International Journal of Advance and Applied Research
www.ijaar.co.in
ISSN – 2347-7075 Impact Factor – 7.328
Peer Reviewed Bi-Monthly
Vol.10 No.4 Mar – Apr 2023

" Antibiogram Pattern and Biofilm Formation by Uropathogenic *E.coli*"

Rani. v. jadhao[1] , S. N. Zodpe[2]
[1,2] Department of Microbiology, Shri Shivaji College of Arts, Commerce and Science Akola 444001 (M.S) India.
***Corresponding Author* - Rani. v. jadhao**
Email- rranivikasjadhao@gmail.com
DOI- 10.5281/zenodo.7965985

Abstract
During the current research work total 51 urine sample were collected form different Pathology Laboratories as well as from Government Medical Hospital Akola All the samples were collected using clean and sterilized Plastic bottles with air tight screw cap tops. The samples were collected and classified as per the sex and age of the patient. Total 22 samples from male and 29 samples from female were collected. Similarly samples were collected for low age group of 7 years to 68 years age group, to check the prevalence of E. coli Out of 40 isolates 15 isolates were found to be biofilm producers i.e. 37.50% whereas, 25 isolates i.e. 62.50 were non biofilm producers. The biofilm formation was confirmed on the basis of Congo Red Agar method and Tube method and is graphically represent in frequency distribution pie chart Further we process for the antibiotics susceptibility test again the E coli isolate was checked from the complete study it was observed that Ampicillin was highly resistant were as chloramphenicol was found to be highly sensitive in controlling the E.coli

Keyword: Uropathogenic E.coli, multidrug resistant

Introduction

A biofilm Is a complex aggregate of microorganisms in which cells are adhere to each other and to a surface. These adherent cells are embedded within a self produced matrix of extracellular polymeric substance (EPS)/slime. Slime is made up of proteins and polysaccharides. In a biofilm, bacteria communicate with one another using chemical signal molecules, termed auto-inducers. This process of chemical communication, called quorum sensing, allows bacteria to monitor the environment for other bacteria and to alter the behavior in response to changes in a community (Waters and Bassler, 2005). Availability of key nutrients, chemotaxis towards surface, motility of bacteria, surface adhesions' and presence of surfactants are certain factors which influence biofilm formation (Thomas and Day, 2007). Both the Gram positive and Gram negative bacteria have the capability to form biofilms.

Biofilm-forming activity is a widespread bacterial feature found on natural and artificial surfaces (Stewart and Franklin, 2008). In natural environments, biofilms represent the preeminent lifestyle of bacteria which can have beneficial effects on plant growth promotion (Naseem et al., 2018). Organic compound degradation (Flemming and Wingender 2010), including different aquatic ecosystems (Costerton et al.,1995 and Basemer, 2015). Moreover, microbial biofilms have been found useful in food fermentation, the production of many bio-based materials, bioremediation, wastewater treatment and microbial fuel cells (Van Houdt and Michiels 2010Edwards and Kjellerup, 2010; Karadag et al., 2015; Santoro et al., 2017 and Moradali and Rehm, 2020)

Bacterial biofilms play a significant role in UTIs, being the cause of both acute and persistent infections. Up to 80% of all infections involve biofilm-forming bacteria, and mainly in the urinary tract, biofilm can become a serious problem. The antimicrobial resistance shown by biofilms is one of the most important concerns of these structures. Biofilm can be resistant to antibiotics up to 1,000 –fold more than planktonic cells as a result of several mechanisms (Soto, 2014).

Escherichia coli is the most common organism associated with asymptomatic Bacteria (ABU) in humans. In contrast to uropathogenic E. coli (UPEC) that cause symptomatic urinary tract infection, very little is known about the mechanisms by which these strains colonize the urinary tract.

Drug resistance to commonly used antibiotics among the uropathogens is on the raise (Pramodhini et al., 2012). Biofilms are a complex aggregation of bacteria with unique properties which facilitate them to evade the host immune response and penetration by antimicrobial agents (Panda et al., 2012). Emergence of antibiotic resistance and biofilm formation among the bacterial pathogens implicated in causing urinary tract infection is of serious concern due to the high recurrence rate and chronicity of infections (Niveditha et al., 2012 and Atray and Atray, 2015) Multidrug resistance and spread of antibiotic resistance are higher among biofilm producers. Biofilm producing pathogenic bacteria with high levels of resistance may make treatment options difficult. The present study aims at isolating and identifying the bacteria causing UTI, detect the biofilm producers and beta lactamase production among the gram negative bacterial isolates and to perform antibiotic susceptibility pattern.

