



International Journal of Medical Research & Health Sciences

www.ijmrhs.com

Volume 4 Issue 2

Coden: IJMRHS

Copyright ©2014

ISSN: 2319-5886

Received: 31st Dec 2014Revised: 27th Jan 2015Accepted: 20th Feb 2015

Research article

BIOFILM FORMATION AND ANTIMICROBIAL RESISTANCE PATTERN AMONG UROPATHOGENS

*DardiCharanKaurG¹, MaralSanjivani.S²¹Department of Microbiology, Maharashtra Institute of Medical Education & Research (MIMER), Talegaon Dabhade, Pune, Maharashtra, India²Assistant Professor Symbiosis Institute of Health Sciences, Pune Maharashtra, India

*Corresponding author email: charan13@rediffmail.com

ABSTRACT

Background: Bacterial biofilms play an important role in urinary tract infections and is responsible for persistence infections and also the higher antimicrobial resistance is seen in biofilm forming uropathogen as compared to free floating bacteria. So the present study was undertaken with the aim to know the prevalence of biofilm formation and antimicrobial resistant pattern of biofilm producer and non-biofilm producing uropathogens. **Materials & Methods:** A total of 146 Gram negative bacilli and 62 S. aureus isolated from patients suspected UTIs were tested for biofilm formation and antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method on Mueller Hinton agar as per CLSI guidelines. **Result:** Out of 208 isolates from urine, Biofilm formation was noted in 122(58.66%) and no biofilm formation in 86(41.35%). [Strong Biofilm formation in 76(36.54%) and weak biofilm formation in 46(22.12%)]. In our study, we noted biofilm and non-biofilm forming microorganism showed mark difference in antimicrobial resistance pattern. In *Staphylococcus aureus* striking difference was noted to ciprofloxacin (100% versus 33.33%) and azithromycin (96% versus 41.67%). Isolates showed no resistance to linezolid. Whereas isolates of *Pseudomonas aeruginosa* to netilin (100% versus 42.86%). And in other Gram negative bacilli difference was noted to gentamicin (87.93% versus 13.43%) and norfloxacin (84.48% versus 37.31%) **Conclusion:** Biofilm forming isolates showed higher antimicrobial resistance as compared to non-biofilm producer. Thus, Uropathogen should be routinely screened for biofilm formation.

Keywords: Uropathogen, Biofilm formation, Antimicrobial resistance pattern

INTRODUCTION

Urinary tract infections (UTIs) are the important causes of morbidity affecting 150 million people globally each year and also continue to be the most common causes of infections in hospitalized patients.^[1, 2] It is the most common bacterial infections in humans both in the community and hospital settings, and in all age groups, and usually requires urgent treatment. Malnutrition, poor hygiene, low socio-economic status is associated with urinary

tract infections and these factors are rife in rural settings.^[3] Microorganism associated with UTI has a property to form biofilm and this biofilm can be formed by one or many bacteria which show antimicrobial tolerance. Host factors like age, diabetes, long term hospitalization and catheterization are the predisposing conditions.^[4] According to the NIH, urology is one of the main areas of concern where biofilm can become a serious problem and

Biofilm are found in the urothelium, prostate stones, and implanted foreign bodies.^[5]

The population of bacteria growing on the biotic and abiotic surfaces is the biofilms. The bacteria embed themselves in a self-produced extracellular matrix of exopolysaccharide (EPS), proteins and some micro molecules such as DNA. This matrix accounts for about 90% biomass.^[6] The extracellular matrix of exopolysaccharide protects the bacteria from host defenses and impedes delivery of antibiotics.^[7] Infact higher antimicrobial resistance is seen in biofilm forming uropathogenas compared to free floating bacteria. Bacterial biofilm is responsible for persistence urinary tract infections and the multidrug resistance so the present study was undertaken with the aim

- To know the prevalence of biofilm formation in uropathogens
- To know the antimicrobial resistant pattern of biofilm producer and non- biofilm producing uropathogens

