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Identification of clinic uropathogen *Escherichia coli* isolates by antibiotic susceptibility, plasmid and whole cell protein profiles

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Abstract

The aim of this research was to evaluate the protein, plasmid and antibiotic resistance patterns among 118 uropathogen *E. coli* strains from infected urinary systems. Plasmids were detected 113 strains (97%). Some isolates harboured up to 10 plasmids, ranging from 1 to 19 kb in size. The total whole cell protein profiles of the strains were obtained using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method. The protein bands were stained with Coomassie-blue and analyzed by statistics package POPGEN. The 118 *E. coli* were also analyzed for their resistance to antimicrobial agents. The highest rates of resistance were against ampicillin (61 %) and amoxicillin-clavulanic acid (46.6 %). The most common antimicrobial resistance of these isolates was ampicillin, amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole, gentamicin, ciprofloxacin, amikacin, cefoxitin, and ceftriaxone. Multiple resistance to all antibiotics except imipenem was observed in 5 isolates. Similarity matrix and dendrograms were generated by using UPGMA algorithm which made it possible to evaluate the similarity or intra-specific polymorphism degrees based on whole-cell protein fingerprinting, plasmid profiles and antibiotic resistance pattern. It was determined that the SDS-PAGE method may provide better criteria than plasmid and antimicrobial susceptibility for the taxonomic and epidemiological studies of *E. coli* isolates.

Key words: *Escherichia coli*, urinary system, antibiotic resistance, plasmid profile, SDS-PAGE.

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Introduction

E. coli is the most frequent urinary pathogen isolated from 50-90 % of all uncomplicated urinary tract infections (Steadman and Topley, 1998). Urinary tract infections are very common infections in humans, with *E. coli* being the dominant pathogen. *E. coli*, the most common member of the family Enterobacteriaceae accounts for 75-90 % of all urinary tract infections in both patients and out patients (Nicolle, 2001). Identification of diarrhaegenic *E. coli* strains requires that these organisms be differentiated from nonpathogenic members of the normal flora. The identification of nonpathogenic members also needs to detect factors that determine virulence of this organism (Toma *et al*., 2003).

Antimicrobial resistance has become an important problem worldwide (Jones and Pfaller 1983). Bacterial resistance to antimicrobial agents has been emerging and rapidly disseminating among many nosocomial and community-acquired pathogens (Tenover, 2001). These organisms have wide variety of antibiotic

sensitivity patterns and treatment must be guided by laboratory investigations (Gross, 1998). The development of antibiotic resistance in *E. coli* has important clinical implications. The development of resistance to older agents such as ampicillin and trimethoprim-sulfamethoxazole, as well as the emerging problem of fluoroquinolone resistance, may substantially limit our antibiotic choices (Karlowsky *et al*., 2002).

Since first reports of transferable resistance to antimicrobials in Japan, the importance of plasmids to both their bacterial hosts and indirectly to man has been progressively appreciated (Platt *et al*., 1984). At the present time, unfortunately, to determine phenotypic profiles, conventional antimicrobial susceptibility testing methods were used in the most centers. 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 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 multi-drug resistant (MDR) pathogens (Pfaller *et al*., 2001).

The molecular characterization of microorganisms is frequently used by physicians, microbiologists, and epidemiologists to provide evidence of genetic relatedness as an aid in the epidemiological investigation of infectious diseases (Sader *et al*., 1995). The need for determining the relatedness of organisms may arise during an outbreak investigation in which a cluster of infections caused by organisms of the same species showing similar antimicrobial resistance profiles and in order to determine clonal spread within a microenvironment, and to determine the source of infection (Sader *et al*., 1993). The application of molecular analyses such as a whole cell protein analysis and plasmid analysis to investigations of infectious disease outbreaks has resulted with the provide of many useful markers that distinguish the epidemic clone of a particular pathogen and helped the identification of specific vehicles of infection (Waschmut *et al*., 1991).

