Study of Biofilm Producing Property and Predisposing Factors in Bacterial Isolates from Community Acquired Urinary Tract Infections

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Research Article

Abstract: **Introduction**: Bacteria with biofilm producing property[BFPP] are associated with chronic and intractable urinary tract infections(UTI),especially with indwelling urinary catheter. Limited data on bacteria with BFPP in community acquired UTI[c-UTI] necessitated the present study. **Aims and Objectives:** To determine prevalence of biofilm producing property, predisposing risk factors and multi-drug resistance in bacterial pathogens from c-UTI. **Material and Methods:** Mid-stream urine specimens from75 patients, attending different OPDs were collected and processed by standard laboratory procedures. Bacterial isolates were tested for BFPP by Tube and Congo red dye tests. Predisposing risk factors and multi-drug resistance by interim guidelines incorporating CDC, EUCAST and FDA criteria were determined. Statistical analysis was done by Fisher's exact test. **Results:** Prevalence of bacterial pathogens with BFPP from c-UTI was 83.75% (67/80). BFPP by Tube method alone, Congo red dye method alone and by both methods was 26.87% (18/67), 28.36% (19/67) and 44.78% (30/67) respectively. Prevalence of BFPP in E.coli, Klebsiella spp., Pseudomonas aeruginosa, Staphylococcus aureus and CONS was 96.43% (27/28), 78.95% (15/19), 85.71% (6/7), 77.78%(14/18) and 66.67%(4/6) respectively. Age, sex, department wise distribution, resistance for individual antibiotics and multi-drug resistance [53.75% (43/80) vs. 8.75%(7/80)] among bacteria with BFPP and non-BFPP was not statistically significant. Association of predisposing risk factors among isolates with BFPP was observed in only 24.61% (16/65) patients. **Conclusions:** Tube test and Congo red agar tests should be used together as screening tests to detect BFPP in bacterial isolates from c-UTI. BFPP was observed as natural property of bacteria rather than a virulence factor. No significant difference in multi-drug resistance observed among bacteria with BFPP and non-BFPP. Predisposing risk factors do not play role in acquisition or expression of BFPP in bacterial isolates from c-UTI.

Key words: Biofilm producing property, Community acquired urinary tract infections, Tube test, Congo red dye test.

Introduction

Urinary tract infection (UTI) is not only a common community acquired infection, but also the most frequently occurring nosocomial infection. Catheter associated urinary tract infections (CAUTI) accounts for 40% of all nosocomial infections and 80% of all nosocomial Urinary Tract Infections (UTIs).[1,2] CAUTI is a representative type of biofilm associated infection usually composed of clusters of diverse, often multi-drug resistant microorganisms with extracellular matrix, formed on both extra-luminal and intraluminal surfaces of urinary catheters.[3]Bacteria in biofilms are protected from antimicrobial agents as well as host defense mechanisms, establishing chronic persistent infections, septicemia and death if not treated, is a well established fact necessitating the removal of catheter as the only treatment modality.[1,2,3] Scanning electron microscopy is the gold standard test for demonstration of biofilms. However, several phenotypic and genotypic methods; Tissue culture plate method, Congo red agar method, tube methods and ica ACD operon detection by PCR have been used routinely to demonstrate biofilm forming property(BFPP) of bacterial isolates as an indirect evidence of presence of biofilms.[4,5] Although, over several decades no significant changes in age, sex, occupation wise, distribution of bacterial flora in community acquired UTI(c-UTI) has been observed, recurrent UTI, relapses, treatment failures and complications are increasingly being reported probably due to increasing drug resistance.[6,7] However, in majority of recurrent UTI and/or relapses no obvious risk factors are identified, which continue to recur in spite of appropriate antibiotic therapy requiring further studies to identify factors involved, especially the role of bacteria with BFPP. Very few studies are available on bacterial isolates from c-UTI with BFPP. Hence the present study was conducted to determine BFPP among bacterial isolates from c-UTI by Tube test and Congo red agar plate method and role of predisposing risk factors.

