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Isolation Identification and Antimicrobial Susceptibility Testing of Salmonella SPP. In Watersources Used for Commercial And Domestic Purposes In Peshawar, Pakistan

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Abstract

Background: Salmonella, a Gram-negative, facultative anaerobic bacterium, is a major zoonotic disease that causes significant illness and mortality worldwide. With approximately 2,500 serotypes divided into two species—Salmonella enterica and Salmonella bongori-this pathogen proves distinct biochemical properties and host specificity. Salmonella is mostly present in the intestines of animals and birds, with transmission to humans often happening through contaminated food and water sources. Non-typhoidal strains usually produce self-limiting gastroenteritis, but typhoidal strains cause severe systemic infections, such as typhoid fever, which is still a serious public health problem. Despite advances in food safety procedures, Salmonella continues to cause an estimated ninety million episodes of gastroenteritis each year, resulting in around 155,000 fatalities globally. Water quality plays a significant role in the spread of Salmonella because of polluted drinking. Methodology: This study was conducted at Department of Microbiology, Rehman Medical Institute, Hayatabad Peshawar, during September to November 2024. A cross-sectional study was designed to investigate the bacteriological analysis of water from different areas of district Peshawar. A total of 384 water samples were collected. The

collected samples were immediately transported to the Department of Microbiology, Rehman Medical Institute Hayatabad, Peshawar, for analysis. The samples were directly transferred in test tubes to the laboratory for further preparation and examination. Nonprobability sampling technique was used. Results: In this study 384 water samples were collected from different areas of Peshawar using convenience sampling. A total of 214 (55.73%) were collected from tape water, 96 (25%) from tube well and 74 (19.27%) were from water tank. Out of 384 samples 43 (11.20 %) samples were found positive for Salmonella. Conclusion: The study highlights the public health implications of antibiotic-resistant bacteria in water, highlighting the need for stringent measures to prevent their occurrence, as the consumption of water containing these bacteria may prolong the treatment of water-borne diseases.

INTRODUCTION

Salmonella is a rod-shaped, facultative anaerobic, Gram-negative bacterium that belongs to the family of Enterobacteriaceae, also referred to as enteric bacteria. Except for *S. pullorum* and *S. gallinarum*, which do not generate spores, *Salmonella* is a motile bacterium. Salmonella has more than 2500 different serotypes (WHO, 2005). *Salmonella* strains have been identified and categorized according to their antigens into groups known as serovars (Snoeyenbos, 1994). Merely two species—*S. enterica* and *S. bonsoir's*—are included in the genus Salmonella according to the most recent nomenclature, which reflects recent advances in taxonomy. Six subspecies of Salmonella enterica can be distinguished from one another based on biochemical traits. According to the Kauffmann–White scheme, Salmonella strains are categorized into serovars based on the wide variety of lipopolysaccharide (LPS) antigens (O) and flagellar protein antigens (H). There are multiple hosts for *Salmonella*. Although mostly animal and bird gut microorganisms. Extensive in the environment, *salmonella* is frequently being discovered from human sewage, farm effluvium, and any material near fecal contamination. It is introduced to people by toxic foods derived from animals. Certain

serovars have notable hosts, such as Salmonella typhi, which is typically found in humans, and Salmonella gallinarum, which is typically found in birds. According to epidemiological and bacteriological data, these animals have the potential to infect humans. Salmonella produce lactose nonfermenting colonies on SS and MAC agar, according to Cheesbrough (1985). The majority of strains exhibit colony blackening as a result of hydrogen sulfide generation. (1) The most prevalent foodborne illnesses are salmonellosis. Salmonella infections should be separated into minor and serious illnesses. Self-constraining diarrhea is the hallmark of minor salmonellosis brought on by nontyphoid Salmonella strains; bacteraemia or meningitis are infrequent outcomes. Enteric fever represents crucial salmonellosis. Typhoid fever manifests as fever, headache, malaise, and occasionally cough. An American bacteriologist D. E. Salmon made the initial discovery of Salmonella in 1884. The bacteria were isolated from the intestinal tract of a pig. Although the intestines of humans and other animals are the primary reservoir of Salmonella, bacteria have also been discovered in insects and reptiles. Salmonella infections can come from a variety of sources, but the most significant ones are vegetables, meat, eggs, dairy products, and water. In advanced countries the most common source of infection is food. The identification of food source causing disease is sometimes difficult, but it is the most important measure to prevent the spread of the infection (2).

