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Molecular Typing the Uropathogenic*Escherichia coli*Clinical Strains using ERIC1b-PCR, and PCR Detection of three Candidate Biomarkers ChuA, YjaA, and TspE4C2

Hameedah Hamzah Ajeel [1], Izdihar Abbas Alwan [2], Sarah KassabShandaway [3]

1Faculty of VeterinaryMedicine , Microbiology Department, AL-Qasim Green University, Babylon , Iraq.

2Faculty of Biotechnology, Biotechnology Department, AL-Qasim Green University, Babylon, Iraq.

3Faculty of Nursing ,Medical Microbiology Department, Telafer University, Telafer, Iraq.

*Corresponding author:

Hameedah Hamzah Ajeel **:**Faculty of Veterinary Medicine , Microbiology Department, AL-Qasim Green University, Babylon , Iraq

Email: hameedahhamza@vet.uoqasim.edu.iq

ORCID =0000-0003-1878-5048

Abstract

The present work does address the molecular typing of ten uropathogen clinical strains isolated from urinary tract infections (UTIs) using: ERIC1b-PCR and PCR detection of ChuA, YjaA, and TspE4C2 virulence genes. Ten out of fifty *E.coli*uropathogen clinical strains were isolated from patients diagnosed as UTIs post culturing of urine samples onto blood agar and Typtone Bile X-Glucuronide (TBX) agar plates. Results of API20E system verified the identity of these ten uropathogens as *E.coli*. ERIC1b-PCR demonstrated high discrimination power to sort the ten clinical strains into six groups according to the obtained banding pattern of DNA. The frequency of occurrence of the three biomarkers ChuA, YjaA, and TspE4C2 among the ten uropathogenic*E.coli* clinical strains was 100%, 90, and 90%, respectively. The phylogenetic grouping of the ten uropathogenic*E.coli* strains based on the presence of these biomarkers revealed 9 strains belonged to group B2 (subgroup B23 : chuA+, yjaA+, TspE4.C2+) and only one strain belonged to group D (subgroup D1: chuA+, yjaA-, TspE4.C2-). The present data would support the superior potential of ERIC1b-PCR for rigorous differentiation among uropathogen*E.coli* clinical strains in comparison to the potential of Clermont et al method .

Keywords: E.coli; UTIs; ERIC1b-PCR; ChuA; YjaA; TspE4C2

Introduction

Escherichia coli is mostly associated with infections in human; intestinal and extraintestinal ones worldwide. These intestinal and the extraintestinal bacteria are abbreviated as IPEC and EXPEC, respectively. The extraintestinal pathogenic E.coli (EXPEC does encompass three main kinds: uropathogenic, sepsis-causing, and neonatal meningitis-causing E. coli,(UPEC), (SEPEC), and(NMEC), respectively. The majority of urinary tract infections (UTIs) in human is accompanied by UPEC (Foxman et al. 2000). Previous epidemiological study addressed that around 50% of nosocomial UTIs (hospitalized inpatients) and 90% community acquired UTIs (non-hospitalized outpatients) are correlated with UPEC allover the world (Kucheria et al. 2005).

This high prevalence and incidence of UPEC addressed the indispensable need to deeply understand the molecular mechanisms underlying this unique epidemiological profile in this pathogen; mainly in the zone of nosocomial UTIs, hospitalized inpatinets and non- hospitalized outpatients. Mostly, the fecal commensal flora is the chief resource for the UPEC strains (Moreno et al. 2008; Russo et al. 1995). The UPEC shows a distinctive pattern regarding the virulence genes in comparison to that of the commensal E.coli (Johnson et al. 2005; Wiles et al. 2008). Though, UTIs could be initiated by typical and atypical UPEC strains. The atypical UPEC could harbor unusual virulence genes repertoire. This might be due to the noteworthy genome plasticity that facilitate the alterations of different virulence genes. Hence, the likelihood of emergence of atypical UPEC strains with unambiguous non-discriminated pattern from DNA fingerprint profile from IPEC and EXPEC (Müller et al. 2007). As a rule of thumb, UPEC based clones would stay for long periods as intestinal tract commensal bacteria and can be transferred among members of families (Johnson et al., 2008; Murray et al., 2004).

