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# Incidence, Antibiogram and MAR Index of *Escherichia coli* from Wastewater Treatment Plant, Reclaimed Forest Soil and Affected Water Bodies

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**ABSTRACT**

Wastewater is a major collector for antibiotic resistant and pathogenic microorganisms posing high risk on public health and environment. Even efficient secondary sewage treatment does not ensure appropriate removal of pathogens especially multi-drug resistant (MDR) *E. coli*. This study aimed to shed light on the prevalence and antimicrobial resistance of *E. coli* in raw wastewater, treated water, sludge, and forest soil irrigated with treated water from the Serapium wastewater treatment plant in Ismailia, Egypt. Samples were collected monthly during the period from February 2018 to January 2019 from different wastewater treatment stages as well as sludge, reclaimed soil and affected water sources. *E. coli* strains were isolated on HiChrome *E. coli* agar and verified by indole reagent system. Kirby-Bauer disk diffusion method was used for antimicrobial susceptibility testing for 19 different antibiotics. *E. coli* mean count ranged between $4.7\times10^4$ and $6.3\times10^5$ MPN/100mL in the influent and $2.1\times10^2$ and $6.3\times10^3$ MPN/100mL for effluent indicating high bacterial load in the effluent. The antimicrobial susceptibility rate for the selected 337 *E. coli* strains was highest for ampicillin (29.2%), tetracycline (22.5%), and ciprofloxacin (16.3%), while the lowest resistance was for ertapenem (2.4%), imipenem (2.3%), meropenem (2.3%), and azithromycin (4.4%). ESBL producing *E. coli* represents 20.67% of the isolates. Notwithstanding, upwards of $10^3$ MPN/100mL *E. coli* with high Multiple Antibiotic Resistance index (MARi) (>0.2) has reached the receiving ecosystem and thus the processes of sewage treatment contribute to the spread of antibiotic resistant bacteria into the environment.

**Key Words:**

MAR index, MDR *E. coli*, Reclaimed soil, Wastewater treatment

## 1. INTRODUCTION

Monitoring the burden of antimicrobial resistance and responding to it with diligence to find practical solutions is currently one of the 4 main priorities of the World Health Organization in the twenty first century [1], [2]. Antimicrobial resistance (AMR) is a serious global concern with far-reaching consequences, with estimates stating that drug-resistant illnesses are responsible for around 5 million deaths per year worldwide [3]. We must act immediately, or common ailments will become incurable and contemporary life-saving operations will become much more deadly unless we take immediate action.

A plethora of investigations have demonstrated antibiotic-resistant bacteria in environmental samples in recent [4]–[6]. As a result of the large amount of wastewater generated by human activities such as agriculture, healthcare facilities, and the general population, wastewater treatment plants (WWTPs) have been found to be unintentional collection points for antimicrobial drugs, antibiotic-resistant bacteria (ARB), and antibiotic resistance genes (ARG) [7]–[9].

WWTPs often retain antimicrobials and other chemicals that have been proven to promote the development of antibiotic resistance, in part because wastewater treatment processes are not designed to eradicate ARB and ARG [10]–[12]. Wastewater treatment plants (WWTPs) can be a breeding ground for antibiotic-resistant bacteria and antibiotic-resistant genes due to their close proximity to humans and the fact that they release treated water into nearby waterways including rivers and reservoirs [12], [13]. The primary source of pathogenic microorganisms in wastewater, including E. coli, is faecal contamination [14]. Wastewater treatment processes are intended to reduce the concentration of contaminants, including pathogens, in effluent before discharge to receiving water bodies or being reused [15], [16]. However, many wastewater treatment plants (WWTPs) discharge such effluents without disinfection [15]. As a result, nondisinfected effluents may still contain high proportions of pathogenic bacteria, posing a risk to public health [16], [17]. E. coli, which has been widely used as a faecal contamination indicator in aquatic environments, is normally considered nonpathogenic; however, some strains can be pathogenic [13], [18], [19].

Recently, as a result of the global water scarcity crisis caused by a variety of factors, including climate change and population growth, in addition to political challenges, water reuse has become an increasingly important global requirement to provide clean water, whether for drinking or for re-use in agricultural production [20]. One of the concerns stated about water reuse is the uncontrolled discharge of contaminants into the environment, which are difficult to remove using traditional sewage systems [21]. Health risk associated with contamination by antibiotic resistant bacteria is another important concern raised when using recycled water for irrigation [6]. The water from the Sarapium treatment facility in Ismailia is partially utilised for experimental agricultural reasons once it has been treated by the plant. While monitoring efforts for recycled water were concentrated on faecal coliform bacteria, the antibiotic resistance index was not included in these monitoring efforts. As a result, the goals of this study are to determine the rate of antibiotic resistance among E. coli strains isolated from raw and treated wastewater, as well as sludge. In order to offer a more thorough evaluation of antibiotic resistance in reuse water, the impact of treatments on eradication of *E. coli* populations was also assessed.

## 2. STUDY AREA

**Site Description:** Water samples were taken at the Sarapium sewage treatment facility in Ismailia, Egypt. Sarapium 's sewage treatment facility opened in 1996 that covers 860 feddans and has a 270,000-cubic-meter-per-day design capacity and a 170,000-cubic-meter-per-day average operational capacity. The facility treats sewage as biological (activated sludge) secondary treatment, and the processed wastewater is safe for drainage in the Al Mahsama drain. The station serves 650,000 people in Ismailia, Abu Sultan, Sarapium, Mostakbal City, Nafisha, and Bahtimi which includes that from many hospitals and a pharmaceutical manufactory. The final effluent is discharged from the terminal in two main directions: to the woodland area. Quantities of ca. 50000 $m^3$/d of treated water are being passed through pre-irrigation filtration system and used to irrigate experimental woodland adjacent to the plant, planted with types of trees producing economically yielding timber. The remaining quantities of treated water (about 155,000 cubic meters / day) are discharged through a slope line up to 11 km long that ends at Al-Mahsama drainage at 4.5 km near the Ismailia-Suez agricultural road.

## 3. MATERIALS AND METHODS

**3.1 Sampling:** Wastewater samples from Serapeum wastewater treatment plant were collected in an air-tight sterile glass bottle. Mixed samples, 500 ml each, of wastewater influent and effluent, pre-irrigation filter, El Mahsama Drainage and Lake El Sayadeen samples were collected, for microbiological analysis. Mixed sludge and woodland soil samples were collected in sealed sterile plastic bags. Samples were collected in a monthly basis from February 2018 to January 2019, transferred to the laboratory in ice box, stored at 4°C processed and processed within 6-12 hours.

