

## Multidrug Resistant Bacteria Colonizing Medical Devices In The I.C.U's Of A Pediatric Tertiary Care Centre : An Observation By Biofilm Detection .

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### Abstract

Biofilms are a group of organisms that attach to a surface in an exopolysaccharide matrix and render them resistant to many group of antibiotics. Indwelling medical devices are often colonized by microorganisms and have a great potential towards biofilm formation. All the medical devices i.e. Intravenous catheters tips (I.V.C.), Endotracheal tubes (E.T.T.), Intercoastal drain tubes (I.C.D.) and Nasogastric tubes (N.G.T. Tubes) were collected aseptically and sent to the Clinical Microbiology Laboratory. The devices were processed by standard microbiological procedures & their biofilm production was determined. In the 100 devices processed, 57% were culture positive. *Staphylococcus epidermidis* (45%), & *Klebsiella pneumoniae* (38%) were the commonest isolates, among which 81% were biofilm producers. All the Gram Positive isolates were found to be Methicillin resistant and in the Gram Negative isolates, 76% were Extended Spectrum Beta Lactamases (E.S.B.L.) and 59% were Carbapenamase producers. From the culture positive devices 81% were biofilm producers. To conclude, among the isolates which colonized the various medical devices, majority of them were Multi Drug Resistant, which were observed to produce strong to moderate biofilms.

**Key words:** Medical devices, colonization, Biofilm Production, Multidrug resistant Bacteria.

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### I. Introduction

Biofilms are sessile communities of microbes which cause a variety of infections. They are characterized by the cells that get irreversibly attached with each other and get embedded in the matrix of Extra Polymeric Substances (E.P.S.) produced by themselves.<sup>1</sup> Biofilms are responsible for many device associated infections, upper respiratory tract infections, urogenital infections & chronic wound<sup>2</sup>. Devices are vulnerable for colonization by the biofilm producing bacteria, and are a source of persistent and resistant infections, unless removed from the patient.<sup>3</sup> Due to the extensive use of these devices in hospitalized patients, incidence of device related infections has increased.<sup>4</sup>

Lowbirthweight, prematurity, congenital malformations, prolonged hospital stay, frequent invasive procedures, and administration of total parenteral nutrition are important factors that increase the risk of infection in neonates & infants.<sup>5</sup> Therefore, this group is prone for life threatening infections. Hence, this study is focused on the microbes colonizing the devices, their potential towards biofilm formation, and their antibiotic resistant patterns among the patients admitted in the N.I.C.U & P.I.C.U. of a Paediatric tertiary care hospital.

### II. Material And Methods

After approval from the ethical committee, this prospective study was carried on a study group of children aged 0-15 years admitted to the N.I.C.U. & P.I.C.U of a Pediatric Tertiary care Hospital (Niloufer Hospital for Women & Children, Hyderabad, T.S. India). The study was conducted over a period of six months i.e., from March 2015 to August 2015. 100 non repetitive indwelling medical devices included I.V.C., E.T.T., I.C.T., N.G.T., received to the clinical microbiological laboratory were the samples processed. Devices placed for more than 12 hours were included in the study.

**Specimen collection and processing:**<sup>6,7,8</sup> The distal 3-5 cm of the tip of the catheter and the terminal end of the tubes was aseptically cut with a sterile scissors & was dropped into a sterile test tube containing brain heart infusion (B.H.I.) broth. Samples were sent immediately to the Microbiology laboratory for processing.

**I.V.C. Catheter tip processing<sup>9</sup>: Maki's semi quantitative culture method** was used for processing the catheter tips. Using a sterile forceps the tip was rolled back and forth across the entire surface of the agar plate using sterile forceps while exerting a slight down ward pressure. The same was repeated again on MacConkey agar plate, and the plates were incubated aerobically at 37<sup>0</sup>C, the colony count was recorded after 24 hours.

**Processing of E.T.T., I.C.T., N.G.T.<sup>10</sup>:**

Under strict aseptic conditions the distal 3-5 cm of the tube tips were sent in a sterile tube containing B.H.I. broth to the Microbiology laboratory immediately for the quantitative culture . These samples were Incubated for four hours , examined microscopically and cultured aerobically on Blood agar & MacConkey agar . The culture plates were incubated at 37<sup>0</sup>C overnight (18-24 hours) and examined for any growth. The organisms isolated were identified by standard biochemical reactions and confirmation of identification was done by Vitek 2 biomérieux automated system.

