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Research Article

Biofilm production and antibiotic resistance among uropathogens causing bacteriuria in diabetic individuals

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Abstract: High glucose in the urine and defective host immune response factors is responsible for predispose to urinary tract infections (UTIs) among diabetes mellitus patients. Asymptomatic bacteriuria refers to the presence of high quantity of uropathogens in the urine of asymptomatic person. Uropathogens have an ability to form a "Biofilm" appearance in the urinary tract. This reservoir of bacteria may be responsible for recurrent UTIs and also hinder the penetration of antimicrobials, resulting in the development of resistant strains. In this study we screened 250 diabetic individuals for asymptomatic bacteriuria and tested the isolated strains for biofilm production and its association to glycemic status as well as drug resistance. A total of 250 urine samples from diabetic patients were collected and processed using standard microbiological techniques. To the strains isolated, biofilm production was detected qualitatively and quantitatively by tube method and optimized microtitre plate assay respectively. Among 250 cases included 176 (70.4%) were uncontrolled and74 (29.6%) were controlled diabetics based on their blood sugar levels and HbA1C levels. Asymptomatic bacteriuria was present in 56 individuals of which 47 (26.7%) isolates were from uncontrolled and 9 (12%) from controlled group. Of the 56 isolates 37 (66%) showed biofilm production by either method. Escherichia coli (44.6%) were the predominant uropathogen and also a major biofilm producer (52.7%). Among the biofilm producing strains, 94.5% (35) were isolated from those with poor glycemic control. Form our study we conclude that blood sugar level plays a role in colonization and causes the biofilm production by the uropathogens in the urinary tract.
Keywords: Biofilm, Asymptomatic bacteriuria, uncontrolled Diabetes mellitus

INTRODUCTION

Diabetes mellitus is a metabolic disease in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because beta cells are resistant to insulin that is produced. Diabetes mellitus has long been considered to be a predisposing factor for urinary tract infection. The main mechanism being defect in the local urinary cytokines (IL8 and IL6) and also hyperglycemia facilitating increased colonization by uropathogens in the urinary tract [1]. Uropathogens have an ability to produce biofilm in the bladder epithelium which forms dormant reservoir inside the bladder. Re-emergence of bacteria from biofilm might be the source of recurrent infection. Inability of the antimicrobials to penetrate the biofilm results in the development of resistant strains. The term asymptomatic bacteriuria refers to the presence of high quantities of an uropathogen in the urine of asymptomatic person [2]. In our study we screened the diabetic individuals attending our OPD for asymptomatic bacteriuria. To the strains the isolated biofilm production was done by tube method and microtitre plate assay. We correlated our findings of asymptomatic bacteriuria with the glycemic status and that of multi-drug resistance with biofilm production.

MATERIALS AND METHODS

A total of 250 patients of known diabetics were participated in the study. Patients were instructed to collect a clean catch mid stream urine in a sterile container under aseptic precussions and the samples were processed within 30 minutes of collection of urine sample. Those samples showing $>10^5$cfu/ml were taken as asymptomatic bacteriuria[3].The isolates were identified using standard microbiological techniques and antibiotic susceptibility testing was done by Kirby – Bauer disc diffusion method for the panel drugs which includes Gentamicin 10µg , Norfloxacin 10 µg, Nalidixic acid 30 µg , Amoxycillin/Clavulanic acid 20/10µg, Ceftazidime 30 µg , Cefuroxime 30 µg, Imipenam 10 µg, Erythromycin 15 µg, Co-trimoxazole 1.25/23.75 µg, Penicillin 10 units, Oxacillin1 µg

[Himedia] as per CLSI guidelines[4]. The pure isolates were stored in nutrient agar slant for further tests.

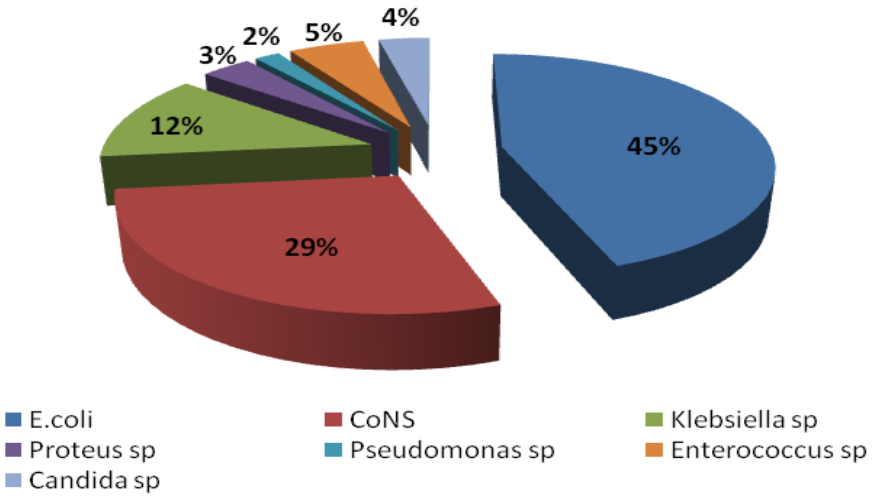
Tests for Biofilm production:

a) Tube method Qualitative assessment of biofilm production: The bacterial strains from overnight growth were inoculated in 10 ml BHI broth and incubated for 24hrs at 37ºC. The tubes were decanted and washed with phosphate buffer saline and stained with 0.1% crystal violet. The excess stain was removed by washing with distilled water. Tubes were dried in inverted position and observed for biofilm production. A uniform violet film lining the wall and bottom of the tube was considered positive for biofilm production [5]. No film or ring formation at the interface of liquid was considered as negative. The test was performed in triplicates and repeated twice to avoid observer bias.

