STUDY ON LOWER URINARY TRACT INFECTIONS AND STENT COLONIZATION IN PATIENTS WITH DOUBLE-J URETERAL STENTS IN A TERTIARY CARE HOSPITAL

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BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled **"STUDY ON LOWER URINARY TRACT INFECTIONS AND STENT COLONIZATION IN PATIENTS WITH DOUBLE- J URETERAL STENTS IN A TERTIARY CARE HOSPITAL"** is the original bonafide work done by **DR.GOWRISREE CHANDURVELAN,** during the period of her Postgraduate study from 2013-2016 under supervision and guidance in Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-600003, in partial fulfillment of the requirement of M.D MICROBIOLOGY degree examination of The Tamilnadu Dr. M.G.R Medical university to be held ***in April 2016.***

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DECLARATION

I, declare that the dissertation titled **"STUDY ON LOWER URINARY TRACT INFECTIONS AND STENT COLONIZATION IN PATIENTS WITH DOUBLE- J URETERAL STENTS IN A TERTIARY CARE HOSPITAL"** submitted by me for the degree of M.D is the bonafide work carried out by me during the period of November 2014 to August 2015 under the expert guidance and supervision of **Prof.Dr.S.VASANTHI, M.D.,** Professor, Institute of Microbiology, Madras Medical College. The dissertation is submitted to the Tamil Nadu Dr. M.G.R Medical University towards partial fulfillment of the university regulations for the award of degree of M.D., Degree (Branch IV) in Microbiology.

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Study on Lower Urinary tract Infections and stent

BY GOWRISREE CHANDURVELAN

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INTRODUCTION

Worldwide production of biomedical devices and engineered medical tissue is rapidly increasing. The [12] insertion of indwelling or implanted foreign polymer bodies, such as prosthetic heart valves, cardiac pacemakers, total artificial hearts and total joint replacements or other orthopaedic devices, as well as intravascular catheters, renal dialysis shunts, cerebrospinal fluid (CSF) shunts or continuous ambulatory peritoneal dialysis catheters, has become an indispensable part of modern medical care.

"Among all currently used medical devices urinary catheters and stents are the most common. [1] The ability to manipulate the urinary tract without the need for an open surgical incision differentiates

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ABSTRACT

Title: Study on Lower Urinary tract Infections and stent colonization in patients with Double- J ureteral stents in a tertiary care hospital.

Introduction: Double-J ureteral stents represent a minimally invasive alternative to preserve urinary drainage whenever ureteral patency is deteriorated or is under a significant risk to be occluded due to extrinsic or intrinsic etiologies. Like all synthetic medical intracavitary devices, Double-J ureteral stent also offers a suitable surface for microbial colonization by biofilm forming microorganisms. Recognizing the colonization is very important for prevention of bacteremia during manipulation.

Aim of the study: To assess the extent of stent colonization and to determine the incidence of urinary tract infections in patients with indwelling Double-J ureteral stents.

Materials and Method: Between November 2014 and August 2015, 100 patients (20-75 years old) who underwent Double J ureteral stent placement for ureteral obstruction were enrolled in this cross sectional study. Urine sample for culture were collected from these patients prior to stent insertion and on the day of stent removal. Stents were removed under sterile conditions with the help of a cystoscope. Double J ureteral stent cultures were also performed on 5% sheep blood agar plate.

Results: Out of the one hundred patients who were treated with Double-J ureteral stents for ureteral obstruction, 67% of patients had Double-J ureteral stent colonization and only 36% had urinary tract infection. The duration of retention of Double-J ureteral stent in the urinary tract had a statistically significant influence on the rate of colonization of the

Double-J ureteral stent and bacteriuria ($p< 0.001$). *Escherichia coli* was the predominant pathogen (32.4%) isolated from Double-J stent culture as well as from the culture of urine samples (34.2%) from patients treated with Double-J ureteral stent.

Conclusion: As microbial ureteral stent colonization does not necessarily lead to bacteriuria, negative urine culture does not rule out biofilm formation, hence urine culture has a low predictive value for ureteral stent colonization.

Key Words: Biofilm, Double-J ureteral stent, Microbial colonization, Urinary tract infection.

INTRODUCTION

Worldwide production of biomedical devices and engineered medical tissue is rapidly increasing. The insertion of indwelling or implanted foreign polymer bodies, such as prosthetic heart valves, cardiac pacemakers, total artificial hearts and total joint replacements or other orthopaedic devices, as well as intravascular catheters, renal dialysis shunts, cerebrospinal fluid (CSF) shunts or continuous ambulatory peritoneal dialysis catheters, has become an indispensable part of modern medical care.

Among all currently used medical devices urinary catheters and stents are the most common. [1] The ability to manipulate the urinary tract without the need for an open surgical incision differentiates urology from other disciplines. Such intervention may be required for diagnostic or therapeutic purposes or both.

The Double-J ureteral stent is a catheter or tube placed within the ureteral lumen in order to maintain its patency due to obstruction by intrinsic or extrinsic etiologies such as ureteral stones, strictures, congenital anomalies, retroperitoneal tumors or fibrosis. The tips of these stents are J-shaped and urologists place them endoscopically over the guidewire. The double coil design at proximal and distal ends

securely anchor the stent in the upper urinary tract (renal pelvis and upper calyx) and the bladder and provide a self-retaining capability .

In the present scenario usage of Double-J ureteral stent has become one of the basic and most valuable tools in day to day urological practice, therefore the complications related to the usage of stents are also more frequent than before. Any implanted medical device may become infected with a bacterial biofilm.[2] Scientists have recently realized that in nature, more than 99% of all bacteria exists as biofilms.[3] Microbial colonization of the indwelling device can be a prelude to both infection and malfunction of the device.

Device-associated infections in urology are complicated by majority of uropathogens including both Gram positive and Gram negative bacteria, as well as yeast which are able to form complex biofilm communities.[4-6] Organisms growing in the biofilm are relatively protected from both antimicrobials and host defenses. [7]

The microorganisms in biofilms are difficult or impossible to treat with antimicrobial agents and their detachment from the device may result in infection. Therefore, it is recommended to prevent their formation rather than treatment. The present study is designed with the aim to isolate and identify the microorganisms which colonize the Double-J ureteral stents and which cause urinary tract infections.

AIMS AND OBJECTIVES

AIM OF THE STUDY

To assess the extent of stent colonization and to determine the incidence of urinary tract infections in patients with indwelling Double-J ureteral stents.

OBJECTIVES

1) To isolate, identify and determine the antimicrobial susceptibility pattern of the microorganisms causing stent colonization in patients treated with indwelling Double- J ureteral stents.

2) To determine the incidence of urinary tract infections in patients with Double-J ureteral stents.

3) To compare the relationship between colonization of the Double-J ureteral stent and the microorganisms isolated from urine samples from these patients.

4) To correlate the relationship between duration of Double-J ureteral stent placement and its colonization.

5) To phenotypically detect biofilm production of the isolates from the colonized Double-J ureteral stents.

REVIEW OF LITERATURE

Hippocrates in 4th century surmised that the kidney had the faculty of extracting and separating moisture from the blood; this moisture descends into bladder. [8]The urinary system is the structure which precisely maintains the chemical environment of the body, performs various excretory, regulatory and secretory functions. The urinary tract allows for the excretion of urine produced by the kidneys.

ENDOSCOPIC ANATOMY OF URINARY TRACT

The knowledge of the normal endoscopic anatomy is a prerequisite for accurate endoscopic screening of the urinary tract. The surface of the distended bladder can be divided into several regions: the vesical neck, which limits the bladder inferiorly, is the major landmark and reference point in the anatomy of the bladder; the trigone corresponds to the area limited by the ureteral orifices and the intravesical urethral opening; the elevation extending between the ureteral orifices is known as the interureteric ridge or mercier's bar. The base (fundus) of the bladder is located posterior to the trigone. The bladder mucosa appears relatively smooth with intravesical protrusions. [9, 10]

The normal non-refluxing ureteral orifice may be prominent on endoscopy; or as an inconspicuous slit that can be identified only on close examination. Starting from the ureteral orifice, the intravesical

section of ureter extends for about 1.5 cm. The intramural part runs obliquely through the bladder musculature at the detrusor hiatus for about 1cm, represents the narrowest part of the ureter -ureterovesical junction (UVJ) which requires dilatation before introduction of large caliber instruments. The other two narrow areas at the pelvic brim and at the ureteropelvic junction (UPJ) appear as slightly stenotic and relatively non-distensible. [9, 10]

The normal renal pelvis is funnel shaped with the apex of the funnel leading into the UPJ. As the ureteroscope enters the renal pelvis, the ostia of the major calyces leading to the upper, middle and lower poles of the kidney appear as circular openings connected to the apex by a long tubular portion, the infundibulum. Carinae separate the major calyces from the renal pelvis. The final structures visible are the minor calyces with their calyceal fornix surrounding the renal papilla. [9, 10]

URINARY TRACT INFECTIONS

Urinary tract infection (UTI) is a spectrum of disease caused by microbial invasion of genitourinary tract that extends from the renal cortex of the kidney to urethral meatus. UTI are common, affect men and women of all ages, and vary dramatically in their presentation and sequelae. Urinary tract infection may involve only the lower urinary tract or both the upper and lower tracts. Lower UTI's are cystitis, urethritis, prostatitis. Upper UTI's are pyelonephritis, intra-renal

abscess and perinephric abscess which is usually a late complication of pyelonephritis.

Uncomplicated UTI – symptomatic urinary tract infection characterized by frequency, urgency, dysuria, or supra pubic pain in a structurally and neurologically normal urinary tract. [11]

Complicated UTI – symptomatic urinary tract infection associated with factors that increase the chance of acquiring bacteria and decrease the efficacy of therapy. The urinary tract is functionally or structurally abnormal, (e.g., indwelling catheters and renal calculi) the host is compromised and or the bacteria have increased virulence or antimicrobial resistance. [11]

INCIDENCE AND EPIDEMIOLOGY

UTI's are considered to be the most common bacterial infection worldwide. In developed countries like USA, it has been estimated that symptomatic UTIs result in as many as 7 million visits to outpatient clinics, 1 million visits to emergency departments, and 100,000 hospitalizations annually. [12]

Urinary tract infections are one of the most common nosocomial infections accounting for approximately 40 % of all hospital acquired infection and 80% of this are associated with use of urinary catheters and indwelling stents.[13] Experts at the Centers for Disease Control and

Prevention have estimated that biofilms are associated with 65% of nosocomial infections.[14]

ETIOLOGIC AGENTS

- Organisms causing UTI are derived primarily from the aerobic members of the fecal flora. Majority of uncomplicated urinary tract infections are caused by a single organism. In contrast, infections among hospitalized patients, patients with urinary catheters, or individuals with structural abnormalities of the urinary tract may be polymicrobial [15]

- The most common pathogens are Gram negative bacilli. *Escherichia coli* cause about 80% of acute infections in patients without urinary tract abnormalities. Other Gram negative bacilli included are *Proteus mirabilis* and *Klebsiella pneumoniae*, which colonize the enteric tract. *Enterobacter spp.*, *Serratia spp.*, and *Pseudomonas aeruginosa* are infrequent in the outpatient population, but they are more frequent in patients with complicated UTI. [15]

- *Staphylococcus saprophyticus*, a Gram positive *coagulase negative staphylococcus*, causes about 10% of infections among young, sexually active women. [15]

- Other less frequently isolated agents are other Gram negative bacilli, such as *Acinetobacter* and *Alcaligenes spp.*, other

Pseudomonas spp., *Citrobacter spp.*, *Gardnerella vaginalis* and *Group B streptococcal spp.* Bacteria such as *Mycobacteria*, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Campylobacter spp.*, *Haemophilus influenza* and certain *Corynebacterium spp.* (e.g., *C. renale*) are rarely recovered urine [15]

- *Candida albicans* is the most common cause of funguria, followed by *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, and other yeasts .[16]

- The most frequently isolated strains from catheterized patients are *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Escherichia coli*, while the strongest biofilm producers are *Proteus mirablis*, *Enterococcus faecalis*, *Candida tropicalis* and *Staphylococcus aureus*.[17]

ROUTES OF INFECTION

The two important routes by which bacteria can invade and spread within the urinary tract are the ascending and hematogenous pathways.

HEMATOGENOUS ROUTE

Infection of the renal parenchyma by blood-borne organisms occurs in humans, but less commonly than by the ascending route. The kidney is frequently the site of abscesses in patients with bacteremia or endocarditis caused by a Gram positive organism, *Staphylococcus*

aureus; infections of the kidney with Gram negative bacilli rarely occur by the hematogenous route. [18]

ASCENDING ROUTE

Most uropathogens originate in the rectal flora and enter the bladder via the urethra. The female urethra is short and proximal to the vulvar and perineal areas, making contamination likely. [18] Urinary tract infections in women develop when uropathogens from the fecal flora colonize the vaginal introitus, which is one of the critical initial steps in the pathogenesis of both acute and recurrent UTI.

Instrumentation of the urinary tract such as urinary catheterization, cystoscopy facilitate ascent of microorganisms and is the most common cause of hospital-acquired UTIs in both sexes. Once the bacteria ascend into the bladder, they may multiply and then pass up the ureters to the renal parenchyma particularly if vesicoureteral reflux is present. [15]

Incomplete emptying of the bladder due to mechanical reasons like bladder neck obstruction, urethral valves, urethral strictures, prostatic hypertrophy or neurogenic malfunction can lead to frequent urinary tract infections due to bladder over distension, which may interfere with local defense mechanisms and also require frequent instrumentation of the urinary tract. [18]

PATHOGENESIS

Symptomatic bacteriuria is highly correlated with the presence of bacteria that mediate attachment to uroepithelial cells. Bacteria with enhanced adherence to vaginal and periurethral cells colonize the anatomic regions adjacent to the urethral orifice. Binding to the uroepithelial surface, in turn, prevents bacterial washout during micturition and is the first step to bacterial invasion. [18]

The adhesive properties of uropathogenic *Escherichia coli* (UPEC) are facilitated by Type 1 and P fimbriae, filamentous surface organelle. The attachment of Type 1 fimbriae is blocked by mannose sensitive (MS) adhesins, while the latter is by mannose resistant (MR) adhesins. The P fimbriae augment the virulence of UPEC by allowing more efficient spread from the intestinal tract to the urinary tract and thereby causing ascending infection. [18]

After entry into the bladder, MS-adhesins which are present on the majority of the Enterobacteriaceae, facilitate attachment to the bladder epithelium. However, when the bacteria ascend to the renal parenchyma, they undergo phase variation and do not express Type 1 fimbriae which enhance phagocytosis. Rather, in the upper urinary tract, P fimbriae are expressed, allowing attachment to renal parenchymal cells resulting in pyelonephritis and inducing bacteremia. [18] *Proteus spp.* is able to hydrolyze urea via urease production which results in an

increase in the pH of urine that is directly toxic to kidney cells and also stimulates the formation of kidney stones. [15, 19] Motile organisms ascend the urinary tract against the flow of urine and cause pyelonephritis. Some organisms demonstrate greater production of K antigen (capsule or outer cell wall antigen); this antigen protects bacteria from being phagocytosed. [18]

In contrast among Gram positive organisms, *Staphylococcus saprophyticus*, which adheres significantly better to uroepithelium than do *Staphylococcus aureus* or *Staphylococcus epidermidis*, is a frequent cause of lower urinary tract infections, whereas *Staphylococcus aureus* uncommonly causes cystitis and ascending pyelonephritis. [18]

In an individual with structural abnormalities of the urinary tract or with a catheter, even organisms of low pathogenicity can cause infection of bladder, kidney, or both, and the above-described properties of the bacteria for pathogenesis are not essential. [18]

URETERAL STENTS

Ureteral stents represent the most mature application of an indwelling endoluminal splint. Stents are hollow tubes that work by draining fluid both around and through their structure and although used commonly in urology, have found applications in cardiac and hepatobiliary surgery.