MATERIAL AND METHODS

The Prospective study was carried out in the department of Microbiology of a tertiary care rural hospital from the period of July 2012 to December 2013. Urine specimen from patients suspected of UTIs was collected. The sample was processed and identification of uropathogen was done by standard microbiological techniques.^[8] A total of 146 Gram negative bacilli and 62 *S. aureus* isolated from patients suspected of UTI were randomly selected. The isolates were tested for biofilm formation by Tube method as described by Christensen et al.^[9]

1. The tube containing TSBglu (10mL) were inoculated with culture of uropathogen and incubated at 37 degree C for overnight.
 2. The tubes were decanted and washed with PBS (pH 7.3) and dried.
 3. Dried tubes were than stained with 0.1% crystal violet.
 4. Excess stain was removed and tubes were washed with deionized water.
 5. Tubes were then placed in inverted position to dry
 6. Tubes were finally observed for biofilm formation
- Assays were performed in triplicate at three different times.

The Isolates were tested for antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method on Mueller Hinton agar as per CLSI guidelines.^[10] The following antimicrobial agents were tested for *Staphylococcus aureus*: amikacin(Ak) (30µg), ampiclox(ACX) 20µg, azithromycin(AZ) 15µg, calithromycin (CLR)15 µg, cefoperazone (CFP)30µg,cefotaxime(CF)30µg,cefuroxime(CR)30µg,ciprofloxacin (CIP)5µg, cotrimoxazole (Cot)5µg, gentamicin (30µg), linezolid (30µg), sparfloxacin (SF)5µg.

The antimicrobial agents tested for *Pseudomonas aeruginosa*: amikacin(Ak) 30µg, cefepime(CPM) 30µg, cefoperazone (CFP)75µg ,ceftazidime(CAZ) 30µg, ciprofloxacin(CIP) 5µg, gentamicin (GEN)10µg,levofloxacin(Le) 5µg, meropenem(MRP) 10µg, netilin(NET) 30µg, Piperacillin(Pi)100µg, ticarcillin(Ti)75µg, tobramycin(TOB)10µg

The antimicrobial agents tested for Gram negative bacilli were amikacin (An) 30µg, cefaclor (CFC) 30µg, cefadroxil (CD) 30µg, ceftriaxone (CTX) 30µg, ciprofloxacin (CIP)5µg, gentamicin (G)10µg, netilin (NET) 30µg, nitrofurantoin (NF) 300µg, norfloxacin (NR) 10µg, ofloxacin (ox) 5µg,

The Antimicrobial disc was obtained from Hi-media Laboratories Pvt. Ltd, Mumbai, India.

RESULTS

Out of 208 isolates from urine, Strong Biofilm formation was noted in 76(36.54%) and Weak Biofilm formation in 46(22.12%) and no biofilm formation in 86(41.35%). (Table No 1). Higher Biofilm formation was seen in females 140(67.31%) as compared to males 68(32.69%),

Table 1: Biofilm producers in uropathogens

Isolates	No of Samples	Strong Biofilm formation	Weak Biofilm formation	Negative Biofilm formation
<i>E. coli</i>	93	27(29.03)	16(17.20)	50(53.76)
<i>Staphylococcus aureus</i>	62	32(51.61)	18(29.03)	12(19.35)
<i>Pseudomonas sps</i>	21	8(38.09)	6(28.57)	7(33.33)
<i>Klebsiellasps</i>	13	5(38.46)	3(23.07)	5(38.46)
<i>Citrobactersps</i>	13	4(30.77)	3(23.07)	6(46.15)
<i>Proteus sps</i>	4	0		4
<i>Morganellamor ganii</i>	1	0		1
<i>Serratiamarcesc ens</i>	1	0		1
Total	208	76(36.54%)	46(22.12%)	86(41.35%)

