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The cytotoxicity effects of liable and stable enterotoxins produced by uropathogenic Escherichia coli

Assist. Prof. Dr. Muthanna Hamid Hassan

Department of Biology, College of Science, university of Anbar, Iraq

ABSTRACT

Objective: The aim of this study, detection of the stable and labile enterotoxins isolated from uropathogenic *Escherichia coli* and carry out cytotoxicity on cancer cell lines .

Methods: through the period extending from February 2019 till June 2019, 50 specimens of urine were collected from patients with urinary tract infections from two hospitals in Anbar and were collected in sterilized containers. Identification of *E. coli* was performed according to Bergy's Manual for Determinative Bacteriology, and API 20E system was used and confirmed by vitek2. Extraction of DNA was carried out by using Plasmid DNA kit (Gene aid, Taiwan) and PCR reaction was used. Two primers were utilized to detect the *lt* and *st* encoding genes. cytotoxicity Performed by MTT.

Results: Out of 50 samples, 22 were not *E. coli* (44%), while 28 were detected as *E. coli* (56%).Infection with the tested bacteria appeared in 6 patients out of 28 and they were males (21.43%), whereas, females were 22 (78.57%). The *st* gene was found in two isolates (7.14%), while no isolates were carrying *lt* gene. The supernatant of bacteria contained biological activity such as anticancer effects against human cancer cell lines such as HeLa, RD and normal cell line (REF).

Conclusion, it was clearly demonstrated that stable toxins had high inhibition ability against cancer cell lines .

Keywords: Escherichia coli , ST, LT, cytotoxicity.

Correspondence:
Assist. Prof. Dr. Muthanna Hamid Hassan
Department of Biology, College of Science, university of Anbar, Iraq

INTRODUCTION

Studies indicated that urinary tract infection is a dangerous and common bacterial disease for humans in the world, About more than 80% of UTIs are caused by Uropathogenic *E. coli* (UPEC) isolates [1]. Uropathogenic *E. coli* (UPEC) are one of the most prevalent strains in infections of the urinary tract [2]. It is generally accepted, that most of the UPEC strains live in the intestine and enter the urinary tract via the urethra. While acute urinary tract infections (UTIs) can be treated with common antibiotics, the chronic recurring UTIs are a bigger threat [3].

Enterotoxigenic *E.coli* (ETEC) recognised as a causative agent of traveller's diarrhoea which characterised by watery diarrhoea with little or no fever [4,5]. This type of diarrhoea is known to occur because of that the cyclic AMP (cAMP) level is known to be elevated which lead to secretion of large amount of electrolytes and water [6,7]. Polymerase chain reaction technique is one of the important methods that are used in ETEC identification in many cases [8].

MATERIAL AND METHODS

Collection of samples

Through the period extending from February 2018 till June 2018, about 50 samples of urine were collected from patients with urinary tract infections in sterilized containers.

Identification of *E. coli*

The samples were cultured on MacConkey, Eosin methylene blue and blood agar, then incubated at 37ºC for 24hrs. The identification of bacteria was performed by standard biochemical tests and confirmed by vitek2 and Api-20 E according to Bergy's Manual for Determinative Bacteriology [9,10,11].

DNA extraction

DNA extraction from uropathogenic *E. coli* was carried out according to the genomic DNA extraction kit (Gene aid, Taiwan). DNA concentration was between 50-56 µg/ml, while DNA purity was between 1.6-1.9.

Detection of Enterotoxin by PCR

Oligonucleotide primers were used to detect enterotoxigenic *E. coli* by amplifying a 696-708 bp of LT1, LT2 genes and 182-186 bp of ST1, ST2 genes [12,13]. As appeared in table (1)

Extraction of toxin from bacterial isolates

Freezing and thawing has been known to be the best method for extraction enterotoxins. Stable toxin was extracted from the isolates that contained *st* gene according to [14].

Cell lines and growth conditions

RD (Rhabdomyosarcoma), HeLa (cervical cancer) cells and normal cell line (REF) were used to determine the effect of enterotoxins extracts. These cells were cultured on RPMI and MEM media which were enhanced with penicillin-streptomycin mixture (1%) and FBS (10%). The experimental conditions was 37 °C and incubation at 5% CO_2 [15].

Cytotoxicity by MTT assay

Cytotoxic impact of the extracted toxin on proliferation of the adherent cells in 96-well microtiter plate was performed according to [16,17].

