Water Treatment Stage Impacts on the Occurrence of Bacteriological Indicators and their Multiple Antibiotic Resistance Index

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Received: 14 February 2024 | Revised: 7 March 2024 | Accepted: 9 March 2024

 DOI: https://doi.org/10.48084/etasr.7069

ABSTRACT

Constant monitoring of the bacteriological indicators of drinking water and the associated Multiple Antibiotic Resistance (MAR) index as impacted by seasonal variations and different stages of Drinking Water Treatment Plants (DWTPs) may assist in understanding the pattern of their seasonal occurrences and the regular operations of the treatment plant that influence their removal. In this paper, the impact of the seasons and of the different stages of DWTPs on bacteriological indicator occurrence and the MAR-index of five treatment plants from three provinces in South Africa were assessed. Colilert-18 and Enterolert Quanti-Tray/2000 IDEXX method were used to enumerate total coliform, *E. coli*, and *Enterococcus* spp. of water samples from the different treatment stages. Kirby–Bauer disc diffusion technique was used to assess the antibiotic susceptibility of the indicator bacteria isolates. All the measured physicochemical parameters were within the permissible limits. All the treatment plants essentially had a very high reduction of the indicator bacteria across all seasons. However, only two plants maintained the microbiological quality of the final treated water in compliance with the standards. A total of 121 isolates

were obtained, and 106 isolates were multidrug resistant with the greatest resistance recorded for the Beta-lactams class of antibiotics. The MAR-index varied across seasons and with different plants. This implied that the usage of antibiotics is season- and site-dependent. The different stages of treatment reduced the indicator bacteria with the most reduction occurring in disinfection and candy stages. These findings extend the knowledge of how the treatment stages and seasons shape indicator bacteria and antibiotic resistance in drinking water.

Keywords-IDEXX Quanti-Tray/2000; antibiotic resistance; coliform; water treatment; antibiotics discs

I. INTRODUCTION

Raw water sources for the Drinking Water Treatment Plants (DWTPs) often face the challenges of quantity and quality changes and associated seasonal variations related to climate and environmental pollution. Authors in [1] highlighted the impact of the economic development of many countries to the environmental pollution due to the increased release of solid and liquid waste that affect the raw water sources and consequently public health. Among the direct impacts of seasonal variation are temperature changes, heavy rainstorms, and longer drought periods which influence the availability of safe drinking water [2, 3]. Increases in temperature have been reported to enhance the eutrophication of surface water and promote the proliferation of diverse microbial communities within aquatic ecosystems which can lead to the emergence of opportunistic pathogenic bacteria [4, 5]. Seasons also cause variation in the antibiotic usage [6]. Studies have shown that the overall antibiotic prescription for outpatients is highest during Winter due to the recurring upper respiratory infections during these months followed by concurrent prescriptions of antibiotics [7, 8]. This intake of antibiotics creates antibiotic resistance in microorganisms which could be present in the feces/urine released by the patients and are transported directly into the surface water or indirectly after improper treatment from wastewater treatment plants. This can pose serious threats and challenges to the DWTPs whose function is to receive and treat surface and underground water to ensure its biological stability to an acceptable standard for human consumption. Authors in [9] define the biological stability of water as the smallest change in the microbial water quality. The measurement of bacteriological indicators such as *E. coli*, *Enterococcus* spp., and total coliforms is a monitoring tool to regulate the level of faecal contamination in water and assess the effectiveness of the DWTPs. The various stages of DWTPs contribute in unique ways to the reduction of the indicator bacteria present in the raw water sources [10]. Stages like flocculation, sedimentation and disinfection have been reported to reduce the occurrence of indicator bacteria [11]. However, while reducing the bacterial load, the release of Antibiotic Resistance Genes (ARGs) from antibiotic resistant bacteria can result in their transfer to other bacteria. This creates a challenging situation to human health arising from the consumption of such water.

In view of the provision of raw water sources including underground water to the DWTPs with less contamination, many studies have been conducted on the evaluation of the quality of raw water to ensure less health risks to humans upon consumption [12-14]. Only a few studies consider the seasonal impact and the stages of treatment on the occurrence of indicator bacteria as well as their antibiotic resistance to different classes of antibiotics. The present study assessed the impact of seasons and different stages of DWTPs on the occurrence of indicator bacteria and Multiple Antibiotic Resistance (MAR) index in five DWTPs in South Africa. The findings provide insights into the temporal change of the bacteriological indicators as well as their changes linked to the different treatment stages in the provinces of Limpopo, Mpumalanga, and Gauteng. The number of antibiotic resistant bacteria isolates across the different treatment plants representing the three provinces and the MAR-index across the seasons highlighting the need for the constant monitoring of the antibiotic intake in the most prone provinces. This will encourage further studies on the preventive strategies that could be adopted prior to or during the seasons when increased intake of antibiotics could occur due to the recurring disease pattern.

II. MATERIALS AND METHODS

A. Study Area and Selection of Sampling Points

This study targeted five treatment plants considered as A, B, C, D, and E from Gauteng (A, D, E), Limpopo (B), and Mpumalanga (C) in South Africa. Consent for sampling was obtained from plant authorities and the study was conducted in compliance to the University of South Africa research ethics requirements. The names of the plants were coded for confidentiality reasons. Replicate samples were taken from the raw water (untreated), each stage of the treatment plants (as shown in Table I), and the final treated water. A total of 336 samples from all the sampling points per site per season were taken. Samples were collected during the Spring of 2022 (in South Africa, the Spring falls within September, October, and November) and the consecutive Summer, Winter, and Autumn of 2023. Sterile 1000 mL bottles were used to collect water samples and transported at 4◦C to the laboratory for analysis within 3–6 h from collection.

B. Physicochemical Analysis of the Water Samples

Electrical conductivity, total dissolved solutes, temperature, and pH were measured in triplicates on-site using Hanna HI9828 multi-parameter ion-specific meter (Hanna Instruments (Pty) Ltd, Bedfordview, South Africa). The mean values were compared to the acceptable standards.

C. Enumeration of Indicator Organisms

Total coliforms, *E. coli*, and *Enterococcus* spp. were quantified using the Colilert-18 and Enterolert Quanti-Tray/ 2000 (ISO 9308-2:2012) (IDEXX Laboratories (Pty) Ltd., Johannesburg, South Africa). All samples were analyzed according to the manufacturer's instructions. Before analysis, all equipment was decontaminated using ultraviolet (UV) radiation. A sachet of the reagent powder (Colilert-18 for *E. coli* and Enterolert-DW for *Enterococcus* spp.) was added to a

100 mL water sample, sealed, and then incubated at different temperatures according to the test, Colilert-18 (35 ±0.5 °C for 18 ± 1 h), Enterolert-DW (41 ±0.5◦C for 24 ± 1 h). Trays were then compared to comparators, and positive wells were counted and transformed to determine Most Probable Numbers (MPNs) using the provided IDEXX MPN charts. Yellow appearance greater than the comparator was recorded positive for total coliform and blue fluorescence under hand-held fluorescent light (4 W, 366 nm) was recorded positive for *E. coli*, and *Enterococcus* spp.

TABLE I. TREATMENT STAGES EMPLOYED AT THE FIVE SELECTED DRINKING WATER TREATMENT PLANTS

A	B	C	D	E
Coagulation/Flocculation	Coagulation/Flocculation	Coagulation/Flocculation	Sedimentation	Pre-chlorination
Sedimentation	Sedimentation	Sedimentation	Filtration	Coagulation/Flocculation
Filtration	Filtration	Candy		Sedimentation
Disinfection	Disinfection	Filtration		Filtration

D. Recovering Isolates and Confirmation test

After incubation and counting of the positive well, the back of the tray was disinfected using 70% ethanol with a sterile swab. A sterile razor blade was deployed to pierce the sterile back of 3 fluorescence-positive wells per tray. Three trays were sampled per water sample and a loop full of well content was streaked on *E. coli* (Eosin methylene blue agar) and *Enterococcus* spp. (ChromAgarTM *Enterococcus*) selective media. The colonies were scraped from the top of the agar with a sterile loop and were used for a confirmation test. Gas and indole production in lactose tryptose lauryl sulphate broth and growth in Brain Heart Infusion (BHI) broth were utilized to confirm *E. coli* and *Enterococci* spp., respectively. The confirmed *E. coli* and *Enterococci* spp. were stored in 20% (v/v) glycerol at −80◦C for future analysis.

E. Antibiotic Susceptibility Testing

Ten antimicrobial agents including Ampicillin (AM) (10 µg), Erythromycin (E) (15 µg), Ciprofloxacin (CIP) (5 µg), Nitrofurantoin (F) (300 µg), Amoxicillin/clavulanic acid (AMC) (30 µg), Gentamicin (CN) (10 µg), Imipenem (IMP) (10 µg), Trimethoprim/sulphamethoxazol (SXT) (25 µg), Levofloxacin (LEV) (5 µg), and Vancomycin (VA) (30 µg) were used for susceptibility testing. The antibiotics were selected based on the Clinical Laboratory Standards Institute [15-17] principles and the former's frequent use in veterinary and human medicine. The Kirby–Bauer disc diffusion technique was deployed, and the measured inhibition zone diameter was interpreted utilizing the Clinical Laboratory Standards Institute (CLSI) breakpoints' values [15-17]. Table II shows a summary of the classes of antibiotics used in this study. Multidrug-resistant isolates (resistant to ≥ three antimicrobial agents) were selected and stored in 20% (v/v) glycerol at −80 °C for future analysis. The *E. coli* strain (ATCC 25922) and *Enterococcus faecalis* (ATCC 29212) were used as control samples according to CLSI control standards.

F. Multiple Antibiotic Resistance (MAR) Index

MAR-index was calculated as (x/y), where "x" is the number of antibiotics to which the isolate was resistant, and "y" is the number of antibiotics to which the isolate was exposed. An isolate from an area of high antibiotic usage will demonstrate an MAR index of >0.2, while one with a value of <0.2 is from a source of lower antibiotics usage.

