## Study of Biofilm Production in Escherichia coli Causing Urinary Tract Infection

Dear Editor,

Urinary tract infections pose a serious health threat with respect to antibiotic resistance and high recurrence rates. *Escherichia coli* is the predominant organism causing urinary tract infections. Uropathogenic *E. coli* forms intracellular bacterial communities with many biofilm-like properties within the bladder epithelium.<sup>1</sup> These intracellular biofilm-like pods allow bacteria to outlast a strong host immune response to establish a dormant reservoir of pathogens inside the bladder cells. Re-emergence of bacteria from this reservoir might be source of recurrent infections.<sup>2</sup>

The present study was aimed at *in vitro* qualitative estimation of biofilm production in *E. coli* and correlates it with antibiotic resistance and surface hydrophobicity.

A total 50 strains of *E. coli* showing significant count (>10<sup>5</sup> cfu /mL) were isolated from cases of urinary tract infections. The organisms were identified by standard biochemical reactions. Antibiotic sensitivity test was performed by Kirby-Bauer disk diffusion method.<sup>3</sup> The results are shown in table 1. Surface hydrophobicity was performed by salt aggregation test (SAT) of Lindahl *et al.*<sup>4</sup>

*E. coli* ATCC 25922 was used as control. Biofilm production was tested by microtitre plate method and quantitated spectrophotometrically using an ELISA Reader.<sup>5</sup> Biofilm production was correlated with antibiotic resistance as well as surface hydrophobicity. Statistical analysis was performed by Kruskal-Wallis test and Mann Whitney U test.

Forty-six out of 50 strains of *E. coli* (92%) showed significant production of biofilm. Twenty-one out of 50 (54%) strains were sensitive to gentamicin followed by tobramycin (50%) and cotrimoxazole (44%) and ciprofloxacin (44%)

Multi-drug resistance pattern of the biofilm producing isolates is shown in Table 2.

Out of the total 50 strains of *E. coli* isolated, there was significant correlation between biofilm production and

isolates tested	
Antibiotic	Number (% sensitive)
	n=50
Amikacin	7 (14)
Ampicillin	10 (20)
Ceftazidine	18 (36)
Ciprofloxacin	22 (44)
Co-trimoxazole	22 (44)
Gentamicin	27 (54)
Nalidixic acid	9 (18)
Netillin	2 (4)
Norfloxacin	9 (18)
Tetracycline	9 (18)
Tobramycin	25 (50)

Table 1: Antibiotic susceptibility results of the 50 E. coli

Table 2: The multidrug resistance pattern of Biofilm   producing E. coli		
Multiple drugs combination	Number (%)	
A,Co,Na,Nx	27 (54)	
A,Na,Nx	7 (14)	
Co,Na,Nx	2 (4)	
A,Co,Na	2 (4)	

A - Ampicillin, Co - Co-trimoxazole, Na - Nalidixic acid, Nx -Norfloxacin

resistance to multiple antibiotics such as ampicillin (A), cotrimoxazole (Co), nalidixic acid (Na) and norfloxacin (Nx). 11 out of 50 strains of *E. coli* showed significant SAT values (> 1.4). These strains were found to show increased biofilm production.

Bacterial biofilms are often associated with long-term persistence of organisms in various environments. Bacteria in biofilm display dramatically increased resistance to antibiotics.<sup>2</sup> The present study also showed significant correlation between biofilm production and multi-drug resistance.

Biofilm production in *E. coli* may promote colonization and lead to increased rate of urinary tract infections. Such infections may be difficult to treat as they exhibit multidrug resistance. A greater understanding of the nature of intracellular bacterial communities in chronic or recurrent urinary tract infections will aid in the development of new and more effective treatments for these problematic diseases.

## References

- 1. Anderson GG, Martin SM, Hultgren SJ. Host subversion by formation of intracellular bacterial communities in the urinary tract. *Microbes Infect* 2004;**6**:1094-101.
- 2. Anderson GG, Palermo JJ, Schilling JD, Roth R, Heuser J, Hultgren SJ. Intracellular bacterial Biofilm-like pods in urinary tract infections. *Science* 2003;**301**:105-7.

- National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disc susceptibility testing. 3<sup>rd</sup> ed. Approved Standard M2-A3. National Committee for Clinical Laboratory Standards. Vellanova, PA: USA; 1984.
- 4. Lindahl M, Faris A, Wadstrom T, Hjerten S. A new test based on salting out to measure relative surface hydrophobicity of bacterial cells. *Biochim Boiphys Acta* 1981;**677**:471-6.
- O'Toole GA, Kolter R. Initiation of biofilm formation in *Pseudomonas fluorescens* WCS 365 proceeds via multiple, convergent signaling pathways: A genetic analysis. Mol Microbiol 1998;28:449-61.

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