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Microbial Qualities of Swimming Pools in Lagos, Nigeria

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Abstract

Twenty different swimming pools in Lagos, Nigeria, were investigated for their microbial qualities. Water samples from the swimming pools were serially diluted and dilution factors of up to 10^{-8} were cultured on the appropriate agar media. The pour plate technique was adopted using standard plate count agar for the determination of the total heterotrophic bacterial and fungal counts. Bacterial and fungal isolates were identified using standard methods. Bacterial isolates include Enterococcus faecalis, Clostridium perfringens, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vulgaris and Staphylococcus epidermidis, while fungal isolates were Penicillium sp, Rhizopus sp, Aspergillus versicolor, Fusarium sp, Trichophyton mentagrophytes, Mucor sp, Candida albicans, Aspergillus niger and Absidia sp. The mean heterotrophic bacterial count ranged from $3.0x10^6$ cfu/ml which occurred in sample R, to $6.6x10^7$ cfu/ml in sample Q while the mean coliform count ranged from a nil count/100 ml to $1.7 x 10^7$ cfu/100 ml (sample S). Fungal counts ranged from a nil count/ml to $2.0x10^7$ cfu/100 ml in sample F. In conclusion, the presence of high levels of coliform and fecal coliform bacteria (E. coli) revelaed that the swimming pools have not met the World Health Organization (WHO) standard for recreational waters. The swimming pools constitute a serious public health hazard, thus necessitating urgent and effective intervention.

Keywords: Bacteria, fungi, swimming pool, water, public, hazard.

1.0 Introduction and Literature Review

Water is one of the most essential needs of man, both for food and for recreation. Life in this planet earth originated in water, water is basic to the life of modern man, no other substances serves man in so many ways as less crucial for the society as a whole than for the well being of each individual (Cabelli, 2003; Alico and Dragonjac, 2006).

It does not only fulfil the basic means of life, water functions in transportation, recreation, power generation, manufacturing industries, cooling, irrigation, food production and processing to mention a few (Mackereth *et al.*, 2003). Natural waters are the major sources of swimming pool water. The potability of swimming pool water is enhanced by frequently changing the water and the use of disinfectant, such as chlorine. The highest possible concentration of about 1 ppm should be maintained because higher chlorine concentration irritates the eyes (Alice, 1977; Fair *et al.*, 2001; Cairns and Dickson, 2003). Swimming pools are concrete tanks, large artificial basins, or large paved holes containing water for swimming. The swimming pool water should meet potable water standard by being transparent, odorless, and tasteless liquid having a freezing point of 0ºC and boiling point of 100ºC (Cairns and Dickson, 2003).

Swimming pool operators prefer iodine to chlorine as a disinfectant because its action is less hindered by organic matter and there is less eye and skin irritation than with chlorine, according to Alice (1977). Bromine is also recommended. Each bather is expected to shower with soap before entering the pool. People with infections of any kind should not be allowed entry into the pool. Despite these operations to ensure potability of the water and maintenance of good hygiene, Cruickshank *et al*. (1975) reported that a 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 bathers. Unless the water is adequately treated, contamination may lead to outbreaks of diseases, such as skin ulcers, gastroenteritis, conjunctivitis, trachoma, ear infection such as otitis media, cholera, dysentery, eczema and skin rashes (Cairncross *et al.*, 2000; UNDP, 2004). 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 (Mood, 2007). As indicators of fecal pollution, their presence is a strong indication of the presence of enteric pathogenic bacteria, such as *Salmonella typhi*, *Salmonella paratyphi*, *Shigella dysenteriae*, *Vibrio cholerae* and parasites in the pool. Skin tuberculosis caused by *Mycobacterium baliteri* has been reported after swimmers had bathed in waters from which a large amount of microorganisms were found (Mood, 2007).

Alice (1977) in her investigation of an outbreak of conjunctivitis in USA during summer camping reported that the incidence of contracting the illness increased by 50% amongst users at the camp swimming pool over non-users. Alcock (1977) highlighted the potential health hazards of *Pseudomonas aeruginosa* in swimming pool waters, adding that this organism is frequently being isolated from the ears of swimmers with otitis media. The principal indicator bacteria are fecal coliform (*E. coli*), total coliform, *Enterococcus faecalis* and *Clostridium perfringens*. Okafor (1985) reported that 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.

