Antimicrobial Susceptibility Pattern of *Escherichia coli* Isolated from Urinary Tract Infected Patients Attending Bir Hospital

Amit Raj Sharma¹, Dwij Raj Bhatta¹, Jyotsna Shrestha² and Megha Raj Banjara¹

¹Central Department of Microbiology, Tribhuvan University, Kathmandu
²Pathology Department, Bir Hospital, Kathmandu

e-mail: amritrsharma@gmail.com

Abstract

Antibiotic resistance among uropathogens is emerging public health problem. This study was done for assessing antibiotic and multidrug resistance (MDR) patterns of *Escherichia coli* at Bir Hospital, Kathmandu, among suspected urinary tract infection (UTI) patients from January to March, 2011. Altogether, 739 urine samples were analysed by semi-quantitative culture method and uropathogens were identified by conventional methods. *E. coli* was tested (109 samples) for antimicrobial susceptibility by Kirby Bauer disc diffusion method as per Clinical and Laboratory Standard Institute (CLSI) guidelines. Out of 739 samples, 27.3% gave significant growth while 3.1% and 29.2% samples gave mixed and non-significant growth respectively. *E. coli* was found to be most predominant isolate (54.0%) followed by coagulase negative *Staphylococci* (CoNS) (21.3%) and *Enterococcus* spp. (7.3%). Nitrofurantoin was found to be the most effective antibiotic followed by ciprofloxacin and ofloxacin while cephalexin was least effective. Out of 109 *E. coli* isolates, 90.8% were MDR strains and most of the isolates had a very high multiple antibiotic resistance (MAR) index, suggesting the origin of the isolates to be of high antibiotic usage. *E. coli* showed higher rate of resistance towards commonly used oral antibiotics. However, nitrofurantoin is still active against organisms. Thus, nitrofurantoin could be the choice for empirical therapy of UTI.

Key words: *Escherichia coli*, multidrug resistance, multiple antibiotic resistance index, urinary tract infection

Introduction

Urinary tract infections (UTIs) are one of the most common infectious diseases ranking next to upper respiratory tract infection. UTIs are often associated with significant morbidity and mortality (Ramesh et al. 2008). Worldwide, about 150 million people are diagnosed with urinary tract infection each year, costing the global economy in excess of 6 billion dollars (Gonzalez & Schaeffer 1999). *E. coli*, the most common member of the family Enterobacteriaceae, accounts for 75.0-90.0% of all UTIs in both inpatients and outpatients (Dromigny et al. 2005). *E. coli* present in the gastrointestinal tract as a commensal provide the pool for initiation of UTI and certain serotypes of *E. coli* responsible for uropathogenicity were traditionally designated as uropathogenic *E. coli* (UPEC) (Raksha et al. 2003). UTI is a common disease ailment among Nepalese population as well as one of the commonest nosocomial infection (Kattel et al. 2008). According to the annual report of fiscal year (2010/2011) published by the Department of Health Services, morbidity of UTI among outpatients were 265,143. The present study was conducted to determine the prevalence and antibiotic susceptibility pattern of *E. coli* associated urinary tract infection among inpatients and outpatients.

Methodology

In a descriptive cross sectional study conducted from January to March 2011, a total of 739 urine samples from suspected UTI patients visiting Bir Hospital, Kathmandu were processed in the microbiology laboratory of this hospital for the isolation of *E. coli*. Each sample was mixed well and aseptically inoculated with 4 mm diameter nichrome wire loop on blood agar and MacConkey agar plates and incubated at 37°C for 24 hours aerobically. Significant UTI was defined as the presence of >10⁵ colony forming unit (CFU)/ml in the culture. All positive cultures were further identified by their cultural characteristics, Gram stain and battery of biochemical reaction. *E. coli* was identified on the
basis of triple sugar iron agar (TSI), sulphide indole motility (SIM) test medium, citrate utilization and urease production. The antimicrobial susceptibility testing (AST) of *E. coli* isolates was done by Kirby Bauer Disc Diffusion Method as per CLSI guideline (CLSI 2011). The antibiotic discs of HiMedia (India) used were ampicillin (10 µg), cephallexin (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), norfloxacin (10 µg), nitrofurantoin (300 µg) and co-trimoxazole (1.25/23.75 µg). A reference strain, *E. coli* ATCC 25922 was used as control. Multiple antibiotic resistance (MAR) index was determined using the formula MAR=x/y, where x was number of antibiotics to which test isolate displayed resistance and y is the total number of antibiotics to which the test organism has been evaluated for sensitivity (Akinjojunla & Enabulele 2010). Data collected were analysed by using PASW (Predictive Analytical Software), version 18.0, the premier vendor for (Statistical Package for the Social Sciences) program. A p-value of less than or equal to 0.05 was considered to be statistically significant (p ≤ 0.05).

