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CORRELATION OF ANTIBIOTIC RESISTANCE AND BIOFILM FORMATION AMONGST UROPATHOGENIC E.COLI

Smita K. Bawankar*

Associate Professor, Department of Microbiology, Shri Shankaracharya institute of Medical Sciences, Durg Chhattisgarh. *Corresponding Author

ABSTRACT

Introduction: Urinary tract infection (UTI) due to E.coli is one of the most common bacterial infections. It forms microcolonies in mucosa lining of urinary bladder known as biofilm which contribute to the development of chronic urinary infections, refractory to antibiotic therapy.

Aim: The objective of this study was to detect the production of biofilm by uropathogens isolated from UTIs and their antimicrobial susceptibility pattern.

Methods and material: A total 426 urine samples from clinically suspected UTI patients were processed by standard microbiological procedures. The isolated uropathogens were tested to biofilm production by microtitre plate method. Antimicrobial susceptibility testing of uropathogens was done by Kirby – Bauer disc diffusion method. The data were analyzed by using SPSS version 17.0. A P-value of less than or equal to 0.05 was considered to be significant.

Results: The antimicrobial susceptibility pattern of biofilm producers showed high resistance to commonly used antibiotics.

Conclusions: The present study showed significant correlation between biofilm production and antibiotic resistance, so it is necessary to screen all isolates for biofilm production.

KEYWORDS : uropathogen, biofilm, antimicrobial susceptibility, UTI

Introduction

Urinary tract infection continues to pose a challenge to the physicians and microbiologists due to their common occurrence, progressive course, leading to serious complications and their increasing resistance to antibiotics (Shahane VD et al., 2006). Urinary tract infection (UTI) represents one of the major nosocomial infections, commonly caused by Escherichia coli, which accounts for 90% of community acquired and 50% of hospital acquired UTI. These E. coli isolates usually originate from patients' intestinal normal flora; however, when fecal E. Coli colonizes the periurethral area and lead to UTI, these isolates are known as uropathogenic E. coli (UPEC) (Seema M et al., 2015) it is one of the most common infectious diseases encountered in clinical practice. Emerging resistance of the uropathogens to the antimicrobial agents due to biofilm formation is a matter of concern while treating symptomatic UTI (Panda PS et al 2016). A biofilm is a thin layer of micro-organisms that adhere to the surface of an organic or inorganic structure, together with their secreted polymers. Biofilms are the predominant phenotype of nearly all the bacteria in their natural habitats, whether they are pathogenic or environmental. (S.Niveditha et al 2012)

The microbial biofilms have been associated with a variety of persistent infections which respond poorly to the conventional antibiotic therapy. This also helps in the spread of antibiotic resistant traits in the nosocomial pathogens by increasing the mutation rates and by the exchange of the genes which are responsible for the antibiotic resistance. The antibiotic therapy against the device associated biofilm organisms often fails without the removal of the infected implant. The physiological heterogeneity is another important characteristic which is observed in the biofilm bacteria. This phenomenon affects the rate of growth and the metabolism of the bacteria and it is reflected by the interbacterial quorum signals, the accumulation of the toxic products and the change in the local micro environment. These so called persister cells are not resistant to the antibiotics per se, but they become resistant when they are associated with the biofilm (Simon AL and Robertson GT.2008). Therefore present study carried out to find out the prevalence of of biofilm production among uropathogens and to study the antimicrobial resistance pattern amongst them.

Material and method

The present study was carried out in the Department of Microbiology, Chandulal Chandrakar Memorial Medical College and Hospital, Durg, Chhattishgarh during July 2014 to August 2015. Hospitalized and OPD patients of all ages, either sexes, with a clinical diagnosis of UTI were included in the study. A total 152 E. coli, isolates recovered from 426 UTI cases. Urine samples were processed immediately and E. Coli isolates were identified by the standard microbiological procedures, as per standard protocols.(Collee JG et al 1996). Biofilm production was detected by microtiter plate (MTP) method,4 in which the ability of microorganisms to form biofilm on abiotic surfaces is detected by growing them in an MTP, which is then detected quantitatively by spectrophotometer using an ELISA reader.

Biofilm formation is detected by :

Tissue Culture Plate Method : (Christensen BB et al.,1999) A loopful of isolated test organisms from overnight cultures were inoculated in 10ml of Trypticase soy broth with 1% glucose and incubated at 37°C for 24 hours. Individual wells of sterile 96 well-flat bottom polystyrene tissue culture treated plate were filled with 200ul of the bacterial suspension corresponding to 0.5 McFarland after further dilution of 1:100 with fresh medium along with control organisms. Only broth served as a control to check sterility and nonspecific binding of media. The plates were inoculated at 37°C for 24 hours. After incubation, contents of each well were removed by gentle tapping and wells were washed three times with 300 µl of sterile saline. The remaining attached bacteria were heat-fixed by exposing them to hot air at 60°C for 60 min. Then150 µl crystal violet (2%) stain was added to each well. After 15 min, the excess stain was rinsed off by decantation, and the plate was washed. 150 µl 95% ethanol was added to each well, and after 30 min, the optical densities (OD) of stained adherent bacterial films were read using a microtitre plate reader at 600nm. The OD values were calculated for all tested strains and negative controls, the cut-off value (ODc) was established.

Table 1. Standard reference table for measurement of biofilm formation.