Infectious diseases which can be transmitted by recreational water include skin, eye and ear infections and gastroenteritis, consequently the levels of microorganisms in recreational water are important for indexing the health hazard associated with swimming (Lagerkvist *et al.*, 2004). The assessment at the time of collection, of some physical-chemical aspects, such as free available chlorine and pH and the estimation of swimmer load could also predict the quality of pool water. In fact, disinfection of swimming pools should control the organisms responsible for many diseases, however, some of them may be relatively protected from disinfection by the filter material or structural features in the pool (Esterman *et al.*, 2004; Palmer *et al.*, 2003).

The best indicator in the assessment of the safety of swimming pool water is disputed. Some researchers emphasize that the microbiological quality of swimming pools is best measured by using bacteria that indicate fecal contamination as fecal coliform and enterococci, while others consider that the risk of infection is more associated with microorganisms derived from the skin, mouth, and upper respiratory tract of bathers rather than fecal contamination (Seyfried *et al.*, 2005b). Some authors consider that microorganisms which indicate hygienic conditions (total coliform and heterotrophic bacteria) and fecal pollution are the best ones. Nevertheless there are doubts if any microorganism can reliably predict the health risks associated with swimming (Galbraith, 2000; Favero *et al.*, 2004; Favero, 2005; Seyfried *et al.*, 2005a; Mossel, 2006; Mood, 2007).

Another important factor to assess bathing water quality is related to the density of the bathers. High density of swimmers leads to a risk of contact with pathogens that are similar to the risk involved in bathing in water considered improper because of fecal pollution (Favero *et al.*, 2004; Mossel, 2006; Dutka, 2008).

Therefore, it has been proposed that no single indicator microorganism is suitable, so fecal 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 (Galbraith, 2000). The use of many indicators and the necessity to evaluate the bather load to assess the quality of swimming pool water could be a constraint for the control of these waters mainly considering cost-benefit analysis and it is important that such a control could be less expensive and simple to be performed. In order to evaluate the best microbiological indicator for swimming pool waters, public outdoor swimming pools in Brazil were microbiological and physical-chemically assessed during four years (Harrigan and McCane, 1976).

Frequency of examination of swimming pool water depends on the size of the pool. In large indoor pools microbiological analyses are performed once per month and chemical analysis at least 2 times per year. Outdoor pools are inspected once per month during the bathing season. Swimming pool water is recycled over more or less specified periods and various chemicals are used to conserve water quality (Bernard *et al.,* 2003; Lagerkvist *et al.*, 2004). Swimming pool water chemistry is gaining interest. The chemistry of swimming pool water completely differs from drinking water chemistry. Some of the chemical components of pool water will be transformed after their release in the pool; others remain stable and can only be removed by water dilution, by addition of fresh water (Erdinger *et al.*, 2001). Inorganic and organic nitrogen containing compounds released into the water by bathers will be transformed, at least partially, to combined chlorine. However, the chemical identity of individual compounds hiding behind this sum parameter is only known to a limited degree. Results show, that compounds forming combined chlorine do not concentrate in the water. Some of them will be transformed to nitrate, others will probably escape (as e.g. nitrogen trichloride) to the atmosphere. Other components of the disinfectant, like chlorate, can concentrate in the water and reach high levels comparable to concentrations of other inorganic components of the water (Erdinger *et al.*, 2005).

Inorganic compounds like sulphate and chloride which are added as disinfectant or in order to stabilize the pH of the water will mainly remain unchanged and concentrate in the water. However, at least a small portion of the chlorine will react with organic or inorganic compounds and some of these reactions products are volatile and will escape from the pool. Hot whirl pools, for example, show lower chloroform concentrations in the water than other pools due to the intensive aeration of the water (Erdinger *et al.*, 2001; Davies and Goldsmith, 2005; Itah and Opara, 2007). The main objective of this work is to determine through cultural techniques whether swimming pools in Lagos metropolis meet WHO standard for recreational waters using microbiological indices, and then suggest control and preventive measures that can be adopted to reduce risk of infections to the minimal.

2.0 Materials and Methods

2.1 Sources and collection of samples

Water samples were collected aseptically from 20 different swimming pools in Lagos Metropolis, Nigeria employing the techniques of Cruickshank *et al.* (1975) and Okafor (1985). All swimming pools are made of glazed tile and have different shapes (square, rectangular, circular, oval and irregular) and sizes (ranged from 50 to 1500 m^2). Samples were labelled A-T based on their collection sites. Sample A flows through pool 2.85 m deep; sample B, flows through pool 2.85 m deep; sample C fills and draws pool 2.2 m deep; sample D flows through pool 2.5 m deep; sample E flows through pool 2.22 m; sample F fills and draws pool 2.85 m deep; sample G fills and draws pool 50 m at its deepest point; sample H flows through pool 2.3 m deep; sample I fills and draws pool 2.2 m deep; sample J fills and draws pool 2.2 m deep. Sample K flows through pool 2.75 m deep; sample L, flows through pool 2.85 m deep; sample M fills and draws pool 2.4 m deep; sample N flows through pool 2.7 m deep; sample O flows through pool 2.32 m; sample P fills and draws pool 2.85 m deep; sample Q fills and draws pool 50 m at its deepest point; sample R flows through pool 2.5 m deep; sample S fill and draw pool 2.3 m deep; sample T fills and draws pool 2.6 m deep. The sampling periods were mostly in the morning before bath, afternoon and evening after bath.