**Results and Discussion**

A total of 739 urine samples, 202 (27.3%) samples showed significant growth, whereas majority of the samples i.e. 298 (40.3%) showed no growth, 216 showed non-significant growth, and out of the total only 23 samples showed mixed growths. *E. coli* (54.0%) was the most predominant uropathogen followed by CoNS (21.3%), *Enterococcus* spp. (7.3%), *Pseudomonas aeruginosa* (4.0%) and *Klebsiella* spp. (2.7%). *Acinetobacter* spp. and *Candida* spp. were 2.2% each. Other bacterial isolates were 1.0% or less than 1.0%. The distribution of *E. coli* were found to be the most frequent in age group 16-49 years in both sexes i.e. 13 and 60 in male and female respectively. In age group 1-15 years only two *E. coli* were isolated from females. The sexwise distribution showed that females (65.3%) were more susceptible to UTI than males (34.7%).

*E. coli*, the most common uropthogen isolated more commonly from the female patients comparatively to the male patients and isolation of *E. coli* among female patients is statistically significant (p<0.05) whereas, isolate of CoNS was more common among male patient; however, isolation of CoNS more among male patients is statistically insignificant (p>0.05) (Table 1).

<table>
<thead>
<tr>
<th>Uropathogens</th>
<th>Male</th>
<th>Female</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>24(34.3)</td>
<td>85(64.4)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Coagulase negative Staphylococcus (CoNS)</td>
<td>22(31.4)</td>
<td>21(15.9)</td>
<td>0.084</td>
</tr>
<tr>
<td><em>Enterococcus</em> spp.</td>
<td>9(12.9)</td>
<td>7(5.3)</td>
<td>0.145</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>4(5.7)</td>
<td>6(4.5)</td>
<td>1.0</td>
</tr>
<tr>
<td><em>Acinetobacter</em> spp.</td>
<td>1(1.4)</td>
<td>4(3.0)</td>
<td>0.654</td>
</tr>
<tr>
<td><em>Candida</em> spp.</td>
<td>2(2.9)</td>
<td>3(2.3)</td>
<td>1.0</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp.</td>
<td>3(4.3)</td>
<td>3(2.3)</td>
<td>0.682</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>2(2.9)</td>
<td>2(1.5)</td>
<td>0.643</td>
</tr>
<tr>
<td><em>Citrobacter</em> spp.</td>
<td>2(2.9)</td>
<td>0(0.0)</td>
<td>0.149</td>
</tr>
<tr>
<td><em>Enterobacter</em> spp.</td>
<td>0(0.0)</td>
<td>1(0.8)</td>
<td>1.0</td>
</tr>
<tr>
<td><em>Salmonella</em> Typhi</td>
<td>1(1.4)</td>
<td>0(0.0)</td>
<td>0.387</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>70(100.0)</strong></td>
<td><strong>132(100.0)</strong></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Sensitive</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>20(18.3)</td>
<td>89(81.7)</td>
</tr>
<tr>
<td>Cephalixin</td>
<td>8(7.3)</td>
<td>101(92.7)</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>23(21.1)</td>
<td>86(78.9)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>55(50.5)</td>
<td>54(49.5)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>55(50.5)</td>
<td>54(49.5)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>51(46.8)</td>
<td>58(53.2)</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>30(45.9)</td>
<td>59(54.1)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>103(94.5)</td>
<td>6(5.5)</td>
</tr>
</tbody>
</table>

