Original Article

Molecular Detection of *PAIsII*$_{J96}$ and *PAIs*$_{III536}$ Genes among *Escherichia coli* Isolates, Babylon, Iraq

Yasameen Riyadh Saeed Al-Azzawi[1,2], **Ibtisam Habeeb Al-Azawi**[1]

[1]Department of Medical Microbiology, College of Medicine, Al-Qadisiyah University, Al Diwaniyah, Iraq, [2]Department of Anesthesia Technologies, College of Health and Medical Technologies, University of Hilla, Babylon, Iraq

Abstract

Background: Pathogenicity islands (PAIs) are distinct genetic elements of pathogens encoding various virulence factors such as protein secretion systems, host invasion factors, iron uptake systems, and toxins. **Objective:** Identification of the *PAIsII*$_{J96}$ and *PAIsIII*$_{536}$ genes through molecular analysis in *Escherichia coli* isolates. **Materials and Methods:** One hundred fifty urine samples were collected from urinary tract infection (UTI) patients at Maternity and Children Babylon Hospital were sampled. Healthy controls provided 100 stool samples. **Results:** One hundred fifty urine samples were collected from UTI patients and 25 healthy stool samples were collected between November 2021 and May 2022 at Al-Maternity Hilla's. 110 samples (73.4%) grew bacteria on varied media. The remaining 40 (26.6%) samples showed no growth. In Eosin methylene blue, 110 *E. coli*-positive cultures. 35 (70.0%) female isolates, 15 (30.0%) male isolates, and 60 (54.5%) other bacteria isolates grew suspected *E. coli*. (60%) of 100 control stool isolates were *E. coli*, 60 (60%) *E. coli* isolates, and 40 (40%) other microorganisms. 50 *E. coli* isolates were studied. 30 *E. coli* isolates were multiple drug resistant (MDR). All 50 isolates were confirmed with ID massage confidence levels ranging from excellent (probability percentages from 94% to 99.7%). *PALIIj96* gene was found in 5/30 (16.7%) MDR *E. coli* urine isolates (190 bp) and (100%) stool isolates. *PAIsIII*$_{536}$ gene was discovered in only 1/30 (6.7%) MDR *E. coli* isolates from urine samples (550 bp), whereas it was not detected in any stool samples (0%). **Conclusions:** *PAIsII*$_{J96}$ and *PAIsIII*$_{536}$ genes showed increased *E. coli* pathogenicity.

Keywords: *E. coli*, molecular gene, *PAIsII*$_{J96}$ and *PAIsIII*$_{536}$ genes

INTRODUCTION

Escherichia coli is one of the few multitasking bacteria. It is employed in recombinant DNA technology because it is a part of the gut microbiota of humans and other animals.[1] However, *E. coli* can both be a deadly disease and a lab instrument.[2]

Several studies have shown that pathogenic *E. coli* strains may be derived from commensal strains by the acquisition of virulence genes.[3] These virulence genes on the chromosome are typically found in specific regions called pathogenicity islands (PAIs). PAIs were described for the first time in uropathogenic *E. coli* strain 536 in the late 1980s by Desvaux *et al.*[4]

PAIs are distinct genetic elements of pathogens encoding various virulence factors such as protein secretion systems, host invasion factors, iron uptake systems, and toxins.[5] PAIs are a subset of genomic islands and can be identified by the large size (>10 kb), frequent association with tRNA encoding genes, a G+C content different from host bacterial core genome, etc. These elements are frequently flanked by repeated sequences and carry many fragments of other mobile and accessory genetic elements such as bacteriophages, plasmids and insertion sequence elements.[6,7]

Seven PAIs have been identified in the prototype strain 536 which was isolated from a patient suffering from pyelonephritis. They had been designated as PAI I$_{536}$ to

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Address for correspondence: Dr. Yasameen Riyadh Saeed Al-Azzawi,
Department of Medical Microbiology, College of Medicine,
Al-Qadisiyah University, Al Diwaniyah, Iraq.
E-mail: yasmeenalazawi1@gmail.com

