Prevalance and Antimicrobial Susceptibility Patterns of *Salmonella Typhi* and *Escherichia Coli* in Drinking Water of Sub-Division Hassan Khel Peshawar

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ABSTRACT

Globally outbreaks of water-borne diseases are extensively threatening public health. In Pakistan, various reports confirmed different diseases regarding drinking water including typhoid fever, diarrhea, urinary tract infection, anemia, and kidney failure. The causative agents associated with diseases are *Salmonella typhi* and *Escherichia coli*. *S. typhi* and *E. coli* both are gram negative rod shaped bacteria spreading through contaminated drinking water by the fecal-oral route due to non-hygienic and poor sanitation environments. Both bacteria are emerging Multi-Drug Resistant (MDR) organisms, increasing morbidity & mortality rates. The current study was designed to evaluate the presence of *S. typhi* and *E. coli* in the drinking water of Sub-Division Hassan Khel Peshawar. Further, to determine MDR bacteria from water and screening ESBL producing bacteria, and detect heavy metals in drinking water. A total of 100 drinking water samples collected from different drinking water sources aseptically, from the studied area from September 2020 to September 2021 at a research laboratory of Abbottabad University of Science and technology. The collected water samples were inoculated on *Salmonella Shigella* Agar and Eosin Methylene Blue Agar. Bacterial Identification was done using gram staining and biochemical tests. Muller-Hinton agar was used for the determination of antibiotic resistance. Furthermore, the analysis of heavy metals (cadmium, copper, and lead) was done by atomic spectrometry. The overall prevalence of *S. typhi* and *E. coli* was 22 and 52% respectively in drinking water. Most of these *S. typhi* were resistant to AMP (90.9%), followed by S (45.4%) SFX, SXT (40.9%), CRO, CXM, FEP, CAZ (31.8%), AMC, CIP (27.3%), C, TE (22.7%). While all isolates were 100% sensitive to AZM. Out of 22 S. typhi isolates 7(31.8%) were ESBL producers. Furthermore, out of 52 isolates of *E. coli,* most were resistant to AMP (82.6%), followed by S (44.2%) SFX (42.3%), TE (34.6%) C, SXT (32.7%), CRO, CXM, FEP, CAZ (30.7%), AMC (26.9%) and CIP (21.1%). While all isolates of *E. coli* were 100%

sensitive to AZM.16 (30.7%) *E. coli* isolates were ESBL producers. Furthermore, overall out of 100 drinking samples, heavy metals were detected in 72% samples. Copper was the most common metal found in 61.0% of samples followed by lead and cadmium 55.0% and 48.0% respectively. This study concluded that water used for drinking purposes in the study area is currently not protected under government authorities. It is highly recommended for government authorities to manage water purification plants that could prevent transmission of pathogenic microbes and save the masses of this territory from water-borne diseases.

Key Words: Prevalance, Antimicrobial Susceptibility Patterns, Salmonella Typhi, Escherichia Coli .

