Detection of Siderophore production in Uropathogenic *Escherichia.coli* in patients with Type 2 Diabetes Mellitus

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Abstract

Introduction: Infection of the urinary tract is a common observation in patients with type 2 diabetes mellitus. UTI can be a severe complication in diabetic patients. The aim of this study was to determine siderophore production as a virulence factor in *Escherichia.coli*, commonly responsible for UTI.

Material and Methods: The study was conducted at the postgraduate department of Botany (Microbiology Unit) of St. Josephs College PG & research center Bangalore India between January 2010 - February 2011. Midstream urine from diabetic patients was collected and *E.coli* was isolated and identified using standard microbiology protocols Growth was examined after 24 hours of incubation at 37^0c. Colonies more than 10^5cfu/ml were considered significant for UTI. Detection of siderophores was performed using Chrome Azurol Assay (CAS)

Results: Siderophore production was observed in 190(95%) of *E.coli* isolates and one of the 20 (5%) among controls.

Conclusion: Iron acquisition or siderophores are a major virulence factor necessary for Uropathogenic *E.coli* in the pathogenesis of UTI in diabetic patients. CAS assay is the most efficient method for the detection of siderophores.

Keywords: Diabetes Mellitus, UPEC, UTI Siderophores.

Introduction

Urinary tract infection (UTI) is the most common and severe complication observed in patients with diabetes often caused by Uropathogenic *E.coli (UPEC)*. Patients with type 2 diabetes are at a greater risk of developing severe UTI due to impaired immune system.[1-2] Autonomic neuropathy in these patients often leads to incomplete emptying of the bladder allowing bacteria to thrive thus contributing to cystitis, pyeleonephrits, and severe sepsis.[3,4,5] Risk factors associated with UTI in diabetic patients in addition to poor glycemic control include diabetic nephropathy and renal abscesses. *E.coli* is the common pathogen isolated in patients with UTI and accounts for 90% of patients with cystitis. UPEC colonizes periurethral areas and enters into urinary tract causing symptomatic UTI. Iron acquisition is necessary for the aerobic metabolism, colonization and maintenance of infection in the host. Aerobactin is the hydroxmate siderophore in *E.coli* and one of the most effective iron chelation system used by this pathogen for iron uptake. Siderophores in bacteria compete for the iron binding proteins, siderophore bound iron is then taken up by the surface receptors of bacteria. Most strains of UPEC associated with UTI produce siderophores as a major Virulence factor[6] The present study elucidates siderophores as a major virulence marker of UPEC in patients with diabetes.

Material and Methods

The study was conducted at the postgraduate department of Botany (Microbiology Unit) of St. Josephs College PG & research center Bangalore India between January 2010 - February 2011. Midstream urine was collected from diabetic patients and *E.coli* strains were isolated and identified using standard microbiology protocols for culture. Growth was examined after 24 hours of incubation at 37^0c. Colonies more than 10^5cfu/ml were considered significant for UTI.

Detection of Siderophores using Chrome azurol assay: Siderophore production in UTI isolates was performed according to the method described by vagarali 2007.[6] First, 60.5mg CAS was dissolved in 50ml deionized water and mixed with 10ml iron (III) solution (1mm FeCl3 – 6H2O, 10mM HCl); by stirring, this solution was slowly mixed with 72.9mg hexadecyltrimethyl ammonium bromide (HDTMA) dissolved in 40ml water. The resultant dark blue solution was autoclaved and mixed with an autoclaved mixture of 900 ml water, 15g agar. 30.24g 1.4-piperazine diethane sulfonic acid (PIPES) and 12g of solution of 50% (w/v) NaOH to raise the pH to the Pka of PIPES.[6,8] The modified CAS agar plate was punched with 2.5–5mm diameter wells by using a gel puncher. Each well was filled with 25–35µl of the broth containing bacterial culture, which was twofold serially diluted from 2.5mm. After incubation of the plate at 37°C or room temperature for 4–8h, orange halo was formed around each well. The result was taken as positive if there was a color change from blue to orange/yellow halo.

Results

Siderophore production was observed in 190(95%) of UPEC isolates and one of the 20 (5%) among controls (Table). Also as shown in the Figure as positive UPEC

isolates with a yellow halo around the well and Control with no halo seen.

Table 1:

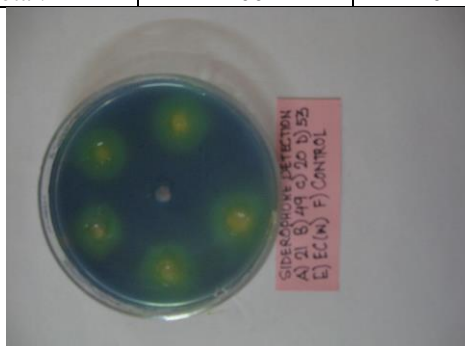| Siderophore production	No of isolates Percentage control	Percentage
+ 190	95%	15
- 105%	19	95
Total:	200	20

Fig. 1: Photo showing the strains of Uropathogenic E.coli producing siderophores with yellow halos around the inoculated wells. Control shows no yellow halos.

Discussion

UPEC has been extensively studied for its various virulence markers. UTI is a among the severe complications observed in diabetic patients during clinical practice, previous studies have suggested UPEC produces significant siderophores during the pathogenesis of UTI. It is therefore necessary to identify UPEC strains in patients with the etiology of Type 2 diabetes mellitus to prevent further complications of severe UTI. Siderophore production has been observed high in patients with acute pyeleonephrits.[7,8,9] The receptors identified for iron uptake are Iro A and Iro N. Other studies suggest *Iro N* as an important siderophore receptor in the virulence of UTI.

References

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