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Assessment of Microbiological Quality of Outdoor Swimming Pools in Ilorin, Kwara State

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Abstract: *Swimming pools have been found to be a reservoir of different types of microorganisms and through this, many contagious infections can be contacted from these pools.*
Aim: the aim of this study is to determine the microbial quality of selected swimming pools and also the antimicrobial resistant pattern of the isolated microorganisms.
Water samples were collected from six different outdoor swimming pools and microbiological analyses using different culturing media were carried out on these samples. Standard antibiotics discs were also used to determine the antibiotic resistant pattern of the isolated microorganisms.
Results obtained from this study showed that the microbial counts for the swimming pools samples ranged from 06×10^3 *-* 98×10^3 *cfu/mL while all the samples examined were feacally contaminated. The isolated microorganisms include; P. cepacia, P. aeruginosa, E. coli, C. fruendii, B. subtilis, S. aureus, S. epidermidis, Pr. vulgaris, Shigella sp., Penicillium notatum, A. niger, Rhizopus sp. and A. flavus. The growth of the isolated organisms in different concentrations of calcium hypochlorite showed that most of the isolated organisms can survive up to 12ppm concentration but P. cepacia and B. subtilis did not show any growth even at 1ppm. All the isolated bacteria showed multi-drug antibiotics resistant pattern except P. cepacia which was only resistant to Nitrofurantoin.*
All the swimming pools examined in this study were contaminated and infections contracted from these pools might be difficult to treat due to the multi-drug antibiotics resistant pattern showed by these microorganisms.
Key words: *antibiotics, calcium hypochlorite, microorganisms, multi-drug resistant, swimming pool*

I. Introduction

Swimming pools are concrete tanks, large artificial basins or large paved holes containing water for swimming {1}. Swimming pools are used for recreational activities, rehabilitative treatment or sport. Swimming pool water should meet potable water standard by being transparent, odourless and tasteless liquid having a freezing point of 0^0C and boiling point of 100^0C {2}.

The quality of swimming pool and spa water can be affected by the transmission of infectious diseases {3}. Infections from swimming pool might be due to inadequate cleaning and / or disinfection measures most of the time {4, 5}. Swimming pool may be infected with pathogenic microorganisms entering the pool directly or indirectly through contaminated air, soil, dust, rain water, sewage, human or animal excrement and individual bather {6}. Feacal matter is introduced into the water when a person has an accidental feacal release or when residual feacal material on swimmers' bodies is washed into the pool {7}, while non-feacal human shedding (e.g from vomit, mucus, saliva or skin) in the swimming pool is also a potential source of pathogenic organisms.

Diseases that include diarrhea, typhoid fever, hepatitis and cholera have been reported from drinking of contaminated water by the swimmers {8}. Improved surveillance data from the United States and Europe have shown that microbial recreational water related illnesses are on the rise {9}.

The portability of swimming pool water is enhanced by frequently changing the water and use of disinfectants, such as chlorine, bromine and iodine. The highest possible concentration of about 1ppm should be maintained because higher chlorine concentration irritates the eyes {10}.

Microbiological evaluation has for many years, been the most significant method for sanitary and quality control of swimming pools. A test for indicator bacteria like enteric pathogenic bacteria as indicators of feacal pollution is a useful tool in identifying contaminated swimming pools {10}.But other researchers reported that the risk of infection is more associated with microorganisms derived from the skin, mouth and upper respiratory tract of bathers rather than feacal contamination {11} while others argued that microorganisms which indicate hygienic conditions {total coliform and heterotrophic bacteria} and feacal pollution are the best {12, 13}. Another important factor to consider when assessing bathing water quality is also related to the density of bathers because high density will lead to a risk of contact with different pathogens {14, 15}. It is then proposed that no single indicator microorganism is suitable, therefore feacal indicators and microorganisms from the mouth, nose and skin areas of bathers should be considered concurrently in assessing the effect of chlorination and the safety of pool water {16}.

Therefore the main purpose of this research work is to determine the microbiological qualities of six different swimming pools from Ilorin, Kwara state.

