

Microbiology and Evaluation of Antibiotic Resistant Bacterial Profiles of Drinking Water in Peshawar, Khyber Pakhtunkhwa

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Abstract: This research work presents a study on drinking water quality in District Peshawar, Khyber Pakhtunkhwa Pakistan, which involved bacteriological analyses and evaluation of antibiotic resistant bacteria from different drinking water sources. The drinking water sources included tube well, hand pump and tap water. Total 30 water samples were analyzed for total plate count, total coliform bacteria, fecal coliform, *E.coli*, *Salmonella typhi*, *Shigella* spp, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Vibrio cholera*, yeast and mold. Drinking water samples contaminated with *E.coli*, *Salmonella typhi*, *Shigella* spp, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Vibrio cholera*, were selected and tested for their susceptibility to fourteen different antibiotics by disc diffusion method. The study showed that minimum TPC values were 25 CFU/ml and maximum values were 5600 CFU/ml, minimum total coliform values were <1.1 MPN/100ml and maximum values were >23 MPN/100ml, 20% samples were contaminated with fecal coliform, 16.67% samples were contaminated with *Salmonella typhi*, 26.67% samples with *Pseudomonas*, 26.67% samples with *Vibrio cholera*, 26.67% samples with shigella spp, 40% samples with *Klebsiella pneumonia*, 56.66% samples with yeast and 13.3% samples with mold. Susceptibility studies showed that *Salmonella typhi* was highly resistance to Amoxicillin, Nalidixic Acid and Cephalothin, *Pseudomonas aeruginosa* was highly resistance to Amoxicillin, Ceftriaxone, Sulphamethoxazole/Trimethoprim and Ceftazidime, *Vibrio cholera* was highly resistance to Amoxicillin, Ceftriaxone, Sulphamethoxazole/Trimethoprim, Tetracycline, Nalidixic Acid and Cephalothin. *Shigella* spp was highly resistance to Amoxicillin, Sulphamethoxazole/Trimethoprim, Nalidixic Acid and Cephalothin and *Klebsiella pneumonia* was highly resistance to Amoxicillin, Sulphamethoxazole/Trimethoprim, Nalidixic Acid and Cephalothin. Based on the results it is suggested that authorities should pay attention to supply safe water and proper sanitary facilities to avoid epidemics of infectious diseases in future.

Key words: Drinking Water Contamination • Coliform Bacteria • Antibiotic Susceptibility Water Borne Diseases.

INTRODUCTION

Water is one of the essential natural resources given by the creator. In Holy Quran it's stated that "We have kept alive everything from water". So it proves that without water life can't go on. We can live without food for many days or weeks, but without water we can die less than a week [1]. Water is a basic requirement for all

living creatures; among them the most important use is drinking [2]. It has been examined that surface water is not suitable for drinking purposes due to organic, inorganic and biological components. Other purposes include cooking, bathing, washing and use in industries and agriculture [3]. The nature of drinking water is very essential to human being, because it is connected to human life [4].

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Clean and safe drinking water is one of the most important requirements for healthy human life [5]. Water quality get weaken due to increase in population, industrial development and unhygienic conditions in all parts of the world [6]. In Pakistan fresh water sources are glaciers, rivers and lakes but due to low rainfall and snowfall, Pakistan is suffering from water deficit. So to solve this problem, people rely on ground water. It is an important natural resource for various human activities [7]. In developing world water pollution is one of the alarming issues, especially caused by animal waste, industrial, household effluent and heavy metals. High concentration of the heavy metals like arsenic and lead has been found in water both in developed and developing countries [8].

Contaminated water may cause direct threat to human health. So the purity and contamination of water is one of the major problems of the world. Rivers, streams and other different reservoir may look clean and have no undesirable odor or taste. Pathogens found in water are not harmful but also invisible to naked eye which may be odorless and tasteless. These bacteria can cause more serious illnesses, such as severe diarrhea, hepatitis or typhoid fever. Water taken from different reservoirs should always be free of contamination or disinfected before being used for drinking or cooking [9].

Analysis of water quality can be done by counting the visible colonies of indicator bacteria, e.g. total coliform, fecal coliform and *E.coli* [10]. *Salmonella typhi* causes typhoid fever and *Vibrio cholera* causes cholera. Both are the important pathogenic bacteria transmitted by water route [11]. *Pseudomonas aeruginosa* found very commonly in nature and can be isolated from a large number of natural sources. An attention has been given to this bacterium because of its increasing significances as a human pathogen and its high resistance to most antibiotics [12].

Staphylococcus aureus is one of the most common agents of food poisoning [13]. *Klebsiella pneumonia* is isolated from the environment, particularly from nutrient-rich industrial effluents such as pulp and paper mill wastes, textile finishing plant effluents and sugarcane wastes [14-16]. *Shigella* is a gram-negative, facultative intracellular pathogen. *Shigella* spp. can clearly be considered waterborne diseases (Other routes of infection are food, soil, person to person, etc.) however, it is an enteric pathogen that may survive but cannot proliferate in treated drinking water [17, 18].

