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# Microbial Load And Antibiotic Susceptibility Profile Of Bacterial Isolates From Drinking Water In Peshawar, Pakistan

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**Abstract**

The health of individual and community are greatly influenced by the quality of water supplied in both developing and underdeveloped countries and has a profound effect on human health. The current study aims to determine and highlight microbial load and their antibiotic susceptibility profile isolated from wells and boreholes drinking water from a distinct area of Peshawar, Pakistan. From November to January 2018, 200 drinking water samples were examined for microbiological load in different areas of Peshawar city. All models were collected from wells and boreholes stored in sterile blue cap bottles and transferred to the laboratory as soon as possible. The spread plate method was used to culture all of the samples. The Kirby-Bauer disc diffusion technique was utilized with CLSI standards to determine susceptibility patterns. Percentage of isolates were Bacillus spp (12.50%), Staphylococcus aureus (6.25%), Escherichia coli (25%), Proteus sp (12.50), Enterobacter sp (12.50%), Klebsiella sp (18.6%), and Pseudomonas sp (12.50%) among 448 different isolated bacteria species respectively. Most isolates were sensitive to Ciprofloxacin, ofloxacin, Ciprofloxacin, augmentin, and norfloxacin, whereas resistance to ampicillin, amoxicillin tetracycline, and cotrimoxazole was high. The isolated pathogenic microorganism indicates sufficient microbial load in drinking water and alarming for public health regulatory bodies. The prevalence of multidrug-resistant isolates, such as Klebsiella sp, Pseudomonas sp, Proteus sp, and E. coli., is a major source of concern due to the risk of infections and the propagation of resistant genes, which restricts the number of chemotherapeutic drugs currently accessible.

**Keywords:** drinking-water, Peshawar, susceptibility profile, disk diffusion method.

**Introduction**

Clean and safe or good quality drinking water is one of the most important requirements for healthy human life (Koelmans et al., 2019). Pure water has a detrimental effect on human health and major public health concern for individuals and the community (Qadri & Faiq, 2020). In developing countries, water pollution is an alarming issue that leads to waterborne diseases. Instead of the latest advancement, waterborne infection is still challenging. According to the World Health Organization (WHO),50% of the population in developing countries is suffering from waterborne infectious diseases (IDs) around the year, and almost 2 billion people consume fecally contaminated water (Daud et al., 2017). Rainfall-runoff and agricultural inputs can contaminate drinking water, causing it to interact with sewage effluents and feces from everyday life, leaving it unsafe for human consumption(Onuoha, 2017; Qadri & Faiq, 2020). Aeromonas, Pseudomonas, and fecal coliforms are indicators of fecal contamination in water(Sagar, Kaistha, Das, & Kumar, 2019). The consumers, especially those who are immunocompromised, may suffer serious health consequences if such isolates are present(Manyi-Loh, Mamphweli, Meyer, & Okoh, 2018; Monapathi, Bezuidenhout, & James Rhode, 2020).

Contaminated water is a profound etiology behind the illness that leads to potential health problems due to the microbial content (Gunarathna, Gunawardana, Jayaweera, Manatunge, & Zoysa, 2018). Enteric pathogens such as enterotoxigenic Escherichia coli, Shigella spp, and Salmonella spp were the most commonly occurring among pathogens disseminated in water sources and are responsible for a variety of diseases such as diarrhea, dysentery, and WHO estimates that by 2025, half of the world's population will live in water-stressed areas, with 785 million people lacking access to clean drinking water(Daud et al., 2017).

Antibiotic resistance has emerged as a significant public health concern(Prigitano, Romanò, Auxilia, Castaldi, & Tortorano, 2018), and literature reported that bacteria isolated from drinking water have detrimental resistance to commonly prescribed antimicrobial agents (Andrade et al., 2020; Le et al., 2016). Bacterial isolates resistant to more than two antimicrobial drugs are known as multiple antibiotic-resistant bacteria (MAR). Various mutations in the bacterial genome were caused by MAR, which has been linked to the patients' excessive, inappropriate, and noncompliance with these medications(Imran, Das, & Naik, 2019). Antibiotic resistance is seen more generally in regions where disproportionate use of antibiotics is practiced. Various studies from Pakistan reported prevalence and widespread resistance to common antimicrobials by isolates from drinking water.

The current study aims to determine the microbial load in water for contamination of potentially harmful bacteria, i.e., isolated and the determination of MAR phenotypes and their susceptibility profile to commonly used antimicrobial agents.

