The Susceptibility Pattern of Urinary Tract Isolates of Escherichia Coli

ENWURU Chika Paulinus, OTOKUNEFOR Kome, OTOKUNEFOR, Tosanwunmi Vincent.

Abstract
Background: Escherichia coli is the major cause of urinary tract infections (UTI) of all ages and gender. The treatment usually follows a known regimen from experience with similar infections over the years. However, the emergence of antimicrobial resistance worldwide has rendered this empirical approach unreliable in the treatment of UTI E. coli, hence the need to strictly determine the antimicrobial susceptibility pattern of each isolate for effective chemotherapy. This study was conducted to isolate and identify E. coli from urine specimens and to determine their antimicrobial susceptibility patterns.

Materials and methods: One hundred and twenty-five urine specimens were collected from patients, processed by standard bacteriological techniques and the resulting isolates identified by biochemical tests. The pattern of antimicrobial susceptibility was determined by disc diffusion method against 11 antibiotics belonging to 7 classes. This includes Gentamicin, Ciprofloxacin, Levofloxacin, Ceftazidime, Cefotaxime, Ceftiraxone, Cefpodoxime, Imipenem, Aztreonam, Amoxicillin/Clavulanate and Ampicillin. Statistical analysis was done using SPSS version 20.

Results: Fifty-six isolates of E. coli were obtained (prevalence rate of 44.8%) from 25 males and 31 females within the ages of 21 – 50 years. Gender was significantly associated with infection (p < 0.05) while age was not associated with infection (p > 0.05). Forty-one (73.2%) of the isolates were multidrug resistant with MDR level of 42.9% (resistant to at least 1 from only 3 classes) to 100% (resistant to at least 1 from the entire 7 classes of antibiotics). The MAR index with respect to the entire 11 antibiotics ranged from 0.18 (resistant to 2 antibiotics) to 0.91 (resistant to 10 out of 11 antibiotics).

Conclusion: The urinary tract isolates of E. coli in this study possess high Multidrug Resistant activity. It is recommended that the antimicrobial susceptibility profile of every urinary E. coli isolate be determined and effectively treated with right choice of antibiotics to avoid treatment failures.

Key words: Multidrug resistance, E. coli, Urinary tract infection, Antibiotics, Resistance, Susceptibility.
Furthermore, the impetus for constant antibacterial susceptibility testing is the fact that antibiotic resistance plasmids are constantly shared among bacteria of the same or different species by conjugation, transduction and transformation. 

This study is therefore aimed at isolating *E. coli* from patients suspected of urinary tract infection and determining their antibacterial susceptibility pattern using available antibiotics.

## II. Materials And Methods

One hundred and twenty-five urine specimens were collected randomly from patients referred for UTI to the laboratory department of Federal Medical Centre, Owerri.

The specimens were collected using sterile universal container and processed using standard microbiological procedure at the laboratory department of Salvation Hospital, Owerri, Imo State. The patients were instructed to collect mid-stream urine aseptically into the sterile screw cap universal container. Biometric data of the patients (age, sex, etc) were collected as well. The urine specimen was mixed thoroughly and using calibrated wire loop of 0.001 ml, a loopful was inoculated on the surface of MacConkey agar and incubated for 24 hours aerobically at 37°C. Lactose fermenting coonies were selected and further identified by biochemical tests for *E. coli* (Gram stain, indole test, Citrate utilization, urease test, catalase, TSIA, Methyl red test, and Voges-Proskauer test). The colonies were counted and recorded as colony forming units per ml urine (CFU/ml). Specimens yielding up to 10⁵ CFU/ml were considered significant for UTI. Also, specimens with bacterial counts less than 10³ but with significant number of pus cell suggestive of UTI were considered significant.

Antimicrobial susceptibility testing was carried out by disc diffusion method using the following antibiotics belonging to 7 classes according to the classification of Magiorakos et al. Gentamicin (Aminoglycoside), Ciprofloxacin and Levofloxacin (Fluoroquinolones), Cefazidime, Cefotaxime, Ceftriaxone, Cefpodoxime (Cephalosporins), Aztreonam (Monobactam), Imipenem (Carbapenem), Amoxicillin/Clavulanate (Beta lactam/Beta lactamase inhibitor), Ampicillin (Penicillin).

