Annual Research & Review in Biology

35(11): 118-125, 2020; Article no.ARRB.56591
ISSN: 2347-565X, NLM ID: 101632869

Antibiotic Sensitivity Patterns and Plasmid Profile of Bacteria Isolated from Some Swimming Pools in Akure, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AKO and EAA designed the study, managed the analyses of the study and wrote the protocol. Author BEF performed the statistical analysis and managed the literature searches. Authors BEF and EAA wrote the first draft of the manuscript. Author BEF performed the research work under close supervision of author AKO. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2020/v35i1130305
Editor(s):
(1) Dr. Ezema Chuka, University of Nigeria, Nigeria.
Reviewers:
(1) Phillip Minnaar, Agricultural Research Council, South Africa.
(2) Igiebor Francis Aibuedefe, Wellspring University, Nigeria.
(3) Oladiran Famurewa, King's University, Nigeria.
Complete Peer review History: http://www.sdiarticle4.com/review-history/56591

Original Research Article

Received 01 April 2020
Accepted 05 June 2020
Published 01 December 2020

ABSTRACT

Aims: To evaluate the antibiotic sensitivity profile of bacteria isolated from swimming pools in Akure, Nigeria.
Place and Duration of Study: Department of Microbiology, The Federal University of Technology, Akure Ondo state Nigeria between May and July, 2018.
Methodology: Water samples were collected in the morning and evening periods including weekends, from ten (10) swimming pools in Akure, Ondo State, Nigeria. The temperature and the pH of the water samples were measured and recorded at the time of collection. The types and loads of bacteria at different times of each day were determined for each of the swimming pools. Characterization and Identification of the various bacterial isolates were based on Gram-staining techniques and biochemical tests. Antibacterial susceptibility profile of the isolates was evaluated using standard methods. *Escherichia coli* and *Staphylococcus aureus* being resistant to multiple antibiotics were subjected to plasmid analysis.
Results: Bacteria isolated include; *E. coli* which had the highest occurrence rate (25%), *S. aureus*

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(18.75%), *Shigella flexneri* (14.50%), *Klebsiella pneumoniae* (14.50%), *Proteus mirabilis* (9.37%), *Citrobacter freundii* (6.25%) *Pseudomonas aeruginosa* (6.25%) *Enterobacter faecalis* (3.13%) and *Salmonella bongori* (3.13%). Ciprofloxacin, pefloxacin and tarivid recorded remarkable zones of inhibition against the isolates. The isolates were notably resistant towards chloramphenicol and septrin. The isolates were further examined for the presence of conjugative plasmids. The results showed that their resistance was chromosomal mediated.

Conclusion: Despite the fact that these pools meet the World Health Organization minimum requirement, Microbiological examination of the swimming pools revealed that most of them are contaminated with various pathogenic microorganisms which are potentially harmful.

Keywords: Plasmid; resistance; antimicrobials; bacteria; antibiotics.

1. INTRODUCTION

Water is very essential for the survival of humans and other life forms. It is required for human daily activities such as drinking, cooking, washing, bathing and also for agricultural, industrial and recreational purposes [1]. Most young Nigerian youths enjoy swimming, hence accounting for the heavy use of swimming pools, especially in urban centres [2]. Swimming as an activity is also a great workout as it helps build endurance, muscle strength and cardiovascular fitness. Although water is a basic requirement for human existence, it can serve as a medium for the transmission of pathogenic microorganisms, if not properly handled, leading to a number of disease outbreaks such as gastroenteritis, conjunctivitis, keratitis, trachoma, otitis, cholera, dysentery, eczema, skin rashes, typhoid, giardiasis, cryptosporidiosis, helminthiasis, hepatitis, rotavirus infection, salmonellosis and central nervous systems associated diseases [3,1]. Research has shown that nearly all swimming pools face the risk of harboring microorganisms which are harmful to human health. Pool water and the surfaces of objects or materials at a swimming facility are exposed to contamination from body fat and human waste materials such as nasal secretions, saliva, sweat, faecal, urine and body lotions and creams [4].