**3.2 *E. coli* count, isolation, and confirmation:** Faecal coliforms and *E. coli* were simultaneously counted in samples of raw and treated sewage water from Sarapium station, filters, Al-Mahsama drain and ElSayadeen lake according to standard method 9221-F by APHA [22]. The method relies on Multiple-Tube technique using EC broth (HiMedia, India).

The presence of faecal coliforms and *E. coli* was determined by standard Multiple-Tube methods, by including an inverted vial (Durham tube) in tubes of EC broth (HiMedia, India). Ten mL of sample or appropriate dilution was added to EC tube, incubated at 44.5 $^{o}$C for 24 h. A loopful of positive cultures showing turbidity and gas is inoculated into EC broth supplemented with 4-Methylumbelliferyl-β-D-glucuronide (MUG) 0.05 g/L, incubated in water bath at 44.5 $^{o}$C for 30 min. Tubes were examined under UV at 365 wavelengths along with positive (*E. coli*) and negative controls (*Klebsiella pneumonia* and an uninoculated medium). The generation of gas during growth is a desirable trait in faecal coliforms. Positive results for *E. coli* are shown by bright blue fluorescence under long UV light [22].

Membrane filtration technique was employed using nitrocellulose membranes (0.45 μm) to isolate *E. coli* cells in samples of raw and treated sewage water from Sarapium station, filters, Al-Mahsama drain and ElSayadeen lake, as well as samples of sludge and woodland soil. The serial dilution method was used, where the water samples were diluted to the appropriate dilution and filtered. As for the sludge and soil samples, 1 gram of the sample was dissolved in 100 ml of phosphate-buffered saline and the samples were diluted to appropriate dilution and filtered. The filters were placed on the mFC agar. and incubated at 44.5°C, colonies of *E.* coli marked in blue were randomly counted and selected. The selected isolates were streaked on TSA agar to obtain pure colonies and confirmed by streaking on high-chrome agar (HiMedia, India), *E. coli* colonies are identified as bluish green colonies and produce pink color by indole reagent test.

**3.3 Antibiotic Susceptibility Testing of the Isolates:** The *E. coli* isolates (n = 337) were further tested for antibiotic susceptibility by Kerby method [23] susceptibilities to ampicillin (30 µg), tetracycline (30 µg), Norfloxacin (10 µg), Ciprofloxacin (5 µg), Azithromycin (15 µg), Erythromycin (15 µg), Ampicillin (10 µg), and Chloramphenicol (30 µg) (oxoid, England) were determined using the disc diffusion method on Muller Hinton agar (HiMedia, India). To ensure the accuracy of antibiotic tests, we use *E. coli* ATCC 25922 as control.

In this study, we used the Krumperman, 1983 [33]-introduced Equation to determine the Multiple Antibiotic Resistance index (MARI) for each *E. coli* isolate. MARI = a / b where a is the number of antibiotics to which the strain is resistant, and b is the total number of antibiotics tested.

**3.4 Statistical analysis:** The SPSS Statistics software version 23.0 was used for all statistical analyses, both descriptive and ANOVA (IBM SPSS Statistics for Windows, NY, USA). Complete linkage protocol was used to generate heatmap at http://www.heatmapper.ca.

## 4. RESULTS AND DISCUSSION

**4.1 Monthly prevalence of *E. coli* at different sample sources:** *Escherichia coli* is one of the most important indicators for measuring environmental contamination with pathogenic bacteria of fecal origin [18]. The traditional wastewater treatment plant at Sarapium-Egypt receives an average of 1.63E+07 MPN/100ml presumptive *E. coli*, which effectively eliminated on average 3 log (99.9%) by the treatment process with an additional 1 log removal by the filtration system before the reuse of the treated water in irrigating 500 acres of experimental woodlands next to the station (Table1). The topic of this research focuses on the traceability of *E. coli* and their antibiotic resistance within the sewage treatment facility, as well as the extent of its impact on the water bodies receiving treated water, as well as the soil of the reclaimed woodlands.

The monthly changes in *E. coli* (MPN/100mL) of the influent, effluent, sludge, pre-irrigation filters, reclaimed soil, El-Mahsama drainage and Lake El-Sayadeen are determined and presented in Table (1). Variations in *E. coli* was assessed using repeated measures ANOVA at 0.05 level. Accordingly, *E. coli* throughout studied timepoints was significantly varied (<0.001) in all sites as revealed by ANOVA. The average *E. coli* throughout the timepoints in influent, effluent and sludge were 1.63E+07, 1.91E+04 and 1.33E+07 MPN/100mL, respectively. However, the filters, soil, El-Mahsama drainage and Lake El-Sayadeen *E. coli* ranged between 1.90E+03 to 3.80E+03, 4.68E+02 to 1.62E+03, 6.90E+02 to 1.60E+03 and 1.84E+02 to 4.65E+02 MPN/100mL with an average of 2.69E+03, 9.36E+02, 1.24E+03 and 3.41E+02 MPN/100mL, respectively. The overall variations in *E. coli* between samples of different site were assessed by paired samples t-test at 0.05 level. A highly significant difference between both influent and effluent sampling sites was revealed. Multivariate analysis of variance was also applied to assess the differences in *E. coli* induced by time (months) and sampling sites and interaction between previous factors. Accordingly, there was a highly significantly difference in *E. coli* between the sludge, filters, soil, El-Mahsama drainage and Lake El-Sayadeen ($p<0.001$***), on the other hand, significant different in timepoints (months) and interaction between months and sampling site was also observed ($p<0.001$***). Changes in temperature, precipitation, as well as differences in water use across the board (in homes, farms, and factories) are all possible as the seasons change [24]. Initial predation, stability of suspended particles, UV inactivation, bacterial activity, and environmental factors also play an effective role in microbial reduction through wastewater treatment process [25], [26]. The overall microbial diversity in wastewater treatment plants may be affected by the temperature of the environment [27], [28]. It was also discovered that the volume of water used, the concentration of that water and the volume of water flowing through plants can all affect the prevalence and diversity of bacteria present in plants [29].

*E. coli* removal efficiency by conventional wastewater treatment systems has been extensively studied across the world [30]. In South Africa two traditional WWTPs showed relatively high efficiency to eliminate *E. coli* contaminants (between 96.0–98.1%) [31]. In Italy 7 biological wastewater treatment systems achieved a removal efficiency of 91.8–96.5% [32]. In general, and due to the nature of biological wastewater treatment systems, the treated water does not meet with the international standards for safe disposal, whether through disposal into natural water sources or reusing treated water in agriculture [33], [34]. Therefore, it is necessary to use one of the methods of water disinfection, such as chlorination or UV, to eliminate pathogenic microbes [35]. It is worth noting that the chlorine disinfection unit at the Sarapium station was not working during the period of this research conduction, therefore, the *E. coli* population in the effluent exceeded the Egyptian Code for Treated Wastewater Disposal (5000 cfu/100ml).

