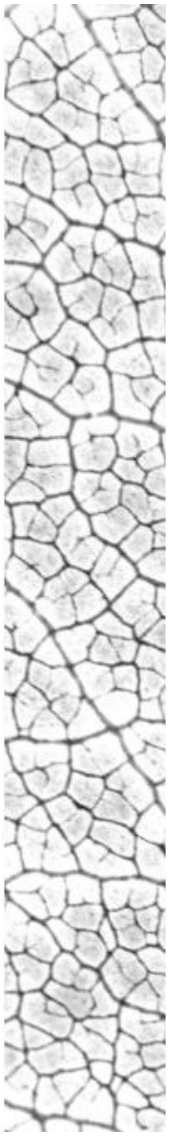

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# Estimation of physicochemical parameters and antibiotics resistance patterns in Almamierh treatment plant in Babylon Province, Iraq

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**Abstract**
Surface water contamination remains a major worldwide public health concern and may contribute to the dissemination of antibiotic-resistant bacteria. The current study dealt with the assessment of physicochemical parameters in the wastewater treatment plant in Almamierh, in the city of Babylon Province, Iraq. The treated water in this plant is used for agricultural purposes. To evaluate water quality, samples were collected from three sites (input, sedimentation, output) for estimation physicochemical parameters that includes (Water temperature, EC, PH, BOD, COD, TDS). the highest concentration recorded in the first site, especially BOD5 (235 Mg/L), COD (761 Mg/L), TDS (626.432 mg/L), while the EC was the highest value in the second location (1008ms/cm). These samples subjected to bacteriological analysis and a number of bacterial species have been isolated, such as: Escherichia coli, Klebsiella pneumonia, Enterococcus faecalis, pseudomonas aeruginosa. Isolates were tested for resistance to 12 classes of antibiotics, the results showed that the highest resistance levels were to β-lactam antibiotics, followed by penicillin, cephem, Fluoroquinolones, tetracycline, glycopeptides, and erythromycin.

**Keywords:** physicochemical parameters, wastewater, antibiotic resistance bacteria

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## INTRODUCTION

Wastewater can originate from many sources such as; homes, businesses and industries, Storm water, surface water and ground water can enter the wastewater collection system and add to the volume of wastewater, the source of a wastewater will determine it's characteristics and how it must be treated (Velho, 2011).Quality of water generally refers to the component of water which present at the optimum level for suitable growth of plants and animals, the quality of water is a vital concern for mankind because it is directly linked with human health (Abdelzaher, et al. 2010, Vibhhute and Ingavale, 2020). Pollution is caused when a change in the physical, chemical or biological condition in the environment harmfully affects quality of human life including other animals and plants' life (Oketola, et al. 2006). Among the pollutants in the water treatment plant are antibiotic resistance bacteria,one of the major problem to human health in this century is the antibiotic resistance, the U.S centers for prevention and control disease and WHO are portraying worldwide emergency and a looming disaster of arrival to the pre-antibiotic era (Liu, et al. 2006). The dissemination of AR among pathogenic bacteria is a serious threat in the natural environment. AR may occur either by mutation or acquisition of antibiotic resistance genes (ARGs) through horizontal gene transfer (HGT). In aquatic environments, HGT is one of the major mechanisms used to spread ARGs from environmental and commensal species to pathogenic ones (Wright, 2010). (von Wintersdorff, et al. 2016; Osodeke, & Akpan, (2018) sulfamethoxazole, tetracycline, ciprofloxacin, erythromycin, and trimethoprim have been detected in several wastewater treatment facilities discharging their treated effluents to both surface and ground waters (Karthikeyan, et al. 2006). Fecal bacteria, mainly E. coli and enterococci, released by wastewaters (treated or not) could play a key role in antibiotic resistance determinants dissemination (Baquero, et al. 2008, Martinez, 2009).

Therefore, the aim of this study was to knowing the status of the pollution in the Almeimerah plant, evaluate the quality of water in by measuring the physicochemical

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parameters and to assess antibiotic resistance patterns between diagnosed isolates during this study.

## MATERIAL AND METHODS

### Study area description

The current study was conducted Almeimerah treatment plant in AL-Hillah city, which is located in the province of Babylon, Iraq. The water source coming to the treatment plant is from the city center, from the lift station F1, the treated water in this plant is drained to the trocars and used for agricultural purpose. The sampling areas of the plant include three important sites during the water treatment process. One sampling site(S1) this includes the water entering the plant before treatment and after separating the large solid components (inlet); Second site(S2) after biological treatment; and the third site(S3) after the final sedimentation basin (outlet). Thirty-six water samples were collected from each site.