2.2 Processing of samples

The pour plate technique of Harrigan and McCane (1976) and Collins and Lyne (1976) was adopted using standard plate count agar (Oxoid, England). This method was used for the determination of the total heterotrophic bacterial and fungal counts. The total coliform and *Escherichia coli* (fecal coliform) counts were enumerated on McConkey agar, as described by Oxoid (1985) and Itah *et al.* (1996).

The fecal coliforms (*E. coli*) were incubated at 44 ± 0.5ºC for 24-48 hours while the total coliform bacterial counts were enumerated at 37ºC for 24-48 hours. Cetrimide agar was used for the isolation of *Pseudomonas aeruginosa* while potato dextrose agar was used for the isolation of fungi. Anaerobic *Clostridium* species and *Enterococcus faecalis* were enumerated on blood agar medium. After primary isolation, pure cultures were obtained by repeated subculture on fresh media using the streak plating technique.

2.3 Characterization and Identification of Isolates

Representative colonies obtained after incubation were sub-cultured on nutrient agar medium which was incubated for 24 hours at 35°C. The cultural characteristics of isolates on the agar plates were observed. The characterization and identification procedures for the microorganisms were carried out using methods of Cowan and Steel (1985) and Holt et al. (2004). Gram staining reactions and cell morphology from heat fixed smears were done. The motility of the isolates was examined using hanging drop technique. Colonies were further characterized using various biochemical tests such as catalase reaction, oxidase reaction, sugar fermentation test, citrate utilization test and urease test. Fungal isolates were identified employing the method of Beneke and Rogers (2005).

3.0 Results and Discussion

The mean heterotrophic bacterial count ranged from $3.0x10^6$ cfu/ml which occurred in sample R, to $6.6x10^7$ cfu/ml in sample Q while the mean coliform count ranged from a nil count/100 ml to 1.7×10^7 cfu/100 ml (sample S). Fungal counts ranged from a nil count/ml to $2.0x10^7$ cfu/100 ml in swimming pool sample F. Fifteen out of the 20 swimming pools, yielded no growth of coliform bacteria. Similarly, fungi were not encountered in thirteen of the pools. However, growths of heterotrophic bacteria were encountered in all the twenty swimming pools studied (Table 1).

The percentage frequencies of bacterial isolates were as *Enterobacter aerogenes* (40%), *Clostridium perfringens* (25%), *Bacillus cereus* (70%), *Escherichia coli* (20%), *Pseudomonas aeruginosa* (65%), *Staphylococcus aureus* (100%), *Staphylococcus epidermidis* (60%) and *Enterococcus faecalis* (40%) (Fig 1). This indicted *S. aureus* as bacterium with the highest percentage frequency while *C. perfringens* showed the least. The percentage distribution of fungal isolates was presented in Figure 2, and this revealed *Fusarium* sp (70%) as having the highest percentage occurrence, followed by *Mucor* sp (65%), *Penicillium* sp (50%), *Aspergillus* niger (45%), *Aspergillus versicolor* (40%), *Candida albicans* (30%), *Trichophyton mentagrophytes* (30%), *Absidia* sp (30%) while the least percentage frequency of fungal isolates was shown by *Rhizopus* (25%),

The presence of significant numbers of any member of the coliforms in swimming pool water indicates either deficiencies in the treatment of the swimming pool or inadequate protection of the source of untreated water (Borchardt and Walton, 1971). Various species of bacteria and fungi, most of which are known human pathogens, were encountered in the various pools, probably as a result of fecal contamination by homoiotherms and poikilotherms. According to Carbell *et al.* (1975) and Stanier *et al*. (1987), *Enterococcus faecalis, Enterobacter, Klebsiella* and *Pseudomonas* species are associated with surface run-off water while *Escherichia coli*, *Staphylococcus aureus* and *Lactobacillus* species are usually contributed by bathers in the pools. Similar reports were made by Robinton and Mood (1986) in their quantitative and qualitative appraisal of microbial pollution of water by swimmers. Bonde (1985) and Itah *et al.* (1996) reported that the presence of *E. coli* in water is a strong indication of the closeness of the sampling site to the source of pollution. *E. coli* is exclusively fecal in origin (Okafor, 1985) and its presence in water is a strong indication of recent fecal pollution because of the extra enteral behavioral pattern of this organism (Itah *et al.*, 1996).

