Microbial contaminants in Pakistan: a review

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Abstract

Worldwide contamination of surface waters with microbial pathogens is of substantial health concern. These contaminants are usually transmitted by improper sanitation measures, unsafe waste disposal, excretions from patients, and physical contacts, i.e., sexual and nonsexual. Majority of these microbial pathogens have been categorized into three classes, i.e., bacteria, viruses and protozoa. Pakistan, being a developing country, is facing a noteworthy threat due to microbial contamination. In Pakistan, bacterial contaminants are reported extensively followed by viral and protozoa contaminants. The health issues associated with bacterial population includes dysentery, abdominal pain, headache, diarrhea etc.; and usually includes faecal and total coliforms, *E. coli*, *Salmonella, Shigella* and *Campylobacter*. The cases related to viral contamination are lesser but chronic and evidenced the presence of HCV, HAV, HEV viruses causing hepatitis, and other hepatic disorders. Lastly, the health impacts associated with protozoans are least reported; and a number of diseases such as giardia, cryptosporidium and toxoplasma have been linked with this class of contaminants. The current review compiles information of these biological contaminants along with their health issues in Pakistan. Moreover, potential sources and fate of microbial contaminants are also discussed.

Keywords: Microbial Pathogens; Bacteria; Viruses; Protozoa, Pakistan

Introduction

Microorganisms are present almost everywhere on earth ranging from oil reservoirs to hot springs (Orphan et al., 2000; Khalil et al., 2015). Their presence in different environment is proxy to environmental conditions, and have been widely exploited to assess the presence of contamination in terms of indicators species (Auer and Niehaus, 1993). The concept of faecal indicators was introduced by early water-microbiologists to assess the faecal pollution indirectly in contaminated waters (Auer and Niehaus, 1993). Since then, bacterial indicators have been proven to be a good tool for pathogens´ probability assessment (Bonadonna et al., 2002; Medema et al., 1996; Teunis et al., 1997).

In modern societies, wastewater generated from industrial and municipal activities contains a wide range of pollutants including organic and inorganic chemicals, and pathogenic microorganisms. Among these pollutants, pathogenic microorganisms are of serious health concerns. Ingestion of such pathogens results into infectious diseases such as gastroenteritis, diarrhea, cholera, dysenteries, and enteric fevers (De Man et al., 2014; Soller et al., 2010). This burden of water-borne diseases is uppermost in low-income settings where the prevalence of diarrhea remains a leading cause of child deaths (Bain et al., 2014). Systematic reviews and outbreak investigations illustrate that the extent of this contamination is expected to increase in the near future mainly due to urbanization, climate change, and population growth.

The microbial pathogens are generally categorized into three major groups as per the classification system i.e. bacteria, viruses, and protozoa. Each group is responsible for a set of diseases according to their innate pathogenicity and infectious potential. For example, bacteria are responsible for diarrhea, cholera and typhoid; viruses can cause hepatitis, polio, and gastrointestinal diseases; and certain protozoa grounds for giardiasis, dysentery, and cryptosporidiosis. Such harmful effects associated with these pathogens have been reported all around the world. For example, in Germany, a disastrous outbreak of diarrhea and the hemolytic–uremic syndrome was reported for the period from May 2011 to July 2011 due to an unusual serotype of Shiga-toxin–producing strain of *Escherichia coli* O104:H4 that resulted into extensive life loss within months (Luna-Gierke et al., 2014). Similarly, in the United States, National Outbreak Reporting System (NORS) reported 81 outbreaks from 2009 to 2010 among which 57 outbreaks were linked to *Cryptos-*

poridium (Craun et al., 2006; Hlavsa et al., 2014). Another study illustrates that infantile diarrhea caused by rotavirus had been responsible for over 3.5 million infections whose outbreak was connected to faecal-oral route (Blacklow and Greenberg, 1991). This mini-review compiles the information on sources and fates of microbial contaminants along with their reporting in different regions of Pakistan.

