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RESEARCH ARTICLE

# Molecular Detection of Some Virulence Genes of *Escherichia coli* Isolated from UTI Patients in Kirkuk City, Iraq

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## Abstract

This study was conducted at Azadi teaching hospital, Kirkuk hospital and children hospital in Kirkuk city, Iraq. A 150 urine samples were collected from both genders and different ages of patients with suspected urinary tract infections according to the clinical manifestations and symptoms diagnosed by the examining physician then, these samples have been examined by an optical microscopy and bacterial cell detection which was a clear evidence of the existence of pathogenic bacteria. The positive samples were cultured and were kept for the purpose of detecting genes encoding for certain virulence factors by a polymerase chain reaction (PCR) and also to test the antibiotic sensitivity of these isolates. Out of 150 samples, 93 of them were positive pathogenic bacteria. 51 (34%) of which infected with E. coli. 37(72.5%) out of 51 were from patients suffering from Cystitis and the other 14(27.4%) were from patients suffering from Pyelonephritis. The isolates were identified by selective media culture and biochemical test and API 20 E. Our study showed that females 39 isolates (76.4%) were infected more than males 12 isolate (23.5%), especially in the age group between (19-47 years). The DNA was extracted from Escherichia coli isolates by gene aid DNA isolation kit. Polymerase Chain Reaction was used for detecting the virulence genes (fimH, *kpsMTII, papC* and *hlyA* ). The result showed that 51 (100%) isolates were carrying fimH gene, 39 (76.4%) isolates were carrying *kpsMTII* gene, 23 (45%) isolates were carrying *papC* gene and 13 (25.4%) isolates were carrying *hlyA* gene.

## Introduction

Uropathogenic *Escherichia coli* (UPEC) are known as the urinary tract infections (UTI) most common cause. Although UPEC has non uniquely defining features, certain electrophoretic types and serotypes predominate [1, 2]. Many UPEC strains contain a number of known virulence factors "VFs", including various encapsulation, adhesins, toxins, a siderophore, and a protease[3].These "VFs" facilitate invasion and colonization of the host, disruption or avoidance of the host defense mechanism, injury to the host tissues, and/or stimulation of a noxious "host inflammatory response"[4, 5].

The severity of disease conditions that is associated with UTIs depends on multiple UPEC VFs and host susceptibility. A wide range of VFs such as adhesins (*fim, sfa, afaI, iha, papC, tsh,* and *papGI,* -II, and also -III), iron acquisition systems ( irp2, and iuc, iroN), protectins (kpsMT, and iss, ompT), and genes encoding for toxins (*astA, cnf1, hlyA, usp, set, vat,* and *cva/cvi*) are definitly involved in the pathogenicity conditions of UPEC[6, 7]. Most of the scientists in the recent time generally accepted this hypothesis that "UPEC have evolved from nonpathogenic strains". And acquire new virulence factors from the accessory DNA horizontal-transfer located at chromosomal or plasmid level is the reason for transformation of non-pathogenic strains to pathogenic one [8]. Progress in molecular technology has actually facilitated studies on UPEC [9].

## Materials and Methods

### Bacterial Strains Collection and Identification Test

Totally 150 of midstream urine samples were collected of hospitalized Pyelonephritis and Cystitis patients from Kirkuk hospitals, in Kirkuk, Iraq. During October 2018 to February 2019.

All samples were cultured on Blood agar, MacConkey agar and Eosin methylene blue agar. The preparation of biochemical tests to confirm differentiation of *E. coli* from other lactose fermenter Enterobacteriaceae by the following: positive for methyl red and indole, negative in the Voges-Proskauer, Simmons citrate, urease production and acid/acid with gas production in the TSI agar [10]. The biochemical tests result of final identification of *E. coli* was dependent on growth morphology on EMB agar and Api 20 E test.

## Bacterial DNA Extraction and PCR Method

### DNA Extraction

Bacterial chromosomal DNAs were extracted with gene aid DNA isolation kit according to the manufacturer's protocols and examined by Electrophoresis apparatus in a 1% agarose stained with ethidium bromide, and examined under UV transilluminator.

### Nano Drop

DNA has been estimated by a Nano drop device at 260/280nm, then preserved at (-20˚C) until further use.