Bacteria that are resistant to antibiotic are common in those areas, where antibiotics are mostly used, but antibiotic resistant bacteria are also common in aquatic

environment [19]. Antibiotics are not only use for the treatment of human illnesses but also commonly used in veterinary practices and routinely supplemented to livestock feed. While antibiotics have proven an effective weapon against bacterial contamination and infection, their extensive use has created a reservoir of antibiotic resistance and multiple antibiotic resistance microorganisms [20]. Multiple antibiotic resistant bacteria that live in various drinking water sources suggest that this contaminated water may be a primary source of severe infectious diseases [21].

MATERIALS AND METHODS

Sample Collection: The water sources in Peshawar were identified and samples were collected from different sources i.e. tube wells, Hand pumps and tap water from different areas of Peshawar. The selected areas were Shaheen town, Sadar, Cantt, City, Gul bahar, Gulberg, Mandra Khail, Charpareza, Kharasaan, Faqir Abad and Budhni. Total 30 drinking water samples were collected. Each sample with the volume of 500 ml was collected in sterilized Duran bottles (Germany) for bacteriological analysis.

Isolation and Identification of Microorganisms:

- Total plate count was determined by the pour plate method using standard method agar and then incubated for 48hrs at 35°C [22].
- The coliform bacteria was determined by multiple tube fermentation techniques using Lactose broth and incubated at 35°C for 48hrs. It was confirmed by brilliant green bile broth and incubated at 35°C for 48hrs [23, 24].
- The fecal coliform bacteria were determined by EC broth. Positive coliforms were inoculated on EC broth at 46°C in water bath 24 ± 2 hrs [25].
- *E.coli* by streaking on Eosin methylene blue Agar and incubated at 35°C for 24 to 48hrs [26].
- *Salmonella typhi* and *Shigella* spp was determined by using peptone water as pre-enrichment medium and incubated at 35°C for 24hrs. Then Streaked on salmonella-shigella Agar as a selective enrichment medium and incubated at 35°C for 24hrs [27]
- *Klebsiella pneumonia* was determined by using Nutrient broth as pre-enrichment medium and incubated at 35°C for 24hrs. Streaked on MacConkey Agar as a selective enrichment medium and incubated at 35°C for 24hrs. Further confirmed by streaking on Simon's citrate agar slant [28]

- *Vibrio cholera* was determined by using peptone water as pre-enrichment medium and incubated at 35°C for 24hrs. Streaked on TCBS medium as a selective enrichment medium and incubated at 35°C for 24hrs [27]
- *Pseudomonas auregenosa* was determined by using nutrient broth as pre-enrichment medium and incubated at 35°C for 24hrs. Streaked on pseudomonas cetrimide agar as a selective enrichment medium and incubated at 35°C for 24hrs. Further confirmed by oxidase test [29]
- *Staphylococcus aureus* was determined by Baird parker agar for the isolation of colonies and was confirmed by streaking on mannitol salt agar slant[30]
- Yeast/Mold was determined by potato dextrose agar for the isolation of colonies and incubated at 35°C for 3 to 5 days [31, 32]

Identification of Antibiotic Resistant Bacteria: The identification was done by using standard Kirby Bauer disk diffusion method [33]. The antibiotics used in this study were: AMINOGLYCOSIDES: Amikacin, Gentamicin, Tobramycin, CEPHALOSPORINS (1st generation): Cephalothin (3rd generation): Cefazidime, Ceftriaxon, MONOBACTAM: Aztreonam, PENICILLINS: Amoxicillin, Piperacillin/tazobactam, QUINOLONES: Ciprofloxacin, Nalidixic acid, SULFONAMIDES: Trimethoprim-Sulfamethoxazole, TETRACYCLINE: Tetracycline, OTHERS: Chloramphenicol

RESULTS

Total 300 drinking water samples were collected from different areas of District Peshawar. The samples were analyzed for microbiology and antibiotic resistant bacteria. For microbiology total plate count, total coliform bacteria, fecal coliform bacteria, *E.coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella* spp, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Vibrio cholera*, yeast and mold were performed. Fourteen antibiotics were used for the identification of antibiotic resistant bacteria.