HISTORY OF STENTS

- Gustav Simon performed the first reported case during the 19th century by placing a tube in the ureter during open cystostomy.[20]

- The era of the modern long-term indwelling ureteral stent began in 1967 when Zimskind reported the use of open-ended silicone tubing inserted endoscopically to bypass malignant ureteral obstruction or ureterovaginal fistulas. [20]

- Finney in 1978, refined the design of ureteral stent by describing a Double-J stent with oppositely directed loops at the renal and vesical ends to prevent migration. Today the Double-J ureteral stent is considered to be the 'Gold standard' of stents. [20]

PROPERTIES OF AN IDEAL STENT

An "ideal" stent should demonstrate optimal flow characteristics, prevent migration and must be well tolerated by the patient. It should also be biocompatible, biodurable, radioopaque, easy to insert and remove and provide cost benefit to the patient and hospital. With such high expectations, tremendous improvements have taken place in the field of stent biomaterials, design and texture; however, no currently available device fulfills all of the criteria for the "ideal" stent. [21, 22]

DOUBLE-J URETERAL STENT

Double-J ureteral stents are made of polyurethane, polyethylene, or silicone. Double-J ureteral stent has both a proximal and distal curl,

designed to retain its position within the ureter and end-holes and multiple side-holes that allow urine to drain freely from the kidney's upper collecting system, down through and around the stent, and into the bladder. [23]

CURRENT STENT BIOMATERIALS

- Initially Polyethylene, a synthetic polymer was used in stent construction, as it was flexible, odorless, translucent, and non-reactive in the body, but was abandoned due to its stiffness, brittleness, and tendency to fragment. [20]

- Currently Silicone one of the most lubricious materials available is the most biocompatible stent material as it is most resistant to biofilm formation, infection and encrustation. However it's softness and elasticity make it difficult to pass through tortuous and tight ureters. [20]

- Polyurethane which is the most common class of polymer currently used in stents, is highly versatile and inexpensive, but has been shown to induce significant epithelial ulceration and erosion than other materials with limited durability and slow in-vivo biodegradation. [20]

INDICATIONS FOR URETERIC STENT INSERTION

The indications for stenting can be broadly divided into three categories [24, 25]

A) For relief of obstructive uropathy

Intrinsic

- Renal or Ureteric calculi
- Stricture
- Ureteropelvic Junction obstruction

Extrinsic

- Hydronephrosis of pregnancy
- Extramural compression of ureter by Retroperitoneal tumors or fibrosis

B) Adjunct to ureteral surgery

Preoperative placement done prior to

- Complicated surgery to identify ureter
- Extracorporeal shock wave Lithotripsy (ESWL) in case of solitary kidney and stone >15mm in diameter.

Intra operative insertion is done following:

- Ureteroscopy
- Steinstrasse (street of stone)post ESWL
- Reconstructive procedures - Ureteroneocystotomy, Ureteroureterostomy, Cystectomy, Endopyelotomy/ pyeloplasty and urinary diversion

C) Management of urine leak

- From trauma or surgery

- Due to ureteral fistula

Urolithiasis

Urolithiasis is a common disease that is associated with significant morbidity and a prevalence of 3-20% worldwide. [26, 27] Stones can either form in the bladder or the kidneys. Renal stones can subsequently move into the ureters where, depending on the size, they will either continue to pass into the urinary bladder or cause obstruction of the ureter causing excruciating pain and potential renal dysfunction. Extracorporeal shock wave lithotripsy (ESWL) and Ureterorenoscopy (URS) are currently the most common treatment options in clinical practice. [28]

Post ureteroscopy stenting

In ureteroscopy the stone is disrupted using holmium laser under direct vision with a rigid or flexible ureteroscope. [29] The principle behind the routine procedure of leaving a stent post ureteroscopy is to avoid ureteral obstruction secondary to ureteral edema and stone fragments. Several studies have suggested that stenting is not routinely required following uncomplicated ureteroscopy without ureteric dilation. [30-32]

Stenting as an adjunct to extracorporeal shock wave lithotripsy (ESWL)

ESWL, utilizes underwater energy wave focused on the stone to shatter it into small passable fragments. ESWL is suitable for stones that are smaller than 2cm and lodged in the upper or middle calyx. [33] Stenting prior to ESWL is thought to preclude renal obstruction from stone fragments following ESWL. Some researchers believe that routine use of ureteral stents in ESWL patients not only lack efficacy to prevent renal obstruction, but may, in fact impede the passage of stone fragments following ESWL. [34]

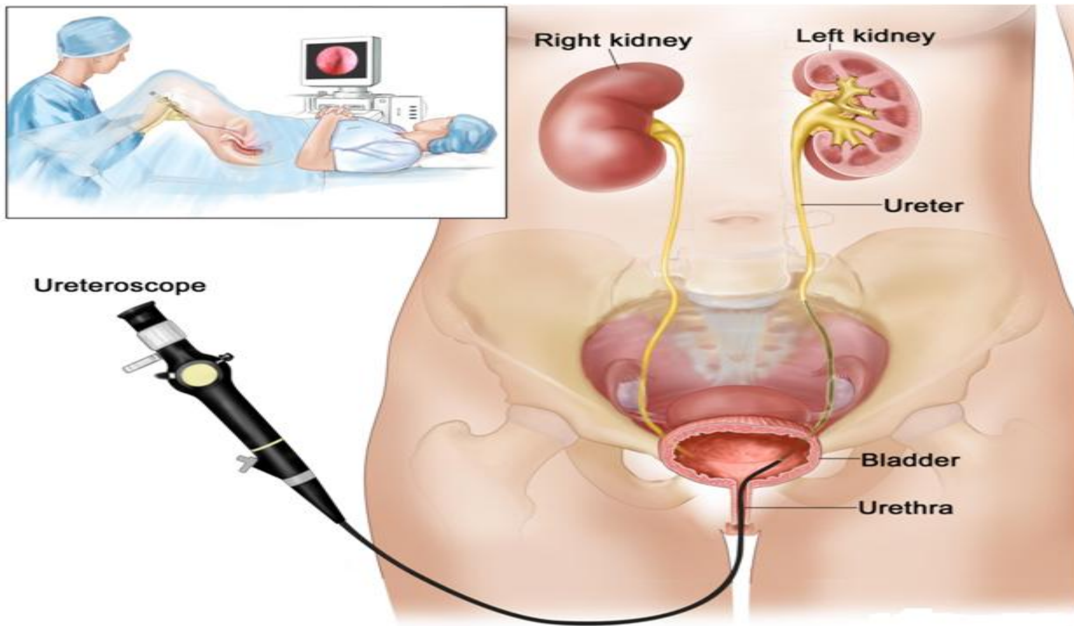
STENT SIZE SELECTION

Double -J ureteral stents are available in sizes from 4.8-5-5.5-6 Fr / 16-24-26-28 cm, where Fr stands for French scale, one Fr is equal to 0.33mm. The most commonly used adult size is 26cm/ 4.8Fr. These generally admit 0.028"-0.035" guide wires. A paediatric patient necessitates the estimation of the ureteric length and then selecting a smaller stent length such as 24/4.8 or 20/4.8. [24]

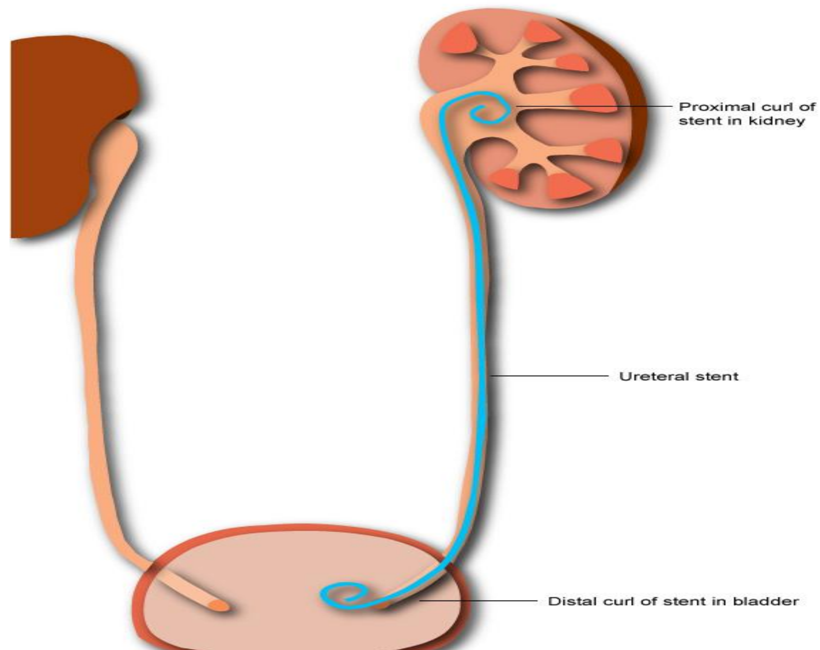
STENTING TECHNIQUE

Stent placement is usually made using either a cystoscope or by percutaneous technique under fluoroscopic guidance. Placement is done by antegrade or retrograde technique. A nephrostomy tube is usually placed first, percutaneously, through the flank and into the kidney prior

STENTING TECHNIQUE



POSITION OF DOUBLE-J URETERAL STENT AFTER PLACEMENT



to an antegrade or retrograde procedure. In this way, the kidney function can be assessed to ensure the kidney's ability to maintain an internal stent before insertion. [23, 35]

ANTEGRADE TECHNIQUE

Double-J stent is placed in an antegrade direction through the nephrostomy tube's track, down through the ureter into the bladder over a guide wire. [23]

RETROGRADE TECHNIQUE

A retrograde placement requires passing a guide wire from the nephrostomy site through the ureter to the bladder, where it is snared and pulled out through the urethra. The stent is passed over the wire in a retrograde direction until it reaches the correct position within the upper collecting system. Once the stent is in place, the guide wire is removed, allowing the ends of the stent to curl into the J shape and anchor the stent within the kidney and bladder. In a variation of this technique, the physician doesn't place the nephrostomy catheter, instead does the retrograde placement cystoscopically, with a wire from below. [23, 35]

Most stents can be safely removed under local anaesthesia using a cystoscope with a bi-prong /triprong forceps.

DURATION OF STENT PLACEMENT

An ideal safe minimal optimal duration for stenting has not been described. Stenting following ureteroscopy or ESWL for ureteric calculi is

generally removed in 2-3 weeks. A difficult percutaneous nephrolithotomy (PCNL) or ESWL is associated with a risk of significant "steinstrasse" and may necessitate stenting for up to 2-3 months. Patients with chronic renal failure due to obstructive uropathy or malignant ureteric obstruction may need lifelong stenting with a 3-monthly serial change. [24]

STENT MONITORING

Stent monitoring includes regular weekly urine culture, serum creatinine and an X-ray KUB showing kidney, ureter and urinary bladder. Internal ureteral stent patency can be evaluated by colour-coded Doppler sonography (CCDS) or by a micturating cystourethrography.[36] CCDS may have sensitivity up to 100% besides being completely non-invasive; a simultaneous KUB ultrasound scan can be done to detect any hydroureteronephrosis.

COMPLICATIONS OF DOUBLE-J STENT INSERTION

1) Stent syndrome

The most common complication is ‘Stent syndrome’. It consists of a constellation of clinical symptoms such as frequency, urgency, flank pain, suprapubic discomfort and sometimes with haematuria and incontinence. Stent acts as a foreign body that irritates the ureteral and bladder wall. [37]

Other complications are (38-43)

2) Malposition of stent

3) Migration of Stent

4) Inadequate relief of obstruction

5) Stent fracture

6) Encrustation

7) Ureteral erosion & Fistulization

8) Forgotten Stent

Urinary tract infections following stent insertion

Urinary tract infection may develop early as a complication of instrumentation of a previously sterile urinary tract, or later as an extension of the underlying disease process. The presence of a foreign body may also lead to colonization of the urinary tract, and ultimately of the stent itself.

BIOFILM

Biofilms and their related complications are a significant cause of morbidity in the patients requiring a urinary device and remain the most common cause of stent failure.[44] Although chronically stented patients are mostly at risk for developing biofilm and the subsequent sequelae, even patients with short-term (7-14days) indwelling stents are at risk, especially those with immunodeficiencies, other concurrent

medical conditions or in those with urinary tract anatomic abnormalities or reconstructions of the urinary tract.

Biofilm structure

Biofilms are defined as "an assemblage of microbial cells that are irreversibly associated with a surface and enclosed in a matrix of primarily polysaccharide material allowing growth and survival in sessile environment".[45]

- ❖ Confocal scanning laser microscope (CSLM) revealed that biofilm is composed primarily of 15% by volume of microcolonies of different species of microbial cells and 85% of matrix material. [44]

- ❖ Extracellular polymeric substance (EPS) is primarily made up of polysaccharides, which may be neutral or polyanionic. The anionic property is due to the presence of uronic acids (D-glucuronic, D-galactouronic and mannuronic acids) and ketal linked pyruvate which helps in the association of divalent cations such as calcium and magnesium, which in turn cross-link with the polymer strands and provide greater binding force in a developed biofilm.[46]

- ❖ Backbone of the EPS contains 1, 3- and 1, 4-β linked hexose residues. The amount of EPS produced varies with different organisms and increases with the age of the biofilm.[47]

Stages in biofilm formation and growth

There are five major stages in biofilm colony formation [48, 49]

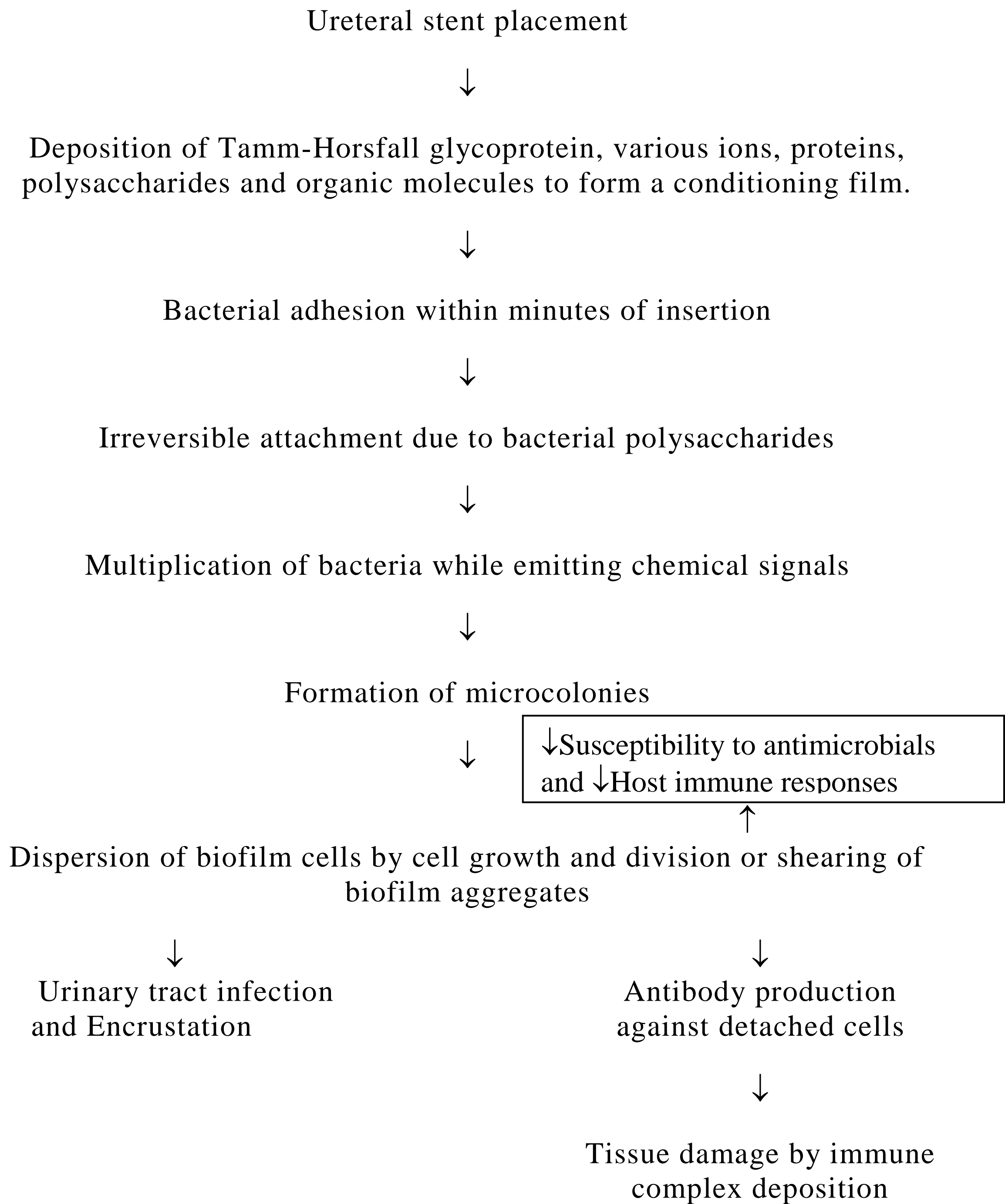
- ❖ Transport and initial attachment of microbes,
- ❖ Irreversible adhesion or attachment,
- ❖ Microcolony formation,
- ❖ Maturation of the biofilm, and
- ❖ Detachment and dispersion of the cells.

