MULTIDRUG RESISTANT (MDR) BACTERIA ISOLATED FROM SELECTED RECREATIONAL WATER IN ILORIN, NIGERIA

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Abstract: In this work, bacteria were isolated, characterized and identified from some selected recreational water samples collected within Ilorin (8.5° N, 4.55° E) using standard bacteriological methods. The frequency of occurrence of isolates was Klebsiella sp. (3.64%), E. coli (5.46%), Enterobacter sp. (7.27%), Salmonella sp. (3.64%), Pseudomonas sp. (18.17%), Proteus sp. (14.55%), Pseudomonas aeruginosa (5.46%), Staphylococcus aureus (14.55%), Staphylococcus sp. (5.46%) and Bacillus sp. (16.36%). In-vitro antibiotic sensitivity patterns were determined using the disc diffusion technique. It was revealed that all bacterial isolates were resistant to amoxillin, except Staphylococcus aureus and Enterobacter sp. with 15.0% and 10.0% inhibition respectively. Pseudomonas aeruginosa 6 (100%), Bacillus sp. 6 (100%) and Staphylococcus sp. 5 (83.3%) were completely resistant to all antibiotic tested except Staphylococcus sp. which was inhibited by septrin (10.0%). Escherichia coli was sensitive to only 2 (33.3%) and it resisted 4 (66.7%) of the antibiotics tested while Proteus sp., Klebsiella sp., and Salmonella sp. were sensitive to only 3 (50.0%) of the antibiotics tested. All the bacterial isolates were resistant to at least 2 (33.3%) antibiotics out of the six antibiotics tested. Chloramphenicol (70.0%) and erythromycin (50.0%) exhibited significant inhibition against the bacterial isolates as compared with amoxillin (20.0%), gentamycin (30.0%) and perfloxacin (30.0%) among the antibiotics tested. The study showed the presence of Multidrug resistant (MDR) bacteria in these recreational water sources. This implies that the swimming pools have not met the World Health Organization standards for recreational water. This calls for urgent attention, as their presence indicates public health risk and possible swimming pools intoxication.

Keywords: Swimming Pool, Isolates, Antibiotics, Multidrug resistance, Sensitivity.

INTRODUCTION

Swimming pool sanitation measure both visual clarity and levels of microflora such as bacteria, protozoans and viruses in swimming pools (Totkova et al., 1994). The goal of sanitation is to prevent the spread of diseases and distribution of pathogens among users (Sule, 2010). Swimming pool water may become contaminated by microorganisms from infected swimmers, incoming water from unhygienic sources, airborne contamination and droppings from birds (Podewils et al., 2007).

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Serious potential health risks associated with to users of poorly maintained swimming pools cannot be overemphasized as it can harbour infectious organisms which can cause mild illness or deadly in some instances. The risk of illness or infection associated with swimming pools and similar recreational water environments is primarily associated with faecal contamination of the water. This may be due to faeces released by the bathers, or direct animal contamination (WHO, 2006). Contact with contaminated water 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., 1980; UNDP, 1989; Onajobi et al., 2013). Many of the outbreaks related to pools and similar environments have occurred because disinfection was not applied or inadequate. Non-faecal human shedding into the pool water or the
surrounding area can be a potential source of pathogenic organisms (WHO, 2006).

Pool operators can help prevent faecal contamination of pools by encouraging pre-swim showering and toilet use. Where possible, they should confine young children to pools, small enough to drain in the event of an accidental faecal release. It was recommended by the World Health Organization (2006) that people with gastroenteritis should not use public or semi-public facilities while ill or for at least a week after their illness.

A number of pathogenic enteric organisms may be transferred through contaminated pool water and the surrounding environment via human shedding. Infected users can directly contaminate pool waters and the surfaces of objects or materials at a facility with primary pathogens in sufficient numbers to lead to skin and other infections in users who subsequently come in contact with the contaminated water or surfaces (WHO, 2006). Swimming pool-related outbreaks of illness have been linked to bacteria and other causative agents. *Shigella* and *Escherichia coli* O157 are two related bacteria that have been linked to outbreaks of illness associated with swimming pools in the recent time.

