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Monitoring the Presence of Bacteria, Fungi and Parasitic Pathogens Associated with Swimming Pools in Port Harcourt Metropolis

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Authors' contributions

This work was carried out in collaboration among all authors. Author EOO designed the study, wrote the protocol and managed field and laboratory analysis of samples. Authors AAA and AAD managed the analysis of data in the study. Author AAD managed the literature searches. All authors read and approved the final manuscript.

Article Information

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Original Research Article

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ABSTRACT

Aims: Ideal water for swimming should meet the portable water standard by being transparent, odorless, and tasteless. However, these qualities can be affected by the presence of infectious agents which directly or indirectly contaminate pool water. This study aims at monitoring the microorganisms (bacteria, fungi and protozoa parasites) and pH associated with swimming pool water.

Study Design: A random sampling technique was adopted to select the five (5) swimming pools for the study based on accessibility and visitation.

Place and Duration of Study: The study was carried out in Port Harcourt Metropolis, Port Harcourt Local Government Area, Rivers State between March 2016 to August 2017.

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Methodology: Five (5) outdoor swimming pools attached to hotels in Port Harcourt Metropolis, Port Harcourt Local Government Area of Rivers State, Nigeria were randomly selected for the study. Water samples were aseptically collected in duplicates and analyzed for total heterotrophic bacterial count, total heterotrophic fungal count, protozoa and pH using appropriate techniques.

Results: The results show that swimming pools with sodium thiosulphate pentahydrate had a mean THBC range from 4×10^{1} cfu/ml - 1.58×10^{3} cfu/ml, mean THFC, 0 cfu/ml - 8×10^{1} cfu/ml and mean pH, 5.9-6.4; for samples without sodium thiosulphate pentahydrate, the mean THBC ranged from 0 cfu/ml - 9.2×10^{1} cfu/ml, mean THF, 0 cfu/ml - 8×10^{1} cfu/ml and mean pH, 5.6-6-2. Protozoa (parasites) were absent in all water samples analyzed. The results further reveals that 95.9% and 100% of the swimming pools samples failed to the meet the <100 cfu/ml and 7.0 – 7.8 World Health Organisation Standards for THBC and pH respectively. The bacteria identified are *Bacillus* spp., *Escherichia coli, Stapylococcus aureus* while the fungi identified are *Aspergillus* spp., *Rhizopus* spp. and *Penicillium* spp.

Conclusion: The study finds the levels of THBC in the samples with sodium thiosulphate pentahydrate, the presence of *Escherichia coli* and the pH range a threat to health of bathers. Therefore, the owners should ensure routine decontamination and assessment of water quality.

Keywords: Bacteria; fungi; parasite; Port Harcourt; potable; swimming pool; water.

1. INTRODUCTION

Microbial pathogens are disease causing microorganisms. They can be of bacterial, viral, fungal or protozoan origin and are host specific, as their portal of entry, mode evading immediate destruction of their host immune system and replication processes are different. They spread via droplets, direct contact; body fluids transmitted by vectors and can be found in soil, air, and water even in swimming pools [1].

Water is used for different purpose such as drinking, washing, cooking, bathing swimming and lots more [2]. Swimming pools are concrete tanks, large artificial basin or large paved holes containing water for swimming or water based recreation. An ideal water for swimming should meet the potable water standard by being transparent, odourless and tasteless having a freezing point of 0°C and a boiling point of 100°C [3].

However, these qualities can be affected by the presence of infectious agents which directly or indirectly contaminate pool water, causing infections/diseases which are of public health importance [4]. The risk of these infections has been linked to faecal contamination of pool water because of some of the activities of bathers, such as passing of excreta during swimming and washing off of residual faecal material on swimmers body into pool water [5]. Pools can also be contaminated by animals especially rodents and birds in open pools. Indirect contamination from air, soil, dust, rain water and sewage is also possible [6,7].

Faecal release by bathers may be unintentional, as in the case of diarrheic stool; other than faecal contamination, non-faecal contamination from human shedding (vomit, mucus, skin or saliva) in pools is also a potential source of pathogenic [6,8,9]. The survival microbes of the microorganisms in pool water maybe due to inadequate cleaning and disinfection, and this could lead to outbreak of diseases that are of public concerns such as gastro- enteritis, conjunctivitis, ear infection, cholera, dysentery etc. [10,11].