MATERIALS AND METHODS

Collection of Samples

Total 40 clinical samples such as urine were collected from Government Medical college and Hospital Akola. Each urine sample cup was labelled with name, age, sex and time of collection.

With the proper precaution and proper handling the clinical specimen were collected by wearing glows, apron and mask. Sterilize plastic container is typically used.

Isolation And Identification of the Isolated Bacteria :

Each of the collected samples without much delay was carried in Microbiology lab for further processing.

The collected urine samples were further processed for isolation of organism.

The selective media used for the isolation of organism was Eosins Methylene Blue Agar (EMB) and MacConkey agar as differential media.

After incubation at 37°C for 24 hrs on EMB Agar green metallic sheen and on MacConkey Agar pink colour colonies were observed.

Identification of the obtained isolates was done on the basis of cultural, Morphological and Biochemical characteristics (Bergey's manual of Determinative Bacteriology, 1939)

Biofilm Detection Methods:

Biofilm detection was carried out by the following methods;

a) **Tube method ™ :**

This is a qualitative method for biofilm detection. A loopful of test organisms inoculated in 10 ml of trypticase soy broth with 1% glucose in test tubes. Incubate the tubes at 37°C for 24 h. After incubation, tubes were decanted and washed with phosphate buffer saline (pH 7.3) and dried. Tubes were then stained with crystal violet (0.1%). Wash excess stain with distilled water. Tubes were dried in inverted position. The scoring for tube method was done according to the results of the control strains. Biofilm formation was considered positive when a visible thick film lined the wall and the bottom of the tube (Christensen et al., 1995).

b)**Congo Red Agar method (CRA**):

CRA medium was prepared with brain heart infusion broth 37 g/L, sucrose 50 g/L, agar 10 g/L and Congo Red indicator 8 g/L. Congo Red stain was prepared as a concentrated aqueous solution and autoclaved separately from the other medium constituents. Then it was added to the autoclaved brain heart infusion agar with sucrose. Inoculate CRA plates with test organisms and incubate at 37oC for 24 h aerobically. Black colonies indicate biofilm production (Freeman et al., 1989).

Antibiotic Susceptibility Test

Antibiotic susceptibility test of biofilm producing bacteria was done on Mueller Hinton agar using the following antibiotic discs: ampicillin colostin, naficillin, nalidixic acid, streptomycin, methicillin, chloramphenicol, clindamycin , cefoxamine, rifampicin, cloxacillin, vancomycin, erythromycin, oxacillin, penicillin. All antibiotic discs were used against E. coli as control strains (Biemer, J. J., 1973).

RESULTS AND DISCUSSION

Table No. 1 : Prevalence and epidermiological studies of *E. coli* from urine sample.

Sr. No.	Sample Collected	Collection Site	Sex	Age
1	Urine	GMC Akola	Male	42
2	Urine	GMC Akola	Male	38
3	Urine	GMC Akola	Female	33
4	Urine	Waichal Pathology, Akola	Female	28
5	Urine	Waichal Pathology, Akola	Male	24
6	Urine	Deshmukh Pathology, Akola	Male	18
7	Urine	Deshmukh Pathology, Akola	Female	23
8	Urine	Deshmukh Pathology, Akola	Male	15
9	Urine	Deshmukh Pathology, Akola	Male	19
10	Urine	Deshmukh Pathology, Akola	Male	27
11	Urine	ICON Hospital, Akola	Male	63
12	Urine	ICON Hospital, Akola	Male	57
13	Urine	ICON Hospital, Akola	Female	29
14	Urine	ICON Hospital, Akola	Female	22
15	Urine	ICON Hospital, Akola	Female	67
16	Urine	ICON Hospital, Akola	Male	56
17	Urine	ICON Hospital, Akola	Male	35
18	Urine	ICON Hospital, Akola	Male	45
19	Urine	ICON Hospital, Akola	Female	7
20	Urine	ICON Hospital, Akola	Female	32
21	Urine	ICON Hospital, Akola	Female	5
22	Urine	ICON Hospital, Akola	Male	39
23	Urine	ICON Hospital, Akola	Male	55
24	Urine	ICON Hospital, Akola	Male	62
25	Urine	ICON Hospital, Akola	Female	25
26	Urine	ICON Hospital, Akola	Female	50
27	Urine	ICON Hospital, Akola	Male	40
28	Urine	ICON Hospital, Akola	Male	26
29	Urine	ICON Hospital, Akola	Female	28
30	Urine	ICON Hospital, Akola	Female	37
31	Urine	ICON Hospital, Akola	Male	56
32	Urine	ICON Hospital, Akola	Male	77
33	Urine	ICON Hospital, Akola	Female	31
34	Urine	ICON Hospital, Akola	Female	24
35	Urine	ICON Hospital, Akola	Male	20
36	Urine	ICON Hospital, Akola	Male	18
37	Urine	ICON Hospital, Akola	Female	12
38	Urine	ICON Hospital, Akola	Female	41
39	Urine	ICON Hospital, Akola	Female	62
40	Urine	ICON Hospital, Akola	Male	21