Statistical analysis

SPSS program was used for Statistical analysis [18].

RESULTS AND DISCUSSION

Isolation and identification

All specimens were cultured on MacConkey agar, EMB agar and blood agar plates. according to the growth characteristics about 28 (56%) samples were identified as *E. coli*. Also, when *E. coli* isolates were cultured on MacConkey agar and incubated at 37 °C for 24h, they

appeared as small, pink and lactose fermenter colonies, while on blood agar they appeared as creamy and α-hemolytic colonies. On EMB agar they appeared as green metallic shine colonies. As appeared in figure (1)

Current results showed that the UTI infections in females were more than that which occurred in males as 78.57% and 21.43% respectively and this can be explained by the anatomical differences between two sexes, so the shortness of female's urethra and Near Slot outlet are responsible to make it easy target of inflammation, while existence of zinc compounds within secretions of prostate gland provide protection for males from infection[19]. *st* gene was 2 out of 28 (7.41%), whereas, isolates did not possess *lt* toxin genes. It was found through the results that the two isolates produced heat- stable enterotoxins were present in the men's samples, while there was no product isolate in the urine samples taken from women despite the large number of *E. coli* isolates isolated from women. Whereas, the most dangerous isolates possessing heat- stable enterotoxins were only in men's urine samples . As appeared in table (2) .This study proved that the molecular method for detection of ETEC isolates is the active method for toxin identification. The results of this study are in agreement with Johnson *et al* [20] .

Cell viability assay

Cytotoxicity was researched by utilizing MTT stain. Dose and time dependency of the cytotoxic extract against HeLa, RD and normal cell line (REF). Cells were tested in three periods (24, 48 and 72 hrs.). As appeared in Figure (2), cancer cells viability was significantly inhibited according to time and dose treatment. It was shown that increasing of incubation time and dose of toxin extract led to decrease the RD and HeLa cancer cells viability and inhibition percentage were 46% and 36% respectively after 24hrs of incubation. Then again, it was shown that all incubation periods and concentrations that were used in this study indicated significant inhibition effect against HeLa ($P<0.05$) cells, and the highest dose (75µg/mL) in the greatest time (72hrs.) gave the best antiproliferative effect on HeLa cells.

The inhibition impact of the toxin extract was more noteworthy against HeLa more than against RD. Additionally the effect of toxin extract against REF normal cells was determent, and it was found that the toxin extract had effects against cancer cells but no effect against REF normal cells. Therefore, over 90% of the REF cells grew well. Production of pro-inflammatory cytokines, in addition to oxidative stress accepted by MTT have the responsibility to cause cells death [21]. The two isolates producing heat –stable enterotoxins were diagnosed by PCR and were of gene size 186, as shown in Figure(3)

Table 1: Primers used in PCR for amplification of Enterotoxigenic *E.coli* genes

Target gene	Primers sequences (5-3)	Amplicon size
ST	5`CTG-TAT-TGT-CTT-TTT-CAC-CT3` 5`GCA-CCC-GGT-ACA-AGC-AGG-AT3`	186
LT R	5`GCG-ACA-AAT-TAT-ACC-GTG-CT3` 5`CCG-AAT-TCT-GTT-ATA-TAT-GT3`	708

Table 2: Number and percentage of infected *Ecoli*

Sex	N. of patient	N. infection with E coli	
		number	Percentage
Male	16	6/28	21.43
Female	34	22 /28	78.57
Total	50	28	100%

