Thi-Qar Medical Journal (TQMJ): Vol. (28), No. (2), 2024
Web Site: https://jmed.utq.edu Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218 ISSN (Online): 3006-4791

Identification and Antimicrobial Resistance of E.Coli Isolated from Patients with Colorectal Carcinoma in Thi Qar Province

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Abstract : Colorectal cancer is a third most prevalent cause of tumorigenesis and several pathogenic bacteria have been correlated with aggressive cases of cancer i.e., genotoxin (colibactin) producing Escherichia coli (E. coli). This study was designed to investigate the pathogenic E. coli isolates from colorectal tissue were characterized based on morphology, antibiotic resistance pattern and risk factors association with CRC. Moreover, E. coli isolates were screened for the presence of the Pks (polyketide synthase) Island specifically targeting colibactin genes (cIbB and uidA). This results revealed a significant increase in the mean of age in CRC patients compared to the benign group (P:0.001). In addition, the CRC incidence was increased significantly in urban areas (72.5%) compared to rural areas (27.5%) (P:0.001). **Moreover,** 100% of these isolates were resistant to Meropenem and 80% of these isolates were resistant to ciprofloxacin, Ceftazidime, Rifampin, and Erythromycin. The presence of cIbB and uidA genes did not show a significant association when comparing colorectal tissues to benign colon tissues. This is the first study on identification and antimicrobial resistance of E.coli among CRC patients in this setting. This work provides local, specific data, which are crucial to understand and combat CRC in Iraq.

Keywords: Escherichia coli (E. coli), Identification, antimicrobial resistance, Colorectal cancer (CRC)

Introduction: Colorectal carcinoma (CRC) is one of the most significant health issues and ranks as the fourth most prevalent cancer globally and stands as the third leading cause of cancer-related mortality [1]. It affects men and women at about the same rate [2]. In the United States, it represents the second most significant cause of cancer-related mortality, resulting in over 600,000 deaths each year [3]. In Iraq, CRC is an essential contributor to deaths from gastrointestinal malignancies and ranks as the third most prevalent, accounting for 2210 new cases [4]. About 95% of CRCs are identified with adenocarcinoma [5], while only 4 % of tumor cases were medullary CRC [6].

The diagnosis of CRC is mostly dependent on the routine diagnostic methods used such as grading and staging systems which offer comprehensive information on tissue abnormalities that is essential for prognosis and therapy planning [7].

Recently, there has been increased focus on the function of the gut microbiota in the onset and progression of CRC. Certain bacterial species, including Fusobacterium nucleatum, Escherichia coli, Bacteroides fragilis, Enterococcus faecalis, and Salmonella sp., have been linked to CRC [9]. Mucosa-associated strains of E. coli are more commonly found in the biopsies of CRC patients compared to individuals without the disease [8].

The previous study found that the presence of a genomic island called polyketide synthetase (pks) in E. coli belonging to the B2 phylogenetic group may have a role in CRC [9]. This island harbors the genetic information necessary for the synthesis of colibactin, which is produced via an assembly line of hybrid non-ribosomal peptide synthetase-polyketide synthase (NRPS-PKS) that is located within the 54-kb pks island and comprises 19 genes (clbA to clbS) [10].

The virulence of E. coli O157:H7 is associated with genes, which produce β-glucuronidase enzyme that encoded by gene uidA gene [11]. Additionally, another study reported that CRC patients in advanced stages had higher colonization of E. coli compared to those in early stages [12]. However, in the south of Iraq, there is no published data on the role of E. coli infection in adults and its associated risk factors with CRC. The current study, therefore, aimed to determine the prevalence, antimicrobial resistance profiles, and risk factors associated with E. coli infection from patients' association with colorectal cancer in Thi-Qar Governorate, south-eastern Iraq.

Materials and Methods

Study setting and design: The present study was conducted at the cancer Research Unit and Microbiology department laboratories, College of Medicine, University of Thi-Qar during the period from August 2023 to March 2024.