**Materials and Methods**

**Sample collection**

About 200 samples were collected from distinct areas of Peshawar (Hayatabad, Sadder, Ring road, Karkhanoo Bazar, Peshtakhara chowk, Kohat road, Kawkshal) from November 2017 to January 2018 and transported in sterilized blue cap bottle or black polyethylene bags within 2hrs of collection. Samples were preserved at $4^0$C if not processed immediately.

**Sterilization of the glassware**

All glassware was wrapped with aluminum foil and sterilized in an autoclave for 15 min at 121°C. The same process was also used to fix distilled water used for serial dilution.

### Serial dilution

For analysis of sample serial dilution, each dilution was poured on Nutrient agar for culture using the spread plate technique. In the test tube, 1 ml of water sample was transferred to 9 ml of sterile distilled water and serially diluted to $10^{-4}$. The plates were then inverted and incubated at 37°C for 24 hours after inoculation.

### Pure culture

For obtaining a pure culture and clear morphology, subculture was performed on Eosin Methylene Blue Agar (EMB), MacConkey Agar (MCA), and Mannitol Salt Agar (MSA) plates and then incubated at 37°C for 24 hours. The same procedure was performed on fresh media for obtaining pure culture.

### Biochemical test

All plates were further processed for colony morphology and differentiation by gram staining and biochemical testing (oxidase, Indole, coagulase, triple sugar iron (TSI), and catalase test) to identify isolated bacteria.

### Antibiotic susceptibility profile

For antimicrobial susceptibility, the disk diffusion method was used using CLSI guidelines. Impregnated antimicrobial disks were placed on inoculated Muller Hinton agar (MHA) media and placed in the incubator for 24 hours at 37°C. The antibiotic tested disk were ciprofloxacin(10 µg), tetracycline(30 µg), Norfloxacin(30 µg), Amoxicillin(30 µg), Ofloxacin(10 µg), Gentamicin(30 µg),Chloramphenicol(30 µg), Augmentin(30 µg), Ceftriaxone(30 µg), Ampicillin(10 µg), Cotrimoxazole(10 µg), Erythromycin(30 µg) and Clindamycin(10 µg). The diameters of the inhibition zone around the disks were measured using a Vernier caliper. The results were recorded as sensitive, resistant, and intermediate.

### 3. Results and Discussion

From drinking water samples, a total of 448 distinct bacterial species were isolated, including five Gram-negative genera, Escherichia coli, Pseudomonas sp, Enterobacter sp, Proteus sp, and Klebsiella sp and two Gram-positive genera. Staphylococcus aureus and Bacillus sp, as shown in table 1.

**Table 1.** Percentage occurrence and bacterial loads of water samples.

| S. No. | Isolates | Gram reaction | No. of isolates | Percentage |
|---|---|---|---|---|
| 1. | Bacillus sp. | Positive | 56 | 12.50% |
| 2. | Staphylococcus aureus | Positive | 28 | 06.25% |
| 3. | Escherichia coli | Negative | 112 | 25.00% |
| 4. | Proteus sp. | Negative | 56 | 12.50% |
| 5. | Enterobacter sp | Negative | 56 | 12.50% |
| 6. | Klebsiella sp. | Negative | 84 | 18.75% |
| 7. | Pseudomonas sp. | Negative | 56 | 12.50% |
| **TOTAL** | | | **448** | **100%** |

**Table 2.** Antibiotic susceptibility patterns of Gram-positive bacterial isolates.

| S.No | Antibiotic | Concentration (µg) | Mean Diameter of Inhibition Zone (in mm) | |
|------|------------|--------------------|-------------|-------------|
| | | | S. aureus | Bacillus sp. |
| 1 | Augmentin | 30 | 19 (S) | 16(I) |
| 2 | Ceftriaxone | 30 | 18 (I) | 15(I) |
| 3 | Ampicillin | 10 | 20 (R) | 19(R) |
| 4 | Cotrimoxazole | 10 | 19 (R) | 18 (I) |
| 5 | Erythromycin | 30 | 18 (I) | 14 (I) |
| 6 | Gentamicin | 30 | 18 (I) | 12 (I) |
| 7 | Clindamycin | 10 | 16 (I) | 14 (I) |

