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IN-VITRO STUDY OF POVIDONE-IODINE AND GENTAMYCIN ON BIOFILM STRAINS OF *IN-SITU* URINARY-CATHETER TO DETERMINE THEIR COMBINED EFFICACY TO PREVENT CATHETER-ASSOCIATED UTI IN A TERTIARY CARE HOSPITAL

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ABSTRACT The biocides are generally used for perineal cleaning prior to urinary tract catheterisation and to lubricate the passage of urinary catheter. These are also included in the urine drainage bags every time they are emptied. The catheter-meatal junction is also cleaned to minimise the periurethral uropathogens. Systemic prophylaxis with suitable antibiotic is also recommended to prevent catheter-associated UTI when indwelling catheters are used. In spite of implementation of this comprehensive policy in patients with long-term indwelling catheter, catheter-associated urinary tract infection (CAUTI) is the usual outcome. In a long-term(>30 days) catheterised patient with continuous drainage, the iatrogenic or ascending infections may not revealed as significant bacteriuria; rather they colonise on indewelling urinary catheter in the form of biofilm & serve as persistent source of treatment refractory infections by time to time dispersion of biofilm fragments. In this study, the biofilm mode of growth on long-term (30 d) indwelling uro-catheters have been studied, sensitivity of biofilm isolates were tested to povidone-iodine (urinary antiseptic) & gentamycin(antibiotic used for UTI prophylaxix) . Analysis of antibiotic resistance pattern and the antiseptic sensitivity of the biofilm strains to povidone-iodine were analysed to decide ,wheather there is a co-relation between antibiotic &biocide resistance, also regarding association of resistant genes. But it is clear that, long-term extensive use of povidone-iodine in catheterised urinary tract, where it has to deal with a rich and varied bacterial flora; was counter-productive and led to infections by drug-resistant Nosocomial pathogens. The study was conducted at IPGME & R, Kolkata in 2011.

KEYWORDS : Catheter-associated UTI, Biofilm, MIC, MBEC

INTRODUCTION:

At least 25-50% of nosocomial infection occur due to combined effect of patient's own flora & invasive devices. In patients with chronic indwelling bladder catheters, 'catheter flora' – i,e microorganisms living on encrustation within the catheter lumen,forms the biofilm; which is central to the pathogenesis of catheter-associated UTI (CAUTI) & affects both therapeutic and preventive strategies. Almost all nosocomial UTIs are associated with preceding instrumentation or indewelling bladder catheters, which create a 3-10% risk of infection each day[6].Use of systemic antimicrobial agents for other purposes decrease risk of UTI during first 4 days of catheterisation, after which resistant bacteria or yeast emerge as a pathogen. Practically 10-50% of patients with short-term catheterisation (up to 1week) develop infection[1] & patients undergoing long-term catheterisetion (>1 month) will inevitably develop bacteriuria . Among the variety of compounds, Povidone-iodine is the most promising solution, used for local instillation into bladder in attempts to decrease bacteriuria and prevent infections. A single dose of povidone-iodine prior to catheter removal seems to be a promising practice, as it will be helpful in reducing rates of NHSN(National Healthcare Safety Network)-defined CAUTI . Furthermore, use of this method as opposed to the suggested use of only systemic antibiotics at time of catheter removal , is potentially preferable from the downstream of less antimicrobial resistance & reduced risk of Clostridium difficile infection .The typical signs and symptoms of UTI have less predictive value for the diagnosis of CAUTI, also fever & asymptomatic bacteriuia in a catheterised patient does not necessarily predict CAUTI[13].Pathological studies reveal that many patients with long-term catheters have occult pyelonephritis[14]. Systemic antibiotics, bladder-acidifying agents, antimicrobial bladder washes, topical disinfectants and antimicrobial drainage bag solutions have all been ineffective at preventing onset of bacteriuia in a long-term catheterised patient, and have been associated with emergence of resistant organisms.It has been studied that, Gentamycin bladder instillations reduce the rate of symptomatic UTI in neurogenic bladder patients on intermittent self-catheterisation, & reduce the need for oral antibiotic [15].