Bacterial Contaminants

The contamination of water bodies with bacterial contaminants is of substantial health concerns since their presence may lead to serious infections and waterborne diseases (Ashbolt, 2004; Smolders et al., 2014). These contaminations are mainly attributed to mishandling of wastes generated from disease carrying humans and animals. Once in the environment, the contaminants may become part of potable-water supplies and subsequently affect the living organisms. According to a survey, an average of 4 billion diarrhea cases per year indicated global disease burden of 5.7% in the year 2000 (Wright et al., 2004). Moreover, if the bacterial contaminants remained untreated, high metabolic activities and short generation times of pathogenic microorganisms may spread the contamination to large geographic areas resulting into disease outbreaks (Hlavsa et al., 2014). The presence of bacterial contamination is not limited to surface water but several other studies have also reported the contamination of groundwater. One reason behind this contamination is the seeping of leachate into the deeper soil while carrying microbial pathogens (Corapcioglu and Haridas, 1983; McCarthy and Zachara, 1989).

The presence of bacterial contaminates has been achieved by different methods however direct enumeration of each group of pathogen is a costly and time-consuming procedure. Therefore, microbiologists have devised an indirect strategy of assessing microbial pollution by identifying corresponding faecal indicators in water bodies. The concept of faecal indicators was introduced by early water-microbiologists to assess the faecal pollution indirectly in contaminated waters (Auer and Niehaus, 1993). Resultantly, presence of indicator species (e.g., faecal coliforms and *E. coli*) is a key of contamination of water with diseases causing germs and pathogens. The presence of faecal coliforms and *E. coli* indicates that the water is contaminated with human or animal excreta.

In Pakistan, various factors are contaminating drinking water with microbial pathogens (Shar et al., 2008). Among them, feces are considered as one of the major sources of bacterial contaminants and therefore their detection in feces is an important priority while checking the quality of drinking water (Sarwar et al., 2004; Azizullah et al., 2011). In Pakistan, majority of the freshwater sources such as rivers, reservoirs, groundwater aquifers, and lakes are exposed to different extents of faecal pollution (Shar et al., 2008). For instance, a study from rural area illustrates that *Campylobacter*, ETEC-LT, *Shigella* and *Salmonella* are normally transmitted with flies and surface water whose primary source of contamination was animal waste. Furthermore, flies further transport the enteropathogens to the nearby areas resulting into offshore contamination. By comparison with the urban areas, it is further re-

vealed that water of rural areas were more contaminated compared to the cities. Nevertheless, *campylobacter* and ETEC-LT are the most commonly observed pathogens in both flies and surface water (Khalil et al., 1994). In Peshawar, 81% of untreated samples are found to be contaminated with coliforms population whereas 19% of contamination was observed even from of the treated samples. Majority of the samples were contaminated with faecal coliforms whereas a few samples were found to be contaminated with faecal streptococci. Moreover, *E. coli* was also found in 43.28% of the samples (Sarwar et al., 2004).

Another study reports the problem of contamination of water supply in Karachi, the largest city in Pakistan with a population of more than 12 million and growing at 6 percent per annum. Majority of the water resources, other than municipal supplies, are found to have coliform bacteria, faecal coliform while their numbers extents higher than national and international norms (Rahman, 1996). In Karachi, the contamination is observed in domestic tanks and community taps of Mahmoodabad and Liaqatabad. Majority of the bacterial pathogens belong to *E. coli, Enterobacter sp, Klebsiella sp.* and *A. faecalis* species; whereas, in Clifton area, species that were found includes *Salmonella para typhi* and *para typhi B, Shigella dysenteriae* and *Aeremonas sp.* (Baqai and Zuberi, 1991).