Illnesses such as diarrhoea, typhoid fever and cholera has been report from drinking of contaminated pool water by swimmers [10]. Improved surveillance data from the United States and Europe have shown that microbial water related illnesses are on the increase [12]. Thus, pool inspectors should be acquainted with the problems caused by contaminated pool water and as well as know how to prevent such contamination. The quality of pool is enhanced by frequent changing of the water and use of disinfectant agents such as chlorine, bromine or iodine [13]. Therefore, this study aimed at evaluating the microbial pathogens and pH associated with selected swimming pools in Port Harcourt Metropolis. Port Harcourt Local Government Area, Rivers State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Port Harcourt Metropolis, Port Harcourt Local Government

Area, Rivers State, Nigeria. It is the capital and largest city of Rivers State, Nigeria and covers an area of 369 km^2 . It lies along the Bonny River and is located in the Niger Delta. Its coordinates are $4^{\circ}49'27'N$ $7^{\circ}2'1'E$ and at the 2006 Census held a population of 1,382,592.

2.2 Site Selection and Sample Size

A cross-sectional study carried out from March 2016 to August 2017. A random sampling technique was adopted and only outdoor swimming pools within the Local Government Area were selected for sampling. Five (5) outdoor swimming pools attached to Hotels in the city were therefore identified and selected for this study. The Hotels are owned by the individuals. They attract people from different places, irrespective of societal status.

2.3 Sample Collection, Storage and Transportation

Water samples were aseptically collected in duplicate from the five different pools at a depth of 25 cm below the surface of the pool using sterile well-labeled wide-mouthed bottles. The sampling was done in the morning when no bather was in the pool. Twenty microliter (20 μ l) of sodium thiosulfate pentahydrate was added into one set of the sample bottles from each swimming pool to dechlorinate the water samples. The samples were then preserved in a cooler containing ice pack and are transported to the Microbiology Laboratory of the Department of Medical Laboratory Science, Rivers State University, Port Harcourt for immediate microbial analysis.

2.4 Total Heterotrophic Bacterial Count and Total Heterotrophic Fungal Count

The swimming pools water samples were analysed for total heterotrophic bacterial count (THBC) and Total heterotrophic fungal counts (THFC) employing spread plate method on Nutrient Agar (NA) and Sabouraud Dextrose agar (SDA) respectively as media for isolation. Using a sterile pipette, 0.1 ml of each sample was plated out on the Nutrient Agar and Sabouraud Dextrose Agar plate with the aid of sterile glass spreader in duplicates. The inoculated plates were then incubated at 37°C for 24 - 48 hours and 30°C for 3 – 7 days for bacteria and fungi respectively. The colonies were reported as colony forming unit per millilitre (cfu/ml) [14]. Identification of bacteria and fungi

was done using the standard method [15]. The bacterial isolates were identified by microscopic (involving Gram staining technique), macroscopic characteristics and biochemical tests and further by using Bergey's manual of Determinative Bacteriology. The fungal isolates were identified on the basis microscopical (using Lactophenol cotton blue) and macroscopical characteristics and taxonomic keys provided by [16].

2.5 Determination of Protozoan Parasites

Each water sample (50-100 ml) was filtered separately through a nitrocellulose membrane (0.45 µm pore size, 142 mm diameter, Millipore) according to the method of [17,18]. The protozoan parasites that might be present on the surface of the membrane filter after sample filtration were collected by soaking and thorough washing of membrane in 20 ml of 5% formal saline (5% formaldehyde in 0.85% sodium chloride). This washing solution was centrifuged at 4000 g for 6 minutes at room temperature and the produced pellet were re-suspended in 1 ml of distilled water. Thereafter, a volume of 500 µl was then used for microscopic examination.

2.6 Determination of pH

The pH of the swimming pool water samples were determined using a pH meter calibrated with 7.0 phosphate buffer solutions according to manufacturer's instruction. This was measured by inserting the pH probe directly into the sample and the reading taken from the digital display.

