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Assessment of environmental factors in expression of heavy metal resistance genes in clinical Pseudomonas aeruginosa isolates associated with urinary tract infections

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Introduction: Environment heavy metals; Cadmium (Cd), Zinc (Zn), Cobalt (Co), Copper (Cu), Chromium (Cr) and Nickel (Ni); have emerged as critical problem for aquatic ecosystems and human health. These are responsible for bladder cancer, cardiovascular illnesses, nervous system and renal disorders due to their continuous intake (Renu et al. 2021; Coetzee et al. 2020). Each metal has a minimum threshold of toxicity due to their bioaccumulation and lack of degradability after becoming a part of food chain (Ahamad et al. 2020) through different resources, and have the ability to remain in the environment for extended periods of time (Chen et al. 2021). Humans utilize cadmium (Cd) in the form of $CdCl_2$; it is highly absorbable and involved in the impairment of the urinary system causing damage to kidney and liver dysfunction (Gasparrini et al. 2020). Nickle (Ni) is carcinogenic when expose to higher concentrations (Yousif et al. 2021). Industrial waste and sewage water are linked with Cu and Zn contaminations. Zinc (Zn) is an essential trace element in humans and is an important cofactor for many enzymes; Zn deficiency causes complications in pregnancy and develops chronic disease. However, it also becomes toxic when the concentration exceeds the permissible concentration by utilizing heavy metals contaminated water (Pavic Vulinovic et al. 2026; Yousif et al. 2021). Cu is essential for hemoglobin synthesis and impaired delivery causes anemia, neutropenia, and bone disease. An increased concentration of Cu due to contaminated water causes lethal diseases, including renal damage (Yousif et al. 2021). Chromium (Cr) is an essential element that is found in sludge, solid waste and contaminate water. Cr is important to reduce body fat and to improve lean body mass. However, an increased concentration has shown harmful effects on human health (Georgaki et al. 2023).

Development of antibiotics and heavy metal resistant genes and their co-resistance are leading cause for more frequent prevalence of UTIs (cystitis, urethritis, pyelonephritis) are making it difficult to treat. Inherent biochemical and structural traits, physiological and genetic adaptation, including morphological alterations and environmental changes to metal speciation are the properties that help microbes to survive in contaminated environment (Turner et al. 2020). Microorganisms are important agents in determining the distribution of metals in the environment and adopts a variety of mechanisms such as metal efflux, intracellular sequestration, and modification of plasmid genes by introducing metal resistance from one cell to another and developing metal resistance (Kumari et al. 2025). Several bacteria such as *Pseudomonas aeruginosa, Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* etc. are found to carry heavy metal resistance genes like *copA, czcA, merA, ncc* and *chrR*. *Pseudomonas aeruginosa*; a non-spore-forming, motile, non-fermenting, gram-negative, facultative anaerobic *Pseudomonadaceae* bacteria. Strains of pseudomonas have shown distinct and notable tolerance against heavy metals (Derakhshan et al. 2020). Therefore, it is crucial to identify the level of

heavy metals to avoid any lethal effects on human health and maintain a permissible level of concentration. The purpose of the current study was to investigate the heavy metal resistance genes and their tolerance in *P. aeruginosa* isolated from UTI samples which is a biomarker for heavy metal accumulation in human body and environment.

Methodology

Study Region

This study was conducted in different regions of Balochistan (Quetta, Muslim Bagh, Zhob, Kalat, Harnai, Qilla Saifullah, and Khuzdar).

Urine and Water Sampling from UTI Patients

Total 200 urine samples were collected and stored at -20°C. After proper labeling, the samples were transferred to Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta for further analyses. 21 water and 21 urine samples; three (3) from each region were used for heavy metal analyses at Central Hitech Laboratory, University of Agriculture, Faisalabad.

Confirmation of Urinary Tract Infections

Keeping in view the clinical signs, physician opinion, scrutinizing samples by urine strip test (Multistix) and Cystine Lactose Electrolyte Deficient (CLED) agar plates (Oxoid, United Kingdom); suspected UTI samples were separated.

Isolation of *P. aeruginosa*

After separation of suspected urine samples; these were cultured at 37°C for 24 hours in brain heart infusion (BHI) broth. These inoculates were streaked and re-streaked each time for 24 hours at 37°C on citramide agar (Oxoid, UK) plates until pure colonies of *P. aeruginosa* were obtained. Gram staining and colony morphology were done for classification, characterization and differentiation of *P. aeruginosa.* These pure colonies were used for further molecular analyses.

DNA Extraction and PCR

The DNA of the bacteria was extracted by Gene JET Genomic DNA purification kit with its lot no. 3208365, manufactured by Thermo-fisher Scientific, Baltics, UAB, Lithuania as per their guidelines. PCR based molecular gene detection of *P. aeruginosa* and different heavy metal resistance genes were amplified through specific marker *16SrRNA, copA, czcA, merA, ncc* and *chrR* gene (Table 1) used PCR based specific forward and

reverse primers under optimal conditions (Table 2). All reactions for these genes were carried out in 25µL volumes with forward and reverse primers 1µL each, Master mix 10µL, PCR grade water 9µL and DNA template 4µL.