Similarly, at Charsadda district, coliform bacterial contamination is found to be 2–5 MPN 100 mL^{-1}; whose harmful are reported equally among infants and elders (Khan et al., 2013). Another study identifies *E. coli*, with *Vibrio cholera* as the most common enteric isolates; however, *Vibrio cholera 01 Ogawa* is found followed by *Campylobacter jejuni* as the most usual enteric pathogen in urban area (Alam et al., 2003). In Khairpur (Sindh) out of 768 tested drinking water samples, 567 (73.83%) are appeared to be contaminated with total coliforms while 351 (45.70%) were contaminated with faecal coliform. Another study in Khairpur reveals that all of water samples collected (100%) are found to be contaminated with total coliforms and faecal coliforms, whereas the samples were obtained from the main reservoir, distribution lines and consumer taps (Azizullah et al., 2011). Similarly, a study conducted between November 1986 to March 1988 in a hospital-based inpatient and outpatient of two hospitals illustrated 1,492 cases of acute lower respiratory tract infection (ALRI); while 378 cases were tested for detection of bacterial antigen among which 277 were contaminated by bacteria (Ghafoor et al., 1990). Over and above, bacterial contamination in Pakistan is not limited to local supplies as water samples obtained from three dams of twin cities of Pakistan (i.e. Rawal Dam, Simly Dam, Khanpur Dam) are found to be contaminated with various pathogenic bacteria. Those pathogenic bacteria were *B. cereus*, *E. aerogens*, *S. aureus*, *E. coli*, *Salmonella* spp., *Shigella* spp. and *Streptobacillus* spp. (Ahmed et al., 2004). Microbial contaminants were also found in water supplies near treatment plants and distribution networks in the area of Ratta Amral, Rawalpindi (Hashmi et al., 2009). Another study is conducted in the valley of Buner in which surface water contained significantly higher numbers of total coliform bacteria, total plate count, total faecal coliform, and *E. coli* compared to the water obtained from natural springs. More precisely, 4 samples out of 12

Table 1: Cases reported for bacterial pathogens in Pakistan

Types of Bacteria	Area from where reported	Reference
Campylobacter, ETEC-LT, *Shigella, Salmonella*	A village of Pakistan	(Khalil et al.,1994)
Faecal Coliform and Total Coliform	Karachi	(Rahman, 1996)
Coliform contamination	Charsadda District	(Khan et al., 2013)
E. coli, Vibriocholera, Campylobacter	South Asia	(Alam et al., 2003)
Faecal and Total Coliform	Khairpur, Sindh	(Azizullah et al., 2010)
Bacillus lereus, E. coli, Salmonella, Shigella, Streptobacillus	Islamabad and Rawalpindi	(Ahmad et al., 2004)
Total Coliform, Faecal Coliform, E. coli	Buner Valley	(Ahmad et al., 2013)
E. coli, Enterobacter, Klebsiella, Salmonella, Aeremonas, Shigella	Mahmoodabad and Liaqatabad, Clifton area (Karachi)	(Baqai and Zuberi, 1991)

studies samples are found to be highly contaminated which afterwards decaled as unsafe based on standards of world health organization (Ahmad et al., 2013). The list of studies conducted to assess the presence of bacterial pathogen are summarized in Table 1.

Viral Contaminants

Virus is a pathogen that harms its host while multiplying its genetic material. The presence of viral genes have been widely exploited as an indicator to determine the host population. Viral infections effect death rate as well as birth rate and therefore can determine the population size of the host (Poss et al., 2002). Wide number of viruses have been identified from water resources which have proven to deteriorate the health of living organisms. For instance, influenza A virus causes acute influenza in human beings and have been found to be responsible for genetic polymorphism in IFITM3 (Lun et al., 2014). Similarly, west Nile is another type of virus which causes diseases in humans as well as animals (Owen et al., 2010). Similarly, in dogs, Canine Influenza virus causes diseases (Karaca et al., 2010). All these types of viruses are generally waterborne in nature and therefore enter to the bodies of living organisms easily. Recent studies reveals that hepatitis C virus (HCV) is the most important cause of chronic and acute hepatitis, cirrhosis and hepatocellular carcinoma, whose deteriorating effects are widely reported in Pakistan as well as in other parts of the world (WHO 1999; Bari et al., 2001). It is important to highlight that pregnant women with hepatic infection can cause abortion, immediate death of infant after birth, premature birth, maternal or fetal death (Smith, 2001).

From December 1993 to March 1994, people living in two sectors of Islamabad (Pakistan) suffered from an epidemic outbreak of Islamabad (Pakistan) suffered from an epidemic outbreak caused due to water contamination by hepatitis E virus. According to a systematic survey, out of 36,705 individuals, 3,827 individuals suffered from severe icteric hepatitis while the attack rate was 10.4%. The situation was further intensified when water treatment plant became out of service and subsequently purification system shifted from slow sand to rapid sand filtration. Initially, water was fed to the plant by a severely contaminated tributary; resultantly, areas where entire water supply was from the purification plant had highest attack rate, i.e., 16.3%. However, areas with 50% of water supply was from the purification plant had attack rate of 12.4%, and of 30% had attack rate of 5.3%. On the other hand, the adjoining areas that are not connected to this source of water supply had the lowest attack rate and that rate was 1.8% (Rab et al., 1997). According to another investigation, 86 samples were taken from various locations of drainage outlets; out of which 47 samples were taken from Rawalpindi and 39 samples were taken from Islamabad. Analysis revealed that 19 samples (44.7%) which were taken from Rawalpindi, and 16 samples (41.02%) taken from Islamabad, got positive retro polymerase chain reaction (RT-PCR). More precisely, these 35 samples were HEV positive; being taken from extremely overfilled area (Ahmad et al., 2010).

