Detection similarity and differences between Uropathogenic Escherichia coli isolated from recurrent urinary tract infections and bladder cancer patients

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ABSTRACT

Background: Urinary tract infections (UTIs) and their complications such as Bladder cancer (Bl. C.) are a health growing problem worldwide.

Objective: To shed light on this subject, present study was done to investigate relationship between recurrent urinary tract infection (RUTI) due to *Escherichia coli* (*E. coli*) and Bl. C.

Type of study: Cross-sectional study.

Methods: This study included 130 patients with RUTI, 50 patients with Bl. C. and 50 control of both sexes (aged 7-85 years) attending Al-Zahra Teaching Hospital in Al-Kut/Wassit governorate and Al-Harery Teaching Hospital of specialized surgeries/Baghdad. The patients were divided into two groups: the first group (n=130) included those who were suffering from recurrent UTI without bladder cancer and diagnosed clinically as having recurrent UTI. The second group(n=50) included those who had bladder cancer. One hundred and thirty morning midstream urine specimens were collected from recurrent urinary tract infection patients and 50 from healthy persons as a control and also 50 biopsy specimens collected from recurrent UTI with bladder cancer(after surgical operation to these patients) during beginning of October 2012 to end of March 2013.

Results: Intracellular bacterial communities (ICBC) (namely *Escherichia coli*) was isolated from (68/130) 53% from patients with RUTI while (12/50) 24% isolated from patients with Bladder cancer In this study, other molecular technique called Repetitive extragenic palindromic (REP) were used for drawing the genetic map of bacteria to know the points of similarity and differences between isolated bacteria. A difference between bacteria in each group were found, but when comparing the genetic map of UPEC isolated from patients with Bl. C. with those isolated from patients with recurrent UTI high difference between them were seen.

Conclusion: Detecting the intracellular bacterial communities (namely *E. coli*) in patients with recurrent UTI, with or without bladder cancer. Detecting similarity and difference in genetic map of UPEC isolated from RUTI and Bl. C. by Repetitive extragenic palindromic DNA (REP) technique, in which found high similarity between UPEC isolated from each group but difference from UPEC isolated from other group.

Keywords: Urinary tract infection, Bladder cancer, Uropathogenic *Escherichia coli*

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Urinary tract infections were typically considered as extracellular infections, it has been recently demonstrated that Uropathogenic *Escherichia coli* bind to invade, and replicate within the murine bladder uroepithelium to form intracellular bacterial communities (IBCs). These IBCs dissociate and bacteria flux out of bladder facet cells, some with filamentous morphology, and ultimately establish quiescent intracellular reservoirs that can seed recurrent infection. Uropathogenic *Escherichia coli* are responsible for up to 85% of these cases [1 2 3]. Uropathogenic *Escherichia coli* express adhesive fibers known as type 1 pili that mediate binding to and invasion of luminal facet cells of the bladder (4,5). This intracellular niche is conducive to UPEC replication and formation of IBCs with biofilm-like properties(6). Intracellular bacterial communities exist only transiently before bacteria dissociate and migrate out of the facet cell (7,8). Upon infection, the host exfoliates and expels bladder epithelial cells into the urine. Epithelial turnover may cause the quiescent bacteria to revert to an actively replicative form, leading to recurrent bacteriuria, this recurrence of UTI with the same serotype might lead to urothelium carcinoma because the IBCs may enhance uroepithelial cells to grow and to divide more than normal rate (9).Bladder cancer is one of the most common cancers worldwide, with the highest incidence in industrialized countries, while it is low in Asia and South America (1,2). In Iraq high incidence of UTI occur, and a research recorded in our country that most of them attributed to *E. coli* infection (10). Bladder cancer accounts for not more than 15% of all malignancies (11). In Iraq Al-Bayati (2006) isolated *E. coli* from 33% of biopsy cultures of bladder cancer.

Method: This study was conducted on 130 urine sample of patients with recurrent urinary tract infection (7-85years old) and 50 healthy person as a control. The *Escherichia coli* were isolated from 68 patient (52.3%) this percentage similar to which obtain by Al-Kuraishy (2005) which reached to 53.24%, and less than results were mentioned by Astal and Shrif (2002) which was 65% and Al-Obaidie (2004) 68%.

