

Bacteriological Examination of Some Public Swimming Pools in Nigeria

C. Onwa Ndunuisi and U. Igwe Grace

Department of Applied Microbiology, Ebonyi State University, P.M.B. 053, Abakaliki, Nigeria

Abstract: The bacteriological analysis of some public swimming pools were carried out in Abakaliki, Ebonyi State. A total of eight public swimming pools were randomly selected for the analysis. Isolation of the bacterial species was carried out using the pour plate method while bacterial identification was carried out using morphological, physiological and biochemical tests. Results of the bacterial load of the samples showed that bacterial contamination of the swimming pools ranged from 1.3×10^1 cfu/ml to 4.1×10^1 cfu/ml. The following bacteria species were isolated from the samples; *Staphylococcus* species, *Streptococcus* species, *Listeria* species, *Enterococcus* species, *Pseudomonas* species, *E. coli*, *Proteus* species and *Bacillus* species. Result of the percentage distribution of the bacterial species showed that *Staphylococcus* species occurred most frequently at 13(21%) while *Bacillus* species had the least occurrence of 5 (8%). The presence of some bacteria species like *E. coli* and other enteric bacteria species isolated from this study suggest the likelihood of faecal contamination of the swimming pools and its unsatisfactory sanitary condition. It is therefore recommended that public swimming pools in Abakaliki and beyond be regularly disinfected /chlorinated.

Key words: Bacteriological • Public • Swimming pool and contamination

INTRODUCTION

Water occupies about 70% of the earth surface and is considered the largest natural resource around us. The importance of water includes drinking, washing, cooking, swimming and also cooling the ecosystem. Therefore, its importance to humans cannot be overlooked. But in spite of the awareness to safeguard our waters, the resource is still contaminated by pathogenic microorganisms [1].

Swimming is generally considered to be a healthy leisure activity for both the young and old. Swimming is even often advised as the most appropriate sport for asthmatic children, mainly on the grounds that inhaling moist air is less conducive to trigger exercise-induced asthma [2]. The growing popularity of swimming and other “in-the-water” activities for sport, fitness, therapy or just enjoyable relaxation has led to the increased use of swimming pools and the establishment of a variety of specific-use pools such as spa pools, waterslides and more recently, hydrotherapy and wave pools. These pools are used by a variety of people of various ages, health status and standards of hygiene [2].

Swimming pools are concrete tanks, large artificial basins or large paved holes containing water for

swimming [3]. Swimming pools have become major recreation facilities for leisure and sports in cities across the world [4]. Swimming pool water should meet potable water standard by being transparent, odourless and tasteless liquid having a freezing point of 0°C and boiling point of 100°C [5].

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 fecal pollution is a useful tool in identifying contaminated swimming pools [6]. 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 [7]. Consequently, pool waters need to be monitored regularly for pathogenic microorganisms originating from fecal contamination e.g. *Escherichia coli* O157, *Campylobacter jejuni*, *Shigella* species, *Cryptosporidium parvum* and Rotaviruses. Non-fecal pathogens like *Legionella pneumophila* and *Pseudomonas aeruginosa* have also been documented to cause recreational water illnesses [8]. Bacterial contamination of swimming pool water poses public health risks to swimmers and others who come into direct contact with such pools [9].

Those who normally take care of these swimming pools have little knowledge about the importance of maintaining the pools to meet both the microbiological and physiochemical standards. Some tend to economize chemicals use for sanitizing the pools due to their scarcity or over chlorinate the pools due to little knowledge of the recommended quantities to apply and hence compromise the quality of the swimming pools [10]. The standard guidelines of swimming pools, particularly in developing countries, are not adhered to because little is known about the contaminants in the pools and the possible health risks involved [11]. Hence, more studies are needed to generate additional information necessary for the development of swimming pool water quality standards. This study was designed to assess the bacteriological examination of some public swimming pools within Abakaliki, Ebonyi state, Nigeria.

MATERIALS AND METHODS

This research was carried out within Abakaliki Metropolis. The population of people in the city has increased as well as the social life of the residents. Building of hotels with recreational centres such as swimming pools had been on increase.

Sample Collection and Transportation: A total of 16 water samples were collected two times from eight different hotels within Abakaliki metropolis. The sampling periods were morning before bath and evening after bath. For anonymity the selected swimming pools were coded SS, SD, AS, CS, SL, OL1, OL2 and VH. Each water sample was collected with a sterile 250 ml wide mouth plastic container at depth of about 30 cm from each swimming pool and transported to the laboratory of Applied Microbiology, Ebonyi State University in a flask containing ice cubes for analysis within 15 minutes.