TABLE II. CLASSES OF ANTIBIOTICS USED IN THIS STUDY

Antibiotics class	Antibiotics used
Beta-lactams	Imipenem
	Amoxicillin/clavulanic
	Ampicillin
	Nitrofurantoin
Quinolone	Levofloxacin
	Ciprofloxacin
Aminoglycoside	Gentamicin
Macrolides	Erythromycin
Sulfonamides	Trimethoprim/sulphamethoxazol
Glycopeptide	Vancomycin

G. Statistical Analysis

The means of triplicate physicochemical measurements were determined in Microsoft Excel. The data obtained from the IDEXX-defined substrate Colilert-18/ QuantiTray 2000 analysis were quantified based on the MPN of *E. coli* and *Enterococcus* spp. and the total coliforms enumerated in the MPN table (IDEXX Quanti-Tray*/2000 MPN Table). Analysis of variance of all the positive wells from sites A, B, C, D, and E as the ones impacted by the seasons and the different treatment stages was calculated in STATISTICA version 12 (StatSoft Inc., Tulsa, OK, USA). Post-hoc analysis was done using the Duncan test to separate the mean values and to show the significant difference between the variables. The percentage resistance of the indicator bacteria across the treatment stages and sites were subjected to analysis using XLSTAT, and a Chi-square test was employed to determine if there were any significant differences between the raw water sources, during the treatment processes and the final treated water as well as the different sampling sites [18].

III. RESULTS

A. Physicochemical Parameters

The results on the mean value of the measured physicochemical parameters of the water samples from different sampling sites collected at different seasons and from different treatment stages can be seen in Appendix I. The pH values ranged from 6.7 to 9.4 across the sampling sites (A-E) with variations in the treatment stages and seasons. However, all the values were within the recommended limit [19]. The water temperature of all the sites was higher during the warmer seasons (Spring and Summer) than the cooler seasons, as expected, and the temperature varied across the treatment stages. The electrical conductivity ranged from 44.1 to 75.6 µS/cm (site A), 40.4 to 81.2 µS/cm (site B), 46.7 to 72.3 µS/cm

(site C), 45.8 to 82.5 µS/cm (site D), and 48.4 to 89.6 µS/cm (site D). The electrical conductivity of site E was slightly higher than that of the other sites for all the treatment stages, and the Winter/Autumn values were higher than those of the other seasons. However, all the values for electrical conductivity for all the sites, seasons, and treatment stages were within the stipulated South African drinking water quality standard [19]. Similarly, the total dissolved solids were higher in Winter (222.4-680 mg/L) and Autumn (325.7-750.2 mg/L) than in Spring (212.4-579.3 mg/L) and Summer (186.2-550.2 mg/L) with variation across the treatment stages and sites. Strikingly, all the values were within the standards [19]. Furthermore, the treatment stages clearly influenced the measured parameters when compared to the raw water sources and the final treated water in all the treatment plants by ensuring that the aesthetic properties of the water were within the stipulated standard.

B. Bacteriological Quality of the Water

The MPN of the indicator bacteria per 100 mL based on IDEXX MPN chart shows variations across the seasons and the treatment stages (Appendix II). The number was also compared to the SANS [19] to assess compliance to the drinking water regulation. In sampling site A, the final treated water met the SANS [19] bacteriological water quality standard for *E. coli*, *Enterococcus* spp., and total coliforms. However, the Autumn water sample from the disinfectant stage did not meet the standard for *E. coli*. The disinfectant stage across the seasons and for all the bacteriological indicators met the stipulated standard [19] in sampling site B but, the final treated water in Summer, Spring, and Autumn was not within the standard for *Enterococcus* spp. Variations in the treatment stages that met the standard were observed at different seasons with the final treated water meeting the standard across the seasons and all the indicators in sampling site C (Appendix II). A similar trend was observed in sampling site D. However, the final treated water did not meet the standard for total coliforms (Spring and Winter) and *Enterococcus* spp. (Winter). The final treated water for sampling site E was within the stipulated standard except for *E. coli* (Summer). The seasonal impacts on the bacteriological indicator based on the mean value at 95% confidence level of all the positive wells of the Colilert-18 and Enterolert Quanti-Tray/ 2000 (ISO 9308-2:2012) across the treatment sites (A-E) are represented in Figure 1 (total coliforms), Figure 2 (*E. coli*), and Figure 3 (*Enterococcus* spp.). For total coliforms (Figure 1), a significant difference ($p<0.05$) was observed in sites A and D, both from Gauteng Province with the highest proliferation of the total coliforms in Summer and Spring seasons, respectively. In sites B (Limpopo Province), C (Mpumalanga Province), and E (Gauteng Province), there were no significant differences across the seasons although the highest proliferation was recorded in Autumn (B) and Spring (C, E) seasons. No significant difference ($p<0.05$) was observed across the seasons for *E. coli* in site B (Figure 2). Across other treatment plants with significant differences ($p<0.05$), varied seasonal response in the proliferation of *E. coli* was observed with the highest proliferation having been evidenced in site A during Autumn, site C during Spring, site D during Spring and site E during Summer. For *Enterococcus* spp., all five sites showed significant differences ($p<0.05$) with the highest proliferation occurring in site A (Autumn), B (Winter), C (Spring), D (Winter), E (Autumn) (Figure 3). At a confidence level of 95%, the mean value of the indicator bacteria based on the overall positive trays of the Colilert-18 and Enterolert Quanti-Tray/ 2000 (ISO 9308-2:2012) was calculated at each stage of the drinking water treatment to access their impact on the removal of indicator bacteria (Figures 4-6). The treatment stages clearly influenced the abundance of the bacteriological indicators that survive during the process. For the total coliforms, a significant difference ($p<0.05$) in their abundance was observed across all treatment sites (Figure 4). The stages with the lowest mean value or no mean value of total coliforms include A and B (disinfection stage and final treated water), C (filtration stage and final treated water), D (sedimentation stage and final treated water), E (flocculation and final treated water). Similarly, a significant difference across the treatment stages in the different treatment plants was observed in the removal of *E. coli* (Figure 5). The stages with very little or no identified *E. coli* included the disinfection stage and the final treated water (site A), disinfection (site B), sedimentation, and final treated water (site C), filtration and final treated water (site D and E). However, the presence of *E. coli* in the final treated water in B and D is a great concern. Figure 6 demonstrates that there is a significant difference across the treatment stages for all the sites in the removal of *Enterococcus* spp. The disinfection stage and the final treated water for sites A and B show a complete removal of the *Enterococcus* spp. It was found that the effect of the treatment stages on the indicator bacteria is site- and treatment method-dependent due to the differences in the abundance of the indicator bacteria at each stage per site.

C. Antibiotic Resistance Profile

Antibiotic resistance profiling of the bacteriological indicator isolated from different treatment stages during Spring, Summer, Winter, and Autumn was done to provide insight into their response to antibiotics. The identified bacteriological indicators from different treatment stages across the four seasons (*E. coli* and *Enterococcus* spp.) and exposed to different classes of antibiotics were 121 (Appendix III). Among the 121 isolates, 15 were not Multidrug Resistant (MDR) with 3 isolates from *E. coli* and 12 isolates from *Enterococcus* spp. (Appendix III). From site A, 26 isolates were tested in total and 2 (1- *E. coli*/Winter/sedimentation and 1- *Enterococcus* spp./Autumn/1- flocculation) isolates were not MDR. Site B had 30 isolates tested and 6 (1- *E. coli*/Winter/raw and 5- *Enterococcus* spp./all in Autumn/1-raw, 1- flocculation, 1- sedimentation,1- filtration, and 1- final water) were not MDR. Twenty-seven isolates were obtained and tested for antibiotics susceptibility in site C. Two isolates (1- *E. coli*/Winter/raw and 1- *Enterococcus* spp./Winter/raw) were not MDR. For site D, out of the 14 tested isolates, 2 (2- *Enterococcus* spp./all in Autumn/1-raw, 1-filtration) were not MDR. At site E, 24 isolates were tested and 3 isolates (3- *Enterococcus* spp./all in Autumn/1- pre-chlorination,1-sedimentation, and 1-filtration) were not MDR (Appendix III).

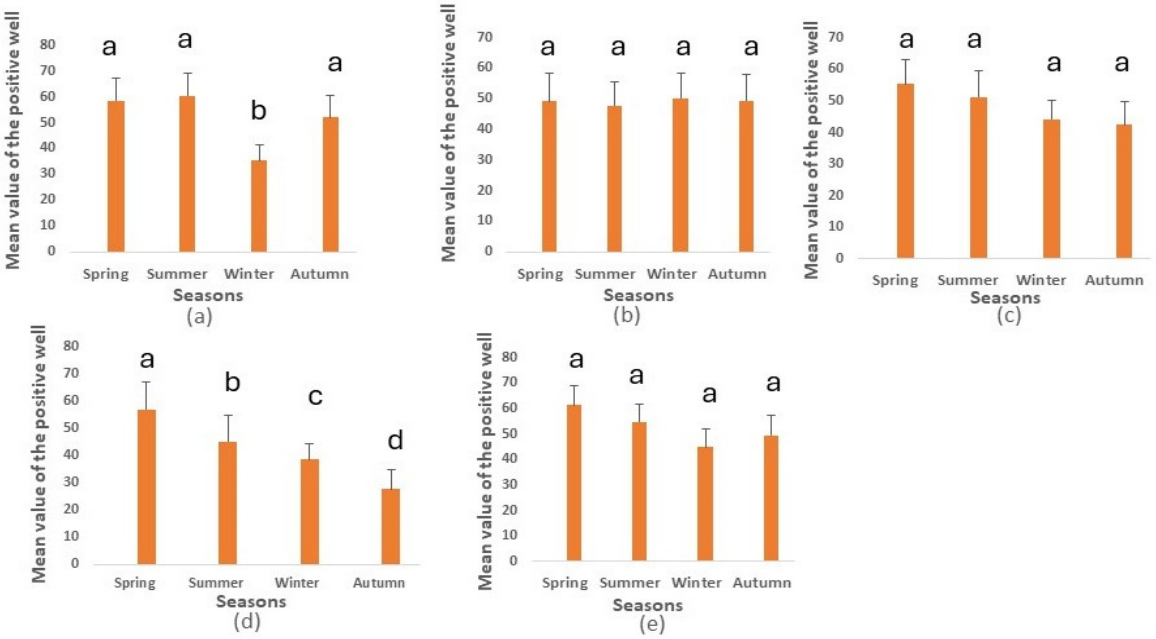

Fig. 1. Impacts of seasonal variation on the mean value of the total coliforms based on the Colilert-18 and Enterolert Quanti-Tray/ 2000 (ISO 9308-2:2012) overall positive results from the tray. Letters (a) to (e) represent the sampling sites. Seasons with the same letter are not significant at $p<0.05$.

