Changing Trends of Antibiotic Resistance in *Escherichia coli*

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ABSTRACT
Escherichia coli is the most frequent urinary pathogen isolated from 50% - 90% of all uncomplicated urinary tract infections. Since the pattern of bacterial resistance is continuously changing, the monitoring of antimicrobial susceptibility becomes more important. The main objective of this study was to update the current antibiotic susceptibility pattern of *Escherichia coli* isolates causing urinary tract infections. A total of 8507 mid stream urine sample were processed during a period of January 2006 to December 2010 at National Public Health Laboratory,Teku, Kathmandu. The urinary isolates from symptomatic urinary tract infections cases were identified by conventional methods and subjected to antimicrobial susceptibility testing by Kirby Bauer's disc diffusion method. Of the total sample,14.51% (1235/8507) showed bacterial growth. *Escherichia coli* comprised 48.7% (602/1235) of the total growth. Proportion of isolation of *Escherichia coli* was higher in female (61%) as compared to that of male (39.1%).Out of the total 55.8% were found to be Multidrug resistant. Most isolates showed increased resistance to Amoxycillin, Amoxyclav and the third generation cephalosporins as compared to resistance in 2006. This study revealed that *Escherichia coli* was common bacterial pathogen causing urinary tract infections. Since the pattern of antibiotic resistance keeps changing, current knowledge on antimicrobial susceptibility pattern is essential in order to improve the empiric treatment.

Keywords: *Escherichia coli, Multidrug resistant, Urinary tract infections*

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INTRODUCTION
Escherichia coli is the predominant cause of both community and nosocomial urinary tract infection (UTI) and also is the commonest cause of urinary tract infection in women and children especially in those with uncomplicated infections.[1] Worldwide, about 150 million people are diagnosed with UTI each year.[2] UTI is challenging, not only because of the large number of infections that occur each year, but also because the diagnosis of UTI is not always straight forward. UTI has to be distinguished from other diseases that have a similar clinical presentation, some UTIs are asymptomatic or present with atypical signs and symptoms, and the diagnosis of UTI in neutropenic patients (who do not typically have pyuria) may require different diagnostic criteria than those used for the general patient population. Because of these factors, much reliance is placed on laboratory tests to augment clinical impressions; even when clinical diagnoses are unequivocal, physicians may order laboratory tests to identify the cause of the infections and/or to provide isolates for anti-microbial susceptibility.[3]

In recent years, management of UTIs has become increasingly problematic due to the emergence of resistance to first-line antibiotics among the causative bacteria, particularly among uropathogenic *E. coli* (UPEC) strains.[4] A variety of virulence factors helps the bacteria to resist the normal host defence system and survive to multiply in the host body. Virulence factors of UPEC include the ability to adhere to uroepithelial cells, certain specific serotypes O and K antigens, resistance to phagocytosis and to the bactericidal action of normal serum. Other factors known to contribute to the virulence are the production of α haemolysis (AH), colicins, aerobactin, cytotoxic necrotizing factor and cell surface hydrophobicity.[5]

The emergence of antibiotic resistance in the management of UTIs is a serious public health issue, particularly in the developing world where apart from high level of poverty, ignorance and poor hygienic practices, there is also high prevalence of fake and spurious drugs of questionable quality in circulation. Emergence of drug resistance in bacteria is important in developing countries, also because all the facilities across country simply cannot afford the cost of culture and susceptibility of isolates, so most of the infections are treated without actual knowledge of resistance, which can make the infection even more worse or complicated. Knowledge about *E.coli,* the most common agent causing responsible for UTIs and their changing susceptibility patterns may help the clinicians to choose the right empirical treatment.