Bacteriological Examination of Samples

Total Aerobic Plate Count: The water samples were 10-fold serially diluted in 9 ml of sterilized peptone water contained in each of the tubes by transferring 1ml of water in the first test tube and mixed; then 1 ml of the first dilution was drawn out into the second tube. This was continued until the 4th tube. Then 100 μ l of two sample dilutions of 10^{-2} and 10^{-4} including the neat (undiluted sample) was plated onto the plate count agar by surface spread using a sterilized glass spreader for uniform inoculation. The plates were incubated at 37°C for 48 hours. Following appropriate length of incubation, all visible colonies were counted and the results were

calculated by multiplying the number of colonies on each plate by the reciprocal of the dilution factor of sample dilution plated and multiplied by ten, which was reported as colony forming units per ml.

$$\text{Number of organisms} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{0.1 \times 1}$$

Coliform Count: The conventional multiple-tube fermentation (MTF) technique was used to determine the most probable number (MPN) of coliform bacteria present in 100 ml of pool water. This technique normally involves three steps: Presumptive, confirmed and completed test.

Presumptive Test: Differential medium for the isolation of coliforms was MacConkey broth. Three broth tube series – the first series containing 3 double strength broth tubes and the remaining two series comprising 6 single strength broth tubes – were inoculated with 10ml, 1ml and 0.1ml of water (ratio 3:3:3) respectively. Tubes were incubated at 37°C and observed at 24 and 48 hours. Presumptive test is positive for coliforms if acid and gas are produced in Durham tubes.

Confirmed Test: To eliminate false-positives from non-coliform organisms, eosin methylene blue (EMB) agar plates were inoculated with a loopful from each positive presumptive broth tube by streaking across the agar surface. Plates were incubated for 24 h at 37°C.

Completed Test: Finally, nutrient agar slants and MacConkey broth tubes were inoculated with distinct colonies picked from cultured isolates on EMB agar plates. After incubation for 24 hours at 37°C, broth cultures were observed for acid and gas production and cultured isolates on agar slants were Gram stain using technique described by Ekopai *et al.* [12].

Identification of Isolated Bacteria: Growth representative of colonies were sub-cultured on nutrient agar medium and incubated for 24 hours at 37°C. The colonial characteristics on agar plates were taken into consideration. The characterization and identification of isolated bacteria were carried out using the procedures of Francois and Schrenzel [13]. The chemical and biochemical tests employed were, Gram's staining reaction, motility, catalase, coagulase, methyl red, oxidase reaction, indole, Voges proskauer, urease, motility, citrate utilization and sugar fermentation.

Statistical Analysis: The frequency of occurrence of the bacteria isolated were calculated using.

$$\text{Frequency (\%)} = \frac{n}{N} \times \frac{100}{1}$$

where *n* = Number of occurrence of bacteria,
 where *N* = Total number of bacteria isolated.

RESULTS

The bacteria load of the water samples showed that the bacteria contamination of swimming pool samples ranges from 1.3×10^1 to 4.1×10^1 Cfu/ml (SS, SD, CS, SL OL1 and OL2) while some of the pool samples had no growth (AS and VH) as shown in Table 1.

The result showed that the bacteria isolated from the swimming pools were identified based on their morphological and biochemical characteristics. A total of

eight (8) bacteria samples were isolated and identified from all the samples. These include: *Listeria* species, *Staphylococcus* species, *Bacillus* species, *Proteus* species, *Pseudomonas* species, *Streptococcus* species and *Escherichia coli* (Table 2).

The result below shows the distribution of the bacterial isolates in swimming pool samples based on their percentage occurrence. *Staphylococcus* species had the highest occurrence with the percentage occurrence of 21.0 %, followed by *Streptococcus* species (16.0 %), while *Bacillus* species had the least occurrence of 8.0 % as shown in Table 3.

The result showed that three water samples (SD, OL1 and OL2), out of the eight swimming pool water sample, the presence of gas and acid/colour change on presumptive test of coliforms on lactose broth were positive as indicated on Table 4.

