# Antibiotics resistance of a strain of *Escherichia coli* isolated from bore hole in Ile Ife, Osun state, Nigeria

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**Abstract:***Escherichia coli* were isolated from water from two boreholes in Ile Ife, Osun state, Nigeria. This was an indication of faecal contamination. These strains of *Escherichia coli* were Gram negative short rods, Catalase positive, Methyl red positive, Voges Proskaeur negative. The strains could ferment glucose galactose, sucrose, lactose, mannitol and maltose with the production of acid and gas but could not hydrolyze starch. A particular strain was resistant to sulfamethoxazole, ampicillin, cotrimoxazole, cephaloridine, streptomycin, carbenicillin, sulfafurazole and tetracycline but sensitive to gentamicin, colistin, nalidixic acid, nitrofurantoin and colistin sulphate. [Ajayi, Adesola Adetutu. **Antibiotics resistance of a strain of *Escherichia coli* isolated from bore hole in Ile Ife, Osun state, Nigeri.** Nature and Science 2011;9(8):6-9]. (ISSN: 1545-0740). http://www.sciencepub.net

**Key words:** Escherichia coli, borehole water, antibiotics resistance.

## Introduction

Water is absolutely essential to all forms of life (Fawole et al., 2002). It is a basic pre-requisite for existence and if not properly treated and handled, can be a medium for breeding some organisms (Ward et al., 1998). These organisms form a bedrock of epidemic and communicable diseases (Brock and Madigan, 1999.) Biological pollutants in drinking water cause the most widespread diseases. They include bacteria, fungi, viruses, actinomycetes, algae and protozoa (Niemi et al., 1982). Bacteria transmitted by drinking water usually grow in the intestinal tract (Greenberg and Hunt, 1985). They are excreted through feaces of an infected person and may find their way via sewage into sources of drinking water (Brock and Madigan, 1999). Enteric Gram negative bacteria (coliforms) are a large heterogenous group of microbes whose natural habitat is the intestinal tract of humans and animals (Greenberg and Hunt, 1985). The genus *Escherichia* is a member of this group (Olutiola *et al.*, 1982). The pathogenic strains of *Escherichia coli* are agents of infantile diarrhea which results in mortality among babies (Antai and Anozie, 1987). It is also a common source of traveler's diarrhea (Brock and Madigan, 1999). Coliforms have been historically used as indicator microorganisms to serve as a measure of feacal contamination and thus potentially of the presence of enteric pathogens in fresh water (Greenberg and Hunt, 1985). This study was designed to assess the safety of drinking water from some boreholes in Ile Ife, Nigeria. *Escherichia coli* was used as an index. Attempts were made to determine the antibiotics resistance pattern of *Escherichia coli* isolated from drinking water from one of these boreholes.

## Materials And Methods

### Sample collection

Water samples were collected from three different boreholes located at different locations in Ile Ife, Osun State, Nigeria. The taps were allowed to run for five minutes to dislodge microbes adhering to the mouth of the taps. The samples were collected into sterile bottles and covered immediately. They were stored in ice packs and transported to the laboratory for immediate analysis (Olutiola *et al.*, 1982).

### Media

All media used were from LAB M, International Diagnostic Group Plc. They were Nutrient agar, MacConkey agar and Eosin Methylene Blue (EMB) agar. They were prepared according to the manufacturer's instructions.

### Biochemical tests and staining techniques

Biochemical tests carried out were Koser's citrate test, catalase test, indole production test, Methyl Red Voges-Prokaeur (MRVP) test and sugar fermentation tests. Other biochemical tests carried out include gelatin liquefaction, Gram strain and spore stain.

### Antibiotic sensitivity test

Antibiotics sensitivity test was carried out on just one positive strain. Diagnostic sensitivity test (DST) agar (Oxoid) and antibiotic multodisks (Gram negative discs and U4 discs) (Oxoid) were used.

**Results**

Water samples were obtained from different boreholes in Ile Ife, Osun state, Nigeria and examined for the presence of *Escherichia coli. Escherichia coli* were observed in water from two of the boreholes but was absent in water from the third borehole.

On nutrient agar, the organisms grew as small colonies with smooth surface and cream color. After incubation at 37˚C for twenty four hours and forty eight hours respectively, the organism grew abundantly at both durations with production of acid and gas in MacConkey broth but with more growth at forty eight hours. On Eosin Methylene Blue (EMB) agar, the colonies appeared as metallic sheen. Slightly turbid growth was observed in peptone water (Table 1).

Gram stain of the organisms showed Gram negative short rods. The isolates were non-spore formers (Table 2). The isolates were unable to utilize citrate as the sole carbon source. The citrate medium remained green in color after incubation at 37˚C for five days (Table 2.) However, within the same period, the isolates produced indole (Table 2).

The organisms examined produced effervescence of gas after emulsifying with a loopful of hydrogen peroxide indicating production of catalase (Table 2). The inoculated MRVP medium was incubated at 37˚C for five days. On adding Methyl Red solution, the formation of red color indicated positive Methyl Red test. However, on addition of Barrit's reagent, the medium remained yellow, indicating negative Voges-Proskaeur test (Table 2).