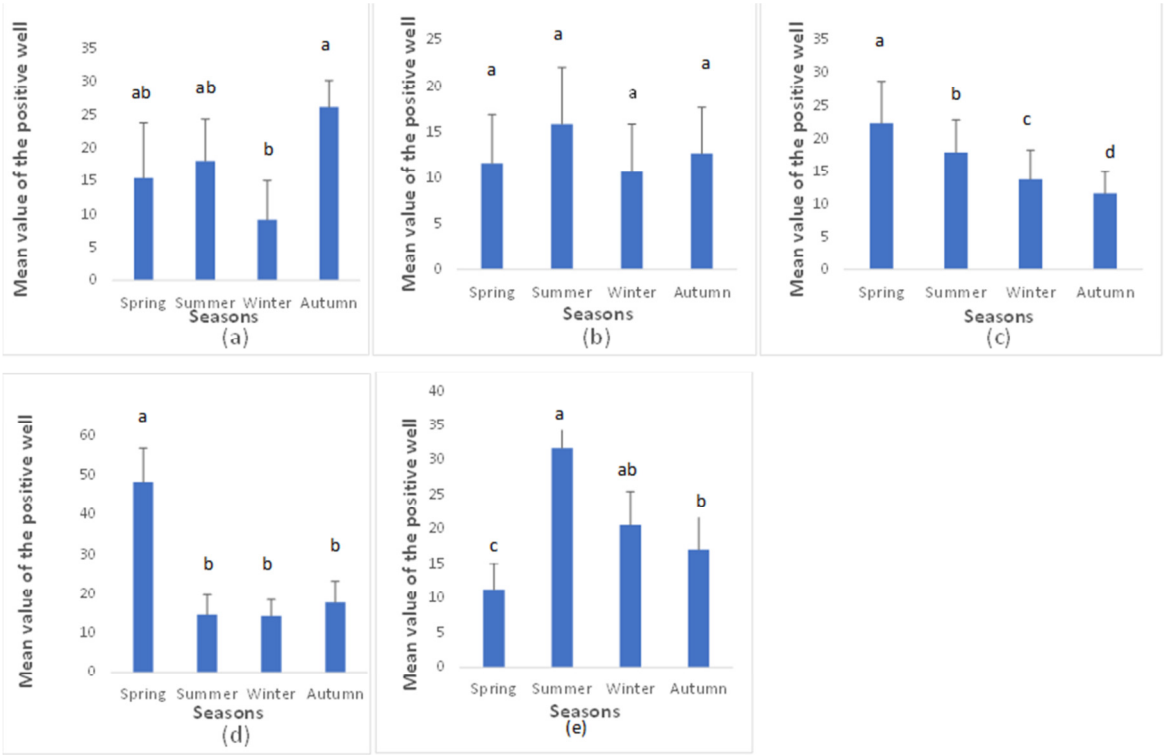

Fig. 2. Impact of seasonal variation on the mean value of *E. coli* based on the Colilert-18 and Enterolert Quanti-Tray/ 2000 (ISO 9308-2:2012) overall positive results from the tray. Letters (a) to (e) represent the sampling sites. Seasons with the same letter are not significant at $p<0.05$.

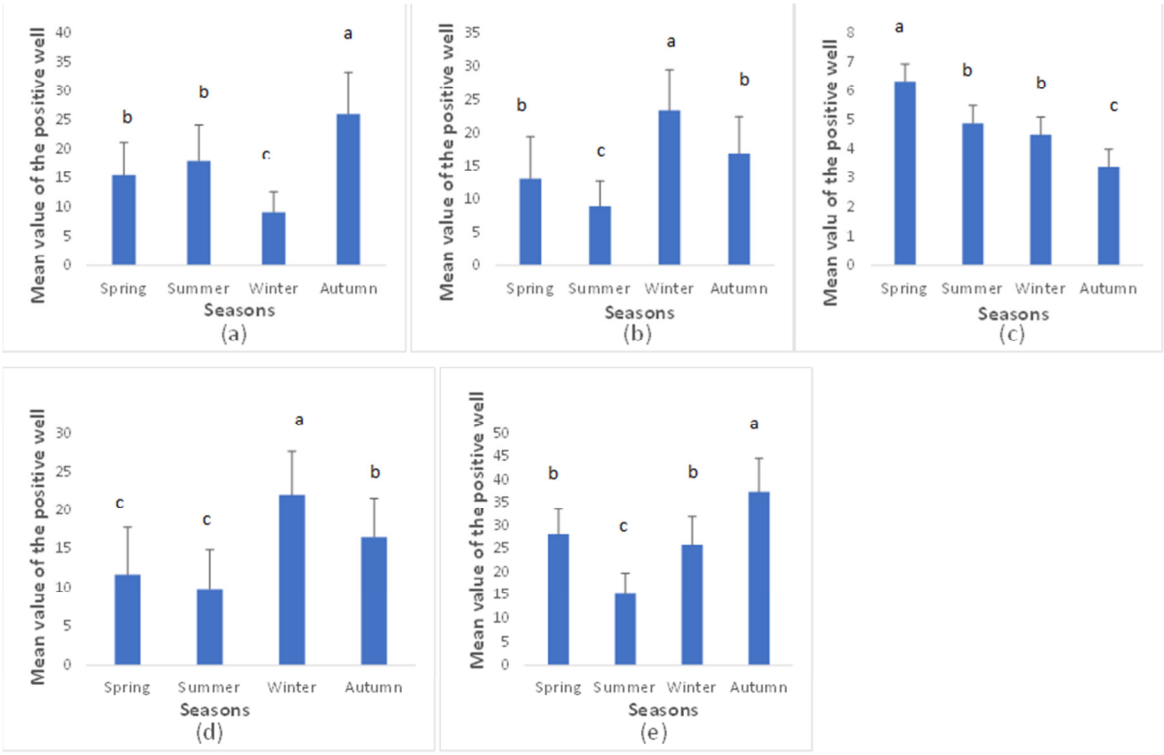

Fig. 3. Impact of seasonal variation on the mean value of *Enterococcus* spp. based on the Colilert-18 and Enterolert Quanti-Tray/ 2000 (ISO 9308-2:2012) overall positive results from the tray. Letters (a) to (e) represent the sampling sites. Seasons with the same letter are not significant at $p<0.05$.

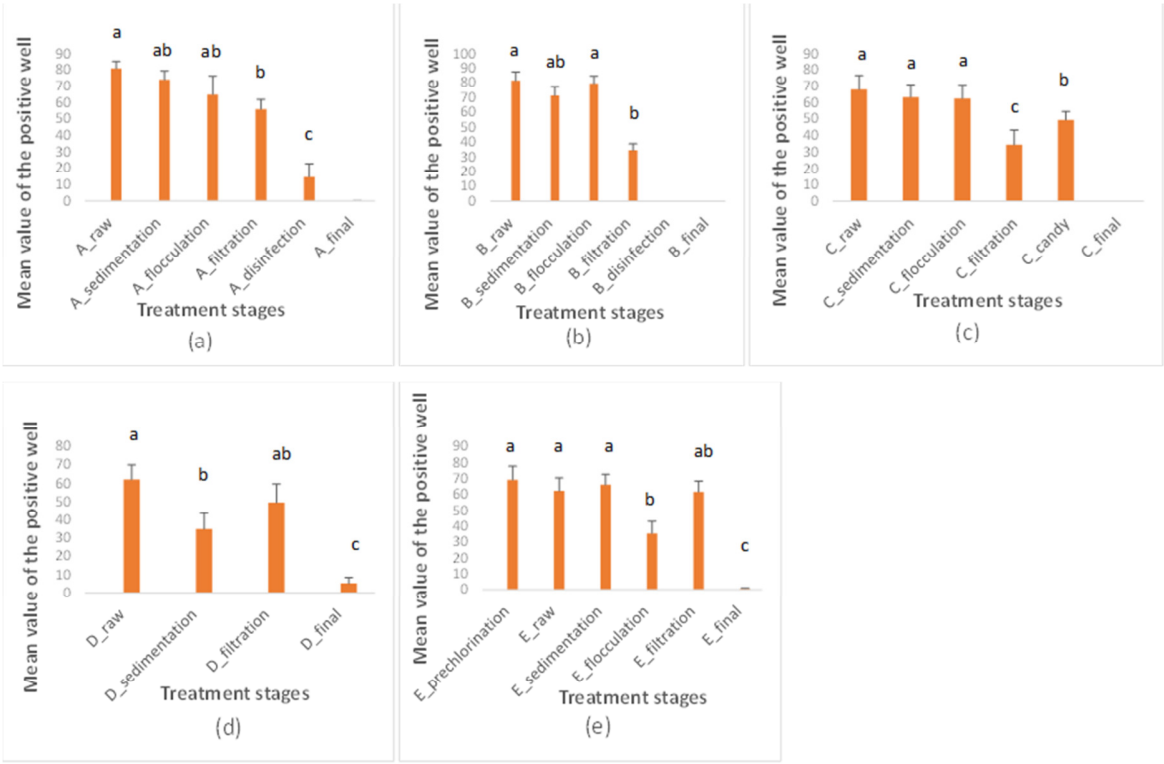

Fig. 4. Effect of the different stages of drinking water treatment adopted by the different treatment plants on total coliforms based on the overall positive results of the trays. Letters (a) to (e) represent the sampling sites. Stages with the same letters are not significant at $p<0.05$.

