ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF ALLIED HEALTH SCIENCES
DEPARTMENT OF MEDICAL LABORATORY SCIENCES

ASSESSMENT OF THE PHYSICO-CHEMICAL AND MICROBIOLOGICAL QUALITY OF SWIMMING POOLS IN ADDIS ABABA, ETHIOPIA

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This is to certify that the thesis entitled with Assessment of the physic chemical and microbiological quality of swimming pools in Addis Ababa, Ethiopia has been prepared by Kokeb Yedeme and complies with the regulations of the university and meets the acceptable standards with respect to originality and quality

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Approved by the Examining Board

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Operational Definitions

Accidental fecal release: is passage of faeces unintentionally by swimmers.

Aerobic plate count: is a count that indicates bacterial population in a sample.

Coli form: is a gram negative aerobic, and rod shaped bacteria that indicate the sanitary condition of foods and water.

Fecal coli form count: is a count of bacteria that are specifically present in the gut of animals or humans.

Indicator organisms: a group of organisms that are used to assess microbiological of water

Most probable number: is a method of counting bacteria in water employing a series of tubes having growth media.

Total coli form count: A count that indicates bacteria from soil, water, air, animal or human waste

Abbreviations

AAU:	Addis Ababa University
AFR:	Accidental fecal release
APC:	Aerobic plate count
CFU:	Colony forming unit
FCC:	Fecal coli form count
MPN:	Most probable number
OAU:	Organization for African Unity
PCA:	Plate count agar
PMM:	Parts per million
TCC:	Total coli form count
UNECA:	United Nations Economic Commission for Africa
WHO:	World health organization

Abstract

Background: *Many potential pathogens could be acquired from swimming pool water during bathing. Hence microbial examination of swimming pool water samples is necessary to ensure that the water is safe for swimming. As it is impractical to screen all samples for all possible pathogens, indicator organisms have been used as surrogate markers of risk.*

Objectives: *The study aimed at assessing the physico-chemical (chlorine, PH, temperature) and microbiological conditions of swimming pools and evaluating the compliance of these pools with the WHO standard.*

Methods: *A cross sectional study was conducted from February for four months(2016-May 2016).A total of 60 swimming pool water samples from 10 swimming pools (6 from each pool)were collected on a weekly basis for physicochemical and bacteriological analysis using the MPN method. PH, residual chlorine and Temperature of the swimming pool water samples were measured. In addition, total viable count and coli form count (total coli form, fecal coli form and (E.coli) were determined. Chi-square test was used for association and data was analyzed using SPSS Version 20.Results were compared with the WHO recommended limits for each parameter.*

Results: *The swimming pool water samples had average pH and temperature of 7.1 and 29°c respectively.58.4%(n=35/60) of the samples had PH in the range of 7.2-7.8 while 58.3%(n=35/60) of samples had temperature in the range of 21°c-32°c.25%(n=15/60)of the swimming pool water samples had residual chlorine in the range of 2-3mg/l.73.3%(n=44/60) of swimming pool water samples had a total viable count below 200cfu/ml and 70 %(n-42/60) of swimming water samples had TCC values less than 2MPN/100ml.More over, 66.7%(n=40/60) of the samples had fecal coli form counts falling bellow 1MPN/100ml.E. coli was present in 30%(n=18/60) of swimming pool water samples while it was absent in 70%(n=42/60) pool water samples. It was also found out that PH and residual chlorine had association with pot ability while temperature was not.*

Conclusion: *It was observed that 70% (n=42/60) of the swimming pool water samples met the WHO microbial standard while 30%(n=18/60) did not.*

Key words*: Swimming pools, microbial contaminations, Addis Ababa, Ethiopia.*

1. Introduction

1.1. Background

The recreational use of water is growing world wide mainly because of its beneficial impact to human health. In the united states alone, over 301 million swimming visits were made by persons aged 7 and above in 2009.However,body contact recreational water has been strongly associated with infectious diseases and artificial water systems (swimming pools and spas) account for more than 90 percent of the outbreaks**(1).**

A swimming pool is a body of water of limited size contained in a holding structure .It is also an enclosed body of water intended for swimming and water based recreation(2). To use the swimming pool water for swimming, it meet potable water standard by being transparent, odorless, and tasteless liquid having a freezing point of 0ºC and boiling point of 100ºC. The pot ability of swimming pool water is enhanced by frequently changing the water and the use of disinfectants (3).The hotels all around the world use chemicals to clean the pool and air conditioner to persist the deadly bacteria in water .Some use liquid form of chlorine, sodium hypochlorite or calcium hypochlorite solution (4).A minimum free available chlorine residual of 1.0 ppm shall be maintained at all times and in all areas of the pool. A maximum of 4.0 ppm shall not be exceeded when the pool is open to the public (5). Swimming pool operators prefer iodine to chlorine as a disinfectant because its action is less hindered by organic matter and there is less eye and skin irritation than with chlorine. Bromine is also recommended (3).

The effectiveness of disinfecting chemicals depends on the PH of the water (6).The PH of swimming pool water should be controlled to ensure efficient disinfection, to avoid damage to the pool fabric and ensure user comfort. The pH should be maintained between 7.2 and 7.8 for chlorine disinfectants and between 7.2 and 7.8 for bromine based and other non chlorine processes. The frequency of measurement will depend upon the type of pool. It is suggested that for public pools, the pH value should be measured continuously and adjusted automatically (7).

