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Biofilm and Extended Spectrum Beta Lactamase (ESBL) Producing Multi Drug Resistant Bacterial Uropathogens: A Challenge to Antibiotic Therapy in Nepal

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Abstract

Background: Urinary tract infection is one of the most common bacterial infections encountered by clinicians in developing countries. A biofilm is a structured consortium of bacteria embedded in a self-produced polymer matrix consisting of polysaccharide, protein and DNA. Antibiotic resistance is a problem of deep scientific concern both in hospital and community settings. This study was aimed to determine the biofilm producers among multidrug uropathogenic bacteria isolated from urine cultures.

Methods: A cross-sectional study was conducted at Manmohan Memorial Medical College and Teaching Hospital, Kathmandu, Nepal by using urine samples from Urinary tract infection patients from June 2020 to January 2021. Identification of the isolates was done by standard microbiological techniques and antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method following Clinical and Laboratory Standard Institute (CLSI) guidelines. Biofilm detection was done by Congo-Red and Tube- adherence Method. Extended spectrum beta-lactamase production was detected by combined disc method using ceftazidime and ceftazidime/clavulanic acid discs and cefotaxime and cefotaxime/clavulanic acid discs. Multi drug resistance was considered for bacteria showing resistance to at least three different classes of antibiotics.

Results: Of total 723 cases of urine sample were processed, 98(13.55%) were found to have significant bacteriuria showing more prevalence in females 74(15.44%). Among the significant bacteriuria of 13.55%, Escherichia coli (45.91%) and CONS (13.27%) were found as most prevalent. In vitro antibiotic susceptibility test showed that Amikacin remains the principle antibiotic of choice based on its effectiveness on both gram positive and gram negative bacteria. Out of 68 MDR gram negative isolates, 69.71% were found to be ESBL producers of which 57.35% were from E.coli followed by 13.23% from Klebisella spp. Similarly, 47.95% of total isolates were found to be biofilm producers with E.coli in highest range. Among 98 uropathogens, 76(77.55%) were multidrug resistant with 42(89.36%) Biofilm Producer. Statistically significant association was observed between biofilm

production and multidrug resistance among uropathogens. Among 76 gram negative isolates, 53(77.55%) isolates were ESBL producers. Of 53 ESBL producers 39(51.31%) were biofilm producers. Statistically, significant association was observed between ESBL and biofilm producers among uropathogens.

Conclusions: This study showed a high frequency of antimicrobial resistance, ESBL producers and biofilm production in uropathogenic strains which necessitate the re-evaluation of first and second line therapies for UTI. Regular monitoring of rates of ESBL production along with multidrug resistance among clinical isolates is very necessary.

Keywords: Biofilm; Multidrug resistant; Extended Spectrum Beta- Lactamase; Nepal

Abbreviations: ESBL-Extended Spectrum Beta- lactamase; MDR-Multi Drug resistant; CLSI-Clinical and Laboratory Standard Institute; UTI-Urinary Tract Infection; CLED-Cystine lactose electrolyte deficient; MHA-Mueller Hinton Agar; HPF-High Power Field

Background

A biofilm is a structured consortium of bacteria embedded in a self-produced polymer matrix consisting of polysaccharide, protein and DNA. Bacterial biofilms cause chronic infections because they show increased tolerance to antibiotics as well as resisting phagocytosis and other components of the body's defence system [1]. Some important pathogenic microorganisms associated with biofilm associated infections are treptococccus spp, Staphylococcus spp Escherichia coli, Proteus mirabilis, Klebsiella spp, Enterococcus faecalis, Pseudomonas aeruginosa, Candida albicans, etc. [2]. Biofilm is also involved in the establishment of Urinary Tract Infections [3].

ESBL producing bacteria do not show resistance only to penicillins, most cephalosporins and aztreonam but also to other classes of antibiotics such as aminoglycosides, cotrimoxazole, tetracycline and fluoroquinolones [4, 5]. There are limited treatment options for the infections caused by ESBL producing bacteria [6] due to which the treatments of such infections are very difficult often resulting into treatment failure.