Opportunistic pathogens can shed from users and transmitted via surfaces and contaminated water. In addition, certain free-living aquatic bacteria and amoebae can grow in the pool, or on other wet surfaces within the facility to a point at which they may cause a variety of respiratory, dermal or central nervous system infections or diseases (WHO, 2006). *Pseudomonas aeruginosa* associated with recreational water use as it is able to withstand high temperatures and disinfectants.

Eric et al. (1982) indicated *Legionella* sp, *Pseudomonas aeruginosa*, *Mycobacterium* sp, *Staphylococcus aureus*, *Leptospira interrogens*, *Molluscipox virus*, *Human Papilloma virus*, *Acanthamoeba* sp, *Trichophyton* sp, and *Epidermophyton floccosum* as non-enteric pathogens that can be found in swimming pools that usually cause dermic or respiratory infections.

According to WHO (2006), the symptoms of *E. coli* O157 infection include bloody diarrhoea (haemorrhagic colitis) and Haemolytic Uraemic Syndrome (HUS), as well as vomiting and fever in more severe cases. HUS, characterized by haemolytic anaemia and acute renal failure, occurs most frequently in infants, young children and elderly people. The symptoms associated with *Shigellosis* include diarrhoea, fever and nausea while the primary health effect associated with the presence of *P. aeruginosa* are dermatitis and folliculitis, an infection of the hair follicles that may result in a pustular rash. *Escherichia coli* have several pathogenic strains which are classified under enterovirulent *E. coli*. They are enterohemorrhagic, enteroinvasive, enterotoxigenic, enteropathogenic, and enteroaggregative.

Routes of transmission of waterborne illnesses can be caused by ingestion or consumption of water, by dermal contact, or by inhalation. *Campylobacter*, *Vibrio cholera* and *Salmonella typhi*, have been isolated from polluted water and are capable of causing diarrheal illness (WHO, 2006). Humans are the reservoir for these pathogens. Prevention strategies for these pathogens include source protection, halogenation of water, and boiling of water. Proper maintenance and disinfection of recreational water systems should be employed as prevention strategies (Onajobi et al., 2013).

The resistance of a microorganism to an antimicrobial agent to which it was originally sensitive is termed as Antimicrobial resistance. Resistant microorganisms are able to withstand attack by antimicrobial drugs, so that standard treatments become ineffective and infections persist to spread to others (WHO, 2013). Drug resistant strains have been reported among the gram negative bacteria including *Salmonella*, *Shigella*, *Klebsiella*, *E. coli*, *Pseudomonas* (Cheesebrough 2006; Riboldi et al., 2009; Inyan 2009; Ibiene et al., 2011).

In recent years, multiple drug resistance is increasing due to abusive usage of commercially available antimicrobial drugs for the treatment of infectious diseases (Bhavanani and Ballow, 2000). Although, the mechanisms of resistance vary among microbes, drug resistant microorganisms are getting out of control worldwide (WHO, 2013). Antibiotic resistance can be a result of horizontal gene transfer (Ochiai et al., 1959).
Microbiological evaluation has been one of the steps that are taken towards improving sanitary quality of swimming pools (Alfred, 2004; Onajobi et al., 2013). The specific aim of this work was to ascertain the sanitary level of selected recreational water (swimming pools) in the light of increasing patronage by tourist, fun seekers and casual users; through enumeration and identification of source bacteria species present and to determine the sensitivity pattern of the suspected bacteria.

**MATERIAL AND METHODS**

Water samples were obtained from ten (10) swimming pools at different sites within Ilorin metropolis as described by Onajobi et al. (2013).

**Isolation of bacteria**

Media used in this study included Nutrient Agar (NA), Peptone Water (PW), MacConkey Agar (MA), Eosine Methylene Blue (EMB), *Salmonella Shigella* Agar (SSA) and Mueller Hinton Agar. These were prepared according to manufacturer’s specification. The standard pour plate method was used as described by Harrigan and McCane (1976), and Collins and Lyne (1976). Discrete colonies were repeatedly sub-cultured until pure cultures of the isolates were obtained. Pure isolates were aseptically transferred onto the nutrient agar slant for subsequent biochemical tests.

