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# Antibiotic Resistance, Heavy Metals and Health Risks in Peshawar's Potable Water: An Integrated Contamination Assessment

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Article Details

**Keywords**: Antibiotic Resistance, Heavy Metals, MPN, COD, BOD, MDR, XDR, E. coli, pH, Biochemical assays

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ABSTRACT

District Peshawar, the capital of Khyber Pakhtunkhwa Province, Pakistan, faces critical challenges in ensuring safe drinking water due to rapid urbanization, inadequate sanitation infrastructure, and anthropogenic pollution. This study employs a comprehensive physicochemical and bacteriological assessment of potable water sources in District Peshawar, Pakistan. Water samples were collected from groundwater, surface sources and municipal supplies (including school systems) and were analyzed for physicochemical parameters (pH, turbidity, TDS, fluoride, heavy metals) via atomic absorption spectrometry and bacteriological indicators (total coliforms, fecal coliforms, E. coli) using Most Probable Number (MPN) and membrane filtration methods. Spatial contamination patterns were mapped via GIS-based interpolation. These Results revealed severe heavy metal pollution, with cadmium (Cd), lead (Pb) and chromium (Cr) exceeding WHO permissible limits, while copper (Cu) and zinc (Zn) remained within thresholds. Bacteriological analysis identified fecal contamination in 44–48% of samples, with nine multidrug-resistant (MDR) bacterial species isolated, though no extensively drug-resistant (XDR) strains were detected, potentially reflecting maintained sanitary conditions in institutional supplies. Physicochemical anomalies included fluoride exceeded (31% of samples >1.5 mg/L), turbidity fluctuations (5–10 NTU), and episodic TDS breaches, while biochemical oxygen demand (BOD) and chemical oxygen demand (COD) consistently remained within acceptable ranges. Contamination hotspots correlated with industrial zones and dense settlements, linking directly to elevated incidences of waterborne diseases and chronic heavy metal toxicity risks. Urgent interventions are necessary, including infrastructure upgrades, point-of-use purification, industrial effluent regulation, and GIS-enabled monitoring systems to align with Sustainable Development Goal targets. Further studies are recommended for better insight.

## 1. INTRODUCTION

Most important natural resource on earth is water. Water is essential for all living organisms, ecological systems, food production and economic development (Postel et al., 1996). Water is the most essential component for human body. About 73 % of the earth surface is cover with water (Ali et al., 2011). After oxygen water is the second important factor of life (Ali et al., 2012).Depend upon the size of human body, the body is composed of 55 % - 78%of water. The percentage in different parts of body are- brain has 90 % of water, blood has 83%, bones 22 % and muscular tissues have 75 % of water (Chandrima et al., 2015). Safe drinking water is required for all living organisms. Availability of fresh drinking water result in significant benefits to human health (Cabral, 2010).The drinking water quality has a strong effect on community health (Tamungang et al., 2016). So, it is essential to monitor drinking water quality regularly as it target person fitness (Hamid et al., 2013). So, physico-chemical and bacteriological investigation of drinking water are important to safe the welfare of the human and permit the practice of a defensive approach to manage potable water (Tamungang et al., 2016). Physical parameter like appearance can be observed with naked eye. Taste may develop due to storage and transportation results from the activity of microorganisms. Odour can also affect the drinking water quality. The color appearance in water is due the metals such as copper, iron and manganese (Tamungang et al., 2016).Different physicochemical parameters are used to check the quality of potable water but bacteriological analysis are also important because due to the presence of enteric pathogens health of an individual is affected directly. Bacteriological analysis has primary importance to control the dangerous diseases. At different administrative levels water health policy are develop through decision makers (Tyagi et al., 2015). Fecal Coliform and Enterococci is the bacterial indicators of fecal pollution. Water quality in developing countries are not safe due to limited resources of water treatment and distribution and poor water supply (Ali et al., 2011).The indicator of fecal contamination is fecal coliform and are usually used to show the microbiological quality of drinking water and also to estimate disease exposure (Abera et al., 2011). Biofilms in drinking water is responsible for the growth of different disease causing microbes, including Legionella spp. and Mycobacterium avium complex (MAC). Type of disinfectant, water temperature, residual concentration, degree of pipe corrosion and biodegradable organic carbon level are some factors that affect the growth of bacteria in biofilm (Ojo et al., 2007). Approximately 80 % deaths and diseases are related to water borne diseases like hepatitis A and cholera (Tyagi et al., 2015). Potable water from different sources like ground water or surface water mainly, through fecal matter pathogens get enter in to human body and infect him and also transmitted to others (Kanth et al.,). Drinking water can be cleaned by physical method like filtration and chemically by chlorination and reverse osmosis. Biochemical test like Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) are performed to check the water quality. Chemical Oxygen Demand is the amount of oxygen required to oxidize organic and inorganic matter by microorganism. The higher oxygen demands means higher amount of pollution in water (Alam et al., 2015). Khyber Pakhtunkhwa is the populated province of Pakistan. Due to less economic progress it is hard to supply 100 % clean water to the community. Mostly the drinking water is supplied through local ground water and municipal tap water (Amir et al., 2015).To test the quality of drinking water the recent study was conducted. Because the drinking water quality have the strong impact on the health of these living organisms (1). Many physicochemical parameters were used to check the water quality. Bacteriological parameters are also important to overcome waterborne diseases (2).The indicator bacteria of fecal contamination are Coliform and Enterococci (3). Presence of indicator bacteria at certain level in drinking water is consider polluted or bacteriologically contaminated (4).Approximately 80% of deaths and diseases in developing countries are caused by contaminated water (5).Majority of community in developing countries have no access to clean water for drinking(6).Some important elements can be taken through water by human being in small amount but sometime the absence of proper amount in water cause mortality, reduce growth and mutagenic effects (3).Heavy metals are poisonous and toxic in nature and having higher density. These heavy metals were consumed by human being through polluted air, food and water. High amount of lead and arsenic affect kidney endocrine damage, skin lesion, affect nervous system and reproductive disorders (7).About 44% of Pakistanis do