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PAI VII$_{536}$.[8] A sequence analysis of PAI I$_{536}$ indicated the presence of α-hemolysin gene cluster along with two genes showing homology to an F17-like and a CS12-like adhesin that exists in enterotoxigenic *E. coli*.[9] Pathogenicity-associated genes (PAGs), such as those that encode P fimbriae, P-related fimbriae, and hemolysin, and that distinguish pathogenic from nonpathogenic strains of a given species by the presence or absence of such PAGs, tend to be clustered in PAIs at relatively few loci in the chromosomes of pathogenic bacteria, as was previously shown for several PAIs in uropathogenic *E. coli*.[10] Pap and P fimbriae, hemolysin, and cytotoxic necrotizing factor 1 toxin are encoded on the *E. coli* J96 chromosome. These J96 PAI-specific DNA sequences may express novel uropathogenic *E. coli* adhesins. Because of the vast number of adhesins, *E. coli* pap knockout mutants in a mouse model of ascending urinary tract infection (UTI) have struggled to colonize or be virulent.[9]

Aim of study

The identification of the *PAIsII*$_{J96}$ and *PAIsIII*$_{536}$ genes through molecular analysis in *E. coli* isolates.

MATERIALS AND METHODS

A total of 150 samples were collected from patients suffering from UTIs were admitted and visit in Maternity and Children Babylon Hospital in Al-Hilla city, during a period from November 2021 to May 2022. One hundred stool samples were collected from healthy persons (as control).

Ethical approval

Before sample collection, all subjects were informed and gave consent for studies and publication. The College of Medicine, University of Al-Qadisiyah, provided guidelines for this study. The study excluded banned biological materials and genetically modified organisms. This study's *E. coli* isolates came from hospitalized patients without any additives.

Clinical specimens

The specimens collected for bacteriological analysis were collected in proper ways to avoid any possible contamination.[11]

Identification of *Escherichia coli* with Vitek2 system

The automatic identification (ID) equipment used in medical microbiology is a Vitek 2 system.

DNA extraction

This method was made according to the genomic DNA purification kit supplemented by the manufacturing company (Geneaid Biotech Ltd, New Taipei City, Taiwan). Primer was provided from Macrogene Company, Korea [Table 1].

RESULTS

Patients with UTIs admitted to Al-Maternity Hilla's and Children's Hospital between November 2021 and May 2022 supplied 150 samples and 100 healthy stool samples.

All samples were aerobically cultured on various media, and 110 (73.4%) showed positive bacterial growth. The fact that no growth was detected in the remaining 40 (26.6%) samples suggests that viruses, fungi, and anaerobic agents are difficult to culture or that the samples varied in size and content. 110 positive cultures showed *E. coli*. 50 (45.5%) of the isolates grew suspected *E. coli* in Eosin methylene blue (selective media), including 35 (70.0%) from women, 15 (30.0%) from men, and 60 (54.5%) from other bacteria. 60 (60%) of the 100 control stool isolates were *E. coli*. Table 2 shows that 60 (60%) of the isolates were *E. coli* and 40 (40%) were other bacteria. This study employed 50 *E. coli* isolates. 30 (60%) *E. coli* isolates were multiple drug resistant (MDR), whereas 20(40%) were not. A Vitek 2 system with GN-ID cards and 64 biochemical experiments confirmed the isolates were *E. coli*. All 50 isolates were confirmed with ID massage confidence

Table 1: The primers sequences used in this study

Genes	Primer sequence (5′–3′)	Size (bp)	Condition	Reference
PAIs II $_{J96}$	F: GA TCC ATG AAA ACA TGG TTA ATG GG R: GAT ATT TTT GTT GCC ATT GGT TAC C	190	95ºC/4 min 95ºC/30 s 55ºC/1 min 72ºC/70 s 72ºC/5 min 4ºC/forever	Sabaté *et al*. (2006)
PAIs III $_{536}$	F: CGG GCA TGC ATC AAT TAT CTT TG R: TGT GTA GAT GCA GTC ACT CCG	550	95ºC/4 min 95ºC/30 s 55ºC/1 min 72ºC/70 s 72ºC/5 min 4ºC/forever	Sabaté *et al.* (2006)

levels ranging from excellent (probability percentages from 94% to 99.7%), confirming the quick detection of bacteria by this approach.

The results showed a high percentage of *E. coli* in the urine and stool 50/110 (45.5%) and 15/25 (60%), respectively. Approximately (38.8%) of all clinically relevant urine isolates in this investigation were found to be *E. coli*. *Escherichia coli* is the most prevalent bacterial pathogen in cases of UTI.