Researchers have found that *E. coli* isolates collected from irrigation water are resistant to the antibiotics of concern [19]. Over a dozen studies suggest that ectotrophic bacteria like *E. coli* and other culturable faecal indicator bacteria like *E. coli* are particularly useful for monitoring the environmental impact of wastewater [36], [36]. The World Health Organization (WHO) Guidelines for the use of wastewater in aquaculture and agriculture and the reuse of drinking water as well as the ISO 16075 Guidelines for the use of treated wastewater from irrigation term are just a few examples of the numerous laws and guidelines at the national and international levels that regulate water reuse [37], [38]. Antibiotic-resistant *E. coli* (or any other pathogen) testing of the reused water, however, is not currently being done in accordance with any existing procedures [11]. Our analytical results not only confirm the presence of these antibiotic-resistant indicator bacteria in the environment, but also offer novel insights into their behavior and ecology.

**4.2 *E. coli* selection and confirmation:** Confirmation and testing for resistance were performed on a total of 381 putative *E. coli* isolates grown on HiChrome agar and confirmed by indole test (Table 2). Out of them, 44 (11.5%), could not be positively identified as *E. coli*, hence they were omitted from the further analysis. As represented in Table 2, the highest number of randomly selected *E. coli* (n=64) was collected from influent followed by effluent (n=54) while lower numbers were from pre-irrigation filters (n=34), ElSayadeen lake (n=41) and ElMahsama drain (n=46).

Conventional methods for identifying fecal coliforms such as *E. coli* are labor-intensive and time-consuming when fecal contamination is suspected [39]. For the simultaneous detection of coliforms and *E. coli*, the use of media containing chromogenic and fluorescent substrates for B-galactosidase (LAC) and B-glucuronidase (GUD) enzymes is in an upward trend [40].In this study, chromogenic and fluorogenic medium (HiChrome) was used for selective isolation of *E. coli* from different sample collection sites. As recommended by APHA, selected *E. coli* strains were further confirmed by Indole test [22].

**4.3 Antibiotic resistance:** In total, 53.11% of all studied *E. coli* isolates were resistant to at least one of the nineteen antibiotics used in the study (Table 2). Influent isolates showed the highest rates of resistance to all included antibiotics (73.44%), whilst isolates from Lake ElSayadeen presented the lowest resistance rates (29.27%) (Table 2). In general, significant percentage of antibiotic resistant strains were detected in all sites.

In this study, we looked specifically at a conventional wastewater treatment plant (WWTP) that its effluent is being used for both irrigation of recreational areas and discharge into local water bodies. Antibiotic-resistant *E. coli* was found in wastewater effluent, irrigated soils, and receiving water bodies. Antibiotic resistance was observed in *E. coli* isolates obtained from different stages of treatment and environmental samples (Figure 1). The highest resistance was to ampicillin with an average of 29.19%. The highest ampicillin resistant strains were observed in influent samples (37.5%) while the lowest resistant was obtained from ElSayadeen lake isolates (14.63%). Among the isolates, tetracycline resistance ranked second (Figure 1). on the other hand, carbapenems- ertapenem, imipenem and meropenems resistant *E. coli* were the least observed isolates (2.49, 2.27 and 2.3%, respectively).

The presence of antibiotic-resistant bacteria in treated wastewater is a serious issue that must be addressed. Several studies have been carried out to determine the presence of antibiotic-resistant bacteria in wastewater and the aquatic environment [7], [41], [42]. Wastewater provides a favorable environment for antibiotic-resistant bacteria that circulate in the surrounding community. A high bacterial density environment with antibiotic residues provides a suitable medium for the selection of antibiotic-resistant bacteria while also providing an optimal environment for the movement of resistance genes [43]. This study revealed the presence of antibiotic-resistant *E. coli* in a conventional sewage treatment facility, the effluent from which is discharged into local waterways and utilized in part to irrigate manmade forests. *E. coli* bacteria were found to be resistant to Ampicillin, Tetracycline, Ciprofloxacin, and Cefotaxime, which are all commonly used antibiotics. This was true both in the treated water and in the affected areas. Other investigations[19], [44], [45] found that *E. coli* isolates found in irrigation water were resistant to these drugs. The influence of effluents on the environment can be tracked with the use of culturable faecal indicator bacteria like *E. coli* [46]. A WWTP in Egypt can only reuse or discharge effluents below faecal indicator bacteria guideline values to ensure minimum adverse environmental impact [47]. This is mandated by the Egyptian code of the reuse of treated wastewater for agricultural purposes (ECP 501-2015) and the Standards and Limits for the drains' water quality to be discharged into watercourses (Law 48/1982) [48]. Nonetheless, there are no established regulations to track the prevalence of antibiotic-resistant *E. coli* in recycled water. Our research establishes a new benchmark for data on the prevalence of these antibiotic-resistant indicator microorganisms.

**4.4 Multiple antibiotic resistance Index (MARI):** The $MARi^{total}$ was higher in comparison to the $MARi^{resistant}$ for all samples (Table 3). Influent and sludge samples displayed relatively higher $MARi^{resistant}$ compared to their $MARi^{total}$ overall populations of sampled isolates (Table 2). This high resistance rates seen among Influent and sludge isolates are to some extent due to the presence of more multiple resistant strains. On the other hands, lower average $MARi^{resistant}$ was obtained for isolates collected from soil, lake ElSayadeen and ElMahsama drainage, due to dilution effect as well as increased salinity of the lake (Table 2).

Resistance to three or more antibiotics (multidrug-resistant) was observed in 109 (61%) of the *E. coli* isolates. resistance to ampicillin was widespread among the multidrug resistant *E. coli* and one of the influent isolates had MARI of 0.737 exhibiting resistance to 11 of the tested antibiotics (Table 2). Reclaimed soil, ElMahsama drain and ElSayadeen lake samples had 15, 10 and 7 isolates with resistance to 3 or more antibiotics, respectively.

Recently, the importance of using the Multiple Antibiotic Resistance Index (MARI) has been pointed out to estimate the degree of environmental risks associated with the spread of antibiotic resistance. A MARI greater than 0.2 is considered an indicator of a public health risk and a highly contaminated environment [7], [49], [50]. In many cases, Multiple Antibiotic Resistance Index (MARI) has significantly increased in the wastewater's course treatment process, showing the proliferation of resistance in the wastewater treatment system [51]. Several studies showed an impressive increase in the MARI in the treated wastewater after disinfection by chlorination or UV, which indicates the ability of multi-drug-resistant bacteria to selectively survive those treatment [52]. As a consequence, the prevalence of resistance is increasing in the wastewater treatment system through the treatment process. Since the chlorination unit in Serapium wastewater treatment system is out of service, we find a decrease in the MARI of the influent samples.