### PCR Analysis

PCR technique was performed for virulence factors genes: type 1 fimbriae (*fimH*), capsule protein (*kpsMTII*), P fimbriae (*papC*) and hemolycin protein (*hlyA*) gene in *Escherichia coli* based on specific primers. PCR amplification was carried out using thermal cycler (MyGene, model MG25+) with specific primers for *fimH, kpsMTII, papC* and *hlyA* genes Table (1).

**Table 1: Primers and gene information**

| Gene(s) | Primer sequences (5'–3') | Primer name | Gene length (nt) | Size of product (bp) | Source of primers | Genebank ID for reference |
|---|---|---|---|---|---|---|
| *fimH* | TGCAGAACGGATAAGCCGTGG | FimH f | 903 | 508 | [12] | AJ225176 |
| | GCAGTCACCTGCCCTCCGGTA | FimH r | | | | |
| *kpsMT*II | GCGCATTTGCTGATACTGTTG | kpsII f | 777 | 272 | | X53819 |
| | CATCCAGACGATAAGCATGAGCA | kpsII r | | | | |
| *hlyA* | AACAAGGATAAGCACTGTTCTGGCT | hly f | 3075 | 1177 | | M10133.1 |
| | ACCATATAAGCGGTCATTCCCGTCA | hly r | | | | |
| papC | GACGGCTGTACTGCAGGGTGTGGCG | pap1 | 2511 | 328 | [13] | X61239.1 |
| | ATATCCTTTCTGCAGGGATGCAATA | pap2 | | | | |

PCR was carried out in 20 µl volume reaction mixtures containing 1 µl of each primer, 3µl of crude template DNA to be added to the ready to use Pre Mix then were completed to 20 µl by adding deionized water according to *AccuPower*® PCR Pre Mix from Bioneer (korea) procedure. The suitable annealing temperature was performed at65°C for all four genes. PCR amplifications was consisting of 25 cycles of a denaturation at 94°C for 2 min, annealing at 65°C for 1 min then extension at 72°C for 2 min in the thermal cycler and some modification for optimization[11].

### PCR Product Analysis

Has examined by Electrophoresis apparatus in a 1.5% agarose substance by using TBE buffer stained with Ethidium Bromide, and have taken a look under UV transilluminator.

## Results

Almost none fairly managed right from their onset, urinary tract infection can in time, develop into a real threat, capable of expanding to renal failure. An enhanced knowledge of the virulence characteristics of the causative microorganism allows the clinician to predict the evolution of infection within the host. In current study, From 150 urine samples 51 (34%)isolates of *E. coli*.37(72.5%) out of 51 were from Cystitis patients and the other 14(27.4%) were from Pyelonephritis patients. Were shown in the following Table (2).

**Table 2**: **Number and percentage of *Escherichia coli***

| Type of samples | Total samples | *E. coli* Positives isolates | | Percent within positives % |
|---|---|---|---|---|
| Cystitis | 150 | 51 | 37 | 72.5 |
| Pyelonephritis | | | 14 | 27.4 |

A total of %76.4(n=39) and %23.5 (n=12) of *Escherichia coli* strains were isolated from Female and Male patients, respectively, Table (3).

**Table 3: Distribution and percentages of E. coli according to gender**

| | Male | Female |
|---|---|---|
| Positive sample | 12 | 39 |
| Percentage % | 23.5 | 76.4 |

The result has revealed that the concentration of all DNA samples of the fifty one *Escherichia coli* isolates were 75 ng/ul in average and the average purity was 1.9, figure (1). Results showed the distribution of virulence genes in *Escherichia coli* which are 39 (76.4 %) isolates were positive for *kpsMT II* and 12 (23.5%) were *kpsMT II* negative, 23 (45%) isolates were carrying *papC* gene and 28 (54.9%) were papC negative, *hlyA* gene were detected in 13 (25.4%) isolates and it was absent in 38 (74.5%) isolate. while the *fimH* gene were detected in all of the 51 (100%) *Escherichia coli* isolates collected, Table (4).