Introduction

The use of antibiotics for treatment of different types of infections and diseases caused by pathogenic bacteria is one of the popular choices. After its introduction in the early 20th century till date, it is used against several life threatening diseases and infections. However, the misuse and overuse resulted in the development of antibiotic resistance. Bacteria with passage of time gained the resistance genes via mutation or by cross horizontal gene transfer[1]. This resistance to antibiotics resulted in the emergence of new infections which are difficult to be treated with the previously drug of choice for the very same infection. This antibiotics resistance has raised the concerns of resistance in future of the current antibiotics if not tackled properly[2]. Antibiotic-resistant genes imparting resistance to a wide range of antibiotics have been identified in a number of water sources including drinking water in both advanced and emerging economies[3, 4]. The major threat for public health is the transfer of resistant genes from bacteria found in environment to those bacteria that are clinically important and are pathogenic in nature. Contamination of drinking water and infection from it is of main concern which can ultimately cause diseases and loss of lives[5, 6]. Furthermore, having access to safe drinking water is critical for avoiding pandemic disease and enhancing overall quality of life [7, 8]. As stated by the World Health Organization, contaminated water is responsible for 80% of all infections[9]. Water-borne illnesses like diarrhoea, which computing for 10% of the disease load in developing countries, are particularly prevalent [10]. *Escherichia coli* is coliforms that can contaminate drinking waterwhen it comes into contact with human or animal faeces.For decades, *E. coli* has been the most reliable indicator of faecal contamination in water quality monitoring.The coliforms may be swept into creeks, rivers, streams, lakes,streams, or ground water during heavy rains. Coliforms can be found in untreated drinking water from these sources. E. coli has demonstrated its propensity to be a substantial repository of antimicrobial drug resistance genes, making it a useful indication of resistance in bacterial groups[11, 12]. Salmonella spp are Gram-negative facultative anaerobe bacteria found in humans, as well as in animals, and environment [13]. Salmonellae are the most common pathogenic bacteria in both humans & animals [14, 15]. Across several countries, they constitute the principal cause of acute gastroenteritis, & salmonellosis is still a major public health concern worldwide [14]. Salmonella species were found in 8.5 percent (342) of river water samples in Canada, which were impacted byrunoff,rainfall, and drainage from agricultural land [16].Salmonella species were found in 16 percent (87) of pond water samples in Czech [17]. The reported prevalence of Salmonella species in multiple river samples in South Africa ranged from 33 to 90% [18].

Typhoid is an infectious disease that is transmitted to humans by contaminated food & drinking water. This infection is spread by fecal-oral rout and causes numerous public health issues in under developing countries[19]**.** Only humans are its natural host. Typhoid fever is endemic in many

developing countries because of poor sanitation, non-hygienic environment and limited access to safe drinking water[20]. It is a severe multi-systemic infectious disease that is the leading cause of death globally with over more than 17 million cases accounted each year. About 80% of morbidity and mortality rate happens in Asia each year[21].

Drinking water usually contaminated by nearby drainage. The majority of the households lack access to safe drinking water. Lack of waste management contaminate both and ground water. Typhoid fever is usually connected with lower and middle-income groups due to these challenging circumstances [22].The growing Multi Drug Resistance in *S. typhi*, which offers resistance to various classes of drugs, is a major barrier to managing Typhoid fever. MDR *S. typhi* has increased the morbidity rate as well as mortality rates all over the world[23]. Chloramphenicol, Trimethoprim-sulfamethoxazole, and Ampicillin were were the first-line medications for typhoid fever. Soon after that, second-line antibiotics the fluoroquinolones became the preferred treatment in regions and resistance to them arose soon as a result of their widespread use. A third-generationCephalosporin, Azithromycin, and Ceftriaxone are currently utilized to treat typhoid fever[24].

Escherichia. coli is a Gram-negative, facultative anaerobic bacterium with a rod shape. The majority of *E. coli* strains colonize the GI tracts of animals and humans as part of a healthy flora. However, some strains contain a potent toxin that can lead to serious illness[25]. Most strains of *E. coli* are harmless, but some strains acquire DNA encoding enterotoxin & invasion factors by and become pathogenic. These pathogenic strains responsible for diarrheal diseases globally, as well as for meningitis in neonates, septicaemia, & urinary tract infections.Human or animal waste may often introduce *E. coli* into pools, streams, and water bodies. People can become infected upon swallowing contaminated water while swimming in a pool, lake, or irrigation canal. *E. coli* can also transmit from one individual to other,when an infected individual does not wash their hands[26].
The development of Multi Drug Resistance Urinary tract infections caused by *E. coli* with a high virulence potential are of great concern[27].

