ENUMERATION AND CHARACTERIZATION OF BACTERIA ISOLATED FROM SWIMMING POOL WATER OBTAINED FROM SELECTED HOTELS AND RECREATIONAL OUTLET IN OFFA METROPOLIS, KWARA STATE, NIGERIA

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ABSTRACT

Swimming pool water serves people in many beneficial ways and the risk of acquiring diseases from the contaminated pool water is as a result of pathogens excreted into the water by bathers among others. This study is aim at assessing the bacteriological quality of swimming pool water. Plate Counts methods and Most Probable Numbers technique were used to determine viable count, and total coliform and feacal coliform counts. Cultural and biochemical characterization were carried out on the isolates. Antibiotics susceptibility test was carried out on the Escherichia coli isolated from the swimming pool water samples obtained at the only recreational outlet studied with highest number of E. coli (4). Contamination of water from the studied swimming pool was as a result of the bathers as well as the inadequate treatment of the pool most especially the recreational outlet swimming pool. Adequate and prompt treatment of the swimming pool water should be encouraged. Pseudomonas sp was found to be more predominant with 31.6% followed by Staphylococcus aureus, Bacillus sp, feacal Streptoccus and E. coli with percentage of occurrence 26.6%, 22.8%, 8.9% and 6.3% respectively. The least percentage of occurrence was observed in Klebsiella, sp with percentage of occurrence of 3.8%.

Keyword: Swimming pool, Contaminations, Microbial Growth, Bathers.

INTRODUCTION

The increase in number of swimming pools available or recreational purposes and other beneficial gains is quite encouraged nowadays. Majority of people in the society go swimming in the pool not only for recreational activities but also for sporting and rehabilitative treatment (Amala and Aleru, 2016). According to Akeju and Awojobi (2015), over 301 million swimming visits were made in the United state by the populace of different age group. A wide range of bacteria can be found in swimming pool and other recreational water environments which may be introduced in a number of ways. Pool water can be contaminated directly by the infected bathers and such contaminations may result into infections in other users who come in contact with such pool water (Ajadi and Thomas, 2017).

Recreational water may be contaminated by excreation from user in the form of vomits, urine and diarrhea directly from the bathers. Also, contaminants can be microbial growth on the filter bed and by external sources water borne pollutants (Yedeme *et al.*, 2017). According to the World Health Organization Guideline for Safe Recreational Water (2006), monitoring of recreational water for deviations from microbial water quality standard is important to public health. This will decrease the incidence of recreational water diseases. Adult swimmers take in 16ml of the pool water on every swimming event and swimmers of the age fifteen and below consume 37ml (Akeju and Awojobi, 2015). Some of the microorganisms associated with the skin, eye, ear and gastrointestinal tract infection are most commonly infectious agents that bring about recreational water diseases (Kiyohara *et al.*, 2010). The main bacterial pathogens causing gastrointestinal disease include *Shigella* species, *Salmonella* species, *Campylobacter* species and *Escherichia coli* for example E. coil 0157:H7.

Aim.

The aim of this study was to assess the bacteriological quality of the swimming pool water collected from selected Hotels and Recreational outlet in Offa Metropolis, Kwara State.

Materials and Methods

Study Area

The study area was Offa which is the commercial centre of Kwara State and the headquarter of Ibolo distinct in Southern part of the state. Based on the commercial activities and availability of quite number of educational institutions, standard hotels and recreational centres with swimming pools are available in Offa town.**Sample Collection**

Sample collection was carried out separately from Avalon hotel swimming pool, Awrab suits and hotel swimming pool and Omigreen recreational center based on the method used by Amale and Aleru (2016). Two hundred milliliter (200ml) wide mouth sterile plastic container was used to collect sample of swimming pool water at the depth of about 30cm for three different points of each pool. The sampling periods were between 10:00am and 2:00pm of each sampling day. The samples were taken immediately to the microbiology laboratory of the Department of Biological Sciences, Federal Polytechnic, Offa.