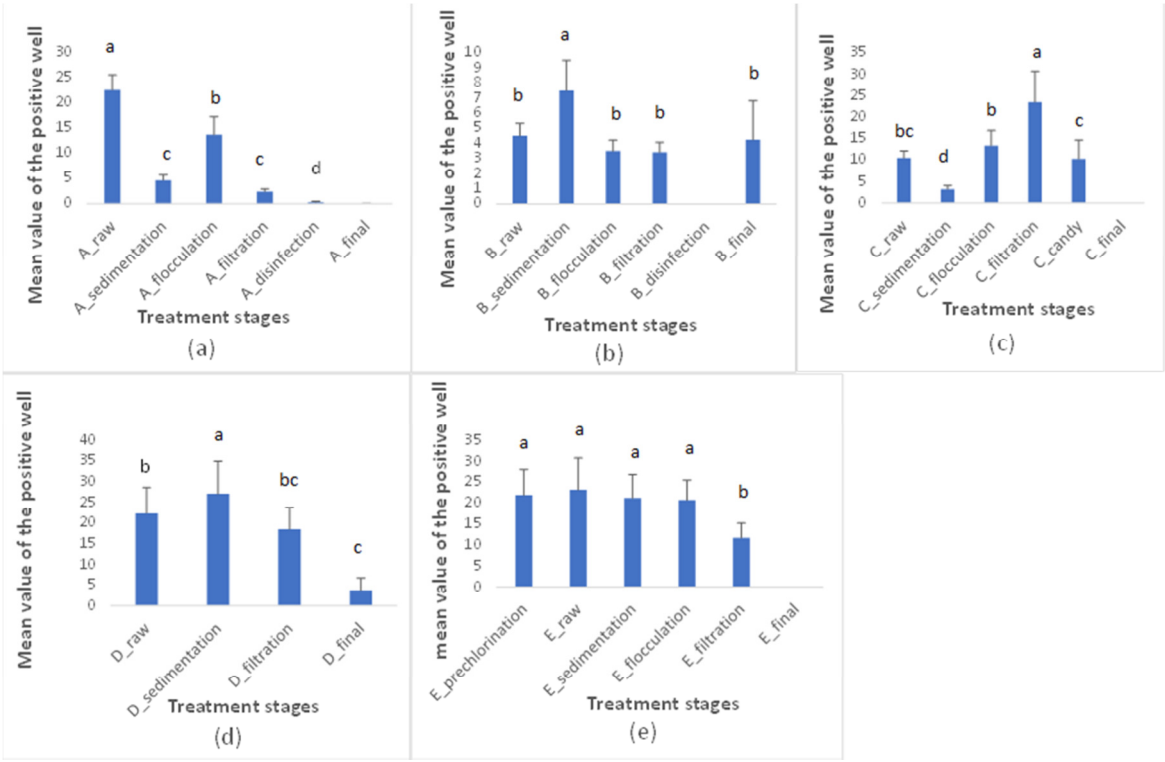

Fig. 5. Effect of the different stages of drinking water treatment adopted by the different treatment plants on *E. coli* based on the overall positive results of the trays. Letters (a) to (e) represent the sampling sites. Stages with the same letters are not significant at $p<0.05$.

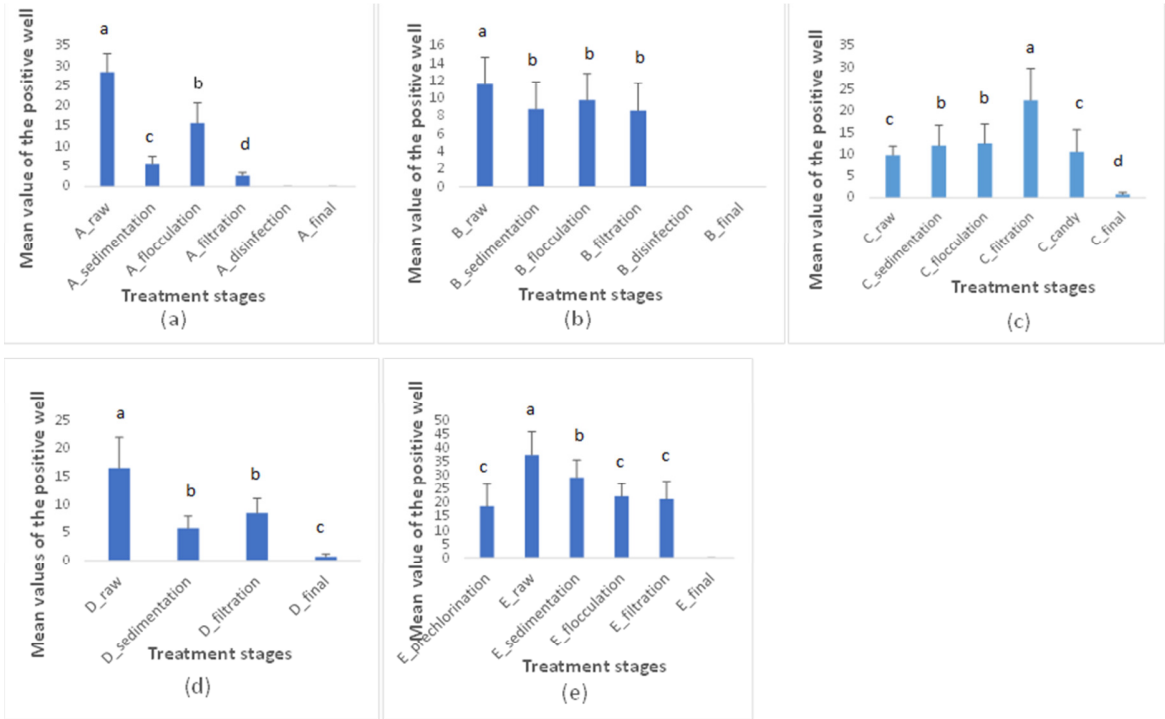

Fig. 6. Effect of the different stages of drinking water treatment adopted by the different treatment plants on *Enterococcus* spp. based on the overall positive results of the trays. Letters (a) to (e) represent the sampling sites. Stages with the same letters are not significant at $p<0.05$.

The percentage of *E. coli* isolates resistant to the different antibiotics applied from the raw water sources, during the treatment processes (this includes all the treatment stages), and the final treated water were significantly different ($p<0.05$)

across the sites and the different treatment stages (Table III). From all the sites, it was observed that the treatment stages contained *E. coli* that showed high resistance to IMP, AMC, CN, AM, F, E, and SXT (site A), all the antibiotics (sites B, C, and D), IMP, AMC, CN, AM, F, E, and SXT (site E). Strikingly, the final treated water from site E (Gauteng) contained *E. coli* that was resistant to AMC, LEV, AM, and E. Similarly, *Enterococcus* spp. isolates from the treatment stages (DTP) exhibited higher antibiotic resistance percentage in all the treatment sites (A-E) (Table SM4). Furthermore, the isolates from the final treated water in site B (Limpopo) displayed higher resistance to IMP, AMC, CN, AM, F, E, and SXT (Table IV).

TABLE III. PERCENTAGE OF *E. COLI* ISOLATES RESISTANT TO ANTIBIOTICS ACROSS RAW WATER, TREATMENT PROCESSES, AND FINAL TREATED WATER

Sitess/Treatment stage		IMP	AMC	LEV	CIP	CN	AM	F	E	SXT	Mean ± SD
A	RW	33.3	37.5	0	0	0	0	33.3	30	33.3	18.6±17.7Bd
	DTP	66.7	62.5	0	0	100	100	66.7	70	66.7	59.2±36.5Ab
	FW	0	0	0	0	0	0	0	0	0	0±0Ce
B	RW	20	37.5	0	0	0	20	28.6	22.2	28.6	17.4±14.1Bd
	DTP	80	62.5	100	100	100	80	71.4	77.8	71.4	82.6±14.1Aa
	FW	0	0	0	0	0	0	0	0	0	0±0Ce
C	RW	20	20	0	0	0	27.3	14.3	27.3	11.1	13.3±11.3Bd
	DTP	80	80	100	100	100	72.7	85.7	72.7	88.9	86.7±11.3Aa
	FW	0	0	0	0	0	0	0	0	0	0±0Ce
D	RW	50	42.9	66.7	33.3	0	42.9	42.9	42.9	50	41.3±17.9Bc
	DTP	50	57.1	33.3	66.7	100	57.1	57.1	57.1	50	58.7±17.9Ab
	FW	0	0	0	0	0	0	0	0	0	0±0Ce
E	RW	16.7	33.3	0	0	0	22.2	33.3	12.5	20	15.3±13.4Bd
	DTP	83.3	50	0	0	100	66.7	66.7	75	80	58.0±35.6Ab
	FW	0	16.7	100	0	0	11.1	0	12.5	0	15.6±32.3Bd

IMP: Imipenem; AMC: Amoxicillin/clavulanic acid; LEV: Levofloxacin; CIP: Ciprofloxacin; CN: Gentamicin; AM: Ampicillin; VA: Vancomycin; F: Nitrofurantoin; E: Erythromycin; SXT: Trimethoprim/sulphamethoxazol; RW: Raw water; DTP: During Treatment Process; FW: Final Water. Means with same letters within the column (Capital letter A-B shows differences between RW, DTP, and FW per sites; Small letters a-h shows differences across the sites and treatment stages) are not significant at $p<0.05$.

TABLE IV. PERCENTAGE OF *ENTEROCOCCUS* SPP. ISOLATES RESISTANT TO THE ANTIBIOTICS ACROSS RAW WATER, TREATMENT PROCESSES, AND FINAL TREATED WATER

Sites/Treatment stage		IMP	AMC	LEV	CIP	CN	AM	VA	F	E	SXT	Mean ± SD
A	RW	42.9	37.5	50	50	0	0	37.5	42.9	52.9	28.6	34.23 ±19.4Be
	DTP	57.1	62.5	50	50	0	100	62.5	57.1	57.1	71.4	56.77 ±24.6Ad
	FW	0	0	0	0	0	0	0	0	0	0	0 ±0Ch
B	RW	20	28.6	50	0	0	18.2	18.2	20	20	20	19.5 ±14.1Bf
	DTP	60	57.1	50	100	50	63.6	72.7	60	60	60	63.34±14.4Acd
	FW	20	14.3	0	0	50	18.2	9.1	20	20	20	17.16 ±14Bf
C	RW	0	20	0	0	0	27.3	30	14.3	30	16.7	13.83 ±13Bg
	DTP	100	80	100	100	100	72.7	70	85.7	70	83.3	86.17 ±13Aa
	FW	0	0	0	0	0	0	0	0	0	0	0±0Ch
D	RW	33.3	33.3	0	0	50	33.3	33.3	50	33.3	50	31.65±18.3Bef
	DT	66.7	66.7	100	100	50	66.7	66.7	50	66.7	50	68.35 ±18.3Ac
	FW	0	0	0	0	0	0	0	0	0	0	0 ±0 Ch
E	RW	28.6	22.2	0	0	0	25	28.6	25	28.6	33.3	19.13 ±13.5Bf
	DTP	71.4	77.8	100	100	100	75	71.4	75	71.4	66.7	80.87 ±13.5Ab
	FW	0	0	0	0	0	0	0	0	0	0	0 ±0 Ch

IMP: Imipenem; AMC: Amoxicillin/clavulanic acid; LEV: Levofloxacin; CIP: Ciprofloxacin; CN: Gentamicin; AM: Ampicillin; VA: Vancomycin; F: Nitrofurantoin; E: Erythromycin; SXT: Trimethoprim/sulphamethoxazol; RW: Raw water; DTP: During Treatment Process; FW: Final Water. Means with same letters within the column (Capital letter A-B shows differences between RW, DTP, and FW per sites; Small letters a-h shows differences across the sites and treatment stages) are not significant at $p<0.05$.

