



Bacteriological Profile, Biofilm Production, and Prevention of Catheter Associated Urinary Tract Infections

Akshay Karyakarte^{1*}, Anil Gaikwad², Jyoti Iravane²

^{1,2}Department of Microbiology,

¹Seth GS Medical College and KEM Hospital, Mumbai, Maharashtra, India

²Government Medical College and Hospital, Aurangabad, Maharashtra, India

***Corresponding Author:**

Akshay Karyakarte

Department of Microbiology, 7th Floor, New Multi-Storeyed Building, Seth GS Medical College and KEM Hospital, Acharya Donde Marg, Parel (E) Mumbai, Maharashtra, India – 400012

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Abstract

Introduction: Catheter Associated Urinary Tract Infection is the representative of biofilm-associated infection, and amounts to over 35% of all healthcare associated infections. Rationale behind this study was to aid in updating guidelines for appropriate treatment, and consequently help reduce the development of multi-drug resistance among organisms.

Material and Methods: This hospital-based cross-sectional study included urine samples from patients having an indwelling urinary catheter, with fever for over two days. Bacterial identification and antibiotic susceptibility were tested by conventional bacteriology. Biofilm production was assessed qualitatively. Impact of infection control practices on development of CAUTI was assessed through detailed history.

Results: Of the 692 samples included, 249 produced bacterial isolates, with 216 of them being Gram-negative organisms. Fisher's exact test was used for statistical analysis; $p < 0.05$ was considered significant. 65.46% of the 249 organisms produced a biofilm ($p < 0.05$). Antimicrobial resistance to Fluoroquinolones and Cephalosporins ranged from 78-93% ($p < 0.05$). 61.13% isolates were from patients with underlying chronic illnesses and 38.87% from acute ones ($p = 0.046$), while 48.22% samples were sterile when empirical antibiotics were started ($p = 0.05$). 95.67% samples from patients with catheter was in situ for over five days produced bacterial growth ($p < 0.05$). 87.16% samples from patients where catheter hygiene was maintained were sterile ($p < 0.05$).

Conclusions: CAUTI leads to a multitude of complications, and represent biofilm-associated infections. Removal of an established biofilm is challenging, additionally, the organisms within biofilms show high resistance. The findings and inferences in this study emphasize that prevention is more effective for CAUTI than treatment.

Keywords: Catheter Associated Urinary Tract Infection, Antimicrobial Resistance, Biofilms, Healthcare Associated Infections, Infection Control Practices

Introduction

The relentless progress of medicine and healthcare has resulted in hazards like Antimicrobial Resistance (AMR) and Healthcare Associated Infections (HAI). Of the four major categories of HAI, i.e., Catheter Associated Urinary Tract Infection (CAUTI), Catheter Related Blood Stream Infection (CRBSI),

Ventilator Associated Pneumonia (VAP), and Surgical Site Infection (SSI), CAUTI amounts to almost 35%, to make it the most common HAI [1]. From a patient-centric point of view, complicated CAUTI causes a myriad of local and systemic complications along with prolonged duration of hospitalization, increased costs, and increased risk of

mortality [2]. CAUTI is widely known as the representative of biofilm-associated infection; biofilms form one of the most important virulence factors of bacteria causing it [3]. Additionally, bacteria within biofilms show a marked increase in AMR, making them difficult to remove from a catheterized urinary tract [4]. Thus, CAUTI poses a two-fold threat, via its extensive occurrence, and the high degree of AMR. This necessitates healthcare professionals to acquire knowledge of current local trend of causative organisms, and their resistance pattern in any healthcare facility. The prospect that it would aid in updating guidelines for appropriate treatment, and consequently help reduce the development of multi-drug resistance among organisms, forms the rationale behind this study.

Material And Methods

This was a hospital-based cross-sectional study conducted over a period of 18 months after approval from the Institutional Ethics Committee. It included urine samples from patients with an IUC, having fever for more than two days. Samples from non-catheterized patients, those from patients having fever or urinary symptoms before catheter insertion and those from patients who did not give valid written informed consent were excluded. The patient's condition was defined as CAUTI only if the definition of HAI was met, the IUC was in situ for more than two calendar days on the date of event, and the IUC was also in place on the date of event or the day before [5].