The presence of *Clostridium perfringens* in some of the pools is an indication of remote pollution (Report 71, 1969) while *Enterococcus faecalis,* when found in water in the absence of *E. coli* because of its extra enteral behavior, is important confirmatory evidence of fecal pollution of swimming pools. The presence of these indicator bacteria indicates the possible presence of enteric pathogenic bacteria in the pool. This incidence constitutes a public health hazard because swimmers can accidently swallow contaminated pool water during swimming which can result in outbreaks of diseases like cholera, shigellosis, typhoid and paratyphoid fever, gastroenteritis and diarrhea. Trachoma, caused by *Chlamydia* species, is known to be associated with eye inflammation and partial or complete blindness.

One cannot rule out the possibility of outbreaks of parasitic infections such as amebiasis, giardiasis, filariasis and scabies. The results of this work are in consonance with an earlier report by Alcock (1977) who isolated *Pseudomonas aeruginosa* from swimming pool waters and highlighted the potential health hazards of this organism, reiterating that it is frequently being isolated from the ears of swimmers with otitis media. Similarly, there are many swimming pool water standards based on physical, chemical and microbiological parameters, but the universally accepted standard is that of the WHO. Nigeria has no swimming pool water standard but has adopted that of the WHO. The high viable bacterial count up to 10^7cfu/ml at 37ºC and high incidence of coliforms and *Escherichia coli* obtained from some pools in this investigation do not meet the recommended criteria for swimming pool safety (Yoder, 2002).

The results differ from the work of Alice (1977) who reported skin tuberculosis caused by *Mycobacterium baliteri* after swimmers had bathed in waters from which large amounts of microorganisms were encountered, as in this work. Mycobacteria were not encountered in this investigation. The results compare favorably with the work of Robinton and Mood (1986), who isolated *Staphylococcus* species from swimming pools with low chlorine residuals. *Bacillus cereus* (Itah and Opara, 1997), *Escherichia coli* (Anozie and Antai, 1987; Itah, 1999) and *Staphylococcus aureus* (Snydman, 1989; Itah and Opara, 1997; Prescott *et al.*, 2002) are known enterotoxin producers when ingested into the body, therefore their presence in pools is a threat to public health because they can be ingested with water by active swimmers. It is important to note that drinking water standards differ from one country to another due to geographical locations and climate. Similarly, there are many swimming pool water standards based on physical, chemical and microbiological parameters, but the universally accepted standard is that of the WHO (2001).

Nigeria has no swimming pool water standard but has adopted that of the WHO (2001). The high viable bacterial count up to 10^7 cfu/ml at 37ºC and high incidence of coliforms and *Escherichia coli* obtained from some pools in this investigation do not meet the recommended criteria for swimming pool safety. This is because, according to Gray (1969), no sample from a pool should contain any coliform organism in 100 ml of water, and in 75% of samples the viable plate count at 37ºC should not exceed 100 orgs/ml. The remaining 25% must not contain more than 100 colonies of bacteria. A standard of fewer than 100 *Staphylococcus aureus* per 100ml of water has been proposed by Mackereth *et al.* (2003) and a free chlorine residual of 0.2 - 0.5 mg/l.

Some fungal pathogens of man were encountered in this work, namely: *Candida albicans, Penicillium, Aspergillus* species and the dermatophyte, *Trichophyton mentagrophyte*. Alice (1977) reported that *Aspergillus niger* is responsible for aspergillosis, usually an infection of the external ear (otomycosis) which may result in ulceration of the lining of the ear canal and perforation of the tympanic membrane. Such fungi normally live in the ear wax. *Candida albicans* causes candidiasis, which is an acute or subacute infection in stools, the vagina, and of the skin of normal persons. It may produce lesions in the mouth, vagina or lungs of infected persons. *Trichophyton mentagrophyte* is the etiologic agent of foot and nail infections. It is also responsible for ringworm of the scalp, beard hair, groin and buttocks. *Fusarium* is known for eye infections in humans and animals according to Prescott *et al.* (2002). In conclusion, none of the swimming pools met the WHO (2001) standard for recreational waters and therefore constitute a serious hazard to public health from a bacteriological view point.

Table 1: Standard plate counts of isolates from twenty different swimming pools in Lagos, Nigeria.