Single species of bacteria (such as *Staphylococcus epidermidis*, *Enterococcus faecalis*, or *E.coli*) are responsible for infection in the early stages of catheterisation. On the other hand mixed communities of mainly gram-ve nosocomial species (such as, *providencia stuartii*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumonia*, *E.coli*) accounts for extensive biofilm formation in lumen of urinary catheters [5] . There is evidence that biofilm strains of bacteria are sensitive to antibiotics by conventional laboratory procedures, but they survive the urinary concentrations of antibiotics produced by standard treatment regimes. In biofilm mode of growth, communication process among the organisms are mediated by diffusible signal molecules or autoinducers (N-acyl homoserine lactone in gm-ve bacteria & oligopeptides in gram+ve bacteria); khown as quorum sensing[16](QS). This QS can be prevented by inhibiting the signal molecule from binding to the receptor via analogues of the signal molecules. Introduction of Usnic acid,a naturally occuring dibenzofuran derivative[17] ,the QS inhibitor RNAIII inhibiting peptide(RIP)[18] have been demonstrated very efficacious in modifying biofilm growth.

AIM & OBJECTIVE:

Aim & objective of this study was to determine, wheather conbined use of an antiseptic & antibiotic would be efficacious to prevent catherter-associated UTI in a long term in-situ urinary catheterised patient.

MATERIALS & METHODS:

The study was carried out with about 29 urinary Foley's catheter sample(proximal part), which were collected aseptically at urology OPD at Institute of Postgraduate Medical Education & Research (IPGME&R),Kolkata; from the patients having indwelling urinary catheter & attending at urology OPD for periodic change of catheter. Patients of all age groups & both sexes were included in this study. In-situ urinary catheters (>1 month) were selected as clinical samples due to their prompt availability of pre-formed biofilms on most of them.

Colonies of planktonic forms of bacteria were obtained by incubating the proximal part of catheters in Brain heart infusion broth (BHIB) followed by subculture in nutrient agar plate. Inoculum suspensions of planktonic forms were prepared by diluting the growth with cation-adjusted Muller-Hinton broth(CAMHB)to the density of 0·5% McFarland standard followed an additional 1:10 dilution. Gentamicin sulphate stock solution were prepared, serial dilutions of Gentamycin were made in the concentrations of 200 µg/ml, 100 µg/ml 50 µg/ ml, 25 µg/ml, 12.5 µg/ml, , 6.25 µg/ml, 3 µg/ml, 1.5 µg/ml . 10% stock solution of povidone-iodine was prepared (1% available iodine). 8 sets of dilution tubes were prepared to get povidone-iodine solutions with available iodine 1%, 0.5%, 0.25%, 0.125%, 0.06%, 0.03%, 0.01%, 0.008%. Cation-adjusted Muller-Hinton broth (CAMHB,pH

7.2-7.4) was added to each dilutions of gentamycin & povidone-iodine.Two sets of tubes were prepared for MIC & MBEC determination. For MBEC determination of sessile forms, proximal part of catheter pieces inoculated into CAMHB-povidone iodine & CAMHB-gentamycin tube sets. After overnight incubation, subculture done in nutrient agar by roll-plate method of the catheter piece & MBEC value obtained. MBEC (minimum biofilm elimination concentration) value denotes the drug concentration required to eliminate highest resistant sub-population within the biofilm.

Loopfull of growth from CAMHB (planktonic form) were inoculated into both sets of tubes & incubated overnight at 37◦C.On next day, subculture done in nutrient agar plate from each tube to get MIC value (minimum inhibitory concentration) of planktonic form. In each set , media with & without antibiotic –antiseptic were included for quality control.

RESULTS :

Oral and parenteral administration generally produce much higher concentration of antibiotic (10 -100 fold)in urine than in blood & tissues. As there is no significant invasion of tissues, a bacterium may be considered sensitive to an antibiotic whose MIC for it is up to 10 or more times as great as that acceptable for the treatment of other kind of infections.