As not all infectious agents are killed by the most frequently used residual disinfectants, and as removal in treatment is slow, it is necessary to minimize accidental fecal releases (AFR) and

vomitus and to respond effectively to them when they occur. The use of pre swim showers and toilets is of great importance in minimizing the introduction of shed organisms (8).

The microbial examination of water is used to monitor and control the quality and safety of various types of waters. These include potable waters (water intended for drinking or use for food preparation), treated recreational waters (swimming pools, spa pools and hydrotherapy pools), and untreated waters used for recreational purposes such as sea, river and lake water. Most water borne disease is related to fecal pollution of water sources, therefore water microbiology testing is largely based on the need to identify indicators of fecal pollution such as coli forms and *E.coli*. As indicators of fecal pollution, their presence is a strong indication of the presence of enteric pathogenic bacteria, such as Salmonella typhi, Salmonella paratyphi, Shigella dysenteriae, Vibrio cholerae and parasites in the pool (3,9).

1.2. Statement of the Problem

The rapid development of various leisure and public pools in the 20th century has led to increased human exposure to chlorinated products. It has been observed that swimmers and pool workers, who are in regular contact with chlorinated indoor pool waters showed an increased risk of respiratory and allergic diseases(4).

Unsafe swimming pool water can transmit infectious diseases. There exists an association between the degree of fecal contamination, as well as contamination from bathers, and the risk of illness resulting from swimming activities (10). Many of the micro organisms in water environments that harm human health are faecal in origin. Faecal pathogens enter the water environment through treated sewage effluent discharge; sewer overflows; urban and rural diffuse pollution; and direct voiding of human, avian, wild life and live stock faeces. The degree of pollution from these sources varies depending on proximity to the source of pollution and the prevailing weather condition (11).

Different microorganisms such as bacteria, fungi, Parasites and viruses such as hepatitis A and E(12)can be found in swimming pool water. Two hundred(200) water samples were collected from four swimming pools, 3 % were positive for coli forms and prevalence of fungal contamination was 27 %(most common were Aspergillus, Penialliumsp, Rhizopussp, and Fusariumsp) while that of bacterial contamination was 9 %(Staphylococcus epidermidis, Bacillus subtilis and Escherichia coli identified (13).Some bacteria, most notably non faecally derived bacteria may accumulate in biofilms and present an infection hazard. In addition, certain free living aquatic bacteria and amoeba can grow in pool waters, pool components and facilities(heating, ventilation and air conditioning systems)or other wet surfaces at a point at which some of them may cause a variety of respiratory, dermal or central nervous system infections or diseases. In addition to infections due to acknowledged pathogens, infections due to opportunistic pathogens are also reported too, which are able to cause a mild to severe disease in immune competent and immune depressed patients(12).

1.3 Significance of the Study

This study:

- Provides information about the current status of microbial quality and physicochemical characteristics of swimming pool.
- will also help hotel owners and swimming pool operators to pay due attention to the over all management of swimming pools including careful disinfection before opening the swimming pool for public service.
- Will also serve as a base line data for other researches in this area.

2. Literature Review

In a study conducted in Iran by *Hamid R*2012, 200 water samples were collected from four swimming pools. Temperature, pH and residual chlorine and turbidity of the pools were examined. Samples were concentrated through a membrane filter and sedimentation, to check for the presence of parasites, fungi, and bacteria. Results indicated that the mean of the physicochemical parameters, except in temperature, was standard in more than 60 % of the pools. Average temperature was higher than standard. The highest chlorine level was recorded in summer. Coli form bacteria was found to be positive in 3 % of the samples. Prevalence of bacterial contamination was 9 %; bacteria isolate included *Staphylococcus epidermidis* .There was a significant association found between fungal and bacterial contamination with residual chlorine (13).

Another study was carried out in Iran in 2012 by Rasti S et al to determine the types of fungal contamination and evaluation of the fecal Streptococcus, total coli form and some physical and chemical parameters of swimming pool waters .In this study, Bacterial and fungal contamination of four public indoor swimming pools was evaluated by standard total coli forms fermentation and using membrane filtration and carpet and swab sampling method with a month interval. In addition, physical and chemical parameters such as residual chlorine, temperature and pH were measured. Data showed that the mean water temperature, pH and residual chlorine were 29.3±1.3, 7.38±0.5 and 0.84±0.5 mg/L, respectively. Mean of total coli form was 1.8±7.7 MPN/100ml and its maximum and minimum were 43 and 0 MPN/100ml, respectively. Mean of fecal streptococcus was 0.3±1.6 MPN/100ml and its maximum and minimum were 9 and 9 MPN/100ml, respectively (14).

A cross sectional study was carried out in Italy by Guida A in 2009 to analyze the microbiological quality of water in rehabilitation and recreational swimming pools and compare the findings with local guidelines. For each facility, water was sampled at the intake point and at two points inside the pool. Total microbial contamination and Pseudomonas aeruginosa contamination were evaluated. According to this study, microbial mesophilic contamination and P. aeruginosa contamination were found in all seven pools. Microbial hemophilic contamination was more common in recreational pools (3–4.2% samples were above threshold values), probably due to the greater number of bathers. *P. aeruginosa* was more common in intake water

than water inside the pool (mean values of 19.3 and 22.5 colony-forming units (cfu)/ml in recreational and rehabilitation pools, respectively). A longer period of contact with chlorine and the dilution process may have led to lower levels of P. aeruginosa in the pool water (range 2–15 cfu/ml)(15).