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 bacterium is usually of primary importance. As indicators of faecal pollution, their presence is a strong indication for the presence of enteric pathogenic bacteria such as *Salmonella typhi, S. paratyphi, Shigella dysenteriae* and *Vibrio cholerae* in the pool. Skin tuberculosis caused by *Mycobacterium baliteri* has been reported after swimmer had bathed in waters from which a large amount of microorganisms was found [5]. Resistance to antimicrobial agents has become important in clinical management and control of many diseases and deserves scientific intervention to bring about some control measures [6,7]. This study therefore sought to determine the bacterial loads of swimming pools in Akure, a south western city in Nigeria; their susceptibility patterns to different antibiotics and to evaluate the plasmid profile of bacterial pathogens that showed multiple antibiotic resistance.

2. METHODOLOGY

2.1 Sample Collection

Water samples were collected in 250 ml bottles. Samples were collected early in the morning (7.00AM to 8.00AM) and evening periods (7.00PM to 8.00PM) including weekends. The swimming pools were not so much used at the early hours of the day compared to evening and weekends. A total of ten (10) swimming pools were sampled within Akure Metropolis and five (5) samples were collected per swimming pool. The samples were collected as described by Zorasi et al. [8]. The swimming pool water was collected at a depth of 20 cm below the water surface. A 1% Sodium thiosulphate ($Na_2S_2O_3$) solution was added to the water sample to deactivate the residual chlorine in the water samples to prevent further disinfectant effects on the microbial population [8,9]. The water samples were transported to the laboratory in an ice packs which read 4°C for analysis within two hours of collection. The temperature and the pH of the water samples were measured and recorded at the time of collection.

2.2 Bacteriological Examination of Samples

Nutrient agar, Salmonella Shigella agar, Eosin Methylene Blue agar and MacConkey agar were

prepared according to the manufacturer's instructions. The samples were 10-fold serially diluted in 9 ml of sterilized peptone water contained in each of the tubes by transferring 1 ml 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 5th tube. A 1 ml aliquot of the pre-enrichment broth of 10^{-3} dilution and undiluted sample (control) was aseptically selected with a sterile 100 μl pipette and plated onto each agar and the surface by the spread plate technique [9]. The plates were incubated at 37°C for 48 hours.

2.3 Identification of Isolated Bacteria

Random 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 based on colonial morphology such as the colour, elevation and margin, cellular morphology such as shape of cell, arrangement of cell and Gram reaction and biochemical characteristics such as indole, methyl red, voges proskaeur, citrate utilization, motility, catalase, coagulate, carbohydrate assimilation, oxidase, urease and spore stain tests.

2.4 Antibacterial Sensitivity Test

The inoculum was standardized prior to antibiotic sensitivity testing. The antibiotic sensitivity test was carried out using Kirby Bauer's disc diffusion method. The test organisms from 18 hours' broth culture were swabbed evenly on a prepared Muller Hinton Agar medium and allowed to dry for 4 hours, antibiotics discs were then placed on the already prepared plates and pressed firmly on it. The plates were incubated at 37°C for 24 hours and zones of inhibition were measured in mm and interpreted accordingly [10].

2.5 Plasmid Analysis

Plasmid curing was carried out in order to determine the location (plasmid-borne or chromosomal) of the resistant marker(s). The curing (elimination) of the plasmids of the resistant isolates was done using sub inhibitory concentration of 10 mg/ml of ethidium bromide [11,12].

2.6 Data Analysis

Each procedure was replicated three times and data obtained were subjected to two-way Analysis of Variance (ANOVA). Means were compared using Duncan's New Multiple Range Test (DNMRT) with the aid of Statistical Package for the Social Sciences (SPSS) at P≤ 0.05 level of significance.

