

## Evaluating the qualitative characteristics and heavy elements of hospital water and their relationship with bio resistance in *p.aeruginosa*.

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### Abstract:

Bacteria use the elements present in the environment to develop their vital defenses, trying to acquire genes from other strains or absorb heavy metals in order to adapt to them and increase their tolerance against high concentrations of heavy elements. *Pseudomonas aeruginosa* is an opportunistic bacterial pathogen that causes infections in hospitals and communities, including in humans and animals. *P. aeruginosa*'s adaptability and endurance in therapeutic settings are cause for concern. Emerging pathogenic strains pose a global threat and cause significant concern. Biocides are commonly used to control the spread of resistant strains of *P. aeruginosa*. However, tolerance to these biocides has been reported, which hinders their effectiveness in clinical settings. This study focused on the factors contributing to the persistence of hospital-acquired *P. aeruginosa*, including its resistance to antibiotics and biocides and the role of heavy metals in the development of increased bacterial resistance to antimicrobials.

**Keywords:** Bioresistance, Chemical-physical factors, NGS, Heavy metals genes, *P.aeruginosa*.

### Introduction:

The environmental situation and the environmental impacts of hospitals contamination institutions are in constant correlation with the situation her general Faye decline and the deterioration suffered by any health institution necessarily leads to her transformation. It is a serious pollutant to the surrounding environment because the waste generated by these institutions is both it was solid or liquid, usually contaminated with chemicals used naturally inside those institutions in laboratories, operating theaters and emergency lobbies, as well as those wastes be exposed to another type of pollution, which is contamination by germs and microbes (Gharaybeh, 2010). Hospitals contamination was not a medical/public health issue nor was it discussed in clinical settings. Since the 1950s, environmental medicine has been discussed more frequently through a greater awareness in public health and preventive medicine; although today, there is now a focus on occupational medicine. Environmental and occupational medicine are however more commonly viewed as an integrated subject, with emphasis

given to industrial issues (Fatiha, 2022). The unscientific and indiscriminate disposal of waste from health institutions inevitably leads to environmental pollution, the spread of epidemics and diseases, and seriously threatens life especially since we know that these institutions thanks to their residence within cities and population centers due to the urgent need for it and that there is a significant expansion in the construction of health institutions hospitals are a natural consequence of the increase in population. The waste of health institutions represents all the waste resulting from hospitals health centers, clinics, laboratories, medical research centers and medical centers anatomy is classified into several types according to their nature and also differ in the way they are processed (Shenawa et al., 2009), (Boyce, 2007) :-

**1-solid waste** :Solid waste from health institutions can be generally divided into several sections differ in their Nature, Methods of dealing with them and methods of disposal, namely :

**2- Liquid residues** : Effluents discharged from health institutions can be generally divided into several sections differ in their Nature, methods of dealing with them and methods of disposal, namely :-

**2-1- Sewage water** : Hospital sewage contains large amounts of infectious disease microbes from bacteria, viruses and worms that are easily transmitted through the water where wastewater is contaminated sanitary departments of communicable and Infectious diseases of patients with intestinal infections or during epidemics.

**2-2- Dangerous chemical liquids**: Caused by the daily sterilization and cleaning process of appliances, equipment, surfaces and floors where there are large amounts of solvents, including organic and inorganic acids and alkalis, it is discharge of general sewage from analytical laboratories and pathological laboratories without treatment.

**2-3- pharmaceutical waste** : Small amounts of medicines are discharged to the public sewers from various medical departments and these drugs may contain antibiotics and toxic drugs for the treatment of tumors (Cytotoxic Drug )and some other types.

**2-4- Radioactive liquid residues** : Small amounts of radioactive liquid residues go to sewage from the sections treatment of oncology .

**2-5- Heavy metal residue waste**: Quantities of heavy metals with high toxicity are discharged, such as Mercury and silver and bullets from dental services centers, from radiography departments, as well as from departments technical assistance in hospitals such as the movement and Mechanical Department .

