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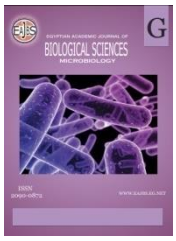

# Bacteriological and Physicochemical Evaluation of Different Wells Water in El-Qalubia Governorate, Egypt

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## ABSTRACT

The provision of potable drinking water is of public health concern, especially in developing countries. This study aimed to test the bacteriological quality of well water in El-Qalubia governorate, Egypt. Untreated well water samples were collected from thirteen sites all over the governorate. The most probable number of tests (presumptive and confirmed tests) revealed that water is unpotable for drinking. Five pathogenic microorganisms were isolated from samples. These isolates were identified by biochemical and phytic tests as *Klebsiella Pneumonia*, *Enterobacter* sp., *Pseudomonas aeruginosa*, *Citrobacter braaki*, and *E. coli*. Antibiotic susceptibility test for these isolates confirmed their multidrug resistance. Thus, this study has shown a high level of bacterial contamination in all the samples. Hence, the need for good maintenance and hygienic practices by households to reduce the risk of disease outbreaks from the organisms encountered in this study.

## INTRODUCTION

Water is a vital and essential commodity to the survival of life, where water is the source of all biological lives and their sustenance (WHO, 2011). It is often used for domestic purposes, especially for drinking (humans, animals, plants) and washing. These different purposes have their own requirements for the composition and purity of water. Thus, water must be analyzed on a regular basis to confirm its suitability (Saxena *et al*., 2011).

Over 75% of the earth's surface is covered by water (Simonovic and Fahmy, 1999), but less than 1% of the world's freshwater is accessible for direct human use. The availability of adequate pure water in terms of both quantity and quality is essential for human survival. Water which was once termed a free gift of nature is becoming scarcer, particularly in developing countries due to unplanned civilization and increasing industrialization (Montgomery and Elimelech, 2007; Zimmerman *et al*., 2008).

The amount of water available to Egypt is limited, and the increased demand for clean water has put pressure on its vital resource. Rapid growth in population, new land reclamation, and industrial development waste production are outstripping the capacity of the Nile to clean itself. This fact has serious public health and economic implications for Egypt. Water from wells is considered contaminated or unsafe for drinking when tested for toxic chemicals or pathogenic microorganisms (Tao *et al*., 2014).

Safe and good quality drinking water is one of the most important human needs. Physical, chemical, and biological characteristics of water are considered as the main health-controlling factor (Kazi *et al*., 2009). According to the findings of the World Health Organization (WHO), about 5 million people die each year because of untreated water and practice of poor hygiene. The unprotected water sources can be contaminated with microbes through rainfall runoff and agricultural inputs, mixing with sewage effluents and feces from wildlife (Obi *et al*., 2014; Sharma *et al.*, 2005) which renders them unacceptable for human consumption. The primary source of contamination by microorganisms could be fecal waste from warm-blooded animals including humans. Different pathogenic bacteria cause enteric diseases in humans while others are non-pathogenic (Lu *et al*., 2014). However, it becomes economically expensive for the communities to dig new wells with a low level of contamination (Abraham *et al*., 2016).

Water is considered unsafe for human consumption when it has pathogenic microorganisms. Water test for bacteriological safety depends on microbiologists' ability to detect coliform bacteria in the wells (David *et al*., 2014. *Escherichia coli* is the most abundant bacteria in the test since it lives longer in water than other intestinal bacteria (Ateba *et al*., 2012). Pathogens like *Escherichia coli*, *Salmonella* sp., *Shigella* spp., *Bacillus* spp., *Pseudomonas* spp., *Streptococcus* spp., *Vibrio* spp. etc. are the groups of bacteria that are responsible for causing diseases like diarrhea, enteric fever, dysentery, and other severe diseases (Munshi *et al*., 2012; Venkatesan *et al*., 2014).

Rural communities use water disinfection methods such as chlorination, coagulation, sedimentation, and boiling (Katakwar *et al*., 2015). Some of these methods become impractical due to the high cost of the required equipment and the low availability of chemical coagulants (Koike *et al*., 2015). Treated water ensures the safety of infants and immunocompromised people who need to avoid exposure to potential pathogens which are a threat to human health (Acharjee *al*., 2013).