Antibiotic resistance is a problem of deep scientific concern both in hospital and community settings. Rapid detection in clinical laboratories is essential for the judicious recognition of antimicrobial resistant organisms. Production of extended-spectrum β-lactamases (ESBLs) is a significant resistance-mechanism that impedes the antimicrobial treatment of infections caused by *Enterobacteriaceae* and is a serious threat to the currently available antibiotic armory [7].

Nepal is a developing country with higher rates of morbidity and mortality due to UTIs. Many researchers have been conducted regarding the prevalence and etiology of UTIs. These researches have recognized UTI as a health burden. Nevertheless, the emergence of multi drug resistance among the etiological agents of UTI has worsened the condition [8]. Biofilm production is related to multi drug resistance [9]. In the context of Nepal, studies on biofilm are extremely limited. This creates a strong necessity for such studies to be carried out to detect the presence of biofilm among clinical isolates. This study is focused on biofilm production in uropathogens. The knowledge about the proportion of the biofilm producers among uropathogens can guide towards effective management of biofilm associated UTIs, its chronicity and reoccurrence.

The regular surveillance of the drug resistance among the clinical isolates will be helpful to know the actual gravity of the situation, hence to formulate the necessary policy to reduce the incidence of drug resistance among the bacteria. Further, the knowledge about the local antimicrobial susceptibility patterns will be helpful to start timely antimicrobial susceptibility patterns will be helpful to start timely proper preliminary treatment [10]. So, in this study we determined the biofilm producers among multidrug uropathogenic bacteria isolated from urine cultures.

Methods

This cross-sectional study was conducted at Manmohan Memorial Medical College and Teaching Hospital (MMCTH), Kantipur College of Medical Science (KCMS), Kathmandu, June, 2020 to January, 2021. Suspected UTI patients (both out and in-patients of (MMCTH) with complaints of urinary problems were requested for voluntary participation after providing the study information and consent was obtained from them prior to their enrollment. A total of 723 patients were enrolled. Approval for this study was obtained from the research committees of MMMCTH and KCMS.

Laboratory Methods

Each patient was properly instructed and requested for 15ml of clean catch midstream urine (CC-MSU) in the provided sterile container with proper labeling. Samples were immediately delivered to the microbiology laboratory for the further processing. The color, appearance and turbidity of urine were noted during macroscopic examination. About 10 ml of urine sample was centrifuged at 3000 rpm for 10 minutes. The pellet was used for wet mount preparation and microscopically examined for the presence of pus cells, erythrocytes, epithelial cells and crystals.

Urine Culture and Identification of Bacterial Isolates [11]

The urine sample (0.01 ml) was cultured semi-quantitatively on Cystine Lactose Electrolyte Deficient (CLED) agar using standard calibrated loop of 4 mm diameter. The plate was then incubated overnight at 37°C. Next day, bacterial colonies were counted (if any) and the number of microorganisms per ml of the original sample was determined. Bacterial colony count $\geq 10^5$cfu/ml of urine was interpreted as 'significant bacteriuria'. Growth negative plates were further incubated for additional 24 hours before concluding as 'culture negative'.

The isolate was identified based on the standard microbiological procedures (colony phenotypes, Gram's staining and biochemical properties. Catalase test and coagulase test results were used to differentiate Gram positive pathogens whereas a set of biochemical tests were implied for identification Gram negative pathogens.

Antibiotic susceptibility test

The antibiotic susceptibility test was performed on Muller Hinton Agar by Modified Kirby Bauer disc diffusion method. Antibiotics were selected depending on the isolates as per Clinical and laboratory Standards Institute guidelines [12]. For gram negative bacilli, Amikacin, Amoxicillin, Cefepime, Ceftriaxone, Cefpodoxime, Chloramphenicol, Cefixime, Tigecycline, Imipenem, Nalidixic Acid, Nitrofurantoin, Norfloxacin, Gentamycin, Ofloxacin, Piperacillin/Tazobactam, Ceftazidime were used, whereas for gram positive bacteria, antibiotics used were Amikacin, Ciprofloxacin, Azithromycin, Cloxacillin, Penicillin-G, Mupirocin, Erythromycin, Cefotaxime, Chloramphenicol, Co-trimaxole, Oxacillin, Gentamycin, Cefixime, Tetracycline, Ceftriaxone, Vancomycin, Clindamycin, Novobiocin, Fucidic acid and PolymyxinB.