MATERIAL AND METHODS
During January 2005 to December 2010, eight thousand five hundred and seven midstream urine specimens were collected from the patients of suspected UTI visiting National Public Health Laboratory and processed according to standard laboratory methods. Samples were inoculated in 5% blood agar and Mac-Conkey agar plates using a calibrated loop and incubated at 37^{0}C for 24 hrs. Plates showing growth of more than 105 CFU/ml bacterial colonies were considered as significant and were further identified using standard bacteriological tests.[6] Antimicrobial susceptibility test was done on Muller Hinton Agar (MHA) using Kirby-Bauer disk diffusion method following Clinical and laboratory Standard Institute (CLSI) recommendations.[7] Isolates showing resistance to more than two classes of antibiotics were considered to be Multi drug resistant (MDR) isolates.

Data were statistically analyzed using SPSS 11.5 version. Chi- square test was done to analyze the data. Results were considered significant if p values were less than 0.005.

RESULTS

Of the total 8507 urine samples processed, only 1235 (14.51%) showed significant bacterial growth and were further proceeded for identification and antibiotic susceptibility test by Kirby Bauer disc diffusion method. Of the total processed sample, *Escherichia coli* alone accounted 48.74% (602/1235) isolates. The year wise distribution of sample, prevalence and proportion of isolation of *Escherichia coli* is shown in Table 1.Throughout the study period, infection by *Escherichia coli* was found higher in female (61%) as compared to that of male (39.1%). The sexwise distribution of *Escherichia coli* isolates in each year (Figure 1).

Infection of *Escherichia coli* was found higher in age group of 60-80 years in case of male patients while in the case of female patients age group of 20-40 years showed high incidence of infection. Age wise distribution of *Escherichia coli* isolates in male and female is demonstrated (Figure 2). However no significant difference was found between age group and incidence of infection.Of the total *Escherichia coli* isolates, 55.8% (336/602) isolates were found to be MDR. The occurrence of MDR isolates was found to be increasing every year.However significant difference between year and occurrence of MDR was not found. Depicts the frequency of occurrence of MDR among the cases (Figure 3). No clear trend of increasing or decreasing antibiotic resistance was observed in particular. The percentage resistance to various antibiotics depicted by *Escherichia coli* in a period of five years (Table 2).