At most, Escherichia coli strains do belong to four phylogenetic categorized groups namely: A, B1, B2 or D (Herzer et al., 1990; Ochman et al., 1984; Selander et al., 1986). Each one of these groups has a distinct ecologicalniche (Gordon and Cowling, 2003) with a different ability to usesugars and antibiotic profile of resistance (Gordon 2004).

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With less potency of gaining membership in the D group, EXPEC has strong adherence to the B2 group (Johnson and Stell 2000; Picard et al., 1999), while A and B1 has the commensal bacteria (Bingen et al., 1998); however, IPEC bacteria are categorized A, B1 and D groups (Pupo et al., 1997). Clermont et al. (Clermont et al., 2000) have developed a PCR instructionsfor phylogenetic-classifying these groups depending on chuA and yjaA genes and TspE4.C2DNA fragment, resulting in subgroups (A0, A1, B1, B22, B23, D1, and D2) based on the use of combinations of these genetic marks (Escobar-Páramo et al., 2006).

ChuA is the haem receptor of the bacterium encoding 69 kDa protein carried on the chromosome and is believed to act a major role in the virulence of this bacterial extraintestinal illnesses (Carlos et al., 2010).

In the aspect of observing the DNA pattern of the uropathogen clinical strains in Iraq, the objective of the current work is to DNA type the uropathogenicE.coli clinical strains (EXPEC) isolated UTIs admitted in Babylon hospitals, Iraq through ERIC1b-PCR and PCR detection of ChuA, YjaA, and TspE4C2 biomarkers .

Patients and methods

Urine samples

During the period of September 2020 to December 2020, fifty urine samples were gathered from patients diagnosed as UTIs, admitted in Babylon hospitals, Iraq. Before the urine sampling, the following characteristics were considered: hematuria, dysuria, proteinuria, and greater than 100 colony forming units (CFU) of bacteria/mL urine. The Urine specimens were collected from the mid-stream by catheterization after intensive antisepsis of the genital area.

Media

Sheep blood agar plates (HiMedia, Canada) were employed in culturing of bacteria from collected urine samples. Typtone Bile X-Glucuronide (TBX) agar plates (HiMedia, Canada) were used in presumptive identification of the bacterial strains as *E.coli* .

Primers and molecular biology reagents

All primers used in this study were synthesized by Integrated DNA Technology, USA. The 2X MyTaq master mix was purchased from Thermo Scientific Fisher Co., USA. DNA ladder, agarose, 6x-gel loading dye, and ethidium bromide were purchased from Bioline Co., USA.

Isolation and identification of bacteria from urine samples

Ten microliters urine samples were overlaid by a micropipette onto the surface of sheep blood agar plates, which were 24h-incubated at 37˚C until the emergence of visible bacterial growth on the plates' surfaces. The presumptive identification of *E.coli* clinical strains was conducted by culturing the suspected colonies on Typtone Bile X-Glucuronide (TBX) agar plates for a period of 24hrs at 37°C. The emergence of blue to blue green colonies on the surface of TBX agar plates was taken as indicative for the strains of *E.coli*. The confirmed identification of this bacteriumimportant clinical strains was performed by the aid of observing the typical profile of *E.coli* on API20E (BioMérieux, Marcy l'Etoile, France system) and IMVIC test).

Genomic DNA

The Genomic DNA isolation was carried out from all clinical strains under study by the QIA amp DNA Mini kit (Qiagen, USA) considering the protocol criteria of the manufacturer. The DNA produced materials were checked for quality on % agarose gel and the visualization was carried out by ultra-violet (UV) visualization (UV-Transilluminator, Clever, UK). Whilst, the concentration of genomic DNA was determined by Nano-drop Spectrophotometer (Applied Biosystem, USA).

