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Research Paper

Bacteriological Profile of Uropathogenes in Catheterized Patients with Special Reference to Detection of Slime Production

Microbiology

KEYWORDS : urinary catheterization, biofilm production and antimicrobial susceptibility.

Safia Maqbool	Dept. of Microbiology Sardar Bhagwan Singh Post Graduate Institute, Balawala, Dehradun, Uttrakhand, 248161
Dr. Anahita V. Bhesania Hodiwala	Professor, Dept of Microbiology,MGM Medical College and Hospital, Navi Mumbai.
Dr. S.A Samant	Associate Professor, Dept of Microbiology, MGM Medical College and Hospital, Navi Mumbai
Junaid Nisar	Dept. of Microbiology Sardar Bhagwan Singh Post Graduate Institute, Balawala, Dehradun, Uttrakhand, 248161

ABSTRACT

OBJECTIVE: *The main objective of this study was to determine the antimicrobial susceptibility of uropathogenes isolated from catheterized patients and to detect the biofilm/slime production of these uropathogenes by the phenotypic method i.e. Tissue culture plate method.*

MATERIALS AND METHODS: *160 uropathogenes were isolated from catheterized patients and were tested for antimicrobial susceptibility as per Clinical Laboratory Standard Institute (CLSI) guidelines, followed by detection of Biofilm/slime production by the Tissue culture plate method.*

RESULT: *160 uropathogenes were isolated and among these 76(47.5%) isolates were biofilm/slime producing and highly resistant to the antimicrobial agents by Kirby-Bauer disc diffusion method as per the Clinical Laboratory Standard Institute (CLSI) guidelines.*

CONCLUSION: *The prevalence of biofilm producing uropathogenes in our study was 47.5%. Our study also showed that rate of biofilm production increases with respect to duration of catheterization and is responsible for developing antimicrobial resistance.*

INTRODUCTION:

UTI may be defined as a condition in which bacteria are established and multiply within the urinary tract. [1] Indwelling urinary catheterization is categorized as either; short-term (in situ less than 28 days), or long-term (in situ greater than 28 days). It has been estimated that the risk of acquiring an infection increases by 5% each day the catheter remains in situ. [2] Biofilms have major medical significance as they decrease susceptibility to antimicrobial agents. The decreased susceptibility to microbial agents within a biofilm arises from multiple factors, including physical impairment of diffusion of antimicrobial agents, reduced bacterial growth rates, and local alterations of the microenvironment that may impair activity of the antimicrobial agent. Furthermore, the proximity of cells within a biofilm can facilitate plasmid exchange and hence enhance the spread of antimicrobial resistance. [3]

MATERIAL AND METHODS: The study was conducted at the Department of Microbiology, M.G.M. Medical College, Kamothe, Navi Mumbai from December 2012 to December 2013. The cases were from M.G.M. Hospital, Kamothe, Navi Mumbai. The inclusion criteria of our study was the patients who were on urinary catheter for more than two days and did not show any signs of UTI before catheterization. Urine samples were collected on 3rd, 5th, and 7th day of catheterization .

Bacteriological procedures: Urine samples were immediately processed. A wet mount was prepared from the urine sample and then immediately inoculated onto blood agar and MacConkeys agar by using calibrated loop (delivering 0.001ml of urine) for semi quantitative method and incubated aerobically at 37^{0}C for 24 hours. A colony count of>10^{3}cfu/ml was considered significant. [4] Further phenotypic identification of isolated organisms was made by colony morphology and various biochemical tests under sterile conditions with controls being satisfactory.[5]

Antimicrobial susceptibility of all uropathogens was tested using Kirby Bauer Disc Diffusion Method by using commercial antibiotic discs (Himedia), according to Clinical Laboratory Standards Institute (CLSI) guide lines 2013.[6]

Tissue culture method:

The isolates were tested by Tissue culture plate method - phenotypic method for biofilm detection. This quantitative test described by Christensen et al. is considered the gold-standard method for biofilm detection. [7]

Procedure:

The organisms isolated from fresh agar plates were inoculated in Brain Heart Infusion (BHI)

with 2% sucrose and incubated for 24hrs at 37^{0}C in stationary conditions. Broth was diluted 1:100with fresh medium. Individual wells of sterile polystyrene 96 well flat bottom culture plate well were filled with 200ul aliquots of diluted cultures. Only medium served as negative control to check sterility and non-specific binding of media and ATCC strain was used as positive control. The Tissue culture plate was incubated for 24hrs at 37^{0}C . After incubation contents of each well was gently removed by taping the plates. The wells were washed four times with 0.2ml of phosphate buffer saline (PBS pH 7.2) to remove free floating planktonic bacteria. Biofilms formed by adherent 'sessile' organisms in plate were fixed with sodium acetate (2%) and stained with crystal violet (0.1%w/v) for one minute. Excess stain was rinsed off by thorough washing with deionized water and plates were kept for drying. Adherent bacterial cells usually formed biofilm on all side wells and were uniformly stained with crystal violet. Optical Density (OD) of stained adherent bacteria was determined with a micro ELISA auto reader at wavelength of 570 nm (OD 570nm). These OD values were considered as index of bacteria adhering to surface and forming biofilms. Experiment was performed in triplicate and repeated three times. [8]

Interpretation:

The interpretation of biofilm production was done according to the criteria of Stepanovicetal.given in table below.[9]

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Table 2. Interpretation of biofilm production

Average OD value	Biofilm production
≤ ODc / ODc < ~ ≤ 2x ODc	Non/weak
2x ODc < ~ ≤ 4x ODc	Moderate
> 4x ODc	Strong

Optical density cut-off value (ODc) = average OD of negative control + 3x standard deviation (SD) of negative control.

RESULT:

Table: 1

Total no. of urine samples obtained	Total no. of urine samples showing growth (86.6%)
150	130(86.6%)

Out of 150 urine samples obtained from catheterized patients, 130 (86.6%) samples showed quantitative culture threshold of $>10^3$ Cfu/ml of urine on culture plates and were subjected to detection of biofilm production and antimicrobial susceptibility testing.

Table: 2

Total no. of samples showing growth	Monomicrobial growth	Polymicrobial growth	Total no. of isolates
130	100 (76.9%)	30 (23%)	160

Table: 3

Microorganisms	Microorganisms isolated (%)
Gram positive cocci (GPC)	34 (21.2%)
Gram negative bacilli (GNB)	126 (78.7%)

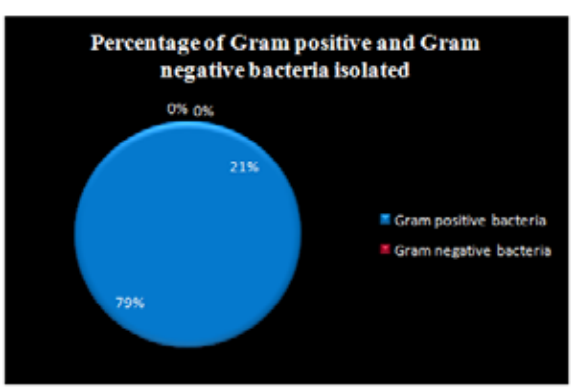

Figure: 1

Table: 4

Bacteriological profile of urine samples from catheterized patients (n=160)

Sr.no.	Microorganisms isolated	Total no.of isolates	Total Percentage (%)
1.	E.coli	65	40%
2.	Pseudomonas aeruginosa	24	15%
3.	Staphylococcus aureus	19	11.8%
4.	Klebsiella pneumonia	12	7.5%
5.	Acinetobacter species	10	6.5%
6.	Coagulasenegative staphylococcus	08	5%
7.	Enterococcus species	07	4.3%
8.	Proteus vulgaris	07	4.37%
9.	Proteus mirabilis	04	2.5%
10.	Citrobacter diversus	02	1.2%
11.	Enterobacter aerogenes	02	1.2%
	TOTAL =	**160**	

Table: 5

Total no. of isolates	Total no. of Biofilm producers	Total no. of non-biofilm Producers
160	76 (47.5%)	84 (52.5%)

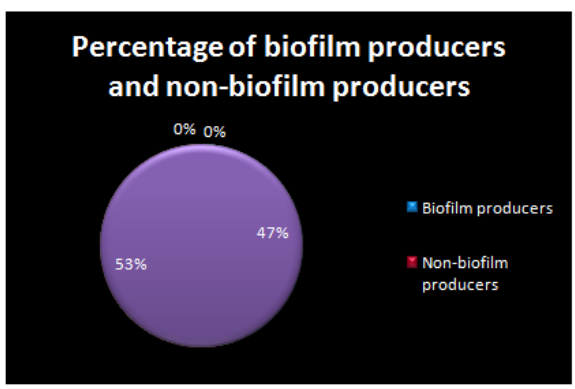

Figure: 2

Table: 6

Isolates positive for biofilm production by Tissue culture plate method (n=76)