Protein profiling by SDS-PAGE is a reliable and reproducible molecular technique that has been used by many workers to type various microorganisms of epidemiological interest. This technique was utilized for differentiating between the pathogenic and the non-pathogenic strains. 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 successully 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 purpose of the study was to investigate the plasmid profiles, antibiotic susceptibility and protein patterns for characterizing and differentiating uropathogenic *E. coli* isolates from patients with urinary infection.

Material and methods

Bacterial isolates

In total 118 *E. coli* strains were isolated from urine samples. The samples were collected between January 2002 to March 2003 from inpatients as well as the outpatient department of the Mersin University Research Hospital. Samples were either midstream urine specimens or catherized urine samples. The midstream urine samples collected from all patients were transported to the laboratory within 30 minutes to one hour. A standard loop technique was used to place 0.01 ml of urine on McConkey's agar (Difco, USA) and blood agar (Difco, USA). Bacteria were cultured on these media in aerobic conditions at 37°C for 24 h (Forbes, 1998) and colony count was performed. More than 10^5 colonies per ml of urine were considered significant. The colonies were identified by standard biochemical tests and the API 20E system (BioMerieux, France).

Antimicrobial resistance testing

Antibiotic susceptibility tests of the collected strains of *E. coli* were made by antibiotic disc diffusion method using filter paper discs. Disc diffusion susceptibility testing on eight antibiotics was performed according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines (2000). The antibiotic discs and their concentrations per disc (mg) included: Trimethoprim-sulfamethoxazole (1.25/23.75); representative antibiotics of aminoglycosides such as gentamicin (10); amikacin (10); quinolones such as ciprofloxacin

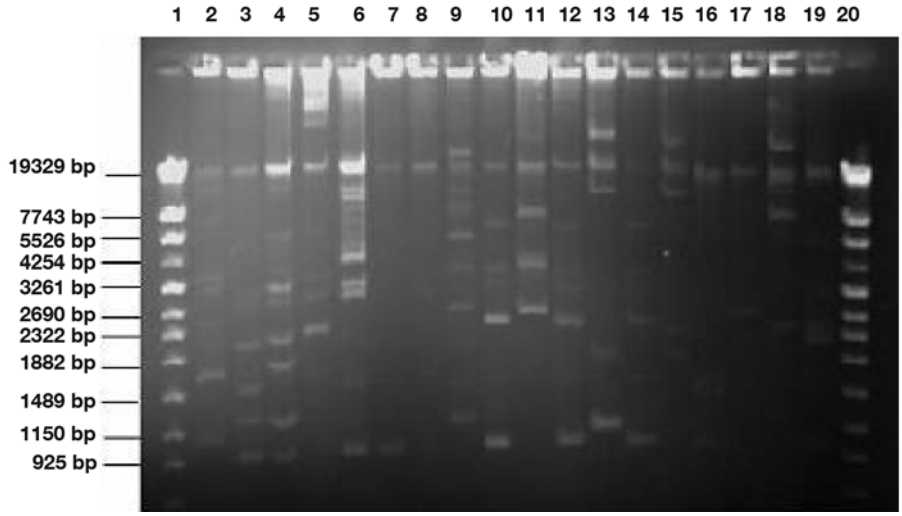

Figure 1. Plasmid patterns of some *E. coli* isolates. 1-Lambda pUC mix Marker, 2- *E. coli* 1, 3- *E. coli* 16, 4- *E. coli* 26, 5- *E.coli* 36, 6- *E.coli* 67, 7- *E.coli* 107, 8- *E. coli* 98, 9- *E. coli* 108, 10- *E. coli* 112, 11- *E.coli* 153, 12- *E. coli* 143, 13- *E.coli* 136, 14- *E. coli* 156, 15- *E. coli* 173, 16- *E. coli* 182, 17- *E. coli* 195, 18- *E. coli* 196, 19- *E. coli* 184, 20- Lambda pUC mix Marker.

