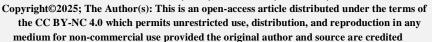


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#### **CASE STUDY**

# A SYSTEMS BASED APPROACH TO MICROBIOLOGICAL **QUALITY ASSESSMENT IN A HEALTHCARE FACILITY'S** WATER DISTRIBUTION NETWORK: A CASE STUDY

Mostafa Essam Eissa<sup>©</sup>

Independent Researcher and Consultant, Cairo, Egypt.

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## \*Address for Correspondence:

Dr. Mostafa Essam Eissa, Independent Researcher and Consultant, Cairo, Egypt. Tel: +201006154853: E-mail:

mostafaessameissa@yahoo.com

#### Abstract

Background: Water distribution systems within healthcare facilities are complex ecosystems that can harbor opportunistic pathogens, posing a significant risk to patient safety. Ensuring the microbiological quality of water requires rigorous monitoring and a deep understanding of the entire system, from source to point-ofuse. This study undertakes a comprehensive statistical analysis of microbiological data from a healthcare facility's water treatment and distribution network to identify contamination hotspots, evaluate the efficacy of critical treatment barriers, and map potential contamination pathways.

**Methods:** This retrospective case study analyzed heterotrophic plate count (HPC) data collected from 29 distinct sampling points throughout a healthcare facility's water system. The points represented various stages, including municipal source water, intermediate treatment steps (softening, ultrafiltration, reverse osmosis), storage tanks, and multiple points-of-use. Non-parametric statistical methods were employed due to the non-normal distribution of microbial data. A Kruskal-Wallis test with Dunn's post-hoc analysis was used to compare microbial loads across all sampling points. A focused Mann-Whitney U test was performed to assess the performance of the ultrafiltration (UF) unit.

Results: The analysis revealed significant variability in microbiological quality throughout the system (Kruskal-Wallis, p<0.0001). One point-of-use and a pre-UF exhibited high median microbial counts and extreme variability, indicating chronic contamination and potential biofilm proliferation. Critically, the ultrafiltration unit failed to demonstrate a statistically significant reduction in microbial load between the pre-filter.

Conclusion: The data reveals variability in microbiological levels across the water system, suggesting that the ultrafiltration barrier's performance is a significant factor influencing downstream water quality. The data points to systemic issues, likely involving widespread biofilm, that compromise water quality at the points-

Keywords: Biofilm, healthcare associated infections, hospital water quality, opportunistic pathogens, ultrafiltration, water treatment.

## INTRODUCTION

Water is an indispensable resource in healthcare facilities, essential for everything from basic hygiene and sanitation to direct patient care, medical device and pharmaceutical reprocessing, preparations. However, the extensive and complex water distribution systems within these facilities can also serve as reservoirs and transmission vectors for a variety of opportunistic waterborne  $(OWPs)^{1}$ . pathogens Organisms such as Legionella pneumophila, P. aeruginosa, M. avium, and various species of Acinetobacter and Stenotrophomonas can colonize these

engineered ecosystems, posing a severe threat, particularly to immunocompromised, elderly, and critically ill patients<sup>2</sup>. Healthcare associated infections (HAIs) linked to contaminated water can lead to increased patient morbidity and mortality, prolonged hospital stays, and substantial economic costs<sup>3</sup>.

The primary mechanism by which OWPs persist in hospital plumbing is through the formation of biofilms. Biofilms are complex, sessile communities of microorganisms encased in a self-produced matrix of extracellular polymeric substances, which adhere to pipe surfaces4. Once established, biofilms provide a protective niche for bacteria, shielding them from

ISSN: 2456-8058 CODEN (USA): UJPRA3 disinfectants, temperature fluctuations, and hydraulic shear forces. They can act as a chronic source of contamination, intermittently sloughing off planktonic cells or biofilm fragments into the bulk water, leading to unpredictable and often high microbial counts at distal outlets<sup>5</sup>. Factors that promote biofilm growth in hospital water systems include large surface-to-volume ratios, complex pipe networks with areas of stagnation (dead legs), nutrient availability, and water temperatures that are often optimal for microbial proliferation (25-45°C).

