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Full Length Research Paper

Antibiotic sensitivity pattern of pathogenic bacterial isolates of public health concern from Lake Hawassa water, Ethiopia

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This study was aimed to identify and test the antibiotic sensitivity of bacterial isolates of public health concern from Lake Hawassa. Human and livestock activities in and around the lake adversely influence the quality of the lake water and pose a health risk for those people which are being exposed to the water. Therefore identifying pathogenic bacterial isolates and their antibiotic sensitivity pattern can be important to manage the associated public health impacts and give information to health workers in the area about the drug of choice. Seventy-nine (79) bacterial isolates such as *Escherichia coli* 22 (27.06%), *Klebsiella* species. 12 (16.47%), Citrobacter 11 (12.94%), *Proteus* species. 9 (10.59%), *Shigella* 9 (10.59%), *Staphylococcus aureus* 8 (9.41%), *Pseudomonas aeruginosa* 6(7.06%), and Salmonella 5 (5.88%), respectively were isolated from the lake. Of the total (79) isolates, 75(94.94), 74(96.20), 2 (2.53%) and 2 (2.53%) of them were resistant to amoxicillin, ampicillin, norfloxacillin, and kanamycin, respectively. High resistance of all bacteria to amoxicillin and ampicillin was seen in the study area which outermost the resistance seen in other similar study. The results indicated that persistent use of antibiotics against human diseases may pollute the lake water and their impact on developing antibiotic resistant may be a serious threat in both health and environment. Therefore, proper measure should be taken against different sources of contaminants that deteriorate the Lake water. Health professionals should pay attention while prescribing amoxicillin and ampicillin to treat patients suffering from infection caused by pathogenic bacteria isolated from the study area.

Key words: Public health, antibiotic sensitivity, lake water, bacterial isolate, Hawassa, Ethiopia.

INTRODUCTION

Water is the most valuable resource which supports the survival of all organisms. People use water for multiple purposes such as drinking, cooking, washing, bathing, irrigation, navigation, power generation, and waste disposal (Fawole et al., 2008). People can be exposed to waterborne microbial contaminants upon drinking water, when water is used for food preparation or when there is body contact during bathing or recreational uses (APHA, 1999).

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Recreational water has an associated health risk due to the presence of pathogenic bacteria (Hatha et al., 2008). During recreational uses, pathogenic bacteria in the water bodies are able to cause infections by accidental ingestion or contact with skin, nasopharyngeal cavity, eyes, ears, respiratory and urogenital passages (WHO, 2003b). According to APHA (1999), the bacteria that have been isolated from recreational waters include *Pseudomonas aeruginosa, Fecal streptococci, Enterococcus* and *Staphylococci*. These bacteria may suggest health risks through body contact and ingestion. The possibility of ingesting small quantities of pathogenic bacteria or pathogenic bacteria that directly entering the eyes, nose, ears or open wounds (UNESCO/WHO/ UNEP, 1996) causes the health hazards for the bathers.

Sources of pathogenic bacteria in recreational water resulted from animals that visit the bathing place, from fecal matter of water birds and birds in water surrounding areas that give access to the water body and the bathers themselves (Roijackers and Lurling, 2007). The problem of water associated health risks are more pronounced in developing countries.

Lake Hawassa is one of the Rift Valley lakes of Ethiopia and utilized for many uses. The lake is one of the tourist destination sites in the country and also used for fishing, small-scale irrigation, laundry, bathing, swimming, live-stock and human drinking and for the disposal of domestic and industrial wastes (Gebremariam and Desta, 2002).

It has been reported that the lake receives huge amount of domestic and industrial effluents from city of Hawassa and the nearby industrial establishments (Fetahi, 2010). Moreover, there are high levels of recreational activity around the lake and is thus exposed to intense human-induced pollution. There are high human and livestock activities in and around the lake which are assumed to adversely influence the quality of the lake water and pose a health risk for those people which are being exposed to the water.

Given that the lake water is also used for human and livestock drinking, bathing and swimming, fishing and small scale irrigation, identifying of pathogenic bacterial isolates and their antibiotic sensitivity pattern can be important to manage the associated public health impacts. In this respect, there is lack of recorded data on identifying of pathogenic bacteria of the water with respect to public health significance in the study area. Therefore, the present study was designed to identify potential pathogenic bacteria of public health concern from the lake and to test their antibiotic sensitivity pattern.