The amplicons were separated by electrophoresis; *16S rRNA* on 1.2% agarose gel, *copA, merA* and *ncc* genes (1.5%), whereas, 1.8% agarose gel was used for *czcA* and *chrR* in 1X-TAE buffer at 80–100 V for 35-45 minutes. DNA bands of *P. aeruginosa 16SrRNA*, *copA, czcA, merA, ncc* and *chrR* were visualized under ultraviolet (UV) light using a UV trans illuminator. Product sizes were determined using the GeneDireX 100-bp DNA ladder RTU Cat No. DM001-R500 as a size marker.

Determination of Heavy Metal Tolerance in *P. aeruginosa*

To find out the susceptibility of heavy metals (copper, nickel, zinc, cobalt, cadmium, chromium and mercury) in *P. aeruginosa* cultures; Kirby-Bauer disc diffusion technique was performed using Mueller- Hinton (MHA) Agar (37 °C for 24 h) using 0.5 McFarland as standard. 1 molar (1M) stock solution of metal salts ($CdCl_2$, $NiCl_2$, $Co(NO_3)_2$, $ZnCl_2$, $Cr_2(NO_3)_2$ and $CuC1_2$) were prepared and was serially diluted (1/2) to get inhibition zones on 5 mm discs. The zone of inhibition was measured from the center of the discs with the help of ruler to the circumference of the zone where a distinct edge was present (Kashif et al. 2026).

Heavy Metals Digestion in Urine Samples

The heavy metals in urine samples were digested using a microwave digestion system (MDS-6G, Sineo) equipped with a Teflon-coated chamber and 8-position carousel. Each sample was prepared by adding 4.0 mL urine + 2.0 mL concentrated nitric acid (HNO_3) into poly-tetra-fluoro-ethylene/per-fluoro-alkoxy-alkane (PTFE/PFA) digestion vials. Vials were tightly sealed and placed inside high-pressure microwave digestion vessels (maximum pressure up to 1.4 Mega Pascal). Digestion was performed using a stepwise heating program: 10 min at 630 W (100% power) and 10 min at 60% power. After digestion, the material was cooled down and final volume was adjusted to an appropriate volume with de-ionized water. The material was filtered with Whatman-42 filter paper and stored in labeled plastic bottles.

Heavy Metal Digestion in Water

Digestion of the heavy metals from water samples of different study regions was conducted with little modifications (Akan 2008) briefly illustrating 5 ml of concentrated hydrochloric acid (HCl) and nitric acid (HNO_3) each were added to a 100 ml of water sample. After incubation at room temperature (25°C) for an hour; samples were then heated at 60°C on a hot plate. The beaker was cool down and another 5ml of concentrated acids

(in equal amount 2.5 ml each) was added. After that samples were heated again until samples turned clear and light in color. Beaker was washed with distilled water from all around the walls and filtered (Whatman-41) to remove any insoluble material. Then deionized water was added upto 100ml of total volume. The samples were analyzed in triplicate for heavy metals determination using deionized water as blank during the whole experiment by atomic absorption spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan). The deionized water of analytical grade used in the experiment was prepared at Hitachi lab. UAF, Faisalabad. Operational conditions and standard (Applichem®) preparations (as per company guidelines) employed in Atomic Absorption Spectrophotometer for determination of heavy metals (AOAC 1990) are provided in supplementary table.

Statistical Analysis

The data collected regarding heavy metal concentrations, pH, total digestible solvents (TDS), and electric conductivity (EC) were tabulated by using Microsoft excel-16. One-way analysis of variance (ANOVA) was used for comparison of the mean of heavy metal concentrations in different regions whereas, Duncan's Multiple range (DMR) test was applied by using SPSS-22 for statistical analyses; at level significant $p<0.05$. Pearson correlation coefficient was used between heavy metal concentration in water and urine.

Results

25 isolates of *P. aeruginosa* were detected from 200 urine samples. The number of positive UTI samples from Quetta, Muslim Bagh, Zhob, Kalat, Harnai, Qilla Saifullah and Khuzdar is provided in table 3.

Minimum Inhibitory Concentration (MIC) of Heavy Metal:

The heavy metal tolerance by *P. aeruginosa* isolates collected from urine samples with minimum inhibitory concentration (MIC) is shown in table 6.

Minimum inhibitory concentration (MIC) of heavy metal tolerance in bacterial isolates from urine samples was detected (Table 4). Interestingly, *P. aeruginosa* showed susceptibility against Cd, Co, Zn, Cr at concentration at 0.0156M, Ni 0.0078M, and Cu 0.0313M. Below these concentrations; no zones were observed. Among all these metals; Ni showed highest inhibitory zones (6.00±1.00mm) whereas, Cu showed lowest values (5.67±1.53mm) among isolates.

