South Asian Journal of Research in Microbiology

9(3): 16-23, 2021; Article no.SAJRM.68063
ISSN: 2582-1989

Phenotypic Characterization of Bacteria Isolated from the Recreational Sites of Two Rivers in Orashi Region, Rivers State, Nigeria

T. Sampson[1*], L. K. Giami[2] and J. A. Okedike[1]

[1]Department of Microbiology, Rivers State University, PMB 5080, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.
[2]Department of Medical Laboratory Science, Rivers State University, PMB 5080, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author TS designed the study and wrote the protocol. Author JAO managed the literature searches, performed the statistical analysis and wrote the first draft of the manuscript. Author LKG and Author JAO managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SAJRM/2021/v9i330209
Editor(s):
(1) Dr. Luciana Furlaneto-Maia, Federal Technological University of Parana, Brazil.
Reviewers:
(1) Hernando José Bolívar Anillo, Universidad Simón Bolívar, Colombia.
(2) Shahrul Razid Sarbini, Universiti Putra Malaysia, Malaysia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/68063

Original Research Article

Received 27 February 2021
Accepted 04 May 2021
Published 07 May 2021

ABSTRACT

Recreational water bodies are water bodies used for recreational activities such as swimming, surfing, water skiing, water diving and sailing. They include rivers, lakes, beaches, spas and swimming pools. This work was therefore aimed at determining the bacteriological profile of recreational water bodies in Orashi region of Rivers State, Nigeria. Surface water samples were collected from two different sites (Orashi River, Mbiama and Sombreiro River, Ahoada) using standard microbiological methods. Upstream, midstream and downstream samples were collected for a period of three months at monthly interval. Standard plate counts were used for total heterotrophic and coliform bacterial counts using standard microbiological media. The total heterotrophic bacterial count ranged from $4.1X10^4$ to $9.5X10^4$ for Orashi River and $3.0X10^3$ to $4.0X10^3$ for Sombreiro River. A significant statistical difference ($p < 0.05$) however, existed between total heterotrophic bacterial counts of the samples collected from Orashi River, while no

**Corresponding author: Email: tonye.sampson@ust.edu.ng, tonye4good62@yahoo.com;*

statistical difference (p > 0.05) was observed in the total heterotrophic bacterial counts of samples from Sombreiro River. In the comparative analysis of the samples from the two water bodies, no statistical difference (p > 0.05) was recorded in the total coliform count in Orashi and Sombreiro Rivers. The phenotypic characterization identified the isolates to include *Staphylococcus* spp., *Klebsiella* spp., *Pseudomonas* spp., *Bacillus* spp., *Enterococcus* spp. and *Micrococcus* spp., with *Klebsiella pneumoniae* as the most occurring (26.1%). *Klebsiella pneumoniae* and *Staphylococcus aureus* are known for their pathogenic potentials, hence their presence in these recreational sites are of public health importance. Provision of standard recreational facilities in localities will however reduce the dependency on river sites for recreational activities, and as well prevent recreational associated illnesses.

Keywords: Bacteriological profile; Orashi region; phenotypic characterization; recreational activities.

1. INTRODUCTION

Water is an important resource for the sustenance of life and the environment [1]. Since the 20th century, the pollution of water bodies has been of great concern to environmentalists and the sources of these pollutions are due to poor and unhygienic practices of man and the release of untreated agricultural as well as industrial wastes [2-5]. Water is regarded as polluted when anthropogenic contaminants are present in it, when it is not suitable for drinking and recreational activities or when it does not support aquatic life [2].

Recreational water bodies which include rivers, swimming pools, lakes, beaches and oceans, contain microorganisms that can cause recreational water illnesses in humans that swim in these water bodies. This problem may be as a result of unawareness, absence of monitoring and/or improper disinfection techniques by the pool managers, or swimming in certain parts of other recreational water bodies where microbes are greater in number [6].

Recreational water illnesses (RWIs) are those illnesses caused by pathogens and chemicals present in water bodies where recreational activities are carried out. RWIs are acquired by swallowing, inhalation of aerosols or having contact with contaminated water in lakes, rivers, streams or improperly disinfected water in swimming pools. They may also be caused by chemicals in these water bodies. Some of the most reported infections associated with recreational water are; gastrointestinal, eye, ear, skin, respiratory, neurologic and wound infections [7].

