Frontiers in Scientific Research and Technology

Online ISSN: 2682-2970
Print ISSN: 2682-2962
http://fsrt.journals.ekb.eg

FSRT J 8 (2024) 57 - 70 10.21608/FSRT.2023.251967.1115

Bioburden Analysis and Microbiological Stability of Municipal Distribution System through Examination of Transformed Total Microbial Count Dataset

Mostafa Eissa[1]*

[1] Pharmaceutical Research Facility, Cairo, Egypt

ARTICLE INFO

Article history:
Received 29 November 2023
Received in revised form 21 December 2023
Accepted 23 December 2023
Available online 30 December 2023

Keywords
Laney Attribute Chart,
Normal Probability Plot,
Correlation Matrix,
Residuals Plot,
Pareto Plot,
Municipal Water.

ABSTRACT

The availability of water suitable for human consumption has become an increasingly critical challenge that faces humanity in the modern period after witnessing dramatic climate changes. Public health authorities in every nation bear the responsibility of monitoring and controlling the quality for fulfilling requests from the community of potable water through valid and stable distribution infrastructure. In this study, a parametric statistical assessment was focused on the microbiological quality of city water in a specific section of the municipal water network that supplies a healthcare plant. The examination period covers one-year duration of collecting qualitative and quantitative testing results. Non-normal microbial count datasets were logarithmically transformed and investigated through the application of a normal probability plot at 95% Confidence Interval (CI). Despite the absence of Out-Of-Specification (OOS) results, the municipal distribution system showed significant variations between its sections suggesting heterogeneity between the investigated segments in the bioburden. However, an initial exploratory Laney attribute control chart did not demonstrate any Out-Of-Control (OOC) points. Nevertheless, a significant variation was evident using One-Way ANOVA (at α 0.05) combined with the Pareto plot and residual analysis. In order to determine the degree of association between different sectors of the water distribution network, the Pearson Correlation Matrix (PCM) (at 95% CI) was used with seven points-of-use pairs with <1.0% two-tailed P-values. There was significant difference in the microbiological density in water between different municipal distribution sections with most points-of-use showing strong association with each other in bioburden count. The transformation enabled the use of the statistical test tools to detect the distribution system efficacy and can mark the microbiological instability within the network.

1. Introduction

Bioburden analysis is a crucial aspect of assessing the quality of municipal water distribution systems (Bruyninckx, 2018). The uncontrolled presence of microorganisms in the water supply system can lead to severe health issues, including waterborne illnesses (Eissa, 2016b). As such, it is essential to understand the bioburden and the stability of the water distribution system.

Quantifying the microorganisms in water is critical for determining the potential risks associated with water consumption. Various bacterial diseases can be transmitted through water (Essam-Eissa, 2018). Therefore, the appropriate analysis of microbiological data is essential for ensuring the safety of municipal water.

* Corresponding author at Pharmaceutical Research Facility

E-mail addresses: mostafaessameissa@yahoo.com (Mostafa Eissa)

The interpretation and application of the total microbiological count results are critical for municipal water management. The total viable aerobic count (TVAC) profile can be used to identify potential sources of defects in the distribution system and to develop appropriate treatment strategies to maintain the biological stability of the water (APHA, 2023).

Bioburden data monitoring and microbial community analysis can also provide valuable information for assessing the biological stability of water (Lyons et al., 2023). Therefore, the appropriate interpretation and application of the total viable count results are essential for ensuring the safety and quality of municipal water.

One of the key issues facing municipalities is the extent to which they can cover their water needs. In order to ensure that municipalities can provide access to safe and quality water, it is important to understand how much water is available to them and how much water is needed to meet their needs (Essam-Eissa, 2017). This paper will discuss the biological quality of the water distribution network from a Total Viable Aerobic Count (TVAC) perspective in

municipal water systems. Furthermore, in this article, we will examine the use of transformed total microbial count in bioburden analysis and its role in assessing the stability of the water distribution system.

1.1. Literature Review

The first step in assessing the biological stability of municipal water is the collection and analysis of microbiological data (Eissa et al., 2022). This involves the collection of water samples from various locations within the distribution system and the subsequent analysis of these samples for the presence and enumeration of microorganisms [as Colony Forming Unit (CFU)] (Essam-Eissa et al., 2023). After the collection and analysis of microbiological data, the next step is to assess the stability of the microbiological count through the water distribution system (Eissa, 2018a). The investigated area could show the pattern in the points-of-use through microbiological examination. The heterotrophic plate count (HPC) could be used as an indicator for the biological stability of city water distribution structure under examination (Favere et al., 2021). In turn, biological stability measures the potential for microorganisms to grow and reproduce in the water (Cabral, 2010).

Water for human consumption is primarily distributed through a municipal water network, although it may also be sometimes obtained directly from wells, streams, or lakes (CDC, 2022). Pipe system network abnormalities may result in the contamination of clean feeding water (Eissa, 2016b). As a result of this possibility, sampling at usage points is critical for monitoring water quality (Eissa, 2018b). In this situation, the sample drawn will be typical of the water that flows through the distribution structure till the output tape (Ahmed-Eissa, 2018b). Furthermore, microbial contamination of oral fluid and topical medicine products remains a serious issue, which is frequently caused by the use of polluted water (Eissa, 2015). Despite the fact that there are no supreme microbiological measures for water (other than water intended to be sterile), the current Good Manufacturing Practice (cGMP) requirements require that appropriate precautions be taken.

Unique statistical tools could be used to detect the status of biological instability in the municipal water distribution system, either in a single line or between multiple segments in the same distribution system (Essam-Eissa, 2015). As a result, proper maintenance is essential to increase the system's microbiological stability and reduce changes in water quality. Accordingly, additional inquiry is required to elucidate areas of failure in light of the several identified probable reasons, either alone or in combination. Another study in the same vein demonstrated the impact of raw water microbiological quality on the final purified water of the pharmaceutical plant produced from the water treatment station loop for medicinal product manufacturing. A group of investigators reported that the income water microbiological count can affect the final product water quality, even though there are barriers to the passage of microbial cells during the drinking water processing stages (Eissa et al., 2015; Eissa, 2016b).

Given that humanity is getting closer to a more advanced stage of water shortage, the microbiological quality of water is essential to its suitability for human consumption. The public health authority must use an efficient examination strategy, including quantitative and illustrative methodologies, to examine the microbial quality of the municipal distribution system due to the issues associated with city water consumption and its microbiologic safety. This article will address a particular problem related to biological stability by measuring the amount of variability of this inspection property in the early phases of monitoring using a small number of samples: bioburden density in tap water.

1.2. Theoretical Framework

Drinking water safety and quality is critical, and as such, it is critical to guarantee that municipal water distribution systems are microbiologically stable (US EPA, 2020). The current in-depth study, which allows us to better grasp the trends and patterns in microbiological data, is one way to analyze this as theorized in Fig. 1. Potential sources of faults should be identified and Drinking water safety and quality are critical, and as such, it is critical to guarantee that municipal water distribution systems are microbiologically stable (Eissa et al., 2022). The current in-depth study, which allows us to better grasp the trends and patterns in microbiological data, is one way to analyze this. Potential sources of faults should be identified and proactive efforts to prevent them taken by assessing critical indicators such as microbial load and change in the count (Griffith, 2016). The total microbial aerobic count was investigated in a specified area encompassing a tape water delivery network (Essam-Eissa, 2017). Bioburden density in 100 mL of city water samples was calculated as CFU for monthly samples from available sites until 2022 (Abu-Sini et al., 2023). Evolved colonies after incubation were identified and counted on the surface of the 0.45 µm sterile membrane filters (Berrang et al., 2017). The sample preparation, collection, and processing procedures, as well as the neutralization of residual disinfectant in water, were carried out in accordance with previous similar study conducted on the same line (Eissa et al., 2022; Essam-Eissa, 2018). Microbial distribution follows distributions other than the well-known Gaussian dispersion (Essam-Eissa, 2016). One of the proven means of database normalization is the logarithmic transformation to the base ten (Essam-Eissa et al., 2023). A unity value might be added to compensate for zero records for some of the bioburden count results in some samples without appreciably distorting the main outcome.