$PAIsII_{J96}$ gene was detected in 5/30 (16.7%) MDR *E. coli* isolates from urine samples, the positive results were detected by the presence of (190 bp) as shown in Figure 1, while this gene was detected in all (100%) of *E. coli* isolates from stool samples as shown in Figure 2.

Table 2: *Escherichia coli* isolates from patients with UTIs

No. of samples	Positive growth	MO isolates	No. of isolates (%)
150 urine samples	110 (73.4%)	*E. coli*	50 (45.5%)
95 female	88 (80%)		35 (70.0%)
55 male	22 (20%)		15 (30.0%)
		Others	60 (54.5%)
		No growth	40 (26.6%)
100 stool samples 60 (60%) *E. coli*, 40 (40%) other M.O			

However, $PAIsIII_{536}$ gene was detected in only 1/30 (6.7%) MDR *E. coli* isolates from urine samples, the positive results were detected by the presence of (550 bp) as shown in Figure 3, whereas this gene was not detected in all (0%) of *E. coli* isolates from stool samples.

In light of the data discussed above, the distribution of toxins encoded and pathogenicity island markers among *E. coli* isolates is shown in Figures 4 and 5.

DISCUSSION

UTIs are the most common diseases encountered in clinical practice worldwide. Despite many efforts, around 150 million people worldwide per year were still affected by UTIs, with considerable morbidity and high medical costs. The uropathogenic *Escherichia coli* (UPEC) mechanism of pathogenesis needs the coordinated expression of a number of virulence genes which cooperate to the establishment of long lasting infections.

The results showed a high percentage of *E. coli* in the urine and stool (45.5%) and (60%), respectively. This may be because the bacteria are naturally present in the gut and urinary tract as part of the normal flora, have adapted to live there, and have strong and potent virulence factors that increase their ability to infect

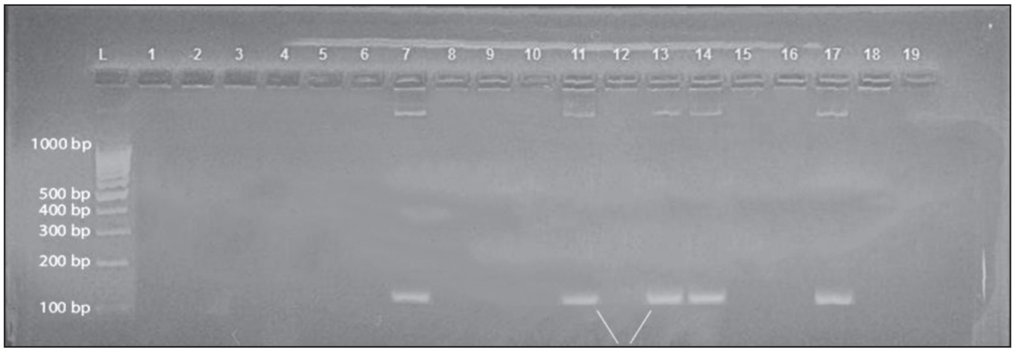

Figure 1: Gel electrophoresis of PCR product of $PAIsII_{J96}$ gene at (190 bp) of MDR *E. coli* isolates from urine samples. L: ladder, lines (7, 11, 13, 14 and 17) positive results for amplification, the electrophoresis was at 70 volt for 80 min