Occasionally, recurrences are due to a persistent focus of infection especially in young women, but the vast majority is thought to represent re-infection (16), since such infection is unusual in the absence of a history of instrumentation or anal intercourse (17).

Biopsy specimens were collected from 50 patients with bladder cancer to isolate intracellular bacterial communities (ICBC). *Escherichia coli* were isolated from 12(24%) patients with Bl. C., and this percentage is more than that obtained by Al-Bayati (2006) which was 10.7%(53 patients). In countries free from Schistosoma spp throughout the world, the peak incidence of Bl.C. is the sixth or seventh decade of life (18), and the disease

reached its peak between the ages of 65 and 75 years (19).

Repetitive extragenic palindromic DNA (REP)

Typical fingerprints were obtained in this study for *E. coli* isolates generated by using rep-PCR performed with primer BOX A1R. Complex fingerprint patterns were obtained for all of the studying isolates. In general, the band patterns of isolates were very similar, and the data indicated that the isolates were closely related. The fingerprint patterns for *E. coli* isolates obtained from the same source were similar, they were not always identical. Lanes that were blank because the PCR failed and lanes in which limited numbers of PCR products were produced not included in the analysis, a few bands were shared by more than 90% of the isolates. Individual lanes generally contained from 5 to 11 PCR product bands, although almost 15 bands were obtained for some *E. coli* isolates and this outcome supported by results obtained by (20). However, genotypic relatedness of the isolates appeared highly similarity and low difference between UPEC isolated from same group but difference in genetic map appeared between *E.coli* isolated from bladder cancer and recurrent urinary tract infection patients that in spite they were belonged to the same origin and that may be due to some mutation that might be happened for these bacteria. The REP-test was done on two stages in order to determine relationship between these isolates:

Stage one include three bladder cancers isolates (a2, a3, a4) and one from recurrent UTI's (a1) after migration on 2% agarose gel for 4 hour at 5 volt /cm and 60AM (Figure 1). More than one band were appeared in each isolate, (a2, a3, a4) were similar but no identical while a1 had some bands shared with (a2, a3, a4) but most bands are differed. Also the genetic tree of these isolates were draw according to Jaccard coefficient (18), in which show high similarity between isolates from recurrent UTI and differ from bladder cancer isolate in most bands but all isolates branches from same root because all of them were *E.coli* (Figure 2).