MARI estimates obtained for isolates from external study sites (influent (0.209) and effluent (0.205) were similar and both were greater than 0.2, indicating that the isolates originated from environments with high antibiotic use or contamination. In spite of other sites developed MARI lower than 0.2, >0.2 MARI for individual strains in all studied sites were found (Table 3). The high MARI values obtained in this study may indicate that the isolates are exposed to antibiotic stress, which may be due to the inappropriate use of antibiotics among the population in the study area and may lead to a further increase in the development of multidrug resistance over time if not Appropriate measures are put in place.

**4.5 Extended beta-lactamase producing *E. coli* (ESBL-EC) :** In this study (Table 2), we found that irrigation water, reclaimed soil and receiving water bodies were contaminated with ESBL-*E. coli* and the highest percentage was found in soil (24%), irrigation water (22.22%) and ElMahsama drain

(26.32%), which confirms the important and emerging role that reclaimed irrigation water, contaminated with wastewater, has in the spread of ESBL *E. coli* [5], [53], [54].

**4.6 Similarities between antibiotic resistant *E. coli* isolates corresponding to sampling sites:** Antibiotic resistance patterns of the resistant isolates from different sampling sites were used to visualize the similarities between antibiotic resistant *E. coli* isolates from different sampling sites. As shown in Figure (2), Hierarchical clustering carried out inside of a heatmap revealed a scattered association between the antibiotic resistance profiles of the isolates and the sampling sites from which they were isolated. Although isolates from influent, sludge, and effluent showed higher similarities to the profiles of resistant *E. coli*, many isolates from soil, ElSayadeen lake, and ElMahsama drain shared similarities with isolates from influent and effluent. This was the case even though influent, sludge, and effluent showed higher similarities. Consequently, it would appear that both the influent and the effluent have some degree of influence on the population of resistant *E. coli* that is released into the environment.

**4.7 linkage between Antibiotic resistances:** In a dendrogram, the relationships between the incidences of resistance to the nineteen antibiotics for all 337 isolates were represented (Figure 3). Most closely associated (0.86), but less frequently, was resistance to carbapenems meropenem and imipenem, while ertapenem exhibited a lower co-occurrence of resistance (0.61). ESBL-producing antibiotics cefotaxime and ceftazidime exhibited a strong association (0.61), while rifampicin and levofloxacin also exhibited substantial co-occurrences (0.59). Tetracycline and ampicillin resistance appeared to be unrelated to other resistances.

## 5. CONCLUSIONS

It appears from the results of this study that eradicating antibiotic-resistant bacteria can be difficult in reclaimed water produced using traditional wastewater treatment procedures. Combating the role of wastewater treatment plants in environmental antibiotic resistance requires addressing a number of factors affecting treatment efficacy, including the microbial community composition entering and leaving the plant, physicochemical factors impacting treatment, environmental factors and overall plant capacity. There is a need to perform more studies before the safety of using reclaimed water that may contain antibiotic-resistant bacteria for human consumption, agriculture, or recreation can be determined. The presence of drug-resistant bacteria in wastewater treatment plants effluent necessitates an immediate revision to existing standards for wastewater disposal, recycling and reuse.

**Table 1.** Monthly records of *E. coli* at different sample sources including Sludge, pre-irrigation filters, soil, El-Mahsama drainage, lake Elsayadeen.

| Month | *E. coli* (MPN/100ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Influent | Effluent | Sludge | Filters | Soil | El Mahsama Drainage | Lake ElSayadeen |
| Jan | 2.10E+07 | 2.60E+04 | 4.03E+06 | 3.10E+03 | 5.12E+02 | 6.90E+02 | 1.84E+02 |
| Feb | 1.26E+07 | 1.90E+04 | 1.60E+05 | 2.60E+03 | 6.75E+02 | 1.10E+03 | 4.36E+02 |
| Mar | 1.04E+07 | 1.60E+04 | 1.09E+07 | 3.10E+03 | 1.02E+03 | 1.25E+03 | 3.60E+02 |
| Apr | 2.11E+07 | 2.20E+04 | 3.63E+06 | 3.80E+03 | 9.46E+02 | 1.40E+03 | 4.52E+02 |
| May | 5.85E+06 | 2.20E+04 | 6.32E+06 | 2.40E+03 | 1.13E+03 | 1.40E+03 | 2.79E+02 |
| Jun | 4.12E+07 | 3.10E+04 | 2.98E+06 | 3.10E+03 | 1.16E+03 | 1.20E+03 | 3.60E+02 |
| Jul | 7.75E+06 | 1.90E+04 | 7.63E+06 | 2.30E+03 | 1.62E+03 | 1.30E+03 | 4.04E+02 |

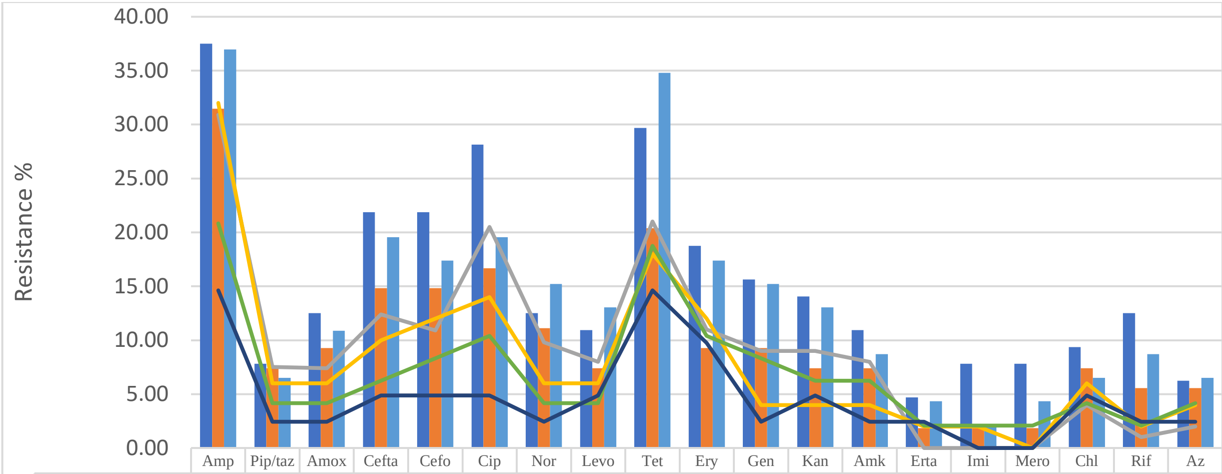
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Aug** | 4.30E+06 | 8.20E+03 | 4.63E+06 | 2.50E+03 | 9.86E+02 | 1.44E+03 | 3.88E+02 |
| **Sep** | 1.51E+07 | 2.70E+04 | 8.67E+07 | 2.60E+03 | 6.58E+02 | 1.25E+03 | 2.16E+02 |
| **Oct** | 3.97E+07 | 1.90E+04 | 1.73E+07 | 2.80E+03 | 1.02E+03 | 1.30E+03 | 2.88E+02 |
| **Nov** | 5.70E+06 | 9.00E+03 | 1.44E+07 | 2.10E+03 | 1.04E+03 | 1.60E+03 | 4.65E+02 |
| **Dec** | 1.15E+07 | 1.10E+04 | 8.82E+05 | 1.90E+03 | 4.68E+02 | 9.00E+02 | 2.60E+02 |
| **Total (average)** | 1.63E+07 | 1.91E+04 | 1.33E+07 | 2.69E+03 | 9.36E+02 | 1.24E+03 | 3.41E+02 |
| **ANOVA (Months)** | <0.001*** | <0.001*** | <0.001*** | <0.001*** | <0.001*** | <0.001*** | <0.001*** |
| **ANOVA (Sites)** | <0.0001*** | | | | | | |