Table 1: Result of MIC versus MBEC of planktonic & sessile forms respectively with povidone-iodine:

Biofilm isolates	Number of biofilm isolates	MIC of planktonic forms (n)	MBEC of sessile forms (N)	interpretation
E.coli	10	n=1 , [0·01%] n=3 [.03%] n=6 , [0·125%]	N=1 , [0·01%] N=3 [0.5%] N=6, [1%]	MIC= MBEC MBEC > MIC
Klebsiella spp.	5	n=4, [0.03%] n=1, [·01%]	N=4, [0·5%] N=1, [·06%]	MBEC > MIC
Proteus mirabilis	2	n= 1 [·125%] n= 1, [·25%]	N= 1, [0·5%] N=1, [>1%]	MBEC > MIC MBEC > MIC
Pseudomonas spp.	4	n=4 , [·5%]	N=4, [>1%]	MBEC > MIC
Staphylococcus aureus	8	n=5 [0·125%] n=3 [0.06%]	N= 5 [0·25%] N=3 [0.5%]	MBEC > MIC

MBEC results revealed uniformly poor sensitivity of pseudomonal biofilms to iodine. On the other hand *E.coli & klebsiella* revealed various MBEC values, either equal or higher with respective MIC value . Equal value of MIC & MBEC may be explained by the fact that, perhaps thickness of respective biofilm is less; so that the physiological and biocide gradient can not be formed yet.

Table 2: Result of MIC versus MBEC of planktonic & sessile forms respectively with Gentamycin:

Biofilm isolates	Number of biofilm isolates	MIC of planktonic forms (n)	MBEC of sessile forms (N)	interpretation
E.coli	10	n=6 [3µg/ml] n=4 [1.5µg/ml	N=3[12.5µg/ml] N=6[6.25µg/ml] N=1[1.5µg/ml]	MBEC>MIC MBEC=MIC
Klebsiella spp.	5	n=4 [3µg/ml] n=1 [1.5 µg/ml]	N=4[12.5 µg/ml] N=1[1.5 µg/ml]	MBEC>MIC MBEC=MIC
Proteus mirabilis	2	n=2[6.25µg/ml]	N=2[25 µg/ml]	MBEC>MIC
Pseudomonas spp.	4	n=1[3µg/ml] n=3[6.25µg/ml]	n=2[12.5µg/ml] n=2 [25µg/ml]	MBEC>MIC
Staphylococcus aureus	8	n=2[3 µg/ml] n=6[6.25 µg/ml]	N=2[12.5µg/ml] N=6 [25µg/ml]	MBEC>MIC

Charts & statistical evaluation:

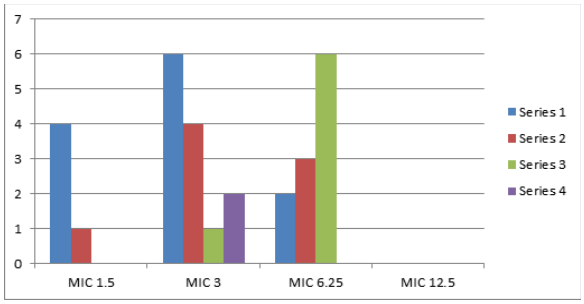

Figure 1: Bar diagram representing MIC values of Gentamycin against the planktonic isolates

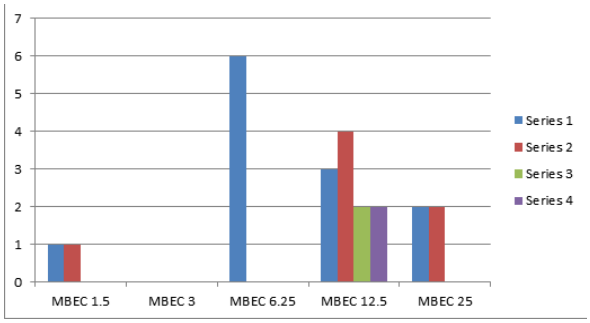

Figure 2: Bar diagram representing MBEC values of Gentamycin against sessile forms of biofilms strains