Heavy metal concentration analysis of water and urine in different regions of Balochistan

21 water samples from each selected Balochistan region (in triplicates) were used for the detection of heavy metal concentration of Cd, Zn, Cr, Cu, Co, and Ni. Statistical analyses of heavy metals concentrations of

water (Table.5[a]) in different regions of study area showed significant difference in concentrations of heavy metals among various parts of the province. The highest levels of Cd (0.95±0.020), Zn (0.93±0.004), Cu (0.86±0.025), Cr (0.97±0.002), Co (0.68±0.005) and Ni (0.52±0.039) ppm were found in Harnai, Kalat, Muslim Bagh, Quetta, Khuzdar and Qilla Saifullah respectively; these concentrations of heavy metals were also beyond the permissible limits of WHO (Anesthesia et al., 2010).

21 urine samples from each selected Balochistan region (in triplicates) were used for the detection of heavy metal concentration of Cd, Zn, Cr, Cu, Co, and Ni. Statistical analyses of heavy metals concentrations of urine (Table.5[b]) in different regions of study area showed significant difference in concentrations of heavy metals among various parts of the province. The highest concentration (ppm) of Cd (0.26±0.018), Zn (0.79±0.002), Cr (0.64±0.009), Cu (0.45±0.016), Co (0.50±0.035) and Ni (0.41±0.021) were found in Harnai, Kalat, Muslim Bagh, Quetta in Khuzdar in Qilla Saifullah respectively.

Correlation of Different Heavy Metals from Urine and Water Samples

Pearson correlation coefficient was used between heavy metal concentration in water (ppm) and urine (ppm). Overall the results indicate that the concentrations of Cd, Zn, Cr, Cu, Co and Ni in water were significantly correlated with their corresponding urinary concentrations. Significant positive correlation was observed in urine and water heavy metal concentrations (Table.5[c]). It was found that there was a moderate positive correlation between CdU vs CdW ($r = 0.457$, $p<0.05$*) and CoU vs CoW ($r = 0.518$, $p<0.05$*), a very strong positive correlation was observed between ZnU vs ZnW ($r = 0.898$, $p<0.01$**), CrU vs CrW ($r = 0.889$, $p<0.01$**) and CuU vs CuW ($r = 0.906$, $p<0.01$**). Whereas, NiU demonstrated strongly positive correlation with NiW ($r = 0.664$, $p<0.01$**).

Comparison of Different Quality Variables of Water Samples in Different Regions of Balochistan

Statistical analyses of different quality variables of water in different regions of study area showed significant difference ($p<0.05$) in pH, TDS, EC among various parts of the province. The highest levels of pH (8.13±0.18) was noted in Muslim Bagh, whereas, TDS (697.67±9.39) and EC (900.67±19.47) were found in Harnai (Table.6a). Pearson correlation coefficient results indicate that the concentrations of these variables in water were significantly $p<0.01$ positive correlated (Table.6[b]).

Molecular Identification

DNA was extracted by kit method, run on agarose gel electrophoresis and were visualized under gel documentation system (Figure 1). Frequency distribution (Table.7) and molecular identification of *P. aeruginosa* (*16SrRNA*) at 926bp (Figure 2) and other heavy metal resistance genes *copA* amplified at 475bp (Figure 3), *czcA* 320bp (Figure 4), *merA* 431bp (Figure 5), *ncc* 457bp (Figure 6) and *chrR* 350bp (Figure 7) under optimized PCR

Discussion

Metals categorization; essential metals (Zn, Fe, Cu, Co, Ni, Cr, and Mo) and non-essential metals (Pb, Hg, Cd, Al, and As) are used for studying toxicity, different biological mechanism and evaluating their harmful effects on human health (Sfakianakis et al. 2015). Excessive use of agricultural pesticides and contaminated drain water discharged from houses are engulfed by human through food chain or drinking water and are biologically accumulated in the body ultimately rising metal concentration in kidneys, liver, urinary bladder etc. leading to gastrointestinal tract (GIT), urinary tract infections (UTI) and skin problems (Wang et al. 2021).

(Satarug 2025; Xie et al. 2025) found Cd (highly toxic non-essential metal) as highly sensitive biomarker for renal injuries which is associated with distressing kidney functions and increase susceptibility to UTI. Zn is required for immune regulation, wound healing and tissue repair etc. During an infection, the availability of Zn is limited due to restriction developed in bacteria, which alters the Zn metabolism and increased concentration of Zn causing nausea, vomiting, diarrhea, kidney disease and UTIs. WHO recommends use of Zn in daily food at permissible level i.e. 5 ppm (Ahmad et al. 2023; Sharma et al. 2025; Satarug 2025). Cr is involved in maintaining normal glucose, protein and lipid metabolisms by enhancing insulin and improving glucose consumption. Continuous exposure of Cr shows a toxic effect on human health especially in kidneys and liver (Singh et al. 2022; Okechukwu et al. 2026). Cu plays an important role in antimicrobial defense mechanism. During infections body secrete more Copper-binding proteins (ceruloplasmin) in urine and its exposure affects the biofilm formation and virulence (Huang et al., 2024). Elevated Cu levels are responsible for crystallization/struvite (infection related stones) in bladder and kidneys at low pH; whereas lower Cu level causes anemia (Prywer et al. 2026). Co is essential component of vitamin B12 and plays a vital role in DNA synthesis, neurological function, erythropoiesis and requiring continuous monitoring because of its potential effects on kidney function and human health. Elevated level of Co is associated with cellular toxicity, renal damage, and alter the urinary microenvironment increasing the susceptibility to UTIs (Liu et al. 2010). Ni; an essential micronutrient, act as a cofactor for specific enzyme (urease) that converts urea into ammonia increasing urine pH creating toxicity and promote the formation of kidney stones (Maier & Benoit 2019). Ni is absorbed by the skin and is up taken through the blood these