Materials and Methods

A prospective observational study of 3 months duration with bacterial isolates from 75 consecutive

patients with c-UTI (without a indwelling urinary catheter) was conducted in a tertiary care hospital to determine prevalence of biofilm producing property and predisposing risk factors among bacterial pathogens with prior approval from Institutional Ethical Committee. Isolation, identification, semi quantitative culture of midstream urine specimens from patients attending different Outpatient Departments were collected and processed according to standard laboratory procedures.[8] Multi-drug resistance was quantitated as per interim guidelines encompassing Centre for Disease Control and prevention(CDC),European Committee on Antimicrobial Susceptibility Testing(EUCAST) and the United states Food and Drug Administration(FDA) criteria.[9] Predisposing risk factors in c-UTI analyzed by questionnaire method. Antimicrobial susceptibility testing was done by Kirby-Bauer's disc diffusion method as per Clinical Laboratory Standards Institute(CLSI) guidelines.[10]

Qualitative Determination of Biofilm Producting Property

Bacterial isolates from c-UTI were subjected to two Qualitative phenotypic screening tests to determine BFPP.

1. **Tube Method**[11]**:**
   The tube method consisted of inoculating 10ml of Brain Heart Infusion broth with 3 to 4 colonies of bacterial isolates from blood agar plate and incubating the broth culture tube overnight (18 hours) at 37˚C. The culture tubes were then emptied of their contents, washed with deionized water several times and stained with safranin 0.1%. Slime production was judged to have occurred if a visible film lines the walls of the tube and the isolate was interpreted as biofilm producer. Ring formation at the liquid-air interface was not considered indicative of slime production.
2. **Congo Red Agar Method (CRA)**[4]**:**
   Biofilm forming colony morphology was detected for organisms on Congo Red Agar plates. Bacteria were cultured in 10ml Brain Heart Infusion broth at 35˚C for 24 hours without shaking, and were then plated onto CRA plates. Incubation was carried out at 35˚C for 24 hours and an additional 24 hours at room temperature before recording the colony morphology. Crusty black colonies with dry filamentous appearance were recorded as biofilm producers, smooth pink colonies as non producers and intermediate colony morphology (pink with dark centers resembling bull's eyes) as potential biofilm producers**.**

Definition of Biofilm Producer: A bacterial isolate was considered as biofilm producer if at least any one of the Phenotypic tests namely Tube test or Congo red dye test yielded positive result for slime production.

Controls for Biofilm Forming Property

Biofilm producing reference strains of Acinetobacter baumannii (ATCC 19606) and Pseudomonas aeruginosa (ATCC 27853) as positive controls and non-biofilm forming reference strains of Staphylococcus aureus (ATCC 25923) E. coli (ATCC 25922) were used.

Inclusion Criteria

1. Patients with classical signs and symptoms of c-UTI were included

Exclusion Criteria

1. Patients suffering from c-UTI with indwelling urinary catheter.

Statistical analysis was done by Fisher's exact test by using free online statistical calculators in www.graphpad.com.

