MICROBIAL QUALITY OF PUBLIC SWIMMING POOLS IN LAGOS

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ABSTRACT

The microbial quality of some public swimming pools in Lagos State, Nigeria was investigated to determine possible risks of infections to *swimmers. Ten swimming pools (Pools A-J) were investigated for their microbial and physico-chemical qualities. The pH of the pools ranged from 5.24-8.71 and the residual chlorine ranged from 0.01-0.07. Water samples from the pools were cultured on differential and selective media. The microbial loads varied with different swimming pools. The fungal population ranged from 0 - 4.7×10^4 cfu ml^{-1}, while the bacterial population ranged from 0 - 1.12 x 10^{5}cfu ml^{-1}. Eight bacterial isolates were obtained and identified using Microbact 24E kit. Fungal isolates (6) were identified by microscopy, Gram's staining and chloramphenicol test. The isolates include Aeromonas hydrophila (4), Burkolderia pseudomallei (2), Serratia marcescens (1), Enterobacter agglomerans (1), Candida albicans (3), Saccharomyces spp. (2) and Aspergillus flavus (1). Antifungal sensitivity test showed 50% susceptibility to nystatin and 50% resistance to voriconazole, while the bacteria were resistant to most of the antibiotic tested. The Multiple Antibiotics Resistance (MAR) index for the isolates ranged from 0.125 to 1.000. The feedback from the questionnaires administered, showed that unhygienic practices and maintenance of pools could contribute to low levels of swimming pool water quality. The high microbial loads and the types of microorganisms isolated from the pools show that contaminated swimming pools can constitute a serious public health hazard to the users.*

Keywords: Bacteria, Fungi, Infection, MAR index, Swimming pools.

INTRODUCTION

A swimming pool is a structure filled with water intended for swimming or water-based recreation. It can be of any size and shape, in ground or above ground. It may be domestic (private), semi-public such as found in hotel, school, health club, housing complex and cruise ship or public including municipal. It may either be supervised or unsupervised (World Health Organization, 2006). Varieties of pathogenic organisms including bacteria, fungi, viruses and parasites have been found in swimming pools and similar recreational water (Centers for Disease Control and Prevention, 2009; Sohrabi *et al.*, 2016). These organisms, often introduced from environmental sources and swimmers, have been reported as causes of infectious diseases. Outbreaks of waterborne gastrointestinal disease

have been linked to faecal contamination of the water, due to faeces released by swimmers (WHO, 2006).

Diarrhoea is the most commonly reported infection associated with pathogenic contaminants in swimming pools (Rabi *et al.,* 2007). Other diseases associated with untreated pools or poorly maintained pools are cryptosporidiosis, otitis externa, commonly called swimmers ear, skin infections and respiratory infections (Centers for Disease Control and Prevention, 2009; Sohrabi *et al.,* 2016). Treatment of swimming pools using pumps, mechanical filters and disinfectants helps to control the transmission of infections, maintain the visual clarity of swimming pool waters and proper sanitation.
There are several reports on the microbial quality of swimming pools. Amala and Aleru (2016) reported that 40% of the swimming pools sampled in Port-Harcourt metropolis, Nigeria, were contaminated with bacteria of the genera *Bacillus, Micrococcus* and *Staphylococcus*. Similarly, Osei-Adjei *et al.* (2014) reported that the bacterial load of the swimming pools sampled in their study in Osu-Labadi Area, Accra exceeded the acceptable limits and were contaminated by *E. coli, Enterobacter faecalis, Enterobacter cloacae, Klebsiella pneumonia, Staphylococcus aureus* and *Streptococcus agalactiae*. Therefore, this study was undertaken to investigate the microbial quality of some public swimming pools in Lagos State, assay for the antimicrobial susceptibility of bacteria and fungi from the pools and relate the findings to hygiene, pool maintenance and possible health implications.

MATERIALS AND METHODS

Study Area

The microbial quality of water from ten different swimming pools (eight hotels and two sporting pools located in Lagos, Nigeria (Table 1) was investigated. All swimming pools were made of concrete covered with glazed tiles, had different shapes (ranging from rectangular to circular shapes) and different sizes ranging from 18 m^2 for the hotel pools to 1250 $m^2$for the sporting pools, and were supplied with fresh ground water.