not have approach to clean water (PCRWR2005-2006).Physical methods through which drinking water can be cleaned include reverse osmosis, chlorination and filtration. To check the water quality, BOD and COD were determined (8).The ratio for waste water and raw water for BOD/COD is higher than 0.5(9) Children have weak immune system due to which bacteria causes infection in them easily (10).The industrial toxic chemicals pharmaceuticals and insects repellents is also one of the main cause of water pollution (3).As compared to the adults children are more exposed to these toxicants (11). This study employs a comprehensive physicochemical and bacteriological assessment of potable water sources in District Peshawar, Pakistan.

**1.1. Research Question:**

- How bacterial contaminations of potable water associated with seasonal variability and water source (e.g. groundwater versus surface water)?
- What are the long term health consequences of contained exposure to the concentrations of the identified heavy metals in drinking water?
- What are the optimum water treatment methods to remove both microbial contaminants and heavy metals without affecting water quality parameters?

**1.2. Microbial Contamination Monitoring:**

This work provides a general overview of bacterial contamination of drinking water, coliform and some pathogenic bacteria detection, which is necessary for water safety management and public health monitoring.

**Antibiotic Resistance Screening:**

Also this work determines the antibiotic resistance of the bacterial isolates ,which helps in an understanding of the occurrence of multi-drug resistant bacteria in sources of drinking water . This contributes to global concerns regarding antimicrobial resistance.

**Heavy Metal Contamination and Risk Assessment:**

Through the analysis of the level of heavy metal concentration(Cd , Pb,Cr ,Cu ,Zn) and comparison against WHO and EPA standards , this research contributes to environmental science in that it marks the points that need interventions for water treatment.

## MATERIALS AND METHODS

**2.1 .Sample collection**

Water samples were collected from different schools of Khyber Pakhtunkhwa at District Peshawar. The source of water sampling was tape water or storage pots. About 50 samples were collected from different schools in sterilized 100ml bottle. The samples were labeled with appropriate code after collection and were immediately transported to Microbiology Laboratory of Abasyn University Peshawar.

**1.2. Sample processing:**

After water samples collection different physical parameters were employed initially to analyze the physical parameters like taste, smell, color, turbidity and pH.

**1.3. Physical parameters**

pH (-log of $H^+$) was determined with pH meter. Turbidity was reported through turbidity meter. While the color and taste were determine with the sense of sight and taste respectively.

**1.4. Biological parameter:**

MPN was performed according to standard protocol of (Chu, 1992).This three steps test includes, (A) Presumptive test, (B) Confirmation test and (C) Complete test

**1.5. Presumptive test:**

Lactose Broth media was prepared and autoclave media and test tubes along with Durham tubes at 121°C and 15 Psi for 15 minutes. Total 10 ml of sample was added to 3 tubes of double-strength of lactose broth media and placed Durham tube in inverted position to each test tube Then 1 ml of sample was added to 3 tubes of single-strength and placed Durham tube in inverted position. Samples of 0.1 ml were added to 3 tubes of single strength and place Durham tube similarly. The tubes were incubated for 48 hours at 37 °C. Appearance of gas was the positive result if no gas appears it indicated negative result (Chandrima et al., 2015).

**1.6. Confirmation test:**

Positive result was processed further through confirmatory test Eosin methylene blue media was prepared and autoclave for 15 minutes at 121°C and 15 Psi, petri dishes was also autoclave in order to sterilize them. Then the media was poured in petri dishes and after cooling of media the sample were streaks on the plates from the tubes having positive results. After streaking petri dishes were placed in the incubator for 24 hours at 37 °C.

**1.7. Complete test (fecal Coliform test):**

Nutrient agar media was prepared and autoclave for 15 minutes also autoclave the test tubes. Then pour the media in the test tube and make slants in test tubes. Then streak the colonies on the slant which was formed on the EMB media then incubate the slant for 24 hours 37 °C. After 24 hours bacterial colonies was observed on the slant. Gram staining was done and examines the slides under microscope.

**Table 3.7 MPN values with codes**

| **Tubes with positive results** | | | **MPNvalue per 100 ml** | **95%confidence** | |
|---|---|---|---|---|---|
| 5tubes with 10 Ml | 5tubes with1ml | 5tubes with 0.1ml | | Lower | Upper |
| 0 | 0 | 0 | <2 | <1 | 7 |
| 0 | 2 | 0 | 4 | <1 | 11 |
| 0 | 1 | 0 | 2 | <1 | 7 |
| 1 | 1 | 0 | 4 | <1 | 11 |
| 1 | 1 | 1 | 6 | <1 | 15 |
| 1 | 0 | 0 | 2 | <1 | 7 |
| 1 | 0 | 1 | 4 | <1 | 11 |
| 2 | 0 | 1 | 7 | 1 | 17 |
| 2 | 0 | 0 | 5 | <1 | 13 |
| 2 | 1 | 1 | 7 | 1 | 17 |
| 2 | 1 | 1 | 9 | 2 | 21 |
| 2 | 3 | 0 | 12 | 3 | 28 |
| 2 | 2 | 0 | 9 | 2 | 21 |
| 3 | 3 | 2 | 100 | 150 | 4800 |
| 3 | 2 | 2 | 210 | 35 | 470 |

| | | | | | |
|---|---|---|---|---|---|
| 3 | 3 | 1 | 460 | 71 | 2400 |
| 3 | 1 | 1 | 75 | 14 | 230 |
| 3 | 1 | 2 | 120 | 30 | 380 |

**3.8. Biochemical assays**

The positive samples for MPN (Most Probable number) were further processed by performing biochemical tests for the species identification. A) Citrate utilization test. B) Urease test. C) Triple iron sugar. D) Catalase test. E) Oxidase test. F) Indol test.

