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Susceptibility of *Staphylococcus aureus*, and *Candida albicans* isolated from an Outdoor Swimming Pool in Abraka, Delta State, Nigeria

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ABSTRACT

The use of outdoor swimming-pools during recreational activities and to practice swimming is on the increase, as against the use of natural water bodies due to increase rate of drowning and the possibility, of contaminations of water-borne diseases. In this study, a well-patronised outdoor swimming pool, in a densely populated town, Abraka community, Delta State, Nigeria, was assessed for the presence of *Staphylococcus aureus*, and *Candida albicans* isolates. The susceptibility potentials of these isolates to some antibiotics were investigated with standard techniques. Water samples were taken from four locations and in three phases (Before use, during use and before discharge) in sterilised sample bottles. During the sampling period, water temperature and pH were also monitored. Standard techniques and procedures were adopted for the isolation and identification of the isolates. The two isolates (*Staphylococcus aureus*, and *Candida albicans*) were present in the sampled water specimen in high frequency. Higher frequencies of the isolates were recorded before changing the water. The pH and water temperature monitored were significantly ($p < 0.05$) different in water samples obtained during water usage and before changing the water with the water before usage. The two isolates were susceptible to Pefloxacin, Rocephin and Ciprofloxacin antibiotics only. Therefore, the occurrence of these isolates in public swimming pool indicated the precarious pool state and a vehicle for the transmission of the isolates. Proper and rotational water hygiene should be practised to curb the presences of the isolates.

Keywords: Bacterium, Fungus, Lentic, Recreation, Public Health.

Introduction

Recreation is seen as a value to individual and society which is unrestricted to swimming. It could be an attitude of mind regarding leisure behaviour that has a direct influence on factors that creates personality (Torkildsen, 1999). Swimming can be performed either in natural or artificial waters (such as swimming pool). Swimming pools are water contained either in concrete tanks, large

artificial basin, or sizeable paved holes (Alcock, 1979). According to Cairns and Dickson (2003), swimming pool waters should meet potable water standard. However, where it is compromised, the water thereof, poises danger to human health and the environment (Cairncross *et al.*, 2000; WHO, 2001). Several studies have been conducted on sanitary and quality control of swimming pools

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using microbiological evaluation (Ayandele *et al.*, 2015).

Studies have shown that *Staphylococcus aureus* (bacteria) and *Fungi Candida albicans* (fungi) isolates are one of most frequently isolated pathogenic organisms in polluted human waters (Adamu *et al.*, 2017; Adamu *et al.*, 2018) as they are indicators of unhealthy ecosystem (Jacob *et al.*, 2016). Studies also revealed that *Staphylococcus aureus* is responsible for a variety of infections such as osteomyelitis, pneumonia, conjunctivitis, urinary tract infections in human (Hussain *et al.*, 2017, Wolff *et al.*, 2013). *Candida albicans* exist as the first-rate causes of invasive fungal infections (Abu-Elteen and Hamad, 2012, Pfaller and Diekema, 2004). *C. albicans* commonly found in the oral cavity, gastrointestinal tracts and genital areas as harmless commensals (Abu-Elteen and Hamad, 2012). However, it is an opportunist pathogen capable of causing wide ranges of localised or superficial and systemic infections (Pfaller and Diekema, 2004). It is the principal candidate in oral candidiasis (Davies *et al.*, 2006) and vulvovaginal candidiasis (Davies *et al.*, 2006).

Therefore, evaluating the potential of *Staphylococcus aureus*, and *Candida albicans* in an outdoor swimming pool, patronised heavily by the public especially students in Abraka, Delta State, Nigeria will provide more information on the health status of the pool and its suitability for the purpose. The study also tends to ascertain the susceptibility of these isolates to some commonly available antibiotics.

Materials and Methods

Study Area

The study was conducted in an outdoor swimming pool, located on Longitude and Latitude of 5.7894°N and 6.1023 °E (Fig. 1), Abraka, Delta State, Nigeria. The pool is floored with white ceramic tiles of different shapes and sizes. It is 9ft deep and 23m x 40m wide.

Sample Collection

The water sample was collected in duplicate from four sampling sites on three occasions (Before public usage, while using and before changing the water). The samples were transferred into a well labelled, sterilised wide-mouthed bottle for *Staphylococcus aureus*, and *Candida albicans* isolation and identification. Sampling was done following the method of Ademorati (1996). The sample-collection bottles were washed out thrice with the sample water before collection of samples. The collection was done by dropping the sample bottles to about 20 cm below the water surface, tilting the mouth of the bottle. The samples were preserved in an ice case and immediately moved to the laboratory for investigation. The water pH and temperature were checked using pH meter (Hanna) and mercury- in - glass thermometer (APHA, 2005), respectively before sampling.

