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# Antibiotics Sensitivity Pattern of Coliform Bacteria Isolated From Well Water Samples in Ipetumodu, Before and After Solar Disinfection

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**ABSTRACT**

Microbial quality of portable water is a major public health concern in developing countries. This is because the primary domestic source of the water is usually from untreated groundwater. This study assessed the bacteriological quality of well water in Ipetumodu, South-western, Nigeria. Additionally, the solar disinfection (SODIS), and antibiotic sensitivity of coliform isolates were evaluated. Five different hand-dug well water samples (in duplicate) were cultured on Eosin Methylene Blue agar using the membrane filtration technique. Parallel aliquots were exposed to direct sunlight for six hours. The pH of the water samples ranged from 5.91 to 6.17. The results revealed that the coliform counts before SODIS ranged from 4.8×10 to $1.2\times10^2$ CFU/100mL and were reduced to 0–8.0 CFU/100mL after SODIS, with complete microbial elimination in two wells. The most recovered isolates were *Klebsiella*, *Salmonella*, *Shigella*, and *Enterobacter* species. Most of the bacteria exhibited high resistance to most of the tested antibiotics like Augmentin (100%), Cefuroxime (100%), and Cefixime (92%). Overall, the findings showed that hand-dug well water in Ipetumodu does not meet national (NAFDAC) or World Health Organization microbiological standards for potable water and is unsafe for consumption without treatment. SODIS represents a feasible, low-cost option for improving water safety but should be integrated with broader interventions and antibiotic stewardship to achieve consistent safety.

**Keywords:** Coliform Bacteria, Well water, Antibiotic Resistance, Solar disinfection

## Introduction

Water is important for human survival. Despite its importance, over 25% of the world population still lack access to portable water, with higher prevalence in developing countries (WHO, 2024). Contaminated water usually serves as a medium ng for the transmission of pathogenic microorganisms. This often leads to communicable diseases such as typhoid, dysentery, cholera, and parasitic infections that place additional pressure on already limited healthcare systems in developing countries. The World Health Organization (WHO, 2017; 2024) has emphasized that safe collection, storage, and treatment of drinking water are necessary to reduce health risks associated with water contamination. Drinking water are mostly seen as bacteriologically safe when indicator organisms are absent (WHO 2011; 2013; Gebrewahd *et al.,* 2020). Water resources are

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widely distributed across the world, yet, only a small portion of freshwater is readily available for human consumption (Völker *et al.,* 2010; Bibi *et al.,* 2016). This has made access to drinkable water to remain a major challenge in many resource-limited countries (Raji & Ibrahim, 2011; Odetoyin *et al.,* 2022). Many rural and peri-urban communities therefore depend on unimproved water sources, which increases their exposure to microbial contamination. In Osun State, a considerable number of residents rely on hand-dug wells, boreholes, surface water sources, and rainwater as their major source(s) of drinking water. In Ipetumodu, hand-dug wells serve as the major source of drinking water for students and residents. These wells are often poorly protected and are exposed to contamination, making regular microbiological assessment necessary. The quality of water can be improved via divers' ways, including chlorination and advanced filtration systems. However, the high cost and technical requirements of these methods limit their use in this locality. Solar disinfection (SODIS) has been identified as a simple, cheap, and eco-friendly method for improving drinking water quality. The method uses sunlight to reduce the number of microorganisms present in water (Luzi *et al.,* 2016; Gebrewahd *et al.,* 2019). Because of its affordability and ease of use, SODIS is being seen as an alternative technique in this resource-limited settings (Nelson *et al.,* 2019). This study assessed the bacteriological quality of well water in Ipetumodu, determined the effectiveness of solar disinfection in reducing coliform bacteria, and evaluated the antibiotic sensitivity patterns of the isolated coliform organisms.

## Materials and Methods

### Study Area and Sample Collection

We carried out the study at Ipetumodu, Osun State, Nigeria, where 5 hand-dug well water samples (both open and close wells) were collected aseptically. All samples were transported in an ice-packed cooler boxes to the microbiology laboratory of Oduduwa University within six hours of collection for processing. Ipetumodu is an ancient town in Osun state with a population of over 211,100 (NPC, 2016).