ERIC-PCR technique

ERIC1b-PCR was used in this study for DNA-profiling of ten clinical uropathogenicE.coli strains; using the ERIC-1b primer: 5'-ATGTAAGCTCCTGGGGATTCAC-3' [Versalovic et al. 1991]. Shortly, the PCR mixture for ERIC1b-PCR included 25 µL of PCR master mix (MyTaq, USA), 1.5 µL (1.5 µM) primer, 11 µL of distilled H_2O, and 2.0µL DNA at 100ng. The thermocyclcer (Biometra, Germany) was programmed as follow: an initial denaturation step at 94 ºC for 2 min, 45 cycles each cycle 94 ºC for 1 min, 40 ºC for 1 min, 72 ºC for 5 min, and a final extension at 72 ºC for 10 min. The resolution and separation of the obtained ERIC1b DNA fragments were conducted on 1.5% agarose gel electrophoresis inpresence of 100 bp DNA ladder (Bioline, USA) at 75 Volt for 45 min using DNA submarine unit (ATTO Co., Japan). Determination of the length of each DNA fragment resulted from ERIC1b-PCR was performed by the calibration to the standard curve of the 100 bp DNA ladder; running parallel with ERIC1b-PCR products on 1.5% agarose gel electrophoresis .

PCR detection of ChuA, YjaA, and TspE4C2 virulence genes

The tracing of the three virulence markersChuA, YjaA, and TspE4C2 was conducted on uropathogenic clinical strains under study by PCR partial amplification of each gene using three gene specific primers

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sets: F-ChuA: 5'GACGAACCAACGGTCAGGAT-3'/R-ChuA:5'TGCCGCCAGTACCAAAGACA -3'(Clermont et al., 2000), F- YjaA:5'-TGAAGTGTCAGGAGACGCTG-3'/R- YjaA:5'-ATGGAGAAATGCGTTCCTCAAC-3'(Clermont et al., 2000),andF- TspE4C2:5'-GAGTAATGTCGGGGCATTCA-3'/R-TspE4C2:5' CGCGCCAACAAAGTATTACG-3' (Clermont et al., 2000). Three polymerase chain reactions were performed for each clinical strain separately using the aforementioned primers. Each PCR reaction mixture (25µL) consisted of 30 ng genomic DNA, 0.15 µM each forward and reverse primer, 12.5µL of PCR Master mix (2X) (MyTaq, USA).The cycling conditions in the PCR thermocycler (Biometra, Germany) were: 95 oC, 5 min for initial denaturation, 30 cycles each cycle: 94 oC, 45 sec for denaturation, 58 oC, 45 sec for annealing, 72 oC, 50 sec for extension, and 72 oC, 10 min for final extension. The PCR produced elements were traced on electrophoresed 1.5% agarose gelUV-visualized. The expected lengths of the elements were: 279, 211, and 152 bp, resulting from the partial amplification of ChuA, YjaA, and TspE4C2 virulence genes, respectively .

Results

Identification of the *E.coli*uropathogenic clinical strains

Ten uropathogenic clinical urine strains were isolated from patients hospitalized in Babylon hospitals, from September 2020 to December 2020. The ten uropathogenic*E.coli* clinical strains displayed a typical pattern of IMVIC test, indole positive, methyl red positive, Voges-Proskauer negative, and citrate utilization negative. Moreover, the confirmatory test conducted by API20 E test on the ten uropathogenicE.coli strains evidenced typical profile for *E.coli*. Accordingly, the collected laboratory data did verify the identity of the clinical strains of quest as E.coli. Furthermore, the ten *E.coli*uropathogenic strains were named as Ec1 to Ec10 .

