Full Length Article

Determination of Antibiotic Resistance and Plasmid DNA Profiles of *Esherichia coli* Strains Isolated from Clinical Materials

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ABSTRACT

In this study, *E. coli* strains, isolated from different clinical materials, were analysed according to their antibiotic resistance patterns and plasmid DNA profiles. When the incidence of *E. coli* infections are considered, more *E. coli* infections were observed in females than in males. Of the all *E. coli* strains isolated from clinical materials, 27% were resistant to only one antibiotic, 25% were resistant to 6 antibiotics and resistance to all 8 antibiotics was established as 6%. In addition to these, resistance rate was highest for ampicillin and ciprofloxacin, whereas lowest resistance was displayed against Piperacillin/Tazobactam. According to the antibiotic resistance patterns of *E. coli* strains, 10 different antibiotypes were generated and the one, which was observed in highest frequency includes ampicillin–resistant property in itself (antibiotype 1). When the plasmid profiles of *E. coli* strains are examined, the profile which includes single band was observed as the most frequent one. It was also established that most of the *E. coli* strains, which were classified in that plasmid profile, acquire the properties of antibiotype 1. Besides it was also observed that; some of the *E. coli* strains, which do not contain any plasmid in their cells showed resistance to various antibiotics. © 2010 Friends Science Publishers

Key Words: *Esherichia coli*; Multidrug resistance; Plasmids; Antimicrobial susceptibility

INTRODUCTION

Escherichia coli that exists in human intestine as a natural flora member, is an opportunistic gram-negative, facultative anaerobic pathogen and it takes place within *Enterobacteriaceae* family (Kaper *et al.*, 2004). When the members of this pathogen leave the regions that they naturally exist in and approach to the other areas of the body, which are suitable for them, they may cause some abenteric infections such as; urinary infections, sepsis and meningitis (Cunin *et al.*, 1999). It has also been stated that these opportunistic pathogens are responsible from ulcerative collitis and Hemolytic Uremic Syndrome (HUS), a potentially fatal kidney disease (Kaper *et al.*, 2004; Rolhion *et al.,* 2007). Appropriate treatment towards the infections caused by *E. coli* variations depends on their antibiotic sensitivities. Some of the antibiotics that are used to eradicate microbial infections caused by them, are listed as; amoxicillin/clavulanic acid, ceftriaxon, gentamicin and ampicillin. Nowadays, antibiotic resistant *E. coli* variations have been a source of every increasing therapeutic problem and the resistance that they display towards the treatments increase day by day (Sahm *et al.*, 2001). Because of containing plasmids in their cells, some of the *E. coli* variations show resistance to various antibiotics (Wiener *et*

al., 1999). The properties gained by plasmids, are the reason of existence of many different resistant profiles and these properties also trigger the spreading of resistant profiles universally. In this study, the antibiotic susceptibilities and the plasmid profiles of various isolated *E. coli* strains were examined and their plasmid profiles were compared with their antibiotic resistance and their antibiotic resistance profile distribution.

MATERIALS AND METHODS

Strains: *E. coli* strains were collected from different clinical materials, which were isolated from different hospitals in Ankara, Turkey. All the samples collected from patients were urine for the services of gynecology, urology, endocrinology, cardiology, nephrology, oncology and neurosurgery, all the samples collected from patients were feces for the services of infection control, thoracic medicine and physical theraphy. And finally, all the samples collected from patients were wound for the service of gastrology. Different from these, both urine and feces were collected for the service of pediatrics, emergency and internal diseases. Isolated *E. coli* strains were inoculated in to the Brain Heart Infusion Broth media including 10% glycerol and stored at -20°C.

Phenotypical identification of *E. coli* strains: In order to accomplish the phenotypical identification of *E. coli* strains, the isolates were examined according to their colony morphologies and microscopic appearance initially. Afterwards, they were identified by standard phenotypical methods (Bopp *et al.*,1999). After the application of standard phenotypical methods; the gram negative isolates, which were lactose, indole, metil red positive and citrate, urease, negative were accepted as *Escherichia coli*. Finally the strains, which were established as *E. coli*, were picked up to be used in the study.

