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Review Article

Antimicrobial Resistance Pollution in Waters of Pakistan: A Narrative Review of Risks and Consequences

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Abstract

This review investigated the antimicrobial resistance (AMR) in the context of environmental waters of Pakistan. The literature was searched through databases *via*; Google Scholar, PubMed, ScienceDirect, ResearchGate, some organization's websites like UNEP, Pak-EPA and Government of Pakistan. This review details the AMR in the context of surface waters (glaciers, glaciated lakes and rivers), wastewaters (wastewater effluents, pharmaceutical wastes entering surface waters) and drinking waters (tap water, pumped water *etc*.) of Pakistan. The pharmaceutical industries, animal husbandry, agriculture and aquaculture practices in Pakistan as a source of AMR have also been discussed. It appeared that the waste effluents (industrial, hospital, domestic, animal farming) are harboring antibiotic-resistant bacteria (ARBs), antibiotics resistant genes (ARGs) and antibiotics residues that are contaminating surface water sources of Pakistan including glaciated lakes, rivers, tributaries, streams and nullahs culminating into the waters used for agriculture or domestic use. The wastewaters of Pakistan should be considered as reservoir of AMR for human where not merely the resistant bacteria/genes and antibiotics, but pollutants like biocides and heavy metals are also drivers of AMR. The governance and legislations need to be strengthened for sustainable protection of waters from AMR menace.

Keywords: Resistant bacteria; Antimicrobial resistance genes; Wastewaters

Introduction

The history of microbial tolerance to antibiotics is older than the discovery of antibiotics to treat and control bacterial diseases. The mechanistic validation of such bacterial resistance could be sought from bacteria dwelling in caves, permafrost and other environments isolated from anthropogenic bacterial contamination. Resistance is a natural mechanism evolved due to sustained competition of resources among microbiome, even against natural secondary metabolites very much similar to antibiotics of this era. Insomuch as, the use of antibiotics as clinical agents had changed the prerequisites for the evolution of resistance by providing selection pressures on microbiome of humans, domestic animals and environments polluted with antibiotics. Since, bacteria and antibiotic resistance genes (ARGs) often cross the species boundaries (Larsson and Falch 2022), the water bodies are key points for understanding the development and spread of ARGs (Calero-Cáceres *et al.* 2024). It is imperative to figure out the spread of AMR bacteria and genes across systems related to environment *i.e.*, *via* surface waters, wastewater and drinking waters.

Pakistan is ranked third among antibiotics consumers after China and India among low-middle income countries (Mirha *et al.* 2024) where self-medication of antibiotics is common. The major contributing factors are lack of knowledge about how and when to use antibiotics. The marginalized population cannot wait for longer recovery period, as they are relying on daily earnings for their subsistence and doctors prefer speedy recovery. However, this statement is based on personal perception of the authors and need further investigation. There is a lack of professional healthcare supervision and consultative healthcare services are bypassed that results in misuse of

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antibiotics (Aslam *et al.* 2022). The situation is even worse in animal husbandry where in addition to treatment of infectious diseases, antibiotics are used as growth promoters/food additives and as prophylactic agents to have maximum growth in least time. Consequently, there is massive production of antibiotics and their irrational use both in human and animal health sectors, ultimately favoring evolution of antibiotic-resistant bacteria (ARBs) and ARGs. This does not end up here, rather these malpractices are contributing to environmental pollution through contaminated industrial wastes, domestic sewage, hospital wastes and animal wastes, ultimately rendering wastewaters as reservoir of AMR. Untreated wastewaters, constantly entering into aquatic environments, employ continuous selection pressure on bacterial populations (Sattar *et al.* 2024). Due to the financial constraints, most of the rural and some urban populations use surface waters or pumped ground waters for drinking purposes, and AMR factors are recycled.

Many studies are conducted on the quality of drinking waters by Pakistan Council for Research on Water Resources (PCRWR) and academia, while few studies are available on AMR in drinking waters, waste waters and natural waters of Pakistan. The present narrative review was conducted to encompass the AMR in environment with special emphasis on surface, waste and drinking waters of Pakistan along with the contributing factors to this menace.

Materials and Methods

This is a narrative review with the focus on AMR in environment viz., surface waters, wastewaters and drinking waters. The sources of AMR including pharmaceutical industries, animal husbandry, agriculture and aquaculture were also explored in the context of Pakistan.

The literature was searched through databases *via*; Google Scholar, PubMed, ScienceDirect and ResearchGate. With few exceptions, only recent literature was included (preferably 2017 onwards), with the understanding that National Action Plan on AMR was available in 2017. Further, the relevant literature was also sought from some organization's websites like UNEP, Pak-EPA, PCRWR and Government of Pakistan. All the sources were selected according to their relevance and appropriateness towards the objectives of this review.