A study conducted in the city Hafizabad, tells that there was 6.5% seroprevalance of hepatitis C virus in the whole area. Approximately 29% and 8% cases of chronic liver disease (CLD) and hepatocellular carcinoma, respectively, were appeared as seropositive for HCV (Bari et al., 2001). A hospital based study conducted from November 1986 to March 1988 reveled that 33,070 of the studied samples had infection of respiratory syncytial virus. Moreover, *Haemophilus influenza* was identified in 9.6070 of cases, and *Streptococcus pneumoniae* was found in 9.9070 of cases. Among

Table 2: Cases reported for viral pathogens in Pakistan

Types of Virus	Area from where reported	References
Hepatitis E virus	Islamabad	(Rab et al., 1997)
Hepatitis E virus	Rawalpindi and Islamabad	(Ahmad et al., 2010)
Hepatitis C virus	Hafizabad	(Bari et al., 2001)
Haemophilus influenza	-	(Ghafoor et al., 1990)
Hepatitis virus	Quetta, Mardan, and Rawalpindi	(Malik et al., 1988)
Hepatitis C virus	Faisalabad	(Ahmad et al., 2007)
Hepatitis A virus and Hepatitis E virus	-	(Baryan et al., 2002)

them, 32070 of the *Haemophilus* isolates were considered due to nonencapsulated *H. influenzae* and the only encapsulated *H. influezae* strain found was called as type b (Ghafoor et al., 1990).

Besides, the infection associated with hepatitis E virus is also observed from different cities of Pakistan. The virus affects the adult individuals mostly and is also reported as an explosive epidemic (Bosan et al., 2010). According to a survey, during an outbreak of hepatitis by E virus, 95% (out of 109 patients) of the individuals had evidences of hepatitis E; whereas, 3% had severe hepatitis E and 2% were suffering from hepatitis A as well (Bryan et al., 2002). Hepatitis A virus seroconversion was found in children to a great extent. In Pakistan, almost 50% to 60% of all the severe viral hepatitis infection in children is due to hepatitis A virus (Bosan et al., 2010). In Quetta, Mardan and Rawalpindi, three epidemics outbreaks were occurred as of acute viral hepatitis. In addition to this, jaundice, anorexia and nausea were also observed in all of the cases (Malik et al., 1988). Last but not least, in Faisalabad, 84 samples were studied among which hepatitis C virus genotype-3 was found in 68 (81%) of the collected samples (Ahmad et al., 2007). Further examples can be seen in Table 2.

Protozoan Contaminants

Protozoa is the third and last class of microbial pathogens which have been reported from diverse environments, i.e., freshwater, marine water, in damp soil, and dry sand etc. Thousands of protozoa species have been reported round the globe (Barker and Brown, 1994). Protozoa have been intensively studied by microbial ecologists mainly due to their potential for marine pollution. They can contaminate the waterfront area of any city, and hence are consumed by fishes. Those fishes are ultimately eaten by animals and human beings causing increased mortality of certain populations. A few examples of such protozoan parasites are *Giardia, Cryptosporidium* and *Toxoplasma* (Fayer et al., 2004). It has been well-established that more than 72 species of protozoan parasites can be transmitted by water and food causing zoonoses. Some parasites are widely abundant whereas some parasites have complex life cycles and they need any host to survive. In the whole taxon, only *Toxoplasma gondii* is the parasite that is transmitted by infected meat and contaminated water and food. Approximately, six groups are reported to be responsible for food contamination and water; *viz Cryptosporidium, Isospora belli, Cyclospora cayetanensis, Giardia duodenalis* and *Entamoeba histolytica/E. dispar* (Pozio 2003).