(5); various cephalosporins such as ceftriaxone (30), cefoxitin (30), cefepim (30); from carbapenem antibiotics such as imipenem (10); penicillin-like antibiotics such as ampicillin (10); and amoxicillin-clavulanic acid (20/10). Zone sizes were interpreted using standard recommendations. *E. coli* ATCC 25922 was used as the reference strain for quality control purposes.

Plasmid DNA analysis

Plasmids DNA were isolated according to the method of Maniatis *et al*., (1989) by alkaline lysis with SDS: Minipreparation). DNA was electrophoresed for 4 hours at 100V on a 0.8 % agarose gel in TAE buffer and the gel photographed under UV illumination using Polaroid film Sigma 667. The approximate molecular weights were determined using plasmids of known size as standards (Lambda-pUC mix Marker 4).

Total protein analysis

The total protein samples were extracted as described by Kishore *et al*. (1996). Total protein analysis was carried out using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described in Laemmli (1970). Each run included marker proteins of known molecular weights (Fermentas). The gels were stained overnight with Coomassie Brillant Blue G-250 according to Bushuk *et al.* (1997) and Demiralp *et al*., (2000).

Cluster analysis

Different fragments on the gel were numbered sequentially and the presence or absence of fragments in each sample was scored (present 1, absent 0) and compared with each other. Cluster analysis of whole cell proteins was performed according to the genetic distance method of Nei (1972)

Results

118 uropathogenic *E. coli* strains were isolated from clinics. Their plasmid profiles and antibiotic resistance were analyzed. Plasmid profiling demonstrated that 113 of 118 isolates contain plasmid DNA. Most of the isolates have from 1 to 10 plasmid bands with sizes ranging from 1 kb to 24 kb. The most common plasmid of 19 kb was detected in almost all strains isolated (Figure 1).

Analysis of the susceptibility testing of 118 *E. coli* strains isolated from inpatients and outpatients with UTI has demonstrated that the rate of resistance to ampicillin (60.2 %) is highest among all the antimicrobials. 52 of the ampicillin resistant strains were simultaneously resistant to trimethoprim-sulfamethoxazole, 26 to gentamicin, 34 to ciprofloxacin, and 12 to ceftriaxone. In the strains, resistance to ampicillin (60.2 %), ciprofloxacin (28.8 %), and trimethoprim sulfamethoxazole (44.1 %) was encountered. A total of 5 (4.2 %) of the resistant isolates were resistant to all drugs studied except imipenem

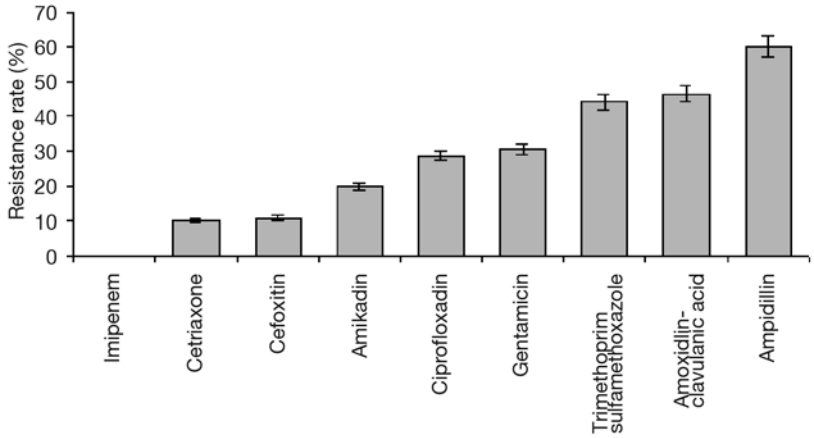

Figure 2. The resistance rates of *E. coli* isolates against to various antibiotics.