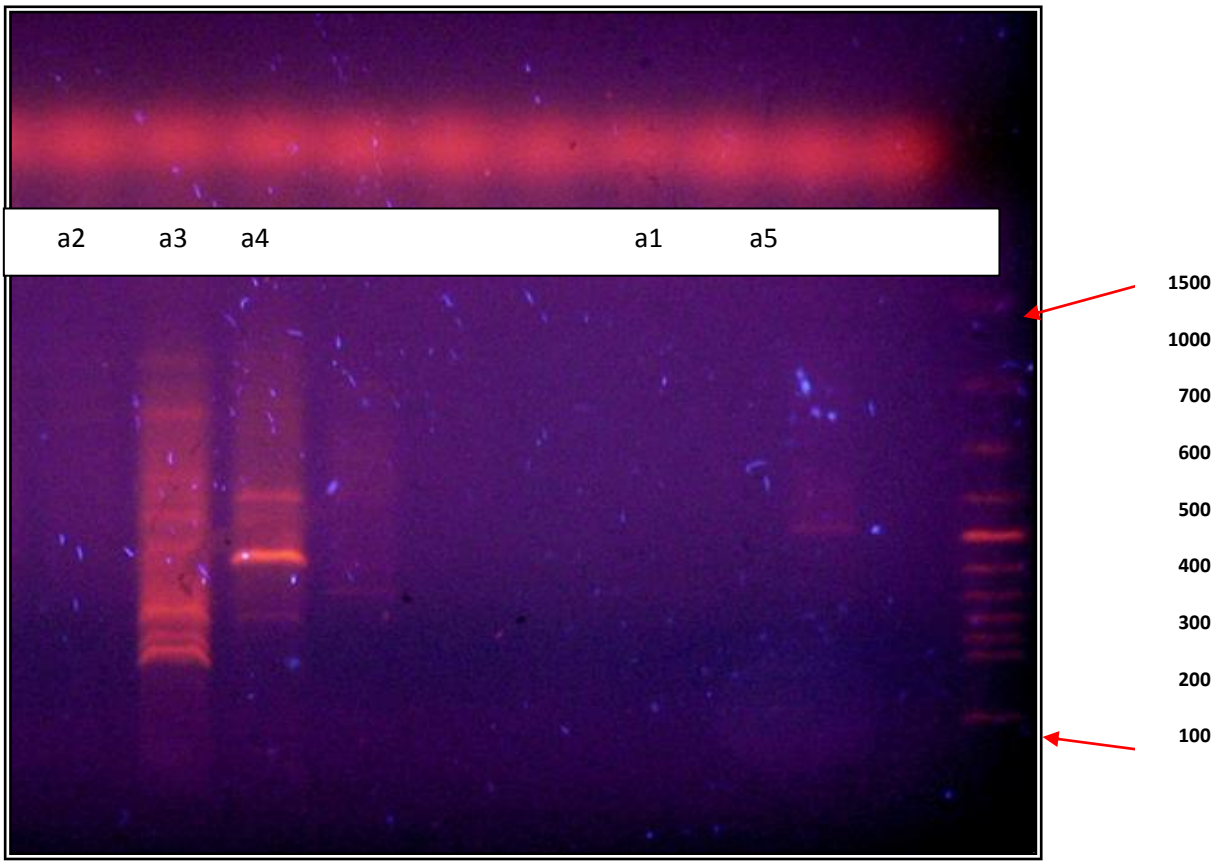

Figure 1: REP test result for (4) samples migration in 2% agarose gel for 4 hour at 5 volt /cm and 60AM.

a2, a3 and a4 bladder cancer isolates

a1 recurrent UTI's isolate

a5 DNA ladder (100bp)