Location	No. of samples	Mean heterotrophic bacterial count (cfu/ml)	Mean coliform count (cfu/ml)	Mean fungal count (cfu/ml)
A	6	3.3×10^6	2.8×10^6	0
B	6	1.9×10^7	0	0
C	6	2.1×10^7	0	0
D	6	1.8×10^7	0	0
E	6	2.5×10^7	0	0
F	6	3.2×10^6	0	1.9×10^7
G	6	3.9×10^6	1.9×10^6	0
H	6	3.3×10^6	2.4×10^6	1.6×10^7
I	6	2.7×10^7	0	0
J	6	3.6×10^6	0	3.5×10^6
K	6	2.2×10^7	0	0
L	6	3.9×10^9	0	0
M	6	4.7×10^6	0	0
O	6	1.5×10^7	2.5×10^5	0
P	6	1.6×10^7	0	7.3×10^6
Q	6	6.6×10^7	0	3.4×10^6
R	6	3.0×10^6	0	2.0×10^4
S	6	3.0×10^6	1.7×10^7	0
T	6	2.8×10^7	0	0

The mean heterotrophic bacterial count ranged from 3.0×10^6 cfu/ml as occurred in sample R, to 6.6×10^7 cfu/ml in sample Q while the mean coliform count ranged from a nil count/100 ml to 1.7×10^7 cfu/100 ml (sample S). Fungal counts ranged from a nil count/ml to 2.0×10^7 cfu/100 ml in swimming pool sample F. Fifteen out of the 20 swimming pools, yielded no growth of coliform bacteria. Similarly, fungi were not encountered in thirteen of the pools. However, growths of heterotrophic bacteria were encountered in all the twenty swimming pools studied