2.7 Statistical Analysis

Data generated from the work was analysed using descriptive statistics (frequencies, percentages and means).

3. RESULTS

The mean total heterotrophic bacterial count of the five swimming pools in which sodium thiosulphate pentahydrate was added to the samples is shown in Table 1. It shows a total of 219 bacterial colonies from all the pools. Pool 4 had the highest plate count of 158 colonies or 1.58×10^3 cfu/ml (72.1%), followed by pool 1 with a count of 31 colonies or 3.1×10^2 cfu/ml (14.2%) followed by Pool 3 with a count of 21 or 2.1×10^2 cfu/ml (9.6%) followed by Pool 2 with a count of 5 or 5.0×10^1 cfu/ml (2.3%) and finally Pool 5 which had the lowest plate count of 4 colonies or 4.0×10^1 cfu/ml (1.8%).

The mean total heterotrophic bacterial count of the five different swimming pool samples without sodium thiosulfate pentahydrate is shown in Table 1. It shows a total of 98 (100%) bacterial colonies from all the pools. Pool 4 has the highest count of 92 colonies or 9.2×10^{1} cfu/ml followed by Pool 1 which had 4 colonies or 4.0×10^{1} cfu/ml (4.1%) followed by Pool 3 and 5 had which had 1 colony or 1.0×10^{1} cfu/ml (1%) while Pool 2 had no bacterial growth (0%).

The mean total heterotrophic fungal count of the different swimming pool samples with sodium thiosulfate pentahydrate is shown in Table 2. It shows that a total of 17 fungal colonies were isolated from the pools under study. Pool 3 had the highest count of 8 colonies or 8.0×10^1 cfu/ml (47.1%) followed by Pool 5 with a count of 4 colonies or 4.0×10^1 cfu/ml (23.5%) followed by Pool 2 which had 3 colonies or 3.0×10^1 cfu/ml (17.6%) followed by Pool 4 which had 2 colonies or 2.0×10^1 cfu/ml (11.8%) while Pool 1 had no fungal growth (0%).

The mean total heterotrophic fungi count of the different swimming pool samples without sodium

thiosulfate pentahydrate is shown in Table 2. A total of 4 fungal colonies were isolated from the pools under study. Pool 1 had no fungal growth (0%), while pool 2, 3 4 and 5 had total plate count of 1 colony each or 1.0×10^{1} cfu/ml (25%).

The bacteria identified from the different swimming pools samples are shown in Table 3. It shows that the Bacillus species was found in pool 1, 2, 3 and 5. Pool 2 also had *Staphylococcus aureus* while pool 4 had *Escherichia coli* and *Staphylococcus aureus*. The fungi identified from the different swimming pool samples are shown in Table 3. It shows that pool 5 had a growth of *Penicillium* spp., *Rhizopus* spp. and *Aspergillus* spp. Pool 4 had *Rhizopus* spp. and *Aspergillus* spp. Pools 2 and 3 had only *Aspergillus* spp. while pool 1 had no fungal growth. No protozoan (parasite) was isolated in all of the pool samples analyzed.

The mean pH values of the different swimming pool samples with and without sodium thiosulfate pentahydrate are shown in Table 4. It shows slightly higher pH values for the core samples with sodium thiosulfate pentahydrate.

Table 1. Total mean bacterial count of the swimming-pool samples with or without sodium
thiosulfate pentahydrate

Station	Total pla	te count (%)	count (%) cfu/ml	
	With STP	Without STP	With STP	Without STP
Pool 1	31(14.2)	4 (4.1)	3.1× 10 ²	4.0×10 ¹
Pool 2	5(2.3)	0	5.0 × 10 ¹	0
Pool 3	21(9.6)	1(1.0)	2.1 × 10 ²	1.0×10^{1}
Pool 4	158(72.1)	92(93.9)	1.58 × 10 ³	9.2×10^{1}
Pool 5	4(1.8)	1(1.0)	4.0 × 10 ¹	1.0 × 10 ¹
Total	219	98		

Cfu/ml-colony-forming units per milliliter

STP – Sodium Thiosulfate Pentahydrate

Table 2. Total mean fungal count of the swimming-pool samples with or without sodium thiosulfate pentahydrate