**Characterization and Identification of Bacterial Isolates**

Pure cultures of the bacterial isolates were carefully examined macroscopically and microscopically for their cultural morphology and cellular characteristics respectively. Isolates were characterized based on their colonial morphology, microscopic techniques and biochemical characteristics, including carbohydrate utilization as described by Cruickshank et al., (1975). All isolates were identified by comparing their characteristics with those of known taxa, as described by Jolt et al. (1994), Cheesbrough (2006), Oyeleke and Manga (2008) and Adebayo-Tayo et al. (2012).

**Antibiotic Susceptibility of Bacterial Isolates**

Sensitivity test was performed using the standard disc diffusion method described by Beathy et al., (2004). The bacterial cell suspension was prepared at 1x10^8 cfu/ml following the McFarland 0.5 turbidity standard. A 0.1ml of the suspension was transferred onto sterile Mueller-Hilton agar plate accordingly. This was swabbed over the entire plate using sterile bent glass rod and allowed to rest for 10 min. Each antibiotic disc was carefully transferred using sterile forceps. The plates were immediately incubated at 37°C for 24hrs. The diameters of the zones of inhibition were determined in mm. The antibiotic discs used were Erythromycin (10 µg), Amoxicillin (30 µg), Septin (30 µg), Chloramphenicol (30 µg), Gentamycin (10 µg) and Perflaxacin (10 µg) manufactured by Cypress diagnostics, Belgium.

**RESULTS AND DISCUSSION**

In this study, enumeration of bacteria present in selected swimming pool water samples collected from different locations in Kwara State, Nigeria was examined. Table 1 showed the biochemical characteristics of isolates obtained. Eight genera of bacteria were isolated from the samples. These were identified as *Bacillus*, *Escherichia*, *Enterobacter*, *Klebsiella*, *Salmonella*, *Staphylococcus*, *Pseudomonas* and *Proteus*. 
Table 1: Biochemical Characteristics of Isolated Bacterial Pathogens

<table>
<thead>
<tr>
<th>S/N</th>
<th>Isolate code</th>
<th>Gram reaction</th>
<th>Oxidase</th>
<th>Catalase</th>
<th>Citrate</th>
<th>Indole</th>
<th>Methyl red</th>
<th>Voges proskauer</th>
<th>Motility</th>
<th>Malhose</th>
<th>Coagulase</th>
<th>Glucose</th>
<th>Lactose</th>
<th>Fructose</th>
<th>Slope</th>
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<td>_</td>
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<td>+</td>
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<td>_</td>
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</tr>
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<td>_</td>
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<td>_</td>
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<td>+</td>
<td>R</td>
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<td>_</td>
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<td>_</td>
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<td>_</td>
<td>_</td>
<td>_</td>
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</tr>
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<td>_</td>
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<td>_</td>
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<td>+</td>
<td>_</td>
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<td>_</td>
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<td>_</td>
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<tr>
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<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>Pseudomonas aeruginosa</td>
</tr>
</tbody>
</table>

Key: GPC - gram positive cocci  GPR - gram positive rod  GNR - gram negative rod

**Percentage Occurrence of the Isolated Microorganisms**

The total 51 bacterial isolates were characterized and identified. Figure 1 revealed the frequencies of occurrence for these suspected bacterial isolates are; *Pseudomonas* sp. 10(18.17%), *Pseudomonas aeruginosa* 3(5.46%), *Escherichia coli* 3(5.46%), *Proteus* sp. 8 (14.55%) *Staphylococcus* sp. 3 (5.46%), *Staphylococcus aureus* 8 (14.55%), *Enterobacter* 4 (7.27%), *Salmonella* sp. 2 (3.64%), *Klebsiella* sp. 2 (3.64%), *Bacillus* sp. 9(16.36%). Figure 2 showed the occurrence for the gram reaction and cellular morphology of the isolates. Gram negative bacilli (70%), gram positive cocci (20%) and gram positive bacilli (10%).

