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Original Article

Identification and Assay of Putative Virulence Properties of *Escherichia coli gyrase* Subunit A and B among Hospitalized UTI Patients in Bangladesh

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Abstract

The effect of virulence genes are both multi-functional and multi-factorial and thus create all major components in the pathogenicity and virulence properties of any isolate. In this study, the *gyrA* and *gyrB* gene encoding *gyrase* specific gene primer allows precise detection of DNA *gyrase* subunit A and B2 (*gyrA* and *gyrB*) of *Escherichia coli* by polymerase chain reaction (PCR). All isolates of *E. coli* were collected from patients suffering from urinary tract infection (UTI). A total of 10 isolates *viz.*, EcoU1, EcoU2, EcoU3, EcoU4, EcoU5, EcoU6, EcoU7, EcoU8, EcoU9, EcoU10 were used in present study in which *gyrA* gene was amplified in 7 isolates (*viz.*, EcoU2, EcoU4, EcoU5, EcoU6, EcoU8, EcoU9, EcoU10) with expected PCR product of 441bp and *gyrB* gene was amplified in all 10 tested isolates and gave the expected 1130bp PCR product after visualization under gel documentation system. At the same time 10 samples were examined *in-vitro* for their putative virulence characteristics *viz.* proteolytic, lipolytic and hemolytic activity. High hemolytic activity was observed for isolates containing both *gyrA* and *gyrB* gene with significant P value of <0.05. Moderate proteolytic and lipolytic activity was observed for isolates containing either *gyrA* or *gyrB* gene with less significant P value of <0.004 to <0.002 respectively.

Keywords: *Escherichia coli,* UTI, *gyrA, gyrB,* PCR detection, virulence factors.

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1. Introduction

E. coli is numerous in all types of environments, cause food spoilage and poisoning and has been associated with a number of diseases in animals and human body as an opportunistic pathogen [5]. Among them urinary tract infection is the most common and 80% of global UTI infection caused by *E. coli* [21]. The disease can spread through

contaminated food products and in some instances from animal contaminates infected with *E. coli* [1]. Epidemiology and pathogenic process of UTI are dictated by an array of factors that act in tandem and ultimately manifest in the typical symptoms of this disease. Among the factors, virulence gene is the vital one. Generally, virulence genes encode products that assist the organisms in expressing its virulence in the host cells [16]. DNA *gyrase* [Eco DNA topoisomerase II (1)] is an ATP-requiring enzyme essential for DNA replication in *E. coli* that introduces negative supertwists into closed duplex DNA, removal of positive super coils ahead of the growing DNA fork, and separation of the two daughter duplexes [7]. *E. coli* have two subunit of DNA *gyrase*: subunit A and B. *Gyrase* catalyzes DNA supercoiling by an interesting and unusual mechanism. It passes a duplex DNA segment through a transient double-stranded DNA break made within a 120 to 150 base-pair (bp) loop of DNA wrapped on the surface of the tetrameric A2B2 *gyrase* complex [12, 14]. Thus, the A subunits appear to promote DNA breakage and reunion during catalysis, a process interrupted by quinolone inhibitors. In contrast, the B subunits bind ATP and are the locus of action of coumarin antibiotics such as novobiocin [14, 23]. The A and B subunits can be individually purified but must be combined to generate the topoisomerase activities of *gyrase* [10]. Both subunits are essential for cell viability and involve in separation of the duplex strands, including DNA replication and transcription and pose β-hemolytic activity: ability to lyses blood agar and thus may contribute into the virulence properties of any isolates [8]. Amplified *gyrB* gene product also been used as a tool in characterization of bacteria and molecular phylogenetic analysis in enterobacteriaceae [3].

2. Materials and Methods

Collection of Bacterial Isolates:

All (10) bacterial isolates of *E. coli* were collected from four different medical colleges and hospitals and diagnostic centers of Sylhet district, Bangladesh *viz.* M. A. G. Osmani medical college and hospital (isolates EcoU1, EcoU3, EcoU7, EcoU10), Popular hospital and diagnostic centre (isolates EcoU5, EcoU6), Ibn Sina hospital and diagnostic centre (isolates EcoU4, EcoU8) and Ragib-Rabeya medical college and hospital (isolate EcoU2, EcoU9). All isolates were collected from UTI patients.

Culture Condition:

The bacterial isolates were streaked in nutrient agar plates and incubate in 37ºC for overnight for appropriate colony formation. After the incubation the single colony of each plate was subjected to re-isolation for pure culture in nutrient agar plate. A single colony was taken carefully and inoculated into nutrient broth for isolation of bacterial genomic DNA.