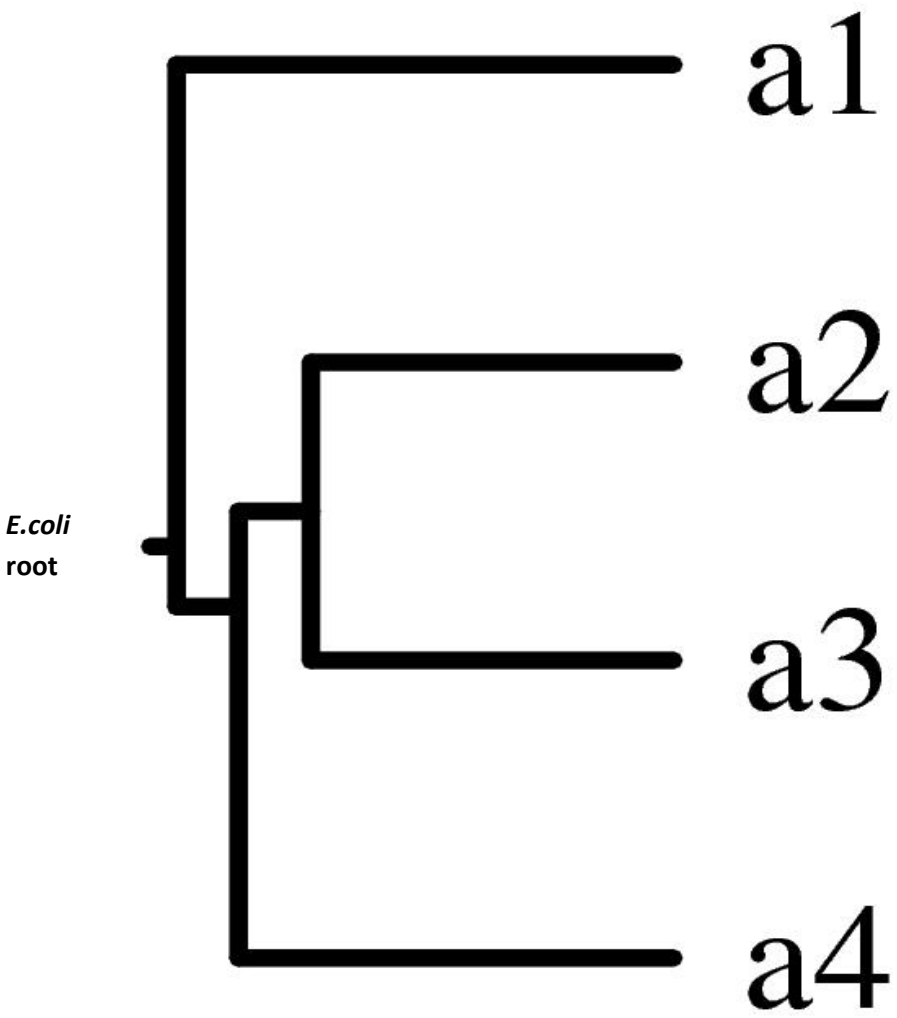

Figure 2: Genetic relativeness drawing to four according to Jaccard coefficient on line on internet.

a2, a3, and a4 bladder cancer isolates.

a1 recurrent UTI isolate.

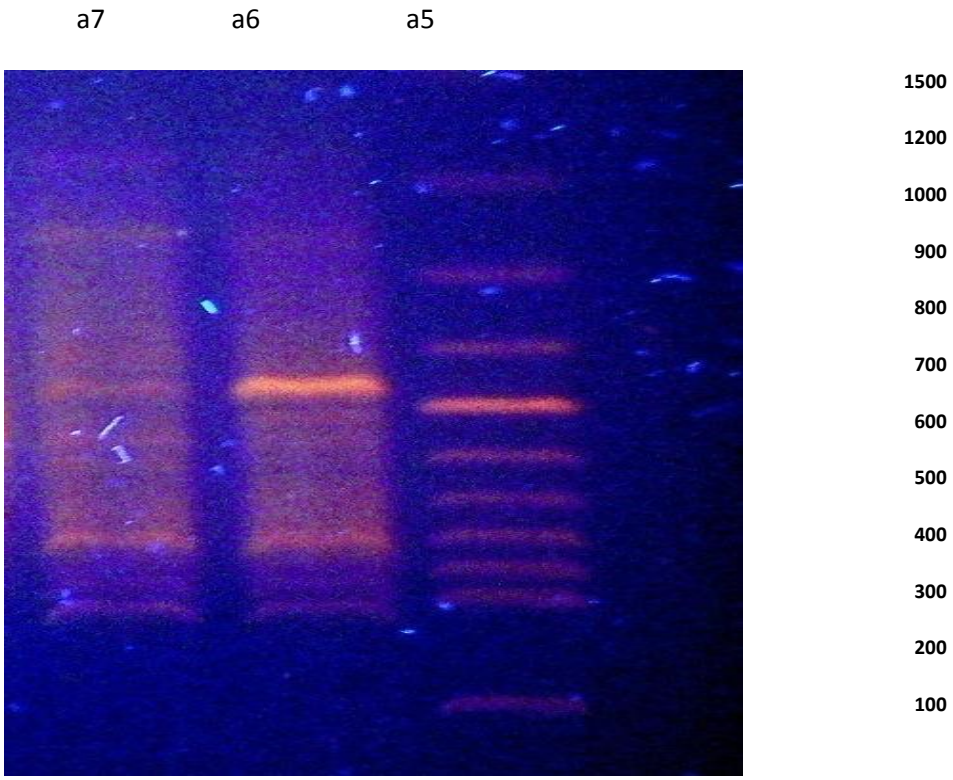

Figure.3: REP test result for (3) samples migration in 2% agarose gel for 4 hour at 5 volt /cm and 60AM.