The size of the biofilm can range from a few isolated monolayers to 400 cells deep, effectively covering the complete luminal area of the device and reaching a population of up to 5×10^9CFU. [11] The distribution of biofilm is influenced by the growth rate of microorganisms on a surface and the strategies used by them to spread over the surface are also important for colonization. [50]

The biofilm is usually built up of three layers, [17]

- ❖ The linking film which attaches to the surface of tissue or biomaterials.
- ❖ The base film of compact microorganisms.
- ❖ The surface film as an outer layer, where planktonic organisms can be released free floating and spreading over the surface

STEPS INVOLVED IN BIOFILM FORMATION AND ITS CONSEQUENCES



MECHANISM OF ANTIMICROBIAL RESISTANCE IN BIOFILM

Biofilms evade anti microbial challenges by multiple mechanisms which are enumerated below,

- ❖ The negatively charged EPS secreted by biofilm bacteria, acts as a physical/chemical barrier preventing penetration of antibodies or antibiotics and also functions as an ion-exchange resin capable of binding a large number of the antibiotic molecules that are attempting to reach the embedded biofilm cells (extrinsic resistance) .[51,52]

- ❖ Bacteria within a biofilm activate many genes which alter the cell envelope, the molecular targets and the susceptibility to antimicrobial agents (intrinsic resistance). [17] Phenotypic changes caused by a genetic switch, play a more important role in the protection from antimicrobial agents than the external resistance provided by the exopolysaccharide matrix.[53]

- ❖ Bacteria embedded in biofilm have reduced growth rates, which in turn minimizes the rate at which antimicrobial agents are taken into the cell and therefore affect inactivation kinetics. [53]

- ❖ Antibiotic degrading enzymes such as β-lactamase may also be immobilized in the EPS matrix, so that the incoming antibiotic molecules can be inactivated effectively. [3]

- ❖ The cell-wall protein composition of bacteria in biofilms is altered by up to 40% from that of its planktonic counterparts. [54] Antibiotic targets may even disappear or membranes of the

biofilm bacteria might be better equipped to pump out the antibiotics before they produce any damage.

- ❖ Bacteria within a biofilm can sense the external environment, communicate with each other and transfer genetic information and plasmids within biofilm which provides a mechanism for selecting and promoting the spread of bacterial resistance to antimicrobial agents.[49]

VARIOUS METHODS OF COLLECTION OF URINE SPECIMEN

Prevention of contamination by normal vaginal, perineal, and anterior urethral flora is the most important consideration for collection of a clinically relevant urine specimen.

Clean-catch midstream urine (CCMU)

The least invasive procedure, the clean-catch midstream urine specimen collection, must be performed carefully for optimal results, especially in females. Good patient education is essential. [15]

Straight catheterized urine

Although this method is slightly more invasive than CCMU, urinary catheterization provides a method for the collection of uncontaminated urine from the bladder. [15]

Suprapubic bladder aspiration

Following proper skin preparation, urine is withdrawn directly into a syringe through a percutaneously inserted needle, thereby ensuring a contamination-free specimen. The bladder must be full before performing the procedure. This collection technique may be indicated when urine sample is difficult to obtain such as in pediatric patients. [15]

Indwelling catheter

Strict aseptic precautions like wearing gloves should be followed while collecting specimen from indwelling catheter. The catheter tubing should be clamped off above the port to allow the collection of freshly voided urine. The catheter port or wall of the tubing should then be cleaned vigorously with 70% ethanol, and urine aspirated via a needle and syringe; the integrity of the closed drainage system must be maintained to prevent the introduction of organisms into the bladder. [15]

Specimen transport

Urine is an excellent supportive medium for growth of most bacteria, so must be transported to the laboratory and processed within 2hours of collection. If a delay occurs specimens may be refrigerated for up to 24 hours. Urine transport tubes containing boric acid preservative has to be used to stabilize the bacterial population at room temperature for 24 hours if refrigeration is not available. [55]

SCREENING PROCEDURES FOR URINE SPECIMEN

Direct Gram stain

A Gram stain of urine is an easy, inexpensive means to provide immediate information as to the nature of the infecting organism to guide empiric therapy. The presence of 1or 2 bacteria of similar morphotype in each oil immersion field (100X objective) correlates with a count of 100,000 or greater by culture. [55] The Gram stain should not be relied on for detecting polymorphonuclear leukocytes in urine because leukocytes deteriorate quickly in urine that is not processed immediately or not adequately preserved.

Pyuria

Patients with more than 400,000 polymorphonuclear neutrophils (PMNs) excreted into the urine per hour are likely to be infected, and the presence of more than 8 PMNs/mm^3 correlates well with this excretion rate and with infection. [15]

Tests for bacterial products

Dipsticks that detect both Leucocyte esterase an enzyme produced by polymorphonuclear neutrophils and Nitrite produced as a result of bacterial nitrate reductase acting on nitrate in urine are available. The test is rapid, inexpensive and simple to perform. [55]

Catalase test

The commercially available uriscreen is a rapid urine-screening system based on the detection of catalase present in most bacterial species commonly causing UTIs except for *Streptococcus spp.,* and *Enterococcus spp*. 30% (V/V) hydrogen peroxide is added to the urine, and the solution is mixed gently. The formation of bubbles above the liquid surface is interpreted as a positive test. [15]

URINE CULTURE

Routine urine cultures should be plated using calibrated bacteriological loops for the semiquantitative method. This method has the advantage of providing information regarding the number of CFU/ml of urine, as well as providing isolated colonies for identification and antibiotic susceptibility testing. MacConkey agar plate and 5% sheep blood agar plate are used to detect the growth of most Gram negative bacilli, *Staphylococcus spp., Streptococcus spp., Enterococcus spp.,* and fungi like *Candida spp*.

INTERPRETATION OF URINE CULTURE RESULTS

The following criteria is used for urine specimens obtained via suprapubic aspiration or straight catheterization, specimens obtained in the operating room, and urine specimens obtained from patients receiving antimicrobial therapy which have a low probability of contamination. [56]

Types of Colonies Isolated	Quantitation CFU/ml	Interpretation
1	$< 10^2$	Probable contaminant
1	$\geq 10^2$	Significant isolate
2	$< 10^2$ for each	Probable contaminants
2	$\geq 10^2$ for each	Significant isolates
2	$\geq 10^2$ for 1	Significant isolate and contaminant
≥ 3	$\geq 10^2$ for 1	Significant isolate and contaminants
≥ 3	$\geq 10^5$ for each	Probable contaminants

The following criteria is used for urine specimens obtained via clean catch technique, from indwelling catheters (urinary or suprapubic), or from nephrostomy tubes, ureterostomy tubes, or ileal loops which have a high probability of contamination. [56]

Types of colonies isolated	Quantitation CFU/ml	Interpretation
1	$< 10^2$	Probable contaminant
1	$\geq 10^2$	Significant isolate
2	$\geq 10^2$ for each	Significant isolates
2	$\geq 10^2$ for 1	Significant isolate and contaminant
2	$< 10^2$ for each	Probable contaminants
≥ 3	$\geq 10^2$ for 1	Significant isolate and contaminants
≥ 3	$\geq 10^2$ for each	Probable contaminants

DIAGNOSIS OF MICROBIAL URETERAL STENT COLONIZATION

The various studies analyzing Microbial ureteral stent colonization (MUSC) used sonication, qualitative broth culture and Maki's semiquantitative roll-plate technique. [57- 60] Maki's roll- plate technique is the international reference and most widely used technique for the diagnosis of catheter-related blood stream infections (CRBSI). [61] Since roll-plate technique is not inferior to sonication in the detection of CRBSI, it is also applied for the diagnosis of MUSC. [62]

Maki's roll-plate technique has the advantages of a higher detection rate of microorganisms and cost-efficiency. It is time saving, requires only 2 minutes when compared with 10 minutes for sonication and there is no need for additional sophisticated technical equipment (i.e. ultrasound bath, hydrophone). [60]

METHODS FOR BIOFILM DETECTION

I. Phenotypic methods

- ❖ **The Microtitre plate method –** The wells of the microtitre plates are inoculated with a bacterial suspension along with positive and negative controls and these are incubated for 24 to 48 hours. Planktonic cells are removed by washing with phosphate buffered saline. Biofilms are fixed with 2% sodium acetate and are stained with 0.1% crystal violet. The excess dye is washed away with

deionised water. 95% ethanol is added to solublize the dye and the optical densities of the stained biofilms are obtained spectrophotometrically.[63]

- **The Tube method –** 10 ml of Trypticase soy broth with 1% glucose is inoculated with a loopful of test organisms, along with positive and negative controls. The broths are incubated aerobically at 37°C for 24 to 48 hours. The culture supernatants are decanted and the tubes are washed with phosphate buffered saline. The tubes are dried and are stained with 0.1% crystal violet. The excess stain is washed away with deionised water. The tubes are dried in an inverted position. Biofilm formation is considered positive when a visible film lined the wall and bottom of the tube. [64]

- **The Congo red agar method –** The Congo red stain is prepared as a concentrated aqueous solution and is autoclaved at 121°C for 15 minutes at 15psi. This is added to autoclaved Brain heart infusion agar with 4% glucose at 55°C. The plates are inoculated with the test organisms along with positive and negative controls and are incubated at 37°C for 24 to 48 hours aerobically. Black colonies with a dry crystalline consistency indicate biofilm production. [65] .The tissue culture plate is better than other

methods since it is easy to perform and helps to assess biofilms, both qualitatively and quantitatively. [66]

II. Genetic assays for clinical diagnostics and epidemiology

Various genetic methods that are used for detection of biofilms are Pulsed field gel electrophoresis (PFGE), Polymerase chain reaction (PCR), Fluorescence in situ hybridization (FISH), Ribotyping, High-resolution melting analysis, DNA sequencing, and DNA arrays. [67]

III. Microscopy in research and explanted device analysis

Microscopy is a commonly used tool for analyzing structural details of biofilms in vitro. [68]

- ❖ Confocal laser scanning microscopy (CLSM) with fluorescent stains, antibodies, and lectins is ideal to characterize biofilms up to 60 µm thickness. [67]

- ❖ Scanning electron microscopy (SEM) helps to study the real physical change in morphology, density, and substructures of biofilms. [67]

- ❖ Atomic force microscopy (AFM) is a useful tool for measuring physical properties of biofilms, for monitoring bacterial adhesion on different surfaces, interactions between cells, and measuring the strength of adhesion by bacterial adhesins and other macromolecules. [67]

- Magnetic resonance imaging and scanning transmission X-ray microscopy are other methods used for successful assessment of biofilms [69]

NEW STENT DESIGNS

- Dual durometer stents involve a transition from a firm biomaterial at the renal end to a soft biomaterial or a fine loop at the bladder end, to facilitate stent placement, reduce migration and minimize patient discomfort due to bladder irritation. [20, 70]

- Thermoexpandable stents are nickel-titanium alloy stents, used where long-term stenting is needed such as in malignant ureteric strictures e.g., Wall stent or Memocath 051™. [20] The stent (unexpanded state) is placed in the ureter after prior dilatation and later expanded by injecting sterile heated water at 50^0C. The shaft diameter is 9F while the proximal end expands to a calibre of 17F.

- Magnetip is a magnetic-material-tipped ureteral stent that can be retrieved without the need for cystoscopy using a magnet on a special retrival catheter. [70]

- Percuflex Helical ureteral stent has a spiral-cut along the entire length of the straight portion of the stent, designed to conform readily to the shape of the ureter to improve patient comfort. [20,70, 71]

- ❖ Biodegradable stents: After placement, stents made of bioabsorbable polymeric materials e.g., Poly-L-lactide-co-glycolide (PLGA), are gradually biodegraded into tissue-compatible compounds that are absorbed and replaced by healing tissue, thereby eliminating the need for cystoscopic removal and the problem of forgotten or neglected stents. [70, 71]

STENT COATINGS

Stent coatings are a part of stent evolution with the most significant development and promising future prospects.

1. Hydrophilic stent coatings

Ureteral stents coated with hydrophilic compounds such as Hydrogel and Polyvinylpyrrolidone (PVP) have excellent lubricant properties and provides smooth and non –adhesive implant surface, which prevents conditioning film formation and bacterial adhesion. [72, 73]

2. Heparin

Heparin is a highly sulfated glycosaminoglycan, with the highest negative charge density amongst all known biologic molecules. Heparin-coated polymeric stents have been shown to provide the stent with an antiadhesive surface that reduces biofilm formation and concomitant stent encrustation. [71]

3. Oxalate-degrading enzymes

Oxalyl coenzyme A decarboxylase (OXC) and formyl coenzyme A transferase (FRC) are oxalate-degrading enzymes derived from anaerobic bacterium *Oxalobacter formigenes* which when coated on to biomaterials were found to reduce encrustation. [74]

4. Diamond-like carbon coating

Stents coated with a plasma deposited diamond like amorphous carbon material are characterized by excellent biocompatibility and were found to decrease stent friction, encrustation tendencies and biofilm formation. [75]

5. Drug eluting stents

Ureteral stents can be loaded with pharmacological agents that continuously release over time to act locally on the urinary tissue.Drug eluting stents that have been developed are Rifampin –soaked ureteral stents, Triclosan coated stents, Ciprofloxacin /N-acetylcysteine impregnated ureteral catheters, Silver nitrate and Ofloxacin-blended copolymer-coated urospiral stents, Ketorolac loaded stents and Paclitaxel eluting metal stents. Drug eluting stents have been found effective in preventing biofilm formation and stent encrustation. [76-81]

6. Biomimetic and Biocovered stents

The development of tissue-engineered stents would be advantageous because of its inherent biocompatibility. Amiel *et al* have

demonstrated the feasibility of using cartilaginous stents created in vitro and in vivo using chondrocyte-seeded polymer matrices. [82]

TREATMENT OF UTI

- Trimethoprim-sulfamethoxazole has been recommended as the first line of treatment for acute cystitis. The use of this drug is considered appropriate in regions with resistance rates not exceeding 20%.[83]

- Nitrofurantoin remains highly active against *Escherichia coli*. [83]

- Fluoroquinolones commonly used for UTI include Ofloxacin, Ciprofloxacin and Levofloxacin. [83]

- Combinations of a β lactam + β lactamase inhibitors like (Ampicillin+ sulbactum, Piperacillin+ Tazobactum) or Imipenem –Cilastin can be used in patients with more complicated histories, previous episodes of pyelonephritis or recent urinary tract manipulations. The treatment of such patients should be guided by urine culture results. [83]

- Fluconazole which achieves high levels in urine is the first line regime for *Candida* infections of the urinary tract. For *Candida* isolates resistant to Fluconazole, oral Fluocytosine or parenteral Amphotericin B can be given. [83]

MATERIALS AND METHODS

Ethical consideration

Approval from the Institutional Ethics committee was obtained before commencement of the study. Informed consent was obtained from patients who satisfied the inclusion criteria.

Study design

Cross sectional study

Study period

Period of study was from November 2014 to August 2015

Sample size

100 patients

Study population

100 patients admitted under the Department of Urology, Rajiv Gandhi Government General Hospital, Chennai and treated with Double-J ureteral stent placement for ureteral obstruction and who satisfied the inclusion criteria were enrolled and evaluated in this study.

Study setting

The present study was carried out in the Institute of Microbiology, Madras Medical College, in association with Department of Urology, RGGGH , Chennai.

Inclusion criteria

- ❖ Male and female patients of more than 18 years of age.
- ❖ Patients who undergo Double-J ureteral stent placement for ureteral obstruction during the study period.
- ❖ Patients with negative urine culture before Double-J ureteral stent placement.

Exclusion criteria

- ❖ Patients less than 18 years
- ❖ Patients currently on antibiotic treatment
- ❖ Patients with malignancy of genitourinary tract
- ❖ Patients with renal transplantation
- ❖ Patients with coagulation disorders
- ❖ Pregnant women

Collection of data

Data was collected from patients who satisfied the inclusion criteria. A detailed history regarding name, age, gender, presenting complaints, past history of any co-morbid illness, recent antibiotic treatment and details of surgery were recorded before collecting the samples. Vital signs were recorded.

SPECIMEN COLLECTION AND TRANSPORT

- **Collection of Urine Sample:**

Two urine samples were collected from each patient, one before Double-J ureteral stent placement and another before stent removal.

Urine samples from patients satisfying the inclusion criteria were collected in a sterile, wide mouthed disposable container under aseptic precautions. Male patients were instructed to thoroughly cleanse and then retract glans penis before voiding and then to collect the clean catch midstream urine sample. Female patients were instructed to wash the genital area with soap and water and collect the clean catch midstream urine sample keeping the labial folds apart with two fingers. Urine samples were transported to the microbiological laboratory within 30 minutes of collection.