2. Materials and methods

The investigated pipeline system is in the Giza governorate at 29.9285° N, 30.9188° E coordinates. The study covered 19 places of usage following the installation of two storage tank units from processed city water (Eissa et al., 2022). The area that contains the municipal water tapes - under examination – were assigned random letters and numbers as in Fig. 1.

Fig. 1. Diagram of the experimentation steps (Eissa et al., 2015; Essam-Eissa, 2017)

2.1. Test Design

To eliminate extraneous contamination, water samples were analyzed microbiologically using aseptic instruments and procedures (Essam-Eissa, 2017). Sterile, single-use, and 0.45 µm disposable membrane filters were used to collect microorganisms in a specific volume of water sample via a vacuum filtration system, where filters were transferred aseptically over agar media for coliform detection and enumeration of the TVAC (Eissa et al., 2022). The plates were incubated and counted to determine the number of CFUs per unit volume of water samples and to detect the pathogen colonies' characteristic look. Groups of researchers offered recommendations for microbiological examination of water samples (Francy et al., 2013; Ngwa et al., 2013). Confirmatory biochemical testing was performed on any suspects using commercial miniaturized kit panels.

2.2. Municipal Water System

The method suggested by Ainsworth (2013) was used to study a healthcare institution with a distribution water network system that received its feed supply of city water from a municipal line (Ainsworth, 2013). The piping network serviced several compartments in the facility, and each portion has its own sampling port from which routine municipal water samples were collected for analysis. Sample collection methods, sample storage, isolation techniques, isolation procedures and the used biochemical testes were done according to Ashour et al (2011).

2.3. Output Record and Analysis

The city water's microbiological limit was determined in accordance with the Environmental Protection Agency's (EPA) recommendations (Bartram et al., 2004). Eissa et al. (2015) described using statistical process control and other investigational techniques for raw water datasets (Eissa et al., 2015). Microsoft Office Excel 2007 was used to collect the primary results of the analyses for generating logarithmically transformed data with one added for each number to compensate for zero results without distorting the originally obtained output. Datasets were tested for distribution, and statistical analysis using GraphPad Prism v6.01 for Windows, while Minitab® v17.1.0 was utilized to create a box plot, control charts and normality plot.

4. Results and Discussion

The initial visualization of the microbiological distribution in municipal water network under examination at different use points is shown in Fig.2 as an average ± Standard Error of the Mean (SEM). The preliminary exploratory Lany U' control chart applied on the original raw data showed that the overall TVAC of the water distribution system was initially under control without any excursions (Fig. 3). The average value of the microbiological count data of the municipal water distribution network in the examined district of Giza governorate in Egypt was 2698 CFU/100 mL. The highest value of the microbiological count data of the city water lines in the examined location of the city was 10931 CFU/100 mL and the lowest value of the investigated group of the use points was 78 CFU/100 mL. Laney-modified process-behavior plot was used instead of the usual U chart because the diagnostic test for Poisson dispersion demonstrated the necessity of the implementation Sigma-Z correction to avoid false control limits and alarms as could be seen in Fig. 3 (Eissa et al., 2023). There were not any signs for the presence of coliforms could be detected in the city water system during the study period.

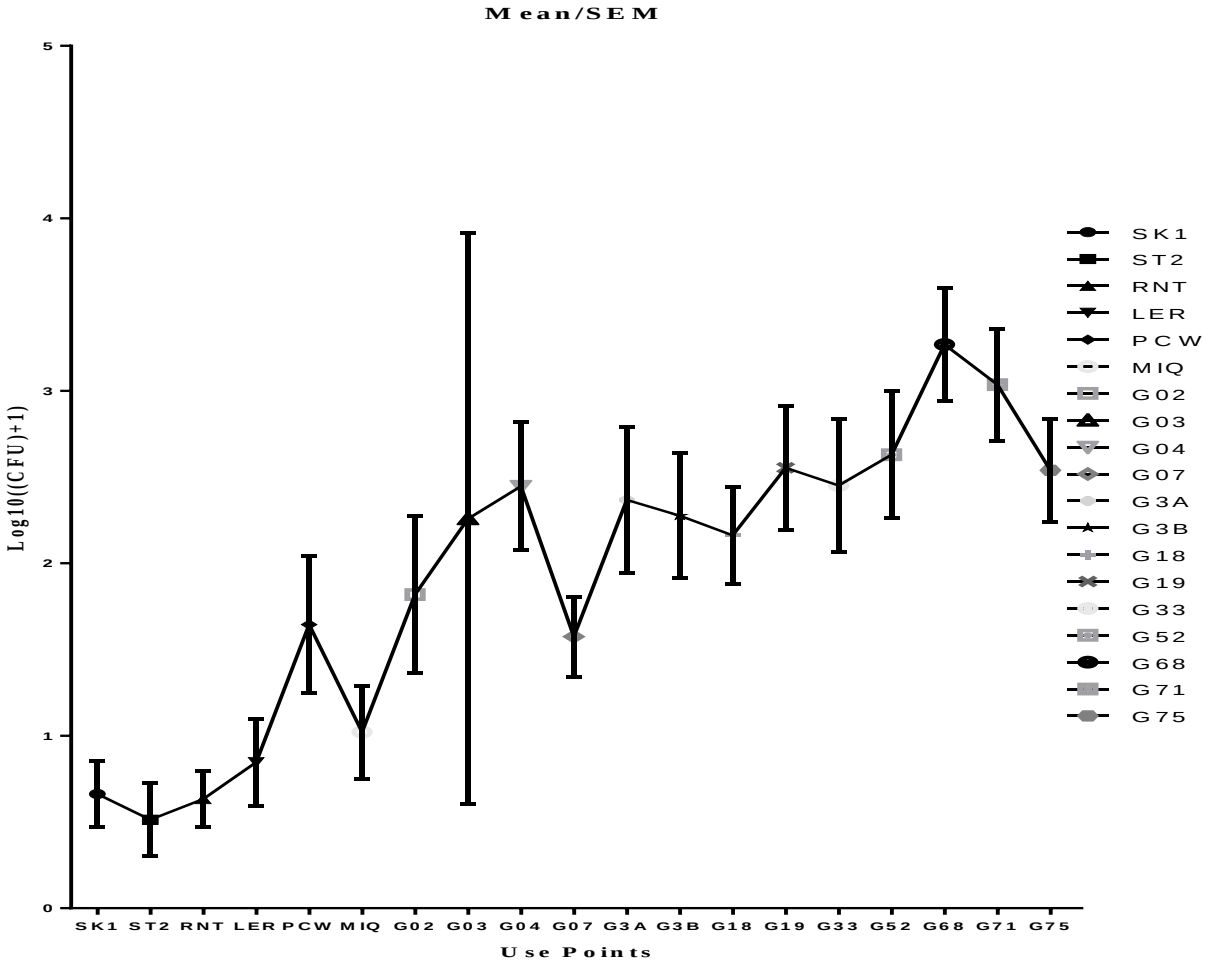


Fig. 2. Network water system showing detailed transformed Mean ± Standard Error of the Mean (SEM) graph for each point of use under examination.