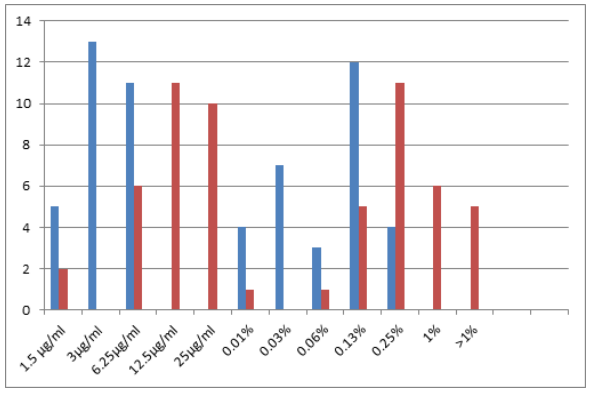

Figure 3 : number of isolates showing various MIC (blue plotted)& MBEC (red plotted) values with Gentamycin & Povidone-iodine.

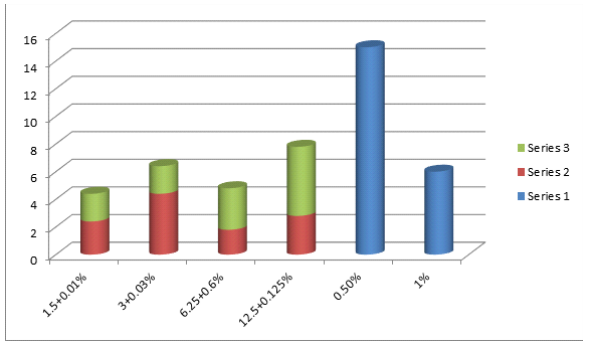

Figure 4: Component bar Chart representing the number of isolates showing the effect of combined MIC-MBEC values of both antibacterials .

Number of biofilms isolates evaluated against the combination of various MIC-MBEC values of both the test drugs ; and it can be concluded that, more number of isolates have shown sensitivity to 12.5µg/ml+ 0.125% concentration & 0.5% povidone-iodine solution.

Calculation of Standard deviation(S.D): Arithmetic mean (µ) of various MIC values (MICs 1.5, 3, 6.25,12.5; mean 5.9) wih gentamycin were calculated. Taking the deviation of each MIC value from the

arithmetic mean followed by squaring each value & adding up all the squared values, required data obtained was 66.96. Standard deviation was obtained by dividing this data by number of observations (here it is 4-1=3, as the sample size 29, i,e <30).

Hence the arithmetic mean & S.D of the MIC values with Gentamycin are 5.9 and 4.8 respectively. Similarly arithmetic mean & S.D of the MBEC values with Gentamycin are 9.65 & 9.57.

Same method was applied for povidone-iodine to get arithmetic mean & S.D of MIC value (0.975 & 0.90 respectively) and MBEC values; (1.975 & 1.75 respectively) ; considering the dilutions; 0.01% ,0.03%, 0.06%, 0.125%, 0.25%, 0.5%, 1%).

Standard error of difference between two means { S.E. (d) } of MIC & MBEC were calculated for both antibacterials. S.E (d) of MIC & MBEC of gentamycin was 5.52. But the actual difference between two means (μ) was 3.75, i.e less than the double of d value(11.04). Also the S.E (d) of MIC & MBEC of povidone-iodine was 0.82 , though the actual difference between two means was 1, again i.e slight less than the double of d value (1.64). So both of the "d" values regarded as " not significant" [for being significant, actual difference of mean should be greater than double of S.E (d) value]

From this finding, we can conclude that, the combined treatment of Gentamycin & Povidone-iodine may not be fully efficacious to prevent catheter-associated UTI, at least in this study group of 29 patients.

DISCUSSION:

The practices of bladder catheterisation, the assessment of its need (based on individual risk assessment of the person);selection of the type of urinary catheter (according to the expected duration; aseptic insertion and maintainance of urinary catheter, along with its correct removal are relevant from the point of view of prevention & control of CAUTI . Chlorhexidine solution was a popular biocide to wash the periurethral skin prior to catheter insertion,in gel form to lubricate the passage of the catheter and, in some cases was being included in the urinary drainage bag.

