Study of antimicrobial susceptibility and plasmid analysis of *Escherichia coli* in Iran, Urmia

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Abstract: **Twenty-five bacterial samples were isolated from buffalo faeces. Twenty strains out of twenty -five strains were identified as *Escherichia coli*. Antibiotic susceptibility tests and plasmid analysis carried on the *E.coli* isolates. Antibiotic susceptibility tests of the isolated strains of *E. coli* were done by antibiotic disc diffusion method. The antibacterial agents were ampicillin, amoxicillin, neomycin, kanamycin, streptomycin, tetracycline, nalidixic acid, flumequine, erythromycin and enrofloxacin. Plasmid DNAs were extracted from each of the drug resistant *E.coli* strains. All of the collected *E.coli* strains were resistant to ampicillin, amoxicillin, neomycin, kanamycin, streptomycin, tetracycline, nalidixic acid, flumequine, erythromycin and enrofloxacin at 90,80,100,50,100,75,75,60,100 and 35% respectively. From the results it was revealed that molecular size of the plasmid DNAs extracted from twenty different drug resistant *E.coli* varied from 9.162 to 13.000 Kb. In this investigation it was revealed that each of the twenty drug resistant *E.coli* harbored a single plasmid. It can be said that increasing incidence of drug resistance in *E.coli* to different antibiotics including the broad spectrum antibiotics tetracycline, nalidixic acid and ... heralds the coming therapeutic problem in the treatment of infections cause by this micro-organism.**

Key words: *Escherichia coli*, antimicrobial susceptibility, plasmid DNA, resistance.

Introduction

Bacterial resistance to antimicrobial agents is a major world wide problem because of introduction of new antimicrobial agents is usually followed sooner or later by emergence of bacterial resistance to these drugs (Patwary, 1994). The drug resistance in bacterial population is may be due to a genetic and non- genetic mechanism(Choudhury, 1988).

Regarding genetic mechanism most drug resistant microbes emerged as a result of genetic changes and subsequent processes by antimicrobial drugs. The Drug resistance may be chromosomal DNA or plasmid DNA mediated. Plasmids are autonomously replicated DNA which are extra- chromosomally located in the micro-organisims. The plasmid mediated drug resistance is caused due to the presence of drug resistance gene(s) harboring on the plasmid DNA. These gene(s) confer the drug resistance phenomenon in the host organism (Meyer's, *et al.*, 1976). Plasmids carrying drug resistance phenotype are known as R-factor which is responsible for the spread of multiple drug resistance among bacteria. R-factor consists of two components i.e. resistance transfer factor (RTF) and resistance determinant 'r'. The complete plasmid (RTF^{+r}) is called R-factor (Patwary, 1994). *Escherichia coli* is one of the serious pathogen that can cause tremendous therapeutic problem by developing resistance against antibiotics. As a result of drug resistance to several antibiotics in *E. coli* it has become a serious problem not only in the developing countries where it is endemic but also an important problem of treating drug resistant *E. coli* infection in

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Table 1: The drug resistance rate of 20 *E.coli* isolates against the10 antibacterial agents.

Antibacterial Agent	% of resistant isolates
Ampicillin	90
Amoxicillin	80
Neomycin	100
Kanamycin	50
Streptomycin	100
Tetracycline	75
Nalidixic acid	75
Flumequine	60
Erythromycine	100
Enrofloxacin	35

Table 2: Multiple drug resistance rate of 20 *E.coli* isolates against the10 antibacterial agents.

number of antibacterial agents	% of resistant isolates
1	100
1<	100
2<	100
3<	100
4<	100
5<	100
6<	100
7<	100
8<	100
9<	14
10<	0

the developed countries (Tauxe *et al.*, 1990).

E.coli is referred to as the colon bacillus because it is the predominant facultative species in the large bowel. The major species of *E. coli* are found in the lower portion of intestine of human and warm blooded animals where it comprises the normal intestinal flora (Pelczer *et al.*, 1993). *E.coli* is the causative agent of many life threatening disease like urinary tract infections, pyelonephritis, bacteremia and diarrhoeal diseases. In this study antimicrobial susceptibility of twenty *E. coli* strains were carried out to determine their antibiotic resistance pattern and characterize the plasmid DNAs.

Materials and Methods

Isolation of bacterial samples: Twenty-five bacterial samples were isolated from buffalo faeces. The isolated bacteria sub- cultured on MacConkey agar plates.

Identification of the bacteria: *E.coli* identified on the basis of gross morphology along with cultural characteristics and the manner in which the bacteria did response to various biochemical tests according to Collins *et al* (1987).