This normalization makes it easier to analyze the data based on the normality assumption and detect any outliers (Ahmed-Eissa, 2018a). The heterotrophic plate count of municipal water shows the logarithmically transformed microbial count of one-year data without any detectable outliers except one with ST2 (Table 1) and the use point G68 with the highest average count while G03 with the most significant observable standard error of the mean. This finding was evident in Fig. 2. The results of the logarithmic transformation of the microbiological count data of the municipal water distribution network in the healthcare plant showed an acceptable normalization upon using normal probability plots (Yuan et al., 2020). Logarithmic transformation of the microbiological data showed significant improvement in the normalization of the count record as could be illustrated from the normal probability plot (Fig. 4). One-Way ANOVA calculated (at alpha (α) = 0.05) for the transformed results showed a significant difference between the examined points of use indicating heterogeneity in the bioburden between different sections of the water distribution network

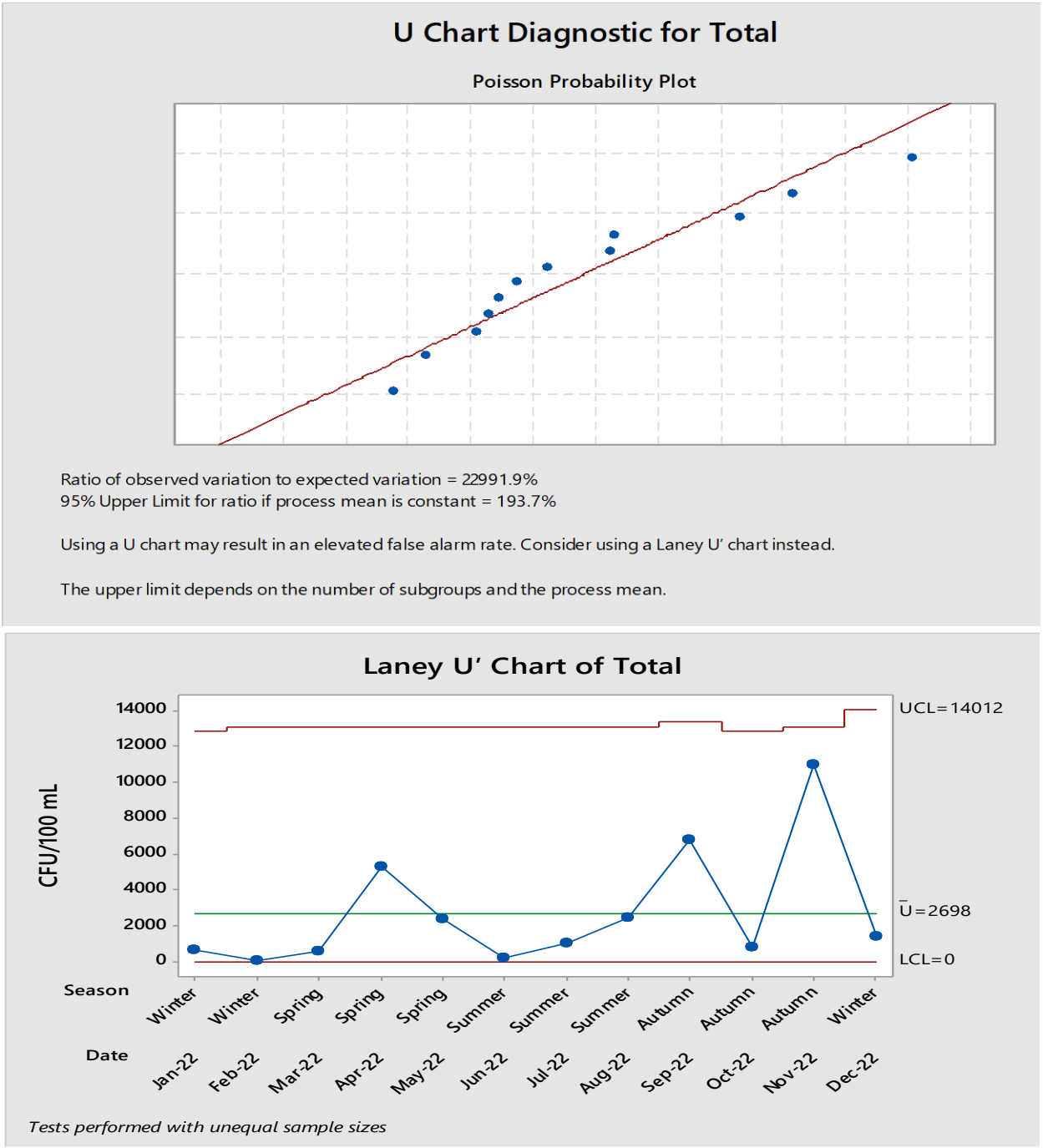


Fig. 3. Poisson probability plot and trending chart for exploratory evaluation of the overall microbiological quality of the distribution network of municipal water.

If the total P-value is minimal, the differences that were noticed are unlikely to be due to random sampling coincidence (Eissa and Nouby, 2016). The notion that all populations have the same means can be rejected (Table 2). This signifies that the variabilities in the microbial densities of city water between some sampling sites are distinct from the others (Eissa et al., 2022). As a result, the findings of the post-tests would provide a hint as to where the differences are. Fig. 5 depicts the uncertainty in the difference between all (or selected) pairs of averages as a 95% confidence interval. This interval contains the genuine difference between the two means 95% of the time (Rashed and Eissa, 2020). With this respect, few pairs showed experimentally interesting variations from each other in bioburden recovered from water samples.

Each value has a residual calculated for it. The discrepancy between a value entered and the mean of all values for a group is known as a residual.

Table 1: Screening of the presence of the aberrant results shown for the logarithmically transformed datasets.

Use Point	SK1	ST2	RNT	LER	PCW	MIQ	G02	G03	G04	G07	G3A	G3B	G18	G19	G33	G52	G68	G71	G75
	Number of points Analyzed																		
Outliers*	12	11	12	12	11	12	12	2	12	12	12	12	12	12	12	12	11	11	12
	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*One outlier was removed in ROUT (Q = 1.0%)

Outlier tests require three or more values. Columns with fewer values were not analyzed.

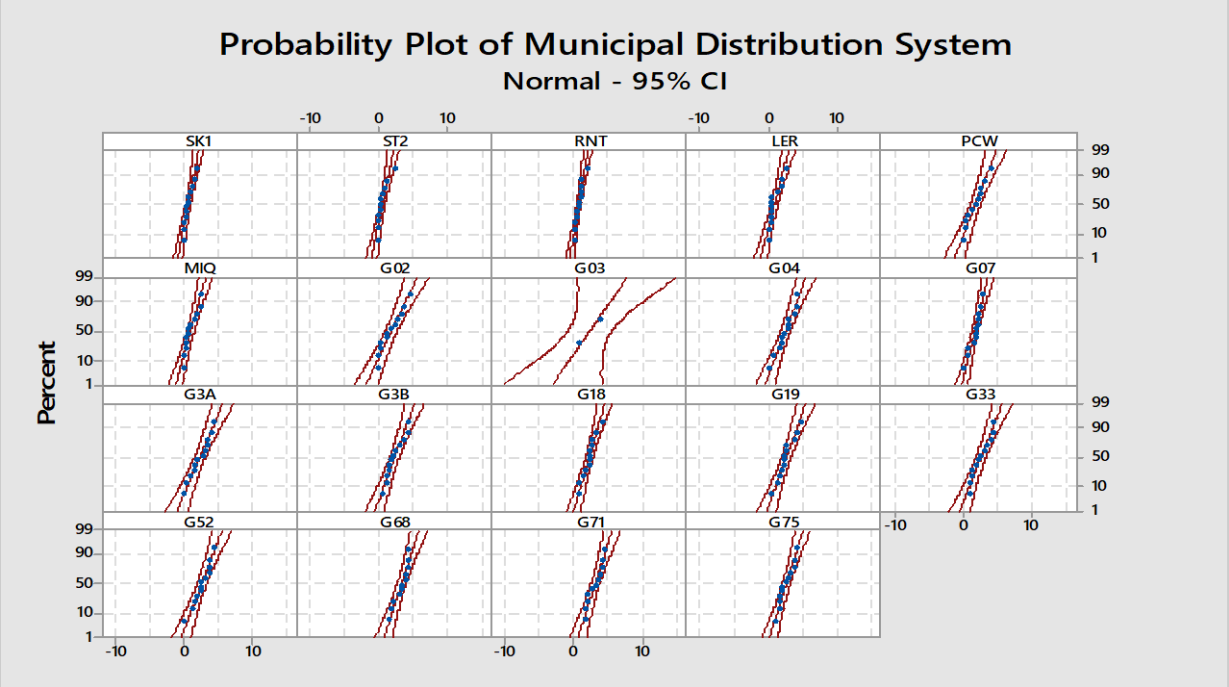


Fig. 4. Normal probability plot demonstrating the effect of the transformation on the normalization of the database of the bioburden count in city water at a 95% Confidence Interval (CI).

