RESEARCH ARTICLE

Identification of Quinolone and Colistin Resistance Genes in *Escherichia coli* Strains Isolated from Mucosal Samples of Patients with Colorectal Cancer and Healthy Subjects

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Abstract: *Introduction*: Antibiotic resistance and extensive use of antibiotics are amongst the major causes of failure in antibiotic treatment. The purpose of this study was to investigate antibiotic resistance patterns and to identify resistance genes of quinolones and colistin in *Escherichia coli*. There are a very few patents on *E. coli* isolated from colorectal cancer. So, this study demonstrates that some bacteria resistant to ciprofloxacin have not resistance genes. Moreover, new patterns for *E. coli* are presented for isolates of patients with colorectal cancer.

Materials and Methods: Of the three healthy people, inflammatory bowel diseases (IBD) patients and colorectal cancer patients, 40 *E. coli* strains isolated after confirmation by biochemical and molecular methods. The susceptibility of isolates to antibiotics was investigated using disk diffusion test. After deoxyribonucleic acid (DNA) extraction, polymerase chain reaction (PCR) was used to identify genes encoding resistance to ciprofloxacin (*qnr A*, *qnr B*) and colistin (*mcr-1*).

Results: The results showed that *E. coli* isolates from colorectal cancer patients had the highest resistance to piperacillin (67.5%), ceftazidime (47.5%), and cefepime (42.5%). Also, *E. coli* strains isolated from IBD patients showed resistance to antibiotic ceftazidime 13%. More than 95% of *E. coli* strains isolated from healthy people were susceptible to antibiotics. Based on the results, 18 (15%) *E. coli* strains showed resistance to ciprofloxacin. The *qnr A* gene was detected in 61.11% isolates; however, *qnr B* was detected in 9 (50%) isolates. Isolates resistant to colistin were not observed.

Conclusion: These findings indicate increased resistance of *E. coli* to ciprofloxacin in comparison with prior studies. Further research in this field will increase our knowledge and more effective exposure to the antibiotic resistance of the pathogenic microorganisms.

1. INTRODUCTION

Enterobacteriaceae family are gram-negative bacilli shape bacteria that are typically considered to be the normal flora of the intestine. These bacteria are one of the common causes of nosocomial infections and human pathogens that can cause diseases such as cystitis, pyelonephritis, septicemia, pneumonia, peritonitis, meningitis, and associated infections with hospital equipment such as catheters [1, 2].

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The most important species of this genus is *Escherichia coli* [2, 3]. The most common infections with *E. coli* include urinary tract infections, intra-abdominal infections, pneumonia, especially hospital pneumonia, neonatal meningitis, soft tissue infections, and ulcers, vascular infections, bacteremia and other infections. With the advent of resistance to fluoroquinolones and cephalosporins, the treatment of infections caused by this organism has become challenging [2, 3].

These resistances in the *Enterobacteriaceae* family, especially *E. coli*, are due to the production of ESBLs (Extended Spectrum B-lactamase) that have increased in most countries in recent years [2]. The organisms that ESBLs also produce can be resistant to other antibiotics, such as aminoglycosides, trimethoprim and sulfamethoxazole, and quinolones [3].

Quinolone resistance genes carrying on a plasmid called plasmid-mediated quinolone resistance (PMQR) are classified into five classes, which include *qnr A*, *qnr B*, *qnr C*, *qnr D*, *qnr S*. These genes encode proteins that prevent fluoroquinolones from binding to topoisomerase II [3].

Colistin belongs to polymyxins (cationic polypeptides) with a wide range of effects on Gram-negative bacteria, especially the *Enterobacteriaceae* family [3]. Recently, in clinical practice, two types of polymyxins (polymyxin B and polymyxin E or Colistin) were used. These polymyxins are similar in biological activity and differ only in their structure in an amino acid. The mechanism of resistance to polymyxin is modifications to lipid A, which is to reduce the affinity to colistin [3]. Colorectal cancer (CRC) is the third leading cause of cancer related deaths and causes mortality of 600,000 people per year in the world [4]. Various factors such as inheritance, environmental factors and inflammatory syndromes associated with infectious agents are effective in creating CRC [5]. Among these factors, infectious agents are associated with cancer by about 20% and play an important role in the development of cancer [6, 7]. In the human gastrointestinal tract, there are about 10^{14}-10^{13} microorganisms that are symbiotic, called microbiota, and are essential for the maintenance of homeostasis [8]. The disorder of this microbial balance has been observed in CRC patients [9]. Resistance to antibiotics among pathogen bacteria is a topic that has been considered today as a worldwide problem. Regarding increasing growth of resistance in hospital infections and treatment centers, the determination of antibiotic resistance patterns in common pathogens can be of great importance in experimental and specific treatments against a particular pathogen. Therefore, due to the importance of this issue, this study was designed. The aim of this study was to investigate antibiotic resistance patterns and to identify genes of quinolones (*qnr A* and *qnr B*) and colistin (*mcr-1*) in *E. coli* isolated from mucosal samples of patients with colorectal cancer (CRC) and inflammation of the intestine and healthy people.