A suspension of the isolate was made in 5 ml sterile normal saline. The turbidity of the suspension was visually matched with the turbidity of 0.5 McFarland Standard. With sterile cotton-tipped swab, the suspension of the isolate was evenly seeded across the plate of Mueller Hinton agar. The antibiotics discs were applied with sterile forceps. The plate was placed in the incubator for 24 hours at 37°C. The diameter of the clearing around each disc was measured (in millimeters), the results interpreted as sensitive (S), intermediate (I) or resistant (R) using CLSI interpretative criteria.

## III. Results

The culture result shows that 56 out of the 125 urine specimens (44.8%) yielded significant growth of *E. coli*. The isolates were obtained from 31 (55.4%) females and 25 (44.6%) males aged 21-50 years. The age ranges 21-30 years and 31-40 years have highest *E. coli* UTI prevalence (Table 1). Gender is significantly associated with having infection (p<0.05). Female patients are more infected than male patients. However, age does not have significant association with infection (p > 0.05).

### Table 1: Age Range and gender of patients with positive cultures

<table>
<thead>
<tr>
<th>Age range</th>
<th>Positive cultures (Males)</th>
<th>Positive cultures (Females)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-20</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>21-30</td>
<td>12</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>31-40</td>
<td>12</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>41-50</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>51-60</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>31</td>
<td>56</td>
</tr>
</tbody>
</table>

The antibiogram revealed that the isolates were moresusceptible to Imipenem (96.4%), none was resistant to it and 3.6% were of intermediate susceptibility to it in comparison to other antibiotics, whereas the least susceptibility was to Ampicillin (1.8%). About 80.4% of the isolates were susceptible to gentamicin while 19.6% were resistant to the drug. Ciprofloxacin and Levofloxacin have 62.5% and 75% susceptibility and 26.8% and 17.9% resistance respectively. The extended spectrum β-lactams have a susceptibility range of 23.2% (cefpodoxime) to 60.7% (ceftriaxone) and resistance range of 23.2% to 51.8% respectively. Aztreonam (a monobactam) had a susceptibility of only 32.1% and resistance of 44.6% as reflected in Figure 1.
Figure 1: Comparative susceptibility pattern of *E. coli* isolates

With respect to the classes of antibiotics, one isolate (1.8%) was resistant to at least 1 antibiotic from the entire 7 classes of antibiotics (100% MDR) whereas 17.9% of the isolates had MDR of 85.1% (resistant to at least 1 antibiotic from 6 out of 7 antibiotic classes (Table 2).

**Table 2:** Percentage Multidrug resistance (MDR) level with the 7 classes of Antibiotics

<table>
<thead>
<tr>
<th>No of Classes of Antibiotics</th>
<th>% MDR</th>
<th>No of Isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>42.9</td>
<td>15 (26.8)</td>
</tr>
<tr>
<td>4</td>
<td>57.1</td>
<td>12 (21.4)</td>
</tr>
<tr>
<td>5</td>
<td>71.4</td>
<td>5 (8.9)</td>
</tr>
<tr>
<td>6</td>
<td>85.1</td>
<td>10 (17.9)</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>1 (1.8)</td>
</tr>
</tbody>
</table>

The Multiple Antibiotic Resistance (MAR) index ranges from 0.18 (resistant to only 2 out of the 11 antibiotics irrespective of the class) to 0.91 (resistant to 10 out of 11 antibiotics (Table 3).

**Table 3:** Multiple Antibiotic Index (MAR) with respect to the entire 11 Antibiotics

<table>
<thead>
<tr>
<th>No of Antibiotics</th>
<th>No of Isolates in the group</th>
<th>MAR Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>6</td>
<td>0.18</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0.27</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>0.36</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>0.45</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>0.55</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>0.64</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>0.73</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>0.82</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>0.91</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>
IV. Discussion

The prevalence rate of urinary tract infection in this study was 44.8%. Similar studies conducted in parts of Nigeria showed varying relatedness with our findings. While some are in agreement with our result, others show dissimilarities. For instance, Rivers State had a mean prevalence of urinary isolates among hospital attendees of 44.9%23, while Oladeinde et al.2 reported 36.69% in Edo State and Iregbu et al.14, reported a low prevalence of 13% in Abuja, Nigeria. In reports review of various prevalence of UTI in children, Uwaezuoke24, reported rates of 6% to 36% among children presenting with severe acute malnutrition among developing countries. The differences in the prevalence of urinary tract infections could be attributed to the socio-economic attributes of the people including their practices and education.

