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# Antimicrobial Resistance profile of *Escherichia coli* from Urine of Patients in Nigerian National Petroleum Corporation Medical Services, Abuja, Nigeria

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**Key Words**
*Escherichia coli*; microbial resistance; urine, patients

**ABSTRACT**

*Escherichia coli* (*E. coli*) is among the most predominant organisms causing urinary tract infections (UTIs) in humans. Studies on antimicrobial resistance in *E. coli* from urine of patients in Nigerian National Petroleum Corporation Medical Services, Abuja, Nigeria was carried out. Exactly 362 urine samples of patients with suspected UTIs were collected and *E. coli* was isolated and identified using standard microbiological methods. The antimicrobial susceptibility testing of the isolates was carried out and interpreted in accordance with Clinical and Laboratory Standards Institute protocol. Of the 362 urine samples, the occurrence of *E. coli* was 45(12.4%); highest age-related occurrence at 21-30yrs of 18(20.5%); and higher in female (15.6%) than male (7.6%). The 45 isolates had highest resistance to ampicillin 43(95.6%) and lowest resistance to gentamicin 13(28.9%). All the 45 isolates were multiple antibiotic resistant (MAR) isolates with MAR indices of > 0.2, and the commonest indices being 0.6 and 0.7 with 10(22.2%) and 8(17.8%) occurrences respectively. The order of occurrence of categories of antibiotic resistance in the 45 isolates was: multi-drug resistance (66.7%) > extensive drug resistance (17.8%) > non-multi-drug resistance (13.3%) > pan drug resistance (2.2%). A further study on molecular diversity of the antimicrobial resistant *E. coli* from urine in the study location is ongoing.

**INTRODUCTION**

Urinary tract infections (UTIs) are one of the most common infections with an estimated annual global incidence of at least 250 millions in developing countries (Gorbani *et al*., 2012; Michael *et al*., 2017) and *Escherichia coli* (*E. coli*) has been reported to cause approximately over 75% of this infection both in community and hospital settings (El bouamri*et al*., 2015; Michael *et al*., 2017). The predisposing factors namely; age, gender and immune status of individual have been reported to affect the prevalence of UTIs (Michael *et al*., 2017).

Antibiotic resistance in urinary *E. coli* to commonly prescribed antimicrobials have been reported to be alarming as a result of inappropriate used and abused of this agent in developing countries (Sedighi *et al*., 2015). The emergence of multidrug resistance *E. coli* isolates causing UTIs have been reported to increase the cost of therapy, morbidity and mortality in developing countries (Pitout, 2012; Assafi *et al*., 2015; El bouamri *et al*., 2015).

Antibiotic resistance of urinary pathogens to different chemical classes of antibiotics namely: beta-lactams, aminoglycosides, quinolones and other classes have been reported (Michael *et al*., 2017). The isolation of *E. coli* from cases of UTIs and analyzing their resistance profile to commonly used antibiotics for UTIs in clinical practice is essential and helpful in the improvement of the efficacy of the empirical treatment. Hence, this study focused on antimicrobial susceptibility of *E. coli* isolated from urine of patients with suspected UTIs in Nigeria National Petroleum Corporation, Medical Services, Abuja, Nigeria.

**Materials and Methods**

**Media**

Bacteriological media that were used in this study include: MacConkey Agar (MCA), Mueller-Hinton Agar (MHA), Nutrient agar (NA), Luria-Bertani (LB) broth, Eosine Methylene Blue (EMB) Agar, Nutrient Broth (NB), Simmons Citrate Agar (SCA), Methyl red/Voges-Proskauer (MR/VP) medium and Peptone water (PW). All the media were sourced from Oxoid Ltd. (U.K.).