### Solar Disinfection (SODIS) Treatment

To test for SODIS, we poured each water sample into clear PET bottles and placed the bottles under direct sunlight on an open rooftop for six hours (García-Gil *et al.,* 2021). Some portions of the water were left untreated to serve as controls. After the sunlight exposure, both the treated and untreated samples were analyzed for bacteria.

### Determination of pH

The pH of the water samples was measured using a pH meter that was calibrated with a 7.0 phosphate buffer solution as described by the manufacturer. The readings were recorded from the digital display.

### Enumeration of Coliforms by Membrane Filtration

The number of coliform bacteria in the water was determined using the membrane filtration method. For each sample, 100 mL of water was filtered through a sterile cellulose nitrate membrane with a 0.45µm pore size. The filters were then placed on Eosin Methylene Blue (EMB) agar and MacConkey agar (Oxoid Ltd., Hampshire, England) plates aseptically and incubated aerobically at 37°C for 24hours. Suspected discrete colonies showing typical coliform characteristics were sub-cultured on Nutrient agar (Oxoid Ltd., Basingstoke, Hampshire, England) plates incubated aerobically at 37°C for 24 hours to obtain pure culture and for further analyses. The isolates were identified by conventional biochemical tests (Cheesbrough, 2006) and Microbact™ 24E identification kit.

### Antibiotic Sensitivity Testing

Kirby-Bauer disc diffusion technique was used to determine the antimicrobial susceptibility pattern of recovered isolates strains following the guidelines set by the Clinical Laboratory Standards Institute (CLSI, 2024). The tested antibiotics were; Ceftazidime (30µg), Cefuroxime (30µg), Gentamicin (10µg), Cefixime (5µg), Ofloxacin (5µg), Augmentin (30µg), and Ciprofloxacin (5µg) (Oxoid Ltd., Basingstoke

Hampshire, England).

## Results

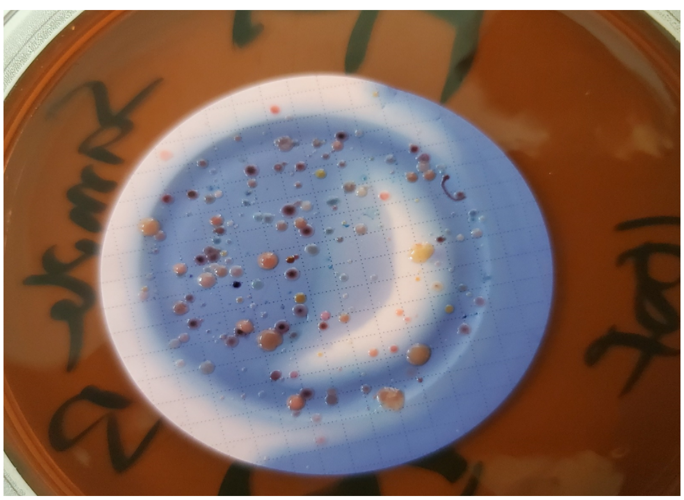
### pH Values of Well Water Samples

The pH values of the five well water samples ranged from 5.91 to 6.17, as shown in Table 1. These values indicate that all the samples were slightly acidic.

**Table 1:** The pH Values of the Water Samples from Well Water in Ipetumodu

| Sample Code | pH Values |
|---|---|
| A | 6.15 |
| B | 6.17 |
| C | 6.11 |
| D | 6.14 |
| E | 5.91 |

**Figure 1.** Eosin Methylene Blue Agar of a Well



Water Sample on Membrane Filter Paper after 24 Hours Incubation

### Cultural, Morphological, and Biochemical Characteristics of Isolates

Green metallic sheen colony, a characteristic of *Escherichia coli* was observed after 24 hours of incubation on EMB agar as shown in Figure 1. *Klebsiella spp., Escherichia coli, Salmonella spp.,* and *Shigella spp.* were the predominant bacteria recovered as presented in Table 2.