### Physicochemical parameters measurement

Field measurements were made at local sites, some physical parameters (i.e., PH, temperature, EC, TDS) were analyzed directly by multiparameter PCSTESTR35, Oakton - U.S.A in in the direct field. While chemical parameters that include;

BOD; Water samples measured for BOD5 by (DO) meter dissolved

oxygen, Oakton –U.S.A in the laboratory after dilution method according to APHA and results expressed as mg/ L.

BOD5 =Di - Df

Di = initial dissolved oxygen concentration before incubation.

Df = final dissolved oxygen concentration after incubation for (5 days) at (210C)

COD; Water samples measured for COD according to closed reflux colorimetric method clarified by(American Public health Association (APHA).(1998)., Lovibond-Germany used for final determination and expressed the units with mg/L.

### Microbial isolation and identification

Water samples were collected from three sites in sterile glass bottles (1000 mL). All samples were stored in a cooler box with ice packs, immediately transported into the laboratory, and kept at 4°C until they were analyzed within 24 hours (h) of sampling. A series of dilutions were performed for each of the collected samples (10-1-10-6) by using a sterile Spreader diffuser, 0.1 ml of each dilution was spread over the prepared culture media; (Nutrient, MacConkey, EMB agar and UTICHROM agar),he dishes were incubated at 37 ° C for 48-24 hours. Laboratory diagnosis procedure can be depended on the steps recommended by (MacFaddin, 2000). for diagnosis. To purify the resulting isolates, they were grown on Hi-crom media. Specific colonies grew in many colors, and each color indicates a specific type of bacteria. Purple colonies were counted as E.coli,mucoid and metallic blue colonies were counted as K. Pneumonia, blue green (small) colonies were counted as Enterococcus faecalis, while P. aeruginosa is colorless and greenish pigment may be observed(Perry, et al. 2007). After purification, the tubes containing brain heart infusion medium were vaccinated with a portion of the young colony of the purified isolates. The tubes were incubated at 37 ° C for 24 hours, then kept with Cleserol at a concentration of 15% and -20 ° C.

### Detection of Antibiotic Resistance among isolates

An antibiotic sensitivity test was used to determine Antibiotic resistance among sampled isolates. A total of 16 antibiotics (Biomaxima, Poland), belonging to 12 categories, were evaluated, including Penicillins: penicillin (P, 10 µg), and piperacillin (pRL, 100 µg); beta-Lactams: ceftazidime-avibactam (CAZ, 20 µg); cephem: cefotaxime (CTX, 30 µg); monobactams: aztreonam (ATM, 30 µg); carbapenems: imipenem (IMP, 10 µg), and meropenem (MEM,10 µg); aminoglycosides gentamicin (CN, 10 µg),tobramycin, (TOB10 µg), amikacin (AK, 30 µg) and streptomycin (S,10µg); fluoroquinolones: ciprofloxacin (CIP, 5 µg); tetracycline (TE, 30 µg); nitrofurans: nitrofurantoin (F, 300 µg); glycopeptides :vancomycin (V,30 µg); macrolides: erythromycin (E, 15 µg); ansamycins: rifampin (R, 5 µg). Disk diffusion method was used to determine the AR patterns among the isolates. 24 h old pure cultures were sub cultured in nutrient broth (NB; Himedia, India) and then incubated for 3 to 6 h at 37C to achieve log phase growth. Next, the turbidity was adjusted in 0.85% sterile normal saline solution to 0.5 McFarland's standard [108 (colony forming unit) CFU/mL] and aliquots were then spread on Mueller Hinton agar (MHA; Himedia, India) with a sterile cotton swab. Antibiotic disks were placed onto the MHA inoculated with the bacteria and gently pressed down to ensure complete contact with the agar, and the plates were then incubated for 24 h at 37°C. the bacterial isolates were designated as resistant, intermediate, and susceptible as recommended by the Clinical Laboratory Standards Institute .