**Antibiotic Discs**

The antibiotic discs and potency that was used in this study include: Amoxicillin (AMX: 10µg)**,** Amoxicillin-Clavulanic acid (AMT: 30µg)**,** Cefotaxime (CTX: 30µg), Cefpodoxime (CPD: 10µg), Ceftazidime (CAZ: 30µg)**,** Ceftriaxone(CRO: 30µg)**,** Cefoxitin (FOX: 30µg), Ciprofloxacin (CIP: 5 µg)**,** Co-trimoxazole (SXT: 25 µg)**,** Gentamicin (CN: 10µg)**,** Ofloxacin (OFX: 5 µg) and Streptomycin (S: 10µg). All the discs were products of Oxoid Ltd (U.K.).

**Equipment**

The equipment that will be used in this study include: Autoclave (Certoclav, Model SM280E, Surgifriend Medicals, England), Oven (Hotbox Size One, Galengkamp, U.K.), Incubator (Model 12-140E, Quincy Lab Inc), Refrigerator/Freezer (Model PRN 1313 HCA, BEKO, Germany), Laminar air flow cabinet (Labcaire, PCR-8 re-circulating laminar flow pre station, product 220/240v) and Microscope (HOVER LABS$^{TM}$ India).

**Chemicals and Reagents**

The chemicals and reagents used in this study include: Acridine orange, Carbol fuschin, Crystal violet, Ethanol, Potassium hydroxide and Kovac's reagents, obtained from BDH Chemical Ltd, England; and Iodine solution obtained from Sigma Chemical Ltd, England.

**Study Location and Sample Collection**

The study location was the Nigerian National Petroleum Corporation Medical Services, Abuja, Nigeria. A total of three hundred and sixty-two (362) early morn-

ing mid-stream urine samples of patients with suspected UTIs attending the health facility were collected using sterile container and transported using ice pack to the Microbiology Laboratory at the Nasarawa State University, Keffi for analysis.

**Ethical Clearance**

The ethical approval for this study was obtained from the Ethical Committee of NNPC Medical Services, Abuja, Nigeria.

**Isolation of *Escherichia coli***

*Escherichia coli* was isolated from urine samples as follows: a loopful of urine sample will be streaked on MacConkey Agar plate and incubated at 37°C for 24 h. Pinkish colonies that grew on MacConkey agar were further streaked on Eosin Methylene Blue Agar and incubated at 37°C for 24h. Greenish metallic sheen colonies that grew on the Eosin Methylene Blue agar plate were selected as presumptive *E. coli.*

**Identification of *Escherichia coli***

The presumptive *E*. *coli* was Gram-stained, and biochemically identified as suspected *E. coli* using *IMViC* (Indole, Methyl red, Voges-Proskauer and Citrate) tests as earlier described (Cheesbrough, 2006). The suspected *E. coli* isolates (Gram negative, rod shape, indole positive, methyl red positive, citrate negative and Voges-Proskauer negative) were using a commercial biochemical testing kit (KB003 H125TM) following the manufacturer's instruction.

**Antimicrobial Susceptibility Testing**

The antimicrobial susceptibility testing of the bacterial isolates was carried out as earlier described by Clinical and Laboratory Standards Institute (CLSI, 2017). Briefly, three (3) pure colonies of the isolates were inoculated in to 5 ml sterile 0.85% (w/v) NaCl (normal saline) and the turbidity of the bacteria suspension will be adjusted to the turbidity equivalent to 0.5 McFarland's standard. The McFarland's standard was prepared as follows: 0.5 ml of 1.172% (w/v) $BaCl_2.2H_2O$ was added into 99.5 ml of 1% (w/v) $H_2SO_4$.

A sterile swab stick was soaked in standardized bacteria suspension and streaked on Mueller-Hinton agar plates and the antibiotic discs were aseptically placed at the center of the plates and allowed to stand for 1 h for pre-diffusion. The plates were incubated at 37°C for 24 h. The diameter zone of inhibition in millimeter was measured and the result was interpreted in accordance with the susceptibility break point earlier described by Clinical and Laboratory Standards Institute (CLSI, 2017).