**3.9. Citrate test:**

Citrate test was used to distinguish between members of Enterobacteriaceae. Simmons citrate agar media was prepared and autoclave for 15 minutes after autoclave, slants were prepared in a sterilized culture tube. Then with the help of sterilized inoculation needle touch the colony that is 18 to 24 hours fresh bacterial cultures. Then streak it on the slants and incubate it for 24 hours at 37$^{o}$C. Green color of the slant indicated positive result while the blue color was the negative result.

**2.8. Urease test:**

The urease test was used to differentiate organisms on the basis of their ability to hydrolyze urea in the presence of urease. Christensen's Urea Agar media was prepared and autoclaved for 15 minutes. After autoclave slants were prepared in a sterilized culture tube. Then slants were streaked with the sterilized inoculation needle and incubated at 37 °C for 24 hours. Pink color of the slant was considered positive result while orange color was negative result.

**3.9. Indol test:**

Indol test was performed to determine the ability of an organism to split amino acid tryptophan to form the compound indol. Tryptophan broth was prepared and autoclaved for 15 minutes and pour it in the test tube, isolated colony were emulsified in the broth and were incubated for 24 hours at 37 °C. After incubation 0.5ml of Kovac's reagent was added to the broth culture. Results were observed by the appearance of pink color in the media.

**3.10. Oxidase test:**

The test was used to identify the bacteria which produce cytochrome c oxidase, which was the enzyme of electron transport chain. The filter paper was soaked with oxidase reagent. Then moisten the paper with distilled water. Isolated colony was picked from slant and spread it on the filter paper. It was observed for 10-30 seconds for the appearance of dark purple color which indicated positive.

**3.11. Catalase test:**

Catalase test was used to determine the presence of catalase enzyme which breaks down the harmful substances hydrogen peroxide in to water and oxygen. First we take a clean glass slide and strike a small amount of bacterial colony on it then add few drops of hydrogen peroxide on it. Then the result was noted. No bubbles were formed in negative result while bubbles were the indication of positive result.

3.12. **Culture susceptibility**

After the identification of species disc diffusion assay were performed according to the protocol of CLSI 2017 through Kirby Bauer method. The antibiotics used for this purpose were Meropenem, Ciprofloxacin, Linezolid, Oxacillin, Vancomycin and Ceftazidime. Multidrug resistant (MDR) and Extreme drug resistant (XDR) were also determined through culture susceptibility.

**4. Biochemical oxygen demand (BOD)**

The BOD was performed in PCSIR Laboratory Peshawar according to the protocol of (Sulieman et al., 2009), Two jars of 100 ml having a cover was used then 25 ml of sample was be taken in every jar and 75 ml of distilled was added to both jars. After that both jars were close well. One jar was kept in the incubator for 5 days at 20-22

°C. Then 2ml of alkali iodide solution was added and 10 ml of manganese sulphate solution to other jar (with the help of disposable syringe).Then close the jar and mix it by inverting the jar many times. When precipitate settle down and clear liquid above the precipitate, again shake the jar by inverting method. When setting produce minimum 50 ml supernatant 8 ml of sulphuric acid was added. Close the jar and mix with inverting method until dissolution occur completely. Then take 100 ml of sample and titrate with Sodium thio-sulphate solution until solution become pale yellow. Then 2 ml of freshly prepared starch solution was added and titrate until blue color was appeared. Repeat the same procedure for blank (Sulieman et al., 2009). The Biochemical Oxygen Demand was calculated as follow.

BOD as mg $O_2/L$=16 ($V_1$-$V_2$).

Where:

*V1* = ml of sodium thiosulphate was for the sample before incubation

*V2*=ml of sodium thiosulphate was used for sample after incubation.

**5. Chemical oxygen demand (COD)**

Total 10 ml of the sample was taken in a 100 ml bottle then 5 ml of conc. Sulphuric acid and 1 g of copper sulphate was added. Add 3 ml of prepared N/40 potassium permanganate solution was added and the flask was placed in boiling water for 30 min. Prepare two salts sodium oxalate and potassium permanganate. Then add 3 ml of N/40 sodium oxalate and titrate with N/40 potassium permanganate until color change to violet. Repeat same procedure for blank. The COD value was determined by the method reported by (Sulieman et al., 2009).The result for COD was calculated as:

COD as mg $O_2/L$= sample of (1/40) x (*A*-*B*) ml of sample.

Where:

*A* = ml of potassium permanganate used for sample.

*B* = ml of potassium permanganate used for blank.

1/40=Molarity of potassium permanganate.