1. **Material and Methods:**

3.1 Samples Collection and isolation of *S. typhi* and *E. coli*

Water samples (100) were collected from Sub-division Hassan Khel Peshawar located about 35 km away from Peshawar city. Samples were collected from primary and secondary sources i.e. Wells, springs, and bores water. Water for bacterial analysis was aseptically collected in sterile caped bottles. All samples were carried out to the laboratory and analysed within 6 hours of its collection followed by bacterial analysis. Both SS (*SalmonellaShigella* agar) agar and EMB (eosin methylene blue) agar plates were prepared according to the manufacturer's instructions. After inoculation, the media were incubated for 24 hours at 37°C and processed further for identification of bacteria.

Bacterial identification:

Samples were processed for colony morphology and Gram staining. Biochemical properties were also tested using biochemical tests.Catalase, glucose and motility test were positive and oxidase, urease and indole test were negative for *S. typhi*. While catalase indole glucose and motility test were positive, and oxidase citrate and urease test were negative for *E. coli*.

Antibiotic Susceptibility test

Antibiotic susceptibility was performed via Kirby bauer disk diffusion method. For this purpose, different antibiotics were used as given in the table. Fresh overnight cultures were used having turbidity of 0.5 mcfarland. The media used for sensitivity was muller hinton agar.

Data analysis

Data was analysed using SPSS version 21 using the descriptive statistical tool. Data was presented in the form of tables.

Results

100 drinking water samples from different water sources, e.g., wells, springs, and tub wells, were screened for S. typhi and E. coli. Out of 100 samples, 58 were collected from bore water, 29 were from open wells, and 13 were collected from stream sources. Out of 100 samples, 22 were positive for *S. typhi* and 52 were positive for *E. coli.* The stream water sources showing high prevalence (84.6%) for *S. typhi* werefollowed by open wells (24.1%) and bore water (6.8%). *E. coli* was found in most stream samples (84.6%), followed by open wells (69.9%) and bore water (36.2%).

S. typhi and E. coli were found in drinking water sources in Sub-Division Hassan Khel.

SN.	Organism	Source	Total sample	Positive Samples	Percentage
1	*Salmonella typhi*	Bore water	58	4	6.8%
		Open wells	29	7	24.1%
		Stream	13	11	84.6%
2	*Escherichia coli*	Bore water	58	21	36.2%
		Open wells	29	20	69.9%
		Stream	13	11	84.6%

Antibiotic susceptibility testing was done by using the Kirby-Bauer method for *S. typhi* and *E. coli*. Most of these *S. typhi* were resistant to Ampicillin (90.9%), followed by Streptomycin (45.4%), Sulfisoxazole, Trimethoprim-Sulfamethoxazole (40.9%), Ceftriaxone, Cefuroxime, Cefepime, Ceftazidime (31.8%), Amoxicillin-Clavulanate, Ciprofloxacin (27.3%), Chloramphenicol, and Tetracycline (22.7%). While all isolates were 100% sensitive to azithromycin, Furthermore, out of 52 isolates of *E. coli,* most were resistant to ampicillin (82.6%), followed by streptomycin (44.2%), sulfisoxazole (42.3%), and tetracycline (34.6%). Chloramphenicol, Trimethoprim-Sulfamethoxazole (32.7%), Ceftriaxone, Cefuroxime, Cefepime, Ceftazidime (30.7%), Amoxicillin-Clavulanate (26.9%) and Ciprofloxacin (21.1%). all isolates of *E. coli* were 100% sensitive to azithromycin.