*, **, ***, significantly different at 0.05, 0.01, 0.001 - NS, non-significant at p>0.05

**Table 2.** Antibiotic resistance, ESBL and Multiple antibiotic resistance index (MARI) according to

| **Source** | **Total isolates** | **Resistant isolates** | | **ESBL** | | **MARI** | |
|---|---|---|---|---|---|---|---|
| | | **No.** | **%** | **No.** | **%** | **MARI$^{total}$** | **MARI resistant** |
| **Influent** | 64 | 47 | 73.44 | 12 | 25.53 | 0.154 | 0.209 |
| **Effluent** | 54 | 28 | 51.85 | 7 | 25.00 | 0.106 | 0.205 |
| **Sludge** | 50 | 30 | 60.00 | 7 | 23.33 | 0.135 | 0.207 |
| **Filters** | 34 | 18 | 52.94 | 4 | 22.22 | 0.094 | 0.189 |
| **Soil** | 46 | 25 | 54.35 | 6 | 24.00 | 0.078 | 0.156 |
| **ElMahsama** | 48 | 19 | 39.58 | 5 | 26.32 | 0.068 | 0.172 |
| **ElSayadeen** | 41 | 12 | 29.27 | 1 | 8.33 | 0.046 | 0.158 |
| **Total** | 337 | 179 | 53.11 | 37.00 | 20.67 | | |

sampling sites.




| | Amp | Pip/taz | Amox | Cefta | Cefo | Cip | Nor | Levo | Tet | Ery | Gen | Kan | Amk | Erta | Imi | Mero | Chl | Rif | Az |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Influent | 37.50 | 7.81 | 12.50 | 21.88 | 21.88 | 28.13 | 12.50 | 10.94 | 29.69 | 18.75 | 15.63 | 14.06 | 10.94 | 4.69 | 7.81 | 7.81 | 9.38 | 12.50 | 6.25 |
| Effluent | 31.48 | 7.41 | 9.26 | 14.81 | 14.81 | 16.67 | 11.11 | 7.41 | 20.37 | 9.26 | 9.26 | 7.41 | 7.41 | 1.85 | 1.85 | 1.85 | 7.41 | 5.56 | 5.56 |
| Sludge | 36.96 | 6.52 | 10.87 | 19.57 | 17.39 | 19.57 | 15.22 | 13.04 | 34.78 | 17.39 | 15.22 | 13.04 | 8.70 | 4.35 | 2.17 | 4.35 | 6.52 | 8.70 | 6.52 |
| Filters | 30.9 | 7.52 | 7.4 | 12.4 | 10.9 | 20.5 | 9.8 | 8 | 21 | 11 | 9 | 9 | 8 | 0 | 0 | 0 | 4 | 1 | 2 |
| Soil | 32.00 | 6.00 | 6.00 | 10.00 | 12.00 | 14.00 | 6.00 | 6.00 | 18.00 | 12.00 | 4.00 | 4.00 | 4.00 | 2.00 | 2.00 | 0.00 | 6.00 | 2.00 | 4.00 |
| ElMahsamah | 20.83 | 4.17 | 4.17 | 6.25 | 8.33 | 10.42 | 4.17 | 4.17 | 18.75 | 10.42 | 8.33 | 6.25 | 6.25 | 2.08 | 2.08 | 2.08 | 4.17 | 2.08 | 4.17 |
| ElSayadeen | 14.63 | 2.44 | 2.44 | 4.88 | 4.88 | 4.88 | 2.44 | 4.88 | 14.63 | 9.76 | 2.44 | 4.88 | 2.44 | 2.44 | 0.00 | 0.00 | 4.88 | 2.44 | 2.44 |

**Figure 1.** Antibiotic resistance percentage of *E. coli* strains according to sampling sites.

**Table 3.** Number of isolates relevant to their resistance to antibiotics with MARI according to different sampling sites

| No. of antibiotic | No. of isolates | | | | | | | | | MARI / isolate |
|---|---|---|---|---|---|---|---|---|---|---|
| | influent | effluent | sludge | Filter | soil | ElMahsama | Elsayadeen | Total | | |
| | | | | | | | | No. | % | |
| 1 | 8 | 6 | 6 | 6 | 7 | 6 | 4 | 43 | 12.8 | 0.053 |
| 2 | 5 | 5 | 6 | 3 | 3 | 4 | 1 | 27 | 8 | 0.105 |
| 3 | 8 | 2 | 2 | 3 | 6 | 2 | 3 | 26 | 7.7 | 0.158 |
| 4 | 11 | 5 | 2 | 2 | 5 | 1 | 1 | 27 | 8 | 0.211 |
| 5 | 5 | 2 | 5 | 1 | 1 | 1 | 1 | 16 | 4.7 | 0.263 |
| 6 | 4 | 4 | 4 | 2 | 3 | 3 | 2 | 22 | 6.5 | 0.316 |
| 7 | 2 | 2 | 2 | 1 | ND | 1 | ND | 8 | 2.4 | 0.368 |
| 8 | 3 | 1 | 2 | ND | ND | 1 | ND | 7 | 2.1 | 0.421 |
| 9 | ND | ND | ND | ND | ND | ND | ND | ND | - | - |
| 10 | ND | ND | ND | ND | ND | ND | ND | ND | - | - |
| 11 | ND | 1 | 1 | ND | ND | ND | ND | 2 | 0.6 | 0.579 |
| 12 | ND | ND | ND | ND | ND | ND | ND | ND | - | - |
| 13 | ND | ND | ND | ND | ND | ND | ND | ND | - | - |
| 14 | 1 | ND | ND | ND | ND | ND | ND | 1 | 0.3 | 0.737 |
| Total | 47 | 28 | 30 | 18 | 25 | 19 | 12 | 179 | 53.1 | |
| ANOVA Site | 0.1271 | | | | | | | | | |
| ANOVA Multiple resistance | <0.001 | | | | | | | | | |