Analysis of tube well water samples from different areas of district Peshawar

Bacteriological analysis of tube well water was shown in Table (1a and 1b). The highest total plate count (TPC) 11800 cfu/ml was found in Mandra Khail BHU Sample 1. The lowest value of (TPC) 4cfu/ml was found in Saibzad Gul road, Cantt. According to World Health Organization (WHO) drinking water standard for TPC is 100 CFU/ml, if

this limit exceeds than the reported standard, the water become unacceptable for drinking purposes. 5 tube well water samples out of 10 showed no growth of total coliform bacteria. Highest TCB i.e. >23 MPN/100ml were found in Mandra Khail BHU Sample 1, Charpareza BHU Sample 1 and Kharasaan BHU Sample 1. The WHO limit of TCB are <1.8 MPN/100ml. Shaheen Town (Softener 1 incoming water, Charpareza BHU Sample 1 and Kharasaan BHU Sample 1 were contaminated with total fecal coliform bacteria (TFC). *E. coli* was absent in all the analyzed water samples. The samples collected from Mandra Khail BHU Sample 1 and Kharasaan BHU Sample 1 have shown positive results of *Salmonella typhi* and the remaining samples were found free from *Salmonella typhi*. The areas Wazirabad City, Gulberg IV City and Mandra Khail BHU Sample 1 were contaminated with shigella spp. All the examined samples were free from *staphylococcus aureus*. Wazirabad City showed growth of *Pseudomonas aeruginosa*. All the examined samples were free from *Klebsiella pneumonia* except Mandra Khail BHU Sample 1 and Charpareza BHU Sample 1, which showed growth of *Klebsiella pneumonia*. Gulberg IV City showed detection of *Vibrio cholera* and the remaining samples were free from *Vibrio cholera*. Shaheen Town (workshop water filter), Saibzad Gul road, Cantt, Lowari lane, City, Wazirabad, City and Gulberg IV, City samples showed yeast growth. Gulberg IV, City and SD House, Qafla road, Cantt samples were of mold and the remaining were free from yeast and mold.

Analysis of Hand Pump Water Samples from Different Areas of District Peshawar:

Bacteriological analysis of hand pump water was shown in Table (2a and 2b). The highest total plate count (TPC) 5600 cfu/ml was found in Budhni 7. The lowest value of (TPC) 75 cfu/ml was found in Budhni 1. Highest TCB i.e. 23 MPN/100ml was found in Budhni 7. All the analyzed samples were free from total fecal coliform bacteria. *E. coli* was absent in all the analyzed water samples. Budhni 2 and Budhni 8 have shown positive results of *Salmonella typhi* and the remaining samples were found free from *Salmonella typhi*. The samples of Budhni 3, Budhni 4 and Budhni 5 showed growth of shigella spp. The remaining samples were free from *Shigella* spp. All the examined samples were free from *staphylococcus aureus*. Budhni 1 and Faqir abad samples showed growth of *Pseudomonas aeruginosa*. Budhni 7, Budhni 8 and Faqir Abad samples were contaminated with *Klebsiella pneumonia*. The rest of the samples were free from *Klebsiella pneumonia*. Budhni 2,

Table 1 (a): Total plate count and total coliform bacteria of the Tube well water source collected from different areas of District Peshawar

S.no	Name of Areas	Total Plate Count cfu/ml	Total coliformMPN/100ml	Fecal coliform MPN/100ml
1.	Shaheen Town (workshop water filter)	30 ±1	<1.1	<1.1
2.	Saibzad Gul road, Cantt	35 ±1	<1.1	<1.1
3.	Lowari lane, City	40 ±1	<1.1	<1.1
4.	Wazirabad, City	38 ±1	<1.1	<1.1
5.	Gulberg IV, City	88 ±2	3.6	<1.1
6.	SD House, Qafla road, Cantt	42 ±1	<1.1	<1.1
7.	Mandra Khail BHU Sample 1	1180 ±10	>23	<1.1
8.	Charpareza BHU Sample 1	690 ±7	>23	16
9.	Kharasaan BHU Sample 1	340 ±4	>23	6.9
10.	Shaheen town 1	320 ±5	23	<1.1

*Each values represent mean ± SD

Table 1 (b): Bacteriological analysis of tube well water of District Peshawar

S.no	Name of Areas	E.coli	Salmonella typhi	Shigella spp.	Yeast and Mold	Staph. aureus	Pseudomonas aeruginosa	Klebsiella pneumonia	Vibrio cholera
1.	Shaheen Town (workshop water filter)	-	-	-	6 ±1 *	-	-	-	-
2.	Saibzad Gul road, Cantt	-	-	-v	35 ±3 *	-	-	-	-
3.	Lowari lane, City	-	-	-	15 ±4 *	-	-	-	-
4.	Wazirabad, City	-	-	+	14 ±2 *	-	+	-	-
5.	Gulberg IV, City	-	-	+	14 ±1 * and 3 ±0 ¹	-	-	-	+
6.	SD House, Qafla road, Cantt	-	-	-	2 ±0 ¹	-	-	-	-
7.	Mandra Khail BHU Sample 1	-	+	+	-	-	-	+	-
8.	Charpareza BHU Sample 1	-	-	-	-	-	-	+	-
9.	Kharasaan BHU Sample 1	-	+	-	-	-	-	-	-
10.	Shaheen town 1	-	-	-	-	-	-	-	-