**Antibiogram of the isolates<sup>11</sup>:**

Kirby Bauer disc diffusion method was performed for antibiotic susceptibility testing. Test organism was sub cultured into peptone water and incubated for 4-6hrs at 37<sup>0</sup>c. Turbidity was standardized with 0.5 McFarlands and swabbed over 90mm Mueller Hinton agar plate. Antibiotic discs were placed at 15mm from the edge of the plate and evenly so that they were no closer than 25mm from centre to centre. Plate was incubated at 37<sup>0</sup>C for 18-24 hrs. Zones of inhibition were measured after incubation with a ruler and interpreted as per CLSI guidelines. The commercially available antibiotic discs were used, supplied by Himedia (Mumbai). Vitek 2 biomérieux automated system was carried out for Minimum Inhibitory Concentration (M.I.C.)

Control : Gram Positive Cocci : Staphylococcus aureus A.T.C.C strain 25923

Gram negative bacilli : E.coli A.T.C.C. strain 25922.

**DETECTION OF BIOFILM FORMATION<sup>3,12</sup>:**

**Tissue culture plate method:** This quantitative test is considered the gold standard method for biofilm detection, described by Christensen et al<sup>3</sup>. Organisms isolated from fresh agar plates were inoculated in 10 mL of trypticase soy broth with 1% glucose and were incubated at 37<sup>0</sup>C for 24 hrs. The cultures were then diluted in 1:100 fresh trypticase soy broth. Individual wells of sterile 96 well-flat bottom polystyrene tissue culture treated plates were filled with 200  $\mu$ L of the diluted cultures. The control organisms were also incubated, diluted and added to tissue culture plate. Negative control wells contained sterile broth. The plates were incubated at 37<sup>0</sup>C for 24 h. After incubation, contents of each well were removed by gentle tapping. The wells were washed with 0.2 mL of phosphate buffer saline (pH 7.2) four times. Biofilm formed by bacteria adherent to the wells were fixed with 2% sodium acetate and stained by crystal violet (0.1%). Excessive stain was removed by using deionized water and plates were kept for drying. Optical density (OD) of stained adherent biofilm was obtained by using micro ELISA autoreader (model 680, Biorad, UK) at wavelength 570 nm. The experiment was performed in triplicate and repeated three times. Isolates were classified as strong, moderate, weak or zero biofilm producers based on their OD<sub>570</sub>.

**Calculation for optical density for detection of Biofilm :**

Strong biofilm producer ( $4 \times \text{ODc}$ ) < OD<sub>570</sub>

Moderate biofilm producer ( $2 \times \text{ODc}$ ) < OD<sub>570</sub>  $\leq$  ( $4 \times \text{ODc}$ )

Weak biofilm producer ODc < OD<sub>570</sub>  $\leq$  ( $2 \times \text{ODc}$ )

OD<sub>570</sub>  $\leq$  ODc = no biofilm producer.

{OD cutoff (ODc) = average OD<sub>570</sub> of negative control + (3 $\times$  standard deviation of negative control)}

Positive Control Biofilm Producer–Pseudomonas aeruginosa A.T.C.C strain 2785

Negative Control: Non Biofilm producer- Staphylococcus aureus A.T.C.C strain 25923

**III. Results:**

Out of the 100 specimen processed , 57% were culture positive. Out of the 36 I.V.C. processed , 56% were culture positive and of the 64 device tubes processed 58% were culture positive.

**Table1 : Microbiological profile of Intra Venous Catheters ( n=20)**

Organism isolated	Percentage %
Methicillin resistant Staphylococcus epidermidis	45%
Methicillin resistant Staphylococcus aureus	15%
Pseudomonas aeruginosa	15%

91% of colonization was seen from the I.V.C. inserted over the lower limb, and out of the 12 I.V.C. collected from the neonates, highest percentage (67%) of colonization was found to be in low birth babies with birth weight less than 2.5 k.g. The duration of I.V.C. was found to be directly proportional to colonization, i.e. 60% of colonization was seen from the I.V.C.'s which were indwelling for greater than 96 hours of duration.

**Table 2 : Microbiological profile of E.T.T. , I.C.T. , N.G.T., (n=37)**

Organism isolated	Percentage (%)
Klebsiella pneumonia	38%
Acinetobacter baumannii	22%
Pseudomonas aeruginosa	19%

**Biofilm Production of Isolates:** Among the 57 positive cultures , 42 (81%) isolates were found to produce biofilm , detected by tissue culture plate method (T.C.P.). Highest number of biofilm producers were isolated from the endotracheal tubes (67%) followed by I.V.C. (38%).

