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BACTERIAL ASSOCIATED WITH SWIMMING POOL WATER IN BENIN CITY, NIGERIA

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ABSTRACT

Swimming pools are man-made recreational water bodies. These water bodies are supposed to be hygienic but due to human activities, they have been considered as sources of infections. The objective of this study was to ascertain the bacterial isolates found in some swimming pools in Benin City. Five swimming pools in Benin City identified as A, B, C, D and E were studied. A total of 15 samples (three from each pool) were collected in duplicates before and after the maximum bather's load, and after the water change. Samples were analysed bacteriologically using standard methods. A total of 51 organisms were isolated with *Staphylococcus epidermidis* having a prevalence of 27.5 %/, *Escherichia coli*; 15.7 %, *Staphylococcus aureus*; 15.7 %, *Enterobacter aerugenes*; 13.7 %, *Klebsiella aerogenes*; 13.7 %, *Pseudomonas aeruginosa;* 5.9 %, *Klebsiella pneumonia*; 3.2% and *Streptococcus faecalis;* 3.2%. The mean viable colony counts of 1.60x 10^2 /ml, 7.33 x 10^2 /ml, 9.00 x 10^2 /ml, 1.60 x 10^2 /ml and 7.30 x 10^2 /ml at 37 ^oC were obtained from A, B, C, D and E pools respectively. It was observed that water from swimming pools B, C and E were contaminated with mean viable counts exceeding the recommended 200 colony counts/ml and detection of *Escherichia coli* in 100 ml. The high bacterial load and the isolation of pathogenic bacteria from the pools demonstrate the need for pool health authorities to improve surveillance, improve pool decontamination standards, and educate swimmers on hygiene before entering pools. This study emphasis the need for proper hygienic maintenance of swimming pools and the need for a bacteriological standard to be drawn up for swimming pools in Nigeria.

Keywords: *Bacteriological Standards, Microorganism, Swimming pools, Water*

INTRODUCTION

Recreation has a substantial role in the life of an ever-increasing number of citizens in the world, and when choosing the scene for it, people tend to couple it with water. With evolving and advancing civilization, man-made water recreational environments are on the boom by offering health promotion and social benefits accompanied by increasing comfort and sophisticated services but also presenting to a certain extent health risk of physical, microbiological, or chemical nature. Recreational waters are a source of microbial infections such as acute gastrointestinal, cutaneous as well as respiratory illnesses (World Health Organization, 2006). Cryptosporidium, noroviruses and enteropathogenic *Escherichia coli* strains are the most important causes of diarrhoea, while *Pseudomonas* and *Staphylococcus aureus* are the main causes of cutaneous infections (Doménech-Sánchez *et al*., 2008). These organisms are partly protected from the action of chlorine in the water; however chlorine-resistant bacteria were the leading cause of pool-related infectious outbreaks between 2000 and 2014 and hotels were the most frequent sites where outbreaks occurred (Hlavsa *et al*., 2018). Several studies have been conducted on quality controls of swimming pools and their microbial load.

Although it is known that swimming pool water should meet potable water standards by being clean, and transparent, in developing countries, there are no maintained standards or regulatory frameworks to ensure that swimming pools are not hubs for pathogens.

Water, in general, is a recognized vehicle for the transmission of pathogens even when the water looks clear and uncontaminated. Apart from the classical water-borne pathogens, there are other intestinal bacteria such as *Clostridium* spp. and faecal streptococci (Wade *et al*., 2003). The role of pool water as a potential common source of infections includes acute otitis external, Trachoma Mollusca contagiosum (a viral disease), foot infection with Trichophyton mentagraphytes var interdigitale; leptospirosis infection, viral pharyngeal conjunctiva fever as well as pharyngitis caused by an adenovirus amongst other diseases. (Bonadonna and la Rosa, 2019; Cohen, 2020).