+ = Positive, - = Negative, * = Yeast, ¹ = Mold

Each values represent mean ± SD

Table 2(a): Total plate count and total coliform bacteria of hand pump water source collected from different areas of District Peshawar

S.no	Name of Areas	Total Plate Count cfu/ml	Total coliformMPN/100ml	Fecal coliform MPN/100ml
1.	Warsak lane, Cantt	272 ±2	<1.1	<1.1
2.	Budhni 1	75 ±1	<1.1	<1.1
3.	Budhni 2	348 ±3	5.1	<1.1
4.	Budhni 3	240 ±2	3.6	<1.1
5.	Budhni 4	180 ±7	<1.1	<1.1
6.	Budhni 5	110 ±3	<1.1	<1.1
7.	Budhni 6	80 ±2	2.2	<1.1
8.	Budhni 7	5600 ±13	23	<1.1
9.	Budhni 8	720 ±5	3.6	<1.1
10.	Faqir Abad	415 ±1	<1.1	<1.1

*Each values represent mean ± SD

Table 2(b): Bacteriological analysis of hand pump water of District Peshawar

S.no	Name of Areas	E.coli	Salmonella typhi	Shigella spp.	Yeast and Mold	Staph. aureus	Pseudomonas aeruginosa	Klebsiella pneumonia	Vibrio cholera
1.	Warsak lane, Cantt	-	-	-	8 ±1 *	-ve	-ve	-	-
2.	Budhni 1	-	-	-	6 ±1 *	-	+	-	-
3.	Budhni 2	-	+	-	24 ±1 *	-	-	-	+
4.	Budhni 3	-	-	+	20 ±2 *	-	-	-	-
5.	Budhni 4	-	-	+	18 ±2 *	-	-	-	+
6.	Budhni 5	-	-	+	6 ±1 *	-	-	-	-
7.	Budhni 6	-	-	-	14 ±2 *	-	-	-	-
8.	Budhni 7	-	-	-	22 ±3 *	-	-	+	-
9.	Budhni 8	-	+	-	32 ±4 *	-	-	+	+
10.	Faqir Abad	-	-	-	-	-	+	+	-

+ = Positive, - = Negative, * = Yeast

Each values represent mean ± SD

Table 3(a): Total plate count and total coliform bacteria of tap water source collected from different areas of District Peshawar

S.no	Name of Areas	Total Plate Count cfu/ml	Total coliform MPN/100ml	Fecal coliform MPN/100ml
1.	Kohat road 1	187 ±3	3.6	2.2
2.	Shaheen town 2	284 ±2	3.6	1.1
3.	Gulberg III Annan baba, Cantt.	1160 ±8	16	5.1
4.	Chamba Peer road, Cantt.	1100 ±7	16	9.2
5.	Cantt: Teaching Hospital, Saddar	1280 ±6	16	<1.1
6.	Opp:Airport main gate, Cantt.	280 ±4	2.2	<1.1
7.	CB Plaza Fakhr-e-alam road, Saddar	135 ±3	1.1	<1.1
8.	Near ASD Airport road, Saddar	190 ±2	<1.1	<1.1
9.	Cantt: Colony Gulberg road, Saddar	180 ±3	<1.1	<1.1
10.	Gulbahar, Saddar	1175 ±7	<1.1	<1.1

*Each values represent mean ± SD

Table 3(b): Bacteriological analysis of tap water of District Peshawar

S.no	Name of Areas	E.coli	Salmonella typhi	Shigella spp.	Yeast and Mold	Staph. aureus	Pseudomonas aeruginosa	Klebsiella pneumonia	Vibrio cholera
1.	Kohat road 1	-	-	+	14 ±3*	-	+	-	-
2.	Shaheen town 2	-	+	-	8 ±2*	-	+	+	+
3.	Gulberg III Annan baba, Cantt.	-	-	+	32 ±2* & 6 ±11	-	-	+	+
4.	Chamba Peer road, Cantt.	-	-	-	8 ±11	-	-	+	+
5.	Cantt: Teaching Hospital, Saddar	-	-	-	-	-	-	-	-
6.	Opp:Airport main gate, Cantt.	-	-	-	-	-	+	+	-
7.	CB Plaza Fakhr-e-alam road, Saddar	-	-	-	-	-	-	+	-
8.	Near ASD Airport road, Saddar	-	-	-	-	-	+	+	-
9.	Cantt: Colony Gulberg road, Saddar	-	-	-	-	-	+	+	-
10.	Gulbahar, Saddar	-	-	-	-	-	-	-	+

+= Positive, - = Negative, *= Yeast, !=Mold, Each values represent mean ± SD

Budhni 4 and Budhni 8 showed detection of *Vibrio cholera* and the remaining samples were free from *Vibrio cholera*. Warsak lane, Cantt, Budhni 1, Budhni 2, Budhni 3, Budhni 4, Budhni 5, Budhni 6, Budhni 7 and Budhni 8 samples showed yeast growth.