- **Double- J ureteral stent proximal tip collection**

Double-J ureteral stents were removed aseptically from patients under local anaesthesia with the help of a rigid cystoscope. Under sterile precautions, about 3- 5 cm of the proximal tip of the Double-J ureteral stent was collected in a sterile plastic screw-capped container and processed in the microbiological laboratory within 6 hours of collection .

SPECIMEN PROCESSING

- **Direct Gram stain**

A smear was prepared from a drop of well mixed uncentrifuged urine on a new glass slide, air dried, heat fixed and stained by Gram stain technique. The smear was first examined with the 40X objective and then with oil immersion objective for the presence of epithelial cells, pus cells, and bacteria. The presence of 1or more bacteria of similar morphology in each oil immersion field correlates with a count of 100,000 or greater by culture.

- **10% potassium hydroxide (KOH) preparation**

A loopful of urine sample was placed on a clean glass slide and a drop of 10% KOH solution was added to it and mixed well. A coverslip was placed over the preparation. The slide was examined carefully first under 10X objective and then with 40X objective to detect hyphal elements, budding yeast cells, spores or conidia.

- **Semiquantitative urine culture**

The collected urine sample was inoculated onto, MacConkey agar plate, 5% sheep Blood agar plate and two Sabouraud dextrose agar (SDA)slopes.

A calibrated bacteriological loop made of nichrome wire (2mm) that delivers a volume of 0.001 ml of urine was flamed and allowed to cool. The loop was inserted vertically into the urine sample mixed well,

without touching the sides of the container and the loopful of sample was spread over the surface of the MacConkey agar plate, by streaking from top to bottom in a vertical line and again from top to bottom perpendicular to this line in a back and forth fashion. Without reflaming, the loop was inserted vertically into the urine again for transfer of a loopful to the 5% sheep blood agar plate. The MacConkey agar plates were incubated at 35° to 37°C aerobically for 24 hours and blood agar plates were incubated at 35° to 37°C in candle jar for 24 hours. One SDA slope was incubated at 25°C and another at 37°C aerobically and examined for growth twice weekly in first week and once a week for next four weeks.

- **Maki's roll-plate semiquantitative culture method for Double -J ureteral stent proximal tip**

The Maki's roll-plate method was performed by rolling the external surface of the ureteral stent tip back and forth on the surface of a 5 % sheep blood agar plate at least three times and then incubating the plate aerobically in candle jar at 37°C for 24 hours, after which the number of colony-forming units (CFU) was quantitated. [60]

INTERPRETATION OF CULTURE RESULTS

After 24 hours of incubation, MacConkey and 5% sheep blood agar plates were observed for growth, colony count and colony morphology. Gram stain was done to confirm the colony morphology.

- The number of colony forming units was calculated by the following formula: Number of colony forming units = colony count × urine dilution factor. Dilution factor is 1000 when 4mm loop which delivers 0.01 ml sample was used, or 100 when 2mm loop which delivers 0.001 ml sample was used. Significant bacteriuria was defined as a count of more than 10^5 colony forming units (CFU) /ml of urine.

- Maki's roll-plate Semiquantitative culture of Double -J ureteral stent tip was considered significant when ≥15 CFU were seen.

IDENTIFICATION OF ISOLATES

- If colony morphology on MacConkey agar and Blood agar plates were suggestive of Gram negative bacilli, preliminary tests such as Gram staining, hanging drop for motility, Catalase and Oxidase tests, were performed. Catalase positive, Oxidase negative, Gram negative bacilli were identified as members of Enterobacteriaceae. The isolates were identified up to the species level by means of biochemical tests such as Nitrate reduction test, Hugh-Leifson's Oxidation fermentation test, Indole test, Methyl red test, Voges Proskauer test, Triple sugar iron agar, Simmon's citrate utilization test, Christensen's Urease test, Phenylalanine deaminase test, Lysine decarboxylase, Ornithine Decarboxylase and Arginine

dihydrolase test and 1% Sugar fermentation tests with Glucose, Sucrose, Lactose, Maltose and Mannitol.[84]

- ❖ Gram negative bacilli, which were motile, catalase and oxidase positive, with alkaline slant and alkaline butt in Triple sugar iron agar and production of bluish green diffusible pigment on Muller Hinton agar plate were identified as *Pseudomonas species*.[85]

- ❖ If Gram negative coccobacilli, which were nonmotile, Catalase positive and Oxidase negative additional tests such as growth at 44°C, 10% OF lactose utilization test and Malonate utilization test were done to identify *Acinetobacter species*.[86]

- ❖ If shiny, white opaque colonies with a zone of hemolysis around them were seen in 5% sheep blood agar plate and Gram staining showed Gram positive cocci in clusters, colonies were subjected to catalase test, coagulase test – Slide and Tube method, modified oxidase test, urease test, Hugh-Leifson's Oxidation fermentation test, mannitol fermentation test, Phenolphthalein phosphatase test and Gelatin liquefaction to identify *Staphylococcus aureus*.[87]

- ❖ If Gram positive cocci in clusters that were catalase positive and coagulase negative were identified, the following additional tests were done for speciation of *Coagulase Negative Staphylococci* (CoNS).[87]

- Carbohydrate fermentation tests using Lactose, Mannitol, Mannose, Xylose, Trehalose.
- Nitrate reduction test
- Ornithine decarboxylase test
- Differential disc diffusion test with Novobiocin(5μg) and Polymyxin B 300 units.

❖ If pin-point magenta colonies were seen in MacConkey agar and Gram staining showed Gram positive oval cocci in pairs, which were catalase negative the following tests were done.[88]

- Heat tolerance test at 60°C
- Growth in 6.5% Sodium chloride
- Bile esculin hydrolysis
- Arginine dihydrolase test
- Carbohydration fermentation tests using Mannitol, Sorbitol, Arabinose, Raffinose
- PYR(Pyrrolidonyl-beta-naphthylamide) test

❖ Colonies seen in SDA slopes were subjected to Gram Staining and Lactophenol cotton blue mount. Creamy white, dry colonies with

Gram positive budding yeast cells and pseudohyphae in Gram Stain were seen, the following tests were done.

- Germ tube test
- Growth in *Candida* CHROM Agar

Yeast colonies were streaked onto *Candida* CHROM Agar and incubated at 37°C for 72 hrs. The various coloured colonies produced by different species of Candida on CHROM Agar were noted and species were identified accordingly. [89]

Colour of *Candida* colonies on *Candida* CHROM agar

Candida spp.	Colour on *Candida* CHROM Agar
Candida albicans	Light- green
Candida dublinensis	Dark –green
Candida glabrata	Pink to purple
Candida krusei	Pink
Candida parapsilosis	Cream to pale pink
Candida tropicalis	Metallic Blue

- **Corn meal agar plate (Dalmau plate)**

With a sterile straight wire, a heavy inoculum of the yeast was streaked across the corn meal agar plate in three parallel lines. Cover slip was kept on it in such a way that the streak lines project beyond the cover slip. Plates were incubated at 22°C to 26°C in dark for 3 days. The edge of the cover slip was examined under the microscope by placing

the agar plate on the stage and observing under the low and high power objectives.

Corn Meal Agar Morphology	Species Identification
Pseudohyphae with terminal chlamydospores; clusters of blastoconidia at septa	*Candida albicans* *Candida dublinensis*
Abundant Pseudohyphae, pine forest arrangement, blastoconidia formed at or in between septa	*Candida tropicalis*
Elongated yeasts, Abundant Pseudohyphae, (matchstick- like appearance)	*Candida krusei*
Blastoconidia along curved pseudohyphae; giant mycelia cells	*Candida parapsilosis*
No pseudohyphae; small cells; terminal budding	*Candida glabrata*

- Sugar fermentation test using 2% sugars – Glucose, Sucrose, Lactose, Maltose, Galactose, Trehalose

- Sugar Assimilation test.

❖ Heavy inoculum of yeast suspension was prepared in 2ml of yeast nitrogen base and added to 18 ml of molten yeast nitrogen base yeast nitrogen base agar and mixed well. Various carbohydrate impregnated discs namely Glucose, Sucrose, Lactose, Maltose, Galactose, Trehalose, Xylose, Raffinose, Celibiose, Ionositol,

Dulcitol were placed with a sterile forceps 30mm apart and incubated at 30°C for 24-48 hours.

❖ Growth around the carbohydrate disc was interpreted as positive for assimilation of the particular sugar.

ANTIBIOTIC SUSCEPTIBILITY TESTING

Antibiotic Susceptibility testing of the isolated organisms was done on Muller Hinton agar plates by Kirby Bauer disc diffusion method as per CLSI document M 100-S24. [90] The antibiotic discs were obtained from HiMedia Laboratories Private limited, Mumbai.

Inoculum preparation

❖ Three to five well isolated colonies were selected from the 5% sheep Blood agar plate, top of each colony was touched with a bacteriological loop and inoculated into 4-5 ml of nutrient broth. The broth culture was incubated at 35°C for 2 hours. The turbidity of broth culture was adjusted with nutrient broth to obtain turbidity optically comparable to that of the 0.5 McFarland standard.

❖ A sterile cotton swab was dipped into the adjusted suspension. The dried surface of a Muller Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. Five antibiotic discs were placed per plate 24 mm apart from center to center and incubated aerobically for 24hours at 37°C.

❖ The diameter of the zone of inhibition was measured and recorded in millimeters and was then compared with zone diameter interpretive standards chart of the CLSI document M 100-S24. [90] The quality control for antimicrobial susceptibility testing was done with the following standard strains; *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853.

Zone diameter interpretive standards for Gram negative bacilli

Antimicrobial Agent	Disc content µg	Gram Negative Bacilli	Zone Diameter Interpretive Criteria (nearest whole mm)		
			Sensi-tive	Inter-mediate	Resis-tant
Ampicillin	10	*Escherichia coli*	≥ 17	14-16	≤ 13
Amikacin	10	Enterobacteriaceae *Pseudomonas aeruginosa* and *Acinetobacter baumanii*	≥ 17	15-16	≤ 14
Gentamicin	10	Enterobacteriaceae *Pseudomonas aeruginosa* and *Acinetobacter baumanii*	≥15	13-14	≤ 12
Trimethoprim/ Sulfamethoxazole	1.25/ 23.75	Enterobacteriaceae and *Acinetobacter baumanii*	≥16	11-15	≤ 10
Ciprofloxacin	5	Enterobacteriaceae *Pseudomonas aeruginosa* and *Acinetobacter baumanii*	≥21	16-20	≤ 15

Antimicrobial Agent	Disc content µg	Gram Negative Bacilli	Zone Diameter Interpretive Criteria (nearest whole mm)		
			Sensi-tive	Inter-mediate	Resis-tant
Cefotaxime	30	Enterobacteriaceae	≥26	23-25	≤ 22
		Acinetobacter baumanii	≥23	15-22	≤ 14
Ceftazidime	30	Enterobacteriaceae	≥21	18-20	≤ 17
		Pseudomonas aeruginosa and *Acinetobacter baumanii*	≥18	15-17	≤ 14
Tetracycline	30	Enterobacteriaceae	≥15	12-14	≤ 11
Imipenem	10	Enterobacteriaceae	≥23	20-22	≤ 19
		Pseudomonas aeruginosa	≥19	16-18	≤ 15
		Acinetobacter baumanii	≥22	19-21	≤ 18
Piperacillin/ Tazobactum	100/ 10	Enterobacteriaceae and *Acinetobacter baumanii*	≥21	18-20	≤ 17
		Pseudomonas aeruginosa	≥21	15-20	≤ 14
Ofloxacin	5	Enterobacteriaceae *Pseudomonas aeruginosa*	≥16	13-15	≤ 12
Norfloxacin	5	Enterobacteriaceae *Pseudomonas aeruginosa*	≥17	13-16	≤ 12
Nitrofurantoin	300	Enterobacteriaceae	≥17	15-16	≤ 14

Zone diameter interpretive standards for S*taphylococcus spp*.

Antimicrobial Agent	Disc content	Zone Diameter Interpretive Criteria (nearest whole mm)		
		Sensitive	Intermediate	Resistant
Penicillin	10 units	≥ 29	-	≤ 28
Trimethoprim/ Sulfamethoxazole	1.25/23.75 µg	≥16	11-15	≤ 10
Linezolid	30 µg	≥23	-	≤ 20
Tetracycline	30 µg	≥19	15-18	≤ 14
Ciprofloxacin	5 µg	≥21	16-20	≤ 15
Norfloxacin	5 µg	≥17	13-16	≤ 12
Nitrofurantoin	300 µg	≥17	15-16	≤ 14

Zone Diameter Interpretive Standards for *Enterococcus spp*.

Antimicrobial Agent	Disc content	Zone Diameter Interpretive Criteria (nearest whole mm)		
		Sensitive	Intermediate	Resistant
Ampicillin	10 µg	≥ 17	-	≤ 16
Penicillin	10 units	≥ 15	-	≤ 14
Linezolid	30 µg	≥23	21-22	≤ 20
Vancomycin	30 µg	≥17	15-16	≤ 14
High level Gentamicin	120 µg	≥10	7-9	≤ 6
Ciprofloxacin	5 µg	≥21	16-20	≤ 15
Norfloxacin	5 µg	≥17	13-16	≤ 12
Tetracycline	30 µg	≥19	15-18	≤ 14
Nitrofurantoin	300 µg	≥17	15-16	≤ 14

ANTIFUNGAL SUSCEPTIBILITY TESTING

- Disc diffusion testing of each isolate was done with Fluconazole and Voriconazole as per CLSI document M44-A. [91] Mueller Hinton agar plates supplemented with 2% glucose and 0.5 μg/ml methylene blue was used.

- Inoculum was prepared by picking 5 distinct colonies of 1mm diameter from a 24 hour old culture of *Candida spp.* and suspended in 5ml of sterile 0.85% saline. The suspension was vortexed for 15 min and the turbidity was adjusted to 0.5 McFarland standards to yield a yeast stock of 1×10^6 to 5×10^6 cell/ml. The cell suspension was inoculated on to the agar plate by streaking with a swab over the entire agar surface.

- Antifungal discs were placed on the inoculated agar plates and incubated at 35°C for 48 hours. Zone diameter is measured to the nearest whole millimeter at the point where there was prominent reduction in growth.

Zone diameter interpretive standards for *Candida species*.

Antifungal Agent	Disc content µg	Zone Diameter Interpretive Criteria (nearest whole mm)		
		Sensitive	Intermediate	Resistant
Fluconazole	25	≥ 17	14-16	≤ 13
Voriconazole	1	≥ 17	15-16	≤ 14

The following Control strains were used for antisusceptibility testing for yeasts; *Candida albicans* ATCC 90028 and *Candida parapsilosis* ATCC 22019.

Determination of Minimum Inhibitory concentration (MIC) for Vancomycin for Methicillin Resistant *Staphylococcus spp.* by Broth Macrodilution Method

Preparation of antibiotic stock solution [92]

Formula: $$W = 1000 \times \frac{V \times C}{P}$$

Where P = potency 950 µg/mg,

V = volume required 10 mL,

C = final concentration of solution 1024 µg/ml.

W = weight of antibiotic in mg to be dissolved in volume V (mL).

Inoculum preparation

- Four to five morphologically similar colonies of the test organism from a 24 hour old culture were picked up with a sterile bacteriological loop and suspended in nutrient broth and

incubated at 35°±2°C. Then the cell suspension was diluted to 1:100 and turbidity was adjusted to 0.5 McFarland standard.

❖ Same procedure was followed for the control organism *Enterococcus faecalis* ATCC 29212.

Procedure

❖ The antibiotic stock solution was prepared by adding required amount of Vancomycin drug substance to 10 ml of 0.85% saline.

❖ Two rows of 12×75 mm tubes each containing 13 test tubes, one row for the test organism and other row for the control organism were arranged. 1 ml of Muller Hinton broth was added to each tube which were labelled as follows: 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 µg/ml.

❖ From the stock solution 1ml of antibiotic solution was added to tube labelled 512 µg/ml with a micropipette then 1ml from 512 µg/ml tube to 256 µg/ml tube, similarly serial dilution was done till the last tube labelled 0.125 µg/ml and 1ml was discarded from the last tube.

❖ 1ml of the 1:100 dilution of the inoculum of test organism was added to each test tube in one set of tubes and control organism was added to another set of tubes and incubated aerobically at 35°-37°C for 18-24 hours.

- Growth control and drug control tubes were included for sterility check.
- The MIC end point was read as the lowest concentration of the antibiotic at which there was no visible growth.
- The MIC of control strain was observed, which was within sensitive range, hence the test was considered to be valid.

Minimum Inhibitory concentration (MIC) interpretive standards of Vancomycin for Staphylococcus spp.