### Coliform Counts Before and After Solar Disinfection

The coliform counts of the untreated well water samples ranged from $4.8\times10$ to $1.2\times10^2$CFU/100mL, as presented in Table 3. Sample C recorded the highest coliform count ($1.2\times10^2$CFU/100mL). After solar disinfection treatment, there was a noticeable reduction in coliform counts across all samples. Complete (100%) elimination of coliforms was observed in Samples A and E. Samples C and D showed partial reductions, while Sample B showed a significant reduction but not complete elimination.

### Antibiotic Sensitivity Patterns

Antibiotics susceptibility testing of the isolates revealed varying resistance patterns, as shown in Figure 2. The isolates exhibited high resistance to Augmentin (100%), Cefuroxime (100%), Cefixime (92%), Ceftazidime (67%), and Gentamicin (67%). However, lower resistance was observed against fluoroquinolones, with Ciprofloxacin showing the highest level of effectiveness against the isolates.

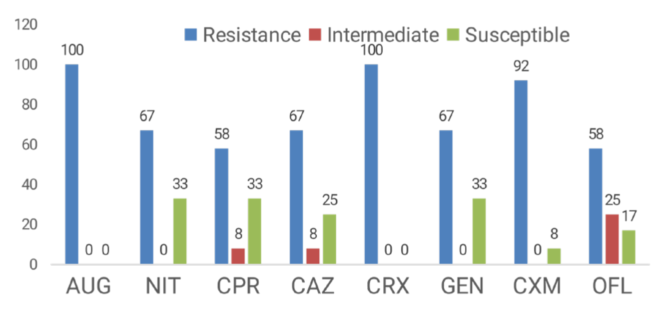



**Figure 2:** Antibiotics Sensitivity Pattern of Isolates from Different Well water in Ipetumodu

LEGENDS for Figure 2: CAZ=Ceftazidime (30µg); CRX=Cefuroxime (30µg); GEN=Gentamicin (10µg); CXM=Cefixime (5µg); OFL=Ofloxacin; (5µg) AUG=Augmentin (30µg); NIT=Nitrofurantoin (300µg); CPR=Ciprofloxacin (5µg); R=Resistant; I=Intermediate; S=Susceptible.

**Table 2:** Cultural, Morphological and Biochemical Characteristics of Dominant Genera of Bacteria Isolated from Well Water

| IC | Cultural Characteristics *Whole colony, Edge, Elevation, Surface, Colour, Opacity* | GR | CS | CT | CUT | IND | MR | VP | OXI | Suspected Organism |
|---|---|---|---|---|---|---|---|---|---|---|
| A1 | Irregular, lobate, Flat, smooth, pink, translucent | -ve | Rod | +ve | +ve | -ve | -ve | +ve | -ve | *Klebsiella* sp. |
| A2 | Circular, entire, rough, flat pink, translucent | -ve | Rod | +ve | +ve | -ve | -ve | +ve | -ve | *Klebsiella* sp. |
| A3 | Circular, entire, raised, smooth, pink, opaque | -ve | Rod | +ve | +ve | -ve | -ve | +ve | -ve | *Klebsiella* sp. |
| A4 | Irregular, lobate, flat, smooth, pink, translucent | -ve | Rod | +ve | +ve | -ve | -ve | +ve | -ve | *Klebsiella* sp. |
| A5 | Irregular, lobate, grow into the medium, black, opaque | -ve | Rod | +ve | -ve | -ve | +ve | -ve | -ve | *Salmonella* sp. |