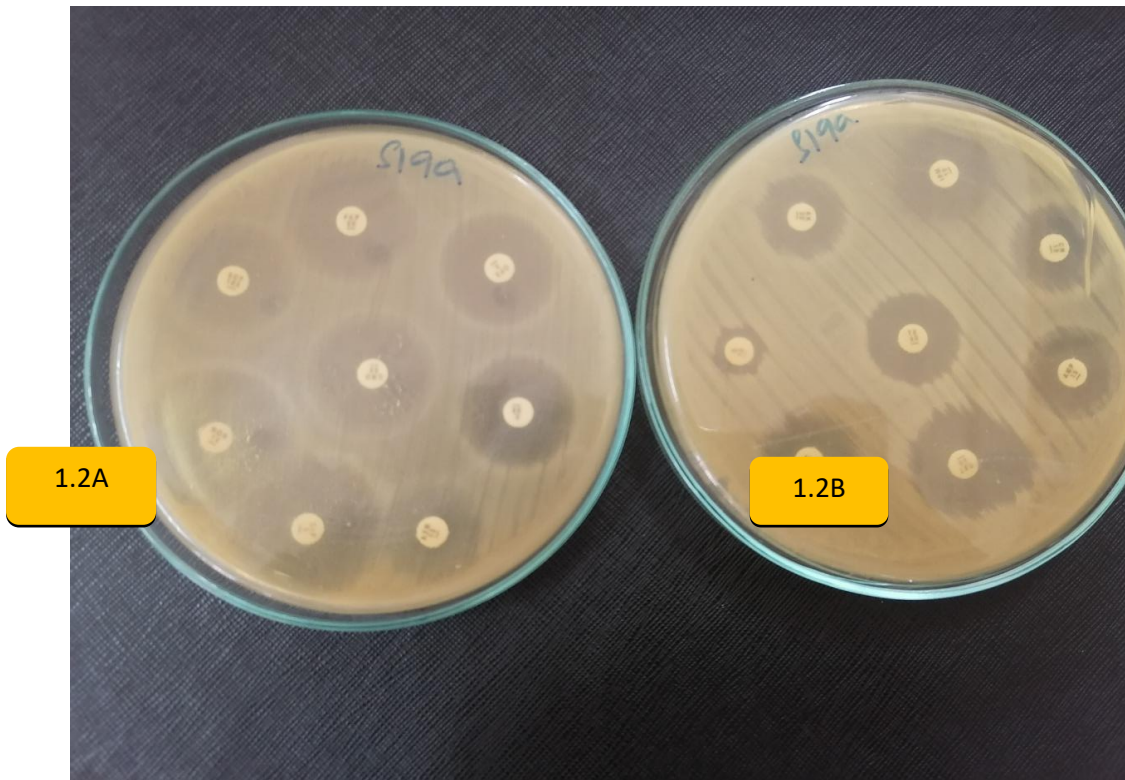**Figure: 1.1** *E. coli* **showing sensitivity on the antibiotics in Muller Hinton agar media.**


Figure: 1.2 *S. typhi* **showing sensitivity on the antibiotics in Muller Hinton agar media.**