**Results and Discussion**

**Isolation and Identification of *Escherichia coli***

The cultural, morphological and biochemical characteristics *E. coli* isolated from urine of the patients is as given in Table 1. Pinkish colony on MCA which grew with greenish metallic sheen on EMB agar was Gram negative rod and had biochemical reactions namely: indole-positive, methyl red-positive, Voges-Proskauer-negative, citrate-negative, ONPG-positive, among others indicates *E. coli*.

**Occurrence of *Escherichia coli***

Out of 362 urine samples, the occurrence of *E. coli* isolates was 45 (12.4%). The occurrences in relation to their age and gender of the patients are as shown in Table 2 and Table 3 respectively. The highest occurrence of *E. coli* in the patients was observed at age 21-30 yrs (20.5%) and the least at age 31-40 yrs (6.9%) as shown in Table 2. In relation to the gender of patients, the occurrence of *E. coli* isolates was higher in female (15.6%) than the male (7.6%) as shown in Table 3. The occurrences of *E. coli* isolates in relation to age and gender of UTIs patients were statistically insignificant (P>0.05).

**Antibiotic Resistance Profile**

The antibiotic resistance profile of *E. coli* isolates from urine is as shown in Table 4. The *E. coli* isolates were more resistant to ampicillin (95.6%), streptomycin (73.3%), cefotaxime (68.9%), ceftazidime (62.2%), sulphamethoxazole/trimethoprim (60.0%) and amoxicillin/clavulanic acid (55.6%) but less resistance to cefoxitin (48.9%), ciprofloxacin (44.4%), imipenem (37.8%) and gentamicin (28.9%).

**Multiple Antibiotic Resistance (MAR) Index**

Multiple antibiotic resistances, defined here as resistance by the isolates to more than two antibiotics tested, were observed in all (100%) the isolates. The MAR indices of the isolates as shown in Table 5 were > 0.2, with the commonest being 0.6 (22.2%) and 0.7 (17.8%).

**Categories of Antibiotic Reasistance**

The distribution of the isolates into different categories of antibiotic resistance namely (Magiorakos *et al.*, 2012): Multi-drug resistance (MDR), extensive drug resistance (XDR) and pan drug resistance (PDR) is as shown in Table 6. The order of percentage occurrence of categories of antibiotic resistance is: MDR (66.7%) > XDR (17.8%) > PDR (2.2%).

Urinary tract infections (UTIs) are one of the most common infections with an estimated annual global incidence of at least 250 million in developing countries (Gorbani *et al*., 2012; Michael *et al*., 2017). Studies on the antimicrobial resistance profile and molecular detection of ESBL resistance in *E. coli* isolated from urine of patients with suspected UTIs in NNPC Medical Services, Abuja, Nigeria was carried out. The occurrence of *E. coli* from urine of suspected UTIs patients observed in this study was expected and this finding is in consistent with the studies earlier reported by Kashyap *et al*. (2013), Giwa *et al*. (2018), Ihsan *et al*. (2016), Wemambu and Ifajeunmu (2016), Adenipekun *et al*. (2016) and Solomon *et al*. (2017) that *E. coli* is one of the most common etiological agent of UTIs. The occurrence of *E. coli* in urine of suspected UTIs patients is an indication that the organism may be one of the bacteria responsible for UTIs in patients attending the health facility. The percentage occurrence of *E. coli* observed in this study was less than 56.0%, 80.9%, 63.0%, 23.0% and 50.0% earlier reported by Giwa *et al.* (2018), Shakya *et al*. (2017), El bouamri *et al*. (2015), Adenipekun *et al*. (2016), Wemambu and Ifajeunmu (2016) and Adenipekun *et al*. (2016).