**Grades of biofilm production by Tissue Culture Plate method :** Out of the 42 isolates which produced Biofilms by T.C.P. Method 23% were strong , 62% were moderate & 14% were weak biofilm producers. 42% of P. aeruginosa isolates , 37.5% of A.baumannii , 25% E.coli, 14% of K.pneumoniae , 7.60% of S.epidermidis were among the strongest biofilm producers.

**Figure 1. TISSUE CULTURE PLATE METHOD**



**Table 3 : Frequency between resistance pattern of Biofilm producers & Non Biofilm producers in Gram Positive isolates**

Antibiotic	Biofilm producer (n=13)		Non Biofilm producer (n=5)	
	Resistant	R%	Resistant	R%
Ampicillin (10mcg)	13	100%	5	100%
Amoxyclav (20/10mcg)	10	77%	3	60%
Amikacin (30mcg)	8	62%	1	20%
Gentamicin (10mcg)	10	77%	2	40%
Levofloxacin (5mcg)	7	53%	1	20%
Cefotaxime (30mcg)	8	62%	2	40%
Cefoperazone +Sulbactam (75/30mcg)	6	46%	1	20%
Linezolid (30mcg)	0	0%	0	0%
Vancomycin (M.I.C.-E Test 0.016-256mcg/ml)	0	0%	0	0%
Cotrimoxazole (25mcg)	11	84%	3	60%
Azithromycin (15mcg)	9	70%	3	60%

Out of the Biofilm producing Staphylococcal species ,all the isolates (100%) were found to be methicillin resistant which was detected by Cefoxitin disc (30mcg) screening method, while all the isolates were susceptible to Vancomycin and Linezolid.

**Table 4: Frequency between resistance pattern of Biofilm producers & Non Biofilm producers in Gram Negative Isolates**

Antibiotic	Biofilm producers n = 29		Non Biofilm producers n = 05	
	Resistant	R%	Resistant	R%
Amoxyclav (20/10mcg)	24	82.7	2	40
Cefotaxime (30mcg)	24	82.7	2	40
Ceftazidime(30mcg)	24	82.7	2	40
Ceftriaxone (30mcg)	24	82.7	2	40
Cefoperazone + Sulbactam (75/30mcg)	19	65.5	1	20
Ciprofloxacin (5mcg)	28	96.5	3	60
s Levofloxacin(5mcg)	23	79.3	2	40
Amikacin(30mcg)	23	79.3	1	20
Imepenem (10mcg)	20	76	0	0
Meropenem(10mcg)	20	76	0	0
Piperacillin/tazobactam (100/10mcg)	20	76	2	40

Out of the 34 Gram Negative Bacilli isolated, 76% were E.S.B.L. producers and 59% were carbapenamase producers . All the G.N.B.'s were susceptible to Colistin. The resistant pattern of the G.N.B's and the susceptibility to antibiotics was confirmed with Vitek 2 biomerieux automated system.

#### IV. Discussion

Medical devices are an integral part of various interventions in treatment on the patients in modern day medicine, especially in the critically ill patients.<sup>13</sup> These invasive devices being important for the patients ,they are prone for bacterial colonization.<sup>14</sup> These devices which are inserted at various body sites bypass the normal defense barriers. By virtue of this, organisms are provided an access to sterile tissues and body fluids.It is hypothesised that the ability of an organism to persist in the environment as well as its virulence is a result of its

capacity to colonize the device and further lead to biofilm formation.<sup>15</sup> Biofilm formation is a pathogenic mechanism for microbial persistence and treatment failure to antibiotics as these biofilm encased organisms are multidrug resistant. Resistance to drugs is attributed to the biofilm producing ability of isolates as they display an altered gene character.<sup>15,16</sup> Hence, due to the above mentioned factors this study was done to emphasise on, the bacteria colonizing medical devices, their potential towards biofilm formation and their antibiotic resistance pattern in patients admitted to N.I.C.U & P.I.C.U. of a Pediatric Tertiary Hospital.

The study group comprised of 100 patients who had medical devices inserted more than 12 hours of duration irrespective of the reason for device insertion. Out of the 100 specimens which were cultured, a total of 57 were reported to be culture positive, while the other 43 did not yield any growth showing a culture positive rate of 57%. Similar rates were observed in the studies done by Ahmed o et al (2008)<sup>17</sup> 52.9% and by Lombardi et al (2014)<sup>18</sup> 59.9% .