8000 = milli equivalent weight of oxygen x 1000m

**6. Heavy metals**

Detection of heavy metals in water samples was done in Soil and environmental sciences department of Agriculture University Peshawar. The detection was done with atomic absorption. It was based upon the absorption of light by free metallic ions. Atomic absorption spectrometers were used to measure the concentration of gas phase atoms by using the absorption of light. The light which was focused into the flame was produced by a hollow cathode lamp, inside which were the sample and an anode. Results were recorded and interpreted**.**

## 6. RESULTS

### 6.1. Physical parameters

Results obtained for physical parameters were within the limits according to the recommended limits of WHO (World Health Organization). The typical value of pH was 6.5-8.5 and the average pH values collected for water samples was 8.2. The standard value for turbidity was 0.1-2 NTU and the average value obtained was 1.1as shown in Table no.1

**Table no 6.1 average values of pH and turbidity**

| S.no | Average value of pH | Average value of turbidity | Total no of samples |
|---|---|---|---|
| 1. | 8.2 ± 0.5 | 1.1 ± 0.5 | 50 |

### 6.2. Bacteriological parameters

MPN test was performed for quantitative and qualitative justification of fecal as well as wild type of coliform bacteria. According to WHO (2006), the drinking water contain less than 1 MPN value per 100 ml. However, in the current research study, it was observed that the MPN value in all samples were very high (9-460 MPN/100 ml) indicating the water reservoirs were highly contaminated with fecal as well as wild type of coliform bacteria as shown in Table 6.2, which was further confirmed by confirmatory tests.

**Table 6.2: Most Probable Number (MPN) values for drinking water collected from educational institutions.**

| r# | amples ode | with 5%ConfidenceLimit ower | pper | IPN ndex | r# | amples ode | with 5%ConfidenceLimit ower | pper | IPN ndex |
|---|---|---|---|---|---|---|---|---|---|
| | -SH | 5 | 70 | 10 | 4 | -KS | ) | 50 | 3 |
| | -ST | 50 | 300 | 10 | 5 | -RA | ) | 40 | 50 |
| | -AK | | 5 | | 6 | -HM | 5 | 70 | 10 |
| | -IC | ) | 40 | 50 | 7 | -IT | 5 | 30 | 3 |
| | -CH | 1 | 400 | 50 | 8 | -RG | ) | 40 | 50 |
| | -GS | 50 | 300 | 10 | 9 | -ES | 5 | 30 | 4 |
| | -SR | | 7 | 1 | ) | -PS | ) | 40 | 50 |
| | -BC | | 5 | 1 | 1 | -AG | 4 | 30 | 5 |
| | -I | | 7 | 1 | 2 | -CS | ) | 30 | 20 |
| ) | -LC | | 5 | 1 | 3 | -SG | ) | 40 | 50 |
| 1 | -CS | | 5 | | 4 | -FS | 4 | 30 | 5 |
| 2 | -AS | 5 | 70 | 10 | 5 | -PS | 5 | 30 | 4 |
| 3 | -PS | | 7 | 1 | 6 | -GP | ) | 30 | 20 |
| 7 | -HS | 1 | 400 | 50 | 7 | -BF | 4 | 30 | 5 |
| 8 | -SP | 50 | 300 | 10 | 8 | -FK | 5 | 30 | 3 |
| ) | -FH | ) | 30 | 20 | 9 | -PF | | 7 | 1 |
| ) | -FA | 5 | 30 | 4 | ) | -CS | ) | 30 | 20 |
| 1 | -MH | ) | 40 | 50 | 1 | -HM | 5 | 30 | 20 |
| 2 | -RW | 4 | 40 | 5 | 2 | -SD | 5 | 30 | 3 |
| 3 | -FA | 5 | 70 | 10 | 3 | -SR | 5 | 30 | 4 |
| 4 | -NH | 1 | 400 | 50 | 4 | -HT | ) | 40 | 50 |
| 5 | -JM | ) | 40 | 50 | 5 | -RS | | 5 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 5 | -SJ | ) | 40 | 50 | 5 | -GP | 5 | 30 | 4 | 6.3. |

Biochemical Tests:

After the determination of MPN values of all samples different biochemical tests were performed in order to identify the species. The biochemical tests performed were Citrate, Urease, Indole, Catalase, Oxidase and TSI. Gram staining was also performed to determine the morphology of specie. The identified species were *Klebsiella spp, S. typhi, P. aeruginosa, S. aureus, E. coli, Enterobacter Spp, Enterococcus spp, P. mirabilis, P. vulgaris.* The results of biochemical tests are shown in Table No 6.3.

**Table 6.3:** Biochemical tests for identification of species.

| S. N 0 | Culture characteristics | Gram Rxn | Shape | Cit test | Urea test | Ind test | Oxida test | Cata test | TSI | Specie Identified |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Abundant white, cluster Colony | - | Bacilli | + | + | - | - | + | A/A | *Klebsiella spp* |
| 2 | Cream chainlike Colony | - | Rod | - | - | - | - | + | K/A $H_2S$ | *S.typhi* |
| 3 | Abundant whitethin Growth | - | Rod | + | - | - | + | + | K/K | *P. aeruginosa* |
| 4 | Mucoid whitish elevated Colonies | + | Cocci | + | + | - | - | + | N/A | *S.aureus* |
| 5 | Abundant, opaque golden growth | - | Rod | - | - | + | - | + | A/AC $O_2$ | *E.coli* |
| 6 | Abundant white scattered colony | + | Cocci | - | - | - | - | - | N/A | *Enterococcus spp* |
| 7 | Abundant, pale whitish | - | Rod | + | - | - | - | + | A/A | *Enterobacter spp* |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | growth | | | | | | | | | |
| 8 | Smaller irregular, smooth, shiny, pale andopaque colony | - | Rod | + | + | - | - | + | K/A | *P. mirabilis* |
| 9 | Smaller irregular, smooth, pale colonies andopaque | - | Rod | + | + | - | - | + | K/A | *P. vulgaris* |

***Key:****GramRnx: Gramstaining,Cittest:Citratetest,Ureatest: Ureasetest,Indtest:Indoletest, Oxidatest: Oxidase test, Cata test: Catalase test.*

**6.4. Isolated Species:**

After performing biochemical tests of all samples which was positive for MPN. The numbers of species isolated from different samples were Klebsiella spp in 5 samples (10.8%), S. aureus in 7 sample (15.2%), S. typhi in 6 samples (13%), P. aeruginosa in 4samples (8.6%), E.coli in 8 samples (17.3%), Enterococcus spp in 8 samples (17.3%), Enterobacter spp in 3 samples (6.5%), P. mirabilis in 2 samples (4.3%) and P. vulgarissamples (6.5%). As shown in fig 6.4 (a) and (b).