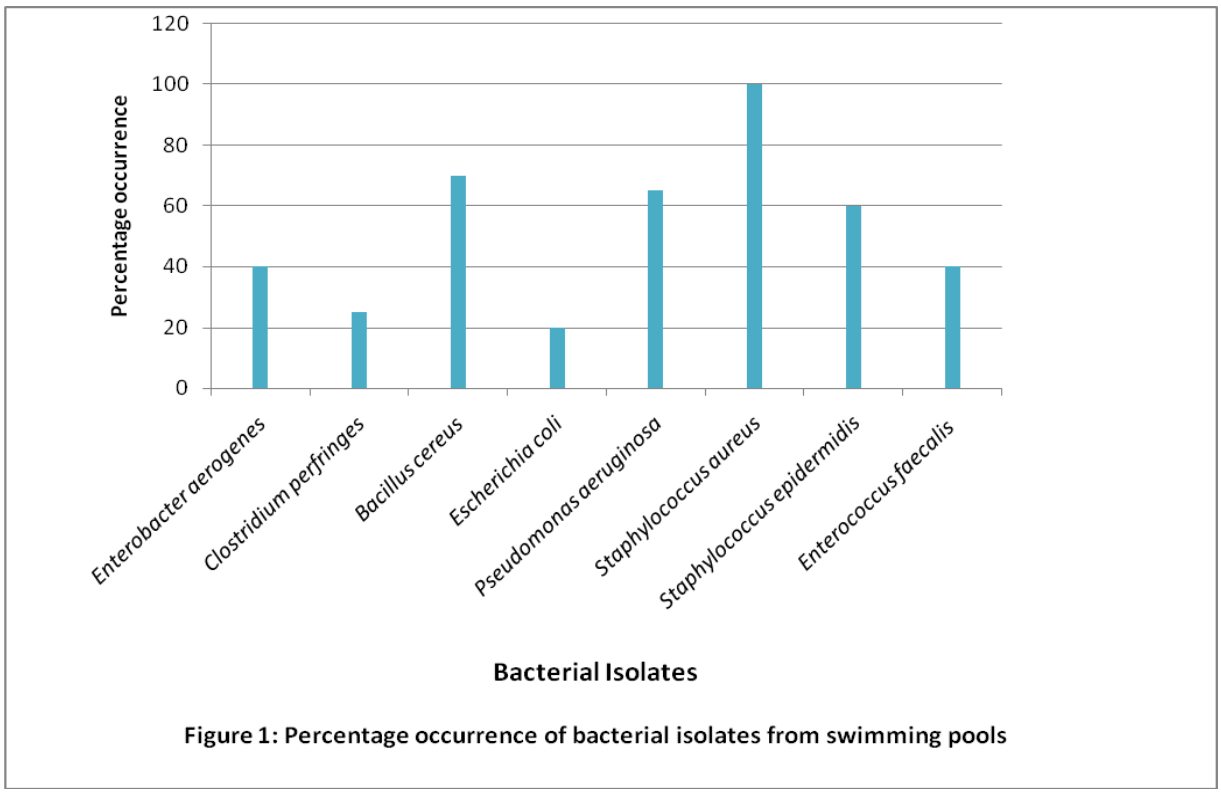

Figure 1: Percentage occurrence of bacterial isolates from swimming pools

Figure 1 shows the percentage frequencies of isolates obtained in this study: *Enterobacter aerogenes* (40%), *Clostridium perfringens* (25%), *Bacillus cereus* (70%), *Escherichia coli* (20%), *Pseudomonas aeruginosa* (65%), *Staphylococcus aureus* (100%), *Staphylococcus epidermidis* (60%) and *Enterococcus faecalis* (40%). This indicated that *S. aureus* had the highest percentage frequencies while the least was exhibited by *E. coli*.

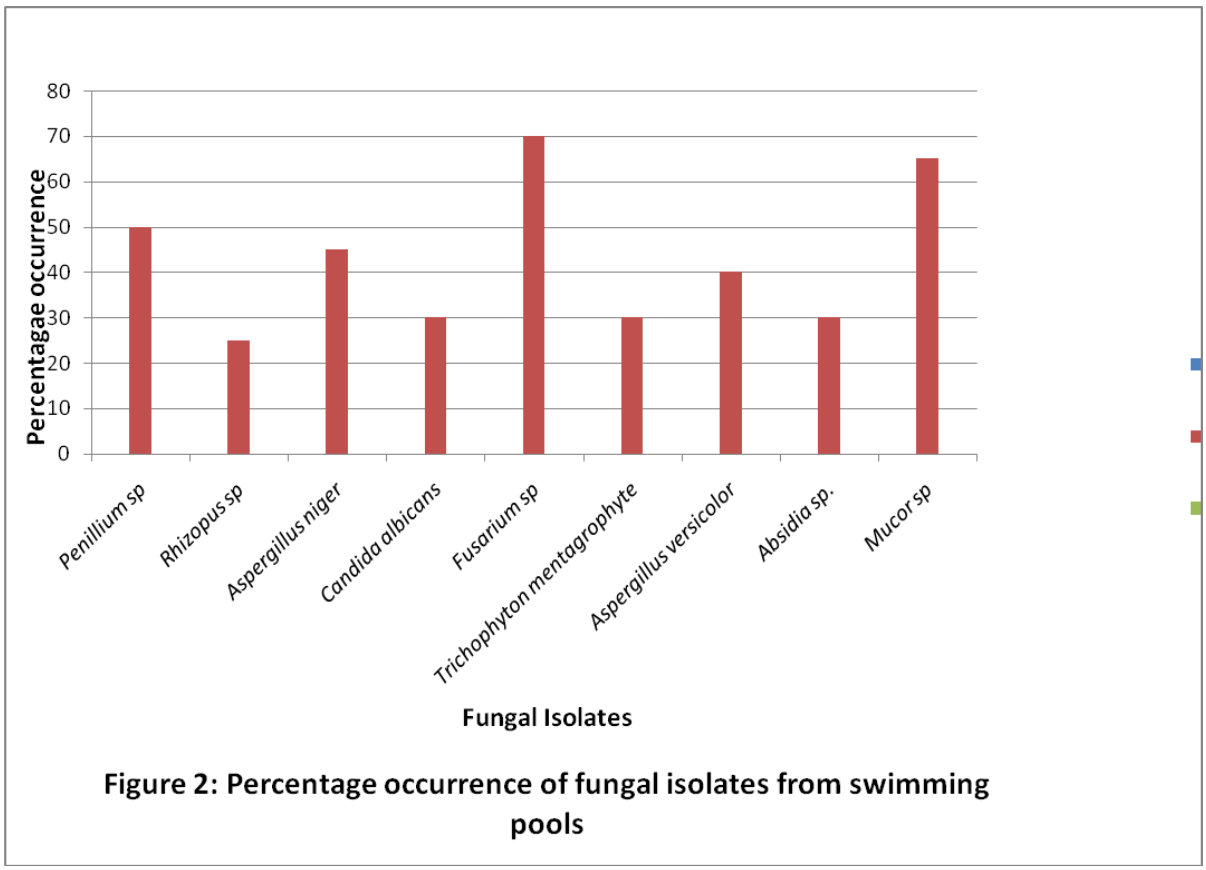

Figure 2: Percentage occurrence of fungal isolates from swimming pools

The percentage distribution of fungal isolates is given in Figure 2 below. This shows *Penicillium* sp (50%), *Rhizopus* (25%), *Aspergillus niger* (45%), *Candida albicans* (30%), *Fusarium* sp (70%), *Trichophyton mentagrophytes* (30%), *Aspergillus versicolor* (40%), *Absidia* sp (30%) and *Mucor* sp (65%).