Station	Total plate count (%)		cfu/ml	
	With STP	Without STP	With STP	Without STP
Pool 1	0	0	0	0
Pool 2	3(17.6)	1(25.0)	3.0 × 10 ¹	1.0 × 10 ¹
Pool 3	8(47.1)	1(25.0)	8.0 × 10 ¹	1.0 × 10 ¹
Pool 4	2(11.8)	1(25.0)	2.0 × 10 ¹	1.0 × 10 ¹
Pool 5	4(23.5)	1(25.0)	4.0 × 10 ¹	1.0 × 10 ¹
Total	17	4		

Cfu/ml-colony-forming units per milliliter

STP – Sodium Thiosulfate Pentahydrate

Station	Bacteria identified	Fungi identified
Pool 1	Bacillus spp.	No fungal growth
Pool 2	Bacillus spp., Staphylococcus aureus	Aspergillus spp.
Pool 3	Bacillus spp.	Aspergillus spp.
Pool 4	Escherichia coli, Staphylococcus aureus	Rhizopus spp.
Pool 5	Bacillus spp.	Aspergillus spp.
		Penicillium spp.,
		Rhizopus spp.,
		Aspergillus spp.

Table 3. Bacteria and fungi identified from the different swimming pool samples

Table 4. Mean pH values of the different swimming pool samples with and without sodium thiosulfate pentahydrate

Name of station	рН
Pool 1	6.4*
	6.2**
Pool 2	6.0*
	5.6**
Pool 3	5.9*
	5.8**
Pool 4	6.3*
	5.6**
Pool 5	6.0*
	5.8**

*- pool samples with sodium thiosulfate pentahydrate **-pool samples without sodium thiosulfate pentahydrate

4. DISCUSSION

The study reveals that among the swimming water samples containing sodium thiosulfate pentahydrate, 95.9% recorded counts such as 3.1×10² cfu/ml, 2.1×10² cfu/ml and 1.58×10¹ cfu/ml which are higher than < 100 cfu/ml [6], the World Health Organization (WHO) standard for swimming pools. Meanwhile, the swimming pool water samples without sodium thiosulfate pentahydrate met the WHO standard of < 100 cfu/ml 100%. The addition of sodium thiosulfate pentahydrate may have aided the growth of bacteria, as a result of its neutralizing activity on chlorine and bromine based disinfectant used in pools, which ordinarily would have impeded the growth of bacteria [6]. However, according to [19] total heterotrophic bacteria count (total aerobic plate count) indicates the culturable organisms present, which could be low or high total bacteria present. In a similar study, [20] recorded that the heterotrophic bacterial load ranged between 0 and 6.35×10⁵ cfu/ml, where 6.35×10⁵ cfu/ml was the highest load and 3×10¹ cfu/ml the least; the highest average TPC was 6.19×10⁵ cfu/ml and the lowest 5.07×10³ cfu/ml. A number of reasons could be attributed to this level of contamination observed in the study. According to [20], owners of swimming pools have little adherence to the national swimming pool or portable water standards because of poor enforcement of laws, ignorance, and negligence. This in their view has resulted in all kinds of uncouth practices in the pool water. Also, there is the case of very high bather density. [21] further revealed that the total heterotrophic bacterial count in an aquatic environment also depends on the availability of growth supporting organic matter.

The bacteria identified in this study were Bacillus spp., Staphylococcus aureus and Escherichia coli. This finding is also in agreement with the findings of [22] that isolated these same organisms including Enterococcus faecalis, Clostridium perfringens. Pseudomonas aeruginosa and Proteus vulgaris. The presence of Escherichia coli indicates faecal contamination; its detection is either as a result of deficiencies in the treatment of the swimming pool or inadequate protection of the source of untreated water [6,23]. According to WHO, for water to be potable, Escherichia coli (faecal coliform) should not be detected; therefore, swimming pool 4 water indicates non-potability. Swimming pools related waterborne illness is commonly attributed to faecal contamination of water either by swimmers or animals that have access to outdoor pools [24,25]. [26] stated that other non-faecal substances, such as vomit, mucus, saliva and skin can also serve as sources of pathogenic microorganisms in swimming pools. Staphylococcus aureus as a normal flora is the most common cause of Staphylococcus infections ranging from minor skin infections to life-threatening diseases such as pneumonia, toxic shock syndrome and septicaemia among others [27]. Bacillus spp., Staphylococcus aureus and Escherichia coli are recognized enterotoxin producers when and they are ingested into the body, therefore their presence in pools is a threat to public health

because they can be ingested with water by active swimmers [22].