Pakistan's climate and AMR risk

Pakistan has diverse topography, ecosystems, and climate zones encompassing high mountainous regions (Hindukush, Himalayas), plains and deserts (Punjab and Sindh) and mountainous/semi-mountainous regions (Baluchistan and Khyber Pakhtunkhwa). The coastal south of Pakistan exhibits tropical to temperate and arid conditions, while northern glaciated regions experience extreme cold and snowfall during winters. The rest of the country have monsoon season from July to September (Ishaque *et al.* 2023).

Pakistan's geographical location has made it more prone to climate change and the mean temperatures is predicted to rise from 21.68°C (2021) to 30°C by 2060, with relatively stable precipitation (Sharif *et al.* 2024). The extent of antibiotic resistance may possibly be enhanced by climate change as has been reported in following studies. According to MacFadden *et al.* (2018), a rise (10ºC) in temperature across the United States was found to be associated with rise in antibiotic resistance of common pathogens *viz*., *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* (4.2%, 2.7%, 2.2%). Another study has reported an association between increase in average ambient temperature (1ºC) with prevalence of carbapenem-resistant *Klebsiella pneumoniae* (1.14-fold) and carbapenem-resistant *Pseudomonas aeruginosa* prevalence (1.06-fold) in China (Li *et al.* 2023).

The year 2022 had faced unprecedented heat wave and drought in summer followed by the abnormal rains and floods during monsoon season (Ishaque *et al.* 2022). The current heat wave (2025) is even more severe specifically in Punjab where temperature is ranging between 4ºC and 7ºC higher than average during the month of June (Gabol 2025). The heat waves are usually followed by heavy rains/floods, the flooded water mixes up with sewage effluents and contaminate surface waters. After receding of floods, the stagnant water remains reservoir of pathogens and diseases (Ishaque *et al.* 2022). It is relevant to mention that the spread of cholera spikes after monsoon floods in Pakistan (Malikzai *et al.* 2023).

AMR in surface waters of Pakistan

River system of Pakistan originates from the biggest glaciers of the world in Himalayas, Karakoram and Hindukush and emerges into five major tributaries *viz*., Jhelum, Chenab, Ravi, Beas and Sutlej, including three small rivers Haro, Soan and Siran that enter the Indus River from eastern side. Many small rivers enter the Indus River from the west sideways including Kunhar, Panjkora and biggest Kabul River. The small rivers joining Indus River from right side include Tai, Kohat, Tank, Gomal, Kurram and many small tributaries. A few small rivers from Azad Jammu and Kashmir also drain into the Indus (Gilani 2021). These rivers are considered the backbone of agriculture and most of the rural population also relies on riverine water for drinking, other domestic purposes and animal rearing. Moreover, Pakistan has 143 large and small water storage reservoirs, among which the Tarbela, Mangla and Chashma are the bigger ones (Ishaque *et al.* 2023).

Table 1 depicts the available information on AMR in surface waters of Pakistan (glaciers, major rivers and tributaries) along with sources and risks of AMR.

Pakistan has more than 5000 glaciers feeding the Indus River through different tributaries. Among these, Passu Glacier is located in Gilgit-Baltistan, the most densely populated non-polar glacier's region of the Karakorum mountain range, covering an area of 72,971 square kilometers.

Table 1: The AMR pollution in inland surface waters of Pakistan

Resource/Location	Bacterial isolates/ARGs	Antibiotics Resistance	ARGs	Antibiotics pollution	Probable source	Risks	Reference
Passu Glacier in Karakorum Mountainous Range	*Brevundimonas diminuta* *Staphylococcus equorum*	Multiple antibiotics	-	-	Hydrological systems, wind, migrating birds, vertebrate feces, tourists, and airborne bacteria	Potential hazard of glacier water as drinking water	Nawaz *et al*. (2023)
Supraglacial lake (Dook Pal Glacier), Hindukush region	Firmicutes Proteobacteria Actinobacteria	Ampicillin Cephalosporins Chloramphenicol Kanamycin Rifampin Tetracyclines		-	Airborne bacteria, migratory birds	Spread of resistant bacteria in downstream environments	Ilahi *et al.* (2023)
Supraglacial lake (meltwater), Dook Pal Glacier, Hindukush region	*Bacillus* sp. *Paenibacillus* sp. *Brevundimonas* sp. *Pseudomonas* sp. *Streptomyces* sp. *Arthrobacter* sp. *Alcaligenes* sp. *Serratia* sp. *Sporosarcina* sp.	-\	*qnrA* *bla*TEM	-	Migratory birds, wind, vertebrate feces and airborne bacteria	Glacial lake outburst may release of ARBs in environment	Sajjad *et al.* (2024)
Siachen Glacier	*Pseudomonas* *Alcaligenes* *Anthinobacterium* *Rhodococcus* *Arthrobacter* *Carnobacterium* *Bacillus* *Lysinibacillus,* *Staphylococcus* *Planomicrobium*	Imipenem Cefotaxime Clindamycin Ofloxacin Colistin sulphate Nalidixic acid Sulfamethoxazole Trimethoprim	-	-	Army patrolling might have harbored pathogenic or opportunistic bacteria	ARBs might transmit resistance genes to environmental bacteria	Rafiq *et al.* (2017)
Kabul River and tributaries (Bara River, Shah Alam River)	-	-		Ciprofloxacin Amoxicillin Cefixime	Wastewater from Peshawar city and northern regions of KP	Antibiotics flux into Indus River	Shams *et al*. (2024)
Indus River System (Indus, Jhelum, Ravi, Chenab and Soan)	*Klebsiella pneumoniae* *E. coli*	MAR Ampicillin Streptomycin Chloramphenicol Tetracycline Nalidixic acid Antibiotics-metal co-existence	-	-	Wastes from major cities, *e.g*., Lahore and Rawalpindi	Reservoirs of resistant bacteria for the public	Sair and Khan (2018)