DNA Extraction:

A total of 10 bacterial isolates were inoculated in nutrient broth culture and incubated overnight at 37ºC and 150rpm in a shaker incubator. The bacterial genomic DNA was extracted by using commercial Genomic DNA Extraction Kit (Bio Basic Inc., 160 Torbay Road, Markham Ontario, Canada) and extracted DNA was preserved at -20ºC in an ultra freezer for further use.

PCR Reaction Mixture Set Up:

The PCR was performed in 25µl reaction mixtures containing DNA template (genomic DNA of bacteria) of 1.2µl, 1µl of 25 mM $MgCl_2$, 5µl of 5x colorless reaction buffer, 0.5 µl concentration of deoxynucleotide triphosphate (dNTP), 1.2µl of each forward primer and reverse primer and 0.15µl DNA polymerase. The amplifications were carried out in a MultiGene gradient thermal cycler (Labnet International Inc. USA).

Amplification Condition:

PCR reaction was optimized with the following parameters: an initial denaturation step of 94°C for 4 min; a denaturation step of 94°C for 1 min, annealing at 58°C for 1 min for g*yrA* and 64ºC for 1min for *gyrB*, and extension at 72°C for 90s; and a final extension step of 72°C for 10 min. 35 serial cycles of reaction was performed.

***In-vitro* Proteolytic Activity:**

The *in vitro* proteolytic activities of the selected strains were measured by using casein as protein source. The activity was detected by casein hydrolysis on agar plates containing YNB (DIFCO) medium supplemented with 0.5% of casein, 0.5% of

Target gene	Primer	Sequence (5'-3')	Product length	Reference
Gyrase Subunit-A	gyrA-F gyrA-R	TCCTATCTTGATTACGCCATG CATGCCATACCTACCGCGAT	441bp	[24]
Gyrase Subunit-B	gyrB2-3F gyrB2-14R	TCCGGCGGTCTGCACGGCGT TTGTCCGGGTTGTACTCGTC	1130bp	[4]

Table no 1: Primer used for Present Study

glucose, and 2% of agar, pH 7.0.Then the plates with bacterial culture were incubated at 37˚C for 72h. Enzyme activity was indicated by the formation of a clear zone around colonies after precipitation with 1 M HCl solution [20]. (Table no. 1)

***In-vitro* Lipolytic Activity:**

Lipolytic activity was assessed by streaking all of the isolates onto Tween 20 (T20) agar [containing (g/l): Peptone, 10; NaCl, 5; CaCl2, 0.1; agar, 20; Tween 20, 1 mL per liter added after autoclaving] and incubating the plates at 37ºC for 72 h. Lipase activity was determined by measuring the halos of precipitated Tween around the streaks [13].

***In-vitro* Hemolytic Activity:**

Hemolytic activity of the isolates was assessed after 48 h incubation at 37ºC on TSA supplemented with 5% (v/v) sheep's blood, isolates being classified according to the diameter of the halos of hemolysis. The zone ratios were calculated as mentioned by [6].

Statistical Analysis

To establish the significance of the results, the Fisher exact test, the Chi-square test, or the Kruskal-Wallis test was used as apposite. The level of significance was set at a P value of <0.05 which only found for isolates containing both *gyrA* and *gyrB*. Thus isolates with both *gyrA* and *gyrB* were considered statistically significant to indicate the association between both variables.

3. Results

Amplified PCR products were detected by agarose gel electrophoresis of each of amplification mixture in 1.2% agarose gels in 0.5% Tris-borate-EDTA (TBE) buffer. Gel was then stained with ethidium bromide solution (10mg/ml) for 30min. The position of each band on gel then visualized and documented in a gel documentation system. Among 10 isolates of *E. coli*, *GyrA* gene has been amplified in 7 isolates *viz.*, EcoU2, EcoU4, EcoU5, EcoU6, EcoU8, EcoU9, EcoU10 with expected PCR product of 441bp and *gyrB* gene amplified in all 10 tested isolates and gave the expected PCR product length of 1130bp (Figure. 1 and 2).

***In vitro* Proteolytic Activity:**

In present study, proteolytic activity fluctuates (Figure. 3) from isolates to isolates but two methods exhibited same result for each of the tested isolates. High proteolytic activity was observed for isolates EcoU9 (11.5mm), EcoU10 (9.2mm), EcoU8 (8.6mm), EcoU4 (7.8mm), whereas isolates EcoU2 (6.6mm), EcoU1 (6.4), EcoU5 (6.4mm), EcoU6 (6.2mm) showed moderate proteolytic activity. Low proteolytic activity was counted for isolates EcoU7 (5.8mm) and EcoU3 (5.2mm).