Screening and ESBL detection

ESBL production was confirmed among the suspected bacterial strain according to the guidelines of CLSI for phenotypic confirmatory testing. The suspected organism was inoculated into Muller Hinton broth and incubated at 370C until the turbidity matched 0.5 Mc farland standards. Using a sterile cotton swab the test organism was carpet cultured on a MHA plate. With the help of sterile forceps, the ESBL detection disc was placed onto the inoculated medium ensuring that they are evenly placed.

The isolates were screened for possible ESBL production using ceftazidime (30 μg) and cefotaxime (30 μg). According to the CLSI guidelines, the isolates showing reduced susceptibility to at least one of these drugs with zone of inhibition for ceftazidime ≤ 22 mm and cefotaxime ≤ 27 mm were considered as the possible ESBL producing strains. The suspected ESBL producing strains

were confirmed for ESBL production by combined disc assay using ceftazidime (30 µg) and ceftazidime/clavulanic acid (30/10 µg) discs and cefotaxime (30 µg) and cefotaxime/clavulanic acid discs (30/10 µg). The zones of inhibition for the ceftazidime and cefotaxime discs were compared to those of the ceftazidime/clavulanic acid and cefotaxime/clavulanic acid discs. An increase in zone diameter of ≥ 5 mm in the presence of clavulanic acid was confirmed as positive for ESBL production [12]. Extended spectrum beta-lactamase production was detected by combined disc method using ceftazidime and ceftazidime/clavulanic acid discs and cefotaxime and cefotaxime/clavulanic acid discs. Bacteria showing resistance to at least three different classes of antibiotics were considered multidrug resistant(MDR)[13].

Interpretation of the Results

An increase in zone diameter of ≥ 5 mm in the presence of clavulanic acid from any or all of the discs indicates the presence of ESBL in the test organism.

Screening of Biofilm Producer

Biofilm production was estimated qualitatively for all the isolates by tube adherence and Congo- red Method.

a) Tube Adherence Method

Suspension of tested strains was incubated in the glass tubes containing Brain Heart Infusion (BHI) broth aerobically at 35°C for the 48 hours. Then the supernatant was discarded and the glass tube was stained by 0.1% safranin solution, washed with distilled water three times and dried. A positive result was defined by the presence of a layer of stained material adhered to the inner wall of the tube. The exclusive observation of a stained ring at the liquid-air interface was considered negative [14].

b) Congo red Agar Method

The medium was prepared (BHI broth, 37 gm/l; sucrose 5gm/l; agar, 10 gm/l), autoclave at 121 C for 15 minutes and cooled down to 55°C.Sterile Congo red dye (0.8 gm/l) was added to the autoclaved BHI agar and plated. Plates were inoculated with test organism and incubated at 37°C for 24 to 48 hours aerobically. Black colonies with a dry crystalline consistency indicated biofilm production; weak producers usually remained pink, though occasional darkening at the center of colonies was observed [15].

Quality Control

Antibiotic disc and MHA plates were monitored for their performance quality with *Escherichia coli* ATCC 25922 and *staphylococcus aureus* 25923. For antimicrobial susceptibility testing standard inoculums (matched with 0.5 Mac-farland solutions) was done under strict aseptic conditions to avoid contamination.

Data Analysis

Data were entered and analyzed by using Statistical Package for Social Science (SPSS) version 21 software package. Statistical analysis was done by calculating percentages and chi- square values (for associations). P value <5 was considered as significant.

Result

During the study period, 723 cases of urine sample were processed from UTI suspected patients. The overall rate of UTI was found to be 13.55%.Among the total processed urine samples, 98(13.55%) were found to have significant bacteriuria showing more prevalence in females 74(15.44%) (Table1).

Table 1: Culture Positivity

Growth	No. of isolates
Significant bacteriuria	98(13.55)
No growth	625(86.45)
Total	**723(100)**

Higher incidence of UTI with significant growth was observed in age group 21-30 years 11(23.91%) followed by age group 41-50 years 13(16.88%). The detail results are shown in the table 2.