### Statistical Analysis

Pearson correlation coefficients were used to analyse the relationships among the bacteriological water quality parameters using the Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, Version 20.0 Armonk, NY). Statistical significance was defined as $p \leq 0.05$, and Analysis of Variance (ANOVA). The significance variation of the concentration of the parameters in each sample were tested at 95% confidence levels.

**Table 1.** Mean ± SD values of physicochemical parameters and bacterial count

| Site | pH | T | EC | TDS | BOD | COD | Bacterial no. |
|---|---|---|---|---|---|---|---|
| | Mean±S.D | | | | | | |
| 1 | 8.4±0.2 | 25.9±7.1 | 978.8±169.3 | 626.432±108.352 | 182.5000±25.9 | 445.8±23.8 | 1.75×106±0.2 |
| 2 | 8.3±0.2 | 24.2±7.1 | 922.0±116.7 | 590.08±74.688 | 45.7500±2.6 | 137.2±15.7 | 0.9×106±0.1 |
| 3 | 8.4±0.1 | 24.6±6.7 | 936.8±150.2 | 599.552±96.128 | 54.2500±15.6 | 102.0±7.9 | 0.4×106±0.08 |
| Pvalue | 0.902 | 0.826 | 0.622 | 0.398* | 0.0002** | 0.0001** | 0.000** |