2. MATERIALS AND METHODS

2.1. Study Design

In this experimental study, sampling was done at Digestive Disease Research Institute, Shahid Beheshti Hospital in Hamedan, Iran. Their study was performed from March to May 2018. The conditions for entering the study population in this research were based on a questionnaire completed by each patient. Items of the questionnaire included: sex, age, history of the disease, gastrointestinal symptoms, native and resident of the region. The patients and control groups included 54 men and 66 women, with a mean age of 56 years (age range 16-81 years) and mean disease duration of 5-13 years provided a signed agreement for this study, and the protocol was approved by the local ethics committee of the Hamadan University of Medical Science (IR.UMSHA.REC.1395.298).

2.2. Clinical Characteristics of Patients

Intestinal biopsies were obtained of the terminal ileum and the colon of 40 patients with CRC and 40 patients with inflammatory bowel diseases (IBD). Moreover, biopsies of the ileum and colon of 40 individuals who had no significant pathological findings following endoscopic examination for changes in stool habits, abdominal pain, upper gastrointestinal bleeding or cancer were considered as controls [10]. During a colonoscopy, two biopsy samples were taken from each person for routine pathological assessment and bacteriological study

2.3. Culture and Isolation of *E. coli*

Biopsy samples (50 to 100 mg) taken by the gastroenterologist from the intestinal tissue of the patients were immediately placed in a tube containing 100 ml of sterile phosphate salt buffer (PBS) and transferred to the microbiological laboratory in the vicinity of the ice. First, the biopsy specimens were three times rinsed with PBS, the purpose of this was to eliminate fecal specimens. After the wash, the biopsy specimens were chopped and turned into a homogeneous state. The aim was to release the *E. coli* bacteria in the intestinal mucosal tissue. The sample chopped with a sterile loop on Blood agar and MacConkey agar was incubated for 24 hours at 37°C. The *E. coli* strains isolated from the biopsy specimens using conventional microbiological (SIM, Methyl-Red/Voges Proskauer (MR-VP) Broth, Simmons citrate agar) tests strains were identified.

E. coli strains from controls were retrieved only from descending colon biopsy specimens.

2.4. Sensitivity to Antibiotics

In this study, sensitivity to 8 antibiotics was determined using disk agar diffusion method, which employed the clinical and laboratory standards institute (CLSI) criteria [11].

2.5. DNA Extraction

The amount of 300 μl of the suspension bacteria in a 1.5 ml microtube was dispensed and centrifuged for 2 minutes for precipitation, containing bacterial culture media at 1400 rpm. To wash the resulting precipitate, 300 μl sterilized distilled water was added and then centrifuged at 1400 rpm for 5 minutes after two steps. Next, 100 μl of 5% NaOH solution was added and placed at 95°C for 30 minutes and then with a round of 1400 rpm for 5 minutes centrifugation.

For final purification of DNA, 100 μl of tris solution was added to the precipitate and centrifuged at 1400 rpm for 5 minutes. The supernatant liquid, which contained pure DNA, was collected in a 0.5 μl microtube. The concentration and absorbance of the purified DNA at 260/280 nm wavelengths were determined by a Nanodrop Spectrophotometer (Thermo Fisher Scientific).

2.6. PCR Reaction to Determine the Resistance Genes

We used three *qnr A*, *qnr B* and *Mcr-1* genes to detect resistance genes with fluqinolones and colistin. PCR was used to amplify these genes Table **1**.

Table 1. The primers used in this study.

Gene		Primer Sequence (5'-3')	Amplicon Size (bp)	References
qnr A	F	ATTTCTCACGCCAGGATTTG	516	[12]
qnr A	R	GATCGGCAAAGGTTAGGTCA		
qnr B	F	TGAAGTGTCAGGAGACGCTG	469	[12]
qnr B	R	ATGGAGAATGCGTTCCTCAAC		
mcr-1	F	CGGTCAGTCCGTTTGTTC	309	[13]
mcr-1	R	CTTGGTCGGTCTGTAGGG		

3. RESULTS

A total of 120 strains of *E. coli* isolated, and 40 isolates from colorectal cancer, were diagnosed, along with 40 isolates from patients with intestinal inflammation. In addition, 40 strains of *E. coli* from healthy were also collected.

The resulting antibiograms showed that *E. coli* strains isolated from patients with colorectal cancer had the highest resistance to piperacillin (67.5%), ceftazidime (47.5%), and cefepime (42.5%). Also, *E. coli* strains isolated from patients with intestinal inflammation showed the highest resistance to ceftazidime (13%). Samples isolated from healthy individuals showed most antibiotics 95% sensitivity. Based on the results of the disk diffusion test, 18 isolates (15%) showed resistance to ciprofloxacin. However, colistin-resistant strains were not detected in *E. coli* (Fig. **1**).

3.1. Distribution of Genes Encoding Resistance to Ciprofloxacin and Colistin in *E. Coli* Strains

According to the results of disc diffusion test, 18 isolates of 15% of group 1: *E. coli* isolated from patients with colorectal cancer (10 isolates), group 2: *E. coli* isolated from patients with inflammatory disease (3 isolates), group 3: *E. coli* isolated healthy (1 isolate) showed resistance to ciprofloxacin. And in the strains of *E. coli*, colistin-resistant strains were not found. PCR test was performed to detect *qnr A* and *qnr B* genes (Fig. **2** and Table **2**).