Water as Universal Solvent

For all living things to function correctly, water is necessary. Water makes up 70–90% of the cells of living things, making it an essential component. The most frequent is drinking water contamination from sewage or other sources, which is brought on by animal and human waste. Microorganisms contaminate water. Runoff from agriculture,

household drainage, and industrial contaminants can all contaminate free-floating water. Runoff from agriculture, household drainage, and industrial contaminants can all contaminate free-floating water. (4) Fecal coliform in water may indicate that it has been contaminated by human or animal feces. Coliform bacteria

should not be present in groundwater from a well that has been built correctly. Faecal coliform bacteria from human, animal, and agricultural waste can pollute well water. Water contamination can result from septic tanks, pastures, feedlots, runoff from wooded areas, and poorly constructed wells. (3)

The Crucial Role of Water in Human Life and Environmental Sustainability

Approximately 71% of the world's surface is covered with water, the most plentiful resource on the planet. It supports all human activity, including household, industrial, agricultural, recreational, commercial, transportation, and energy uses, and is necessary for the survival of life. Water makes up around 70% of the human body and is essential for everyday physiological functions. Only around 2.5 percent of the Earth's enormous water resources are fresh water, with the majority being contained in glaciers and ice caps. While just a small portion (about 1% of all freshwaters) is easily usable by humans in rivers, lakes and reservoirs, over 30% of the worlds freshwater are stored underground. Ocean saltwater makes up the remaining 97.5% of the water on Earth. This lack of accessible freshwater emphasizes how crucial it is to regularly assess the availability and quality of water. Ensuring the sustainability of this essential resource to meet the many demands of ecosystems and humans is becoming more and more important as pollution and overuse threaten it. For the sake of agriculture, human health, and the integrity of the environment, water quality must be preserved. When water is not in its typical colorless, odorless, tasteless, and sparkling state, it is considered contaminated.

Running and stagnant surface waters, as well as subsurface water below, can become contaminated by septic tanks, excreta leaks, effluent from treatment plants and slaughterhouses, hospitals, decontamination stations, and industrial and agricultural wastes. These forms of pollution cause water bodies to have higher concentrations of turbidity, suspended sediments, biological oxygen demand, microbial pathogens, and parasites When compared to surface waters, ground waters—such as those from wells and boreholes—are thought to be the safest and purest sources of drinking water. They are located around 100 meters below the surface of the earth and are caused by water seeping into the ground and being stored between soil particles, rock well fissures, or crevices.(4)

Chemical Pollutants in Drinking Water

Laboratory toxicity studies and epidemiological studies are the two main methods used to evaluate the risks of consuming chemical contaminants found in drinking water. Some drinking water supplies, especially those that do not use coagulation and filtration, frequently have finely divided solid particles of mineral and organic origin suspended in them. The occurrence, content, and characteristics of these compounds were examined to determine whether prolonged consumption of them in water is likely to have a negative impact on human health. Because many cancers have lengthy induction periods, epidemiological studies have not shown an increase in cancer death rates over time that can be attributed to fibrous contamination of the drinking water. However, these negative findings do not rule out the possibility that such an increase may be discovered in the future. Research on the analysis of fibrous mineral particles in water and the toxicity of these materials when consumed should be vigorously pursued for these and other reasons, which are described elsewhere. (5) Barium, cadmium,

chromium, lead, mercury, and silver are the six metallic elements for which the Interim Primary Drinking Water Regulations specify maximum levels. Bronze, cobalt, copper, magnesium, manganese, molybdenum, nickel, tin, vanadium, and zinc were among the ten additional metals that were analyzed. Because sodium poses health risks, it was assessed separately. Arsenic, selenium, fluoride, nitrate, and sulfate were among the other inorganic components of drinking water that were examined.(5)

Antibiotic Sensitivity Patterns in Isolated Bacterial Strains

The sensitivity of these isolated strains was tested using a total of five antibiotics: cefixime, levofloxacin, clindamycin, augmentin, and amoxicillin. The most effective antibiotic against Salmonella and *E. Coli* is cefixime, which has a zone of inhibition of 30 cm and 34 cm, respectively, but both bacteria demonstrated resistance to DA and amoxicillin. 87% of coliforms in groundwater supplies were found to be resistant to at least one antibiotic in a previous study, with resistance most frequently targeting Novobiocin, Cephalothin, and Ampicillin. Shigella exhibited a wide zone of inhibition (27 cm) against levofloxacin, but Klebsiella was the most susceptible to its effects. Shigella is a vulnerable bacterium that all drugs produce a zone of inhibition against, although E. Coli and Salmonella are the most resistant, exhibiting resistance to medicines such as clindamycin and amoxicillin. (6) Klebsiella was the most vulnerable to the effects of levofloxacin, while Shigella showed a broad zone of suppression (27 cm). All medications create a zone of inhibition against Shigella, a susceptible bacterium; however, *Salmonella* and *E. Coli* are the most resistant, showing resistance to medications like amoxicillin and clindamycin. The goal of this research Endeavor is to comprehend and manage infections linked to Salmonella. High rates of mortality and morbidity are caused by acute diarrheal diseases. Over two billion people worldwide suffer from