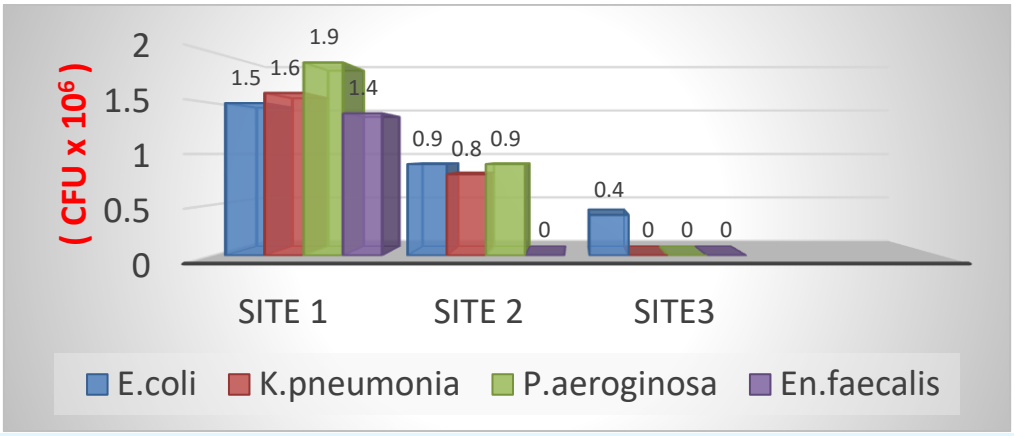


**Fig. 1.** Distribution of coliform bacteria during three sampling site

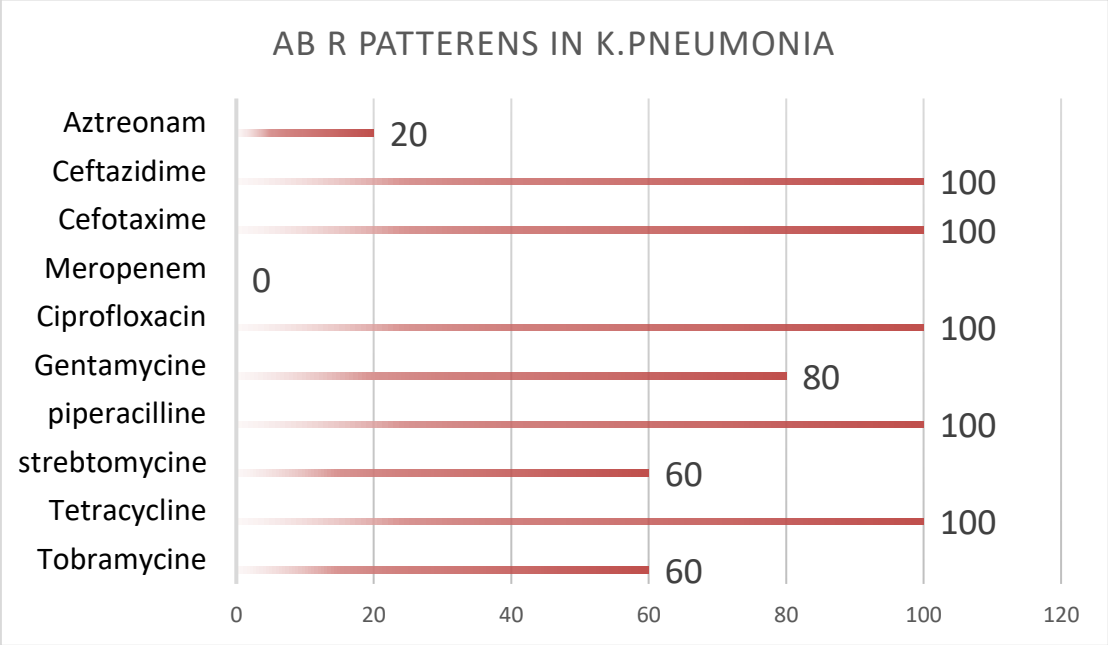


**Fig. 2.** Patterns of antibiotic resistance in klebsiella pneumonia

## RESULTS

### Distribution of physicochemical parameters

Mean and standard deviation values for physicochemical variables (PH,T, TDS,EC,COD,BOD) along all sampling sites are shown in **Table 1**.

pH: The value of pH have direct influence on other physicochemical parameters, according to Fakayode (Fakayode, 2005)., the pH of a water body is very important in assessing water quality because it affects other chemical reactions such as solubility and metal toxicity. In the present study the pH values at S1,S2and S3 are 8.4±0.2, 8.3±0.2 and 8.4±0.1 .

T: Temperature is an important and essential indicator of the fact that it affects other properties of wastewater. Temperature of the sites under study were recorded 25.9±7.1 ºC, 24.2±7.1ºC, 24.6±6.7ºC, at S1,S2 and S3 Respectively

TDS: Dissolved solids represent the amount of organic and inorganic dissolved compounds that may remain constant and thus lead to a cumulative toxic effect (Environnemental 2001). Average TDS