Among the I.V.C. Tips colonized *S.epidermidis* was the most common organism isolated (45%) in our study. **Larke et al**<sup>19</sup> have reported that skin of 80-90% of people is colonized with *S.epidermidis* and most of the Coagulase Negative Staphylococci (CONS) infections are acquired from patients own flora. According to the data of the National Health Care Safety Network<sup>20</sup>, major risk factor for infection with CONS is the presence of implanted biomedical devices. Next common isolates were *Staphylococcus aureus* (15%), and *Pseudomonas aeruginosa* (15%).

Out of the 12 I.V.C's inserted in the lower extremity 11 showed colonization (91%). Increased colonization in the lower limb insertion site is due to the high density of local skin flora.<sup>21</sup> This was in correlation with Rao et al (2005)<sup>22</sup>, Ahmed O Shafik et al (2008)<sup>17</sup>, Monil Singhai et al (2012).<sup>23</sup>

Out of the 12 Intravenous catheter tips colonized from the neonates (66.6%) were Low Birth Weight babies. Low birth weight infants are at increased risk for device associated infections due to their immature immune system.<sup>24</sup>

60% of colonization was observed in greater than 96 hours of stay of the device insitu. Thomas et al (2013)<sup>24</sup> too reported in their study that increased duration of catheterization was a significant factor in development of catheter related blood stream Infections.

The present study showed predominance of gram negative bacilli colonizing in the endotracheal tube, Intercostal drain tubes and nasogastric tube. *K. pneumoniae* (38%) was the commonest isolate obtained followed by *Acinetobacter baumannii* (22%) and *Pseudomonas aeruginosa* (19%). Feldmann C (1999)<sup>25</sup> documented that the interior of the endotracheal tube of the patients on mechanical ventilation was colonized rapidly with gram negative bacilli and that colonization of the E.T.T. may begin as early as 12 hours and it is more abundant at 96 hours, hence the rate of colonization is a 'Time dependant process'. In a study by JMacFie et al<sup>26</sup> the commonest organisms isolated were *Candida sp.* and *E.coli* from the nasogastric tubes. In our study we observed that *Acinetobacter baumannii* was the commonest organism isolated from the Intercostal drain tubes.

In the present study, out of the 57 bacterial isolates, 81% were biofilm producers and the highest number of biofilm producers were from the E.T.T.'s 67%, followed by I.V.C.'s 38%. *K.pneumoniae* 91% was found to produce biofilm from E.T.T. All the isolates (100%) of *S.epidermidis* from I.V.C's were biofilm producers. Similarly out of the 4 isolates from N.G.T'S 1 (25%) *klebsiella pneumoniae* formed biofilm. Out of the 6 isolates from the I.C.D. tubes 1 (17%) *Acinetobacter baumannii* isolate, showed biofilm production. Out of the 42 biofilm producers detected by T.C.P. method, 23%, 62%, 14%, were strong, moderate, weak biofilm producers respectively. 42% of *P. aeruginosa* isolates, 37.5% of *A.baumannii*, 25% *E.coli*, 14% of *K.pneumoniae*, 7.60% of *S.epidermidis* were among the strongest biofilm producers. T.C.P. method is an accurate & reproducible method for detection of biofilm producers (Mathur T et al (2006)).<sup>12</sup> In their study T.C.P. method detected 53.9% of biofilm producers. An advantage with the T.C.P. method is that it obviates the difficulty of categorization by taking the measurement of the Optical Density (O.D.) value which helps in quantification of the biofilm which is produced.

In the present study, all the Biofilm producing Staphylococcal species (100%) were Methicillin resistant. 100% sensitivity was seen with Linezolid & Vancomycin. In a study conducted by Maya Nandkumar et al (2013)<sup>27</sup>, 82% of biofilm producing *Staphylococcus* species were Methicillin resistant and 14% of isolates were resistant to Vancomycin. In the studies conducted by De Arajo et al (2006)<sup>28</sup>, Agarwal et al (2012)<sup>29</sup> 'CONS isolates producing biofilms had increased resistance with antibiotics than non-biofilm producing organisms. 76% of the gram negative bacilli were E.S.B.L. producers and 59% were found to be Carbapenemase producers. In a study done by Monil Singhai et al (2012)<sup>23</sup> 81% of the Gram negative bacilli were E.S.B.L. producers and in a study conducted by Sangita et al (2012)<sup>7</sup>, 23% were E.S.B.L. producers while 34% were Carbapenemase producers. All the G.N.B.'s were susceptible to Colistin. This resistant pattern of antibiotics and the susceptibility was confirmed with Vitek 2 biomerieux automated system. In a study done by Mulla Summaiya et al (2012)<sup>31</sup>, all the isolates were susceptible to Colistin. An elevated Efflux pump expression mechanism, physiological heterogeneity, increasing mutation rates & exchange of genes are responsible for

antibiotic resistance in biofilm producing organism. Biofilm producers mostly being nosocomial have been associated with variety of persistent infections, leading to treatment failure.<sup>31</sup>