Foreign and local tourists spend about two (2) billion days at coastal recreational sites yearly as recreational use of inland and marine waters increase in many countries [8]. According to Alexander and Heaven [9], exposure to recreational water hazards such as pathogenic microorganisms does not always result in infection, nor does the infection always result to clinical illness. It is the dose and physiological condition of the exposed individual that determines whether or not the pathogens are capable of causing illnesses. While most recreational water illnesses are mild, like self-limiting diarrhoea, some severe illnesses may also occur. Some viruses, bacteria and protozoa associated with recreational water illnesses may cause severe diseases that may be fatal [10].

The Orashi region of Rivers State, Nigeria is comprised of four local government areas; Abua/Odual, Ahoada East, Ahoada West and Ogba/Egbema/Ndoni and seven ethnic groups; Abua, Egbema, Ekpeye, Engenni, Ndoni, Odual and Ogba. The Orashi region houses two major rivers, the River Orashi and River Sombreiro which accounts for the region's occupation in fishing and diving (including sand diving). The region is known to possess crude oil and may perhaps be regarded as one of the largest oil and gas producing region in Nigeria. The region's vegetation is a tropical rain forest with an annual rainfall of about 180 centimeters and an average temperature of 26^{o}C [11].

Due to the absence of synthetic recreational water bodies in majority of the communities in this region, most of the inhabitants depend on the use of designated sites in the river for such purposes, thereby exposing them to the risk of recreational water associated illnesses. Earlier researchers [12-13] on the river water quality focused majorly on the portability and domestic importance of the water samples from this region. Little or not much has been reported regarding the bacteriological quality of water collected from stations used for traditional recreational purposes in this region. This present study was therefore targeted at evaluating the bacteriological quality of traditional recreational water sites from two different rivers (Orashi River, Mbiama Community and Sombreiro River, Ahoada) in Orashi region, Rivers State Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area and Duration

The study was conducted in the Orashi Region of Rivers State, Nigeria. Water samples were collected from two water bodies (Orashi River, Mbiama and Sombreiro River, Ahoada) used for recreational activities. The study was conducted for a period of 6 months from May to November, 2020.

2.2 Method of Collection of Recreational Water Samples

Water samples were collected from two water bodies (Orashi River, Mbiama and Sombreiro River, Ahoada) used for recreational activities. Surface water samples were collected from upstream, midstream and downstream of the two recreational water bodies using sterile sample bottles. The upstream represented the stations designated or used mainly for swimming and other recreational activities; the midstream was 500 meters away from the upstream; the downstream was a region 1000 meters away from the upstream, along the direction of flow of the river.

2.3 Microbiological Analysis

2.3.1 Serial dilution

A serial 10-fold dilution was carried out for each water sample. From the sample, 1ml was transferred into a test tube containing 9ml normal saline and repeated to obtain a dilution of up to 10^{-2} used in the study.

2.3.2 Inoculation and incubation

For the quantitative analysis, 0.1ml of the dilution was inoculated into Petri dishes containing appropriate bacteriological media such as nutrient agar for heterotrophic bacteria, MacConkey agar for coliforms and mannitol salt agar for *Staphylococcus* species characterization. The plates were incubated at 37℃ for 24 to 48 hours after which the plates were examined for growth. Distinct colonies were sub-cultured aseptically on nutrient agar plates and incubated at 37℃ for 24hours in order to obtain pure cultures of the isolates.

2.3.3 Characterization and identification of isolates

The isolates were identified based on microscopy and their cultural as well as biochemical attributes. The bacterial species were identified phenotypically by examining their cultural identities based standard microbiological procedures, as reported by Sampson et al. [14] and Akani et al. [15]. This involved spreading aliquots of the serially diluted samples over the surface of solid agar plates and incubated at 37ºC for 24-48 hours. Bacterial growths following incubation of the samples were subjected to microscopy, cultural characterization, alongside standard biochemical test procedures [16], to identify the bacterial isolates present in the water samples.