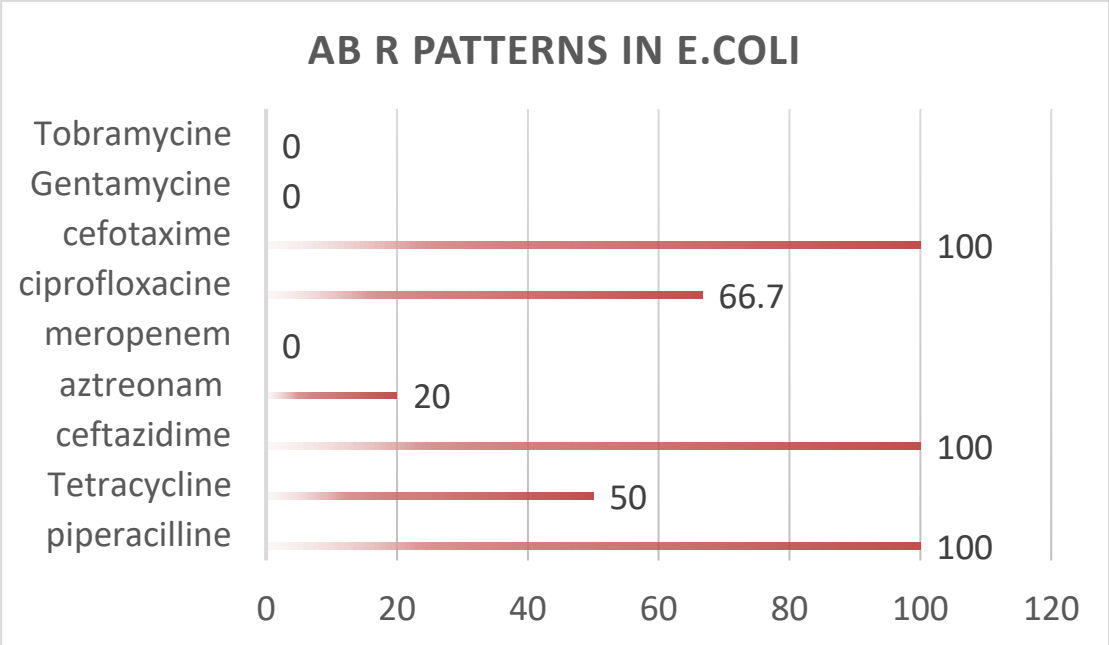


**Fig. 3.** Patterns of antibiotic resistance in Escherichia coli

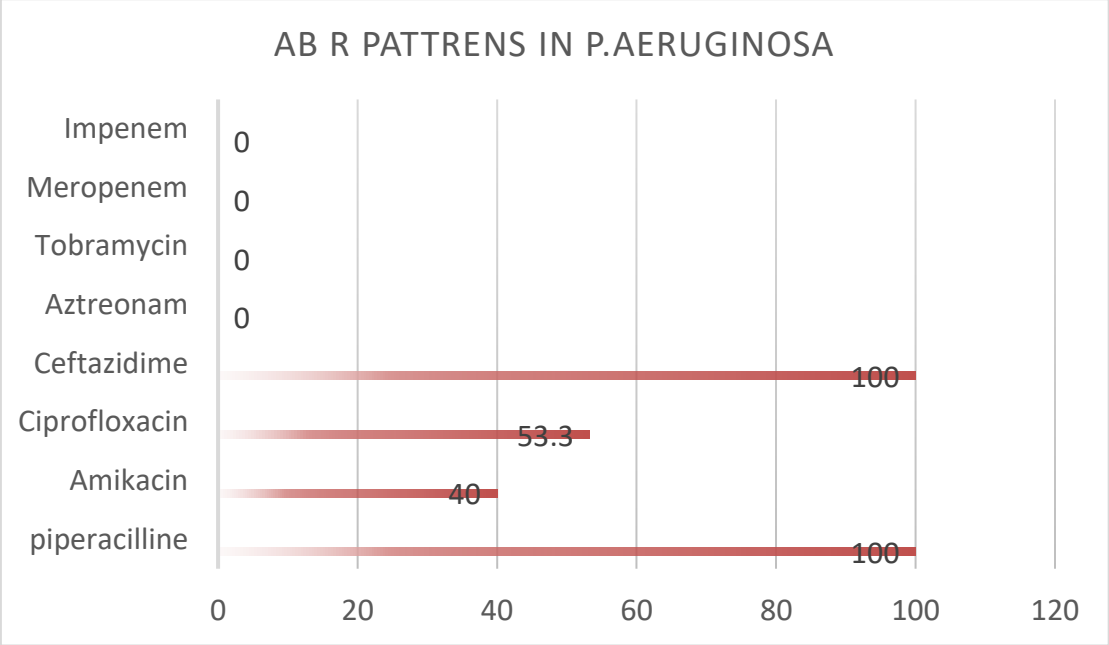


**Fig. 4.** Patterns of antibiotic resistance in pseudomonas aeruginosa

concentration at S1,S2 and S3 is 626.432±108.352 mg/L, 590.08±74.688 mg/L, 599.552±96.128 mg/L.

EC: The value of E.C usually gives an indication of the presence of dissolved ions in water such as chlorides, calcium, sulphates (Odiyo, et al. 2012). In the current study, the following values of EC were recorded 978.8±169.3µs/cm, 922.0±116.7 µs/cm, 936.8±150.2 µs/cm at S1, S2, and S3 respectively.

BOD: It is a measure of the oxygen required by microorganisms while destroying organic matter(Singh, et al. 2012). The current study show the concentration of BOD at S1,S2,and S3 is 182.5000±25.9 mg/L, 45.7500±2.6 mg/L, 54.2500±15.6 mg/L.

COD: COD is another measure of organic material contamination in water specified in mg/L. COD is the amount of dissolved oxygen required to cause chemical oxidation of the organic material in water (Patil, et al. 2012). The concentration of COD in the current study is 445.8±23.8 mg/L, 137.2±15.7 mg/L, 102.0±7.9 mg/L, at S1, S2 and S3 respectively.

**Distribution coliform bacteria**

The total coliform counts were observed in the samples collected from S1(1.75×106CFUL/mL),