| IC | Cultural Characteristics *Whole colony, Edge, Elevation, Surface, Colour, Opacity* | GR | CS | CT | CUT | IND | MR | VP | OXI | Suspected Organism |
|---|---|---|---|---|---|---|---|---|---|---|
| A6 | Circular, entire, flat, smooth, cream, translucent | -ve | Rod | +ve | -ve | -ve | +ve | -ve | -ve | *Shigella* sp. |
| A7 | Circular, entire, flat, smooth, brown, opaque | -ve | Rod | +ve | +ve | -ve | +ve | -ve | -ve | *Salmonella* sp. |
| B8 | Irregular, lobate, flat, smooth, pink, translucent | -ve | Rod | +ve | +ve | -ve | -ve | +ve | -ve | *Klebsiella* sp. |
| B9 | Irregular, convex, raised, smooth, pink, translucent | -ve | Rod | +ve | +ve | -ve | -ve | +ve | -ve | *Klebsiella* sp. |
| B10 | Circular, entire, raised, smooth, pink, opaque | -ve | Rod | +ve | +ve | -ve | -ve | +ve | -ve | *Klebsiella* sp. |
| B11 | Circular, entire, raised, smooth, cream, translucent | -ve | Rod | +ve | -ve | -ve | +ve | -ve | -ve | *Shigella* sp. |

| IC | Cultural Characteristics *Whole colony, Edge, Elevation, Surface, Colour, Opacity* | GR | CS | CT | CUT | IND | MR | VP | OXI | Suspected Organism |
|---|---|---|---|---|---|---|---|---|---|---|
| B12 | Circular, entire, flat, smooth, black, opaque | -ve | Rod | +ve | -ve | -ve | +ve | -ve | -ve | *Salmonella* sp. |
| C15 | Filamentous, rhizoid, raised, rough, pink, opaque | -ve | Rod | +ve | +ve | -ve | -ve | +ve | -ve | *Klebsiella* sp. |
| C16 | Circular, entire, flat, convex, pink | -ve | Rod | +ve | +ve | -ve | -ve | +ve | -ve | *Klebsiella* sp. |
| C17 | Filamentous, rhizoid, raised, rough, black | -ve | Rod | +ve | -ve | -ve | +ve | -ve | -ve | *Salmonella* sp. |
| C18 | Circular, entire, flat, smooth, brown | -ve | Rod | +ve | +ve | -ve | +ve | -ve | -ve | *Salmonella* sp. |
| C19 | Circular, entire, raise, smooth, pink, opaque | -ve | Rod | +ve | +ve | -ve | -ve | +ve | -ve | *Enterobacter* sp. |
| D20 | Circular, entire, raised, smooth, pink, opaque | -ve | Rod | +ve | +ve | -ve | -ve | +ve | -ve | *Klebsiella* sp. |
| D21 | Circular, entire, raised, smooth, pink, opaque | -ve | Rod | +ve | +ve | -ve | -ve | +ve | -ve | *Klebsiella* sp. |

| IC | Cultural Characteristics *Whole colony, Edge, Elevation, Surface, Colour, Opacity* | GR | CS | CT | CUT | IND | MR | VP | OXI | Suspected Organism |
|---|---|---|---|---|---|---|---|---|---|---|
| D22 | Irregular, lobate, flat, smooth, black, opaque | -ve | Rod | +ve | +ve | -ve | +ve | -ve | -ve | *Salmonella* sp. |
| D23 | Irregular, entire, flat, smooth, cream, translucent | -ve | Rod | +ve | -ve | -ve | +ve | -ve | -ve | *Shigella* sp. |
| D24 | Circular, entire, raised, smooth, pink, opaque | -ve | Rod | +ve | +ve | -ve | -ve | +ve | -ve | *Enterobacter* sp. |
| D25 | Circular, entire, flat, smooth, black, opaque | -ve | Rod | +ve | -ve | -ve | +ve | -ve | -ve | *Salmonella* sp. |
| E26 | Irregular, filamentous, flat, rough, pink. Opaque | -ve | Rod | +ve | +ve | -ve | -ve | +ve | -ve | *Klebsiella* sp. |
| E27 | Circular, entire, raised, smooth, pink, opaque | -ve | Rod | +ve | +ve | -ve | -ve | +ve | -ve | *Klebsiella* sp. |
| E31 | Irregular, entire, raised, smooth, cream, opaque | -ve | Rod | +ve | -ve | -ve | +ve | -ve | -ve | *Shigella* sp. |

LEGENDS for Table 2: (-ve) Negative, (+ve) Positive, CUT= Citrate Utilization Test, CT= Catalase Test, GR= Gram Reaction, CS= Cell Shape, IND= Indole test, MR= Methyl Red test, VP= Voges-Proskauer test, OXI= Oxidase test.