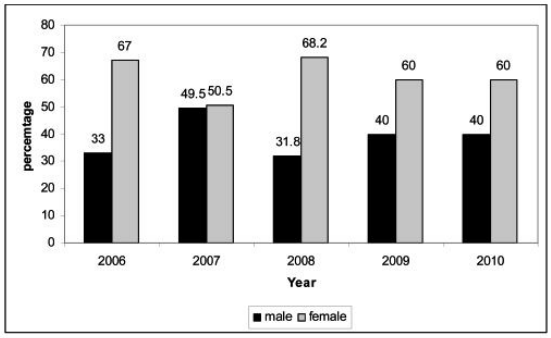

Figure 1: Sex wise distribution of *E.coli* isolates

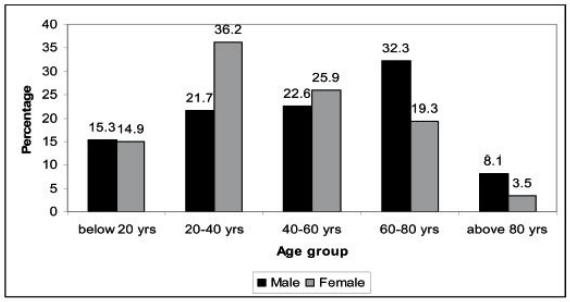

Figure 2:Age wise distribution of *E.coli* isolates

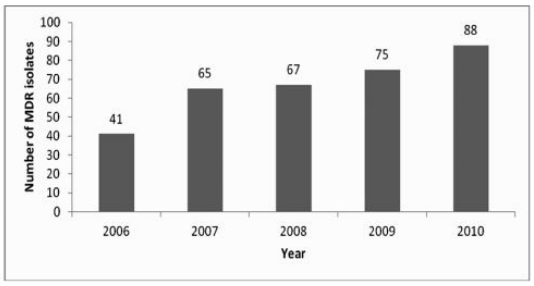

Figure 3: Increasing trend of Multidrug resistance in *Escherichia coli*

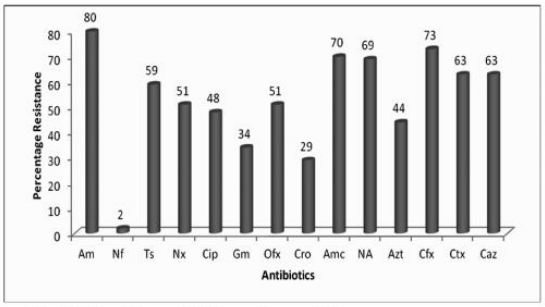

Figure 4: Antibiotic resistance exhibited over 5 year period.

In overall, Amoxycillin exhibited 79.9% resistance followed by Cefixime (72.5%), Amoxyclav (70.1%), Nalidixic Acid(69.2%), Ceftazidime (63.3%), Cefotaxime(62.8%), Cotrimoxazole (59.0%) and so on. Nitrofurantoin (1.52%) was found to be most effective drug against *Escherichia coli*. The overall resistance against various antibiotics is demonstrated (Figure 4).

Table 1: Year wise distribution of sample, growth and proportion of isolation of Escherichia coli

Year	Total sample received	Growth observed	*Escherichia coli* isolates
2006	1551	268(17.3%)	100(37.3%)
2007	1606	273(16.9%)	105(38.5%)
2008	1438	186(12.9%)	107(57.5%)
2009	1658	253(15.3%)	140(55.3%)
2010	2254	255(11.3%)	150(58.8%)

Table 2 : Percentage resistance to various antibiotics

Antibiotics	Percentage resistance observed in each year				
	2006	2007	2008	2009	2010
Amoxycillin	67.2%	83.7%	72.9%	83.7%	82.17%
Nitrofurantoin	1.5%	19.1%	11.2%	7.57%	30.4%
Cotrimoxazole	49.3%	65.1%	61.5%	61.9%	57.6%
Norfloxacin	52.7%	53.3%	45%	50%	53.5%
Ciprofloxacin	33.7%	47.9%	50.5%	51.6%	53.2%
Gentamycin	15.3%	39%	36.7%	37.2%	44.4%
Ofloxacin	36.3%	47.8%	56.7%	50.4%	54.1%
Ceftriaxone	16.3%	32.2%	31.7%	25.7%	35.2%
Amoxyclav	25%	57.1%	60%	88.5%	90%
Nalidixic Acid	72.7%	67.7%	71.9%	66.6%	63.2%
Azithromycin	33.3%	29.2%	36.6%	54.1%	50%
Cefixime	50%	63.6%	60%	91.3%	92.8%
Ceftazidime	-	43.7%	75.8%	64.8%	60.8%
Cefotaxime	-	50%	78.1%	68.4%	53.8%

DISCUSSION

Although UTI ranks the most common infection in the developing countries, in the present study only 1235 of the total 8507 samples (14.5%) were proven by culture. This indicates that urine culture is essential for a definitive diagnosis of urinary tract infection. The low positivity of culture is in accordance to other studies done in Nepal or elsewhere.[10,11,12]

Isolation of *Escherichia coli* as the most common pathogen in UTI has been extensively reported in many studies.[13,14] Of the total, 1235 isolates, *Escherichia coli* alone accounted 48.74% (602/1235). Although, the rate of *E. coli* isolation in our setting is changing, it is comparatively high in comparison to study made by Karki *et al.,* but low in comparison to study done by Basnet *et al.,* in Nepal.15,16 However, the rate of isolation of *E. coli* is found varying geographically from various institutes in various time periods.[15-20]

The findings that females had higher prevalence of UTI as compared to males agree to previous studies.[21-23] Close proximity of the female urethral meatus to the anus, shorter urethra and sexual intercourse have been reported as factors that influences higher prevalence in females.[24] Females of the sexually active age group i.e 20-40 years were found prone to urinary tract infection in comparison to males of that age. This is in agreement with Orett and Das.[25,26] Males of age group 60-80 years and above 80 years showed high rate of infection. This result is in accordance with study done by Das *et al.,* who reported[27]72% males of age >60 years prone to infection but contradict with Basnyat *et al.,* which shows high rate of infection in males of age group 16-60 years.[17,26]

Resistance rates of *E.coli* to many commonly used antimicrobial agents have increased over the years and these resistance rates vary from country to country.*27* The proportion of antibiotic resistance is found increasing in the year 2010 as compared to 2006 however no particular trend could be established annually.