MAR; Multiple antibiotics resistance

The antibiotic-resistant bacteria (ARB) with ARGs and class 1 integron integrase have been reported among bacteria of this region. The glacial melted water is the only source for domestic use in some areas and therefore of public health concern (Nawaz *et al.* 2023). Another glacier, Dook Pal is one of the best-known glaciers in the Chitral Valley (Hindukush region) that feeds two glacial lakes. The meltwater of this glacier has been reported to harbor ARBs and ARGs. The probable source of AMR in these extreme climatic conditions are suggested to be hydrological systems, wind, migrating birds, vertebrate feces, tourists, and airborne bacteria (Ilahi *et al*. 2023; Sajjad *et al*. 2024). Another glacier, Siachen is well known as it sets division between China and Pakistan and is considered world's highest non-polar glacier and world's biggest garbage dump. It has also been explored for culturable bacterial diversity and different gram-positive and gram-negative bacteria along with their antibiogram has been reported. The authors have speculated that the antibiotics resistance in this region could be due to transmission of pathogenic or opportunistic bacteria by army patrolling, heavy metals pollution and production of antimicrobial compounds (Rafiq *et al.* 2017).

Kabul River originates in Afghanistan and drains into the Indus River (Khuram *et al.* 2017). This river along its tributaries *viz*., Bara River with Shah Alam River, Sardaryab River, Kalpani stream, receives wastewater from major districts/suburbs of Peshawar, Swat, Charsadda, Mardan and Nowshera (Schilling 2023). In an investigation, antibiotics (ciprofloxacin, amoxicillin, cefixime) pollution was detected in the Kabul River and its two major tributaries, Bara River and Shah Alam River. The combined flux of the antibiotics in the Kabul River was estimated as 59 tons/year. The higher antibiotics pollution in the Kabul River was attributed to the wastewaters originating from areas of Peshawar. The study reported higher risk of antimicrobial resistance development in the Kabul River and its major tributaries (Shams *et al.* 2024).

Indus, the longest river (3200 km) receives major tributaries (Soan, Jhelum, Chenab and the Ravi) and drains

Table 2: AMR pollution in wastewaters/effluents and streams of Pakistan

Resource/Location	Bacterial isolates/ARGs	Antibiotics Resistance	Antibiotics Resistance genes	Antibiotics pollution	Probable source	Risks	Reference
Wastewater efflux into Sutlej River	*E. coli* *Klebsiella pneumoniae* *Pseudomonas aeruginosa* *Salmonella* spp.	MAR Ciprofloxacin Co-trimoxazole Ampicillin Tetracycline	Aminoglycosides (aadA) Sulfonamides (Sul1, Sul3) Tetracyclines {Tet (A/B/D)} Cephalosporins (Bla_CTM X)	-	Municipal waste discharge into Sutlej River	Spread of AMR through drinking river water	Rahim *et al.* (2024)
Wastewater efflux into Soan River	-	-	-	Ciprofloxacin Ofloxacin Ampicillin Levofloxacin Sulfamethoxazole.	Discharge of untreated wastewater into river	Entrance of resistance bacteria and resistant genes into food chain	Zafar *et al.* (2021)
Waste water from Hattar Industrial State, Industrial area, Kahuta, Islamabad	-	-	-	Ciprofloxacin Enrofloxacin Levofloxacin	Untreated waste water from the pharmaceutical industries	Contamination of food chain through irrigation of crops	Riaz *et al.* (2017)
Wastewater streams, Rawalpindi-Islamabad, Faisalabad	*Aeromonas* *Escherichia* sp.	Ampicillin Amoxicillin Ofloxacin Ciprofloxacin Levofloxacin	*qnrS2* *blaTEM*	-	Sewage from major hospitals entering into Nullah Lai (RWP-ISB); Local drain, Faisalabad	Spread of ARGs through *Aeromonas* vector into River Soan, Ravi and Chenab	Saima *et al.* (2020) Fiaz *et al.* (2021)
Korang River and its tributaries (downstream Baharakahu, downstream G-6), Lai stream, wet markets and wastewater treatment plant	ESBL *E. coli*	Cephalosporins Monobactam Carbapenems	blaTEM blaCTX-M	-	Wet markets (poultry meat shops), animal dung, WWTP (microbial decontamination is not being carried out at WWTP)	Transmission of AMR bacteria through Korang River to Rawal Lake and residents of RWP- ISB	Ahsan *et al.* (2022)
Wastewaters (sewage, household, canals) throughout Pakistan	*E. coli* *K. pneumoniae*	Cefotaxime Ciprofloxacin Imipenem Gentamicin	bla NDM bla OXA	-	Sewage and domestic wastes in major cities of Pakistan (Punjab, KP, Sindh, Baluchistan)	Transmission of ARB and ARGs through contaminated water entering streams/used in agriculture	Sattar *et al.* (2024)