Antibacterial susceptibility testing and antibiotyping: The susceptibilities of *E. coli* strains to 8 different antibacterial agents (Amoxicilin/Clavulonic acid: 30 μg, Ceftriaxon: 30 μg, Gentamicin: 10 μg, Ciprofloxacin: 5 μg, Piperacilin/Tazobactam: 10 μg, Cefazolin: 30 μg, Cefuroxim: 30 μg, Ampicillin: 10 μg) were assessed by disc - diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS). Mueller Hinton agar plates were inoculated with a bacterial suspension corresponding to a 0.5 McFarland optical density standard (Kõljalg *et al.*, 2009). Antibiotic discs were placed on the surfaces of the agar plates and then the plates were incubated at 37°C. After 24 h, the zone sizes of inhibited areas were calculated and the strains were classified as Resistant (R), Intermediate (I) or Sensitive (S), according to the zone table, constituted by Clinical and Laboratory Standards Institute (CLSI). Depending on the results of antibiogram tests, different antibiotype groups were generated. The strains which contain the same antibiotic susceptibility, were indicated with the same antibiotype number. According to the values obtained from antibiogram tests, percentage resistance levels of *E. coli* strains were calculated. The occurrence of *E. coli* infections in different age, sex, sample and service groups and the percentages of resistance to various antibiotics by sex of patients were determined.

Isolation and analysis of plasmids: The isolation of plasmid DNA of *E. coli* strains was performed by EZ-10 Spin Column Plasmid DNA MiniPreps Kit (Bio Basic Inc.). After the isolation stage, plasmids were transfered in to 1% agarose gel at 50V for 5 h. Finally, the gel, including plasmids in itself, were screened by Gel Logic 200 Molecular Imaging System (Kodak, Rochester).

RESULTS

In this study, clinical isolates of *E. coli* collected from different hospitals in Ankara, were identified phenotypically and examined according to their drug resistance pattern. The percentages of occurrence of *E. coli* infections in different age, sex, sample and service groups are shown in Table I.

According to the results, more *E. coli* infections were observed in woman (77.1%) than in man (22.9%). When the quantity of urine samples that *E. coli* strains were observed in (77.2%) and the amount of patients, existing in urology

Table I: Occurence of *E.coli* in different groups

Gender:	% of Strains Tested
Woman:	77.08
Man:	22.92
Age Interval:	
0-15	18.75
16-30	6.25
31-45	8.33
46-60	20.83
61-75	25.00
76-90	14.58
Samples:	
Urine:	79.17
Feces:	20.83
Wound:	2.08
Services:	
Gynecology :	10.42
Paediatrics:	10.42
Emergency Service:	14.58
Internal Diseases:	6.25
Urology:	18.75
Gastrology:	2.08
Endocrinology:	6.25
Infection Control:	2.08
Cardiology:	4.17
Thoracic Medicine:	2.08
Nephrology:	6.25
Physical Therapy:	2.08
Oncology:	4.17
Neurosurgery:	2.08
Family Practice:	2.08

Table II: The Percentage of multi–drug resistance among *E. coli*

Resistance to different antimicrobial agents	Strains resistant (%)
Fully Sensitive	0
Resistance to 1 antibiotic	27.08
Resistance to 2 antibiotics	20.83
Resistance to 3 antibiotics	0
Resistance to 4 antibiotics	16.67
Resistance to 5 antibiotics	0
Resistance to 6 antibiotics	25.00
Resistance to 7 antibiotics	4.17
Resistance to 8 antibiotics	6.25

Table III: Resistance of *E. coli* strains by sex of patients to 8 different antibiotics

	Resistant strains (%)	
Antibiotic	**Male**	**Female**
Amoxicillin/Clavulanic acid	36.36	40.54
Ceftriaxon	36.36	35.14
Gentamicin	54.55	35.14
Ciprofloxacin	36.36	62.16
Piperacillin/Tazobactam	9.09	5.41
Cefazolin	54.55	51.35
Cefuroxim	36.36	35.14
Ampicillin	100	91.89

unit, because of *E. coli* infections (18.8%), were considered, it was obvious that the frequency of urinary infections caused by *E. coli* strains was greater than the frequency of other infections caused by them and it was also seen that the age interval at which *E. coli* infections occured more frequently, was 61-75 (25.0%).

Table IV: Frequencies of 10 different antibiotype patterns

AMC	CRO	CN	CIP	TPZ	CZ	CXM	AMP	Antibiotype pattern	Antibiotypes (%)
S	S	S	S	S	S	S	R	**ANT1**	22.92
S	S	R	S	S	S	S	S	**ANT2**	4.17
R	R	S	R	S	R	R	R	**ANT3**	14.58
S	S	S	R	S	S	S	R	**ANT4**	20.8
R	S	R	S	S	R	S	R	**ANT5**	12.5
I	R	R	R	S	R	R	R	**ANT6**	6.25
R	R	R	R	S	R	R	R	**ANT7**	4.17
R	S	S	S	S	R	R	R	**ANT8**	4.17
S	R	R	R	S	R	R	R	**ANT9**	4.19
R	R	R	R	R	R	R	R	**ANT10**	6.25