The risk of infection in swimming pools as man-made water recreational environments can be reduced to a minimum by running the recreation facilities with the application of informed risk management measures (World Health Organization, 2006). However, there is a need to examine the pools on a routine basis. Hence, this study aimed at investigating the bacterial isolates associated with swimming pools and identify if swimming pools can be a vehicle for the transmission of infections in our study locality.

STUDY AREA

Site Selection and Sample Size

A cross-sectional study was carried out on five outdoor swimming pools in Benin City between June and July 2021 with a goal-directed sampling strategy employed. The swimming pools were labelled A, B, C, D and E. The samples were examined to show the microbiological quality of the swimming pool water before and after maximum bathers' load and after the pool water change.

Sample Collection, Storage, and Transportation

A total of fifteen samples were collected in sterile well-labelled 500ml wide-mouthed bottles. Water samples were collected, when no bathers were in the pools (access was denied during the maximum bather density). The dichlorination of water samples was done using sodium thiosulphate ($Na_2S_20_3$) (100 mg/l) and Sodium thiosulphate pentahydrate by adding to each bottle. The samples were transported to the laboratory at 4^{o}C in an insulated cooler. The samples were processed within an hour of the collection based on the method of Ibe and Okplenye (2008).

Laboratory Analysis

The presumptive coliform count was done using the multiple tube fermentation method for total coliform. A varying quantities of water (50 ml, 10 ml, and 1 ml) were added to equivalent volumes of Double and single strength MacConkey broth. The initial set of three tubes had 10ml of each double-strength broth with the second and third sets having 10ml single strength broth with each containing Durham's tube before sterilization was done to show gas production at 37^{o}C ± 0.5^{o}C for 24hrs (Uhuo *et al*., 2014). Bottles showing acid and gas production were recorded as positive and the most probable number (MPN) of coliform /100ml read off from the MacCrady probability table. Known positive (*Escherichia coli*) and negative (*Alcaligenes faecalis*) controls were put up.

Coliform Test: Coliform tests were carried out by transferring a loopful from a positive presumptive test to sterile Brilliant green lactose bile broth fermentation tubes as a confirmation test. Tubes were incubated at 37 ± 0.5°C for 24hrs – 48 hrs for the total coliforms and 44.5^{o}C for faecal coliforms. The formation of gas in any amount in the inverted vial of the brilliant green lactose bile broth fermentation tube at any time within 48 ± 3 hrs constitutes a positive coliform test.

The Completed Test: The completed test was carried out by streaking a loopful from the positive tube onto Eosin Methylene Blue (EMB) agar plates from each tube of brilliant green lactose bile broth showing gas. The plates were incubated at 37°C for 24 – 48hrs. Colonies developing on EMB agar were further identified as faecal coliforms.

Differential coliform Count: This was done to ascertain whether the coliform detected in the presumptive test was *Escherichia coli*. This was done by Eijkman test for faecal coliform described by Cowan and Steel, (1994). Tubes showing acid and gas production at 44^{o}C ± 0.5oC were recorded as positive for *Escherichia coli* and its probable number was read off the MacCrandy Probability Table.

Screening for Salmonella Species: 1ml of water sample was incubated into 10ml of sterile Selenite F broth and incubated at 37oC for 24hrs. Subcultures were made onto sterile MacConkey Agar and Deoxycholate citrate agar and incubated at 37oC overnight. Non-lactose fermenting colonies were investigated biochemically. Examination for *Streptococcus faecalis* subcultures of bottles showing acid or acid and gas fermentation in the presumptive coliform tests were made into tubes containing 5ml of sterile sodium azide medium (Hannay and Norton, 1947).

Characterization of Isolates

Stock cultures of the isolate were identified by standard techniques as described by Cowan and Steel (1994) for identification of medical bacteria. This includes colonial appearances on the media, morphologic characteristics and specific biochemical tests were performed for further identification of the organisms. Gram's staining technique was also done from the colonies to act as a guide for identification.