a5, a6 bladder cancer isolates

a7 recurrent UTI's isolate

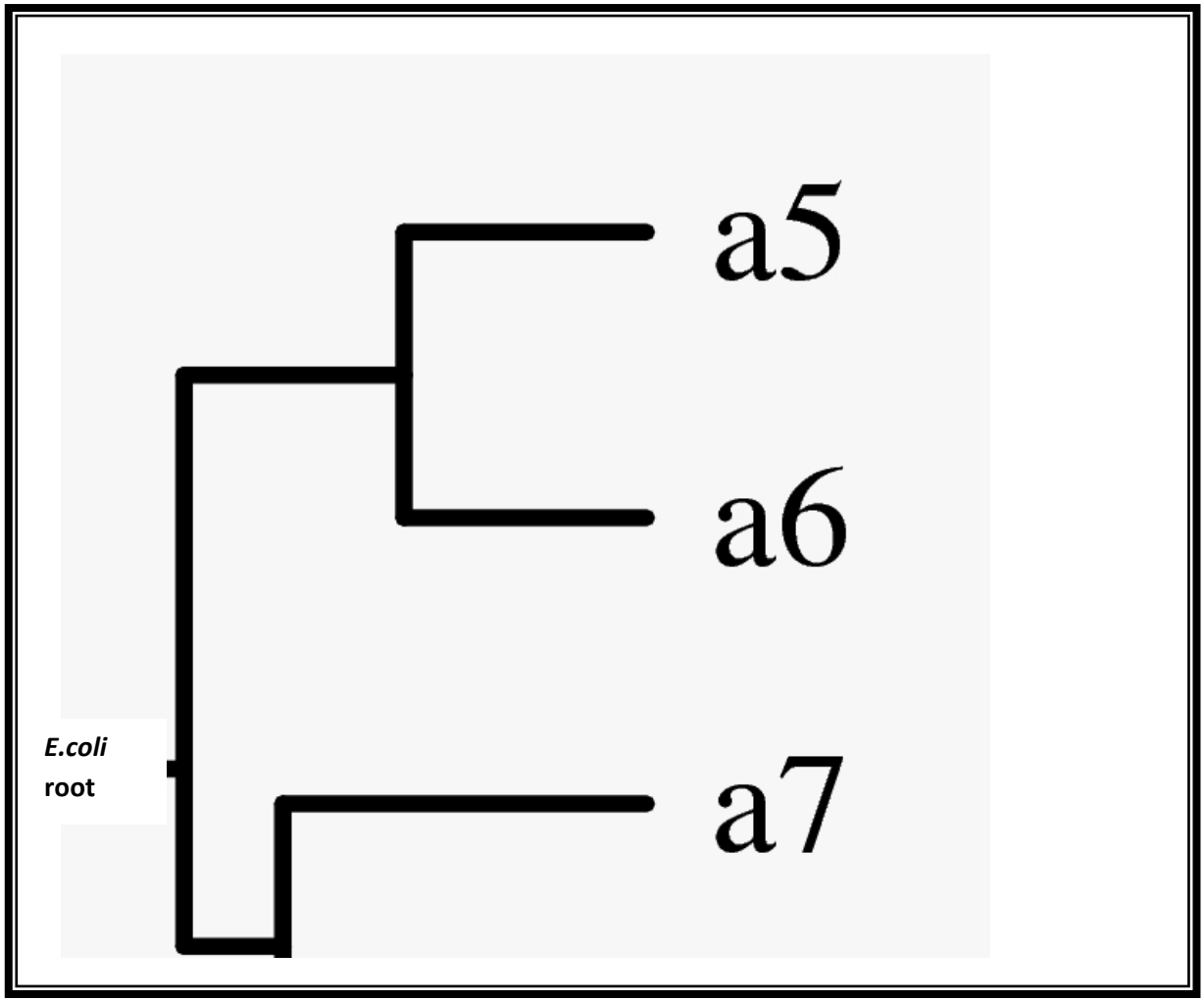

Figure.4: Genetic relativeness drawing to three isolates according to Jaccard coefficient on line on internet.

a5 and a6 bladder cancer isolates.

a7 recurrent UTI isolate.

Stage two included two bladder cancer's isolates (a5 and a6) and one recurrent UTI's isolates (a7) after migration in 2% agarose gel (3), also more than one band appeared in each isolate high similarity were obtained between isolates of RUTI and also between Bl. C. isolates but when comparing bands between this two group high difference were obtained, also the genetic tree of these isolates were draw according to Jaccard coefficient(18), in which show high similarity between isolate from recurrent UTI and differ from bladder cancer isolates in most band (figure 3) and genetic tree of this stage (figure 4), was done by (21) in America which used it to comparing genetic map of *Enterococcus* spp. with *E. coli* isolates, and also done by (22) in America to comparing genetic map of *Burkholderia cepacia* isolates.

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