diarrhea every year, according to the World Health Organization (WHO). One-third of infections are caused by food. One of the most common food borne infections is salmonellas is. There are two categories for *salmonella* infections: minor and serious. Non-typhoid Salmonella strains that cause mild salmonellas is rarely cause bacteraemia or meningitis, instead causing self-limiting diarrhea. One important symptom of salmonellas is is typhoid fever. Symptoms of typhoid fever may include fever, coughing, headaches, and malaise. (3)

Route of Transmission

The five "—F ‖ s" of salmonella transmission are commonly used as a mnemonic to emphasize the basic ways the bacteria may spread.

1. Food: Salmonella may be found in a variety of foods, including raw chicken, eggs, and unpasteurized dairy products. Contamination can happen at any point along the food supply chain.

2. Fingers: Human hands may easily transport salmonella bacterium, especially if regular hand washing practices are not used. Touching contaminated surfaces or handling infectious food can cause transmission.

3. Faeces: These bacteria are frequently detected in the intestines of diseased animals and humans. When contaminated faeces come into touch with food, water, or surfaces, the virus spreads.

4. Fluids: Water infected with salmonella, either through faecal contamination or poor sanitation measures, can aid transmission, particularly in places with inadequate water treatment.

5. Fomites: Inanimate things, such as kitchen utensils, cutting boards, and worktops, can carry the germs if they come into touch with infected food or surfaces. Understanding

these routes is critical for avoiding salmonella infections with adequate hygiene and food safety standards.

WASH System

Enhancing the quality of water in commercial environments is essential for both environmental sustainability and public health. Here are a few tactics: To provide long-term monitoring, regulations, and funding, WASH systems, like health systems, use technology, behavior modification, and system strengthening. Promptly and affordably implemented, with an emphasis on institutions and homes.

Safe Drinking Water

Developing supply infrastructure, such as piped water, public standpipes, boreholes, and rainwater collection, is necessary to increase access to safe drinking water.

Sanitation

By using safe toilets, adequate containment, and ways of transport, a safe sanitation system isolates human waste from contact.

Hygiene

The goal of hygiene programs is to promote hand washing with soap especially significant occasions. (7)

Epidemiology

Access to a clean and safe drinking water supply is crucial for human survival, as detailed in SDG 6. However, the World Health Organization reports that diarrheal illnesses claim the lives of 525,000 children under the age of five each year. Additionally, according to the United Nations Children's Fund, 2.1 billion people worldwide do not have access to safely managed drinking water supplies. According to estimates, using water with low

microbiological quality is responsible for 80% of all infections in poorer nations. (WHO, 2002) (3).

An estimated 90 million instances of gastroenteritis and 155,000 fatalities are caused by *Salmonella* spp. each year, making it a significant zoonotic food borne pathogen of economic importance in both people and animals. Although most cases of salmonellas is are classified as food borne, it has been estimated that direct contact with animal's accounts for around 10% of cases.

Both humans and animals' digestive tracts are home to the genus *Salmonella*, which contains a wide variety of bacteria. Because they can adapt and thrive in harsh environments, they are widely distributed in the environment. Both serovar and host variables (breed, age, gender, nutrition, and/or immunity) influence clinical symptoms and mortality among the more than 2600 Salmonella serovars that have been identified. (8)

TABLE 1.1: EPIDEMIOLOGY OF *SALMONELLA* IN ASIA

S#	Country	Prevalence
1	Pakistan	14
2	India	24
3	China	64
4	Indonesia	15

The incidence of typhoid and paratyphoid cases in particular high-risk populations in "Karachi, Pakistan; Kolkata, India; North Jakarta, Indonesia; and Hechi City, China" will be examined through one-year surveillance research. In the four sites mentioned above, 285 *S. typhi* episodes and 84 S. Paratyphi A episodes were recorded during the

surveillance period. China showed the lowest prevalence of *S. typhi* (15.2/100,000/year), whereas Pakistan had the highest incidence (394/100,000/year). (9)

Four Canadian provinces experienced an outbreak of frozen raw chicken in 2015, with 44 cases reported. Uncooked mung bean sprouts were implicated in 230 Salmonella infections that were reported in South Australia in 2016. Rockmelon consumption was linked to 97 instances in New South Wales. Salmonella Virchow was found in several states in 2016, and consumption of raw food or organic shakes was connected to more than twenty-seven cases. In the same year, Salmonella Poona was discovered in the USA. (2)

MATERIAL AND METHODS

This study was conducted from September to November 2024 at the Rehman Medical Institute's Microbiology Department in Hayatabad, Peshawar, Pakistan.