![Figure 1: Percentage Occurrence of Isolated Bacteria](image-url)
Various species of bacteria were encountered in the swimming pools, they include; *Klebsiella* sp., *Escherichia* sp., *Enterobacter* sp., *Salmonella* sp., *Staphylococcus aureus* and *Proteus* sp. According to Carbell *et al.* (1975) and Stainer *et al.* (1987), *Enterobacter* and *Klebsiella* species are associated with surface runoff waters. *Enterobacter* isolated from water samples are examples of non-faecal coliform and can be found in soil which serves as a source by which the pathogens enters the water (Schlegel, 2002).

![Figure 2: % Gram reaction of the isolates in swimming pool samples. GNB – Gram Negative Bacilli, GPC – Gram Positive Cocc, GPB – Gram Positive Bacilli](image)

*Escherichia coli* 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 the presence of *E. coli* in water as a strong indication of the closeness of the sampling site to the source of pollution. *E. coli* is exclusively faecal in origin and its presence in water is a strong indication of recent faecal pollution (Itah *et al.*, 1996). The presence of these indicator bacteria indicates the possible presence of enteric pathogenic bacteria in the pool. The incidence constitutes a public health hazard because swimmers can accidentally swallow contaminated pool water during swimming which can result in an outbreak of diseases.

Moreover, *E. coli* are known to be 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. The presence of *Salmonella* species is not in agreement with the WHO standard for recreational use because they are associated with gastrointestinal infections; typhoid fever/enteric fever, diarrhea, abdominal pain, dysentery (EPA, 2003). *Proteus vulgaris* are also of public health significance since they belong to the intestinal flora.

Table 2 shows the results of the in-vitro antibiotic sensitivity pattern of the bacterial isolates. All bacterial isolates were resistant to amoxillin, except *Staphylococcus aureus* and *Enterobacter* sp. with 15.0% and 10.0% inhibition respectively. *Pseudomonas aeruginosa* 6 (100%), *Bacillus* sp. 6 (100%) and *Staphylococcus* sp. 5 (83.3%) were completely resistance to all antibiotics tested except *Staphylococcus* sp. which was inhibited by septrin (10.0%). All the bacterial isolates were resistant to atleast 2 (33.3%) antibiotics out of the total antibiotics tested in this study.

*Escherichia coli* was sensitive to only 2(33.3%) and it resisted 4 (66.7%) of the antibiotics tested. In the same vein, *Proteus* sp., *Klebsiella* sp., and *Salmonella* sp. were sensitive to only 3 (50.0%) and resistance to 3 (50.0%) of the antibiotics tested. Highest percentage sensitivity was exhibited by *Enterobacter* sp., *Pseudomonas* sp., and *Staphylococcus aureus*. They were inhibited by 4 (66.7%) out of the antibiotics tested, though they showed resistance to 2 (33.3%) of the antibiotics tested.
In this study, chloramphenicol (70.0%) and erythromycin (50.0%) exhibited higher inhibition against the bacterial isolates as compared with amoxillin (20.0%), gentamycin (30.0%) and perfloxacin (30.0%) among the antibiotics tested. In the light of this, chloramphenicol and erythromycin were the most effective while amoxillin, gentamycin and perfloxacin were the least effective against the test bacterial isolates. Amoxillin inhibited only *Enterobacter* sp. (10.0%) and *Staphylococcus aureus* (15.0%), Gentamycin inhibited only *Proteus* sp. (12.0%), *Klebsiella* sp. (15.0%) and *Pseudomonas* sp. (25.0%), while perfloxacin inhibited *Salmonella* sp. (10.0%), *Enterobacter* sp. (22.0%) and *Pseudomonas* sp. (11.0%).

*Staphylococcus aureus* was susceptible to 4 (66.7%) and resistance to 2 (33.3%) of the antibiotics tested. This is at variance with what was reported by Nkang *et al.* (2009), who reported the resistivity of *Staphylococcus aureus* to chloramphenicol and sensitive to gentamycin. However, sensitivity of *Staphylococcus aureus* erythromycin, amoxillin and septrin as reported by Inyang (2009) and Nkang *et al.* (2009) is similar to these present findings.