Results

Prevalence of BFPP in bacterial isolates from c-UTI was higher, 83.75 %(67/80) compared to no BFPP,16.25%(13/80). Bacterial isolates with BFPP were higher among males, 95% (9/20) than in females 83.63%(46/55)[P=0.2724 NS] In the present study E.coli , 35% (28/80)was observed as most common pathogen of c-UTI with 96.43%(27/28) of the isolates with BFPP, followed by Klebsiella spp. 23.75%(19/80) with 78.95%(15/17) and Staphylococci 22.5%(18/80) with 77.78%(14/18). **[Table 1]**Among 80 isolates from c-UTI 67 bacterial isolates were observed to be possessing BFPP. 44.77% (30/67) were tested positive by both Tube and Congo red dye test, 28.35%(19/67) by Congo red dye test alone and 26.87%(18/67) by Tube test alone. **[Table 2]** 28.35%(19/ 67) patients had a predisposing risk factor compared to 75.38%(49/65) without any predisposing risk factor for recurrent UTI. However, association of predisposing risk factors among bacterial isolates with BFPP than isolates with non-BFPP was not statistically significant[Fisher's exact test, [P =0.7070,NS] **[Table 3].** Although, higher resistance was observed for individual antibiotics tested among Gram positive cocci and Gram negative bacilli with biofilm producing and non-biofilm producing property was observed, difference in antibiotic resistance pattern was not statistically significant in majority of the antibiotics tested**. [Appendix 1 & 2]** In the present study 62.5%(50/80) were observed to be multi-drug resistant isolates as per guidelines used. MDR status was observed to be higher in isolates with BFPP, 53.75%(43/80) than in isolates with non-BFPP, 8.75%(7/80)[*P*=0.5393 NS]

Discussion

Present study reported a high prevalence of BFPP among bacterial pathogens from c-UTI, even in the absence of predisposing risk factors without statistically significant difference in age and sex wise distribution. Bacterial flora of c-UTI in the present study was comparable with other published studies with E.coli as the commonest isolate followed by others. However, failure to detect or attempting to detect biofilm producing property among bacterial pathogens of c-UTI in other studies probably is a result of stereotypic thinking of researchers regarding association of BFPP only in UTI associated with indwelling urinary catheter and/or Hospital acquired UTI.[6,12] Majority of the published studies on c-UTI have not determined BFPP and few studies reported a lower prevalence of BFPP in c-UTI than Hospital acquired UTI by different phenotypic and genotypic methods**.**[13]Many studies have equated BFPP of bacterial isolates with presence of biofilm without being demonstrated by electron microscopy. Biofilms are responsible for more than 65% of human infections and are often linked to indwelling devices like prosthetic heart valves, orthopedic implants, contact lenses, intrauterine devices and especially urinary and central venous catheter being found on inner as well as external surface resulting in infections at localized site of insertion or disseminated infections like bacteremia, septicemia and death.[14]Biofilms are also known to occur in chronic and difficult resolve infections without indwelling devices like cystic fibrosis, infectious kidney stones, dental caries, periodontal disease, gingivitis, necrotizing fasciitis, chronic prostatitis, osteomyelitis, and otitis media.[14,15]However, increasing trend has been observed in detecting and reporting chronic and resistant to treat infections due to biofilms in nosocomial infections. BFPP of bacterial isolates, a potential to form biofilms is tested by phenotypic tests; Tube test, Congo red agar test and Tissue culture plate test and Genotypic tests for detection of genes in ***ica*** ACD operon. However, conclusive evidence, the demonstration of biofilm is done by Electron microscopy**.