In Pakistan, protozoa contamination is less investigated however some studies reports their harmful effects to living organisms. According to a study conducted in three areas of Khyber Pakhtunkhwa namely Kohat, Karak and Hangu; protozoan oocysts (eggs) were present in all of the localities. The study involved 450 samples collected from different sources i.e. Tap water, ponds and drain water among which protozoan evidences were recorded in approximately 65.5% of the whole sample. Moreover, *Giardia spp.* were observed to be nearly 14.1% and *Cryptosporidium spp.* to be 19.5% (Ayaz et al., 2011). Although, their direct effects on human population were not recorded nevertheless both groups (*Giardia* and *Cryptosporidium*) are well known as cause of gastroenteritis and many other waterborne diseases (Guerrant, 1997; Furness et al., 2000). These protozoan along with the *Entamoeba histolytica* cause diarrheal diseases (Black et al., 1977; Hassan et al., 2015). *Giardia* was also found in 65 samples collected from different sources i.e. bore water, tube well, and drainage; in which maximum number of *Giardia* (10.76%) was found in tube well (Hassan et al., 2015). Similarly, *Entamoeba histolytica* and *E. coli* were found in excreta and sewage water (Ayaz et al., 2011). According to another study conducted in Toba Tek Singh during April 2009 to March 2010, 275/584 samples of excreta were found to be contaminated by *Eimeria spp*. (Rehman et al., 2011). Likewise, 8850 stool samples from patients of hospitals of Rawalpindi and Islamabad revealed presence of several protozoan species namely *Entamoeba histolytica, Iodamoeba bütschlii, Giardia lamblia,* and *Pentatrichomonas hominis* (Table 3) (Pal and Rana, 1983).

Insights on Sources and Fates of Microbial Pathogens

Wastewater originating from residential areas can be the major source for biological contamination (Nebel and Wright, 1996). Availability of clean water is becoming challenge for everyone especially due to geometric increase in population. Water contamination by bacteria can be due to leakage of pipes, pollution from sewerage pipes, problems in water supply system, irregular water supply, and shallow water tables due to human activities. The conditions are even more critical in thickly colonized cities like Karachi, Lahore, Rawalpindi, Peshawar, Faisalabad, Qasur, Sialkot and Gujrat in which several surface water spots had strong human-source contamination originating from anthropogenic activities (Shar et al., 2008; Azizullah et al., 2010; Kelsey et al., 2003). On

Table 3: Cases reported for protozoan contaminants in Pakistan

Types of Protozoa	Area from where reported	References
Giardia, *Cryptosporidium* and *Entameoba histolytica*	Khyber pakhtunkhwa	(Ayaz et al., 2011)
Giardia	Different sources of area in Pakistan	(Hassan et al., 2015)
Eimeria spp.	Toba Tek Singh	(Rehman et al., 2011)
Entameoba histolytica, Iodameoba butschlii, Giardia, Pentarichomonas	Rawalpindi and Islamabad	(Pal and Rana, 1983)

the other hand, lack of awareness in rural population especially about the distribution network render the higher extent of microbial pollution in rural areas (Ahmad et al., 2013). Microbial contaminants in water supplies at treatment plants and distribution networks (Ratta Amral, Rawalpindi) highlights the significance in more systematic way (Hashmi et al., 2009). Another source of water contamination can be industrial effluents and other agrochemicals. Development in urban areas and increased demand of food has become the reason for increased use of pesticides and other agrochemicals which help proliferation of specific microbial pathogens (Naidu et al., 1996).

In under-developed countries, viral transmission is mainly linked with blood transfusion without appropriate screening procedures. Hepatic patients, workers at medical workplace, infants of HCV infected mother, patients of hemodialysis, and their sexual partners strengthens the viral transmission mechanism (Tillmann and Manns 1996). For instance, healing injections by workers of hospitals are appeared to one of the major source of HCV transmission (Luby et al., 1997). Other sources for hepatitis C virus include piercing, drawing tattoos, and shaving by barbers that do not consider necessary precautions. Besides, nonsexual contacts such as tooth brushes, shaving blades, and nail-cutters are also a way of viral transmission (Alter et al., 1990). Nevertheless, important source of hepatic virus is usually transition by drinking water contaminated with patient feces (Smith 2001; Malik et al., 1988). It has been reported that the adults of age 15 to 30 years are main focus of hepatitis E virus.