To mitigate these risks, healthcare facilities employ multi-barrier water treatment systems designed to remove or inactivate microorganisms. These systems often include several stages, such as water softening to remove hardness, activated carbon filtration for chlorine and organic removal, reverse osmosis (RO) for producing high-purity water, and ultrafiltration (UF) as a physical barrier against bacteria and viruses. Ultraviolet (UV) disinfection is also commonly used as a final polishing step. The effectiveness of this entire treatment train is paramount. A failure at any single point can compromise the integrity of the entire system, allowing microbial intrusion and subsequent colonization of downstream components, including storage tanks and the final distribution loop<sup>6</sup>.

Despite the presence of these sophisticated engineering controls, ensuring consistent microbiological quality remains a significant challenge. HPCs, while not a direct measure of pathogenic risk, serve as a crucial indicator of general water quality and the potential for biofilm proliferation<sup>7</sup>. High variability or a sudden increase in HPCs can signal a loss of microbiological control within the system. Therefore, a robust, data-driven approach to analyzing monitoring results is essential for proactive risk management. Statistical analysis of long-term monitoring data can move facilities from a reactive "find-and-fix" approach to a predictive, systems-based understanding of their water network's performance.

This study presents a comprehensive microbiological quality assessment of a large, operational healthcare facility's water system. By applying rigorous statistical analysis to a substantial dataset of HPC results from across the treatment and distribution network, this research aims to characterize the microbiological burden and its variability at numerous points from source to use. Statistically evaluate the performance of a critical treatment barrier the ultrafiltration unit. Identify potential contamination pathways and hotspots by analyzing the correlations between different parts of the system. Provide evidence-based, actionable recommend-dations for engineering interventions and enhanced monitoring strategies to improve water safety and protect patients.

### MATERIALS AND METHODS

#### **Study Design and Data Source**

This study was a retrospective; descriptive analysis of microbiological monitoring data collected from a single healthcare facility's water system in Asian country covering the region embracing India, Pakistan and Bangladesh<sup>8</sup>. The anonymized dataset consisted of heterotrophic plate count (HPC) results, recorded as

colony-forming units per milliliter (CFU/100 mL), from routine water quality surveillance<sup>9</sup>. The data covered a period of approximately three years, providing a robust basis for assessing temporal trends and system performance.

## **Description of the water system and sampling points**

The facility operates a complex, multi-barrier water treatment and distribution system. Water samples were collected from 29 distinct locations chosen to represent all critical stages of the network.

The sampling points (and their symbolic codes used in the dataset) included <sup>10-12</sup>:

**Source Water (SW):** SW-01 - Municipal water entering the facility.

**Pre-Treatment (PT):** PT-01 - Water post-ion exchange softening; PT-02-Point of dichlorination by chemical neutralizer addition.

**Primary Treatment (TR):** TR-01A and TR-01B - Water immediately before and after the ultrafiltration (UF) unit; TR-02 - Water post-reverse osmosis; TR-03 - Water post-electrode ionization; TR-04 - Water post-ultraviolet disinfection.

**Storage (ST):** ST-01 - Central storage tank for purified water; ST-02 and ST-03 - City water storage tanks; ST-04 - A separate purified water storage tank.

**Distribution Loop (DL):** DL-01 - Water returning to the central system from the main distribution loop.

**Points-of-Use (POU):** A series of 16 points designated POU-01 through POU-16, representing various outlets in quality control, operation area, clinical support, and general facility areas.

This comprehensive sampling plan allowed for a thorough evaluation of each component's contribution to the final water quality.

## **Data Analysis**

All statistical analyses were conducted using a standard statistical software package. A p-value of < 0.05 was considered statistically significant for all tests<sup>13-15</sup>.

## Descriptive statistics and normality testing

Descriptive statistics, including the number of samples (N), minimum, maximum, mean, median, standard deviation (SD), and interquartile range (IQR), were calculated for HPCs at each of the 29 sampling points. The distribution of the data was assessed for normality using different statistical tests, including Shapiro-Wilk, and Kolmogorov-Smirnov tests 16,17.

