ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# Comparative Analysis of Escherichia Coli Strain Plasmid DNA Profile, Isolated From Normal and Malignant Intestinal Epithelium of the Patients with Colorectal Cancer.

**Nga Thi Nguen[1]*, Naila Ibragimova[1], Ilgiz Gataullin[2], Ramil Vafin[1] and Olga Ilynskaya[1].**

[1]Kazan Federal University, Russia.
[2]Kazan State Medical Academy, Russia.

**ABSTRACT**

Optionally cultured aerobic bacteria were isolated from the biopsy materials from the colorectal cancer patients (CCP). The species belonging was determined using MALDI-TOF mass spectrometry. Escherichia coli bacteria were the main representatives of patient microflora. 16 strains of E. coli were selected from normal and oncologically transformed epithelium to characterize a set of their plasmids. An antagonistic activity against E. coli K12 was found only in 3% of the strains. There is the difference in the profile of E. coli strain plasmid DNA, colonizing normal and oncologically transformed epithelium, both by size and by the number of plasmids and by assumed mobile genetic elements. Among the strains isolated from the patient, 63% had a differing plasmid composition depending on the bacteria association with normal and malignant epithelium. At that no marker plasmid, a concomitant E. coli, colonizing only normal or only malignant epithelium was revealed. PCR analysis of plasmid DNA samples of the studied E. coli concerning the presence of lthB- and STa- genes encoding heat-labile and heat-stable enterotoxin, did not reveal their presence, which excludes the belonging of enterotoxigenic (ETEC) subgroup strains of E.coli.

**Keywords:** Colorectal cancer, Escherichiacoli, plasmid, antagonistic activity, colicin, electrophoresis, toxigenicity.

***Corresponding author***

ISSN: 0975-8585

## INTRODUCTION

E. coli bacteria are the main representatives of facultative anaerobes in a man. Only highly adapted E. coli clones are known, which acquired certain attributes of virulence, increasing the ability to adapt to new niches; at that they cause a wide spectrum of diseases. The carcinogenesis of colorectal cancer occurs over many years. At that it is proposed that intestinal microflora is adapted to the altered microenvironment and provides the shift in the normal balance of bacteria. It is known that intestinal group bacteria produce toxins - colicins which present a special form of quite a successful adaptation to the change of the ecological niche state. Colicins as well as other bacteriocins play an important role in the relationship of bacteria towards the development of bacterial community and the dynamics of its diversity [1,2,3]. These protein toxins catalyze the gap of ß-1,4 bonds between N-acetylglucosamine and N-acetylmuramic acid in the glycan layer of bacterial cell wall or inhibit the synthesis pf peptydoglycanamurein with the formation of spheroplasts and the subsequent lysis of the bacterial cell, which gives the advantage to colicin-synthesizing bacteria in a competitive struggle [12].

6 categories of pathogenic bacteria are well described among E. coli: enteropathogenic (EPEC), enterohaemorrhagic (EHEC), enterotoxigenic (ETEC), enteroaggregative (EAEC), enteroinvasive (EIEC) and diffuse adhesion (DAEC) ones. All these groups contain plasmids associated with virulence. Commensal bacteria may induce deletions, point mutations, or other changes in DNA, which contribute to the emergence of virulence or its level increase [4.5]. Despite the constantly growing number of well-known plasmid replicons, not all of them are related to virulence. Most of the plasmids, associated with E.coli virulence, belong to the F-group of plasmids [4,5]. In addition to large associated virulence plasmids (over 60 thousand of nucleotide pairs), small virulence - associated plasmids are found (less than 10 thousands of nucleotide pairs) [5]. Enterobacteriaceae also contain small cryptic plasmids, whose function is still not completely defined [5].

In this regard, the studies conducted to detect the characteristics and enterobacterial plasmids and in particular, E. coli, with a view to the future improvement of diagnostic and therapeutic combat opportunities with intestinal diseases are quite valid and current.