3. RESULTS

3.1 Bacterial Loads

From this study, a total of 32 species of the genera *Citrobacter*, *Enterobacter, Escherichia, Klebsiella, Proteus, Pseudomonas, Salmonella, Shigella* and *Staphylococcus* were isolated. Of the total bacterial isolates recovered, *E. coli* had the highest occurrence (25%), followed by *S. aureus* (18.75%), *Sh. flexneri* (14.50%), *K. pneumoniae* (14.50%), *P. mirabilis* (9.37%), *C. freundii* (6.25%) *Ps. aeruginosa* (6.25%) *Ent. faecalis* (3.13%), *Sal. bongori* (3.13%) (Fig. 1).

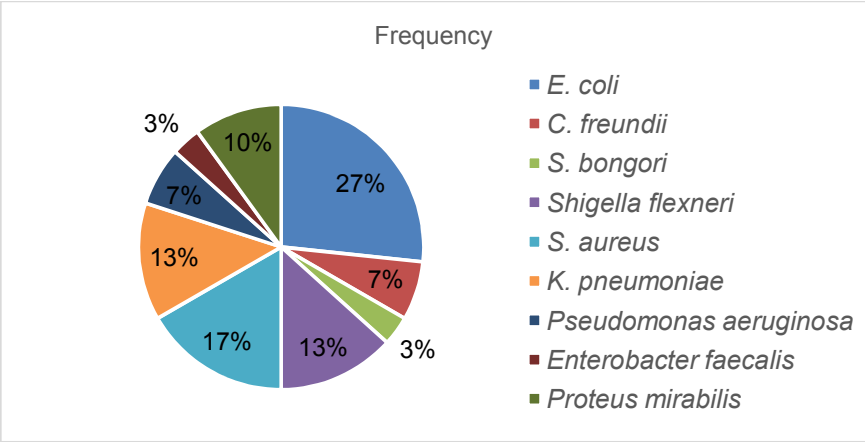

Fig. 1. Frequency of occurrence of bacteria isolated across the swimming pools in Akure

3.2 Antibiotic Sensitivity Profile

Figs. 2 and 3 show the antibiotic sensitivity patterns of each isolate. Most of the bacterial isolates were found resistant to commonly used antibiotics such as ampicillin, tetracycline, ofloxacin, chloramphenicol and gentamicin. *Escherichia coli* and *Staphylococcus aureus* showed high resistance to multiple antibiotics and as such were subjected to plasmid analysis. The *Escherichia coli* Isolated from the selected swimming pools showed 100% resistance to streptomycin, septrin and chloramphenicol (Fig. 2). While the Gram-positive bacterium (*S. aureus*), also showed multiple-antibiotic resistance to ampicillin, erythromycin and gentamycin (Fig. 3). Ofloxacin was the most effective antibiotic against Gram-negative isolates while augmentin was the least effective (Fig. 1). In the case of Gram-positive isolates, ciprofloxacin was the most effective while pefloxacin was the least effective (Fig. 2).

3.3 Plasmid Profile

In order to determine whether the observed resistance pattern in the isolates, was plasmid or chromosomal mediated, *Escherichia coli* and *Staphylococcus aureus* isolates were examined for the presence of conjugative plasmids (Fig. 1). The results showed that their resistance was chromosomal mediated (Figs. 4 and 5).