**3- The impact of hospital contamination on the vital community and ecosystem** : The pollution of hospitals and the resulting dangers of medical waste or, as some call it, waste of health care services pose a danger to the environment in general and the human being as an organism lives in it in particular, as it contains a small percentage manufactured waste is dangerous, but it sometimes leads to deaths. Medical waste contains highly contained infectious waste, which in turn contains many types of pathogenic microbes by entering the human body by many means, needles may be and acute surgical machines or inhaled through the air, the category of nurses and health care providers shall be the most vulnerable group to these infections and diseases, especially since they are in the first and direct row to use these tools the means. Some of the contaminated materials for hospitals pose a danger in general to health care providers and this is in case they are not disposed of 'exposure to such substances, whether during their

preparation or treatment, by inhaling their dust, spray or ingress transdermal is a great danger to the health of these may lead to their death. such substances are capable of killing human cells and causing deformations in addition to causing dermatitis, headache, nausea (Fatiha,2022). Medical waste affects the environment especially if it is not disposed of or we do not deal with it in the way correct and proper, whether in collecting, transporting or disposing of it, to cause pollution in this way this is due to the dumping and backfilling of waste in deep wells or in underground complexes as in the Clark Fork Complex of Montana State (Al-Maghrabi, 2001) . Medical waste also poses a danger to the environment by containing water coming from health care providers have significant amounts of chemicals that are distributed through networks sewage, such as Mercury and cadmium, which contribute to the contamination of sludge in the sewage treatment plant health (Shehata,2000) . This waste also poses a danger to the air, as the emission of toxic smoke and dust from incinerator chimneys and waste chimneys contribute to air pollution due to the amount of carbon gas and carbon monoxide produced( Co<sub>2</sub>. Co<sub>3</sub>) . Therefore, in general, medical waste poses a danger and a direct impact on human health, especially on health care providers in public and private institutions also threaten the environment by contributing significantly to pollution soil, groundwater and air, either because they are not discharged properly or in the process of disposal in by themselves (Fatiha,2022).

#### 4-Heavy metals Genes

Heavy metals are natural elements of the Earth's crust. They cannot decompose or break down to a small extent, they enter our body through drinking water, food and air. It is true that trace elements , some heavy metals (such as copper , selenium, zinc ) are necessary to maintain the metabolism of the human body. However, its increased concentration leads to intoxication. Examples of heavy metal poisoning include lead contamination of drinking water from lead pipes, high ambient air concentrations near emission sources, or via the food chain(Azeez Namuq, 2020). These metals are considered one of the most dangerous pollutants, as most of them have the character of accumulation, where they accumulate in the bodies of aquatic animals such as fish, birds and plants and reach humans by eating these foods, and they also do not have the ability to decompose or chemical or bacteriological breakdown in the environment when they collect in the bodies of living organisms. Particularly bacteria are negatively impacted by heavy metals. When metal concentrations are higher than the organism's minimal inhibitory concentration, their most noticeable consequence is the disintegration of cell membranes; additionally, metals frequently interact with other parameters including pH and temperature (Al-Khafaji,2008). Heavy metals bind to thiol groups in proteins and enzymes when they are present in organisms, particularly positively charged ions with big atomic weights like cadmium and mercury. Furthermore, they have the ability to bind to phosphate or hydroxyl groups in DNA, which modifies the structure of the protein. The malfunction of nucleic acids, etc. B. Single-strand breaks due to Cd's nonspecific binding to DNA (Fashola et al.2016). Ionic interactions allow heavy metal ions to disrupt and even destroy similarly charged alkali metal ions. Ionic interactions allow heavy metal ions to disrupt similarly charged alkali metal ions and cause their destruction. Alkali metals are vital to bacterial cells because they bind and replace enzymes with zinc and Cd. To suppress enzyme activity, cadmium can take the role of calcium and magnesium (Johansen, et al.2018). Heavy metals' interaction with the glutathione complex, which stops it from doing its function, is another impact. Furthermore, oxidative phosphorylation and membrane permeability are impacted by heavy metals. (Mendez et al. 2019). Uncontrolled industrial and agricultural wastewater discharge exposes bacteria to heavy metals present in the environment, resulting

