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# Antibiotics Resistant Profile Of Microrganims Isolated From Different Water Body Samples In Ibadan Metropolis

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**Abstract:** Water is essential to life so an adequate, safe and accessible supply must be available to all. Episodes of patients with contaminated water related infections not responding to treatments with antibiotics known to be effective against the infective agents have been reported. In Ibadan, a major problem is inaccessibility to potable water by most inhabitants. This study took sample of water from water bodies and assessed their microbial profile as well as antibiotics susceptibility and resistance patterns. Water samples from sources at various locations in Ibadan metropolis were evaluated to determine the microbial load and distribution in the samples, as well as antibiotic resistance profile of isolates. Total Faecal Coliform Count (TFCC) ranged from log mean of 49±0.03 cfu/ml to 82±0.03 cfu/ml. Biochemical analyses on isolates obtained showed the presence of *Klebsiella* spp., *Aeromonas hydrophilia*, *Salmonella* spp., *Enterobacter* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Chromobacterium* spp., and *Bacillus lichenformis*. Antibiotic sensitivity using disc tests were carried out with antibiotics which included; Amoxicilin, Cotrixomazole, Nitrofuratoin, Gentamicin, Nalidixic Acid, Ofloxacin, Augmentin, Tetracycline. *Pseudomonas* spp, *Chromobacterium* spp *Aeromonas hydrophilia* and *Salmonella* spp were sensitive to Nitrofurantoin while *Enterobacter spp,* and *Aeromonas hydrophilia* were sensitive to Nalidixic acid. Gentamycin was effective against 5 out of the 8 bacteria isolates within this study and those isolates included; *Enterobacter spp, Aeromonas hydrophilia Bacillus licheniformis*, *Acinetobacteri* spp, *Chromobaterium* spp and *Salmonella* spp. Ofloxacin was also seen to be effective against some the isolates such as *Enterobacter spp*, *Acinetobacteria* spp, *Chromobaterium* spp and *Salmonella* spp. Of serious concern is a *Klebsiella* spp which is resistant to all the antibiotics tested. Results revealed water bodies in Ibadan to posses very high content of microbial pathogens. Results from the antibiotics and sensitivity tests were quite disturbing as most of the isolates showed multiple and extended drug resistance to the antibiotics of interest. Researchers note with dismay that some of these water bodies serve as water sources for residents within Ibadan metropolis and thus may serve as reservoirs for spread of resistant microorganisms which could be pathogenic.

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## Introduction

Water is essential to life as an adequate, safe and accessible supply must be available to all. Improving access to safe drinking water can result in significant benefits to health. (WHO, 2014). According to the WHO, the mortality of water associated diseases exceeds 5 million people per year. Wastewater discharges in fresh waters and costal seawaters are the major source of faecal microorganisms including pathogens (Grabow, 1996).

Acute microbial diarrheal diseases are a major public health problem in developing countries. People affected by diarrheal diseases are those with the lowest financial resources and poorest hygienic facilities. Children under five, primarily in Asian and African countries, are the most affected by microbial diseases transmitted through water (Seas *et al*., 2000).

Hospital sewages are usually partially closed and treated regularly yet sewage effluents contaminated with pathogenic microorganisms find their way from these sewers into water bodies around the hospital vicinities. In most rural areas, these water bodies provide water for cooking, washing, bathing, and other household uses. The microorganisms are therefore introduced into their body systems through all these materials and cause all manner of diseases (Rice, 2009).

Hospitals, particularly intensive care units (ICUs), are breeding grounds for the development and spread of antibiotic resistant bacteria due to heavy antibiotic use and the attendant risk of cross infections between hospital staff and patients (WHO, 2016).

The specific objectives for this study were to isolate and identify bacterial isolates from samples of hospital effluents as well as water samples from various sources within the city of Ibadan and determine the antibiotic susceptibility profile of Gram negative bacteria obtained.

**Materials and Methods**

Sewage water samples were collected from two different hospital effluent sites in Ibadan, one at the Adeoyo maternity hospital, Yemetu effluent site, while the second was sewage outlet of the University college hospital, Ibadan. Six water samples from different sources were also collected within Ibadan metropolis. The sites of sample collection included two uncovered wells at Iwo road and Challenge axis, two streams at Dugbe and Iwo road respectively and two Municipal water treatment plants at Eleyele and Asejire areas of Ibadan in Oyo state Nigeria.