Study Design:

A cross-sectional study was created to investigate the bacteriological analysis of water from various parts of the Peshawar district. A total of 384 samples of water were gathered. The Rehman Medical Institute's Microbiology Department/Laboratory in Hayatabad, Peshawar, received the collected samples right away for examination. The samples were sent straight to the lab in test tubes for additional processing and analysis.

Study Settings

Department of Microbiology, Rehman Medical Institute Hayatabad Peshawar.

Study Duration:

The duration of study was three months, from September to November 2024.

Population and Sample

The sample size was calculated via using Openepi sample size calculator, variability in prevalence P, estimated prevalence=50%, E is margin of error taken as 5%. And Z for Confidence level of 95% is 1.96.A samples of 384 water were collected from different areas of district Peshawar, Khyber Pakhtunkhwa, Pakistan. (6)

Sampling Technique

Nonprobability sampling technique was used.

- **Inclusion criteria:** Samples were collected from restaurants, Houses, Tube wells and storage water tanks used for commercial and domestic purposes.
- **Exclusion criteria:** Fresh water (Which is directly collected from Bore)

Methodology Procedure

Collection and Transportation: All water samples for this investigation were obtained aseptically with sterile tools and properly transferred to suitable containers. The samples were handled carefully. Aseptic precautions were maintained throughout transit, and the samples were preserved in sterile containers until they were ready for bacteriological investigation.

Preparation of Culture Media: The commercial media (Blood and McConkey (MC) agar) were prepared according to the direction of the Manufacturers in the laboratory.

Isolation of Bacteria by Culturing of Sample

The collected water samples were streaked in Blood and MaConkey agar followed by incubation at 37℃ for overnight. The cultural examination of different sources of water samples for bacteriological analysis was done on the basis of biochemical tests.

Confirmatory Tests

1. **Catalase Test:** This test was used to differentiate those bacteria that produced the enzyme catalyse, such as staphylococci, from non-catalase producing bacteria

such as streptococci. To perform the test an amount of 2-3 ml of 3% hydrogen peroxide solution was poured into a test tube. Using a sterile wooden stick or a glass rod, a good growth of the test organism was immersed into the solution. If the organisms are catalase producer, bubbles of oxygen are released.

2. Indole Test: Two milliliter of peptone water was inoculated with the 5ml of bacterial culture and incubated at 37°C for 48 hours. Kovac's reagent (0.5 ml) was added, shacked well and examined after one minute. A red color in the reagent layer indicated indole. In negative case there is no development of red color.

ANTIBIOTIC SENSITIVITY TEST (AST)

Muller Hinton Agar (MHA): After suspending thirty-eight grams of dehydrated Muller Hinton Agar Medium in 1000 milliliters of cold distilled water and boiling it until it was completely dissolved, the mixture was autoclaved at 121 degrees Celsius and 15 pounds of pressure for 15 minutes. The autoclaved materials were then lowed to cool to 45 degrees Celsius in a water bath before being transferred to sterile Petri dishes. Following solidification, the Petri dishes were placed in an incubator set at 37 degrees Celsius for 24 hours to ensure sterility, and they were then kept in a refrigerator at 4°C until they were needed.

In accordance with the Clinical and Laboratory Standards Institute's (CLSI) guidance, the disc diffusion technique was employed to detect antimicrobial susceptibility test. Disc diffusion was utilized to test for antimicrobial medication sensitivity to five widely used antibiotics.

The procedure of disc diffusion method is presented below:

i. The colony was selected from Blood and MaConkey agar plate.

ii. After touching the colony with a sterile loop, it was moved into a tube filled with sterile physiological saline and extensively vortexed.

iii. A sterile cotton swab was dipped into the bacterial suspension. The excess fluid of swab was removed by pressing firmly against the inside of the tube just above the fluid level.

iv. The swab was streaked over the entire surface of Mueller-Hinton agar (Himedia, India) medium three times, rotating the plate approximately 60 degrees after each application to ensure an even distribution of the inoculums.

v. The antimicrobial discs were placed individually using sterile forceps and then gently press down onto the agar.

vi. The plates were inverted and incubated at 37°C temperature for overnight. After incubation the diameter of the zone of complete inhibition (including diameter of the discs) was measured in millimeters with a ruler.

Interpretation of the Results: After placing the discs on the plate, the plates were inverted and incubated at 37°C for 12 hours, at which point the diameter of the zones of full inhibition (including the disc diameter) was measured and recorded in millimeters. Measurements were taken with a ruler on the plate's underside without lifting the lid. The zones of growth inhibition were compared to the Clinical and Laboratory Standards Institute's zone-size interpretive table (2024). Antimicrobial testing findings were reported as susceptible, intermediate, and resistant according to the zone diameter interpretative guidelines supplied by CLSI (2024).

