Prevalence and antibiotic resistance patterns of diarrheagenic *Escherichia coli* isolated from adolescents and adults in Hamedan, Western Iran

Mohammad Yousef Alikhani¹, Seyyed Hamid Hashemi²*, Mohammad Mehdi Aslani³, Safar Farajnia⁴

¹Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamedan, Iran. ²Department of Infectious diseases, Faculty of Medicine, Hamadan University of Medical Sciences, Hamedan, Iran. ³Department of Microbiology, Pasteur Institute of Iran, Tehran,Iran. ⁴Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Received: May 2012, Accepted: October 2012.

ABSTRACT

**Background and Objectives:** Pathogenic strains of *Escherichia coli* are a common cause of acute infectious diarrhea. The aim of this study was to investigate the frequency, virulence markers and antibiotic resistance patterns of diarrheagenic *E. coli* (DEC) isolated from adolescents and adults in Hamadan, west of Iran.

**Materials and Methods:** A total of 187 stool samples were collected from adults with acute diarrhea. Stool culture was performed by conventional methods for enteropathogenic bacteria. Virulence factor genes for DEC were detected by polymerase chain reaction. Antimicrobial susceptibility was tested using the disk diffusion method.

**Results:** Among the 187 patients, 40 (21.4%) were positive for DEC. The most frequently identified DEC was enteropathogenic *E. coli* (47.5%), followed by enteroaggregative (20%), enterotoxigenic (17.5%) and shiga-toxin producing *E. coli* (15%). No isolates of enteroinvasive *E. coli* were detected. All STEC strains were stx⁺ / eae⁻. Out of the seven ETEC strains, five (71.4%) produced ST, one (14.3%) produced only LT and one (14.3%) of the isolates produced both ST and LT encoded by est and elt genes, respectively. Among the 40 DEC strains 27(67.5%) were multidrug resistant.

**Conclusion:** DEC contribute to the burden of diarrhea in adults in Hamadan. Enteropathogenic *E. coli* was the most commonly identified DEC strain in the region studied.

**Keywords:** Diarrhea, *Escherichia coli*, Antimicrobial resistance, Hamadan

INTRODUCTION

Infectious diarrheal diseases are a major cause of morbidity and mortality in developing countries. In the past decades several new enteric pathogens, including bacterial, viral, and parasitic agents have been described. However, since most of the burdens of diarrheal diseases are incurred among infants and children in developing countries, the majority of diarrhea etiology studies are performed on children in such regions (1-7).

Pathogenic strains of *Escherichia coli* are a common cause of acute infectious diarrhea. *E. coli* can cause diarrhea by different mechanisms. Each type of *E. coli* diarrhea is associated with a different pathotype of *E. coli*, and each pathotype has characteristic virulence determinants that contribute to its pathogenic mechanisms (8). Five pathotypes of diarrheagenic *E. coli* (DEC) which have been identified in different countries include: enterotoxigenic *E. coli* (ETEC), Shiga toxin–producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAEC). Many types of STEC and EPEC have the *eae* chromosomal gene, encoding the outer membrane...
protein intimin, and both strains elicit attaching and effacing lesions on the intestinal mucosa (9). EPEC and STEC are distinguished by the presence of the Shiga toxin–encoding gene, being present only in STEC. ETEC is a common cause of traveler’s diarrhea (10) which is caused by the production of heat-labile (LT) and/or heat-stable (ST) enterotoxins. EIEC is similar to Shigella species by means of genetic traits of virulence. EAEC have been associated with acute and persistent diarrhea in children and adults in developed and developing areas (11, 12).

Numerous studies have reported the role of diarrheagenic E. coli in childhood diarrhea. However, only a few reports have involved the etiologic role of E. coli pathotypes in teenagers and adults’ diarrhea (13). Of important notice is the development of multidrug resistance in all pathotypes of E. coli isolated from children with diarrhea (14, 15). In the last decades, resistance against the commonly used drugs for the treatment of enteric infections including ampicillin, tetracycline, and co-trimoxazole has increased among DEC (16). This resistance in the developing countries has been attributed to the extensive use of antibiotics and poor prescription practices. This problem may also affect the isolates from adults with diarrhea.