Bacterial Media Preparation

The laboratory media for bacterial growth were reconstituted and sterized according to the manufacturer's descriptions. The media used for this study include Centrimide agar for *Pseudomonas* count, Tryptic soy agar for *Bacilli* count, Manitol Salt agar for Staphylococcal count and Nutrient agar for sub culturing prior to gram staining process.s

Enumeration of Table Viable Count

Bacterial analysis of pool water samples was carried out by pour plate methods as described by Ajadi and Thomas (2017). Nutrient agar, Mannitol Salt agar, Centrimide agar, Tryptic soy agar were used for the enumeration of viable counts using standard pour plate technique.

One milliliters (1ml) of serially diluted of each water sample was inoculated into already sterilized Petri dish and the sterile medium was poured on the inoculum aseptically. This was gently rocked back and forth and allowed to solidify. This was repeated for all media used. All the plates were incubated at 37^0C for 24 hours. The isolates from all the media were sub-cultured on sterile nutrient agar plates for morphological and biochemical assessments.

Enumeration of Feacal Bacterial Indicators

The Total Coliform Bacteria and Feacal Coliform Bacteria were determined by Most Probable Number (MPN) using the multiple tube fermentation test as described by Oyedun, *et al.,* (2016) The multiple tube fermentation test was performed in three steps using lactose broth medium. The steps include: (1) Presumptive test (2) Confirmatory test and (3) Completed test.

The MacConkey broth was weighed and dispensed in distilled water based on the manufacturer's description to prepare both double and single strength media. Ten milliliter of the double strength broth was dispensed separately in five tubes and the single strength broth in 10 tubes. The inner vial (Durham's tube) was inserted in each of the fifteen tubes in inverted position and they were examined to be full of the medium with no air bubble. All the fifteen tubes were sterilized inside the autoclave at 121 ^{o}C for 15 minutes.

Presumptive test: After sterilization of the medium, the tubes and content were allowed to cool down and inoculated with water sample. Ten milliliter each of water sample was inoculated in the five tubes containing 10ml double strength broth using sterile pipette. One ml each of water sample was added to 5 out of 10 tubes containing single strength broth while 0.1ml each of water sample was inoculated into the remaining 5 tubes. All the tubes were incubated at 37 ^{o}C for 24 hours. The number of positive tubes (the tubes that showed both acid production as a result of colour change in the medium and gas production as trapped with inverted Durham's tubes) were counted and recorded as MPN/100ml of the sample. This procedure of presumptive test was repeated to enumerate the feacal coliform (thermotolerant) bacteria of each water sample by incubating the tubes at elevated temperature of 44.5 ^{o}C for 24 hours. The results were then recorded as MPN/100ml of the sample.

Confirmatory Test: Sterilized wire loop was used to transfer 2 drops of feacal coliform culture medium from each of the fermentative tubes with presumptive positive result to:

1. Three (3) ml of lactose broth inside the fermentative tube containing inverted Durham's tubes.
2. Nutrient agar slant.

3. Three (3) ml of tryptone water. The inoculated lactose broth fermentation tube and the tryptone water were incubated at 44.5 ^{o}C for 24hours while the inoculated agar slant was incubated at 37 ^{o}C for 24 hours. Gram stained preparation was made from the slant and viewed under the microscope to reveal Gram-negative non-spore forming rods. Kovacs reagent (0.1 ml) was added to the tryptone water after incubation and was gently mixed. The presence of indole was indicated by a red colour forming a film over the aqueous phase of the medium.

Completed test: The inoculum from each positive tube of the confirmatory test was streaked on plates of Eosin methylene blue (EMB) agar which had previously prepared according to the manufacturer description. The plates were incubated at 44.5 ^{o}C for 24 hours.

Cultural Characteristics

The cultural characteristics of all the isolated bacteria and the number of the colonies were determined. The total colony count were multiplied with the dilution factor. The bacterial counts were expressed as colony forming units per ml (cfu/ml). The observed cultural characteristics which were used to classify the isolates were pigmentation edge, shape, elevation, consistency and surface.

Biochemical Characterization

Each of the isolates was subjected to various biochemical tests such as Citrate, Indole, Urease, Methylred, Voger proskauer, CSoagulase and Oxidase.

Antibiotic Susceptibility test.

The antibiotic susceptibility test was carried out on the isolates of the *Escherichia coli* being the indicator of the prsesence feacal pathogens.This was determined using Kirby-Bauer disk diffusion technique as reported by Akeju and Awojobi (2015).