Overall strong biofilm formation was 36.54%, weak in 22.12% and 41.35% were Negative for Biofilm. Strong biofilm formation and weak biofilm formation was significantly less for *E.coli*. (Chi²test =5.99; P<0.05). As against that Strong biofilm formation was significantly more in *Staphylococcus aureus* (Chi²test =12.44; P<0.05). Outcome of *P. aeruginosa* was comparable to the overall outcome. The above table depicts highest Biofilm producers were *Staphylococcus aureus* 50/62 (80.65%) followed by *P. aeruginosa* 14/21(66.67%)

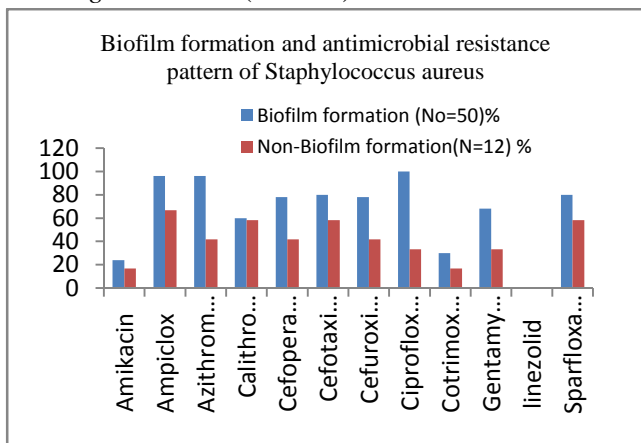


Fig 1: Biofilm formation and antimicrobial resistance pattern of *Staphylococcus aureus*

The above chart depicts Biofilm formation and non-Biofilm producer showed mark difference in antimicrobial resistance pattern to ciprofloxacin and azithromycin. Isolated showed no resistance to linezolid. On the X axis are the antimicrobial agent tested and on Y axis is the percentage of resistance shown by the isolate

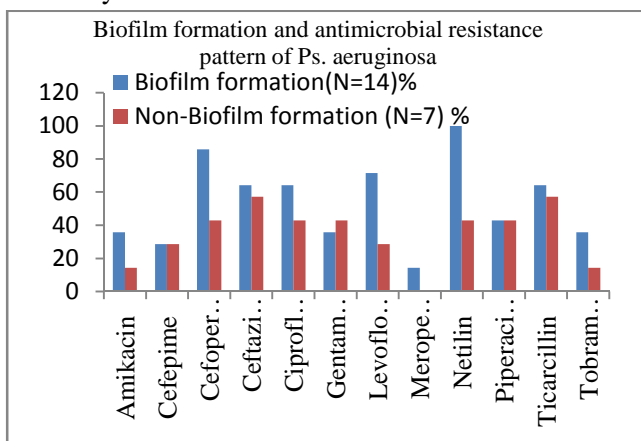


Fig 2: Biofilm formation and antimicrobial resistance pattern of *Ps. aeruginosa*

In the above table it is observed Biofilm formation and non-Biofilm producer *Pseudomonas aeruginosa* showed significant difference in antimicrobial resistance pattern to netilin. On the X axis are the

antimicrobial agent tested and on Y axis is the percentage of resistance shown by the isolates.

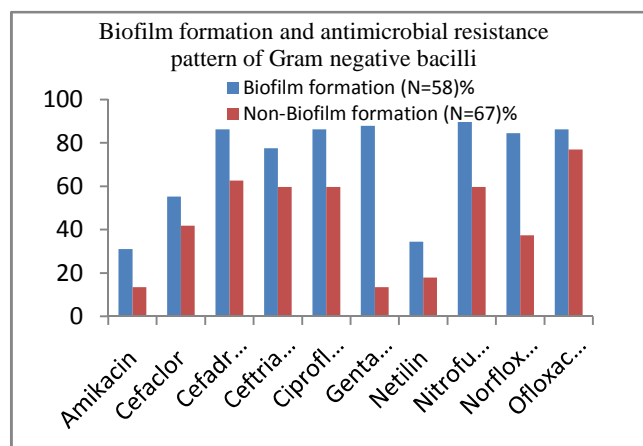


Fig 3: Biofilm formation and antimicrobial resistance pattern of Gram negative bacilli