Recently there has been growing attention on the incidence of antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs) in treated and untreated drinking water. Therefore, the purpose of the present study was to determine the microbiological quality of untreated commercial drinking water samples collected from various untreated wells in El-Qalubia governorate, Egypt.

## MATERIALS AND METHODS

### Study Area and Collection of Samples:

The study was carried out in El-Qalubia Governorate (30°19'45.25"N, 31°13'0.65"E Egypt. Thirteen water samples were collected from untreated wells. These samples were obtained from different sites all over the governorate namely (S1, S2, S3, S4, N1, N2, N3, N4, T1, M1, M2, E1, E2). Physical and chemical analysis of different water samples was determined as shown in Table 1. An overview of the work done in this study is depicted in Figure 1.

### Microbiological Assessment of Well Water Samples:

***1-Enumeration of Total Viable Bacteria:*** One ml of water samples was added to Reasoner's 2A (R2A) agar. plates. These plates were incubated for 48 h. at 37°C. The bacterial count was detected as cfu/ml.

***2-Multiple-tube Fermentation Technique:***

**a. Presumptive Phase:**

Lauryl tryptose broth triple-strength media was used in this phase of the multiple-tube test. Fermentation tubes in rows of five each were arranged in a test tube rack. Twenty ml of water samples were inoculated into 10 ml of lauryl tryptose broth. Tubes were shaken vigorously and incubated at 35 ± 0.5°C for 24 ±2 h. After the incubation period, tubes were swirled and examined for growth and acidic reaction (shades of yellow color). Positive tubes were directed to the confirmed test.

**b-Confirmed Phase:**

Brilliant green lactose bile (BGLB) broth fermentation tubes were used for the confirmed phase. All presumptive tubes showing growth, any amount of gas, or acidic reaction within 24 ± 2 h of incubation were submitted to the confirmed phase. With a sterile loop, one or more loopful of culture were transferred to a fermentation tube containing BGLB broth. The inoculated BGLB broth tubes were incubated at 35 ± 0.5°C. Any amount of gas formed in the inverted vial of the BGLB broth fermentation tube at any time within 48 ± 3 h constitutes a positive confirmed phase.