A study was carried out in Iran by M.A. B et al in 2006indicated that 51.3% of the total samples were contaminated with Pseudomonas. Furthermore, 16.6, 11 and 7% of the total samples were contaminated with Escherichia coli, fecal coli forms and streptococci, respectively .A mean value of residual chlorine 0.45 mg L-1 was found in Pseudomonas contaminated samples. However, the corresponding value for non-contaminated samples was 1.052 mg l-1.Similarly, 26.3% of the samples collected from the covered pools and 53.9% of those collected from exposed pools were contaminated with Pseudomonas.. Additionally, the data showed that the source of water supply was also a major determinant of the degree of contamination. Surprisingly, public pools filled with well water were found to be less contaminated with different germs as compared to those filled with normal tap water. Exposed pools were found to be more contaminated with E. coli than covered pools. Similar observations were made for contaminated and non-contaminated samples with fecal streptococci and coli forms. There was an inverse relationship between the number of coli forms and the mean value of residual chlorine in the pools(16).

A study done in Hamadan by Rasool Y et al in 2003showed that opportunistic and pathogenic bacteria contaminated all swimming pool waters, but pathogenic fungi were not detected. Out of 48 samples, 40 samples (83.3%) were contaminated by opportunistic and pathogenic bacteria. Indoor swimming pools showed more bacterial contamination. The pH of all swimming pools was between 6to 8 and the level of chlorine was between 0.3 - 0.46 ppm(17).

A study involving 160 public swimming pools in Atlanta was done to determine how common two parasites occur in public swimming pools. The result showed that two microbial parasites Cryptosporidium and Giardia were present in one out of twelve swimming pools. These parasites are found in human feces. They are spread when someone swallows swimming pool water. They are also spread if a person does not wash his or her hands after handling a dirty diaper or eats contaminated food (18).

A study in Colorado by Capello M.A in 2011 suggested that 11% of the public swimming facilities were in excess of public health standards for total coli form bacteria and that 18.5% of the public swimming facilities were in excess of public health standards for HPC bacteria. The results indicate that the contamination observed in excess of public health standards was most likely the result of inadequate water treatment operations (19).

A study in South California by Gregory D.et al in 2007 showed that, out of 66 samples from Doheny State Beach, CA, 40.1% were positive for *V. cholera* and 27.3% were positive for V. parahaemolyticus, and 1 sample (1.5%) was positive for the *V. parahaemolyticus* toxin gene. Of the 96 samples from Avalon Harbor, CA, 18.7% were positive for *V. cholerae*, 69.8% were positive for *V. parahaemolyticus*, and 5.2% were positive for the *V. parahaemolyticus* toxin gene(20).

A study conducted in Atlanta by Jon T in 2013 indicated that 58 % of the water samples showed signs of *E. coli*. Though the researchers could not definitively blame human waste for the results, they wrote that it "signifies that swimmers introduced fecal material into pool water." "It is time to stop treating the swimming pool as a toilet," –CDC remarks (21)!

According to a study in Greece by S. Alexiou D in 2010 showed that a total of 16.6% (45/271) of the samples were positive for P. aeruginosa. Of the amenities examined, the most contaminated were hydrotherapy pools (25% of samples positive). A small percentage of isolates (20.0%) showed resistance to antibiotics. The study showed that compared with other studies, the prevalence of P. aeruginosa in swimming pools was relatively low, while the antibiotic resistance pattern of these community isolates was not high(22).

A study by in Nigeria by Itah AYin 2004 showed that total viable count of microorganims in Ibeno (B) and Uyo (E) swimming pools were 6 x 106cfu/ml and for Calabar (H) swimming pool, 3.3 x 107cfu/ml. The total coli form counts were 106cfu/100 ml for Calabar (G) swim- ming pools and 2 x 107cfu/100 ml for Calabar (H) swimming pools while the fungal count ranged from 5 x 106cfu/ml to 3 x 107cfu/ml. Physical and chemical parameters known to be hazardous to health were also identified. The presence of high levels of coli form and fecal coli form bacteria (E. coli) revealed that the swimming pools have not met the World Health Organization (WHO) standard for recreational waters (23).

3. Objectives

3.1 General objective

To assess the physico-chemical and microbiological qualities of swimming pools in Addis Ababa, Ethiopia

3.2 Specific Objectives

- To assess the physico- chemical condition (residual chlorine, PH, temperature) of swimming pool water
- To determine total viable count, total coli form count, fecalcoli form count and *E.coli*
- To evaluate the microbial safety of swimming pools against the WHO standard

4. Materials and Methods

4.1 Study Design

A cross sectional study design was used and all the swimming pools which were active during the study period were included

4.2 Study Area and Period

This study was carried out from February, 2016-May, 2016 in Addis Ababa, the capital city of Ethiopia. It is the largest city in Ethiopia, with a population of 3,384,569 according to the 2007 population census with annual growth rate of 3.8%. This number has been increased from the originally published 2,738,248 figure and appears to be still largely underestimated. As the largest city, Addis Ababa has many hotels with different amenities like swimming pools. About nine hotels and one recreation center are known to have swimming services for the public. Two out of the swimming pools were indoor while the rest were out door.

4.3 source population: All swimming pools in Addis Ababa were used as source population.
4.4. Study population: All the swimming pools which were active during the study period
4.5. Inclusion and exclusion criteria
Swimming pools recognized by Addis Ababa culture and tourism bureau were included and those not recognized by the bureau were not included.