bacteria control intracellular Ni via efflux system. Ni resistance may enhance bacterial survival in urinary system along with use of long term catherization (Kiran et al. 2021).

Urinary tract infections (UTIs) remained a significant health problem, particularly due to growing prevalence of multi drug resistant bacteria (Pipit et al. 2025). In previous studies *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus* were reported as leading bacteria responsible for this infection (Abed et al. 2025). Among them *P. aeruginosa,* a significant opportunistic uropathogen, having major clinical importance due to its intrinsic resistance mechanisms and ability to acquire heavy metal resistance genes (Saad et al. 2020). *P. aeruginosa* being prominent resistant bacteria showed resistance, to the diversity of heavy metals, that is why it has been extensively studied under the metals stress conditions due to the presence of HMRGs.

In the current study, UTI were found positive in 19.38% isolates for *P. aeruginosa*. whereas (Khatoon et al. 2023; Ahmad et al. 2022; Pirkani et al. 2020; Shah et al. 2015) also reported 12.70%, 10.3%, 7.53% and 5.4% respectively in different region of Pakistan. Little variations in existence of *P. aeruginosa* may be attributed to differ in population, sample size, geographical location and environmental exposure.

In the present study heavy metal tolerance in *P. aeruginosa* isolates from urine samples was detected with minimum inhibitory concentration (MICs) for Cd, Co, Zn, Cr at 0.0156M, whereas MIC for Ni and Cu was detected 0.0078M and 0.0313M. Similarly, (Hassan et al. 2008) reported MICs for Cu, Co, Ni, Zn, Cr and Cd were 6.3, 5.9, 6.8, 9.2, 5.8 and 4.4mM respectively. These outcomes also support the findings of recent studies. Similar studies reported MIC for Cu, Cr, Co, Cd and Zn as 1.2mM, 1.2mM, 1.4mM, 1.5mM and 1.5mM (Hassen et al. 1998). Whereas, other researchers determined MIC of Cd, Co, Ni, Zn, Cr and Cu as 6mM, 2mM, 3mM, 15mM, 2mM and 9mM (Dziewit et al. 2015). (Teitzel & Parsek 2003) found MIC of Cu and Zn as 2mM and 8mM respectively using micro titer method.

In this study, 21 water and 21 urine samples (in triplicates) were collected from seven different regions of Balochistan that were used for the detection of heavy metal concentrations. Present study revealed that concentration (ppm) of heavy metals Cd, Zn, Cr, Cu, Co and Ni in water and urine were found significantly different $p<0.05$, $p<0.01$ the highest in Harnai, Kalat, Muslim Bagh, Quetta, Khuzdar and Qilla Saifullah respectively. Furthermore, heavy metal concentrations (ppm) were different in different regions of Balochistan and mostly beyond the permissible heavy metal concentrations limits of WHO in drinking water. It was also found

that water and urine heavy metal concentrations were moderate positive to very strong positive correlated with correlation coefficient (r) ranging +0.40 to +0.59 and +0.80 to +1.00 respectively.