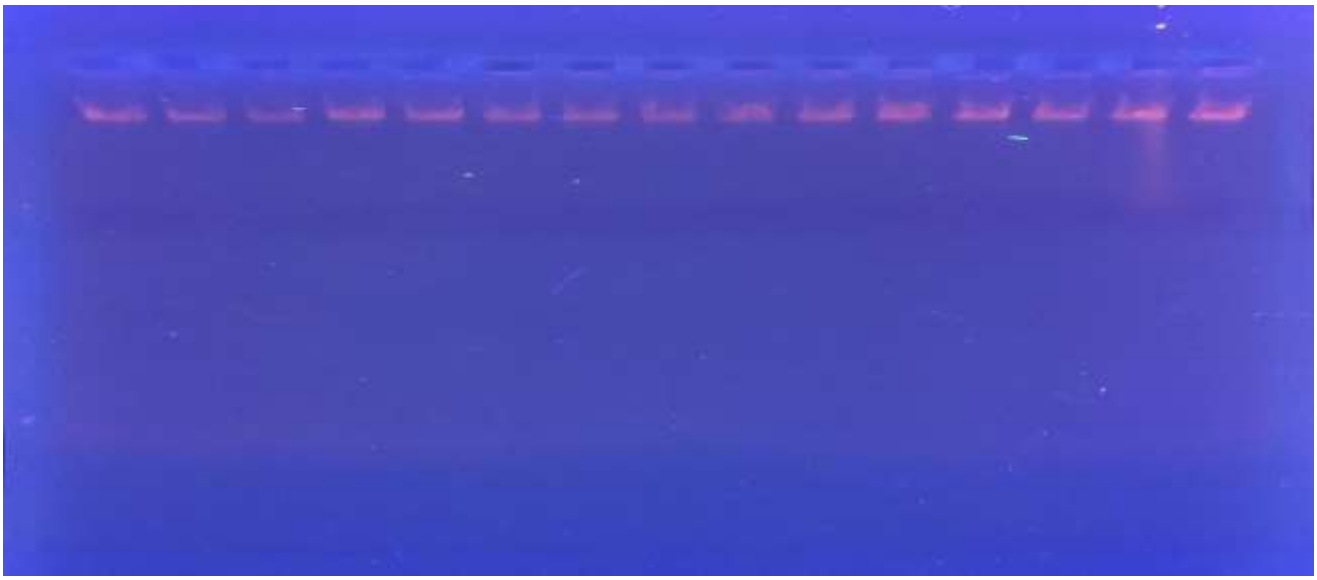

**Figure 1: A run of agarose gel electrophoresis of the DNA samples**

**Table 4: Number and percentage of virulence genes of positiveisolates**

| Name of gene | Positive isolates | Percentage |
|---|---|---|
| *fimH* | 51 | 100% |
| *papC* | 23 | 45% |
| *kpsMT II* | 39 | 76.4% |
| *hlyA* | 13 | 25.4% |

**Table 5: Distribution of genes according to type of infection, pyelonephritis&Cystitis**

| Gene / Case | No | *fimH* | *kpsMT II* | *hlyA* | *papC* |
|---|---|---|---|---|---|
| **Cystitis** | 37 | 37 (100%) | 32 (86.4%) | 12 (32.4%) | 17 (45.9%) |
| **Pyelonephritis** | 14 | 14 (100%) | 7 (50%) | 1 (7.1%) | 6 (42.8%) |
| **Total** | 51 | 51 (100%) | 39 (76.4%) | 13 (25.4%) | 23 (45%) |

For detection of virulence factor genes of *Escherichia coli* (*fimH, papC, kpsMTII* and *hlyA* ) PCR reactions were done and the following results were obtained.

PCR results of *fimH* gene (508bp), *papC* gene (328bp), *kpsMT II* gene (272bp) and *hlyA* gene (1177bp) expression are shown in Figures 2, 3, 4 and 5 respectively.

