**Antibiotic susceptibility of *Escherichia coli* isolated in cases of urinary tract infection in Nsukka, Nigeria**

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Abstract

The study was carried out to determine the occurrence and antibiotics susceptibility profile of *Escherichia coli* recovered from patients with urinary tract infection (UTI) in Nsukka, southeastern Nigeria.

Materials and method. Midstream urine samples were obtained from 266 outpatients. Standardized questionnaires were used to collect relevant information from the subjects. Urine samples were plated on MacConkey and eosin methylene blue agar. *E. coli* isolates were identified using standardized procedure. Kirby-Bauer disc diffusion method was used to determine the susceptibility of the isolates.

Results. An overall UTI prevalence of 18.8% with 29 (19.30%) from females and 21 (18.10%) from males was observed. Females and males within the age range of 20–29 and 50–59 had the highest prevalence rate of 23.2% and 15%, respectively. The highest prevalence rate (26%) occurred in the Igbo-Etiti local government area, compared to the other four local government areas within the study locations. Urinalysis showed that 16 urine samples contained protein, 29 were nitrite positive and 31 were acidic. Most of the isolates were susceptible to nitrofurantoin (94%), ciprofloxacin (70%) and ofloxacin (78%) while all of them were completely resistant to cefuroxime, cefazidime and augmentin (100%). All 50 (100%) *E. coli* isolates showed multiple resistance to three or more antibiotics with a resistance index ranging from 0.375–1.00. The MIC results showed that 17 (34%) isolates were sensitive to gentamycin (MIC ≤ 0.25µg/ml, MBC ≥ 128µg/ml), resistant to ciprofloxacin and ofloxacin.

Conclusion. *E. coli* is predominantly associated with UTI in the study area and was susceptible to nitrofurantoin.

Key words

*Escherichia coli*, urinary tract infection, Nsukka, antibiotics, southeastern Nigeria

INTRODUCTION

Every year, over 150 million cases of urinary tract infections are reported [1]. This is estimated to cost the world economy over six billion US dollars [2]. The increasing resistance to bacteria etiological agents of UTI, especially in developing countries, has become a huge public health issue and a major obstacle in resolving the problem posed by UTIs. Monitoring bacteria etiological agents of UTI worldwide, as well as their resistance patterns to antibiotics, is important since the world has become a global village. Area-specific studies are useful in this regard because it can shed light about the type of pathogens responsible for UTI in different regions, and their patterns of antibiotic sensitivity/resistance.

*Escherichia coli* is the major etiological agent of UTI [3, 4] and responsible for up to 90% of all UTI cases reported so far [5]. In most cases, *E. coli* is harmless and forms a part of the normal flora of the gut and thus disrupts the establishment of pathogenic bacteria within the intestine. UTI has been reported to be more prevalent in females than in males, especially during youth and adulthood, due to the proximity of the female urethral opening to the anus, thus enabling easier access of *E. coli* to the bladder [6]. The urinary tract comprises the lower and upper urinary tracts, and the infection is named after the part that is infected. UTI can also be symptomatic or asymptomatic [7]. UTI can cause inflammatory diseases as a result of the multiplication of the pathogen at the opening of the urinary tract, and this in turn alters the normal functioning of the urinary tract and kidneys [7].

The introduction of antimicrobial therapy has led to improvement in the management of UTI [8]. However, the emergence of antibiotic resistance by a growing number of antibiotic resistant pathogens in the management of UTI has become a serious public health problem, particularly in developing countries like Nigeria where factors such as a high poverty level, ignorance, and poor hygiene practices contribute to the incidence of UTI, as well as a high prevalence of fake and spurious drugs of questionable quality in circulation [9]. Due to the lack of a rational antibiotic policy in clinical medicine, antibiotics are misused more frequently in many developing countries which results in the emergence of many multidrug resistant (MDR) strains of pathogenic bacteria, especially *Escherichia coli* [10]. Hence, there is the need for a sound knowledge of the antibiotic resistance patterns of these pathogens, especially *Escherichia coli*, in order to ensure efficient treatment and eradication, as well as the monitoring of the epidemiology of UTI. Moreover, a better knowledge of the virulence characteristics of the main pathogen causing urinary tract infection in developing countries, such as Nigeria, will be useful in monitoring the evolution of infection in the host organism.