### **Comparative Analysis**

To compare microbial loads across the system, the following non-parametric tests were employed:

- Kruskal-Wallis test: This test, the non-parametric equivalent of a one-way ANOVA, was used to determine if there were statistically significant differences in the median HPCs among all 29 sampling groups<sup>18</sup>.
- **Dunn's multiple comparisons test:** Following a significant Kruskal-Wallis result, Dunn's test was used as a post-hoc analysis to perform pair wise comparisons between sampling points, identifying exactly where the significant differences lay<sup>19</sup>.
- Mann-Whitney U test: This test, the nonparametric equivalent of an independent t-test, was used for a focused comparison of HPCs between

two independent groups: the Pre-UF and Post-UF samples<sup>20</sup>. This was done to specifically evaluate the log-reduction performance of the ultrafiltration unit

#### **Correlation analysis**

To investigate the relationships and potential contamination pathways between different locations in the water network, Spearman's rank correlation coefficient ( $\rho$ ) was calculated for all pairs of sampling points<sup>21</sup>. This non-parametric measure assesses the strength and direction of a monotonic relationship between two variables.

## **RESULTS**

As is typical for environmental microbial data, the HPC counts were found to be non-normally distributed across

most sampling points, necessitating the use of non-parametric statistical methods for subsequent analyses.

#### Descriptive analysis of microbial contamination

The analysis of descriptive statistics revealed extreme variability in microbiological quality across the water system (Table 1). The incoming municipal water (SW-01) was of high quality, with a low median HPC (3.0 CFU/100 mL) and a tight distribution (IQR: 0.0-9.25 CFU/100 mL). Nevertheless, several points within the facility demonstrated significantly higher levels of microbial content and fluctuations. Importantly, skewness of data was found to be 4.803 (highly right-skewed  $\rightarrow$  frequent low counts with extreme outliers), 90<sup>th</sup> percentile: 27.5 CFU/100 mL (critical for quality thresholds) and 10<sup>th</sup> percentile: 0.0 CFU/100 mL  $\rightarrow$  10% of samples had detectable counts. All 29 parameters failed normality tests (D'Agostino, Shapiro-Wilk, KS; p < 0.0001) justifying the use of non-parametric tests.

Table 1: Descriptive statistics for selected key sampling points.

Abbreviation	Description	Median	Std. Deviation
	•	(CFU/100 mL)	
SW-01	Source Water (Municipal)	3.0	36.97
TR-01A	Pre-Ultrafiltration	68.0	2136.0
TR-01B	Post-Ultrafiltration	26.0	675.1
TR-02	Reverse Osmosis	1.0	548.7
ST-01	Central Purified Water Tank	20.0	63.91
ST-04	Purified Water Tank	2.0	50.46
DL-01	Distribution Loop Return	0.0	34.58
POU-01	Point of Use 01	240.0	2893.0

For instance, the point-of-use POU-01 showed a median count of 240.0 CFU/100 mL, but a mean of 1311.0 CFU/100 mL and a very high standard deviation of 2893.0 CFU/100 mL. Moreover, geometric mean was found to be 200.6 (better measure for skewed data). The large discrepancy between the mean and median, coupled with the high standard deviation, indicates the presence of frequent, extreme contamination events. Similarly, the pre-UF had a median of 68.0 CFU/100 mL but a maximum recorded value of 8900.0 CFU/100 mL, again pointing to significant process instability. The main circulation loop (DL-01), representing the overall quality of water circulating in the system, had a median of 0.0 CFU/100 mL but a very wide range, with a maximum value of 300.0 CFU/100 mL across 354 samples. This suggests a persistent, low-level background of contamination punctuated by periodic increases.

# System-wide comparison of microbial loads

The Kruskal-Wallis's test confirmed that there were highly significant differences in the median HPCs among the 29 sampling locations (H=593.5, p<0.0001). This result validates that the observed variations in

microbial contamination across the system are not due to random chance but reflect true differences in the performance and condition of the network's various components. Dunn's multiple comparisons test provided a granular view of these differences. Key significant findings included: The municipal water point was not generally significantly cleaner than most downstream points, including the return of distribution loop (DL-01), a storage tank (ST-04), and one point-of-use (POU) outlet except the first point-of-use and softener unit but not with UF and unit.

#### Performance evaluation of the UF unit

A critical finding of this study emerged from the focused analysis of the ultrafiltration unit (Table 2). The Mann-Whitney U test was used to compare the HPCs immediately before (Pre-UF) and after (Post-UF) this filtration step. The median HPC for Pre-UF water was  $68.0 \, \text{CFU}/100 \, \text{mL}$  (N=59), while the median for Post-UF water was  $26.0 \, \text{CFU}/100 \, \text{mL}$  (N=52). Despite this apparent reduction in the median value, the difference was not statistically significant (Mann-Whitney; U= 1318, p=0.2031).