Hypothesis

1. Pathogenic bacterial isolates are found in Hawassa Lake water.
2. All bacterial isolates have similar resistance to all antibiotics.

MATERIALS AND METHODS

Lake Hawassa is one of the lakes in Rift Valley of Ethiopia and located at about 275 km south of Addis Ababa. The lake borders Hawassa city on the western side, situated at an altitude of 1685 m above sea level. It is estimated that the lake has a width, length, and depth of 62, 16 and 21 m, respectively, covering a total surface area of 90 km^2 and a catchments area of 1250 km^2. The lake is a closed basin, where there is no known outflow but primarily fed by a small river named TikurWuha that originates from a swampy area in the north part of the lake (Desta, 2003).

Study design

The study was used to identify potential pathogenic bacterial isolates from Lake Hawassa. The study was conducted from February to July 2011.

Sample site selection

The sample sites were selected by judgment sampling method based on sampling factors recommended by APHA (1999) for fresh surface waters, (lakes and rivers). Public activities on the lake such as fishing practices, cloth washing, animal washing and car washing, bathing and swimming, municipal waste discharge to lake and run-off water during the rainy season to the lake were considered during site selection (Figure 1 and Table 1).

Sample size determination

Samples were taken by considering different sources of contamination, public activities on the lake, animals visit on the lake, fishing activity, run-off water during rainy season and irrigation activities. Equal amount of samples were taken from all sampling sites in triplicates and a total of 72 water samples were collected.

Sample collection protocol and transportation

Water samples of 100 ml were collected from all sites of the lake based on APHA (1999) procedure for a sampling of bathing beach using sterilized plastic bottles. The collected samples were properly labeled with full details of the source, time, and date of collection and aseptically taken to Hawassa University Veterinary Medicine Microbiology Laboratory in ice box within an hour and immediately analyzed (Figure 2).

Isolation and characterization of pathogenic bacteria

Pathogenic bacteria such as *Staphylococcus aureus*, *Pseudomonas aueroginosa* and *Proteus species* were isolated by culturing the samples on Nutrient agar, MacConkey agar, and Mannitol salt agar (Figure 3). After incubation at 37°C for 24 h, pure colonies of *P. aueroginosa* were identified by gram staining, catalase test and TSIA test while pure colonies of *S. aureus* were obtained by subculturing on Mannitol salt agar.

Colonies showing swarming growths were recorded as *Proteus species*. *E. coli,* and *Klebsiella species* were isolated using MacConkey and characterized using biochemical tests gram stain