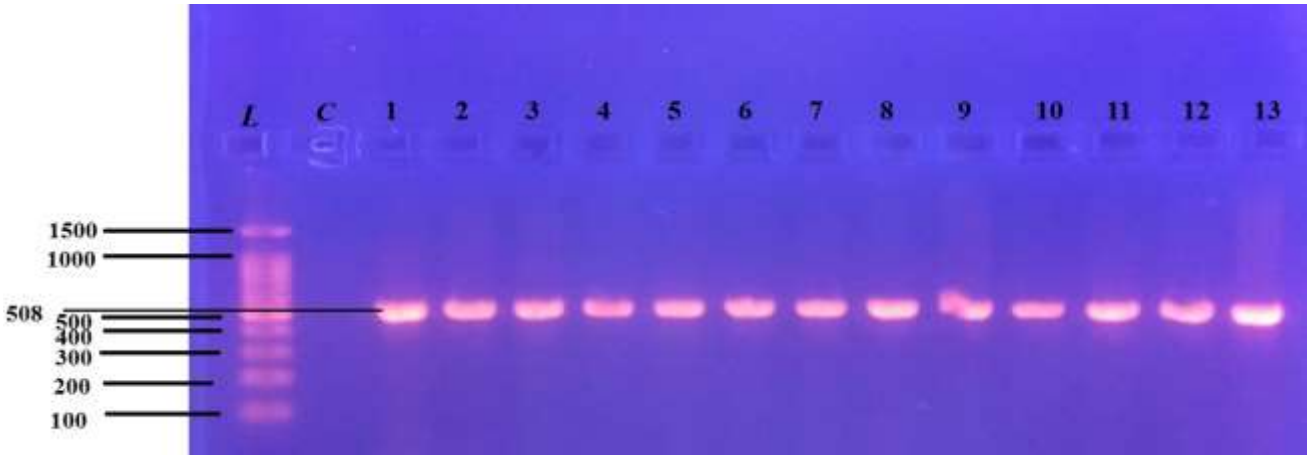


**Figure 2: Agarose Gel electrophoresis of PCR product for the detection of *fimH* gene (508bp) using 1.5%agarose for 60 min at 70 volt, stained with ethidium bromide, L: 100bp DNA Ladder, Lanes C negative control. Lanes (1-13): Positive for *fimH* gene (508bp)**

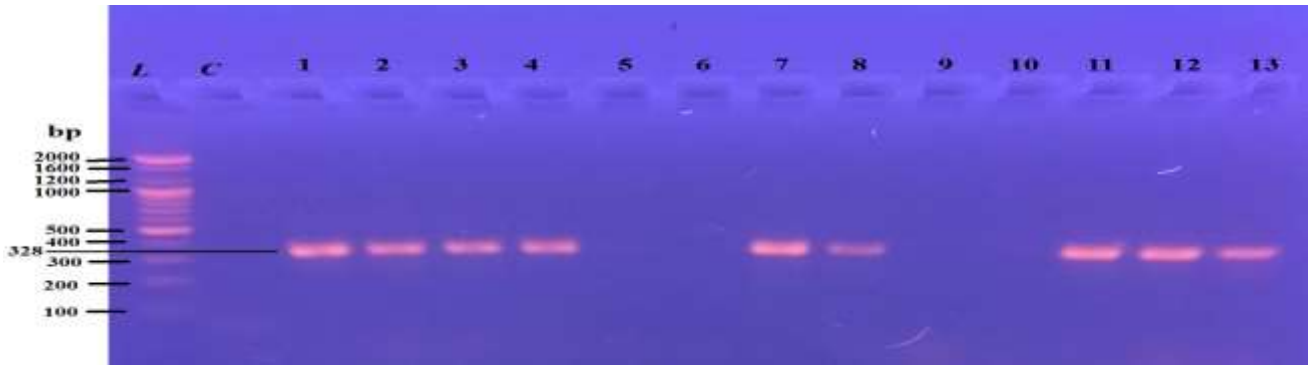


**Figure 3: Agarose Gel electrophoresis of PCR product for the detection of *papC* gene (328bp) using 1.5%agarose for 60 min at 70 volt , stained with ethidium bromide, L: 100bp DNA Ladder, Lanes C: negative control, lanes (1,2,3,4,7,8,11,12and 13):Positive for *papC* gene (328bp) and lanes (5,6,9 and 10): negative for the gene**