Phylogenetic group determination

The distribution of ChuA, YjaA, and TspE4C2 virulence markers in the ten uropathogenicE.coli clinical strains was investigated by partial amplification of each gene using uniplex PCR. For ChuA, all tested uropathogenicE.coli clinical strains displayed PCR product with the expected size of 279 bp (Fig.1). Conversely, the other two virulence genes YjaA, and TspE4C2 nine out of ten clinical strains demonstrated PCR products with the expected sizes of 211 and 152 bp, respectively as shown in Fig.1. Consequently, the frequency of occurrence of ChuA, YjaA, and TspE4C2 among the ten tested strains was 100 % (n=10/10), 90 % (n=9/10), and 90% (n=9/10), respectively. Based on these findings, nine out of ten uropathogenic EXPC strains belonged to group B2 (subgroup B23 : chuA+, yjaA+, TspE4.C2+) and only one strain belonged to group D (subgroup D1: chuA+, yjaA-, TspE4.C2.(-

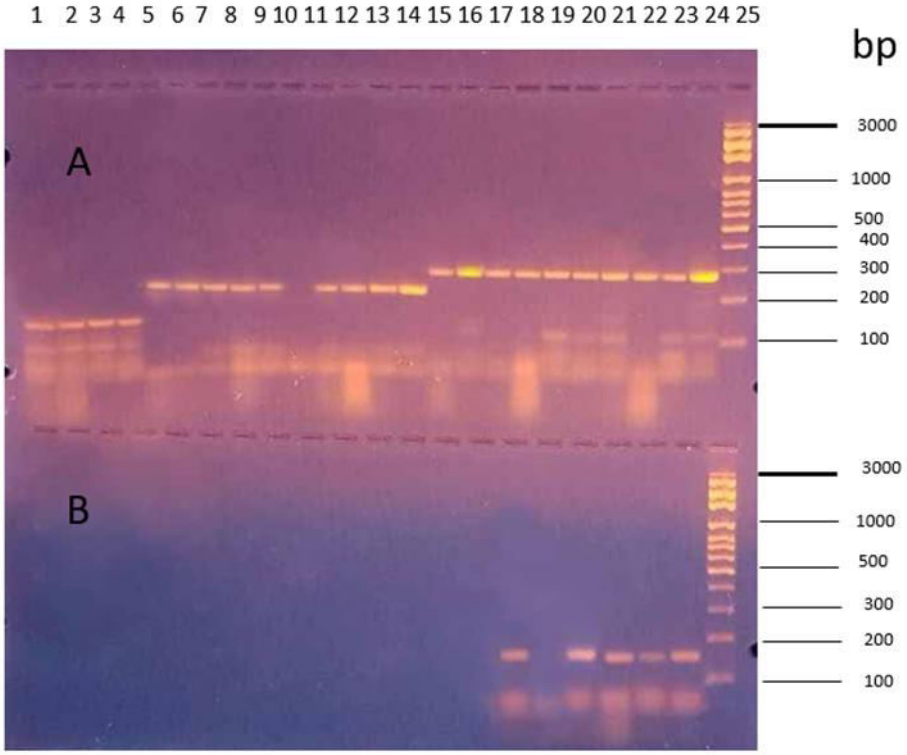

Fig 1: Agarose gel electrophoresis (1.5%) displaying the PCR produced elements of ChuA, YjaA, and TspE4C2 after partial amplification using gene specific primers from the ten uropathogenE.coli strains.

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Panel A, Lane 25: 100 bp DNA ladder. Lanes (15-24): PCR products of ChuA gene partial amplification (279 bp) from ten E.coliuropathogenic clinical strains named Ec1 to Ec10 serially. Lanes (5-14): PCR products of YjaA gene partial amplification (211 bp) from ten E.coliuropathogenic clinical strains named Ec1 to Ec10 serially. Lanes (1-4): PCR products of TspE4C2 gene partial amplification (152 bp) from four E.coliuropathogenic clinical strains named Ec1 to Ec4 serially. Panel B, Lane 25: 100 bp DNA ladder, Lanes (19-24): PCR products of TspE4C2 gene partial amplification (152 bp) from six E.coliuropathogenic clinical strains named Ec5 to Ec10 serially.