Patients suffering from colorectal cancer, confirmed by the histopathologists, attending Al-Amel, Al-Hadarat, and Al-Hussein teaching hospitals were enrolled in the study. This research study used the malignant and benign colon and rectum tissues from patients aged above 20 years old to detect the presence of different E coli genes using a conventional PCR.

Patient samples collection: This study included 74 colon and rectum tissue samples. These samples are divided into two cohorts, CRC and benign colon and rectum tissues. Fifty tissue samples were CRC. The control cohort included 24 tissue samples obtained from non-cancerous people admitted to the same hospitals by colonoscopy. A tissue sample from each patient was collected, kept in 9% normal saline, and the transported to the laboratory on the ice box where testing started on the same day. The Patient data was collected either directly from the patients as a questioner or from the histopathological reports.

Ethical consideration : The study was approved by the health directorate committee in Thi-Qar province (No.162 on 7/8/2023). Permission to conduct this study was also obtained from the committee of publication ethics at the College of Medicine, University of Thi-Qar/

Iraq. Verbal consent was obtained from the patient at hospitals and clinics to take a biopsy from tissue.

Isolation and identification of E. Coli: The isolation and identification of E coli were performed according to [13]. Briefly, each tissue sample was suspended in 9% normal saline. A loopful of enrichment broth was streaked on MacConkey agar and Eosin Methylene Blue agar, and the plates were incubated at 37 ºC for 24 hours. Presumptive E. coli colonies from each plate were determined based on the morphological characteristics such as shape, size, margin, consistency, and color of colonies. The identification of E. coli colonies was confirmed by using Gram's staining and various biochemical tests (i.e. Oxidase, Indole Test, Methyl red test, and Voges –Proskauer). Pure E. coli cultures confirmed by morphological and biochemical tests were processed and preserved in 20% glycerol and stored at -20C until further processing.

DNA extraction and PCR detection : DNA was extracted from each E. coli isolate using the Genomic DNA Extraction Promega kit USA, according to the manufacturer's instructions. A conventional PCR assay was performed for detection of two pks island of E. coli: (cIbB and uidA) according to the procedure previously described by Shimpoh et al. [14]. Primers to amplify a 283 bp sequence in cIbB gene with the forward primer 5-GCGCATCCTCAAGAGTAAATA-3 and the reverse primer 5-GCGCTCTATGCTCATCAACC-3, while for uidA gene using amplify a 147 bp sequence with the forward primer 5-TGGTAATTACCGAC GAAAACGGC-3 and the reverse primer 5-ACGCGTG GTTACAGTCTTGCG-3.

Antimicrobial susceptibility test: Antimicrobial susceptibility testing of E. coli isolates was determined using the disc diffusion method on Mueller-Hinton agar plates according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2015). The following 9 antimicrobials were tested: Amikacin (25µg) Ceftazidime (30µg) Ciprofloxacin (10µg) Erythromycin (10µg) Imipenem (10µg) Meropenem (10µg) Nitrofurantoin (300µg) Rifampin (5µg) Tetracycline (30µg)
Antibiotic susceptibility was ascertained after 18–24 hours of incubation at 37℃. E. coli strains were classified as resistant, susceptible and intermediate by comparison of diameter of inhibition to the CLSI, 2015 guidelines. [15].

Statistical Analysis: The data of the current study was statistically analysis by using SPSS (Statistical Package of Social Science version 26), based on using both Non-parametric and Cross-table chi-square, independent sample t-test, one-way ANOVA and LSD, and Kendall's tau-b correlation at p. value < 0.05.

Results

Bacterial culture and biochemical tests: The current study was designed to isolate and identify the E. coli from CRC and benign tissues and screen and identify the pks genes in E. coli isolates in both groups of tissues, using PCR. The results showed the different morphology characteristics of E. coli which grow on different media (Table 1).