The limitation of our study was that a proper clinical followup could not be performed due to transfer of these patients to other set ups, or leaving against medical advice and in death of the patients.

## V. Conclusion

Medical devices have unquestionable benefits in the medical practice, yet they come with many life threatening complications. Biofilm production has been implicated as one of the potential virulence factor of bacteria resulting in multidrug resistant device associated infections. This results in decreased quality of health care provided to the patient & causes prolonged hospital stay. Therefore, it is essential to review the patient daily and removal of the device should be recommended as soon as possible. Subsequently, proper isolation practices and meticulous hand hygiene has to be implicated for prevention of cross contamination which will result in decrease in the colonization of biofilm producing multidrug organisms in the devices as well in the hospital environments. Further it is important to know about the local resistance pattern of these organisms in critical areas of the hospital, as this can be helpful for the implementation & formulation of **Hospital infection policy**. The rationale use of antibiotics will reduce device associated infections with multidrug resistant organisms and a better patient outcome.

**Conflicts of Interest: The authors declare no conflicts of interest**

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## References:

- [1]. Donlan RM, Costerton W. Biofilm: Survival mechanisms of clinically relevant Microorganisms. *Clin Microbiology Rev* 2002;15(2):167-93
- [2]. Reid G. Biofilms in infectious diseases and on medical devices. *Int. Journal Antimicrob* 1999;223-26
- [3]. Sangitha rewdiwala, Bhaumesh M. Rajdev, Summaiya Mulla. Characterization of bacterial etiological agents of biofilm formation in medical devices in critical care setup; *Critical care research and practice*. 2012 vol. 2012.
- [4]. Costerton J.W., Stewart PS, Greensberg EP. Bacterial biofilms. A common cause of persistent infections. *Science* 1999;284:1318-22
- [5]. Yalaz M, Altun-Köroğlu Ö, Ulusoy B, Yıldız B, Akisu M, Vardar F, Özinel MA, Kültürsay N. Evaluation of device-associated infections in a neonatal intensive care unit. *Turkish Journal of Pediatrics*. 2012;54:128-135.
- [6]. Parameswaran R, Sherchan JB, Varma DM, Mukhopadhyay C, Vidyasgar S. Intravascular catheter related infections in an Indian tertiary care hospital. *J infect DEV countries*, 2011;5:452-8.
- [7]. Khanna V, Mukhopadhyay C, Vandana KE, Dabke P. Evaluation of central venous catheter associated blood stream infections: A microbiological observational study. *J Pathog* 2013;936864.
- [8]. Patil HV, Patil VC, Ramteerthkar MN, Kulkarni RD (2011): Catheter venous catheter blood stream infections in the intensive care unit. *Indian crit care Med* 115:213-223.
- [9]. Maki D.G., Weise C.E., Sarafin H.W. A semiquantitative culture method for identifying I.V.C. related infections. *N England J M* 1977; 296:1305-9.
- [10]. Tullu S., Deshmukh T., Baveja S.M. Bacterial profile and antimicrobial susceptibility pattern in catheter related nosocomial infections. *J. Postgraduate Med* 1998;44:7-13
- [11]. CLSI Performance Standards for anti microbial susceptibility testing. 24<sup>th</sup> Informational supplement. *CISI document M 100-S24*. Wayne, PA: Clinical and laboratory Standards institute; 2014
- [12]. Mathur T, Singhal S, Khan S, Upadhyay D J, Fatma T, Ratan A. Detection of biofilm formation among clinical isolates of Staphylococci: An evaluation of three different screening methods. *I.J.M.M.* 24:25-25.; 2006.
- [13]. Vidya pai, Muzafar Amin, Sunil Rao P. Indwelling Catheters, I.V. Cannulae and Endotracheal tubes: A den for microbes. *International Journal of Pharmacy and Biological Sciences*. 2015;5(2):75-79
- [14]. Vasanthi R., Karthikeyan D., Jeya M. Study of biofilm production and anti microbial resistance pattern of the bacterial isolated from invasive devices. *Int. J Res Health Sci [Internet]*. 2014 Jan 31;2(1):274-81
- [15]. Paula Ramirez, Sara Gil Perotin, Veronica Marti, Jose Miguel Sahuquillo, Eva Gonzalez, Isabel Calleja, Rosario Menendez and Juan Bonastre. Implications of endotracheal tube biofilm in ventilator-associated pneumonia response: a state of concept. *Critical Care* 2012, 16:R93.
- [16]. Koenig SM, Truitt JD. Ventilator associated pneumonia: Diagnosis, treatment, and prevention. *Clin Microbiol Rev* 2006; 19(4):637-657.

