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Analysis of Antibiotic Sensitivity of *Salmonella Typhi* Isolated fromTap Water at District Peshawar

Muhammad Khan[1,4], Hamid Hussain Afridi[2], Khawaja Ejaz Ul Haq[1], Hafiza Misbah Ahmad[1], Irfan[1], Abdul Malik[3], Asghar Ali[1], Muhammad Haroon[1], Faraz Ahmad Khan[4]and Rizwan Arif[1]

[1]Center of Biotechnology & Microbiology, University of Peshawar, **Pakistan**
[2]Shaheed Benazir Bhutto University, Sheringal Dir, **Pakistan**
[3]Department of Biotechnology, Abdul Wali Khan University Mardan, **Pakistan**
[4]Institute of Integrative Biosciences, CECOS University, Peshawar, **Pakistan**

***Correspondence:** khanmuhammad0892@gmial.com **Received: 31-08-2023, Revised: 04 November 2023, Accepted: 06- November 2023 e-Published: 11 November -2023**

This research study presents the antibiotic sensitivity of *Salmonella Typhi (S. Typhi)* isolated from drinking water. Water-borne disease outbreaks are a major hazard to public health worldwide. Therefore, we collected 30 samples from different locations in Peshawar to screen for *S. Typh* and their pattern of antibiotic sensitivity. Antibiotic resistance is nowadays a major threat to public health. The specimens through the spread plate technique grew on Xylose Lysine Deoxycholate (XLD) and Salmonella Shigella (SS) media. Gram staining, TSI, catalase, indole, citrate, urease, and oxidase tests were used for identification. While the Kirby-Bauer technique was used to assess antibiotic sensitivity. XLD and SS agar showed black-centered colonies. The triple sugar iron and catalase test showed positive results, while the indole, urease, citrate, and oxidase tests exhibited negative results. The prevalence of *S. Typhi* was recorded at about 60% in tap water. Our research led us to the conclusion that the water is not fit for drinking. On emergency footing, actions should be made to halt the potential spread of *S. Typhi* to stop the emergence of typhoid.

Keywords: Antibiotic sensitivity, multi-drug resistance, Antibiotics, Typhoid

INTRODUCTION

The UN Sustainable Development Goals state that by 2030, everyone should have access to clean and affordable drinking water (Karkey et al. 2016). Water is an essential need of all living creatures and is directly related to human life which makes the investigation of quality water important. According to studies, organic, inorganic, and biological components make surface water unfit for drinking. Moreover, global population growth, industrial expansion, and unsanitary circumstances are making water quality worse. In Asia, water and food sources are frequently contaminated with various pathogens, including the infectious microorganism *S. Typhi.* It is a gram-negative, non-spore-forming, rod-shaped bacteria that grows on differential and selective media and is anaerobic (Ranjbar et al. 2017). In Pakistan, many people depend on contaminated groundwater for daily use, leading to illnesses and fatalities. In addition to being dangerous and invisible to the naked eye, pathogens in water may also be tasteless and odorless. Serious infections including typhoid fever, hepatitis, or severe diarrhea can be brought on by *S Typhi* (Jahan et al. 2022). Antibiotics were prescribed to treat various bacterial infections. However, misuse or overuse of antibiotics develops antibiotic resistance in bacteria. Acquiring a resistant gene by beneficial bacteria from resistant species is a threat to the environment and for the coming generation (Banin, Hughes, and Kuipers 2017). *S. Typhi* is a disease-causing bacterium found in water and food. It causes typhoid fever in humans characterized by an increase in temperature, headache, upset stomach, and loose stool or constipation(Papadopoulos et al. 2016)