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OBJECTIVE
The aim of this study was to determine the prevalence and antibiotic susceptibility of *E. coli* recovered from urinary tract infections in the Nsukka geopolitical zone in southeastern Nigeria.

MATERIALS AND METHOD

Sample collection. Urine samples were collected from 266 patients of different age groups in the outpatient’s department of each selected hospital in the six local government areas in the Nsukka geopolitical zone. All participants provided written consent after thorough explanation of the rationale for the study. Fresh voided mid-stream urine specimens (10ml) were obtained from patients into sterile universal screw–capped bottles. Participants were instructed on how to collect the urine samples. Each participant was given a standardized questionnaire on socio-economic status, residence, occupation, age, gender, antibiotics prophylaxis, previous UTI, and presenting symptoms. The specimens were labeled and transported immediately in iced bags to the microbiology laboratory of the University of Nigeria in Nsukka for analyses.

Urinalysis. The urine samples were checked for colour and turbidity. Quantitative urine analysis was performed using a commercially available urine dipstick Combi 9 (CombustestH, Roche Diagnostics, Mannheim, Germany) following the manufacturer’s recommendations. The pH, presence of glucose, protein, ascorbic acid, blood, ketone, bilirubin, urobilinogen and nitrite were noted for each sample.

Microscopy. Ten millimeters of well–mixed urine sample was centrifuged at 5,000rpm for five minutes. The supernatant was discarded, and a drop of each urine sample was placed on a glass slide and viewed at high magnification through a calibrated wire loop, a loopful (0.01ml) of each urine sample was inoculated on chromogenic agar for further identification. Discrete colonies were further subcultured onto eosin methylene blue agar (Oxoid, Hampshire, United Kingdom) for isolation of the isolate recovered, after subjecting them to a panel of eight antibiotics. The broth dilution method was used to determine the MIC of three antibiotic, namely ciprofloxacin, ofloxacin and gentamicin against *E. coli*. *E. coli* was inoculated into Mueller-Hinton broth containing a two-fold serial dilution of an initial antibiotics solution of 256µg/ml. An uninoculated tube was used as the control. The setup was incubated for 18 hours at 37°C. Antibiotic concentration ranged from 0.06–128µg/ml for ofloxacin, 0.008–128µg/ml for ciprofloxacin and 0.03–128µg/ml for gentamicin. The minimum bactericidal concentration (MBC) was determined by subculturing on antibiotics-free Mueller Hinton agar plates, 0.01ml of the broth culture of the organism/antibiotic’s mixture (at MIC) that showed no visible growth. The lowest concentration that showed no growth on the antibiotics-free medium after overnight incubation (18–24) hours was recorded as the MBC.

Multiple antibiotics resistance (MAR) indexing. Multiple antibiotics resistance index (MAR) was calculated for each *E. coli* isolate recovered, after subjecting them to a panel of eight antibiotics. The formula used was $x/y$ where ‘$x$’ represents the number of antibiotics to which the isolate is resistant, and ‘$y$’ represents the total number of antibiotics to which the isolate was exposed [14]. The *E. coli* isolates resistant to three or more antibiotics were classified as multiple antibiotics resistant (MAR) isolates, while those resistant to less than three antibiotics were classified as non-multi-antibiotic resistant (NMAR) isolates.