I = Intermediate sensitivity, S=Sensitive, R=Resistant, Zone of Inhibition: 0-13 mm = resistance; 14 - 18 mm = Intermediate sensitivity; 18 mm and above = Sensitivity.
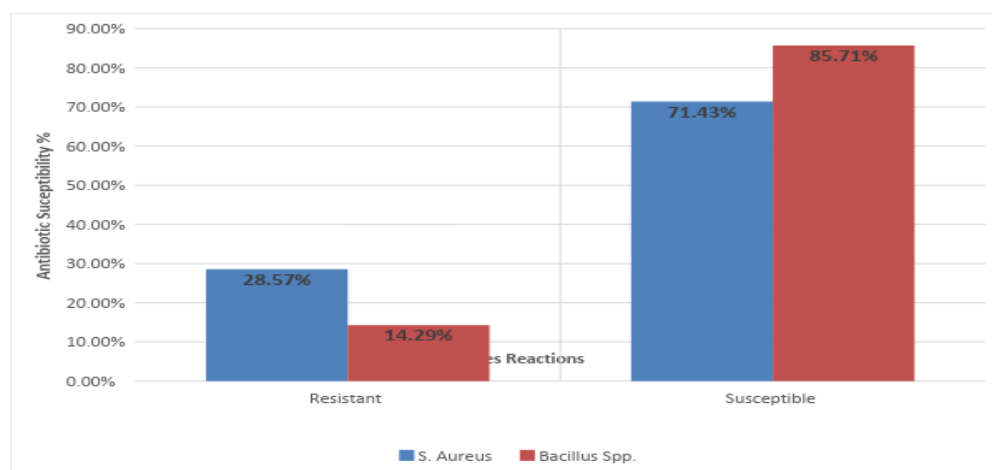


**Fig 2.** Mean Percentage antibiotic susceptibility pattern of gram-positive bacteria.

Antimicrobial resistance bacteria emerge in environments due to unnecessary antimicrobial use (Caniça, Manageiro, Abriouel, Moran-Gilad, & Franz, 2019). In our study, Gram-positive isolates were shown to be sensitive to Amoxicillin-clavulanic acid, Ceftriaxone, and Gentamicin but resistant to Ampicillin and Cotrimoxazole. This observation is consistent with the findings (Ogunleye, Omoya, & Ayantola, 2016). who published that Bacillus sp and Staphylococcus aureus isolated from well waters in Iworoko-Ekiti, Nigeria, were Ampicillin resistant. The early study shows (Mian et al., 2020) 100% resistance to Ampicillin isolated bacteria from sachet drinking water. Another study reported a similar finding to our study and reported resistance of streptomycin, amoxicillin, and Ciprofloxacin for Bacillus while Cotrimoxazole, Gentamicin, Ceftriaxone for S. aureus(Foxlee, Townell, McIver, & Lau, 2019).

Table 3 and Figure 1 (a, b) show the antibiotic susceptibility pattern of Gram-Negative bacteria, i.e., Enterobacter sp, Proteus sp, Klebsiella sp, Pseudomonas sp, and E. coli.

**Table 3.** Antibiotic susceptibility patterns of Gram-negative bacterial isolates.

| Antibiotic | Conc. (µg) | Mean Diameter of Inhibition Zone (in mm) | | | | |
|---|---|---|---|---|---|---|
| | | Enterobacter sp. | Proteus sp. | Klebsiella sp. | Pseudomonas sp. | E. coli |
| Ciprofloxacin | 10 | 17 (I) | 19 (S) | 21 (I) | 16 (I) | 07 (R) |
| Tetracycline | 30 | 13 (I) | 10 (R) | 15 (I) | 08 (R) | 11(R) |
| Norfloxacin | 30 | 16 (I) | 10 (R) | 19 (S) | 09 (R) | 14 (I) |
| Amoxicillin | 30 | 10 (R) | 13 (I) | 11 (R) | 06 (R) | 08 (R) |
| Ofloxacin | 10 | 11 (R) | 15 (I) | 18 (I) | 19 (S) | 21(S) |
| Gentamicin | 10 | 16 (I) | 20 (S) | 17 (I) | 19 (S) | 19 (S) |
| Chloramphenicol | 30 | 14 (I) | 19 (S) | 10 (R) | 16 (I) | 08 (R) |