Antibiotic susceptibility testing of the bacteria: Antibiotic susceptibility testing of the isolated strains of *E. coli* were done by antibiotic disc diffusion method using filter paper discs (Bauer *et al.*, 1966). A 24hr. culture of the isolate grown at 37°C were spread on a Mueller-Hinton agar (Oxoid) plate by using sterilized glass spreader. The inoculated plates were allowed to stand for 3-5 minutes. The discs placed onto the agar surface using sterile forceps. The plates incubated at 35°C within 15 minutes of applying for 16-18 hours. After incubation the plates were observed in order to calculate the diameter of clear zone produced around each disc. Ten commonly used antibiotics, viz. ampicillin, amoxicillin, neomycin, kanamycin, streptomycin, tetracycline, nalidixic acid, flumequine, erythromycin and enrofloxacin (Padtan Teb). In order to establish antibiotic susceptibility profile of the isolated *E. coli* strains, the clear zone produce around each disc were measured in millimeter.

Extraction of plasmid DNA: Plasmid DNAs analyses on each resistant *E.coli* strains were done according to Sambrook *et al* (1989).

Agarose gel electrophoresis of the extracted DNA: DNA extracted from each of the sample strain was subject to gel electrophoresis with 0.8% agarose gel according to Meyer's *et al* (1976). In this study λDNA (Hind III digested) was used as marker DNA (Rezina *et al.*, 2001).

Results

Identification of the collected bacterial samples: Identification of bacterial samples for different morphological and biochemical tests were performed according to the procedures described by Collins *et al* (1987). Twenty strains out of twenty -

Table3: Drug resistance pattern of 20 *E.coli* isolates against the10 antibacterial agents. Amp=ampicillin, Amo=amoxicillin, Neo= neomycin, Kan= kanamycin, St= streptomycin, Te= tetracycline, Na=nalidixic acid, Flu= flumequine, Ery= erythromycin, Enr= enrofloxacin.

NO. of Strains	Resistance pattern
1	Amp, Amo, Neo, Kan, St, Ery
2	Amp, Amo, Neo, Kan, St, Ery
3	Neo, St, Te, Na, Flu, Ery, Enr
4	Neo, St, Te, Na, Flu,Ery, Enr
5	Amp, Amo, Neo, Kan, St, Te, Na, Ery, Enr
6	Amp, Amo, Neo, Kan, St, Te, Na, Ery
7	Amp, Amo, Neo, St, Te, Na, Ery
8	Amp, Amo, Neo, St, Te, Na, Flu, Ery
9	Amp, Neo, Kan, St, Te, Na, Flu, Ery, Enr
10	Amp, Neo, Kan, St, Te, Na, Flu, Ery
11	Amp, Amo, Neo, St, Flu, Ery
12	Amp, Amo, Neo, St, Te, Na, Flu, Ery, Enr
13	Amp, Amo, Neo, Kan, St, Te, Na, Flu, Ery
14	Amp, Amo, Neo, Kan, St, Te, Na, Flu, Ery
15	Amp, Amo, Neo, St, Flu, Ery
16	Amp, Amo, Neo, St, Ery, Enr
17	Amp, Amo, Neo, St, Te, Na, Ery
18	Amp, Amo, Neo, Kan, St, Te, Na, Flu, Ery
19	Amp, Amo, Neo, St, Te, Na, Ery, Enr
20	Amp, Amo, Neo, Kan, St, Te, Na, Flu, Ery

five strains were identified as *E.coli* on the basis of their biochemical behavior and morphological characteristics.

Establishment of antibiotic susceptibility profile: All the *E.coli* strains were resistant to ampicillin, amoxicillin, neomycin, kanamycin, streptomycin, tetracycline, nalidixic acid, flumequine, erythromycin and enrofloxacin at 90, 80, 100, 50, 100, 75, 75, 60, 100 and 35% respectively (Table1).

The multiple drug resistance of 20 *E.coli* isolates against the 10 antibacterial agents are shown in table 2. The drug resistance pattern in *E.coli* isolates are also shown in table 3).

Characterisation of plasmid DNAs by agarose gel electrophoresis: it was revealed that molecular size of the plasmid DNAs extracted from twenty different drug resistant *E.coli* varied from 9.162 to 13.000 Kb.

Table 4: Molecular size of different plasmid DNAs extracted from *E.coli* strains.