Table 2: Mann-Whitney Test for Ultrafiltration Efficacy (TR-01A vs. TR-01B).

Parameter*	Value	
Median of Pre-UF (TR-01A)	68.00 CFU/100 mL	
Median of Post-UF (TR-01B)	26.00 CFU/100 mL	
<i>p</i> -value	0.2031	
Hodges-Lehmann median difference	-16.0CFU/100 mL	
Conclusion	Not Significant	

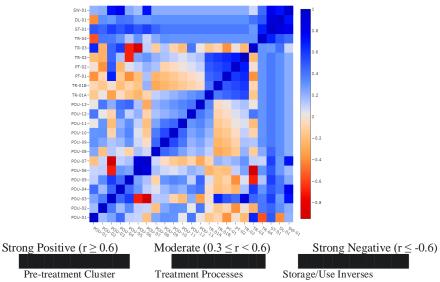


Figure 1: Correlation matrix as heatmap for different locations in water distribution and processing system.

This lack of statistical significance indicates that the UF unit is failing to perform its function as a reliable and effective microbial barrier. While it may be removing some bacteria, its performance is inconsistent and insufficient to guarantee a significant improvement in water quality. Nevertheless, the effect size measure by Hodges-Lehmann estimate shows a median difference of -16.00 CFU/100 mL (Pre-UF>Post-UF). Thus, while statistically non-significant, the UF unit reduced median counts by 23.5% (68.0→52.0 CFU/100 mL post-filtration).

## Correlation analysis of contamination pathways

The Spearman's rank correlation analysis provided insights into the dynamic relationships between different parts of the water system. The correlation matrix revealed several strong, positive correlations (p>0.5) that suggest potential pathways for the spread of contamination (Figure 1):

**A. pretreatment interdependencies (high correlation)** Softener (PT-01) ↔ Sodium Metabisulfite (PT-02): r=+0.68 (n=44), Indicates coordinated microbial behavior between chemical pretreatment units.

#### B. storage-loop dynamics

Central Tank (ST-01)  $\leftrightarrow$  Loop Return (DL-01): r=+0.68 (n=54), Reflects bidirectional contamination exchange.

# C. source water influences

Municipal Source (SW-01)  $\leftrightarrow$  Sodium Metabisulfite (PT-02): r=+0.70 (n=16), Suggests source quality directly impacts chemical pretreatment efficacy.

## D. unexpected inverse relationships

Purified Water Tank (ST-04) ↔ Restroom POU (POU-14):r=-0.31 (n=87), indicates possible cross-connection issues.

# **Quality-control anomalies**

Artifactual Correlations (excluded from analysis): RO (TR-02)  $\leftrightarrow$  Water Tank (ST-02): r=+1.00 (n=5, insufficient samples) and UV (TR-04)  $\leftrightarrow$  gown change POU (POU-16): r=+0.87 (n=22, marginal significance).

## Heatmap-derived system insights

**Pretreatment cluster:** PT-01/PT-02/Pre-UF show moderate intercorrelations (r=0.35-0.60) which suggests shared vulnerability to microbial breaches. Treatment

**Barrier Decoupling:** RO/EDI/UV show minimal correlation with downstream points ( $|r| \le 0.25$ ) confirms treatment inefficacy identified in Mann-Whitney tests. Point-of-Use Hotspots: POU-01/POU-05/POU-14 form subcluster with r=0.31-0.43 indicates the possibility of localized biofilm development.

Conversely, the lack of correlation between the highquality RO water and many of the contaminated downstream points further reinforces the conclusion that contamination is being introduced by components within the distribution system (i.e., tanks and pipe work) rather than passing through the primary treatment train.

## DISCUSSION

This comprehensive statistical analysis of a healthcare water system's microbiological data provides a detailed and concerning picture of its operational state<sup>22</sup>. The findings move beyond simple pass/fail metrics to reveal systemic vulnerabilities and specific points of failure that pose a potential risk to patient safety.