In the above table it is depicted that Gram negative bacilli (Biofilm formation and non-Biofilm producer), showed significant difference in antimicrobial resistance pattern to gentamicin and norfloxacin. On the X axis are the antimicrobial agent tested and on Y axis is the percentage of resistance shown by the isolates.

DISCUSSION

Biofilms are estimated to be responsible for over 65% of nosocomial infections and 80% of all microbial infections as stated by U. Römling.^[11] *E. coli*, *Staphylococcus aureus*, *Pseudomonas sps*, *Klebsiellasps*, *Citrobactersps*, *Proteus sps*, *Morganellamorganii*, *Serratiamarcescens* are the pathogen isolated from urine similar were the findings of Sara M. Soto.^[12] In our study *E. coli* was the predominant organism agent from urinary tract infections whereas in a study by Lucchetti et al *P. aeruginosa*. According to epidemiologic data, 35.0% to all acquired nosocomial infections are urinary and 80.0% are related to catheter use.^[13]

In our study higher prevalence of UTI was seen in females as compared to the males 68 (32.69%), thus showing a female predominance. Our study is similar to the findings of Syed M A, Devanand P et al.^[14,15] Kamat US et al in their study noted females are more prone to develop UTIs, probably due to their anatomical physiological changes like short urethra, its proximity to the anus, dilatation of the urethra and the stasis urine during pregnancy.^[2]

In our study we observed for Biofilm formation among the uropathogen. We noted highest Biofilm producers were *Staphylococcus aureus* 50/62 (80.65%) followed by *P. aeruginosa* 14/21(66.67%) and *E.coli* 43/93(46.24%).

The bacteria from the bowel move to the bladder and adhere to the uroepithelium and form biofilm which can invade the renal tissue causing pyelonephritis. The clinical spectrum of complicated UTIs may range from cystitis to urosepsis with septic shock and relapse is due to the biofilm forming capacity of the microorganism.^[16, 17]

Alicia Valéria Zaranzain their study showed biofilm production by the Congo Red Agar method in 52.0% & biofilm formation by 86% on polystyrene microplates. Among them strong biofilm formation was found in 22.1%, moderate in 47.7% and weak in 30.2%. Carlos J et al reported biofilm formation in *P. aeruginosa* in 83% of clinical strains & that biofilm formation was higher in MDR isolates.^[18,19]

The components of the EPS involved in the formation of *P. aeruginosa* biofilm are encoded mainly by different genes located in three independent operons: algU, psl, and pel and in *S. aureus* by gene icaABDC.^[20, 21]

The persistent cells shows reduced metabolism leading to higher antimicrobial resistance. Biofilm are difficult to eradicate so combined therapy is recommended for the treatment of biofilm-associated infections.

In our study, we noted Biofilm and non-Biofilm forming *Staphylococcus aureus* isolates showed marked difference in antimicrobial resistance pattern to ciprofloxacin (100% versus 33.33%) and azithromycin (96% versus 41.67%). Isolates showed no resistance to linezolid. *Pseudomonas aeruginosa* isolates showed significant difference to netilmicin (100% versus 42.86%). In the Gram negative bacilli (Biofilm formation and non-Biofilm producer), significant difference in antimicrobial resistance pattern was observed to gentamicin (87.93% versus 13.43%) and norfloxacin (84.48% versus 37.31%). (Chart 1-3) Fatima Khan et al found ciprofloxacin was effective against biofilm producers and Zheng Z et al noted rifampicin has putative antibiofilm properties, to penetrate Staphylococcal Biofilm.^[22, 23] Donlan R.M., et al in their study on Biofilm's Survival mechanisms of clinically relevant microorganisms observed the age of the biofilm also

affects the susceptibility to antibiotics. In their study they highlighted 10-day-old biofilms are more resistant than 2-day-old biofilms. This emphasizes the need for prompt diagnosis and treatment.^[24]