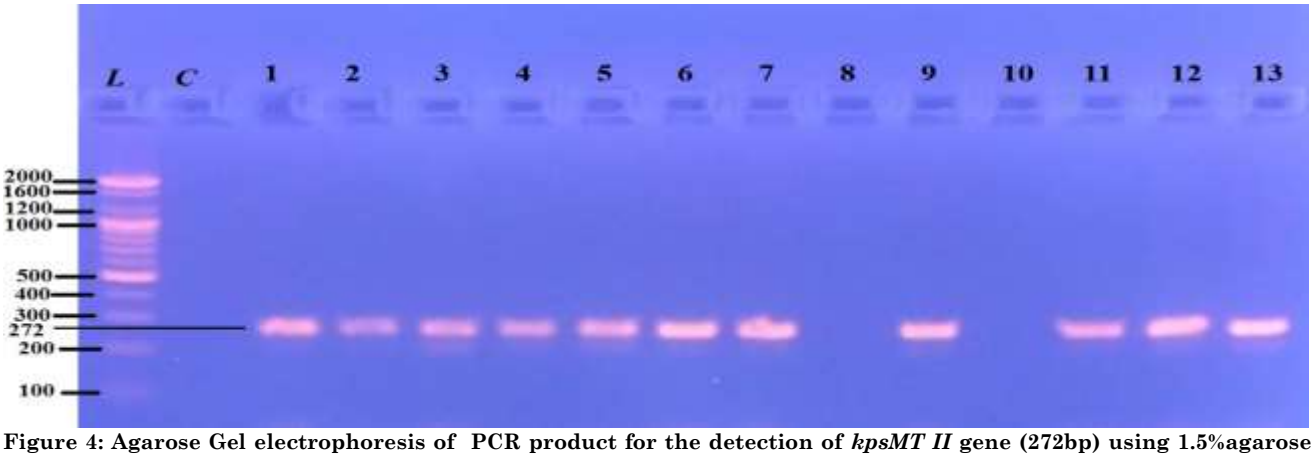


**Figure 4: Agarose Gel electrophoresis of PCR product for the detection of *kpsMT II* gene (272bp) using 1.5%agarose for 60 min at 70 volt , stained with ethidium bromide, L: 100bp DNA Ladder, Lanes C: negative control, lanes (1,2,3,4,5,6,7,9,11,12 and 13):Positive for *kpsMT II* gene (272bp) and lanes (8 and 10): negative for the gene**

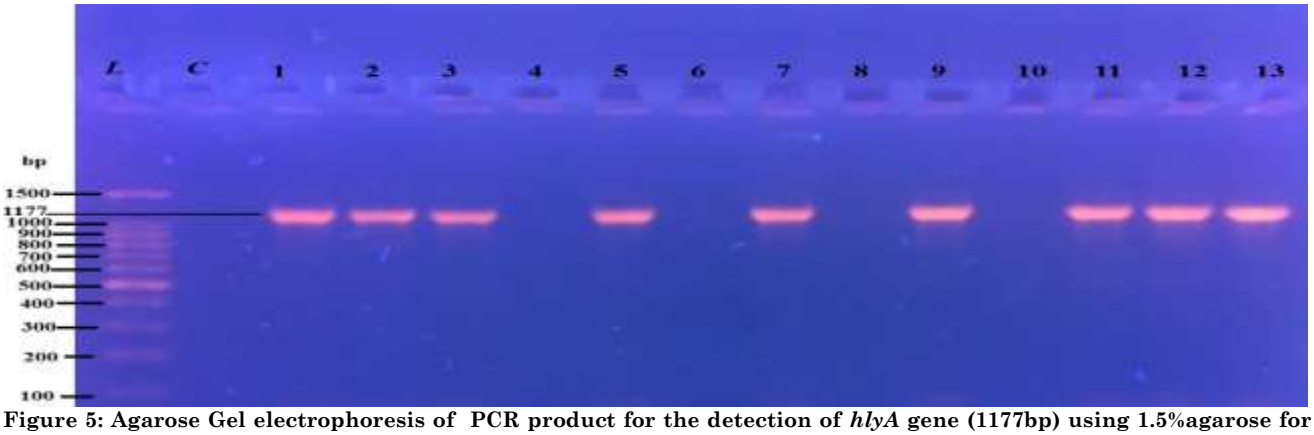


**Figure 5: Agarose Gel electrophoresis of PCR product for the detection of *hlyA* gene (1177bp) using 1.5%agarose for 60 min at 70 volt , stained with ethidium bromide, L: 100bp DNA Ladder, Lanes C: negative control, lanes (1,2,3,5,7,9,11,12 and 13):Positive for *hlyA* gene (1177bp) and lanes (4,6,8 and 10): negative samples for *hlyA* gene**

 

## Discussion

Current technology has definitely made the identification of potential virulence genes a relatively simple quest. Infection models that is studying isogenic strains that differ by a certain virulence gene provide a strong demonstration for pathogenicity if infectivity was determined by one single characteristic, but uropathogenicity is defined by various traits mostly with functional redundancy. Therefore, epidemiological associations with some clinical syndromes of UTI define the virulence determinants that have contributed to uropathogenicity.

