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International Journal of Applied Sciences and Biotechnology

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Research Article

International Journal of Applied Sciences and Biotechnology

ISSN: 2091-2609

PLASMID PROFILING OF MULTIDRUG RESISTANT *ESCHERICHIA COLI* STRAINS ISOLATED FROM URINARY TRACT INFECTION PATIENTS

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ABSTRACT

Introduction- Urinary tract infection is a common community-acquired bacterial disease. *Escherichia coli* is reported to be the major cause of urinary tract infection. **Aim & Objective-** The study was conducted with the aim of determining the antibiotic resistance pattern and plasmid profile of multidrug resistant *Escherichia coli* isolated from Urinary Tract Infection patients. **Materials and Method-** Antibiotic susceptibility tests were performed against *E. coli* following the protocol for the Kirby-Bauer disc diffusion method. Plasmid DNA was isolated following the protocol of Kado and Liu. **Results-** Multidrug resistant isolates exhibited high resistance to drugs like Amoxicillin, Cefixime, Ciprofloxacin, Cotrimethoxazole, Norfloxacin and Ofloxacin. The plasmid profiling showed that all, except one, isolate contained at least one plasmid. A band of approximately 23 kb was seen in most of the isolates.

Key words- urinary tract infection, *Escherichia coli*, antibiotic resistance**,** plasmid profile, antibiotic susceptibility

Introduction

Urinary tract infection (UTI) is a common community-acquired bacterial disease (Dromigny et al., 2005). *E. coli* is reported to be the major cause (85-95%) of urinary tract infection (Russo et al., 2003). Urinary tract infections are typically treated with medications called antibiotics or antimicrobials that have antagonistic effect on the bacteria. The selective pressure of the antimicrobials selects those strains that are resistant to the applied antimicrobials causing the resistant strains to multiply and spread. Therefore, multidrug resistant (MDR) organisms are frequently found in urinary tract infection (Calbo et al., 2006). Antimicrobial therapy of UTI caused by *E. coli* is often impaired due to the resistance to commonly- used antimicrobial agents (Chakupurakal et al., 2010; Giamarellou, 2010).

There is a large reservoir of resistant genes, in bacterial genomes and in extra-chromosomal pieces of DNA (plasmids) that encode different mechanisms of drug resistance (Soulsby, 2005). The transmission of antibiotic resistance, often to several drugs simultaneously, from one bacterium to another is attributed to plasmids. Understanding antibiotic resistance patterns and molecular characterization of plasmids is epidemiologically useful (Hassan, 1985). Although conventional antimicrobial susceptibility testing methods are useful methods for detecting resistance profiles and for selecting potentially useful therapeutic agents, they are insensitive tools for tracing the spread of individual strains within a hospital or region. Molecular methods like plasmid profiling provide powerful tools to track bacterial strains and contribute to the evaluation of nosocomial infection outbreaks, recurrent infection and clonal dissemination of specific pathogens (Sader et al., 1995). They are also used as a means of providing additional information, to detect and evaluate the mode of dissemination of MDR pathogens (Pfaller et al., 2001). Plasmid analysis has also proved a useful method for differentiating bacterial isolates (Waschmut et al., 1991; Dorn et al., 1992). The number and size of the plasmids present is used as the basis for strain identification. This strain typing technique has been used successfully for analysis of outbreaks of nosocomial infections (Schaberg et al., 1981) and community acquired infections (Fornasini et al., 1992) caused by a variety of species of Gram negative rods.

The present study was undertaken to determine the antibiotic resistance pattern and plasmid profile of MDR *E. coli* isolated from Urinary Tract Infection patients and to analyze the probable link between them. Our data might be informative to both of the health professionals and the scientific community, which may help to make a positive contribution to current understanding and knowledge of the situation in UTI caused by MDR bacterial pathogens.

Materials and Methods

The study was conducted among UTI patients visiting Kathmandu Model Hospital, Kathmandu, Nepal during May 2012. Antibiotic susceptibility tests were performed against *E. coli* following the protocol for the Kirby-Bauer disc diffusion method according to *Performance Standards for antimicrobial susceptibility testing* (National Committee for Clinical

Laboratory Standards, 2002) at Pathology Department, Kathmandu Model Hospital, Kathmandu, Nepal. *E. coli* isolates with intermediate susceptibility classification were considered not to be resistant to that drug and multi-resistance was defined as resistance to more than one drug. MDR *E. coli* strains isolated from the patients were collected in nutrient agar slants and cultured for 24 hours. Single colonies from the slants were transferred to LB broth to conduct plasmid isolation. Plasmid DNA was isolated following the protocol of Kado and Liu (Kado et al., 1981). Electrophoresis of plasmid DNA was performed on 1% agarose gel, run for 60 minutes at 100 V on TAE buffer system, stained with ethidium bromide and observed under UV trans-illuminator. Molecular analyses were performed at Department of Biotechnology, College for Professional Studies, Maitidevi, Kathmandu, Nepal.