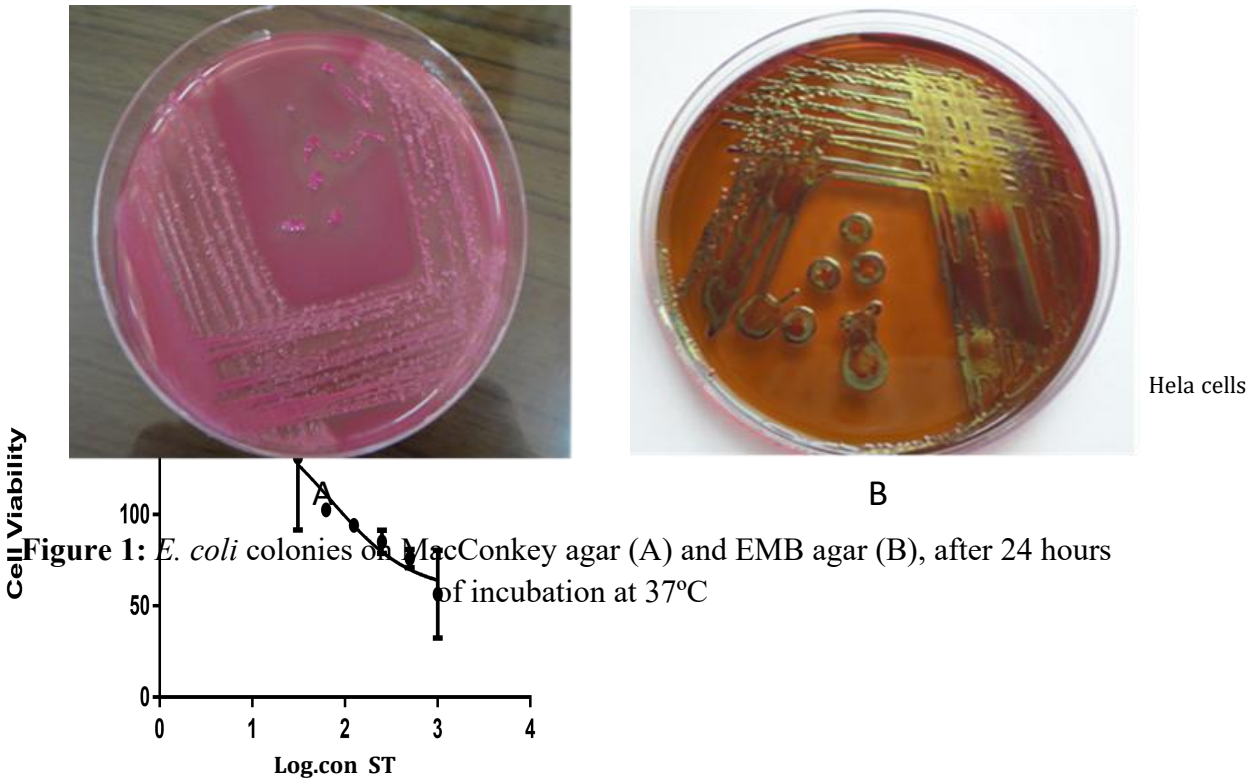

Figure 1: *E. coli* colonies on MacConkey agar (A) and EMB agar (B), after 24 hours of incubation at 37ºC

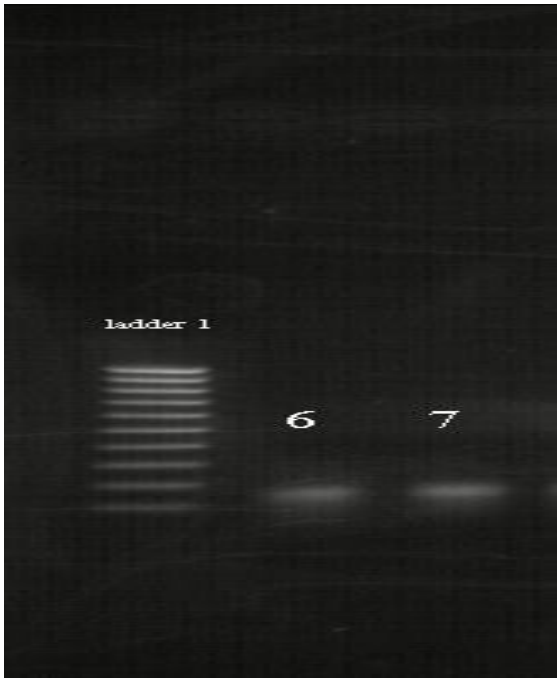

Figure (3) : Agarose gel electrophoresis of enterotoxin genes produce fragments that amplified by specific primers STI, STII. of heat-stable enterotoxin. Fragments were fractionated by electrophoresis on 1.5% agarose at 80v for 1 hr and visualized by Ethidium bromide.

CONCLUSION

it was clearly demonstrated that heat-stable enterotoxins are present in *Escherichia coli* isolates from urine samples men only and had high inhibition ability aginst cancer cell lines .

Conflict of interest

No conflict of interest

Funding: Self

Ethical Clearance: This study is ethically approved by the Institutional ethical Committee.

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