Sample Collection

Water samples were collected from the various swimming pools between the hours of 8 am and 9 am. Each water sample was collected into a sterile one litre wide mouth plastic container at a depth of about 30 cm from ten different regions of each swimming pool. The samples from the different regions for each pool was mixed together, labelled with codes and immediately transferred to the laboratory for analysis.

Table 1: Time of collection of water samples from the swimming pools to the time of cleaning of pools

Sample	Source	Time of collection	Time of cleaning of pool
Pool A	Sport centre	Before cleaning	2 weeks
Pool B	Hotel	Before cleaning	2 weeks
Pool C	Hotel	Before cleaning	1 week
Pool D	Hotel	Before cleaning	2 weeks
Pool E	Hotel	After cleaning	1 week
Pool F	Hotel	Before cleaning	1 week
Pool G	Hotel	After cleaning	1 week
Pool H	Hotel	After cleaning	1 week
Pool I	Sport centre	After cleaning	2 weeks
Pool J	Hotel	After cleaning	2 weeks

Questionnaire

One hundred questionnaires with eleven questions were distributed among swimmers on the sampling sites, the pool operators and among students on campuses to know the habits exhibited by swimmers such as urinating and defecating and the type of infections swimmers contact when swimming in a contaminated pool. The responses were used to speculate the type of organisms isolated in this work and the possible health implications.

Physico-chemical Analysis

The pH and the residual chlorine of the water samples were assayed using a standard pH meter and the Mohr's method for chloride ion determination respectively (American Public Health Association, 2005). The glass electrode was first inserted in a standard phosphate buffer solution of pH 7.0 and pH 4.0. The electrode was then immersed into each sample and the readings were taken. The chloride in neutral or weakly saline solution containing chromate was titrated against silver nitrate. Silver chloride precipitated and end point was reached by the formation of silver chromate.

Microbiological Analysis

Total Heterotrophic Bacterial (THB) and Total Heterotrophic Fungal (THF) Population: THB was determined as described by Cruickshank *et al*. (2000) using Nutrient agar (NA).The THF was carried out using Potato Dextrose Agar (PDA). Aliquots of 0.1 ml of serially diluted water sample were transferred to sterile Petri-dishes containing solidified agar. A sterile glass rod was used to spread the suspension and the plates were incubated in inverted position aerobically at 28^{o}C for 24 h for bacteria and 48-72 h for fungi. After the incubation period, the number of colonies were counted.

Coliform Counts: Total coliforms count was estimated by the Most Probable Number (MPN) technique using MacConkey (MAC) broth. Faecal coliforms were estimated using Eosin Methylene Blue (EMB) Agar prepared according to manufacturer's instructions. Seeded plates were incubated at 37^{o}C and 44^{o}C for about 48 h for coliforms and faecal coliforms respectively. The 5-tube MPN Technique as described by Collee *et al*. (1989)

was used to determine the total coliform counts of the swimming pools. The result was interpreted using the five tubes MPN table and recorded as MPN/100 ml.

Isolation and enumeration of bacteria: *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas* species were isolated and enumerated using Eosin Methylene Blue Agar, Mannitol salt Agar (MSA) and *Pseudomonas* Base Agar (PBA) respectively. Aliquots of 0.1 ml of serially diluted water samples were transferred to sterile Petri-dishes containing solidified agar. A sterile glass rod was used to spread the suspension and plates were incubated in inverted position aerobically at 37^{o}C for 24 h. After the incubation period, number of colonies between 30-300 were counted. For *Salmonella*, 1ml of the water samples was inoculated into 4 ml of Selenite F broth and incubated for 24 h. Aliquots of 0.1 ml obtained from inoculated Selenite F broth were transferred to sterile Petri-dishes containing solidified agar. A sterile glass rod was used to spread the suspension and plates were incubated in inverted position aerobically at 37^{o}C for 24 h. After the incubation period, number of colonies between 30-300 were counted (Anonymous, 1995).