**[5] Prevalence of bacterial pathogens with BFPP from c-UTI in the present study was 83.75%(67/80) with a higher prevalence among Gram negative bacilli, 96.36%(53/55) than Gram positive cocci 72%(18/25). Prevalence of BFPP was highest in E.coli followed by Klebsiella spp., Pseudomonas aeruginosa, Staphylococcus aureus and CONS. Tube method alone or Congo red dye test alone as a screening test to detect BFPP would have missed28.36%(19/67) and 26.87%(18/67) bacterial isolates with BFPP respectively in the present study. Caution has to be exercised in analyzing and interpreting these results since potential to produce biofilm does not indicate the presence of biofilm in c-UTI due to bacterial isolates with BFPP. In the present study, Tube test detected 71.64%(48/67) of the bacterial isolates with BFPP compared with 73.13%(49/67) by Congo red agar method. Oliveira *et al* have reported that among 100 Coagulase negative Staphylococci, 82% tested positive by PCR, 82% by the tube test, 81% by the Tissue culture plate test assay, and 73% by the CRA method. Using PCR as a reference, the tube test showed the best correlation with detection of the ***ica***genes, presenting high sensitivity (100%) and specificity (100%).Oliveira *et al* have proposed the tube adherence test for the routine detection of biofilm production in Coagulase negative Staphylococci(CONS) because of its easy application and low cost and because it guarantees reliable results with excellent sensitivity and specificity. [5]However, Aricola *et al* have reported better agreement between CRA plate method and ***ica*** gene carriage than Tube method or Tissue culture plate method. [4] Gene detection responsible for biofilm production, ***ica*** ACD operon and others, indicate the potential for biofilm production rather than a specific test to forecast biofilm production. On the contrary, expression of***ica*** m-RNA has been shown to occur in biofilm negative S. epidermidis. Bacterial isolates with BFPP detected by ***ica*** ACD operon, not resulting in biofilm formation suggests other regulatory mechanisms.[16] Tissue culture plate method is a quantitative assay usually discriminating clearly between strongly adherent strains and nonadherent strains but is less reliable with bacterial isolates in the weakly adherent range.[17] Although tissue culture plate method is sensitive, accurate and reproducible phenotypic screening test for detection of BFPP, was not used since it is cumbersome and requires spectrophotometric measurement of density of stained bacterial biofilms adherent to plastic surfaces. TCP is a quantitative test with cut off values based on biofilms produced by bacterial isolates from clinical infections and indicates an objective measurement of degree of adherence, but still not suitable as routine screening test.[18] Further large scale studies are required to asses in vivo factors responsible for high degree of expression of BFPP in bacterial isolates from c-UTIs. However, a high prevalence of BFPP observed among bacterial isolates represents a strong potential to form biofilms if conditions are favorable. This is further strengthened by the fact that BFPP is a natural phenomenon possessed by commensal bacteria, Staphylococcus epidemidis as reported by Araujo et al, however with excessive slime production in similar isolates from clinical infections. Several workers have reported BFPP among bacterial isolates from healthy individuals and also in bacteria in