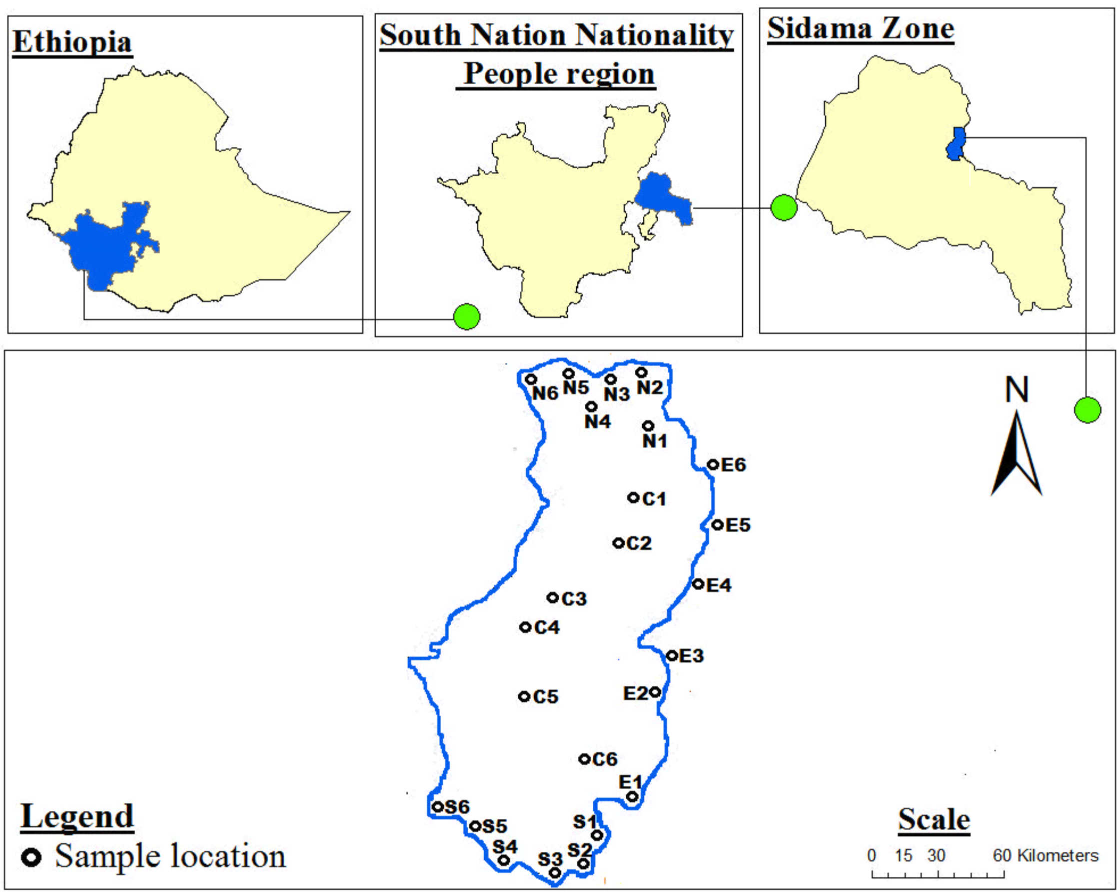

Figure 1. Study area Map (Modified from Google map).

Table 1. Sampling sites.

Sampling sites	Sampling points
Lake water	
Site 1	$E_1 - E_6$ (Eastern side)
Site 2	$N_2 - N_6$ (Northern Side)
Site 3	$S_1 - S_6$ (Southern Side)
Site 4	$C_1 - C_6$ (Center of the Lake)

tests, catalase test, oxidase test, IMViC test, urease test and TSI agar test.

Isolation and characterization of *Salmonella* and *Shigella*

Salmonella and *Shigella* were isolated by culturing 25 ml of water samples in 225 ml of lactose broth (pre-enrichment media for *Salmonella*) and nutrient broth (pre-enrichment media for *Shigella*) and then incubated at 37°C for 24 h. After 24 h of incubation, one milliliter of the broth was inoculated into 10 ml of tetrathionate broth (enrichment media for both *Salmonella* and *Shigella*) and incubated at 37°C for 18 h. A loopful from tetrathionate was streaked on *Salmonella-Shigella* agar and incubated at 37°C for 24 h. *Citrobacter* was identified from Salmonella using TSI agar test (Arora and Arora, 2007). Finally, eachcolony characteristics were determined by their color and morphology on each culture medium.

Antibiotic sensitivity test

All bacterial isolates were tested for the sensitivity to commonly used antibiotics in the study area such as Amoxicillin, Ampicillin, Norfloxacillin and Kanamycin (Oxoid) using Kirby-Bauer disc diffusion method (Duncan et al., 2005). Zone of complete growth inhibition around each of the discs formed after incubation of the isolated culture on Muller Hinton agar was carefully measured using transparent plastic ruler and zone size was interpreted as sensitive, intermediate and resistance based on the standard chart on Duncan et al. (2005) and WHO (2003a).

Data entry, analysis, and interpretation

The raw data were entered into excel spreadsheet. Data analysis was done using SPSS version 16.0. Descriptive statistics such as mean, standard deviation, percentage, and tables were used.

RESULTS

Isolation and characterization of bacteria from the lake

A total of 79 bacterial isolates were identified from the

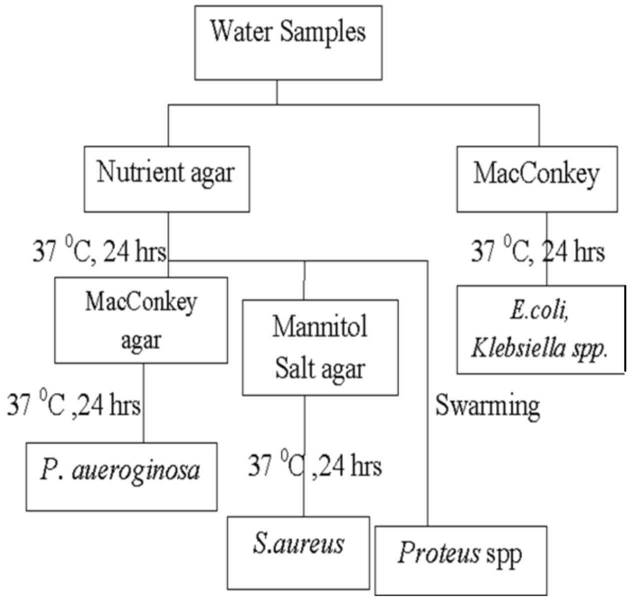

Figure 2. Identification procedure of pathogenic Bacteria (Drawn using procedure stated on Arora and Arora, 2007).