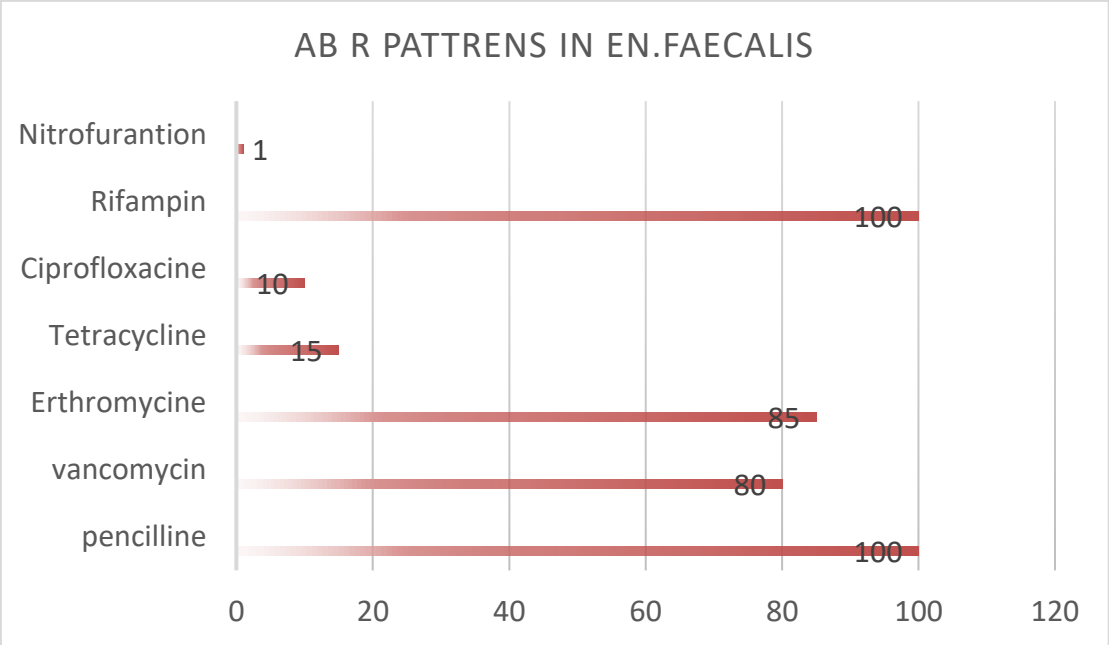


**Fig. 5.** Patterns of antibiotic resistance in Enterococcus faecalis

followed closely by S2 (0.9×106 CFUL/mL), and S3(0.4×106 CFU/mL).

**Pattern of Antibiotic Resistance**

Antibiotic resistance patterns were demonstrated for all types of the resulting isolates. The following tables show the resistance ratios of the resulting isolates for each type of antibiotic.

## DISCUSSION

Quality of water generally refers to the component of water which present at the optimum level for suitable growth of plants and animals, the quality of water is a vital concern for mankind because it is directly linked with human health.(Abdelzaher, et al. 2010).physicochemical parameters are the limiting factors for the survival of aquatic organisms (Lawson, 2011). Pollution is caused when a change in the physical,

Chemical or biological condition in the environment harmfully affects quality of human life including other animals and plants' life.(EPA. 2012). Physicochemical parameters values that shown in **Table 1** were above the advisory limits (Hailu, et al. 2018). pH value was alkalinity and the first site recorded the highest pH value and this agreement with (Hailu et al Alabaster &, Lloyd 1980). where the pH value in S1 was 8.833 ± 0.058. The higher value of pH is may be due to the usage of alkaline materials for different activities, and there is no significant difference among pH values at three sampling sites(P value is 0.902) . Temperature is an important indicator of water quality in regards to existence and survival of aquatic organisms. There is no significant difference in temperature value among sites (p value is 0.826),S1 also record the high value of temperature was 25.9±7.1

Consistent with our result, Alabaster and Lloyd (Iloms, et al. (2020). reported that temperature of natural inland waters in the tropics generally varies between 25 and 35 C. As for electrical conductivity (EC) and TDS, there was no significant change in EC in the water sampled at the different sites (p value 0.622), and S1 recorded the highest value 978.8±169.3, this result is in coincidence with (Cañedo-Argüelles, 2013).Higher values of EC in wastewater may be attributed to the presence of contaminants such as Na+, Ca2+, Mg2+, K+, Cl -, SO4, HCO3-,and other metal salts (EPA, 1996). As for TDS there was a significant difference among sites (p value is 0.398).The highest percentage of TDS was (626.432±108.352m g /L in S1. TDS values for wastewater are appropriate values for the use of water in irrigation of agricultural crops, as recorded in (EPA 1996). These results were in agreement with Wetzel (Gray, F. N. (2002). where high values of TDS indicate to increase the salts in water. COD have ahigh significant difference among sampling sites where (pvalue is 0.0001) .COD in this study was higher than BOD values, and this agreement with the previous studies (Gray, 2002). COD is higher than BOD because COD measures substances that are both chemically and biologically oxidized where ratio of COD: BOD provides a useful guide to the proportion of organic material present in wastewaters (Edokpayi, 2016)(Edokpayi, 2016). There was a high significant difference in BOD values among sites (p value is 0,0002) and this agreement with (Adefisoye, & Okoh, 2019)The release of manure effluents and sewage wastewater containing different bacterial pathogens into aquatic environments