Table (1): The results of culture characteristics of E. Coli

Culture Media	Morphology Of Colonies
Emb Agar	Small, Rounded, Green Metallic Sheen
Macconkey Agar	Small, Rounded, Pink In Color

The results of the biochemical tests showed that these isolates gave negative results for oxidase, and Voges –Proskauer tests, while gave positive results for indole and methyl red test as shown in table (2).

Table (2): Shows the biochemical results for E. Coli isolates.

Biochemical Test	Result Of E. Coli
Indole	Positive
Methyl Red	Positive
Voges –Proskauer	Negative
Oxidase	Negative

Antimicrobial susceptibility testing: The results of antimicrobial susceptibility of **E. coli** isolates indicate that all these isolates were sensitive to Amikacin and Nitrofurantoin. In contrast, all these isolates were resistant to Meropenem. Furthermore, 80% of these isolates

were resistant to ciprofloxacin, Ceftazidime, Rifampin, and Erythromycin. In addition, 60% of these isolates were also resistant to Imipenem and tetracycline (Table 3).

Table (3): Antimicrobial susceptibility patterns of *E.coli* against 9 antibiotic discs

NO	Antibiotic	Resistant		Intermediate		Sensitivity	
		NO	%	NO	%	NO	%
1	Amikacin	0	0	0	0	20	100
2	Nitrofurantoin	0	0	0	0	20	100
3	Meropenem	20	100	0	0	0	0
4	Ciprofloxacin	16	80	0	0	0	0
5	Ceftazidime	16	80	0	0	0	0
6	Rifampin	16	80	0	0	0	0
7	Erythromycin	16	80	0	0	0	0
8	Imipenem	12	60	0	0	0	0
9	Tetracycline	12	60	0	0	0	0

Prevalence of Age, Sex, and Residency in benign and malignant colorectal tissues

The present study showed a significant increase in the mean of age in CRC patients compared to the benign group (P:0.001). In addition, the CRC incidence was increased significantly in urban areas (72.5%) compared to rural areas (27.5%) (P:0.001). In the benign

group, both males and females have an equal percentage of distribution. There was no significant difference in the incidence of CRC and benign groups according to sex group (P:0.887) Table 4.

Table (4): Shows prevalence of age, sex and residency in CRC patients and control

Characteristics		Patients No. 80	Control No. 24	P. Value
		Mean ± S. D		
Age		53.3 ± 14.3	42.0 ± 15.1	0.001
Sex	Sex	No. & %	No. & %	0.887
	Male	44 (55.0)	13 (54.17)	
	Female	36 (45.0)	11 (45.83)	
Residency	Urban	58 (72.5)	12 (50.0)	0.001
	Rural	22 (27.5)	12 (50.0)	

Prevalence of cIbB and uidA in E Coli isolates from malignant and benign tissues

The presence of the bacterial genes, cIbB and uidA, showed no significant differences when comparing CRC patients to the benign group. Both malignant and benign tissues were free from cIbB gene. In contrast, uidA gene was found in all tissue samples except one of the benign samples (Table 5). These were diagnosed by molecular methods to identify genes "cIbB and uidA" as illustrated in (Figures 1).

Table (5): Prevalence of cIbB and uidA genes in E Coli isolates from malignant and benign tissues.

Bacterial Gene	Test Result	Patients		Benign		P. Value
		No.	%	No.	%	
Cibb	Positive	0	0.0	0	0.0	1.00
	Negative	50	100	24	100	
Uida	Positive	50	100	23	95.65	0.775
	Negative	0	0.0	1	4.35	