Sr.no.	Microorganisms	No. of microorganisms isolated	No. of biofilm producing microorganisms	Percentage of biofilm producing microorganism
1.	Klebsiella pneumonia	12	08	66.6%
2.	E.coli	65	37	56.9%
3.	Pseudomonas aeruginosa	24	12	50%
4.	Staphylococcus aureus	19	09	42.3%
5.	Proteus vulgaris	07	03	42%
6.	Acinetobacter species	10	04	40%
7.	Enterococcus species	07	02	28%
8.	Proteus mirabilis	04	01	25%
9.	Coagulase negative staphylococcus	08	00	0%
10.	Citrobacter diversus	02	00	0%

Sr.no.	Microorganisms	No. of microorganisms isolated	No. of biofilm producing microorganisms	Percentage of biofilm producing microorganism
11.	Enterobacter aerogenes	02	00	0%
	TOTAL =	**160**	**76**	

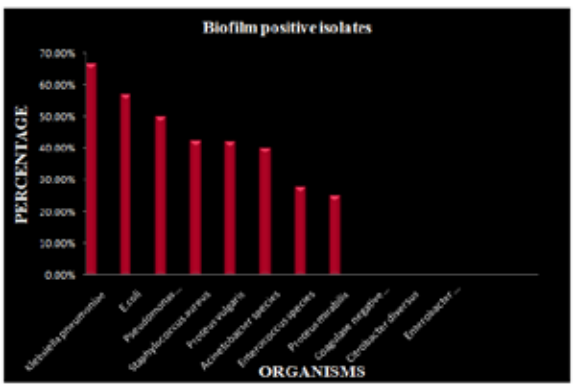

Figure: 3

Table: 7
Rate of biofilm formation with respect to duration of catheterization:

Duration (days)	Total isolates studied	Total biofilm producers
3rd day	81	11 (13.5%)
5th day	74	60 (81%)
7th day	05	05 (100%)
TOTAL =	**160**	**76**

Table: 8
Antibiotic resistance pattern (%) of biofilm producing and non-biofilm producing organisms:

Antimicrobial agents	Biofilm producing(%) organisms	Non-biofilm producing(%) organisms
Amikacin (AK)	76.9%	50%
Ciprofloxacin (CIP)	76.9%	44%
Cefotaxime (CTX)	61.5%	45.9%
Cefuroxime (CXM)	76.9%	60%
Augmentin (AMC)	92.3%	70%
Lomefloxacin (LOM)	76.9%	49%
Ceftazidime (CAZ)	84.6%	70%
Cefoperazone (CPZ)	64.6%	40.9%
Gentamicin (GEN)	86%	70%
Netillin (NET)	65%%	41%
Pfloxacin (PF)	66%	47.5%
Ofloxacin (OF)	72%	60%
CO-Trimoxazole (COT)	63.6	39%
Cloxacillin (COX)	45.5%	30%
Roxithromycin (RO)	63%	35%
Cefalexin (CN)	63%	30%
Levofloxacin(LE)	45%	26%
Tetracycline (TE)	63%	34%
Lincomycin (L)	72%	34%
Ampicillin/ Sulbactam(A/S)	60%	50%
Linezolid (LZ)	63.6.%	53%
Cefaperazone/ Sulbactam (CFS)	51%	38.5%
Meropenem (MRP)	41%	28.5%
Imepenem(IMP)	29.4%	14%
Ticarcillin/clavulanic acid (TCC)	58.8%	42.8%
Ceftriaxone (CTR)	58.8%	28.5%
Pipracillin/ Tazobactam (PIT)	76%	42.8%
Azithromycin (AZM)	40%	25%
Cefazolin (CZ)	40%	25%
Vancomycin (VA)	0%	0%
Cefaclor (CF)	50%	35%
Rifampicin (RIF)	80%	40
Teicoplanin (TEI)	55%	30%
Polymyxin –B (PB)	75%	50%
Etrapenem (ETP)	62.5%	50%
Aztreonam (AT)	75%	50%
Tigecycline (TGC)	62.5%	50%