4. DISCUSSION

In the current study, antibiotic resistance patterns (with an emphasis on fluoroquinolones) of *E. coli* isolated from patients with colorectal cancer and IBD and healthy subjects as control were assessed. Out of 120 strains, 18 strains of 15% *E. coli* were resistant to ciprofloxacin. Ten strains of 35% of *E. coli* isolated from patients with colo-

rectal cancer showed resistance to ciprofloxacin. Also, antibiotic susceptibility test results showed that among the 120 strains of *E. coli in* this study, the high susceptibility to antibodies to colistin, amikacin and imipenem was 100%, 90% and 90%, respectively. Resistance to these antibiotics in strains isolated from patients with colorectal cancer was observed as follows: resistance to pipracillin was 67.5%, to ceftazidime being 47.5%, and to cefepime being 42.5%. The results of this study, similar other studies in different areas of Iran and Europe, showed a high level of resistance to fluoroquinolones. Aibinu *et al.*, isolated 103 strains of *E. coli* from cancer patients, their results showed resistance to ciprofloxacin being 21.4% that was consistent with our study [14]. In another study, Kheirabadi *et al.*, reported resistance to ciprofloxacin being 22.7% [15]. Also, Jamshidi *et al.*, investigated *E. coli* isolated from people with urinary tract infections, which reported resistance rate to ciprofloxacin 40% [16].

Since fluoroquinolones are commonly used antibiotics in the treatment of urinary tract infections in Iran, the increasing resistance to these antibiotics has raised concerns about the choice of treatment for these infections. In various studies, the rate of resistance has been reported to be between 3% and 10%, but there are many differences between the results of studies that have been responsible for the causes of *E. coli* infection. Resistance to fluoroquinolones, especially ciprofloxacin, up to 20% has also been reported to confirm the results of resistance to this antibiotic in our study area [17]. According to some studies, resistance to fluoroquinolones has increased from 3% to 23% over 8 years [18, 19]. Ruiz *et al.*, suggested the treatment of urinary tract infections with fluoroquinolones. In addition, resistance to fluoroquinolones 10% to 20% have been reported which is similar to the results of our study [20]. Given the high resistance to ciprofloxacin in this study and other studies conducted in Iran, this
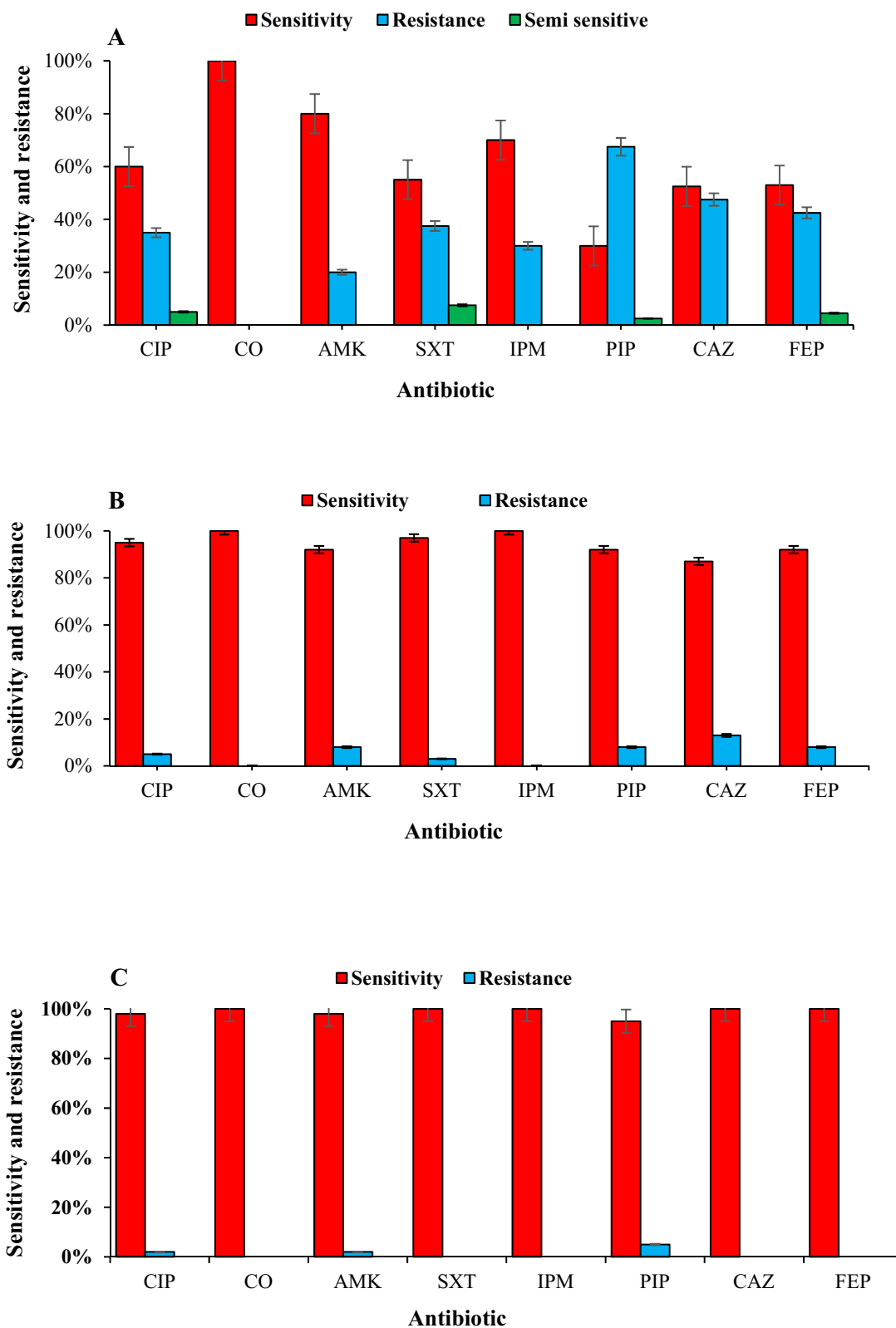
Fig. (1). Resistance and sensitive of *E. coli* strains isolated (**A**); Patients with colorectal cancer (**B**); Patients with inflammatory bowel diseases (**C**); Healthy subjects. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Abbreviations: CIP; Ciprofloxacin. CO; Colistin. AMK; Amikacin. SXT; Trimethoprim/sulfamethoxazole. IPM; Imipenem. PIP; Piperacillin. CAZ; Ceftazidime. FEP; Cefepime.

