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Evaluation of the Bacteriological Quality of Outdoor Public Swimming Pools in Awka, Anambra State, Nigeria

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Abstract: Fifteen outdoor public swimming pools in Awka, Nigeria were assessed bacteriologically before and after use by bathers to determine their suitability for bathing purposes. The total bacterial, total coliform, faecal coliform, Staphylococcal and Pseudomonas counts were carried out using standard methods. The total bacterial count before and after use respectively was 10-160 cfu/ml and 100-280 cfu/ml; total coliform count, 3-87cfu/100ml and 40-120 cfu/100ml; Staphylococcal count, 0-70 cfu/ml and 0-169 cfu/ml while faecal coliforms and Pseudomonas were not detected in the samples. The bacteria were identified as *klebsiella pneumoniae, Proteus mirabilis, klebsiella oxytoca, Enterobacter cloacae, Citrobacter freundii, Salmonella typhi, Bacillus licheniformis Staphylococcus aureus* and *Staphylococcus epidermidis*. *Klebsiella pneumoniae* was detected in majority of the samples before and after use while *Bacillus licheniformis, Citrobacter freundii and Salmonella typhi* were each detected in one sample only before and after use. *Klebsiella pneumoniae* also had the highest occurence of 25.0% and 17.1% before and after use while *Bacillus licheniformis* had the lowest occurrence of 4.8% and 7.3% before and after use. None of the pools met the World Health Organization standard for coliforms therefore adequate and frequent treatment as well as regular bacteriological analyses of such pools are recommended.

Keywords: Bacteriological, Evaluation, Swimming Pools, Outdoor, Quality

1. Introduction

The first step in providing a safe swimming environment is to provide healthy swimming pools as well as a swimming pool environment free from bacteria. This type of policy protects the swimmers and prevents them from contracting communicable infectious diseases. Research has shown that nearly all swimming pools face the risk of harboring microorganisms which are harmful to human health [1]. The majority of people attend swimming facilities for recreational activities, rehabilitative treatment or sport. In recent years, there have been many reported cases of infectious diseases caused by the in advertent swallowing of swimming pool water that was contaminated with bacteria while swimming [2]. A variety of microorganisms can be found in swimming pools which may be introduced in the pool water in a number of ways. In many cases, the risk of illness has been linked to faecal contamination of the water due to faeces released by bathers or contaminated source water or in outdoor pools may be the result of direct animal contamination [3]. Faecal matter is introduced into the water when a person has an accidental faecal release or when faecal material on swimmers bodies is washed into the pool [4]. Non-faecal human shedding in the swimming pools is also a potential source of pathogenic organism. Bacteria can also be shed from users and transmitted through contaminated water. Some bacteria, most notably non- faecally derived bacteria may accumulate biofilms and present an infection hazard. In addition, certain free living aquatic bacteria can grow in pool waters, in pools components or facilities or other wet surfaces within the facility to a point at which some of them may cause a variety of respiratory, dermal or central nervous system infections or diseases [3].

Swimming pools are often associated with out breaks of waterborne infections. The infectious agents recovered from