The samples were all collected in sterile bottles, put into containers with ice packs and transported safely to the Lead City University Microbiology Laboratory for analysis. Physico- chemical properties of the samples were determined using parameters such as temperature, pH, total hardness, total alkalinity, Chloride levels, total dissolved solids, Total suspended Materials, Total Coliform & Faecal coliform count and total bacteria count (CLI, 2009).

Media used to culture organisms from the samples included Salmonella-shigella agar, Eosin Methylene blue Agar, McConkey Agar and Nutrient Agar. All media were prepared according to their manufacturers' instructions. Serial dilution was carried out on all samples and the dilution factor $10^{-1}$, $10^{-4}$, $10^{-6}$ were selected to be inoculated onto petri dishes using pour plate method and incubated at 23°C for 24 hours after which growth was observed. After incubation, the colonies on each plate were observed and recorded.

After incubation of the inoculants on nutrient agar for 24 hours, identification of the bacterial population from the samples was carried out in a stepwise manner using cultural, morphological and biochemical characteristics of isolates. API-20E test strips were also used in this study to standardize identification system for one of the isolates which was observed to have resistance against all the antibiotics tested. Twenty one miniaturized biochemical tests kits were used and the results compared to the available database matrix for its proper identification.

Antibiotic sensitivity test was done to determine the level of susceptibility of isolates to specific antibiotics using disc diffusion method. Twenty four hour old pure culture of each isolate grown on nutrient broth (optical density adjusted to McFarland 0.5) was subcultured on Mueller-Hinton agar plate (Becton-Dickinson, USA) and incubated for 24 hours at 37°C (CLSI, 2010). Gram negative multidisc (Oxoid) was used. The antibiotic on the disk included: Amoxicilin, Cotrixomazole, Nitrofuratoin, Gentamicin, Nalidixic Acid, Ofloxacin, Augmentin and Tetracycline. The zones of inhibition were measured and interpreted as resistant or sensitive according to CLSI guidelines.

**Results**

Water samples taken from streams and rivers, results showed that the total Faecal Coliform Count (TFCC) ranged from log mean of 49±0.03 cfu/ml in Stream 1 at Iwo road to 82±0.03 cfu/ml in Municipal water 1 at Asejire. Total Coliform Count ranged from 1.84±0.01 cfu/ml ($Log_{10}$) in Municipal Water 1 at Iwo road to 190±0 cfu/ml in Stream 1 at Iwo road and Total Aerobic Count was between 6.25±0.02 cfu/ml in Stream 1 at Iwo road and 6.96±0 cfu/ml in Uncovered Well 2 at Iwo. For the samples from the sewage ranges were between $9.79^{a}$±0.02 Cfu to $9.77^{a}$±0.07Cfu for TFCC for both UCH and Adeoyo hospitals respectively. TCC was between $2.00^{b}$±0.00 to $3.00^{a}$± 0.00 for the samples from UCH and Adeoyo sewage respectively. road. Bacteria isolates were obtained from the two samples of sewage effluent that were gotten from these two hospitals.

**Table 1: Physicochemical Parameters of Water and Sewage samples Collected in Ibadan Metropolis**

| S/N | Parameters | M1 | M2 | S1 | S2 | UCW1 | UCW2 | SUCH | SADY |
|---|---|---|---|---|---|---|---|---|---|
| 1 | pH | 5.78 | 7.21 | 6.83 | 6.46 | 7.25 | 7.26 | 7.12 | 6.23 |
| 2 | Temperature (°C) | 30 | 26 | 24.5 | 29 | 27 | 26 | 24 | 23 |
| 3 | Alkalinity test (mg/I) <75=normal | 25 | 39 | 100 | 23 | 47 | 200 | 57 | 50 |
| 4 | Total hardness (mg/I) <100=normal | 194 | 208 | 140 | 116 | 128 | 186 | 125 | 148 |
| 5 | Chloride content (mg/I) | 5.5 | 1.6 | 5.0 | 2.3 | 0.9 | 0.6 | 0.5 | 0.2 |
| 6 | Total suspended solid (mg/I) | 4.2 | 4.4 | 0.2 | 0.2 | 6.8 | 7.0 | 2.7 | 3.5 |
| 7 | Total dissolved solid (mg/I) | 10 | 4 | 7 | 4 | 5 | 3 | 5 | 6 |