The objectives of this work were the identification of E.coli strains, producing colicin, the search for lthB and STa-genes encoding heat-labile and heat-stable enterotoxin and also the characteristics of E.coli strain plazmid profile, isolated from the biopsy samples of normal and transformed intestinal epithelium among the patients with colorectal cancer.

## MATERIALS AND METHODS

Antagonist activity as an indicator of colicine presence. The studied cultures of E. coli were sown by lawn method on a nutrient medium in a Petri dish. The crops were incubated at 37 °C during the day. Then the studied cultures were transitioned in the form of a cut-out portion with agar on new petri dishes, where a 6-hour broth culture of E.coli K12 was sown with the lawn in advance. The result was taken into account after 24 hours of incubation at 37 °C along the growth inhibition zones of E.coli strain K12 around the studied cultures.
The analysis of the strain plasmid profile. The bacterias in 15 ml of LB liquid medium were incubated for 16 hours at 37 C and at forced aeration with 200 rpm. In order to isolate plasmids the kit GenJETPlasmidminiprepKit of the cat. №: K0503 (Fermentas) was used according to the manufacturer's instructions. We performed an electrophoretic analysis of obtained plasmid solutions in a 1% agarose gel within TBE buffer (pH 8.0) containing ethidium bromide, followed by the visualization of the results under ultraviolet transilluminator (λ = 310 nm). Dimensions of plasmid DNA was evaluated in comparison with standard DNA markers.

PCR analysis of gene toxigenicity. We analyzed the samples of plasmid DNA concerning the studied E. coli samples on the presence of lthB- and STa- genes encoding thermolabile and thermostable enterotoxins. PCR was performed using the amplifier MJ MiniGradientThermalCycler (Bio-Rad, USA). The detection of PCR analysis results was performed by horizontal electrophoresis method in 2.5% agarose gel within TBE buffer (pH 8.0) containing ethidium bromide.

ISSN: 0975-8585

**Table 1: Oligonucleotide primers used in operation**

| Item № | *Gene locus* | The name of oligonucleotide primers, their sequence and length (n.) | PCR-product (p.n.) | Reference |
|---|---|---|---|---|
| 1 | *lthB* | LT-f: 5/-ACGGCGTTACTATCCTCTC-3/ (19 n.)<br>LT-r: 5/-TGGTCTCGGTCAGATATGTG-3/ (20 n.) | 273 | [6] |
| 2 | *STa* | STa-1: 5/-GCTAATGTTGGCAATTTTTATTTCTGTA-3/ (28 n.)<br>STa-2: 5/-AGGATTACAACAAAGTTCACAGCAGTAA-3/ (28 n.) | 190 | [7] |

The statistical processing of results was performed using standard methods in Microsoft Excel 2007.

## RESULTS AND THEIR DISCUSSION

Among 32 examined strains of E. coli, isolated from the patients with CRC, an antagonistic activity against E.coli K12 was found only in one strain (3%); Perhaps it is this strain which can be colicinogenic [8,9].

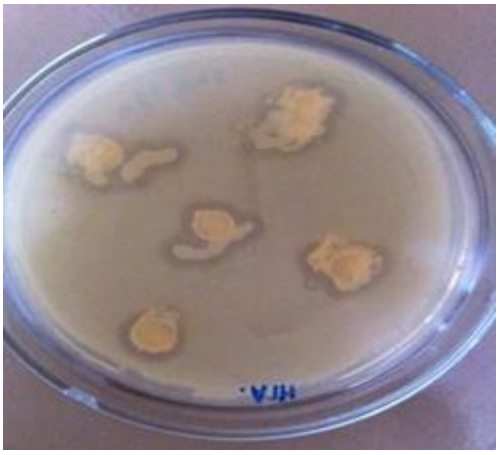

**Figure 1: E. coli strain isolated from the biopsy of the patient with colorectal cancer inhibits the growth of E. coli K12 strain.**