References

Alcock, S.R. (1977). Acute otitis externa in divers working in the North Sea. A microbiological survey of seven saturation divers. *Journal of Hygiene*; 18: 359-409.

Alice, L.S. (1977) Principles of microbiology. 5th ed. London: CV Mosby: 661-710.

Alico R. K. and Dragonjac M. F. (2006). Evaluation of culture media for recovery of Staphylococcus aureus from swimming pools. *Applied Environmental. Microbioiogy*. 51, 699 702.

Anozie, S.O. and Antai, S.P. (1987). Incidence of infantile diarrhea due to enteropathogenic *E. coli* in Port Harcourt Metropolis. *Nigerian Journal of Microbiology*; 7: 66-70.

Beneke, E. S. and Rogers, A. L. (2005). Medical mycology manual. 3rd ed. Minneapolis: Burgers Publishing, pp 226.

Bernard, A. C., Michel O. S., Buchet, J. P., Hermans, C. G., Dumont, X. R. and Doyle, I. J. (2003). Lung hyper-permeability and asthma prevalence in schoolchildren: unexpected associations with the attendance at chlorinated swimming pools. *Occupy Environ Med* 60, 385-394

Bonde, G. J. (1985). Bacteriological methods of estimation of water pollution. Public Health Laboratory Services, New York, 15: 124-8.

Borchardt, J.A. and Walton, G. (1971). Water quality and treatment: a handbook of public water supply. American Water Works Association: 1-52.

Cabelli, V. J. (2003). Swimming--associated illness and recreational water quality criteria. *War. Sci. Technol*. 21, 13 21.

Cairncross, S. C., Curtis, D. G., Feahem, R. L. and Bradley, G. H. (2000). Evaluation for village water supply planning. Chichester: John Wiley and Sons, 220-55.

Cairns J. J. and Dickson, K. L. (2003). Biological methods for the assessment of water quality. American Water Works Association Bulletin, 13-5.

Carbell, U.J., Levin, M.A., Dufour, A. and McCabel, J. (1975). The development of criteria for recreational water. Progress in water technology. London: International Symposium on Discharge of Sewage from Sea Outfalls.: 37-47.

Collins, C. H. and Lyne, P. M. (1976): Microbiological methods. Great Britain: Butterworth, 151-79.

Cowan, S.T. and Steel, K.J. (1985): Manual for the identification of bacteria. Cambridge University Press, Verlage, New York, p. 502.

Cruickshank, R., Duguid, J.P., Marmion, B.P. and Swain, R.H.A. (1975): Medical microbiology: a guide to laboratory diagnosis and control of infections. 12th ed. Vol 1. Edinburgh: Churchhill Livingstone: 585pp.

Davies O. L. and Goldsmith P. L. (2005): Statistical Methods in Research and Production, 4th edn. Longman, London.

Dutka, B. J. (2008). Methods for microbiological analysis of waters, wastewaters and sediments, pp. II.44-11.47. Inland Waters Directorate, Ontario.

Erdinger, L. J., Kirsch, F. H. and Sonntag, H. G. (2001): Haloforms in spas. *Zentralbl Hyg Umweltmed*. 200, 309-317.

Erdinger, L. J., Kirsch, F. H. and Sonntag, H. G. (2005): Chlorate as an inorganic disinfection by product in swimming pools. *Zentralbl Hyg Umweltmed* 202, 61-75.

Esterman, A., Roder, D. M., Cameron, A. S., Robinson, B. S., Waiters, R. P., Lake, J. A. and Christy, P. E. (2004): Determinants of the microbiological characteristics of South Australian swimming pools. *Applied Environmental Microbiology*. 47, 325 328.

Fair, G. M., Gerger, J. C. and Okun, D. A. (2001): Water purification and waste water treatment disposal. New York: John Wiley and Sons, 192-31.

Favero, M. S. (2005): Microbiological indicators of health risks associated with swimming. *American Journal of Public Health* 75, 105I I054.

Favero, M. S., Drake, C. H. and Randall, B. (2004): Use of staphylococci as indicator of swimming pool pollution. *U.S. Public. Health Report*. 79, 61 70.

Galbraith, N. S. (2000): Infections associated with swimming pools. *Environmental Health* 15, 31-33.

Gray, R.D. (1969). Public health laboratory service on the bacteriological examination of water supplies. *Journal of Hygiene*; 62: 195-201.