Organism	MIC Interpretive Criteria (µg/ml)		
	Sensitive	Intermediate	Resistant
Staphylococcus aureus	≤ 2	4-8	≥ 16
Staphylococcus epidermidis	≤ 4	8-16	≥ 32

Determination of Minimum inhibitory concentration (MIC) of Meropenem by Broth Macrodilution Method for Imipenem resistant Gram negative Bacilli

Preparation of antibiotic stock solution [92]

Formula: $W = 1000 \times \frac{V \times C}{P}$

Where P = potency 750 µg/mg,

V = volume required 10 mL,

C = final concentration of solution 1024 mg/L, and

W = weight of antibiotic in mg to be dissolved in volume V (mL).

❖ The required amount of drug was calculated and dissolved in 10ml of 0.85% saline.

❖ Same procedure was followed as for Vancomycin MIC and the control organism was *Escherichia coli* ATCC 25922.

Minimum Inhibitory concentration (MIC) interpretive standards of Meropenem for Gram negative bacilli

Organism	MIC Interpretive Criteria (µg/ml)		
	Sensitive	**Intermediate**	**Resistant**
Enterobacteriaceae	≤ 1	2	≥ 4
Pseudomonas aeruginosa	≤ 2	4	≥ 8

Determination of Minimum inhibitory concentration (MIC) of Amphotericin B by Broth Microdilution Method for Yeast

Preparation of antifungal stock solution [93]

Formula: $W = 1000 \times \frac{V \times C}{P}$

Where P = potency 750 µg/mg,

V = volume required 10 mL,

C = final concentration of solution 1600µg/ml, and

W = weight of antibiotic in mg to be dissolved in volume V (mL).

❖ Broth microdilution method for yeasts was performed as per the CLSI guidelines M27-A3. [93] Dimethyl sulfoxide (DMSO) was used as solvent and RPMI 1640 (with glutamine, without bicarbonate and phenol red as indicator) was used as broth medium. The concentration of Amphotericin B tested was in the range of 0.0313 to 16 µg/ml.

Inoculum preparation

❖ Five morphologically similar colonies of the *Candida* species from a 24 hour old culture were picked up using a sterile bacteriological loop and suspended in 5 ml of 0.85% saline and incubated at 35°±2°C. The cell suspension was vortexed for 15 seconds and cell density was adjusted with a spectrophotometer by adding sufficient sterile 0.85% saline to increase the transmittance equivalent to that produced by a 0.5 McFarland standard.

❖ A working suspension was made by 1:100 dilution followed by a 1:20 dilution of the stock suspension with RPMI 1640 broth medium which results in 5×10^2 to 2.5×10^3 cells/ml.

❖ Same procedure was followed for the control organism *Candida albicans* ATCC 90028.

Procedure

- ❖ The broth microdilution was performed by using sterile disposable U shaped 96 well microdilution plates. 100 µl of varying drug concentrations were dispensed in each row from 1to 10 wells.

- ❖ 11th column of microdilution plate was the growth control with 100 µl of sterile drug free medium and inoculated with the corresponding inoculum suspension. 12th column of microdilution plate was the drug control with 100 µl of sterile drug free medium and 100 µl drug.

- ❖ The microdilution plates were incubated for 24 hours at 35°C. After 24 hours the microdilution were scored with the aid of a reading mirror. The growth in each well was compared with that of the growth control well.

- ❖ The MIC was interpreted as the well with lowest concentration of drug with no visible growth.

- ❖ If the MIC is ≥ 1 µg/ml for a *Candida spp*. then it is likely to be resistant to Amphotericin B.

DETECTION OF BETA-LACTAMASE PRODUCTION IN GRAM NEGATIVE BACILLI

❖ Screening test for Extended-Spectrum Beta-Lactamases (ESBL's)

Each isolate was screened for the ESBL production against Cefotaxime (30µg) and Ceftazidime (30µg) discs by disc diffusion

method. Isolates with zone size of ≤ 27 mm for Cefotaxime or ≤ 22 mm for Ceftazidime were suspected to be ESBL producer.[90]

- **Phenotypic Confirmatory test for ESBL**

All isolates suspected to be ESBL producers in the screening test were further confirmed as per the CLSI guidelines by the disc potentiation test by using Ceftazidime (30µg) and Ceftazidime-clavulanic acid (30/10µg) discs and Cefotaxime (30µg) and Cefotaxime-clavulanic acid (30/10µg) discs.[90]

A ≥ 5-mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus the zone diameter of the agent when tested alone signified a positive ESBL. Escherichia coli ATCC 25922 was used as the quality control strain.[90]

Detection of the Metallo- β- lactamases (MBL's)

- The isolates which were resistant to Imipenem were suspected of metallo-β lactamase production.

- The metallo-β lactamase production was detected by the Imipenem – Ethylene diamine tetraacetic acid (IPM+ EDTA) double disc synergy test.[94]

- Suspected isolate adjusted to 0.5 McFarland standard was swabbed onto a plate of Mueller Hinton agar. 10µg Imipenem (IPM) disc and a IPM+ EDTA disc were placed at a distance of 10

mm edge to edge. The plates were incubated overnight at 35°C for 24 hours.

- ❖ The organisms were considered to be MBL producers if the increase in the inhibition zone of the IPM+EDTA disk was ≥ 5 mm than the plain IPM disc.

Screening test for AmpC β -lactamases

- ❖ All the Gram negative isolates were screened for AmpC β lactamase production by the disc antagonism test using Cefoxitin (30µg) disc by disc diffusion method. The isolates which showed a reduced susceptibility to Cefoxitin ≤ 18 mm were tested for confirmation by the AmpC Disc test.

Detection of AmpC β -lactamases by Disc test

- ❖ The isolates resistant to cefoxitin were subjected to AmpC Disc test.[95]

- ❖ A lawn culture of E. coli ATCC 25922 was prepared on Muller Hinton agar (MHA) plate. Sterile disks (6 mm) were moistened with sterile 0.85% saline (20µl) and inoculated with several colonies of the test organism.

- ❖ The inoculated disc was then placed beside a Cefoxitin disc (almost touching) on the inoculated plate. The plates were incubated overnight at 35°C.

- ❖ A positive test appeared as a flattening or indentation of the Cefoxitin inhibition zone in the vicinity of the test disc.
- ❖ A negative test had an undistorted zone around the Cefoxitin disc.

Screening test for Detection of Methicillin Resistance in *Staphylococcu*s species

The *Staphylococcal* isolates were tested for methicillin resistance using Cefoxitin (30µg) by disc diffusion method in MHA plate. Cefoxitin is used as a surrogate marker for mec-A mediated oxacillin resistance. Zone size of ≤21mm for *Staphylococcus aureus* and *Staphylococcus lugdunensis,* ≤ 24mm for CoNS *species* around Cefoxitin disc were considered as methicillin resistant as per CLSI guidelines. *Staphylococcus aureus* ATCC 25923 was used as quality control strain. [90]

<u>Phenotypic detection of Biofilm production by isolates from colonized Double -J ureteral stents detected by Microtitre plate method</u>

- ❖ The procedure was done as described by Stepanovic et al.[63]
- ❖ Isolates from recently subcultured 5% sheep BAP plates were inoculated in trypticase soy broth with 1% glucose media and incubated for 18 hours at 37oC and then diluted 1in100 with trypticase soy broth.

- Individual wells of sterile polystyrene, 96 well-flat bottom microtitre plates were filled with 200 µl of the diluted cultures and uninoculated trypticase soy broth broth served as control to check sterility and non-specific binding of media. The test was conducted in triplicate for concurrence.

- The microtitre plates were incubated for 18 - 24 hours at 37°C. After incubation, the content from each well was gently removed by tapping the plates. The wells were washed four times with 200 µl 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 200 µl sodium acetate (2%) . 200 µl of 0.1%(W/V) aqueous crystal violet solution was added to each well and allowed to stand for 15 minutes.

- Excess stain was rinsed off by thorough washing with deionized water and plates were kept for drying. 200 µl of 95% ethanol was added to solubilize bound crystal violet.

- Adherent cells usually form biofilm and the microtitre wells were uniformly stained with crystal violet. Optical density (OD) of stained adherent bacteria was determined with a ELISA auto reader at wavelength of 570 nm.

- ❖ Quality Control Strains: Pseudomonas aeruginosa ATCC 27853 a strong biofilm producer was used as positive control. Escherichia coli ATCC 25922 a non biofilm producer was used as negative control.[96]

Interpretation

- ❖ The average OD values were calculated for all tested strains and negative controls, since all tests were performed in triplicate.
- ❖ The cut-off value (ODc) is defined as three standard deviations (SD) above the mean OD of the negative control:
- ❖ ODc=average OD of negative control + (3× SD of negative control).
- ❖ Isolates were categorized as

Non biofilm producer	:	$OD \leq OD_c$
Weak biofilm producer	:	$OD_c \leq OD \leq 2ODc$
Moderate biofilm producer	:	$2OD_c \leq OD \leq 4\ OD_c$
Strong biofilm producer	:	$4ODc < OD$

STATISTICAL ANALYSIS

Results were analyzed statistically using statistical package of social sciences (SPSS) version 20.0. Pearson's chi square test and fisher's exact test were used and a p value of < 0.05 was considered significant.

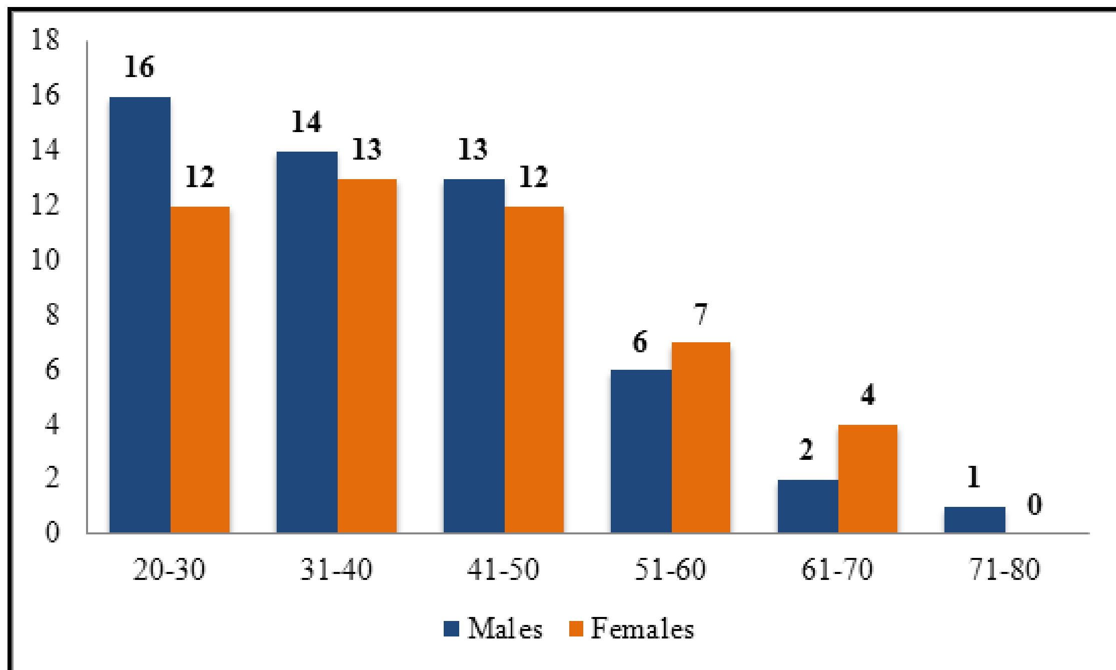
RESULTS

This cross sectional study was carried out in the Institute of Microbiology, Madras Medical College, in association with the Department of Urology, Rajiv Gandhi Government General Hospital, Chennai. A total of 100 patients who were treated with Double-J ureteral stents for relief of ureteral obstruction and who satisfied the inclusion criteria were included in this study for from November 2014 to August 2015.

Table-1: ANALYSIS OF AGE AND GENDER DISTRIBUTION OF PATIENTS WITH DOUBLE-J URETERAL STENT PLACEMENT (n=100)

Age group	Number of patients	Gender		Percentage
		Male	Female	
20-30	28	16	12	28%
31-40	7	14	13	27%
41-50	25	13	12	25%
51-60	13	6	7	13%
61-70	6	2	4	6%
71-80	1	1	0	1%
Total	100	52	48	100%

FIGURE 1: DISTRIBUTION OF PATIENTS WITH DOUBLE-J URETERAL STENT PLACEMENT ACCORDING TO AGE AND GENDER

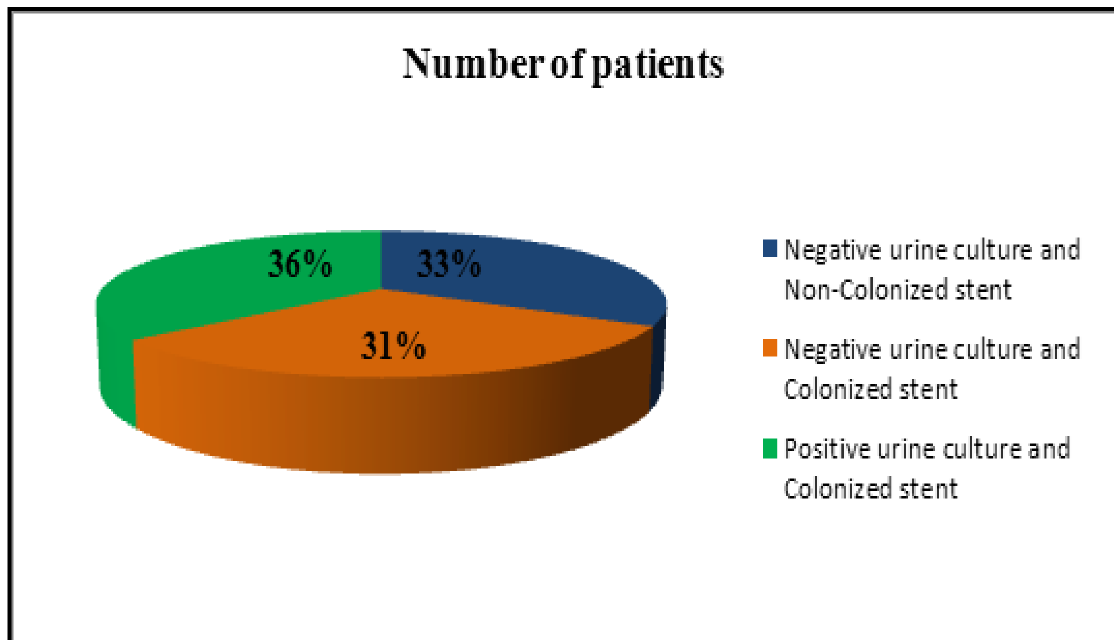


The age (in years) ranged from 20 to 75 years. The mean ± SD of age (in years) of patients treated with indwelling Double-J ureteral stents was 40.45± 12.534.Majority of the patients were in the age group of 31-50 years. Out of 100 patients 52 were males and 48 were females.

TABLE 2: ANALYSIS OF PATIENTS BASED ON DOUBLE-J URETERAL STENT AND URINE CULTURE RESULTS (N=100)

Double-J ureteral stent colonization (n=100)	Urine Culture (n=100)		Total
	Negative	Positive	
Present	31	36	67
Absent	33	0	33
Total	64	36	100

Figure 2: DISTRIBUTION OF PATIENTS BASED ON DOUBLE-J URETERAL STENT AND URINE CULTURE RESULTS



Out of 100 patients with Double-J ureteral stent placement, 67 patients had colonization of Double-J ureteral stent surface while only 36 patients had positive urine culture. In 33 patients both Double-J ureteral stent and urine culture were negative.