(AMC: Amoxicilin/Clavulonic acid, CRO: Ceftriaxon, CN: Gentamicin, CIP: Ciprofloxacin, TPZ: Piperacilin/Tazobactam, CZ:Cefazolin, CXM: Cefuroxim, AMP: Ampicillin
S: Sensitive, I: Intermediate, R: Resistant)

The antibiogram tests of *E. coli* strains were done according to the National Committee for Clinical Laboratory Standards (NCCLS) Disc-Diffusion method and the antibiotic drug resistance patterns of them were examined. According to the results, none of the strains were sensitive to all eight antibiotics. It was found that; the strains which were isolated from different patients display resistance to at least one antibiotic and the number of strains displaying resistance to only a single antibiotic was greater than the number of other strains showing resistance to more than one antibiotic (27.1%). In addition, the percentage of strains resistant to 6 antibiotics was found as the second highest result (25%). The percentage of strains displaying resistance to eight different antibiotics are listed in Table II.

On the other hand, it was seen that all the *E. coli* strains obtained from male patients, were resistant to ampicillin (100%). But when the female patients infected by *E. coli* are considered, it was seen that not all of the strains of *E. coli* were sensitive to ampicillin and the percentage of strains resistant to ampicillin in females was established as 91.9%. By considering both of the results, it is obvious that *E. coli* strains showed highest resistance to ampicillin. The percentages of resistances to various antibiotics in different sex groups are shown in Table III.

The second highest resistance was developed against ciprofloxacin and the rate of resistance developed against ciprofloxacin reveals that; ciproflaxacin-resistant strains, isolated from females (62.16%) are more than the ciproflaxacin-resistant strains, isolated from males (36.36%). Additionally except for gentamicin and ciprofloxacin, similar resistance rates were displayed agains other antibiotics for both females and males.

According to the antibiotic resistances displayed against various antibiotics by *E. coli* strains, 10 different antibiotypes were generated. Antibiotypes and their frequencies are listed in Table IV. Within 10 antibiotypes generated according to different resistance levels of *E. coli* strains, antibiotype1 was observed in highest frequency (22.9%) and the antibiotype, which shows the second highest frequency was found as antibiotype4 (20.8%).

Finally when the plasmid DNA profiles of *E. coli* strains were analysed, existence of 9 different plasmid DNA profiles was found and 1-9 plasmid bands were determined. In addition, the profile which includes only one band was observed as the most frequent one. It was also established that the *E. coli* strains, which were classified in that plasmid profile, acquire the properties of antibiotype 1, which includes ampicillin-resistant property in itself. The antibiotic test results, antibiotic resistances to various antibiotics that *E. coli* strains developed and the plasmid profile types of them are all evaluated together.

DISCUSSION

Bacterial resistance is a growing-problem worldwide (Chehregani *et al.*, 2007). As a result of developing resistance to new antibiotics, microorganisms can easily adapt themselves to various conditions. Some of the recent developments suggest that the information carried by extrachrosomal DNA fragments known as plasmids, play an important role upon this issue (Saunders, 1984; Urban & Rahal, 1997). Whereas; possession of various virulence factors is also suggested to be related with antimicrobial resistance (Rushdy *et al.*, 2007). *E. coli* is one of the main casual agents of some extremely important infections and as a result of this, this study is carried out to reveal the relationship between antibiotic sensitivities and plasmid profiles of *E. coli* strains isolated from different patients in various age and sex groups.

When the infections caused by *E. coli* strains are examined according to different parameters, it is seen that our findings are in agreement with those of other recent studies suggesting; the infections caused by *E. coli* are observed mostly in females than in males (Manges *et al.*, 2001) and the incidence of *E. coli* infections is greater for elder people (Raum *et al.*, 2007). It is also stated that, urinary tract is the most frequent system, as the origin of infection (Peralta *et al.*, 2007). The number of patients in urology unit, infected by *E. coli* strains revealed that the frequency of urinary tract infections strains is greater than the frequency of other *E. coli* infections.

The results related with elder woman show that; females whose age intervals are between 61 and 75, are predisposed to urinary tract infections caused by *E. coli*

strains. It is expected to be due to the postmenopausal situation that every woman face with and decreasing of estrogen hormone secreted by woman. But according to some of the recent studies carried out with estrogen hormone; the higher frequency of urinary tract infections for elder females does not depend on the lack of estrogen hormone. Instead of it, it is stated that estrogen treatment increases the susceptibility to the infections caused by *E. coli* (Curran *et al.*, 2007).