2.4 Hemolytic Assay on Blood Agar

This test is used to differentiate between bacterial species that produce extracellular enzymes (hemolysins) that lyse red blood cells. This destruction of the red blood cells is determined by the pattern of hemolysis produced by the isolate on blood agar.

Blood agar was prepared following standard microbiological procedures for the preparation of blood agar and allowed to cool. Using sterile wire loop, a colony of the test isolate was inoculated unto the freshly prepared blood agar aseptically and incubated at 37oC for 24 hours, then observed for hemolytic pattern. A greenish-grey to brownish colour around the colony indicated alpha hemolysis (partial lysis of the red blood cells) while the clearance of blood on the medium indicated beta hemolysis (the complete lysis of the red blood cells). Gamma hemolysis is represented by no change on the growth medium, indicating no lysis of red blood cells.

2.5 Statistical Analysis

Statistical analysis of the data obtained was carried out on the total heterotrophic bacterial count and coliform count of Orashi and Sombreiro Rivers. Analysis of variance (ANOVA) was used to check for significant difference between the upstream, midstream and downstream of the two rivers for total heterotrophic bacterial and coliform counts.

3. RESULTS

The result of the total heterotrophic bacterial count for Orashi and Sombreiro Rivers is shown in Table 1

The total Heterotrophic bacterial count for Orashi River ranged from $4.1x10^4$ to $9.5x10^4$ with the upstream having the highest count of $9.5x10^4$, followed by the Midstream ($6.0x10^4$) and downstream as the least ($4.1x10^4$cfu/ml). The mean THBC for Orashi River was $6.5x10^4$±2.7. Statistical analysis, showed that there was no significant difference ($p > 0.05$) in the total heterotrophic bacterial count for Orashi River. The total heterotrophic bacterial count for Sombreiro River ranged from $3.0x10^3$ to $4.0x10^3$. Sombreiro upstream had the highest THBC of $4.0x10^3$cfu/ml, followed by the Midstream ($3.4x10^3$), and $3.0x10^3$ was recorded for downstream. The mean THBC for Sombreiro River was $3.5x10^3$±0.5. The ANOVA showed a significant difference ($p < 0.05$) in the total heterotrophic bacterial count for Sombreiro River.

The result for the total coliform count for Orashi and Sombreiro Rivers is shown in Table 1. Orashi Upstream recorded the highest coliform count of $4.8x10^3$cfu/ml, no coliform count was recorded at Orashi midstream whereas Orashi downstream had the least ($4.2x10^3$cfu/ml). The mean coliform count of Orashi River was $3.0x10^3$±0.4. For the total coliform count, Sombreiro upstream recorded the highest number of count with $3.4x10^3$cfu/ml, followed by Sombreiro midstream with $3.1x10^3$cfu/ml. Orashi downstream had no coliform count. The mean coliform count for Sombreiro River was $2.2x10^3$±0.2. The ANOVA, $p>0.05$ showed that there was no significant difference in the total coliform counts for Orashi and Sombreiro Rivers. The results obtained from the study showed that there were more heterotrophic bacteria than coliform in both rivers with Orashi River having more heterotrophic bacterial and coliform counts than the Sombreiro River as shown in Fig. 1.

The phenotypic identification of the isolates revealed that *Klebsiella pneumoniae* was the most occurring with a percentage frequency of 26.1%, followed by *Bacillus spp*. and *Staphylococcus spp.* with 21.7%, *Pseudomonas spp*. with 17.4%, *Enterococcus spp*. with 8.7% and *Micrococcus spp*. with the least occurrence (4.4%) as shown in Fig. 2.

The hemolytic pattern of the isolates is shown in Table 2. *Staphylococcus spp*., *Bacillus spp.* and *Enterococcus spp.* showed gamma hemolysis whereas, *Klebsiella spp*. and *Micrococcus spp*. showed alpha hemolysis.

4. DISCUSSION

Recreational water is any water body used for recreational activities such as diving, swimming, surfing, bathing or sailing. These water bodies include; Rivers, lakes, beaches, swimming pools and spas. Recreational activities are great sources of fun, exercise and relaxation. However, with these activities come the risk of recreational water illnesses or injuries [17-19].