Table 2.   Distribution of resistance genes to ciprofloxacin and colistin antibiotics in *E. coli* strains.

Strains Resistant to Ciprofloxacin		Genes Studied			Antibiotic Patterns
		qnr A	*qnr B*	*mcr-1*	
E. coli isolated from patients with colorectal cancer	S1	+	-	-	CIP (R), AMK(S), CO(S), SXT(R), CAZ(S), FEP(S), IPM(S), PIP(R)
	S2	+	+	-	CIP (R), AMK(R), CO(S), SXT(S), CAZ(S), FEP(R), IPM(S), PIP(R)
	S3	-	+	-	CIP (R), AMK(S), CO(S), SXT(R), CAZ(S), FEP(S), IPM(S), PIP(R)
	S4	+	-	-	CIP (R), AMK(R), CO(S), SXT(S), CAZ(R), FEP(R), IPM(S), PIP(R)
	S5	-	+	-	CIP (R), AMK(S), CO(S), SXT(S), CAZ(S), FEP(R), IPM(S), PIP(R)
	S6	+	+	-	CIP (R), AMK(R), CO(S), SXT(R), CAZ(S), FEP(S), IPM(S), PIP(R)
	S7	-	+	-	CIP (R), AMK(R), CO(S), SXT(S), CAZ(R), FEP(R), IPM(S), PIP(R)
	S8	+	-	-	CIP (R), AMK(S), CO(S), SXT(S), CAZ(R), FEP(S), IPM(S), PIP(R)
	S9	+	+	-	CIP (R), AMK(R), CO(S), SXT(R), CAZ(S), FEP(R), IPM(S), PIP(R)
	S10	+	+	-	CIP (R), AMK(S), CO(S), SXT(S), CAZ(R), FEP(S), IPM(S), PIP(R)
E. coli isolated from patients with IBD	S11	+	+	-	CIP (R), AMK(S), CO(S), SXT(R), CAZ(S), FEP(R), IPM(S), PIP(R)
	S12	+	-	-	CIP (R), AMK(R), CO(S), SXT(S), CAZ(R), FEP(S), IPM(S), PIP(S)
	S13	+	-	-	CIP (R), AMK(S), CO(S), SXT(S), CAZ(S), FEP(S), IPM(S), PIP(R)
E. coli isolated healthy subjects	S14	+	+	-	CIP (R), AMK(R), CO(S), SXT(S), CAZ(S), FEP(S), IPM(S), PIP(S)

Abbreviations: CIP; Ciprofloxacin. CO; Colistin. AMK; Amikacin. SXT; Trimethoprim/sulfamethoxazole. IPM; Imipenem. PIP; Piperacillin. CAZ; Ceftazidime. FEP; Cefepime S: Sensitive R: Resistance.
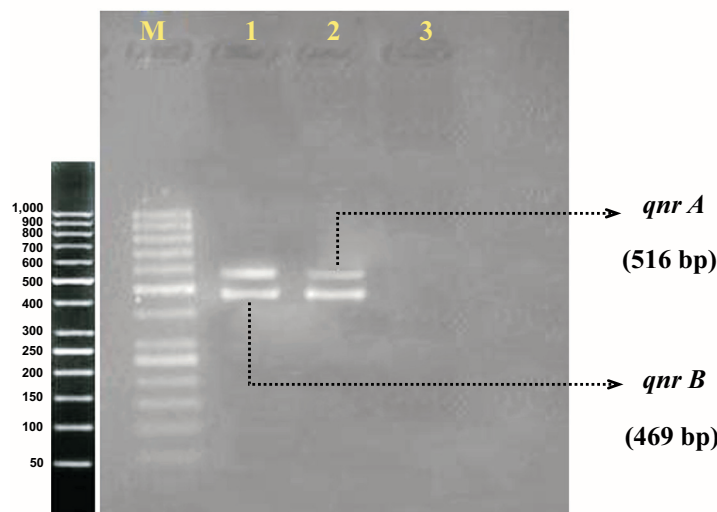
Fig. (2). Agarose gel electrophoresis of *qnr A, qnr B* gene in ciprofloxacin resistant strains PCR products. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Lane 1; (PCR product of *qnr A* and *qnr B* gene) and **Lane 2;** (positive control of *qnr A* and *qnr B* gene), **Lane 3;** (negative control), **M**; size of DNA marker (50 bp).

