INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

EVALUATION OF VARIOUS METHODS FOR DETECTION OF BIOFILM FORMATION AMONG MULTI DRUG RESISTANT UROPATHOGENIC *KLEBSIELLA PNEUMONIAE* ISOLATES

Devinder kaur*, Kamaljeet, Dr.Varsha A Singh, Dr. Ruhi bunger
Maharishi Markandeshwar Institute of Medical Science and Research (MMIMSR), Mullana, Ambala, Haryana, India.

ARTICLE INFO

Article history
Received 21/08/2015
Available online
31/08/2015

Keywords
Biofilms,
Klebsiella Pneumoniae,
Multi Drug Resistance,
Tissue Culture Plate Method(TCPM),
Tube Adherence Method(TAM),
Modified Congo Red Agar Method(MCRAM) .

ABSTRACT

Urinary tract infection is a serious health threat with respect to antibiotic resistance and biofilm formation being the prime cause for the antibiotic resistance, the present study aimed to detect biofilm formation in Multidrug-resistant uropathogenic *Klebsiella pnemoniae* isolates by using Tissue Culture Plate Method, Tube adherence method and Modified Congo Red Agar Method. The study was conducted in the department of Microbiology of Maharishi Markandeshwar institute of medical science and research, Mullana .Out of total 550 samples, 23.2%(128) was uropathogenic. Out of 128 samples 60 samples were *Klebsiella pnemoniae* strains. They were multidrug resistant (cephalosporins and floroquinlones). The rate of biofilm producers is detected by three methods which were 38(63.33%). In which, Modified Congo Red Agar Method was best 36(60%) followed by Tube adherence method 33 (55%) and Tissue Culture Plate Method 28 (46.67%).In present study, higher biofilm producing isolates were detected in indoor and urban patients 76.31%(29) and 57.89 %(22) respectively. Furthermore, regarding the age highest percentage of biofilm producing isolates was found between 50-79 years (55.26%) followed by 20-49 years of age (23.68%). Dominancy of female (71.05%) were established over the male of only (28.94%). The data on antibiogram revealed that Imipenem (73.68%) followed by Amikacin (68.42%) and Nitrofurantoin(52.63%) were most effective antibiotic for biofilm producing Multidrug-resistance *Klebsiella pneumoniae* .So at last we can conclude that the urine isolates specially Multidrug-resistance *Klebsiella pneumoniae* should be screened for biofilm production and should put for antibiotic sensitivity to determine antibiotic policy in the hospital.

Corresponding author
Devinder kaur
Department of Microbiology.
Maharishi Markandeshwar Institute of
Medical Science and Research (MMIMSR),
Mullana, Ambala, Haryana, India.
E.MAIL ID:devinder.dvd@gmail.com.

*Please cite this article in press as **Devinder kaur** et al. Evaluation of Various Methods For Detection of Biofilm Formation Among Multi Drug Resistant Uropathogenic Klebsiella Pneumoniae Isolates. Indo American Journal of Pharmaceutical Research.2015:5(08).*

INTRODUCTION

The inclination of biofilms has skyrocketed in recent years due to increased responsiveness of the pervasiveness and impact of biofilms on natural and industrial systems, as well as human health. A biofilm is a well-organized, cooperating community of microorganisms. Biofilm associated cells are differentiated from the suspended counterpart by reduced growth rate, up and down regulation of gene and generation of extracellular polymeric matrix. According to the recent public announcement from National institute of Health, more than 80% of all microbial infections are caused by Biofilm.[1] Over the past few years, a notable increase in antibiotic resistance among microorganism recovered from hospitalized patients has been reported, especially for critically ill patients[2]. Urinary tract infection (UTI) is the second most common infectious presentation in community practice.[3]*K.pneumoniae* infections are one of the most important nosocomial infections currently facing health care professionals today. Mortality and morbidity resulting from *K. pneumoniae*-associated infections is often the product of bacteraemia where part of the biofilm can become dislodged from the main entity to form a secondary site, or where cells detached from the biofilms can cause such diseases as septic shock, and respiratory and acute renal failures.[4]

MATERIAL AND METHODS

A cross sectional study was conducted in the Microbiology Department of Maharishi Markandeshwar institute of medical science and research, Mullana Ambala. A total of 550 urine samples were collected from the outdoor patient and indoor patients of Maharishi Markandeshwar institute of medical science and research, Mullana Ambala. Urine samples were processed within one hour of the collection by inoculating on Cysteine Lactose Electrolyte Deficient (CLED) agar plats and incubated at 37°C for 24 hrs. After 24hrs out of550 samples128 samples are Klebsiella pneumoniae. On the basis of colony morphology and biochemical reaction. These samples were proceeds for antibiotic sensitivity test as per Modified Stokes method on Muller Hinton agar plates according to the National Committee for the Clinical Laboratory Standard. A 60 urine samples are detected for Multidrug Resistant for cephalosporins and floroquinlones. These 60 urine isolates of *Klebsiella pneumoniae* strains were subjected to detection of biofilm by: Tissue Culture Plate Method, Modified Congo Red Agar Method and Tube Adherence Method.