The percentage occurrence of *E. coli* isolates from urine of suspected UTIs patients in relation to age was highest at age 21-30 yrs, consistent with a study earlier described by Shakya *et al*. (2017) where 26.0% occurrence was reported at age 21-30 yrs. The high occurrence of *E. coli* in urine of suspected UTIs patients at age 21-30 yrs may be due to the fact that individuals at this age group are sexually active and may be more prone to UTIs. Our finding also shows that the occurrence of *E. coli* in urine of suspected UTIs patients was higher in female than the male and this also is in agreement with the study earlier reported Thakur *et al*. (2013), Parajuli *et al*. (2017) and Shakya *et al*. (2017). The high occurrence of the isolates in female than the male may be due to anatomical differences where women have short reproductive tract and may be more prone to UTIs than the male (Shakya *et al*., 2017). The differences in the occurrence of *E. coli* in urine of suspected UTIs patients in relation to gender and age were statistically insignificant, implying that age and gender of patients may not necessarily be a factor for occurrence of urinary *E. coli*.

The high resistance of *E. coli* isolates to ampicillin, cefotaxime, ceftazidime, streptomycin and sulphamethoxazole/trimethoprim was expected and this may be due to misused, ineffective empiric treatment, poor dosage schedules and prolong therapy using single agents which is more common in developing countries (Assafi *et al*., 2015). From this study we also observed that the isolates were less resistance to amoxicillin/clavulanic acid, ciprofloxacin, gentamicin and imipenem and this may be an indication that such antibiotics may not have been misused or abused in the study location. The low resistance to amoxicillin/clavulanic acid and ciprofloxacin observed in this study justify their used for empirical therapy of UTIs caused by Enterobactericeae. The low resistance of the isolates to imipenem observed in this study was in agreement with the study earlier reported by Polse *et al.* (2016) and Shakya *et al*. (2017). The percentage resistance of the isolates to gentamicin and imipenem was higher than 10.6% and 13.9% reported by Shakya *et al*. (2017).

The occurrence of multi-drug resistance, extensive drug resistance and pandrug resistance urinary *E. coli* isolates observed in this study was not surprising. The occurrence of MDR *E. coli* isolates observed in this study agree with the study earlier reported by Parajuli *et al*. (2017) and Shakya *et al*. (2017) that, MDR *E. coli* isolates are responsible for most cases of UTIs and are widely spread both community and hospital settings. The percentage occurrence of MDR *E. coli* isolates observed in this study was less than 78.9% reported by Shakya *et al*. (2017) and higher than 64.9% reported by Parajuli *et al*. (2017).

The high resistance of *E. coli* isolates to cefotaxime and ceftazidime observed in this study was not different from the study earlier described by Ngwai *et al.* (2013), Nkene *et al*. (2015), Ihsan *et al*. (2016) and Shakya *et al*. (2017). The resistance of the isolates to the third-generation cephalosporin observed in this may be due to the production of extended spectrum β-lactamase (ESBL) as observed in the study previously described by Nkene *et al*. (2015) that most urinary *E. coli* resistance to cefotaxime and ceftazidime were ESBL producers. Further studies on the detection of ESBL resistance genes as well as other antibiotic resistance in the antibiotic resistant isolates should be carried out.

## Conclusion

The *Escherichia coli* isolated from urine of patients with suspected UTI in NNPC Medical Services, Abuja, Nigeria harbored high resistance to antibiotics, with many of them being multi-drug resistant.

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**Table 1:** Cultural, Morphological and Biochemical characteristics of *Escherichia coli* from urine of patients with suspected Urinary Tract Infections in Nigerian National Petroleum Corporation Medical Services, Abuja, Nigeria

| Cultural Characteristics | Morphological Characteristics | | Biochemical Characteristics | | | | | | | | | | | | Inference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gram stain | Morphology | ONPG | Ornithine | UR | LYS | NT | $H_2S$ | CT | TDA | VP | MR | IND | MAL | |
| Pinkish colony on MCA and greenish metallic sheen colony EMB agar | - | rod | + | + | - | + | + | - | - | - | - | + | + | _ | *E. coli* |

MCA = MacConkey agar; EMB = Eosin methylene blue; UR = Urease; LYS = Lysine; $H_2S$ = Hydrogen Sulphide; CT = Citrate; TDA = Phenylalanine deaminase; VP = Voges-Proskauer; IND = Indole; MAL = Malonate; - = Negative; + = Positive