S.N	Antimicrobial agent	Disc code	Potency μg	Resistant	Intermediate	Susceptible
1	Ampicillin	AMP	10	≤13	14-16	≥17
2	Amoxycillin-Clavulanate	AMC	20/10	≤13	14-17	≥18
3	Azithromycin	AZM	15	≤ 16	N/A	≥ 32
4	Ceftriaxone	CRO	30	≤19	20-22	≥23
5	Ceftazidime	CAZ	30	≤17	18-20	≥21
6	Cefuroxime	CXM	30	≤14	15-17	≥18
7	Ciprofloxacin	CIP	5	≤20	21-30	≤31
8	Cefepime	FEP	30	≤14	15-17	≥18
9	Chloramphenicol	C	30	≤12	13-17	≥18
10	Streptomycin	S	10	≤11	12-14	≥15
11	Sulfisoxazole	SFX	300	≤12	13-16	≥17
12	Tetracycline	TE	30	≤11	12-14	≥15
13	Trimethoprim-Sulfamethoxazole	SXT	1.25/23.75	≤10	11-15	≥16
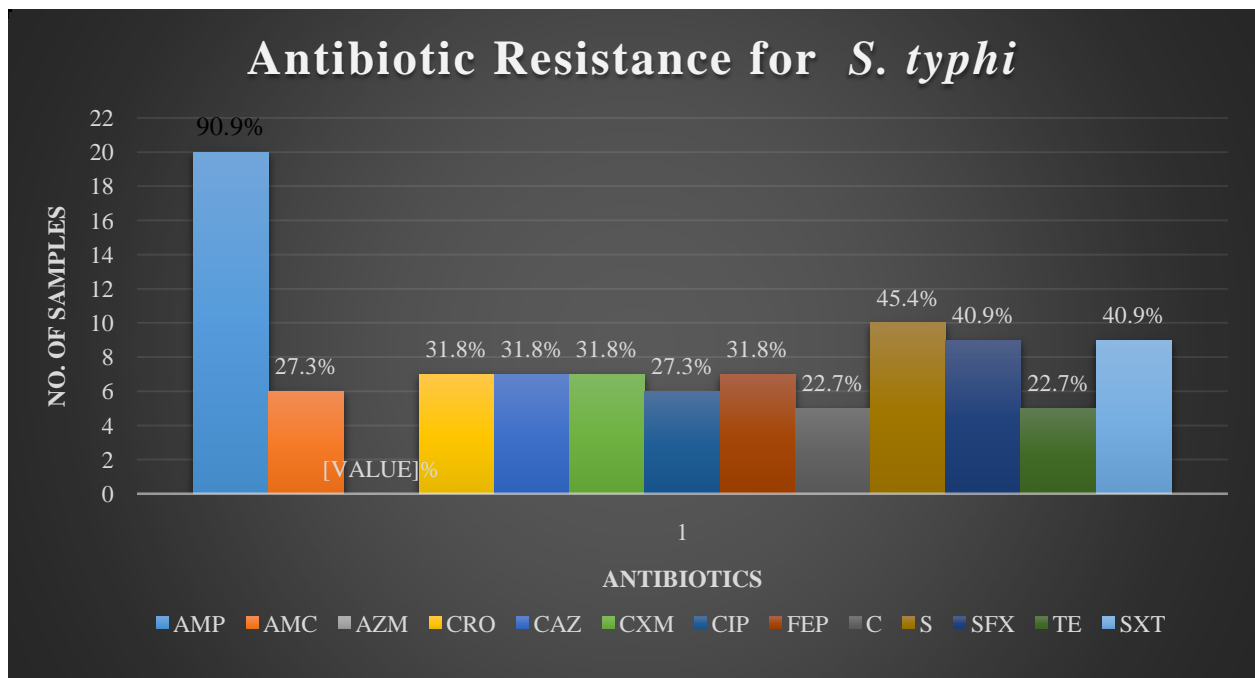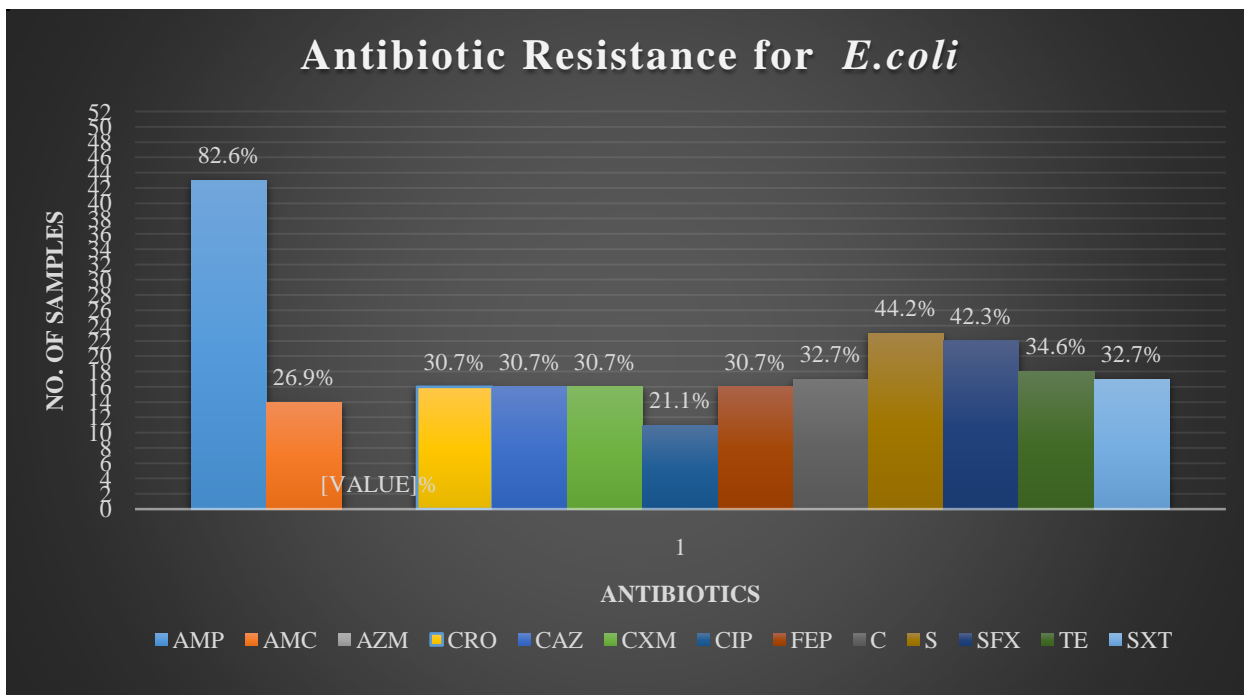
4. Discussion:

Several diseases arise from pathogenic oragnisms including bacteria, viruses, fungi and parasites are due to drinking of contaminated water. Contamination of water is due to several different reasons including anthropogen activities as well as due to natural causes and contamination by animals. This results in the transmission of diseases when used for drinking and other purposes to humans[28].several Studies reported microorganisms responsible for outbreaks caused by drinking water contaminated by *Vibrio cholerae*, *Shigella sp.*, *S. typhi*, *Campylobacter*, *Escherichia coli*, *Cryptosporidium parvum*, *Entamoeba Entamoeba histolytica*, *Giardia* sp. *Balantidium coli*, rotavirus, hepatitis A virus, hepatitis E virus, *Leptospira interrogans* poliovirus[29].

The current study focused on the find the prevalence of *S. typhi* and *E. coli* and their antimicrobial susceptibility in drinking water of sub division Hassan Khel. For this purpose, hundred water samples were collected from different areas of Hassan Khel. This Study also focuses on detection of heavy metals like cadmium, lead and copper. Results showed that out of 100 samples, 22% were positive for *S. typhi,* while 52% samples showed presence of *E. coli*. After analysis, it was found that cadmium was present in 48%, copper was found in 61%, and Lead was found in 55% collected samples.

In 2018 one of the study reported by Qamar, Farah Naz, et al., from Pakistan the presence of *S. typhi* in water samples. Results showed that the prevalence was high in the samples collected near sewage lines. The epidemic curve, implying ongoing contamination of water sources, maybe due to sewage water mixing with municipal water [30]. Another study conducted in 2021 showed the same prevalence results as the current study[31]. In Nigeria in 2020, a study was published, revealing the highest number of *S. typhi* in drinking water samples[32]. As reported in the current study, the prevalence of *S. typhi,* was reported by Osei, Frank B., et al.[31]. In 2021 a study showed 64% of *S. typhi* reported in drinking water samples[33].

According to several reports, Pakistani travellers have exported XDR typhoid to the United States, the United Kingdom, Australia, Denmark, Taiwan, and Canada. It is endemic in many developing

countries where water and food are potentially contaminated, and sanitation is inadequate. Several million cases occur each year, resulting in more than 0.2 million deaths[34]. Fortunately, typhoid fever has become relatively uncommon in developed countries. From 2008–2015, about 350 confirmed cases of typhoid fever were reported to the Center for Disease Control and Prevention in the United States, with a rate of fewer than 0.5 cases per 0.1 million people[35]. This rarity has been linked to the continued sensitivity of most *S. typhi* to antibiotics such as third-generation cephalosporin[36].

