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# Antibiotics Susceptibility of *Escherichia coli* Isolated from Female Students with Urinary Tract Infection

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## Abstract

Uropathogenic strains of *Escherichia coli* were isolated from urine of female students in Madona University Elele, Rivers state, Nigeria and subjected to antibiotics sensitivity test using agar diffusion method. One hundred and twenty-five samples of early morning mid-stream urine were collected and inoculated by streaking directly into sterile eosine methylene blue (EMB) agar and incubated at 37°C for 24 hours. Black colonies with green metallic sheen on EMB, which is typical of *E. coli* were purified and inoculated on nutrient agar impregnated with multi disc antibiotics using spread plate method. The plates were incubated at 37°C for 24 hours. Observation of clear zone of inhibition indicated sensitivity while absence of any cleared zone indicated resistance. Result indicates that there was significantly more chances of isolating *E. coli* from urine ($p > 0.05$). Antibiotics susceptibility result shows that *E. coli* strains were more likely to be resistant to the antibiotics tested than those that were sensitive. Out of the 50 positive cases, the highest observed resistance was tetracycline (94%) and the least was ciprofloxacin (28%). 80% of isolates were resistant to sulphamethoxazole, 80% to penicillin, 76% to streptomycin and 60% to chloramphenicol. This study reveals that there is an increased rate of drug resistance among patients with urinary tract infection. Since antimicrobial resistance are constantly evolving and present global public health concerns, measures should be taken to prevent *E. coli* infection by organizing enlightenments programmes regularly to warn the students of the risk of drug abuse, poor personal hygiene and environmental conditions.

## Keywords

Antibiotics, *E. coli,* Urine, Uropathogenic, Resistance, Sensitive

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## 1. Introduction

*Escherichia coli* is a Gram- negative bacterium found in the human and animal lower intestines. *E. coli* belongs to member of enterobacterioacea family. There are many strains of *E. coli* and are classified based on the portal of infection, mode of pathogenesis, change in their physiological and life cycle, ability to use unique carbon source, ability to inhabit particular ecological niche and resistance to antimicrobial agent (Davis, 2008). At the molecular level, genes of the following strains have been identified, namely: uropathogenic *E. coli* (UPEC), enterotoxigenic *E. coli*, Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enterohemorrahagic *E. coli* (EHEC) also known as shiga toxin-producing *E. coli* (STEC), Enteroaggregative *E. coli* (EAEC) and Adherent-Invasive *E. coli* (AIEC) (Davis, 2008; Carl et al, 2015; CDC, 2015; MRL, 2016; Wikipedia 2016).

Uropathogenic *E. coli* (UPEC) causes urinary tract infections (UTI) (Ronald, 2003). The mode of pathogenesis has been

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described and virulence genes identified (MRL, 2015, Carl et al, 2015). UPEC is known to use a protein, adhesin and bind at specific receptor sites to endothelial cells of the urinary tract and colonizes the bladder. It produces alpha-and beta-hemolysin, which causes lysis of urinary tract cells (Nester et al, 2004; Wikipedia, 2016). UPEC also has the ability to form biofilms, which confer resistance to antibiotics and human immune defenses (Nester et al, 2004; Akinubi et al, 2009). This may account for transferable antibiotic resistance (Alex et al, 2011). An individual's susceptibility to UTI caused by *E. coli* can be attributed to lack of UPEC's receptor, which occurs in approximately 1% of individuals.

Control of bacterial infection by antibiotics treatment either kills or inhibits the growth of microbial pathogen. Non-susceptible microbes have developed resistance to such antimicrobial, which are part of organism's genetic makeup (Cedric, 1998; Frye and Jackson, 2013). Antibiotic-resistant *E. coli* may also transfer the genes responsible for antibiotic resistance to other species such as *Staphylococcus aureus* when they are in close proximity particularly as biofilms. *E. coli* often carry resistant plasmids and under stress, readily transfer those plasmids to other species.

Most enterobacteriaceae are susceptible to antibiotics such as aminoglycosidess, penicillin and cephalosporin (Egri-Okwajietal, 2005). In this study, uropathogenic strains of *E. coli* isolated from urine were tested against an array of antibiotics to determine the extent of its sensitivity and resistance.

# 2. Materials and Methods

## 2.1. Specimen Collection

A total of 125 samples of early morning mid-stream urine were collected in a sterile container from female student of Madonna University, Elele, Nigeria and immediately transported to the Department of Microbiology Laboratory.

## 2.2. Culturing and Isolation of Bacteria

A loopful of urine was inoculated by streaking directly into sterile Eosine methylene blue agar (EMB) (Oxoid) and incubated at 37°C for 24 hours. *E. coli* forms typical black colonies with green metallic sheen on EMB. Isolates were sub-cultured on sterile nutrient agar and incubated at 37°C for 24 hours and the cultured plates were examined for growth (Cheesbourgh, 2006; Todar, 2007).

## 2.3. Characterization of Bacteria Isolates

Based on colonial morphology, cultural characteristics and biochemical tests, each isolates were identified. The cultural characteristics observed included colony size, shape, colour edge and growth pattern. Microscopy of Gram stained smears of E. coli pure isolates was carried out to determine morphological characteristics while biochemical tests were used for confirmation. Biochemical tests included Indole test and citrate utilization test as described by Todar (2007) as well as catalase and urease test as described by Cheesbrough (2006).