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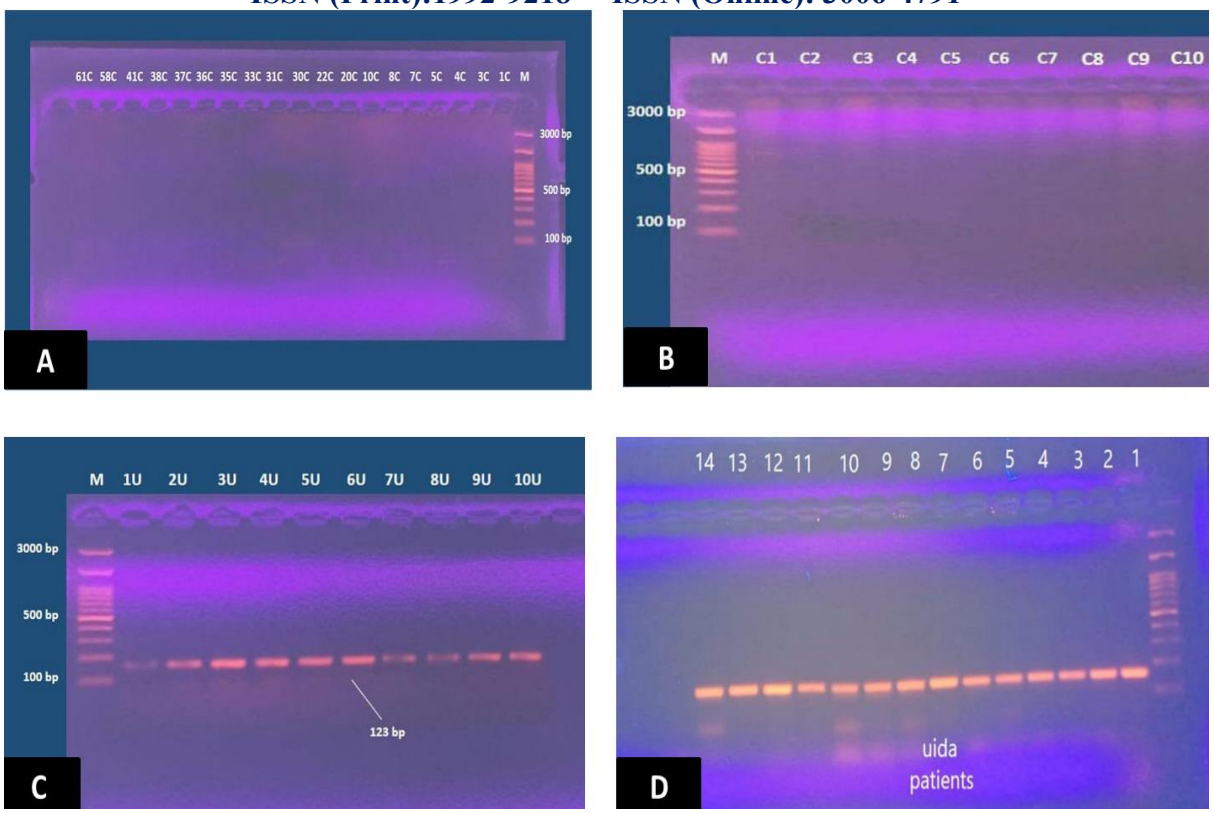

Figure (1): Shows the presence of E.coli genes, cIbB and uidA, in malignant and benign tissue of colon. A) The benign colon tissue was free from cIbB gene. B) The CRC tissue was free from cIbB gene. C) uidA gene was found in benign tissue of colon. D) uidA gene was observed in CRC tissue. The size of the PCR product is (283, 147bp) respectively. The gel was 1.5% at 80 volt / 80 min. DNA ladder (100-3000).

Discussion: Several potential risk factors for CRC development have been reported, including bacterial infections. A previous study by Farahani et al. indicated that a specific type of pathogenic E. coli, which produces colibactin, may contribute to CRC formation, particularly in patients with bowel diseases such as ulcerative colitis.