into Arabian Sea near Karachi. A study explored single sites from Indus and its tributaries (Chenab, Jhelum, Ravi and the River Soan). The study reported that *Klebsiella* and *Escherichia coli* strains were abundant and most strains exhibited resistance towards ampicillin (44.7%), streptomycin (37.3%) and chloramphenicol (33.9%). Multiple antibiotic resistance and metal-resistance was also reported that was attributed to co-resistance amongst the bacterial populations in rivers downstream of large cities where sewage effluents are directly contaminating the waters. The most worrisome is, the river water is not only used for irrigation but used for drinking by population residing in rural areas (Sair and Khan 2018).

Briefly, the rivers serve as a fundamental water source for irrigation and domestic use in most areas of Pakistan. Therefore, defined legislation and implementation strategies are urgently required for containment of antibiotics pollution at production, use and disposal sites.

AMR in wastewater effluents

The amount of wastewater generation once approximated was 962,335 million gallons per day (MGD), however the recent figures are not available as per our information. The current literature on AMR in wastewaters of Pakistan, the sources and risks are presented in Table 2. According to available information, the wastewater is either released into the main rivers and surface water bodies or used for irrigation purposes while, only 1% of waste water is reported to be treated (Mahfooz *et al.* 2020). Even the 1% treatment of water is doubtful as an investigation has reported that all of the water samples from wastewater treatment plant (WWTP) at Nullah Lai (Rawalpindi, Pakistan) and its following stream (Lai stream) were contaminated with AMR bacteria. Nullah Lai drains into Korang River that along with its tributaries, wet markets, and WWTP were reported to have extended-spectrum beta-lactamase-producing *E. coli* (ESBL *E. coli*) resistant to

cephalosporins, monobactam and carbapenems. The contamination source was anticipated to be wet markets (poultry meat shops), animal dung, and WWTPs. Surprisingly, wastewater was merely treated for heavy metals while microbial sanitization methods were not being adopted at WWTP, Nullah Lai, Rawalpindi (Ahsan *et al.* 2022).

River Sutlej collects effluents from treatment plants around the Bahawalpur metropolitan, Pakistan. Recently, water samples from wastewater efflux and receiving waters in this river were found to contain ARBs; *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella spp*, resistant to ciprofloxacin, co-trimoxazole, ampicillin and tetracycline. The ARGs; aadA, Sul1, Sul3, Tet (A/B/D), Bla CTM X were also reported. Fortuitously, lower resistance of these isolates was found against imipenem and gentamicin, an indication that these antibiotics still retain efficacy and need to be secured for clinical use. This investigation depicted that ARBs and ARGs in wastewater are entering River Sutlej, a source of antibiotic resistance (ABR) to the general population as most of the villages use this resource as drinking water (Rahim *et al.* 2024).

Ravi, Chenab and Soan rivers receive steams, drains and tributaries from major cities *viz*., Lahore, Faisalabad and Rawalpindi, respectively. Wastewaters from tributaries to these rivers were found to contain ARB; *Aeromonas sp and Escherichia* sp., resistant to ampicillin, amoxicillin, ofloxacin, ciprofloxacin, levofloxacin and harbored ARGs; *qnrS2* and *blaTEM*. In fact, the major hospital effluents from the twin cities, Rawalpindi and Islamabad are transferred to River Soan through Nullah Lai. Similarly, hospital wastes from major hospitals of Faisalabad are transmitted through local drain to River Ravi and Chenab. These studies have postulated the spread of ARGs through *Aeromonas* vector into to River Soan, Ravi and Chenab (Saima *et al.* 2020; Fiaz *et al.* 2021).