**Table 3: Coliform Counts in Well Water Samples Before and After Solar Disinfection**

| Sample | Coliform Count Before Solar Disinfection (CFU/100ml) | Coliform Count After Solar Disinfection (CFU/100ml) | Remarks |
|---|---|---|---|
| A | 7.0 x 10 | 0 | Complete Elimination |
| B | 8.2 x 10 | 3.0 | Significant Reduction |
| C | $1.2 \times 10^2$ | 4.0 x 10 | Partial Reduction |
| D | 4.5 x 10 | 8.0 | Partial Reduction |
| E | 6.3 x 10 | 0 | Complete Elimination |

## Discussion

The results of this study indicate that hand-dug well water sources in Ipetumodu are contaminated with coliform bacteria at levels exceeding the permissible limits recommended by World Health Organization (WHO, 2017). WHO (2017) described drinking water to be free from faecal coliforms in any 100mL sample, while total coliforms should ideally be absent. The coliform counts recorded in this study therefore represent a significant public health concern. The detection of *Escherichia coli* in the water samples is a strong indication of faecal contamination which is similar to what was reported by Odetoyin *et al.* (2022) that reported different diarrheagenic *Escherichia coli* from well water sources in Ile-Ife. *Salmonella* spp. and *Shigella spp.* were also recovered from this study, these are pathogens that has been linked with illnesses such as typhoid fever and dysentery. Continuous consumption of contaminated water from these wells may increase the risk of waterborne infections, particularly among individuals with weakened immune systems like children, the elderly (Egberongbe *et al.,* 2012; Akinlabi *et al.,* 2022; Ali *et al.,* 2023).

SODIS was carried out in this study as a simple and low-cost method for improving water quality. The results showed that SODIS was effective in reducing coliform counts in all treated samples. Complete elimination of coliform bacteria was achieved in two samples, while partial reductions were observed in the remaining samples. The differences in treatment effectiveness may be related to factors such as the initial level of contamination and the clarity of the water. High turbidity can reduce the effectiveness of sunlight penetration, thereby lowering the disinfection efficiency. Despite these limitations, the overall reduction in coliform counts supports previous findings and confirms the usefulness of SODIS as a practical household water treatment option in resource-limited communities (Bibi *et al.,* 2016).

The antibiotic susceptibility results further raise public health concerns. Although some of the coliform isolates were susceptibility to fluoroquinolones such as Ciprofloxacin and Ofloxacin, high levels of resistance were observed against commonly used antibiotics, including Augmentin, Cefuroxime, Cefixime, and Ceftazidime; probably as a result of its increased availability of multiple generic formulations in the markets (Von Baum and Marre 2005; Holmes *et al.,* 2016; Cavany *et al.,* 2023). Resistance to these frequently prescribed antibiotics limits treatment options for waterborne infections and reflects the growing problem of antibiotic resistance. The convergence of unsafe drinking water and antibiotic-resistant bacteria poses a serious health challenge. Contaminated water increases the risk of infection, while antibiotic resistance reduces the effectiveness of available treatments.

## Conclusion

This study shows that hand-dug well water sources in Ipetumodu are not microbiologically safe for direct consumption, as coliform contamination levels exceeded recommended safety standards. The identification of pathogenic coliform bacteria indicates a significant health risk to the local population. Solar disinfection (SODIS) was found to be an effective, affordable, and simple method for reducing bacterial contamination in well water, making it suitable for use in resource-limited settings. However, the high level of antibiotic resistance observed among the bacterial isolates presents an additional public health concern. To reduce these risks, there is a need for regular water quality assessment, increased adoption of household water treatment methods such as SODIS, and improved antibiotic stewardship practices. Ensuring access to safe drinking water and promoting responsible antibiotic use are essential for protecting public health in Ipetumodu community.

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