Harrigan, M. G. and McCane, M. E. (1976): Laboratory methods in food and dairy microbiology. London: Academic Press, 33-200.

Holt, G. H., Krieg, N. R. Sneath, P. H. Staley, J. T. and Williams, S. T. (2004): Bergey's manual of determinative bacteriology. 9th ed. Baltimore: Williams and Wilkins, 787pp.

Itah, A. Y. and Opara, A. A. (2004). Enterotoxigenicity profile of Bacillus cereus strains contaminating canned foods in Calabar using the ligated rat ileal loop technique. *Journal of Medical Laboratory Sciences* 4: 91-6.

Itah, A. Y. and Opara, A. A. (2007). Enterotoxin production by Staphylococcus aureus strains contaminating canned foods in Calabar, Nigeria. *Journal of Science, Engineering and Technology* 4: 740-50.

Itah, A.Y. (1999). Ileal loop reactive Escherichia coli serotypes isolated from infantile diarrheal stools in Calabar, *Nigerian Journal Science Engineering and Technology*; 6: 1577-88.

Itah, A.Y. and Opara, A.A. (1997) Enterotoxin production by *Staphylococcus aureus* strains contaminating canned foods in Calabar, *Nigeria Journal Science, Engineering and Technology;* 4: 740-50.

Itah, A.Y., Etukudo, S.M. and Akpan, E.J. (1996): Bacteriological and chemical analysis of some rural water supplies in Calabar, Nigeria. *West African Journal Biology and Applied Chemistry*; 41: 1-10.

Lagerkvist, B. J., Bernard, A. B., Bergstrom, E. F., Holmstrom, K., and Karp, K. G. (2004). Pulmonary epithelial integrity in children: relationship to ambient ozone exposure and swimming pool attendance. *Environ Health Perspect* 112, 1768-1771

Mackereth, F. H., Heron, J. and Talling, J. F. (2003). Water analysis. Ambleside: Fresh Water Biological Association Scientific Publication, 43-69.

Mood, E. W. (2007). Bacterial indicators of water quality in swimming pools and their role. In Bacterial Indicators Health Hazards Associated with Water. ASTM STP635, pp. 239 246. Am. Soc. Test. Mater., Philadelphia, Pa.

Mossel, D. A. (2006). Microbiological markers for swimming associated infectious health hazards. *American Journal of Public Health*76, 297.

Okafor N. (1985): Aquatic and waste microbiology. Ibadan: Fourth Dimension Publishers, 198pp.

Oxoid (1985). Oxoid manual of dehydrated culture media, ingredients and other laboratory services. Basingstoke, England: Oxoid,.

Palmer, M. D., Lock, J. D. and Gowda, T. P. (2003). The use of bacteriological indicators for swimming water quality. Water Pollution Control 122, 14-18.

Prescott, L.M., Harley, J.P. and Klein DA. Microbiology (2002). 5th ed. Report 71. The Bacteriological examination of water supplies. 4th ed. London: HMSO, 1969. New York: McGraw-Hill Book: 1026pp.

Robinton, E.D. and Mood, E.W. (1986). Quantitative and qualitative appraisal of microbial pollution of water by swimmers: a preliminary report. *Jounal of Hygiene*; 31: 489-99.

Seyfried P. L., Tobin R. S., Brown N. E. and Ness P. F. (2005b) A prospective study of swimming-related illness, Morbidity and the microbiological quality of water. *American Journal of Public Health* 75, 1071 1074.

Seyfried, P. L., Tobin, R. S., Brown, N. E. and Ness, P. F. (2005a). A prospective study of swimming-related illness Swimming-associated health risk. *American Journal of Public Health* 75, 1068 1070.

Snydman, D. R. (1989). Foodborne diseases. In: Schaechter M, Medoff G, Schlessinger D, eds. Mechanism of microbial diseases. Baltimore: Williams and Wilkins, 785-6.

Stanier, R.Y., Ingraham, J.L., Wheelis, M.L. and Painter, P.R. (1987) General microbiology. 5th ed. London: Macmillan Press: 689p.

UNDP (2004). Water as a source of sickness. United Nation Development Programme Source: a short guide. 1: 11-3.

WHO (2001). Expert Committee on International Standards for Drinking Water. International standard for drinking water. 3rd ed. Geneva: WHO, 130-40.

Yoder, J. S., Blackburn, B. G., Craun, G. F., Hill, V. P., Levy, D. A. and Beach, M. J. (2002). Surveillance for waterborne disease outbreaks associated with recreational water – US, MMWR Surveill Summ 53, 1-22.