RESULTS

In the study, the overall prevalence rate of UTI was 18.8% with 50 (29 females and 21 males) of the 266 patients positive for UTI. Females within the age range 20–29 years had the highest prevalence rate (23.0%), followed by those in the age range 30–39 years (22.7%), 10–19 years (20.0%), while those within the age range 50–59 years (2.5%) had the least prevalence. Among the males, the highest prevalence was also observed in the age range 50–59 years (15%), followed by those above 70 years (13.0%) and 60–69 years (10.7%), while the least was found in the age range 20–29 years (3.6%). Within the study area the Igbo-Etiti local government area was observed to have the highest prevalence rate (26.0%), followed by Igbo-Eze North (24.0%), Nsukka (18.0%), Igbo-Eze South 16%, and Uzo-Uwani (9.5%), while the Udenu local government area had the lowest rate (6.0%).

Participants in the rural communities showed the highest prevalence rate (84%) while those in the urban community had a low prevalence (16%). Participants who were single at the time of the study had the highest prevalence (64%), followed by those who were married (32%), while the widows/widowers had the least prevalence (4%). With respect to occupation, students showed the highest prevalence (58%), while farmers had the least prevalence (4%). In the same vein, participants with tertiary level of education had the highest prevalence (58%), while those with no formal education had the least prevalence (4%). Among the positive samples, 58%
presented symptoms of UTI at the time of study, while 42% were without symptoms.

Urine analysis showed that of 50 of positive urine samples, 29 were positive for nitrite, 16 were protein positive, 14 had ascorbic acid, and two urine samples had a small glucose concentration. The urinalysis also showed high acidity in 31 urine samples (11 males and 20 females) and a turbid appearance of urine samples was observed in 31 urine samples. Microscopy of centrifuged urine samples showed a high number of calcium oxalate crystals in five urine samples (two males and three females), sulfonamide crystals in two female urine samples while cystine crystal and triple phosphate crystal were found in two urine samples (a male and a female). Fourteen (14) urine samples (two males and 12 females) had numerous epithelial cells, and red blood cells were found in two female urine samples. There were a significant number of pus cells (pyuria) in 32 urine samples (15 males and 17 females). Hyaline casts were also found in five urine samples (two males and three females) while granular cast was found in a male urine sample.

Among the antibiotics tested in the study, the highest rate of resistance of *E. coli* was found for cefuroxime (100%), ceftazidime (100%), augmentin (100%) and cefixime (74%), while the sensitivity patterns of *E. coli* to the antibiotics tested were highest in nitrofurantoin (94%), ofloxacin (78%), ciprofloxacin (70%) and gentamicin (66%). *E. coli* recovered from females had the highest sensitivity rate to nitrofurantoin (56%), ofloxacin (48%) and ciprofloxacin (46%) while males had sensitivity rate of nitrofurantoin (38%), ofloxacin (30%) and gentamicin (28%). *E. coli* recovered from the various study areas were observed to have almost the same sensitivity rate to nitrofurantoin, ofloxacin, gentamicin, ciprofloxacin and gentamicin. All isolates from the study areas (local government areas) were 100% resistant to cefuroxime, ceftazidime and augmentin.

All the *Escherichia coli* isolates recovered exhibited an overall multiple antibiotic resistance (MAR) index range of 0.375 - 1.0. A total of fifty *E. coli* isolates were subjected to MIC determination, of which 38 (76%) of the isolates were resistant to ciprofloxacin, while 24% were sensitive. Also, 37 (74%) were resistant to ofloxacin while 13 (6%) were sensitive. Lastly, 33 (66%) of the isolates were resistant to ciprofloxacin and 17 (34%) were sensitive to it. MIC<sub>50</sub> was found to be between 32µg/ml and 64µg/ml, while MIC<sub>90</sub> was observed to be at 128µg/ml for the antibiotics tested.