Analysis of Tap Water Samples from Different Areas of District Peshawar: Bacteriological analysis of tap water was shown in Table (3a and 3b). The highest total plate count (TPC) 1280 cfu/ml was found in Cantt: Teaching Hospital, Saddar. The lowest value of (TPC) 135 cfu/ml was found in CB Plaza Fakhr-e-alam road, Saddar. Highest TCB i.e. 16 MPN/100ml were found in Gulberg III Annan baba Cantt, Chamba Peer road Cantt and Cantt: Teaching Hospital Saddar. Kohat road 1, Shaheen town, Gulberg III Annan baba Cantt and Chamba Peer road Cantt samples were contaminated with total fecal coliform bacteria (TFC). *E. coli* was absent in all the analyzed water samples. *Salmonella typhi* was found in Shaheen town 2. Kohat road 1 and Gulberg III Annan baba Cantt showed *Shigella* spp growth. The remaining samples were free from *Shigella* spp. All the examined samples were free from *staphylococcus aureus*. Samples of Kohat road 1, Shaheen town 2, Opp:Airport main gate Cantt, Near ASD Airport road Saddar and Cantt: Colony Gulberg road Saddar showed growth of *Pseudomonas aeruginosa*. Most of the examined samples were contaminated with

Klebsiella pneumonia. The areas included: Shaheen town 2, Gulberg III Annan baba Cantt, Chamba Peer road Cantt, Opp:Airport main gate Cantt, CB Plaza Fakhr-e-alam road Saddar, Near ASD Airport road Saddar and Cantt: Colony Gulberg road Saddar. Samples of Shaheen town, Gulberg III Annan baba Cantt, Chamba Peer road Cantt and Gulbahar Saddar showed detection of *Vibrio cholera* and the remaining samples were free from *Vibrio cholera*. Kohat road 1, Shaheen town 2 and Gulberg III Annan baba, Cantt, samples showed yeast growth. Gulberg III Annan baba, Cantt and Chamba Peer road, Cantt samples showed mold growth.

Antibiotic Resistance: Total 5 *Salmonella typhi*, 8 *Pseudomonas aeruginosa*, 8 *Vibrio cholera*, 8 *Shigella* spp. and 12 *Klebsiella pneumonia* isolates were assayed for resistance to fourteen antibiotics (Table 4 and Figures 1).

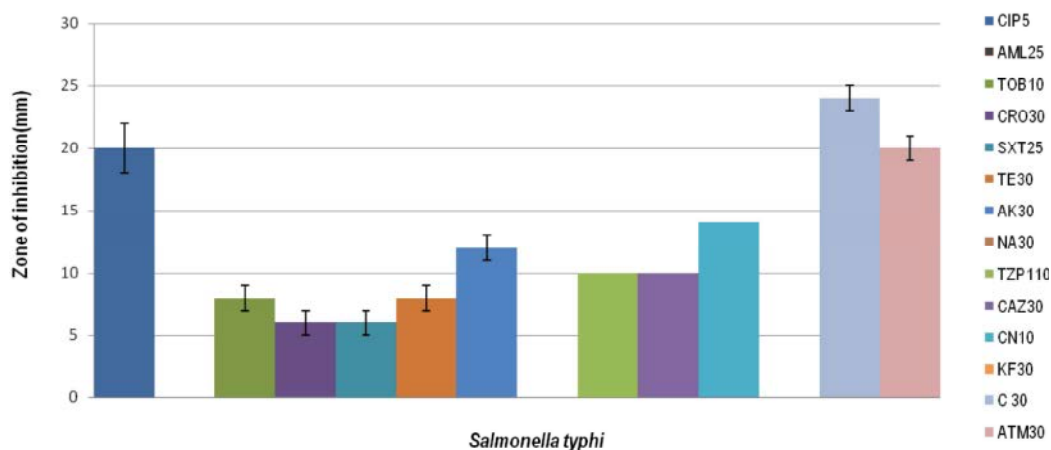
The overall resistance observed was most frequently observed to Ciprofloxacin, Tobramycin, Amikacin, Piperacillin/tazobactam, Gentamicin and Chloramphenicol. Isolates that exhibited resistance to at least three antibiotics were regarded as multiple antibiotic-resistant strains. The results showed that all the isolates were resistant to more than three antibiotics. Ciprofloxacin was more resistant to *pseudomonas aeruginosa*. Chloramphenicol and Aztreonam showed same resistance