RESULTS

*Fig I S*hows out of total 550 samples ,128(23.2%) were uropathogenic *Klebsiella pneumoniae. Fig II* shows out of those 128 Uropathogenic *Klebsiella pneumonia* , 60(46.8%) showed resistance to Cephalosporins and Floroquinolones group of antibiotics. *Fig III* shows out of 60 multi drug resistant uropathogenic *Klebsiella pneumoniae* isolates ,38(63.33%) were biofilm producers. *Fig IV* Shows that the detection of biofilm the highest rate was by Modified Congo red agar (60%)and Tube Adherence method (55%) followed by Tissue Culture plate method (46.67%)..*FigVI* Shows that Imepenem came out to be the most sensitive drug against biofilm producing MDR *Klebsiella pneumoniea*. The other two most sensitive drugs were Amikacin (68.42%) and Nitrofurantoin (52.63%) respectively. *FigVII* Revealed that among biofilm producing *Klebsiella pneumoniae* 52.6% were isolated from catheterized patients and 47.3% in non catheterized patients.*Fig XII* Depicted that higher number of biofilm producing isolates were seen in indoor patients (76.31%) as compared to patients from outpatient department (23.6%). *FigIX* Depicts the correlation of age with the occurrence of biofilm producing *Klebsiella pneumoniae* in urinary tract infections. Highest percentage of isolates was found between 50-79 years of age (55.26%) followed by 20-49 years(23.68%).*FigXI* Demonstrated that female predominance (71.05%) was seen among patients diagnosed with biofilm producing uropathogenic *Klebsiella pneumoniae* when compared with males (28.94%). *FigXI* Depicted that higher number of biofilm producing isolates were seen in patients residing in urban areas urban areas (57.89%) as compared to the ones from rural areas (42.1%).

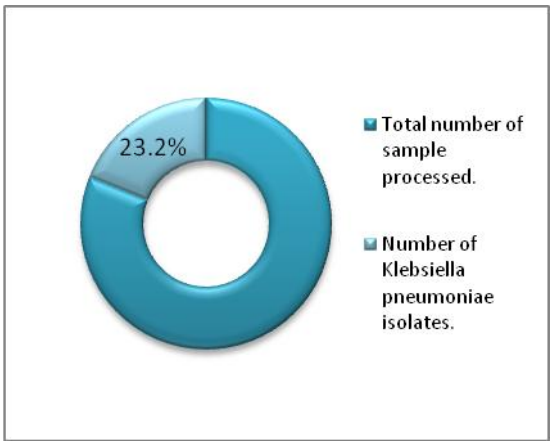


FIG I: Rate of *Klebsiella pneumoniae* isolates in urine samples.

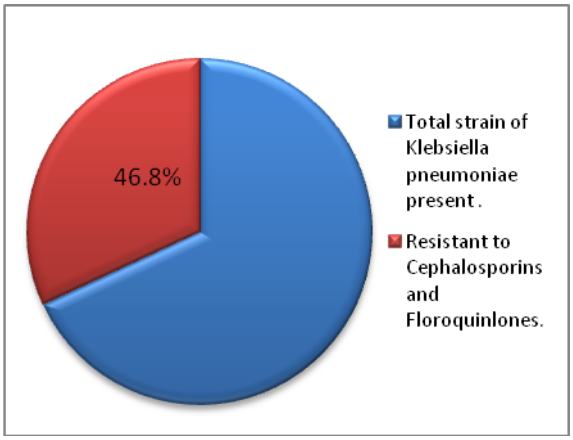


FIG II: Rate of uropathogenic *Klebsiella pneumoniae* isolates resistant to Cephalosporins and Floroquinlones.