Recreational water users can be exposed to a range of pathogens, those naturally present in water as well as pathogens introduced by other users of recreational water. Faecal contamination is responsible for a majority of recreational water illnesses. The sources of faecal contamination include sewage, surface runoff, domestic animals, wildlife, and human faeces or leftover particles that get into the water while swimming [20]. The hazards associated

Table 1. Total heterotrophic bacterial count

Sample ID	Sample description	THBC (cfu/ml)	TCC (cfu/ml)
O1	ORASHI UPSTREAM	$9.5X10^4$	$4.8X10^3$
O2	ORASHI MIDSTREAM	$6.0X10^4$	-
O3	ORASHI DOWNSTREAM	$4.1X10^4$	$4.2X10^3$
MEAN		**$6.5X10^4$±2.7**	**$3.0X10^3$±0.4**
S1	SOMBREIRO UPSTREAM	$4.0X10^3$	$3.4X10^3$
S2	SOMBREIRO MIDSTREAM	$3.4X10^3$	$3.1X10^3$
S3	SOMBREIRO DOWNSTREAM	$3.0X10^3$	-
MEAN		**$3.5X10^3$±0.5**	**$2.2X10^3$±0.2**
	WHO STANDARD	1.0×10^2	0/100 ml

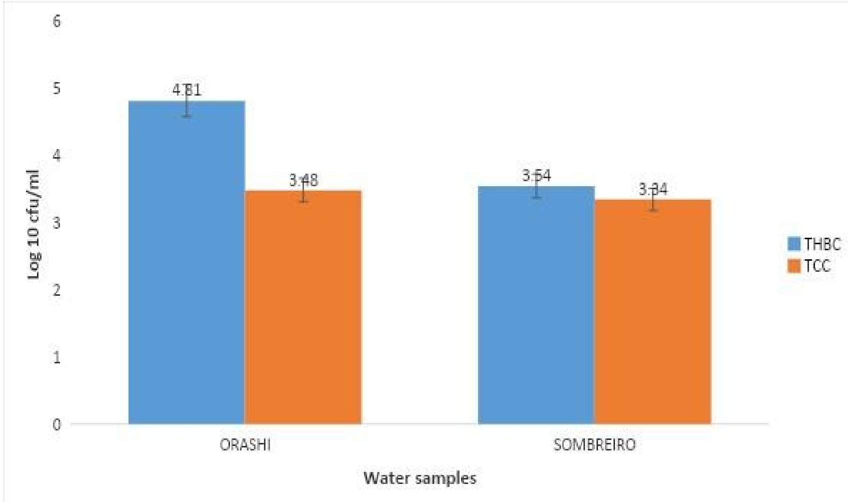

Fig. 1. Comparison of the bacterial population in Orashi and Sombreiro Rivers

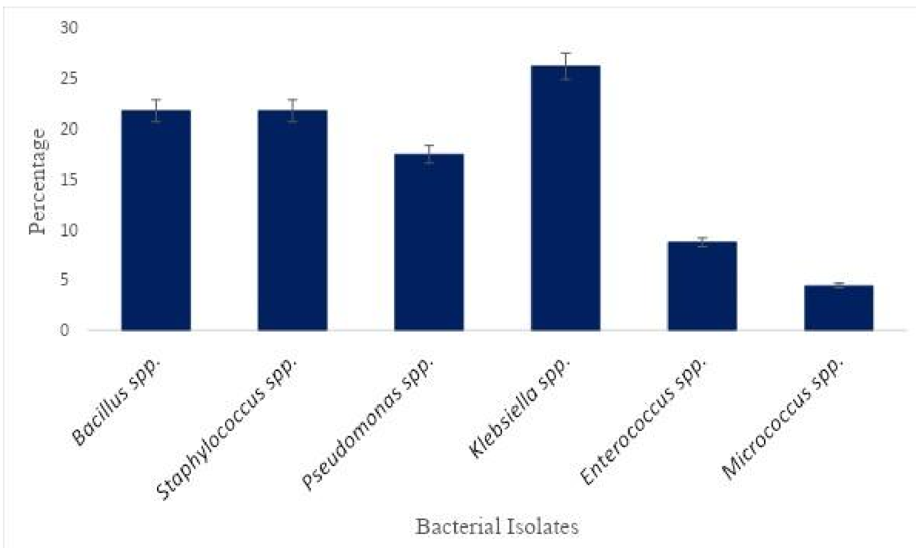

Fig. 2. Frequency distribution of bacterial isolate

Table 2. Hemolytic characteristics of the isolates

Isolate	Hemolysis Pattern
Staphylococcus spp.	Gamma Hemolysis
Klebsiella spp.	Alpha Hemolysis
Bacillus spp.	Gamma Hemolysis
Enterococcus spp.	Gamma Hemolysis
Micrococcus spp.	Alpha Hemolysis

with recreational water bodies differ between points in the water. This is particularly true for swimming pools, rivers and lakes as they are more vulnerable to water contamination than the coastal beaches where contaminants are more rapidly diluted and dispersed by currents and large volumes of water [6].

From the study, it was observed that the bacterial counts at the three different sampling points (Upstream, Midstream, Downstream) varied for both recreational sites (Table 1) with the upstream, (points mainly used for recreational events) having more bacterial counts for both Orashi and Sombreiro rivers, and downstream having the least. This may be due to the difference in sampling points [6] or as a result of more human activities taking place at the upstream of the water bodies. This result corresponds with the findings of [21], where they reported higher bacterial counts at the upstream of Thenkasi River and suggested mass visiting of the water body to be responsible for the higher bacterial load at the upstream. Thus, implying that the upstream may be associated with higher risk of recreational water illnesses.