Uncentrifuged urine was examined microscopically before inoculating semi-quantitatively on Cystine Lactose Electrolyte Deficient (CLED) agar and incubated overnight at 37°C aerobically. Colony count was performed after incubation to ascertain its significance according to the Kass Concept of Significant Bacteriuria [6]. If significant, the bacterial agents were provisionally identified, for selection of antibiotics for sensitivity testing, by colony characteristics and microscopic examination of a Gram's-stained smear of the growth. Antibiotic susceptibility was tested by Modified Kirby-Bauer Disk Diffusion method. Antibiotics were selected and breakpoints were determined using the Clinical and Laboratory Standards Institute (CLSI) 2020-M100 guidelines [7]. Definitive identification of organisms was made using conventional bacteriological

techniques of biochemical reactions like Catalase and Coagulase tests for Gram-positive organisms, Oxidase, Indole, Methyl Red, Voges-Proskauer, Citrate Utilization, Urease Production, and Triple-Sugar Iron Test along with Decarboxylation and Oxidative-Fermentative tests for *Enterobacteriaceae* and Non-Fermenting Gram-Negative bacilli [8]. Biofilm production was assessed qualitatively by use of tube adherence tests, using Brain Heart Infusion Broth and Safranin [9]. Details regarding administration of empirical antibiotics, duration of catheterization, and adherence to bundle care practices were obtained via detailed clinical and treatment history. All data were evaluated and tabulated in Microsoft® Excel whereas statistical analysis was done using OpenEpi online software.

Results

A total of 692 samples were included in the present study, of which 335 (48.41%) showed no growth on CLED Agar. Also, 108 samples grew *Candida* species, which were excluded. Thus, 249 isolates were processed further for determining their bacteriological profile. Of these, 216 isolates were Gram-Negative organisms (143 from the *Enterobacteriaceae* family and 73 non-fermenting Gram-Negative Bacilli), while 33 were Gram-Positive. The organisms isolated were – *Klebsiella pneumoniae* (n=91; 36.54%), *Pseudomonas aeruginosa* (n=57; 22.89%), *Escherichia coli* (n=49; 19.67%), *Enterococcus* species (n=31; 12.45%), *Acinetobacter baumannii* (n=16; 6.42%), *Proteus mirabilis* (n=2), Methicillin Resistant *Staphylococcus aureus* (MRSA) (n=2), and *Citrobacter koseri* (n=1). Fisher's exact test was applied to study the significance of various parameters within the study; a p-value of <0.05 was considered significant across the board.

Antibiotic Susceptibility Test results showed high degree of resistance across the board, regardless of the class of antibiotics tested. High resistance to Fluoroquinolones and Cephalosporins (R=80%) was particularly alarming, as these are the most commonly prescribed antibiotics in most healthcare settings. The susceptibility patterns of the antibiotics were calculated for individual organisms isolated according to CLSI 2020 M100 guidelines [7] (Table 1).

Out of the 249 isolates included, 163 were biofilm-producing by tube adherence test (65.46%; $p < 0.001$). Biofilm production was seen in non-fermenting Gram-Negative bacilli and a few members of the *Enterobacteriaceae* family, but was entirely absent in Gram-Positive organisms (Table 2).

Clinical history also revealed that 423 of the samples were received patients suffering from chronic illnesses (61.13%) while 269 were from those suffering from acute ones (61.13% vs 38.87%; $p = 0.04639$). On the other hand, over half the samples from patients with acute illnesses were sterile (147 of 269; 54.65%) while the ratio was much less in samples from patients with chronic ones. (188 of 423; 44.45%) (Graph 1).

On analysing details from the history, it was found that in absence of empirical antibiotics, the number of samples turning out to be sterile was markedly low ($n=4$; 8.89%), which increased when empirical antibiotics were administered. The number of such samples when multiple antibiotics were given ($n=145$; 50.87%) was greater than when a single antibiotic was given ($n=167$; 46.13%). Thus, nearly half the samples turned out to be sterile when some form of empirical antibiotic therapy was instituted ($n=312$; 48.22%; $p < 0.05$) (Graph 1).

Assessment of clinical history revealed that the occasion of obtaining a sterile sample was noticeably high when catheter was in-situ for less than five days ($n=335$; 72.67%) while the occasion of obtaining bacterial growth was quite low ($n=28$; 6.07%). Drastically opposite, there were no sterile samples obtained when duration was over five days ($n=0$). Also, the occasion of obtaining bacterial growth was extremely high ($n=221$; 95.67%; $p < 0.05$) (Graph 2).