ND = not detected


**Figure 2.** Heatmap showing hierarchical clustering of the 337 *E. coli* isolates' resistance to various antibiotics is depicted to sampling sites. Each cell in a row represents a single susceptibility test result for a given *E. coli* isolate. Green tiles represent resistance, black tiles represent sensitive patterns

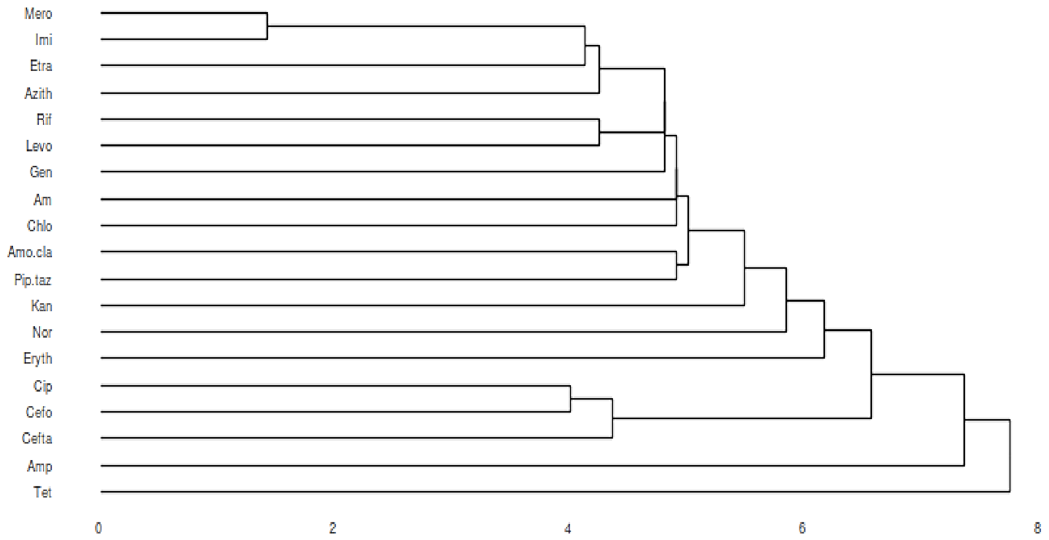



**Figure 3.** Dendrogram showing linkage between Antibiotic resistances in *E. coli* isolates of the current study

## 6. REFERENCES


[1] D. A. Goff *et al.*, "A global call from five countries to collaborate in antibiotic stewardship: united we succeed, divided we might fail," *The Lancet Infectious Diseases*, vol. 17, no. 2, pp. e56–e63, Feb. 2017, doi: 10.1016/S1473-3099(16)30386-3.

[2] C. Elias, L. Moja, D. Mertz, M. Loeb, G. Forte, and N. Magrini, "Guideline recommendations and antimicrobial resistance: the need for a change," *BMJ Open*, vol. 7, no. 7, p. e016264, Jul. 2017, doi: 10.1136/bmjopen-2017-016264.

[3] D. Jasovský, J. Littmann, A. Zorzet, and O. Cars, "Antimicrobial resistance—a threat to the world's sustainable development," *Upsala Journal of Medical Sciences*, vol. 121, no. 3, pp. 159–164, Jul. 2016, doi: 10.1080/03009734.2016.1195900.

[4] D. G. J. Larsson and C.-F. Flach, "Antibiotic resistance in the environment," *Nat Rev Microbiol*, vol. 20, no. 5, pp. 257–269, May 2022, doi: 10.1038/s41579-021-00649-x.

[5] P. Vital *et al.*, "Antibiotic Resistance and Extended-Spectrum Beta-Lactamase Production of Escherichia coli Isolated from Irrigation Waters in Selected Urban Farms in Metro Manila, Philippines," *Water*, vol. 10, no. 5, p. 548, Apr. 2018, doi: 10.3390/w10050548.

[6] S. Khalid *et al.*, "A Review of Environmental Contamination and Health Risk Assessment of Wastewater Use for Crop Irrigation with a Focus on Low and High-Income Countries," *IJERPH*, vol. 15, no. 5, p. 895, May 2018, doi: 10.3390/ijerph15050895.

[7] L. Wengenroth *et al.*, "Antibiotic Resistance in Wastewater Treatment Plants and Transmission Risks for Employees and Residents: The Concept of the AWARE Study," *Antibiotics (Basel)*, vol. 10, no. 5, p. 478, Apr. 2021, doi: 10.3390/antibiotics10050478.

[8] M. Mukherjee, E. Laird, T. J. Gentry, J. P. Brooks, and R. Karthikeyan, "Increased Antimicrobial and Multidrug Resistance Downstream of Wastewater Treatment Plants in an Urban Watershed," *Front. Microbiol.*, vol. 12, p. 657353, May 2021, doi: 10.3389/fmicb.2021.657353.

[9] A. M. Motlagh and Z. Yang, "Detection and occurrence of indicator organisms and pathogens," *Water Environment Research*, vol. 91, no. 10, pp. 1402–1408, Oct. 2019, doi: 10.1002/wer.1238.

[10] M.-U. Alam *et al.*, "Effective Treatment Strategies for the Removal of Antibiotic-Resistant Bacteria, Antibiotic-Resistance Genes, and Antibiotic Residues in the Effluent From Wastewater Treatment Plants Receiving Municipal, Hospital, and Domestic Wastewater: Protocol for a Systematic Review," *JMIR Res Protoc*, vol. 10, no. 11, p. e33365, Nov. 2021, doi: 10.2196/33365.

[11] A. Khaled Abdella Ahmed, M. Shalaby, O. Negim, and T. Abdel-Wahed, "Comparative Study of the Egyptian Code for Reusing Treated Wastewater for Agriculture," *Sohag Engineering Journal*, vol. 0, no. 0, pp. 0–0, Feb. 2022, doi: 10.21608/sej.2022.115242.1006.