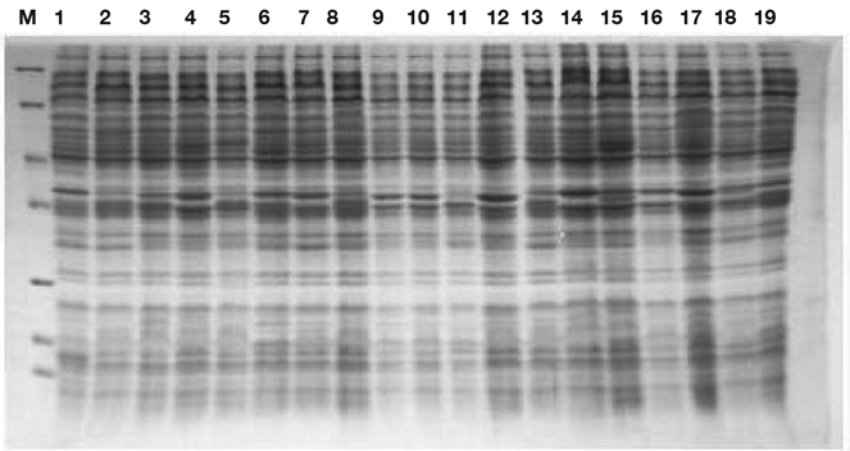

Figure 3. SDS-PAGE protein profiles of *E. coli* isolates. M-molecular weight standards (kDa) (116-β-galaktosidase, 66- Bovine serum albumin, 45- ovalbumin, 35- lactate dehydrogenase, 25- restriction endonuclease *Bsp*981, 18-β-lactoglobulin, 14-lysozym), 1- *E. coli* ATCC 35218, 2- *E. coli*-172, 3- *E. coli*-133, 4- *E. coli*-156, 5- *E. coli*-100 6- *E. coli* 155, 7- *E. coli* 19, 8- *E. coli* 136, 9- *E. coli* 93, 10- *E. coli* 182, 11- *E. coli* 147, 12- *E. coli* 183, 13- *E. coli* 82, 14- *E. coli* 5, 15- *E. coli* 7, 16- *E. coli* 8, 17- *E. coli* 18, 18- *E. coli* 23, 19- *E. coli* 26.

(Figure 2). Most of the *E. coli* isolates showed resistance to two or more antibiotics and were therefore MDR.

Whole-cell protein profiles of urinary *E. coli* isolates obtained by SDS-PAGE are shown in Figure 3. The protein profiles were inspected visually and compared with each other. The protein profiles of all *E. coli* isolates exhibited different banding patterns; molecular weights varied between 6.5-200 kDa. A particularly high degree of similarity was concentrated in the region between 25-116 kDa (may be species specific). The genetic distance of the strains based on whole protein profiles of *E. coli* isolates was calculated and dendrograms were constructed using the POPGEN statistical program (Figure 4). The dendrogram allows two main clusters to be distinguished. The clusters were again subdivided into subclusters having genetic similarity between 0 % and 94 %.