Detailed clinical history revealed that most samples were sterile when proper care bundles were followed during and after catheter insertion ($n=285$; 87.16%; $p < 0.0000001$). Conversely, when care bundles were not followed, the number of samples turning out sterile dropped remarkably ($n=50$; 13.7%) (Graph 2).

Discussion

Urinary Tract Infection accounts for 40% of all HAIs, of which 70- 80% are attributable to an IUC. Risk for developing CAUTI is around 3-5% for each day of in-situ catheter, which rises to about 25% when catheter is in-situ for a week, and to 100% after

completion of one month of catheterization. The incidence of CAUTI in intensive care units is up to 5.3%, in general wards is up to 3.1% and overall, in India, is 1.63 – 2.1% [10]. There are three criteria for diagnosis of CAUTI – history of catheterization within 48 hours, presence of significant bacteriuria, and presence of signs and symptoms of CAUTI not attributable to any other condition. As signs and symptoms of UTI in a catheterized patient are vague and non-specific, high index of clinical suspicion is necessary [11].

The rate of obtaining growth from samples was evaluated analytically. These findings show much variance across places and times, where 44.4% growth was obtained a hospital in Indonesia in 2019, while only 25% in a hospital in India in 2021. [12, 13] The present study found bacterial growth in 249 of the included samples (35.98%; $p < 0.05$) This reiterates the initial proposition that causative organisms and their susceptibility patterns vary through locations and times, and it is imperative to acquire knowledge of current local trend of causative organisms, and their resistance pattern in any healthcare facility. Out of the 249 bacterial isolates, 216 were Gram-negative organisms (86.74%) and 33 were Gram-positive (13.26%). These findings were comparable to various studies internationally and nationally, with a study in Nairobi and two concurrent studies in India having a 92% rate of growing Gram-negative organisms. [14, 15, 16]. Furthermore, these findings agree with many academic volumes as well [6, 11, 10].

Most of the isolated organisms showed a high degree of resistance across the board, regardless of the class of antibiotics tested. As most of the isolates were Gram-negative organisms, the resistance patterns were analysed by clubbing the tested antibiotics into five groups and two individual drugs. In addition to being highly resistant to commonly used antibiotics, the organisms can also overcome specific urinary antibiotics like Cefazolin, Cotrimoxazole, and Nitrofurantoin. Findings in the study were compared globally and nationally showing comparable results [(Table 1).

Biofilms are defined as a microbially derived sessile community having cells that are attached to an interface or to each other, are embedded in an extracellular polymeric matrix that they have

produced and demonstrate altered phenotype associated with differential gene expression. This definition also applies to cells that have broken off a biofilm and circulate in the body fluids, and possess the ability to establish itself in another niche. Externally, biofilms form usually on abiotic surfaces; in the human body, they form on the skin, oropharynx, nose, and especially, on indwelling medical devices. Though there are various methods for detection of biofilms, they can be broadly classified into Quantitative and Qualitative Detection Methods [20, 21, 22]. Since the rate of biofilm production in this study was statistically significant ($p < 0.05$), it can be inferred that 65.46% of suspected CAUTI samples having bacterial growth will consist of a biofilm-producing isolate. Similar findings were observed in southern India, with two separate studies showing the rate of biofilm production to be 60%. A study in central India, however, reports this rate to be much higher, with 81.25% isolates being biofilm-producing [15, 16, 23].

The instance of obtaining a sterile sample was found to increase when empirical antibiotic therapy was instituted. Since the findings were statistically significant ($p < 0.05$), it begs to infer that CAUTI is preventable in 48.22% cases if some form of empirical antibiotic therapy is instituted. A noteworthy study conducted in Boston, Massachusetts in 2019 showed that using empirical antibiotics to prevent CAUTI culminated in reducing costs and increasing the Quality-Adjusted Life-Years. The flip side of that coin is that the protective effect of empirical antibiotics is usually transient, and can cause selection of antibiotic-resistant bacteria [24, 25]. Hence, there is much controversy regarding the value of empirical systemic antibiotics, and institution of such measures should be a cautious decision.

Duration of catheterization was found to be directly proportional to development CAUTI, which is reiterated by the fact that no samples received after five days of catheterization were sterile ($p < 0.05$). Similar findings were obtained nationally and globally as well [12, 13]. It follows, therefore, that removing or changing the IUC within five days can aid in the preventing CAUTI.