The studies cited above have indicated that the carbapenem-resistant bacteria (CRB) are circulating in environment/wastewater samples with evidence of their spread in human population (Mills and Lee 2019). A recent investigation has confirmed this and highlighted that the wastewaters of Pakistan are major source of antibiotic-resistant bacteria and hotspots for the antibiotic-resistant gene exchange. The study investigated bacterial isolates from wastewaters (sewage, household, canals) throughout Pakistan and reported CRB, *E. coli* and *K. pneumoniae*, resistant to cefotaxime, ciprofloxacin, imipenem and gentamicin. The ARGs, bla NDM and bla OXA were reported from these isolates. Therefore, sewage/domestic waste in major cities of Pakistan (Punjab, Khyber Pakhtunkhwa, Sindh, Baluchistan) was supposed to be the major source of CRB that pose serious threat of transmitting ARB and ARGs into streams and rivers or being used in agriculture (Sattar *et al.* 2024).

Unregulated disposal and use of antibiotics is also a serious contributing factor of AMR through antibiotics pollution of surface waters. The wastewater channels entering Soan River were reported to harbor antibiotics pollution with highest concentration of ciprofloxacin followed by levofloxacin, ofloxacin, ampicillin and sulfamethoxazole. The antibiotics pollution of Soan River might have been due to non-existence or improper function of wastewater treatment plants and discharge of municipal, industrial and hospital effluents into the streams/channels entering Soan River (Zafar *et al.* 2021). Another study reported contamination of ciprofloxacin, enrofloxacin and levofloxacin in wastewater water/sludge samples of Hattar Industrial State, Khyber Pakhtunkhwa and Hummak Industrial area, Islamabad, Pakistan (Riaz *et al.* 2017). Similarly, the wastewater released from the pharmaceutical industry and in its receiving environment along Sheikhupura road, Lahore, Pakistan was found polluted by ofloxacin, ciprofloxacin, moxifloxacin, sparfloxacin and gemifloxacin (Ashfaq *et al.* 2017).

The antibiotics pollution serves as surrogate for bacterial resistance, whereby sensitive and beneficial bacteria are killed while resistant microbes survive in the wastewaters heavily contaminated with antibiotics. The low levels of antibiotics also serve the same through providing an opportunity to the microbes, develop resistance slowly (Zafar *et al.* 2021). Some studies have also reported presence of antibiotics residues in aquatic organisms, fish and shrimp that could transfer to human through food chain (Liu *et al.* 2018). This is the reason that wastewater stream contaminated with antibiotics serves as a reservoir of ARB (Zafar *et al.* 2021).

In Pakistan, wastewater is extensively used in agriculture, especially for growing vegetables and fodder crops, therefore the risk of AMR transmission to human and animals becomes fortified. It is alarming that farmers prefer to use untreated wastewater compared to treated wastewater in many parts of Pakistan, as it gives more profitable production (Parveen and Khan 2023). In this situation, the regulations and standards need to be revised with mandatory inclusion of wastewater disinfection before use in agriculture or drainage into surface waters. However, the ultimate goals could only be achieved through prioritizing the global issue of environmental AMR in national agendas, international collaboration, financing and strict implementation of the legislations.

AMR in drinking water

Pakistan ranks third among countries with inadequate water supplies and poor sanitation, with 80% of rural areas (Batool and Shahzad 2021), around 2.1 million people have no access to clean drinking water (Muhammad *et al.* 2024). In most of the cities of Pakistan, the major source of drinking water is ground water (Daud *et al.* 2017), that is vulnerable to quality deterioration due to mismanagement of industrial and sewage waste. The water quality in major cities is rapidly worsening as sewage and industrial wastewater is directly entering into rivers without prior treatment/decontamination resulting in the formation of