Antibiotics were considered first drug of choice for the treatment of infections till the end of last century. However, interest in typhoid fever has gradually declined in industrialized countries. For example, surveillance data from Karachi, Pakistan, revealed an increase in the prevalence of MDR *S. typhi* resistance to ceftriaxone which was reported in two isolates collected from children between 2009 and 2011[37]. Since 2016, a large proportion of cephalosporin-resistant isolates have been reported from Sindh, primarily from the big cities i.e. Karachi and Hyderabad. Furthermore, another case reported from UK in a patient with recent travel history to Pakistan[38].

After antibiotic sensitivity via disk diffusion method of the current study showed that *S. typhi* was resistant to ampicillin, ceftriaxone, sulfamethoxazole/trimethoprim, and chloramphenicol, 90.9%, 31.8%, 40.9%, and 22.7% respectively. Similarly, accordingto the previous report published by *Osei, et al.,*in 2021 showing similar resistance to the same antibiotics [31].Another study confirmed the presence of MDR strain of *Salmonella enterica serovar typhi* in the drinking water samples[39]. In 2020 Pakistani authors Umair, Masab, and Shajee Ahmad Siddiqui, published a report which showed highest number of resistance to amoxicillin 57.6%, co-trimoxazole 61.4%, chloramphenicol 46.9%) and ciprofloxacin (62.7%). Antibiotic resistance rates were lowest for ceftriaxone (4.4%)[40]. In 2021 *Anjum et al.,* collected data about antibiotic resistance profile of S. typhi, revealed resistance of 98.7% to chloramphenicol and trimethoprimsulfamethoxazole, 96% resistance to ampicillin, 94.7% to ceftriaxone, 82.2% to ciprofloxacin, 2.63% to azithromycin[41].

According to *Khan et al.,* the resistance ratio of *S. typhi* for ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole were 82%, 88% and 89% respectively. Percentage of MDR isolates in *S. typhi* was found to be 74%. Resistance to ciprofloxacin against *S. typhi* was also found to be high (53% isolates). XDR isolates in *S. typhi* were significant (27% isolates). All isolates were found sensitive to azithromycin [42]. while current study also found *S. typhi* 100% sensitive to azithromycin. A similar high resistance was reported by *Abbasi et al.,* in 2021 showing resistance to Tetracycline 55.6%, Cotrimoxazole, Ampicillin, and Chloramphenicol 33.3% for each[43]. A similar study was conducted in Anhui, China in July 2021. Study reported the prevalence of resistance was 87.5% for ampicillin, 62.5% for tetracycline, 50.0% for Sulfisoxazole, and most of the isolates were sensitive to ciprofloxacin (87.5%) [44].

In this study, all of *S. typhi* were sensitive to azithromycin. As a result, the only therapeutic choice for typhoid fever is azithromycin. The only antibiotic that can be used to treat XDR *S. typhi* is azithromycin. Azithromycin resistance in *S. typhi* has already been identified, but resistance in XDR *S. typhi* isolates will represent a significant issue.[45]. Preliminary research of azithromycin for thr treatment of SARS-CoV-2 led to abuse and misuse of azithromycin in even suspected COVID-19 patients[46]. This will increase the menace of azithromycin non-susceptibility in XDR *S. typhi* due to the unprecedented use of azithromycin in Pakistan. If the use of azithromycin is not regulated, it will be a ticking time bomb in Pakistan, with typhoid fever becoming untreatable.

In June 2016, a survey was done byLyimo, Beatus, et al.*et al* which was focused on the prevalence and antimicrobial pattern of *E. coli* in drinking water. Study reported that among 878 samples, 41% was positive toward *E. coli* among which 28% *E. coli* were multi drug resistant. The current study showed similar results of prevalence as well as antimicrobial resistance[47]. A study conducted by Ahmed, Jamil, et al. on behalf of the assessment of drinking water quality showed around 1/2 of the water samples were contaminated with *E. coli* about 49% [48]. A similar prevalence study was conducted by Tahri at el., In Tadla Morocco, 31.37% has been identified as *Escherichia coli*. Similarly, the antimicrobial activity of *E. coli* strains reveals a high level of resistance among all antibiotics tested[49].