Table 1: Bacterial load of the swimming pool water samples

Sample Code	Bacteria load ($\times 10^1$) Cfu/ml
SS	2.4
SD	3.7
AS	-
CS	4.1
SL	2.0
OL1	1.3
OL2	2.8
VH	-

Key: - = No growth

Table 2: Characteristics of the bacteria isolates from swimming pool

Cell Morphology	Gram Reaction	Motility	Catalase	Indole	Urease	Oxidase	Coagulase	Citrate	Methyl red,	Voges proskauer	Sugar Fermentation			Suspected Organism	Sample
											Glucose	Fructose	Lactose		
Rod shaped	+	-	+	-	-	+	-	+	-	-	+	-	-	<i>Bacillus</i> species	
Rod shaped	-	-	+	-	-	+	-	+	-	-	+	-	-	<i>Pseudomonas</i> species	
Cocci	+	-	+	-	+	-	+	-	+	-	+	-	-	<i>Staphylococcus</i> species	
Rod shaped	-	+	+	+	-	-	-	-	+	+	+	+	+	<i>Proteus</i> species	
Cocci	-	+	+	-	-	-	-	+	-	-	+	+	+	<i>Streptococcus</i> species	
Rod shaped	+	-	+	+	-	-	-	+	-	+	+	+	-	<i>Enterobacter</i> species	
	-	-	+	+	-	-	-	+	+	-	+	+	-	<i>Escherichia coli</i>	
Rod shaped	+	+	+	-	-	-	-	+	+	+	+	+	+	<i>Listeria</i> species	

Key: - = Negative, + = Positive

Table 3: Percentage distribution of the organisms in the swimming pool samples

Isolates	Number of Occurrence	Percentage Distribution (%)	Sample Codes
<i>Listeria</i> species	7	11.0	SS, OL2, CS
<i>Staphylococcus</i> species	13	21.0	CS, SD, OL1, OL2
<i>Enterococcus</i> species	8	13.0	SD, OL1, OL2
<i>Bacillus</i> species	5	8.0	SS, CS, OL2, SL
<i>Proteus</i> species	7	11.0	OL1, OL2, SD
<i>Pseudomonas</i> species	6	10.0	SS, CS, SD, OL2
<i>Streptococcus</i> species	10	16.0	SS, OL1, OL2, SL
<i>E. coli</i>	6	10.0	SD, OL1, OL2
Total	62	100	

Table 4: Presumptive identification of Coliforms on lactose broth

Sample Codes	Presence of Gas	Presence of Acid/Colour Change
SS	-	-
SD	+	+
AS	-	-
CS	-	-
SL	-	-
OL1	+	+
OL2	+	+
VH	-	-

Key: - = Negative, + = Positive

Table 5: Confirmatory test of coliforms on Eosin methylene blue (EMB) agar

Sample Codes	Presence of Growth	Characteristics on Agar Plate
SD	+	Dark purple to black colony
OL1	+	Dark purple to black colony
OL2	+	Dark purple to black colony

Key: + = Growth present

The confirmatory test of coliforms from the selected swimming pools shows that the water from swimming pools designated SD, OL1 and OL2 showed positive with Eosin methylene blue agar as shown in Table 5.

DISCUSSION

The bacterial load of six swimming pools (SS, SD, CS, SL, OL1 and OL2) water analyzed was relatively high as shown in Table 1. This high bacterial load might be due to the low residual chlorine level or because the bacteria have become resistant to the calcium hypochlorite that is used in treating those swimming pools, similar reports were also made by Francois and Schrenzel [13], Itah and Ekpombok [14], Klapes and Vesley [15], Lumb *et al.* [16]. The results obtained in this work with bacterial count that ranged from 1.3×10^1 Cfu/ml to 4.1×10^1 Cfu/ml showed that the swimming pools did not meet the WHO standard that has been accepted by Nigeria. Also, samples in AS and VH were acceptable according to WHO standard for aerobic plate count as a result of no growth in them (WHO, 2004).

The following bacteria species were isolated from the samples; *Staphylococcus* species, *Streptococcus* species, *Listeria* species, *Enterococcus* species, *Pseudomonas* species, *E. coli*, *Proteus* species and *Bacillus* species (Table 2). The isolation of different species of bacteria which are known human pathogens from these pools might be due to faecal contamination from both humans and animals [17, 18]. *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 [19].

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 [20]. The presence of pathogens in water for drinking and swimming purposes is of public health concern [21]. Swimmers can accidentally swallow contaminated pool water during swimming which can result in outbreaks of diseases like cholera, shigellosis, typhoid fever, gastroenteritis and diarrhea [22, 23].