indicates that the resistance to antibiotics of fluoroquinolones, especially ciprofloxacin, is increasing in Iran [21]. In a study conducted in the United States in 2006, resistance to *E. coli* isolates was also reported for quinolones to be 21% and 12% for fluoroquinolones, which is less relevant to the current study [22]. In an investigation accomplished in Pakistan in 2011, *E. coli* resistance rate to ciprofloxacin was 36.45%, which is similar to the results of the present study [23]. Kheir Abadi *et al.*, found the resistance of *E. coli* isolates to ciprofloxacin 22.7%, which is similar to the results of our study [15]. Sanchez *et al.*, evaluated the antimicrobial resistance of *E. coli* between 2002 and 2010, ad found that the resistance to ciprofloxacin increased from 3% to 17.1%, and resistance to co-trimoxazole increased from 17.9% to 24.2%; the resistance to these antibiotics in the current study also increased much higher in comparison with previous studies [24]. Mohajeri *et al.*, reported that *E. coli* were resistant to cefotaxime, ceftazidime and ciprofloxacin in 27%, 22.5%, and 26%, respectively [25]. Farshad *et al.*, reported an antibiotic resistance pattern in *E. coli* isolated from the urinary tract infection patient. *E. coli* isolates were resistant to co-trimoxazole, amikacin and ciprofloxacin, in 76%, 3% and 3.8%, respectively, and resistance to imipenem was not observed [26].

Resistance to various antibiotics varies based on the therapeutic patterns that occur in different areas. By comparing the results of antibiograms and evaluating the percentage of resistance to antibiotics in diverse regions, we found that in this study, resistance to pepracillin was more than in similar studies, while in the case of ciprofloxacin, amikacin and cotrimoxazole, variable results were observed and resistance to antibiotics such as cefotaxime and cefipime was more than that observed in the present study.

When comparing various antibiotic percentages with similar tests, it was noted that regional variation in different parts of the world or even one country provides different therapeutic responses to antimicrobial drugs.

The results conclude that sensitivity to antibiotics is due to factors such as irrational antibiotics prescription, enzymatic mutations, as well as transfer of resistance through plasmids has decreased [27, 28]. The results of statistical analysis of the chi-square test showed that the resistance level of isolated *E. coli* in cancer patients was higher than in healthy subjects with IBD ($P < 0.05$).

Based on the findings of antibiograms, 15% of *E. coli* isolates showed resistance to ciprofloxacin. And in the strains of *E. coli*, colistin-resistant strains were not found. The PCR test was performed to identify the genes *qnr A* and *qnr B*.

The results showed that the prevalence of *qnr A* and *qnr B* genes in isolates resistant to ciprofloxacin was 11 (61%) and 9 (50%) respectively.

Jamshidi *et al.*, reported resistance to ciprofloxacin (46%), although the rate of resistance to ciprofloxacin was higher than their study, but the presence of *qnr A* gene was lower than our study [16].

Zhou *et al.*, showed that the prevalence of *qnr A*, *qnr B* genes in *E. coli* strains was reported to be 0.4%, and 1.2%, respectively, which showed a lower outbreak than the present study [29]. Robicsek *et al.*, in between 1999 and 2004 reported the presence of an abundance of *qnr A* genes in isolates of *E. coli* (4%) [12].

In the study by Akya *et al.*, the antibiotic resistance to ciprofloxacin 56 (84.8%) was observed to be high. The prevalence of *qnr A* and *qnr B* genes was 28.7% and 40.9%, respectively [30]. Although the main cause of resistance to quinolones is mutation in quinolone target genes, in recent years, plasmid-mediated quinolone resistance genes (PMQRs) have contributed to the high horizontal transmissivity in the development of quinolone-resistant isolates [31].

In the current study, *qnr B* was the most abundant gene, as reported in other studies [30]. In the

investigation of Rajaei *et al.*, the prevalence of *qnr A* and *qnr B* genes was 10.66% and 8.33%, respectively [32]. Soleimani *et al.*, found the prevalence of *qnr A* gene in the isolates resistant to ciprofloxacin to be 14.3%, being less prevalent than our study. Studies have shown that there is a direct correlation between the amount of consumption, quinolones and the rate of resistance to ciprofloxacin [33].

In studies conducted around the world, the prevalence of ciprofloxacin-resistant strains was different. Corkill *et al.*, reported an incidence of *qnr A* gene in 32% of the isolates studied [34]. In another study Oktem *et al.* found that out of the 34 isolates of *E. coli* resistant to ciprofloxacin, 5 isolates contained 6.3% of the *qnr A* gene [35].