4.6. Sample Size and Sampling Method

A total of 60 swimming pool water samples (6 samples from each source) were collected from nine hotels and one recreational center swimming pool facilities that were active during the study period.

4.7 Study Variables

4.7.1 Dependent Variable

Microbial quality of the swimming pools was the dependent variable

4.7.2 Independent Variables

PH, residual chlorine and temperature were the independent variables

4.8 Sample Collection

Water samples were collected at three points (from deeper point, shallow point and intake point) from each swimming pool in Addis Ababa. A 250 ml sterile bottle with sodium thiosulphate was immersed to an elbow depth with its opening facing the water. Its opening was then inverted so that water could get in to it. After the bottle was full, it was withdrawn from the water. PH, residual chlorine and temperature of samples obtained from each point were measured at the time of collection. A total of 60 water samples were collected from ten swimming pools, six samples from each swimming pool, on a weekly basis.

4.9 Sample Transport and Storage

Sample containing bottle was transported in ice box to microbiological laboratory with in two hours of collection. All the samples were analyzed on the day of collection.

4.10 Microbiological Analysis of Swimming Pool Water

4.10.1 Total Viable Count: Sample bottle was mixed by gentle inversion. Then, 1ml of sample was poured in to a sterile culture plate. 20 ml of plate count agar (PCA) was poured in to the plate. The sample and the PCA were mixed and were made to stay at room temperature until the mixture solidified. Plates were incubated for 48 hours at 37 degrees Celsius. Total viable counts were then counted by using digital colony counter (24).

4.10.2 Total Coli form Count: Three sets of test tubes (one 50ml, five 10ml, five 5 ml) containing Durham's tubes within them were arranged. Double strength MacConkey broth was poured in to the 50 ml and the 10 ml test tubes (50ml in to the50 ml and 10 ml in to the 10 ml) while 1 ml of single strength MacConkey broth was added in to each of the 5ml test tubes. Pool water was then added to the three sets of test tubes(50ml in to 50 ml test tube ,10 ml in to each 10 ml,1ml in to each 5 ml test tubes).The contents of each test tube were then mixed. Every test tube was observed for the presence of air bubbles in the Durham's tubes and air bubbles were removed fro test tubes which had them. The lids of each test tube were then loosened and all the tubes were incubated at 37 degrees Celsius. After 48 hours, each test tube was examined for gas production and for change in color. Test tubes which became yellow and produced gas were considered positive for total coli form and those with no color change and gas production were considered negative for total coli form. Finally, the total coli form count was obtained from the Most Probable Number (MPN) Index (25).

4.10.3 Fecalcoli form: Each positive test tube from each set of test tubes were sub cultured in to test tubes containing an *Escherichia coli* broth with Durham`s tube. Contents were mixed and air bubbles were removed from test tubes with air bubbles. Lids of each test tube were loosened and the test tubes were incubated at 44.4 degrees Celsius for 24 hours. After 24 hours, the test tubes were examined for turbidity and gas production. Those with turbidity and gas production were taken as positive for fecal coli forms and the fecal coli form count was determined from the Most Probable Number (MPN) index (25).

4.10.4 *E.coli*: Samples from positive test tubes for fecal coli form were inoculated on to nutrient broth and incubated at 44.4 degrees Celsius for 24 hours. After 24 hours, a drop of Kovacs reagent was added. Formation of red ring was used as a proof for Indole positivity and hence for the presence of *E.coli (26).*

4.10.5 Quality Control

E. coli ATCC25922 was used as a positive control while sterile media incubated with out sample was used as a negative control. Standard operating procedures were followed strictly in every step of the work. The functionality of instruments was checked before use. Unexpired media were used and Manufacturer`s instructions regarding media preparation were followed meticulously.

4.11 Data Analysis

Data were entered, cleaned and analyzed by SPSS version 20.Descriptive statistics (mean, frequency, percent and range) were calculated and a Chi square test was used to check association between variables. Results were then presented in words and depicted in tables and diagrams.

4.12 Data Quality Assurance

To check sterility of the Hi Media Mac Conkey broth, broth with out any sample and broth with a positive sample were incubated in every step of the culturing process. Absence of growth on broth with out sample and presence of growth on broth with a positive sample were used as measures of broth appropriateness. Data entered in to SPSS V.20 was checked for completeness and cleanness.

4.14 Ethical Considerations

An ethical clearance was obtained from departmental research and review committee of School of Medical Laboratory Science, AAU. Purpose of the research was then clearly explained by the principal investigator to hotels with swimming pools in Addis Ababa. Persons in charge of the hotels were also told that information fetched from the swimming pools would be kept confidential. Finally, permission was obtained from the respective hotels and data collection was started there after.

5 Results

A total of 60 water samples were collected on a weekly basis for a period of three months .The physico-chemical parameters (residual chlorine, PH and temperature) and microbiological parameters (APC, TCC FCC and *E.coli*) of the swimming water samples were determined using the most probable number method(MPN).

5.1 Physico-chemical Parameters of the Swimming Pool

5.1.1 PH

The PH value of the swimming pool water samples ranged from 5-10 with an average of 7.1.More than half i.e.58.4%(n=35/60)of the swimming pools had PH values in the range of 7.2-7.8 which is the WHO recommended limit for swimming pool water. Among the tested swimming pool water samples, 20 % (n=12/60) had PH values below 7.2 while 21.7 %(n=13/60) had values above 7.8.Compared to WHO recommended PH values, 41.6 %(n=25/60) of the water samples had pH values out of the recommended limits.