Molecular typing using ERIC1b-PCR

The ten uropathogenic*E.coli* clinical strains were grouped in clades based on their ERIC1R-PCR banding pattern displayed in Fig 2 and Table 1. ERIC1R-PCR showed a large number of DNA bands (8-12) in some E.coli strains named Ec2, Ec3, Ec6, and Ec10. Conversely, the remaining strains showed a small number of ERIC1R-PCR banding pattern (3-5) in Ec1, Ec4, Ec5, Ec7, Ec8, and Ec9. The molecular weight of each DNA band in the ERIC1R-PCR pattern was identified using DNA standard curve (Fig 3, Table 1). The ERIC1R-PCR could cluster the ten uropathogenic clinical strains into six clades (clusters) with five different ERIC profiles named P1to P6. The most frequent ERIC1b bands were 3000, 1500, and 1000 bp demonstrated in almost all E.coli strains (i.e., in the five detected clusters) (Fig.2 and Table 1). In contrast, the lowest frequently identified band was 270 bp; existed in the cluster P1 (Table 1).

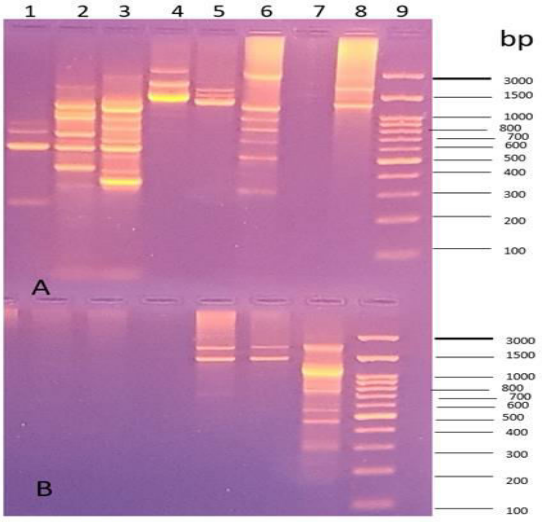

Fig 2: Agarose gel electrophoresis (1.5%) displaying the banding pattern of ERIC1R-PCR for the ten uropathogenicE.coli clinical strains using ERIC-1R. Panel A, Lane 9: DNA ladder, Lanes (1-6, 8): ERIC1R-PCR DNA banding pattern for the seven E.coli clinical strains named Ec1 to Ec7 serially. Panel B, Lane 9: 100bp DNA ladder, Lanes (6-8): ERIC1R-PCR DNA banding pattern for the three E.coli clinical strains named Ec8 to Ec10 serially.

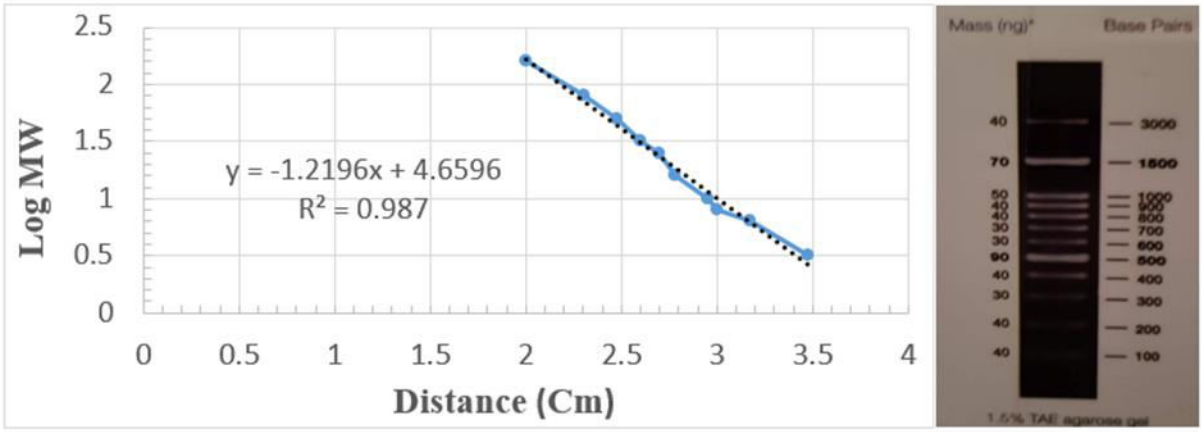

Fig 3: A standard curve, drawn by Excel, for 100 bp DNA ladder (abm, Canada). The R2 value was near to 1.0 that indicated the small variation between the predicted values and the experimental values. MW: molecular weight of DNA ladder bands.