. The ratio of typhoid fever in underdeveloped states is higher as compared to developed states. It is transmitted through the fecal-oral route (Das et al. 2018). From the above notions, the researchers refer to it as the disease of poor countries. According to WHO, 21,000,000 cases of typhoid and 222,000 fatalities are reported each year (Watson and Edmunds 2015). This bacterium has received attention due to the causative agent of human diseases and their strong potential of susceptibility to antibiotics (Marchello, Carr, and Crump 2020). It has developed resistance to a variety of antibiotics, causing concern for 21 million people worldwide. A significant obstacle to treating typhoid fever is *S. Typhi* developing multi-drug resistance, which offers tolerance to several different treatment classes. It has increased morbidity and mortality rates throughout the world(Saxena, Ravinder, and Randhawa 2021). Our research study examines the resistance of *S. Typhi* to various antibiotics found in water.

MATERIALS AND METHODS

A total of 30 specimens were gathered in autoclaved capped bottles from Peshawar. All specimens were brought to the research and subjected to culture.

Preparation of SS Medium for Identification of *S. Typhi*

The water was autoclaved at 121℃ under 15 psi for 15 minutes for the preparation of SS medium in which bile salts (8.50 g) gram's iodine brilliant green (0.0003g), neutral red (0.025 g) sodium citrate (8.5 g) mixture of peptone (5 g), ferric citrate (1 g), lactose (10 g), Beef extract (5.0 g), sodium thiosulfate (8.5 g) and bacteriological media (13,5 g) were added with frequent spinning until homogenous mixture form. The media was cooled down to 50℃ in a water bath before pouring in sterilized Petri dishes. Subculturing was done using fourth quadrant streaking to obtain isolated colonies, the plates were incubated at 37℃ for 1-2 days (Sakagami et al. 2021).

Preparation of XLD Agar for Bacterial Identification

1000ml water was autoclaved at 121℃ under 15 psi for 15 minutes for the preparation of the XLD medium. in which sodium chloride (1g), xylose yeast extract (3g), ferric ammonium citrate (0.8g), lactose (5g), lysine hydrochloride (0.08g), phenol red (5g), sucrose (3.75g), sodium thiosulfate (7.5g), sodium deoxycholate (6.5g) were added and frequently spin until the homogenous mixture form. Media was cooled down to 50℃ before pouring in sterilized Petri dishes. Using the spread plate streaking technique, the samples were inoculated in SS agar plates and allowed for incubation at 37°C for 1-2 days.

Gram Staining

Using a sterilized dropper, a single droplet of normal saline was added to a sterile glass slide. Using a disinfected loop, a single colony was mixed in it to prepare a smear and heat fixed. After fixing the slide, crystal violet solution was added for 30-60 seconds. The smear was covered for 30 seconds, the slide was then washed with 95 % ethyl alcohol to unbind color. Counter-stain (safranin) was added for a half minute. The slide was again washed with distilled water and allowed to dry in the air. The slide was then observed under the microscope.

Biochemical Testing

Triple Sugar Iron Test

It is used to confirm the presence of *S. Typhi* phenotypically. It tests for acid and gas production from glucose and sucrose fermentation, as well as lactose and hydrogen sulfide production. The media was sterilized and poured into clean test tubes to allow for slant-position solidification. After solidification, the tubes were butt inoculated and then streaked on the surface of the agar slant. The results were recorded after the tubes had been incubated overnight.

Citrate Test

The citrate medium was prepared by autoclaving water at 121℃ for 15 minutes. The agar was added to sterilized distilled water and boiled at 100℃ on a plate. The same medium was cooled down to 50℃and poured into sterilized tubes. A well-isolated colony is taken from a whole night culture using a disinfected inoculating loop. The citrate agar tubes are inoculated by streaking the slant's surface. The loop helped the slant streak back and forth. The tubes were incubated for 1-2 days at 37℃

Indole Test

It was used to confirm the presence of *S. Typhi*. For this purpose, a drop of Kovacs indole reagent was added to the test tube. A single colony was mixed in it. The results were noted in between 1-3 minutes