Table 3: AMR pollution in drinking waters of Pakistan

Drinking water source/ Location	Bacterial isolates/ ARGs	Antibiotics Resistance	Probable source	Risks	Recommendations	Reference
Drinking water, Barkhan District, Baluchistan	*Salmonella typhi*, *E. coli* *P. aeruginosa*	Ampicillin Chloramphenicol Ceftriaxone Gentamicin Sulfamethoxazole Trimethoprim	Unscrupulous dumping of domestic wastes, poor drainage facilities, open defecation, and a water-logging environment	Waterborne disease outbreaks	Implementation of globally accepted guidelines for treatment and prevention policies through national authorities and at the individual level	Muhammad *et al.* (2024)
Tap water, storage tanks, tube well, District Mansehra, KP	*E. coli* *Salmonella* spp.	Clindamycin Amoxicillin	Lack of disinfection, poorly-managed pipelines, infiltration of contaminated sewage, and backsiphonage	Inappropriate treatment of water-borne diseases with these antibiotics	Stringent measures be taken to prevent contamination of drinking waters	Mian *et al.* (2020)
Hand pumped (underground) water in different towns, Dera Ismail Khan, KP	*Enterobacteriaceae*, *Serriata* sp. *Proteues* sp. *Pseudomonas* sp.	Chloramphenicol Amoxicillin	Horizontal gene transfer between microorganisms in environment	Transfer of antibiotic resistant to normal flora or other pathogens	-	Alam *et al.* (2018)
Household water, Peri-urban areas, Karachi	*E. coli* *Enterobacter* spp. *Klebsiella pneumoniae*	Ceftazidime Trimethoprim/ sulfamethoxazole	Insufficient water pressure, interruption, contamination from leaky sewage lines	Childhood diarrhea is water-borne	NQS should include HPCs and consider high HPC counts (> 500 CFU/mL) to indicate water is unsafe for consumption Point-of-use disinfection Chlorination of water	Shakoor *et al.* (2018)
Drinking water around Bolan Medical Complex Hospital, Provincial Sandeman Hospital, Quetta	*E. coli*	Tetracycline Trimethoprim-sulfamethoxazole Cefixime Amoxicillin	Cross contamination and bacterial transfer from clinical samples to water and vice versa	Drug resistance (*ESBL blaCTX-M-15*, *tetA*, and *TMP-SMX dfrA1*) and pathogenicity associated *PAP* genes are of serious health concern	Strict quality control procedures to ensure the wastewater treatment	Akbar *et al.* (2022)

pollution plumes, making aquifers unusable for domestic consumption (Quddoos *et al.* 2024). The leakage in water supply lines and effluence from sewage lines entering into drinking water supplies is blamed for microbial contamination of drinking water. Multiple studies have been conducted by PCRWR that revealed contamination of drinking waters by heavy metals, other chemical pollutants and bacteria across the provinces of Pakistan (Ahsan *et al.* 2021; Rasheed *et al.* 2021, 2022), while few studies are available on AMR contamination of drinking waters (Mian *et al.* 2020; Saima *et al.* 2020) as presented in Table 3.

Drinking water sources in Barkhan, Baluchistan (April 2022 to September 2022) were reported to be contaminated with pathogenic bacteria *Salmonella typhi*, *E. coli* and *P. aeruginosa*. Among these, *S. typhi* isolates were reported to be highly resistant to ampicillin, chloramphenicol, ceftriaxone, gentamicin, sulfamethoxazole/trimethoprim, while *E. coli* isolates were found resistant against ampicillin followed by ceftriaxone, chloramphenicol, azithromycin and sulfamethoxazole/trimethoprim. *P. aeruginosa* isolates were found resistant against ampicillin, ceftriaxone, chloramphenicol, azithromycin and sulfamethoxazole/ trimethoprim. Fortuitously, all these pathogenic isolates were reported to be highly sensitive to imipenem (Muhammad *et al.* 2024). Another study on drinking water from/ around different hospitals in Quetta, Baluchistan has reported the contamination with ESBL *E. coli* having resistance against tetracycline, trimethoprim-sulfamethoxazole, cefixime and amoxicillin (Akbar *et al.* 2022). Dumping of domestic wastes, open defecation, poor drainage facilities, and a water-logging environment was speculated to be the main cause of AMR contamination of drinking waters in Barkhan district, while cross contamination and bacterial transfer from clinical samples to water and vice versa was blamed to be the cause of AMR in and around hospitals of Quetta, Baluchistan.

The situation of Khyber Pakhtunkhwa (KP) was considered better as more than 80% of the people of province were thought to be utilizing clean drinking water from surface and ground sources (Daud *et al.* 2017), however, this information is more than two decades old. The recent studies have revealed that the tap water, storage tanks, tube well water that was used for drinking purposes in District Mansehra, KP was contaminated with *E. coli* and *Salmonella* sp resistant to clindamycin and amoxicillin (Mian *et al.* 2020). Similarly, hand pumped (underground) water in different towns of Dera Ismail Khan, was found to be contaminated with chloramphenicol and amoxicillin resistant bacteria (*Enterobacteriaceae*, *Serriata* sp. *Proteues* sp. *Pseudomonas* sp.) (Alam *et al.* 2018). Lack of disinfection, poorly-managed pipelines, infiltration of contaminated sewage, and backsiphonage were reported to be

the possible drivers of AMR pollution of drinking waters in KP.

Punjab has best water supply system among all the provinces of Pakistan (Daud *et al.* 2017). A number of studies investigated the quality of drinking water in metropolitan and rural areas of Punjab. The water quality parameters in most of these reports include heavy metals and other pollutants along with presence of *E. coli* or coliform bacteria. Studies on AMR in drinking waters could not be retrieved after repeated searches by the authors.