Phenomenon of chlorhexidine resistance was first observed during a study of the development of urinary tract infections in patients undergoing intermittent catheterisation in the early stages of paraplegia [7]. Among the gm-ve flora developed around urethral meatus the resistant organisms mainly were Providencia stuartii, Proteus mirabilis & pseudomonas aeruginosa. Stickler and Thomas et al [2] further examined the MICs of gm-ve flora isolated from UTI patients of both the community & local hospitals observed that the higher MIC (>500μg/ml) was limited to above mentioned organisns.

Chlorhexidine resistance of Proteus mirabilis is most vulnerable as their encapsulated microcolonies become the foci for stone formation in bladder & kidney. They also form extensive crystalline biofilms followed by catheter encrustation and blockage. Urinary retention can facilitate vesico- urethral reflux followed by episodes of pyelonephritis [9]. from this study, it is clear that long-term extensive use of antiseptic in the catheterised urinary tract, where it has to deal with a rich and varied flora, was counter-productive and led to infection by drug-resistant nosocomial pathogens.

Firehammer et al showed that,the recent isolates of Proteus mirabilis obtained from encrusted catheters were extremely sensitive to triclosan(MIC 0.5μg/ml) [10].

Gentamycin concentration in urine may be 3·2-600 μg/ml during therapy of UTI. A study performed in a rabbit model on the efficacy of gentamycin-releasing urethral catheters showed inhibition of CAUTIs for 5 days, suggesting a role for these devices in short-term catheterisation[4]. Waites et al demonstrated that oral antibiotics change the urinary, perineal and urethral flora of neurogenic bladder patients [12]. Moreover, it may not be possible to safely achieve adequate urine concentrations via oral or IV administration . Diffusion of aminoglycosides across the uroepithelium is even more limited due to their polar cationic nature [11].

Another study on Intravesical administration of gentamycin for recurrent UTI in patients with clean intermittent bladder catheterisation, were carried out by C.Van Neuwkoop et al in Leiden University Medical center Netherland(2010) & published in International journal of Antimicrobial agents.Patients with multidrug-resistant E.coli, treated with 80mg/d intravesical gentamycin;became free from UTI in 8-9m. On the other hand, Berkelman et al showed the increased bactericidal activity by dilute preparation of povidone-iodine solution[3] .

In this study , biofilms isolates showing different MICs & MBECs with gentamycin and povidone-iodine. Planktonic Isolates have lower MICs, but biofilms-forming sessile isolates have raised MBECs with gentamycin. Povidone-iodine instillation to the uro-catheter & bladder may be promising, as most of the sessile isolates showing sensitivity to 1% solution. Furthermore, to some extent bactericidal activity of povidone-iodine solution depend upon presence of organic matter at local site [8] .

CONCLUSION:

The sterility of the urinary tract is maintained in part by the outflow of urine, which prevents pathogenic organisms from ascending into the bladder. Prolonged use of an urinary catheter in a patient's management impair this protective mechanism. Povidone-iodine (Betadine) is a complex of the potent bactericidal agent iodine & the carrier molecule povidone. On contact with tissues, the carrier complex slowly releases free iodine. Gradual release decreases tissue irritation and reduces potential toxicity while preserving the agent's germicidal activity. This study led an attempt to evaluate the effectiveness of Betadine irrigation solution (10% povidone-iodine), instilled into the bladder immediately prior to indewelling catheter removal to decrease the risk of subsequent bacteriuria, along with prophylactic gentamycin, leading to decreased rate of NHSN defined CAUTI. Despite promising results, the efficacy of bladder irrigation has been limited by toxic effect of the solutions used & difficulty to combat with more resistant biofilm strains on urocatheters.

Attempts have been made to prevent bacterial colonisation by biofilm strains on uro-catheters, by incorporating antibiotics or biocides into the catheter material (metallic silver-coated surfaces).

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