The relative abundance of bacteriological indicators (*E. coli* and *Enterococcus* spp.) resistant to different classes of antibiotics, such as Beta-lactams (IMP, AMC, AM, and F), Quinolone (LEV and CIP), Aminoglycoside (CN), Macrolides (E), Sulfonamides (SXT), and Glycopeptide (VA) is represented in Figure 7. The isolated indicator bacteria were more resistant to the Beta-lactams class of antibiotics across all the sampling sites with sites A and D (Gauteng Province) posing more than 55% resistant isolates to the Beta-lactams class of antibiotics. Macrolides were the most resisted class of antibiotics after the Beta-lactams group followed by the Sulfonamides group.

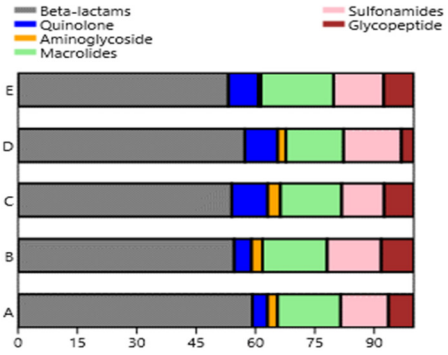

Fig. 7. Relative abundance of bacteriological indicator isolates resistant to different classes of antibiotics tested in this study.

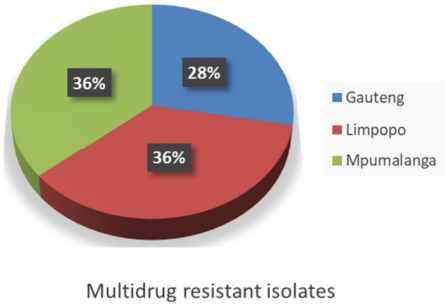

Fig. 8. Mean percentage of multidrug resistant isolates present in water samples from the selected drinking water treatment plants in Gauteng, Limpopo, and Mpumalanga Provinces.

The least resisted class of antibiotics was the Aminoglycoside with no resistance observed at site E. The mean percentage of MDR isolates from the water samples obtained from plants in Gauteng, Limpopo, and Mpumalanga is summarized in Figure 8. The results revealed that these three provinces were exposed to indicator bacteria that were multidrug resistant, but both Limpopo and Mpumalanga Province had the highest MDR isolates

D. Multiple Antibiotic Resistance Index

The MAR index was calculated based on the resistances to the different antibiotics by the bacteriological indicators, as observed in the Appendix. From the 121 bacteriological indicator isolates, 6 isolates showed an MAR-index < 0.2. This indicates high usage of the different antibiotics in the area where the sampling sites are located. The percentage of all the MAR-index > 0.2 was calculated based on the seasons (Figure 9) to assess the season with the higher usage of antibiotics relative to the sampling sites and the surrounding environment. From the information in Figure 9, variation in the usage of antibiotics across the seasons in the different sampling sites was observed.

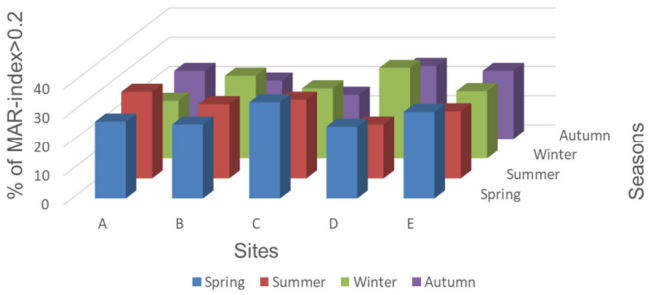

Fig. 9. Seasonal impact on the antibiotic usage across the areas where the sampling sites are located.

In the area surrounding site A (Gauteng Province), the usage of antibiotics was high in Summer and Autumn whereas in site B (Limpopo Province), high usage of antibiotics occurred in Winter. Spring season exhibited a high usage of antibiotics for site C (Mpumalanga Province), Winter and Autumn for site D (Gauteng Province), and Spring and Autumn for site E (Gauteng Province). This indicates that seasons affect the usage of antibiotics in the area surrounding the sampling sites differently. Notably, in Gauteng Province, all the sites showed high usage of antibiotics in Autumn season.

IV. DISCUSSION

The persistence of bacteria during drinking water treatment stages and their constant regrowth in the final treated water is a constant global challenge. The regrowth of the bacteria is linked to the physicochemical and biological properties of the water and the impact of the treatment stages. Seasons and different water treatment stages affect the physicochemical parameters of water [20, 21]. Hence, there is a need for constant monitoring of the water physicochemical parameters including groundwater sources to ensure that they meet the stipulated standards [22]. All physicochemical parameters (pH, temperature, electrical conductivity, and total dissolved solutes) measured in the present study were within the SANS [19] standard across all seasons with variations in the measured value observed across the seasons. Similarly to this study's findings, authors in [22] observed that the pH of the raw water sources (groundwater) was within the stipulated standard. Furthermore, authors in [12] observed that pH, temperature, and total dissolved solutes were within the stipulated standards across raw water sources, treatment stages, and seasons except for their electrical conductivity, which was above the permissible limit. The reason behind this difference could be attributed to the selected different treatment plants and the adopted treatment processes. Among the factors that affect the aesthetic properties of water, an increase in the electrical conductivity and the total dissolved solutes produce a remarkable change in the water aesthetic properties making it unfit for human consumption. Increased electrical conductivity is linked to increased minerals in the water samples [23]. Generally, as expected, the bacteriological indicators (total coliform, *E. coli*, and *Enterococcus* spp.) decreased from the raw water to the final treated water with variations in the number of indicators across the treatment stages and the final treated water in different seasons. The differences in the seasonal proliferation of the indicator bacteria in different sites could be linked to environmental parameter changes in the different sites or different anthropogenic activities occurring in the area surrounding the sampling sites as well as the seasonal impact on the infrastructure [24]. Other studies have shown that seasons influence the proliferation of the indicator bacteria during the treatment of drinking water. Proliferation of the measured indicator bacteria during Winter and Summer seasons increased, but not in Autumn and Spring [12]. In South Africa, the Summer season is characterized by rainfall more than Winter, and runoffs from settlements and agricultural activities into the raw water sources occur. In addition, fluctuations in air temperature associated with the different seasons also drive bacteria growth, regrowth, and proliferation in surface water and groundwater [24-26]. This could account for the increased proliferation of *E. coli* and total coliforms during the Summer and Spring seasons at the related sites. Authors in [27] highlighted that the proliferation of the indicator bacteria could be linked to fecal pollution seen to be varied across different areas and different seasons depending on the human activities. Fecal pollution occurs due to direct sewage deposition into raw water sources, discharged from wastewater treatment plants as well as runoffs during rainfalls

from agricultural and other anthropogenic activities. This could also account for the site-seasonal patterns of the proliferation of the indicator bacteria observed.

The treatment stages in the DWTPs have specific roles in establishing water quality standards through the removal of organic matter and pathogenic microorganisms from the raw water sources. However, changes that could occur during operational processes in the different treatment stages alter the removal of microbial populations including bacteriological indicators. In the DWTPs, the type of coagulants used in the coagulation process, the method of sedimentation, the filtration types and infrastructure, and the oxidant used in the disinfection process are linked to the expected water quality and microbiological status of the water [28]. Processes, such as oxidation, filtration, soil infiltration, coagulation and flocculation, and adsorption have been reported to play a vital role in the removal of pathogenic microorganisms (including indicator bacteria) from water samples [11, 29, 30]. In the current study, although the total coliforms, *E. coli*, and *Enterococcus* spp. in most of the treatment stages were not within the stipulated standards (Appendix), there was an enhanced reduction of the indicator bacteria by the end of the treatment of the final water that met the standards despite the starting raw water quality. To obtain further insight into the different treatment stages that could contribute more to the reduction of the indicator bacteria, the mean value of the positive IDEXX colilert trays was calculated. Significantly, the treatment stages reduced the abundance of the indicator bacteria with variations across the sites and of the individual indicator bacteria (total coliforms, *E. coli*, and *Enterococcus* spp.). Disinfection stage (sites A and B), filtration stage (site C), sedimentation and flocculation stages (site D and E, respectively) play a major role in the reduction of the coliforms. Authors in [31] observed high bacteria indicators during preliminary coagulation in settling tanks, filtration on sand filters, and 100% reduction during the disinfection stage. This finding is congruent to the level of bacteria indicator reduction in disinfection stages of most of the sites in this study. This could be attributed to the disinfecting effect of the different oxidant used, such as chlorine, chloroamine, ozone, and UV light [32, 33]. Provision of potable water with good microbiological and aesthetical properties is necessary for the promotion of agricultural production and reduction of health risk associated with the delivery of contaminated water to the populace [34]. Provincially, the final treated water from DWTPs in only one of the provinces recorded no indicator bacteria. All the raw water sources from all the provinces were above the stipulated standard limits. This could be attributed to agricultural activities, groundwater overexploitation, and other human activities that lead to the contamination of raw water sources [35]. Site B is from the Limpopo Province which has many rural communities and often a shortage of proper sanitation, thus water resources are prone to fecal contamination. Rural and informal settlement of people in these areas rely on pit latrines as their primary means of sanitation and this has a health impact associated with microbiological and chemical contamination of groundwater tables as well as of other natural water bodies in their proximity [36]. Mpumalanga (site C) and Gauteng Province (A, D, and E) are not exceptions to the water quality problems. The two provinces faced water shortage due to increased urbanization, migration, and industrialization along with aging infrastructure issues, especially in the Mpumalanga Province [37]. The increase in population comes along with increased production of sewages and with the occurrence of water shortage. Improper disposal of sewage wastewater could result in the pollution of the raw water resources used by DWTPs. This creates great challenges to treatment plants especially those with poor infrastructure and lack of good maintenance practices [38]. All this information could account for the increased pollution of the raw water sources as observed in this study and the consequent presence of the indicator bacteria in the final treated water of most of the sites. In addition, one could say that infrastructural challenges and robustness in the treatment plants could also be the reason behind the presence of the indicator bacteria in the final treated water.