in the formation of metal-resistant cells and bacterial mutations (Al-Musharafi, 2016 and Masindi, 2018). The acquisition of extrinsic variables is associated with microbial resistance to heavy metals in the context of survival. Antimicrobial resistance genes have been shown to be linked to integrin and class 1 gene cassettes in wastewater treatment plant samples (Goss et al. 2018 and Kotlarska, et al. 2015). This suggests that some genes are being selected for at the same time. Heavy metal resistance can develop in bacteria through a number of mechanisms, such as precipitation, active transport through efflux pumps, extracellular permeability barriers, and intercellular sequestration or storage (Prabhakaran, et al. 2016). A study was detected that genes *copA*, *copB*, *czcA*, and *merA*, which encode resistance to metal in *P. aeruginosa*. These genes are responsible about the pan-resistance operon in the *Pseudomonas aeruginosa* (Muslim and Al-Saadi, 2023). Ferripyoverdine receptor A (*fpvA*); Receptor for the siderophore ferripyoverdine, located in cell outer membrane of *P. aeruginosa* (Mridha and Kümmerli, 2022). Tributyltin (TBT) resistance gene PA0320; homologous to YgiW. PA0320 plays a certain role in stress tolerance against TBT as well as in stressors producing reactive oxygen species (Fukushima, et al. 2012). Chromate ( $\text{CrO}_4$ ) transport protein (*ChrA*) Responsible for the inducibility of the resistance (Branco, et al. 2008). Copper-containing azurin-like protein (*cinA*) *CinA* is similar to the *P. fluorescens* DF57 *Cot* protein which is involved in copper tolerance; *cinA* Influences the ability of *Pseudomonas aeruginosa* PAO1 to resist the bactericidal effect of copper (Grace, et al. 2022) Based on these considerations, the present study aimed evaluation of the impact of changes in chemical physical factors and heavy metals on the bioresistance of bacteria in some hospitals of Iraq.

## Materials and Methods

### 1. Environmental sampling

The current study was conducted on two public hospitals and the study included taking environmental samples for the hospital, where the chemical physical factors of wastewater in the hospitals were measured for three important sites and three repetitions of each examination for the purpose of controlling the quality of the results and the samples were transferred directly to the laboratory to complete obtaining the results. The tests included air temperature (AT), water temperature (WT), pH, electrical conductivity (EC), dissolved oxygen (DO), total dissolved sediment (TDS), Biological oxygen demand 5 (BOD5), turbidity (Turb), total hardness (TH), magnesium (Mg), calcium (Ca), sulfate ion ( $\text{SO}_4$ ), phosphate ion ( $\text{PO}_4$ ), chloride (Cl), in addition to heavy metals such as cadmium (Cd), chromium (Cr), zinc (Zn), lead (Pb), silver (Ag), copper (Cu).

### 2. Microbiological Sampling

Bacterial samples were taken from the same sites from which environmental samples were taken by swabs and transported by transporting media to the laboratory and cultivated on selective media for the growth of *Pseudomonas aeruginosa* such as the *macConkey* medium and the *Pseudomonas chromogenic Agar* medium. After obtaining pure isolates, they were diagnosed by the Vitek compact 2 device to confirm the diagnosis of isolates and test their sensitivity to antibiotics.

### 3. NGS Analysis

The NGS technique was used to obtain the whole genome sequence of XDR-resistant bacterial isolates isolated from the same sites mentioned above. Antibiotic and heavy metal resistance genes have been identified in some isolates.

**Results and Discussion**

The results of o-Chemicalphysical factors showed a clear discrepancy among the values through the Duncan test as shown in Table 1, which represents the least significant range of the arithmetic averages of the values of Chemicalphysical factors .

Table 1: The difference in the average of some chemicalphysical variables for the study sites in hospitals.

Hospital	sites	AT	WT	pH	EC	DO	TDS	BOD5	Turb	T H	Mg	Ca	SO4	PO4	Cl
H1	S1	13.5 a	15.67 a	8.267 A	1256 c	9.467 a,b	688.33 d	3.040 a	3.067 d	436.7 c	55.67 c	112.0 and	317.67 c	0.203 c	165.0 And
	S2	12.0 B	13.67 b	7.667 B	1760 a	8.500 a,b	1070.0 a	0.890 c	8.233 A	630.0 a	66.33a	157.7 a	377.00 A	0.440 a	310.0 A
	S3	11.6 c	12.83 c	7.033 c	1420 b	7.000 B	978.33 b	0.687 d	6.300 B	565.0 b	61.00 B	140.0 b	341.00 B	0.340 b	285.7 b
H2	S1	12.0 B	14.30 b	8.600 A	1050 and	10.200 a,b	595.00 F	2.400 b	4.400 C	352.5 and	47.00 D	122.0 d	232.5. D	0.185 c	303.0 A
	S2	11.5 c	13.95 B	7.650 C	1350 b,c	11.100 A	640.00 And	0.775 c,d	3.050 D	397.5 d	44.50 And	131.5 c	205.00 And	0.128 d	272.0 C
	S3	10.1 d	12.90 c	6.700 c	1325 c,d	8.850 a,b	795.00 c	0.350 and	2.600 and	439.0 c	34.50 f	128.0 c	232.50 d	0.197 c	192.0 d

