

Uropathogenic *Escherichia coli* biofilm-forming capabilities are not predictable from clinical details or from colonial morphology

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Introduction

Antibiotic resistance is increasing to an extent where efficacy is not guaranteed when treating infection. Biofilm formation has been shown to complicate treatment whereby formation of biofilm is associated with higher minimum inhibitory concentration values of antibiotic.

Urinary tract infections (UTI) are among the most common infections affecting both men and, particularly, women, 50-60% of whom will at some time become infected with uropathogenic bacteria [1]. Enterobacteriales, including *Escherichia coli* are the most common uropathogens, accounting for 80% of all reported infections [2].

While most cases of UTI can be successfully treated with oral antibiotics, recurrent infections are not uncommon, and several studies have pointed to biofilm formation and the associated antimicrobial resistance as a key factor leading to recurrence in UTIs. The majority of recurrent infections have been shown to be caused by biofilm positive strains [3].

Objectives

As inappropriate antibiotic treatment is associated with the development of antibiotic resistance, and the efficacy of antibiotics are significantly reduced when treating biofilm associated infections, we sought to investigate the prevalence and strength of biofilm formation among uropathogenic *E. coli* (UPEC) from patients with UTI whose clinical details suggested either recurrent UTI or non-recurrent infection, and to investigate whether colonial phenotypes might be a predictor of biofilm forming propensity.

Materials and Methods

62 urines from patients with laboratory-confirmed UTI were provided by the Department of Clinical Microbiology, Cork University Hospital. Twenty-nine of the urines were from patients with no clinical details indicating recurrent urinary tract infection (Unspecified population) while the remaining 33 urines were from patients whose clinical details indicated a recurrent infection.

Specimens were cultured using Cysteine Lactose Electrolyte Deficient (CLED) agar and isolates were photographed to record colonial morphology in each case. Biofilm formation was determined using the microtitre plate method [5]. *E. coli* ATCC 25922, previously described as a strong biofilm former [4], was used as a positive control strain for biofilm formation.

Isolates which formed biofilms between 90% and twice the level of the control strain were noted as strong biofilm formers, with isolates forming more than twice the level being noted as very strong. Weak biofilm formers were determined to be strains which generated less than 90% but at least 40% of the biofilm formed by *E. coli* ATCC 25922, and those that generated less than 40% of the biofilm of the control strain were designated as +/- (indeterminate)biofilm-formers.

The colony morphology of each isolate as it appeared on CLED agar was noted along with the biofilm formed to determine whether biofilm forming propensity was predictable based on colony appearance.

Results

Fig.1 (a) Biofilm formation of unspecified UPEC

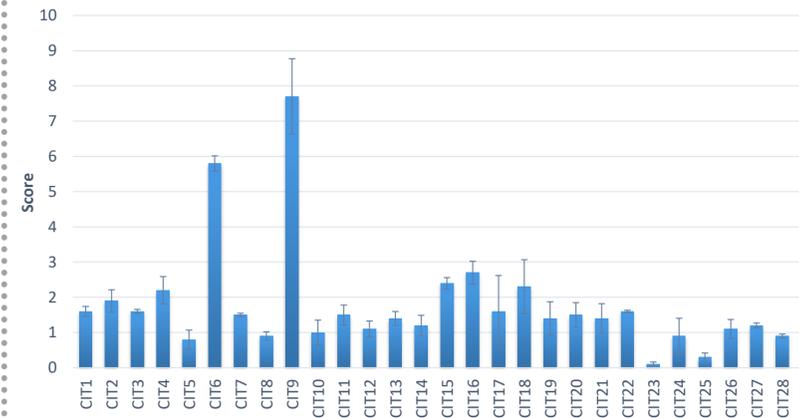


Fig.1 (b) Biofilm formation of Recurrent UPEC

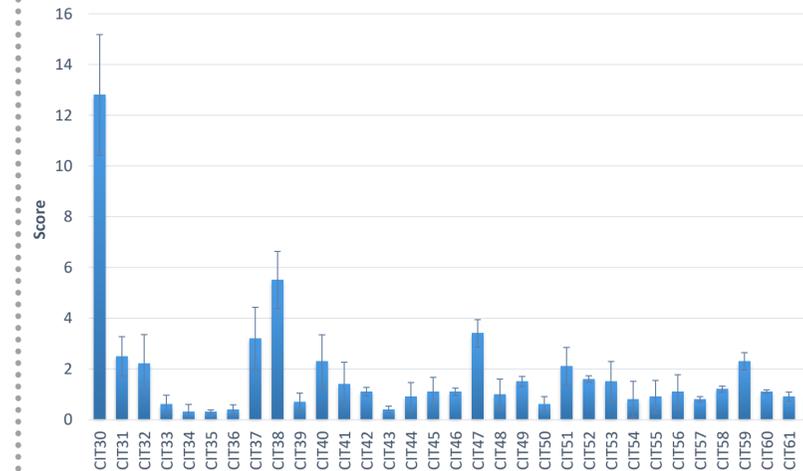


Fig. 1(a) Biofilm formation of UPEC isolates from patients whose clinical history of urinary tract was unspecified. Fig 1(b) Biofilm formation of isolates from patients with a history of recurrent urinary tract infections.

Note: Biofilm formation is expressed relative to the control strain. A score of 1 indicates biofilm formation equal to the control strain, while a score of 2 indicates biofilm formation twice that of the control strain.

Conclusions

- Strong or very strong biofilm formation was observed in 81% of isolates studied. A total of 93.6% of all test isolates were found to be positive for biofilm formation, with the remainder (four isolates) showing indeterminate or very weak biofilm formation (See Figure 1(a) and 1(b)).
- Clinical details indicating a recurrent infection were not predictors of strength of biofilm formation, however. The effect of antibiotic concentration on biofilm-forming propensity in UPEC needs to be examined.
- A Kruskal-wallis test was performed, finding no significant correlation between colony size and biofilm formation ($p = 0.1$). With only two strains of UPEC noted as being mucoid, and most strains being lactose fermenting, statistical analysis of these phenotypes in relation to biofilm formation could not be assessed.

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