various ecosystems in nature to overcome predation, niche domination and survival strategy rather than a virulence factor. [19,20] Eftekhar *et al* have reported almost equal prevalence of BFPP in bacterial isolates with by Congo red dye method, from nasal passage/healthy skin and various infections i.e. UTI, wound infections, Surgical site infections, blood stream infections [68% vs. 64%] from equal number of isolates examined[50 from each group] further indicating BFPP as a almost universal phenomenon.[16] High prevalence of BFPP in the absence of predisposing risk factors from c-UTI isolates, raised a doubt as to whether higher sensitivity of Tube test and CRA test used in the present study was a result of false positivity which lead to discarding of initial 36 bacterial isolates from c-UTI. However, restarting of the study with new glass tube for every bacterial isolate, freshly prepared stains, running parallel duplicate tests and interpretation of results by more than 3 observers confirmed that high sensitivity of Tube test in detecting BFPP was in fact true and not false positivity. Possible explanations for false positivity are imperfections of glass test tubes, traces of grease, or minor media variations may influencing the ability of bacteria to attach to and colonize surfaces and the subjectivity associated with the visual assessment of adherence affecting the reliability of the tube assay. [17]

Previous antibiotic therapy for unrelated infections, undiagnosed infections and only a subset of patients with c-UTI seeking microbiological investigation and medical care in our tertiary care center raises doubt as to whether sub inhibitory dose of antibiotic treatment could have lead to stimulation or derepression of BFPP in bacterial isolates from c-UTI, a factor known to stimulate production of exo-polysaccharidesin both Gram-negative and Gram-positive bacteria.[19,21] However, chances appear remote in the present study. Long term follow up of patients with bacterial isolates with BFPP for recurrent UTI was not planned and was not done due to administrative constraints. This tempts us to conclude that a subset of our patients of c-UTI with bacterial isolates possessing BFPP should succumb to recurrent UTI, considering all these cases of c-UTI as first and index cases of UTI by bacteria with BFPP. However, this probability appears remote since some basic and special investigations clearly ruled out the possibility of any predisposing factors for biofilm production or acquisition of biofilm producing bacteria or chronic infections associated with biofilm producing bacteria probably indicating BFPP as a natural phenomenon than a virulence factor as documented from bacterial isolates from several natural environmental niches in the ecosystem. Multi-drug resistance(53.75%(43/80) vs. 8.75%(7/80)) in bacteria with BFPP and non-BFPP was not statistically significant[*P*=0.5393 NS]. Findings of present study confirms several published studies reporting increasing drug resistance in c-UTI as a result of selection bias since very few UTIs are cultured routinely and culture results are available from patients with complicated UTI, recent treatments, recurrent UTI or suspected drug resistance cases. Slightly higher MDR among bacteria with BFPP and non-BFPP was due to guidelines which recommend testing all antibiotics in a given antibiotic class. Several studies have proved BFPP as one of the virulence factor in recurrent UTI, similar studies are not available in first episodes of c-UTI for comparison.[22] In the present study E. coli was the most common bacterial isolates from c-UTI with 96.43%(27/28) of the isolates with BFPP. Soto SM *et al* have reported recurrent UTI with E. coli in 24 of the 43 females with c-UTI followed prospectively. BFPP was observed as a significant risk factor for recurrent UTI along with Yersiniabactin [fyu] and aerobactin [aer] detected by PCR using gene specific primers**.**[23]Mulvey*et al*demonstrated that uropathogens can persist in bladder tissue in underlying epithelia cells, a phenomenon analogous to biofilm and may act as source of recurrent UTI.[24]Anderson *et al* observed that intracellular bacteria mature into biofilms, creating pod-like bulges on the bladder surface which explains the persistence of bladder infections despite robust host defenses and appropriate antibiotic therapy. These studies indicate role of biofilm in recurrent c-UTI.[25] However, such findings were not sought in the present study. Association of predisposing risk factors in patients of isolates with BFPP was observed in only 24.61%(16/65) patients with no predisposing risk factors in 75.38%(49/65)[Fisher's exact test, P=0.7070 NS].One risk factor each in 16 patients with bacteria possessing BFPP was observed contrary to 3 patients with single risk factors in isolates with non-BFPP. Although predisposing risk factors were analyzed and interpreted objectively, the impact of these risk factors on patients was by and large subjective and to some extent arbitrary since their role was observed to be clinically not significant. Influence of risk factors on BFPP or acquisition of biofilm producing bacteria in community acquired UTI could not be analyzed due to small study population. However,Soto SM et al have proved association of in-vitro biofilm producing property along with Yersinibactin gene as a cause of relapses in c-UTI among patients with multiple predisposing risk factors by logistic regression analysis.[23] Microbiologists need to learn from population biologists and ecologists who have been thinking and working on bacterial communities and communication of bacteria in biofilm for decades. At present, medical microbiologists are at cross roads of ***natural science, biology and medicine***as

far as the BFPP of bacterial isolates from c-UTI are concerned, being glorified by medical microbiologists as a major virulence factor, which is however, a natural phenomenon as reported by natural biologists. Present study reports higher prevalence of BFPP in the absence of predisposing risk factors without statistically significant difference in MDR status and resistance in individual antibiotics among bacterial pathogens with BFPP and non-BFPP. Further research with coordinated approach to unravel the enigma of BFPP of bacterial isolates from c-UTI with its implications, is the need of the hour to implement preventive and therapeutic measures.

Implications of the Study

- Implications of bacteria with BFPP in our patients with respect to relapse or recurrent UTI needs prospective longitudinal studies by long term follow up.