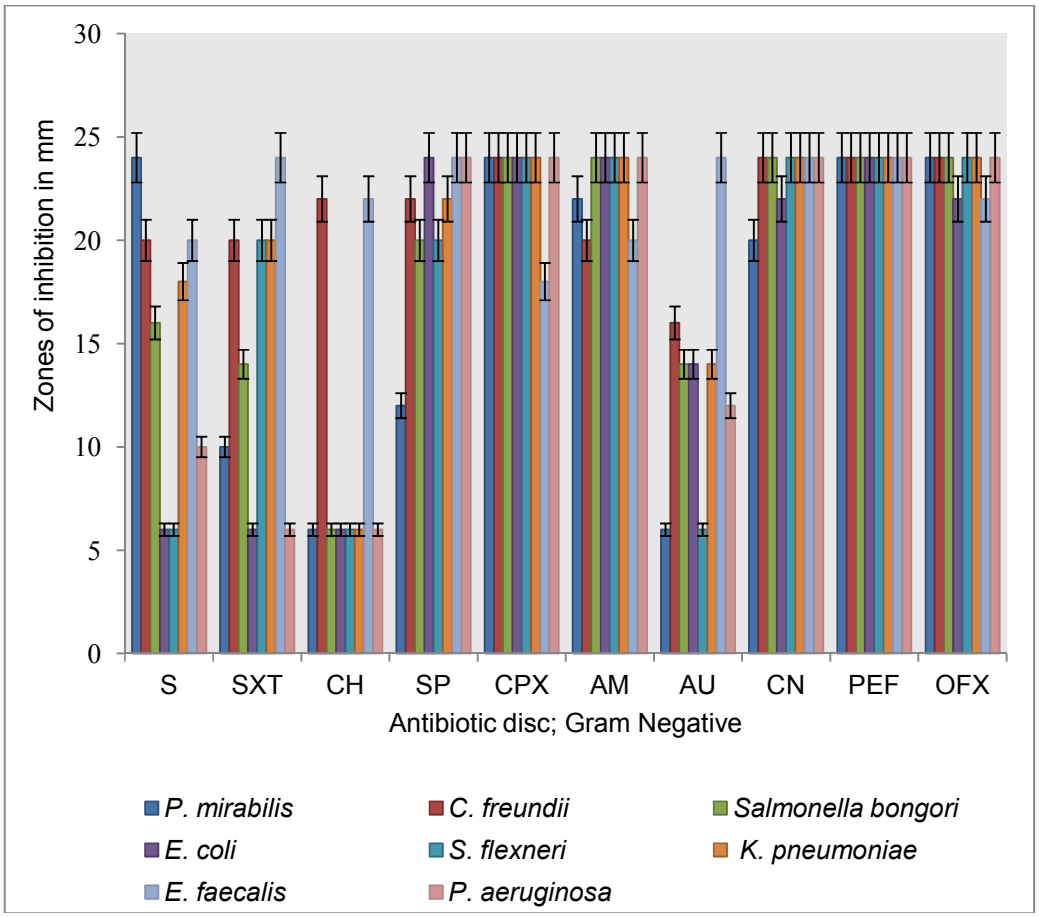

Fig. 2. Antibiotic sensitivity patterns of bacteria isolated from swimming pool water samples (Gram Negative)

KEY:
CN = Gentamycin
S = Streptomycin
SP = Sparfloxacin
AU = Augmentin
PEF = Pefloxacin
SXT = Septrin
CPX = Ciprofloxacin
OFX = Ofloxacin
CH = Chloramphenicol
AM = Ampicillin

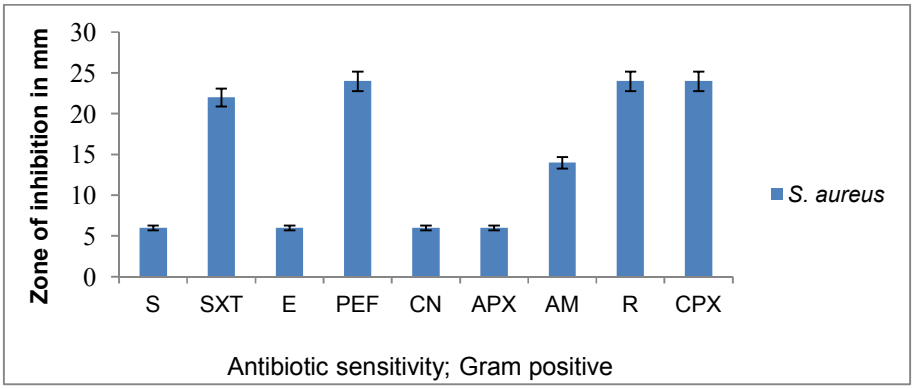

Fig. 3. Antibiotic sensitivity patterns of bacteria isolated from swimming pool water

KEY:
S = Septromycin *SXT = Septrin* *E = Erythromycin*
PEF = Pefloxacin *CN = Gentamycin* *AM = Ampicillin*
CPX = Ciprofloxacin