Fig. 1 showed the comparison between total heterotrophic bacterial count and total coliform count in Orashi and Sombreiro rivers. It revealed that the total heterotrophic count was higher than the total coliform count for the two rivers with Orashi River having more heterotrophic bacterial count than Sombreiro River. Analysis of variance carried out on the microbial counts showed that there was no significant difference at probability level of 0.05% ($p>0.05$) between Orashi and Sombreiro Rivers.

This result is in agreement with the work of Amanidaz *et. al*., [22]. An increase in heterotrophic bacteria in a water body may be due to problems with treatment, effect of microbial growth in the flow system or the presence of biofilm and it may also increase the risk of gastroenteritis [23-24]. Fig. 2 revealed that six [6] bacterial species were isolated and identified to be *Staphylococcus* spp*.,* *Klebsiella* spp., *Pseudomonas* spp., *Bacillus* spp., *Enterococcus* spp. and *Micrococcus* spp. The bacterial species isolated from this region were not entirely the same with the work of earlier researchers [25-26] that relied on *Escherichia coli* and *Enterococcus* spp. in evaluating recreational water quality. However, similar bacterial species have been isolated from public swimming pools located in Port Harcourt, Rivers State, Nigeria [27]. This indicates that recreational water quality is regularly threatened by both enteric and non-enteric heterotrophic bacterial species. These organisms have been widely reported for their public health importance [28-29], as they cause different disease conditions in humans.

Enterococci are Gram positive cocci that occur in pairs as diplococci or as short chains and are thus difficult to distinguish from streptococci using only physiological or phenotypic characteristics. Enterococci are mostly found in human and animal faeces. The presence of enterococci in water indicates faecal contamination and possibly suggests the presence of enteric pathogens [30]. Fig. 2 further revealed that *Klebsiella* spp. was the most occurring isolate (26.1%) in these recreational sites.

Staphylococcus spp. and *Bacillus* spp. had the second highest percentage occurrence of 21.7%, followed by *Pseudomonas* spp. (17.4%), *Enterococcus* spp. (8.7%) and *Micrococcus* spp. (4.4%) as the least. The bacterial population seen in this study have been widely reported as potential human pathogens capable of causing different disease in humans, including swimmers ear as well as skin infections and have been linked with some major outbreaks [31].

The presence of these bacterial genera is attributable to the various human activities taking place at the river, including sand diving. Other possible sources could be from sewage and domestic waste disposal practices found around these locations. The pathogens could therefore be from point pollution from the different swimmers, or from nearby sources, dispersed by river current to these locations [6].