Table 2: Urine specimens with significant growth

Age group	Male total	Significant growth(%)	Female total	Significant growth(%)	Total	Significant growth (%)
NB-10	18	4(9.52)	28	28(15.38)	46	32(14.28)
11-20	17	3(17.64)	49	5(10.20)	66	8(12.12)
21-30	42	3(16.67)	182	8 (28.57)	224	11(23.91)
31-40	49	5(10.20)	76	13(17.10)	125	18(14.40)
41-50	30	3(10)	47	10(21.27)	77	13(16.88)
51-60	20	0(0)	34	3(8.82)	54	3(5.55)
61-70	22	2(9.09)	16	3(18.75)	38	5(13.15)
71-80	27	2(7.40)	14	2(14.28)	41	4(9.75)
>80	19	2(10.52)	33	2(6.06)	52	4(7.69)
Total	**244(33.74)**	**24(9.83)**	**479(66.25)**	**74(15.44)**	**723**	**98(13.55)**

Out of 723 sample 158 samples were collected from Inpatient and 565 samples were collected from Outpatient sample. Highest percentage of the significant growth isolates were obtained in Inpatient sample with 27(17.08%) followed by Outpatient sample with 71(12.56%). Among 98 patients with significant bacteriuria, 76 patients were female (66.25%) and 24 were male (33.74%) (Table3)

Table 3: Patient type and gender wise distribution

Patient type	Total	Significant Growth	Gender	Total	Significant Growth
Inpatient	158	27(17.08%)	Male	244	24(9.83%)
Outpatient	565	71(12.56%)	Female	479	74(15.44%)
Total	**723**	**98(13.55%)**	**Total**	**723**	**98(13.55%)**

Out of 98 significant growth of isolates maximum number of growth found as E. coli with 45(45.91%) in which 15(62.5%) male patients and 30(40.5%) female patients followed by CONS with 13(13.27%) in which 3(12.5%0 male patients and 10(13.51%) female patients (Table4).

Table 4: Distribution of the isolates

Isolated Organism	Total frequency	Male	Female
E.coli	45(45.91)	15(62.5)	30(40.5)
K.pneumoniae	4(4.09)	0(0.00)	4(5.40)
K.oxytoca	6(6.12)	0(0.00)	6(8.10)
Pseudomonas spp	3(3.06)	1(4.17)	2(2.70)
Proteus spp	5(5.10)	1(4.167)	4(5.40)
S. Paratyphi	2(2.04)	0(0.00)	2(2.70)
S. Typhi `	1(1.02)	0(0.00)	1(1.35)
C.freundii	7(7.14)	2(8.33)	5(6.75)
C.koseri	2(2.04)	0(0.00)	2(2.70)
Edwardsiella	1(1.02)	0(0.00)	1(1.35)
S.aureus	9(9.19)	2(8.33)	7(9.45)
CONS	13(13.27)	3(12.5)	10(13.51)
Total	**98**	**24**	**74**

Antibiotic Resistance Patterns of the Isolates

The lowest rate of resistance was seen toward Tigecycline followed by Chloramphenicol/Imipenum/Amikacin in gram negative isloates. All other isolates were found to be resistant to amoxicillin except to E. coli (Table5).

Table 5: Antimicrobial resistance pattern of gram negative isolates

0rganism	*E.coli*(n=45)	*Klebsiella* spp(n=10)	*Pseudo*(n=3)	*Proteus*(n=5)	*Salmonella* spp(n=3)	*Citrobacter* (n=9)	*Edwardsiella* (n=1)
IMP	4(8.88)	0(0.00)	0(0.00)	1(20)	0(0.00)	2(22.22)	0(0.00)
CPD	38(84.44)	10(100)	2(66.66)	4(80)	3(100)	8(88.89)	0(0.00)
GEN	14(31.11)	6(60)	0(0.00)	1(20)	1(33.33)	2(22.22)	0(0.00)
AK	4(8.88)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
PIT	7(15.55)	2(20)	1(33.33)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
NA	21(46.66)	7(70)	2(66.66)	2(40)	2(66.66)	0(0.00)	0(0.00)
OF	15(33.33)	2(20)	0(0.00)	1(20)	0(0.00)	4(44.44)	1(100)
C	3(6.66)	2(20)	0(0.00)	3(60)	0(0.00)	1(11.11)	0(100)
AMX	39(86.66)	2(20)	3(100)	3(60)	2(66.66)	9(100)	1(100)
CPM	20(44.44)	6(60)	1(33.33)	1(20)	0(0.00)	5(55.55)	0(0.00)
CFM	33(73.33)	5(50)	2(66.66)	1(20)	1(33.33)	6(66.66)	0(0.00)
CAZ	23(51.11)	3(30)	1(33.33)	4(80)	2(66.66)	7(77.77)	0(0.00)
NX	17(37.77)	5(50)	1(33.33)	4(80)	3(100)	2(22.22)	0(0.00)
CTR	36(80.00)	4(40)	3(100)	4(80)	3(100)	8(88.88)	0(0.00)