Table no. confirmation of Biofilm forming isolate by Tube Test and Congo Red Agar Test

Sr. No.	Isolates	Biofilm formation organism positive	
		Tube Test	Congo Red Agar Test
		Dye on wall of test tube	Black Colour Colonies
01	U1	+ve	+ve
02	U2	+ve	+ve
03	U3	+ve	+ve
04	U4	+ve	+ve
05	U5	+ve	+ve
06	U6	+ve	+ve
07	U7	+ve	+ve
08	U8	+ve	+ve
09	U9	+ve	+ve

10	U10	+ve	+ve
11	U11	+ve	+ve
12	U12	+ve	+ve
13	U13	+ve	+ve
14	U14	+ve	+ve
15	U15	+ve	+ve

Table No. 3 :Frequency distribution of Biofilm forming isolates

Sr. No.	Total Sample Collected	Positive Biofilm formation Test	Negative Biofilm formation Test
1	40 (100%)	15 37.50 %	25 62.50%

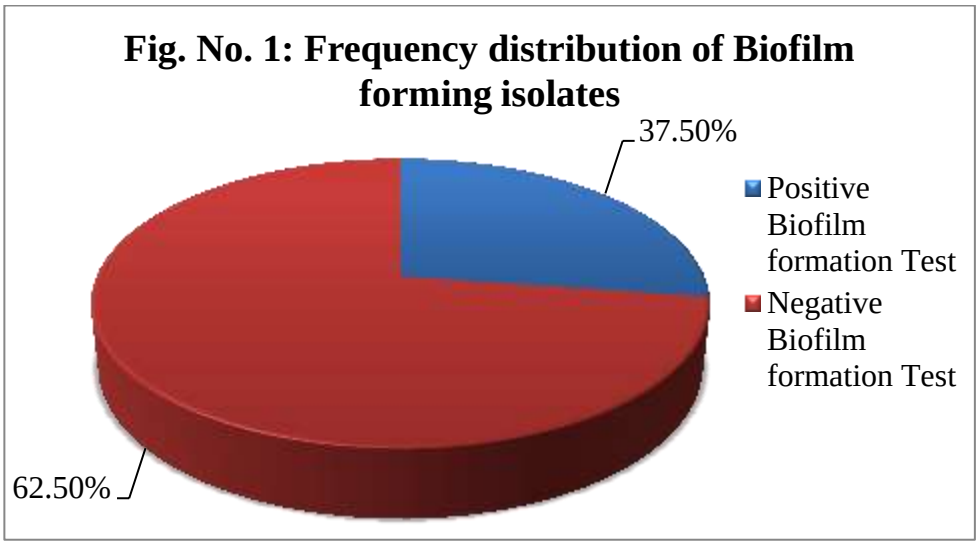
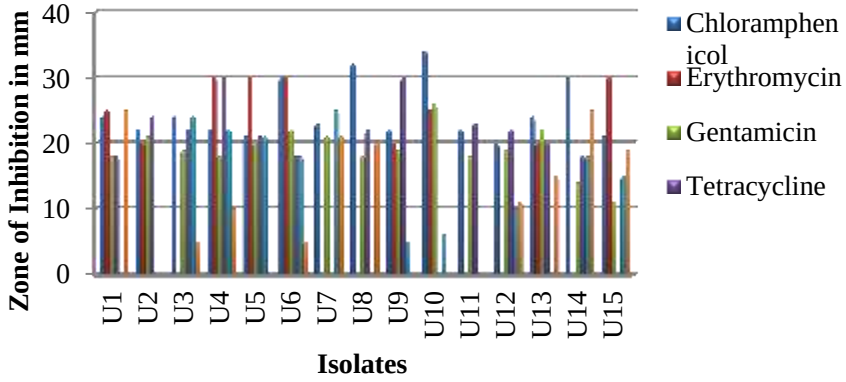