The aim of this study was to determine the frequency of diarrheagenic E. coli strains in the teenagers and adults’ diarrheal diseases by polymerase chain reaction (PCR) technique and antibiotic resistance patterns.

MATERIALS AND METHODS

During July 2008-July 2009, a total of 187 stool specimens were collected, from adolescents and adults with acute diarrhea who attended the outpatient clinic of Sina Hospital in Hamadan. Among the 187 samples, 112(59.9%) were from males and 75(40.1%) from female patients. The age range of patients was from 12 to 65 years (with a mean age of 39.3 years). Diarrhea was defined as a history of more than one liquid stool per day or three or more stools of loose consistency during the previous 24 hours.

Microbiologic methods. Fresh stool specimens obtained from patients were inoculated into the Cary-Blair transport medium (BD BBL™, USA), which was refrigerated on the day of collection and transported within two days to the microbiology laboratory of Hamadan University of Medical Sciences. All the specimens were cultured for pathogenic bacteria using standard methods (17). Three individual lactose-fermenting and 2 non-lactose-fermenting colonies from MacConkey medium were pooled and subjected to direct PCR for E. coli virulence genes (elt, est, eae, ial, and stx and pCVD432 plasmid).

Polymerase chain reaction assays. The PCR for diarrheagenic E. coli was performed in patients whose stools grew E. coli. To detect STEC (stx and/or eae genes), ETEC (elt and est genes), EIEC (ial gene), EPEC (eae gene), and EAEC (EAEC-associated plasmid pCVD432) PCR assays were performed using the primer pairs listed in Table 1 (18-22). DNA was isolated from whole organisms by boiling method (23). Bacteria were harvested from an overnight broth culture, suspended in 1 mL of sterile distilled water, and incubated at 100°C for 10 minutes. Following centrifugation of the lysate, 1 μL (approximately 50 ng DNA) of the supernatant was used in the PCRs. Diarrheagenic E. coli reference strains EIEC/ Shigella ATCC 43893 (ial), EPEC 2348/69 (eaeA’), EAEC E-17-2 (pCVD432’), ETEC ATCC 35401 (elt’ and/or, est’), STEC EDL933 (stx’ and/or eaeA’) were used as positive controls and E. coli K12 (ATCC 10798) as negative control.

Antibiotic susceptibility testing. Antimicrobial susceptibility testing was performed by the disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines (24). Antimicrobial agents tested were ampicillin (10 µg), tetracycline (30 µg), co-trimoxazole (25 µg), chloramphenicol (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefexime (5 µg), gentamycin (10 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), and norfloxacin (10 µg) (HiMedia Laboratories Co., India). The E. coli strain ATCC 25922 was included as a quality control in all assays.

Statistical analysis. Data were analyzed using SPSS version 11 software. Percentages were compared using a Pearson chi-square test for dependent samples or Fisher’s exact test when appropriate.

RESULTS

Out of the 187 patients, 81(43.3%) had positive stool culture including 40(21.4%) E. coli, 26
Shigella, 7(3.7%) Salmonella, 6(3.2%) Klebsiella, 1(0.5%) Yersinia enterocolitica, 1(0.5%) Campylobacter jejuni. As shown in Table 2, EPEC was the most prevalent (47.5%) DEC isolated in patients, followed by EAEC, ETEC and STEC. Isolates of EIEC were not detected. All the STEC strains were stx+/eaeA-. Out of the 7 ETEC strains, 5(71.4%) produced ST, 1(14.3%) produced LT, and 1(14.3%) of the isolates produced both ST and LT, encoded by est and elt genes, respectively. Out of the 40 DEC strains, 19(47.5%) were recognized as EPEC, 8(20%) as EAEC, 7(17.5%) as ETEC and 6(15%) strains as STEC.