Another study was conducted by Odonkor, et al., in 2018, according to their results, Multidrug-resistant *E. coli* was found in 49.48% of cases. Antibiotic resistance patterns in *E. coli* isolates were found to be very high. Penicillin 32.99%, cefuroxime 28.87%, and tetracycline resistance were the most common 21.45% [50]. While the current study revealed, the prevalence of *E. coli* was 52% similar to the above study. Another recent study conducted by Odetoyin et al., in Nigeria, reported 40.6% wells were contaminated by *E. coli causing* diarrhea[51] which were similar results to the current study. Similar study was done by Cho et al., in Georgia in 2018, Tetracycline resistance was most often detected, followed by ampicillin, streptomycin Sulfisoxazole[52]. Current study showed resistance to ceftriaxone and chloramphenicol 34.6% and 32.7% respectively. While a study conducted by Mahmoud et al., the isolates showed a high rate of tetracycline resistance 97.7%, followed by, ciprofloxacin 46.6%, while similar results to ceftriaxone 35.5%, and chloramphenicol 31.1% [53]. According to Rayasam et al., out of the performed samples, 51 contained coliforms, and 19 of the 51 were *E. coli*. All of the isolates showed resistance to ampicillin. Resistance to Ceftazidime was observed in 52 isolates, sulfamethoxazole–trimethoprim in 46, ciprofloxacin in 30 [54].

Another study in the year 2019 was conducted by L.D.Lawwyne. The main purpose of the study was to assess the prevalence of *E. coli* and *Salmonella* spp. in poultry drinking water and cloacal swabs, as well as their antibiotic susceptibility. Study reported that 10.7% of samples in 2018 and 7.1% in 2017. Study further reported that *E. coli* isolated was highly resistant to ampicillin and tetracycline. Talking about current study, the current study reported the ratio of *E. coli* to be 52% which were highly resistant to ampicillin[55].

Furthermore, the current study also evaluated the presence of heavy metals (cadmium, copper and lead) in drinking water of Sub-Division Hassan Khel. The levels of cadmium, copper and lead were found in range of 0.0-0.11, 0.0-0.13 and 0.0-0.09mg/L respectively. Another study reported by Pudza et al., in 2020 revealed concentration of cadmium, copper and lead in a range of 0.01-0.27, 0.01-0.27 and 0.01-0.2mg/L respectively[56]. similar study was conducted in Kerman city, Iran by Sarvestani et al., in 2019, reported concentration of cadmium 0.0-0.002, copper 0.0-0.03 and lead 0.0001-0.21[57].

Conclusion

Our Current study Conclude that the overall prevalence of *S. typhi* and *E. coli* was 22 and 52% respectively in drinking water. Most of these *S. typhi* were resistant to AMP (90.9%), followed by S (45.4%) SFX, SXT (40.9%), CRO, CXM, FEP, CAZ (31.8%), AMC, CIP (27.3%), C, TE (22.7%). While all isolates were 100% sensitive to AZM. Out of 22 S. typhi isolates 7(31.8%) were ESBL producers. Furthermore, out of 52 isolates of *E. coli,* most were resistant to AMP (82.6%), followed

by S (44.2%) SFX (42.3%), TE (34.6%) C, SXT (32.7%), CRO, CXM, FEP, CAZ (30.7%), AMC (26.9%) and CIP (21.1%). While all isolates of *E. coli* were 100% sensitive to AZM.16 (30.7%) *E. coli* isolates were ESBL producers. Furthermore, overall out of 100 drinking samples, heavy metals were detected in 72% samples. Copper was the most common metal found in 61.0% of samples followed by lead and cadmium 55.0% and 48.0% respectively. This study concluded that water used for drinking purposes in the study area is currently not protected under government authorities. It is highly recommended for government authorities to manage water purification plants that could prevent transmission of pathogenic microbes and save the masses of this territory from water-borne diseases.

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