Staphylococcus species is known to produce enterotoxin. *Proteus* species belongs to the intestinal flora but is also widely distributed in soil and water Reali *et al.* [24]. *Enterobacter aerogenes* isolated from the water samples are examples of non faecal coliform and can be found in vegetation and soil which serves as sources by which the pathogens enters the water Saba and Tekpor [25]. Schlegel [26] suggested that pool operators should drain the water from the pools and do disinfection after use. In line with the report of this study, Shittu, Olaitan and Amusa [27] in Accra, Ghana isolated *E. coli*, *Enterobacter faecalis*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Staphylococcus epidermidis* from swimming pools. Also, in a related study on swimming pools in Ilorin, Nigeria, *Micrococcus*, *Aeromonas*, *Pseudomonas*, *Klebsiella*, *Lactobacillus*, *Bacillus*, *Citrobacter*, *Corynebacterium*, *E. coli* and *Staphylococcus aureus* were equally isolated [28]. Another similar study which was investigated on swimming pools in Lagos, the bacteria isolated were *Enterococcus faecalis*, *Clostridium perfringens*, *Bacillus cereus*, *E. coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus* and *Staphylococcus epidermidis* [29]. *Staphylococcus aureus* and *Bacillus*

cereus could be associated with gastroenteritis when ingested with swimming pool water and both organisms are enterotoxin producers [30]. The presence of *Pseudomonas* species found in this study is likely to be attributed to the growth of *Pseudomonas* species being supported by the warm, moist environment on decks and floors as previously reported by the Saskatchewan Ministry of Health (2010). The *E. coli* isolates obtained in this study is most probably derived from swimmers, human origin is presumed.

Staphylococcus species had the highest occurrence with the percentage occurrence of 21.0 %, followed by *Streptococcus* species (16.0 %), *Enterococcus* species (13.0 %), while *E. coli* and *Pseudomonas* species had the least occurrence of 10.0 % (Table 3). The findings that the species of *Staphylococcus* species isolated from the swimming pools were more than other bacterial isolates was not surprising because this organism exist in large number on the human skin, in other words, they are normal flora of the skin. Similarly, Yang *et al.* [31] also reported that the major contaminating bacteria of swimming pools have been *Staphylococci* species. Yoder *et al.* [32] reported that the major contaminants of swimming pools and other recreational waters with high bather density are *Staphylococcus epidermidis* and *Staphylococcus aureus*. The result of this study is also similar to that of Alcock [3] who reported *Staphylococcus aureus* as the highest bacterial isolated obtained from swimming pools in Accra, Ghana.

Three swimming pools (SD, OL1 and OL2) out of the eight sampled for presumptive test were coliforms positive on lactose broth shown in Table 4. This three swimming pool designated SD, OL1 and OL2 were also positive for coliforms with Eosin methylene blue agar as the confirmatory test (Table 5). The presence of coliforms in swimming pools is as a result of low free chlorine concentrations which are usually below the minimum permissible limit set by the National Swimming Pool Foundation [30]. Microbes can quickly multiply in water because of lack cleansing ability due to the lower concentrations of chlorine [24]. It has been previously reported that there is a significant association between pool contamination and free residual chlorine concentration [5]. However, even though the chlorine levels were not measured in this study, all results for the total coliform, *E. coli* and other bacteria load can be attributed to the level of free residual chlorine concentrations in the swimming pools water. [31], reported that when the concentration of free chlorine is too low, the risk for bacterial growth and infection disease

to swimmers will increase because the concentration of chlorine is not enough to eliminate the microorganisms in the pool. Anyim *et al.* [7] stated that a swimming pool may be infected with pathogenic microorganisms such as coliforms entering the pool either directly or indirectly through contaminated human or animal excrement such as feces and urine from the swimmers. Tate, Mawer and Newton [28] stated that the treatment of water for drinking and swimming purposes should focus on the elimination of coliform bacteria so as to prevent an epidemic of water related diseases.

CONCLUSION

The study indicated that most swimming pools within the Abakaliki metropolis were contaminated. The isolation of pathogenic enteric bacteria and *Staphylococcus aureus* from this study is an indication of poor bather hygiene and poor compliance of the standards. Thus, there is a need to increase monitoring of the recreational facilities, increased bather hygiene education, as well as pool staff education and improved pool circulation. Furthermore, studies should be carried out on other microbial contaminants apart from bacteria that contaminate swimming pool water and also on pool architecture and bather population that contribute to poor compliance of the standards.

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