Majority of *E. coli* showed susceptibility towards nitrofurantoin (94.5%) followed by ciprofloxacin and ofloxacin with the susceptibility of 50.5% for each drug. Cephalixin (7.3%) was found least effective drug followed by ampicillin (18.3%). Norfloxacin, nalidixic acid and co-trimoxazole were found effective only for less than half of the isolates of *E. coli* (Table 2).
Taking resistant to two or more classes of antibiotics as MDR, it was detected in 90.8% isolates. Among the 99 (90.8%) MDR strains, 21 (19.3%) were resistant to two antibiotics and 78 (71.6%) were resistant to three or more antibiotics (Table 3).

### Table 3. Multidrug resistance (MDR) pattern of *E. coli*

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>No. of isolates</th>
<th>Resistance to antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>109</td>
<td>Total 99</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MDR Strains</th>
<th>0 Drug</th>
<th>1 Drug</th>
<th>2 Drug</th>
<th>&gt;2 Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
<td>21</td>
<td>78</td>
</tr>
</tbody>
</table>

Sixteen multidrug resistance patterns were observed in *E. coli* for the eight antimicrobial agents tested. Resistance to Amp-Na-Cp-Cip-Of-Nx-Nit was the most frequent pattern observed in 30.3% of *E. coli* isolates, whereas Na-Cp-Cot, Amp-Na-Cp-Nx, Amp-Na-Cp-Cip-Nx, Na-Cip-Of-Nx-Cot and Amp-Na-Cp-Cip-Of-Nx-Nit were the least frequent pattern observed in 1.0% of *E. coli* for each (Table 4).

### Table 4. Antibiotic resistance pattern of MDR *E. coli* strains

<table>
<thead>
<tr>
<th>Antibiotic resistant pattern</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amp-Cp</td>
<td>13(13.1)</td>
</tr>
<tr>
<td>Na-Cp</td>
<td>4(4.0)</td>
</tr>
<tr>
<td>Na-Cot</td>
<td>4(4.0)</td>
</tr>
<tr>
<td>Amp-Cp-Cot</td>
<td>2(2.0)</td>
</tr>
<tr>
<td>Amp-Na-Cp</td>
<td>5(5.1)</td>
</tr>
<tr>
<td>Na-Cp-Cot</td>
<td>1(1.0)</td>
</tr>
<tr>
<td>Amp-Na-Cp-Nx</td>
<td>1(1.0)</td>
</tr>
<tr>
<td>Amp-Na-Cp-Cot</td>
<td>12(12.1)</td>
</tr>
<tr>
<td>Amp-Na-Cp-Nx-Cot</td>
<td>2(2.0)</td>
</tr>
<tr>
<td>Amp-Na-Cp-Cip-Nx</td>
<td>1(1.0)</td>
</tr>
<tr>
<td>Na-Cip-Of-Nx-Cot</td>
<td>1(1.0)</td>
</tr>
<tr>
<td>Amp-Na-Cp-Cip-Of-Nx</td>
<td>15(15.2)</td>
</tr>
<tr>
<td>Amp-Na-Cp-Of-Nx-Cot</td>
<td>2(2.0)</td>
</tr>
<tr>
<td>Amp-Na-Cp-Of-Nx-Cot</td>
<td>30(30.3)</td>
</tr>
<tr>
<td>Amp-Na-Cp-Of-Nx-Nit-Cot</td>
<td>1(1.0)</td>
</tr>
<tr>
<td>Amp-Na-Cp-Of-Nx-Nit-Cot</td>
<td>5(5.1)</td>
</tr>
<tr>
<td><strong>Total MDR isolates</strong></td>
<td><strong>99(100.0)</strong></td>
</tr>
</tbody>
</table>

Note: *Amp-Ampicillin, Cp-Cephalexin, Na-Nalidixic acid, Cot-Co-trimoxazole, Nx-Norfloxacin, Cip-Ciprofloxacin, Of-Ofloxacin, Nit-Nitrofurantoin*

Out of 109 *E. coli* isolates, only 10 showed MAR index of 0.1 i.e. these isolates were only resistant to one antibiotic. However, five isolates showed MAR index of 1 i.e. these isolates were resistant to all the antibiotics used in antibiotic susceptibility testing (Table 5).