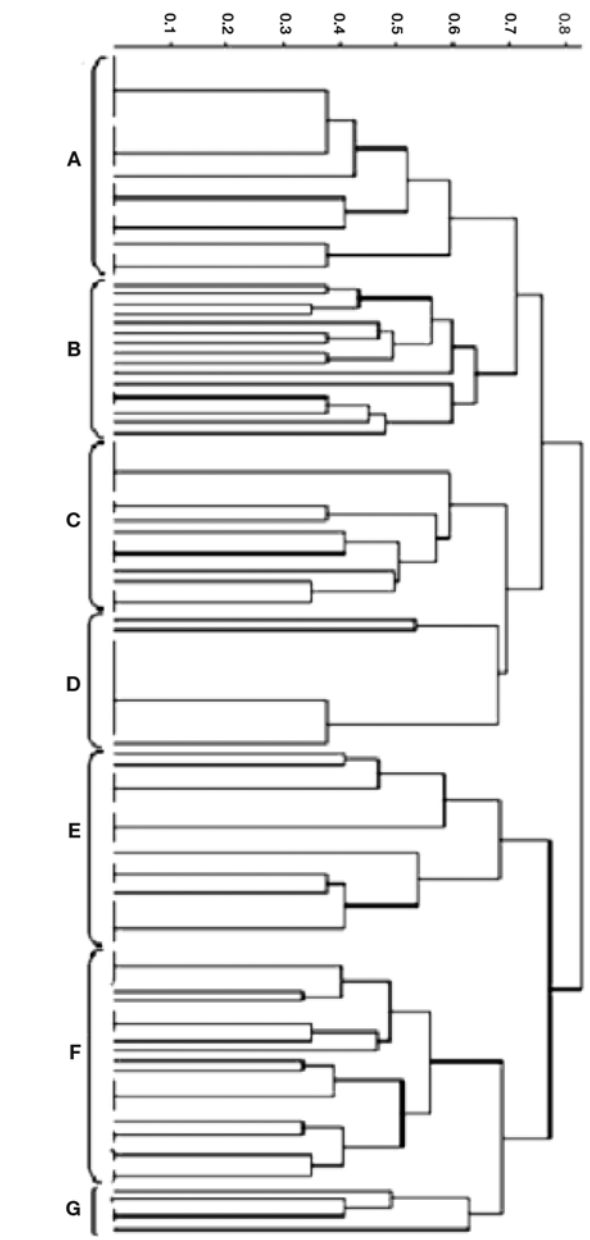

Figure 4. The dendrogram based on whole cell protein profiles of *E. coli* isolates. . **A)** *E. coli* ATCC 35218, *E. coli* 156, 19, 147, 31, 80, 85, 172, 133, 100, 155, 136, 106, 93, 182, 34, 35, 75, 79, 27, 121, 126, 127, **B)** *E. coli* 5, 8, 82, 7, 23, 26, 160, 139, 140, 177, 131, 134, 162, 138, 152, 165, **C)** *E. coli* 183, 18, 29, 32, 78, 83, 104, 112, 110, 120, 146, 148, 149, 128, 137, 142, 143, 144, **D)** *E. coli* 118, 173, 166, 168, 169, 170, 176, 184, 185, 187, 188, 198, 180, **E)** *E. coli* 179, 101, 64, 196, 3, 59, 38, 28, 67, 69, 63, 36, 91, 87, 16, 65, 66, 68, 1, 92, **F)** *E. coli* 25, 116, 123, 103, 153, 159, 97, 200, 74, 86, 88, 150, 105, 107, 108, 117, 122, 98, 115, 163, 94, 95, 76, 77, **G)** *E. coli* 114, 171, 96, 195, 141.