TABLE 3: ANALYSIS OF AGE WISE DISTRIBUTION OF PATIENTS WITH COLONIZED DOUBLE-J URETERAL STENT AND POSITIVE URINE CULTURE (n=100)

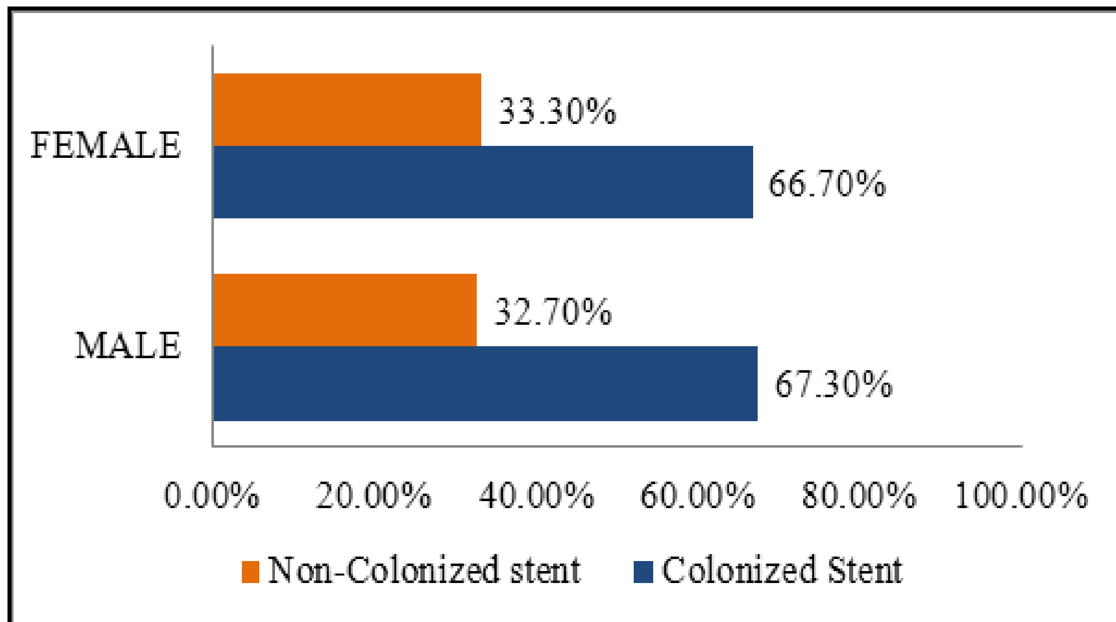
Age group (years)	Total number of patients	Number of patients with colonized Double-J ureteral stent	Number of patients with positive urine culture
20-30	28	20 (71.4%)	10 (35.7%)
31-40	27	15 (55.6%)	8 (29.6%)
41-50	25	19 (76%)	10 (52.6%)
51-60	13	9 (69.2%)	7 (53.8%)
61-70	6	3 (50%)	0
71-80	1	1 (100%)	1(100%)
Total	100	67	36

The colonization of Double-J ureteral stent is higher in the 41-50 years age group followed by those between 20-30 years of age. Patients between 51-60 years had higher percentage of positive urine culture.

TABLE 4: GENDER WISE DISTRIBUTION OF COLONIZED AND NON-COLONIZED STENTS (n=100)

Double-J ureteral stent	Gender						Pearson Chi-Square
	Male		Female		Total		
	N	%	N	%	N	%	
Colonized	35	67.3	32	66.7	67	67	P-Value 0.946
Non-colonized	17	32.7	16	33.3	33	33	
Total	52	100	48	100	100	100	

FIGURE-3: GENDER WISE DISTRIBUTION OF COLONIZED AND NON-COLONIZED STENTS



There was statistically no significant relationship between Double-J ureteral stent colonization and gender ($p > 0.05$).

TABLE 5: INDICATIONS FOR DOUBLE-J URETERAL STENT PLACEMENT (n=100)

Indications	Number of Cases (n)	Percentage (%)
Ureteroscopic (URS) stone Extraction/Lithotripsy	62	62
After Percutaneous nephrolithotomy (PCNL)	17	17
Prior to Extracorporeal shockwave Lithotripsy (ESWL)	14	14
Hydronephrosis	2	2
Open Urolithiasis surgery	5	
Total	100	100

In majority of the patients (62%) Double-J stents were placed following Ureteroscopic stone extraction/ Lithotripsy, followed by ureteral stenting in patients who have undergone PCNL (17%) and prior to ESWL (14%).

TABLE 6: CORRELATION BETWEEN DURATION OF STENT PLACEMENT AND COLONIZATION OF DOUBLE-J URETERAL STENTS AND URINE CULTURE

Cuture Results	Duration Of Stent Placement							
	< 4 WKS		4 - 6 WKS		>6 WKS		Total	
	N	%	N	%	N	%	N	%
Negative urine culture and non colonized stent	31	43.1	2	11.1	0	0	33	33
Negative urine culture and colonized stent	29	40.3	2	11.1	0	0	31	31
Positive urine and colonized stent	12	16.7	14	77.8	10	100.0	36	36
Total	72	100	18	100	10	100	100	100

Chi-Square Test	Value	P-Value
Fisher's Exact Test	40.349	<0.001

The duration of stent placement had statistically significant influence on the rate of bacteriuria and Double-J ureteral stent colonization (p <0.001). When the duration of stent placement is > 6 weeks urine culture and Double-J ureteral stent colonization is 100% positive.

TABLE-7: MICROBIOLOGICAL PROFILE IN PATIENTS WITH POSITIVE DOUBLE-J URETERAL STENT CULTURE (n=100)

Type of isolate	Organism	Total number of isolates (n)	Percentage
Gram Negative Bacilli	*Escherichia coli*	22	32.4%
	Klebsiella oxytoca	11	16.2%
	Klebsiella pneumoniae	2	2.9%
	Pseudomonas aeruginosa	18	26.5%
	Acinetobacter baumannii	1	1.5%
Gram Positive Cocci	*Staphylococcus aureus*	4	5.9%
	Staphylococcus epidermidis	2	2.9%
	Enterococcus faecalis	4	5.9%
Yeast	*Candida tropicalis*	3	4.4%
	Candida krusei	1	1.5%
	Total	68	100.0%

The most commonly isolated pathogen in Double-J ureteral stent culture was *Escherichia coli* (32.4%). Pure growth was isolated from the Double-J ureteral stent surface in 66 patients and one patient had colonization with 2 microorganisms namely *Klebsiella pneumoniae* and *Staphylococcus epidermidis*.

FIGURE-4: MICROBIOLOGICAL PROFILE IN PATIENTS WITH POSITIVE DOUBLE-J URETERAL STENT CULTURE (%) (TABLE-7)

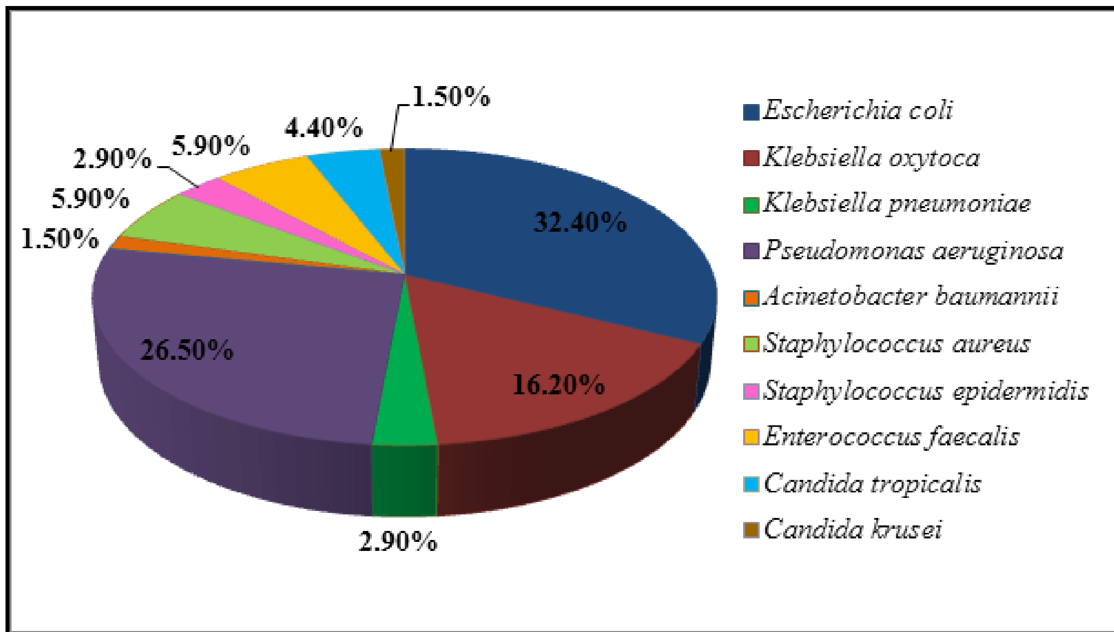


FIGURE-5: MICROBIOLOGICAL PROFILE IN PATIENTS WITH POSITIVE URINE CULTURE (%) (TABLE 8)

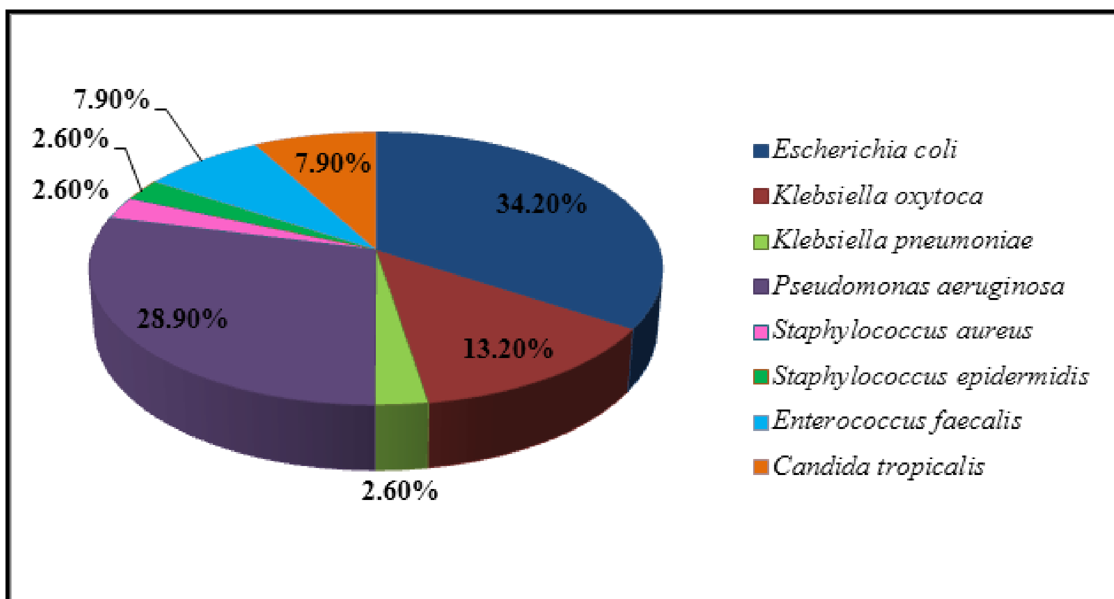


TABLE 8: MICROBIOLOGICAL PROFILE OF URINE CULTURE IN PATIENTS WITH DOUBLE-J URETERAL STENT PLACEMENT

Type of isolate	Organism	Total number of isolates (n)	Percentage
Gram Negative Bacilli	*Escherichia coli*	13	34.2%
	Klebsiella oxytoca	5	13.2%
	Klebsiella pneumoniae	1	2.6%
	Pseudomonas aeruginosa	11	28.9%
Gram Positive Cocci	*Staphylococcus aureus*	1	2.6%
	Staphylococcus epidermidis	1	2.6%
	Enterococcus faecalis	3	7.9%
Yeast	*Candida tropicalis*	3	7.9%
	Total	38	100%

Microbiological profile of urine culture showed *Escherichia coli* to be the commonest pathogen (34.2%) followed by *Pseudomonas aeruginosa* (28.9%). Out of the 36 patients who showed growth in urine culture, 34 patients had growth of single microorganism. Mixed growth in the urine sample of two patients were *Pseudomonas aeruginosa* + *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* + *Enterococcus faecalis.*

TABLE-9: ANTIBIOTIC SENSITIVITY PATTERN OF GRAM NEGATIVE BACILLI

S. NO	ANTIBIOTIC	*Escherichia coli* (n=22)		*Klebsiella oxytoca* (n=11)		*Klebsiella pneumoniae* (n=2)		*Pseudomonas aeruginosa* (n=18)		*Acinetobacter baumannii* (n=1)		Total Percentage Sensitivity
		S	%	S	%	S	%	S	%	S	%	%
1	Ampicillin (10µg)	1	4.5	-	-	-	-	-	-	-	-	4.5
2	Gentamicin (10µg)	6	27.3	3	27.3	0	0	10	55.6	1	100	37
3	Amikacin (30µg)	11	50	3	27.3	2	100	10	55.6	1	100	50
4	Ciprofloxacin (5µg)	4	18.2	2	18.2	1	50	5	27.8	1	100	24.1
5	Trimethoprim-sulfamethoxazole (1.25/23.75µg)	6	27.3	5	45.5	1	50	-	-	1	100	36.1
6	Cefotaxime (30µg)	3	13.6	2	18.2	0	0	-	-	1	100	16.7
7	Ceftazidime (30µg)	6	27.3	4	36.4	0	0	7	38.9	1	100	33.3
8	Imipenem (10µg)	18	81.8	9	81.8	2	100	16	88.9	1	100	84.9
9	Piperacillin-Tazobactum (100/10µg)	12	54.5	6	54.5	2	100	10	55.6	1	100	56.6
10	Tetracycline (30µg)	8	36.4	6	54.5	2	100	-	-	1	100	47.2
11	Ofloxacin (5µg)	7	31.8	2	18.2	1	50	4	22.2	-	-	25
12	Norfloxacin (10µg)	9	40.9	5	45.5	1	50	10	55.6	-	-	46.2
13	Nitrofurantoin (300µg)	14	63.6	6	54.5	0	0	-	-	-	-	57.1

S- Sensitive
All the identical isolates identified from Double-J ureteral stent culture and urine culture had same susceptibility pattern.

All the Gram negative bacilli were highly susceptibile to Imipenem (84.9%) and Nitrofurantoin (57.1%).

TABLE-10: ANTIBIOTIC SENSITIVITY PATTERN OF GRAM POSITIVE COCCI

S.NO	ANTIBIOTIC	*Staphylococcus aureus* (n=4)		*Staphylococcus epidermidis* (n=2)		*Enterococcus faecalis* (n=4)		Percentage Sensitivity
		S	%	S	%	S	%	
1	Ampicillin (10µg)	-	-	-	-	0	0	0%
2	Pencillin (10 Units)	0	0	0	0	0	0	0%
3	Trimethoprim-sulfamethoxazole (1.25/23.75µg)	0	0	1	50	-	-	16.7%
4	Linezolid (30µg)	4	100	2	100	4	100	100%
5	Vancomycin (30µg)	-	-	-	-	4	100	100%
6	Tetracycline (30µg)	4	100	1	50	3	75	80%
7	High-level Gentamicin (120µg)					4	100	100%
8	Ciprofloxacin (5µg)	0	0	1	50	1	25	20%
9	Norfloxacin (10µg)	2	50	1	50	0	0	30%
10	Nitrofurantoin (300µg)	4	100	1	50	2	50	70%

S-sensitive

The Gram positive organisms were highly susceptible to Linezolid (100%), Tetracycline (80%) followed by Nitrofurantoin (70%) and least susceptible to Trimethoprim-sulfamethoxazole (16.7%). All the isolates tested were resistant to Ampicillin and Penicillin.