- [17]. Ahmed O, Shafik, Mossad A, Morgan ,Somya M, Desouky, Gamal A, Amer and Shereen H. Ahmed . Vascular Catheter Related Bacteremia In Pediatrics. *Egyptian Journal Of Medical Microbiology* 2008;17(2)
- [18]. Lombardi S, Scutell M , Felice V, Di Campli E, Di Giulio M, et al. Central vascular catheter infections in a hospital in Italy. *New Microbiol* 2014; 37:41- 50.
- [19]. Larke RL, VanderHyde K, Deeb GM, Dietrich S, Massey J P et al. A outbreak of coagulase negative Staphylococcal surgical site infections following aortic valve replacement. *Infect Control HospEpidemiol* 2001;22:618-23.
- [20]. Moriarty TF ,Zaat SA , and Busscher HJ (Eds.). *Biomaterials associated infection: immunological aspects and anti microbial strategies*. Springer .2013, XIV..
- [21]. Khosravi AD, Parhizgari N, Montazeri EA, Mozaffari A, Abbasi F. The prevalence of Bacteria isolated from Endotracheal tubes of patients in Golestan Hospital , Ahvaz , Iran and Determination of their antibiotic susceptibility patterns. *Jundishpur Journal of Microbiology* 2012;6:67-71.
- [22]. Rao Sd, Joseph MP,Lavi R, Machaden et al .Infections related to vascular catheters in a pediatric intensive care unit. *Indian Pediatr*. 2005; 42:667-672
- [23]. MonilSinghai, Abida Malik , Ashraf Malik , Vinita Raawat. Colonization of peripheral intravascular catheters with biofilm producing microbes: An evaluation of risk factors. *Niger Med J*. 2012 Jan – March:53(1);37-41.
- [24]. Thomas D, Parameswaran P, Harish BN. Catheter related blood stream infections in the pediatric intensive care unit. A descriptive study . *Indian J CritCare Med*.2013;17:135-9.
- [25]. C Feldman, M.Kassel , J. Cantrell, S.Kaka , R.Morar, A.Goolam Mahomed ,JI Philips. The presence and sequence of endotracheal tube colonization in patients undergoing mechanical ventilation. *EurRespir J* 1999; 13:546-551.
- [26]. J Macfie, C O'Boyle, Cj Mitchell, PM Bukley, D Johnstone, D Sudworth. Gut Origin of sepsis: A prospective study investigating association between bacterial translocation , gastric micro flora and septic morbidity. *gut* 1999;45:223-228.
- [27]. Pradeep kumar SS, Eawer HV, Maya Nandkumar A. Multiple drug resistant bacterial biofilms on implanted catheters - a reservoir of infection. *J Assoc Physician India* 2013 Oct;61(10):702-7.
- [28]. De Araujo GL, Coelho LR, De Carvalho CB, Maciel RM, Coronado et al. Commensal Isolate of Methicillin resistant *Staphylococcus epidermidis* are also well equipped to produce biofilm on polyesterene surfaces. *J Antimicrob Chemother* 2006;57:855-64.114.
- [29]. Agarwal A., Jain A , . Association between drug resistance & production of biofilm in *Staphylococci*. *Indian J Med Res*.2012;135:562-4.
- [30]. Mulla S. A., Jetwaani U.N. Assesment of biofilm formation by the causative organism of ventilator associated pneumonia at intensive care unit of a tertiary care hospital . *National journal of medical research* 2012;2:1
- [31]. Subramaniyam P., Shanmugam N , Sivaraman U, Shailesh K, Selvaraj S. Antibiotic resistance pattern of biofilm forming uropathogens isolated from catheterized patients in Pondicherry , India. *Australian Medical Journal [AMJ]*2012;5:7344-348