TABLE 2. THE ZONE-SIZE OF SALMONELLA SPP INTERPRETATIVE TABLE PROVIDED BY CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI, 2024) DIAMETER OF ZONE OF INHIBITION EXPRESSED IN MM

Antimicrobial agent	Resistant	Intermediate	Sensitive
Cefixime 5 µg	⩽ 19	20-22	⩾23
Ampicillin 10 µg	⩽13	14–16	⩾17
Ciprofloxacin 5 µg	⩽20	21–30	⩾31
Trimethoprim/Sulfamethoxazole 1.25/23.75 µg	⩽10	11–15	⩾16
Gentamycin 10 µg	⩽12	13–14	⩾15
Chloramphenical 30 µg	⩽12	13-17	⩾18

AST of Ciprofloxacin

A total of 384 samples were analyzed, revealing a Salmonella spp. positivity rate of 11.20% (n=43). Antimicrobial susceptibility testing demonstrated that 9.90% (n=38) of the samples were sensitive, 0.26% (n=1) exhibited intermediate susceptibility, and 0.78% (n=3) were resistant to Ciprofloxacin.

AST to Gentamycin

A total of 384 samples were analyzed, revealing a Salmonella spp. positivity rate of 11.20% (n=43). Antimicrobial susceptibility testing demonstrated that 6.77% (n=26) of the samples were sensitive, 3.39% (n=13) exhibited intermediate susceptibility, and 1.04% (n=4) were resistant to Gentamicin.

AST to Ampicillin

A total of 384 samples were analyzed, revealing a Salmonella spp. positivity rate of 11.20% (n=43). Antimicrobial susceptibility testing demonstrated that 4.43% (n=17) of the samples were sensitive, 3.39% (n=13) exhibited intermediate susceptibility, and 3.39% (n=13) were resistant to Ampicillin.

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AST to Cefixime

A total of 384 samples were analyzed, revealing a Salmonella spp. positivity rate of 11.20% (n=43). Antimicrobial susceptibility testing demonstrated that 9.90% (n=38) of the samples were sensitive, 0.52% (n=2) exhibited intermediate susceptibility, and 0.78% (n=3) were resistant to Cefixime.

AST to Trimethoprim/Sulfamethoxazole

A total of 384 samples were analyzed, revealing a *Salmonella* spp. positivity rate of 11.20% (n=43). Antimicrobial susceptibility testing demonstrated that 10.16% (n=39) of the samples were sensitive, 1.04% (n=4) exhibited intermediate susceptibility, and 0% were resistant to Trimethoprim/Sulfamethoxazole.

Sample Collected from Different Area Chloramphenicol AST 30ug

A total of 384 samples were analyzed, revealing a Salmonella spp. positivity rate of 11.20% (n=43). Antimicrobial susceptibility testing demonstrated that 10.94% (n=42) of the samples were sensitive, while 0.26% (n=1) exhibited resistance to Chloramphenicol (AST 30µg).

Source Wise AST Ciprofloxacin AST 5ug

A total of 384 samples were analyzed, revealing a Salmonella spp. positivity rate of 11.20% (n=43). Frequency distribution analysis demonstrated that 6.25% (n=24) of tap water, 2.60% (n=10) of water tank, and 1.04% (n=4) of tube well samples were sensitive to antimicrobial agents. Intermediate susceptibility was observed in 0.26% (n=1) of tap water samples. Resistance to Ciprofloxacin (AST 5µg) was detected in 0.78% (n=3) of water tank samples.

Source of Sample Collected Gentamycin AST 10ug

A total of 384 samples were analyzed, revealing a Salmonella spp. positivity rate of 11.20% (n=43). Frequency distribution analysis demonstrated that 4.95% (n=19) of tap water, 1.30% (n=5) of water tank, and 0.52% (n=2) of tube well samples were sensitive to antimicrobial agents. Intermediate susceptibility was observed in 1.30% (n=5) of tap water, 1.56% (n=6) of water tank, and 0.52% (n=2) of samples. Resistance to Ciprofloxacin (AST 5µg) was detected in 0.52% (n=2) of tap water and 0.52% (n=2) of water tank samples.