swimming pools water include a variety of pathogens including bacteria [5, 6]. In addition to infections due to acknowledged pathogens, infections due to opportunistic microorganisms such as *Mycobacterium fortuitum, Mycobacterium chelonei* and *Mycobacterium marinum* are reported too which are able to cause mild to severe disease in immuno competent and immuno depressed individuals [7, 8]. Most of the waterborne outbreaks of gastrointestinal diseases such as Salmonellosis have been associated with recreational exposure [9]. Approximately, 40% of the microorganism isolates from swimming pools are Bacillus which is due to the fact that they contain spores which are resistant to disinfecting substances. in the majority of cases, Bacillus subtilis is considered to be a non-pathogen but due to its dominant flora, under some special circumstances, it is thought to be the cause of conjunctivitis, meningitis, pneumonia and septicemia [10]. Pseudomonas is resistant to sodium hypochlorite that is used for disinfecting pools and it is considered to be an opportunistic microorganism that is involved in urinary tract infections, wound infections, sepsis and bed sores [10]. Despite the fact that this bacterium is not considered a major threat and plays no direct role in producing infections in humans, should not be ignored when checking the water quality of swimming pools [1]. Microbiological evaluation has for many years been the most significant method for sanitary and quality control of swimming pools. For effective quality control, a test for indicator bacteria is usually of primary importance. As indicators of faecal pollution, their presence is a strong indication of the presence of enteric pathogenic bacteria such as *Salmonella typhi, Salmonella paratyphi, Shigella dysenteriae* and *Vibrio cholerae* in the pool. Skin tuberculosis caused by *Mycobacterium baliteri* has been reported after swimmer had bathed in waters from which a large amount of microorganisms was found [11].

The principal indicator bacteria are faecal coliforms, total coliforms, *Enterococcus faecalis* and *Clostridium perfringens*. Although, bathing places are traditionally examined by total plate count and a coliform test, they do not provide specific information regarding Staphylococcus aureus and Pseudomonas aeruginosa both of which are more resistant to disinfection [11]. Adequate knowledge of the bacteriological quality of swimming pools is imperative to guide their safety for use, minimize the spread of contagious diseases and determine the effectiveness of the pools treatment processes, therefore in this study, the bacteriological quality of outdoor public swimming pools in Awka, Nigeria was assessed.

2. Materials and Methods

2.1. Sample Collection

Samples were collected between January and February, 2016. From fifteen privately-owned hotels Awka, Anambra State in sterile containers in the morning before use and in the evening after use by bathers. The samples were labeled A-O and conveyed to the microbiology laboratory of Nnamdi Azikiwe University on ice pack. Bacteriological analyses were carried out within 24 hours of collection. between January and February, 2016.

2.2. Heterotrophic Plate Count

Nutrient agar was weighed and prepared based on the manufacturer's instruction. It was sterilized in an autolave at 121°C for fifteen minutes, allowed to cool to 45°C and aseptically dispensed into Petri dishes. 0.1ml of each water sample was collected using a sterile pipette and dropped on the center of the nutrient agar plates. A sterile glass rod sterilized by dipping in ethanol and flaming was used to spread the sample evenly. The plates were incubated for 24 hours at 37°C and the colonies that grew were counted. Each water sample was cultivated in duplicates and the average colony count recorded.

2.3. Total Coliform Count

MacConkey agar was prepared based on the manufacturer's instruction. It was sterilized by autoclaving at 121°C for fifteen minutes and dispensed aseptically into sterile petri dishes. One hundred milliliters of each sample were aseptically passed through a membrane filter and the filter paper aseptically transferred to the medium using sterile forceps. The medium was incubated in an inverted position at 37°C for 24 hours and the pink colonies of coliforms were recorded.

2.4. Faecal Coliform Count

Eosin methylene blue agar was weighed and prepared according to the manufacturer's instruction. It was sterilized by autoclaving at 121°C for fifteen minutes, allowed to cool to 45°C and aseptically dispensed into sterile petri dishes. 100 ml of each water sample was passed through a membrane filter and thereafter transferred aseptically on the surface of the solidified medium. The dishes were incubated in an inverted position at 37°C for 24 hours and the colonies of faecal coliforms with their greenish metallic sheen were recorded.

2.5. Staphylococcal Count

The spread plate method was used. Mannitol salt agar was weighed, prepared according to the manufacturer's instruction and sterilized by autoclaving at 121°C for fifteen minutes. It was allowed to cool to 45°C and introduced into sterile petri dishes. 0.1ml of each water sample was introduced into the petri dishes and evenly spread with a sterile glass rod. Incubation was carried out in an inverted position at 37°C for 24 hours after which the colonies that developed were counted. Each sample was cultured in duplicates and the average colony count was recorded.