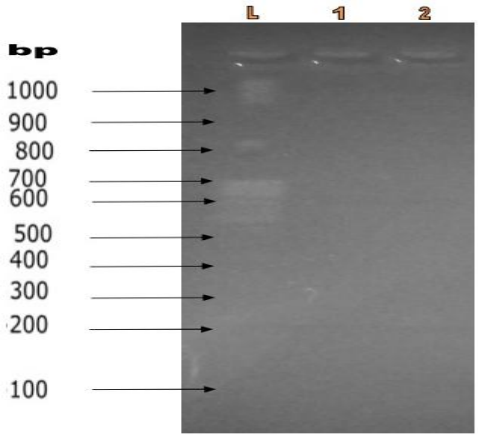

Plate 1. Agarose gel electrophoresis showing plasmid profile of *Staphylococcus aureus* (1) and *Escherichia coli* (2) isolated from swimming pool: both showing chromosomal resistance

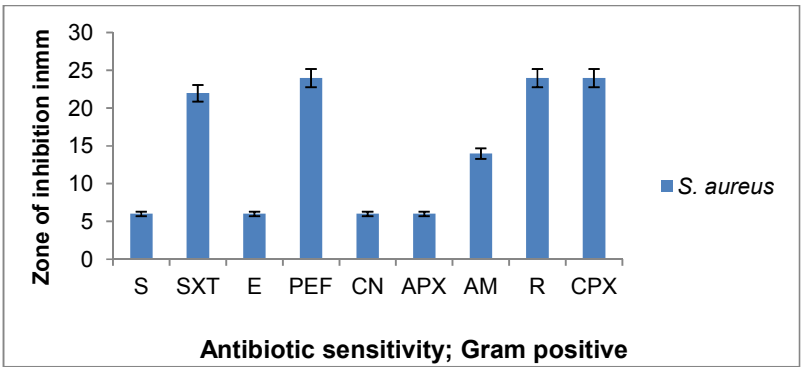

Fig. 4. Post curing antibiotic sensitivity patterns of *Staphylococcus aureus*

KEY:
AMX = Amoxycillin *OFL = Ofloxacin* *STR = Streptomycin*
CHL = Chloramphenicol *CEF = Ceftriaxone* *GEN = Gentamycin*
PEF = Pefloxacin *CPX = Ciprofloxacin*
ERY = Erythromycin

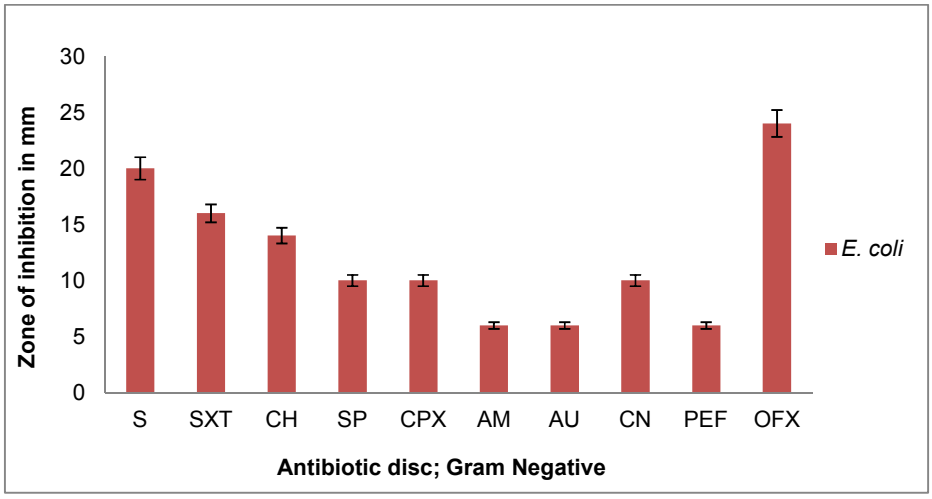

Fig. 5. Post curing antibiotic sensitivity patterns of *Escherichia coli*

KEY:

CN = Gentamycin *PEF = Pefloxacin* *OFX = Ofloxacin*
S = Streptomycin *SXT = Septrin* *CH = Chloramphenicol*
SP = Sparfloxacin *CPX = Ciprofloxacin* *AM = Ampicillin*
AU = Augmentin

4. DISCUSSION

Swimming pools examined were found to be highly contaminated by bacteria which are pathogenic. It was observed that *Escherichia coli* and *Staphylococcus aureus* were frequently isolated from the swimming pools samples and this observation was supported by [1,10]. Other bacteria encountereed in this study belonged to the genera *Escherichia, Staphylococcus, Citrobacter*, *Enterobacter, Klebsiella, Proteus, Pseudomonas, Salmonella,* and *Shigella*. Most of which are pathogenic to man, some are non-pathogenic while some may cause opportunistic infections [13,1]. The presence of *Escherichia coli* indicates faecal contamination of the swimming pool. This signifies that the pools fall below the microbiological quality standard specified by the world health organization. This constitute public health hazard because swimmers can accidentally swallow pool water during swimming which can lead to a variety of diseases such as Typhoid fever, Cholera, Diarrhea and other gastrointestinal disorders. Asides the fact that Pathogenic organisms enter the water through bathers, high bacterial count may also be as a result of contaminated water source as well as the location of the pool and the season of the year. These factors adversely affect the quality of swimming pools.

Results obtained from this study is similar to that of Ajadi et al. [1], Ekopai et al. [9] and Joyce et al. [14]. However, it was observed that the researchers did not isolate *Escherichia coli* which is an enteric organism. This indicates that the samples obtained were not feacally contaminated. Nevertheless, this study showed a similar results with that of Ayandele et al. [10]. which reveal the frequency of isolation of *Escherichia coli* to be 60%. The *Escherichia coli* Isolated from the selected swimming pools showed 100% resistance to streptomycin, septrin and chloramphenicol, while *S. aureus* also showed multiple-antibiotic resistance to ampicillin, erythromycin and gentamycin. Ofloxacin was the most effective antibiotics against Gram-negative isolates while augmentin was the least effective. In the case of Gram-positive isolates, ciprofloxacin was the most effective while pefloxacin was the least effective. Plasmid analysis of the multiple resistance organisms, *E. coli* and *S. aureus* showed the absence of plasmids, their resistance is chromosomal mediated. Hence, the bacteria isolates still showed multi-drug resistance after curing. According to Alaofin and Onifade [12], and Tsaku et al. [15], antibiotic resistance in some bacterial isolates which seem not to possess plasmids was associated with chromosome and/or transposons [16]. The implication of these resistances is that many bacterial diseases that could be treated with inexpensive antibiotics, has recently been made more expensive. It can also lead to change in first line treatment. It should be noted that

susceptibility of bacteria to antibiotics is not static and resistance may be due to antibiotic abuse, antibiotic over use or may be chromosomally or plasmid mediated.

Many of the outbreaks related to swimming pools and similar environments have occurred because disinfection was not applied or was inadequate (World Health Organization, 2006). Hence, The operators should follow recreational water guidelines for proper management of swimming pools. Users should adhere to good sanitary practices and the various health authorities should monitor swimming pool facilities and ensure strict compliance to guidelines for sanitation and proper pool management in order to stem the incidence of recreational diseases.

5. CONCLUSION

The microbiological qualities of some of the swimming pools examined were poor, with the water bodies being contaminated with various pathogenic microorganisms which are potentially harmful. Since the use of recreational water bodies is on high increase especially among youths, this calls for stricter surveillance measures by responsible health authorities for protection of swimmers' health. Continued microbiological water analysis of the pools' will give an insight into pool hygiene and help to prevent infection outbreaks among swimmers. The general public should be sensitized on the potential health risks associated with swimming pools as this will alleviate the mismanagement of the pools by swimmers.

ACKNOWLEDGEMENT

The authors acknowledge the immense contributions of Mr. Babajide Ajayi of the Department of Microbiology, Federal University of Technology Akure, Ondo state, Nigeria during the course of this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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