The results of heavy metals showed a clear discrepancy through their presence in the wastewater coming out of the hospitals under study, where differences in their levels were recorded, ranging from their absence to pollution levels representing the threshold limit of toxicity . In table 2, a comparison was made between the levels of heavy metals in the sites for the two hospitals mentioned It was found that there was a similar relationship between most of the tests at the sites, as the levels of both cobalt and chromium increased at the final estuary of the treatment unit in the second hospital, while the value of cobalt decreased at the first sites of both hospitals, and the value of chromium increased at the first site of the second hospital (Zhang,et al.2016). This result may be due to the cumulative effect of cobalt contamination. Because the second hospital operates with a system of underground tanks into which the hospital’s liquid treatment units are poured, while in the first hospital, cobalt levels decreased at the final outfall of hospital waste. On the contrary, chromium levels increased(Kumari,et al.2020) .

Table 2: The difference in the average of some heavy metals variables for the study sites in hospitals.

Hospital	sites	Cd	Cr	Zn	Pb	At the	With
H1	S1	0.0367d	0.5533a	0.1251c	0.1258d	0.0013d	0.0813c
	S2	0.0384c	0.5465a	0.2057b,c	0.2377a	0.00617b	0.198a
	S3	0.0269e	0.4370b	0.1511c	0.1651c	0.00537b	0.0955b
H2	S1	0.0207f	0.4183b	0.3940a,b	0.11975d	0.0105a	0.053d
	S2	0.2035b	0.3389c	0.5975a	0.0990e	0.0062b	0.036e
	S3	0.3785a	0.59975a	0.2100b,c	0.2215b	0.0026c	0.0875c

It also showed a clear decrease in silver levels in both hospitals and fluctuating levels of copper and zinc, and this can be represented illustratively as in Figure 1.

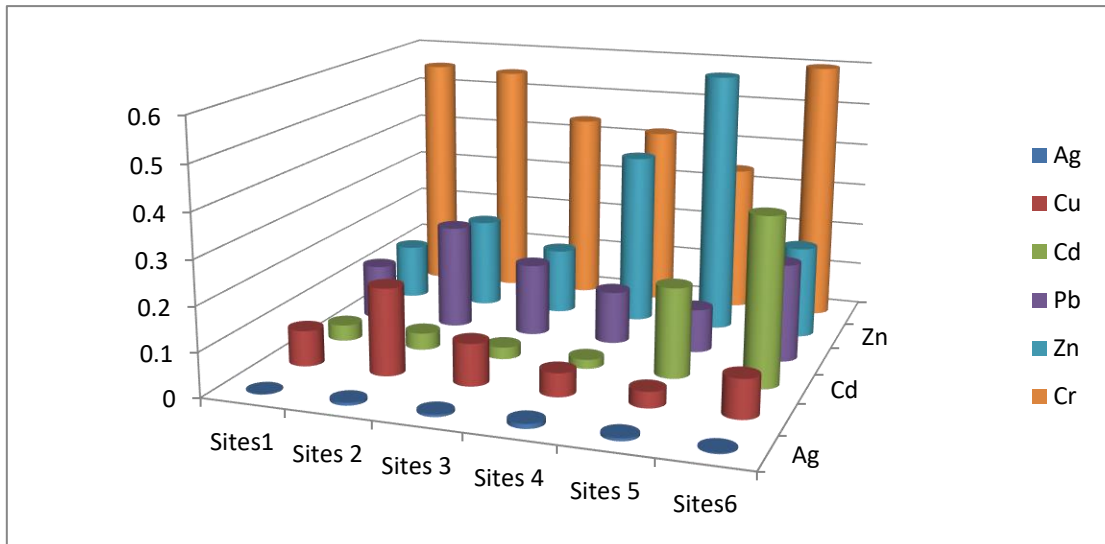


Figure 1: The levels of Heavy Metals in the two hospitals

Based on the above values of some factors and after analyzing the results, it was found that there is a direct relationship between both the temperature of air and water, as well as pH levels, DO and BOD5, as shown in diagram (1).