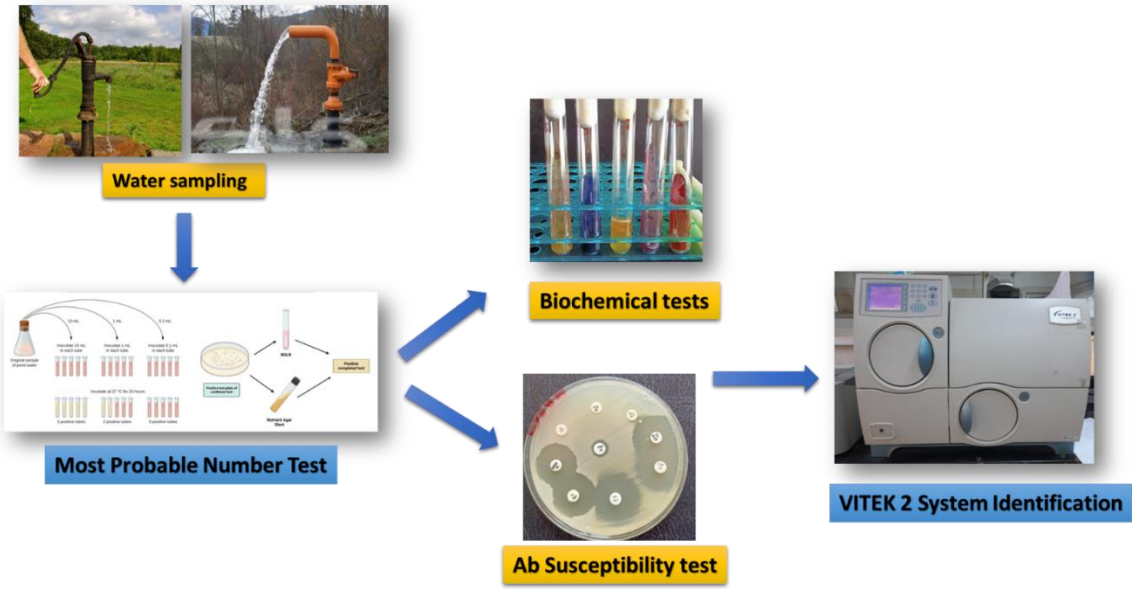


**Fig. 1.** Schematic representation for bacteriological examination of well water samples.

**Table 1.** Physical and chemical analysis of collected water samples.

| Physicochemical parameters | Unit | Standard limit | Sample code | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | S1 | S2 | S3 | S4 | N 1 | N2 | N3 | N4 | T1 | M1 | M2 | E1 | E2 |
| Turbidity | NTU | >1.0 | 2.3 | 2.5 | 2 | 5 | 6.5 | 5.6 | 5.4 | 3.5 | 4.1 | 3 | 2.1 | 2.7 | 3.9 |
| pH | – | 6.5-8.5 | 7.5 | 7.5 | 7.4 | 7.3 | 7.4 | 7.5 | 7.5 | 7.6 | 7.6 | 7.6 | 7.6 | 7.6 | 7.6 |
| Conductivity | Ms | – | 1046 | 1000 | 1259 | 990 | 952 | 1121 | 1100 | 1065 | 997 | 1034 | 1029 | 1017 | 900 |
| Tds | mg/L | >1000 | 669 | 660 | 831 | 653 | 628 | 740 | 726 | 703 | 658 | 682 | 678 | 674 | 564 |
| Chloride | mg/L | >250 | 140 | 130 | 130 | 135 | 125 | 130 | 155 | 150 | 140 | 140 | 140 | 146 | 128 |
| Total hardness | mg/L | >500 | 380 | 400 | 300 | 360 | 360 | 400 | 400 | 400 | 380 | 380 | 380 | 380 | 360 |
| Total calcium | mg/L | >350 | 240 | 260 | 240 | 220 | 220 | 280 | 260 | 280 | 220 | 250 | 250 | 240 | 220 |
| Mg | mg/L | >150 | 140 | 140 | 60 | 140 | 140 | 120 | 140 | 120 | 60 | 130 | 130 | 140 | 140 |
| Sulphite | mg/L | >250 | 64 | 63 | 64 | 66 | 66 | 72 | 71 | 68 | 60 | 58 | 46.6 | 48.3 | 42 |

* S1,S2,S3,S4: Senhera N1,N2,N3,N4: Namool, T1: Toukh, M1,M2: Meet Kenana, E1,E2: El-Dir.

**Isolation and Biochemical Characterization of Bacterial Isolates:**

Loopful of cultures from different samples grown on BGLB broth media was transferred to Eosin methylene blue (EMB) media. The plates were incubated for 24-48 h at 37 °C.The phenotypic characterization of bacterial isolates was carried out through morphological and biochemical observations as described by Bergy's manual of systemic

bacteriology (Holt *et al.,* 1994). Morphological characterizations such as Gram staining, colony morphology, shape, and size were studied under a light compound microscope. Biochemical characterizations such as catalase, indole, citrate, urease, triple sugar iron, and lysine iron agar tests were determined as described by (Holt *et al.,* 1994).

The amount of water available to Egypt is limited, and the increased demand for clean water has put pressure on its vital resource. Rapid growth in population, new land reclamation, and industrial development waste production are outstripping the capacity of the Nile to clean itself. This fact has serious public health and economic implications for Egypt. Water from wells is considered contaminated or unsafe for drinking when tested for toxic chemicals or pathogenic microorganisms (Tao *et al*., 2014).

**Antibiotic Susceptibility Testing:**

Antibiotic susceptibility testing was performed using the disc diffusion method (Biemer, 1973) for the following antibiotics Amoxicilln (AM), Piperacillin+ tazobactam (TPZ), Amikacin (AK), Tobramycin (TOB), Tigecyclin (TGC), Ceftrixone (CRO), Ceftazidine (CAZ), Cefoperazon (CPZ), Ampicillin+Sulbactam (SAM), Levofloxacin (Levo), Cefoxitin (FOX), Norfloxacin (NOR), Trimethoprim+ sulfamethoxazole (COT), Streptomycin (S), Amoxicillin+ Clavularat (AMC), Cefatoxime (CTX), Gentamycin (CN), Cefoprazone/sulbactam (CES), Ertapenem (ETP), Neropenem (Mem), Peflofloxacin (Pef), Ciprofloxacin (Cip), Aztreonam (ATM), and Colistin (Cl).