Identification of Bacterial Isolates: The bacterial isolates were identified by morphological characteristics, Gram's reaction, motility test, catalase test, oxidase test and Microbact 24E kit.

Fungal Identification: The fungal isolates were identified by morphological characteristics, microscopic examination using lacto phenol cotton blue stain, Gram's reaction and chloramphenicol test for yeasts.

Antibiotic Sensitivity Test of Bacterial Isolates: Susceptibility of the bacterial isolates to antibiotics was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton medium. The results were read and interpreted based on the guidelines of Clinical and Laboratory Standards Institute Guidelines (CLSI, 2012). The antibiotics tested were ceftazidime (30µg/ml), cefuroxime (30 µg/ml), gentamicin (10 µg/ml), cefixime (5 µg/ml), ofloxacin (5 µg/ml), augmentin (30 µg/ml), nitrofuratoin (300 µg/ml) and ciprofloxacin (5 µg/ml).The Multiple Antibiotic Resistance (MAR) Index and Percentage Resistance of MAR of each isolate were calculated (Krumperman, 1983).

Antifungal Susceptibility Test: One millilitre of each standardized spore suspension (105spores/ml) was evenly spread on the surface of solidified PDA plates. Nystatin (100 units) and voriconazole (1 µg) were placed on the surface of the inoculated plate. The plates were incubated at ambient temperature for 48 h and observed for growth. Anti-fungal activities were measured and recorded as means of diameter of zones of inhibition from four replicates discs.

Statistical Analysis
The results were subjected to statistical analysis using GraphPad Prism 5.

RESULTS
Out of one hundred questionnaires that were circulated, only 73 responses were returned. The number of positive (yes) responses to habits such as urination and defecation in swimming pools and ingestion of swimming pool water where greater than the negative responses. Similarly, higher number of the respondents checked swimming pool appearance and maintain hygienic practices in and around swimming pool area (Fig.1). Many of the respondents have contacted abdominal (diarrhoea), eye, ear and skin infections from swimming. They also engaged in the use of antimicrobial agents to control infections that may arise from swimming.

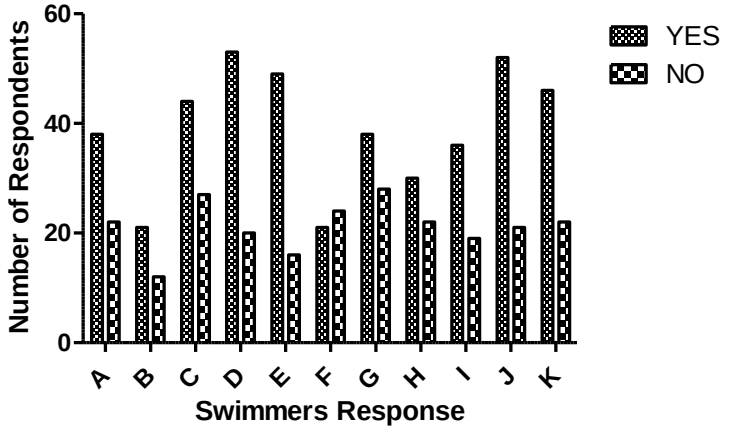

Fig. 1: Survey of swimmers responses to sanitary conditions and habits around swimming pools

A, urinate in pool; B, defecate in pool; C, contacted skin infections; D, contacted abdominal infections (diarrhoea); E, eye infection; F, ear infection; G, ingest swimming pool water; H, use antifungal agents; I, use antibiotics; J, check physical appearance of pool; K, Maintain hygienic practices in and around swimming pool area.

The result of the physico-chemical analysis of swimming pools is shown in Table 2. The pH of the pools was within the range of 5.24-8.71. Pools A, B, C, D, E and F were lower than the acceptable pH limit, whereas Pools G, H, I and J were within acceptable pH limit of 7.00-8.50 (WHO, 2003). The residual chlorine of all the pools (0.01-0.07 ppm) was below the acceptable limit of 0.2-0.5 ppm (WHO, 2003).