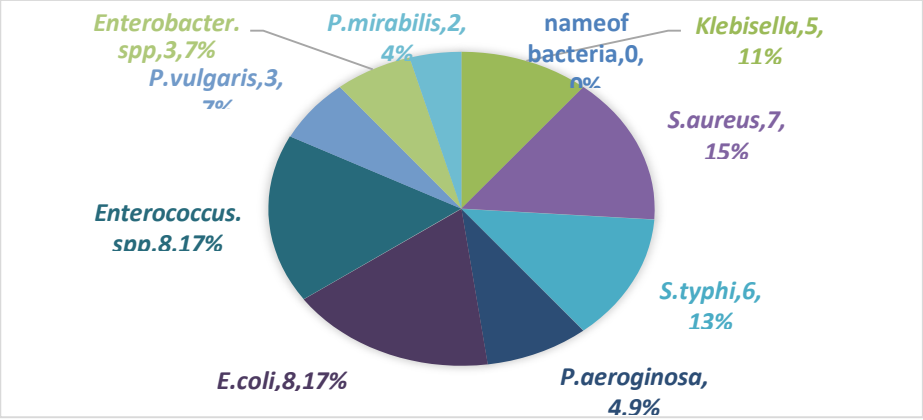


**Fig 6.4 (a)** No of species isolated and identified through biochemical tests.

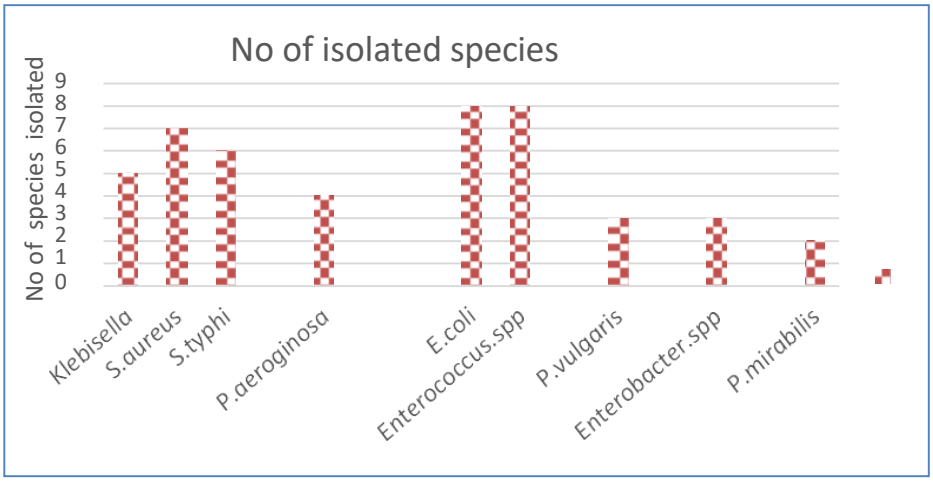


**Fig 6.4**(b) No of species isolated and identified through biochemical tests.

6.5. Culture Sensitivity:

Different antibiotics were used against Klebsiella spp, Enterococcus spp, P. mirabilis, Enterobacter spp, P. vulgaris and S. typhi. Meropenem formed inhibition zone of 21.8 mm against P. aeruginosa so was intermediately susceptible to P. aeruginosa, while Klebsiella spp with zone of 15.5 mm, S. typhi with zone of 12.8 mm, E.coli with zone of 17.33 mm, P. mirabilis with zone of 14.5 mm, S. aureus with zone of 14.6 mm, P. vulgaris with zone of 12 mm, Enterobacter spp with zone of 18.5 mm were resistant against Meropenem. Linezolid were susceptible to P. aeruginosa and S. aureus formed inhibition zone of 27mm and 21.8mm respectively. Klebsiella spp, S. typhi, Enterococcus spp, E.coli, P. mirabilis, P. vulgaris and Enterobacter spp with the inhibition zones 0.33 mm,0.66 mm,0.33 mm, 0.33mm,9.5mm,9.5mm and 0.33 were resistant against Linezolid. Ciprofloxacin was the most potent antibiotic towards P. aeruginosa and E.coli.The inhibition zone against P. aeruginosa was 21.8 mm and E.coli was 21.8 mm. Klebsiella spp, S. typhi, Enterococcus spp, P. mirabilis, P. vulgaris, S. aureus and Enterobacter spp with zone of17.3mm,0.3.mm,9.5mm,19.5mm,14.5mm, 19.5mm and 14.6mm were resistant to Ciprofloxacin. Ceftazidime was resistant to all species. Oxacillin and Vancomycin were only effective against S. aureus. Isolated S. aureus was Methicillin Resistant Staphylococcus aureus (MRSA). No XDR bacteria were detected. Results was shown in Table 4.4 and fig 4.4(a) and (b)