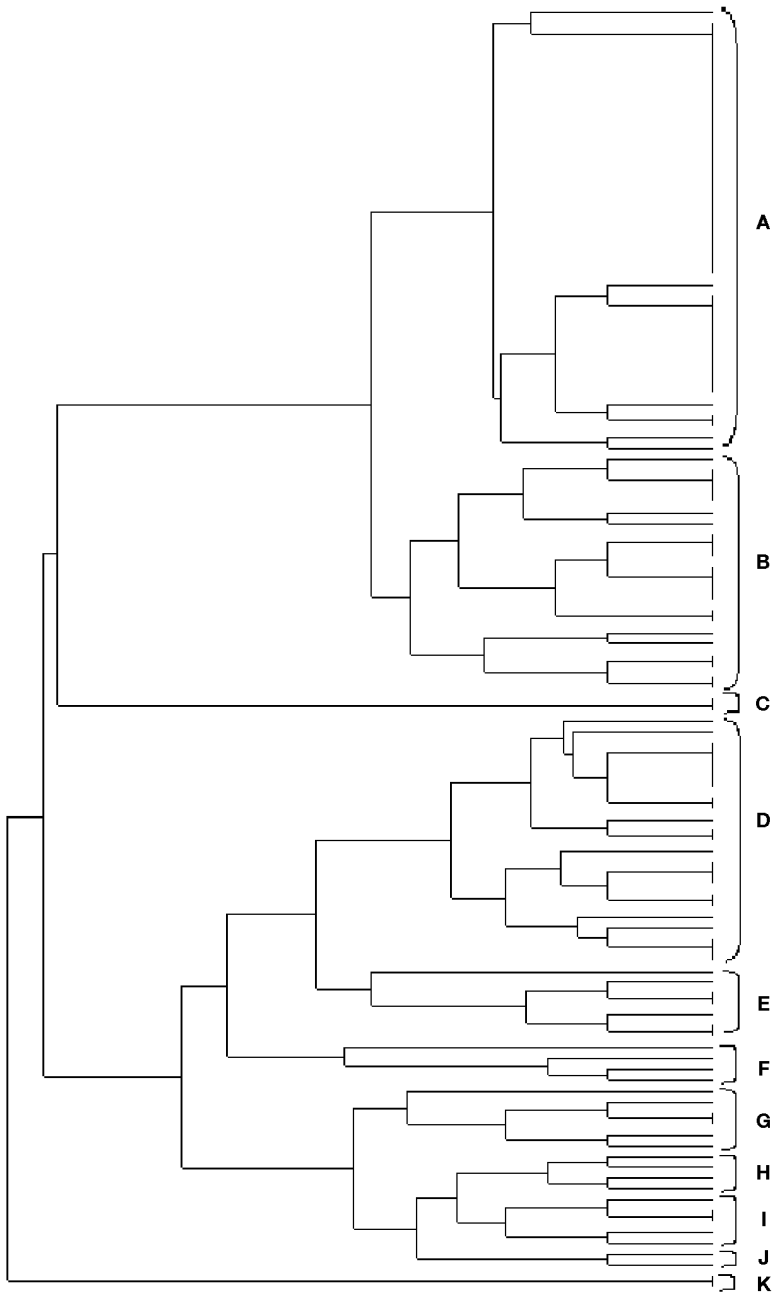

Figure 5. The dendrogram based on antibiotics resistance of *E. coli* isolates. . **A)** *E. coli* 1,102, 95, 52, 43, 41, 40, 39, 38, 37, 36, 35, 34, 33, 27, 24, 23, 22, 21, 20, 19, 18, 17, 12, 16, 8, 113, 112, 11, 73, 71, 70, 62, 58, 25, 31, 28, 29, 49, 9, 32, **B)** *E. coli* 2, 114, 105, 54, 60, 117, 118, 66, 30, 63, 74, 72, 50, 69, 51, 76, 26, 94, 67, 93, 80, 97, **C)** *E. coli* 7, 87, **D)** *E. coli* 100, 5, 65, 48, 15, 3, 11, 4, 56, 116, 103, 106, 88, 104, 10, 42, 46, 47, 96, 75, 109, 55, 59, **E)** *E. coli* 6, 13, 14, 53, 108, 44, 107, **F)** *E. coli* 77, 84, 45, 78, **G)** *E. coli* 85, 86, 57, 61, 64, 110, **H)** *E. coli* 79, 83, 98, 115, **I)** *E. coli* 82, 90, 98, 95, 92, **J)** *E. coli* 81, 89, **K)** *E. coli* 68, 101.