**Bacterial Identification by Vitek 2 Compact System:**

Identification of bacterial isolates was done by Vitek 2 compact (Ling *et al* 2001; Darbandi, 2010). This system uses colorimetric reagent cards that are incubated and interpreted automatically (Pincus, 2005).

## RESULTS AND DISCUSSION

**Enumeration of Total Viable Bacteria:**

Water is necessary for life on Earth, but it may also act as an optimum medium for various pathogens. Every human being has the right to drink safe and healthy water (Mohammad. N.F., *et al* 2021).

Data in Table 2**.** Shows the colony forming unit per millimeter for different organisms isolated on R2A agar media from different untreated wells. The untreated wells included a great number of total bacterial counts that is greater than 50 cfu/ml indicating microbial contamination of water. The highest average was observed in the S2 sample equal 200 cfu/ml and the lowest mean count 60 cfu/ ml was observed in T1 sample.

Unsafe drinking water and unsanitary circumstances enhance the risk of different health hazards like typhoid fever, shigellosis, and other diseases. According to WHO (2019) 60% of all deaths due to diarrhea in low- and middle-income countries are attributable to inadequate drinking water (35%), sanitation (31%) and hygiene (12%).

**Table 2.** The average number of colony-forming units (cfu/ml) for different wells water.

| Site | cfu/ml |
|---|---|
| S1 | 140 |
| S 2 | 200 |
| S3 | 148 |
| S4 | 140 |
| N1 | 100 |
| N2 | 112 |
| N3 | 110 |
| N4 | 112 |
| T1 | 60 |
| M1 | 102 |
| M2 | 70 |
| E1 | 60 |
| E2 | 64 |

**Multiple-Tube Fermentation Technique:**

Lactose-based broth medium was used to detect the metabolic end products of lactose fermentation. Bacterial density can be estimated by the formula given or from the table using the number of positive tubes in the multiple dilutions. The number of sample portions selected will be governed by the desired precision of the result. MPN Index/100 ml for tested isolates was recorded according to data in Table 3.

**Table 3.** MPN index and 95% confidence limits for various combinations of positive and negative results when five 20 ml portions are used (APHA, 1992).

| NO. of Tubes Giving Positive Reaction Out of 5 of 20 ml Each | MPN Index/100 ml | 95%Confidence Limits (Approximate) | |
|---|---|---|---|
| | | Lower | Upper |
| 0 | <1.1 | 0 | 3.0 |
| 1 | 1.1 | 0.05 | 6.3 |
| 2 | 2.6 | 0.3 | 9.6 |
| 3 | 4.6 | 0.8 | 14.7 |
| 4 | 8 | 1.7 | 26.4 |
| 5 | >8 | 4.0 | Infinite |