**Table 6.5** Inhibition zones form by antibiotics against different species of bacteria.

| **acterial olates** | **ntibiotics and zone of inhibition measured in mm** | | | | | |
|---|---|---|---|---|---|---|
| | EM | ZD | N | AZ | X | A |
| *lebsiellaspp* | 5.5±0.40 | 33±0.5 | 7.3±0.5 | 9.5±0.5 | 33±0.5 | 33±0.5 |
| *typhi* | 2.8±0.76 | 66±0.5 | 33±0.5 | 33±0.5 | 33±0.5 | 9.5±0.5 |
| *aeruginosa* | 1.8±0.76 | 7±0.76 | 1.8±0.7 | 33±0.5 | 33±0.5 | 4.6±0.5 |
| *nterococcus spp.* | 33±0.577 | 33±0.5 | 5±0.5 | 5±0.5 | 33±0.5 | 33±0.5 |
| *coli* | 7.33±0.57 | 33±0.5 | 1.8±0.7 | 33±0.5 | 33±0.5 | 33±0.5 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *mirabilis* | 4.5±0.40 | 5±0.5 | 3.5±0.5 | 5±0.5 | 33±0.5 | 33±0.5 |
| *aureus* | 4.6±0.5 | 4.8±0.763 | 3.5±0.5 | 33±0.5 | 32.8±0.2 | 35.8±0.2 |
| *vulgaris* | 32±0.816 | 5±0.5 | 4.5±0.4 | 33±0.5 | 5±0.5 | 33±0.5 |
| *nterobacter spp.* | 3.5±0.5 | 4.6±0.57 | 33±0.5 | 33±0.5 | 33±0.5 | 33±0.5 |

***Key:****Meropenem:MEMLinezolid: LZD,Ciprofloxacin:CN,Ceftazidime:CAZ,Oxacillin:OX, Vancomycin: VA*

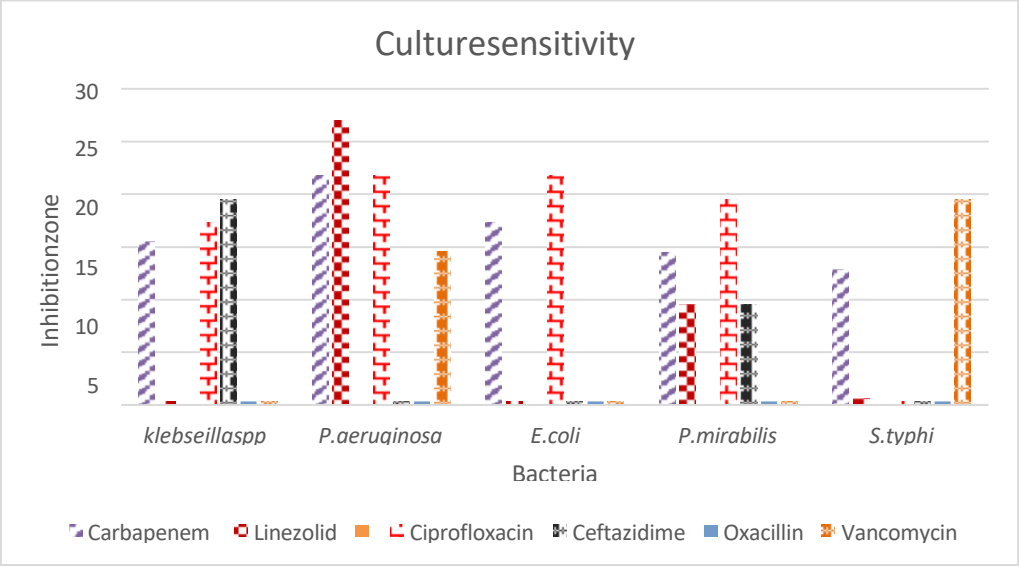


**Fig6.5 (a)** Inhibition zones form by antibiotics against different species of bacteria.

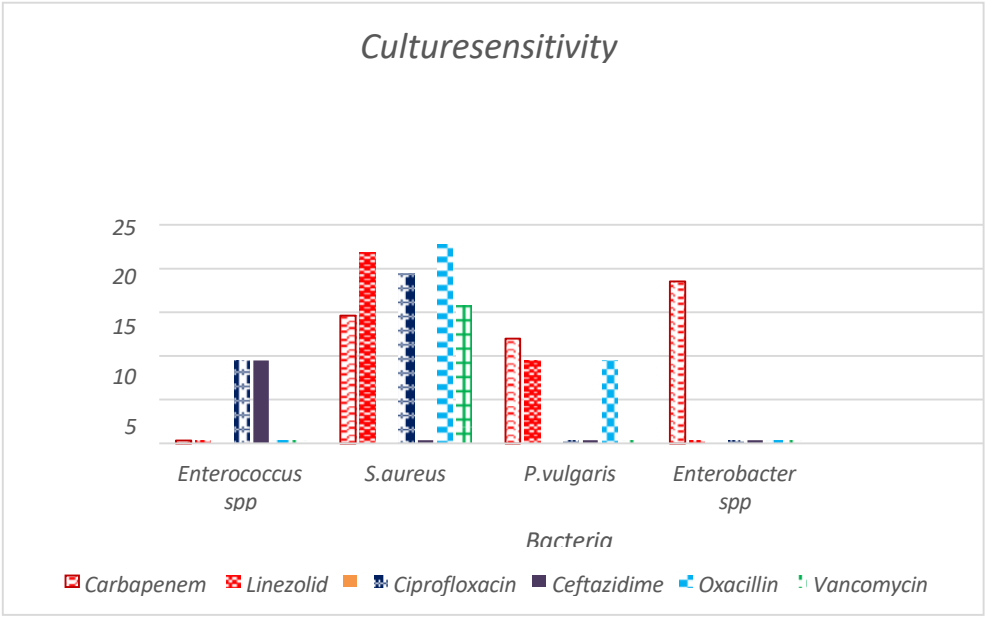


**Fig6.5 (b)** Inhibition zones form by antibiotics against different species of bacteria.

**6.6. Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD):**

According to WHO(World Health Organization) 2006 and EPA(Environmental Protection Agency) 2007,the COD value for drinking water was 8mg/l. BOD values for drinking water was lie between 5-8 ppm. Sample code CH-4, HM-3, ST-2, SH-1 and HT-5 have the COD values of 4.45mg/l, 3.67mg/l, 3.56mg/l, 2.34mg/l and 2.23mg/l and BOD value of 3mg/l, 2.49mg/l, 5.24mg/l, 1.59mg/l, 1.51mg/l respectively. The result was shown in fig 4.5.