b) Optimized Microtitre plate assay Quantitative assessment of biofilm production : In a flat bottomed sterile microtitre plate, 20µl of 24hrs broth culture and 180µl of trypticase soy broth were added and incubated at 37ºC for 24 hours. Negative control was put up with only 200µl of trypticase soy broth. After 48 hours the bacteria were removed by inverting the plate followed by vigorous tapping on absorbent material. The adhered organisms which form the biofilm are fixed by keeping the plate in a water bath at 80ºC for 30 minutes. After fixation the adhered cells were stained by adding 0.5% crystal violet (220µl) for 1 minute. Then the plate was washed using distilled water and allowed to dry[6]. In order to quantify the biofilm production 220µl of decolorizing solution (ethanol: acetone; 80:20) was added to the wells. After 15 minutes the eluted solution was observed for optical density at 590nm using ELISA reader. The OD value < 0.1 is negative, 0.1 – 0.2 is weakly positive and OD value > 0.2 is strong positive for biofilm production.

RESULT

Of the 250 known diabetics patients among them 124 were males and 126 were females. Those individuals whose fasting blood sugar >140 mg/dl, postprandial blood sugar >200mg/dl and HbA1C levels >7 were grouped as uncontrolled diabetes; while those with fasting blood sugar <140 mg/dl, postprandial blood sugar <200mg/dl and HbA1C levels <7 were grouped as controlled diabetes. We found that 176(70.4%) persons had uncontrolled diabetes and 74 (29.6%) had controlled diabetes.Asymptomatic bacteriuria was present in 26.7% (47) of uncontrolled diabetes and 12% (9) among controlled diabetes. *Escherichia coli* (44.6%) were the predominant pathogen isolated followed by *coagulase negative Staphylococci.*



Graph: 1 shows the distribution of uropathogensis among diabetic patients:

Out of the 56 strains isolated, 37 (66%) showed biofilm production by either method. Only 18 strains were identified as biofilm producers by tube method where as microtitre plate method identified 31 strains as biofilm producers. The sensitivity of tube method is 38.17% and specificity is 76% with positive predictive value 66.67% and negative predictive value 50% when compared to microtitre plate method. Among the biofilm producing strains 94.5% (35) were isolated from those with poor glycemic control (uncontrolled diabetes).The association of glycemic status with that of asymptomatic bacteriuria and biofilm production is shown in the Table (1). The multiple drug resistance pattern of the biofilm producers are shown in Table (2) whereas non biofilm producers were found sensitive to most of the drugs tested.

Table 1: **Association of glycemic status with that of asymptomatic bacteriuria and biofilm production.**

Glycemic status	Asymptomatic bacteriuria		Biofilm production	
	Present	Absent	Present	Absent
Uncontrolled diabetes	47	129	35	12
Controlled diabetes	9	65	2	7
Total	56	194	37	19

Legend: 1 Shows the Analysis of data showed significant correlation of asymptomatic bacteriuria in uncontrolled group with p value - 0.018 (Chi-square test) and increased biofilm production was seen among strains from uncontrolled group with p-value: 0.0048

Table 2: Multiple drug resistance pattern of the biofilm producers

S.No	Drug resistance pattern	Number of resistance strains
1.	Amoxycillin/Clavulanic acid + Gentamicin	4
2.	Cefuroxime + Norfloxacin + Amoxycillin/Clavulanic acid	4
3.	Amoxycillin/Clavulanic acid + Norfloxacin + Nalidixic acid + Gentamicin	5
4.	Amoxycillin/Clavulanic acid + Norfloxacin + Nalidixic acid + Cefuroxime + Gentamicin	2

Legend: 2 Shows the Analysis of data showed significant correlation of Multiple drug resistance pattern of the biofilm producers in uncontrolled group with p value - 0.018 (Chi-square test) and increased biofilm production was seen among strains from uncontrolled group with p-value: 0.0048

DISCUSSION

In the present study, asymptomatic bacteriuria is more common (26.7%) among the subjects who have very poor glycemic control, whereas it is 12% among those with good glycemic control. There is no significant gender variation, both male and female are equally affected as compared to other studies were there is more preponderance of asymptomatic bacteriuria in females[7] . As of other studies *Escherichia coli* is the most frequent uropathogen isolated and responsible for asymptomatic bacteriuria in about 44.6% of the diabetics. When compared to tube method, optimized microtitre plate method identified biofilm production with more specificity and less observer bias[8]. Our study also confirms that the biofilm producing uropathogens are mostly multidrug resistant when compared to non biofilm producers. It is evident from the above findings that asymptomatic bacteriuria is frequently encountered in uncontrolled diabetesThis study highlights that high blood sugar levels play a role in colonization and biofilm production by the uropathogens and insist upon adequate glycemic control to avoid complications such as recurrent UTI, emphysematous cystitis, pyelonephritis, bacteraemia secondary to UTI and other urological problems.

CONCLUSION

A high prevalence of asymptomatic bacteriuria (ASB) was established among uncontrolled diabetic individuals. The main pathogen was *E. coli* is beginning to acquire resistance to some of the clinically used antibiotics. The authors suggested that screening for ASB is warranted in diabetic patients particularly if pyuria is detected in urine analysis since ASB has been found to be a risk factor for developing symptomatic urinary tract infection and thereby prevent renal complications.

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