NIT	26(57.77)	1(10)	0(0.00)	1(20)	1(33.33)	1(11.11)	0(0.00)
TGC	1(2.22)	2(20)	0(0.00)	2(40)	0(0.00)	0(0.00)	0(0.00)

Note: The antibiotics written in short forms: AK-Amikacin, AMX–Amoxicillin, CPM-Cefepime, CTR-Ceftriaxone, CPD- Cefpodoxime, C-Chloramphenicol, CFM-Cefixime, TGC - Tigecycline ,IPM- Imipenem NA-Nalidixic Acid, NIT-Nitrofurantoin, NX-Norfloxacin, GEN-Gentamycin, OF-Ofloxacin, PIT-Piperacillin/Tazobactam, CAZ- Ceftazidime.

In isolates of gram positive, the lowest rate of resistance was seen toward Amikacin followed by Gentamycine/Chloramphenicol. All S. aureus isolated were resistance to ceftriaxone and Co-trimaxole (Table 6).

Table 6: Antibiotic resistant pattern of gram positive isolates

Organism	*S.aureus*(n=9)	CONS(n=13)
AK	0(0.00)	0(0.00)
CIP	1(11.11)	7(53.84)
AZM	2(22.22)	2(15.38)
COX	4(44.44)	4(30.76)
P	8(88.88)	2(15.38)
MU	8(88.88)	5(38.46)
E	5(55.56)	5(38.46)
CTX	2(22.22)	3(23.07)
C	1(11.11)	1(7.69)
COT	9(100)	5(38.46)
OX	8(88.89)	3(23.07)
GEN	1(11.11)	1(7.69)
CFM	7(77.79)	7(53.84)
TE	6(66.66)	4(30.76)
CTR	9(100)	4(30.76)
VA	5(55.55)	2(15.38)
CD	7(77.79)	6(46.15)
NV	Not used	1(7.69)
FC	Not used	3(23.07)
PB	Not used	4(30.76)

Note: The antibiotics written in short forms: AK-Amikacin, CIP-Ciprofloxacin, AZM- Azithromycin, COZ-Cloxacillin, P-Penicillin-G, MU-Mupirocin,, E-Erythromycin, CTX-Cefotaxime, C-Chloramphenicol, COT- Co-trimaxole, OX- Oxacillin, GEN- Gentamycin, CFM-Cefixime, TE- Tetracycline, CTR-Ceftriaxone, VA- Vancomycin, CD-Clindamycin, NV-Novobiocin, FC- Fucidic Acid, PB- Polymyxin Acid.

Of the 98 isolates 47(47.95%) produced Biofilm. Among the isolates 21(21.42%) E.coli produced Biofilm production in both Congo-red method and Tube-adherence method followed by 6(6.12%) CONS/6(6.12%) K. pneumonia/4(6.12%) C. freundii (Table 7).