# Potential biofilm proliferation and system instability

The most pervasive theme emerging from the data is that of widespread and uncontrolled microbial growth, characteristic of extensive biofilm colonization. The high standard deviations and the significant positive skew (mean > median) observed at numerous sampling points, particularly at point-of-use (POU-01) and in storage (ST-04), are classic signatures of a system contaminated with mature biofilm<sup>23</sup>. For UP1 (POU-01), the median was 240.0 CFU/100 mL while the mean was 1311.0 CFU/100 mL (SD=2893.0), confirming extreme skewness (skewness=2.726).ST-04 showed similar divergence (median=2.0 CFU/100 mL, mean=20.82 CFU/100 mL, skewness=3.221). In such systems, the bulk water may often have low microbial counts, but the biofilm acts as a persistent reservoir. Hydraulic disturbances, temperature changes, or disinfectant decay can trigger sloughing events, where fragments of biofilm and large numbers of planktonic cells are suddenly released into the water, causing the sporadic, highmagnitude spikes observed in the data<sup>24</sup>. This instability makes it impossible to guarantee safe water quality at the tap at any given moment and represents a significant departure from a state of microbiological control.

### Critical failure of a key engineering barrier

Perhaps the most alarming finding of this study is the statistically demonstrated failure of the ultrafiltration unit. UF is employed as a physical barrier, with a pore size designed to reliably remove bacteria, viruses, and other particulates<sup>25</sup>. The finding that there is no significant difference in HPCs between the pre-UF and post-UF water (p=0.2031) is a critical indictment of this unit's performance. The Hodges-Lehmann estimate confirmed a non-significant median difference of -16.0 CFU/mL. Several engineering or operational factors could account for this failure. These includes:

**Membrane integrity breach:** A loss of integrity in the membrane fibers or seals would allow raw water to bypass the filtration barrier, a phenomenon known as channeling.

**Fouling and biofilm growth:** The UF membrane itself can become fouled with organic matter and colonized by biofilm. If not properly backwashed and chemically cleaned. Moreover, the biofilm on the "clean" side of the membrane can slough off and contaminate the filtered water<sup>26</sup>.

**Post-filter contamination:** It is possible that the sampling point for Post-UF is located downstream of a contaminated section of pipe or fitting, although this is less likely if the sampling port was installed correctly.

The observed 23.5% reduction contrast against typical 99.99% (4-log) industry expectation. Regardless of the specific cause, this finding invalidates a key assumption about the system's safety<sup>27,28</sup>. A facility relying on a UF unit for microbial control that is not, in fact, performing this function is operating with a significant and unmitigated risk.

#### Storage tanks as microbial incubators and amplifiers

The correlation and comparative analyses demonstrate conclusively that storage tanks function as critical microbial reservoirs for proliferation, amplifying contamination levels beyond their feed water sources<sup>29</sup>. This pattern is most evident in the purified water storage tank (ST-04), which exhibited significantly higher microbial counts than the RO-treated water supplying it. While RO (TR-02) produced water with a median of 1.0 CFU/100 mL, ST-04 showed a median of 2.0 CFU/100 mL and extreme spikes up to 280.0 CFU/100 mL (maximum value from descriptive statistics). The kurtosis for ST-04 was 10.56, confirming particle-driven nutrient hotspots.

A self-sustaining contamination cycle was identified between the central purified water tank (ST-01) and the distribution loop return (DL-01), characterized by a strong positive correlation (*p*=0.679, *p*<0.0001)<sup>30</sup>. This interdependence manifests through two reciprocal mechanisms: First, ST-01 seeds the distribution loop with microorganisms during routine operations, evidenced by synchronized contamination spikes (ST-01 max: 255.0 CFU/100 mL; DL-01 max: 300.0 CFU/100 mL). Second, biofilm fragments sloughed from the loop during hydraulic disturbances inoculate water returning to ST-01, creating a feedback loop that perpetuates contamination. This cyclical dynamic was further

validated by DL-01's 446 statistically significant pair wise differences with other sampling points in Dunn's test, confirming its role as a system-wide contamination nexus.

Critical hydraulic linkages to point-of-use outlets were also revealed<sup>31</sup>. ST-04 correlated weakly with restroom points (POU-14; p=0.224). Conversely, ST-01 showed a strong negative correlation with preparation point (POU-05; p=-0.696), suggesting preferential routing of contaminated water to high-risk clinical zones. These pathways expose differential patient safety risks: while ST-04 influences non-critical areas, ST-01 directly impacts water used for sensitive processes.