When the matching value exceeds the sample mean, the residual is positive; when it is below the sample mean, it is negative (Fig. 6). The plot showed random dispersion without any trend, observable clustering or particular pattern around the reference zero line.

The distribution of multiple comparisons in terms of pairwise significance of difference and the q-statistics was shown by significance interpretation (Fig. 7). The program classifies if the P-value is not significant (ns) and less than 0.05, 0.01, 0.001, or 0.0001 (denoted by the number of the asterisks (*) in the graph) for each pair of means. It is improbable that the observed difference is caused by a chance of random sampling if the P-value for a post-test is minimal. It is incorrect to assume that the means of those two populations are the same.

ANOVA divides the variability of all the values into two components: one caused by treatment-related variation in group averages, and the other by residual variation, which is variance within the groups (Eissa, 2019). The difference between each value and its group mean, or the total of the squares of those differences is used to measure variation within groups (within the columns) (Eissa, 2016a) and hence the residual sum of squares could be computed. The sum of the squares of the differences between the group means and the grand mean (the mean of all values in all groups) is used to calculate the variation between groups (owing to treatment). This changes to the treatment sum-of-squares when the sizes of each group are considered.

Table 2: One-Way ANOVA Tukey's correction of confidence interval and significance for multiple comparisons test including 171 comparisons per family.

ANOVA Summary	
Alpha	0.05
F	6.448
P value	< 0.0001
P value summary	****
Are differences among means statistically significant? (P < 0.05)	Yes
R square	0.3731

ANOVA table§	**SS**	**DF**	**MS**	**F* (DFn, DFd)**	**P value**
Treatment (between columns)	147.2	18	8.178	F (18, 195) = 6.448	P < 0.0001
Residual (within columns)	247.3	195	1.268	α	0.05
Total	394.6	213			
Model comparison	SS	DF	**Probability it is correct**		
Null H. All population means identical	394.6	213	0.00%		
Alternative H: Distinct population means	247.3	195	100.00%		
Ratio of probabilities			0.0		

* The F-ratio, which is derived from the ANOVA table, is used to calculate the P-value along with the two degrees of freedom (df) values.

SS: Sum of Squares. MS: Mean Square.

§ MS is calculated by dividing the sum-of-squares by the appropriate number of degrees of freedom. Each sum-of-squares relates to a specific number of degrees of freedom (df, determined from number of subjects and number of groups). These can be regarded as variations. The pooled standard deviation can be understood as the square root of the mean square residual.

**** means P ≤ 0.0001

The Pearson correlation matrix was used to examine the association of the HPC between different segments of the municipal distribution network at 95% CI (Essam-Eissa and Mohamed-Mahmoud, 2015). Normalization of the data allowed for the application of the parametric tests, while one-way ANOVA identified several segments of the water network with significantly different microbial densities in water (Eissa, 2019). The Pearson correlation matrix was used to study the correlation of HPC between the different points of use with very strong correlations observed between ST2, SK1, LER and G3B, MIQ. Other segments with lower variable degrees of association could be found in the matrix with descending levels of the shades. The p-value is a metric for the strength of the evidence the data give against the null hypothesis; the lower the p-value, the stronger the evidence. The following scale is employed: Very strong evidence is p(X) 0.01; strong evidence is p(X) (0.01, 0.05); weak evidence is p(X) (0.05, 0.1); and little or no evidence is p(X) > 0.1.

The output of the r-table works reasonably in the same line as the P-table. Details of this study can be seen in Tables 3a and 3b for r- and P-values, respectively. Therefore, it can be concluded that the distribution system requires appropriate maintenance in terms of microbial concentration in some sections even if there were no Out-Of-Specifications (OOS) results could be spotted. Significant variation in bioburden density of municipal distribution water system from Fig. 5 could be projected into Table 3a which showed that very strongly and most strongly correlated segments of the network did not vary appreciably (α 0.05) with the exception of one port (G52) with the points of use SK1 and ST2. Moving forward, such bioburden analysis and correlations should be maintained to continue the monitoring and enforcement of a safe and secure water distribution system. An extended period of microbiological monitoring is essential to better assess the situation.

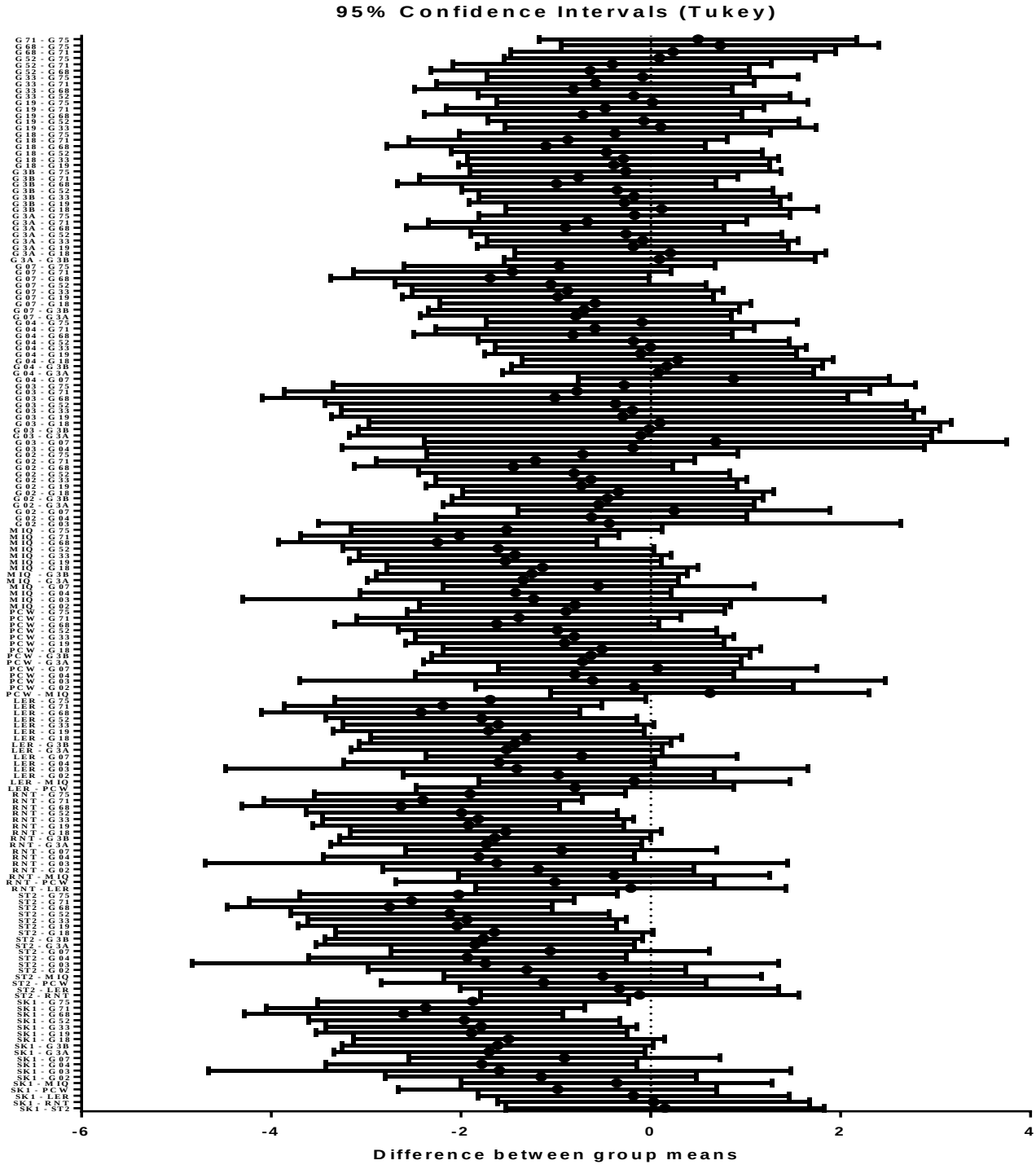


Fig. 5. Confidence Intervals (CIs) graphing of the ordinary one-way ANOVA for pairwise comparisons in Tukey's multiple comparisons test (TMCT).