The Total Heterotrophic Bacterial Fungal (THB) Count (cfu/ml) and Total Heterotrophic Fungal (THF) populations (cfu/ml^{-1}) of the swimming pools is shown in Fig. 2. The

bacterial loads varied among the swimming pools. The THB counts of pools A,B, D,E and F were high according to WHO standard of <200/100 ml and pools C,G, H, I and J were low (WHO 2003, 2006). The mean heterotrophic count (cfu/ml) ranged from 0 in pools C, G, H, I and J to $1.12x10^5$in pool A. Fungi were not isolated from pools D, E, and G, while the THF population in pools A, B, C, F, H, I and J was between $2.0x10^4$cfu/ml^{-1} and 4.7 x 10^4cfu/ml^{-1}. There was no visible bacterial growth when the swimming pool samples were cultured on the following selective media: EMB (*Escherichia coli*), SSA (*Salmonella*), PBA (*Pseudomonas* spp.) and MSA (*Staphylococcus aureus*) (Table 3). The results obtained were within the acceptable microbial limits for *Escherichia coli, Salmonella, Pseudomonas* and *Staphylococcus aureus* according to World Health Organization (2003). Also, the total coliform counts (cells/100ml) for all the samples were within acceptable limit of <10/100 ml (WHO, 2003). The least coliform count of 2/100 cfu/ml^{-1} was observed in almost all the samples except for B which was 6/100 cfu/ml^{-1}.

Table 2: Physico-chemical analysis of some swimming pools in Lagos State

Parameters	Swimming pools										WHO Limits (2003)
	Pool A	Pool B	Pool C	Pool D	Pool E	Pool F	Pool G	Pool H	Pool I	Pool J	
Hydrogen ion concentration (pH)	5.36	6.00	5.28	5.24	5.61	5.30	8.71	8.40	7.20	7.45	7.00-8.50
Residual chlorine(ppm)	0.01	0.03	0.02	0.02	0.03	0.01	0.04	0.04	0.07	0.05	0.2-0.5

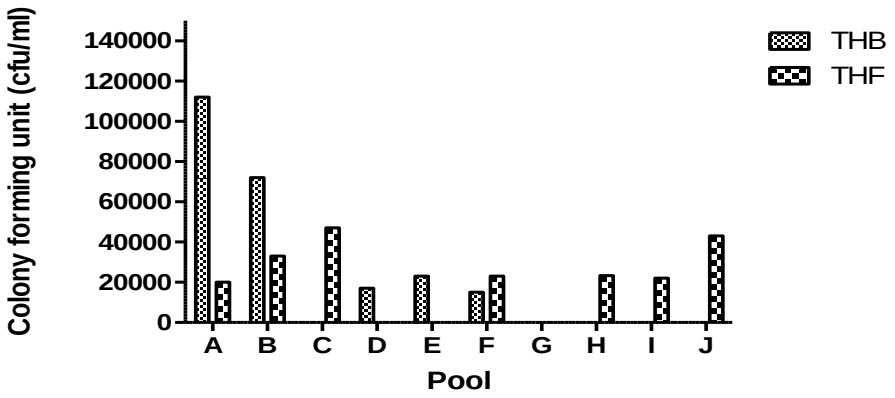

Fig. 2: Microbial populations of some public swimming pools in Lagos State

THB, Total Heterotrophic Bacteria; THF, Total Heterotrophic Fungi**;** World Health Organization acceptable limit for bacteria is <200/100 ml (WHO, 2006).

Table 3: Bacterial isolates of public health importance from some swimming pool samples in Lagos State

S/N	Sample codes	*Escherichia coli*	*Salmonella Shigella* Agar	*Pseudomonas* species	*Staphylococcus aureus*	Faecal coliforms	Total coliforms (MPN) (cells/100ml)
1	Pool A	ND	ND	ND	ND	ND	<2
2	Pool B	ND	ND	ND	ND	ND	6
3	Pool C	ND	ND	ND	ND	ND	<2
4	Pool D	ND	ND	ND	ND	ND	<2
5	Pool E	ND	ND	ND	ND	ND	<2
6	Pool F	ND	ND	ND	ND	ND	<2
7	Pool G	ND	ND	ND	ND	ND	<2
8	Pool H	ND	ND	ND	ND	ND	<2
9	Pool I	ND	ND	ND	ND	ND	<2
10	Pool J	ND	ND	ND	ND	ND	<2
11	WHO Acceptable limit	0/100 ml	<1/100 ml	<10/100 ml	<50/100 ml	0/100 ml	<10/100 ml