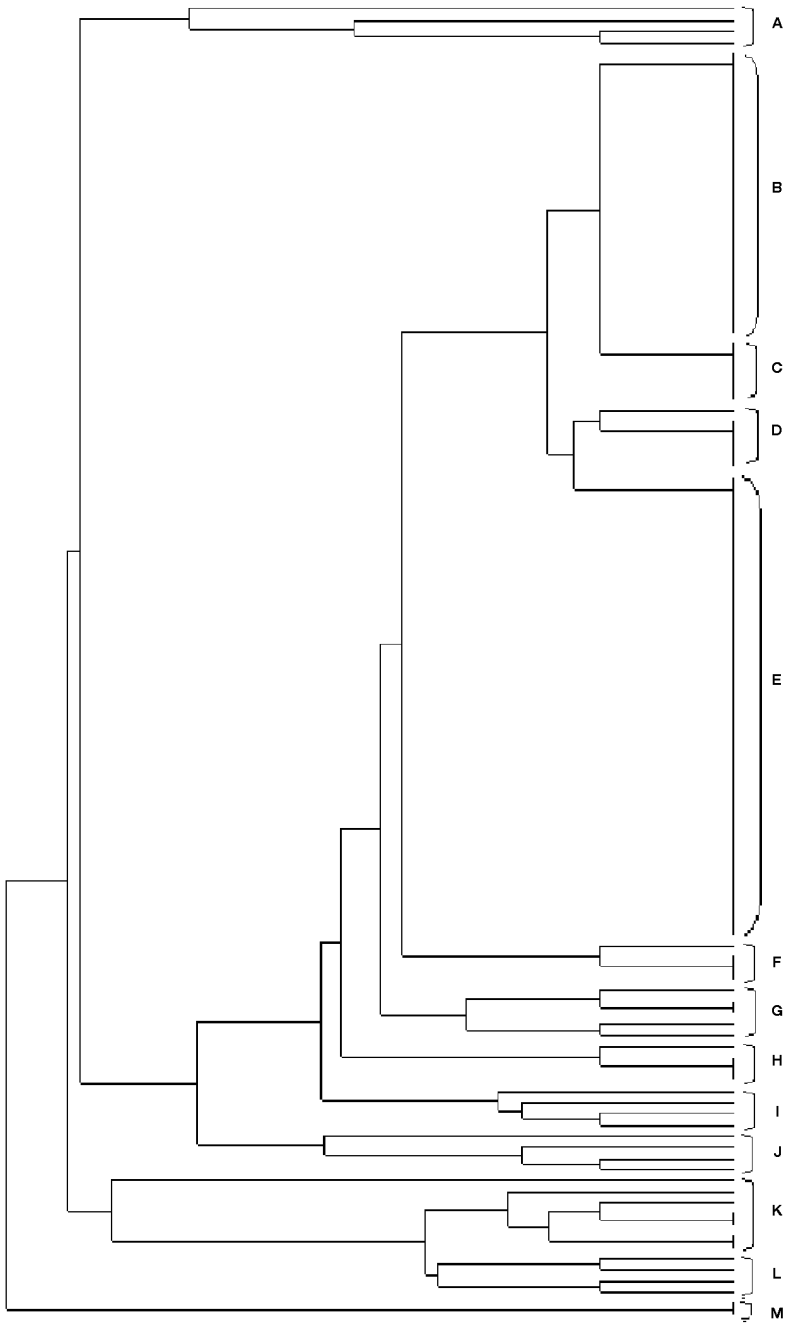

Figure 6. The dendrogram based on plasmid profiles of *E. coli* isolates. **A)** *E. coli* 1,20, 81, 95, **B)** *E. coli* 110, 90, 77, 70, 64, 61, 52, 51, 44, 43, 39, 32, 31, 30, 29, 28, 27, 16, 14, 13, 12, 10, 8, 5, 3, 2, **C)** *E. coli* 93, 68, 67, 11, 6, 7, **D)** *E. coli* 4, 102, 92, 84, 63, 73, **E)** *E. coli* 118, 117, 114, 113, 112, 111, 107, 10, 104, 101, 100, 99, 98, 97, 96, 80, 79, 76, 75, 72, 71, 69, 66, 62, 60, 59, 57, 55, 49, 47, 46, 40, 38, 37, 36, 34, 33, 25, 23, 22, 9, 21, **F)** *E. coli* 24, 87, 85, 86, **G)** *E. coli* 53, 19, 42, 103, 115, **H)** *E. coli* 94, 88, 82, 83, **I)** *E. coli* 109, 74, 18, 35, **J)** *E. coli* 17, 65, 54, 116, **K)** *E. coli* 89, 91, 15, 45, 50, 41, 58, **L)** *E. coli* 26, 56, 48, 78, **M)** *E. coli* 106, 108.

