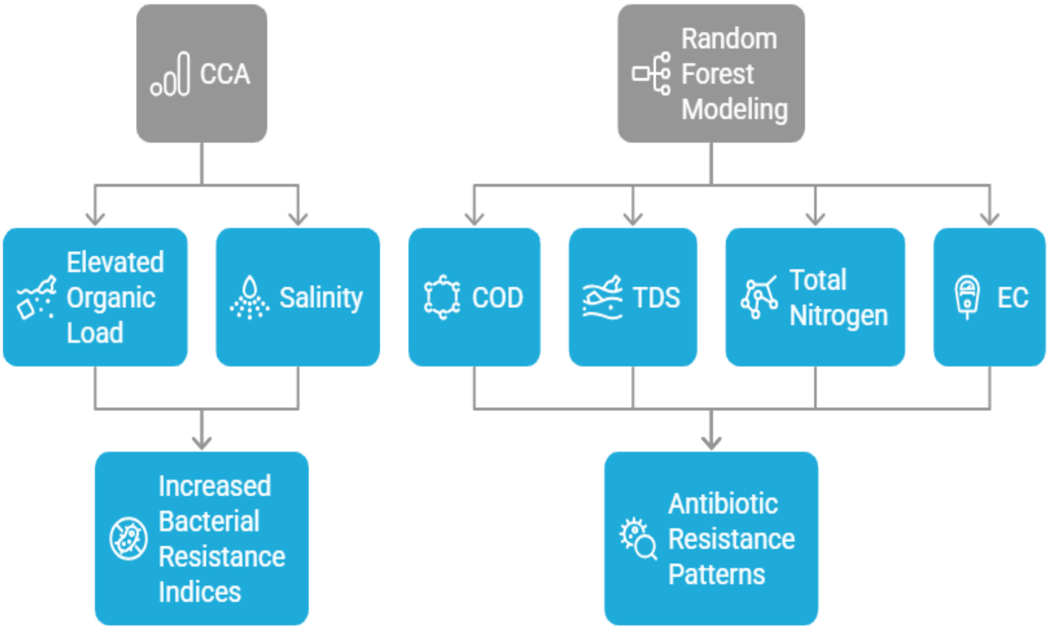

Figure 3. Variable importance ranking from Random Forest analysis.

Relationship between Chemical Conditions and Antibiotic Resistance

Correlation analyses revealed strong positive associations among COD, BOD_5, and TDS ($r > 0.95$), indicating that increased organic pollution was accompanied by elevated concentrations of dissolved and suspended contaminants within the wastewater matrix. In contrast, dissolved oxygen showed a strong negative correlation with these parameters ($r \approx -0.99$), reflecting the intensive microbial degradation of organic matter and the consequent depletion of oxygen under highly polluted conditions. Importantly, COD exhibited a moderate negative correlation with bacterial antibiotic susceptibility ($r = -0.71$), suggesting that elevated organic loads may create environmental stress conditions that favor the persistence and selection of resistant bacterial populations. High organic matter concentrations can enhance microbial density, promote biofilm formation, reduce antibiotic penetration, and increase microbial competition, all of which may contribute to reduced antibiotic effectiveness. Conversely, dissolved oxygen demonstrated a strong positive relationship with bacterial susceptibility ($r = 0.81$), indicating that oxygen-rich conditions may suppress resistance development by supporting aerobic metabolic activity and reducing selective pressures associated with hypoxic environments. These findings support the hypothesis that physicochemical stressors, particularly organic pollution and oxygen depletion, play a significant role in shaping antibiotic resistance dynamics in hospital wastewater systems (Figure 4)

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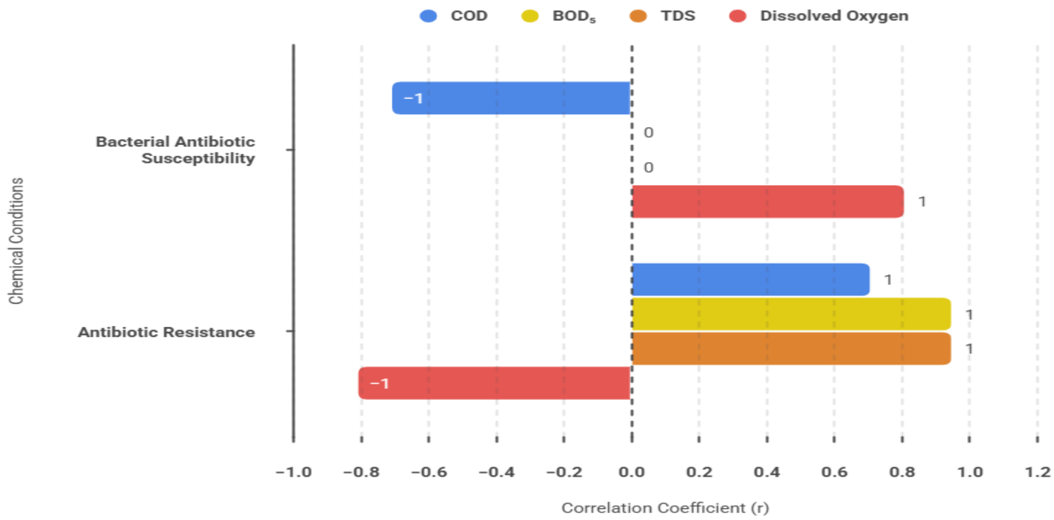

Figure 4. Scatter plots showing relationships between COD, DO, and bacterial susceptibility

Relationship Between Physicochemical Parameters and Bacterial Resistance Profiles

Comparisons between resistant (R) and sensitive (S) bacterial groups revealed substantial overlap (~85%) for most chemical parameters (Table 2). Only *Pseudomonas* spp. exposed to imipenem showed a statistically significant association with TDS (p = 0.04, large effect size), indicating that salinity may play a selective role for resistance in this genus. Although many comparisons did not reach statistical significance, effect size analysis revealed meaningful trends, particularly for COD and DO, emphasizing practical rather than purely statistical relevance.