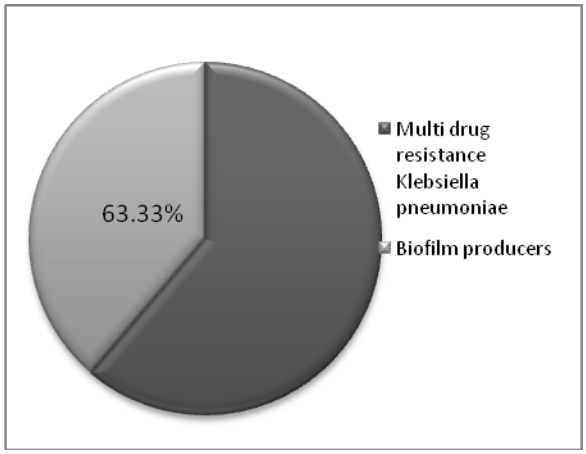


FIG III: Rate of biofilm producing multidrug resistance uropathogenic *Klebsiella pneumoniae*.

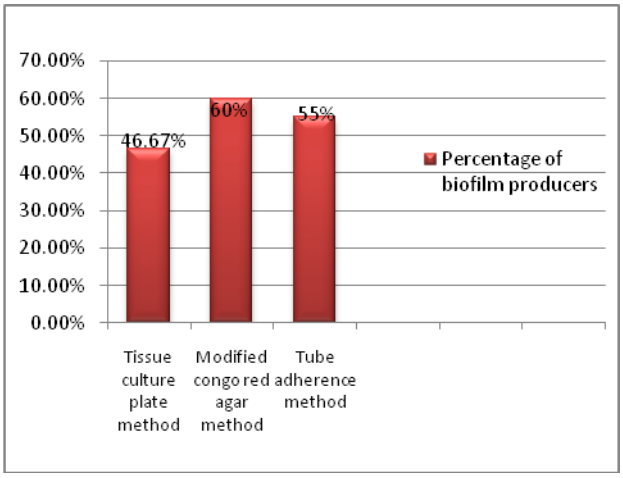
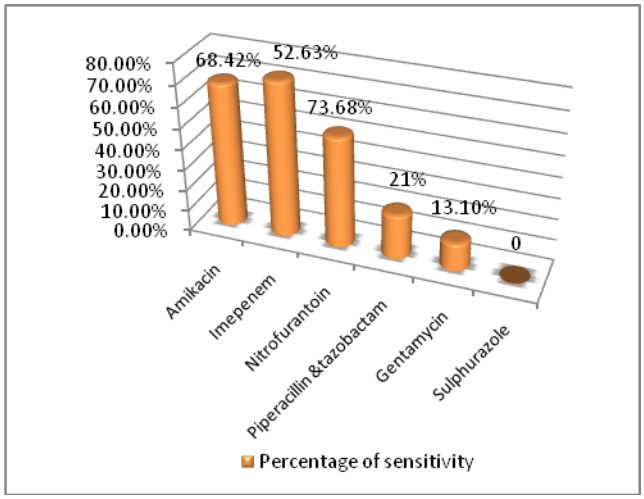


FIG IV: Detection of biofilm in Multi-drug resistant uropathogenic *Klebsiella pneumoniae* isolates by three phenotypic methods.



FIGVI: Antibiotic sensitivity pattern of biofilm producing uropathogenic *Klebsiella pneumoniae* .

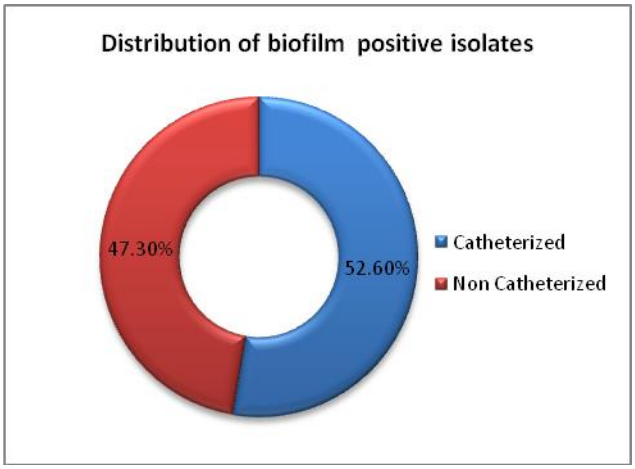


FIG VII: Association of rate of biofilm production in uropathogenic *Klebsiella pneumoniae* isolates with Catheterization.