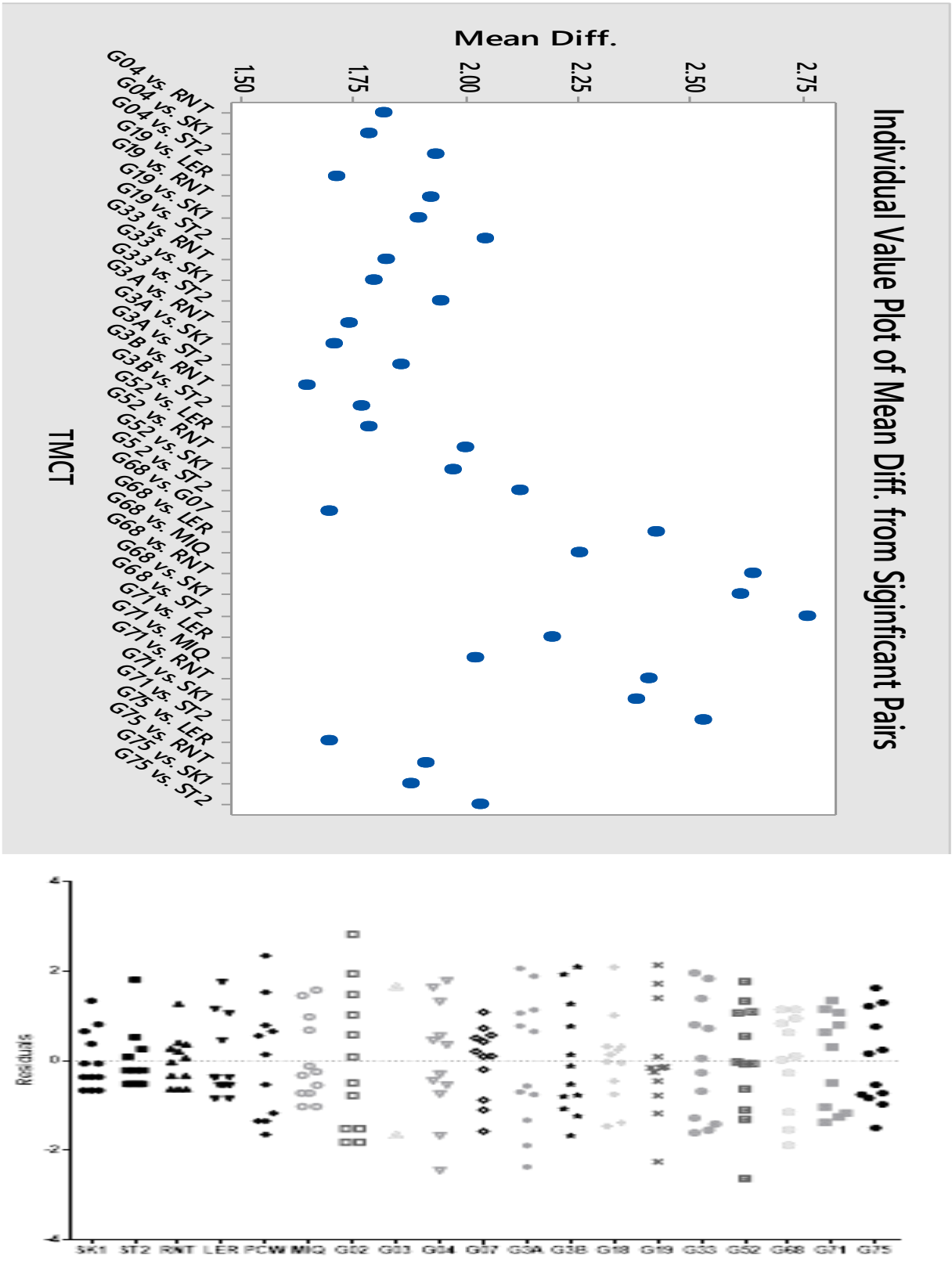
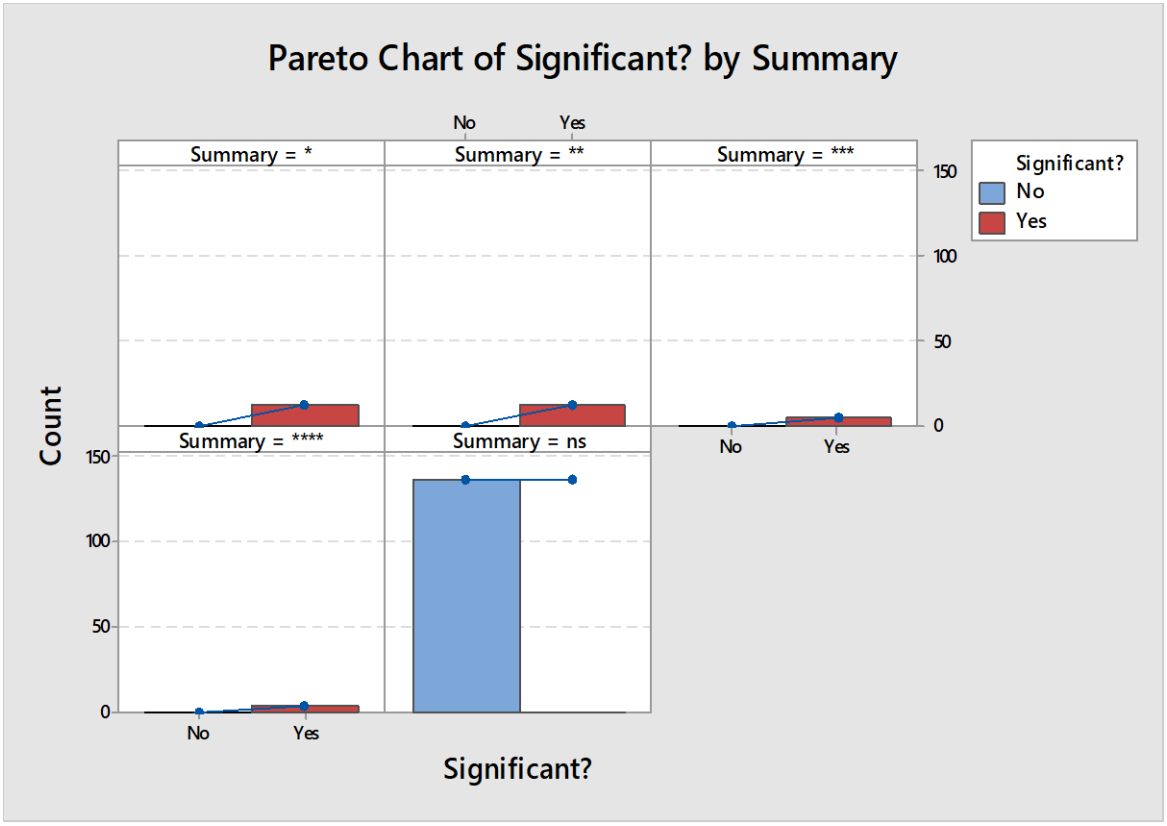


Fig. 6. Residuals plot of the ordinary one-way ANOVA of the bioburden of municipal distribution system.