The tanks' operational deficiencies were quantifiable through distribution metrics<sup>32</sup>. ST-01's interquartile range (0.75-90.0 CFU/100 mL) was 2.5 times wider than its RO feed source (0.0-35.0 CFU/100 mL), indicating inconsistent turnover. Meanwhile, ST-04's 90 percentile value (78.0 CFU/100 mL) far exceeded its median (2.0), demonstrating frequent high-amplitude contamination events consistent with the biofilm detachment. These findings necessitate targeted engineering interventions: ultrasonic sediment monitoring in ST-04, hydraulic recalibration to eliminate ST-01's dead zones, and real-time biofilm sensors at loop endpoints to disrupt the contamination cycle<sup>33</sup>. Without such measures. storage infrastructure will continue compromising the entire water system's microbiological integrity.

## Implications for patient safety and public health

While HPCs are not direct measures of pathogens, they are a validated indicator of the conditions that allow OWPs like *P. aeruginosa* and *Legionella* to thrive<sup>7</sup>. A system with high and variable HPCs, compromised filtration barriers, and extensive biofilm is an ideal environment for the proliferation of these dangerous organisms. The water from such a system, when used for patient hygiene, wound cleaning, respiratory therapy equipment, or even hand washing by healthcare workers, can become a direct vector for HAIs<sup>34</sup>. The findings of this study, therefore, have direct and serious implications for infection prevention and control within the facility.

## Scope for future work

The current study provides a robust statistical foundation based on HPC. Future work could build upon these findings to create an even more comprehensive picture the system's microbial ecology.<sup>35</sup> investigations could focus on identifying the specific microbial species present using culture-based or molecular methods (e.g., 16S rRNA sequencing), which would allow for a more direct assessment of pathogenic risk<sup>36</sup>. Additionally, future studies could integrate the microbiological data presented here with operational parameters, such as disinfectant residuals, water temperatures, and flow rates. Correlating these physical and chemical data points with the HPC results could help build predictive models to identify the root causes of contamination events and further refine control strategies. Finally, the existence of biofilm needs further investigation to confirm either its presence or not in the present situation. Thus, HPC data cannot confirm pathogen presence or biofilm composition; future studies should integrate species identification.

#### **CONCLUSIONS**

This data-driven investigation of a healthcare water system was aimed to move beyond simple compliance monitoring to provide a deep, evidence-based diagnosis of its microbiological health. Thus, the conclusion is unequivocal: the system requires a significant improvement in microbiological control, characterized by a potential widespread biofilm, unstable water quality, and the failure of a critical engineering control in the ultrafiltration unit. Based on this comprehensive analysis, the following actionable recommendations are proposed to mitigate the risks and restore the system to a state of control.

For facility engineering and management: firstly, Immediate UF system remediation; conduction of an urgent and thorough investigation of the ultrafiltration unit to restore its function. This must include integrity testing of the membranes, a review of operational parameters (e.g., backwash frequency, transmembrane pressure), and an assessment of the chemical cleaning regimen. The unit must be repaired or replaced to ensure it provides a statistically verifiable reduction in microbial load. System-wide shock disinfection; Planning and execution of system-wide shock disinfection are necessary, particularly targeting the identified hotspots. The choice of disinfectant and procedure should be based on efficacy against biofilms. and modification; Thirdly, engineering review Conduction of a full engineering review of the system is crucial to identify and eliminate "dead legs" and other areas of stagnation that promote biofilm growth, along with design improvement. For infection prevention and quality management; enhance the monitoring program: The monitoring plan should be updated based on these findings. Increase the frequency of sampling at critical control points and at high-risk locations. Implement a water management plan: formalize a comprehensive water management plan as recommended by public health bodies. This plan should be multidisciplinary, involving facility engineers, infection preventionists, and clinicians, and should use this data analysis as its foundational risk assessment. Continuous data analysis: establish a routine for the statistical analysis of monitoring data. This will enable the early detection of trends, anomalies, and emerging risks, allowing for proactive intervention rather than reactive crisis management.

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None to declare.

## **AUTHOR'S CONTRIBUTIONS**

Eissa ME: conceived the idea, writing the manuscript, literature survey, formal analysis, critical review.

## DATA AVAILABILITY

The accompanying author can provide the empirical data that were utilized to support the study's conclusions upon request.

#### CONFLICT OF INTEREST

No conflict of interest associated with this work.

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