Wang *et al.*, in a total of 78 isolates of ciprofloxacin-resistant *E. coli* in Brazil between 2002 and 2003 reported only one *qnr A* positive [36]. Since the two recent studies have a difference in time for about a decade with our study, the results of these studies confirm the increasing trend of *qnr* genes. Pakzad *et al.*, reported a total of 14 isolates of *E. coli* in the 24 resistant strains of *qnr A* gene (37.5%) [37].

Mohamadbigi *et al.*, similar to our study, found resistance to ciprofloxacin to be 72.77% and also the prevalence of *qnr A* and *qnr B* genes was found to be 37.33% and 67.92%, respectively [38].

Given that between quinolones, ciprofloxacin has the most effective action against gram-negative bacteria and gram-positive bacteria [39]. Ciprofloxacin inhibits bacterial DNA from DNA repair and translates by inhibiting DNA gyrase in gram-negative bacteria and topoisomerase IV in gram-positive bacteria [40, 41].

Yosofi *et al.*, investigated the relationship between *qnr* genes in inducing resistance to ciprofloxacin in *E. coli*. The results of their study showed that 139 ciprofloxacin-resistant patients were present in 309 samples. Out of these, 103 samples 74.1% were observed to carry the *qnr B* gene and 8.5% carriedthe *qnr A* gene [42].

Bouchakour *et al.*, studied 39 strains of *E. coli*; the frequency of *qnr A* and *qnr B* genes was found to be 2.65% and 10.25, respectively [31]. The difference between the results of the study and the present study in terms of the observed resistance level can be due to more precise monitoring programs in that country and the inaccessibility of these important drugs.

CONCLUSION

Considering the results of this study and other studies and due to increasing resistance to various types of antimicrobial agents such as ciprofloxacin and imipenem, which are the therapeutic indices for *E. coli* infections, further studies in this field as well as reduction in the use of antibiotics and irrational antibiotics prescription by physicians are necessary. The results of this study showed that *qnr* genes play an important role in resistance to quinolones in the virulence of *E. coli*. The high prevalence of these genes in strains as well as the presence of plasmids, which facilitate their rapid transfer and spread, is clinically important. Therefore, there is a need to monitor and investigate the genetic pattern of resistance to fluoroquinolones, the existence of screening programs and serious preventive measures. The specific invention has not been made in this study; only in this study, new patterns for *E. coli* are presented in samples of patients with colorectal cancer.

CURRENT AND FUTURE DEVELOPMENTS

There is need to monitor and investigate the genetic patterns of resistance to fluoroquinolones and colistin, the existence of screening programs and serious preventive measures. It is necessary to control the prophylactic use of antibiotics, especially those that are critical for human health, to reduce the abundance of resistance organisms. At the same time, systematic surveillance of antimicrobial resistance considering a One Health approach should be implemented to guide the intervention strategies directed to control antimicrobial resistance.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The present study was ethically approved by the Hamadan University of Medical Sciences, Institutional Review Board (9508114597).

HUMAN AND ANIMAL RIGHTS

No animals were used for studies that are the basis of this research. All human procedures followed were in accordance with the Helsinki Declaration of 1975.

CONSENT FOR PUBLICATION

Informed consent was obtained from the participants of this study.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

LIMITATIONS

One of the most important limitations of this study was the number of *E. coli* isolated from patients with colorectal cancer.

LIST OF ABBREVIATIONS

E. coli	=	*Escherichia coli*
CRC	=	Colorectal Cancer
GI	=	Gastroenteritis
MEM	=	Meropenem
IMI	=	Imipenem
CIP	=	Ciprofloxacin
CO	=	Colistin
PCR	=	Polymerase Chain Reaction

REFERENCES

[1]   Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. Lancet Infect Dis 2009; 9(4): 228-36.
http://dx.doi.org/10.1016/S1473-3099(09)70054-4
PMID: 19324295

[2]   Patel JB, Rasheed JK, Kitchel B. Carbapenemases in enterobacteriaceae: activity, epidemiology, and laboratory detection. Clin Microbiol Newsl 2009; 31(8): 55-62.
http://dx.doi.org/10.1016/j.clinmicnews.2009.03.005

[3]   Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clin Microbiol Rev 2005; 18(4): 657-86.
http://dx.doi.org/10.1128/CMR.18.4.657-686.2005
PMID: 16223952

[4]   Bonnet M, Buc E, Sauvanet P, *et al.* Colonization of the human gut by *E. coli* and colorectal cancer risk. Clin Cancer Res 2014; 20(4): 859-67.
http://dx.doi.org/10.1158/1078-0432.CCR-13-1343
PMID: 24334760

[5]   Antonic V, Stojadinovic A, Kester KE, *et al.* Significance of infectious agents in colorectal cancer development. J Cancer 2013; 4(3): 227-40.
http://dx.doi.org/10.7150/jca.5835 PMID: 23459622

[6]   Zur Hausen H. The search for infectious causes of human cancers: where and why. Virology 2009; 392(1): 1-10.
http://dx.doi.org/10.1016/j.virol.2009.06.001   PMID: 19720205

[7]   Dalton-Griffin L, Kellam P. Infectious causes of cancer and their detection. J Biol 2009; 8(7): 67.
http://dx.doi.org/10.1186/jbiol168   PMID: 19678917