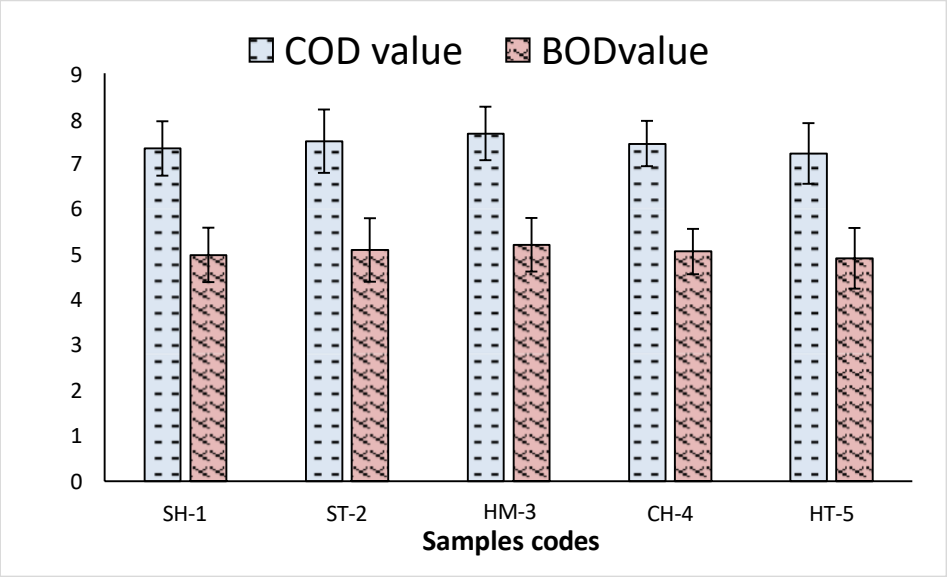


**Fig 6.6.** COD vs BOD of some selected samples.

6.7. Heavy Metals:

Heavy metals were detected through atomic absorption in Agriculture University Peshawar. The metals which was detected in the recent study was Cadmium (Cd), Copper (Cu), Lead (Pb), Zinc (Zn) and Chromium (Cr) in drinking water. The normal range recommended by WHO in Geneva, 1993 was also mentioned with each metal (Edition .F, 2011). Cd, Pb, Cr were in larger amount then normal range in all samples. Cu, Zn was in less concentration in all samples then the normal range.The result was shown in fig 4.6.