Table 4: Antibiotic Assay

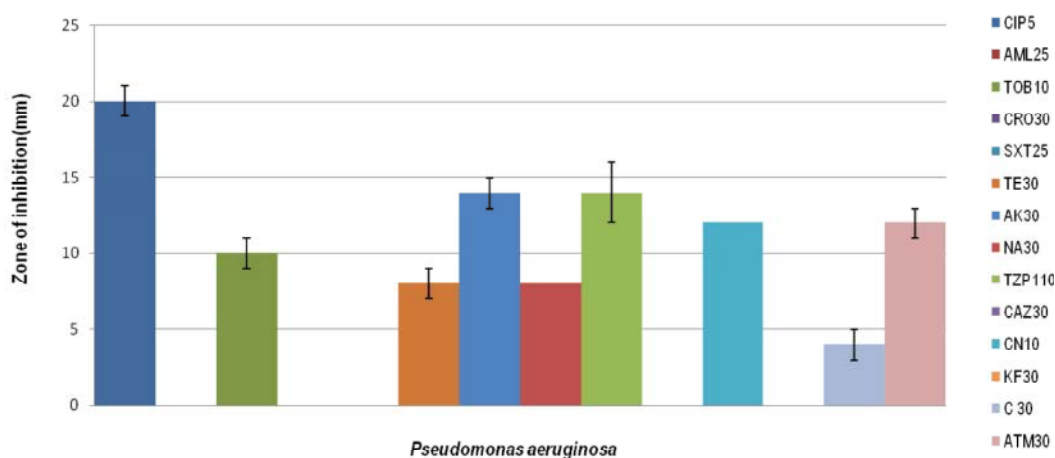
Zone of Inhibition (mm)														
Bacteria	CIP 5µg	AML 25 µg	TOB10 µg	CRO 30 µg	SXT 25 µg	TE 30 µg	AK 30 µg	NA 30 µg	TZP 110 µg	CAZ 30 µg	CN 10 µg	KF 30 µg	C 30 µg	ATM 30 µg
Salmonella typhin= 5	20 ± 2mm	-	8 ± 1mm	6 ± 1mm	6 ± 1mm	8 ± 1mm	12± 1mm	-	10 ± 0mm	10 ± 0mm	14 ± 0mm	-	24 ± 1mm	20 ± 1mm
Pseudomonas aeruginosan= 8	20 ± 1mm	-	10 ± 1mm	-	-	8 ± 1mm	14± 1mm	8 ± 0mm	14 ± 2mm	-	12 ± 0mm	-	4 ± 1mm	12 ± 1mm
Vibrio cholera= 8	18 ± 2mm	-	8 ± 1mm	-	-	-	12± 1mm	-	12 ± 0mm	-	12 ± 0mm	-	20 ± 1mm	20 ± 1mm
Shigella spp.n= 8	22 ± 1mm	-	4 ± 0mm	14 ± 1mm	-	20 ± 1mm	12 ± 1mm	-	12 ± 0mm	8 ± 0mm	12 ± 1mm	-	18 ± 1mm	20 ± 2mm
Klebsiella pneumonia= 12	16 ± 1	-	6 ± 1mm	8 ± 0mm	-	16 ± 1mm	8 ± 1mm	-	14 ± 0mm	6 ± 0mm	8 ± 0mm	-	16 ± 1mm	18 ± 1mm

*Each values represent mean ± SD Where as:

Ciprofloxacin (OXOID) = CIP 5, Amoxicillin (OXOID) = AML 25, Tobramycin (OXOID) = TOB 10, Ceftriaxone (OXOID) = CRO 30, Sulphamethoxazole/Trimethoprim (OXOID) = SXT 25, Tetracycline (OXOID) = TE 30, Amikacin (OXOID) = AK 30, Nalidixic Acid (OXOID) = NA 30, Piperacillin/tazobactam (OXOID) = TZP 110, Ceftazidime (OXOID) = CAZ 30, Gentamicin (OXOID) = CN 10, Cephalothin (OXOID) = KF 30, Chloramphenicol (OXOID) = C 30 and Aztreonam (OXOID) = ATM 30.

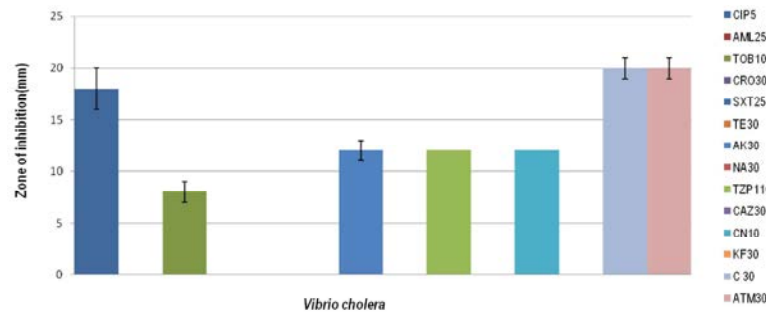


Bacteria	CIP5µg	AML25 µg	TOB10 µg	CRO30 µg	SXT25 µg	TE30 µg	AK30 µg	NA30 µg	TZP110 µg	CAZ30 µg	CN10 µg	KF30 µg	C 30 µg	ATM30 µg
Salmonella typhim= 5	20 ± 2mm	-	8 ± 1mm	6 ± 1mm	6 ± 1mm	8 ± 1mm	12 ± 1mm	-	10 ± 0mm	10 ± 0mm	14 ± 0mm	-	24 ± 1mm	20 ± 1mm