2.6. Pseudomonas Count

Cetrimide agar was prepared according to the

manufacturer's instruction and sterilized by autoclaving at 121°C for fifteen minutes. The medium was introduced into sterile Petri dishes and allowed to cool. Upon cooling, 0.1ml of each water sample was introduced into the dishes and evenly spread with a sterile glass rod. The Petri dishes were incubated in an inverted position at 37°C for 24 hours and the colonies that developed were counted. Each sample was cultured in duplicates and the average number of colonies was recorded.

2.7. Characterization and Identification of the Isolates

The bacterial isolates were characterized based on colonial morphology such as the colour, elevation and margin, cellular morphology such as shape of cell, arrangement of cell and gram reaction and biochemical characteristics such as indole, methyl red, voges proskaeur, citrate utilization, motility, catalase, coagulate, carbohydrate assimilation, oxidase, urease and spore stain tests.

2.8. Analysis of Data

The data obtained before and after use were subjected to correlation analysis using t- distribution.

3. Results

The bacteriological quality of the swimming pools before use is shown in Table 1. The heterotrophic plate count ranged between 10 and 160 cfu/ml; total coliform, 3-87 cfu/100ml; Staphylococcal count, 0-70 cfu/ml while faecal coliform bacteria and Pseudomonas were not detected in any of the pools. The bacteriological quality of the swimming pools after use is presented in Table 2. The heterotrophic plate count was 107-208 cfu/ml; total coliform count, 38-120 cfu/100ml; Staphylococcal count, 0-169 cfu/ml while the faecal coliforms and Pseudomonas were not isolated from the samples. The morphological and biochemical characteristics of the bacterial isolates are shown in Table 3. Organisms identified include *klebsiella pneumoniae, Proteus mirabilis, klebsiella oxytoca, Enterobacter cloacae, Citrobacter freundii, Salmonella typhi, Bacillus licheniformis, Staphylococcus aureus* and *Staphylococcus epidermidis*.

The number of swimming pools with the bacterial isolates before and after use is presented in Table 4. *Klesbiella pneumoniae* was detected in eleven (73.33%) of the pools while *Citrobacter freundii*, *Salmonella typhi* and *Bacillus Licheniformis* respectively were detected in one pool (6.67%) only before use while *Klebsiella pneumoniae* was isolated from twelve (80.00%) of the pools after use. In addition, *Citrobacter freundii, Salmonella typhi* and *Bacillus Licheniformis* respectively were isolated from one pool (6.67%) only. The distribution of the bacterial isolates in the swimming pools before and after use is shown in Table 5. *Klebsiella pneumoniae* occurred most frequently in the pools before and after use while *bacillus liheniformis* had the least occurrence in the pools before and after use.

Table 1. *Bacteriological quality of the swimming pools before use.*

Pool	Heterotrophic plate count (cfu/ml)	Total coliform (cfu/100ml)	Fiscal coliform count (cfu/100ml)	Staphylocci count (cfu/ml)	Pseudommas count (cfu/ml)
A	160	76	ND	ND	ND
B	90	39	ND	ND	ND
C	14	6	ND	ND	ND
D	41	30	ND	ND	ND
E	50	34	ND	ND	ND
F	110	78	ND	ND	ND
G	120	87	ND	ND	ND
H	131	65	ND	ND	ND
I	70	45	ND	ND	ND
J	50	38	ND	ND	ND
K	138	62	ND	ND	ND
L	10	3	ND	5	ND
M	80	40	ND	30	ND
N	18	10	ND	6	ND
O	140	70	ND	50	ND
WHO standard	100	10	0	30	0