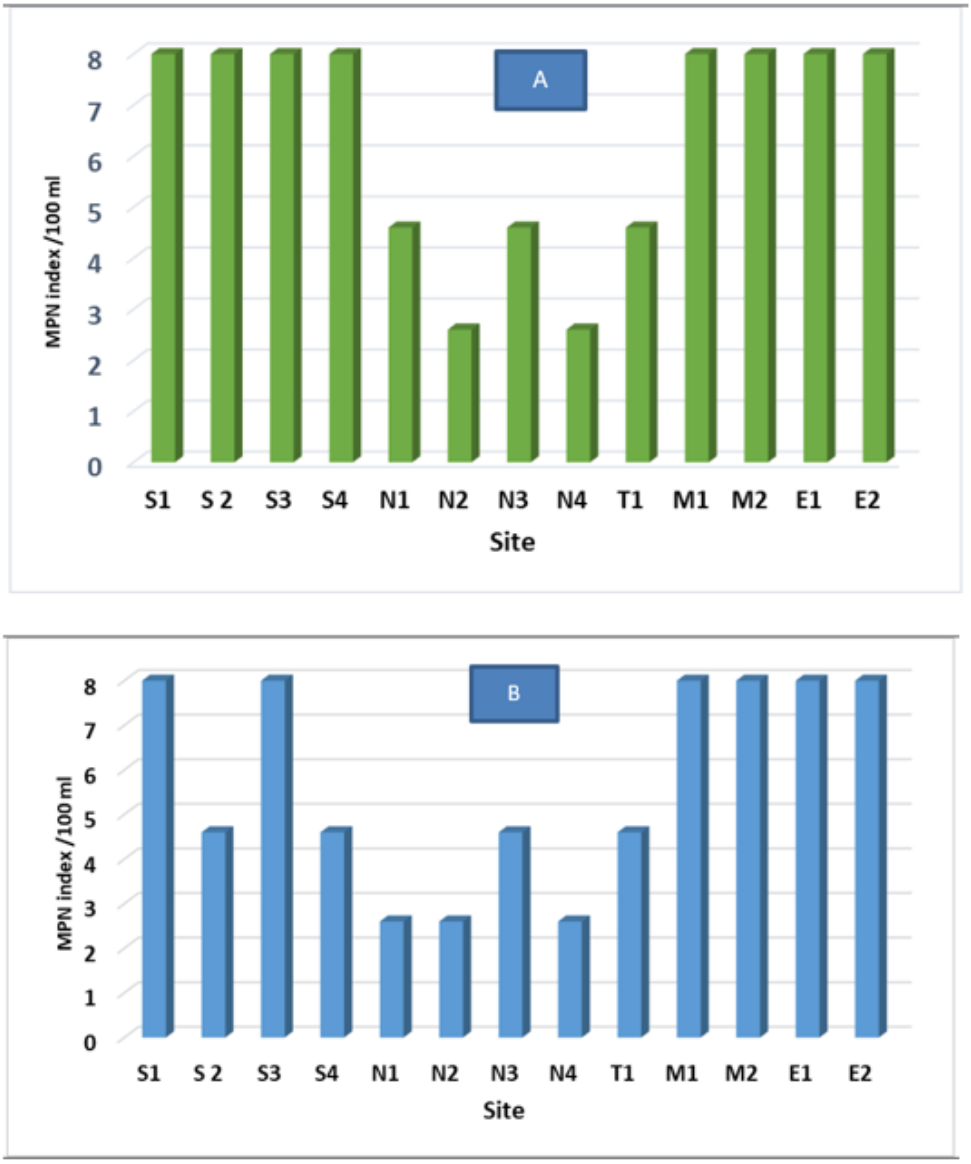


**Fig. 2.** Results of (a) Presumptive test and (b) confirmed test for different collected samples.

Results of the presumptive test showed that 61.5 % gave 8 MPN index /100 ml. while 23% and 15.3 % gave 4.6 and 2.6 MPN index /100 ml (Fig 2a). The presence of coliforms must be confirmed in a lactose- and bile salt-containing medium [brilliant green lactose bile (BGLB) broth]. So, the coliforms are defined as all facultative anaerobic, Gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose with gas and acid

production in the presence of bile salts within 48 h at 35°C. Results of the confirmed test showed that 46% gave 8 MPN index /100 ml. while 30 and 23% had 4.6 and 2.6 MPN index /100 ml (Fig 2b).