I = Intermediate sensitivity, S=Sensitive, R=Resistant, Zone of Inhibition: 0-13 mm = resistance; 14 - 18 mm = Intermediate sensitivity; 18 mm and above = Sensitivity
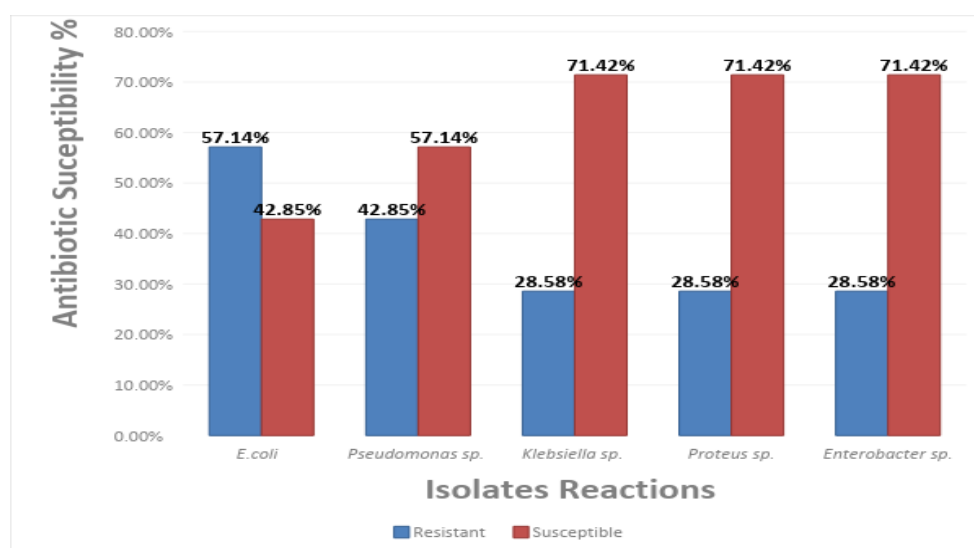


**Fig 3.** Mean Percentage antibiotic susceptibility pattern of gram-Negative bacteria

Among Gram-negative bacteria, E. coli were the most resistant, followed by Pseudomonas sp, Klebsiella sp, Proteus sp., (33%), and Enterobacter sp, respectively. While sensitive to ofloxacin and gentamicin, E. coli show a resistant pattern to Ciprofloxacin, tetracycline, amoxicillin, and chloramphenicol. Pseudomonades sp in our study were resistant to tetracycline, norfloxacin, amoxicillin, and chloramphenicol. In their findings, a similar pattern observed by T.A. Ayandiran et al. (Ayandiran, Ayandele, Dahunsi, & Ajala, 2014) reported resistance to all antibiotics for Pseudomonas sp.

**(a)**                                    **(b)**

**Figure 1.** The result in both figures indicate the antibiotic susceptibility profile of bacterial isolates

## Conclusions

Drinking water sources in our study setting are of poor microbial quality. The existence of multidrug-resistant bacteria, such as E. coli, Klebsiella sp., Pseudomonas sp., and Proteus sp., is significant due to the risk of infectious disease and horizontal transmission of resistance genes might compromise the efficacy of currently available chemotherapeutic drugs. In the presence of antibiotic-resistant microbial isolates in drinking water, physicians may find it difficult to treat waterborne illnesses, which calls for care in the excessive use of antibiotics.

## Conflicts of Interest

The authors declare no conflict of interest.

## References

Andrade, L., Kelly, M., Hynds, P., Weatherill, J., Majury, A., & O'Dwyer, J. (2020). Groundwater resources as a global reservoir for antimicrobial-resistant bacteria. Water Research, 170, 115360.

Ayandiran, T., Ayandele, A., Dahunsi, S., & Ajala, O. (2014). Microbial assessment and prevalence of antibiotic resistance in polluted Oluwa River, Nigeria. The Egyptian Journal of Aquatic Research, 40(3), 291-299.

Caniça, M., Manageiro, V., Abriouel, H., Moran-Gilad, J., & Franz, C. M. (2019). Antibiotic resistance in foodborne bacteria. Trends in Food Science & Technology, 84, 41-44.

Qamar, Z., Anwar, F., Ahmad, R., Haq, I., Khan, A. M. K., Hussain, R., ... & Khan, J. (2021). Prevalence of Hepatitis C virus and determination of its genotypes in subjects of Tehsil Daggar District Buner, KP, Pakistan. Clinical Epidemiology and Global Health, 12, 100809.

Foxlee, N. D., Townell, N., McIver, L., & Lau, C. L. (2019). Antibiotic resistance in pacific island countries and territories: a systematic scoping review. Antibiotics, 8(1), 29.