In this study, level of heavy metals were increased in water as well as in urine simultaneously, the highest concentration of Cd in water and urine was detected in Harnai. Similarly, (Mustafa et al. 2017; Ullah and Muhammad 2020) reported elevated Cd concentrations in drinking water, soil and plants from jhal magsi, Zhob and Loralai; another part of Balochistan province. (Khanam et al. 2020; Vacchi-suzzi et al. 2018) also reported urinary Cd levels in healthy adults; however, urinary biomonitoring in Pakistan have also confirmed detectable Cd in human urine indicating environmental exposure of this metal within the population. The highest level of Zn was noted in Kalat among the study regions. (Cybulski et al. 2022; Mustafa 2017) determined higher concentration of Zn, Whereas; other researchers detected Zn concentrations in water samples below the permissible level (Mergia et al. 2025; H. Rehman et al. 2025). Similar findings have been reported by (Moore et al. 2018) found low urinary Zn concentration strongly influenced by renal excretion, nutritional habits, regional differences and varies within Pakistani population; conversely elevated urinary Zn occur during infection, inflammation, oxidative stress and alter urinary functions. (Salih, Jaafar et al. 2013) found the impact Zn in urine which also effects the woman fertility. While studying Co concentration in different areas of Balochistan, higher concentration was found in Khuzdar. Similarly, (Ahmad et al. 2023; Chandio et al. 2021; Bradham et al. 2023) reported Cr, Pb, Cu, Co, Zn, As and Ni concentrations exceeded the allowable limits of the WHO standards these differences of metal concentrations might be due to different metal rich areas, human exposure, mining and industrial activities, these findings were in line with the outcomes of current studies. Contrary to current findings (Ayub & Ahmad 2020) also conducted a study in different areas of Balochistan and found low Co level reason behind this contradiction might be difference in study regions. (Kettelarij et al. 2018; Moore et al. 2018) reported Co as a urinary biomarker and increased level of Co exposure through inhalation or dermal contact results in elevated Co concentration causing UTI. Cu concentration in drinking water was higher according to WHO, whereas; higher concentration of Cu was recorded in Quetta in study area. These results are in accord with the findings of other studies that were conducted in India and Nigeria; reported Cu concentration in drinking water (Ayenimo & Adeloju 2016; Sharma et al. 2020; Mustafa 2017; Cybulski et al. 2022; Mergia et al. 2025). Urinary Cu has been associated with nephrotoxicity, inflammatory responses, increased urinary excretion may also indicate enhances renal elimination in UTI (Kuo et al. 2022; Schmied et al. 2021). The highest water and urinary level of Ni was noted in Qilla Saifullah under study area. Similarly, Ni was found at lower levels in water indicating environmental exposure as a potential source of Ni (Cybulski et al. 2022; Mergia et al. 2025; Moradnia et al. 2024). Urinary Ni has been

linked to nephrotoxicity, inflammatory reactions and higher urine excretion might also suggested to enhanced renal elimination in UTI, reported in healthy population, supporting its use as a biomarker of Ni exposure (Kuo et al. 2022; Schmied et al. 2021). During this study, the highest level of Cr was noted in the water and urine samples of Muslim Bagh simultaneously. Similarly, (Chandio et al. 2021) reported elevated Cr concentration in drinking water of Muslim Bagh, highlighting substantial environmental Cr contamination in the region. (Khanam et al. 2020; Yanan et al. 2025) reported significant Cr exposure in drinking water that were associated with increased Cr level in urine among populations of floodplains.

In the present study significant positive correlation was observed heavy metal concentrations. It was found that there was a moderate positive correlation between water and urine Cd and Co metal concentrations; Whereas, a very strong positive correlation of water and urine heavy metals Zn, Cr, Ni and Cu was also detected. In this study, it was also revealed that different concentrations of heavy metals are found in different regions of Balochistan. Similarly, (Farkhondeh et al. 2021) demonstrated the relationship between heavy metals in drinking water and chronic kidney diseases, exposure to chromium, cadmium, lead and arsenic through drinking water and incidence of kidney problems. These findings are in line with the results of this study. Negative (-ive) correlation was found between different metals and glomerular filtration rate. Moreover, positive association was observed between chromium, cadmium and arsenic exposure and urinary N-acetyl-β-D-glucosaminidase concentrations and kidney injury. Other researchers, (Islam et al. 2023) reported that higher levels of different heavy metals in ecosystem and give them more exposure to these metalloids. Despite finding no correlations among age and gender they found associations with heavy metal concentration in drinking water and urine along with the type of jobs they were doing. These findings also validate the outcomes of current studies. Further studies conducted by (Rehman et al. 2018) are also in agreement with existing studies which explored that the potential toxic elements through drinking water is responsible for their bioaccumulation in hair, nail, urine and blood. Pearson's correlation analyses of this data revealed strong positive correlation to daily water intake and urine.

In the current study statistical analyses of different quality variables of water in different regions of study area showed significant difference (p<0.05) in pH, TDS, EC among various parts of the province. Study revealed considerable regional variations in physicochemical characteristics of water samples such as pH, Total digestible solvents (TDS) and Electric conductivity **(**EC) among various regions of Balochistan. The highest pH of water (8.13±0.18) was noted in Muslim Bagh, pH values were within the WHO-recommended range of 6.5-8.5 for drinking water. (Mohammad 2020) reported pH 7.38 in Quetta. Current studies revealed TDS levels as 697.67±9.39 (mg/L) were found in Harnai. Similar findings were reported by (Mohammad 2020; Oyem et al.

2014) in ground water. In this study, the highest level of EC (900.67±19.47) was found in Harnai. Similarly, EC for different ground drinking water samples were recorded 784 and 14.46 µs/cm (Mohammad 2020; Oyem et al. 2014). In the present study, TDS-EC ($r = 0.942$, $p<0.01$) indicated strong positive correlation between them. These finding are in consistent with (Butt et al. 2026; Talib et al. 2019; Saalidong et al. 2022) who also reported strong positive correlation ($r = 0.98$), ($r = 0.995$) and ($r = 0.963$) in ground water. Contrast to the findings of present studies, (Ullah et al. 2022) reported very weak positive association ($r = 0.178$), this difference may be due to variations in geological characteristics, ground water chemistry and dissolved ionic composition among studied region.