RESULTS

Table 1 shows the distribution of organisms isolated from the pools with *Staphylococcus epidermidis* having a prevalence of 27.5 %, *Escherichia coli;* 15.7 %, *Staphylococcus aureu;s* 15.7 %, *Enterobacter aerugenes*; 13.7 %, *Klebsiella aerogenes*; 13.7 %, *Pseudomonas aeruginosa;* 5.9 %, *Klebsiella pneumonia*; 3.2 % and *Streptococcus faecalis;* 3.2 %. Table 2 presents the mean viable count of the pools represented as colony forming units (cfu/ml). The results show that pool C had the highest count (900 cfu/ml), followed by pools B (733 cfu/ml) and E (730 cfu/ml). Pool A and D, are observed to be low in mean viable count of bacteria 160 cfu/ml respectively.

Table 3 shows the average presumptive and differential coliform counts obtained from five swimming pools. From the presumptive coliform count for the estimation of the most probable number of coliform organisms present in 100ml of the sample it was observed that more coliform organisms were introduced into the swimming pools during maximum bathers load. The differential coliform counts showed that B, C and E swimming pools had *Escherichia coli*.

Table 4 shows the prevalence of each of the isolates in each pool and it was observed that *Staphylococcus epidermidis* and *Klebsiella aerugenes* were isolated from the five swimming pools studied and *Pseudomonas aeruginosa* and *Streptococcus faecalis* were each isolated from only one out of the five swimming pools. While Table 5 shows the number of isolates from each swimming pool with C swimming pool having 7 (87.5 %), B has 6 (75.0 %) and closely followed by E 5 (62.5 %).

Table 1: Bacterial isolates and their percentage occurrence

Isolates	Number	Percentage %
Staphylococcus epidermidis	14	27.5
Escherichia coli	8	15.7
Staphylococcus aureus	8	15.7
Klebsiella aerogenes	14	27.7
Pseudomonas aeruginosa	3	5.9
Klebsiella pneumonia	2	3.9
Streptococcus faecalis	2	3.9
TOTAL	**51**	**100%**

Table 2: Mean viable counts of bacteria in the different swimming pools

Swimming Pool	Total bacterial counts			
	Before Heavy Bathers Load (cfu/ml) (10^2)	After Heavy Bathers Load (cfu/ml) (10^2)	After Water Change (cfu/ml) (10^2)	Mean of three Samples (cfu/ml) (10^2)
A	1.50	2.00	1.30	1.60
B	6.00	8.50	7.50	7.33
C	9.00	9.20	8.70	9.00
D	1.80	2.00	1.00	1.60
E	6.00	8.90	7.00	7.30

Table 3: The average presumptive and differential coliform counts obtained from five swimming pools.

Water Sources	Presumptive Coliform Count			Differential Coliform Count		
	Before Heavy Bathers Load	After Heavy Bathers Load	After Water Change	Before Heavy Bathers Load	After Heavy Bathers Load	After Water Change
A	1	2	0	0	0	0
B	6	7	6	2	3	3
C	5	7	7	2	3	3
D	0	1	0	0	0	0
E	3	4	3	0	2	2

Table 4: Prevalence of bacterial isolates in each pool.

Isolate	Sources of Bacterial Isolates				
Staphylococcus epidermidis	A	B	C	D	E
Escherichia coli	C	B	E	-	-
Staphylococcus aureus	A	B	C	E	-
Enterobacter aeruginosa	C	D	E	-	-
Klebsiella aerugenes	A	B	C	E	D
Pseudomonas aeruginosa	C	-	-	-	-
Klebsiella pneumonia	B	C	-	-	-
Streptococcus faecalis	B	-	-	-	-

Table 5: Bacterial isolates from swimming pools studied

Swimming Pools	Number of Isolates	Percentage %
C	7	87.5
B	6	75
E	5	62.5
A	3	37.5
D	3	37.5

DISCUSSION AND CONCLUSION

Swimming pools are man-made recreational water bodies found in private homes, recreational centres or in hospitality industries. People patronize these centres for leisure and recreational purposes, especially during the festive period. This has resulted in increased usage which has given rise to some reported infections of the users (Amalu *et al*., 2019). This study sought to examine some swimming pools to ascertain the bacteria associated with them if there are any.