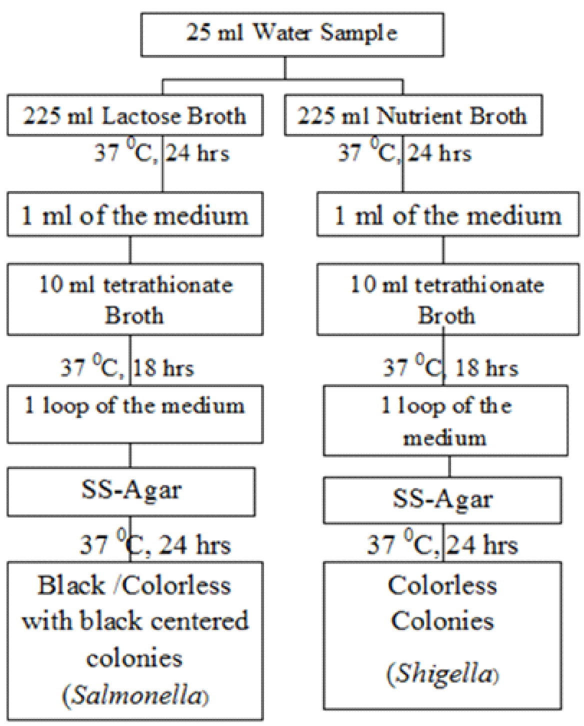

Figure 3. Isolation Procedure Salmonella and Shigella (Drawn using procedure stated on Arora and Arora, 2007).

total of 72 collected water samples, which consists of eight bacterial *species*. These include *E.coli, Klebsiella species*, *Salmonella, Shigella, P. aeruginosa*, *Proteus* spp., *Citrobacte*r and *S. aureus* (Table 2). Of the total 79 bacterial isolates, 22 (27.85%) were *E.coli* followed by *Klebsiella* species*,* 12 (15.19%), *Citrobacter,* 11(13.92%),

Table 2. Distribution of Bacterial Isolates from four sampling Sites of the Lake.

Bacterial isolates	Sampling sites				
	Site 1	**Site 2**	**Site 3**	**Site 4**	**Total isolates**
E. coli	7(31.81%)	4(18.18%)	6 (27.27%)	5(22.73%)	22 (27.85%)
Klebsiella spp.	3 (25%)	0 (0.00%)	8 (66.66%)	1 (8.33%)	12 (15.19%)
Salmonella	1 (20%)	2 (40%)	1 (20%)	1 (20%)	5 (6.33%)
Proteus spp.	0 (0.00%)	5 (55.55%)	0 (0.00%)	4 (44.44%)	9 (11.39%)
Shigella	1 (11.11%)	2 (22.22%)	1 (11.11%)	5 (55.55%)	9 (11.39%)
S. aureus	1 (20%)	1 (20%)	3 (60%)	0 (0.00%)	5(6.33%)
Pseudomonas aerogenosa	3 (50%)	2 (33.33%)	1 (16.67%)	0 (0.00%)	6 (7.59%)
Citrobacter	7 (63.63%)	2 (18.18%)	0 (0.00%)	2 (18.18%)	11(13.92%)
Total	23(29.11%)	18 (22.79%)	20(25.32%)	18 (22.78%)	79

Proteus spp., 9 (11.39%), *Shigella* 9 (11.39%), *P. aeruginosa* 6 (7.59%) *S. aureus* 5(6.33%) and *Salmonella*, 5 (6.33%), respectively in decreasing order. *E. coli*was found to be the highest isolate whereas *Salmonella* and *S. aureus* was isolated in lowest number.