**Table 2: The number and an approximate size of E. coli plasmid DNA**

| Patient | Plasmids with E coli conditionally healthy epithelial tissue | Plasmids from E coli transformed epithelial tissue |
|---|---|---|
| 1 | 23130<br>3000 | 23130<br>3000 |
| 2 | 23130 | 23130 |
| 3 | 3500 | >10000 |
| 4 | 23130<br>2500<br>1500-1100 | 23130 |
| 5 | >10000<br>1700<br>1500 | >10000<br>1700 |
| 6 | 23130<br>3000 | нет |
| 7 | 23130 | 23130<br>3000 |
| 8 | 23130 | 23130<br>5000<br>3200-3500<br>2000<br>1600<br>1400 |
| 9 | 9416<br>3000 | 9416<br>3000 |
| 10 | 9416<br>4361 | 9416 |
| 11 | 23130<br>3500<br>3000 | 23130<br>3500<br>3000 |
| 12 | - | 9416 |
| 13 | 23130<br>3500<br>3000 | 23130<br>3500<br>3000 |
| 14 | - | 9416 |
| 15 | 9416<br>750 | - |
| 16 | >23130<br>9416<br>4000<br>3500<br>2322<br>2000<br>1400-1500<br><1400 | 4000 |

ISSN: 0975-8585

It was shown that 41% of E. coli strains isolated from healthy people produce colicins [10]. In this study, there was a low content of colicinogenic E. coli among the patients with colorectal cancer.

The size of E. coli plasmid strain, isolated from oncologically transformed epithelium and the epithelium, unaffected by tumor was determined electrophoretically. The summary data on the number and an approximate size of E. coli DNA plasmids, isolated from oncologically transformed epithelium and the epithelium, unaffected by tumor are presented in Table 2.

Thus, the significant differences were revealed in the spectrum of E. coli plasmids, colonizing normal and oncologically transformed epithelium. 63% of the strains isolated from the same patient, but with different sections of the gut (normal and malignant epithelium) differed in plasmid preparations. At that a characteristic marker plasmid concomitant with E. coli, colonizing only normal or only malignant epithelium is not revealed. According to the literature data it is known that the size of plasmids must be at least 2000 n.p. [11]. DNA fragments with the number of less than 2000 n.p., encountered on electrophoregrams are probably the insertion sequences, which may have different numbers among E. coli: 768 n.p. (IS 1), 1327 n.p. (IS 2), 1258 n.p. (IS 3), 1195 n.p. (IS 5), 1329 n.p. (IS 10), 1531 n.p. (IS 50) [12]. These fragments do not make the part of larger plasmids, as the technique of plasmid isolation protects the plasmid material from restriction [GeneJETPlasmidMiniprepKit].

The distribution of plasmid amount and anticipated mobile genetic elements by the rate of incidence among E. coli isolates, isolated from normal and malignant intestinal epithelium is shown in Table 3.

**Table 3: Is the distribution of plasmids and mobile genetic elements by the frequency of occurrence among E. coli isolates, isolated from normal and malignant intestinal epithelium.**

| The range of plasmid and mobile genetic element amount | Frequency of occurrence | |
|---|---|---|
| | Normal epithelium | Malignant epithelium |
| > 20000 n.p. | 37% | 25% |
| 2000-20000 n.p. | 93% | 93% |
| <2000 n.p. | 6% | 0% |

The distribution of plasmid amount and assumed mobile genetic elements by the frequency of E. coli isolate occurrence, from normal and malignant intestinal epithelium is shown on Figure 2.

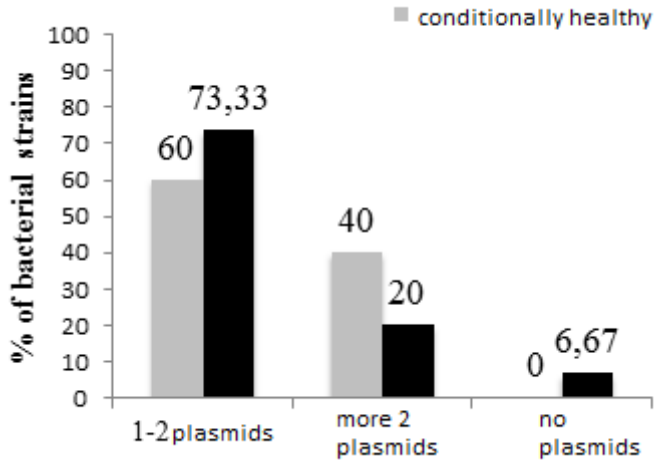


**Figure 2: Distribution of plasmids and mobile genetic elements of E. coli isolates, isolated from normal and malignant intestinal epithelium by the frequency of occurrence.**