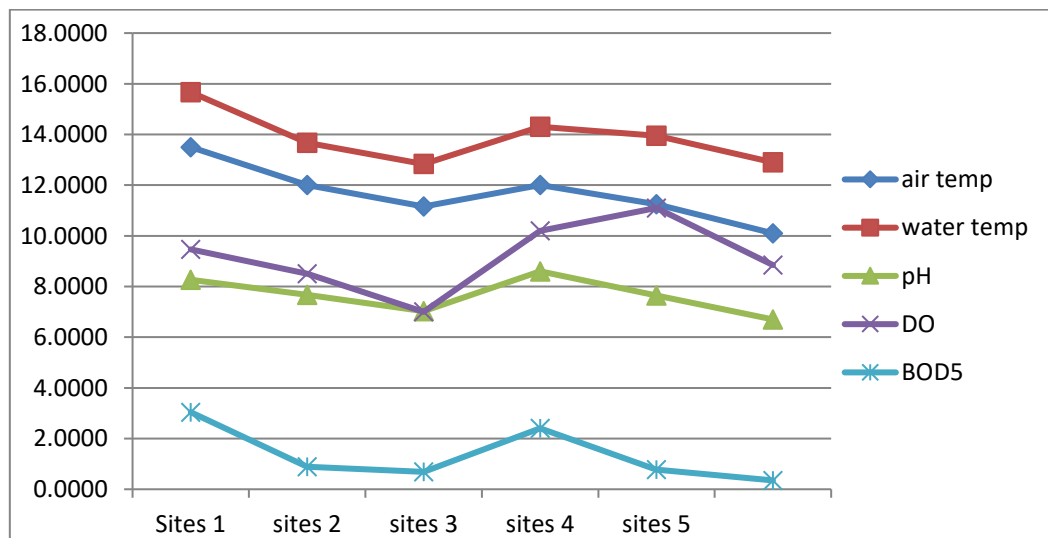


Diagram 1 : Direct relationships Among BOD5,DO,pH,WT and AT in the sites of hospitals

The increase in air temperatures is attributed to the increase in water temperature by a small difference, and this is confirmed by previous studies (Rice, *et al* 2015) and the current study agrees with it, if we note through Scheme 1 that the temperature index of water rises relatively with the air, which is a logical explanation for what is happening in the open environment . We also note the direct relationship between

both PH and the biological oxygen demand<sup>5</sup> and dissolved oxygen, as it is known according to previous studies that the biological oxygen demand<sup>5</sup> decreases the lower the PH due to the decomposition of organic substances and the increased acidity of the medium, but the change in oxygen solubility should decrease according to previous studies in open aquatic environments, perhaps due to the presence of some types of cyanobacteria or other algae that produce oxygen and thus its quantity increases in a direct relationship with PH and BOD<sup>5</sup> (Yaqin Guo et al 2019).

The current study showed that dissolved salts such as magnesium, calcium, phosphates, sulfates and chlorides are directly proportional to the total hardness, and this result is consistent with previous studies ( Dey *et al.* 2024) despite the changes in closed environments, some variables have maintained the environmental constant of some kind, as in diagram 2

