# RESEARCH NOTE

## Implication of biofilm formation in the persistence of urinary tract infection caused by uropathogenic *Escherichia coli*

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## ABSTRACT

*Escherichia coli* is the most frequent microorganism involved in urinary tract infection (UTI). Acute UTI caused by uropathogenic *E. coli* (UPEC) can lead to recurrent infection, which can be defined as either re-infection or relapse. *E. coli* strains causing relapse ($n$ = 27) and re-infection ($n$ = 53) were analysed. In-vitro production of biofilm, yersiniabactin and aerobactin was significantly more frequent among strains causing relapse. Biofilm assays may be helpful in selecting patients who require a therapeutic approach to eradicate persistent biofilm-forming *E. coli* strains and prevent subsequent relapses.

**Keywords** Aerobactin, biofilm formation, *Escherichia coli*, relapse, urinary tract infection, yersiniabactin

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*Escherichia coli* is the most frequent cause of urinary tract infection (UTI). Uropathogenic *E. coli* (UPEC) strains have a number of virulence factors that increase their ability to colonise and persist in the urogenital tract [1]. Acute UTI caused by UPEC can lead to recurrent infection, which is defined as 're-infection' when it involves a strain other than that causing the original infection, or as 'relapse' when it is caused by the same strain as that involved in the original UTI. Approximately 25% of women with an episode of acute cystitis later develop recurrent UTI, which represents a substantial burden to the healthcare system. Consequently, studies are underway to elucidate the factors predisposing to recurrent UTI in order to develop effective methods of prevention and therapy [2]. In the present study, patients were followed prospectively for several months in order to determine the nature of any recurrence. The *E. coli* strains isolated were analysed to determine any possible relationships among relapse/re-infection, biofilm formation and the presence of virulence factors.

In total, 43 ambulatory female patients aged >18 years were included in the study following an index episode of UTI (cystitis or acute pyelonephritis), irrespective of any history of recurrent UTI. Women with renal or hepatic insufficiency, and those receiving immunosuppressive therapy, were excluded. The patients were followed clinically for at least 6 months, with urine cultures every month. Urine samples were analysed in the Clinical Microbiology Laboratory of the Hospital Clinic, Barcelona, Spain. Eighty urine samples positive for *E. coli* were included in this study. Clinical variables recorded were: presence of urinary incontinence; diabetes mellitus; indwelling urethral catheter; renal insufficiency and menopause; history of renal colic; urinary tract abnormalities; previous UTI or urinary instrumentation; and exposure to antibiotics in the 3-month period before the index infection. Urinary tract abnormalities included bladder diverticuli, cystocele, congenital malformations, stones and renal cyst, as well as functional disorders such as neurogenic bladder and vesicoureteral reflux.

The UPEC isolates collected from each patient were analysed by repetitive extragenic palindromic (REP)-PCR [3] and pulsed-field gel electrophoresis of chromosomal DNA digested with *Xba*I [4] to distinguish between re-infection and relapse. Isolates with the same REP-PCR and pulsed-field gel electrophoresis fingerprint patterns were considered to be the same strain. Virulence factors were detected by PCR using gene-specific primers [5] for haemolysin (*hlyA*), cytotoxic necrotising factor-1 (*cnf1*), toxin autotransporter (*sat*), type 1 fimbriae (*fimA*), yersiniabactin (*fyuA*), aerobactin (*aer*), S-fimbriae (*sfaS*), P-fimbriae (*papA*, C, G, EF and *prs*) and Ag43 (*flu*). Detection of biofilm production was based on a protocol described

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previously [6]. The strains were grown overnight in Luria–Bertani broth [7] at 37°C without shaking. A 1.25-μL aliquot of an overnight culture was then subcultured in 125 μL of M63 medium [7] containing Luria–Bertani broth 1% v/v in a well of a polystyrene microtitre plate and incubated at 30°C overnight without shaking. A 1.25-μL of each culture was then subcultured in 125 μL of M63 medium in a new polystyrene microtitre plate, and re-incubated as described above. After 24 h, the culture was removed from the plate and the biofilm was stained with 175 μL of crystal violet for 1 min, washed with phosphate-buffered saline, and air-dried for *c.* 1 h. The retained stain was solubilised in dimethylsulphoxide and the absorbance was measured at 550 nm. A strain was considered to be positive for biofilm production when the absorbance was greater than four-fold the value for a control well without bacteria.

Proportions and means were compared using the chi-square test and *t*-test. Logistic regression was used to identify factors associated independently with relapse or re-infection. Two logistic models were constructed: the first, not including clinical characteristics, in which the entire collection of *E. coli* strains was considered; and the second, including clinical characteristics, in which only the isolate causing the index episode of UTI was considered.