5.1.2 Residual Chlorine

The data has shown that the residual chlorine falls between 0.0 and 4.0 mg/l where 73.3%(n=44/60) of swimming pool water samples had residual chlorine values in the range of 0.0-1.9mg/L while 25%(n=15/60)had values falling between 2.0 and 3.9mg/l.Only 1.7% (n=1/60) of the swimming pool water sample was found to have residual chlorine values of 4mg/l.73.3% (n=44/60)of the swimming pool water samples had residual chlorine values below 2.0 which is the WHO minimum recommended limit where as 25%(n=15/60) had values within the WHO recommended range(2-3mg/l).

Table 1 Frequency distribution of PH and residual chlorine of swimming pools in Addis Ababa, 2016.

PH range	Frequency	Percent	Cumulative percent
5-7.1	12	20	20
7.2-7.8	35	58.3	78.3
8-10	13	21.7	100
Total	60	100	
Residual chlorine	Frequency	percent	cumulative percent
0-1.9	44	73.3	73.3
2-3.9	15	25.0	98.3
4-4.9	1	1.7	100.0
Total	60	100.0	

5.1.3 Temperature

The study has shown that swimming pool water samples had temperature values ranging from 20 ^{0}c-37^{0}c with an average of 29^{0}c.More than half i.e. 58.3% of (n=35/60)the swimming pool water samples were found to have temperatures ranging from 21^{0}c-32^{0}c.However,11.7% (n= 7 /60) of the swimming pool water samples had a temperature of 20^0 c

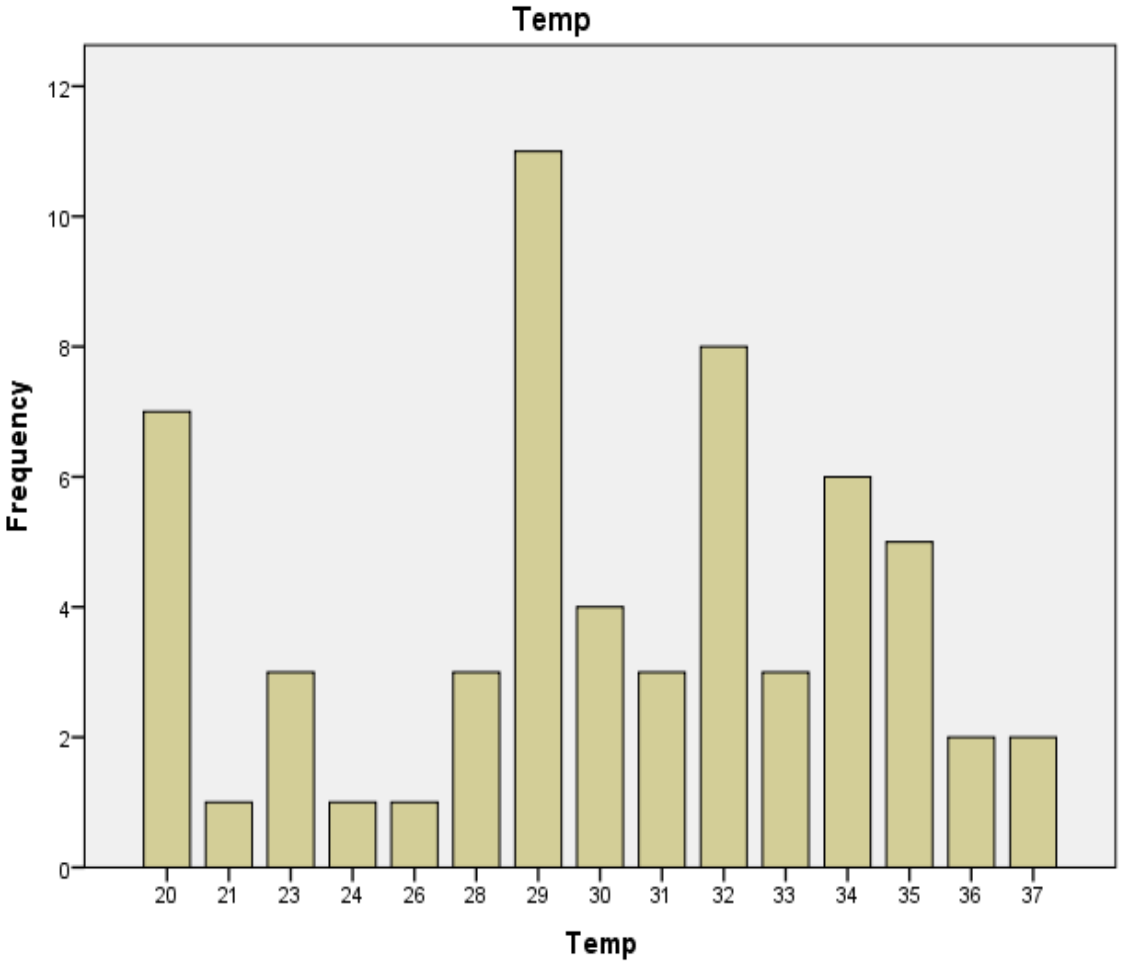

Figure1: Temperature of swimming pools in Addis Ababa, 2016

5.2 Microbial Assessment of Swimming pool Water Samples

5.2.1 Total Viable Count/Aerobic Plate Count (APC).

Data regarding the total viable count revealed that the swimming pool water samples had values in the range of 0cfu/ml-76000cfu/ml. Majority i.e.73.3%(n=44/60) of swimming pool water samples had a total viable count below 200 cfu/ml while 26.7%(n=16/60) of the swimming pool water samples had a total viable count above 200 cfu/ml.