II. Materials And Methods

2.1 Study Area

The study area used in this work is Ilorin, Kwara state. Six outdoor swimming pools in the city were randomly selected for this study.

2.2 Sample Collection

Water samples were collected aseptically in triplicate from 6 different swimming pools at a depth of 200mm below the surface of the pool using sterilized wide-mouthed bottles and kept on ice pack.

Nutrient Agar, potato Dextrose Agar, MacConkey Agar and Manitol Salt Agar were used for the isolation of microorganisms present in the swimming pools by using standard microbiological methods {17, 18}. The pour plate technique was used for isolation, bacterial plates and fungal plates were incubated at 37^0C for 24 hours and 25^0C at 48 hours respectively. Pure isolates were stored and identified by microscopy and biochemical tests {19}.

2.3 Determination of the Resistant of the isolated Microorganisms to Different Concentrations of Calcium Hypochlorite

Modified Minimal salt medium {MSM} that contains different concentrations of sodium hypochlorite was prepared. These concentrations were 1ppm, 3ppm, 5ppm, 7ppm, 12ppm and 15ppm. 0.5mL of each isolate at McFarland standard was introduced into sterilized MSM containing different concentration of sodium hypochlorite and incubated at 37^0C for 24 hours and 25^0C for 48hours for bacterial and fungal isolates respectively.

2.4 Antimicrobial Susceptibility Test for Isolated Microorganisms

2.4.1 Antibacterial Test

Agar disc diffusion method was used to determine susceptibility or resistance pattern of the isolated bacteria against selected antibiotics. The test organisms from 18 hours broth culture were swabbed evenly on a prepared Nutrient Agar medium and allowed to dry for 4 hours, antibiotics discs were then placed on the already prepared plates and pressed firmly on it. The plates were incubated at 37^0C for 24 hours and zones of inhibition were measured in mm and interpreted accordingly {20}.

2.4.2 Antifungal Test

The resistance or susceptibility of the fungal isolates against standard antifungal agents, Mycoten and Nystatin (100, 200, 250 and 500mg/ml) in vitro by agar diffusion method was also determined. Lawn culture was prepared using the test organisms on Potato Dextrose Agar. The inoculated plates were kept aside for a few minutes, sterile What man's disc of 5 mm in diameter containing the different concentrations of these antifungal agents were placed at perpendicular equidistant to each other on already prepared plates. It was then incubated at room temperature for 48 hours. The activity of the extract was determined by measuring the diameters of zone of inhibition.