The antimicrobial susceptibility profile of the diarrheagenic E. coli to 11 antibiotics is shown in Table 3. DEC isolates were resistant to most of the antibiotics tested. Antimicrobial resistance patterns for patients who had DEC included 87.5% resistant to ampicillin and 75% to co-trimoxazole. Out of the 40 DEC strains 27(67.5%) were multidrug resistant (resistance to > 3 antimicrobial drugs), and the major resistance profile was ampicillin/co-trimoxazole/tetracycline. Moreover, 29 (72.5%) isolates were resistant to tetracycline, 20(50%) to cefexim and cefotaxime, and 1(2.5%) to ciprofloxacin and norfloxacin. Comparison of resistance patterns between patients belonging to different DEC groups showed that STEC was less resistant to ampicillin and co-trimoxazole than ETEC, EPEC and EAEC. ETEC was significantly more resistant (p < 0.05) to ciprofloxacin and norfloxacin than other DEC groups.

**DISCUSSION**

The frequency, virulence markers and antibiotic resistance patterns of several pathotypes of DEC, not routinely studied in adults, has been described in this study. The molecular targets used in this study have been substantiated as important virulence factors for each of the organisms and/ or have been correlated with diarrhea in epidemiologic studies.

In this study, enteropathogenic bacteria were...
isolated from 43.3% of adolescents and adults with acute diarrhea. Previous studies indicate that bacteria are the etiologic agents of 20% to 60% of the total diarrhea cases. Although viruses are the most common causes of acute diarrhea in children in industrial countries, bacteria are the most prevalent enteropathogens in children and adults in developing countries (13).

In this study, DEC was the most common isolated bacteria. DEC has been reported as the most frequently identified pathogen in various studies throughout the world. Some studies, however, have reported different bacteria as the leading entropathogen in adults, such as _Salmonella_ spp. in Tunisia and New Caledonia (25, 26) and _Campylobacter jejuni_ in Sweden (13). Moreover, in a study in Austria, no cases of DEC were diagnosed (27). Some of these regional differences may be related to study population or stool culture techniques. Indeed, in agreement with this study, DEC was frequently isolated in developing countries such as India (28), Nigeria (29), Thailand (30), and Korea (31).

The most common pathotype of DEC in the current study was EPEC, followed by EAEC. These two pathotypes are among the most common enteric pathogens isolated from children less than five years in developing countries (14, 15, 32). In adults, ETEC and EAEC are the most common causes of travelers’ diarrhea, respectively (33). In a large study of bacterial enteropathogens among hospitalized diarrhea patients in all age groups from India, EPEC was the most frequent DEC pathotype (28). On the other hand, a study of DEC infection in two regions in the United States implicated EAEC as the most common bacterial cause of diarrhea in all age groups (34). A large study in the United Kingdom also identified EAEC as the second most common bacterial enteropathogen after _Campylobacter_ species (35). These studies indicate the important role of EAEC and EPEC in diarrhea both in children and adults.

In this study, no cases of infection with EIEC were diagnosed. Most other studies also rarely reported this pathotype in teenagers and adults. Only one study in recent years reported EIEC as the predominant diarrheagenic bacteria in Ecuador (36).

Most DEC strains isolated in this study were resistant to ampicillin and co-trimoxazole (87.5% and 75% resistance, respectively). Similar results reported in other studies indicate the universal resistance of DEC to these agents. Higher rates of resistance have been reported both in children and adults in recent years (14, 34, 35, 37).

In the current study, 67.5% of the DEC isolates were multidrug resistant (MDR). The most resistance pattern was ampicillin/co-trimoxasole/tetracycline. The incidence of diarrhea due to MDR _E. coli_ has increased in developing countries in the last decade. Higher rates have been reported from 50 to 75% in recent years (11, 16, 38), and the highest rate up to 75% have been reported from India (38, 39). Selective antibiotic pressure associated with the inappropriate use of antibiotics may be responsible for the antimicrobial resistance. Also, dissemination of transferable plasmids encoding multidrug resistance has been observed in the DEC isolates from diarrheal stools and surface waters. Use of surface waters for drinking or irrigation raises an important hazard for dissemination of MDR DEC in the developing countries (40).

At present, because of increased frequency of MDR DEC, fluoroquinolones are considered as the first-line drugs for the treatment of diarrhea. Indeed, several studies have documented the emergence and spread of fluoroquinolone resistant enteric pathogens (28, 39) and thus, a monitoring of drug susceptibility of DEC seems to be a critical issue in Iran.