No.of strains	Molecular size(Kb)
1	10.180
2	11.190
3	9.188
4	9.162
5	11.190
6	11.198
7	10.120
8	12.216
9	10.180
10	12.180
11	11.190
12	9.300
13	10.180
14	11.198
15	9.162
16	11.198
17	11.198
18	12.216
19	13.000
20	12.450

Discussion

Plasmid DNAs, possibly carring drug resistant gene(s) were characterized and twenty-five bacterial samples were collected from bufallo feaces in Urmia,Iran. Twenty out of twenty -five bacterial strains were screened as *E.coli* on morphological characteristics. All these twenty *E.coli* were subjected to antimicrobial susceptibility tesing in attempt to establish their antimicrobial susceptibility profile. Ten commonly used antimicrobial drugs were used in the susceptibility testing. It was revealed that different strains were resistant to different antimicrobial drugs and their efficiency varied from antibiotics to antibiotics. From enrofloxacin to neomycin, streptomycin and erythromycin the range varied from 35 to 100%. These findings were in accord with the Azad *et al* (1999). They have documented reports of isolation of multi drug resistant *E.coli* which were resistant to at least eight

commonly used antibiotics including ampicillin, tetracycline and chloramphenicol. Antimicrobial susceptibility testing of intestinal micro-organisms like *E.coli* is important consideration because the administration of antimicrobial substances can alter the intestinal microbial balance and resulted in the suppression of certain beneficial bacterial disorders (Kobayashi *et al.*, 1973).

Plasmid DNAs were isolated from each of the twenty drug resistant *E.coli* strains by protocol according to Sambrook *et al* (1989). The DNAs were subjected to agarose gel electrophoresis with 0.8% agarose according to Meyer's *et al* (1976).

From the pattern of bands observed in the gel after staining with etidium bromide solution (EtBr), The molecular size of the plasmid DNAs were calculated. The molecular size of the plasmid DNAs isolated from twenty *E.coli* strains were varied from 9.162 to 13.000 Kb. In this case we have used λDNA (Hind III digested) as marker DNA.

These findings were in consistent with the findings of others. (Flint *et al.*, 1987; Martinez *et al.*, 1987; Rezina *et al.*, 2001; Wachsmuth *et al.*, 1983). They reported that about strains of *E.coli* harbored plasmid of varying molecular weight and molecular size. In this investigation it was revealed that each of the twenty drug resistant *E.coli* harbored single plasmid.

However, more research is necessary in order to determine the exact mechanism of drug resistance in these *E.coli* and to identify the resistance transfer factor (RTF) and 'r' determinant of these plasmid DNAs. In conclusion it can be said that increasing incidence of drug resistance in *E.coli* to different antibiotics including the broad spectrum antibiotics tetracycline, nalidixic acid and chloramphenicol heralds the coming therapeutic problem in the treatment of infections cause by this micro-organism.

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مطالعه حساسیت نسبت به داروهای ضد میکروبی و تعیین الگوی پلاسمیدی اشریشیاکلی در ارومیه-ایران

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بیست و پنج نمونه باکتریایی از مدفوع گاومیش جدا گردید و ۲۰ نمونه به عنوان اشیریشیاکلی شناسایی شد. آزمایش حساسیت آنتی‌بیوتیکی و شناسایی پلاسمید بر روی ۲۰ نمونه انجام گرفت. آنتی بیوتیک‌های مورد استفاده آمپی‌سیلین، آموکسی‌سیلین، نئومایسین، کانامایسین، استرپتومایسین، تتراسیکلین، نالیدیکسیک اسید، فلومکوئین، اریترومایسین و انروفلوکساسین بودند. استخراج DNA پلاسمیدی از سویه‌های مقاوم اشریشیاکلی به عمل آمد. کلیه سویه‌های اشریشیاکلی مورد آزمایش نسبت به آمپی‌سیلین، آموکسی سیلین، نئومایسین، کانامایسین، استرپتومایسین، تتراسیکلین، نالیدیکسیک اسید، فلومکوئین، اریترومایسین و انروفلوکساسین به ترتیب به میزان ۹۰، ۵۰، ۱۰۰، ۸۰، ۱۰۰، ۷۵، ۷۵، ۶۰، ۱۰۰ و ۳۵ درصد مقاومت نشان دادند. نتایج استخراج DNA پلاسمیدی نشان داد که اندازه پلاسمیدها در سویه‌های مختلف از ۹/۱۶۲ تا ۱۳/۰۰۰ کیلوباز متفاوت بوده و هریک از سویه‌های مقاوم تنها حامل یک پلاسمید می‌باشند. نتایج مطالعه نشان داد که هر یک از سویه‌های مقاوم تنها حاوی یک پلاسمید می‌باشند که احتمالاً مقاومت نسبت به چند دارو را اداره می‌نمایند. افزایش مقاومت‌های دارویی در سویه‌های اشریشیاکلی به ویژه نسبت به آنتی‌بیوتیک‌های وسیع الطیف مانند تتراسیکلین، نالیدیکسیک اسید و... درمان عفونت‌های حاصل از این ارگانیسم را در آینده با مشکلات جدی مواجه خواهد کرد.

واژه‌های کلیدی: اشریشیاکلی، حساسیت آنتی بیوتیکی، DNA پلاسمیدی، مقاومت.

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