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Table 1: ERIC1R-PCR profile and molecular weight of DNA bands for the ten uropathogenicE.coli clinical strains

DNA band size (bp)	***E.coli* strains with DNA band size**	**ERIC1R-PCR profile no in relation to the strain**
270, 600, 800, 1000	Ec1	P1
2000, 1500, 1300, 1200, 1000, 800, 600, 500, 300	Ec2	P2
3000, 1500, 1300, 1200, 1000, 800, 700, 600, 500, 450, 400, 350, 302	Ec3	P2
3000, 2000, 1500, 1000, 800, 600, 500, 300	Ec6	P3
2000, 1000, 1200, 800, 600, 500, 300	Ec10	P3
2000, 1300	Ec7	P4
2000, 1300	Ec8	P4
2000, 1300	Ec9	P4
1500, 1400, 1250	Ec4	P5
3200, 2000, 1500, 1000	Ec5	P6

Discussion

The EXPEC E.coli strains have exhibited a high prevalence of occurrence among UTIS worldwide. Several studies have addressed the search for a powerful method to help discriminate among these uropathogenic EXPEC strains. The molecular method that has the potential to differentiate between two very closely related EXPEC uropathogenic strains is considered the gold standard method in this issue. From the standpoint of epidemiology, sorting the uropathogemicE.coli strains prevailing in a certain country is a good step on the way to control their prevalence and avoid the emergence of multidrug resistant strains derived from their progeny. In this context, the objective of the current work is to compare between two well established molecular methods: ERIC1b genotyping and Clermont et al method (Clermont et al., 2000) for phylogenetic group of ten EIPEC uropathogenicE.coli strains isolated from UTIs in Iraq. As per our findings, the Clermont et al., method succeeded to sort the ten uropathogenic strains into two groups. The group B2 was the major group included 9 strains; group B2 (subgroup B23: chuA+, yjaA+, TspE4.C2+) and the group D was the minor group included only one strain; group D (subgroup: chuA+, yjaA-, TspE4.C2-). Conversely, the ERIC-1b technique was successful to sort the ten uropathogenic strains into 6 group namely P1-P6 .

Ramazanzadeh et al. did use the ERIC1b-PCR to understand the genetic-based diversity among EXPEC E. coli clinical strains from hospitalized inpatients (Ramazanzadeh et al., 2013). According to their work, 230 clinical strains and 205 were successfully grouped in 20 cluster groups, in whichhigh number is being categorized to the D group (Ramazanzadeh et al. 2013). Another study did conclude that fingerprinting technique ERIC1b-PCR has a high potential in the grouping of human pathogens (Versalvic et al., 1991). A previous study addressed the ERIC1b- PCR method to genotype 49 clinical strains recovered from chickens with septicemia with clustering into four main clusters, A to D (da Silveira et al. 2002). The ERIC1b-PCR method did succeed to genotype 37 E. coli clinical strains isolated from cows as follow: 7 and 17 clinical strains in the A and B groups, respectively (Prabhu et al. 2010). A previous study highlighted the genotyping enterotoxigenic E. coli strains from cows with affiliation to the phylogenetic group D (Xiu-Yan Lang et al., 2013.(

Our findings did confirm that ERIC PCR procedure was a quick, reproducible tool for exploring the genetic-based diversity among different EXPEC clinical. We can conclude that the Clermont et al., method could be exploited for the discrimination of E.coli clinical strains on the level of the four

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reported phylogenetic groups namely A, B1, B2, and D. Further subtyping and discrimination among the clinical strains belonging to any of these phylogenetic group should depend on another method with superior discrimination power like ERIC1b-PCR. Conclusively, the present study does recommend the ERIC1b-PCR as the gold standard methodology for discrimination among the clinical strains of E.coliuropathogens. In addition, meta-analysis data would greatly support our conclusion regarding the superiority of ERIC1b-PCR method in the discrimination of E.coli clinical strains in comparison to another methods.

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