### Table 1. Age and gender distribution of UTI

| Age groups | Number tested | Number positive (%) | Total prevalence (%) |
|------------|---------------|---------------------|----------------------|
| 0–9        | 17            | 0 (0.0) 2 (11.8)    | 2 (11.8)             |
| 10–19      | 5             | 0 (0.0) 1 (20.0)    | 1 (20.0)             |
| 20–29      | 56            | 2 (3.6) 13 (23.2)   | 15 (26.8)            |
| 30–39      | 22            | 0 (0.0) 5 (22.7)    | 5 (22.7)             |
| 40–49      | 61            | 3 (7.3) 3 (7.3)     | 6 (14.6)             |
| 50–59      | 40            | 6 (15.0) 1 (2.5)    | 7 (17.5)             |
| 60–69      | 28            | 3 (10.7) 1 (3.6)    | 4 (14.3)             |
| ≥ 70       | 57            | 7 (12.2) 3 (5.3)    | 10 (17.5)            |
| Total      | 266           | 21 (42.0) 29 (58.0) | 50 (18.8)            |

### Table 2. Socio-demographic characteristics of the study participants

| Status                  | Total number Sampled (%) | UTI positive Samples (%) |
|-------------------------|--------------------------|-------------------------|
| **Locality**            |                          |                         |
| Rural                   | 216 (81)                 | 42 (84)                 |
| Urban                   | 50 (19)                  | 8 (16)                  |
| **Marital status**      |                          |                         |
| Single                  | 112 (42)                 | 32 (64)                 |
| Married                 | 136 (51)                 | 16 (32)                 |
| Divorced                | 3 (1)                    | 0 (0)                   |
| Widower/Widower         | 15 (6)                   | 2 (4)                   |
| **Occupation**          |                          |                         |
| Students                | 67 (25)                  | 29 (58)                 |
| Civil servants          | 88 (33)                  | 8 (16)                  |
| Traders                 | 56 (21)                  | 7 (14)                  |
| Farmers                 | 19 (7)                   | 2 (4)                   |
| Unemployed              | 29 (11)                  | 4 (8)                   |
| Others*                 | 7 (3)                    | 0 (0)                   |
| **Literacy level**      |                          |                         |
| Basic/Primary*          | 87 (33)                  | 12 (24)                 |
| Secondary*              | 67 (25)                  | 7 (14)                  |
| Tertiary*               | 55 (21)                  | 29 (58)                 |
| None                    | 57 (21)                  | 2 (4)                   |
| **Symptoms of UTI**     |                          |                         |
| Yes                     | 125 (47)                 | 29 (58)                 |
| No                      | 141 (53)                 | 21 (42)                 |

*Others – includes artisans; Basic/Primary – school for children aged between about five and eleven where they receive elementary education; Secondary – school for children aged between 11–18 where they receive secondary education, e.g. technical and vocational courses; Tertiary – post-secondary education which focuses on learning endeavors in specialized fields, including academic and higher vocational education.
DISCUSSION

Urinary tract infection (UTI) is one of the most common infections, especially in hospital settings. UTI is usually high among young females at reproductive age [15, 16]. E. coli was found to be the most common cause of UTI in all age groups. The purpose of the presented study was to describe the susceptibility profiles and patient demographics of E. coli isolates recovered from UTI from all six local government areas in Nsukka geopolitical zone in southeastern Nigeria.

The overall prevalence rate of UTI obtained in the study (18.8%) is similar to the findings in other studies [17, 18, 19, 20, 16] which reported similar prevalence rates of 23.3%, 20%, 25%, 22% and 21.1%, respectively, and these appeared to be high when compared to those obtained elsewhere. For instance, a study in southeast Nigeria [21] reported a prevalence of 13.9%, another in Benin City, Nigeria, reported a prevalence of 11% [22]; although an 8.25% prevalence was also reported in Benin City [23]. In the current study, the low incidence of urinary tract infection reported in Nsukka may be due to different clinical profiles, geographical location, and good hygiene level of the patients. E. coli was the common causative agent of UTI as reported in this study and in Rother studies [24, 25, 26]. This could be because it is the predominant bacteria in the gastrointestinal tract of humans, and other previous researches have shown E. coli to be the cause in 80–90% of cases of UTI.