Seven bacterial spp. were recovered from Site 1, of which *E.coli* 7 (31.81%) and *Citrobacter* was the highest in number 7 (63.63%). *Salmonella, Shigella*, and *S.aureus* were isolated in lowest number. Eighteen bacterial isolates recovered from Site 2 and *Proteus species* were the highest in number 5 (55.55%) than the other bacteria. Six bacterial *species* were recovered from Site 3, of which, *Klebsiella* species*,* were isolated in the highest number 8 (66.66%). *Salmonella, Shigella*, and *P. aeruginosa* were isolated in lowest number. Six bacterial *species* were recovered from Site 4 and *E. coli* 5(22.73%) and *Shigella* 5 (55.55%) were the highest in number while *Klebsiella* species and *Salmonella* was the lowest in number. Of the total (22) *E. coli* isolates, highest number 7(31.81%) were recovered from Site 1.

Antibiotic sensitivity pattern of pathogenic bacterial isolates

The susceptibility test was conducted for all bacterial isolates recovered from all sampling site using four commonly used antibiotics in the study area such as Amoxicillin, Ampicillin, Norfloxacillin, and Kanamycin.

All isolates were tested with amoxicillin and 75(94.94) of them were found to be resistant. Similarly, all isolates were tested with ampicillin and 74(96.20) of them were resistant while of the isolates tested with norfloxacillin and kanamycin, an equal number of isolate 2 (2.53%) were found to be resistant. The resistance of the isolates to four antibiotics were in the order of amoxicillin >ampicillin >norfloxacillin = kanamycin (Table 3).

All isolates of *Citrobacter, Shigella, S. aureus* and *P. aeruginosa* tested with ampicillin were resistant. The result showed that *Salmonella* were less resistant to amoxicillin 4 (80%) and ampicillin 3 (60%), respectively. *E. coli* was highly resistant to amoxicillin 21(95.45) while none of *E. coli* isolate was resistant to both norfloxacillin and kanamycin. All *Klebsiella species* were tested against the four antibiotics and the highest resistance was recorded for amoxicillin 11(91.67) but none of the isolates were resistant to both norfloxacillin and kanamycin.

The highest resistances of *S. aureus* were recorded for amoxicillin and ampicillin while lowest resistance was recorded for norfloxacillin. Similarly, the highest resistance of *P. aeruginosa* was recorded for ampicillin but none of the isolates was resistant to both norfloxacillin and kanamycin. All *Salmonella* isolate were tested against the four antibiotics and highest resistance was recorded for amoxicillin but no resistance pattern was observed against norfloxacillin and kanamycin.

DISCUSSION

Identification of pathogenic and opportunistic pathogens from the lake water indicates that the water is highly contaminated with fecal matter, domestic and urban run-off water. The impact of effluent or untreated wastewater discharged into receiving water bodies can be detrimental to water availability and security. The pathogenic bacterial isolates recovered from the lake is similar to pathogenic bacterial isolates recovered by Lateef et al. (2005) from water samples collected in Southwest Nigeria and these bacterial isolates include *E. coli, S. aureus*, *P.aeruginosa, Klebsiella* species. The isolates are also comparable with bacterial isolates from water samples of Aksu River in Kahramanmaras in Turkey, which consists of *E. coli, Pseudomonas species*, *Klepsiealla species* and *Citrobacter* of which *E. coli* is the highest isolate (Toroglu et al., 2005).

Identification of highest number bacterial isolate from site 1 of the lake comes from the different public activities load on this site such as public bathing, cloth washing, animal washing, animal watering, public recreation, boat activities and large fishing. Figure 4 show human bathing with full body contact with water at site 1 of the Lake.

Table 3. The resistant pattern of bacterial isolates from Lake Hawassa.

Bacterial isolates	Number (%) of isolates resistant to antibiotics			
	Amoxicillin	Ampicillin	Norfloxacillin	Kanamycin
E.coli(n=22)	21(95.45)	20(90.91)	_	_
Klebsiella(n=12)	11(91.67)	10(83.33)	_	_
Citrobacter(n=11)	11(100)	11(100)	_	2(18.18)
Proteus (n=9)	9(100)	8(88.89)	_	_
Shigella(n=9)	9(100)	9(100)	_	_
S.aureus(n=5)	5(100)	5(100)	2(40)	_
P. aeruginosa(n=6)	5(83.33)	6(100)	_	_
Salmonella (n=5)	4(80)	3(60)	_	_
Total (%)	**75(94.94)**	**74(93.67)**	**2(2.53)**	**2(2.53)**

Figure 4. Human Bathing in the lake at Site 1 (photo taken during study).