***In vitro* Lipolytic Activity:**

In the present study, all isolates exhibited positive result for the *in vitro* lypolytic activity (Figure. 3). The highest lypolytic activity was observed for the isolate EcoU10 (8.2mm), EcoU4 (8.2mm) and EcoU2 (8.0mm) followed by EcoU9 (7.8mm), EcoU5 (7.7mm),

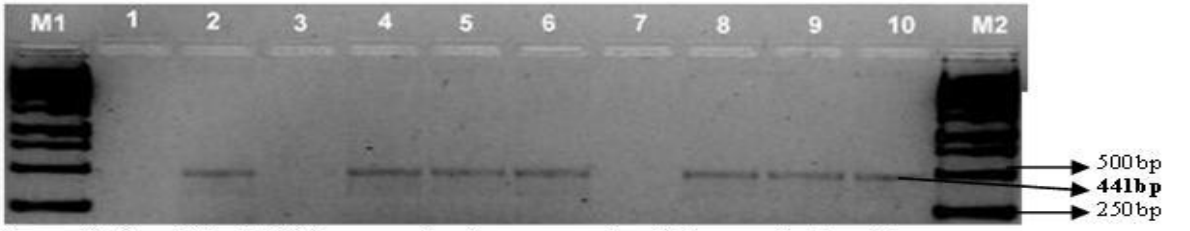

Figure 1. Amplified PCR fragments of *gyrase* subunit A gene in *E. coli*.

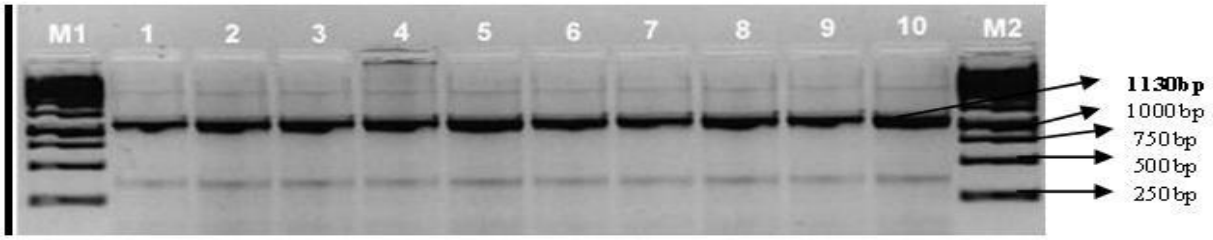

Figure 2. Amplified PCR fragments of *gyrase* subunit B gene in *E. coli*.

M1 and M2= 1kb DNA ladder (Fermentus, GeneRuller™)
Lane 1-10= EcoU1, EcoU2, EcoU3, EcoU4, EcoU5, EcoU6, EcoU7, EcoU8, EcoU9 and EcoU10.