ND = Not Detected

Table 2. *Bacteriological quality of the swimming pools after use.*

Pool	Heterotrophic plate count (cfu/ml)	Total coliform (cfu/100ml)	Fiscal coliform count (cfu/100ml)	Staphylocci count (cfu/ml)	Pseudommas count (cfu/ml)
A	280	780	ND	169	ND
B	225	100	ND	ND	ND
C	142	40	ND	68	ND
D	100	38	ND	51	ND
E	174	60	ND	80	ND
F	203	85	ND	ND	ND
G	163	99	ND	ND	ND

Pool	Heterotrophic plate count (cfu/ml)	Total coliform (cfu/100ml)	Fiscal coliform count (cfu/100ml)	Staphylocci count (cfu/ml)	Pseudommas count (cfu/ml)
H	226	83	ND	ND	ND
I	145	51	ND	90	ND
J	180	75	ND	ND	ND
K	200	74	ND	ND	ND
L	140	50	ND	85	ND
M	182	60	ND	113	ND
N	107	80	ND	24	ND
O	202	120		73	
WHO standard	100	10	0	30	0

ND = Not Detected

Table 3. *The morphological and biochemical characteristics of the bacterial isolates are shown.*

Isolate	Morphology	Gram reaction	Indole test	Mathyl rod test	Voges Proskaeur test
A	ROD	-	-	+	-
B	ROD	-	-	+	-
C	ROD	-	+	-	+
D	ROD	-	-	-	+
E	ROD	-	-	+	-
F	ROD	-	-	+	-
G	ROD	+	-	-	-
H	COCCUS	+	-	-	-
I	COCCUS	+	-	-	-

Table 3. *Continue.*

Isolate	Urine test	Motolity test	Glucose formation	Sucrose formation	Maltrose formation	Raffine formation
A	+	-	AG	AG	A	A
B	+	+	AG	AG	A	-
C	+	-	AG	AG	AG	A
D	+	+	AG	AG	A	A
E	+	-	AG	AG	A	AG
F	-	+	AG	-	-	-
G	-	-	-	-	-	-
H	-	-	-	-	-	-
I	-	-	-	-	-	-

Table 3. *Continue.*

Isolate	Lactose formation	Coagulase test	Citale utilization	Spre test	Ceta lacen	Identify
A	AG	-	+	-	-	*Klabsiella Preumminae*
B	-	-	+	-	-	*Proteus Mirabilis*
C	AG	-	+	-	-	*Klebsiella Oxytoca*
D	AG	-	+	-	-	*Enterrobacter Cloacae*
E	AG	-	+	-	-	*Citrabacter Freundii*
F	-	-	+	-	-	*Salmonella Typhi*
G	-	-	-	+	+	*Bacillus Lincheiformis*
H	-	+	-	-	+	*Staphylacoccus Aureus*
I	-	-	-	-	+	*Staphyloccus Epidermidis*

Table 4. *Number of swimming pools with the bacterial isolate before and after use.*

Bacterial Isolates	Number of pools with the isolate before use (%)	Number of pools with the isolate after use (%)
Klebsiella prenmamiac	11 (73.33)	12 (80.00)
Proteus miralilis	3 (20.00)	4 (26.67)
Klebsiella oxytoca	2 (13.33)	3 (20.00)
Enterobacter cloacae	2 (13.33)	3 (20.00)
Salmonella typhi	1 (6.67)	1 (6.67)
Bacillus licheniformis	1 (6.67)	1 (6.67)
Staphylococcus aureus	1 (6.67)	1 (6.67)
Staphylococcus epidermidis	6 (40.00)	9 (6.00)
Citrrobacter freundii	2 (13.33)	3 (20.00)

Table 5. *Distribution the bacterial isolate in the swimming pools before and after use.*