Current study was conducted on urine samples of 200 suspected UTI patients, out of these 129/200 (64.5%) were found positive for UTI through CLED and Multistix test whereas, 25/129 (19.38%) isolates were found positive for *P. aeruginosa*. This research validated presence of heavy metal resistance genes *copA, czcA* (Cd, Zn, Co), *merA, chrR* and *ncc* (Ni, Cd, Co) in *P. aeruginosa* from urine samples of UTI. Several heavy metal resistant genes (*copA, czcA, merA, ncc* and *chrR*) resist the bacteria against different heavy metals (Ture et al. 2018). *P. aeruginosa* co-express HMRGs and ARGs that can exert selective pressure in favorable bacterial strains carrying HMRGs and there coexistence makes UTI more difficult to treat (Gillieatt & Coleman 2024). (Hrbacek et al. 2025) found *P. aeruginosa* retains copper resistance system by developing *copA* gene to survive in Cu induced clinical isolates stress coexisting with antibiotic resistance genes. (Darwiche et al. 2025) demonstrated synergistic toxicity of Cu and Ni due to disturbing bacterial metabolism, their findings suggested that metal exposure may influence bacterial physiology and development of metal stress. *P. aeruginosa* isolated from UTI can tolerate Cr through resistance genes *(chrR*) these gene help bacteria to survive in contaminated environment by reducing intracellular Cr toxicity (Okechukwu et al. 2026).

In this study, *copA, czcA, merA, ncc* and *chrR* heavy metal resistance genes were also detected in 88%, 84%, 52%, 28% and 32% in *P. aeruginosa* isolates respectively. These findings were in accord with the research conducted by (Muslim & Al-Saadi et al. 2023) who also detected *czcA* (100%), and *copA* (85.7%) from clinical samples supporting that Cd, Zn, Co, Cu resistance is highly conserved; whereas, *merA* (40%) was reported as low conserved among clinical *P. aeruginosa* isolates. For *ncc* and *chrR* limited data was available on prevalence of these genes in UTI isolates. Similar to the outcomes of present research, *P. aeruginosa* reported *copA* (100%) and *merA* (77%) as highly conserved resistant genes for hospitals and environmental isolates (Deredjian et al. 2011). The findings of (Ture et al. 2021; Malik et al. 2023; Hossain et al. 2022) found *copA* (46.2%, 9% and 33.3%), *czcA* (35.8%, 36.3 and 51.5%), *ncc* (17.9% and 36.3%), *chrR* (18%) and *merA* (21.2%) isolated from fish

and mussel in coliform bacteria also validated the outcomes of current studies. Hence, increased level of nickel and cobalt significantly affected presence of *ncc* gene in bacteria. In other studies *copA, czcA, ncc* and *merA* genes were identified ranging from 1.20% to 32.53% from fish (Ture et al. 2018).

Conclusion

From this study it was concluded that different regions of Balochistan contain noticeable higher levels of heavy metals, TDS, and EC in water that ultimately enhanced urinary heavy metals reflecting an association in human UTI. Moreover, susceptibility of these metals to *P. aeruginosa* was also recorded. Determination of heavy metal resistance genes in urine samples is due to increased contaminations of water, food and environment that may adversely affect human health.

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Assessment of environmental factors in expression of heavy metal resistance genes in clinical *Pseudomonas aeruginosa* isolates associated with urinary tract infections

Figures

conditions (Table 2) and visualized under UV.