Bundle care practices for CAUTI prevention are manifold; they involve conformity to all aseptic

precautions during catheter insertion, regular meatal care, and proper placement of the drainage bag. A significant study conducted at the Mayo Clinic in Minnesota found that rate of CAUTI development reduced by 70% with proper implementation of bundle care. [26] These findings translate nationally as well, where a hospital in India reports reduction in CAUTI by 51.4% on proper implementation of care bundles [27]. The high statistical significance of the findings in the present study helps postulate that strict adherence to bundle care definitively prevents CAUTI, while overlooking these practices eventually causes it.

In conclusion, the treatment of established CAUTI is challenging, irrespective of the causative organism. In addition to being highly resistant to most antibiotics, the organisms can overcome antibiotics with high urinary concentration as well. Since CAUTI is the representative of biofilm-associated infection, treatment options need to be calibrated keeping biofilms in mind. Institution of empirical antibiotic therapy has a significant effect its prevention CAUTI. However, since the effect of empirical antibiotics is transient and mostly leads to selection of drug-resistant bacteria, the institution of empirical antibiotic therapy should be a cautious decision. This study also finds that duration of catheterization and appropriate implementation of bundle care practices for insertion and maintenance of an IUC has a decisive impact on prevention of CAUTI.

All the findings and inferences in the present study emphasize that prevention strategies are more effective in case of CAUTI as compared to treatment options. Consequently, the adage *Prevention is better than Cure* holds true.

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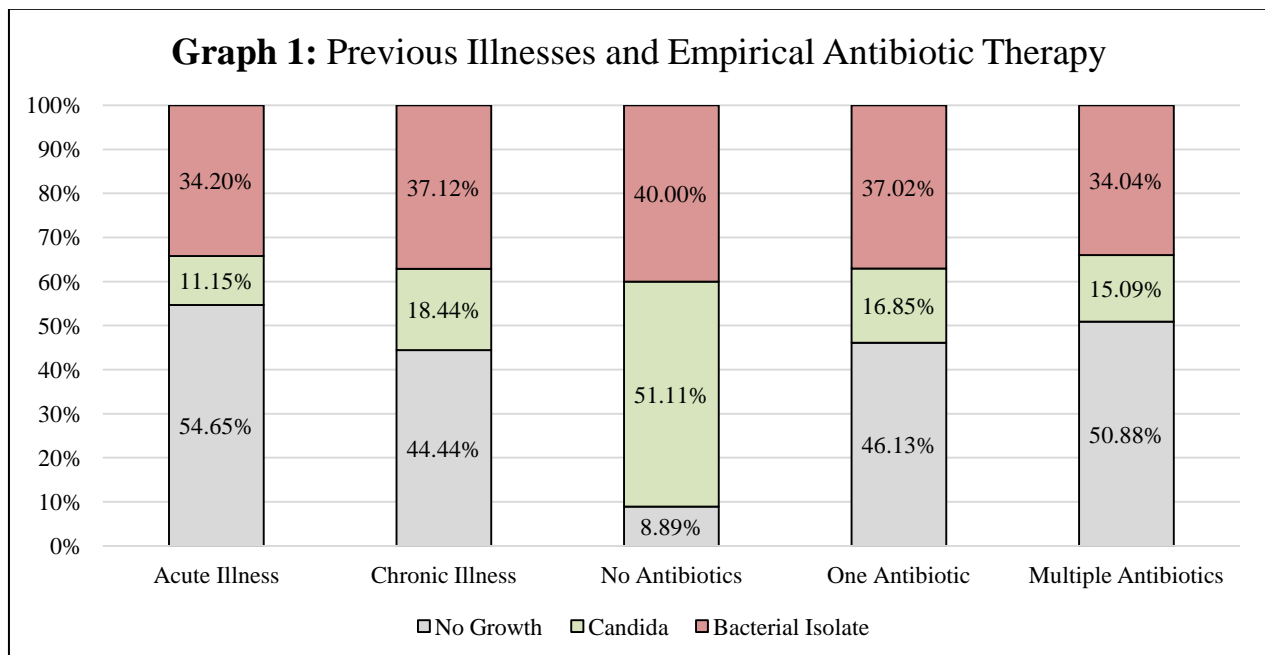
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Figure Legends

Graph 1:

Title – Previous Illnesses and Empirical Antibiotic Therapy

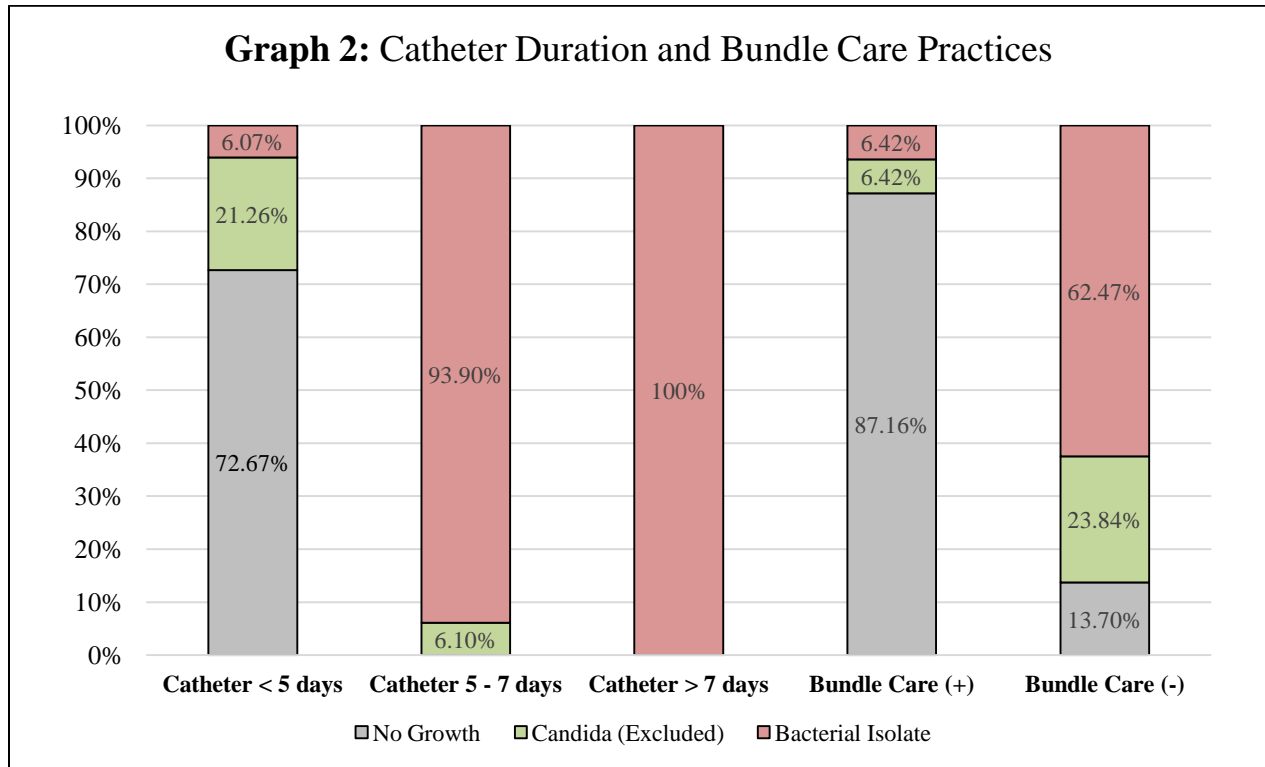
Legend – Shows the distribution if isolates with respect to previous illnesses and empirical antibiotics. 61.13% samples were received from patients with underlying chronic illnesses (statistically significant). Also, 48.22% samples turned out to be sterile when empirical antibiotic therapy was instituted, which was statistically significant as well.



Graph 2:

Title – Catheter Duration and Bundle Care Practices

Legend – Showing the distribution with respect to duration of catheterization and catheter hygiene practices. No sterile samples were found when duration of catheterization was longer than five days. Conversely, 87.16% samples were sterile when appropriate bundle care practices were followed. Both findings were statistically significant.



Tables

Table 1: Antibiotic Susceptibility Pattern (%R)