### Table 5. Multiple antibiotic resistance (MAR) indices of *E. coli*

<table>
<thead>
<tr>
<th>MAR index</th>
<th>Frequency of MAR index <em>E. coli</em> (n=109)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>0.1</td>
<td>10(9.2)</td>
</tr>
<tr>
<td>0.2</td>
<td>21(19.3)</td>
</tr>
<tr>
<td>0.3</td>
<td>8(7.3)</td>
</tr>
<tr>
<td>0.4</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>0.5</td>
<td>13(11.9)</td>
</tr>
<tr>
<td>0.6</td>
<td>4(3.7)</td>
</tr>
<tr>
<td>0.7</td>
<td>17(15.6)</td>
</tr>
<tr>
<td>0.8</td>
<td>31(28.4)</td>
</tr>
<tr>
<td>0.9</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>1.0</td>
<td>5(4.6)</td>
</tr>
</tbody>
</table>

Our study was conducted among outpatients and inpatients suspected of urinary tract infection, attending Bir Hospital, Kathmandu. In this study, 27.3% urine specimens from suspected UTI patients gave significant growth. The results are in agreement with other investigators from Nepal (Chhetri et al. 2001, Karki et al. 2004, Kumari et al. 2005, Rai et al. 2008, Acharya et al. 2011) and rest of the world (Levitt 1993, Bashar et al. 2009). The majority of urine specimens showed no growth (40.3%). This may be due to patients undergoing antibiotics therapy which has inhibited or destroyed the bacterial growth (Okonofua & Okonofu 1989), or slow growing organisms, and which were not able to grow on the routine culture media used (Kattel et al. 2008). This study showed that the commonest isolates were *E. coli* (54%), CoNS (21.3%), *Enterococcus* spp. (7.3%), *Pseudomonas aeruginosa* (4.0%) and *Klebsiella* spp. (2.7%). In a study from Kathmandu, in combined group, outpatients as well as inpatients, *E. coli* (59.6%) was the commonest one followed by *Staphylococcus aureus* (12.5%), *Klebsiella* spp. (10.8), *Enterococcus* spp. (7.9%) and *Pseudomonas aeruginosa* (5.0%) (Kattel et al. 2008). *E. coli* was isolated as the most predominant isolate and accounted for 54.0% of the total uropathogens.

E. coli, the most common uropthogen isolated more commonly from female patients comparatively to the male patients and isolation of E. coli more among female patients is statistically significant (p<0.05) whereas, isolate of CoNS was more common among male patient; however, isolation of CoNS more among male patients is statistically insignificant (p>0.05). The reason for the higher prevalence of CoNS in males is not clear, though lack of circumcision, receptive anal intercourse and HIV infection recognised as risk factor for males (Orrett & Davis 2006). The study revealed that females (65.3%) were more susceptible to UTI than males (34.7%), which is also similar to other studies (Dimitrov et al. 2003, Akram et al. 2007, Arjunan et al. 2010, Manjunath et al. 2011, Alzohairy & Khadri 2011, Acharya et al. 2011). The increased incidence of the urinary tract infection in women is conditioned by favouring anatomic factors, by hormonal changes and by the urodynamic disturbance occurring with age (Bobos et al. 2010).