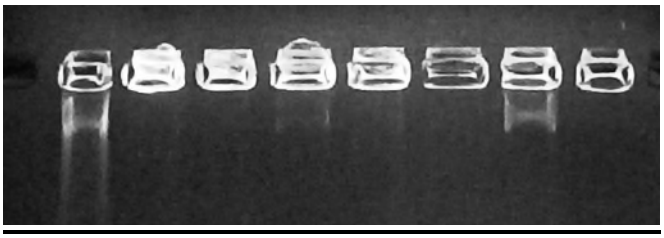


Figure 1. Extraction of genomic DNA from *P. aeruginosa* isolates

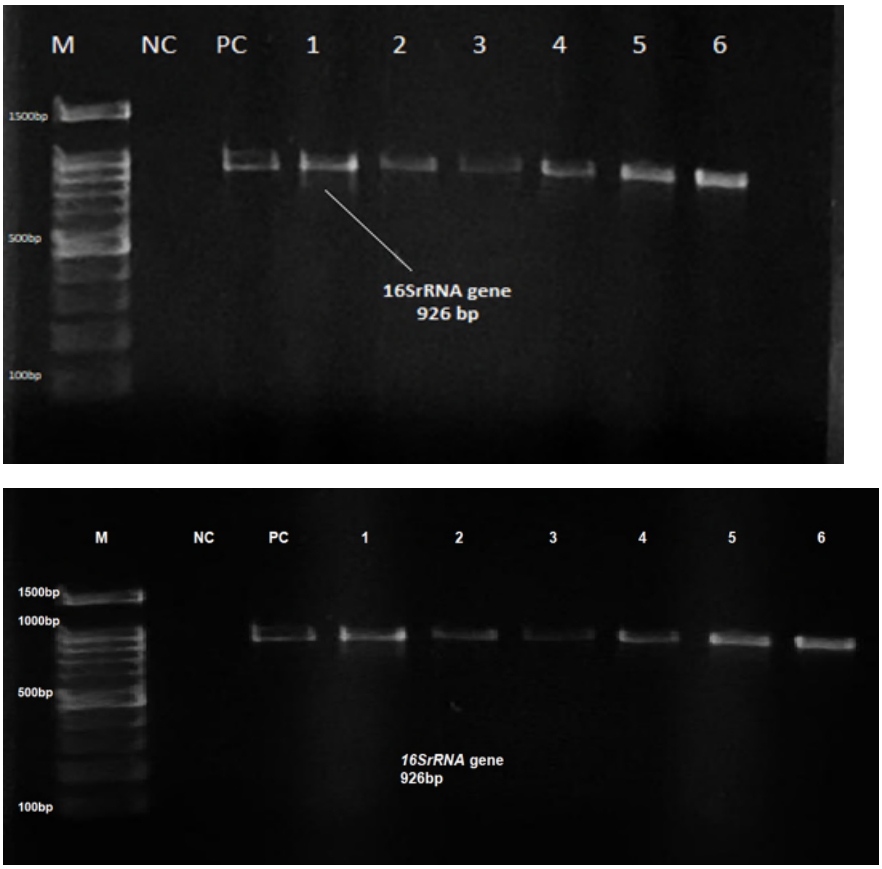


Figure 2. *P. aeruginosa 16SrRNA* amplification at 926 bp, using 1.2% agarose gel in 1X TAE buffer. A size marker (GeneDireX 100-bp DNA ladder RTU Cat No. DM001-R500) was used for the size difference. M ladder, PC positive control, NC negative control

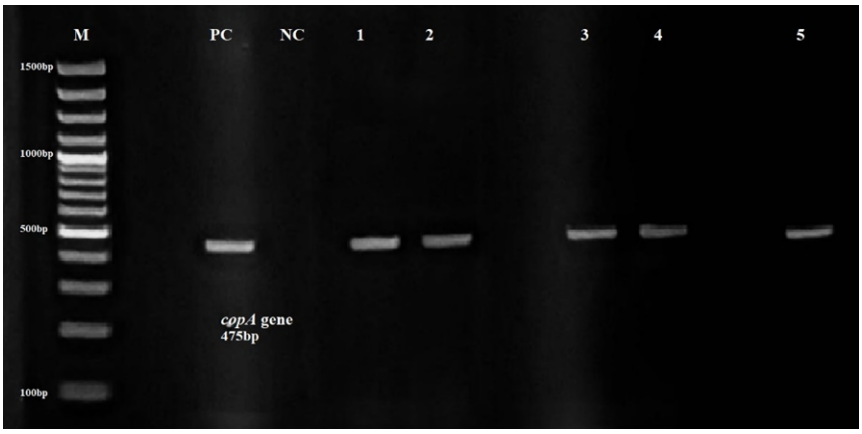


Figure 3. Heavy metal resistance gene amplification *copA* at 475 bp, using 1.5% agarose gel in 1X TAE buffer. A size marker (GeneDireX 100-bp DNA ladder RTU Cat No. DM001-R500) was used for the size difference. M ladder, PC positive control, NC negative control

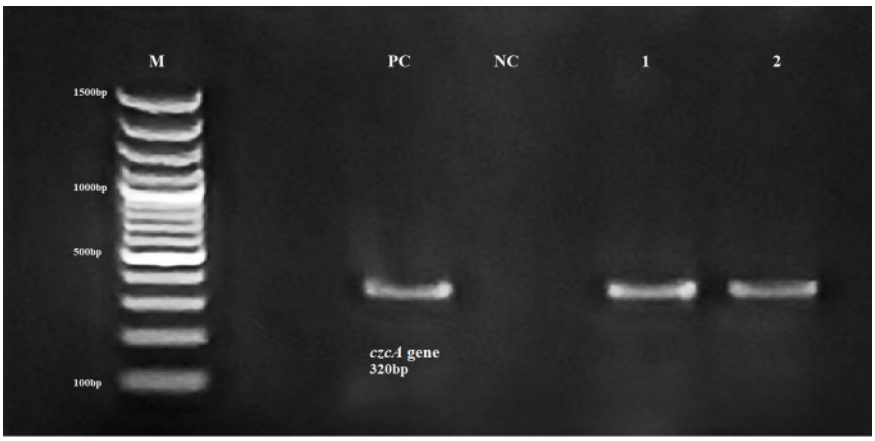


Figure 4. Heavy metal resistance gene amplification *czcA* at 320 bp, using 1.8% agarose gel in 1X TAE buffer. A size marker (GeneDireX 100-bp DNA ladder RTU Cat No. DM001-R500) was used for the size difference. M ladder, PC positive control, NC negative control