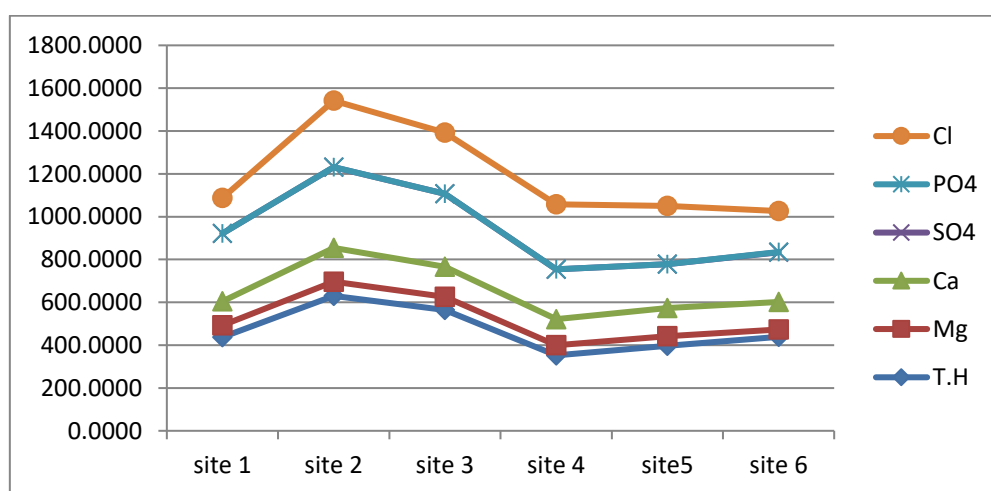


Diagram 2 : Relationship among TH ,Mg,Ca,SO4,PO4 and Cl in the sites of hospitals .

After obtaining bacterial isolates of *Pseudomonas aeruginosa* and accurately diagnosing them by the Vitek device, the isolates resistant to antibiotics and disinfectants used in both hospitals were sorted, where one of the isolates with excessive resistance was selected and its DNA was extracted and then subjected to the protocol adopted in the NGS technique for total genome sequencing . Bioinformatics algorithms have been used to identify resistance genes in *Pseudomonas aeruginosa* . The analysis showed that the bacterial genome contains genes resistant to some heavy metals that are present in the same medium from which these bacteria are isolated, It were (recG, ruvA, ruvB, ruvC, fptA, fpvA, fpvB, fpvR, fpvI, mexI) . These genes have been identified in previous studies as genes resistant to high concentrations of heavy metals acquired by bacteria either through jumping genes or as a result of continuous exposure to these metals, bacteria develop a certain mechanism to produce virulence factors resistant to these metals . This isolation was registered on the website of the University of Oxford and was marked with its own (St 4426) and registered in the name of the researcher through the website and the following link

[https://pubmlst.org/bigsgdb?page=profileInfo&db=pubmlst\\_paeruginosa\\_seqdef&scheme\\_id=1&profile\\_id=4426](https://pubmlst.org/bigsgdb?page=profileInfo&db=pubmlst_paeruginosa_seqdef&scheme_id=1&profile_id=4426)

Bacteria use a mechanism called adaptive resistance to momentarily strengthen their resistance to the effects of drugs and other stresses. It entails modifications in the expression of genes and proteins in

response to external stimuli. But as the surrounding circumstances improve, this kind of resistance is typically reversible (Pang, et al.2019).

Biocides are effective against both active and dormant bacteria due to their multitarget antimicrobial action, which is not affected by metabolic state (Fernandes et al.2022). Excessive and incorrect use of biocides might lead to tolerance or even antimicrobial cross-resistance (Capita et al.2014). Biocides' effectiveness can be impacted by a variety of factors, including chemical properties like concentration, pH, and composition, as well as environmental conditions like temperature, organic matter, and contact time (Gerald, et al.2022). Bacteria's susceptibility to biocides can be influenced by their antibiotic resistance features, whether inherent or acquired. Disinfectants, unlike antibiotics, target non-specific processes or locations within bacterial cells, making them highly effective. *P. aeruginosa* has multiple antimicrobial tolerance mechanisms, and overexpression of efflux pumps is often linked to this problem ( Rozman, et al.2021). Research has shown efflux-pump-mediated resistance to many biocides, including as phenolic chemicals, cationic biocides( ElDein, et al.2021) alkylating agents (Charlebois, et al.2017) and oxidizing compounds ( Martin, et al.2008). The mexAB-oprM, mexCD-oprJ, and mexEF-oprN genes in *P. aeruginosa* are some of the most researched efflux pump regulators linked to biocide tolerance (Morita, et al.2003). The qac genes, particularly qacE and qacED1, found in plasmids and integrons, have been linked to resistance to biocides, particularly quaternary ammonium compounds, in *P. aeruginosa* strains from clinical and environmental settings( Namaki, et al.2021). Through the acquisition of heavy metal genes by bacteria, it is possible to activate adaptive resistance that can survive in harsh environments even if the environmental conditions are not suitable for living (Moradali, et al.2017).. Scientists are concerned about the potential link between biocide tolerance and antibiotic resistance ( Martin, et al.2008). Surface disinfection failures may result in the spread of microorganisms resistant to antibiotics and disinfectants in healthcare settings. Co-resistance, where biocide tolerance and antibiotic resistance genes are on the same mobile genetic element or cross-resistance, where the same mechanism is responsible for both antibiotic resistance and biocide tolerance, such as efflux pumps and changes in outer membrane permeability (Chen, et al.2021), can cause this issue. *P. aeruginosa*'s continuous exposure to subinhibitory concentrations of benzalkonium chloride, as demonstrated in 2010 by (Mc Cay et al.2010), increased the bacterium's tolerance to the chemical agent but also significantly increased its resistance to fluoroquinolones through mutations in gyrA. ( Tandukar et al.2013) also showed that this exposure is linked to increased resistance to other clinically relevant antibiotics, like tetracycline and penicillin . Overall, selective pressure brought on by repeatedly exposing bacteria to biocides at subinhibitory concentrations appears to contribute to the emergence of antibiotic resistance, though more investigation is required to fully comprehend its implications for the development of co- and cross-resistance (Jones and Joshi,2021).