The resistivity (100.0%) of *Bacillus* sp. to all antibiotics tested in this research is in agreement with the work of Iyang (2009), who reported the resistivity of *Bacillus* sp. to chloramphenicol, erythromycin, and gentamycin. This finding deviated from the report made by Umar *et al.* (2006) and Ibiene *et al.* (2011), who reported the sensitivity of *Bacillus* sp. to chloramphenicol and erythromycin. Concentration of the antibiotic, source of isolates and horizontal resistance gene transfer could be attributed to the variation in susceptibility and resistance of the isolates to different antibiotics (Inyang, 2009; Okonko *et al.*, 2010 and Ibiene *et al.*, 2011).

Furthermore, the resistivity pattern of *Escherichia coli* (66.7%), *Staphylococcus* sp. (83.3%), *Bacillus* sp. (100.0%) and *Pseudomonas aeruginosa* (100.0%) highly satisfied the MDR pattern of resistance to >3 antibiotics tested in this study. High percentage resistance 66.7% of *Escherichia coli* in this study is comparable to 62.5% reported by Ibiene *et al.* (2009) but at variance with sensitivity to...
septin and gentamycin reported. Okonko et al. (2010), reported E. coli resistance to gentamycin and MDR pattern of 5(62.5%) of the test antibiotic similar to this present findings. However, the MDR pattern reported on E. coli in this study is in conformity to a previous study of Dolejska et al. (2007). Pathogenic isolates of E. coli has relatively large potential for developing resistance and (Karlowsky et al., 2004; Okonko et al., 2010 and Ibiene et al., 2011).

It was further observed that Klebsiella sp. was sensitive to chloramphenicol, erythromycin, and gentamycin. This is comparable to what has been previously reported by Reish et al. (1993) and Nkang et al. (2009) but at variance with the sensitivity to amocillin and septrin. Reish et al. (1993) and Aiyegoro et al. (2007) reported resistance to 66.7% against amocillin by Klebsiella sp. which is equally comparable to these present findings.

The study further highlights the most alarming situation of highly diverse antibiotics resistance rates by P. aeruginosa. Pseudomonas aeruginosa showed resistance to 6 (100.0%) antibiotics in-vitro. This is comparable to Multi-Drug Resistance (MDR) reported by Aiyegoro et al. (2007) and Okonko et al. (2009a). The resistivity of P. aeruginosa to chloramphenicol is similar to the findings of Nkang et al. (2009) but deviates slightly in the sensitivity to amocillin, septrin and gentamycin reported. Multi-drug resistance has important implications for the empiric therapy of infections caused by Klebsiella sp., P. aeruginosa, E. coli and S. aureus and for the possible co-selection of antimicrobial resistance mediated by multi-drug resistance plasmids (Oteo et al., 2002; Sherley et al., 2004 and Nkang et al., 2009). It was documented that gram negative bacilli harbour series of antibiotic resistant genes which can be transferred to other bacteria horizontally (Piddock, 2006; Depardieu et al., 2007; Leavitt et al., 2007; Lockhart et al., 2007 and Nkang et al., 2009).

CONCLUSION

Some swimming pool has been reported by various researchers to harbour coliform organisms in numbers greater than the required WHO recreational water standard for pool water. Other pathogenic bacteria have also been isolated from various swimming pools. It is therefore imperative that operators of swimming pools should ensure proper changing of the water and also warn the swimmers from emptying bowel or bladder contents into the water. Swimmers should always prioritize their health over recreation.

Infections caused by resistant microorganisms often fail to respond to conventional treatment, resulting in prolonged illness, greater risk of death and higher costs of treatments. According to World Health Organization, a high percentage of hospital-acquired infections are caused by highly resistant bacteria such as methicillin-resistant Staphylococcus aureus (MRSA) or multidrug-resistant Gram-negative bacteria. New resistance mechanisms have emerged, making the latest generation of antibiotics virtually ineffective. The evolution of resistant strains is a natural phenomenon that happens when microorganisms are exposed to antimicrobial drugs, and resistant traits can be exchanged between certain types of bacteria.

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