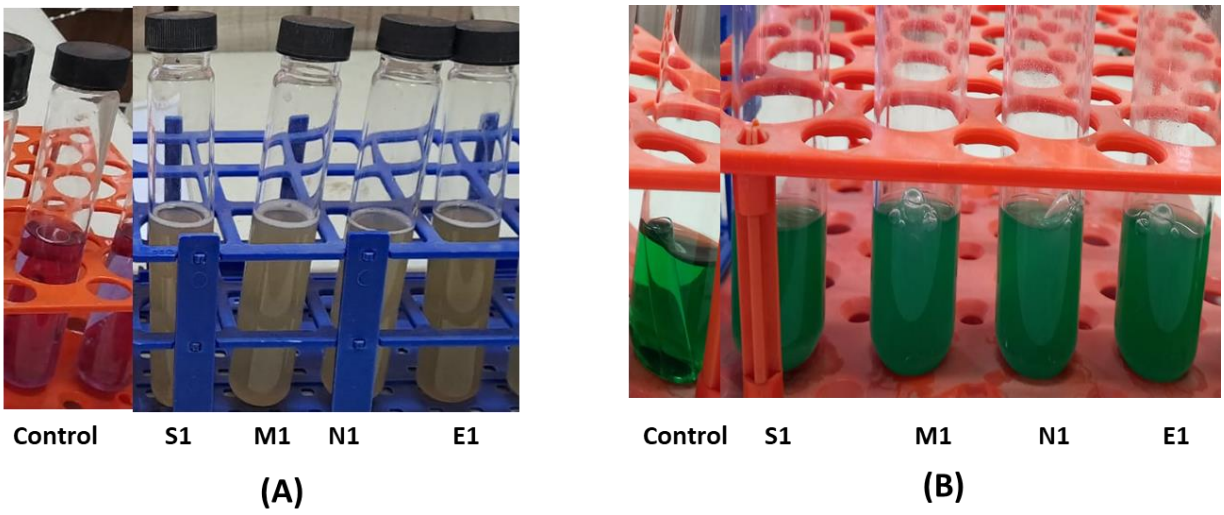


**Fig. 3.** Results of (A) Presumptive test and (B) confirmed test for different collected samples.

**Identification of the Pathogenic Bacteria (Morphological and Biochemical Identification of Bacterial Isolates):**

According to Bergey's manual of systematic bacteriology Holt *et al*. (1994) and Vos *et al*. (2011) respectively, the morphological and biochemical tests are shown in Table 4. Microscopic morphology confirmed that all isolates were gram-negative bacilli. According to Vitek 2 compact system, isolates code P1, P2, P3, P4 and P5 were identified as *Klebsiella pneumonia*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Citrobacter braaki*, and *E. coli* respectively. All isolates were indole negative except *E. coli* which was indole positive. Also, all isolates were citrate positive except *E. coli* which was citrate negative. Additionally, all isolates were urease negative except *Klebsiella pneumoniae* which was positive.

Dawwam *et al.* (2022) confirmed biochemical tests of *E. coli* that gave methyl red and indole positive, urease and citrate negative.

**Table 4.** Different biochemical tests for different bacterial isolates.

| Isolate code | Site | Gram staining | Bacterial shape | TSI | LIA | Indole | Urease | Citrate | Identified organism |
|---|---|---|---|---|---|---|---|---|---|
| P1 | S1 | Negative | Bacilli | A*/A | k*/k | *- | *+ | + | *Klebsiella Pneumonia* |
| P2 | N1 | Negative | Bacilli | A/A | - | - | - | + | *Enterobacter cloacae* |
| P3 | T1 | negative | Bacilli | k/k | - | - | - | + | *Pseudomonas Aeruginosa* |
| P4 | E2 | negative | Bacilli | variable | - | - | variable | + | *Citrobacter Braakii* |
| P5 | M2 | negative | Bacilli | + | + | + | - | - | *E.coli* |

*A: acidic, k: Alkaline, -: negative, +: positive.

Rawy *et al.* (2020) found that *Klebsiella pneumonia* is oxides negative, indole negative, urease positive, triple sugar iron test with yellow slant yellow butt, citrate positive. Also, Rady *et al.* (2012) reported that *Enterobacter cloacae* were positively reacted with catalase and citrate utilization. And negatively reacted with Lysine decarboxylase, Indole, and oxidase. Banerjee *et al*. (2017) found that *pseudomonasa eruginosa* was positive for oxidase, catalase, citrate, nitrate reduction, and negative for indole whereas, *Citrobacter braaki* was positively reacted with citrate, while in urease and indole is variable (Janda *et al*,1994).

**Antibiotic Susceptibility of The Bacterial Isolates:**

The wide distribution of antibiotic-resistant bacteria in surface and ground waters has been reported in previous studies (Harakeh *et al.* 2005; Kummerer, 2009)**.** Data in Table 5 showed the resistance/sensitivity of the isolated microbes toward twenty-four popular antibiotics. The isolated *Klebsiella pneumoniae* and *E. coli* were highly sensitive to fourteen antibiotics and resistant against the other ten.