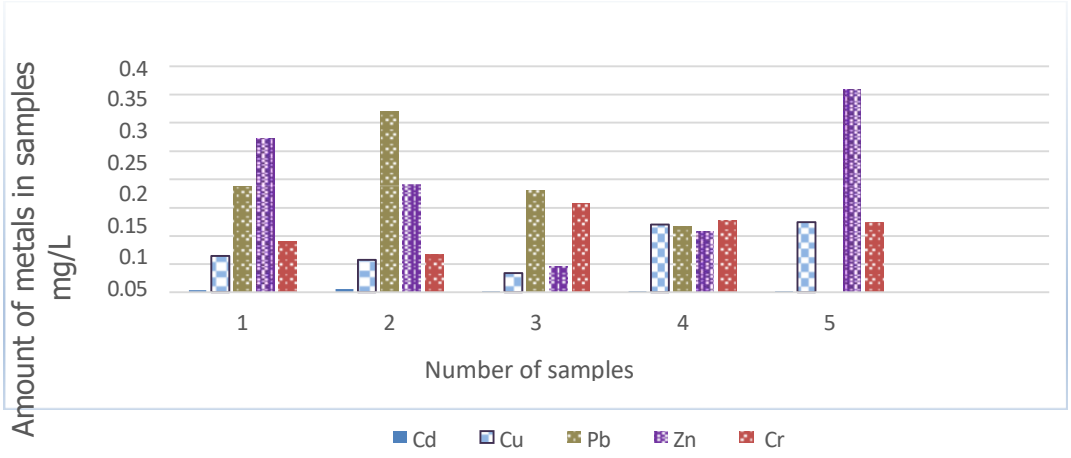


**Fig 6.7 .**Heavy metals detection in water samples.

## 7. Discussion

Water, a fundamental component of life, necessitates rigorous safety assessment, particularly in high risk regions like Peshawar a critically polluted district in Pakistan. This study targets school water systems due to children's intensified immunological vulnerability, analyzing 50 samples for physicochemical and bacteriological parameters. Physically, all samples exhibited acceptable organoleptic properties (colorless, tasteless, and odorless) except one from Shakardar (Kohat district) with objectionable taste (Iqbal et al., 2013). pH values (8.1–8.6; average 8.2) aligned with WHO guidelines (6.5–8.5) (Singare et al., 2010), though sustained exposure to elevated pH may cause dermal irritation (Amir et al., 2015). Turbidity (0–3 NTU; average 1.1 NTU) complied with WHO standards (<5 NTU) (Amir et al., 2015). Bacteriological analysis revealed pervasive fecal contamination, with 92% (46/50) of samples testing positive for coliforms via MPN (210–4800 MPN/100mL), indicating sewage infiltration consistent with findings in Khirpur's municipal supplies (Ali et al., 2011; Shar et al., 2008). Isolates included Escherichia coli (8 samples), Salmonella typhi (6), Pseudomonas aeruginosa (4), Enterococcus spp. (8), Enterobacter spp. (3), Proteus mirabilis (2), and Proteus vulgaris (3). Antibiotic susceptibility testing demonstrated widespread multidrug resistance: Meropenem and Vancomycin each resisted 7/9 species (e.g., Meropenem effective only against P. aeruginosa; Vancomycin against S. typhi and S. aureus), while Linezolid, Ciprofloxacin, Ceftazidime, and Oxacillin showed variable resistance (Ciprofloxacin susceptible to E. coli and P. aeruginosa; Oxacillin to S. aureus alone). Heavy metal analysis identified alarming exceedances of WHO limits for lead (Pb: 0.1–0.3 mg/L), cadmium (Cd: 0.002–0.006 mg/L), and chromium (Cr: 0.001–0.09 mg/L), contrasting with compliant zinc (Zn: 0.04–0.3 mg/L) and copper (Cu: 0.03–0.12 mg/L) levels. These metals pose bioaccumulation risks, corroborated by Egyptian studies linking tap water exposure to elevated blood Cr/Pb and renal dysfunction (Badr et al., 2011). Organic pollution indicators (BOD: 1.51–5.24 mg/L; COD: 2.23–4.45 mg/L) remained within EPA (2006) limits across samples (e.g., SH-1: COD 2.34, BOD 1.59; HT-5: COD 2.23, BOD 1.51) distinct from Bangladesh's Turag River, where BOD reached 180 mg/L near industrial discharges (Rahman et al., 2012). Collectively, the co-occurrence of toxic metals and antibiotic-resistant pathogens in Peshawar's school water underscores an urgent need for infrastructure upgrades, point-of-use filtration, and enhanced antimicrobial stewardship to safeguard children's health.

## 8. Conclusions

This integrated study reveals that drinking water in Peshawar's schools complies with WHO standards for physicochemical parameters (taste, odor, color, turbidity, pH), it exhibits critical co-contamination of multidrug-resistant (MDR) pathogens and toxic heavy metals. Fecal contamination was detected in 92% of samples (46/50 MPN-positive; 210–4800/100mL), with isolation of nine pathogenic species including Salmonella typhi, MDR Escherichia coli, and Pseudomonas aeruginosa. Antibiotic resistance profiling revealed universal MDR phenotypes notably resistance to Meropenem (effective against only 2/9 species) and Vancomycin (2/9) though extensively drug-resistant (XDR) strains were absent. Concurrently, cadmium (Cd: 0.002–0.006 mg/L), lead (Pb: 0.1–0.3 mg/L), and chromium (Cr: 0.01–0.09 mg/L) consistently exceeded WHO thresholds, posing chronic toxicity risks, while zinc (Zn: 0.04–0.3 mg/L) and copper (Cu: 0.03–0.12 mg/L) remained acquiescent. Biochemical oxygen demand (BOD: 1.51–5.24 mg/L) and chemical oxygen demand (COD: 2.23–4.45 mg/L) conformed the (EPA 2007) and (WHO 2006) standards. This dual contamination paradigm synergistically combining metal carcinogenic and antibiotic-resistant pathogenesis demands urgent interventions: immediate deployment of point-of-use filtration (activated carbon/UV), infrastructure rehabilitation to prevent sewage infiltration, and policy reforms integrating antimicrobial resistance monitoring into water safety frameworks to protect immune-compromised pediatric populations and achieve UN Sustainable Development Goal (SDG-6) targets in Pakistan's rapidly urbanizing landscapes.

### 8.1 Recommendations

1. It is recommended that drinking water must be boiled or physical filtration with 8-fold clean cloth processing before consumption at school and home level at Peshawar.
2. Chlorine tablets may also be utilized to clean water in water tanks.
3. Maintenance of drinking water pipe line should be done on regular basis and water tanks for storage of clean water must be properly sealed to avoid contamination

8.2 **Limitation**

**1.** Boiling of water remove only bacteria but heavy metals and other chemicals are still present in water.
**2.** Level of chlorine should be 4mg/l are safe for drinking purpose while large amount of chlorine in drinking water causes diseases like bladder cancer.

**Scientific Significance**

This study proposes critical information and clear evidence on drinking water quality assessments in District Peshawar schools, which includes a thorough physical, chemical and biological analysis. Evaluation of the results is alarming as it shows that most of the water samples were found unsafe for drinking, raising the turbidity, mean pH, unpleasant taste and colored water. Moreover, bio assessment identified the presence of fecal coliforms and pathogenic bacteria, specifically using a Most Probable Number (MPN) approach with unacceptable MPN sterile water column or serial dilutions that also dwelled in multi-drug resistant forms. Furthermore in assessing most of the metals contaminants tended to point to cadmium, lead and chromium levels through all water sample profile which exceeded acceptable limits. These finding open the door to a potential risk for the public health and advocate for the need for research or more serious the uptake of working systems and proper monitoring protocols in the interventions that include schools and all forms of educational institutions. This study has created a void with available data that can help inform policymakers and stakeholders in areas where drinking water is a major problem for means to establish and drink water safety or apply for sustainable development which will mostly likely improve the health of students in Peshawar and cities in the province.

**Conflict of Interests and Compliance with Ethics Guidelines:**

The authors declare no conflict interest and all the Authors contributed equally in this study

**Authors Contribution:**

**Wajiha Usman:** Writing, review, editing, conceptualization, formal analysis, visualization, validation.
**Dr Riaz Muhammad**: supervision, resource, project administration, funding acquisition, conceptualization
**Nabila Jabbar**: Writing, review, software, methodology, writing, editing, formal analysis, original draft, conceptualization.

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