III. Results

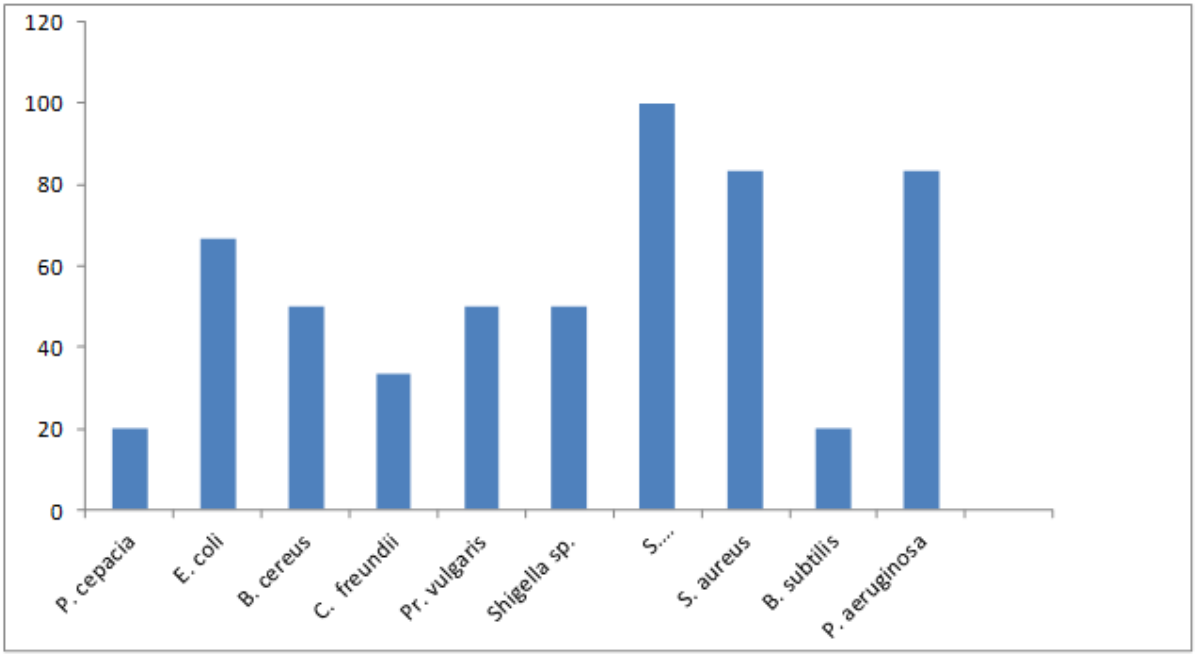
Table 1 showed the Total Microbial Counts of microorganisms present in the swimming pools samples. Total bacterial counts ranged from 14 to 68 × 10^3 cfu/mL, while the Total Fungal count is from 11 to 25 × 10^3 cfu/mL. The Total Coliforms Count showed that all the swimming pools studied were feacally contaminated with counts ranging from 06 to 16 ×10^3 cfu/mL while the Staphylococcal Count ranged from10 to 16 × 10^3 cfu/mL. The percentage of occurrence of bacterial isolates showed that *S. epidermidis* had the highest occurrence of 100%, *P. aeruginosa* and *S. aureus* had 83.3% occurrence, while *E. coli* showed 66.7%, *Pro. vulgaris*, *Shigella* sp and *B. cereus* had 50% occurrence, *Citrobacter fruendii* had 33.3% with *P. cepacia* and *B. subtilis* had the least occurrence of 20% {Fig 1}. Fig 2 showed the occurrence of fungal isolates from the swimming pools. *A. niger* and *A. flavus* had the highest occurrence of 83.3%, followed by *Rhizopus* sp with 50% occurrence and *Penicillium notatum* had the least occurrence of 33.3%.

Table 2 showed the growth of isolated microorganisms in different concentrations of calcium hypochlorite. *P. cepacia* and *B. subtilis* showed no growth in all the different concentrations of calcium hypochlorite, while *S. epidermidis, S. aureus, E. coli* and *B. cereus* showed growth in concentrations of up to 12ppm. *P. cepacia* was only resistant to Nitrofurantoin while the remaining nine bacterial isolates showed multi-antibiotics resistant pattern ranging from two {2} to five {5} {Table 3}.Table 4 showed the Antifungal Susceptibility test of the isolated fungi to two antifungal agents; Mycoten and Nystatin at different