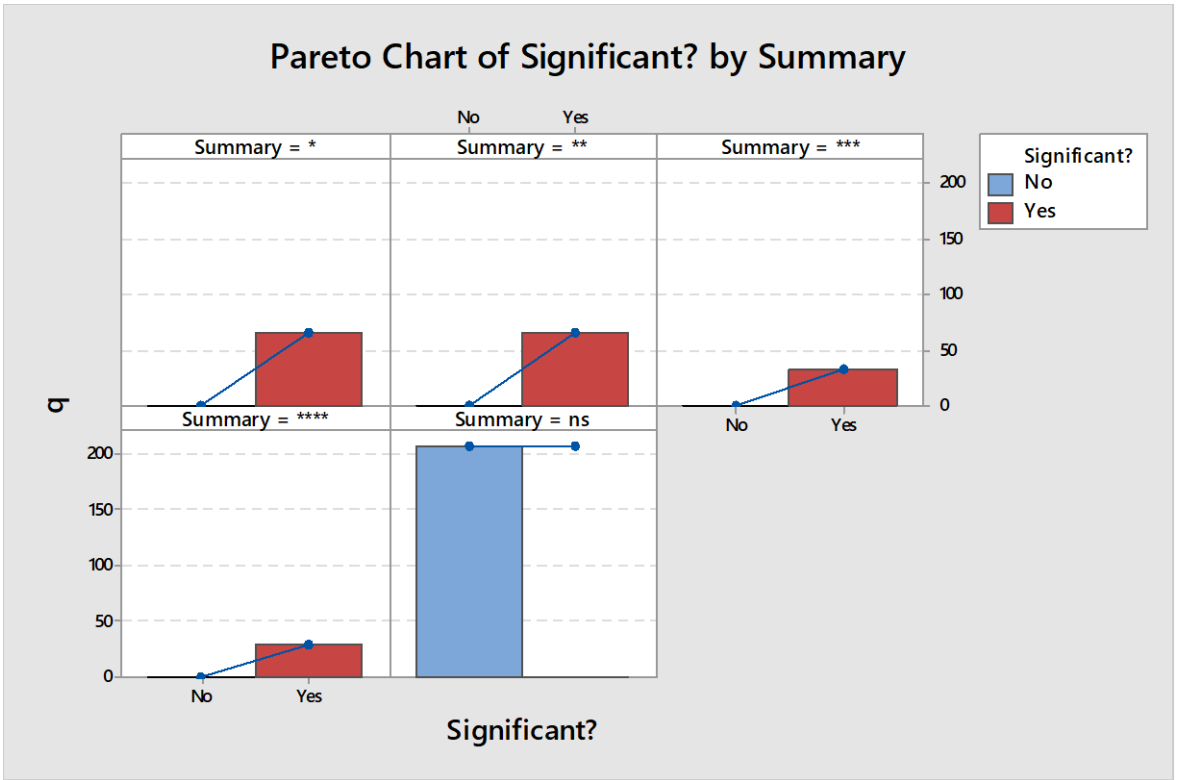


Fig. 7. Pareto analysis for the significance of the difference between the pairwise comparisons showing the final outcome summary and the q statistics.

Table 3a: Pearson Correlation Matrix (PCM) showing the level of association between the examined use points: Correlation Coefficients (CC) at 95% Confidence Interval (CI).

r	SK1	ST2	RNT	LER	PCW	MIQ	G02	G04	G07	G3A	G3B	G18	G19	G33	G52	G68	G71	G75
SK1		0.82	0.36	0.89	-0.33	0.44	0.30	0.27Y	-0.26	0.38Y	0.49	-0.33	0.04Y	0.21Y	0.73Y	0.45Y	0.38Y	0.09Y
ST2	0.82		0.23	0.83	-0.53	0.60	0.33	0.28Y	-0.46	0.30Y	0.51Y	-0.27	0.44Y	0.31Y	0.72Y	0.36Y	0.25Y	-0.05Y
RNT	0.36	0.23		0.21	0.44	-0.15	-0.17	0.21Y	0.07	-0.06Y	-0.03Y	-0.61	-0.20Y	-0.35Y	0.47Y	0.29Y	0.21Y	-0.15Y
LER	0.89	0.83	0.21		-0.61	0.42	0.20	0.25	-0.22	0.32	0.28	-0.29	0.04Y	0.27	0.59Y	0.25Y	0.28Y	0.04Y
PCW	-0.33	-0.53	0.44	-0.61		-0.38	0.04	0.13	0.39	-0.19	-0.17	-0.12	-0.31	-0.24	-0.35	-0.01	0.04	-0.13
MIQ	0.44	0.60	-0.15	0.42	-0.38		0.69	0.14	-0.71	0.51	0.85	0.44	0.42	0.77	0.52	0.29Y	0.00Y	0.47
G02	0.30	0.33	-0.17	0.20	0.04	0.69		0.29	-0.38	0.17	0.59	0.41	0.38	0.74	0.05	0.00	0.26	0.09
G04	0.27Y	0.28Y	0.21Y	0.25	0.13	0.14	0.29		-0.05	0.21	0.11	0.09	0.07	0.34	0.25	-0.16	0.10	-0.12
G07	-0.26	-0.46	0.07	-0.22	0.39	-0.71	-0.38	-0.05		-0.51	-0.73	-0.20	-0.50	-0.61	-0.58	-0.46Y	-0.10	-0.12
G3A	0.38Y	0.30Y	-0.06Y	0.32	-0.19	0.51	0.17	0.21	-0.51		0.72	0.39	-0.02	0.66	0.45	0.73	0.30	0.32
G3B	0.49	0.51Y	-0.03Y	0.28	-0.17	0.85	0.59	0.11	-0.73	0.72		0.38	0.39	0.68	0.62	0.63	0.19	0.39
G18	-0.33	-0.27	-0.61	-0.29	-0.12	0.44	0.41	0.09	-0.20	0.39	0.38		0.22	0.67	-0.27	-0.11	-0.25	0.28
G19	0.04Y	0.44Y	-0.20Y	0.04Y	-0.31	0.42	0.38	0.07	-0.50	-0.02	0.39	0.22		0.34	0.17	-0.15	-0.41	-0.42
G33	0.21Y	0.31Y	-0.35Y	0.27	-0.24	0.77	0.74	0.34	-0.61	0.66	0.68	0.67	0.34		0.12	0.20	0.18	0.17
G52	0.73Y	0.72Y	0.47Y	0.59Y	-0.35	0.52	0.05	0.25	-0.58	0.45	0.62	-0.27	0.17	0.12		0.57	0.20	0.27
G68	0.45Y	0.36Y	0.29Y	0.25Y	-0.01	0.29Y	0.00	-0.16	-0.46Y	0.73	0.63	-0.11	-0.15	0.20	0.57		0.61	0.32
G71	0.38Y	0.25Y	0.21Y	0.28Y	0.04	0.00Y	0.26	0.10	-0.10	0.30	0.19	-0.25	-0.41	0.18	0.20	0.61		0.16
G75	0.09Y	-0.05Y	-0.15Y	0.04Y	-0.13	0.47	0.09	-0.12	-0.12	0.32	0.39	0.28	-0.42	0.17	0.27	0.32	0.16	

Correlation coefficient (r)	|Lower|	|Upper|
Very weak	0.00	0.19
Weak	0.20	0.39
Moderate	0.40	0.59
Strong	0.60	0.79
Very Strong	0.80	1.00

Y: Denotes significantly Different use points in Microbial count Identified by One-Way ANOVA

Table 3b: Pearson Correlation Matrix (PCM) showing the level of association between the examined use points: Two-tailed P-values.