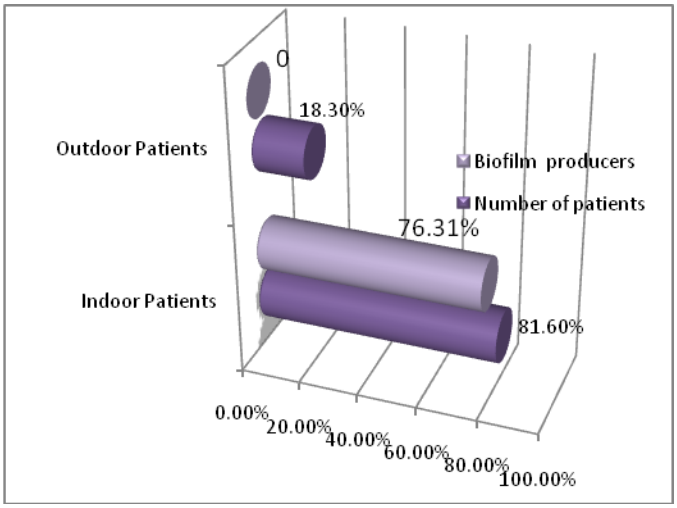
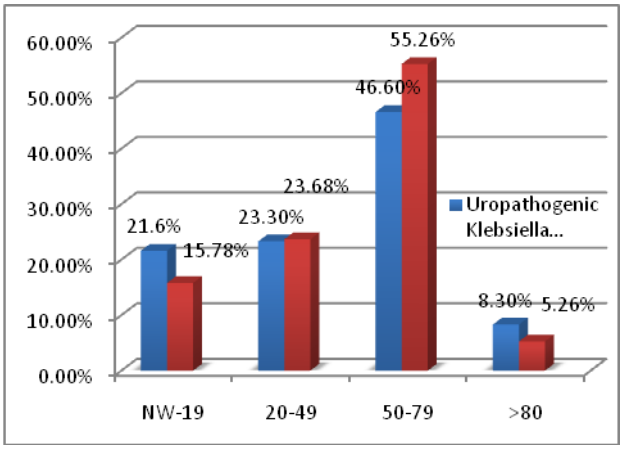
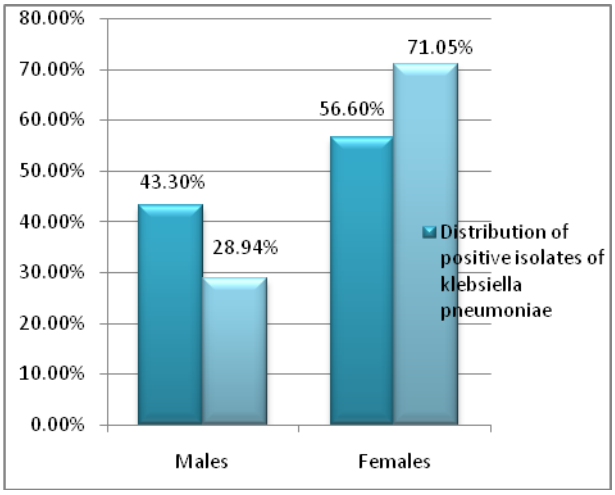


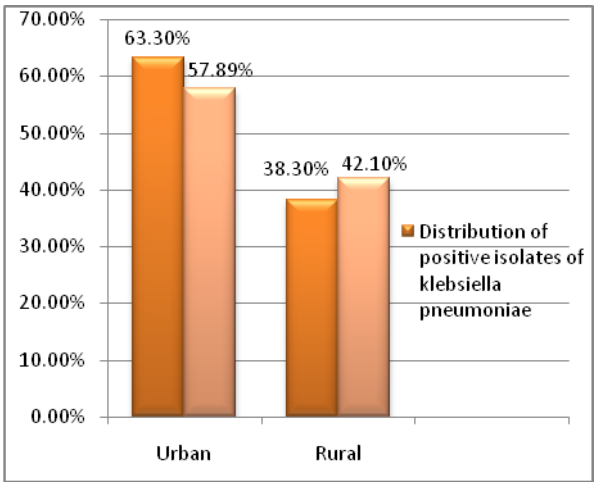
FIG VIII: Association of rate of biofilm production in uropathogenic *Klebsiella pneumoniae* isolates with respect to hospitalization.



FIGIX: Age wise distribution of biofilm production in uropathogenic *Klebsiella pneumoniae* isolates.



FIGX: Gender wise distribution of biofilm production in uropathgenic *Klebsiella pneumoniae* isolates.



FIGXI: Rate of biofilm producing uropathogenic *Klebsiella pneumoniae* isolates with respect to Rural and Urban population.