To ensure the safety of the water for recreational purposes, the entry and proliferation of these pathogens should therefore be prevented and controlled. Measures to ensure this safety would include stoppage of waste, including sewage disposal into river water bodies, regulation of sand diving and dredging activities as well as educating the rural dwellers and swimmers on the importance personal hygiene among swimmers.

According to Encyclopedia [32], hemolysis is the breakdown of red blood cells by a bacterial protein known as hemolysin. These compounds are responsible for membrane damage, cell lysis and destruction of neighboring cells and tissues in order to provide nutrients, mainly iron, for the toxin-producing bacteria [33]. Iron is an essential element for living organisms as it plays catalytic, regulatory and structural roles in the cell and many authors have also pointed out its important role as a virulence regulator for commensal and pathogenic microorganisms [34-35]. The result from the study revealed that some of the isolates were alpha hemolytic. This indicates that these organisms, even though they are environmental isolates, may pose heath treats to individuals exposed to them at these recreational sites.

5. CONCLUSION

The study showed that the points used for recreational activities of both the Orashi and Sombreiro rivers had more heterotrophic bacteria and coliform counts than the other sites, and thus may be associated with higher risk of recreational water illness.

The study has also shown that *Klebsiella pneumoniae* was the most prevalent isolate in this area. *Klebsiella,* alongside *Staphylococcus spp*. are known for their pathogenic potentials, hence their presence in these recreational sites are of public health importance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dikio ED. Water quality evaluation of Vaal River, Sharpeville and Bedworth lakes in the Vaal Region of South Africa. Res. J. Appl. Sci. Eng. Tech. 2010;2(6):574-579.
2. Ekubo AT, Abowei JFN. Aspects of Aquatic pollution in Nigeria. Research Journal of Environmental and Earth science. 2011;3(6):673-693.
3. Dulo SO. Determination of some physicochemical parameters of the Nairobi River, Kenya. J. Appl. Sci. Environ. Manage. 2008; 12(1):57-6.
4. Adesuyi AA, Njoku KL, Akinola MO. Assessment of Heavy Metals Pollution in Soils and Vegetation around Selected Industries in Lagos State, Nigeria. Journal of Geoscience and Environment Protection. 2015;3:11-10.
5. Dan UI, Ekpo FE, Etim DE. Influence of heavy metals pollution in borehole water collected within abandoned battery industry, Essien Udim, Nigeria. J.Environ. Sci. Water Resour. 2013;2(1):22-26.
6. Ministry for the Environment. Environment New Zealand. Wellington: Ministry for the Environment; 2007
7. CDC. Healthy Swimming; 2017. Available:www.cdc.gov/healthywater/swimming/rwi. Accessed July 31, 2019. 19:23.
8. Shuval H. Estimating the global burden of thalassogenic diseases: human infectious Diseases caused by wastewater pollution of the Marine environment. Journal of water and health. 2003;1(2):53-64.
9. Alexander LM, Heaven A. Health Risks Associated with Exposure to Seawater contaminated with Sewage: the Blackpool Beach Survey 1990. Environmental Epidemiology Research Unit, Lancaster University, Lancaster, Uk. 1991;67.
10. WHO. Water recreation and disease. Plausibility of Associated Infections: Acute effects, Sequelae and Mortality by Katty Pond. Published by IWA publishing, London, Uk; 2005.
11. Daminabo D. School Dropout and Educational Development in Orashi Region, Rivers State, Nigeria. Social Research. 2019;3:102-119.
12. Seiyaboh EI, Angaye TCN, Okogbue BC. Physicochemical quality assessment of River Orashi in Eastern Niger Delta of Nigeria. Journal of Environmental Treatment Techniques. 2016;4(4):143-148.
13. Seiyaboh EI, Kolawole EP. Diversity and Levels of Bacteriological Contamination in Orashi River, Mbiama Community, River State, Nigeria. Journal of Advances in Microbiology. 2017; 4(3):1-6.
14. Sampson T, Ogugbue CJ, Okpokwasili GC. Determination of the Bacterial Community Structure in a Crude Oilinundated Tropical Soil Using Next Generation Sequencing Technique. Journal of Advances in Microbiology. 2018;11(1):1-18.13.
15. Akani NP, Hakam IO, Sampson T. Prevalence and antibiogram of Pseudomonas aeruginosa isolated from West African Mud Creeper (*Tympanotonus fuscatus*). South Asian Journal of Research in Microbiology. 2019; 5(2):1- 8.14.
16. Holt JG, Kreig NR, Sneath PHA, Stanley JT, Willams ST. Bergey's manual of determinative bacteriology-Ninth Edition. Lippincott, Williams & Wilkins, Baltimore; 1994.
17. Agbagwa OE, Young-Harry WM. Health Implications of some Swimming Pools Located in Port Harcourt, Nigeria. Public Health Research. 2012;2(6):190-196.
18. EPA. Retrieved from; 2018. Available:www.epa.gov/report-environment/recreational waters#note1.