Key: M1 = Municipal water 1, Municipal water 2, Stream 1, Stream 2, Uncovered well 1, Uncovered well 2, SUCH-Sewage in UCH, SADY- Sewage from Adeoyo

Colony Morphologies of isolates were determined and results shown on Table 1. Eight organisms were obtained from pure colonies. Antibiotic sensitivity test was carried out on these isolates, and the result of the test is shown in Table 3. Isolate coded ADY/UCH3 was identified as *Klebsiella oxytoca* using API strip kit, results are shown on Table 5.

The results revealed that sewage from UCH and Adeoyo's microbial loads were significantly different ($P<0.05$) with log means of 9.79±0.02 and 9.77±0.07 on Nutrient Agar. On MA, Adeoyo had the higher log mean total coliform count of 3.00±0.00 and UCH had a mean total coliform count of 2.00±0.00 in log form which is significantly different ($P<0.05$) from that of Adeoyo. However, on SSA, total aerobic bacterial count of Adeoyo sample was 6.36 ±0.06 and UCH had log mean count of 6.83±0.01 which are significantly different ($P<0.05$).

**Table 2a: Bacteria Counts from Water samples in Ibadan ($Log_{10}$), 1st Sample Collection**

| S/N | Description/ Location | TFCC Cfu/ml (log10) | TCC Cfu/100ml (log10) | TAC Cfu/ml (log10) |
|---|---|---|---|---|
| 1. | Municipal Water 1 (Asejire) | 82±0.03$^{(d)}$ | 1.84±0.01$^{(d)}$ | 6.71±0.03$^{(b)}$ |
| 2. | Municipal Water 2 (Eleyele) | 77±0.03$^{(cd)}$ | 1.78±0.04$^{(e)}$ | 6.77±0.05$^{(b)}$ |
| 3. | Uncovered Well 1 (Challenge) | 79±0.03$^{(cd)}$ | 1.78±0.02$^{(e)}$ | 6.86±0.06$^{(c)}$ |
| 4. | Uncovered Well 2 (Iwo road) | 74±0$^{(c)}$ | 1.95±0$^{(c)}$ | 6.96±0$^{(d)}$ |
| 5. | Stream 1 (Iwo road) | 55±0.03$^{(b)}$ | 2.34±0.00$^{(a)}$ | 6.21±0.01$^{(a)}$ |
| 6. | Stream 2 (Dugbe) | 47±0.05$^{(a)}$ | 2.23±0$^{(b)}$ | 6.25±0.02$^{(a)}$ |
| 7. | Sewage (UCH) | 9.79$^{a}$±0.02 | 2.00$^{b}$±0.00 | 6.83$^{a}$±0.01 |
| 8. | Sewage (Adeoyo) | 9.77$^{a}$±0.07 | 3.00$^{a}$± 0.00 | 6.36$^{b}$± 0.06 |

Values represent Log average and Log standard deviation of bacteria counts from triplicates.
Means on the same column with different superscript are significantly different while the means with the same superscript are not significantly different ($p<0.05$).

**Table 2b: Bacteria Counts Of Bacterial Isolates From Water Bodies In Ibadan $Log_{10}$ on Samples, 2nd Sample Collection**