The bacteria isolated were *Aeromonas hydrophila* strain 1, *Burkolderia pseudomallei* strain 1, *Aeromonas hydrophila* strain 2, *Serratia marcescens*, *Aeromonas hydrophila* strain 3, *Burkolderia pseudomallei* strain 2, *Enterobacter agglomerans* and *Aeromonas hydrophila* strain 4. Most of the bacteria isolate were resistant to several of the antibiotics used as shown in Table 4. The Multiple Antibiotic Resistance (MAR) index for all isolates ranged from 0.125 to 1.000. *Candida albicans* strain B and *Saccharomyces* sp. strain B were resistant to both nystatin and voriconazole antifungal agents, while *Candida albicans* strains A and C were sensitive to both agents. *Aspergillus niger* and *Saccharomyces* sp. strain A were resistant to voriconazole and nystatin respectively (Table 5).

Table 4: Antibiotics sensitivity test of bacteria isolated from swimming pools in Lagos State

Isolates	Antibiotics (µg/ml) Diameter of zone of inhibition (mm)								
	CAZ (30 µg/ml)	CRX (30 µg/ml)	GEN (10 µg/ml)	CXM (5 µg/ml)	OFL (5 µg/ml)	AUG (30 µg/ml)	NIT (300 µg/ml)	CPR (5 µg/ml)	MAR Index
A. hydrophila strain 1	15(R)	11(R)	13(I)	15(R)	17(R)	0(R)	14(I)	18(I)	0.625
B. pseudomallei strain 1	15(R)	12(R)	10(R)	9(R)	17(R)	15(R)	11(R)	20(S)	0.875
A. hydrophila strain 2	11(R)	7(R)	10(R)	0(R)	16(R)	10(R)	12(R)	16(R)	1.000
S. marcescens	12(R)	0(R)	14(S)	0(R)	16(R)	11(R)	10(R)	20(S)	0.750
A. hydrophila strain 3	18(S)	19(S)	22(S)	18(S)	25(S)	19(S)	18(S)	22(S)	0.000
B. pseudomallei strain2	10(R)	0(R)	19(S)	0(R)	21(S)	10(R)	20(S)	21(S)	0.625
E. agglomerans	11(R)	0(R)	17(S)	9(R)	20(S)	0(R)	20(S)	22(S)	0.625
A. hydrophila strain 4	15(I)	17(S)	21(S)	17(S)	22(S)	16(S)	7(R)	24(S)	0.125

S, Susceptible; R, Resistant; I, Intermediate; MAR Index, Multiple Antibiotics Resistance index;CAZ, ceftazidime; CRX, cerufixime; GEN, gentamicin; CXM, cefixime; OFL, ofloxacin; AUG, augmentin; NIT, nitrofuratoin; CPR, ciprofloxacin.

Table 5: Antifungal susceptibility test of fungi isolated from public swimming pools in Lagos State

Antifungal agent	Diameter of zone of inhibition (mm)					
	***Candida albicans* strain A**	***Candida albicans* strain B**	***Candida albicans* strain C**	***Saccharomyces* sp. Strain A**	***Saccharomyces* sp. Strain B**	***Aspergillus niger***
Nystatin (100 units)	22.25	-	22.50	-	-	25.25
Voriconazole(1µg)	17.25	-	17.25	16.75	-	-

DISCUSSION

Many of the outbreaks related to swimming pools and similar environments have occurred because disinfection was not applied or was inadequate (WHO, 2006). This report was validated by the physico-chemical (residual chlorine and pH) data obtained in this study, the microbial analysis and respondents from questionnaires. The low residual chlorine level in the pools may be as a result of inadequate chlorination or the high presence of bacteria in them. Most of the pools sampled had pH values lower than WHO (2003) acceptable limit of 7.00-8.50.The pH has a direct impact on the recreational users of water only at very low or very high values (American Public Health Association, 2005). Problems of high pH in swimming pools include primary irritation of the skin such as dry, itchy skin and scalp, burning eyes and nose, drop in disinfection potential of chlorine resulting in bacterial growth. Low pH may have effects on the pools such as in corroding of the metal pool accessories, staining resulting from corrosion, burning eyes and skin, destroying of swim wears and accessories (American Public Health Association, 2005; Bilajac *et al.*, 2012).