There was a clear zone on the plate of inoculated nutrient gelatin medium upon addition of mercuric chloride solution. This indicated a negative result for hydrolysis of gelatin (Table 2).

The sugars employed, glucose, galactose, lactose, mannitol and maltose were fermented with production of acid and gas (Table 3). Antibiotics sensitivity test was carried out on just one strain of the isolated *Escherichia coli* . For Gram negative multodisk (Oxoid), the organism was resistant to ampicillin cotrimoxazole, streptomycin and tetracycline but sensitive to colistin sulfate, gentamycin, nalidixic acid and nitrofurantoin. For U4 multodisk (oxoid), the organism was sensitive to gentamicin, but resistant to cephaloridine, tetracycline, sulfamethoxazole, sulfafurazole and ampicillin (Table 4).

**Table 1: Cultural and morphological characteristics of organisms isolated from borehole water samples**

| Medium | Characteristics |
|---|---|
| Growth on nutrient agar | Smooth surface, small, low convex and cream coloured features. Abundant growth, production of acid and gas. |
| Growth on MacConkey broth | |
| Growth on Eosin Methylene blue (EMB) agar. | Smooth surface, low convex with metallic green sheen, small and low convex. |
| Growth in peptone water | Slightly turbid |

**Table 2: Biochemical characterization**

| Test | Observation |
|---|---|
| Gram stain | Short rods |
| Catalase | Positive |
| Citrate | Negative |
| Methyl red | Positive |
| Voges-Proskaeur | Negative |
| Indole | Positive |
| Gelatin liquefaction | Negative |
| Spore formation | Negative |

**Table 3: Sugar Fermentation Test**

| Test | Observation |
|---|---|
| Glucose | AG |
| Galactose | AG |
| Sucrose | AG |
| Lactose | AG |
| Mannitol | AG |
| Maltose | AG |
| Starch | Nil |

Key: Acid production; G- Gas production ; AG- Acid and Gas production

**Table 4: Antibiotics sensitivity of *Escherichia coli* isolated from water from borehole**

| Antibiotics code | Concentration (µg) | Zones of inhibition (mm) |
|---|---|---|
| COT | 25 | 0 |
| STR | 25 | 0 |
| TET | 25 | 0 |
| PN | 25 | 0 |
| COL | 25 | 10 |
| GN | 10 | 21 |
| NAL | 30 | 7 |
| NIT | 200 | 14 |
| CR | 25 | 0 |
| GN | 10 | 17 |
| TE | 50 | 0 |
| SXT | 25 | 0 |
| PY | 100 | 0 |
| PN | 25 | 0 |
| SF | 500 | 0 |
| CT | 10 | 13 |

**Key:**

**Gram negative Discs**
COT – Cotrimoxazole
STR – Streptomycin
TET –Tetracycline
PN – Ampicillin
COL – Colistin
GN – Gentamycin
NAL – Nalidixic acid
NIT – Nitrofurantoin

**U4 – Discs**
CR – Cephaloridine
GN – Gentamicin
TE – Tetracycline
SXT – Sulfamethoxazole
PY – Carbenicillin
PN – Ampicillin
SF – Sulfafurazole
CT – Colistin sulfate

**Discussion**

The results of this study showed that two of the three boreholes examined had *Escherichia coli*. *E.coli* in water samples depicts feacal contamination (Akinluyi and Odeyemi, 1984) The water could have been contaminated by pathogens or disease producing bacteria or viruses which can exist in feacal materials (Wilker,1989). Although most strains of *Escherichia coli* and other coliforms are a collection of relatively harmless microorganisms that live in large numbers in the intestines of warm-blooded (for instance man) and cold-blooded animals but some of them are pathogenic (Greenberg and Hunt, 1985). Feacal contamination of ground water supplies occur most frequently as a result of the seepage of domestic sewage, either of animals or humans in the ground (Pelczar and Reid, 1993). It is therefore of great importance that the supply ground water be located at a safe distance from possible

sources of contamination, such as pit latrines, cesspools, septic tanks and barn yards (Ogedengbe, 1980). Antibiotics sensitivity tests carried out in this study showed that colistin, nitrofurantoin, gentamycin, colistin sulfate and nalidixic acid could be effective against this particular strain of *Escherichia coli*. Tetracycline, ampicillin, cotrimoxazole, sulfamethazole, cephaloridine, streptomycin, carbenicillin, sulfafurazole were ineffective. The *Escherichia coli* were resistant to these antibiotics. The resistance of *Escherichia coli* to commonly used antibiotics such as ampicillin, tetracycline and streptomycin has been attributed to the acquisition of resistance plasmids (Zo *et al*., 1999). Such antibiotics resistance has been found to be prevalent among young children in Nigeria (Laminkanra *et al.,* 1989). This observation is somewhat of urgent concern since exposure to antibiotics is common in adults and older children. This high incidence of resistance in children may also be attributed to the transfer of resistance plasmids passively from their mothers (Lederberg, 1992).

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