The development of antimicrobial resistant bacteria and their spread is associated with environmental factors which include seasonal variations [39, 40]. This makes constant monitoring of the antimicrobial resistance pattern, the spread, as well as the factors that enhance the development of resistant bacteria in the final treated water necessary. The antimicrobial resistance response of the bacteria indicator shows 106 out of 121 isolates, being MDR. Site B recorded the highest MDR isolates (see Appendix). Site B is a DWTP located in Limpopo Province and surrounded by rural settings and livelihoods of less developed water and sanitation conditions. This could cause the use of untreated water by the population around the area leading to an outbreak of diseases that could warrant regular use of particular antibiotics. The number of MDR isolates varied with seasons and the different treatment plants (A-E). In treatment plants C (Mpumalanga Province) and E (Gauteng Province), the highest MDR isolates were recorded in Spring. In plants A and D (both in Gauteng), the highest MDR isolates occurred in Summer and Winter, respectively, while in plant B, the highest MDR isolates were observed in Spring, Summer, and Winter seasons. This also agrees with the MAR-index (Figure 9), which indicates the high usage of antibiotics in the area surrounding the treatment plants in those seasons. Authors in [41, 42] highlighted the misuse and overuse of similar antibiotics in human medicine and agriculture as a factor that promotes the development and spread of MDR in microorganisms. This accounts for the increase in the MDR isolates in areas with high usage of antibiotics. Author in [6] assessed the seasonal variation between the antibiotic use and resistance. Although the seasonal peaks varied with the class of antibiotics used, high resistance to the combination of antibiotics used was observed more during Winter and Spring. This could be associated with high disease outbreaks during Winter that results in the increased intake of the antibiotics as well as the disease outbreak associated with the change of weather conditions from low temperature to a higher temperature. This aligns with this study's findings, where high resistance to antibiotics was observed in Winter and Spring in the treatment plants B, C, D, and E. Beta-lactam classes of antibiotics and macrolides are the most prescribed antibiotics in the treatment of respiratory infections during cold and flu seasons (Winter and Spring). This could account for the

increased usage of the antibiotics in the area and the development of MDR isolates. The percentage of indicator bacteria (*E. coli* and *Enterococcus* spp.) resistant to different antibiotics was higher during the flocculation, filtration, sedimentation, disinfection, and candy treatment stages when compared to the raw water source and final treated water. This increase in the antibiotic resistance and genes could be attributed to the harsh treatment conditions and their capability of disrupting the cell wall of the bacteria. This disruption could lead to the release of ARGs which can be transferred to other bacteria resulting in the development of resistance to antibiotics [43]. The different treatment processes could alter the temperature gradient of the system, which directly or indirectly affects the proliferation of the indicator bacteria and promotes the spread of ARGs and the development of Antibiotic Resistant Bacteria (ARB). Studies have shown increased proliferation of ARB in the different treatment stages of DWTPs when compared to the raw water sources [43-46]. This could be linked to the conditions in the different treatment stages that could enhance the release of ARGs and the development of ARBs. Authors in [47] observed a positive correlation between the temperature of the DWTPs and the prevalent ARGs, especially the aminoglycoside ARG, sulfonamide ARG, and total ARG. This implied that the increase in the antibiotic resistance observed in this study could also be a factor of the temperature change within the different treatment stages. Based on the MDR isolates from the different provinces, it was noted that Gauteng, Limpopo, and Mpumalanga provinces are laden with MDR indicator bacteria. Both Limpopo and Mpumalanga recorded the highest MDR isolates (38%). Although not a lot of work has been done in this area to ascertain the level of MDR isolates from Gauteng, Limpopo, and Mpumalanga Provinces, the work performed in [48] on the prevalence of MDR isolates from two major hospitals in Limpopo, indicated high occurrence of MDR isolates in the area. This is an indication of an increased use of antibiotics causing an abundance of ARGs that could be channeled to the DWTPs. This creates a challenge to the DWTPs. In view of the poor or overburdened infrastructure and the necessary technical know-how required in the removal of these ARGs and pathogenic indicator bacteria, there is a possibility of high occurrence of MDR isolates in the DWTPs as observed in the current study.

V. CONCLUSION

The study revealed that seasons and different stages of DWTPs influence the occurrence of indicator bacteria as well as the usage of antibiotics in all the studied provinces (Gauteng, Limpopo, and Mpumalanga) of South Africa. The measured physicochemical properties across the seasons and different treatment stages were within the standards indicating good aesthetic properties of the water sampled. All the treatment plants played an essential part in the reduction of the indicator bacteria across all seasons. However, only two sites (A-Gauteng Province and C-Mpumalanga Province) maintained the microbiological standard of the final treated water across all seasons. Increased proliferation of total coliforms and *E. coli* as observed in Spring and Summer and *Enterococcus* spp. in Winter and Autumn shows that changes in environmental factors and other anthropogenic activities could account for seasonal variations in the indicator bacteria. The different treatment stages also affect the indicator bacteria that survive the process, as the disinfectant and candy stages followed by the filtration and coagulation/flocculation stages caused a reduction in the occurrence of the indicator bacteria. The usage of the antibiotics in the sampling area is season-dependent and the spread of the antibiotic resistance in the indicator bacteria is highly impacted by the treatment stages of DWTPs.

Among the contributions of this study to the field is the provision of insights into the temporal factors within the treatment stages of DWTPs, including their impact on the enhancement of the proliferation of the indicator bacteria and an increase in the antibiotic resistance in the three considered provinces. Furthermore, an understanding of the level of antibiotic usage is pertinent as these provinces are linked to many economic activities in the country. This will lead to the development of strategies to battle the increased usage of antibiotics and the cost of drinking water treatment as well as the treatment of ailment arising from poor water treatment as diverse disease outbreaks may occur from drinking water contamination. This calls for more research work to be done to unveil the inherent factors within the treatment stages that contribute to the spread of antibiotic resistance in the indicator bacteria. In addition, in-depth assessment of antibiotic usage in different seasons and water quality needs further research which may guide the development of strategies in preventing the development of antibiotic resistance in water resources and treatment plants.

ACKNOWLEDGMENT

The authors wish to acknowledge the Water Research Commission (South Africa) for funding under the research grant 2022/2023-00801.

APPENDIX

MEAN PHYSICOCHEMICAL PARAMETERS FROM ALL THE SITES THROUGHOUT THE SAMPLING PERIOD

Season		**pH**					**Temperature (°C)**					**EC (µS/cm)**					**TDS (mg/L)**				
	SANS [19] limit	**≥5 to ≤9.7**					**25**					**≤170**					**≤1200**				
	Sites	**A**	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**
Spring	Raw	6.7	8.2	7.4	8.5	7.5	24	25	24	23	25	69.2	67.3	55.6	67.3	72.8	520.1	451.5	361.4	500.2	556.2
	Coagulation/Flocculation	8.4	7.9	9.2	-	8.4	25	22	24	-	24	71.1	65.6	58.2	-	71.3	432.4	393.2	373.7	-	579.3
	Sedimentation	9.2	8.0	8.3	7.7	6.8	24	24	24	22	21	65.7	56.3	57.7	59.2	73.9	224.4	420.4	356.9	426.7	452.9
	Filtration	8.6	7.9	8.0	6.8	9.2	21	22	24	20	22	56.8	63.7	57.8	50.5	67.5	361.8	331.2	368.6	335.7	444.0
	Disinfection	6.5	6.2	-	-	6.9	22	24	-	-	22	48	57	-	-	60	255	301	-	-	338
	Final	7.6	7.1	7.8	7.4	7.9	23	23	22	21	20	59.4	54.7	57.9	49.6	66.9	256.2	212.4	376.4	229.4	385.6
Summer	Raw	7.5	8.0	7.3	8.2	7.2	26	25	25	25	26	45.2	43.2	48.6	45.8	56.0	398.3	433.2	299.7	421.5	501.5

	Coagulation/Flocculation	7.9	8.1	9.3	-	7.7	23	24	25	-	22	44.1	45.6	50.1	-	55.4	420.3	470.5	261	-	550.2
	Sedimentation	7.4	7.6	7.0	7.4	8.2	25	22	24	23	24	50.2	40.4	46.7	50.2	48.4	375.2	356.9	243	470.5	374.2
	Filtration	8.0	7.8	7.7	7.7	8.4	22	20	25	22	22	51.4	51.2	47.7	50.8	50.2	225.9	240.3	228	356.2	333.4
	Disinfection	6.9	6.1	-	-	6.2	21	21	-	-	23	49	51	-	-	51	254.6	236.9	-	-	303.5
	Final	7.3	7.8	7.4	7.6	7.5	23	20	24	20	21	48.9	50.4	55.6	48.4	46.7	186.2	197.4	315.9	288.8	204.6
Winter	Raw	9.4	8.9	9.2	9.1	8.6	19	19	18	20	18	75.6	81.2	72.3	82.5	89.6	590.3	530.2	680	589.4	612.0
	Coagulation/Flocculation	8.3	9.1	6.9	-	7.9	20	18	17	-	19	68.9	67.5	66.2	-	82.7	592.8	557.9	432.1	-	610.4
	Sedimentation	8.8	7.8	7.1	8.9	8.0	21	20	20	19	20	66.4	75.7	70.5	69.6	78.5	450.2	456.2	504.7	408.8	483.8
	Filtration	7.9	8.0	7.4	8.2	8.2	18	19	21	20	19	64.2	72.4	64.9	78.3	79.4	245.7	378.9	326.3	382.1	362.4
	Disinfection	6.6	6.8	-	-	6.3	19	18	-	-	20	60.4	67.2	-	-	66.8	232.7	352.8	-	-	335.8
	Final	7.7	7.3	7.9	7.9	7.4	19	18	20	18	18	62.7	68.3	54.4	60.4	72.3	250.8	259.4	253.9	222.4	279.3
Autumn	Raw	7.5	8.4	8.8	9.0	8.7	20	19	21	22	19	77.2	62.8	77.9	92.2	93.9	562.6	600.4	720.9	610.1	750.2
	Coagulation/Flocculation	7.9	8.1	7.8	-	8.3	19	19	20	-	19	73.4	65.9	63.7	-	88.4	580.4	598.9	699.5	-	629.2
	Sedimentation	8.2	7.6	7.4	8.6	7.7	18	17	21	19	20	67.8	70.3	65.8	78.4	85.8	524.6	498.4	650.5	578.5	472.8
	Filtration	8.0	7.3	7.1	7.8	7.3	18	18	19	18	20	69.1	66.0	66.1	74.1	83.9	490.3	400.4	490.4	429.8	398.0
	Disinfection	6.5	6.9	-	-	6.2	19	20	-	-	19	57.4	62.8	-	-	80.1	466.3	306.6	-	-	368.2
	Final	7.2	7.1	7.0	7.3	7.0	17	17	18	17	18	64.0	64.1	60.3	76.5	79.3	365.7	359.0	325.7	385.8	350.3