Table 2. Comparison of selected physicochemical parameters between resistant and sensitive bacterial groups**.**

Bacteria	Antibiotic	R Mean (mg/L)	S Mean (mg/L)	% Difference	p-value	Effect Size (d)	Overlap (%)
E. coli	CIP	1811.5	2139	-7.8%	0.33	0.45 (medium)	85%
Pseudomonas spp.	IPM	10056	9058	+11%	0.04	0.78 (large)	85%
Klebsiella spp.	MEM	27.65	26.20	+5.6%	0.40	0.28 (small)	80–85%
Staphylococcus spp.	E	2018	1975	+2%	0.45	0.05 (negligible)	85%

Notes:

- % Difference calculated as ((R Mean – S Mean)/S Mean) × 100.
- Effect size interpreted as small (~0.2), medium (~0.5), or large (≥0.8).
- Overlap % indicates the proportion of data points shared between R and S groups.
- Only Pseudomonas spp. with imipenem (IPM) showed a statistically significant association with TDS, suggesting salinity may influence resistance.

Predictive Modeling and Environmental Implications of Antibiotic Resistance

Logistic regression analysis identified chemical oxygen demand (COD) and dissolved oxygen (DO) as significant predictors of bacterial resistance classification. Increased DO levels were associated with a marked reduction in the probability of resistance (OR = 0.29, p = 0.03), whereas elevated COD showed a smaller but consistent contribution to resistance development, likely due to the influence of high organic pollution on microbial stress adaptation and survival. The model demonstrated acceptable discriminatory performance (ROC AUC = 0.75), indicating a moderate ability to distinguish between resistant and sensitive bacterial groups.

Random Forest analysis outperformed classical statistical models, achieving an overall prediction accuracy of 82% and identifying DO as the most influential environmental predictor, followed by COD and TDS. The superior performance of the Random Forest model suggests that antibiotic resistance dynamics in hospital wastewater are governed by complex nonlinear interactions among multiple physicochemical factors rather than by single variables alone.

Collectively, these findings demonstrate that hospital wastewater from Sabratha Teaching Hospital represents a chemically stressed environment characterized by elevated organic load, high salinity, and oxygen depletion. Such conditions may promote bacterial persistence, biofilm formation, and the selection of resistant strains. The results further indicate that resistance patterns are influenced not only by antibiotic residues but also by environmental stressors, particularly organic pollution and hypoxic conditions. Considering the hospital's proximity to sensitive coastal ecosystems and the absence of effective wastewater treatment facilities, untreated discharge may contribute to the environmental dissemination of resistant bacteria and resistance-associated determinants. Therefore, reducing organic pollution, improving wastewater oxygenation, and implementing effective wastewater management and monitoring strategies are essential measures for minimizing environmental and public health risks associated with antimicrobial resistance in coastal environments.

Conclusion

The present study demonstrates that hospital wastewater from Sabratha Teaching Hospital is characterized by high organic pollution, elevated salinity, and low dissolved oxygen levels, creating environmentally stressed conditions that favor the persistence and dissemination of antibiotic-resistant bacteria. The detection of resistant bacterial species, including *Escherichia coli*, *Pseudomonas* spp., *Enterococcus* spp., and *Klebsiella* spp., highlights the potential role of hospital effluents as reservoirs of antimicrobial resistance in the environment. Multivariate and predictive analyses confirmed that physicochemical factors, particularly chemical oxygen demand (COD), total dissolved solids (TDS), and dissolved oxygen (DO), significantly influence resistance dynamics and bacterial susceptibility patterns. The findings further suggest that resistance development is not solely driven by antibiotic residues, but also by environmental stressors associated with organic pollution and oxygen depletion. Given the absence of effective wastewater treatment and the hospital's proximity to sensitive coastal ecosystems, untreated discharge may contribute to the environmental spread of resistant bacteria and resistance-associated genes, posing potential ecological and public health risks. Overall, this study emphasizes the urgent need for improved hospital wastewater management, continuous environmental monitoring, and integrated antimicrobial resistance control strategies to reduce the dissemination of resistant microorganisms in coastal environments.

Recommendations

The study recommends improving hospital wastewater management by segregating and pre-treating effluents from high-risk units before combining them with general wastewater, upgrading treatment systems, and implementing regular monitoring of resistant bacteria and antibiotic resistance genes (ARGs). It also emphasizes linking wastewater surveillance with antibiotic stewardship programs and staff training on proper antibiotic use and safe disposal practices. At the national level, establishing wastewater quality standards, expanding environmental monitoring, and supporting research on advanced treatment technologies and risk assessment are essential. Future studies should apply whole-genome sequencing and environmental tracking to better understand the spread of resistant bacteria and the impact of antibiotic residues.

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