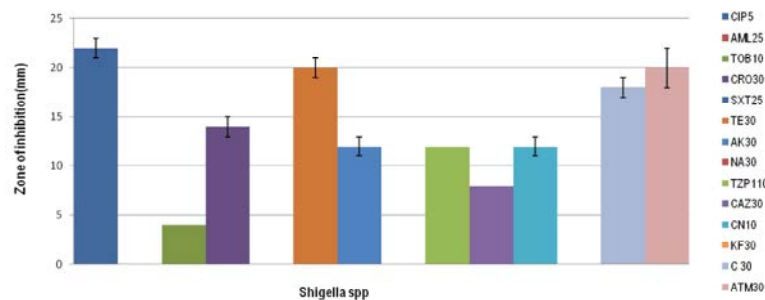
Fig. 1(a): Antibiotic resistance pattern of *Salmonella typhi* in Drinking water of district Peshawar

Bacteria	CIP5µg	AML25 µg	TOB10 µg	CRO30 µg	SXT25 µg	TE30 µg	AK30 µg	NA30 µg	TZP110 µg	CAZ30 µg	CN10 µg	KF30 µg	C 30 µg	ATM30 µg
Pseudomonas aeruginosan= 8	20 ± 1mm	-	10 ± 1mm	-	-	8 ± 1mm	14 ± 1mm	8 ± 0mm	14 ± 2mm	-	12 ± 0mm	-	4 ± 1mm	12 ± 1mm

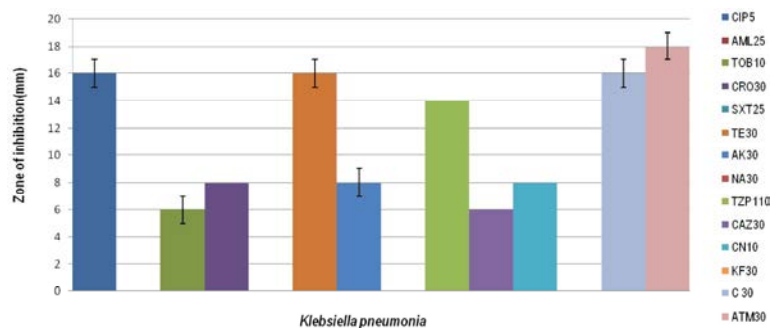
Fig. 1(b): Antibiotic resistance pattern of *Pseudomonas aeruginosa* in Drinking water of district Peshawar



Bacteria	CIP5µg	AML25 µg	TOB10 µg	CRO30 µg	SXT25 µg	TE30 µg	AK30 µg	NA30 µg	TZP110 µg	CAZ30 µg	CN10 µg	KF30 µg	C 30 µg	ATM30 µg
Vibrio cholera n= 8	18 ± 2mm	-	8 ± 1mm	-	-	-	12 ± 1mm	-	12 ± 0mm	-	12 ± 0mm	-	20 ± 1mm	20 ± 1mm

Fig. 1(c): Antibiotic resistance pattern of *Vibrio cholera* in Drinking water of district Peshawar

Bacteria	CIP5µg	AML25 µg	TOB10 µg	CRO30 µg	SXT25 µg	TE30 µg	AK30 µg	NA30 µg	TZP110 µg	CAZ30 µg	CN10 µg	KF30 µg	C 30 µg
Shigella spp. n= 8	22 ± 1mm	-	4 ± 0mm	14 ± 1mm	-	20 ± 1mm	12 ± 1mm	-	12 ± 0mm	8 ± 0mm	12 ± 1mm	-	18 ± 1mm

Fig. 1(d): Antibiotic resistance pattern of *Shigella* spp in Drinking water of district Peshawar

Bacteria	CIP5µg	AML25 µg	TOB10 µg	CRO30 µg	SXT25 µg	TE30 µg	AK30 µg	NA30 µg	TZP110 µg	CAZ30 µg	CN10 µg	KF30 µg	C 30 µg	ATM30 µg
Klebsiella pneumonia n= 12	16 ± 1	-	6 ± 1mm	8 ± 0mm	-	16 ± 1mm	8 ± 1mm	-	14 ± 0mm	6 ± 0mm	8 ± 0mm	-	16 ± 1mm	18 ± 1mm

Fig. 1(e): Antibiotic resistance pattern of *Klebsiella pneumonia* in Drinking water of district Peshawar

against *Vibrio cholera*. Ciprofloxacin showed more resistant to *Shigella* spp. Aztreonam showed more resistant to *Klebsiella Pneumonia*.