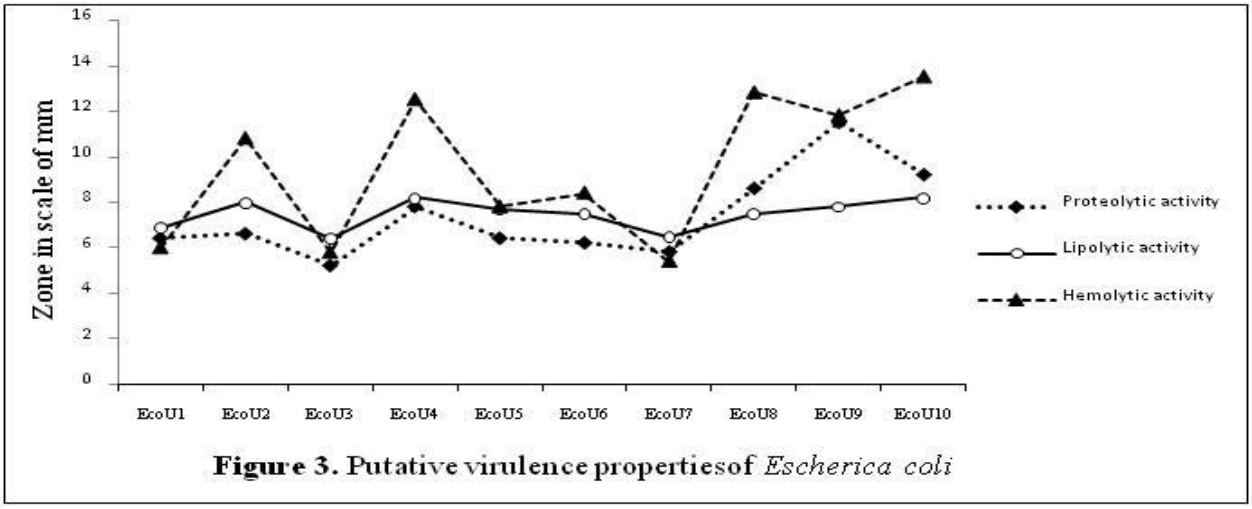

Figure 3. Putative virulence propertiesof *Escherica coli*

EcoU6 (7.5mm), EcoU8 (7.5mm), EcoU1 (6.9mm), EcoU7 (6.5mm) and EcoU3 (6.4mm).

***In vitro* Hemolytic Activity:**

E. coli showed a divergence pattern of hemolytic activity (Figure. 3) on red blood cell (RBC). High hemolytic activity was observed for isolates EcoU10 (13.5mm), EcoU8 (12.8mm), EcoU4 (12.5mm), EcoU9 (11.8mm) and EcoU2 (10.8mm). On the other hand low hemolytic activity was recorded for isolates EcoU6 (8.4mm) and EcoU5 (7.8mm) while EcoU1 (6.0mm), EcoU3 (5.8mm) and EcoU7 (5.4mm) posed very low hemolytic activity when compared with other *E. coli* isolates.

Statistical Importance

From the statistical analysis, it was clearly noticed that hemolytic activity has the higher contribution (P value of <0.05) in the progression of *E. coli* associated disease followed by lipolytic activity (P value of <0.004). While proteolytic activity comparatively has lower level of significance (P value of <0.002).

4. Discussion

E. coli species are found to be associated with a wide range of infection, both in human and other animals. Among those, typhoid fever and urinary tract infections are two most common illnesses caused by *E. coli* and account for 75%-90% of total UTI infections [21]. DNA *gyrase* is an essential enzyme that helps maintain the equilibrium level of negative supercoiling in the *E. coli* chromosome [18]. Pathogenesis of *E. coli* infection depends upon a large number of factors, products or distinct bacterial properties controlled by an array of genes that contributes into the actual virulence of *E. coli* species because they help the organism overcome host defenses and colonize or invade the urinary tract [11]. In present study, *gyrA* gene amplified in 7 isolates *viz.*, EcoU2, EcoU4, EcoU5, EcoU6, EcoU8, EcoU9, EcoU10 with expected PCR product of 441bp whereas *gyrB* gene amplified in all 10 tested isolates and gave the expected PCR product length of 1130bp. *Gyrase* subunit A (*gyrA*) bearing isolates exhibited higher proteolytic lipolytic and hemolytic activity compared to non-*gyrA* bearing isolates. On the other hand, *gyrB* gene is a single-copy gene, present in all bacteria which encode the ATP*ase* domain of DNA *gyrase*, an enzyme essential for DNA replication [17]. Two subunits of DNA *gyrase* can be found together in *E. coli* where both pose hemolytic activity and an alteration or mutation in subunit A can also reflect in subunit B [2]. This reports revealed its contribution to disease progression with β-hemolytic activity as like it contribute in *Klebsiella* spp. [22] and *Pseudomonas* spp. [4]. Earlier studies reported that *gyrase* subunit B gene contribute the major virulence properties of many bacterial species and has been used as a molecular tool for identification of bacterial species and phylogenetic analysis [3, 25]. *GyrB* gene also contributes in progression of enterobactericeae associated disease in human [19]. It has been reported that the isolates containing *gyrase* subunit A has the ability to haemolyse sheep blood [8] and *gyrase* subunit B encodes sequence pose β-hemolytic activity in horse/sheep blood agar [9]. This result indicates that *gyrA* together with *gyrB* possibly contribute the disease progression process of urinary tract infection. In conclusion, both *gyrA* and *gyrB* may have significant contribution in disease progression process of UTI. Because of *E. coli* infection and occurrence of resistance to commercial antibiotics increases day by day [5], this report will help researchers for further molecular investigation of virulent genes or factors in contribution of disease *E. coli* associated disease prophylaxis and treatments.

Conflict-of-Interest:

The authors declare no conflict of interest including any financial, personal or other relationships with other people or organizations for the study.

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