DISCUSSION

Urinary tract infections(UTI) accounts for a significant part of the work load in clinical microbiology laboratories and enteric bacteria particularly, the second most common bacteria to produce biofilm is Klebsiella pneumoniae and remain the second most frequent cause of UTI ,although the distribution of pathogens that cause UTI is changing .The relationship between antibiotic use and resistance is complex . Antimicrobial resistance offered by different uropathogens is one of the barricades that might hinder a successful treatment. In the present study the rate of uropathogenic *Klebsiella pneumoniae* came out to be 23.2% which is in accordance with Shaifai I et al and Akram M et al where the positivity rate of uropathogenic Klebsiella was 21.6% and 22% respectively.[4]In the present study, the rate of multidrug resistant uropathogenic *Klebsiella pneumoniae* came out to be 46.8%. It is supported by the studies done by Chatterjee M et al[5] and Kumar CN et al[6] in which the resistance rates to the above mentioned groups came out to be 48.5% and 50.4% respectively. , In the present study, positivity rate of biofilm producing uropathogenic *Klebsiella pneumoniae* came out to be 63.33% which is well in accordance with the studies done by Nivedhita S[7] and Promodhini S et al[8] in which Klebsiella pneumoniae as biofilm producers have come out to be 63% each.In the present study, the results with combination of all three methods showed 63.1% positivity followed by the rate was decreasing as the less number of test were evaluated By supporting the result, nobody had mentioned the positivity rate including with the three methods in biofilm production by uropathogenic *Kebsiella pneumoniae*. In the present study, the rate of multi drug resistant *Klebsiella pneumoniae* was higher in patients with urinary catheters (52.6%) It is further supported by the study done by Abdalla NMA et al[9]in which the biofilm forming uropathogens in catheterized patients were 55.5% and in non catheterized patients were 44.4%. In the present study, biofilm producing isolates were detected in 76.31% of patients residing in the hospital and 23.6% in outdoor patients.Accordingly, the age group most prone to biofilm producing *Klebsiella pneumonia* came out to be 50-79 years (55.26%) followed by 20-49 years (23.68%).In the present study, biofilm production was primarily seen in females (71.05%) as compared to males (28.94%). In the present study, the urban population constituted 57.89% of the patients whereas the rural population constituted 42.1% in which biofilm formation was detected.

CONCULISION

To conclude, In the present study rate of biofilm formation in urinary tract infection came out to be significantly high 63.33% at least to an extent which should not be ignored anyway. Hence,Whenever encounter multi-drug resistant (cephalosporins and floroquinolones) *Klebsiella pneumoniae*, subject it for detection of biofilm formation. There are many methods of screening but the phenotypic methods rapid, simple and cheap. These three methods are Tissue culture plate method, Modified Congo red agar and Tube adherence method which every laboratory can afford. Out of these three methods Modified Congo red agar method has been show the best result 60% whereas Tissue culture plate method was gold standard . Biofilm formation is one of the well documented etiology of urinary tract infection and in the currently study has affected females 71.05% as compared to males with almost all age groups . In diabetes mellitus(43.24%) and catheterizated paitents (81%) there are more chances of biofilm formation so such patients should also be screening for biofilm detection. The multi-drug resistant biofilm producing *Klebsiella pneumoniae* are usually sensitive to Imipenem and Amikacin.

REFERENCES

1. Donlan R M. Biofilms: Microbial life on surfaces. Emerg Infect Dis. 2002 Sep;8(9):881-90.
2. *Marhova.M*[1], Kostadinova.S[1], S. Stoitsova[2]. Biofilm-forming capabilities of Urinary Escherichia coli isolate. 2010; 24(2): 589-93.
3. Fridkin SK, Gaynes RP. Antimicrobial resistance in intensive care units. Clin Chest Med. 1999;20(2):303-16.
4. Mohammed A, Mohammed S, Khan AU. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in J N M C Hospital Aligarh, India. Ann of Clini Microb and Antimicrob. 2007; 6: 1-7.
5. Chatterjee M, Banerjee IM, Guha IS, Lahiri A,Karak K. Study of drug resistance pattern of principal ESBL producing urinary isolates in an urban hospital setting in Eastern India Sri Lankan. Journal of Infectious Diseases. 2012; 1: 36-41.
6. Chaudhary NK, Mahadeva M. Extended expectrum betalactamases in uropathogen .asian J pharm clin res 2013;6(3):207-210.
7. Niveditha S., Pramodhini S., Umadevi S., Kumar S., Stephen S. The isolation and the biofilm formation of uropathogens in the patients with catheter associated urinary tract infections (UTIs). J Clin and Diag Res 2012; 6(9):1478-82.
8. Subrmanian P, Shanmugam N, Sivaraman UD, Kumar S, Stpphen S. Antiobiotic resistance pattern of biofilm-forming uropathogens isolated from catheterised patients in Pondicherry. AMJ .2012; 5(7): 344-8.
9. Abdallah AMN, Elsayed BS, Mostafa YMM, El-gohary MG. Biofilm for`ming bacteria isolated from urinary tract infection, relation to catheterization and susceptibility to antibiotics. Int J for Biotech and Mole Bio Res. 2011; 2(10): 172-8.