A study on household water from peri-urban areas of Karachi viz., Cattle Colony, Rehri Goth, in the Bin Qasim town that receive piped water supply from the Kinjhar lake system was contaminated with *E. coli*, *Enterobacter* spp. and *Klebsiella pneumoniae* resistant to ceftazidime, trimethoprim/sulfamethoxazole. The reasons for this contamination are believed to be insufficient water pressure, system interruption and contamination from leaky sewage lines (Shakoor *et al.* 2018).

The drinking water contamination with resistant bacteria calls for implementation of globally accepted guidelines/policies through government and non-government organizations and at the individual level. Stringent measures are required to prevent water contamination. Point-of-use disinfection like chlorination of water need to be implemented.

AMR pollution contributors

Pharmaceutical industries: There are more than 700 pharmaceutical industries that are geographically spread all over Pakistan. At provincial level, highest number of pharmaceutical units are concentrated in Punjab (440), followed by Sindh (183), and KP (114), while few (15) units in Baluchistan and Azad Jammu and Kashmir (7) (PPMA, 2017). These industries are producing excessive amount of waste, non-complying with National Environmental Quality Standards (NEQS), releasing wastewater into domestic wastewater systems without any decontamination process (Rehman *et al.* 2015). It is surprising to note that River Ravi had highest concentration of pharmaceutical waste among world rivers (Wilkinson *et al.* 2022). An investigation has revealed the existence of antibiotics; ofloxacin, ciprofloxacin, moxifloxacin, sparfloxacin and gemifloxacin in waste/soil samples near the pharmaceutical units along Sheikhupura road, Lahore, Pakistan (Ashfaq *et al.* 2017). Similarly, water and sludge samples from the drains in Hattar Industrial State, Taxila-Haripur Road, Khyber Pakhtunkhwa and Hummak Industrial area, Kahuta-Islamabad Road, Islamabad, were found contaminated with ciprofloxacin, enrofloxacin and levofloxacin (Riaz *et al.* 2017).

Animal husbandry: Pakistan has 67.3 million large ruminants, 89.6 million small ruminants, and 1,230 million poultry. Veterinary antibiotics (VAs) are commonly used as food additives/growth promoters (FAs/GPs) and in the prevention/treatment of infectious diseases in poultry and livestock farming (Sardar *et al.* 2021). It has been reported that tons of critically important antimicrobials with highest priority were imported for the years 2017–19 that include tylosin, enrofloxacin, colistin, tilmicosin, spiramycin, norfloxacin, and ceftiofur. The resultant antimicrobial consumption was 10.05 mg/kg of animal biomass. The estimated poultry FAs/GPs consumption was 96.53 mg/kg of biomass that contained zinc bacitracin, enramycin, bacitracin methylene disalicylate, tylosin, tiamulin, colistin, lincomycin, streptomycin, flavophospholipol, tilmicosin and penicillin (Umair *et al.* 2022). Poultry wastes are directly used as fertilizer or disposed into municipal drains or nearby open land (Rousham *et al.* 2018; Habiba *et al.* 2022). Antibiotics existing in poultry wastes are usually bioactive and result in high ABR in bacteria exposed to the close environment (Habiba *et al.* 2022). Insomuch as, the antibiotics used in animal husbandry results in AMR pollution of the environmental soil, wastewaters and find their way towards surface water bodies without treatment. Even, if treated, antibiotics and ARGs in livestock waste water cannot be removed completely and are discharged into the water environment (Wu *et al.* 2024).

Agriculture: Agriculture is the backbone of Pakistan's economy and is dominated by crops; wheat, rice, sugarcane, cotton, vegetables and pulses. The irrigation system is based on rivers, tributaries and canals or pumped water. It is critical to mention that Pakistan is facing scarcity of water as resources are shrinking due to construction of dams and barrages and population is expanding. Climate change/warming is further escalating the issues of water shortage and pollution. The economic conditions of local farmers are worsening due to high production costs and low prices of the crops set by the government. Livestock is the major sector of agriculture that plays significant role in livelihood of farmers and contributes to national gross domestic product (GDP). The intimate link between livestock and agriculture is further strengthened by the fact that livestock manure is the major source of organic fertilizer. The animal dung is stored and used directly in the agriculture farms to increase/maintain the soil fertility, while chemical fertilizers is the next level option due to high costs to the local farmers. Animal fertilizer (livestock and poultry) is the prime entry route of antibiotics in the agricultural ecosystem of Pakistan. It is relevant to mention that 49% of farmyard manure is directly applied in the fields as organic fertilizer. Further, slaughterhouses are common in Pakistan, and approximately 8,000 tons of blood meal that is produced annually is being used as soil conditioner without any pre-treatment (Rahman and Mohsin 2019).