During the study period, 80 unrelated *E. coli* strains were collected from 43 females with recurrent UTI. Twenty-four patients had the same *E. coli* strain involved in at least one recurrent episode, while 19 patients were infected by different *E. coli* strains in each episode. Hence, 27 *E. coli* strains were considered to cause relapse, and 53 were categorised as causing re-infection. The mean age of the patients was 48.3 ± 20.5 (range 19–89) years, and 19 (44%) were post-menopausal. Twenty-seven (63%) patients had at least one episode of symptomatic UTI before enrolment in the study, six (14%) had a history of renal colic, two (5%) had been subject to urinary instrumentation, 16 (37%) had a urinary tract abnormality, 22 (51%) had some degree of incontinence, and four (9%) suffered from diabetes mellitus. Thirty-three (77%) patients had been exposed to antibiotics within 3 months of the index UTI, but none had renal insufficiency or an indwelling urethral catheter.

The prevalence of in-vitro biofilm formation and virulence factors among strains causing relapse and re-infection is shown in Table 1.

**Table 1.** Distribution of virulence characteristics among *Escherichia coli* isolates causing relapse or re-infection

| Characteristic | Relapse ($n$ = 27) No. (%) | Re-infection ($n$ = 53) No. (%) | p |
|---|---|---|---|
| Biofilm-positive | 20 (74) | 22 (42) | 0.005 |
| *flu* | 15 (56) | 32 (60) | 0.67 |
| *hlyA* | 7 (26) | 19 (36) | 0.37 |
| *cnf1* | 5 (19) | 13 (25) | 0.54 |
| *sat1* | 8 (30) | 13 (25) | 0.62 |
| *fimA* | 24 (89) | 47 (89) | 0.97 |
| *fyu* | 20 (74) | 27 (51) | 0.04 |
| *aer* | 20 (74) | 27 (51) | 0.04 |
| *sfaS* | 9 (33) | 11 (21) | 0.21 |
| *papA* | 10 (37) | 21 (40) | 0.8 |
| *papC* | 13 (48) | 24 (45) | 0.8 |
| *papG* | 7 (26) | 17 (32) | 0.6 |
| *papEF* | 14 (52) | 22 (42) | 0.37 |
| *prs* | 7 (26) | 8 (15) | 0.3 |

Three characteristics were significantly more frequent among strains causing relapses, namely in-vitro biofilm formation (p 0.005), the presence of a yersiniabactin (*fyu*) gene and the presence of an aerobactin (*aer*) gene (both p 0.04). Logistic regression selected only biofilm formation (OR 4.96, 95% CI 1.65–14.9) and the presence of a yersiniabactin gene (OR 3.6, 95% CI 1.18–11) as factors that were associated independently with strains involved in relapse.

When the analysis was restricted to the strains involved in the index episode of UTI, again only in-vitro biofilm production (OR 11.4, 95% CI 2–64.6) and the presence of a yersiniabactin gene (OR 6.37, 95% CI 1.05–38.7) were associated independently with relapse. In this respect, none of the patient characteristics seemed to be important.

Recurrent UTIs are common among young, healthy women, despite the fact that they generally have anatomically and physiologically normal urinary tracts [8]. The data from the present study indicate that the only factors associated consistently with UTI relapse in women are of microbial origin, i.e., the capacity to form biofilm *in vitro* and the presence of a gene for yersiniabactin. Recurrence has been associated previously with several virulence determinants present in UPEC, and with behavioural or other factors that facilitate vaginal colonisation or entry of colonising uropathogens into the bladder. However, Mulvey *et al*. [9] demonstrated that uropathogens can persist within the bladder tissue in underlying epithelial cells and may be a source of recurrent UTI. Anderson *et al*. [10] observed that intracellular bacteria mature into biofilms, creating pod-like bulges on the bladder surface. This bacterial structural organisation may explain the persist-

ence of bladder infections despite robust host defences. Yersiniabactin and aerobactin, two virulence factors related to iron-uptake systems, have also been associated with relapse [11]; the present data confirmed these observations, which could be related to the need of the bacteria to capture iron for growth in a stressful environment. However, biofilm production may be the key determinant for the persistence of UPEC in either the vaginal reservoir, the bladder epithelial cells or both. An in-vitro biofilm assay could therefore be useful in clinical practice to select patients who may require a therapeutic approach directed at erradicating persistent biofilm-forming *E. coli* strains in order to prevent subsequent relapses.

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# RESEARCH NOTE

## New phage type among methicillin-resistant *Staphylococcus aureus* associated with a local outbreak in Belgium during 2002

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## ABSTRACT

In total, 150 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates collected during 2002 from a general Belgian hospital were phage-typed at routine test dilution ×100. The majority (45%) belonged to phage group (J)*, while 10% were classified as a new phage type 29/(42E)/54/(D11)*. The isolates belonging to this new type carried the *aac(6′)-aph(2″)* and the *aph(3′)* aminoglycoside resistance genes and showed high-level resistance to oxacillin. Molecular typing revealed that they belonged to the multiresistant clonal pulsed-field gel electrophoresis (PFGE) type D8. PFGE group D, characterised as genotype ST228-MRSA-I, has been present in Belgian hospitals since 1999.

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