Sara M. Soto in the review article analyzed some workers observed Macrolides (erythromycin, clarithromycin, and azithromycin) present high "in vitro" and "in vivo" activity, against biofilm-forming organism *P. aeruginosa*, other Gram-negative bacteria, and *Staphylococcus* spp. Other workers reported macrolides enhances biofilm formation in Gram-positive bacteria with the explanation that there is increase in the expression of biofilm-related genes (icaAatIE fruA, pyrR, sarA, and sigB).^[12]

In our study we found higher antimicrobial resistance in biofilm producers as compared to the negative biofilm producers. Similar were the findings of other workers Fatima Khan et al, Bijayini Behera et al^[22,25]

Sara M. Soto in study, noted higher antimicrobial resistance by biofilm may be due to the some antimicrobial agents are not able to diffuse through the matrix or sometimes the time taken to diffuse through is longer than the duration of treatment or the antibiotic lifetime. Or an antimicrobial agent that diffuses can be inactivated by the pH inside biofilm.^[12]

CONCLUSION

Biofilm forming isolates showed higher antimicrobial resistance as compared to non-Biofilm producer. This is due to metabolically inactive persister cells. Antimicrobial resistance is a global issue, so uropathogen should be routinely screened for biofilm formation and antimicrobial resistance before initiating the treatment.

Conflict of Interest: Nil

REFERENCES

1. Orenstein R, Wong ES. Urinary tract infections in adults. *Am Fam Physician*. 1999; 59:1225–34.
2. Kamat US, Fereirra A, Amonkar D, Motghare DD, Kulkarni MS. Epidemiology of the hospital acquired urinary tract infections in a medical college hospital in Goa. *IJU*. 2009; 25(1):76.
3. Ahmed SM, Avasara AK. Urinary tract infections (UTI) among adolescent girls in rural Karimnagar District, AP K.A.P. STUDY. *Indian J Pre Soc Med*. 2008;(1)39