In the study of Sahm *et al.* (2001), rates of multidrug resistance were demonstrated to be higher among males (10.4%) than females (6.6%) and simmilar to our results rates of multidrug resistance were found to be higher among patients > 65 years of age (8.7%) than patients < 17 (6.8%) and 18 to 65 (6.1%) years of age. In addition; 5 different antibiotics (ampicillin, cephalothin, ciprofloxacin, nitrofurantoin & trimethoprim-sulfamethoxazole) had been used to asses the current breadth of multidrug resistance among urinary isolates of *E. coli* (Sahm *et al.*, 2001).

On the other hand, Arikan and Aygan (2009) suggest that, bacterial resistance to antimicrobial agents is increasing worldwide due to several reasons. In parallel with this, the results of our study confirm the statement related with multidrug resistance. Because similar to their suggestion, the percentage of strains resistant to 6 antibiotics was found as the second highest result (25%). According to Sahm *et al.* (2001), concurrent resistance to antimicrobials of different structural classes have arisen in a multitude of bacterial species and may complicate the therapeutic management of infections. When the results of the study carried out by them are examined; 7.1% *E. coli* strains had been established to be resistant to three or more agents and considered as multidrug resistant (Sahm *et al.*, 2001).

According to the antibiotic resistance tests, *E. coli* strains displayed highest resistance against ampicillin. In parallel with our study, Celebi *et al.* (2007) demonstrate that the highest rates of resistance to antimicrobial agents by *E. coli* strains were against ampicillin and amoxicillin-clavulanic acid. In our study, different from the study carried out by Celebi *et al.* (2007) the second highest resistance was displayed against ciprofloxacin. But according to Kõljalg *et al.* (2009) the highest resistance is developed against Sulfamethoxazole and the rate of resistance to ampicillin takes the second place. When the studies carried out by Reinthaler *et al.* (2003) and Peralta *et al.* (2007) are considered; the highest resistance rate was found again for ampicillin. These recent studies, suggesting that the highest rate of resistance is displayed against ampicillin are in agreement with our findings.

When the rates of resistance to other antibiotic groups by *E. coli* strains were examined in various studies, some differences were observed. The different results may depend on the geographical condition of patients from where the microorganisms were isolated. Vorland *et al.* (1985) demonstrate that the incidence of urinary tract infections caused by *E. coli*, depends on climatic conditions but the rate of resistance to antibiotics does not follow this pattern and is rather constant whatever the climate is.

In one of the recent studies, antibiotic resistance profiles were generated according to three key antibiotics (ampicillin, ciprofloxacin & cotrimoxazole) and 8 different antibiotic resistance profiles had been established (Gobernado *et al.*, 2007). In this study; within 10 antibiotypes generated according to different resistance levels of *E. coli* strains to 8 different antibiotics, antibiotype 1 was observed in highest frequency and the antibiotype, which showed the second highest frequency was antibiotype 4.

Inside of 9 different plasmid DNA profiles, the profile which includes only one band was observed as the most frequent one. When the plasmid profiles of *E. coli* strains were compared with their antibiotic resistance profiles, a significant relationship was obtained only for ampicillin resistance. Results show that, some of the *E. coli* strains, whose plasmids include single band (the most frequent plasmid profile), acquire the properties of antibiotype 1. When the antibiotic susceptibility of *E. coli* strains, which were classified in antibiotype 1, are taken into consideration, it is seen that the only resistance displayed by them is against ampicillin. As a result, it is concluded that the only resistance displayed against ampicillin by some of the *E. coli* strains may be because of plasmid existence. In addition to this, 3 of the *E. coli* strains, which were isolated from urine of the patients existing in urology unit, showed same resistance profiles for the antibiotics of cefazolin, cefuroxim and ampicillin. As a result, the plasmid profiles of these strains were also examined and according to our findings, simmilar plasmid profiles were observed for three of them. According to Myaing *et al.* (2005) 3 of 131 isolates containing 8 plasmids, had been resistant to all 12 antibiotics.

Besides, in this study, simmilar with the study carried out by Celebi *et al.* (2007), it was also observed that; as some of the *E. coli* strains, which do not contain any plasmid in their cells showed resistance to various antibiotics, significant close relation between plasmid occurrence and multiple antibiotic resistance were not accurate for all of the *E. coli* strains isolated.

In conclusion, the frequency of *E. coli* infections is greater in females between 61-75 and urinary infections are the most common infections caused by *E. coli* strains. On the other hand, the highest resistance rates were found to be displayed against ampicillin and ciprofloxacin. Additionally, within 10 antibiotypes, generated according to different antibiotic resistance profiles, ampicillin-resistant strains, which were classified in antibiotype 1, were established in highest frequency and it was also observed that, some of the *E. coli* strains whose plasmids include single band (the most frequent plasmid profile), acquire the properties of antibiotype 1. Except ampicillin resistance, there was no significant relation between antibiotypes and plasmid profiles of *E. coli* strains isolated from different clinical materials.

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