As far as antibiotic concern all isolates have divided into 11 clusters, while 13 clusters have seen with plasmid profiles (Figure 5-6).

Discussion

In this study, we tried to distinguish *E. coli* strain using plasmid profiles, SDS-PAGE and antibiotics susceptibility. In order to improve control and prevention strategies against infectious diseases, microbial pathogens need to be identified quickly and accurately. Microorganisms can be identified both phenotypically and genotypically. Conventional methods such as morphological, biochemical, and physiological tests that are used for identification and characterization of bacterial strains are mainly based on phenotypic traits (Fantasia, 1990). Today's clinical microbiology techniques for isolation and phenotypic characterization of etiological agents rely on culturing samples under the artificial conditions of a laboratory (Eisenstadt and Washington, 1996; Kunin, 1997). Traditional methods used to differentiate closely related organisms are typically not sensitive enough and are influenced by physiological factors. In urinary tract infections, delays between specimen collection and laboratory diagnosis lead to the prescription of an antibiotic therapy relying solely on the physician's experience, which is usually inappropriate (Stamm, 2002). In clinical settings, molecular techniques provide more sensitive, faster, and easier tools than conventional microbiological methods of diagnosis (Relman *et al*., 1992; Relman, 1999). Alternative approaches such as plasmid analysis and SDS-PAGE electrophoresis are used for fast and reliable identification of bacterial strains (McClure, 2000; Shi *et al.,* 1996). SDS-PAGE is used in studies to discriminate the bacterial strains.

Plasmid analysis is also used method for differentiation among some bacterial strains (Tanner *et al.,* 1996; Wallia *et al.,* 1988). This study showed that antibiotic susceptibility, plasmid DNA and SDS-PAGE analysis of whole cell proteins have a discriminatory power to distinguish the *E. coli* strain. However, when we look at the dendrogram created separately using plasmid profiles, antibiotic susceptibility and whole cell protein profiles, the most variation have seen in whole cell protein profiles.

Antimicrobial resistance plasmids have been increasingly associated with both Gram-positive and Gram-negative bacterial infections (Watanabe and Fukasawa, 1960). This trend is accelerated by the fact that *E. coli* is a common enteric commensal of mammals and a common cause of human infections. As such, *E. coli* strains are routinely exposed to a wide range of antimicrobial agents. *E. coli* also has a very wide natural distribution (Selander and Levin, 1980) and a propensity for plasmid carriage (Sherley *et al,* 2003). Resistance to various antibiotics is relatively common in clinical pathogens in Turkey and also common in *E. coli* strains (Elçi *et al*., 1998; Tekerekoğlu *et al.,* 1998; Özden *et al.,* 2003) and it is frequently plasmid-mediated (Neu, 1992). In this study, plasmids were screened to determine their antibiotic resistance profiles. It was observed that there is not a close relation between plasmid occurrence and multiple antibiotics resistance for all of the isolates because some of the isolates, without plasmid has antibiotic resistance.

SDS-PAGE analyses was the most efficient method for characterizing *E. coli* species used in this study, because these species showed differences in their electrophoretic protein patterns. To differentiate *E. coli* strains present in urinary tract infections, more than one method should be used, since care in handling of these strains is very important factor in accuracy of research involving clinical, epidemiological and taxonomic studies. After initial screening for *E. coli* strains, this information may be associated with SDS-PAGE of whole cell proteins. This method could be used as a routine procedure for distinguish *E. coli* strains from urinary tract infections.

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