4. <http://www.infectioncontrolday.com/news/2013/10/women-most-often-suffer-utis-but-men-more-likely-to-be-hospitalized.aspx>
5. P. Tenke, B. Kovacs, M. Jäckel, and E. Nagy, "The role of biofilm infection in urology," *World Journal of Urology*. 2006; 24(1):13–20.
6. H.-C. Flemming and J. Wingender, "The biofilm matrix," *Nature Reviews Microbiology*. 2010; 8(9):623–33.
7. Stewart, P.S. Mechanisms of antibiotic resistance in bacterial biofilms. *Ind. J. Med. Microbiol*. 2002; 29: 107-13.
8. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic microbiology. 12th edition, chapter 57. Infection of the urinary tract. Mosby Elsevier; 2007:842-55
9. Gordon D, Christensen W, Andrew Simpson, Alan L. Bisno, And Edwin H. Beachey. Adherence of Slime- Producing Strains of *Staphylococcus epidermidis* to Smooth Surfaces. *Infection and Immunity*, July 1982; Vol. 37(1):318-26.
10. Clinical and Laboratory Standard Institute. Performance standards for antimicrobial disc susceptibility tests, twentieth supplement. 2012; 32(3):100-21.
11. U. Römling and C. Balsalobre, "Biofilm infections, their resilience to therapy and innovative treatment strategies," *Journal of Internal Medicine*. 2012; 272(6):541–61.
12. Sara M. Soto. Importance of Biofilms in Urinary Tract Infections: New Therapeutic Approaches. *Advances in Biology*. 2014; 13 [http:// dx.doi. org/ 10.1155/2014/543974](http://dx.doi.org/10.1155/2014/543974)
13. G. Lucchetti, A. J. Silva, S. M. Y. Ueda, M. C. D. Perez and L. M. J. Mimica, "Infecções Do Trato Urinário: Análise da Frequência e Do Perfil de Sensibilidade Dos Agentes Causadores de Infecções Do Trato Urinário Em Pacientes Com Cateterização Vesical Crônica," *Journal Brasileiro de Patologia e Medicina Laboratorial*. , 2005; 41(6) 383-89.
14. Syed M A, Ramakrishna P J, shaniyakoyakutty, Arya B, Shakir VPA. Urinary Tract Infections – An overview on the Prevalence and the AntibioGram of Gram Negative Uropathogens in a Tertiary Care Centre in North Kerala, India. *Journal of Clinical and Diagnostic Research*. 2012; 6(7): 1192-95.
15. Devanand P and Ramchandra S S. Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in urban community of Meerut city, India. *ISRN Microbiology* 2013, 13
16. J. C. Nickel, I. Ruseska, J. B. Wright, and J. W. Costerton, "Tobramycin resistance of *Pseudomonas aeruginosa* cells growing as a biofilm on urinary catheter material," *Antimicrobial Agents and Chemotherapy*/ 1985;27(4):619–24.
17. S. M. Soto, A. Smithson, J. P. Horcajada, J. A. Martinez, J. P. Mensa, and J. Vila, "Implication of biofilm formation in the persistence of urinary tract infection caused by uropathogenic *Escherichia coli*," *Clinical Microbiology and Infection*. 2006; 12(10): 1034–36.
18. Alicia Valéria Zaranza, Francielle Costa Morais, Monique Santos do Carmo, Adriana de Mendonça Marques, Cristina Andrade-Monteiro, Thiago Feitosa Ferro, Valério Monteiro-Neto, Patrícia de Maria Silva Figueiredo. Antimicrobial Susceptibility, Biofilm Production and Adhesion to HEp-2 Cells of *Pseudomonas aeruginosa* Strains Isolated from Clinical Samples. *Journal of Biomaterials and Nanobiotechnology*, 2013, 4, 98-06.
19. Carlos J Sanchez Jr, Katrin Mende, Miriam L Beckius, Kevin S Akers, Desiree R Romano, Joseph C Wenke and Clinton K Murray, *BMC Infectious Diseases*. 2013;13:47-
20. Xicohtencatl-Cortes J, Monteiro-Neto V, Saldaña Z, Ledesma MA, Puente JL, Girón JA. The type 4 pili of enterohemorrhagic *Escherichia coli* O157:H7 are multipurpose structures with pathogenic attributes. *J Bacteriol* 2009; 191:411-21.
21. Ammendolia, M.G., R.D. Rosa, L. Montanaro, C.R. Arciola and L. Baldassarri. Slime production and expression of slime associated antigen by staphylococcal clinical isolates. *J. Clin. Microbiol*. 1999; 37: 3235-38.
22. Fatima Khan, Indu Shukla, Meher Rizvi, Tariq Mansoor and S.C. Sharma. Detection of Biofilm Formation in *Staphylococcus aureus*. Does it have a role in Treatment of MRSA Infections? *Trends in Medical Research*. 2011; 6: 116-23.

23. Zheng Z, Stewart PS. Penetration of rifampin through *Staphylococcus epidermidis* biofilms. *Antimicrob Agents Chemother* 2002; 46:900-03.
24. Donlan R.M., Costerton W., Biofilms: Survival mechanisms of clinically relevant microorganisms. *Clin. Microbiol. Rev.*, 2002, 15(2): 167-93.
25. Bijayini Behera, Anupam Das, Purva Mathur & Arti Kapil. High prevalence of carbapenem resistant *Pseudomonas aeruginosa* at a tertiary care centre of north India. Are we under-reporting? *Indian J Med Res.* 2008; 128:324-25.