Most E. coli isolates in our study were resistant to ampicillin (81.7%) which resembles other studies (Rashedmarandi et al. 2008, Behroozi et al. 2010, Farshad et al. 2010a, Farshad et al. 2011). Overall quinolone resistance of E. coli were 57.7%. Increased resistance in quinolones against E. coli may reflect the overuse of these drugs for the treatment of UTI (Saleh et al. 2009). Another factor could be the generalized use of fluoroquinolones in animals feed (especially in poultry), and the subsequent transmission of resistant to strains from animals to humans (Miller & Tang 2004). In our study, only 5.5% isolates were resistant to nitrofurantoin. These findings are in agreement with other workers (Moniri et al. 2003, Bean et al. 2008, Biadglegne & Abera 2009, Raza et al. 2011, Bahadin et al. 2011, Baral et al. 2012). Nitrofurantoin was also found as the most effective antimicrobial in UTI caused by E. coli from studies in Nepal (Karki et al. 2004, Shrestha et al. 2007, Sharma et al. 2011, Rijal et al. 2012) and rest of the world (Sahm et al. 2001, Jamie et al. 2002, Alos et al. 2004, Okonko et al. 2009, Behroozi et al. 2010, Eryilmaz et al. 2010). However, Akram et al. (2007), Kausar et al. (2009), and Arjunan et al. (2010) were reported higher rate of E. coli resistance to nitrofurantoin. In case of the present study, nitrofurantoin was found to be the most effective antimicrobial. Resistance to nitrofurantoin among E. coli isolates from UTIs remained low despite of more than 50 year’s widespread use of the drug (Kahlmeter 2000, Mazzulli et al. 2001). Reason for the lack of emerging resistance are not fully understood, but likely include restricting use to indication for urinary infection, limited systemic absorption, and the need for multiple genetic mutations for the bacteria to develop resistance (Nicolle et al. 2006). Most of the E. coli isolates showed the multidrug resistant (90.8%) in agreement with other studies that found multidrug resistant E. coli ranging from 67.0 to 100.0% (Bashar et al. 2009, Moyo et al. 2010, Farshad et al. 2010a, Hassan et al. 2011). The higher MDR may be due to large portion of E. coli isolates being previously exposed to several antibiotics. In this study the antimicrobial agents showed that E. coli were highly resistant to commonly used antibiotics i.e. cephalaxin, ampicillin, nalidixic acid, ciprofloxacin, ofloxacin, norfloxacin, and co-trimoxazole. The resistance rate of E. coli to commonly used antibiotics was: ampicillin (81.7%), cephalaxin (92.7%), nalidixic acid (78.9%), ciprofloxacin (49.5%), ofloxacin (49.5%), norfloxacin (53.2%), co-trimoxazole (54.1%) and nitrofurantoin (5.5%). The increasing resistance of co-trimoxazole to E. coli has been reported in other studies from Nepal and other countries (Kattel et al. 2008, Rai et al. 2008, Bashar et al. 2009, Biadglegne & Abera 2009, Aboudobara et al. 2010, Jadhav et al. 2011). They were higher than the rate reported in our study. Ampicillin resistance among E. coli was 81.7%, which is comparable with other studies (Rafique et al. 2002, Biadglegne & Abera 2009, Beheroozi et al. 2011, Alzohairy & Khadri 2011). On the basis of our finding, antimicrobials such as ampicillin and co-trimoxazole should no longer be recommended for initial empirical therapies for UTIs.

Multiple antibiotic resistance (MAR) index is a tool that reveals the spread of bacterial resistance in a given population (Krumpermann 1983). An MAR index greater than 0.2 implies that the strains of such bacteria originate from an environment where several
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antibiotics are used (Ehimmudu 2003). The MAR indices of E. coli obtained in this study is a possible indication that a very large proportion of the bacterial isolates have been exposed to several antibiotics.

Higher resistance rate to all antibiotics used in this study except nitrofurantoin may be explained by high and uncontrolled use of these antibiotics in our institutions.

Most E. coli isolates are highly resistant to commonly prescribed antibiotics (ampicillin, cephalexin, quinolones and co-trimoxazole), but are still susceptible to nitrofurantoin which should be considered as preferred therapeutic agent once the organism is identified.

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