In conclusion, this study reveals that DEC strains contribute to the burden of adult diarrheal diseases and

---

**Table 3. Antimicrobial resistance profile of diarrheagenic _E. coli_ isolates.**

| DEC (n) | AMP | CTX | CRO | CFM | TET | GM | CL | SXT | NA | NOR | CIP |
|--------|-----|-----|-----|-----|-----|----|----|-----|----|-----|-----|
| EPEC (19) | 16 (84.2) | 14 (73.6) | 2 (10.5) | 2 (10.5) | 12 (63.2) | 2 (10.5) | 6 (31.6) | 14 (73.7) | 3 (15.8) | 0 (0.0) | 0 (0.0) |
| EAEC (8) | 8 (100) | 7 (87.5) | 1 (12.5) | 7 (87.5) | 7 (87.5) | 6 (75) | 4 (50) | 6 (75) | 2 (25) | 1 (12.5) | 1 (12.5) |
| ETEC (7) | 6 (85.7) | 5 (71.4) | 3 (42.8) | 2 (28.6) | 6 (85.7) | 2 (28.6) | 1 (14.3) | 5 (71.4) | 2 (28.6) | 2 (28.6) | 1 (14.3) |
| STEC (6) | 5 (83) | 4 (66) | 17 (17) | 4 (67) | 5 (83) | 1 (17) | 3 (50) | 4 (67) | 2 (28.6) | 0 (0.0) | 0 (0.0) |
| Total (40) | 35 (87.5) | 30 (75) | 7 (17.5) | 15 (37.5) | 30 (75) | 11 (27.5) | 14 (35) | 29 (72.5) | 9 (22.5) | 3 (7.5) | 2 (5)

AMP, ampicillin; CRO, ceftriaxone; CTX, cefotaxime; CFM, cefixime; TET, tetracycline; GM, gentamycin; CL, chloramphenicol; SXT, co-trimoxazole; NA, nalidixic acid; NOR, norfloxacin; CIP, ciprofloxacin.
EPEC is the most commonly identified DEC strain in Hamedan. To stop the increasing prevalence rate of MDR DEC, the indiscriminate use of antibiotics needs to be avoided and the guidelines for proper use of antibiotics for the treatment of diarrhea in this region needs to be established.

ACKNOWLEDGEMENTS

This study was supported by the Vice-Chancellor of Research and Technology, Hamedan University of Medical sciences, Hamedan, Iran.