Urease Test

Urea agar was prepared by autoclaving water at 121℃ under 15 psi for 15 minutes. Media was added into the sterilized distilled water and boiled at 100℃.It was then cooled down to 50℃and poured in sterilized tubes. A well-isolated colony was taken from the culture with a germ-free inoculating loop. The urease agar tubes were inoculated by streaking the surface of the slant and incubated for 1-2 days at 37℃. The results were recorded

Oxidase Test

A drop of oxidase reagent was added to filter paper. A single colony was mixed in it. The result was noted after a few minutes

Antibiotic Sensitivity Test

The Kirby-Bauer technique was used to examine the sensitivity of antibiotics. A single colony of *S. Typhi* was taken and mixed in saline solution and compared with a turbidity of 0.5 MFU using a Wickerham card. By using sterilize cotton swabs a bacterial lawn was prepared on MHA agar petri plates and allowed to dry then sterilized antibiotics discs were placed on MHA medium cultured plates and incubated at 37℃ overnight. The results were then observed. For disc diffusion, the antibiotics used in this study are given below. Ampicillin (10µg), Ciprofloxacin (5 µg), amoxicillin (10µg), Cefotaxime (30µg), Cefoxitin (30 µg), Aztreonam (30 µg), Gentamicin (10 µg), Amikacin (30 µg). The results were analyzed and recorded.

RESULTS

Growth of *S. Typhi* on SS Agar

SS growth medium was used for the selective isolation and characterization of *Salmonella* in water specimens. All the samples showing black-centered colonies on SS agar medium confirmed the presence of *S. Typhi*, as shown in Figure 1.

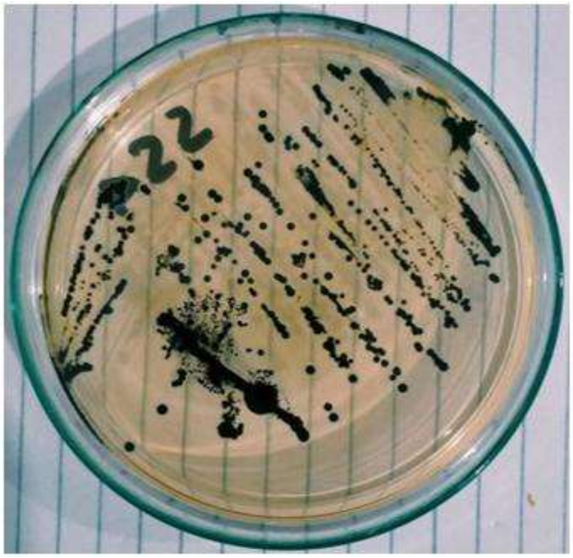

Figure 1: showing growth of *S. Typhi* on SS agar

Growth of *S. Typhi* on XLD Agar:

XLD is the media for the selective growth of *S. Typhi*. Therefore, the presence of *S. Typhi* in the sample was confirmed after the formation of black colonies in XLD media when spread on XLD agar plates as shown in Fig 2a and 2b

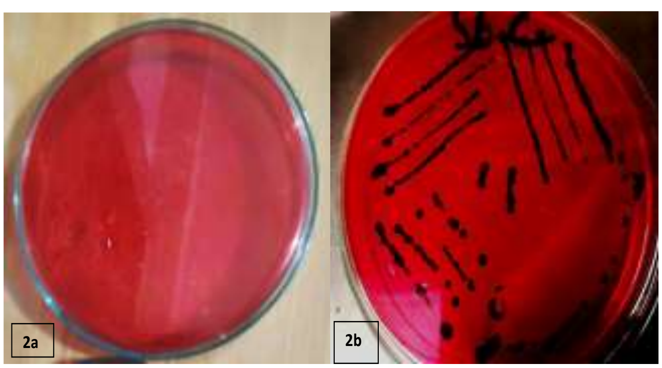

Fig 2a: Showing XLD negative plate. **Fig 2b**. Black-centered colonies showing growth of *S. Typhi* on XLD growthmedium

Biochemical Tests

Fig 3 shows the triple sugar iron and catalase test showed positive results, while the indole, urease, citrate, and oxidase tests exhibited negative results.