Gender was significantly associated with infection (p < 0.05) while age was discovered to have no significant association with infection; (p >0.05). These findings corroborate the findings of Oladeinde et al.2, who reported that sex and acquisition of UTI are significantly related while no significant relationship exists between age and infection. The observation of higher female than male rate of UTI in this present study (55.4% female versus 44.6% male) is also in consonant with other studies23, 25. This is attributable to many factors like the female urogenital tract anatomical features; its closeness to the anal opening, the short urethral length and more frequent sexual activity as postulated by Omorogie et al.26. Some physiological activities in females such as monthly menstrual cycle, hygienic conditions, activities like vaginal douching and contraceptives use, all help to disorganize the normal microbial population around the female urogenital area, and pre-dispose her to colonization/infection with urogenital pathogens. The age ranges 21-30 years and 31 to 40 years have the highest frequency of infection in our study which corresponds to the report of Oladeinde et al.2. Although not statistically significant, it could be pointed out that these affected groups are very active age groups in social considerations and have greater chances of involvement in risky sexual acts, such as unprotected sex, plurality of sexual partners and anal sex that in turn could result to UTI.

This study revealed that among all antibiotics used the highest susceptibility was to Imipenem (Carbapenem) with 3.4% intermediate susceptibility and none that was out rightly resistant. Penicillins recorded the highest resistance by all the isolates with Ampicillin showing 98.2% resistance and only 1.8% susceptibility. The next antibiotics that attracted lower resistance from the isolates include Aminoglycoside, Gentamicin (19.6% resistance versus 80.4% susceptibility), Levofloxacin (17.9% resistance versus 70% susceptibility), and Ciprofloxacin (26.8% resistance versus 62.5% susceptibility). The overall resistance to the 3rd generation Cephalosporins (Cefazidime, Cefotaxime, Ceftriaxone and Cefpodoxime) ranged from 23.2% to 51.8%. The findings agreed significantly with reports of Iregbu and Nwajiobi14 in Abuja Nigeria who reported all tested bacteria as highly susceptible to Imipenem. The reason for such low resistance to Imipenem may possibly be that carbapenems are expensive, not readily accessible indiscriminately and has also not been abused like other relatively cheap antibiotics. This view agrees with the observation by Troillet et al.27, that wherever resistance to Imipenem occurs, therapy with Imipenem was responsible for the developing and disseminating resistance to same drug. There is also a possibility that the resistance mode of the isolates precludes carbapenemase production which answers for most observed resistances to carbapenem antibiotics28, 29. Wade and Benjamin30 attributed the extensive susceptibility of pathogens to Imipenem to the drug’s efficiency in penetrating the organisms’ outer membrane plus its strong affinity for PBP-2 (Penicillin Binding Protein-2). Resistance to Gentamicin and the 3rd generation cephalosporins in this study is also in consonant with the report of Iregbu and Nwajiobi14. Most isolates demonstrated non-susceptibility to Ampicillin, a Penicillin which is the oldest antibiotic with the oldest history of abuse and misuse in the developing countries31. Mohammed et al.32 reported similar resistance pattern to Ampicillin (94.1%). The observed non-susceptibility to Ampicillin therefore is not unexpected. Several reports have emphasized resistance of isolates especially Enterobacteriaceae to Ampicillin33, 34.

The Multiple Antibiotics Resistance Index of all isolates ranged from 0.18 (resistance to 1 antibacterial) to 0.91 (non-susceptibility to 10 out of 11 used). With respect to the 7 classes of antibiotics, the Multidrug resistance (MDR) level of 42.9% (resistant to at least one agent in 3 different classes as described by Magiorakos et al.33) to 100% (resistant to the entire 7 classes of antibiotics). The import of this is the reality of the threat of the continued resurgence of generations of bacteria that are continually non-susceptible to commonly used antibiotics. This has seriously challenged the capability of healthcare providers to effectively curtail the morbidity and death rate associated with infectious agents including E. coli.

V. Conclusion

The urinary tract isolates of E. coli in this study possess high multidrug resistant activity. While most isolates were susceptible to Imipenem, most are resistant to Ampicillin. The high level multidrug resistance makes it difficult to predict drugs for empirical therapy in E. coli urinary tract infections. It is therefore recommended that the antimicrobial susceptibility profile of every urinary E. coli isolate be determined before...
being effectively treated with right choice of antibiotics to avoid treatment failures and selection of multi-drug resistance pathogens.

References


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