Source wise AST Ampicillin AST 10ug

A total of 384 samples were analyzed, revealing a Salmonella spp. positivity rate of 11.20% (n=43). Frequency distribution analysis demonstrated that 3.39% (n=13) of tap water, 0.78% (n=3) of water tank, and 0.26% (n=1) of tube well samples were sensitive to antimicrobial agents. Intermediate susceptibility was observed in 2.08% (n=8) of tap water, 1.04% (n=4) of water tank, and 0.26% (n=1) of tube well samples. Resistance to Ampicillin (AST 10µg) was detected in 1.30% (n=5) of tap water, 1.56% (n=6) of water tank, and 0.52% (n=2) of tube well samples.

Source wise AST Cefixime AST 5ug

A total of 384 samples were analyzed, yielding a Salmonella spp. positivity rate of 11.20% (n=43). Frequency distribution analysis revealed that 6.51% (n=25) of tap water, 2.34% (n=9) of water tank, and 1.04% (n=4) of tube well samples were sensitive to antimicrobial agents. Intermediate susceptibility was observed in 0.78% (n=3) of water tank samples. Resistance to Cefixime (AST 5µg) was detected in 0.26% (n=1) of tap water and 0.26% (n=1) of water tank samples.

Source Wise AST Trimethoprim/Sulfamethoxazole AST 25/23.75ug

A total of 384 samples were analyzed, revealing a Salmonella spp. positivity rate of 11.20% (n=43). Frequency distribution analysis demonstrated that 6.25% (n=24) of tap water, 2.86% (n=11) of water tank, and 1.04% (n=4) of tube well samples were sensitive to antimicrobial agents. Intermediate susceptibility was observed in 0.52% (n=2) of tap water and 0.52% (n=2) of water tank samples. Notably, all samples exhibited susceptibility to Trimethoprim/Sulfamethoxazole (AST 25/23.75µg), with no resistance detected.

Source wise AST Chloramphenicol AST 30ug

A total of 384 samples were analyzed in this study, revealing a prevalence of 11.20% (n=43) positive for Salmonella spp. The distribution of Salmonella-positive samples was as follows: 6.77% (n=26) from tap water, 3.13% (n=12) from water tanks, and 1.04% (n=4) from tube wells. Notably, 0.26% (n=1) of water tank samples exhibited intermediate resistance to Chloramphenicol (AST 30µg).

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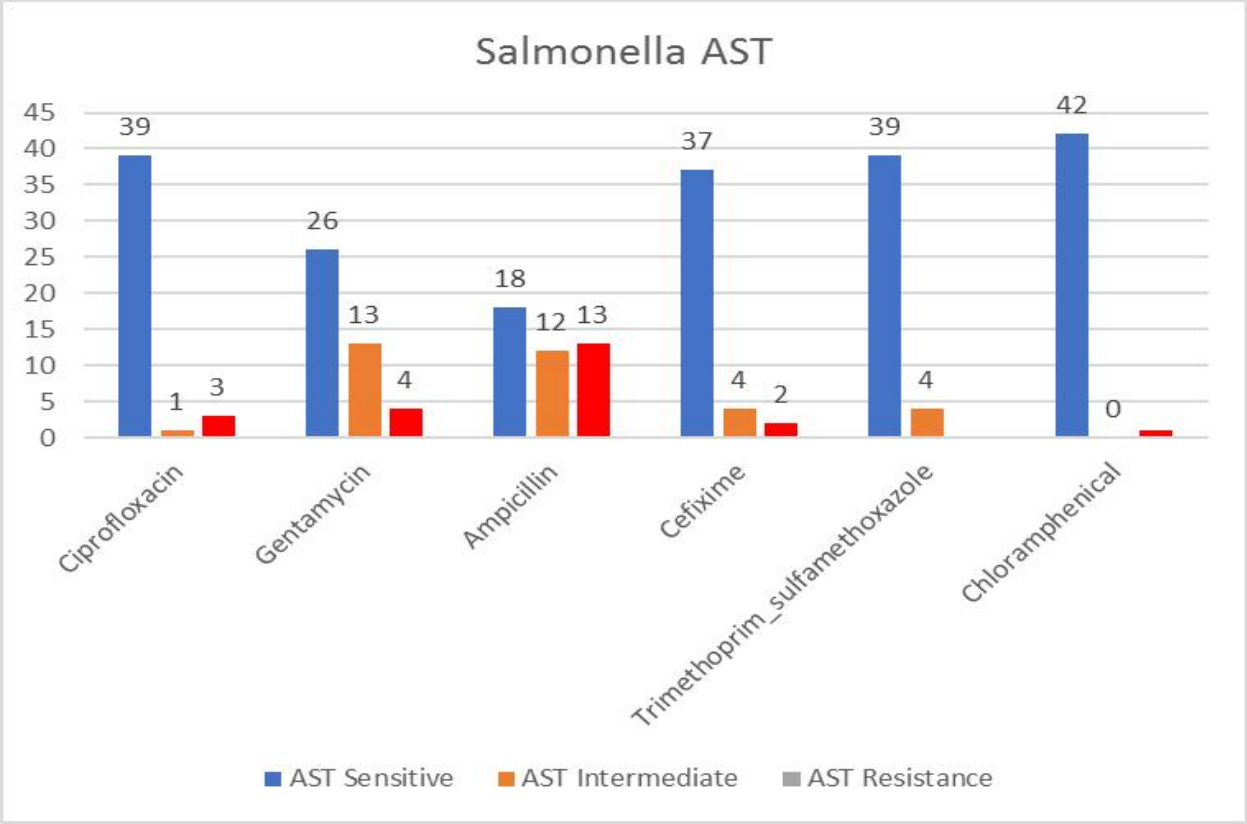