FIGURE-6: ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *ESCHERICHIA COLI* ISOLATES FROM PATIENTS WITH DOUBLE-J URETERAL STENT PLACEMENT (TABLE 9)

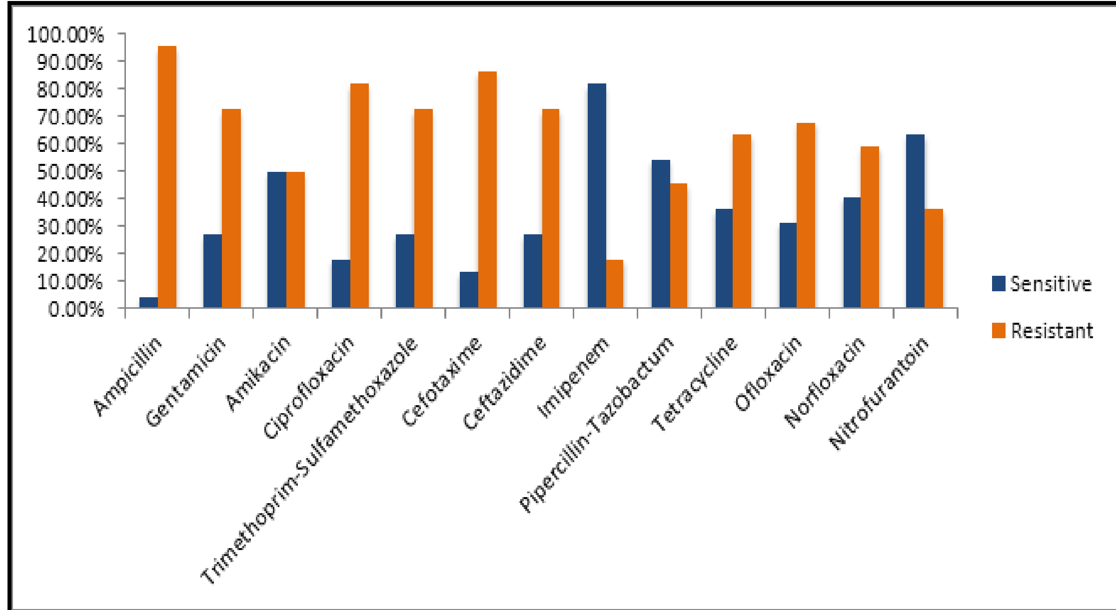


FIGURE-7: ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *KLEBSIELLA OXYTOCA* ISOLATES FROM PATIENTS WITH DOUBLE-J URETERAL STENT PLACEMENT (TABLE 9)

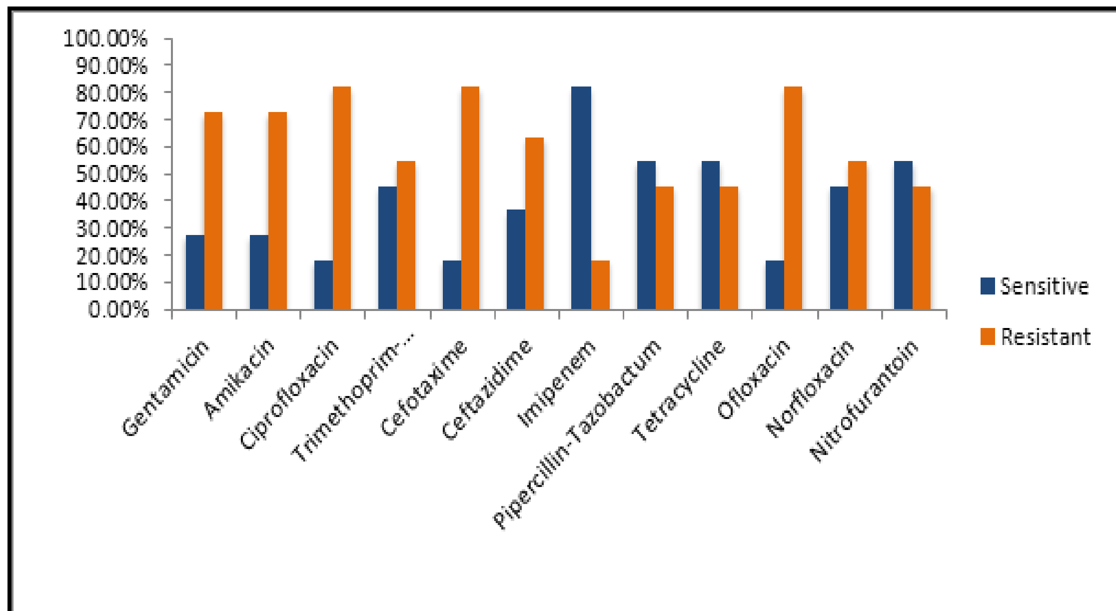


FIGURE 8: ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *KLEBSIELLA PNEUMONIAE* ISOLATES FROM PATIENTS WITH DOUBLE-J URETERAL STENT PLACEMENT (TABLE 9)

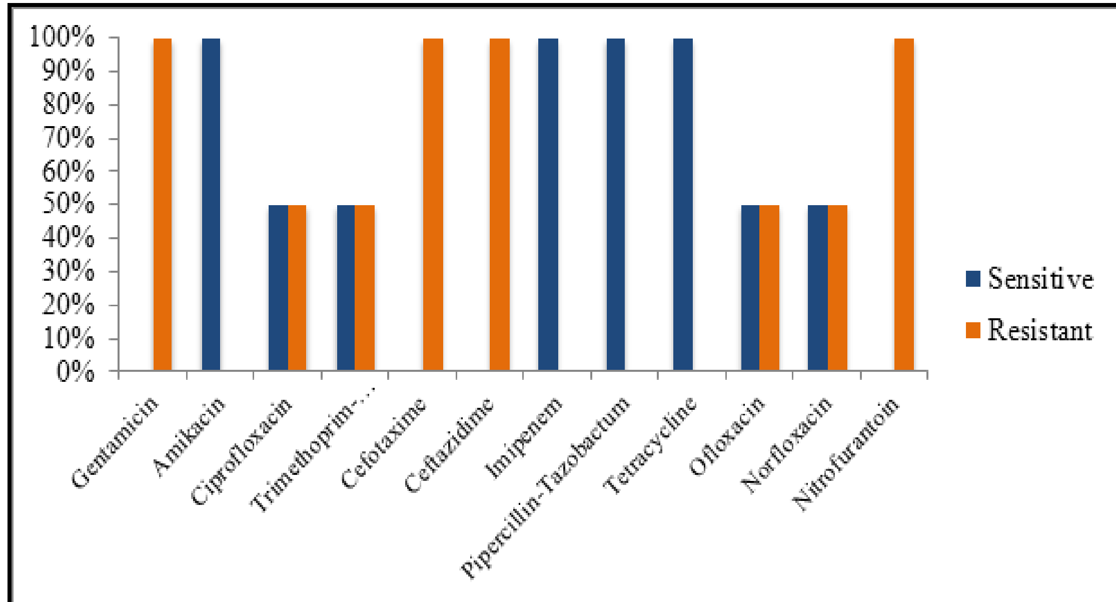


FIGURE 9: ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *PSEUDOMONAS AERUGINOSA* ISOLATES FROM PATIENTS WITH DOUBLE-J URETERAL STENT PLACEMENT (TABLE 9)

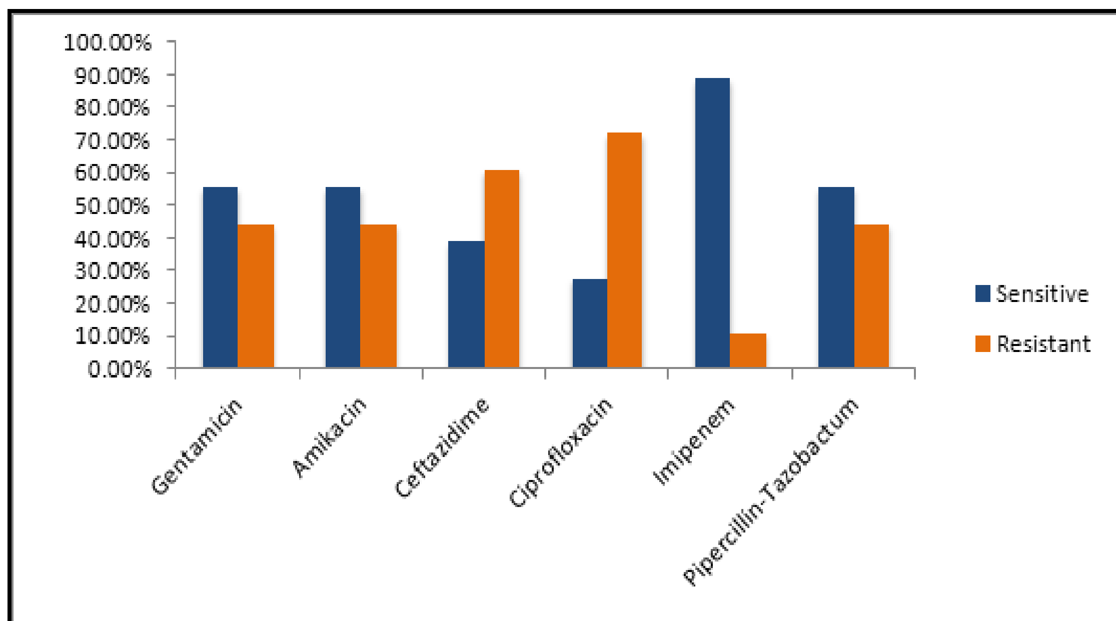


FIGURE 10: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *ENTEROCOCCUS FAECALIS* ISOLATES FROM PATIENTS WITH DOUBLE-J URETERAL STENT PLACEMENT (TABLE 10)

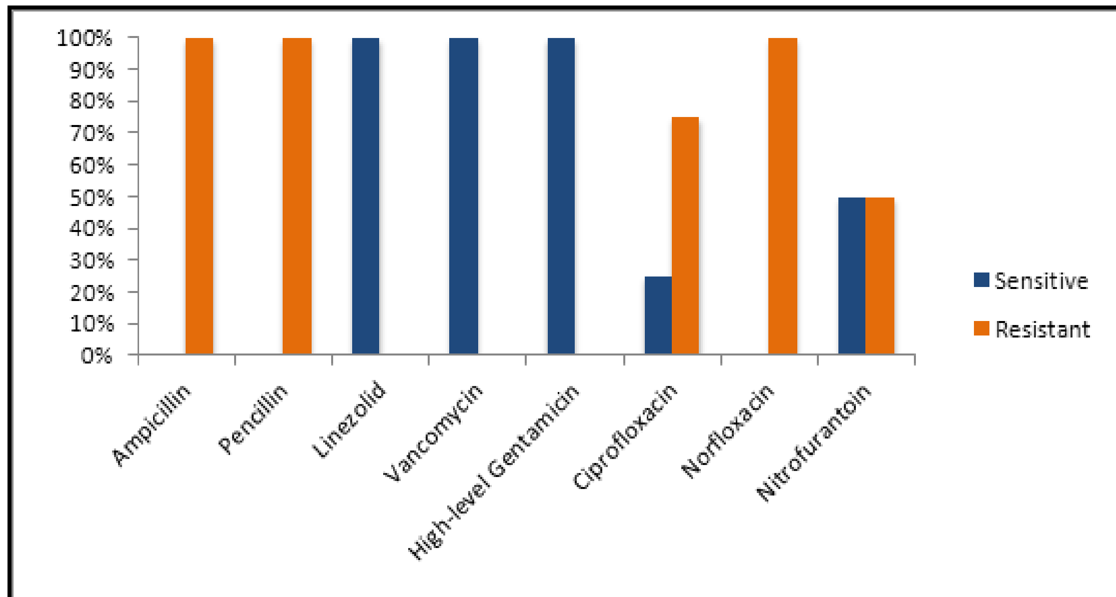


FIGURE-11: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *STAPHYLOCOCCUS AUREUS* ISOLATES FROM PATIENTS WITH DOUBLE-J URETERAL STENT PLACEMENT (TABLE 10)

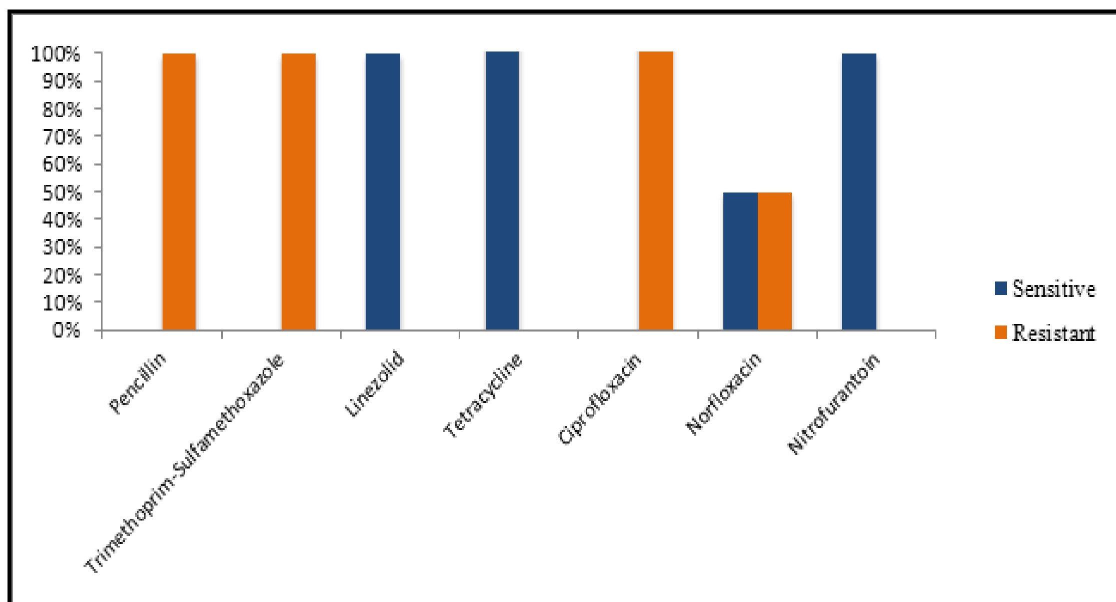


TABLE 11: ANALYSIS OF DISTRIBUTION OF RESISTANCE PATTERN IN GRAM NEGATIVE BACILLI (N=53)

GRAM NEGATIVE BACILLI (n=53)	Extended-Spectrum Beta Lactamases (ESBL)		Metallo-Beta-lactamases (MBL)		AmpC Beta-lactamases	
	N	%	N	%	N	%
Escherichia coli (n=22)	16	72.7	4	18.2	1	4.5
Klebsiella oxytoca (n=11)	6	54.5	1	9.1	2	18.2
Klebsiella pneumoniae (n=2)	1	50%	0	0	0	0
Pseudomonas aeruginosa (n=18)	-	-	2	11.1	3	16.7
Total	23	65.7%	7	13.2%	6	11.3%

Out of 35 isolates tested for ESBL production 23 isolates (65.7%) were found to be ESBL producers. Among the 53 isolates tested, 7(13.2%) were MBL producers and 6 (11.3%) were AmpC β-lactamases producers.

FIGURE-12: DISTRIBUTION OF RESISTANCE PATTERN IN GRAM NEGATIVE BACILLI ISOLATED FROM PATIENTS WITH DOUBLE-J URETERAL STENTS

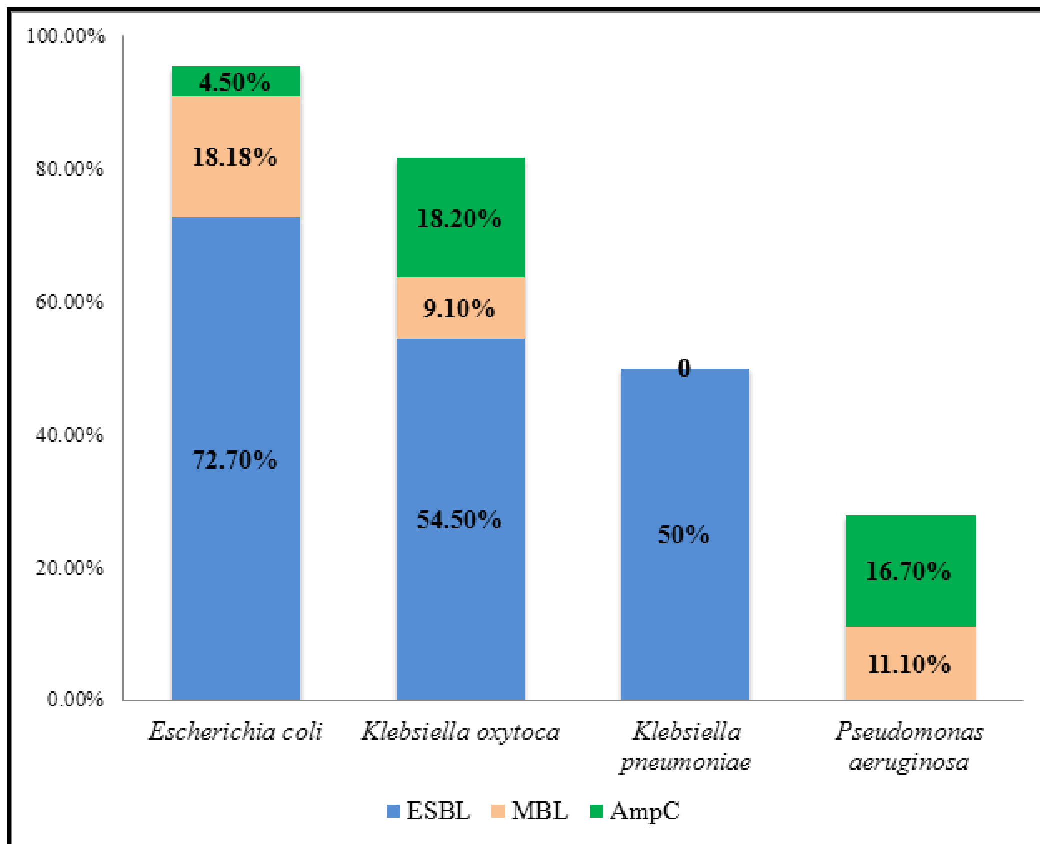


TABLE-12: DETECTION OF RESISTANCE PATTERN OF *STAPHYLOCOCCUS SPP.* BY CEFOXITIN DISC DIFFUSION TEST

Organism	Number of isolates (n)	Methicillin sensitive		Methicillin resistant	
		N	%	N	%
Staphylococcus aureus	4	2	50	2	50
Staphylococcus epidermidis	2	1	50	1	50

Out of the 4 *Staphylococcus aureus* isolates (50%) were methicillin resistant and one *Staphylococcus epidermidis* isolate (50%) was methicillin resistant.