[12] D. Rodríguez-Molina, P. Mang, H. Schmitt, M. C. Chifiriuc, K. Radon, and L. Wengenroth, "Do wastewater treatment plants increase antibiotic resistant bacteria or genes in the environment? Protocol for a systematic review," *Syst Rev*, vol. 8, no. 1, p. 304, Dec. 2019, doi: 10.1186/s13643-019-1236-9.

[13] E. Li, F. Saleem, T. A. Edge, and H. E. Schellhorn, "Biological Indicators for Fecal Pollution Detection and Source Tracking: A Review," *Processes*, vol. 9, no. 11, p. 2058, Nov. 2021, doi: 10.3390/pr9112058.

[14] C. Chahal, B. van den Akker, F. Young, C. Franco, J. Blackbeard, and P. Monis, "Pathogen and Particle Associations in Wastewater," in *Advances in Applied Microbiology*, vol. 97, Elsevier, 2016, pp. 63–119. doi: 10.1016/bs.aambs.2016.08.001.

[15] S. Naidoo and A. Olaniran, “Treated Wastewater Effluent as a Source of Microbial Pollution of Surface Water Resources,” *IJERPH*, vol. 11, no. 1, pp. 249–270, Dec. 2013, doi: 10.3390/ijerph110100249.

[16] R. Al-Sa`ed, “Pathogens Assessment in Reclaimed Effluent Used for Industrial Crops Irrigation,” *IJERPH*, vol. 4, no. 1, pp. 68–75, Mar. 2007, doi: 10.3390/ijerph2007010011.

[17] N. J. Rowan, “Defining Established and Emerging Microbial Risks in the Aquatic Environment: Current Knowledge, Implications, and Outlooks,” *International Journal of Microbiology*, vol. 2011, pp. 1–15, 2011, doi: 10.1155/2011/462832.

[18] A. M. Motlagh and Z. Yang, “Detection and occurrence of indicator organisms and pathogens,” *Water Environment Research*, vol. 91, no. 10, pp. 1402–1408, Oct. 2019, doi: 10.1002/wer.1238.

[19] M.-T. Gekenidis, W. Qi, J. Hummerjohann, R. Zbinden, F. Walsh, and D. Drissner, “Antibiotic-resistant indicator bacteria in irrigation water: High prevalence of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli,” *PLoS ONE*, vol. 13, no. 11, p. e0207857, Nov. 2018, doi: 10.1371/journal.pone.0207857.

[20] R. G. Abd Ellah, “Water resources in Egypt and their challenges, Lake Nasser case study,” *The Egyptian Journal of Aquatic Research*, vol. 46, no. 1, pp. 1–12, Mar. 2020, doi: 10.1016/j.ejar.2020.03.001.

[21] I. Bashir, F. A. Lone, R. A. Bhat, S. A. Mir, Z. A. Dar, and S. A. Dar, “Concerns and Threats of Contamination on Aquatic Ecosystems,” in *Bioremediation and Biotechnology*, K. R. Hakeem, R. A. Bhat, and H. Qadri, Eds. Cham: Springer International Publishing, 2020, pp. 1–26. doi: 10.1007/978-3-030-35691-0_1.

[22] L. L. Bridgewater *et al.*, Eds., *Standard methods for the examination of water and wastewater*, 23rd edition. Washington, DC: American Public Health Association, 2017.

[23] M. P. Weinstein, Ed., *Performance standards for antimicrobial susceptibility testing*, 29th edition. Wayne, Pennsylvania: Clinical and Laboratory Standards Institute, 2019.

[24] J. L. Hatfield and C. Dold, “Water-Use Efficiency: Advances and Challenges in a Changing Climate,” *Front. Plant Sci.*, vol. 10, p. 103, Feb. 2019, doi: 10.3389/fpls.2019.00103.

[25] S. Naidoo and A. O. Olaniran, “Treated wastewater effluent as a source of microbial pollution of surface water resources,” *Int J Environ Res Public Health*, vol. 11, no. 1, pp. 249–270, Dec. 2013, doi: 10.3390/ijerph110100249.

[26] C. Chahal, B. van den Akker, F. Young, C. Franco, J. Blackbeard, and P. Monis, “Pathogen and Particle Associations in Wastewater: Significance and Implications for Treatment and Disinfection Processes,” *Adv Appl Microbiol*, vol. 97, pp. 63–119, 2016, doi: 10.1016/bs.aambs.2016.08.001.

[27] G. Płaza, Ł. Jałowiecki, D. Głowacka, J. Hubeny, M. Harnisz, and E. Korzeniewska, “Insights into the microbial diversity and structure in a full-scale municipal wastewater treatment plant with particular regard to Archaea,” *PLoS One*, vol. 16, no. 4, p. e0250514, 2021, doi: 10.1371/journal.pone.0250514.

[28] Y. K. Kim *et al.*, “The capacity of wastewater treatment plants drives bacterial community structure and its assembly,” *Sci Rep*, vol. 9, no. 1, p. 14809, Oct. 2019, doi: 10.1038/s41598-019-50952-0.

[29] P. K. Pandey, P. H. Kass, M. L. Soupir, S. Biswas, and V. P. Singh, “Contamination of water resources by pathogenic bacteria,” *AMB Express*, vol. 4, p. 51, 2014, doi: 10.1186/s13568-014-0051-x.

[30] F. F. Reinthaler *et al.*, “Antibiotic resistance of E. coli in sewage and sludge,” *Water Research*, vol. 37, no. 8, pp. 1685–1690, Apr. 2003, doi: 10.1016/S0043-1354(02)00569-9.

[31] O. Osuolale and A. Okoh, "Assessment of the Physicochemical Qualities and Prevalence of Escherichia coli and Vibrios in the Final Effluents of Two Wastewater Treatment Plants in South Africa: Ecological and Public Health Implications," *IJERPH*, vol. 12, no. 10, pp. 13399–13412, Oct. 2015, doi: 10.3390/ijerph121013399.

[32] M. Raboni, R. Gavasci, and V. Torretta, "Assessment of the Fate of Escherichia coli in Different Stages of Wastewater Treatment Plants," *Water Air Soil Pollut*, vol. 227, no. 12, p. 455, Dec. 2016, doi: 10.1007/s11270-016-3157-8.

[33] T. A. Elbana, N. Bakr, and M. Elbana, "Reuse of Treated Wastewater in Egypt: Challenges and Opportunities," in *Unconventional Water Resources and Agriculture in Egypt*, vol. 75, A. M. Negm, Ed. Cham: Springer International Publishing, 2017, pp. 429–453. doi: 10.1007/698_2017_46.

[34] M. Helmecke, E. Fries, and C. Schulte, "Regulating water reuse for agricultural irrigation: risks related to organic micro-contaminants," *Environ Sci Eur*, vol. 32, no. 1, p. 4, Dec. 2020, doi: 10.1186/s12302-019-0283-0.