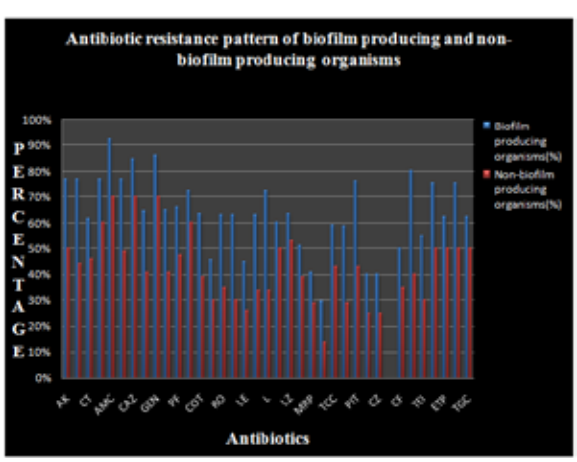

Figure:4

DISCUSSION:-

Out of 150 samples included in our study 130(86.6%) showed growth of uropathogens (Table:1) which indicates that catheterization is one of the most important predisposing factor for development of UTI in hospitalised patients. A study by Muraggan S. et al. reported 64% uropathogens from 135 urine samples of catheterized patients. [10]

Amongst 130 samples 100 (76.9%) samples showed monomicrobial growth and 30 (23%) showed growth of two organisms (Table:2). A study by Gad and El-Feky showed that a total of 292 bacterial isolates were recovered from100 patients.[11] A study by Ouslander JG et al. and Warren JW et al. reported that long term catheter users (>month) have high concentration of bacteria in the urine that tend to be polymicrobial .[12,13] A study by Hooton T. et al. also reported that in contrast to patients with short term catheterization, UTI's in patients with long term catheterization are usually polymicrobial .[14] This was comparable with our study in which we found more samples showing monomicrobial growth as none of our patients were on catheterization for more than 7 days.

Amongst these 160 isolates, the majority were Gram negative bacilli 126 (78.7%) as compared to Gram positive bacteria 34 (21.2%). Isolation rate of Gram negative bacilli (80%) was seen to be higher in catheterized patients as compared to Gram positive bacteria as per the study of Tenke et al. as well. [15] However a study by Rewatkar et al. reported that out of 160 isolates, 30 were Gram positive bacteria and 30 were Gram negative bacilli. [16]

In this study out of 160 isolates, E.coli (40%) was the most common uropathogen (Table:4). A study by El-Feky MA and Hittinahalli et al. have reported similar findings where they showed E.coli as a uropathogen in 64% and 56% cases respectively. [17,8]

In our study 76 (47.5%) isolates were detected positive for biofilm production using tissue culture plate method (Table:5). This was higher as compared to the studies by Khan et al. and Venkitanarayanan et al. who have reported 60% uropathogenic isolates as biofilm producers.[18,19] On the other hand a study by Swarnakar et al. have reported biofilm production in as high as 89.5% uropathogens. [20]

This study also showed that, the biofilm production was highest i.e. 66.6% and 56.9% in K.pneumoniae and E.coli respectively (Table: 6). Another study by Cox et al. and Morris et al. reported that >60% K. pneumoniae isolates from catheterized patients showed biofilm production.[21,22] Also a study by Sharma et al and Nivedeta et al reported biofilm production in 67.5% and 60% E.coli isolates respectively.[23,24] Another study by Anand kumar et al. reported biofilm production by 84% K.pneumoniae and 76% E.col isolate.s[25]

Also this study showed that biofilm production increases with the duration of catheter in situ. On 3rd, 5th and 7th day of catheterization the total biofilm producers were 13.5%, 81% and 100% respectively (Table:7) as bacteriuria occurs at the rate of 3%-10% per/ day.[26]

A study by Cardenas et al. reported biofilm production of 32%, 64% and 100% on 4th, 6th and 7th day respectively. [27]

This study also detected that antimicrobial resistance is higher in biofilm producing organisms as compared to non-biofilm producing organisms which is similar to findings in the Hassan et al.[9], Devanand et al. [28] and Rawatkar and Wadhera JB.[16].

CONCLUSION:

This study showed that biofilm production increases with duration of catheterization and is responsible for developing antimicrobial resistance.

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