Study in	Organism	AG	CS	PD	CP	FQ	CT	NF	DX	VM	LZ
Nairobi (2012) [14]	<i>E. coli</i>	18.2	33.5	NA [#]	7.40	14.8	11.4	9.10	NA [#]	NA [#]	NA [#]
	<i>K. pneumoniae</i>	33.6	35.2	NA [#]	16.4	15.8	9.20	NA	NA [#]	NA [#]	NA [#]
	<i>P. aeruginosa</i>	38.5	18	NA [#]	41	29.6	0.00	0.00	NA [#]	NA [#]	NA [#]
	<i>Enterococcus sp.</i>	NA [#]	NA [#]	NA [#]	NA [#]	NA [#]	NA [#]	NA [#]	30.6	11.1	0.00
India (2014) [17]	<i>E. coli</i>	33.3	88.9	40.7	44.4	59.2	81.5	40.7	NA [#]	NA [#]	NA [#]
	<i>K. pneumoniae</i>	54.5	100	45.4	18.2	81.8	99.9	54.5	NA [#]	NA [#]	NA [#]
	<i>P. aeruginosa</i>	37.5	75	50	50	75	87.5	87.5	NA [#]	NA [#]	NA [#]
India (2015) [18]	<i>E. coli</i>	18	86.5	50	0.00	55	NA [#]	NA [#]	NA [#]	NA [#]	NA [#]
	<i>K. pneumoniae</i>	50	100	68	9	100	NA [#]	NA [#]	NA [#]	NA [#]	NA [#]
	<i>P. aeruginosa</i>	22.3	100	100	75.5	50	NA [#]	NA [#]	NA [#]	NA [#]	NA [#]

	<i>A. baumannii</i>	100	50	100	100	100	NA [#]	NA [#]	NA [#]	NA [#]	NA [#]
India (2017) [13]	<i>E. coli</i>	35.5	84.3	73.1	3.75	82.5	27.5	11.3	NA [#]	NA [#]	NA [#]
	<i>K. pneumoniae</i>	75	98.4	75	0.00	93.8	68.8	43.8	NA [#]	NA [#]	NA [#]
	<i>P. aeruginosa</i>	23.3	23.3	6.66	0.00	33.3	26.7	NA [#]	NA [#]	NA [#]	NA [#]
India (2018) [20]	<i>E. coli</i>	50	NA [#]	100	0.00	100	NA [#]	NA [#]	NA [#]	NA [#]	NA [#]
	<i>K. pneumoniae</i>	100	100	100	0.00	NA [#]	NA [#]	NA [#]	NA [#]	NA [#]	NA [#]
	<i>P. aeruginosa</i>	50	NA [#]	NA [#]	0.00	NA [#]	NA [#]	NA [#]	NA [#]	NA [#]	NA [#]
China (2020) [19]	<i>K. pneumoniae</i>	45.6	45.6	82.2	30	62.2	NA [#]	NA [#]	NA [#]	NA [#]	NA [#]
Present Study (2022)	<i>E. coli</i>	72.5	95.3	84.6	80	93.4	90.1	82.4	NA [#]	NA [#]	NA [#]
	<i>K. pneumoniae</i>	71.3	78.4	73.7	80.7	80.7	94.7	91.2	NA [#]	NA [#]	NA [#]
	<i>P. aeruginosa</i>	45.9	90.3	59.2	56.1	93.9	91.8	63.3	NA [#]	NA [#]	NA [#]
	<i>A. baumannii</i>	66.7	78.1	65.6	62.5	81.2	68.8	81.2	NA [#]	NA [#]	NA [#]
	<i>Enterococcus sp.</i>	NA [#]	NA [#]	NA [#]	NA [#]	NA [#]	NA [#]	NA [#]	51.6	12.9	100

* AG – Aminoglycosides, CS – Cephalosporins, PD – Penicillin Derivatives, CP – Carbapenems FQ – Fluoroquinolones, CT- Cotrimoxazole, NF – Nitrofurantoin, DX – Doxycycline, VM – Vancomycin, LZ – Linezolid | NA[#] – Not Applied | Values in **bold** are statistically significant (p<0.05)

Table 1: Showing the Antibiotic Susceptibility Pattern.

Table 2: Biofilm Production

Organism	Total	Biofilm Produced in	Rate	p-value
<i>Pseudomonas aeruginosa</i>	57	52	91.23%	0.00000062
<i>Acinetobacter baumannii</i>	16	14	87.50%	0.04354
<i>Klebsiella pneumoniae</i>	91	65	71.43%	0.08559
<i>Escherichia coli</i>	49	32	65.31%	0.0001833
<i>Citrobacter koseri</i>	1	0	0.00	NA
<i>Enterococcus sp.</i>	31	0	0.00	NA
MRSA	2	0	0.00	NA
<i>Proteus mirabilis</i>	2	0	0.00	NA
TOTAL	249	163	65.46%	<0.05

Table 2: Showing biofilm production among the isolates. The total rate of biofilm production in the present study turned out to be 65.46%, which was statistically significant.