Antibiotics entering the soil with animal manure stay mostly on the soil surface, however, in rice cultivation practices, the standing water serves as a source of antibiotics/residues leaching into the soil. Once the antibiotics enter the soil atmosphere, they are prone to sorption/desorption, sequestration and transportation through

surface water run-off, flood irrigation or groundwater aquifers after these get dissolved in the soil solution (Sardar *et al.* 2021). These antibiotics interact with natural soil microbiome, create imbalance in microbe community and increase antimicrobial resistance genes (Knapp *et al.* 2011). The sustained use of animal manure results in buildup of hazardous content in terrestrial environment, plants and ultimately enter the food chain. The bioaccumulation of antibiotics depends upon the type of antibiotics, the exposure time, the plant and its tissues or organs. The root crop is more prone to bioaccumulation of antibiotics as these are directly exposed to soil. Briefly, the irrational use of animal manure is a great threat to staple crops like potato, wheat, corn and millet (Sardar *et al.* 2021). It is believed that pretreatment of animal manure, like composting, biogas or biochar application could reduce the risks of antibiotics/residues contamination of the agricultural soils and commodities.

Aquaculture: Aquaculture includes culture of aquatic organisms including finfish, shellfish and plants in fresh, brackish and marine waters. In Pakistan, marine fisheries are mainly based on capture fisheries, while inland aquaculture is practiced in all provinces/regions that mostly relies on extensive or semi-intensive fish farming practices. Freshwater fish species *Catla catla, Labeo rohita, Cirrhinus mrigala, Cyprinus carpio* and *Oreochromis niloticus* are cultured under extensive or semi-intensive culture systems of warm waters, while *Oncorhynchus mykiss* is cultured in cold waters. Under extensive culture, animal manure (cow/buffalo and poultry) are being used at fish farms to increase the natural productivity of the ponds, while artificial feed is used in addition to manuring in semi-intensive systems. The animal manures are a source of AMR, both bacteria and antibiotics residues (30-90% of antibiotics residues) that are eliminated through ruminant excreta. The animal manures if treated (composting, biogas production), the incidence of diseases can be reduced. No regulations regarding use of animal manure in aquaculture are yet available. Therefore, the legislations/policies need to be devised for effective and hygienic utilization of animal manure as fertilizer in fish farming. Further, the utilization of fertilizer, manure (excretes of animals), supplements, and other materials used at fish farms should pass through prescribed safety measures for hygienic production and reducing the AMR burden in aquaculture sector (Ullah *et al.* 2023a).

According to available information, antibiotics are not used as feed additives in fish culture, nor as growth promoters, exceptions; however, exist not being documented yet. It is critical to mention that water for the aquaculture comes either from surface water sources (reservoirs, streams, canals etc.) or is pumped (ground water). These waters are not free of the contamination by ABR, ARGs, antibiotics residues (described in previous section) that are even multiplied during monsoon season due to floods. A number of studies are available that reported resistant bacteria from riverine fish (Mahmood *et al.* 2024) and healthy or diseased farmed fish (Ullah *et al.* 2023a, b).

Conclusions

The wastewaters of Pakistan have become major source of ARB and hotspots for the antibiotic-resistant gene exchange. These waste effluents (industrial, hospital, domestic, animal farming) are contaminating surface water sources of Pakistan including glaciated lakes in the north mountainous regions, rivers, tributaries, streams and nullahs that are recycling ARBs, ARGs or antibiotics residues into the waters used for agriculture or domestic use. This never-ending vicious cycle of AMR could be interrupted to some extent if wastewaters are treated before entering into the surface waters. Currently, the wastewater plants are far less in numbers and only 1% waste waters are reported to be treated before entering the surface water resources. Most of these WWTPs are even non-functional or not complying with AMR containment. Protection of waters (glaciers, rivers, streams, nullahs) from AMR pollution (ARBs, ARGs and antibiotics/residues) is the main requirement for containment of AMR spread. Strict legislation and law enforcement, with particular emphasis on AMR is required. It is required to increase in numbers and efficiency of wastewater treatment plants with mandatory protocols for AMR containment. Point-of-use decontamination may also serve the supply of drinking water free of AMR. The situation calls for involvement of government and NGOs in terms of financial and technical investment to control this menace of global concern.

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Author Contributions

SA and QA conceived the idea, finalized methodology and supervised the study. MAA, MTA AH and JA reviewed the literature and written different sections of review article. BAR and MHK critically analyzed the literature and made tabulated illustrations.

Conflict of Interest

The authors have no conflict of interest to declare.

Data Availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Ethics Approval

Not applicable to this paper.

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