**Table 2:** Occurrence of *Escherichia coli* from urine in relation to age of patients with suspected urinary tract infections in Nigerian National Petroleum Corporation Medical Services, Abuja, Nigeria

| Age | No. of Samples | No. (%) *E. coli* |
|---|---|---|
| ≤10 | 11 | 1 (9.1) |
| 11-20 | 49 | 6 (12.2) |
| 21-30 | 88 | 18 (20.5) |
| 31-40 | 129 | 9 (6.9) |
| 41-50 | 58 | 8 (13.8) |
| >50 | 27 | 3 (11.1) |
| Total | 362 | 45 (12.4) |

**Table 3:** Occurrence of *Escherichia coli* from urine in relation to gender of patients with suspected urinary tract infections in Nigerian National Petroleum Corporation Medical Services, Abuja, Nigeria

| Gender | No. of Samples | No. (%) *E. coli* |
|---|---|---|
| Male | 144 | 11 (7.6) |
| Female | 218 | 34 (15.6) |
| Total | 362 | 45 (12.4) |

**Table 4:** Antibiotic Resistance Profile of the *Escherichia coli* from urine of patients with suspected urinary tract infections in Nigerian National Petroleum Corporation Medical Services, Abuja, Nigeria

| Antibiotics | Disc content (µg) | No. (%) Resistance |
|---|---|---|
| Amoxicillin/Clavulanic acid (AMC) | 30 | 25 (55.6) |
| Ampicillin (AMP) | 10 | 43 (95.6) |
| Cfefoxitin (FOX) | 30 | 22 (48.9) |
| Cefotaxime (CTX) | 30 | 31 (68.9) |
| Ceftazidime (CAZ) | 30 | 28 (62.2) |
| Gentamicin (CN) | 10 | 13 (28.9) |
| Ciprofloxacin (CIP) | 5 | 20 (44.4) |
| Imipenem (IPM) | 30 | 17 (37.8) |
| Streptomycin (S) | 30 | 33 (73.3) |
| Sulphamethoxazole/Trimethoprim (SXT) | 25 | 27 (60.0) |

**Table 5:** Multiple Antibiotic Resistance (MAR) index of *Escherichia coli* from urine of patients with suspected urinary tract infections in Nigerian National Petroleum Corporation Medical Services, Abuja, Nigeria

| No. of Antibiotic Resistance to (a) | No. of Antibiotic tested (b) | MAR Index (a/b) | Frequency (%) MAR Isolates |
|---|---|---|---|
| 10 | 10 | 1.0 | 1 (2.2) |
| 9 | 10 | 0.9 | 3 (6.7) |
| 8 | 10 | 0.8 | 7 (15.6) |
| 7 | 10 | 0.7 | 8 (17.8) |
| 6 | 10 | 0.6 | 10 (22.2) |
| 5 | 10 | 0.5 | 4 (8.9) |
| 4 | 10 | 0.4 | 6 (13.3) |
| 3 | 10 | 0.3 | 6 (13.3) |
| 2 | 10 | 0.2 | 0 (0) |



**Table 6:** Categories of Antibiotic Resistance in the *Escherichia coli* from urine of patients with suspected urinary tract infections in Nigerian National Petroleum Corporation Medical Services, Abuja, Nigeria

| Categories of Antibiotic Resistance | Frequency (%) *E. coli* (n=45) |
|---|---|
| NMDR | 6 (13.3) |
| MDR | 30 (66.7) |
| XDR | 8 (17.8) |
| PDR | 1 (2.2) |

NMDR=Non-multi-drug resistance; MDR=Multi-drug resistance(non-susceptible to ≥1 agent in ≥3 antimicrobial categories); XDR=Extensive drug resistance (non-susceptible to ≥1 agent in all but ≤2 antimicrobial categories); PDR=Pan drug resistance (non-susceptible to all antimicrobial listed) (Magiorako*set al.*, 2012).