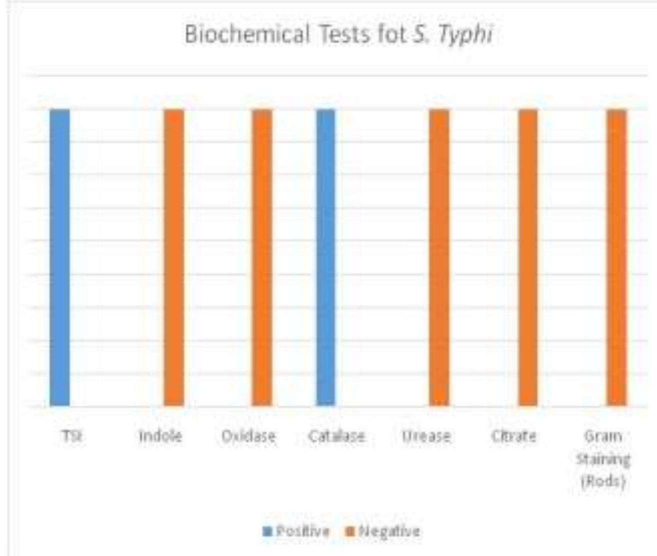

Figure 3: Showing results of Biochemical Tests

Antibiotic Sensitivity

Antibiotic sensitivity was performed for all the samples using the Kirby Bauer techniques as shown in figure 4. The antibiotics used and the diameter zones according to CLSI for the antibiotics and other results are given in table 1 and 2. Within 30 specimens, 18 were found positive for *S. Typhi*. The results showed that, Gentamicin, was (5 %) resistant, (5%) intermediate and (90%) sensitive, Cefotaxime was (100%) sensitive, and resistant, Ciprofloxacin was (5%) intermediate and (95%) sensitive, Penicillin was (100 %) resistant, Aztreonam and Amikacin were (100%) sensitive

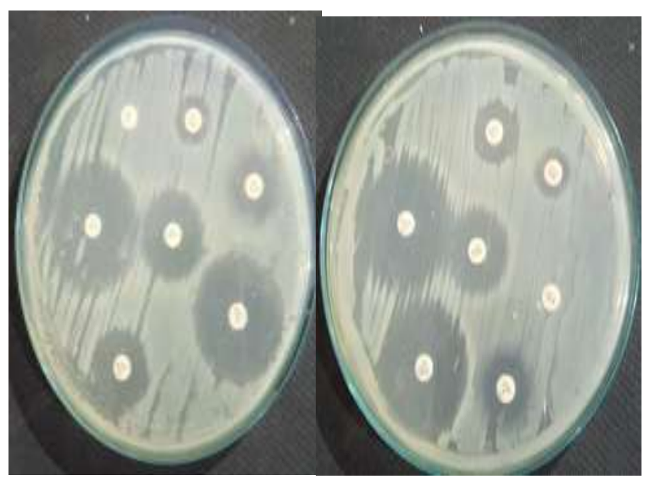

Figure 4: *S. Typhi* showing the sensitivity on the antibiotics in Muller Hinton agar media

Table 1: Results of Antibiotic Sensitivity Tests

Antibiotics	Disc weighted in µg	Resistance	Intermediate	Susceptible
ciprofloxacin	5	≤17	16-20	≥21
gentamicin	10	≤13	13-14	≥15
amikacin	30	≤15	15-16	≥17
cefoxitin	30	≤14	15-17	≥18
cefotaxime	30	≤14	23-25	≥26
aztreonam	30	≤17	8-20	≥21
penicillin	10	≤28	-------	≥ 29

Table 2: Inhibition zones of antibiotics used against *Salmonella typhi* isolates