REFERENCES

1. Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. Bull World Health Organ 2003; 81: 197-204.
2. Okeke IN, Lamikanra A, Czecezulin J, Dubovsky F, Kaper J, Nataro J. Heterogeneous virulence of enteroaggregative Escherichia coli strains isolated from children in Southwest Nigeria. J Infect Dis 2000; 181: 252-260.
3. Presterl E, Zwick RH, Reichmann S, Aichelburg A, Winkler S, Kremsner PG, et al. Frequency and virulence properties of diarrheagenic Escherichia coli in children with diarrhea in Gabon. Am J Trop Med Hyg 2003; 69: 406-410.
4. Aslani MM, Alikhani MY. Serotypes of Enteropathogenic Escherichia coli isolated from children Under 5 Years of Age. Iranian J Publ Health 2009; 38: 70-77.
5. Alikhani MY, Masoumi Asl H, Khairkhah M, Farajnia S, Aslani MM. Phenotypic and genotypic characterization of Escherichia Coli O111serotypes. Gastroenterol Hepatol From Bed to Bench 2011; 4: 147-152.
6. Aslani MM, Alikhani MY. Molecular and phenotypic characterization of atypical enteropathogenic Escherichia coli serotypes isolated from children with and without diarrhea. J Microbiol Immunol Infect 2011; 44: 27-32.
7. Alikhani MY, Mirsalehian A, Fatollahzadeh B, Pourshafie MR, Aslani MM. Prevalence of Enteropathogenic and Shiga Toxin-producing Escherichia coli among Children with and without Diarrhea in Iran. J Health Popul Nutr 2007; 25: 88-93.
8. Donnenberg MS. Enterobacteriaceae. In: Mandell GL., BennettJE, Dolin R, (eds). Principles and practice of infectious diseases. 7th ed. Philadelphia, Elsevier Churchill Livingstone, 2010, pp. 2815-2834.
9. Donnenberg MS. Enteropathogenic Escherichia coli. In: Blaser MJ, Smith PD, Ravdin JI, Greenberg HB, Guerrant RL., (eds). Infections of the gastrointestinal tract. New York, Raven Press, 1995, pp. 709-726.
10. Qadri F, Svennerholm AM, Faruque AS, Sack DA. Enterotoxigenic Escherichia coli in developing countries: epidemiology, microbiology, clinical features, treatment and prevention. Clin Microbiol Rev 2005; 18: 465-483.
11. Oberhelman RA, Laborde D, Mera R, Starszak E, Saunders P, Mirza A, et al. Colonization with enteroadherent, enterotoxigenic and enterohemorrhagic Escherichia coli among day-care center attendees in New Orleans, Louisiana. Pediatr Infect Dis J 1998; 17: 1159-1162.
12. Presterl E, Nadrchal R, Wolf D, Rotter M, Hirsch AM. Enteroaggregative and enterotoxigenic Escherichia coli among isolates from patients with diarrhea in Austria. Eur J Clin Microbiol Infect Dis 1999; 18: 209-212.
13. Svennungsso B, Lagergren A, Ekwall E, Evenga˚rd B, Hedlund KO, Ka˚rnell A, et al. Enteropathogens in adult patients with diarrhea and healthy control subjects: A 1- Year Prospective Study in a Swedish Clinic for Infectious Diseases. Clin Infect Dis 2000; 30: 770-778.
14. Vila J, Vargas M, Casals C, Urassa H, Mshinda H, Schelllemberg D, et al. Antimicrobial resistance of diarrheagenic Escherichia coli isolated from children under the age of 5 years from Ifakara, Tanzania. Antimicrob Agents Chemother 1999; 43: 3022-3024.
15. Usein CR, Tatu-Chitoiu D, Ciontea S, Condei M, Damian M. Escherichia coli pathotypes associated with diarrhea in Romanian children younger than 5 years of age. Jpn J Infect Dis 2009; 62: 289-293.
16. Aslani MM, Alikhani MY, Zavari A, Yousef R, Zamani AR. Characterization of enteroaggregative Escherichia coli (EAEC) clinical isolates and their antibiotic resistance pattern. Int J Infect Dis 2011; 15: e136-e139.
17. Forbes BE, Sahm DF, Weissfeld AS. Bailey & Scott’s Diagnostic Microbiology. 12th ed. USA: The C.V. Mosby Company, 2007; 363-378.
18. Beaudry M, Zhu C, Fairbrother JM, Harel J. Genotypic and phenotypic characterization of Escherichia coli isolates from dogs manifesting attaching and effacing lesions. J Clin Microbiol 1996; 34: 144-148.
19. Blomen I, Lofdal S, Stenstrom TA, Norberg R. Identification of enterotoxigenic Escherichia coli isolates, a comparison of PCR, DNA hybridisation, ELISAs and bioassays. J Microbiol Methods 1993; 17: 181-191.
20. Lin Z, Kurazono H, Yamasaki S, Takeda Y. Detection of various variant Verotoxin genes in Escherichia coli by polymerase chain reaction. Microbiol. Immunol 1993; 37: 543-548.
21. Schmidt H, Knop C, Franke S, Aleksie S, Heesemann J, Karch H. Development of PCR for screening of enteroaggregative Escherichia coli. J Clin Microbiol 1995; 33: 70-75.
22. Frankel G, Riley L, Giron JA, Valmossi J, Friedmann A, Strockbine N, et al. Detection of Shigella in feces using DNA amplification. J Infect Dis 1990; 161: 1252-1256.
23. Nessa K, Dilnuba A, Jobirul I, Lutful Kabir FM, Anowar Hossain M. Usefulness of a Multiplex PCR for Detection of Diarrheagenic Escherichia coli in a Diagnostic Microbiology Laboratory Setting. Bangladesh J Med
DIARRHEAGENIC E. COLI STRAINS IN ADULTS

24. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disc susceptibility tests. Approved standard. 7th ed. Wayne, PA: NCCLS; 2003.
25. Al-Gallas N, Bahri O, Bouratbeen A, Ben Haassen A, Ben Aissa R. Etiology of acute diarrhea in children and adults in Tunis, Tunisia, with emphasis on diarrheagenic Escherichia coli: prevalence, phenotyping, and molecular epidemiology. Am J Trop Med Hyg 2007; 77: 571-582.
26. Germani Y, Morillon M, Begaud E, Dubourdieu H, Costa R, Thevenon J. Two-year study of endemic enteric pathogens associated with acute diarrhea in New Caledonia. J Clin Microbiol 1994; 32: 1532-1536.
27. Huhulescu S, Kiss R, Brettelecker M, Cerny RJ, Hess C, Wewalka G, et al. Etiology of acute gastroenteritis in three sentinel general practices, Austria 2007. Infection 2009; 37: 103-108.
28. Samal SK, Khuntia HK, Nanda PK, Satapathy CS, Nayak SR, Sarangi AK, et al. Incidence of bacterial enteropathogens among hospitalized diarrhea patients from Orissa, India. Jpn J Infect Dis 2008; 61: 350-355.
29. Nweze EI. Aetiology of diarrhoea and virulence properties of diarrheagenic Escherichia coli among patients and healthy subjects in southeast Nigeria. J Health Popul Nutr 2010; 28: 245-252.
30. Kalnauwakul S, Phengmak M, Kongmuang U, Nakaguchi Y, Nishibuchi M. Examination of diarrheal stools in Hat Yai City, South Thailand, for Escherichia coli O157 and other diarrheagenic Escherichia coli using immunomagnetic separation and PCR method. Southeast Asian J Trop Med Public Health 2007; 38: 871-880.
31. Cho SH, Shin HH, Choi YH, Park MS, Lee BK. Enteric bacteria isolated from acute diarrheal patients in the Republic of Korea between the year 2004 and 2006. J Microbiol 2008; 46: 325-330.
32. Jafari A, Aslani MM, Bouzari S. Escherichia coli: a brief review of diarrheagenic pathotypes and their role in diarrheal diseases in Iran. Iran J Microbiol 2012; 4: 102-117.
33. Shah N, DuPont HL, Ramsey DJ. Global etiology of travelers’ diarrhea: systematic review from 1973 to the present. Am J Trop Med Hyg 2009; 80: 609-614.
34. Nataro JP, Mai V, Johnson J, Blackwelder WC, Heimer R, Tirrell S, et al. Diarrheagenic Escherichia coli Infection in Baltimore, Maryland, and New Haven, Connecticut. Clin Infect Dis 2006; 43: 402-407.
35. Tompkins DS, Hudson MJ, Smith HR, Eglin RP, Wheeler JG, Brett MM, et al. A study of infectious intestinal disease in England: microbiological findings in cases and controls. Commun Dis Public Health 1999; 2: 108-113.
36. Vieira N, Bates SJ, Solberg OD, Ponce K, Howsmon R, Cevallos W, et al. High prevalence of enteroinvasive Escherichia coli isolated in a remote region of northern coastal Ecuador. Am J Trop Med Hyg 2007; 76: 528-533.
37. Irajian G, Jazayeri-Moghadas A, Beheshti A. Prevalence of extended-spectrum beta lactamase positive and multidrug resistance pattern of Escherichia coli and Klebsiella pneumoniae isolates, Semnan, Iran. Iran J Microbiol 2009; 1: 49-53.
38. Raju B, Ballal M. Multidrug resistant enterovaggregative Escherichia coli diarrhoea in rural southern Indian population. Scand J Infect Dis 2009; 41: 105-108.
39. Vaishnavi C, Kaur S. The epidemiological and resistogram patterns of enteropathogenic and enterotoxigenic Escherichia coli isolated from diarrhoeal stools in a north Indian hospital. Trop Gastroenterol 2003; 24: 70-72.
40. Chigor VN, Umoh JV, Smith SJ, Igbinosa EO, Okoh AI. Multidrug resistance and plasmid patterns of Escherichia coli O157 and other E. coli isolated from diarrhoeal stools and surface waters from some selected sources in Zaria, Nigeria. Int J Environ Res Public Health 2010; 7: 3831-3841.

http://ijm.tums.ac.ir