Limitations of the present study

- Gene detection, *ica*ACD operon, responsible for BFPP was not done.
- Age wise, sex wise distribution and MDR status among bacterial isolates with Biofilm producing and non-biofilm producing property could not be analyzed with certainty, due to small study population.

Conclusions

1. Both Tube test and Congo red agar test should be used together to detect majority of bacterial isolates with biofilm producing property from c-UTI.

2. E.coli is the most common bacterial isolate with biofilm producing property followed by Klebsiella species in c-UTI.

3. Predisposing risk factors do not play any role in expression and/or acquisition of bacteria with Biofilm producing property

4. Whether biofilm producing property is a natural survival strategy possessed by majority of bacteria is to be confirmed by large multi-centric longitudinal study.

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Tables

Table 1: Distribution of bacterial pathogens with biofilm producing and non-biofilm producing property from community acquired urinary tract infection

Bacterial pathogen	Total no. of isolates	No. of isolates with BFPP	No. of isolates with non-BFPP
E. coli	28	27(96.43%)	1(3.56%)
Klebsiella	19	15(78.95%)	4(21.05%)
Staphylococcus aureus	18	14(77.78%)	4(22.22%)
Pseudomonas spp.	7	6(85.71%)	1(14.28%)
Coagulase Negative Staphylococcus	6	4(66.67%)	2(33.33%)
Gram negative non fermenter	1	0	1(100%)
Micrococcus	1	1(100%)	0
Total	80	67(83.75%)	13(16.25%)

Note: No.-Number, BFPP- Biofilm producing property,
Bacteria with BFPP -Tested positive by any one of the two test,
Bacteria with non-BFPP-Tested negative by both tests.

Table 2: Phenotypic test wise distribution of bacterial isolates with BFPP

Bacterial pathogen	No. of bacterial isolates	Congo red dye test alone (A)	Tube test alone (B)	Positive both tests(C)	Positive by either or both tests (A+B+C)
E. coli	28	5(17.86%)	2(7.14%)	20(71.43%)	27(96.43%)
Klebsiella spp.	19	6(31.58%)	8(42.10%)	1(5.27%)	15(78.95%)
Staphylococcus aureus	18	4(22.22%)	7(38.89%)	3(16.67%)	14(77.78%)
Pseudomonas spp.	7	1(14.28%)	1(14.28%)	4(57.15%)	6(85.71%)
Coagulase Negative Staphylococcus	6	2(33.33%)	1(16.67%)	1(16.67%)	4(66.67%)
Gram Negative non fermenter	1	0	0	0	0
Micrococci	1	0	0	1	1
TOTAL	80	18	19	30	67

Note: No**.-**Number, BFPP- Biofilm producing property

Table 3: Distribution of predisposing risk factors among bacterial Isolates with BFPP and non-BFPP

Risk factor	No. of patients	Risk factor in bacterial isolates with BFPP	Risk factor in bacterial isolates with non-BFPP
Diabetes	3	2	1
Benign hypertrophy of prostate	2	2	0

Pregnancy	9	7	2
Left sided multiple cystic kidney	1	1	0
Molar pregnancy	1	1	0
Recurrent UTI	2	2	0
Pelvic inflammatory disease	1	1	0
	19	16	3

Note: No.**-**Number, BFPP- Biofilm producing property

Table 4: Distribution of Multi-drug resistance among bacterial isolates with BFPP and non-BFPP

Bacterial isolate (n=Number)	**No. of isolates with MDR**	**MDR in isolates with BFPP**	**MDR in isolates with non-BFPP**
E. coli (28)	22(78.57%)	21(75%)	1(3.57%)
Klebsiella spp. (19)	16(84.21%)	13(68.42%)	3(15.79%)
Staphylococcus aureus (18)	10(55.55%)	8(44.44%)	2(11.11%)
Pseudomonas (7)	0	0	0
Coagulase Negative Staphylococcus (6)	1(16.66%)	1(16.66%)	0
Gram Negative non fermenter (1)	1(100%)	0	1(100%)
Micrococci (1)	0	0	0
TOTAL (80)	50(62.5%)	43(53.75%)	7(8.75%)