In the current study, of the UTI positive samples, 29 (58%) of the participants were females while 21 (42%) were males. The study also showed a higher incidence of urinary tract infection in females than males. The results obtained are similar to those in other studies reported in Nigeria [27, 16, 28] and in Yemen [29]. The high prevalence of infection in females is usually related to anatomical and pathogenic factors, such as the short length of the urethra, hence a shorter distance for bacteria ascending up the tract, hormonal changes affecting the adherence of bacteria to the mucosa, and urethra trauma during sexual intercourse. Among the females, the highest prevalence was seen in the reproductive age group20–29 years (23.0%), followed closely by those aged 30–39 years (22.7%). This may be as a result of increased sexual intercourse in these populations [30].

Among males, an increased prevalence of UTI was observed in those aged 50–59 years (15.0%), followed by those aged ≥70 years and above (13.9%). The increasing...
frequency of prostate disease and diabetes mellitus in males are responsible for the increase in the incidence of UTI in elderly patients. This report is similar to some studies [31, 16] in which a prevalence of 23.3% was reported in females aged 26–30 years, and in disagreement with another study that showed that female patients within the age range 37–47 years had the highest prevalence rate of 46.2%, followed by 26–36 years (31.1%), 15–25 years (18.2%) and above 48 years of age, showed the lowest prevalence of 4.5%, while among the male patients, 37–47 years showed the highest prevalence rate of 51.3%, followed by 26–36 years (29.5%), 15–25 years (14.1%), and above 48 years of age showed the lowest (5.1%) [25]. Statistical analysis showed that there was no significant difference between age and gender at 5% (P < 0.05).

Participants in the rural communities in this study showed the highest UTI prevalence of 84% while the urban communities had a prevalence of 16%. Those who were single at the time of the study had the highest prevalence of 64%, followed by those who were married – 32%, while widows/widowers had 4%. This is similar to a previous report [25] which reported that single individuals had the highest prevalence (44%), followed by those who were married (27%), divorced/separated (18%) and widows/widowers (11%). His is contrary to a study that reported the highest prevalence of UTIs among married women (66%), followed by those who were single (24%) [32]. The highest prevalence of UTIs among single individuals might be attributed to multiple sex partners and unfaithfulness to one sex partner, both of which are common within the study areas. The lower incidence rate of urinary tract infection reported among those who were married and widow/widower in this study may be attributed to adherence to single partners and a high degree of hygiene. With respect to occupation, students showed the highest prevalence (58%), followed by civil servants (16%), traders (14%) and the unemployed (8%), while farmers had the least prevalence (4%). This is similar to a report by [25] that showed students having the highest prevalence rate (42%), followed by civil servants (30%), small traders (15%), and casual labourers who showed the least prevalence (13%). This report is not in accordance with findings in [33] where small traders showed the highest prevalence rate (33%), followed by casual labourers (30%), civil servants (21%), and students showed the least (17%).

In the same vein, participants with tertiary education had the highest prevalence (58%), while those with no formal education had the least prevalence (4%). The high prevalence rate of UTI observed in those who had tertiary education may be due to the lack of exposure to health education. This result coincides with reports from [33] who reported a high prevalence rate of UTI among those in high school (35%) and higher education (tertiary) students (25%), and in disagreement with Rother studies [34] which showed that women with little or no education and low socio-economic status had a high prevalence of UTI. A study also reported the highest prevalence of UTIs among students in high schools (35%), followed by students in higher education (25%), illiterates (18%), and educated individuals who recorded the least (3%) [35]. The highest prevalence rate of UTI among those with no formal education was observed to be 33% while those in high school was the least with a prevalence rate of 13% [36]. The prevalence of UTI in this study was also observed to be high (58%) in participants having symptoms of UTI while 42% were without symptoms as at the time of the study. This is as a result of the symptoms being good pointers to the infection.