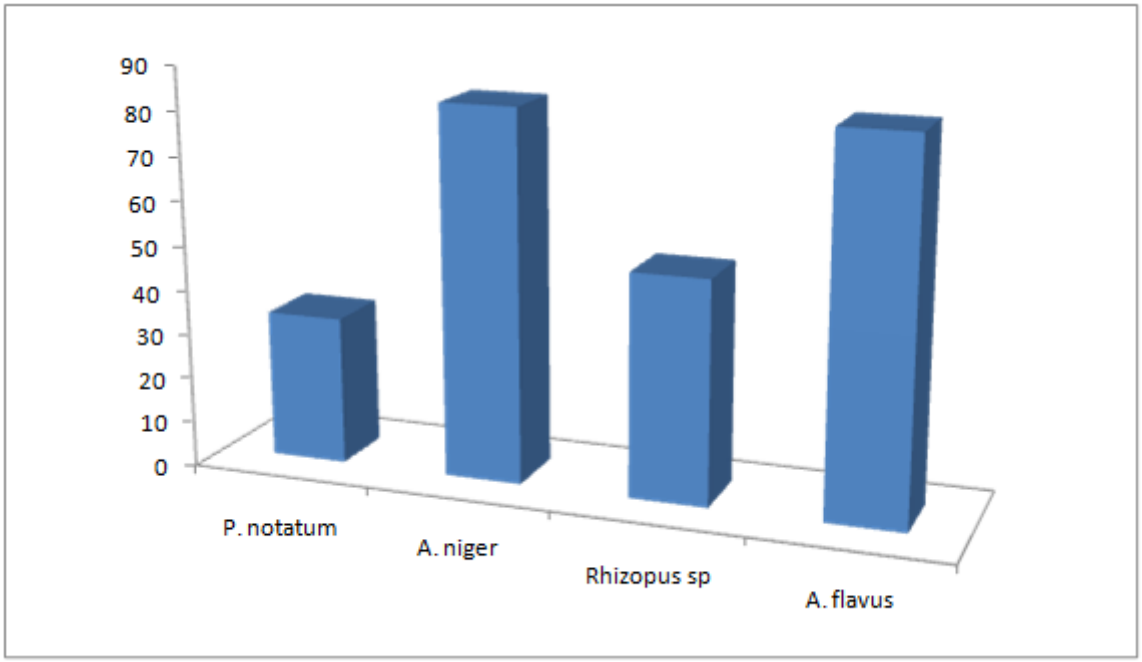
concentrations. *Penicillium notatum* was resistant to all the concentrations of Mycoten, while the three other fungi were susceptible to the Mycoten at different concentrations. All the fungal isolates were resistant to different concentrations of Nystatin except at 500mg that they were all susceptible to the antifungal agent {Table 4}.

3.1 Table 1: Total Microbial Counts of the Water Samples from the Swimming Pools

Sample	Total Bacterial Count (cfu/mL × 10^3)	Fungal Count (cfu/mL × 10^3)	Coliforms Count (cfu/mL × 10^3)	Staphylococcal Count (cfu/mL × 10^3)
1	68	12	12	10
2	47	20	10	15
3	27	25	16	21
4	16	13	06	12
5	14	11	09	15
6	22	16	12	17



3.2 Fig 1: Occurrence of bacterial isolates in the swimming pools



3.3 Fig 2: Occurrence of fungal isolates in the swimming pools

3.4 Table 2: Growth of the Isolated Microorganisms in the Different Concentrations of Calcium Hypochlorite

Organism	1ppm	3ppm	5ppm	7ppm	12ppm	15ppm
P. aeruginosa	+	+	+	+	-	-
P. cepacia	-	-	-	-	-	-
Pr. vulgaris	+	+	+	-	-	-
S. epidermidis	+	+	+	+	+	-
S. aureus	+	+	+	+	+	-
E. coli	+	+	+	+	+	-
Shigella sp.	+	+	+	+	-	-
Bacillus cereus	+	+	+	+	+	-
B. subtilis	-	-	-	-	-	-
Citrobacter freundii	+	+	+	+	-	-
Penicillium notatum	+	-	-	-	-	-
Aspergillus niger	+	+	+	-	-	-
Rhizopus sp	+	+	-	-	-	-
flavus	+	+	+	+	-	-

3.5 Table 3: Phenotypic Pattern of Antibiotics Resistant among the Isolated Bacteria

Isolate	Antibiotics Resistant Pattern
P. cepacia	NIT
E. coli	NIT AUG
Bacillus cereus	AUG CAZ
Citrobacter freudii	AUG CAZ
Pr. vulgaris	AUG CAZ GEN
Shigella sp.	NIT AUG CAZ
S. epidermidis	NIT AUG CAZ CPR
S. aureus	NIT AUG CAZ CPR
B. subtilis	NIT AUG CAZ CPR
P. aeruginosa	NIT AUG CAZ CPR GEN

KEY: NIT: Nitrofurantoin, AUG: Augmentin, CAZ: Ceptazidine, GEN: Gentamycin, CPR: Ciprofloxacin.