DISCUSSIONS

Thirty samples of drinking water were collected from tube wells, hand pump and Tap water from different areas of District Peshawar KPK. The samples were analyzed for total plate count, total coliform bacteria, fecal coliform

bacteria; *E.coli*, *Staphylococcus aureus*, *Salmonella typhi*, *shigella* spp, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Vibrio cholera*, yeast and mold were performed. Fourteen antibiotics were used for the identification of antibiotic resistant bacteria.

It was observed that total plate count (TPC) of hand pump water samples collected from Budhni 7 was estimated to be very high 5600 CFU/ml, as compare to tube wells and tap water. Abid *et al.* [34] who had mentioned 12000 CFU/ml in drinking water of Peshawar.

Later on Alizai *et al.* [35] showed high bacterial count (33000 CFU/ml) in drinking water of Peshawar. Total coliform bacteria minimum (<1.1 MPN/100ml) to maximum (>23 MPN/100ml) was found in this investigation. Most of Tube wells water samples showed high coliform bacteria i.e. >23 MPN/100ml, which is higher than the limit of WHO drinking water standard. Most of Tap and hand pump coliform results were within the permissible limit of WHO drinking water standard. Cheema *et al.* [36] concluded that 60% water samples were unfit for drinking purposes because of coliform bacteria and total plate count. Total 23.3% water was contaminated with fecal coliform and 8.7% was contaminated with *E.coli*. In different regions of the world *E. coli* was also isolated from drinking water sources. Similar studies were carried out by Franciska *et al.* [37] analyzed the quality of drinking water from private water supplies in Netherlands. Total 144 samples were collected for bacteriological analysis. Their results show that 10.9% samples were contaminated due to *E. coli* and *Enterococci* presence. Hasan *et al.* [38] studied that the drinking water from different sources such as river, ground and well water showed that 67% of total water samples were found contaminated with coli forms and fecal coliforms.

The present study showed *Salmonella typhi* 16.67%, *Pseudomonas aeruginosa* 26.67%, *Vibrio cholera* 26.67%, shigella spp 26.67%, *Klebsiella pneumonia* 40%, Yeast 56.6% and Mold 13.3%. Prasai *et al.* [39] conducted a study to evaluate the quality of drinking water of Kathmandu valley. During the study, 238 isolates of enteric bacteria were identified, out of which 26.4% were *Escherichia coli*, 6.3% were *Pseudomonas aeruginosa*, 5.4% were *Klebsiella* spp, 4.0% were Shigella spp, 3.0% were *Salmonella typhi*, 1.0% were *Vibrio cholera* and other bacteria. Ahmed *et al.* [9] analyzed drinking surface water from different dams in Rawalpindi/Islamabad region (Pakistan). Water samples contain different pathogenic bacteria like *Staphylococcus aureus*, *E.coli*, salmonella spp and Shigella spp.

Fourteen antibiotics were used for the identification of antibiotic resistant bacteria. The overall resistance observed was most frequently observed to Ciprofloxacin, Tobramycin, Amikacin, Piperacillin/tazobactam, Gentamicin and Chloramphenicol. The results showed that all the isolates were resistant to more than three antibiotics. Afiukwa *et al.* [40] found that *E. coli* was highly resistance to trimethoprim, sulphamethoxazole/trimethoprim, ampicillin, aztreonam, ceftazidime and cefpirome but susceptible to gentamicin, ciprofloxacin, ceftiofur and cefotaxime. Enterobacter and *Klebsiella* spp. were totally resistance to all the antibiotics tested. Okeke

et al. [41] found that Antibiotic resistance in bacteria is a serious problem facing society today and one of the reasons responsible for this problem is overuse of antibiotics in humans

CONCLUSIONS

The results of this investigation revealed that bacteriological quality of the given sources failed to meet the standard for drinking water. It is a serious threat to the people of the area if proper measurements are not taken by the concerned authorities. Total plate count and total coliform bacteria range was higher than WHO limit, so this is unacceptable for drinking. The water sources were contaminated with *Salmonella typhi*, shigella spp, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Vibrio cholera*, yeast and mold. Antibiotic resistance is considered to be a major problem because many disease causing bacteria are becoming more resistant to the commonly used antibiotics. The bacteria *Salmonella typhi*, Shigella spp, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Vibrio cholera*, isolated from tube wells, tap water and hand pump showed greater multiple antibiotic resistances. The overuse and misuse of antibiotics can create the conditions for the development of antibiotic resistant bacteria.

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