Retrieved May 24th, 2020. 18:15.
19. National Research Council. Clean Coastal Waters: Understanding and Reducing the Effects of Nutrient Pollution. Washington DC: National Academics Press; 2000.
20. Fewtrell L, Kay D. Recreational water and Infection: a review of recent findings. Current Environmental Health Report. 2015;2:85-94.
21. Vignesh S, Dahms P, Rajendran A, Kim B, James RA. Microbial Effects on Geochemical Parameters in a Tropical River Basin. Environ. Process. 2015;2:125-144.
22. Amanidaz N, Zafarzadeh A, Mahvi AH. The Interaction between Heterotrophic Bacteria and Coliform, Fecal Coliform, Fecal Streptococci Bacteria in the Water Supply Networks. Iranian Journal of Public Health. 2015;44(12):1685-1692.
23. Ainsworth R, Water S, WHO. Safe Piped Water: Managing Microbial water Quality in Piped Distribution Systems. The International Water Association Publishing. 2004;25-130.
24. Prevost M, Rompre A, Coallier J, Servais P, Laurent P, Clement B, et al. Suspended Bacterial Biomass and Activity in Full-Scale Drinking Water Distribution Systems: Impact of water Treatment. Water Research. 1998;32:1393-1406.
25. Staradumskyte D, Paulauskas A. Indicators of Microbial Drinking and Recreational Water Quality. Biologija. 2012;58(1).
26. Adeniji OO, Sibanda T, Okoh AI. Recreational Water Quality Status of the Kidd's Beach as determined by its Physicochemical and Bacteriological Quality Parameters. Heliyon. 2019;5(6): e01893.
27. Agbagwa OE, Young-Harry WM. Health Implications of Some Public Swimming Pools Located in Port Harcourt, Nigeria. Public Health Research. 2012;2(6):190-196.
28. Ahmad A, Dada A, Usup CG, Heng LY. Validation of the Enterococci indicator for bacteriological quality monitoring of beaches in Malaysia using a multivariate approach. Springer Plus. 2013;425:1–18.
29. Elmanama AA, Fahd MI, Afifi S, Abdallah S, Bahr S. Microbiological beach sand quality in Gaza Strip in comparison to seawater quality. Environ. Res. 2005;1:1–10.
30. Oshiro RK. Enterococci in Water by Membrane-Enterococcus-Esculin Iron Agar (mE-EIA). U.S. Environmental Protection Agency. 2002;1106:1.
31. Hlavsa MC, Cikesh BL, Roberts VA. Outbreaks Associated with Treated Recreational Water-United States, 2000-2014. MMWR Morb Mortal Wkly Rep. 2018;67:547-551.
32. Encyclopedia. Blood Agar, Hemolysis and Hemolytic Reactions; 2019. Available:www.encyclopedia.com/science/encyclopedia-almanacs-transcripts-and-map/blood-agar-hem olyisis-and-hemolytic-reactions.
Accessed April 10, 2021. 13:04.
33. Bullen JJ, Rogers HJ, Spalding PB, Ward CG. Iron and infection: the heart of the matter. FEMS Immunol. Med. Microbiol. 2005;43:325–330.
34. Litwin CM, Calderwood SB. Role of iron in regulation of virulence genes. Clin. Microbiol. Rev. 1993;6:137–149.
35. Zughaier SM, Cornelis P. Editorial: role of iron in bacterial pathogenesis. Front. Cell. Infect. Microbiol. 2018;8:34.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/68063