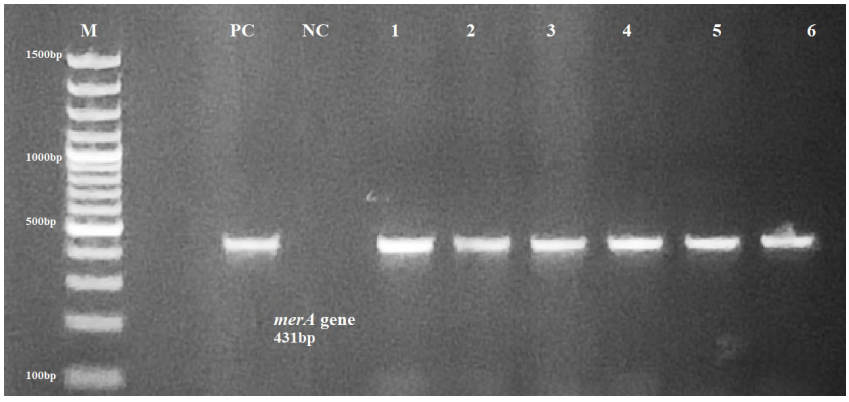


Figure 5. Heavy metal resistance gene amplification *merA* at 431bp, using 1.5% agarose gel in 1X TAE buffer. A size marker (GeneDireX 100-bp DNA ladder RTU Cat No. DM001-R500) was used for the size difference. M ladder, PC positive control, NC negative control

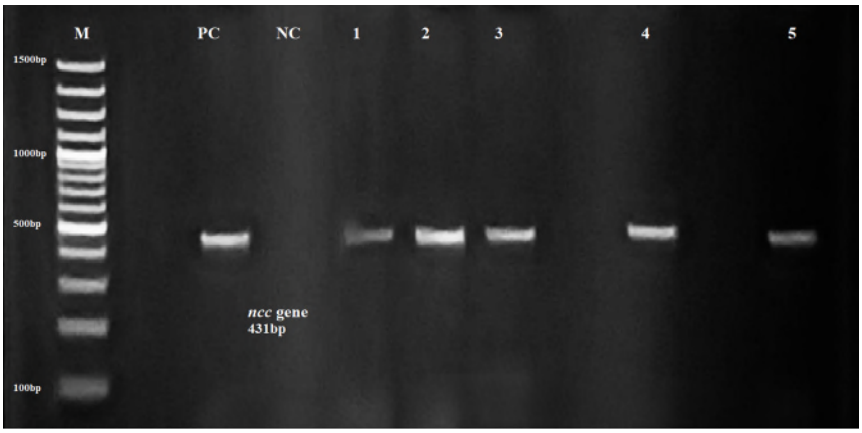


Figure 6. Heavy metal resistance gene amplification *ncc* at 457 bp, using 1.5% agarose gel in 1X TAE buffer. A size marker (GeneDireX 100-bp DNA ladder RTU Cat No. DM001-R500) was used for the size difference. M ladder, PC positive control, NC negative control

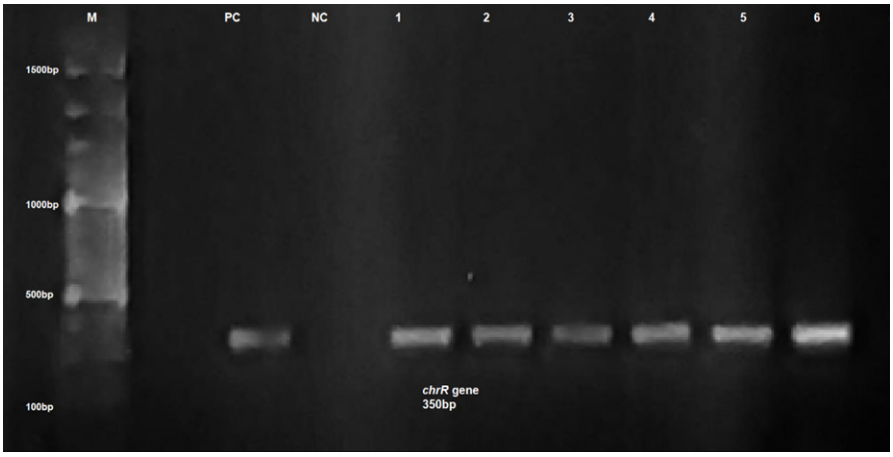


Figure 7. Heavy metal resistance gene amplification *chrR* at 350bp, using 1.8% agarose gel in 1X TAE buffer. A size marker (GeneDireX 100-bp DNA ladder RTU Cat No. DM001-R500) was used for the size difference. M ladder, PC positive control, NC negative control

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