Bacterial Isolates	Distribution before use (%)	Distribution after use (%)
Klebsiella prenmamiac	305 (25.0)	456 (17.1)
Proteus miralilis	238 (19.5)	423 (15.9)
Klebsiella oxytoca	105 (12.3)	220 (8.2)
Enterobacter cloacae	136 (11.1)	213 (8.0)
Salmonella typhi	67 (5.5)	203 (7.6)
Bacillus licheniformis	59 (4.8)	195 (7.3)
Staphylococcus aureus	75 (6.1)	435 (16.3)
Staphylococcus epidermidis	100 (8.2)	318 (11.9)
Citrrobacter freundii	92 (7.5)	206 (7.7)
Total	1222 (100.0)	2669 (100.0)

4. Discussion

The outdoor pools assessed were tiled, located in privately-owned hotels with the volume of the water ranging between 54m^3 and 475m^3. The water sources came from the respective hotels borehole facilities while the average number of bathers per day ranged between 2 and 30. The bacteriological assessment showed that the pools were contaminated with bacteria before use (Table 1). This could be as a result of poor pool treatment, infrequent changing of the pools water and dirty pools environment. Sixty percent of the pools assessed met the WHO standard of less than 100 cfu/ml in terms of heterotrophic bacteria. 20% met the standard in terms of total coliforms per 100ml; 86.7% in terms of Staphylococci; per 100ml while all met the WHO standard of zero faecal coliforms and Pseudomonas before use. This result agreed with the observation of Onajobi *et al* [12] who observed appreciable number of heterotrophic bacteria and coliforms in the pools they assessed before use.

The assessment showed that the heterotrophic bacterial count, total coliform count and Staphylococcal count were higher after use (Table 2) while faecal coliforms and pseudomonas aeruginosa were also not detected. This result could be attributed to the increased temperature, the shedding of bacteria through the sweat, saliva, urine and sputum of the bathers which are freely introduced into the pools as well as the multiplication of such bacteria with time. This result is also in agreement with the observation of Onajobi et al [12]. 6.7%, and 46.7% met the WHO standard in terms of heterotrophic bacteria and Staphylococci respectively while none met the standard in terms of faecal coliforms, total coliforms and Pseudomonas after use.

The bacterial isolates from the pools before and after use were *klebsiella pneumoniae, Proteus mirabilis, klebsiella oxytoca, Enterobacter cloacae, Citrobacter freundii, salmonella typhi, Bacillus licheniformis, Staphylococcus aureus* and *Staphylococcus epidermidis.* Anyim et al [13] isolated *klebsiella sp, Salmonella typhi* and *Staphylococcus aureus* from the water used for drinking and swimming purposes in Ishiagu community, Ebonyi state. Anake et al [14] detected total coliforms, Proteus sp and Salmonella sp in the different water sources they assessed in Ota, Ogun state Nigeria. Itah and Ekpombok [11] isolated *Staphylococcus aureus* and *Staphylococcus epidermidis* and total coliforms from the swimming pools in the south-south zone of south-eastern Nigeria. The presence of coliforms in the swimming pools indicated inadequate treatment of such pools, contamination of the source of the pools water as well as contamination by bathers. None of the pools met the WHO standard of zero coliforms per 100ml of water. *Klebsiella pneumoniae* was detected in majority of the swimming pools before and after use. The bacterium also occurred most frequently in the pools before and after use (Table 5). The heterotrophic plate count, total coliform count and Staphylococcal count before and after use were significant at 5% significance level using t-distribution. Since these bacteria isolated from the pools assessed are known pathogens, adequate treatment measures must be put in place to safeguard the health of the bathers.

5. Conclusion

Most of the pools did not comply with the World Health Organization standard for swimming pools with regards to the total coliforms, therefore maintenance of the free residual chlorine standard is imperative. Frequent changing of the pools water must be undertaken. In addition, bathers should be advised to take their both before entering the pools. In addition, the sanitary conditions of the pools surroundings must be improved.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

Onuorah samuel, Ginika-Osuorji Joy and Odibo Frederick particularly designed the study, carried out the laboratory work and penned down the results and discussion and Ojiagu C gathered research literature. All authors read and approved the final manuscript.

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