Experimental Procedures

Following sterile procedures, ten-fold successive dilution of the water sample was made; from which 0.1ml was plated into a sterile Petri dish. Nutrient Agar (NA) (Titan Biotech) preparation was based on the manufacturer's specification. The medium was poured onto a sterile petri dish containing 0.1ml of the sample employing pour plate techniques. After that, the plate was incubated at 37°C for 24hours to obtain mixed culture. Isolated colonies were recorded and then purified to acquire pure culture achieved repetitively through sub-culturing on new media for primary isolation in the manner represented/ delineated/described by Chikezie (2015). Pure stock cultures obtained were inoculated on NA slant and preserved in the refrigerator at 4°C before sensitivity test. The identification was based on its biochemical characteristics (gram stain, production of catalase and coagulase, urease, citrate, oxidase, indole, methyl red, glucose, lactose and sucrose) that were performed as described in Cheesebrough (2000) and Adeoye (2007).

Germ Tube Formation was used to identify the fungus isolate grown on Potato Dextrose Agar as described in Adamu *et al.* (2018). After that, the use of Lactophenol cotton blue-green before observation with x100 objective lenses was employed to confirm the isolates. Antibiotic susceptibility was carried out on the isolates using disc diffusion method with inoculated solidified nutrient agar and incubated at 37°C for 24 hours and was observed for susceptibility and resistance profile (Cheesbrough, 2000).

Statistical Analysis

Past statistical software 2000 was used to employ the One-way ANOVA to explore significant differences in the pH and temperature of the pool at the different designated period of use.

Results

The mean and standard error values of the determined pH and temperature of the swimming water are presented in Table 1. It was observed that as the usage of the water continues before the change, the pH of the water was acidic with a higher temperature value of 31.34±1.04°C. Table 2 reveals the phenotypic methods used for the identification of the isolates. Table 3 presents the total bacterial and fungal counts of three sampling phases. Table 3 also compares the isolates abundance with the WHO acceptable limit. The susceptibility test performed with fourteen (14) commonly available antibiotics revealed only three (3) (pefloxacin, rocephalin and ciprofloxacin) were the only antibiotics that the isolates were susceptible to (Table 4).

Table 1: Mean and standard error of pH and water temperature monitored in an outdoor swimming pool in Abraka, Delta State.

| Sampling Period | pH | Temperature (°C) |
|-----------------|------------|------------------|
| Before Use | 6.89±0.38 | 29.25±0.45 |
| While in Use | 5.04±1.24* | 30.64±1.44* |
| Before Change | 4.12±0.34* | 31.34±1.04* |

*P<0.05

Table 2: Phenotypic identification methods adopted for the identification of the isolates.

| Parameters | <i>Stapylococcus aureus</i> | <i>Candida albicans</i> |
|----------------|-----------------------------|-------------------------|
| Catalase | + | NA |
| Coagulate | + | NA |
| Methyl red | - | NA |
| Gram reaction | + | NA |
| Gem tube | NA | + |
| Spore staining | NA | + |

+: positive, -: negative, NA: not applicable

Table 3: Total Bacterial and Fungal counts in the three phases sampled in an outdoor swimming pool in Abraka, Delta State.

| Isolates | Before Use CFU/ml | During Use CFU/ml | Before Discharge CFU/ml | WHO Acceptable Limit |
|--------------------|----------------------|----------------------|----------------------------|-------------------------|
| <i>S. aureus</i> | 7 | 2 | 18 | ≤1CFU/100ml |
| <i>C. albicans</i> | 3 | 5 | 6 | ≤1CFU/100ml |

Table 4: Antibiotics susceptibility/Resistance profile of the bacterium and fungus to some selected antibiotics tablets

| Antibiotics | Concentration | Isolate | |
|-----------------|---------------|------------------|--------------------|
| | | <i>S. aureus</i> | <i>C. albicans</i> |
| Pefloxacin | 10µg/ml | + | + |
| Gentamycin | 10µg/ml | + | - |
| Ampliclox | 30µg/ml | - | - |
| Zinnacef | 20µg/ml | - | - |
| Amoxicillin | 30µg/ml | - | - |
| Rocephin | 25µg/ml | + | + |
| Ciprofloxacin | 10µg/ml | + | + |
| Streptomycin | 30µg/ml | + | - |
| Erythromycin | 10µg/ml | + | - |
| Chloranphenicol | 30µg/ml | + | - |
| Augumentin | 30µg/ml | + | - |
| Tarivid | 10µg/ml | - | - |
| Septtrin | 10µg/ml | - | - |
| Sparfloxacin | 30µg/ml | + | - |