Note: No. **-** Number, MDR; Multi-drug resistance, BFPP- Biofilm producing property

Appendix 1: Difference in antibiotic resistance among bacteria with BFPP and non-BFPP among Gram positive cocci

Antibiotic	**Resistance among GPC [n=25]**	**Resistance among isolates with BFPP (n=19)**	**Resistance among isolates with non-BFPP (n=6)**	***P* value**
Penicillin	18/25 (72%)	15/19 (78.95%)	3/6 (50%)	0.2985, NS
Oxacillin	11/25 (44%)	8/19 (42.11%)	3/6 (50%)	1, NS
Ampicillin	12/25 (48%)	9/19 (47.37%)	3/6 (50%)	1, NS
Ampicillin- cloxacillin	8/25 (32%)	5/19 (26.32%)	3/6 (50%)	0.3442, NS
Amocicillinclavulanic acid	5/25 (20%)	3/19 (15.79%)	2/6 (33.33%)	0.5623, NS
Piperacillin	10 (40%)	8/19 (42.11%)	2/6 (33.33%)	1, NS
Piperacillintazobactum	5 (20%)	3/19 (15.79%)	2/6 (33.33%)	0.5623, NS
Imipenem	3 (12%)	2/19 (10.53%)	1/6 (16.66%)	1, NS
Meropenem	6 (24%)	4/19 (21.05%)	2/6 (33.33%)	0.6061, NS
Cefuroxime	8 (32%)	6/19 (31.58%)	2/6 (33.33%)	1, NS
Cefoxitin	9 (36%)	7/19 (36.84%)	2/6 (33.33%)	1, NS
Cefotaxime	6(24%)	4/19 (21.05%)	2/6 (33.33%)	0.6061, NS
Ceftriaxone	5 (20%)	4/19 (21.05%)	1/6 (16.66%)	1, NS
Ceftriaxone sulbactum	3 (12%)	2/19 (10.53%)	1/6 (16.66%)	1,NS
Ceftazidime	10 (40%)	8/19 (42.11%)	2/6 (33.33%)	1,NS
Cefoperazone	4 (16%)	3/19 (15.79%)	1/6 (16.66%)	1,NS
Cefepime	9 (36%)	7/19 (36.84%)	2/6 (33.33%)	1,NS
Ceftazidimetazobactum	6 (24%)	4/19 (21.05%)	2/6 (33.33%)	0.6061,NS
Cefepimetazobactum	4 (16%)	3/19 (15.79%)	1/6 (16.66%)	1,NS
Amikacin	4 (16%)	2/19 (10.53%)	2/6 (33.33%)	0.2340,NS
Gentamycin	5 (20%)	3/19 (15.79%)	2/6 (33.33%)	0.5623,NS
Netilmycin	4 (16%)	2/19 (10.53%)	2/6 (33.33%)	0.2340,NS
Tobramycin	7 (28%)	5/19 (26.32%)	2/6 (33.33%)	1,NS
Erythromycin	13 (52%)	11/19 (57.89%)	2/6 (33.33%)	0.3783,NS
Azithromycin	9 (36%)	7/19 (36.84%)	2/6 (33.33%)	1,NS
Clindamycin	15 (60%)	12/19 (63.16%)	3/6 (50%)	0.6532,NS
Vancomycin	5 (20%)	3/19 (15.79%)	2/6 (33.33%)	0.5623,NS
Nitrofurantoin	5 (20%)	4/19 (21.05%)	1/6 (16.66%)	1,NS
Cotrimoxazole	12 (48%)	9/19 (47.37%)	3/6 (50%)	1,NS
Ciprofloxacin	11 (44%)	8/19 (42.11%)	3/6 (50%)	1,NS
Norfloxacin	10 (40%)	7/19 (36.84%)	3/6 (50%)	0.6532, NS
Ofloxacin	9 (36%)	7/19 (36.84%)	2/6 (33.33%)	1, NS
Gatifloxacin	6 (24%)	4/19 (21.05%)	2/6 (33.33%)	0.6061, NS