5.2.2 Total Coli Form Count (TCC)

From the total coli form counts done on the 60 water samples, 66.7%(n=40 /60) of the swimming pool water samples had TCC values of 0MPN/100ml while 5(8.3%) had TCC values of 180MPN/100ml with a rang of 0MPN/100ml-180MPN/100 ml. 66.7% (n=40 /60) of the swimming pool water samples had TCC values of 0MPN/100ml while 5(8.3%) had TCC values of 180MPN/100ml. 70.0 %(n=42 /60) of the swimming water samples met the WHO standard with a TCC value of less than 2MPN/100ml while 30%(n=18/60) did not with TCC values above 2MPN/100ml.

Table 2 Frequency distribution of total viable count and total coliform count of swimming pools in Addis Ababa, 2016

Total coliform count	Frequency	Percent	Cumulative Frequency
0	40	66.7	66.7
1	2	3.3	70.0
2	2	3.3	73.3
3	1	1.7	75
5	1	1.7	76.7
7	1	1.7	78.4
8	3	5.0	83.4
11	2	3.3	86.7
18	1	1.7	88.4
20	1	1.7	90.1
25	1	1.7	91.8
180	5	8.3	100.0
Total	60	100.0	
Total viable count	Frequency	Percent	cumulative percent
<200	44	73.3	73.3
>200	16	26.7	100.0
Total	60	100.0	

5.2.3 Fecal Coli Form Count (FCC)

The fecal coli form counts ranged from 0MPN/100ml -180MPN/100ml.66.7% (n=40/60) swimming pool water samples had fecal coli form counts falling bellow 1MPN/100ml while 33.3%(n=20/60) had counts above 1MPN/100ml. 8.3%(n=5/60) swimming pool water samples were found to have a fecal coli form count of 180MPN/100ml and 5%(n=3/60) swimming pool water samples had fecal coli form counts of 25MPN/100ml,20MPN/100ml and 18MPN/100ml while the rest had counts below the aforementioned values.

Table 3 Frequency Distribution of Fecal Coliform Counts of Swimming Pools in Addis Ababa, 2016.

Fecal coliform count	Frequency	Percent	Cumulative frequency
0	40	66.7	66.7
1	3	5.0	71.7
2	2	3.3	75.0
3	1	1.7	76.7
7	1	1.7	78.4
8	3	5.0	83.4
11	2	3.3	86.7
18	1	1.7	88.4
20	1	1.7	90.1
25	1	1.7	91.8
180	5	8.3	100.0
Total	60	100.0	

5.2.4 E. *Coli*

The swimming pool water samples were checked for the presence or absence of *E.coli.* Accordingly, *E. coli* was present in 30 %(n=18/60)of swimming pool water samples while it was absent in 70%(n=42/60) of the pool water samples.

5.3 Evaluation of Swimming Pool Water samples against the WHO Standard (potability)

Compliance of swimming pool water samples was evaluated against the WHO standard. 68.3 % (n=41/60) of the swimming pool water samples which had neither *E. coli* nor fecal coli form showed compliance, 31.7 %(n=19/60) of samples which had either *E. coli* or fecal coli formdid not show compliance with the WHO standard.

Table 4 Compliance of swimming pool water samples in Addis Ababa, 2016 with WHO standard.

E.coli/Fecal coliform	Frequency	Percent	Cumulative Percent
Present	19	31.7	31.7
Absent	41	68.3	100.0
Total	60	100.0	

5.3 Association between Physico-Chemical Variables and Potability

Association between residual chlorine and pot ability of swimming pool water samples was done using Chi-square test. Accordingly, When the residual chlorine was 1ppm 19 water samples were non potable and when residual chlorine values were 2ppm and 3ppm,the corresponding water samples were all potable.(df=2, p-value =0.006).

Association between PH and pot ability was also done by using Chi-square test(α:0.5,df:16,CI:95% ,p-value=0.004).At PH (5.0-7.2),5 non potable and 12 potable swimming water samples, at PH (7.3-8.0),7 non potable and 24 potable swimming pool water samples were obtained.

Association between temperature and pot ability was done using chi-square test (α: 0.5, p-value=0.194, df: 14, CI: 95%).For temperatures ranging from 20-26^{0}C, 7 non potable and 6 potable samples were obtained and for temperatures that range from 280c-34,12^{0}c non potable and 26 potable water samples were found and 9 potable and 0 none potable water samples were obtained at temperatures in the range of 35^{0}c-40^{0}c.

Table 5 Association between residual chlorine, PH, Temperature and Portability

Residual chlorine	Pot ability		Total
	Non potable	Potable	
1ppm	19	25	44
2ppm	0	15	15
3ppm	0	1	1
Total	19	41	60
PH range	Non potable	Potable	Total
5-7.2	5	12	17
7.3-8.0	7	24	31
>8.0	7	5	12
Total	19	41	60
Temperature range	Non potable	Potable	Total
20-26	7	6	12
28-34	12	26	38
35-40	0	9	9
Total	19	41	60

6. Discussion

Data from this study showed that all the swimming pool water samples had PH values ranging from 5-10 with average PH value of 7.1.This result was comparable to results of a study by Rasti S, Assadi M, Iranshahi L et al in 2012 which showed a mean PH value of 7.38±0.5.A study by RasoolY, Mohammed R, Samarghandi et al in 2003 indicated that all the swimming pools had PH values ranging from 6 to 8.This PH range was almost similar to this study which had PH values in the range of 5to 10.WHO recommends that PH values of a swimming pool fall in the range of 7.2 to 7.8.In line with this,58.4% of the swimming pool water samples (PH:7.2-7.8) comply with the WHO`s standard for PH while 41.6% had values out of the standard,i.e.20% had values below 7.2 and 21.6% had values above 7.8.This may be attributed absence of educated pool operators ,bathers not taking pre swim showers and not changing pool water frequently.