P-value	SK1	ST2	RNT	LER	PCW	MIQ	G02	G04	G07	G3A	G3B	G18	G19	G33	G52	G68	G71	G75
SK1		0.2	25.7	0.0	31.4	14.8	34.3	39.1	41.7	22.2	10.7	29.2	90.0	50.9	0.7	16.6	25.2	78.3
ST2	0.2		50.5	0.1	11.2	5.2	31.5	41.1	15.4	36.5	11.1	42.4	18.0	36.1	1.2	27.2	45.8	89.3
RNT	25.7	50.5		51.9	17.9	65.2	60.0	50.6	82.2	85.8	93.8	3.7	53.1	25.8	12.4	38.9	54.4	63.5
LER	0.0	0.1	51.9		4.5	16.9	52.7	42.7	48.4	31.5	38.1	35.7	90.0	40.2	4.2	46.4	41.0	89.6
PCW	31.4	11.2	17.9	4.5		24.8	90.4	70.9	23.6	57.5	62.6	72.2	34.9	46.8	29.4	97.0	91.2	70.7
MIQ	14.8	5.2	65.2	16.9	24.8		1.4	65.5	0.9	8.7	0.0	14.8	17.2	0.4	8.3	38.4	99.2	12.7
G02	34.3	31.5	60.0	52.7	90.4	1.4		35.8	22.0	59.8	4.5	18.2	22.3	0.6	87.2	99.3	43.9	77.4
G04	39.1	41.1	50.6	42.7	70.9	65.5	35.8		88.2	50.7	72.7	78.3	83.0	28.6	43.0	64.3	77.4	72.1
G07	41.7	15.4	82.2	48.4	23.6	0.9	22.0	88.2		9.1	0.7	52.4	9.9	3.7	4.8	15.2	77.0	71.5
G3A	22.2	36.5	85.8	31.5	57.5	8.7	59.8	50.7	9.1		0.9	21.2	95.3	2.0	14.2	1.1	36.8	31.2
G3B	10.7	11.1	93.8	38.1	62.6	0.0	4.5	72.7	0.7	0.9		22.2	21.5	1.6	3.3	3.8	57.1	21.4
G18	29.2	42.4	3.7	35.7	72.2	14.8	18.2	78.3	52.4	21.2	22.2		48.7	1.8	39.4	75.7	46.6	37.0
G19	90.0	18.0	53.1	90.0	34.9	17.2	22.3	83.0	9.9	95.3	21.5	48.7		28.1	59.4	65.8	21.7	17.5
G33	50.9	36.1	25.8	40.2	46.8	0.4	0.6	28.6	3.7	2.0	1.6	1.8	28.1		70.4	55.4	59.8	60.6
G52	0.7	1.2	12.4	4.2	29.4	8.3	87.2	43.0	4.8	14.2	3.3	39.4	59.4	70.4		6.5	56.0	39.4
G68	16.6	27.2	38.9	46.4	97.0	38.4	99.3	64.3	15.2	1.1	3.8	75.7	65.8	55.4	6.5		4.8	33.5
G71	25.2	45.8	54.4	41.0	91.2	99.2	43.9	77.4	77.0	36.8	57.1	46.6	21.7	59.8	56.0	4.8		64.7
G75	78.3	89.3	63.5	89.6	70.7	12.7	77.4	72.1	71.5	31.2	21.4	37.0	17.5	60.6	39.4	33.5	64.7	

P-value	P-value%
More than 0.1	>10%
Between 0.1 - 0.05	5% -10%
Between 0.05 - 0.01	5% -1%
Less than 0.01	< 1%

Conclusion

To achieve informative results, statistical components such as the Laney U' control chart, logarithmic transformation of microbiological data, multiple comparison tests, and the correlation matrix are all essential. The control chart and the outlier detection results are particularly useful in identifying any possible deviations in the microbiological quality that require immediate attention. The practical work conducted using simple and inexpensive microbiological methods is particularly advantageous for public health authorities in developing nations. However, regardless of the methods used, regular bioburden analysis is critical to ensuring the safety and quality of municipal water distribution systems.

Recommendations

It seems that based on the results of the bioburden analysis of the municipal distribution system, there is a need for continuous monitoring to maintain stability. The heterophilic plate count results and parametric statistical tests applied to the one-year dataset suggest the importance of controlling municipal water quality during the distribution phase to ensure that the delivered water is safe for human consumption. Public health authorities should establish continuous monitoring programs to ensure the quality and safety of water from the use ports. It is recommended that similar studies be conducted to include other important quality aspects such as physical and chemical properties of water. This will contribute to the increased reliability and safety of the assessment of the public's water supply. Future investigations should explore the possible association between Heterophilic Plate Count

(HPC) and other components and parameters to enhance system stability.

References

Abu-Sini, M. K.; Maharmah, R. A.; Abulebdah, D. H. and Al-Sabi, M. N. S. (2023). Isolation and Identification of Coliform Bacteria and Multidrug-Resistant *Escherichia coli* from Water Intended for Drug Compounding in Community Pharmacies in Jordan. *Healthcare*, *11*(3): 299. https://doi.org/10.3390/healthcare11030299

Ahmed-Eissa, M. E. (2018a). Food Outbreak: An Overview on Selected Cases over Long-Term Web-Based Monitoring. *SM Journal of Nutrition and Metabolism*, *5*(1): 1029. https://www.jsmcentral.org/sm-nutrition-metabolism/fulltext_smjnm-v5-1029.pdf

Ahmed-Eissa, M. E. (2018b). Microbiological Quality of Purified Water Assessment Using Two Different Trending Approaches: A Case Study. *Sumerianz Journal of Scientific Research*, *1*(3): 75–79. https://www.sumerianz.com/?ic=journal-homeandjournal=29andinfo=archive-detailandmonth=09-2018andissue=3andvolume=1

Ainsworth, R. (2013). Safe Piped Water: Managing Microbial Water Quality in Piped Distribution Systems. *Water Intelligence Online*, *12*. https://doi.org/10.2166/9781780405841

American Public Health Association, American Water Works Association, Water Environment Federation. ((2023)*.). Standard methods. Standard Methods for the Examination of Water and Wastewater. https://www.standardmethods.org/*

Ashour, M. S. E. D., Mansy, M. S., & Eissa, M. E. (2011). Microbiological environmental monitoring in pharmaceutical facility. Egyptian Academic Journal of Biological Sciences, G. Microbiology, 3(1), 63-74. https://doi.org/10.21608/eajbsg.2011.16696

Bartram, J.; Cotruvo, J.; Exner, M.; Fricker, C. and Glasmacher, A. (2004). Heterotrophic plate count measurement in drinking water safety management. *International Journal of Food Microbiology*, *92*(3): 241–247. https://doi.org/10.1016/j.ijfoodmicro.2003.08.005

Berrang, M. E.; Meinersmann, R. J. and Cox, N. A. (2017). Passage of Campylobacter jejuni and Campylobacter coli Subtypes through 0.45- and 0.65-Micrometer-Pore-Size Nitrocellulose Filters. *Journal of Food Protection*, *80*(12): 2029–2032. https://doi.org/10.4315/0362-028X.JFP-17-211

Bruyninckx, H. (2018). Clean water is life, health, food, leisure, energy. European Environment Agency. *European Environment Agency*. https://www.eea.europa.eu/signals/signals-2018-content-list/articles/clean-water-is-life-health

Cabral, J. P. S. (2010). Water Microbiology. Bacterial Pathogens and Water. *International Journal of Environmental Research and Public Health*, *7*(10): 3657–3703. https://doi.org/10.3390/ijerph7103657

Eissa, M. (2015). Diversity of bacteria in pharmaceutical water: Significance and impact on quality. *European Pharmaceutical Review*, *20*(3): 54–57. https://www.europeanpharmaceuticalreview.com/article/33069/microbiology-in-depth-focus-2015/

Eissa, M. (2016a). NOVEL APPROACH TO THE ASSESSMENT OF PRESERVATION EFFECT FOR ORAL LIQUID PHARMACEUTICAL PRODUCTS. *Panacea Journal of Pharmacy and Pharmaceutical Sciences*, *5*(1): 17–31.