In the current study, microscopy of the urine samples showed the presence of many pus cells (white blood cells), epithelia cells and casts in samples that were positive for UTI. The presence of epithelia cells may be due to vaginal contamination during sample collection. Different crystal types indicate the presence or absence of an infection. The presence of calcium oxalate crystals can be due to chronic dehydration or hyperparathyroidism, in turn, can lead to urinary tract infection due to the presence of calculi (stones) in the urinary tract. Cystine crystals are found in cystinuria which is a rare congenital metabolic disorder. Urinalysis showed a large presence of protein, nitrite and ascorbic acid in urine samples. The presence of protein in urine (proteinuria) is usually an indication of kidney disorders, including glomerulonephritis and urinary tract infection. Nitrites are found in normal urine and are the result of consuming a diet containing vegetables which are reduced to nitrates by organisms such as Escherichia coli and Klebsiella spp in large quantities.

The E. coli isolates recovered in the presented study showed high sensitivity to fluoroquinolones, as was observed in another study [16]. E. coli isolates recovered in the current study were 94% sensitive to nitrofurantoin, 78% to ofloxacin and 70% to ciprofloxacin. The antibiotics sensitivity test result in this study shows that nitrofurantoin was the most effective antibiotic, followed by ofloxacin and ciprofloxacin. This is similar to the finding where sensitivity of E. coli to nitrofurantoin was 95.6% [37]. In other studies performed in developing countries, E. coli demonstrated a lower sensitivity to ciprofloxacin of between 15–43.2% [38]. Gentamicin was active against 66% of the E. coli isolates recovered. The antibacterial activity of gentamicin against the isolates in the presented study is comparable to the finding in Maiduguri, Northern Nigeria, where less than 70% of E. coli isolates were sensitive to this antibiotic [39]. The resistance of the isolates tested showed high resistance rates to cefixime (74%), augmentin (100%), ceftazidime (100%) and cefuroxime (100%), which is similar to a study in India [40].

The Escherichia coli isolates in the current study had an MAR index ranging from 0.375–1.0, which is an indication that the E. coli isolates had been exposed to several antibiotics. All E. coli isolates (100%) in this study were multidrug resistant to the antibiotics tested. This is similar to a study that recorded an overall prevalence of MDR of 73% [16]. This is similar to study in southeastern Nigeria [25] and in disagreement with the current study [41]. The high prevalence of MDR in this study might be the result of difference in the definition used for multidrug resistance in this study, and the multiple resistant genes possessed by the isolates on their mobile genetic elements [42]. Self-medication, which is a common practice in the study area [2], also might have a major role in the development of such a higher prevalence of MDR [43].

The MIC results showed that 76%, 74% and 66% of the isolates were resistant to ciprofloxacin, ofloxacin and gentamicin, respectively, which is similar to a study reported [44]. This could be the result of the misuse and frequent exposure to fluoroquinolones, leading to an increase in the MDR of this antibiotic. Also, fluoroquinolone resistance in E. coli can emerge, particularly in patients with urinary tract infections, who have received fluoroquinolone prophylaxis
[45]. Usually, the prevalence of fluoroquinolone resistance is related to the intensity of antibiotic use [45]. An association between the increase in quinolone prescriptions and an increase in bacterial resistance has been reported from several countries [46].

**CONCLUSION**

The overall prevalence of UTI in the Nsukka geopolitical zone was 18.8%. The increasing level of antibiotic resistance, especially to the fluoroquinolones and cephalosporins, has become a major health problem globally and calls for an urgent review and implementation of effective antibiotic policy in Nsukka and its environs in southeastern Nigeria. This will assist clinicians in the rational choice of antibiotics therapy in avoiding the misuse of these antibiotics. Antibiotic analysis and good stewardship practice in antibiotics by patients in this study area will also help bring about the complete elimination of the surge of antibiotic resistance.

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