The culture results showed that the E.coli bacteria on Eosin Methylene Blue Agar, the isolates produced shiny, metallic green colonies. This characteristic, indicative of E. coli, is due to the medium containing eosin and methylene blue pigments, which precipitate in an acidic environment created by lactose fermentation, as described [15]. In addition, the biochemical tests showed that E. coli tested positive for Indole and Methyl Red but negative for Voges-Proskauer and oxidase, corroborating the previous result [16].

In the present study, the results of antimicrobial susceptibility of E.coli isolated from CRC indicate that all isolates were resistant to Meropenem (100%), a result that contrasts with the findings of [17], which reported high sensitivity to this antibiotic. The current result showed that 80% of these isolates were resistant to ciprofloxacin, Ceftazidime, Rifampin, and Erythromycin. This is agreed with the previous findings [18]. This data showed 60% of these isolates resistant to both tetracycline and Imipenem. This is agreed with findings from Abdelhamid and Abozahra (2017), who reported that 68% of isolates were resistant to

tetracycline, and with Al-Atabi (2013), who found that 100% of isolates were sensitivity to Imipenem [18]. Additionally, all isolates were resistant to amikacin and nitrofurantoin (100%), a result that contrasts with [19], which reported high sensitivity to these antibiotics. The misuse and overuse of antimicrobials in may contribute to the development of resistance [20], is the main factor may explain the differences between these results and previous findings.

This study found an increased risk of CRC in elderly individuals compared to other age groups. In the present study setting, indicated that the risk for developing the disease begins at the age of 40 and significantly increases between the ages of 43 and 52 for both sexes. Age is considered an equally relevant risk factor for both men and women. This is consistent with previous findings, which showed an increase in CRC patients within the age range of 50-70 years [21].

Another factor that may have a role in malignancy is sex. This study found that the incidence of CRC was not associated with sex. This is agreed with the previous findings [17 ,22]. This data, however, was in consistent with previous finding which indicated increased the risk of CRC in males compared to females, our data showed increased [23]. The potential reason is that compared to men, women have noticeably more significant amounts of estrogen, which is found to play an essential role in preventing CRC development. They also found that the protective impact is likely to be facilitated by estrogen receptor ß (ERB) [24].

The current study also identified significant differences in CRC incidence between urban and rural residents when compared to benign groups. These findings are consistent with those of Wen et al., who reported notably higher rates of CRC in Shijiazhuang (urban) compared to Shexian (rural) for both men and women [25].

This study evaluates the presence of cIbB in both benign and malignant tissues using PCR and detect if this gene could be used as a possible biomarker for CRC diagnosis and prognosis. This study showed that there was no significant difference in the presence of cIbB gene in CRC tissues compared to benign tissues of colon. This result was supported by Iwasaki etal who reported that no significant in pks E.coli genes was observed between CRC and control group [26]. In addition, this data was agreed with the earlier results [22]. but was disagreed with previous findings [17,18]. The observed variation could have emerged from the utilization of different tissue samples for molecular analysis, potentially exerting an influence on the resultant outcomes. Therefore, further research is warranted to elucidate the correlation between colibactin exposure from pks E. coli and the incidence of CRC [26].

The presence of uidA gene was observed in all malignant (50) and 23 out 24 benign colon tissue samples. However, this study confirmed that there was no significant association in uidA gene when comparing benign vs. malignant tissues. These findings are consistent with previous research [26], although they contrast with other studies [27]. This difference could be because of sample size.

Conclusion: E. coli is one of several pathogenic bacteria have been correlated with serious cases of cancer which is producing Escherichia coli genotoxin (colibactin). This study indicates that E. coli isolates are found to be multidrug-resistant. A high prevalence of the

antimicrobials-resistant isolates is a significant public health concern for treating diseases. advanced age and urban area are found the common risk factor associated with CRC incidence. The presence of pks genes bacteria may not associated significantly with the CRC progression. It will be worth checking the production of colibactin in these isolates and also comparative genomics analysis for a better understanding of their role in CRC. This work provides local, specific data, which are crucial to understand and combat CRC in Iraq.

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