are a leading cause of the deterioration of aquatic resources . Faecal bacteria, especially E. coli, are used as an indicator of possible pathogen presence in surface water due to its ability to persist in aquatic environments for a considerable period of time (Chitnis,V, et al. 2000). Most of the values of the microbial parameters obtained from three Al-Muaymarah treatment plant sites were above the advisory limits (Yamina, et al. 2014). The results shown in **Fig. 1** showed th at the highest percentage of contamination by gram negative bacteria and isolated from the treatment plant was in the first site(1.75×106CFUL/mL),because the water coming to the treatment plant is untreated and coming from the city center and contaminated with many microbes,and this agreement with (Baron, & Finegold, 1990). The results also showed (**Fig. 1**) that the P.aeruginosa is the most present in the treatment plant followed by the species E.coli,K. Pneumonia, E.faecalis,

Our results were in accordance with Yamina et al(Oberhofer, 1985). who found that (75%) of pathogenic wastewater bacteria were Gram negative bacteria including E. coli and K. pneumoniae. This diversity in the rates of bacterial contamination is due to the difference in physiological and genetic characteristics between the different bacterial species, as these species possess many virulence factors that increase their diseases and qualify them to be present in aquatic environments, and the most important of these factors; Production of enzymes that break down complex organic compounds and convert them into simple substances such as the production of an enzyme of carboxylase, urease, gelatinase, catalase, stratase and oxidase(Mara, 1974, Howard, 1987 Whitton, 1980. Hazen, & Toranzos, 1990). These species also have a high tolerance to various ranges of pH, temperature, and dissolved oxygen (Blaak, 2015. Rupinder, et al.2013. Abhijit, et al. 2013). The result s revealed high resistance (100%) of Gram-negative bacterial isolates to Beta-lactamase (ceftazidime) Cephem (cefotaxime), penicillin(piperacillin), while resistance to tetracycline was (50%) and resistance ciprofloxacin was(66.7%), and resistance to aztreonam was 20% and all isolates was sensitive to meropenem. Our results were consistent with several studies on antibiotic resistance among Gram-negative pathogens in wastewater (Majda, et al. 2013).,(Kritu, et al. 2013).Developing of resistance to β-lactams can be clarified as a result to carrying genes encodes for extended spectrum β-lactamases (ESBLs) like TEM-1, OXA-1, CTX-M and SHV. ESBLs genes located on bacterial chromosomes or may be exchanged among species and genus via transposable elements like plasmids. Production of extended-spectrum β-lactamases (ESBLs) is a significant resistance-mechanism that impedes the antimicrobial treatment of infections caused by Enterobacteriaceae and is a serious threat to the currently available antibiotic armory (Cornejova, et al. 2015),(Chang, et al. 2000)..Also, many studies have found that 52% of the isolates of E. coli bacteria possess first-class integrons carried on their plasmids and encode for resistance to beta-lactamase and other antibiotics(Murray,et al. 1985).

As for the resistance of penicillins in the negative types, a Gram stain is subjective to the occurrence of genetic mutations, or as a result of receiving genetic elements through the transfer of plasmids or leaping genes, and this resistance results from a change in the production of penicillin-binding proteins (PBPs) that have a low affinity for binding to β-lactams antibiotic . Ultimately, antibiotic resistance is likely to be the result of selective anthropogenic pressures imposed by the release of antibiotics and / or AR determinants present in clinical environments. Therefore, urgent care is needed to prevent inappropriate and indiscriminate use of antibiotics as medications and for other preventive purposes, especially In developing countries like Iraq, where medicines can be obtained without a prescription due to the lack of drug regulation.

## CONCLUSION

In this study, a great diversity of bacterial species was observed in the treatment plant, in addition to observing resistance patterns of many antibiotics in wastewater, which may be the main reason for the emergence of resistance genes in wastewater and the inability to use this treated water for agricultural purposes.

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