Eissa, M. (2016b). Study of Microbial Distribution from Different Processing Stages in Purified Water Production Plant of Pharmaceutical Manufacturing Facility. *Research and Reviews: A Journal of Microbiology and Virology*, *6*(1), 31–45.

Eissa, M. (2018a). Evaluation of microbiological purified water trend using two types of control chart. *European Pharmaceutical Review*, *23*(5): 36–38. https://www.europeanpharmaceuticalreview.com/article/80649/purified-water-trends/

Eissa, M. (2018b). Evaluation of microbiological purified water trend using two types of control chart. *European Pharmaceutical Review*, *23*(5): 36–38. https://www.europeanpharmaceuticalreview.com/article/80649/purified-water-trends/

Eissa, M. (2019). Application of control charts for non-normally distributed data using statistical software program: A technical case study. *World Journal of Advanced Research and Reviews*, *1*(1): 039–048. https://doi.org/10.30574/wjarr.2019.1.1.0013

Eissa, M. E. (2019). APPLICATION OF STATISTICAL Process Control On Analysis Of Surgery-Related Infection Record: An Extended Study Of Three Selected Countries Using Statistical Software. Current Trends in Pharmaceutical Research, 6(1).

Eissa, M. E. and Nouby, A. S. (2016). Recovery of Gram-Positive Cocci and *Candida albicans* from Peroxygen/Silver-Based Disinfectants. *Polish Journal of Microbiology*, *65*(1): 13–22. https://doi.org/10.5604/17331331.1197271

Eissa, M. E.; Rashed, E. R. and Eissa, D. E. (2022). Dendrogram Analysis and Statistical Examination for Total Microbiological Mesophilic Aerobic Count of Municipal Water Distribution Network System. *HighTech and Innovation Journal*, *3*(1): 28–36. https://doi.org/10.28991/HIJ-2022-03-01-03

Eissa, M.; Rashed, E. and Eissa, D. (2022). Principal component analysis in long term assessment of total viable plate count of municipal water distribution network system in healthcare facility. *Environmental Research and Technology*, *5*(2): 165–171. https://doi.org/10.35208/ert.1062683

Eissa, M.; Rashed, E. and Eissa, D. (2022). Principal Component Analysis in Long Term Assessment of Total Viable Plate Count of Municipal Water Distribution Network System in Healthcare Facility. *Environmental Research and Technology*. https://doi.org/10.35208/ert.1062683

Eissa, M.; Rashed, E. and Eissa, D. (2023). Microbiological Stability Assessment of Municipal Distribution Line Using Control Chart Approach for Total Bioburden Count. *Sağlık Akademisi Kastamonu*, *8*(2): 363–383. https://doi.org/10.25279/sak.1035879

Eissa, M.; Seif, M. and Fares, M. (2015). *Assessment of purified water quality in pharmaceutical facility using six sigma tools* [International Journal of Pharmaceutical Quality Assurance]. https://impactfactor.org/PDF/IJPQA/6/IJPQA,Vol6,Issue2,Article4.pdf

Essam-Eissa, M. (2015). Shewhart Control Chart in Microbiological Quality Control of Purified Water and its Use in Quantitative Risk Evaluation. *Pharmaceutical and Biosciences Journal*, 45–51. https://doi.org/10.20510/ukjpb/4/i1/87845

Essam-Eissa, M. (2016). Study of parameters affecting infection risk from contaminated injectable products using multiple spot contamination model: A case study of insulin vials. *Journal Of Chinese Pharmaceutical Science*, *25*(11). https://doi.org/10.5246/jcps.2016.11.093

Essam-Eissa, M. (2017). Determination of the Microbiological Quality of Feed City Water to Pharmaceutical Facility: Distribution Study and Statistical Analysis. *ATHENS JOURNAL OF SCIENCES*, *4*(2): 143–160. https://doi.org/10.30958/ajs.4-2-4

Essam-Eissa, M. (2018). Investigation of Microbiological Quality of Water from the Feed Source to the Terminal Application in the Healthcare Facility: A Case Study. *Health Research*, *2*(1), 16. https://doi.org/10.31058/j.hr.2018.21002

Essam-Eissa, M. and Mohamed Mahmoud, A. (2015). DEVELOPMENT OF METHODS FOR MICROBIAL RECOVERY: PHARMACEUTICAL DOSAGE FORMS INCLUDING DRUGS WITH ANTIMICROBIAL PROPERTIES (STUDY III). *EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH*, *2*(4): 537–549.

Essam-Eissa, M.; Refaat Rashed, E. and Essam-Eissa, D. (2023). Case of Preferential Selection of Attribute over Variable Control Charts in Trend Analysis of Microbiological Count in Water. *Acta Natura et Scientia*, *4*(1): 1–9. https://doi.org/10.29329/actanatsci.2023.353.01

Favere, J.; Barbosa, R. G.; Sleutels, T.; Verstraete, W.; De Gusseme, B. and Boon, N. (2021). Safeguarding the microbial water quality from source to tap. *Npj Clean Water*, *4*(1): 28. https://doi.org/10.1038/s41545-021-00118-1

Francy, D. S.; Stelzer, E. A.; Brady, A. M. G.; Huitger, C.; Bushon, R. N.; Ip, H. S.; Ware, M. W.; Villegas, E. N.; Gallardo, V. and Lindquist, H. D. A. (2013). Comparison of Filters for Concentrating Microbial Indicators and Pathogens in Lake Water Samples. *Applied and Environmental Microbiology*, *79*(4), 1342–1352. https://doi.org/10.1128/AEM.03117-12

Griffith, C. (2016). Surface Sampling and the Detection of Contamination. In *Handbook of Hygiene Control in the Food Industry* (pp. 673–696). Elsevier. https://doi.org/10.1016/B978-0-08-100155-4.00044-3

Lyons, K. J.; Ikonen, J.; Hokajärvi, A.-M.; Räsänen, T.; Pitkänen, T.; Kauppinen, A.; Kujala, K.; Rossi, P. M. and Miettinen, I. T. (2023). Monitoring groundwater quality with real-time data, stable water isotopes, and microbial community analysis: A comparison with conventional methods. *Science of The Total Environment*, *864*, 161199. https://doi.org/10.1016/j.scitotenv.2022.161199

Ngwa, G. A.; Schop, R.; Weir, S.; León-Velarde, C. G. and Odumeru, J. A. (2013). Detection and enumeration of E. coli O157:H7 in water samples by culture and molecular methods. *Journal of Microbiological Methods*, *92*(2): 164–172. https://doi.org/10.1016/j.mimet.2012.11.018

Rashed, E. R. and Eissa, M. E. (2020). *Long-term monitoring of Cancer Mortality Rates in USA: A descriptive analysis using statistical process control tools*. https://doi.org/10.5281/ZENODO.3740610

US EPA, O. (2020, November 23). *Drinking Water Distribution System Tools and Resources* [Overviews and Factsheets]. https://www.epa.gov/dwreginfo/drinking-water-distribution-system-tools-and-resources

Water Sources | Public Water Systems | Drinking Water | Healthy Water | CDC. (2022, April 6). https://www.cdc.gov/healthywater/drinking/public/water_sources.html

Yuan, C.-C.; Chung, W.-H.; Cai, C. and Sung, S.-T. (2020). Application of Statistical Process Control on Port State Control. *Journal of Marine Science and Engineering*, *8*(10), 746. https://doi.org/10.3390/jmse8100746