[35] E. R. Blatchley *et al.*, "Effects of Wastewater Disinfection on Waterborne Bacteria and Viruses," *Water Environment Research*, vol. 79, no. 1, pp. 81–92, Jan. 2007, doi: 10.2175/106143006X102024.

[36] A. Korajkic, B. McMinn, and V. Harwood, "Relationships between Microbial Indicators and Pathogens in Recreational Water Settings," *IJERPH*, vol. 15, no. 12, p. 2842, Dec. 2018, doi: 10.3390/ijerph15122842.

[37] F. Shoushtarian and M. Negahban-Azar, "Worldwide Regulations and Guidelines for Agricultural Water Reuse: A Critical Review," *Water*, vol. 12, no. 4, p. 971, Mar. 2020, doi: 10.3390/w12040971.

[38] U. J. Blumenthal, D. D. Mara, A. Peasey, G. Ruiz-Palacios, and R. Stott, "Guidelines for the microbiological quality of treated wastewater used in agriculture: recommendations for revising WHO guidelines," *Bull World Health Organ*, vol. 78, no. 9, pp. 1104–1116, 2000.

[39] H. B. Doğan-Halkman, İ. Çakır, F. Keven, R. W. Worobo, and A. K. Halkman, "Relationship among fecal coliforms and Escherichia coli in various foods," *Eur Food Res Technol*, vol. 216, no. 4, pp. 331–334, Apr. 2003, doi: 10.1007/s00217-002-0647-2.

[40] K. P. Brenner, C. C. Rankin, Y. R. Roybal, G. N. Stelma, P. V. Scarpino, and A. P. Dufour, "New medium for the simultaneous detection of total coliforms and Escherichia coli in water," *Appl Environ Microbiol*, vol. 59, no. 11, pp. 3534–3544, Nov. 1993, doi: 10.1128/aem.59.11.3534-3544.1993.

[41] M. Mukherjee, E. Laird, T. J. Gentry, J. P. Brooks, and R. Karthikeyan, "Increased Antimicrobial and Multidrug Resistance Downstream of Wastewater Treatment Plants in an Urban Watershed," *Front. Microbiol.*, vol. 12, p. 657353, May 2021, doi: 10.3389/fmicb.2021.657353.

[42] C. Mutuku, Z. Gazdag, and S. Melegh, "Occurrence of antibiotics and bacterial resistance genes in wastewater: resistance mechanisms and antimicrobial resistance control approaches," *World J Microbiol Biotechnol*, vol. 38, no. 9, p. 152, Jul. 2022, doi: 10.1007/s11274-022-03334-0.

[43] M. Haenni *et al.*, "Environmental contamination in a high-income country (France) by antibiotics, antibiotic-resistant bacteria, and antibiotic resistance genes: Status and possible causes," *Environ Int*, vol. 159, p. 107047, Jan. 2022, doi: 10.1016/j.envint.2021.107047.

[44] R. S. Dungan and D. L. Bjorneberg, "Antimicrobial Resistance in Escherichia coli and Enterococcal Isolates From Irrigation Return Flows in a High-Desert Watershed," *Front. Microbiol.*, vol. 12, p. 660697, May 2021, doi: 10.3389/fmicb.2021.660697.

[45] M. U. Rasheed, N. Thajuddin, P. Ahamed, Z. Teklemariam, and K. Jamil, "Antimicrobial drug resistance in strains of Escherichia coli isolated from food sources," *Rev Inst Med Trop Sao Paulo*, vol. 56, no. 4, pp. 341–346, Aug. 2014, doi: 10.1590/s0036-46652014000400012.

[46] C. Uluseker *et al.*, "A Review on Occurrence and Spread of Antibiotic Resistance in Wastewaters and in Wastewater Treatment Plants: Mechanisms and Perspectives," *Front. Microbiol.*, vol. 12, p. 717809, Oct. 2021, doi: 10.3389/fmicb.2021.717809.

[47] T. A. Elbana, N. Bakr, and M. Elbana, "Reuse of Treated Wastewater in Egypt: Challenges and Opportunities," in *Unconventional Water Resources and Agriculture in Egypt*, vol. 75, A. M. Negm, Ed. Cham: Springer International Publishing, 2017, pp. 429–453. doi: 10.1007/698_2017_46.

[48] T. A. Elbana, N. Bakr, and M. Elbana, "Reuse of Treated Wastewater in Egypt: Challenges and Opportunities," in *Unconventional Water Resources and Agriculture in Egypt*, vol. 75, A. M. Negm, Ed. Cham: Springer International Publishing, 2017, pp. 429–453. doi: 10.1007/698_2017_46.

[49] Ts. Singh, D. Tsering, and S. Poonia, "Antibiotic susceptibility profile of bacteria isolated from natural sources of water from rural areas of East Sikkim," *Indian J Community Med*, vol. 39, no. 3, p. 156, 2014, doi: 10.4103/0970-0218.137152.

[50] V. Chigor, I.-A. Ibangha, C. Chigor, and Y. Titilawo, "Treated wastewater used in fresh produce irrigation in Nsukka, Southeast Nigeria is a reservoir of enterotoxigenic and multidrug-resistant Escherichia coli," *Heliyon*, vol. 6, no. 4, p. e03780, Apr. 2020, doi: 10.1016/j.heliyon.2020.e03780.

[51] A. Teshome, T. Alemayehu, W. Deriba, and Y. Ayele, "Antibiotic Resistance Profile of Bacteria Isolated from Wastewater Systems in Eastern Ethiopia," *Journal of Environmental and Public Health*, vol. 2020, pp. 1–10, Sep. 2020, doi: 10.1155/2020/2796365.

[52] M. Mukherjee, E. Laird, T. J. Gentry, J. P. Brooks, and R. Karthikeyan, "Increased Antimicrobial and Multidrug Resistance Downstream of Wastewater Treatment Plants in an Urban Watershed," *Front. Microbiol.*, vol. 12, p. 657353, May 2021, doi: 10.3389/fmicb.2021.657353.

[53] L. Montero, J. Irazabal, P. Cardenas, J. P. Graham, and G. Trueba, "Extended-Spectrum Beta-Lactamase Producing-Escherichia coli Isolated From Irrigation Waters and Produce in Ecuador," *Front. Microbiol.*, vol. 12, p. 709418, Oct. 2021, doi: 10.3389/fmicb.2021.709418.

[54] A. M. Motlagh and Z. Yang, "Detection and occurrence of indicator organisms and pathogens," *Water Environment Research*, vol. 91, no. 10, pp. 1402–1408, Oct. 2019, doi: 10.1002/wer.1238.