The study showed that the residual chlorine was in the range of 0 to 4mg/lwith an average value of 1.16 mg/L. This value agreed with the finding by Rasti S, Assadi M, Iranshahi L et al in 2012 which had an average residual chlorine value of 0.84±0.5 mg/l. Another study by Neghab M,Georgi H. A, Baghapour M.A et al indicated a residual chlorine value to be in the range of 0.3-0.46mg/l .This value was also in close agreement with the finding of this study. WHO has specified the residual chlorine values of swimming pools to range from 2-3mg/l.In line with this,73.3%(n=44/60) of the swimming pool water samples had residual chlorine values in the range of 0-1.9mg/l which is below the lower WHO recommended limit. 1.7 %(n=1/60) of the swimming pool water sample were found to have residual chlorine value of 4 mg/l which also deviated the upper WHO recommended limit. Only 25% of the water samples had residual chlorine values falling between 2 and 3mg/l which is within the WHO recommended range. This suggested that there was no careful monitoring of residual chlorine in the majority of swimming pools.

With regard to temperature, the study indicated that swimming pool water samples had an average temperature of 29^0c.This result was in close agreement with the result of a study by Rasti S, Assadi M, Iranshahi L et al in 2012 which had an average value of 29.3 ± 1.3^0c.11.7%(n=7/60) of the swimming pool water samples had a temperature of 20^0 c which is below the WHO minimum recommended limit while 30%(n=18/60) had temperatures

above the WHO maximum recommended limit for pool water temperature. How ever,58.3%(n=35/60) of swimming pool water samples were found to have temperatures ranging from 21^0c-32^0c which is the WHO`s recommended temperature range for swimming pool water. This implies that there was still a problem in monitoring the temperature of swimming pool waters.

In our study 73.3% swimming pool water samples with a count of <200cfu/ml met the standard while 26.7% with a count above 200cfu/ml did not meet the set standard. The finding of 26.7% of the swimming pool water samples with a total viable count above 200 cfu/ml was less comparable with the finding of a study conducted by Cappello M in 2011 that indicated 18.5% of the swimming pool water samples to be in excess of public health standards. The disparity in result may be attributed to differences in sample size and differences in pool monitoring.

With regard to total coli form count, data from this study showed that total coli form counts ranged from 0MPN/100ml to 180 MPN/100ml. A study by Rasti S, Assadi M, Iranshahi L et al in 2012 showed TCC to range from 0MPN/100ml to 43MPN/100ml.Interms of mean value for TCC, this study had a value of approximately 2.5 while that of Hamid R, Leila I, Mohamed P et al in 2012 had a value of 1.8±7.7 MPN/100ml.This shows that the two studies had comparable results. With regard to the minimum and maximum values of TCC, how ever, a gap exists. The gap may be because of variation in the study period, sample size and pool water treatment. Another study by Itah AY in 2004 also showed TCC value of 106cfu/100 ml which is not comparable with result of this study.70 % of the swimming pool water samples had values below 2MPN/100ml while 30% had values above 2MPN/100ml.When this is evaluated in line with the WHO standard, 70% of the samples met the standard with TCC values below 2MPN/100ml while 30% did not meet the standard with TCC values above 2MPN/100ml.

Data from this study indicated that 33.3% of the swimming pool water samples were positive for fecal coli forms. This finding was much higher than that of Stamatiina L, Catherin D, Jourge F et al in 2007 which had 3% fecal coli form counts out of 200 samples. Another study by Neghab M Georgi H. A, Baghapour M.A, et al in 2003 showed that 11% of the total water samples were positive for fecal coli forms which was also much lower than that of the finding from this study. The variation in result could be due to differences in study period, sample size, pool treatment.

The world health organization recommends that fecal coli forms be below 0MPN/100ml.66.7% of the samples in this study met the WHO standard with a fecal coli form count of 0MPN/100ml while 33.3% did not meet the standard with their values being >0MPN/100ml.Out of the33.3%, 8.3% had fecal coli form counts of 180MPN/100ml while 5% had values of 25MPN/100ml, 20MPN/100ml and 18MPN/100ml.

30 %(n=18/60) of the samples were positive for *E.coli* while 70 %(n=42/60) were negative. In a study by Rasool Y, Mohammed R, Samarghandi et al in 2003, 16.6% of the samples were positive for *E.coli*. This result was lower than the finding of this study. In another study by John T in 2013,58% of the samples were positive for *E.coli* which was much larger than the result in this study. *E.coli* should not be detected in swimming pools, according to WHO standard. In 70% of the swimming pools, *E.coli* was not detected thus 70% met WHO standard while E.*coli* was detected in 30% of the pools thus 30% of the samples did not meet the set standard. The non conformance to the standard might be due to differences in sanitation practice, bather attitude, sample size and study period.

Swimming pools should meet potable water standard. According to WHO, water to be potable, *E.coli*/fecal coli forms should not be detected. With this respect, 68.3 % (n=41/60) of the samples had no *E.coli* thus were potable while 31.7% (n=19/60) had *E.coli* and/or *E.coli* indicating non pot ability.