Mean OD values	Adherence	Biofilm formation
<0.120	Non/ weak	
0.120 - 0.240	Moderately	Moderate
>0.240	Strong	High

The antibiotic susceptibility testing was performed by using standard antimicrobial agents as per CLSI guideline. E. coli (ATCC 25922) was used as control strain.

Statistical Analysis

Statistical analysis was done by using SPSS version 17.0. A P-value of less than or equal to 0.05 was considered to be significant.

Results

Of the 426 urine specimens of urinary tract infection processed, E. coli was 152 (53.71%). 152 E. coli strains subjected to biofilm production, by Tissue Culture Plate Method (TCP),98 (64.47%) showed biofilm

production whereas 54(35.52%) strains were non biofilm producer. 12(12.24%) strains showed highly positive and 86 strains (87.75%) showed moderate biofilm production.

The multi-drug resistant pattern of the biofilm producing E. coli is shown in Table 1. All the biofilm forming strains showed maximum resistance to amoxyclav (100%) & ampicillin (100%) followed by ciprofloxacin (98.97%),ceftazidime (97.95%) co-trimoxazole (89.79%), cephotaxime(88.77%) , piperacillin/tazobactam(85.71%), gentamicin(86.74%), tetracycline (80.61%) and amikacin (59.18%). Both biofilm producer and non- biofilm producer were highly resistant to Amoxyclv, Ampicillin followed by gentamicin, ciprofloxacin ,& piperacillin/tazobactam. However, resistance to other antibiotics such as co-trimoxazole, (89.79% vs. 37.03%), tetracycline (80.61% vs. 48.14%) Amikacin,(59.18% vs. 33.33%) and Norfloxacin(62.24% vs. 44.44%), was comparatively higher among biofilm producer than non-biofilm producer. Nitrofurontoin was found to be 100% sensitive amongst nonbiofilm producer, whereas 7(7.14%) biofilm producer were found to be resistant. These biofilm positive UPECs were found to be multidrug resistant, which was proved to be statistically significant (P # 0.05)

Table 2: Distribution of Antimicrobial resistance amongst biofilm producer UPEC

Antibiotic	Biofilm producer N=98	Biofilm nonproducer N=54
Nitrofurantoin	7(7.14%)	19(35.18%)
Norfloxacin	61(62.24%)	24(44.44%)
Cotrimoxazole	88(89.79%)	20(37.03)
Ampicillin	98(100%)	49(90.74%)
Amoxclav	98(100%)	50(92.59%)
Ceftazidime	96(97.95%)	29(53.70%)
Cefotaxime	87(88.77%)	41(75.92%)
Imipenem	29(29.59%)	0
Gentamicin	85(86.74%)	44(81.48%)
Amikacin	58(59.18%)	18(33.33%)
Tetracycline	78(80.61%)	26(48.14%)
Ciprofloxacin	97(98.97%)	42(77.77%)
Piperacillin-Tazobactum	84(85.71%)	39(72.22%)

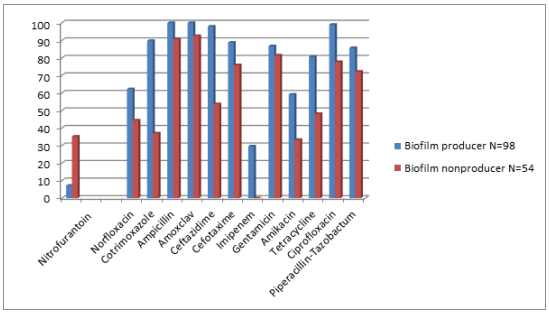

Figure 1 Antibiotic resistance pattern of biofilm and non biofilm producers uropathogenic E.coli (n=152)

Discussion

Urinary tract infection (UTI) is the most commonly acquired bacterial infection and antibiotic resistance of uropathogens has been known to increase worldwide with biofilm production being the prime cause. In our study significant production of biofilms was seen in 64.47% isolates of *E. coli*, which is similar to other studies . Sharma *et al.,* reported (67.5%) ,Ponnusamy et al. showed (72%) ,S.Niveditha et al . reported 60% strains , whereas Abdagire NV et al. Reported (60.15%) of biofilm forming E.coli.

In current study antibiotic resistance was higher among biofilm producers to commonly used antibiotics as compared to non biofilm producers. (fig. 1). The beta-lactam antibiotics cefotaxime and ceftazidime, and the aminoglycosides gentamicin and amikacin were hardly effective. There are several reasons why these antibacterial agents are not as effective on biofilm cells as they are on planktonic cells. Some antibiotics, such as beta-lactams, require rapid bacterial growth to kill cells (Anderson GG et al. 2003). Thus the reason behind antibiotics resistance is due to long term persistence of bacteria in biofilm in various environments, decreased bacterial growth rate in a biofilm, expression of resistence genes, and restricted penteration of antibiotics into biofilm , similar results were obtained by Rewatkar et al 2013 & Eman A et al 2015.

Thus there is an urgent need to regulate the overuse of antibiotics. This would limit the spread of resistant microorganisms in the community as well as in hospital settings.

More researchs are needed to find out easier methods for diagnosing and quantifying biofilm infection, to develop more specific antimicrobial agents and ideal device surfaces that would surely help the fight against biofilm formation.

Conclusion

The present study showed significant correlation between biofilm production and drug resistance. So it is necessary to screen all urinary isolates for biofilm production. This will help our clinician in prescribing an appropriate antibiotic against urinary tract infection.

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