For the isolates isolated from normal epithelium, the incidence of major plasmids with the amount of more than 20000 n.p., capable of carrying toxigenicity and pathogenicity genes [13], and also colicins [14] made 37%, while this value for the isolates with malignant epithelium does not exceed 25%. The incidence of

plasmid occurrence isolated from normal and malignant epithelium with the amount of 2,000 - 20,000 n.p. made 93%. The estimated mobile genetic elements with the amount of less than 2000 n.p., isolated from isolates of unaffected intestine epithelium, occurred with the frequency equal to 6%, and did not occur in isolates of malignant epithelium.

The data obtained by us indicate a significant difference in the profile of the plasmid isolates from normal and malignant epithelium.

PCR analysis of plasmid DNA samples within the studied samples of E. coli and lthB- STa-genes encoding heat-labile and heat-stable enterotoxins, did not reveal their presence that excludes their belonging to enterotoxigenic (ETEC) E.coli subgroup (Fig. 3).

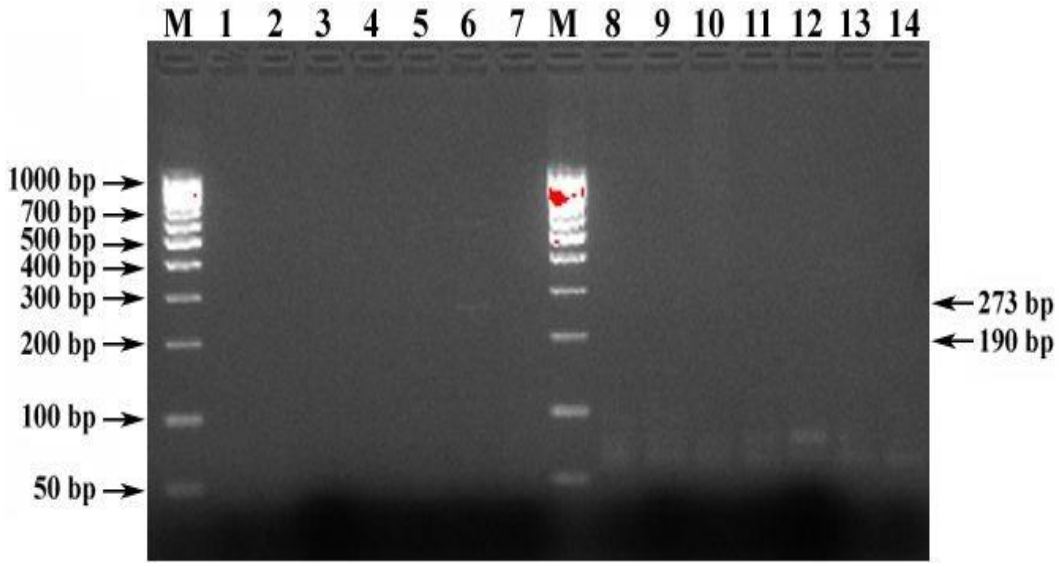


**Figure 3: Electrophoregram of plasmid PCR analysis result with LT-f + LT-f primers (E. coli lthB-locus gene amplification (heat labile enterotoxin)) and with STa-STa-1 + 2 primers (amplification of E. coli STa-gene locus (thermostable enterotoxin))**

## CONCLUSION

Colicin-producing strains made only 3% of all E. coli strains, isolated from an oncologically transformed epithelium and the epithelium, unaffected by tumor.

The plasmid DNA of examined E. coli did not reveal genetic determinants controlling toxigenic activity associated with heat-labile and heat stable enterotoxins.

Plasmid profile of E. coli bacteria strains, isolated from normal and malignant intestine epithelium of the same patient differed in 63% of cases.