## 2.4. Antibiotics Susceptibility Test

Antibiotic susceptibility test was done on nutrient agar (NA) using multi-disc antibiotics. Prepared NA was poured in Petri dish and allowed to solidify. Isolated organisms were inoculated into peptone water and allowed for 6 hours. A sterile swap stick was used to transfer organisms in the broth and spread plated. The multi-disc antibiotics impregnated paper was centrally placed and gently pressed into the agar with a pair of sterile forceps. The plate was inverted and incubated at 37°C for 24 hours. Observation of clear diameter zone of inhibition indicated sensitivity while absence of any cleared zone indicated resistance. The interpretation of results was done using the zone sizes. Zone of inhibition greater than 10mm was considered sensitive, 5-10mm moderate, and no zone of inhibition or less than 5 mm resistant (Akinyemi et al, 1997).

# 3. Results and Conclusion

The frequency (%) of *Escherichia coli isolated* from the urine samples according to age brackets of the students is shown in figure 1.

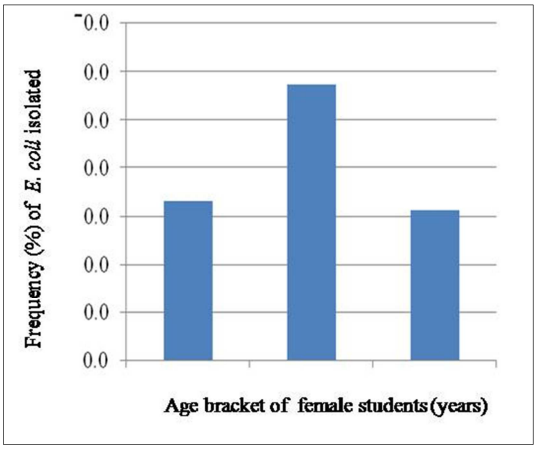


**Figure 1.** Frequency (%) of E. coli positive cases isolated from urine of female students with urinary tract infection.

Fifty (50) positive *E. coli* cases were recorded. Percentages of the age brackets with positive cases were 33.3, 57.5 and 31.3 for 16-20, 21-25 and 26 age brackets respectively. There was significantly more chances of isolating *Escherichia coli* from urine.

Table 1 shows the percentage susceptibility of *E. coli* isolated from students' urine to various antibiotics. It shows that *E. coli* strains were more likely to be resistant to the antibiotics tested than those that were sensitive. High resistance to tetracycline was observed, 28% was resistant to ciprofloxacin, 60% to chloramphenicol, 80% to sulphamethoxazole, 76% to streptomycin and 80% to penicillin, while resistance to tetracycline was 94%.

**Table 1.** Antibiotics Susceptibility Patterns of *E. coli* Isolated from Clinical Specimen.

| Antibiotics Tested | Sensitive (%) | Resistant (%) |
|---|---|---|
| Ofloxacin | 20 (40%) | 30 (60%) |
| Nalidixic | 16 (32%) | 34 (68%) |
| Chloramphenicol | 20 (40%) | 30 (60%) |
| Gentamycin | 20 (40%) | 30 (60%) |
| Cefoxitin | 22 (44%) | 28 (56%) |
| Ciprofloxacin | 36 (72%) | 14 (28%) |
| Sulphamethoxazole | 10 (20%) | 40 (80%) |
| Streptomycin | 12 (24%) | 38 (76%) |
| Penicillin | 10 (20%) | 40 (80%) |
| Tetracycline | 3 (6%) | 47 (94%) |

Out of 125 female students that volunteered their urine for examination, 50 had positive isolates of *Escherichia coli representing 40%*. This is lower than the 59% of *E. coli* positive cases from urine reported by Umolu et al (2006). Isolates of UPEC have relatively high potentials for developing resistance to antimicrobial agents tested. This was observed by Omigie et al (2006) who also reported resistance of *E. coli* isolates to tetracycline and sulphomethaxazole.

Antibiotic resistance is a growing problem. Some of this is due to overuse of antibiotics in humans, but some of it is probably due to use of antibiotics as growth promoters in animal feeds (Oteo et al, 2005; Adenipekun et al, 2015). In recent years, the use of quinolones has increased in many countries and emergence of bacterial resistance to quinolones has been observed. Consistent step-wise increase in Escherichia coli resistant to ciprofloxacin was observed from 1995 (0.7%) to 2001 (2.5%) by Bolon et al, (2004). Ciprofloxacin resistance in Portugal was 25% and Italy 24.3% and 6.8%. Albinu et al (2004) observed that *E. coli* is highly resistant to ampicillin, amoxicillin, tetracycline and trimethoprim - sulfamethoxazole. Also, Umolu et al (2006) reported very high resistance levels (>75%) of *E. coli* against tetracycline, augmentin and amoxicillin while nitrofurantoin and ofloxacin recorded the least resistance levels of 6% and 19% respectively among the isolates. Stelling et al (2005) equally reported high resistance of *E. coli* to antimicrobials in South Africa, Israel, Philippines and Hong Kong. Oteo et al (2005) also reported resistance to Ciprofloxacin ranging from. 6.2 to 25.8% in Portugal, Italy, Germany and Netherlands. Isolates in this study were highly resistant to tetracycline.

The study revealed that urinary tract infection is mainly attributed to *E. coli*, and that girls within the age brackets of 24-25 years are the most affected. This study as well as reviewed literature reveals that there is an increased rate of drug resistance among patients, globally. Since these antibiotics resistant bacteria are constantly evolving and present global public health problem, measures should be taken to minimize *E. coli* infection by organizing enlightenments programmes regularly to educate students, and particularly females of the risk of drug abuse, poor personal hygiene and environmental conditions. Good toilet, bathroom and portable water should be made available. Proper ventilation should be also allowed within the hostels to avoid spread of infection.

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