The questionnaires administered assessed the swimmers' habits and knowledge of the infections that could be contacted during swimming. There was a consensus among the respondents that swimmers can contact diarrhoea, eye infection, ear infection and skin infections during swimming. The high microbial load in the pools could be from contaminated water sources, faecal and non-faecal contaminants from swimmers or inadequate treatment of pools. Le-chevalier *et al*. (1988) reported the occurrence of coliforms in the presence of residual disinfectant in pools and Craun *et al*. (2005) corroborated that swimmers tends to shed bacteria from faecal and non-faecal sources in pools. The absence of coliforms from some of the pools shows that they were not contaminated by faecal sources. *Escherichia coli*, *Salmonella/Shigella*, *Pseudomonas* spp. and *Staphylococcus aureus* were also absent from the pools.

The presence of *Aeromonas hydrophila, Burkolderia pseudomallei, Serratia marcescens, Enterobacter agglomerans, Candida albicans, Saccharomyces cerevisiae* and *Aspergillus niger* in swimming pools is not desirable and are of public health importance. Other researchers have isolated similar organisms. Sule and Oyeyiola (2010) isolated *Aeromonas* spp. and *Burkolderia pseudomallei* from swimming pools in Ilorin.

Aeromona shydrophila can cause eczema and gastroenteritis in humans most especially in people who have compromised immune systems and young children (Venkat *et al*., 2000). *Burkolderia pseudomallei* is a major cause of bronchitis, pneumonia, and meliodosis which normally infects the lungs and causes abscess (cavity of pus) (Cunha, 2010). *Serratia marcescens* is one of the bacteria that can cause urinary tract infections, eye infections (conjuctivitis) and wound infections; it is commonly found in the respiratory and urinary tracts of hospitalized adults (Muñoz *et al.*, 2004; Khanna *et al.*, 2013). *Enterobacter agglomerans* is an opportunistic pathogen in the immunocompromised, causing wound, blood and urinary tract infections (Mardaneh and Dallal, 2013). In agreement with our findings, Agbagwa and Young-Harry (2012) reported the isolation of *Enterobacter agglomerans* from public pools in Port- Harcourt, Nigeria.

Candida albicans is a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections in humans (Ryan and Ray, 2004; dEnfert and Hube, 2007). *Saccharomyces cerevisiae* is acknowledged as an emerging infectious agent, with an incidence of up to 4% among blood culture germs (Enache-Angoulvant and Hennequin, 2005; da Silva *et al.*, 2011). Person *et al.*, (2010) stated that infections due to *Aspergillus* species cause significant morbidity and mortality. They also reported a case of an *Aspergillus niger* that caused invasive pulmonary aspergillosis. Rasti *et al.*, (2012) have reported the prevalence of saprophytic and opportunistic fungi in some public swimming pools in Kashan, Iran.

The antimicrobial susceptibility test revealed that majority of the isolates were resistant to most of the antibiotics and antifungal agents used for treating infection. The implication is that it could be difficult to treat an infection acquired from the swimming pools arising from such organisms (Son *et al*., 1997). In addition, the resistant strains could spread to the community. The MAR index for most of the bacterial isolates was over 20% which implies that the swimming pools were contaminated through human sources.

In conclusion, most of the swimming pools did not meet the physio-chemical and bacteriological standards of swimming pools as specified by World Health Organization, (WHO, 2003). This therefore calls for urgent actions by all concerned. The operators should follow recreational water guidelines for proper management of swimming pools. Users should adhere to good sanitary practices and the various health authorities should monitor swimming pool facilities and ensure strict compliance to guidelines for sanitation and proper pool management in order to stem the incidence of recreational diseases.

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