+: Susceptible; -: Resistance

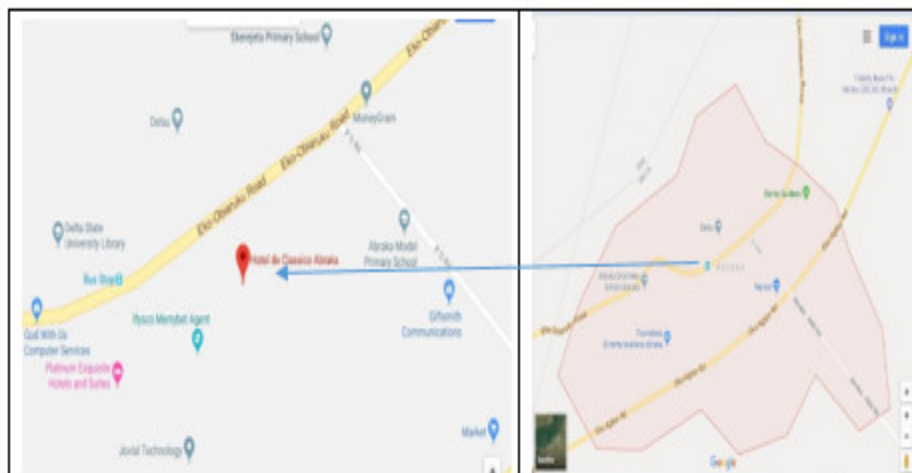


Fig 1: Map of Study Site using google earth location map (2019)

Discussion

The isolation of these pathogens in swimming pools has been reported by other researchers (Olorunjuwon, 2012; Abu-Elteen and Hamad, 2012). The affinity for one another could be due to the adherence of *Staphylococcus aureus* to the filamentous fungus; *Candida albicans* (Peters *et al.*, 2012) and thus both found drifting in the swimming pool. The co-isolation of *Candida albicans* and *Staphylococcus aureus* has been reported by several researchers and synergistically causing morbidity and deaths in humans (Todd *et al.*, 2019). The concomitant existence of these pathogens could be symbiotically related to their indigenesness in man. The acidic water with its corresponding high-temperature value may be attributed to the polluted state of the water as a result of the presences of *Candida albicans* and *Staphylococcus aureus* amongst other pathogens that may be present in the water. The elevated frequency (count) of these isolates in the swimming pool after usage is a strong indication that the pool was impacted by the swimmers, which are latent carriers of these pathogens. Besides the mucosal contaminations (Todd *et al.*, 2019), the faecal-en route could be another

possible source of these pathogens when pool users do not observe utmost sanitary measures (Ekopai *et al.*, 2017). As a spherical bacterium, *S. aureus* often found embedded in man en route the nose and skin of people have survived to a great extent outside the human. More so, notably infected persons with skin infections as well as life-threatening diseases caused by these pathogens are not screened out from using the pool (Ajadi and Thonda, 2017). Similarly, *C. albicans*, a diploid filamentous fungus; is opportunistic in nature indicted in causing oral and genital infection diseases in humans (Ryan and Ray, 2004) invading men using hyphae (Peters *et al.*, 2012; Onifade *et al.*, 2019). The reduced number of isolates after the change of water has exonerated biofilms formations along the pipes as another probable means of contaminating the pool. The concomitant occurrence of both organisms is therefore of great public health message as they may be responsible for the persistence, resistance and advancement of so many diseases (Shirtliff and Leid, 2009; Melton and Anderson, 2018; Jamel *et al.*, 2018). Thus, their presence in a public swimming pool may provide an accessible vehicle for its transmission. The aggregation of 60-100 bathers

at a time in the given size of the studied pool was worrisome and considered a public nuisance. This clustering, which is an express spread of diseases must be stopped. The antibiotic susceptibility test revealed that the isolates were resistant to most of the antibiotics and antifungal agents used for treating infections. Thus, it could be challenging to treat an infection acquired from the swimming pools arising from such organisms (Son *et al.*, 1997). Researchers have reported on the susceptibility of the tested antibiotics on the pathogens (Onwubiko and Sadip, 2011; Al-Zoubi *et al.*, 2015). Swimming pool users must endeavour not to gulp in the water while swimming and be quick to wash after swimming with disinfectant.

The study thereof revealed that the swimming pool did not meet the standards of WHO and EPA for recreational water quality. Despite the acclaimed disinfection of the pool water weekly with 100g chlorine/150Ash/1kg Alum (Questionnaire). The intensity of the isolates during use of pool suggests improper or no treatments as well as the use of substandard disinfectants (Ekopai *et al.*, 2017). The presence of *Staphylococcus aureus* and *Candida albicans* discovered in the swimming pool is not desirable for recreational water.

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Conflict of Interest

There is no known contention.

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