Results and Discussion

The isolated bacterial species include; *Klebsiella* sp*, Staphylococcus aureus, Pseduomonas* sp*, Bacillus* sp, faecal *Streptococcus, Escherichic coli.* It was observed that the highest occurrence of colony was recorded for Pseudomonas, followed by *Staphylococcus sp, Bacilli* sp*, Streptococcus* sp *and Escherichia coli* while the least number of colony was recorded for *Kubsiella* sp with respective percentage of occurrence as shown in table 1.

This result was corroborated with the findings of Ali shtayeh *et al*., (2002). Table 1 also revealed that some of the isolated bacteria are enteric pathogen which can lead to gastroenteritis. Also, some normal flora were equally isolated and the findings of Ajadi and Thomas (2017) justify the findings of this research.

The result of various biochemical tests carried out and the cultural characteristics of each of the isolates were shown in the table 3.

Table 1: Frequency and Percentage Distribution of the Bacterial Isolates from the Swimming Pool water samples

Probable Bacteria	Frequency	Percentage Occurrence (%)
Bacillus sp	18	22.8
Escherichia coli	5	6.3
Feacal *Streptococcus*	7	8.9
Staphylococcus sp	21	26.6
Klebsella	3	3.8
Pseudomonas aeruginosa	25	31.6
Total	79	100

Table 2 revealed the results of the antibiotics susceptibility tests carried out on the *E. coli* isolates obtained from the swimming pool water collected from the recreational outlet. The results obtained corroborate with the findings of Ajadi and Thomas (2017) where Streptomycin, Ampicillin, Nalicidic acid were mostly inhibit the growth of *Escherichia coli* isolated from swimming pool water.

The highest zone of inhibition was observed in the Tetracycline with 38.1mm while the least was observed with chloramphenicol with 7.4mm zone of inhibition.

Table2: Antibiotic susceptibility Tests on E.coh isolated from swimming pool water.

Antibiotics	Code	Concentration Mg	Zone of inhibition (mm)
Streptomycin	STR	25	33.2
Tetracycline	TET	25	38.1
Ampicillin	AMP	25	28.3
Nalicidic acid	NAL	25	32.6
Penicillin	PEN	25	8.1
Chloramphemicol	CHL	25	7.4

The result of various biochemical tests carried out and the cultural characteristics of each of the isolates were shown in table 3.

Table 3: Morphological and Biochemical Characteristics of the Isolates

Tentative organisms	Elevation	Surface	Cons	Pig	Gram staining	Shape	Spore staining	Motility	Catalase	Oxidase	Glucose	Indole	Coag	MR	VP	Cit	Ure
E. coli	Raised	Smooth	Mucoid	Greyish white	- ve	Short rod	- ve	+ ve	+ve	-ve	+ve	+ve	-ve	+ ve	-ve	-ve	-ve
Bacillus	Flat	Rough	Mucoid	Yellow	+ ve	Rod	+ ve	+ ve	+ve	-ve	+ve	-ve	-ve	+ ve	-ve	+ve	- ve
Staphlococcus	Convex	Smooth	Soft	Golden yellow	+ ve	Sphere (cluster)	- ve	- ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve
Pseudomonas	Flat	Rough	Mucoid	Blue green	- ve	Rod	- ve	+ ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
Feacal Streptococcus	Raised	Rough	Mucoid	Dark red	+ ve	Sphere (chain form)	- ve	- ve	-ve	-ve	+ve	-ve	-+ve	-ve	+ve	-ve	+ve
Klebsiella sp	Raised	Smooth	Mucord	Blue	- ve		- ve	- ve	- ve	+ ve	+ve	- ve	- ve	+ ve	- ve	+ ve	- ve

Key: MR: Methyl red, VP: Voger proskauer, Cit.: Citrate, Ure: Urease, Pig: Pigmentation, Cons: Consistency, Coag.: Coagulase - ve = Negative, + ve = Positive

The cultural and biochemical characteristics of the isolates reported by Ajadi and Thomas (2017). Justify the observation of this study.

CONCLUSION AND RECOMMENDATION

The studied swimming pool water samples were polluted with pathogens of importance which can bring about transmission of swimming pool water borne infections. Prompt and urgent interventions inform of adequate treatment of the swimming pool water with disinfectant such as hypochlorite solution should be adopted.

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