MOST PROBABLE NUMBER OF THE INDICATOR BACTERIA PER 100 ML BASED ON IDEXX MPN CHART DEDUCED FROM THE POSITIVE TRAYS

Site	**Teatment stage**	**Total coliform (MPN/100 mL) SANS 241-1:2015. ≤10**				***E. coli* (MPN/100 mL) SANS 241-1:2015. 0**				***Enterococcus spp.* (MPN/100 mL) SANS 241-1:2015. 0**			
		Spring	**Summer**	**Winter**	**Autumn**	**Spring**	**Summer**	**Winter**	**Autumn**	**Spring**	**Summer**	**Winter**	**Autumn**
A	Raw	>2419.6	>2419.6	378.4	>2419.6	54.6	16	25.6	53.8	20.1	52.1	68.4	116.2
	Coagulation/Flocculation	>2419.6	>2419.6	378.4	>2419.6	35.9	9.8	12.2	62.7	4.1	39.5	65.7	109.0
	Sedimentation	1299.7	1299.7	47.3	248.1	4.2	2	4.1	8.6	-	5.2	1	9.7
	Filtration	248.1	248.1	378.4	25.9	5.2	3	-	2.0	-	-	-	1.0
	Disinfection	-	-	-	-	-	-	-	75.9	-	-	-	-
	Final water	-	-	-	-	-	-	-	-	-	-	-	-
B	Raw	>2419.6	>2419.6	1732.9	>2419.6	5.1	5.1	1	2.0	2.0	-	19.3	17.5
	Coagulation/Flocculation	>2419.6	648.8	1986.3	-	-	6.3	4.1	1.0	1	7.1	19.9	6.3
	Sedimentation	1299.7	177.2	1203.3	>2419.6	4.1	16	7.1	-	2	1	21.6	8.5
	Filtration	14.4	79.4	82.3	90.0	2	2	2	3.1	-	-	6.1	31.7
	Disinfection	-	-	-	-	-	-	-	-	-	-	-	-
	Final water	-	-	-	-	-	-	-	-	1	1	-	30.7
C	Raw	>2419.6	>2419.6	36.6	>2419.6	39.9	9.5	5.1	19.7	18.9	3	10.9	21.6
	Coagulation/Flocculation	>2419.6	>2419.6	83.1	>2419.6	34.5	2	6.3	11.0	8.6	5.2	3.1	4.1
	Sedimentation	1299.7	1299.7	25.8	59.4	47.9	5.2	1	2.0	2	1	2	1.0
	Candy	248.1	248.1	69.7	87.8	3.1	1	-	-	4.1	-	-	-
	Filtration	2	2	257.2	49.6	-	-	-	-	3.1	-	-	1.0
	Final water	-	-	-	-	-	-	-	-	-	-	-	-
D	Raw	>2419.6	>2419.6	86.5	214.3	235.9	8.6	11.7	2.0	-	-	18.7	10.7
	Sedimentation	>2419.6	>2419.6	41.1	179.3	21.8	-	4.1	-	-	-	30.9	2.0
	Filtration	>2419.6	>2419.6	3	45.0	24.6	1	-	-	-	-	4.1	1.0
	Final water	1	-	32.7	-	-	-	-	-	-	-	3.1	-
E	Raw	>2419.6	>2419.6	>2419.6	>2419.6	-	214.2	93.4	4.1	3.1	-	185	>2419.6
	Pre-chlorination	>2419.6	>2419.6	-	>2419.6	-	235.9	-	67.0	-	-	2	870.4
	Coagulation/Flocculation	>2419.6	>2419.6	-	>2419.6	20.3	48.1	-	43.5	16.1	-	8.6	7.1
	Sedimentation	>2419.6	>2419.6	248.9	>2419.6	1	24.6	-	1.0	36.4	-	4	53.8
	Filtration	>2419.6	>2419.6	76.7	82.2	-	17.3	-	-	93.3	-	-	3.0
	Final Water	-	-	-	-	1	-	-	-	-	-	-	-

IDEXX Quanti-Tray*/2000 MPN Table

TABLE V. RESPONSE OF THE *E. COLI* AND *ENTEROCOCCUS* SPP. ISOLATES FROM THE POSITIVE TRAYS TO ANTIMICROBIAL AGENTS AND THEIR MULTIPLE ANTIBIOTIC RESISTANCE INDEX

Site	**Treatment stage**	**Bacteria**	**Season**	**Zone of Inhibition (mm)**										**Status**	**MAR-index**
				Imp	**AMC**	**Lev**	**CIP**	**CN**	**AM**	**VA**	**F**	**E**	**SXT**		
A	Raw	*E. coli*	Spring	13	11	29	20	15	0	-	12	0	24	MDR	0.6
	Raw	*E. coli*	Summer	12	10	30	30	22	0	-	0	0	0	MDR	0.7
	Raw	*E. coli*	Winter	14	8	25	20	19	0	-	0	0	10	MDR	0.7
	Raw	*E. coli*	Autumn	0	0	23	23	20	0	-	0	0	0	MDR	0.7
	Raw	*Enterococcus* spp.	Spring	13	14	30	30	20	0	0	18	11	0	MDR	0.6

	Raw	*Enterococcus* spp.	Summer	12	13	10	8	15	0	0	0	0	0	MDR	0.9
	Raw	*Enterococcus* spp.	Winter	9	10	25	20	15	10	6	0	10	17	MDR	0.6
	Raw	*Enterococcus spp.*	Autumn	0	25	10	12	12	18	19	17	16	0	MDR	0.6
	Coagulation/Flocculation	*E. coli*	Spring	0	0	20	20	18	0	-	0	0	0	MDR	0.7
	Coagulation/Flocculation	*E. coli*	Summer	12	11	22	21	13	0	-	0	15	21	MDR	0.6
	Coagulation/Flocculation	*E. coli*	Winter	8	13	28	38	21	0	-	0	0	0	MDR	0.7
	Coagulation/Flocculation	*E. coli*	Autumn	0	0	25	25	15	0	-	12	0	23	MDR	0.6
	Coagulation/Flocculation	*Enterococcus* spp.	Spring	15	0	0	0	19	0	0	0	0	0	MDR	0.8
	Coagulation/Flocculation	*Enterococcus* spp.	Summer	12	12	29	27	18	7	12	0	20	0	MDR	0.6
	Coagulation/Flocculation	*Enterococcus* spp.	Winter	10	9	30	33	17	8	0	0	12	0	MDR	0.7
	Coagulation/Flocculation	*Enterococcus* spp.	Autumn	30	25	21	22	10	20	23	21	23	29	MDR*	0.1
	Sedimentation	*Enterococcus* spp.	Summer	11	14	25	28	16	0	0	0	0	0	MDR	0.7
	Sedimentation	*Enterococcus* spp.	Winter	12	10	27	30	18	0	0	15	0	0	MDR	0.6
	Sedimentation	*Enterococcus* spp.	Autumn	0	22	19	19	12	18	20	0	22	21	MDR	0.3
	Sedimentation	*E. coli*	Spring	19	15	25	26	17	0	-	0	0	0	MDR	0.5
	Sedimentation	*E. coli*	Summer	14.	13	20	24	15	0	-	17	0	0	MDR	0.6
	Sedimentation	*E. coli*	Winter	19	18	34	33	26	12	-	19	0	25	MDR*	0.2
	Sedimentation	*E. coli*	Autumn	9	0	34	28	15	0	-	15	0	22	MDR	0.5
	Filtration	*E. coli*	Spring	10	8	20	22	20	10	-	0	0	19	MDR	0.6
	Filtration	*E. coli*	Summer	14	15	23	27	15	0	-	16	0	30	MDR	0.4
	Filtration	*E. coli*	Autumn	0	0	32	40	16	0	-	15	-	22	MDR	0.5
B	Raw	*E. coli*	Spring	0	12	29	30	15	0	-	10	0	0	MDR	0.7
	Raw	*E. coli*	Summer	11	8	20	25	18	20	-	7	30	13	MDR	0.4
	Raw	*E. coli*	Winter	15	10	22	20	15	13	-	16	9	16	MDR*	0.3
	Raw	*E. coli*	Autumn	0	17	25	30	20	13	-	0	0	22	MDR	0.5
	Raw	*Enterococcus* spp.	Spring	6	0	33	32	15	0	5	0	0	0	MDR	0.7
	Raw	*Enterococcus* spp.	Winter	12	12	0	21	16	0	0	19	0	0	MDR	0.7
	Raw	*Enterococcus spp.*	Autumn	0	30	22	20	0	20	21	18	23	25	MDR*	0.2
	Coagulation/Flocculation	*E. coli*	Summer	12	0	22	28	17	0	-	14	0	0	MDR	0.7
	Coagulation/Flocculation	*E. coli*	Winter	10	0	24	22	15	12	-	0	0	19	MDR	0.6
	Coagulation/Flocculation	*E. coli*	Autumn	0	0	25	32	17	0	-	12	0	25	MDR	0.6
	Coagulation/Flocculation	*Enterococcus* spp.	Spring	12	10	15	20	20	0	0	15	0	19	MDR	0.5
	Coagulation/Flocculation	*Enterococcus* spp.	Summer	13	11	20	22	20	0	0	12	0	0	MDR	0.7
	Coagulation/Flocculation	*Enterococcus* spp.	Winter	10	10	0	0	15	0	10	0	20	0	MDR	0.8
	Coagulation/Flocculation	*Enterococcus* spp.	Autumn	15	20	29	25	15	15	0	18	0	25	MDR*	0.2
	Sedimentation	*E. coli*	Spring	11	17	18	22	16	0	-	17	0	0	MDR	0.5
	Sedimentation	*E. coli*	Summer	10	19	19	21	15	0	-	23	0	0	MDR	0.5
	Sedimentation	*E. coli*	Winter	10	17	19	28	15	0	-	20	0	0	MDR	0.5
	Sedimentation	*Enterococcus* spp.	Spring	12	21	25	29	22	0	0	20	0	0	MDR	0.5
	Sedimentation	*Enterococcus* spp.	Summer	14	17	23	25	19	0	0	18	0	0	MDR	0.5
	Sedimentation	*Enterococcus* spp.	Winter	9	20	30	32	21	0	0	17	0	0.	MDR	0.5
	Sedimentation	*Enterococcus* spp.	Autumn	20	21	23	21	0	20	20	22	24	30	MDR*	0.1
	Filtration	*E. coli*	Spring	12	10	16	22	14	0	-	0	0	17	MDR	0.7
	Filtration	*E. coli*	Summer	0	0	0	10	17	0	-	0	10	0	MDR	0.9
	Filtration	*E. coli*	Winter	10	9	20	22	14	0	-	0	17	20	MDR	0.6
	Filtration	*E. coli*	Autumn	8	0	28	31	15	0	-	13	0	24	MDR	0.5
	Filtration	*Enterococcus* spp.	Winter	18	11	27	26	17	0	0	0	0.	0	MDR	0.6
	Filtration	*Enterococcus* spp.	Autumn	20	25	0	20	18	21	21	19	30	29	MDR*	0.1
	Final	*Enterococcus* spp.	Spring	0	20	37	35	12	0	0	10	0	0	MDR	0.7
	Final	*Enterococcus* spp.	Summer	11	12	19	25	20	0	0	17	0	0	MDR	0.6
	Final	*Enterococcus* spp.	Autumn	10	21	20	20	0	18	20	19	23	25	MDR*	0.2
C	Raw	*E. coli*	Spring	0	0	29	24	20	0	-	0	0	0	MDR	0.7
	Raw	*E. coli*	Summer	16	9	18	22	16	0	-	16	0	20	MDR	0.4
	Raw	*E. coli*	Winter	16	15	27	22	19	9	-	15	0	24	MDR*	0.3
	Raw	*E. coli*	Autumn	14	0	10	12	17	0	-	10	0	0	MDR	0.8
	Raw	*Enterococcus* spp.	Spring	15	10	20	24	15	0	0	0	0	0	MDR	0.6
	Raw	*Enterococcus* spp.	Summer	16	9	18	22	16	0	0	16	0	20.	MDR	0.4
	Raw	*Enterococcus* spp.	Winter	16	15	27	22	19	9	0	15	0	24	MDR*	0.3
	Raw	*Enterococcus* spp.	Autumn	0	20	35	12	13	18	0	25	20	25	MDR	0.4
	Coagulation/Flocculation	*E. coli*	Spring	17	8	25	24	13	10	-	0	0	9	MDR	0.7
	Coagulation/Flocculation	*E. coli*	Summer	16	7	25	27	15	0	-	25	0	0	MDR	0.5
	Coagulation/Flocculation	*E. coli*	Winter	15	0	20	20	15	0	-	0	0	0	MDR	0.6
	Coagulation/Flocculation	*E. coli*	Autumn	8	0	30	23	19	0	-	0	0	0	MDR	0.7
	Coagulation/Flocculation	*Enterococcus* spp.	Spring	0	0	0	7	21	0	0	0	0	0	MDR	0.9
	Coagulation/Flocculation	*Enterococcus* spp.	Summer	15	10	20	30	20	0	0	0	0	25	MDR	0.5
	Coagulation/Flocculation	*Enterococcus* spp.	Winter	10	8	8	7	12	0	9	0	19	19	MDR	0.8
	Sedimentation	*E. coli*	Spring	0	0	22	20	16	0	-	10	0	0	MDR	0.7