To obtain an organism's pathogenic pathway requires some information on relative prevalence of virulence genes. In this study, 150 urine samples were collected from patients suspected to have UTI based on clinical manifestations sampling was based on urinary tract conditions like the existence of the congenital abnormalities or presence of stones in parts of the urinary system. Pregnant women and diabetic patients were excluded as they were more susceptible to diseases than others as describe by Schneeberger [14].

The laboratory test results indicate that all positive samples 93 (100%) were positive for leukocyte esterase. The number of males infected with the UTI caused by *E. coli* during the study period, were 12(23.5%) whereas 39(76.4%) in females, this results were compatible with most local and global results such as Huda[11] which showed that (86.2%) females were infected with UPEC in comparison with males (13.8%)and Gebissa [15]. That presented (26.8%) were infected males. This might be because of the anatomical differences of the urogenital organs in the two sexes. This difference have made female more susceptible to urogenital pathogens and allow the bacteria to access to the bladder [15, 19].

Fever, flank pain, dysuria, frequency, urgency and suprapubic pain has been the observed clinical symptoms of our study and is comparable with report of Bent [16]. In the current study, UTI was classified based on infection site according to physician diagnosis, upper UTI infection (pyelonephritis) was presented in males 5(41.66%) and in females 7(17.94%) in contrast to lower infection (cystitis) was presented in males 7(58.33%) and in females 32(82.05%).

This study agrees with Frazaneh [17] who had shown cystitis was a predominant infection than pyelonephritis in Iran as well as Huda in Iraq[11]. Within this study four virulence genes were inspected, In adhesion type 1 fimbriae which recognized terminally located D-mannose, was absolutely frequent in all isolates *fimH*(n=51,100%) this result is a match with Johnson[12], but Aljanaby revealed that *fimH* detection doesn't have to be 100%, as he presented (92%) of *fimH* positive isolates[18]. Our result has shown the role of *fimH*in adhesion but its role in pathogenicity remain unclear and this agrees with bien [20].

Synthesis of capsule protein *kpsmII* (n=39, 76.4%) was increased may be because of damage of inner liner of the bladder cells by any of the other virulence factors this agree with Ejrnaes[21] and this result is close to Johnsons result[12] and also agrees with yun[22], but Yamamoto had relatively different results, by showing (91.1%) of isolates carrying the gene encoding for capsule[23].The gene *hlyA* that encodes for hemolysin exotoxins had the lowest result in current study, (n=13, 25.4%) this decrease may be due to increase of other product genes such as *fyuA* as Huda presented [11].

This result agrees with Yun [22], who showed that (20.3%) of his isolates were carrying *hlyA* gene, it's also almost a match to what Tiba presented [24]. He showed (25.3%) of his isolates were positive for *hlyA* gene, Johnson in the other hand had somewhat different result [12]. He demonstrated that (41%) of his isolates were *hlyA* positive. The gene *papC* that takes part in encoding for P fimbriae, plays an important role in the UTI and pyelonephritis and their role is to adhere to mucosal and tissue matrix, in the current study the results have shown relatively low percent of *papC* (n=23, 45%), this result is almost a match with Abe [25] who showed (45.8%) of *papC* positives isolates. And disagrees with Johnson [12], who presented (77%) of *papC* positives isolates.

The distributions of genes were shown that *fimH* was the absolute most frequent; followed by *kpsmII* then *papC*, the gene *hlyA* was less abundant among the isolates than the other three genes. This agrees to Firoozeh [17].

 

## Conclusion

According to our study results we conclude:

- *E.coli* was the most predominant isolates among the other type of bacteria that's causing UTI.
- Females are more susceptible to UTI than males.
- Cystitis is more likely to occur than pyelonephritis.
- There is a relationship in *fimH* with UTI.
- There is a relationship in *kpsmII* with UTI.

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