| S/N | Description/ Location | TFCC Cfu/ml (Log10) | TCC Cfu/100ml (Log10) | TAC Cfu/ml (Log10) $10^8$ |
|---|---|---|---|---|
| 1. | Municipal Water 1 (Asejire) | 0.19±0.02$^{(c)}$ | 90± 1$^{(d)}$ | 6.82± 0.06$^{(bc)}$ |
| 2. | Municipal Water 2 (Eleyele) | 0.18±0.0$^{(c)}$ | 60± 2$^{(e)}$ | 6.77± 0.03$^{(b)}$ |
| 3. | Uncovered Well 1 (Challenge) | 0.19±0.02$^{(c)}$ | 90± 2$^{(d)}$ | 6.82± 0.03$^{(bc)}$ |
| 4. | Uncovered Well 2 (Iwo road) | 0.17± 0.01$^{(c)}$ | 100±0$^{(c)}$ | 6.85±0$^{(c)}$ |
| 5. | Stream 1 (Iwo road) | 0.35±0.05$^{(a)}$ | 190± 0$^{(a)}$ | 6.26± 0.03$^{(a)}$ |
| 6. | Stream 2 (Dugbe) | 0.28±0.02$^{(b)}$ | 180±0$^{(b)}$ | 6.22±0.01$^{(a)}$ |
| 7. | Sewage (UCH) | 6.30$^{a}$ | 0.10$^{b}$ | 6.70$^{a}$ |
| 8. | Sewage (Adeoyo) | 6.00$^{a}$ | 1.00$^{a}$ | 2.30$^{b}$ |

Values present Log average and Log standard deviation of bacteria counts from triplicates.
Means on the same column with different superscript are significantly different while the means with the same superscript are not significantly different ($p<0.05$).

**Table 3: Cultural description of Bacterial Isolates from Samples**

| S/N | Isolates Code | Isolates Code | Colonial Description |
|---|---|---|---|
| 1 | | ADY/ UCH 1 | Smooth black colony on SSA |
| 2 | | ADY/ UCH 2 | Mucoid pale pink colony on MA |
| 3 | | ADY/ UCH 3 | Smooth black colony on SSA |
| 4 | | ADY/ UCH 4 | Watery pale pink colony on SSA |
| 5 | | UC1 | Mucoid pinkish cream colony on MA |
| 6 | | UC2 | Creamy whitish colony on NA |
| 7 | | ADY/ UCH 6 | No pigmented domed and mucoid in nature |
| 8 | | ADY/ UCH 5 | Opaque to white colony like growth with hair like growth |

**Table 4: Biochemical Characteristics of the Isolates**

| Gram Staining | Citrate | Catalase | $H_2S$ | Growth in KCN | Indole Test | Methyl Red Test | VP Test | Urease Test | **Probable identity of isolates** |
|---|---|---|---|---|---|---|---|---|---|
| - | + | + | - | + | - | - | + | | *Enterobacter* spp |
| - | + | + | + | + | - | + | - | | *Aeromonas hydrophilia* |
| - | + | - | + | + | - | | | | *Salmonella* spp |
| | | | | | | | | | *Pseudomonas* spp |
| - | + | + | - | | - | - | + | + | *Klebsiella* spp |
| - | - | + | - | + | + | + | - | - | *Chromobacterium* spp |
| | + | + | - | - | + | - | - | - | *Acinetobacter* spp |
| + | - | - | + | - | - | - | + | - | *Bacillus Licheniformis* |

**Table 5: Antibiotics Susceptibility Profile of the Isolates**

| Isolates | AUG | OFL | GEN | NAL | NIT | COT | AMX | TET |
|---|---|---|---|---|---|---|---|---|
| *Pseudomonas spp* | R | R | R | R | 14 | R | R | R |
| *Enterobacter spp* | R | 22mm | 11mm | 20mm | R | R | R | R |
| *Klebsiella spp* | R | R | R | R | R | R | R | R |
| *Aeromonas hydrophilia* | R | R | 19mm | 21mm | 11mm | R | R | R |
| *Bacillus licheniformis* | R | R | 15mm | R | 26mm | R | R | R |
| *Acinetobacteri* spp | R | 20mm | 70mm | R | R | R | R | R |
| *Chromobaterium* spp | R | 25mm | R | R | 25mm | R | R | R |
| *Salmonella* spp | R | 17mm | 09mm | R | 14mm | 12mm | R | R |

Where R stands for resistance and sensitivity is measured in mm,

**Table 6: API Kit Biochemical Test Results of *Klebsiella* spp**

| | | |
|---|---|---|
| ONPG | 1 | + |
| ADH | 2 | - |
| LDC4 | | - |
| ODC | 1 | - |
| CIT | 2 | - |
| $H_2S$ | 4 | - |
| URE | 1 | - |
| TDA | 2 | - |
| IND | 4 | - |
| VP | 1 | - |
| GEL | 2 | - |
| GLU | 4 | + |
| MAN | 1 | + |
| INO | 2 | + |
| SOR | 4 | + |
| RHA | 1 | + |
| SAC | 2 | - |
| MEL | 4 | + |
| AMY | 1 | + |
| ARA | 2 | + |
| OX | 4 | - |
| $NO_21$ | | + |
| $N_2$ | 2 | - |
| MOB | 4 | - |
| McC | 1 | + |
| OF-O | 2 | + |
| OF-F | 4 | + |

+ represents positivity to test while – represents negativity to test.