## SUMMARY

Within this study low levels of colicinogenic E. coli among the patients with colorectal cancer were determined. The difference is in the spectra of E. coli plasmid strains, colonizing normal and oncologically transformed epithelium. Thus no characteristic marker plasmid of concomitant E. coli is detected, colonizing only normal or only malignant epithelium. Toxigenicity genes encoding thermostable and thermolabile enterotoxins in plasmid DNA of isolated strains were not detected.

## ACKNOWLEDGMENTS

The research was performed within the Russian Government Program of Competitive Growth of Kazan Federal University and supported by the Russian Research Foundation (grant № 14-14-00522).

**REFERENCES**


[1] Cramer, W. A. The best offense is a good defense / W. A. Cramer, M. Lindeberg, R. Taylor // Nat. Struct. Biol. – 1999. – V. 6. – P. 295–297.

[2] Lazdunski, C. J. Colicin import into Escherichia coli cells / C. J. Lazdunski, E. Bouveret, A. Rigal, L. Journet, R. Lloubes, H. Benedetti // J. Bacteriol. – 1998. – V. 180. – P. 4993–5002.

[3] Cursino, L. Recent updated aspects of colicins of enterobacteriaceae / L.Cursino, J. Šmarda, E.Chartone-Souza1, A. M. A. Nascimento // Brazilian Journal of Microbiology – 2002. – V. 33. – P. 185-195.

[4] Kaper, J. B. Pathogenic Escherichia coli / J. B. Kaper , J. P. Nataro, H. L. T. Mobley // Nature Reviews Microbiology. – 2004. February – V. 2. – P. 123-140.

[5] Johnson, T. J. Pathogenomics of the Virulence Plasmids of Escherichia coli / T. J. Johnson, L. K. Nolan // Microbiology And Molecular Biology Reviews. – Dec. 2009. – V. 73, N. 4. – P. 750–774.

[6] Tobias, J. Simple and rapid multiplex PCR for identification of the main human diarrheagenic Escherichia coli / J. Tobias, S. R. Vutukuru // Microbiol. Res. – 2012. – V. 167, N 9. – P. 564-570.

[7] Kim, M. Bacterial Interactions with the Host Epithelium / M. Kim, H. Ashida, M. Ogawa, Y. Yoshikawa, H. Mimuro, C. Sasakawa // Cell Host Microbe. - 2010 Jul 22. – V. 8, N. 1. – P. 20-35.

[8] Ozeki, E. T. Production of colicin by single bacteria [Text] / T. E. Ozeki, H. Stocker, D. A. B. De Margerie // Nature. – 1959. – V.184. – P. 337-339.

[9] Papadakos, G. Nuclease colicins and their immunity proteins / G. Papadakos, J. A. Wojdyla, C. Kleanthous // Quarterly Reviews of Biophysics – 2012. – V. 45. – P. 57–103.

[10] Smarda, J. Incidence of colicinogenic strains among human Escherichia coli / J. Šmarda, V. Obdrzálek // J. Basic Microbiol. – 2001. – V. 41. – P. 367-374.

[11] Casali, N. E. Coli Plasmid Vectors: Methods and Applications / N. Casali, A. Preston // Humana Press. – 2003. – V. 235. – P. (316).

[12] Starlinger, P. IS-Elements in Microorganisms / P. Starlinger, H. Saedler / Biochimie. – 1972. V. 54. – P. 177-185.

[13] Pérès, S. Y. A new cytolethal distending toxin (CDT) from Escherichia coli producing CNF2 blocks HeLa cell division in G2/M phase / S. Y. Pérès, O. Marchès, F. Daigle, J. P. Nougayrède, F. Herault, C. Tasca, J. De Rycke, E. Oswald // MolMicrobiol. – 1997. Jun. – V. 24, N. 5. – P. 1095-107.

[14] Smarda, J. Colicins-exocellular lethal proteins of Escherichia coli / J. Smarda, D. Smajs // Folia Microbiol – 1998. – V. 43. – P. 563–582.