As far as this site is boundering the city, many recreation centers were there in which different activities are common. A comparable number of bacterial isolates was recovered from site 3 because in this site is exposed to small irrigation, fishing and bird's fecal matter living nearby the lake at this site. But less number of isolates were recovered from site 2 and 4 compared to others. This is because since site 2 is far from the exposure of human and animal activities on the northern side of the lake and is the site in which even the countryside pupil used the lake water for drinking and domestic uses. Site 4 the center of the lake where exposure is less except some swimmers and boat recreation.

The presence of pathogenic bacterial isolates such as *Salmonella* and *Shigella* in the lake could be related to contamination of the lake, as a result, public activity such as fishing, irrigation, and house utensils washing in the lake. *P. aeruginosa* and *S. aureus* in the lake most likely comes from uncontrolled public bathing activity in the lake. Similarly, *P. aeruginosa* and *S. aureus* were recovered from Water and Bottom Sediments in the Czarna Hańcza River (Niewolak and Opieka, 2000). This may be attributed to full-contact large mass swimming in the lake and other activities such as animal washing in the water.

Identification of the highest number of *E. coli* from the lake indicates high contamination of the lake with feacal matter from human and animal origin which similar to a report of Toroglu and Toroglu (2009) which states that isolation of this pathogen from Lake Golbasi water is an indication feacal contamination of the lake. This renders the water dangerous for human consumption and other domestic uses. Similarly, Onifade and Ilori (2008) reported that *E. coli* were isolated from water sources in Akure metropolis of Ondo state in Nigeria which indicates that the water is contaminated with the feacal material.

Figure 5. Municipal waste canal directed to the lake at site 1 (Photo taken during study).

The percentage of *E. coli* reported by Itah and Ekpombok (2004) is comparable with *E. coli* isolated from Lake Hawassa but the percentage of *P. aeruginosa* and *S. aureus* outsmart the percentage of *P. aeruginosa* and *S. aureus* from the lake in the current study. *Klebsiella species* were also isolated which indicates the level of contamination of the lake. Isolation high *E.coli* from site1 could be related to contamination of the lake at this site by animal and human feacal matter as well as the discharge of domestic wastewater, urban wastewater to the lake in which figure 5 is an evidence for this.

Drug resistances to bacterial isolates recovered from the lake water may result from the phermeutical effluent discharged into the lake from the city, waste discharged from Hawassa ceramic factory, waste water discharge from nearby Hawassa referral hospital or public bathing and cloth washing in the lake. The highest resistances of all isolates to amoxicillin and ampicillin may be a result of discharge fromnearby Hawassa referral hospital which may contain these drugs. It also indicates the drugs are frequently used the study area. In addition waste water discharged from Hawassa textile factory, Etab soup factory, and St. George beer factory which mixed to Shallo swamp and enter into the lake through TikurWuha River contributes for the resistance of these bacterial isolates in the study area.

The high resistant strains of *Proteus species, Shigella, S. aureus* and *Citrobacter* to amoxicillin indicated that amoxicillin is not a drug of choice for treatment of patients suffering from an infection of these bacteria in the study area. The drugs observed to be most effective in the study are Norfloxacillin and Kanamycin.

Similarly, the high resistance of bacterial isolates to the antibiotics was reported byToroglu et al. (2005). This may be is a reflection of misuse or abuse of antibiotics in the environment (Lateef, 2004). Antibiotic prescriptions in hospitals are given without clear evidence of infection or adequate medical indication. In developing countries, drugs are available to the public and thus people may practice self-administration of antibiotics and further increase the prevalence of drug-resistant strains (Lateef, 2004).

Conclusion

Isolation of pathogenic bacteria such as *Salmonella, Shigella, P. aeruginosa and S. aureus* from the lake are potential causes of illness in humans in the study area and thus indicates a poor bacteriological quality of the lake. All water sources from all Sites of the Lake tested were found to be contaminated and the bacteriological sources of contamination have been identified. According to this study, all isolates developed resistance against amoxicillin and ampicillin whereas kanamycin and norfloxacillin appeared to be a drug of choice to treat patients suffering from these bacterial infections in the study area. Generally ineffective protection of the Lake from different sources of contamination, affect the bacteriological quality of the Lake. This causes the Lake water out of its intended use and in all case pose a public health risk. Therefore, proper measure should be taken against different sources of contaminants that deteriorate the Lake water. Health professionals should pay attention while prescribing amoxicillin and ampicillin to treat patients suffering from infection caused by pathogenic bacteria isolated from the study area.

Conflict of Interests

The authors have not declared any conflict of interests.

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