Results

Twenty five MDR *E. coli* strains were isolated. From the Antibiotic Susceptibility data it was found that more than 80% multidrug resistant *E. coli* isolates were resistant to Amoxicillin, Cefixime, Ciprofloxacin, Cotrimoxazole, Norfloxacin and Ofloxacin. 80% isolates showed resistance against Cefotaxime. All isolates showed resistance to Amoxycillin and Ciprofloxacin. 68% to 76% isolates were resistant to Ceftazidime and Ceftriaxone respectively. Most isolates were sensitive to Amikacin and Cefoperazone/sulbactum. None of the *E. coli* isolate was resistant to Nitrofurantoin (Fig.-1).

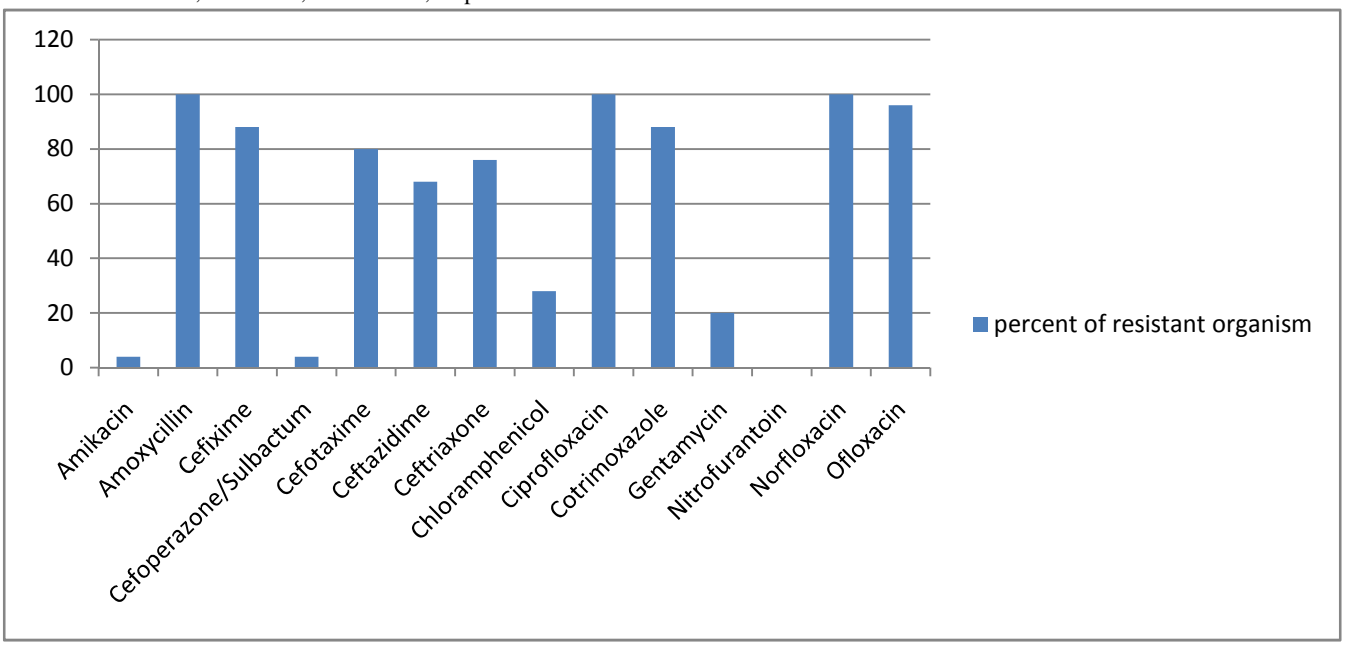

Fig. 1: Percentage of resistance to various antibiotics

Table 1: Multiple drug resistance pattern of *E. coli* isolates

Number of drugs the organism is resistant to	Number of resistant isolates
5	25
6	3
7	2
8	3
9	10
10	4
11	2

Table 1 shows the number of isolates that are resistant to particular number of drugs. All twenty five isolates were resistant to at least five drugs tested. Three isolates were resistant to six drugs. Two isolates were resistant to seven drugs. Three isolates were resistant to eight drugs. Ten out of twenty five MDR isolates were resistant to nine drugs. Four isolates were resistant to 10 drugs tested and 2 were resistant to 11 drugs which was maximum resistance.

Plasmid Profiling showed that all the isolates, except one, contained at least one plasmid. The isolates had from one to five plasmids. Five had two plasmids. Three had three plasmids. One had four, and one had five plasmids. Plasmid of size approximately 23 kb was common in all isolates. Only one isolate out of 25 contained plasmid sized bigger than 23 kb (Fig. 2).