**Table 5.** Resistance/ sensitivity of the isolated bacteria to different antibiotics

| Antibiotics | Concentrations (µg/disc) | Antibiotic susceptibility pattern | | | | |
|---|---|---|---|---|---|---|
| | | *Klebsiella pneumoniae* | *Enterobacr complex* | *Pseudomonas aeruginosa* | *Citrobacter braakii* | *E.coli* |
| Amoxicilln | 10 | R | R | S | R | S |
| Piperacillin+tazobactm | 100/10 | S | R | R | R | S |
| Amikacin | 30 | S | R | S | S | R |
| Tobramycin | 10 | S | S | R | R | R |
| Tigecyclin | 15 | S | R | R | R | R |
| Ceftrixone | 30 | R | S | S | R | S |
| Ceftazidine | 30 | R | S | S | S | R |
| Cefoperazone | 75 | R | R | R | S | R |
| Ampicillin+Sulbactam | 20 | R | R | S | R | S |
| Levofloxacin | 5 | S | S | R | S | R |
| Cefoxitin | 30 | R | R | R | R | S |
| Norfloxacin | 10 | S | S | S | R | S |
| Trimethoprim+sulfamethoxazole | 25 | R | R | R | S | R |
| Streptomycin | 10 | R | S | R | R | S |
| Amoxicillin+Clavulart | 20/10 | R | R | S | R | R |
| Cefatoxime | 30 | R | S | R | R | R |
| Gentamycin | 20 | S | S | S | S | S |
| Cefoprazone /sulbactam | 20 | S | S | S | S | S |
| Ertapenem | 25 | S | R | S | R | S |
| Neropenem | 20 | S | R | R | R | R |
| Peflofloxacin | 5 | S | S | S | S | S |
| Ciprofloxacin | 5 | S | R | R | S | S |
| Aztreonam | 5 | S | S | S | S | S |
| Colistin | 25 | S | R | S | S | S |

***** R: resistant, S: sensitive**.**

Both *Citrobacter braakii* and *Enterobacr complex* were sensitive to eleven antibiotics and resistant to thirteen, while *pseudomonas aeruginosa* was sensitive to thirteen antibiotics and resistant to eleven. Resistance could be attributed to heavy contamination from sewage effluent, surface runoff, agricultural activities, wildlife, industrial pollution, and so forth. Considering the high incidence of HIV/AIDS in South Africa, the importance of such findings cannot be overemphasized. The results of this study on bacterial resistance profiles are consistent with previous studies in other surface and drinking water systems (Pavlov., *et al* 2004; Shrivastava *et al*., 2004; Moore., *et al.*, 2010)

A motivation for this study was the numerous reports about the occurrence of pathogenic microorganisms in drinking water and the associated diseases (Obi *et al*., 2002; Schafer., *et al* 2009; Carter., *et al* 2009).

In addition to this, the resistance of microorganisms to antibiotics of clinical

interest has previously been reported (Mulamattathil *et al.,* 2000; Kinge *et al.,* 2010; Ateba *et al.,* 2008). In likewise, several authors also isolated *E. coli*, *Shigella,* and *Salmonella* from samples of water (Talukder *et al.,* 2013; Mian *et al.,* 2020)**.** A study conducted by Nguendo-Yongsi *et al*. (2004) identified 1242 isolates of *Enterobacteriaceae* family from a variety of drinking water, of which *Shigella* species had 0.24% incidence.

The finding of this study confirmed the prevalence of pathogenic bacteria in well water might be due to poor sanitation, mixing of sewage effluents with well water, and due to fecal contamination. In Pakistan, 74% of tape, storage tanks, and tube well were identified as contaminated with *E. coli*, followed by *Salmonella* spp. (54%) and *Shigella* spp. (40%) (Mian *et al*., 2020). Thus, we must pay attention to the control of *pathogenic bacteria* and follow WHO standards of drinking water.

**Conclusion**

The presence of Multiple antibiotic resistance organisms in well water is an important health concern due to the risk of developing waterborne diseases and the health risks associated with immunocompromised patients living in the area. It is therefore imperative to monitor the quality of water and strict quality control measures should be put in place to ensure the effective treatment of water.

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