In our study, among the β-lactam antibiotics, increasing rate of resistance against amoxicillin is observed. Similar to this observation various other studies have reported that the overall resistance rates to amoxicillin are between 45%–100% among the different urinary isolates.[28,29] This clearly states that using amoxicillin would not cover as a single agent for empirical treatment of a suspected UTI. Amoxycillin clavulanate was found to be performing relatively better in the similar situation. But, according to our study, also Amoxycillin clavulanate could not be used as the choice drug because of the rising resistance rate exhibited by this combination drug over 5 year period.

Trimethoprim-sulfamethoxazole another commonly used 1st-line antimicrobial agent had very high resistance rates against *Escherichia coli* (49%–63%). This is in keeping with similar increase in resistance to trimethoprim-sulfamethoxazole reported in other countries and indicates that the use of this antibiotic as a single agent for the treatment of UTI is not appropriate in our setting.[30,31]

Alternative regimens such as fluoroquinolone are widely accepted as better empiric choice of UTI treatment in many countries.[30] We found that the commonly used fluoroquinolones provided moderate effectivity against most *Escherichia coli* with susceptibility rate ranging between 49% to 52%. Similarly, low level resistance was reported to nitrofurantoin. This drug exhibited low resistance rate in the major part of the world (0%–5.4%), despite of it's being used for many years.[32] It could be used as drug of choice to treat empiric cases of UTI.

Among the third generation cephalosporins, Ceftriaxone had a better spectrum of activity as compared to Cefixime, Cefotaxime and Ceftazidime. However, these third generation cephalosporins should be recommended to patients with complicated pyelonephritis and patients who are unable to take oral antimicrobial therapy.[27] Because the third generation cephalosporins can related to cause a wide range of adverse drug reactions like diarrhea, vomiting, headache, dizziness, oral and vaginal candidiasis, pseudomembranous colitis, superinfection, eosinophilia, and/or fever, nausea, rash, electrolyte disturbances, and/or pain and inflammation at injection site, the use of third generation cephalosporins should be encouraged only in severe cases.

Aminoglycosides like gentamicin are known to have good coverage against Pseudomonas and other bacteria that are resistant to other antibiotics are usually reserved for serious UTI and used in combination with other antibiotics.[32] In our study, *Escherichia coli* were found to be moderately covered by gentamicin (with susceptibility of 45-85%).Macrolides like azithromycin are not reported to be commonly used against uropathogens, however our study reveals moderate efficacy of azithromycin with around 50% susceptibility range.

CONCLUSION

In conclusion, this study provided the much needed information on the prevalence of E. coli, the most important bacterial pathogen of UTI and their resistance trend to commonly used antimicrobials. It also demonstrated an increasing resistance to commonly used 1st- line antimicrobials like amoxicillin, trimethoprim-sulfamethoxazole, third generation cephalosporins by these isolates. Currently, in our scenario Nitrofurantoin remained the most active agent and as it can be administered orally and is highly concentrated in urine, it may therefore be the most appropriate agent for empirical use in uncomplicated UTI. The increasing number of MDR E.coli isolates annually should draw our attention. The resistance pattern, though not different from rest of the world is ever increasing due to inappropriate use of available antibiotics. We believe this information would provide a baseline for continuous surveillance of causative agent of UTI and their resistance pattern to ensure appropriate treatment and prevent further development of drug resistance.

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