The total fungal colonies from the pool samples containing sodium thiosulfate pentahydrate than that isolated from the two samples without sodium thiosulfate pentahydrate were in the range of 0 - 8 x 10^{1} cfu/ml and 0 - 1.0 x 10^{1} cfu/ml respectively. This result is similar to findings by [28] who reported average fungal counts of 0 cfu/ml - 8 cfu/ml and 1 cfu/ml - 15 cfu/ml. This could be attributed to poor disinfection. lack of treatment, human activities and contaminated pools environment. A higher fungal count of 5x10⁶ cfu/ml - 3x10⁷ cfu/ml was reported by [29] for the swimming pools they assessed in Calabar, South-South Nigeria. The difference might be due to medium used, sample size, density of bathers, the sanitary status of the pools environment, the hygienic state of the bathers before using the pools and the source of the pools water [28]. The fungi isolated from the pools in this study where Aspergillus spp., Rhizopus spp. and Penicillium spp. These fungi can be hazardous in specific conditions especially in immunocompromised individuals and cause infections such as allergy and otomycosis [30]. In a similar study by [31], Penicillium notatum, Aspergillus niger, Rhizopus species and Aspergillus flavus were isolated from some pools in Kwara State of Nigeria. [32] also isolated Penicillium spp., Aspergillus versicolor, Rhizopus spp., Fusarium spp., Trichophyton mentagrophytes, Mucor species, Candida albicans, Aspergilluis niger and Absidae species from some swimming pools in Lagos state, Nigeria.

In recreational waters, especially swimming pool waters, Cryptosporidium and Giardia were common findings, which often caused outbreaks [33]. The study showed that no parasite was found in all of the swimming pool samples analyzed. In a similar study, [31] isolated Cryptosporiduim species and Giardia species from pool samples analyzed. Also, [34] demonstrated that swimming pools and hot tub water in Qazrin Province were contaminated with Azanthamoeba and Naegleri species. Acanthamoeba spp., Naegleria folweri and Balamuthia mandrillaris are important free-living amoeba which can cause some fatal diseases such as granolumatose amoebic encephalitis (GAE) and primary amoebic meningoencephalitis (PAM) [35]. The absence of protozoan may be due to the season and low swimming density as well as pools used mainly by adults [33]. [36] revealed that, notwithstanding the reassuring result obtained in this study, the risk of parasitic contamination of the swimming pools should not underestimated; the contamination may be underestimated by the technique and procedures employed.

The pH of the swimming pool water has a direct impact on the recreational users only at very low or very high values [37]. Under non-compliance circumstances, pH may have effects on the skin and eyes. The pH values of 5.8 – 64 for all pool samples recorded in this study shows 100% noncompliance to the WHO standard for pH (7.0 -7.8) [38]. [39] also reported a pH range of 4.48-7.70 in selected swimming pools in Osogbo metropolis, Nigeria. This low pH probably indicates excessive chlorination [39]. This may be attributed to the absence of educated pool operators, bathers not taking pre-swim showers and less frequent changing of the pool water [40]. Low pH conditions according to [41] have been associated with problems such as itching, chlorine loss, skin spots and sore in swimmers.

5. CONCLUSION

The findings of this study showed that most of the swimming pools exceeded the recommended limit for total heterotrophic bacteria especially when *Escherichia coli* was isolated in one of the pools, but fungal and protozoa counts are within limit. Also, pH was below the acceptable range for all the swimming pools. Therefore, the swimming pools can still constitute serious health problems to the bathers. This should prompt hotel owners with swimming pools to properly and routinely decontaminate their pools and carry out microbial assessment to ensure World Health Organisation standard for recreational waters.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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