	Sedimentation	*E. coli*	Summer	0	0	0	26	25	0	-	17	0	0	MDR	0.7
	Sedimentation	*E. coli*	Winter	0	13	6	0	16	0	-	8	0	0	MDR	0.9
	Sedimentation	*E. coli*	Autumn	10	10	25	21	8	10	-	12	0	20	MDR	0.7
	Sedimentation	*Enterococcus* spp.	Spring	10	10	25	26	15	8	0	15	12	0	MDR	0.6
	Sedimentation	*Enterococcus* spp.	Summer	0	12	22	23	19	0	0	0	0	0	MDR	0.7
	Sedimentation	*Enterococcus* spp.	Winter	10	10	25	26	15	8	0	15	12	0	MDR	0.6
	Sedimentation	*Enterococcus* spp.	Autumn	0	0	0	0	12	0	20	0	12	20	MDR	0.8
	Candy	*E. coli*	Spring	15	10	28	30	15	0	-	0	0	0	MDR	0.6
	Candy	*E. coli*	Summer	12	9	30	32	20	0	-	0	0	0	MDR	0.7
	Candy	*Enterococcus* spp.	Spring	13	9	8	8	15	0	15	0	9	30	MDR	0.7
	Filtration	*Enterococcus* spp.	Spring	15	13	16	22	15	8	8	9	0	0	MDR	0.6
D	Raw	*E. coli*	Spring	0	0	0	0	20	0	-	0	0	0	MDR	0.9
	Raw	*E. coli*	Summer	0	0	20	20	18	0	-	0	0	0	MDR	0.7
	Raw	*E. coli*	Winter	0	10	10	22	19	0	-	0	0	0	MDR	0.8
	Raw	*E. coli*	Autumn	7	0	21	30	15	0	-	0	0	0	MDR	0.7
	Raw	*Enterococcus* spp.	Winter	0	7	15	21	10	0	0	6	0	0	MDR	0.8
	Raw	*Enterococcus spp.*	Autumn	30	10	21	22	20	20	18	20	25	0	MDR*	0.2
	Sedimentation	*E. coli*	Spring	0	0	27	30	17	0	-	0	0	0	MDR	0.7
	Sedimentation	*E. coli*	Winter	0	0	20	0	19	0	-	0	0	0	MDR	0.8
	Sedimentation	*Enterococcus* spp.	Winter	13	13	20	21	13	0	0	16	0	22	MDR	0.6
	Sedimentation	*Enterococcus* spp.	Autumn	0	0	17	18	25	0	20	20	22	0	MDR	0.4
	Filtration	*E. coli*	Spring	8	0	0	0	22	0	-	0	0	15	MDR	0.8
	Filtration	*E. coli*	Summer	15	10	20	20	15	0	-	0	0	0.	MDR	0.6
	Filtration	*Enterococcus* spp.	Winter	13	0	0	0	15	0	0	0	0	0	MDR	0.9
	Filtration	*Enterococcus* spp.	Autumn	30	20	20	20	22	19	20	17	22	30	MDR*	0
E	Pre-Chlorination	*E. coli*	Summer	15	0	25	23	15	0	-	10	0	0	MDR	0.6
	Pre-Chlorination	*E. coli*	Autumn	18	15	23	27	20	0	0	20	0	0	MDR	0.4
	Pre-Chlorination	*Enterococcus* spp.	Winter	13	17	25	25	17	0	0	13	0	0	MDR	0.6
	Pre-Chlorination	*Enterococcus* spp.	Autumn	18	30	20	30	25	0	30	20	0	21	MDR*	0.2
	Raw	*E. coli*	Summer	15	10	18	22	15	0	-	14	18	0	MDR	0.5
	Raw	*E. coli*	Winter	8	14	22	22	15	0	-	8	8	19	MDR	0.6
	Raw	*E. coli*	Autumn	20	13	25	30	22	20	-	18	0	0	MDR	0.4
	Raw	*Enterococcus* spp.	Spring	10	12	27	24	17	0	0	10	0	0	MDR	0.7
	Raw	*Enterococcus* spp.	Winter	10	10	18	30	15	0	0	0	0	0	MDR	0.7
	Raw	*Enterococcus* spp.	Autumn	15	20	22	25	21	0	0	18	10	19	MDR	0.3
	Coagulation/Flocculation	*E. coli*	Spring	12	16	16	26	17	0	-	10	10	14	MDR	0.6
	Coagulation/Flocculation	*E. coli*	Summer	0	0	20	25	17	0	-	0	0	0	MDR	0.7
	Coagulation/Flocculation	*Enterococcus* spp.	Spring	0	10	0	0	15	0	0.	0	0	0	MDR	0.9
	Coagulation/Flocculation	*Enterococcus* spp.	Winter	10	8	8	7	12	0	9	0	19	19	MDR	0.8
	Sedimentation	*E. coli*	Spring	9	8	29	30	15	0	-	0	7	0	MDR	0.6
	Sedimentation	*E. coli*	Summer	10	16	20	30	20	8	-	15	0	0	MDR	0.5
	Sedimentation	*E. coli*	Autumn	13	20	17	25	17	0	-	19	0	20	MDR	0.4
	Sedimentation	*Enterococcus* spp.	Spring	10	0	0	0	22	0	0	0	0	0	MDR	0.9
	Sedimentation	*Enterococcus* spp.	Winter	10	0	15	15	15	0	20	10	0	0	MDR	0.6
	Sedimentation	*Enterococcus* spp.	Autumn	15	21	19	26	23	18	25	20	0	21	MDR*	0.1
	Filtration	*E. coli*	Summer	10	15	20	23	15	0	-	13	10	18	MDR	0.5
	Filtration	*Enterococcus* spp.	Spring	8	7	8	8	17	0	0	9	0	0	MDR	0.9
	Filtration	*Enterococcus* spp.	Autumn	18	19	25	24	24	18	25	22	0	26	MDR*	0.1
	Final	*E. coli*	Spring	15	10	12	17	14	0	-	15	0	28	MDR	0.6
Control	ATCC25922	*E. coli*		27	18	29	34	20	18	-	22	0	23		
Control	ATCC29212	*Enterococcus faecalis*		15	25	21	23	12	20	20	20	20	25		

MDR*- not multidrug-resistant, MDR- multidrug-resistant

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