Table 7: Biofilm production by Congo red and Tube adherence method

Isolates	Total(%)	Congo-red method		Tube-adherence method		Biofilm Production
		Weak	Strong	Weak	Strong	
E.coli	45(45.91)	4	17	4	17	21(21.42)
K. pneumonia	6(6.12)	2	4	2	4	6(6.12)
K. oxytoca	4(4.08)	0	0	0	0	0(0.00)
Pseudomonas spp	3(3.06)	0	1	0	1	1(1.02)
Proteus spp	5(5.01)	1	3	2	2	4(4.08)
S. Typhi	2(2.04)	0	1	1	0	1(1.02)
S. Paratyphi	1(1.02)	0	0	0	0	0(0.00)
C. freundii	7(7.14)	2	2	2	2	4(6.12)
C. koseri	2(2.04)	2	0	1	1	2(2.04)
Edwardsiella	1(1.02)	0	0	0	0	0(0.00)
S.aureus	9(9.19)	1	1	1	1	2(2.04)
CONS	13(13.26)	2	4	3	3	6(6.12)
Total	**98**	**12**	**35**	**15**	**32**	**47(47.95)**

MDR and ESBL Production among the Isolates

Out of 45 isolates of E.coli, 39 isolates found with MDR and 32(42.10%) with ESBL confirmed. Out of 76 isolates 68 were found with MDR. Out of 61 ESBL screened 53(69.71%) were ESBL confirmed (Table 8).

Table 8: MDR and ESBL production profile among isolates

Organism	Total isolates	No. of MDR	ESBL screening	ESBL confirmed
Gram - ve rods				
E.coli	45	39	35	32(42.10)
K.pneumoniae	6	5	5	5(6.57)
K.oxytoca	4	4	3	2(2.63)
Pseudomonas	3	3	2	2(2.63)
Proteus	5	5	5	5(6.57)
*S.*Typhi	2	2	1	1(1.31)
*S.*Paratyphi	1	1	0	0(0.00)
C.koseri	2	2	1	0(0.00)
C.freundii	7	6	6	6(7.90)
Edwardsiella	1	1	3	0(0.00)
Total	**76**	**68**	**61**	**53(69.71)**

Biofilm and Multi Drug Resistance

Among 98 uropathogens, 76(77.55%) were multidrug resistant with 42(89.36%) Biofilm Producer. Statistically significant association was observed between biofilm production and multidrug resistance among uropathogens (Table 9)

Table 9: Biofilm and Multi Drug Resistance

	Multi-drug Resistance		Total(%)	P-value
Biofilm	**Yes(%)**	**No(%)**		
Producer	42(89.36)	5(10.63)	**47(47.95)**	0.001
Non-producer	34(66.66)	17(33.33)	**51(52.05)**	
Total	**76(77.55)**	**22(22.44)**	**98(100)**	

Biofilm production in ESBL producers

Among 76 gram negative isolates, 53(77.55%) isolates were ESBL producers. Of 53 ESBL producers 39(51.31%) were biofilm producers. Statistically, significant association was observed between ESBL and biofilm producers among uropathogens (Table 10)

Table 10: Biofilm production in ESBL producers of gram negative isolates

	ESBL Production		Total(%)	P-value
Biofilm	**Yes(%)**	**No(%)**		
Producer	35(46.05)	4(5.26)	**39(51.31)**	0.001
Non-producer	18(19.73)	19(25.00)	**37(48.69)**	
Total	**53(77.55)**	**23(30.26)**	**76(100)**	

Discussion

Out of 723 urine specimens received for culture in the laboratory, only 98 (13.55%) showed significant growth. The low percentage of culture positivity among the urine specimens was due to the non- specific symptoms of UTI which created a suspicion for the presence of UTI and need for the request of urine culture [16] and also due to self-medication by the patient [17]. The highest frequency of growth was of *E.coli* (45.91%) followed by *K. pneumoniae* (11.21%) which was similar to the result carried by Moland et al., 2006. *E. coli* and *K. pneumoniae* are clinically important members of the Enterobacteriaceae commonly causing infections of different sites [18].

The greater incidence of UTI among females as compared to males accounts to Close proximity of the female urethral meatus to the anus, shorter urethra, and sexual intercourse have been reported as factors that influences this higher prevalence in women [19]. Though UTI was found to be prevalent in all the age- groups, the age- group largely to be infected was observed to be 21-30 years. The reason behind the outcome is that these age -group are the sexually active age group and are involved in sexual activities as well as use of spermicidal contraceptives which are identified risk factors for UTI among females [20]. The occurrence of UTI among inpatients and outpatients was 17.08% and 12.56% respectively. Many studies show significant difference among inpatient and outpatient [21] whereas some does not [22].