Cefotaxime (30u)	Gentamicin (10ug)	Cefoxitin 30ug	Ciprofloxacin (5ug)	Penicillin (10ug)	Aztreonam (30ug)	Amikacin (30)
29mm (S)	10mm (R)	22mm (S)	21mm (S)	00mm (R)	35mm (S)	24mm (S)
32mm (S)	16mm (S)	22mm (S)	22mm (S)	4mm (R)	39mm (S)	25mm (S)
38mm (S)	14mm (I)	20mm (S)	16mm (I)	2mm (R)	34mm (S)	24mm (S)
31mm (S)	31mm (S)	19mm (S)	29mm (S)	5mm (R)	30mm (S)	20mm (S)
28mm (S)	22mm (S)	22mm (S)	28mm (S)	00mm (R)	24mm (S)	22mm (S)
31mm (S)	26mm (S)	26mm (S)	24mm (S)	3mm (R)	26mm (S)	27mm (S)
29mm (S)	25mm (S)	24mm (S)	26mm (S)	6mm (R)	29mm (S)	23mm (S)
30mm (S)	18mm (S)	20mm (S)	28mm (S)	6mm (R)	24mm (S)	31mm (S)
34mm (S)	21mm (S)	24mm (S)	30mm (S)	2mm (R)	28mm (S)	22mm (S)
31mm (S)	24mm (S)	22mm (S)	24mm (S)	00mm (R)	23mm (S)	29mm (S)
29mm (S)	19mm (S)	26mm (S)	27mm (S)	5mm (R)	26mm (S)	25mm (S)
27mm (S)	25mm (S)	21mm (S)	30mm (S)	3mm (R)	24mm (S)	28mm (S)
30mm (S)	23mm (S)	24mm (S)	28mm (S)	5mm (R)	31mm (S)	34mm (S)
33mm (S)	20mm (S)	20mm (S)	31mm (S)	2mm (R)	26mm (S)	30mm (S)
36mm (S)	23mm (S)	19mm (S)	34mm (S)	4mm (R)	28mm (S)	23mm (S)
34mm (S)	26mm (S)	23mm (S)	29mm (S)	1mm (R)	30mm (S)	27mm (S)
31mm (S)	22mm (S)	25mm (S)	27mm (S)	5mm (R)	24mm (S)	22mm (S)
34mm (S)	25mm (S)	22mm (S)	31mm (S)	4mm (R)	31mm (S)	28mm (S)