[8]   Zhu Q, Gao R, Wu W, Qin H. The role of gut microbiota in the pathogenesis of colorectal cancer. Tumour Biol 2013; 34(3): 1285-300.
http://dx.doi.org/10.1007/s13277-013-0684-4   PMID: 23397545

[9]   Buc E, Dubois D, Sauvanet P, *et al.* High prevalence of mucosa-associated *E. coli* producing cyclomodulin and genotoxin in colon cancer. PLoS One 2013; 8(2): e56964.
http://dx.doi.org/10.1371/journal.pone.0056964
PMID: 23457644

[10] Serfaty L, De Leusse A, Rosmorduc O, *et al.* Ursodeoxycholic acid therapy and the risk of colorectal adenoma in patients with primary biliary cirrhosis: an ob-

servational study. Hepatology 2003; 38(1): 203-9. http://dx.doi.org/10.1053/jhep.2003.50311   PMID: 12830003

[11]   CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-eight Informational Supplement. CLSI document M100-S28 Clinical and Laboratory Standards Institute 2018.

[12]   Robicsek A, Strahilevitz J, Sahm DF, Jacoby GA, Hooper DC. qnr prevalence in ceftazidime-resistant Enterobacteriaceae isolates from the United States. Antimicrob Agents Chemother 2006; 50(8): 2872-4. http://dx.doi.org/10.1128/AAC.01647-05   PMID: 16870791

[13]   Liu Y-Y, Wang Y, Walsh TR, *et al.* Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 2016; 16(2): 161-8. http://dx.doi.org/10.1016/S1473-3099(15)00424-7 PMID: 26603172

[14]   Aibinu IE, Adenipekun EO, Nwaka D, Adelowotan AO, Ajekigbe A, Adeyemi O. Emergence of cross-resistance to fluoroquinolones in gram-negative isolates from cancer infections in a tertiary hospital in Nigeria. J Am Sci 2008; 4: 14-2.

[15]   Kheirabadi SA, Najafipour S, Kafilzadeh F, Abdollahi A, Jafari S, Moravej A. Evaluation of drug resistance pattern of *Escherichia coli* strains isolated from Fasa Vali-e-Asr hospital patients. Journal of Fasa University of Medical Sciences 2013; 2(4): 273-8.

[16]   Mansory Jamshidi N, Pakzad e, Tabaraee b, Hadadi a. Frequency of qnr genes in *Escherichia coli* strains resistant to quinolones isolated from Ilam Imam Khomani hospital and Tehran Milad hospital. Sci J Ilam Univ Med Sci 2013; 21(6): 16-22.

[17]   Heisig P, Schedletzky H, Falkenstein-Paul H. Mutations in the gyrA gene of a highly fluoroquinolone-resistant clinical isolate of Escherichia coli. Antimicrob Agents Chemother 1993; 37(4): 696-701. http://dx.doi.org/10.1128/AAC.37.4.696   PMID: 8388197

[18]   Chapman JS, Georgopapadakou NH. Routes of quinolone permeation in *Escherichia coli*. Antimicrob Agents Chemother 1988; 32(4): 438-42. http://dx.doi.org/10.1128/AAC.32.4.438   PMID: 3132091

[19]   Conrad S, Oethinger M, Kaifel K, Klotz G, Marre R, Kern WV. GyrA mutations in high-level fluoroquinolone-resistant clinical isolates of *Escherichia coli*. J Antimicrob Chemother 1996; 38(3): 443-55. http://dx.doi.org/10.1093/jac/38.3.443   PMID: 8889719

[20]   Ruiz J. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. J Antimicrob Chemother 2003; 51(5): 1109-17. http://dx.doi.org/10.1093/jac/dkg222   PMID: 12697644

[21]   Siasi E, Hossieni F, Rahiminia P. Relation of gyrA and parC genes mutations with fluoroquinolonesresistance in *Escherichia coli* of urinary tract infections. New Cell Mol Biotechnol J 2017; 7(25): 91-100.

[22]   Moreno E, Prats G, Sabaté M, Pérez T, Johnson JR, Andreu A. Quinolone, fluoroquinolone and trimethoprim/sulfamethoxazole resistance in relation to virulence determinants and phylogenetic background

among uropathogenic *Escherichia coli*. J Antimicrob Chemother 2006; 57(2): 204-11. http://dx.doi.org/10.1093/jac/dki468   PMID: 16390858

[23]   Muhammad I, Uzma M, Yasmin B, Mehmood Q, Habib B. Prevalence of antimicrobial resistance and integrons in *Escherichia coli* from Punjab, Pakistan. Braz J Microbiol 2011; 42(2): 462-6. http://dx.doi.org/10.1590/S1517-83822011000200008 PMID: 24031655

[24]   Sanchez GV, Master RN, Karlowsky JA, Bordon JM. *In vitro* antimicrobial resistance of urinary *E. coli* among US outpatients from 2000 to 2010. Antimicrob Agents Chemother 2012; 56(4): 2181-3.

[25]   Mohajeri P, Izadi B, Rezai M, Falahi B, Khademi H, Ebrahimi R. Assessment of the frequency of extended spectrum beta lactamases producing *Escherichia coli* isolated from urinary tract infections and its antibiotic resistance pattern in kermanshah. J Ardabil Univ Med Sci 2011; 11(1): 86-94.