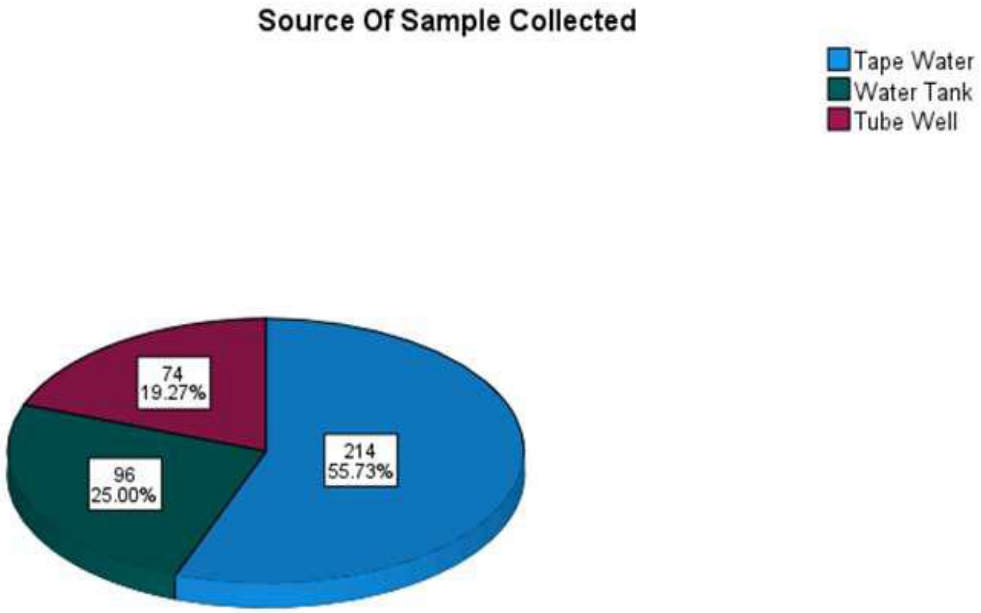

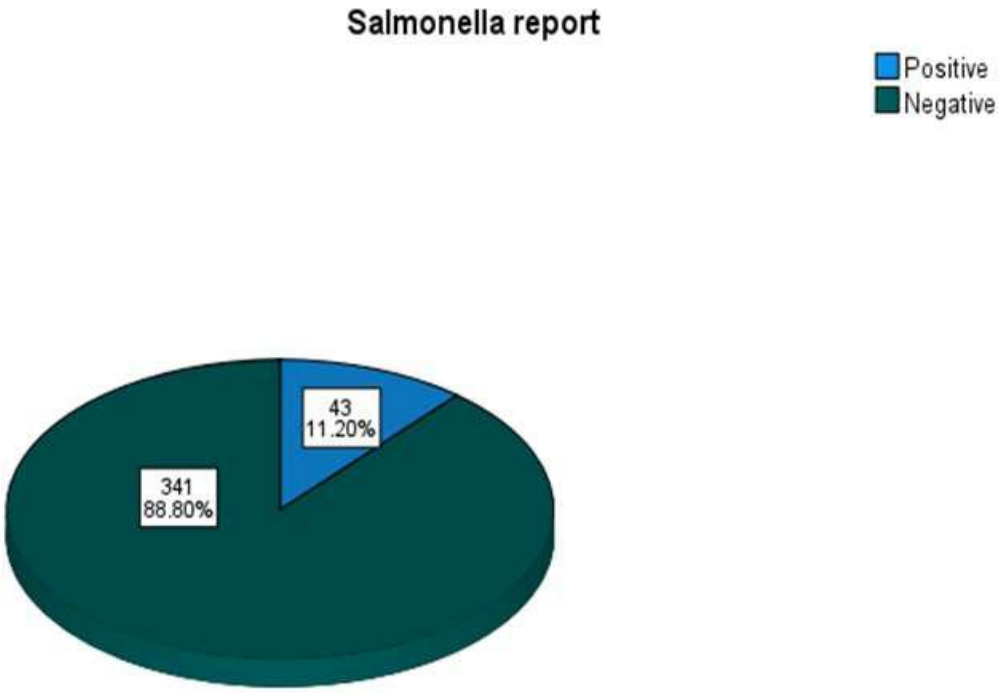

DISCUSSION

The aim of the present research was the isolation, identification, and determination of antibiotic of Salmonella spp. isolated from different areas of Peshawar. The isolates were confirmed as Salmonella spp. by cultural through media culture and biochemical test. This study has shown the Salmonella spp. in various sources and locations. Finally, the antibiotic sensitivity and resistance patterns of the isolates of different sources were identified in this study.

Water from various sources i.e. tap water, tube well water and water/storage tank water were subjected for bacteriological examination. Among these sources, the contamination (based on waterborne pathogens) was observed highest in tap water i.e., 6.77% as compared to water/storage tanks i.e., 3.38% and tube well water i.e. 1.04%.

Hussain *et al.* (2020) collected water samples from around 25 different areas of district Mansehra. Where they found 54% samples positive for salmonella collected from various sources such as tape water, water tank and tube well. The highest percentage found positive was tape water 80.36%. our results shows that the highest percentage of positivity found in tape water 6.77% as compare to storage water 3.38% and tube well water 1.04% collected from different area of district Peshawar.(6).

In another study of Mahfuzul Islam *et al.* (2021) studied that out of one hundred samples collected from wastewater of different hospitals, in which eighty-seven were found positive for salmonella. Our result is nearly similar to this in which we found 43 samples positive for salmonella. (1).

According to WHO wash guidelines drinking water should be free from all types of microorganisms. In the study Salmonella spp. were found to be distributed widely in the water samples analyzed. The overall occurrence of *Salmonella* spp.(S) was 11.20% in the positive samples 6.77% were from tape water, 3.38% from water tanks and 1.04 were from tube well. Our result is not matching, where there had severe lack of hygienic measures. (7)

In another study Popa *et al.* (2021) reported that 5377 cases were positive for *Salmonella* in Pakistan and USA. In the light of it, it's clear that Salmonella is a prominent cause of food and water borne diseases worldwide. (2)

In the book of ―Safe Drinking Water Committee‖ by National Research Council says that Deficiencies in treatment and contamination of groundwater were responsible for most of the outbreaks (65%) and cases (63%) in 1971-1974. Inadequate or interrupted chlorination was involved in 31% of the outbreaks and 44% of the cases. our