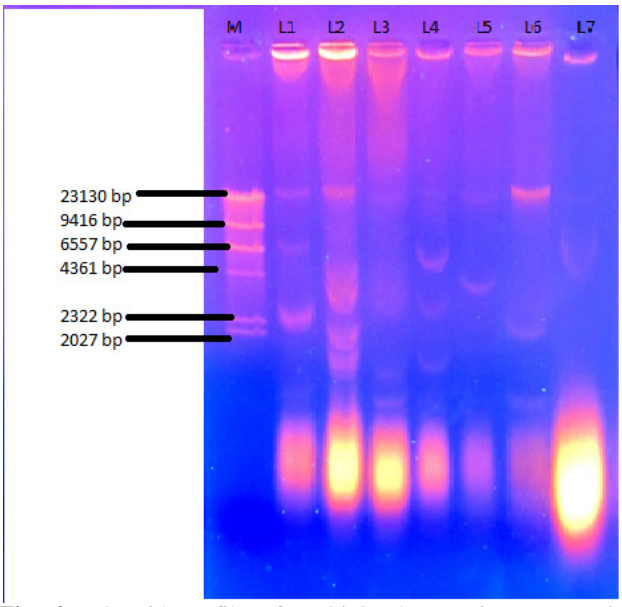

Fig. 2: Plasmid profile of multiple drug resistant *E. coli* isolates.

Discussion

Since their discovery, antimicrobials have been proved remarkably effective for the control of bacterial infections. However, it was soon evidenced that bacterial pathogens were unlikely to surrender unconditionally, and some pathogens rapidly became resistant to many of the first effective drugs (Freeman, 1985). Over the past few decades, antimicrobials have become increasingly available for a broad range of pathogens. Due to the widespread use of these drugs, new forms of antimicrobial resistance have emerged (Giamarellou, 2010). Indiscriminate use of antimicrobial by healthcare providers or by way of self-prescribing and over-the-counter availability are major risk factors for the development of high levels of antimicrobial resistance, which is common in both developed and developing countries. Some other factors contributing towards resistance include incorrect diagnosis, unnecessary prescriptions, improper use of antibiotics by patients, and the use of antibiotics as livestock food additives for growth promotion (Bouza et al., 2002). Antibiotic resistance profile for clinical *E. coli* isolates have well been documented by various workers (Shao et al., 2004; Sadechi et al., 2005; Rijavec et al., 2006; Celebi et al., 2007; Lina et al., 2007; Jan et al., 2009; Baral et al., 2012). The present study provides the information about the antibiotics resistance pattern of the multidrug resistant *E. coli* isolated from the UTI patients of a hospital in Nepal. It was found from our study that multidrug resistant *E. coli* exhibited resistance to large number of antibiotics (up to 11 in our study) and also exhibited wide range of resistance pattern. Amikacin, Cefoperazone/sulbactum and Nitrofurantoin were observed to be the most active antimicrobials against the organism while high resistance were observed against Amoxicillin, Cefixime, Ciprofloxacin, Cotrimethaxazole, Norfloxacin, Ofloxacin and Cefotaxime.

Plasmid profiling showed that almost all MDR *E. coli* isolates contained plasmids and many of them share some common plasmids too. No relation could be detected between the antibiotic resistance pattern and the plasmid profile analysis in the present study due to technical limitations. But further study on the isolates by resistance transfer testing and plasmid curing to assign resistance genes to plasmid DNA may confirm the plasmid origin of MDR pattern.

Conclusion

Urinary Tract Infection is a disease that is common. It keeps affecting many at all times. The most important cause of UTI is considered to be the bacteria *E. coli*. Antibiotics that are used to treat UTI are becoming ineffective due to emergence of resistance. Antibiotic resistance has become a worldwide problem. In the present study we studied antibiotic resistance pattern and plasmid profiles of MDR *E. coli* (25 samples) isolated from UTI patients. It was observed that the MDR isolates showed high degree of resistance to multiple drugs. It was observed that the isolates exhibited high resistance to drugs like Amoxicillin, Cefixime, Ciprofloxacin, Cotrimethoxazole, Norfloxacin and Ofloxacin. The organisms were found to be sensitive to drugs like Amikacin, Cefoperazone/sulbactum and Nitrofurantoin. The plasmid profiling showed that all, except one, isolate contained at least one plasmid. A band of approximately 23 kb was seen in 22 isolates. Although *E. coli* has been reported to be MDR by possessing the antibiotic resistant genes in its transferable R-plasmids, detection of this feature in UTI isolates from Nepal is largely unknown (Vaidya, 2011). Further study on the isolates at molecular level may be beneficial in ruling out the cause of MDR pattern which may help to make a positive contribution to current understanding and knowledge of the situation in UTI caused by MDR bacterial pathogens and for the development of better treatment strategy and prevention of the disease.

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