[26]   Farshad S, Ranjbar R, Anvarinejad M, Shahidi MA, Hosseini M, Eds. Emergence of multi drug resistant strains of *Escherichia coli* isolated from urinary tract infection. Open Conf Proc J 2010; 1: 192-6. http://dx.doi.org/10.2174/2210289201001010192

[27]   Jabeen K, Zafar A, Hasan R. Frequency and sensitivity pattern of extended spectrum beta lactamase producing isolates in a tertiary care hospital laboratory of Pakistan. J Pak Med Assoc 2005; 55(10): 436-9. PMID: 16304852

[28]   Poole K. Resistance to β-lactam antibiotics. Cell Mol Life Sci 2004; 61(17): 2200-23. http://dx.doi.org/10.1007/s00018-004-4060-9 PMID: 15338052

[29]   Zhou T-L, Chen X-J, Zhou M-M, Zhao Y-J, Luo X-H, Bao Q-Y. Prevalence of plasmid-mediated quinolone resistance in *Escherichia coli* isolates in Wenzhou, Southern China, 2002-2008. Jpn J Infect Dis 2011; 64(1): 55-7. PMID: 21266756

[30]   Akya A, Chegenelorestani R, Elahi A, Hamzavi Y. Frequency of plasmid-mediated quinolone resistance genes in extended-spectum β -lactamase-producing *Escherichia coli*. Majallah-i Danishgah-i Ulum-i Pizishki-i Mazandaran 2017; 27(151): 41-51.

[31]   Bouchakour M, Zerouali K, Gros Claude JD, *et al.* Plasmid-mediated quinolone resistance in expanded spectrum beta lactamase producing enterobacteriaceae in Morocco. J Infect Dev Ctries 2010; 4(12): 779-803. PMID: 21252459

[32]   Rajaei S, Kazemi-Pour N, Rokhbakhsh-Zamin F. Frequency of plasmid-mediated quinolone resistance genes among clinical isolates of *Pseudomonas aeruginosa* in Kerman, Iran. Indian J Med Microbiol 2017; 11(3): 10-8.

[33]   Soleimani-Asl Y, Zibaei M, Firoozeh F. Detection of qnrA gene among quinolone-resistant *Escherichia coli* isolated from urinary tract infections in Khorram Abad during 2011-2012. Feyz J Kashan Univ Med Sci 2013; 17(5): 488-94.

[34]   Corkill JE, Anson JJ, Hart CA. High prevalence of the plasmid-mediated quinolone resistance determinant qnrA in multidrug-resistant Enterobacteriaceae from blood cultures in Liverpool, UK. J Antimicrob Chemother 2005; 56(6): 1115-7. http://dx.doi.org/10.1093/jac/dki388   PMID: 16260446

[35]   Oktem IMA, Gulay Z, Biçmen M, Gur D, Group HPS. qnrA prevalence in extended-spectrum beta-lactamase-positive Enterobacteriaceae isolates from Turkey. Jpn J Infect Dis 2008; 61(1): 13-7. PMID: 18219128

[36]   Wang M, Tran JH, Jacoby GA, Zhang Y, Wang F, Hooper DC. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. Antimicrob Agents Chemother 2003; 47(7): 2242-8.   http://dx.doi.org/10.1128/AAC.47.7.2242-2248.2003 PMID: 12821475

[37]   Pakzad I, Ghafourian S, Taherikalani M, *et al.* Qnr prevalence in extended spectrum beta-lactamases (ESBLs) and none-ESBLs producing *Escherichia coli* isolated from urinary tract infections in central of Iran. Iran J Basic Med Sci 2011; 14(5): 458-64. PMID: 23493061

[38]   Mohamadbigi M, Akbarmehr J, Jafari B. Evaluation of the frequency of plasmid-mediated quinolone resistance genes in clinical isolates of *Escherichia coli* and *Klebsiella* spp in Tehran 2016. J MICRO WORLD 2016; 9: 199-207.

[39]   Bansal S, Tandon V. Contribution of mutations in DNA gyrase and topoisomerase IV genes to ciprofloxacin resistance in *Escherichia coli* clinical isolates. Int J Antimicrob Agents 2011; 37(3): 253-5. http://dx.doi.org/10.1016/j.ijantimicag. 2010.11.022 PMID: 21236644

[40]   Shin JH, Jung HJ, Lee JY, Kim HR, Lee JN, Chang CL. High rates of plasmid-mediated quinolone resistance QnrB variants among ciprofloxacin-resistant Escherichia coli and *Klebsiella pneumoniae* from urinary tract infections in Korea. Microb Drug Resist 2008; 14(3): 221-6.
 http://dx.doi.org/10.1089/mdr.2008.0834   PMID: 18707554

[41]   Mirmostafa S, Sabet M, Amir N. Determination of plasmid profile and antibiotic resistance pattern in clinical *Escherichia coli* strains, isolated from different region of Karaj city. New Cell Mol Biotechnol J 2014; 3(12): 93-7.

[42]   Yosofi S, Mojtahedi A, Shenaghari M. Association to qnr genes with ciprofloxacin resistant *E. coli*. Kordestan Univ Med Sci 2015; 20(5): 52-60.