Using these methods, 47.95% of total urinary isolates were found to be biofilm producers. Previous studies have also proposed the importance of bacterial biofilm formation in UTIs [23]. Among the diverse urinary isolates, *E.coli, Klebsiella* spp, *Pseudomonas* spp, *Proteus* spp, *Citrobacter Freundii, S aureus* and CONS were shown to produce biofilm in-vitro. Among total *E.coli* isolates, 21.42% of UPEC were observed to produce biofilm.

Multi Drug Resistance (MDR) isolates are the major problematic concern in the community. It is worth mentioning that in the present study 89.47% of the samples were resistant to different classes. Among the MDR organisms isolated in the present study *E.coli* is the leading organism with 57.35% whereas least MDR was observed in Edwardsiella. Various other investigators have supported the high rate of MDR in E.coli [8]. The prevalence of ESBL-producing enterobacterial isolates evaluated in the present study is 69.71% which is similar to the findings of other investigator [24]. The present study suggests that ESBL producing isolates had a higher ability to form biofilm in comparison with non-ESBL producing isolates. The clinically relevant observation noted in our study was high resistance of biofilm producers to commonly used antibiotics than biofilm non-producers similar to other studies [25].

Among the isolates of *E.coli*, Amikacin, Imipenam, tigecycline, Chloramphenicol were found to be the most effective drug whereas amoxicillin is least effective followed by cefpodoxime. This result is comparable to the study conducted by [26] and also reported amikacin to be the most effective for CONS and *S.aureus*. However these are used as alternative therapeutic agents in the absence of the first line drug [24]. The present study suggest CONS resistant towards Novobiocin, Gentamicin, Chloramphenicol (7.69%) followed by Vancomycin, Azithromycin and Penicillin G (15.38%).

Antimicrobial Resistance among biofilm producers appeared to be higher as compared to non-biofilm producers. The increase in antimicrobial resistance among biofilm producers is due to the presence of the protective covering of exopolysaccharide which alters the penetration of antimicrobial agents through the biofilm and hinders the activity of antimicrobial agents against the bacterial cells.

The ability to form biofilm was higher in ESBL producing isolates in comparison with non biofilm producing isolates. The present study reports 71.69% of ESBL producer showed biofilm producers. This study was in accordance to the study carried out by [27]. It is possible that higher mortality and severity of infection caused by ESBL producing isolates is due to the expression of several virulence genes simultaneously, rather than gaining new virulence genes. There was significant association among biofilm producers and ESBL producers.

In our study high rate of ESBL production was observed among the isolates and ability to form biofilm was higher in ESBL producing isolates, which is very serious and shows the dissemination of ESBL producing bacteria to the community [10]. The prevalence of multiple drug resistance bacteria may not only vary from countries to countries but also from institutions to institutions and this can be partially explained by the difference in local antibiotic prescribing habits and difference in effectiveness of infection control program in different health institutes.

As the study conducted in Manmohan Memorial Medical College and Teaching Hospital, which does not represent whole scenario of country. The surveillance should be carried out throughout the year covering wide geographical region in order to obtain information regarding variation of pathogen and their antibiotic sensitivity profile.

Conclusion

UTI status is being Conventional antibiotic therapy targeted against planktonic bacteria would certainly be ineffective towards biofilm. As a result, biofilm exists and serve as a reservoir for planktonic bacteria rendering infection to reoccur and persist. Antibiotic concentration required for the elimination of biofilm appears to be high and is impossible to be attained *in- vivo*. Therefore, it is the need of the hour to search for alternatives to fight against biofilm associated UTIs. ESBL production by common pathogenic organisms poses problems for therapeutics. It is necessary to know the prevalence of ESBL and biofilm producing stains in a hospital so as to formulate a policy of empirical therapy in high risk units where infections due to resistant organisms are much higher.

Author's contributions

SS and LB designed and conceived the study, carried out the research works, analysed data, and prepared the final manuscript. LB and BS carried out the research works and analyzed the data. SS and PS monitored the study. All authors read and approved the final manuscript.

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Conflict of interest

None declared

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