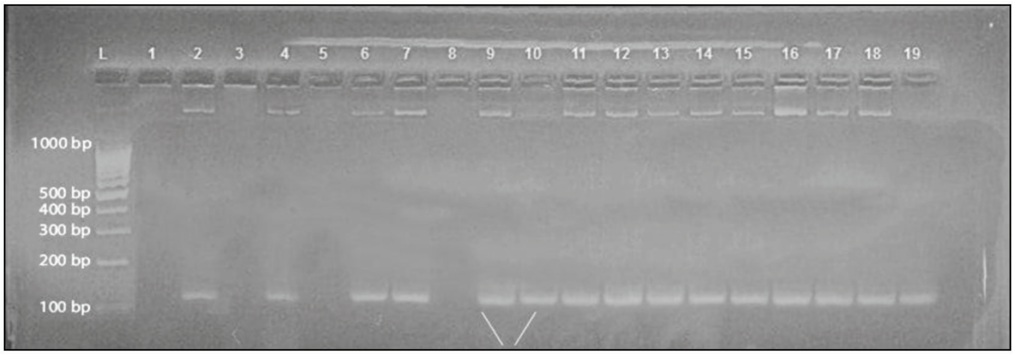

Figure 2: Gel electrophoresis of PCR product of $PAIsII_{J96}$ gene at (190 bp) of *E. coli* isolates from stool samples. L: ladder, lines (2, 4, 6, 7, and 9–19) positive results for amplification, the electrophoresis was at 70 volt for 80 min

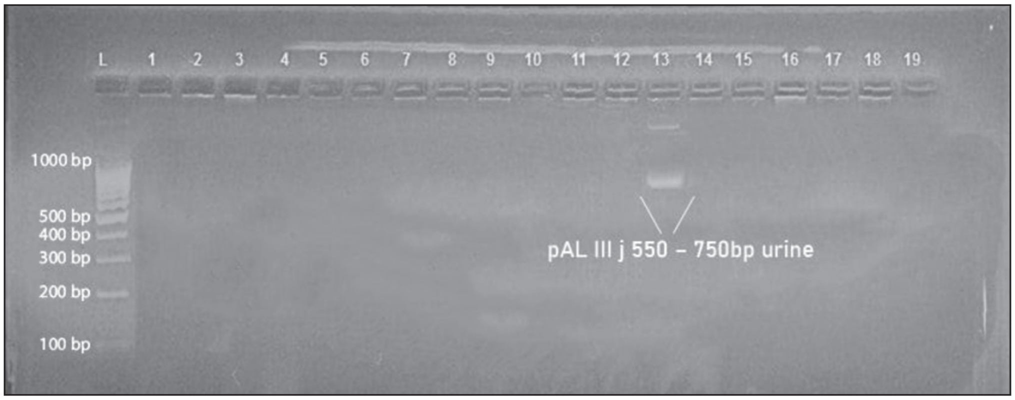

Figure 3: Gel electrophoresis of PCR product of *PAIsIII*$_{536}$ gene at (550 bp) of MDR *E. coli* isolates from urine samples. L: ladder, line (10) positive results for amplification, the electrophoresis was at 70 volt for 80 min

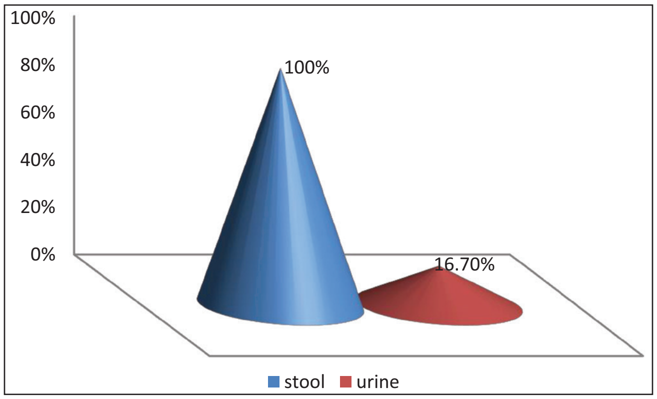

Figure 4: Prevalence of *PAIsII*$_{J96}$ gene among uropathogenic and intestinal *E. coli*

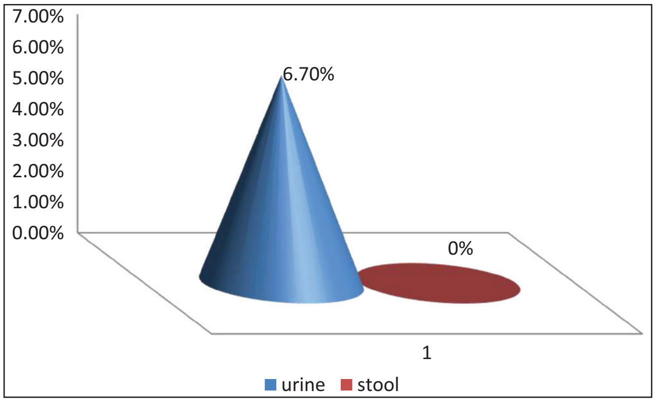

Figure 5: Prevalence of *PAIs III*$_{536}$ gene among uropathogenic and intestinal *E. coli*

people.[12] All of the isolates in this investigation tested negative for the oxidase assay and produced gas during glucose fermentation on Triple Sugar Iron Agar (acid/acid). Additionally, the isolates tested positively for catalase and indole, findings that are consistent with those of Osuntokun.[13]