Levofloxacin	5 (20%)	3/19 (15.79%)	2/6 (33.33%)	0.5623, NS
Sparfloxacin	7 (28%)	4/19 (21.05%)	3/6 (50%)	0.2985; NS
Linezolide	6 (24%)	4/19 (21.05%)	2/6 (33.33%)	0.6061; NS

Note: NS-Not significant

Appendix 2: Difference in antibiotic resistance among bacterial isolates with biofilm and non-biofilm producing property among Gram negative bacilli

Antibiotic	**Resistant isolates (n=55)**	**Resistance in isolates with BFPP (n=48)**	**Resistance in isolates with non-BFPP (n=7)**	***P* value**
Ampicillin	46(83.64%)	40(82.33%)	6(85.71%)	1; NS
Ampicillin cloxacillin	47(85.46%)	41(85.42%)	6(85.71%)	1; NS
Amocicillinclavulanic acid	30(54.55%)	25(52.08%)	5(71.43%)	0.4363, NS
Piperacillin	33(60%)	28(58.33%)	5(71.43%)	0.6895; NS
Piperacillintazobactum	12(21.81%)	7(14.58%)	5(71.43%)	0.0037, S
Imipenem	3(5.45%)	1(2.08%)	2(28.57%)	0.0398, S
Meropenem	4(7.28%)	2(4.16%)	2 (28.57%)	0.0745; NS
Mefuroxime	39 (70.90%)	34 (70.83%)	5(71.43%)	1; NS
Cefotaxime	31(56.4%)	28(58.33%)	3(42.86%)	0.6862, NS
Ceftriaxone	31(56.4%)	27(56.25%)	4(57.14%)	1; NS
Ceftriaxone sulbactum	10(18.18%)	7(14.58%)	3(42.86%)	0.1041; NS
Ceftazidime	24 (43.63%)	20(41.66%)	4(57.14%)	0.6862; NS
Cefoperazone	5(9.09%)	2(4.16%)	3(42.86%)	0.0118, S
Cefepime	25(45.45%)	22(45.83%)	3(42.86%)	1, NS
Ceftazidimetazobactum	8(14.54%)	4(8.33%)	4(57.14%)	0.0059, S
Cefepimetazobactum	9(16.36%)	6(12.5%)	3(42.86%)	0.0776; NS
Amikacin	9(16.36%)	6(12.5%)	3(42.86%)	0.0776; NS
Gentamycin	11(20%)	8(16.66%)	3(42.86%)	0.1342; NS
Netilmycin	7(12.72%)	4(8.33%)	3(42.86%)	0.0367; S
Tobramycin	10(18.18%)	7(14.58%)	3(42.86%)	0.1041; NS
Azithromycin	18(32.73%)	13(27.08%)	5(71.43%)	0.0317; S
Nitrofurantoin	4(7.28%)	2(4.16%)	2(28.57%)	0.0745; NS
Ciprofloxacin	37(67.27%)	33(68.75%)	4(57.14%)	0.6713; NS
Norfloxacin	37 (67.27%)	34 (70.83%)	3 (42.86%)	0.1996; NS
Ofloxacin	30 (54.55%)	27 (56.25%)	3 (42.86%)	0.6894; NS
Gatifloxacin	23 (41.82%)	21 (43.75%)	2 (28.57%)	0.6862; NS
Levofloxacin	30 (54.55%)	27 (56.25%)	3 (42.86%)	0.6894; NS
Sparfloxacin	35 (63.63%)	31 (64.58%)	4 (57.14%)	0.6960; NS

Note: NS-Not significant, S-Significant