3.6 Table 4: Antifungal Susceptibility Test for the Fungal Isolates

Antifungal Agent	Different Concentration of Antifungal Agent 100mg	200mg	250mg	500mg	Isolate
Nystatin	R	R	R	13mm	
Mycoten	R	R	R	R	Penicillium notatum
Nystatin	R	R	R	9mm	
Mycoten	17mm	19mm	20mm	25mm	Aspergillus niger
Nystatin	R	R	R	15mm	
Mycoten	22mm	24mm	27mm	30mm	Rhizopus sp
Nystatin	R	R	R	15mm	
Mycoten	21mm	22mm	23mm	24.5mm	A. flavus

IV. Discussion

Total Viable Count (TVC) of all the pools water analyzed is relatively high. This high TVC might be due to the low residual chlorine level or because the microorganisms have become resistant to the calcium hypochlorite that is used in treating those swimming pools, similar reports were also made by {9, 21, 22}. The results obtained in this work with bacterial count of 14 to 68 × 10^3cfu/mL showed that the swimming pools did not meet the WHO standard that has been accepted by Nigeria. The presence of Coliforms in those swimming pools showed that there is deficiency in the treatment of the swimming pools or in adequate protection of the swimming pool {10}. Though Nigeria does not have a particular standard yet but {23} reported that heterotrophic plate count should not exceed 200 cfu/mL and total coliform count should not be greater than 50 cfu/mL. Therefore those swimming pools greatly deviated from the accepted standards from some countries.

The isolation of different species of bacteria and fungi which are known human pathogens from these pools might be due to feacal contamination from both humans and animals {22, 24}. *Pseudomonas* species are associated with surface run-off water, while *E. coli, S. epidermidis* and *S. aureus* are usually contributed by

bathers in the swimming pools {10}. Most of these bacterial isolates are known enterotoxin producers when ingested into the body, therefore the presence of these bacteria in pools is a threat to public health {25}. The presence of *E. coli, Shigella sp* and *C. fruendii* is a strong indication of feacal contamination {26}. It has also been reported by some workers that *E. coli* should be totally absent in 100ml of pool water {27}. The presence of these organisms may constitute a public health hazard because swimmers can accidentally swallow contaminated pool water during swimming which can result in outbreaks of diseases like cholera, shigellosis, typhoid fever, gastroenteritis and diarrhea {9, 28}.

Some fungal pathogens of humans that include *Penicillium* and *Aspergillus* species were encountered in this work. The presence of *Aspergillus, Penicillium* and *Rhizopus* were also detected from swimming pools water in Greece {27}.*Aspergillus niger* has been reported to cause aspergillosis which is an infection of the external ear {29}. *Aspergillus* has also been reported as a starting point for dissemination of infections in immune compromised patients {30}. The antibiotic resistance of bacterial strains isolated from recreational waters, like swimming pools, lake etc. have been reported by many workers {31, 32}.*P. aeruginosa* showed the highest multidrug resistant in this study and several researches have shown that this organism is naturally resistant to many antibiotics because of their relatively impermeable membrane, constitutively expressed and inducible efflux systems and a chromosomally encoded inducible β lactame {33}. High multi drug resistance in *E. coli* had also been reported {34}. Many of the organisms isolated from this work were able to tolerate high concentration of calcium hypochlorite. High elevated microbial resistance to antiseptics and disinfectants has been reported from public swimming pools {35}. *Acinetobacter baumannii* was able to tolerate all the concentrations, up to 4ppm of chlorine used in their work {36}, *E. coli* isolated from a chlorine treated swimming pool were found to be resistant to chlorine for up to nine passages {37}. High tolerance of bacteria to disinfectants could either be intrinsic or resulting from mutations {38}. Additionally, wide spread use of disinfectants has been reported to trigger the selection of resistant strains {38}. The high resistant to calcium hypochlorite and possession of multi drug resistant by many of these microorganisms might be due to many intrinsic factors. In addition, the surviving chlorine tolerant bacteria might also be antibiotic resistant {39, 40}.

V. Conclusion

The results obtained in this study showed that swimming pools can constitute serious problems to the bathers due to high microbial counts, resistant of those isolated microorganisms to calcium hypochlorite commonly used for treatment of the swimming pools and also to commonly available antibiotics. Therefore, there is need for the owners of swimming pools to take proper care of their pools. This study is limited to few samples because many of the hotels managers refused to give us permission to collect samples from their swimming pools.

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