study is in correlation to this, in which we find that the 11.20% positive samples are from those area where had lack severe hygienic measures. (5)

Salmonella spp. Isolates were most commonly resistant as in previous study the findings are consistent with previous research by Abrar Hussain Mian *et al.* (2020), which reported high levels of antibiotic resistance in groundwater supplies, particularly against Novobiocin, Cephalothin, and Ampicillin.(6) While in our study low level of antibiotics were resistance in tape water, water tanks and tube wells, the resistance drugs were ciprofloxacin, gentamicin and ampicillin.

CONCLUSIONS

The rise of drug-resistant organisms poses a substantial problem to doctors, and drinking water polluted with these antibiotic-resistant germs can delay the treatment of water-borne infections. This shows that traditional antibiotics may be ineffective, necessitating the adoption of newer, more costly antimicrobial medicines.

This study uncovers the presence of antimicrobial-resistant bacteria in home drinking water, highlighting the significant public health implications. As a result, it is critical to implement and enforce stringent preventative measures to avoid the establishment and transmission of antibiotic-resistant microbes in drinking water sources, thereby maintaining public health and preventing the proliferation of waterborne diseases.

RECOMMENDATION

Consuming water tainted with antibiotic-resistant microbes may lengthen the time needed to cure waterborne illnesses, and the spread of these organisms is a significant concern for medical professionals. This implies that the use of new and more costly antimicrobial drugs may be required to treat aquatic illnesses as the effectiveness of

traditional antibiotics may be weakened. The current study emphasizes the existence of bacteria resistant to antibiotics in water sources used for residential and commercial reasons, highlighting the serious public health consequences. As a result, we support the adoption of strict policies to stop the growth and spread of bacteria that are resistant to antibiotics in water sources.

Furthermore, we recommend that boiled or filtered water be utilized for drinking and other purposes, rather than untreated and unboiled water, to mitigate the risk of waterborne disease transmission. This precautionary measure can help to reduce the likelihood of exposure to antibiotic-resistant bacteria, thereby minimizing the potential for prolonged treatment durations and the need for alternative, more expensive antimicrobial therapies.

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