The source of protozoan parasite is the human or animal excreta that is released or dumped in the environment. It brings encysted protozoan with it, whose ultimate fate can be seen in different water bodies (Fayer et al., 2004). The majority of protozoan infections are due to socioeconomic values, improper sanitation and poor food habits (Pozio 2003). However, faecal pollution, improper cleanliness measures, and poor treatment of water further trigger the parasites eggs to be transmitted at higher rates (Pal and Rana, 1983).

The infiltration of leachate carrying microbial pathogens to the drinking water resources is another dilemma of water pollution in under-developed countries. The situation generally happens when septic tanks are designed without and/or improper lining especially in the areas where variety of waste is being dumped every day. Natural porosity of soil allow variety of microbial pollutants to pass through along with other chemical pollutants (Petrovic et al., 2003). The waste ultimately enters in groundwater system where microbial pathogens proliferate depending on the environmental conditions. Moreover, active nature of biological contaminants allow them to travel long distances through the earth (Corapcioglu and Haridas, 1983); whereas smaller viruses or bacteria follow the same phenomenon of adsorption, as of synthetic compounds, for their transportation. On the other hand some microorganisms are transported by colloidal clay or harmless organic matter present in soil (McGechan and Lewis, 2002). Mack et al., (1972) found polio virus in a well present in the area 90 m away from a wastewater drain. The virus was isolated from the depth of 30 m whose prima-

ry source was later confirmed as wastewater drain. Similarly, Schaub and Sorber, (1977) and Moore et al., (1981) found viruses from the groundwater under the area receiving waste effluents. In their study, virus particles penetrated to the depth of 30 m whereas dispersal was strong even at distance of 180 m. Vaughn et al., (1981) also reported that enteroviruses was proliferating even at the distance from of 45 m from the wastewater drain while the depth was about 12 m. The transmission within soil was also observed for bacteria as well because colonies of coliform were recorded in the groundwater from 1 to 450 m of original contamination source (Gerba et al., 1975). Similarly, Hagedorn (1981) conducted many studies in which he reported that the movement of bacterial contaminants in groundwater from the lateral distances ranging from 1 to 830 m (Corapcioglu and Haridas, 1983). The harmful bacterial contaminants which have ability to travel through long distances in groundwater include *Salmonella* sp., *Shigella* sp., *Escherichia coli*, *Vibrio* sp., whereas viral contaminants include Norwalk-like agent, poliovirus, coxsackievirus, hepatitis, and echovirus (Corapcioglu and Haridas, 1983). In certain cases, harmless biological entities are also responsible for transportation of various contaminants (Blais et al., 2007).

Conclusions and Recommendations

Globally, safe water is not available to about 1.1 billion people and 400 children of age less than 5 die per hour due to biological contamination (Gadgil, 1998). The current review elucidates that the presence of microbial pathogens in the environment can cause significant damages compared to any other source of pollution. In Pakistan, bacterial contaminants are widely investigated compared to other pathogen types however the harmful effects of each group cannot be undermined in any case. Harmful impacts associated with the microbial contaminants are more in areas with poor sanitation and untreated water supply; which indicates their presence in untreated or poorly treated water, sewage water, and in some cases water bodies in densely populated areas such as ponds and lakes etc. This surface water when seeps down in soil, contaminates the groundwater reservoirs which are then if supplied for drinking with poorly treated or untreated quality, affects the consumers. Among the contaminants reported in Pakistan are *E. coli*, *Salmonella*, *Shigella*, *Campylobacter*, total and faecal coliform, hepatitis E virus, hepatitis A virus, hepatitis C virus, *Haemophilus influenza*, *Giardia*, *Cryptosporidium*, *Entameoba histolytica*, *Eimeria* spp., and *Pentarichomonas*; and these contaminants are the cause of dysentery, abdominal pain, diarrhea, headache problem, hepatitis, hepatocellular carcinoma, chronic liver disease, along with some respiratory disorders. It is therefore high recommendations are needed especially for this class of contaminants and detailed investigations shall be done in order to avoid high mortality in future.

Compliance with ethical standards

Conflict of Interest

The authors declare that they have no conflict of interests.

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