**Discussion**

Water is essential to life and the presence of microorganisms that are highly resistant to antibiotics in water is quite alarming. Results within this study show a high number of microbial isolates showing all manners and degrees of resistance to antibiotics of interest. Results show that bacterial species that were found in the water samples included: *Klebsiella oxytoca*, *Aeromonas Spp.*, *Salmonella Spp.*, *Enterobacter Spp.*, *Pseudomonas spp Acinetobacter spp*.

Running water bodies are considered especially relevant as putative reservoirs of multi-resistant bacteria, since they collect surface waters containing materials from different origins, e.g., wastewater plants, water of urban or industrial effluents, agricultural activities, or rain (EPA 2009). Thus, they provide an immense resistome, including pathogenic and non-pathogenic antibiotic-resistant bacteria (Lupo *et al*., 2012). The *Enterobacteriaceae* group isolated from water samples of water bodies used from this research work, were all resistance to Amoxillin, Augmentin, and Tetracycline. *Pseudomonas* spp, *Acinetobacte spp, Chromobacterium violaceum* and *Klebsiella* spp were all resistant to Cotrimoxazole.

*Pseudomonas* spp, *Chromobacterium* spp *Aeromonas hydrophilia* and *Salmonella* spp were sensitive to Nitrofurantoin while *Enterobacter spp,* and *Aeromonas hydrophilia* were sensitive to Nalidixic acid. Gentamycin was effective against 5 out of the 8 bacteria isolated from samples within this study and those isolates included; *Enterobacter spp, Aeromonas hydrophilia Bacillus licheniformis*, *Acinetobacteri* spp, *Chromobaterium* spp and *Salmonella* spp. Ofloxacin was also seen to be effective against some the isolates such as *Enterobacter spp*, *Acinetobacteri* spp, *Chromobaterium* spp and *Salmonella* spp.

Coliforms are bacteria are generally considered a risk to health, and infection due to coliform may be fatal for infants, elderly and immune-compromised people (www.emedicinehealth.com). Attahiru *et al*., (2016) reaffirmed in their study that the presence of coliform such as *Klebsiella*, indicates that water is contaminated by dangerous faecal matter. WHO standard aerobic bacteria in the potable water states that the total aerobic bacteria count should not be more than 100cfu/ml (WHO, 2006).

The presence of counts exceeding the WHO limits indicates that the water samples contain high concentration of bacteria that could make the water unsafe for drinking. Ranges of coliform contamination of water within this study were quite high indicating that a lot of these water bodies had feacal contamination and were thus unsafe for human consumption. Yet, sadly they form sources of water for Ibadan residents to wash household tools, do laundry and sometimes drink.

Hospital acquired infections (HAIs) are also a major safety concern for both health care providers and the patients. According to EPA standard, every water sample that has coliform must be analysed for either faecal coliforms or *Klebsiella* (EPA, 2009) with a view to ascertaining contamination with human and animal waste and possibly pathogenic bacteria or organism. The presence of coliform bacteria in the stream and water samples were a clear indication that these water samples were not fit for human consumption and domestic use,.

**Conclusion**

This aim of this study was to determine the antimicrobial profile of isolates obtained from sewages and some water samples in different locations in Ibadan metropolis. Results showed that Bacterial isolates within the study areas were highly resistant to a lot of antibiotics which are being used to treat water related infections. Nigerian scientists must continue to

battle bacterial resistance in antibiotics because if not, it could be our undoing as issues as little as cuts and scrapes could lead to death. We may go back to the pre-antibiotic age, where activities of daily routine brought with them the risk of death.

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