## Conclusion

*P. aeruginosa* is a very robust pathogen that can create new survival strategies in addition to having a broad range of innate defensive mechanisms against external aggressions. *P. aeruginosa* appears to be able to flourish in the unfavorable conditions found in hospital settings because of the virulence factors it expresses, which appear to contribute not only to its pathogenicity but also to its high ability to adapt to various external aggressors, such as antibiotics and biocides, and to colonize inert materials. This bacterium poses a serious threat to public health because to its well-known multi-resistant character against a variety of antibiotics, including last-generation medications. *P.aeruginosa* has also been observed to exhibit biocide tolerance and possibly co- and cross-resistance to antibiotics. The

aforementioned behaviors appear to be associated with the ongoing exposure to subinhibitory doses of biocides, a common occurrence stemming from their routine usage in various contexts such as hospital, industrial, and residential settings. All of these factors encourage the selection of extremely resistant strains, which will proliferate in the environment and subsequently spread to other hosts. In order to better understand *P. aeruginosa's* resistance mechanisms and, as a result, create novel management techniques against this bacterium with the goal of limiting its dispersion in hospital environments, it is imperative that scientific study in this subject be continued.

**Statistical Analysis**

All data were analyzed with Statistical Package for Social Science software (SPSS; IBM Corp., Armonk, New York, US; version 25). The t test was used to compare median values of chemicalphysical factors between two hospitals as shown in table (4.A,B)

**Table 4.A : The difference between the arithmetic mean and the T-value calculated to indicate the differences in some chemical-physical qualities in the first and second hospital.**

	AT	WT	pH	EC	DO	TDS	BOD <sub>5</sub>	Turb	T.H	Mg	Ca	SO4	PO4	Cl
H1	12.22 20	14.05 60	7.65 60	1478.88 90	8.322 0	912.22 20	1.53 89	5.86 67	543.88 89	61.00 00	136.55 56	345.22 22	0.32 78	253.5 56
H2	11.11 67	13.71 67	7.65 00	1241.66 67	10.05 00	676.66 67	1.17 50	3.35 00	396.33 33	42.00 00	127.16 67	223.33 33	0.17 02	255.6 67
t-value	2.447	0.685	0.01 6	2.638	- 1.780	3.592	0.74 2	3.12 9	4.690	7.621	1.362	11.548	4.28 8	- 0.076
P-value	0.026	0.503	0.98 7	0.018	0.094	0.002	0.46 9	0.00 6	0.000	0.000	0.192	0.000	0.00 1	0.941

Table 4.A : The difference between the arithmetic mean and the T-value calculated to indicate the differences in some heavy metals qualities in the first and second hospital.

	Cd	Cr	Zn	Pb	At the	With
H1	0.031719	0.513456	0.160667	0.176178	0.004278	0.124956
H2	0.200900	0.452317	0.400500	0.146750	0.006433	0.058833
t-value	-3.274	1.364	-3.124	1.167	-1.560	3.316
P-value	0.005	0.192	0.007	0.260	0.138	0.004

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