Table No. 4: Antibiotic Susceptibility Test against *E. coli* Isolates

Isolates	Zone of Inhibition in mm					
	Chloramphenicol	Erythromycin	Gentamicin	Tetracycline	Amoxiclave	Ampicillin
U1	24.0	25.0	18.0	18.0	R	25.0
U2	22.0	20.0	21.0	24.0	R	R
U3	24.0	R	19.0	22.0	24.0	5.0
U4	22.0	30.0	18.0	30.0	22.0	10.0
U5	21.0	30.0	20.0	21.0	21.0	R
U6	30.0	30.0	22.0	18.0	18.0	5.0
U7	23.0	R	21.0	R	25.0	21.0
U8	32.0	R	18.0	22.0	20. .0	20.0
U9	22.0	20.0	19.0	30.0	5.0	R
U10	34.0	25.0	26.0	R	6.0	R
U11	22.0	R	18.0	23.0	R	R
U12	20.0	R	19.0	22.0	10.0	11.0
U13	24.0	20.0	22.0	20.0	R	15.0
U14	30.0	R	14.0	18.0	18.0	25.0
U15	21.0	30.0	11.0	R	15.0	19.0

R – No zone of inhibition

Fig. 2 : Antibiotic Susceptibility Test against *E. coli* Isolates



Photoplate
Antibiotics Susceptibility Test

Photoplate
Biofilm Detection Method

Tube Method

Biofilm formation Positive

During the current research work total 40 urine sample were collected form different Pathology Laboratories as well as from Government Medical Hospital Akola (Table No. 1). All the samples were collected using clean and sterilized Plastic bottles with air

tight screw cap tops. The samples were collected and classified as per the sex and age of the patient. Total 22 samples from male and 29 samples from female were collected. Similarly samples were collected for low age group of 7 years to 68 years age group, to check the prevalence of E. coli organism.

The confirmation of Biofilm formation isolate were done on the basis of Biofilm forming ability of the isolate and it was noticed that two different method tube method and Congo red Agar method. Black colour colonies on Congo Red Agar media will confirmed biofilm production ability of isolates whereas visible thick film was obtained inside the wall of tube indicate positive result for tube test (Table No.3)

Out of total 40 sample Positive biofilm test was found for 15 (37.50%) samples and for Negative biofilm test 25 (62.50%) samples were observed (fig.no1)

Further we process for the antibiotics susceptibility test again the E coli isolate which where the biofilm Producer regarding the Choramphenicol u8 show maximum zone of 32 mm were the list zone of 21mm was showed by 2 isolate U5 and U15 indicating that this particular antibiotics is having the ability control E.coli organism regarding the erythromycin we observed U7 U8 and near about 5 isolate show complete resistance toward the erythromycin were as some of the isolate show better sensitivity in zone of 30 mm and list zone of 20mm regarding the gentamicin we observed U10 show maximum zone of 25mm were the list zone of U15 mm was showed by 2 isolate U6 and U13 indicating that this particular antibiotics is having the ability control E. coli organism

Gentamicin U10 show maximum zone of 26mm was showed were the list of zone of 11.mm was showed by 2 isolates U2 and U7 indicating that this particular antibiotics is having the ability control E. coli organisms.

Tetracycline we observed the U7, U10. U15 near about 3 isolate show complete resistance toward the tetracycline we as some of the Isolate the better sensitivity in zone of 30 mm and list zone of 18mm. Amoxyclave we observed the u1. U2, U11, U13 near about 4 isolate show complete resistance toward the amoxyclave we as some of the isolate the better sensitivity in zone of 25mm and list zone of 5.0 mm ampicillin

Amoxyclave we observed U1 U2 u11 near about 3 isolate show complete resistance we as some of the isolate the better sensitivity in zone of 24 mm and list zone of 5.0 mm

Ampicillins we observed U2 U5 U9 U10 U11 near about 5 isolate show complete resistance we as some of the isolate the better sensitivity in zone of 25mm and list zone of 0.5 mm (Table No. 7)

Conclusion

In This Study it has been founded that Gram Negative Bacteria are the commonest Organism isolated from UII Patient

E.Coli are one of the leading causes of the urinary Tract infection in humans the finding of this study Reviled that E.Coli was observed as the most Common etiologic agent of UII the Prevalence of UTIs was High in Female then in Male

Biofilm producing bacteria are responsible for many recalcitrant infections and are difficult to eradicate Biofilm Production in E. coli promotes colonization and lead to increased UTI

Antibiotic susceptibility pattem showed that many isolates showed the multiple drug resistance

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