Association between PH and pot ability was done by using Chi-square test (α:0.5,df:16,CI:95%). At PH (5.0-7.2), 5 non potable and 12 potable swimming water samples, at PH (7.3-8.0), 7 non potable and 24 potable swimming pool water samples were obtained. P-value of0.004 was found showing a statistically significant association between PH and pot ability.

Association between temperature and pot ability was done using chi-square test (α: 0.5, df:14, CI:95%).For temperatures ranging from 20-26^{0}C,7 non potable and 6 potable swimming pool water samples were obtained and for temperatures that ranged from 28^{0}c-34^{0}c,12 non potable and 26 potable swimming water samples were found and 9 potable and 0 none potable swimming pool water samples were obtained at temperatures in the range of 35^{0}c-40^{0}c.A p-value >0.05 shows pot ability and temperature had no statistically significant association(P-value =0.194).

Strengths and limitations of the study

Strengths

- This study has shown the counts of coli forms in swimming pools
- It also shades light regarding the status of swimming pools in Addis Ababa as weighed against WHO standard**.**

Limitations

- The study did not assess individual organisms in swimming pools
- The findings of the study are based on samples collected for short period of time which may lack representativeness of samples in a year.

7. Conclusion

The study found out that 58.4% of the swimming pool water samples (PH: 7.2-7.8) complied with the WHO`s standard for PH while 41.6% did not. Only 26.7% of the water samples complied with the WHO standard with regard to residual chlorine while 58.3% of the swimming pool water samples complied with the standard with regard to temperature. PH and residual chlorine had association with potability while temperature had no association. In general, it was evident from the study that a good number of swimming pools did not meet the WHO standard. It has also been found out that good monitoring of residual chlorine and PH had a significant impact on the microbial safety of swimming pools

8. Recommendations

Based on the gaps noted from the study, the following recommendations are worth noting.

- Culture and tourism bureau of Addis Ababa city government should use swimming pool safety as a requirement for licensing hotels.
- Owners of hotels with swimming pool facility should closely follow the over all swimming pool operation activity
- FMHACA should establish a professional's group that periodically regulates the compliance of swimming pools with the WHO standard.
- Further research about the specific pathogens found in swimming pools and their pattern of drug resistance should be done as it is difficult to judge if the observed non compliance with the WHO`s standard was due to a problem in the disinfection of the swimming pools or to drug resistance.

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Annex

Annex I Laboratory procedures

1.1 Bacteriological characterization of swimming pool water samples

Total viable count (procedure)

1.Transfer I ml of well mixed sample in to a sterile culture plate

2. Pour 20 ml of plate count agar over the sample and mix.

3. Wait until the mixture solidifies and incubate it at 37℃ for the 48 hours.

4. Count number of colonies using digital colony counter and report the result as the number of colonies in a given volume of the sample as follows.

CFU/ml=Colonies counted÷ Actual volume of sample in plate.

Total coli form count (Most probable number method)

1. Prepare Mackkonkey broth in single and double strength.
2. Assemble three sets of test tubes (one 50 ml, five 10mland five 5ml)
3. Transfer 50 ml broth in to the 50ml, 10ml in to each 10 ml test tube and 1 ml in to each of the five test tubes with 5ml capacity and sterilize the test tubes in an autoclave
4. Mix the sample very well and transfer 50 ml in to 50 ml test tube,10 ml sample in to each 10 ml test tubes and 1 ml of sample in to ach of the 5 ml capacity test tubes.
5. If any, remove air bubbles in Durhams tube within test tubes and incubate at 37℃ of 48 hours and observe gas production and appearance of yellow color which indicate positivity.
6. Count number of tubes giving a positive reaction get the corresponding MPN from the MPN Index report as MPN/100ml.

Fecal coli form count

1. Innoculate an EC broth by taking sample from each positive test tube and check absence of air bubbles within Durham`s tubes
2. Incubate the inoculated test tubes at 44℃ for 24 hours.
3. Count the positive test tubes and get the MPN/100ml of the sample by referring to the MPN Index table.

E. coli

1. Take loop full of sample from all the positive test tubes for fecal coli form and inoculate it on nutrient agar at 44^0.5C for 24 hours.
2. Add a drop Kovax reagent to positive test tubes and observe the formation of a red ring on the surface of the nutrient broth indicating the presence of E.coli and report as E.coli present.

1.2 Swimming pool water sample format

Type of water sample	piped	well	spring	reservoir	Swimming pool	chlorinated	unchlorinated	other
Sample code								

Client`s name		
Contact person		
Tel no		
Time sample collected		
Time sample analysis started		
PH		
Residual chlorine		
Temperature		
Sampled by		
Signature		

Parameters	Method reference	Quality control	result	Acceptable limit
APC at 35	APHA,1995 19(Edition	(P/F)	CFU/ml	
Coliform count	WHO,1971/2004,Eijkman test	P	MPN/100ml	
Fecal coliform	WHO,1971/2004,Eijkman test	P	MPN/100ml	
E.coli type 1	ICMSF,1988,(2 Edition)	P	present	absent

Declaration

I the undersigned, declare that this is my original work and has not been presented for a degree in this or any other university and all sources of materials used for this thesis have been acknowledged.

Name: Kokebe Yedeme

Signature _____

Place_____

Date of submission _____

This thesis has been submitted with my approval as University advisor.

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