Anwar, F., Tayyab, M., Haq, I., & Shah, O. U. (2021). Viral overload of COVID-19 pandemics: Overweight people a soft target to get an infection. International Journal of Clinical Virology, 5(2), 070-071.

Imran, M., Das, K. R., & Naik, M. M. (2019). Co-selection of multi-antibiotic resistance in bacterial pathogens in metal and microplastic contaminated environments: An emerging health threat. Chemosphere, 215, 846-857.

Haq, I., Muhammad, A., Fazli Zahir, M. K., Anwar, F., Akhtar, M. S., & Ullah, F. (2020). Serological and Epidemiology study of Helicobacter pylori infection among Dyspeptic patients in District Peshawar Pakistan. Adv. Biores, 11(3), 81-85.

Le, T.-H., Ng, C., Chen, H., Yi, X. Z., Koh, T. H., Barkham, T. M. S., . . . Gin, K. Y.-H. (2016). Occurrences and characterization of antibiotic-resistant bacteria and genetic determinants of hospital wastewater in a tropical country. Antimicrobial agents and chemotherapy, 60(12), 7449.

Rehman, A., Haq, I., Asghar, M., Afridi, G. Z., & Faisal, S. (2020). Sero-epidemiological Identification of Dengue Virus in Individuals at District Shangla, Khyber Pakhtunkhwa. Pakistan. J Biomedical Sci, 9(3), 10.

Mian, A. H., Fatima, T., Qayyum, S., Ali, K., Shah, R., & Ali, M. (2020). A study of bacterial profile and antibiotic susceptibility pattern found in drinking water at district Mansehra, Pakistan. Applied Nanoscience, 1-5.

Jan, Z., Ahmad, S. U., Amara Qadus, Y. A., Sajjad, W., Rais, F., Tanveer, S., ... & Haq, I. (2021). 19. Insilico structural and functional assessment of hypothetical protein L345_13461 from Ophiophagus hannah. Pure and Applied Biology (PAB), 10(4), 1109-1118.

Ogunleye, A., Omoya, F., & Ayantola, K. (2016). Bacterial antibiogram and physicochemical parameters of well water in Iworoko-Ekiti, Nigeria. Journal of Applied Life Sciences International, 1-10.

Asif, A., Asghar, M., Khan, H. U., Haq, I., Shuaib, S. L., Khalid, F., ... & Rehman, N. (2021). Antibiotic susceptibility pattern of clinical isolates of methicillin resistant staphylococcus aureus in Peshawar, Pakistan. Annals of the Romanian Society for Cell Biology, 25(6), 20116-20131.

Prigitano, A., Romanò, L., Auxilia, F., Castaldi, S., & Tortorano, A. M. (2018). Antibiotic resistance: Italian awareness survey 2016. Journal of infection and public health, 11(1), 30-34.

Anwar, F., Khan, M., Salman, M., Ahmad, S., Ullah, F., Khan, J., ... & Abbas, M. (2021). Seroprevalence of hepatitis B virus in human population of district Buner Khyber Pakhtunkhwa Pakistan. Clinical Epidemiology and Global Health, 10, 100688.

Sagar, S., Kaistha, S., Das, A. J., & Kumar, R. (2019). Antibiotic Resistant Bacteria: A Challenge to Modern Medicine: Springer.

Ahmad, S. U., Khan, M. S., Jan, Z., Khan, N., Ali, A., Rehman, N., ... & Zahir, F. (2021). Genome wide association study and phylogenetic analysis of novel SARS-COV-2 virus among different countries. Pakistan Journal of Pharmaceutical Sciences, 34(4).

Khalid, F. (2021). Comparative Diagnostic Analysis and Biochemical Profile in Patients with Covid-19, Dengue and Acute Febrile Illness: Suggestions for Patient Controlling. Annals of the Romanian Society for Cell Biology, 25(7), 1733-1744.

Bashir, Z., Ahmad, S. U., Kiani, B. H., Jan, Z., Khan, N., Khan, U., ... & Mahmood, T. (2021). Immunoinformatics approaches to explore B and T cell epitope-based vaccine designing for SARS-CoV-2 Virus. Pak. J. Pharm. Sci, 34(1), 345-352.

ul Haq, I., Khan, M., Rehman, Z., Anwar, F., Ullah, H., & Ullah, N. (2018). HBV prevalence in the volunteer blood donors in Peshawar, Khyber Pakhtunkhwa Pakistan. Int J Biosci, 13(5), 50-54.