Approximately (38.8%) of all clinically relevant urine isolates in this investigation were found to be *E. coli*. This is in line with the results of a previous study by Choi *et al.*,[14] which found that *E. coli* was the most common pathogen isolated from patients with UTIs.

González-Villalobos *et al.*[15] observed that *E. coli* was isolated from UTI infections in (32%) of cases, while Hasan[16] reported that *E. coli* accounted for (76%) of UTI cases. According to research by Lyer *et al.*[17], *E. coli* accounts for a very high percentage (70%) of UTIs. When compared to earlier research, the data presented here were roughly in line with Tornic *et al.*[18] findings that *E. coli* was prevalent among UTI infections at a rate of (32%).

Escherichia coli is the most prevalent bacterial pathogen in cases of UTI, which is one of the most common bacterial diseases in humans.[19] It was discovered by Al-Shami *et al.*[20] that *E. coli* makes up the largest percentage of the genus *E. coli* (36.28%) because of its ability to thrive in the human urinary tract. This adaptation is the result of *E. coli* resistance to the harsh conditions found there and its ability to stick to the surface of the urinary tract.

PAI IV536 is the most common PAIs in both commensal and UPEC isolates, and is said to be the most common PAIs in all of the Enterobacteriaceae family.[21] PAI IV536, also known as high-pathogenicity island (HPI), was found in 30% of *E. coli* isolates in a prior research of Zakaria *et al.*[8] Ghosh *et al.*[22] has been hypothesized that, HPI is a fitness island rather than a pathogenicity island due to the prevalence of PAI IV536 in commensal isolates. HPI is not essential for the virulence of *E. coli* that causes disease outside of the intestines, although *in vivo* investigations conducted by Galardini *et al.*[23] have demonstrated otherwise. PAI IV was shown to be stable in *E. coli* 536 by Gati *et al.*,[24] which may account for its prevalence.

All the other PAI markers were found in equal numbers in both commensal and UPEC isolates, albeit at much greater frequencies in the latter.

Sabaté *et al.*[25] observed PAI-like domains in 92% of UPEC isolates; Najafi *et al.*[26] discovered PAI IIJ96 in

24%, and PAI I536 in 1%; however, Navidinia *et al.*[27] discovered no evidence of PAI II536 or PAI IJ96 in any *E. coli* isolates from UTIs. Consistent with the current study, Ostblom *et al.*[28] found that, PAI IV536 was extremely common, but that PAI I536 was far less common (19% *vs.* 73%) and that PAI IIJ96 was significantly more common. PAI II536 and PAI IJ96 may not play a significant role in the development of UTIs, it has been hypothesized. Gultekin *et al.*[29] developed two novel multiplex PCR techniques for the detection of eight PAIs linked to pathogenic *E. coli* isolates. The percentage of commensal isolates with PAIs was high (40%); however, it was significantly lower than the rate for UPEC isolates (93%). Intestinal niche may be home to *E. coli* isolates, predominantly from phylogenetic Group B2 that have amassed a high number of PAIs, according to some research.[21]

Equal numbers of PAIs were found in *E. coli* isolates from both healthy women and patients with UTIs, and these isolates showed the same combination of PAIs regardless of whether they were from the same or different phylogenetic groups.[30-33]

Gultekin *et al.*[29] found that, a PCR-positive for a specific region of PAI III536 was present in 64.5% of isolates producing UTIs and in 39.3% of nonpathogenic *E. coli*, drawing the conclusion that PAIs III536 was more prevalent than PAIs II536 or PAI I536. These discrepancies may be explained by the observation that PAIs II and III of *E. coli* 536 are the most unstable and therefore the easiest to lose.[4]

CONCLUSION

PAIsII$_{J96}$ and *PAIsIII*$_{536}$ genes were increased *E. coli* pathogenicity.

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Conflicts of interest

There are no conflicts of interest.

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