The result of this study shows a 27.5 % prevalence of both *Staphylococcus epidermis* and *Klebsiella aerogenes*. This is in tandem with an earlier report by Aleru *et al*. (2021) who did similar work in Port Harcourt and Obiakpor Local government area of Rivers State, Nigeria. This implies that the water bodies lack maintenance by the operatives, and this would be a matter of public health significance. Coagulase-negative staphylococci, especially *Staphylococcus epidermidis*, have long been known as a major source of health-care-associated infections. Moreover, *Staphylococcus epidermidis* is a frequent contaminant in clinical samples, rendering diagnosis difficult. *Staphylococcus epidermidis* was the most prevalent isolate (27.5 %), as earlier reported by Widerström, (2016).

This study observed a mean viable microbial count from B (7.33×10^2 cfu/ml) C (9.0×10^2 cfu/ml) and E swimming pools (7.30×10^2 cfu/ml) that exceed the recommended values (for microorganisms by bacteriological standards) for swimming pools which are 200 cfu/ml indicating that they were contaminated.

Using the most probable number presumptive and differential coliform count as an index of pollution it is observed from Table 3 that B and C swimming pools were polluted with *Escherichia coli* and also using faecal streptococci as an index of water pollution it is observed that B and C swimming pools were polluted with *Streptococcus faecalis*. Enumeration of the coliform of microbial aquatic eco-system has been universally applied to document the sanitary quality of any water. Coliforms are defined as aerobic Gram-negative, oxidase-negative non-sporulating and rod-shaped bacteria that are capable of growing in the presence of bile salts and fermenting lactose with gas formation within 48 hours. The coliform bacteria constitute a large part of the normal intestinal flora especially *Escherichia coli* within the intestine most of the strains generally do not cause disease and may even contribute to the normal functions of faecal pollution. The American Society of Public Health stated that coliform standards are a major public health factor in judging the sanitary quality of swimming pools (Payus *et al*., 2018). Motlagh and Yang (2019) in their study stated that the presence of

coliforms and faecal streptococci suggests faecal contamination of the pool while the usefulness of the total microbial count as an index of bacteria water pollution has been questioned partly because of coliform detected or coliform suppression on presumptive media.

The study found that swimming pools with heavy bather loads had higher levels of viable microorganisms, including coliform bacilli. The results also showed that swimming pools B and C had higher levels of these microorganisms compared to pools A and D. These findings agree with previous studies conducted by Elmar *et al*. (2007) and Petterson *et al*. (2021), which also found increased levels of microorganisms after heavy bather loads.

The prevalence of *Pseudomonas aeruginosa* a pathogen frequently found in the case of otitis externa is very low in this study but this may, however, turn into a problem in a swimming pool environment if proper maintenance requirements are not met. This may predispose bathers to otitis media as earlier reported by Hajjartabar (2004).

This study observed that chlorination of the polluted pools may have failed or was insufficient to provide the recommended residual chlorine levels of 0.2 - 0.5 parts per million (PPM). Also, the duration of the change of pool water was too long, especially in B where the authorities used the presence of algae on the walls of the pool as an index of the need to change the water. It was observed that humans may not necessarily be the sole source of contamination of the pool because wind carrying dust particles also settle on the water however, this study observed growth only at 37^0C indicating that the organisms were of human origin (Itah and Ekpombok, 2004).

CONCLUSION

In conclusion, it is pertinent to note that swimming pools are contaminated with bacteria of human origin and these swimming pools are not maintained according to standard operating procedures hence the high prevalence of bacteria. It is therefore, recommended that operators of swimming pools should be trained on the importance of pool maintenance to avert diseases outbreak of diseases of public health consequences.

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