DISCUSSION

Water is essential for every living creature, hence there is no concept of life without it. Water purity and pollution are two of the biggest issues in the world today since contaminants in water can directly endanger human health. Water can spread a variety of pathogens to consumers, thus it is important to ensure early detection of contamination(Israr et al. 2022). The presence of pathogenic microorganisms in drinking water causes a wide variety of diseases. According to the WHO, 3.4 million children die from water-borne diseases per annum. In the last 20 years, multi-drug resistant (MDR) Salmonella enterica strains have spread throughout the world. Overconsumption of antibiotics has dramatically accelerated the evolution of their resistivity in humans(Robertine et al. 2021). Antibiotic resistance, which is thought to be responsible for 700,000, annual fatalities, has increased pressure on human healthcare during the previous few decades. Resistant genes have frequently been linked to the environment, however, it is unknown how many antibiotics in the environment are responsible for this development (Sun et al. 2020). Several reports claim that tourists from Pakistan have transmitted XDR typhoid to other countries. Its prevalence increases in advanced states when sanitation conditions are poor and water is at risk of contamination. Each year, there are several million cases and more than 0.2 million fatalities. Thankfully, typhoid illness is now quite rare in advanced countries. According to the record from 2008 to 2015, about three hundred fifty cases of typhoid were registered in the US which is 1 case in every 0.2 million individuals. This decrease in cases is due to *S. Typhi*'s ongoing susceptibility to drugs like third-generation cephalosporin (Israr et al. 2022). Since the end of the last century, these antibiotics were considered the first choice for infection treatment. However, the ratio of typhoid fever cases has gradually reduced in developed countries (da Silva et al. 2022). On the other hand, the record of typhoid fever from Karachi showed an increase in the ratio of MDR *S. Typhi*. In 2009 and 2011, two young patients were reported to have sporadic resistance to ceftriaxone (Bengtsson-Palme and Larsson 2016). In the current study, 30 samples were obtained for the screening, where after screening 60% of specimens were found *S. Typhi* positive. Moreover, the results showed that Gentamicin was (5 %) resistant, (5%) intermediate and (90%) sensitive, Cefotaxime was (100%) sensitive, and resistant, Ciprofloxacin was (5%) intermediate and (95%) sensitive, Penicillin was (100 %) resistant, Aztreonam and Amikacin were (100%) sensitive. Israr et al in their study for antibiotic sensitivity used the disc diffusion method where the resistance of *S. Typhi* was shown to be 90.9% (ampicillin), 31.8% (ceftriaxone), 40.9% (sulfamethoxazole), and 22.7% (chloramphenicol). Further, the research study of Anjum et al in 2021, records evidence of the resistivity of *S. Typhi* against antibiotics and showed 96%, resistance to ampicillin,94.7% to ceftriaxone, 82.2% to ciprofloxacin,98.7% to chloramphenicol, and 2.63% to sulfamethoxazole. According to research published by Umer Rashid et al., typhoid is 42.32 % prevalent in people aged 21 to 30 years, followed by 30.42 % in people aged 11-20 years. In children below 10 years, it is about 8.46 % prevalent (Rasul et al. 2017). Furthermore, the research study published by Agha Khan University, shows the rise in the prevalence of MDR *S. Typhi* from 34.2% to 48.5% (Britto et al. 2018). Our research aims to evaluate the existence of *S typhi* in the drinking water sources of Peshawar city. Among the samples, *S. Typhi* which produced an enzyme, showed a high level of resistance to ampicillin and amoxicillin. While cefotaxime, gentamicin, cefoxitin, ciprofloxacin, aztreonam, and amikacin antibiotics were found to be effective against *S. Typhi*. In previous studies, the resistant *S. Typhi* to ampicillin and azithromycin was reported (El-Prince, Hussein, and Abd El-Rahman 2019).. The findings in Metropolis declare *S. Typhi* intermediate resistant to Gentamicin. Based on these findings their water may be possibly polluted with *S. Typhi*, while in the current study in contrast *S. Typhi* showed a sensitive pattern against Gentamicin.

CONCLUSION

Currently, the diseases are challenging to treat because of bacterial resistance to antibiotics. This research project was designed to evaluate the resistant profile of *Salmonella typhi* in drinking water. The results disclose the presence of resistant *S. Typhi* in water. For the expanding population, the sudden rise in such germs poses a serious health risk because antibiotics cannot effectively combat them. Requires immediate attention to overcome this problem which requires following sanitation measures and personal sterility. Installation of a water filter is highly recommended to prevent water-borne diseases

Supplementary materials

All relevant materials are included in this manuscript.

Author contributions

Dr. Hamid Hussain Afridi supervised the project. Hamid Hussain Afridi, Muhammad Khan, Khawaja Ejaz Ul Haq, Hafiza Misbah Ahmad, and Irfan perform the experiment. Abdul Malik, Asghar Ali, Muhammad Haroon, Faraz Ahmad Khan and Rizwan Arif review, validate the analysis, statistics and Grammer. At final stage all the authors have written the manuscript and agreed to submit it.

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Institutional Review Board Statement

Not applicable

Informed Consent Statement
Not applicable.

Data Availability Statement
All of the relevant data is included in the paper.

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Conflict of interest
All the authors have declared no conflict of interest.

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