TABLE 13: INTERPRETATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) OF VANCOMYCIN FOR METHICILLIN RESISTANT *STAPHYLOCOCCUS SPP.* BY BROTH MACRODILUTION METHOD

Organism	Number of Methicillin resistant isolates	MIC values µg/ml	Interpretation
Staphylococcus aureus (n=4)	2	≤ 2	Sensitive
Staphylococcus epidermidis (n=2)	1	≤ 4	Sensitive

All the Methicillin resistant *Staphylococcal* isolates were 100% sensitive to Vancomycin.

TABLE-14: INTERPRETATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) OF MEROPENEM FOR IMIPENEM RESISTANT GRAM NEGATIVE BACILLI BY BROTH MACRODILUTION METHOD

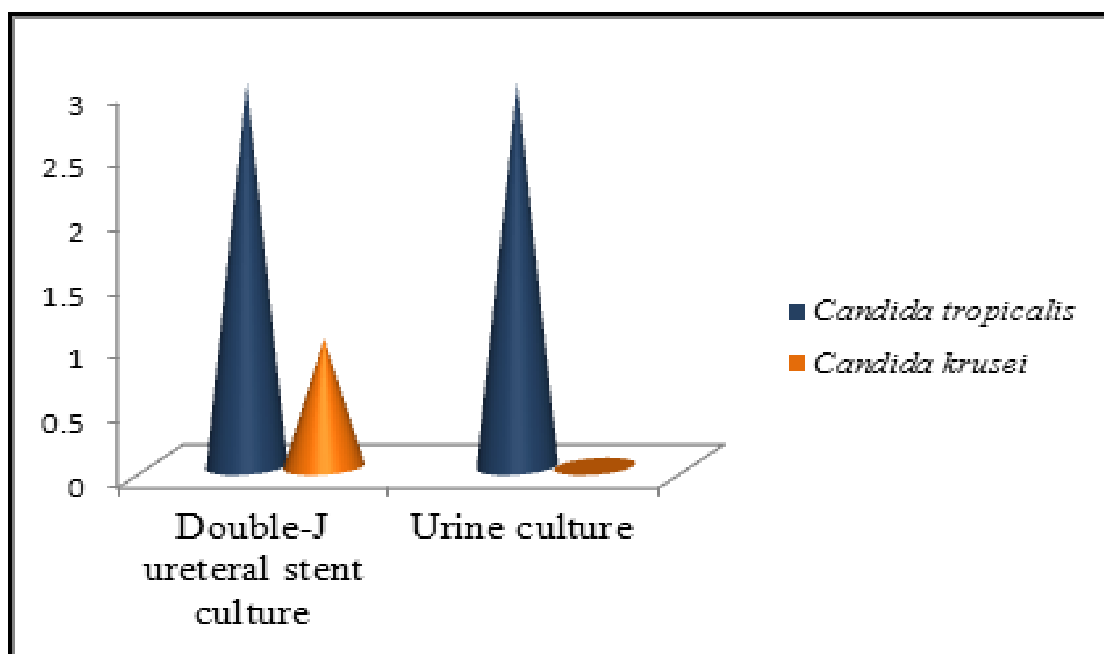
Gram Negative Bacilli	Imipenem resistant isolates	MIC values µg/ml						Interpretation
		≤ 2	4	8	16	32	64	
Escherichia coli (n=22)	3	-	-	-	1	2	-	Resistant
Klebsiella oxytoca (n=11)	2	-	-	-	1	1	-	Resistant
Pseudomonas aeruginosa (n=18)	2	-	-	-	1	1	-	Resistant

All the 7 Imipenem resistant Gram negative bacilli (100%) were found to be resistant to Meropenem.

TABLE-15: DISTRIBUTION OF *CANDIDA* ISOLATES IN DOUBLE-J URETERAL STENT AND URINE CULTURE

Candida species (n=4)	Double-J ureteral stent culture	Urine culture
Candida tropicalis (n=3)	3	3
Candida krusei (n=1)	1	0

FIGURE-13: DISTRIBUTION OF *CANDIDA* ISOLATES IN PATIENTS WITH DOUBLE-J URETERAL STENT



All the three *Candida tropicalis* isolated from patients with Double-J ureteral stent placement were present in both Double-J ureteral stent surface and urine culture. *Candida krusei* was present in Double-J ureteral stent surface only.

TABLE-16: ANTIFUNGAL SENSITIVITY PATTERN OF *CANDIDA SPP*. BY DISC DIFFUSION METHOD

Candida species	Number of isolates	Fluconazole (25µg)		Voriconazole (1µg)	
		S	%	S	%
Candida tropicalis	3	1	33.3%	1	33.3%
Candida krusei	1	0	0	0	0

Only one *Candida tropicalis* isolate was sensitive to both Fluconazole and Voriconazole (33.3%), other 2 isolates of *Candida tropicalis* and one isolate of *Candida krusei* were resistant to both antifungal agents.

TABLE-17: INTERPRETATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) OF AMPHOTERICIN-B FOR *CANDIDA SPP*. BY BROTH MICRODILUTION METHOD

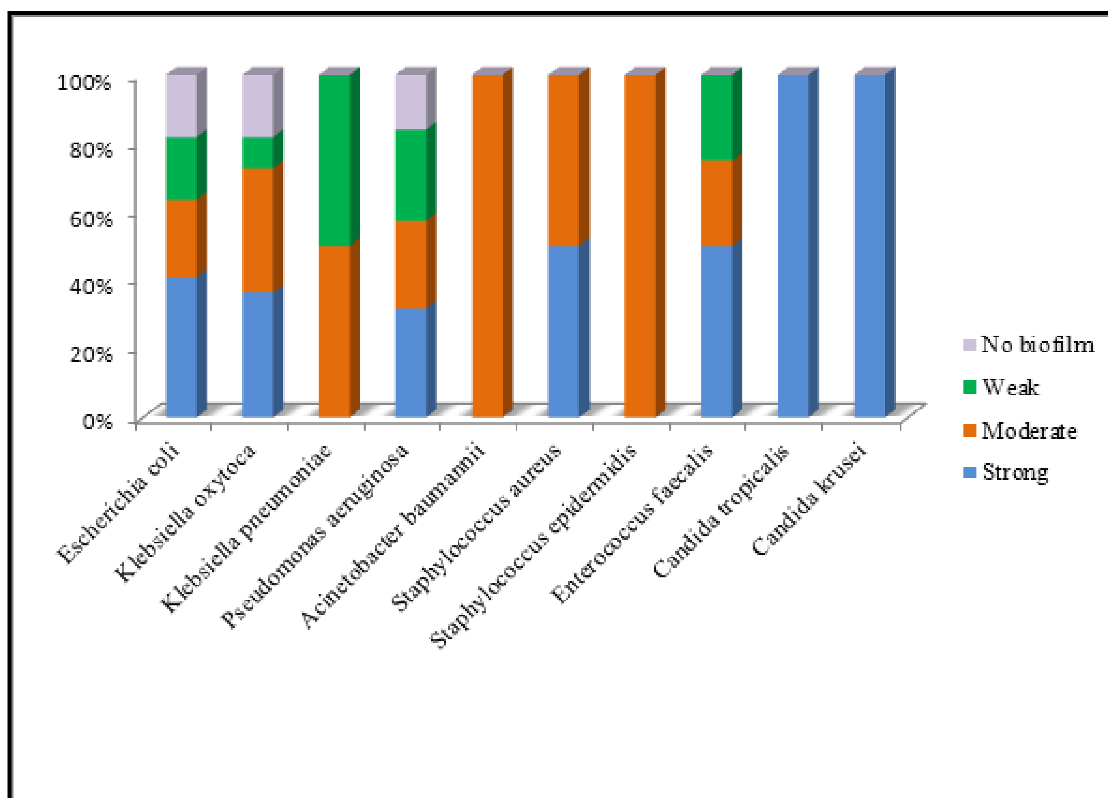
Candida species	Number of Isolates	MIC Values		Interpretation
		Sensitive ≤ 1 µg/ml	Resistant >1 µg/ml	
Candida tropicalis (n=3)	3	3	0	Sensitive
Candida krusei (n=1)	1	1	0	Sensitive

All the four *Candida* isolates were found to be 100% sensitive for Amphotericin B.

TABLE-18: PHENOTYPIC DETECTION OF BIOFILM PRODUCTION BY MICROTITRE PLATE METHOD

Organism	Strong Biofilm Producers	Moderate Biofilm Producers	Weak Biofilm Producers	Non-biofilm producers
Escherichia coli (n=22)	9 (41%)	5(22.7%)	4(18.2%)	4(18.2%)
Klebsiella oxytoca (n=11)	4 (36.4%)	4(36.4%)	1(9.1%)	2(18.2%)
Klebsiella pneumoniae (n=2)	0	1 (50%)	1 (50%)	0
Pseudomonas aeruginosa (n=18)	6(33.3)	4(26.7%)	5(27.8%)	3(16.7%)
Acinetobacter baumannii (n=1)	0	1(100%)	0	0
Staphylococcus aureus (n=4)	2 (50%)	2 (50%)	0	0
Staphylococcus epidermidis (n=2)	0	2 (100%)	0	0
Enterococcus faecalis (n=4)	2 (50%)	1(25%)	1(25%)	0
Candida tropicalis (n=3)	3 (100%)	0	0	0
Candida krusei (n=1)	1 (100%)	0	0	0
TOTAL	27 (39.7%)	20(29.4%)	12(17.6%)	9(13.2%)

Figure-14: Phenotypic Detection of Biofilm Production by Microtitre Plate Method

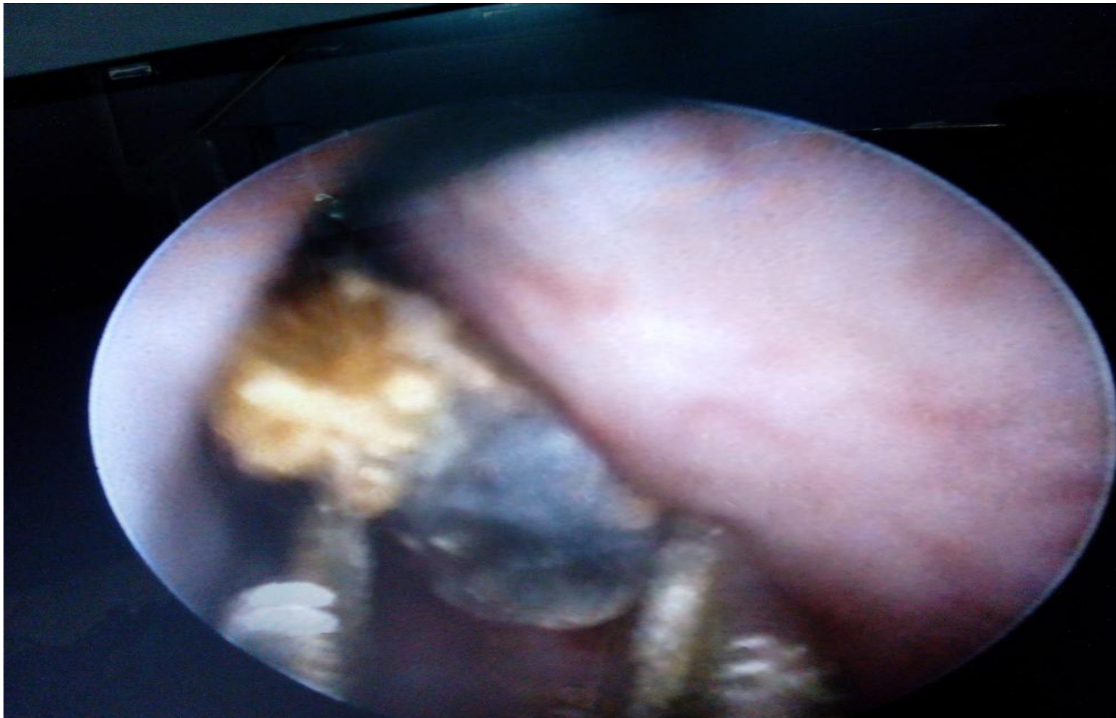


Out of the 68 isolates 59 isolates (86.8%) were biofilm producers and only 9 (13.2%) were non-biofilm producers. All the four *Candida* isolates (100%) were strong biofilm producers.

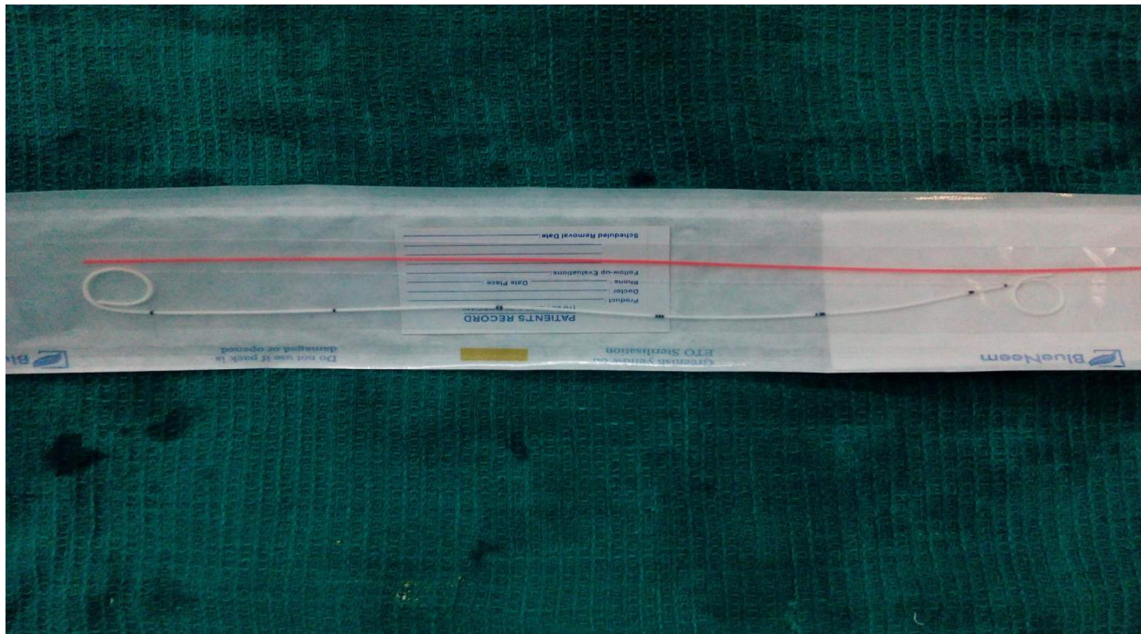
COLOUR PLATE 1: VIDEO ENDOSCOPIC UNIT

COLOUR PLATE 2: URETEROSCOPE

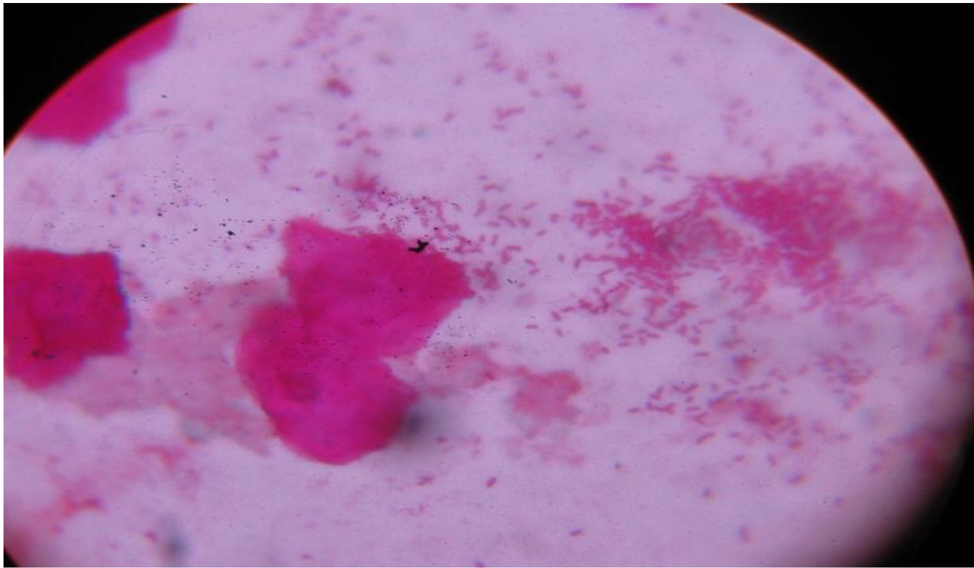
COLOUR PLATE 3: ENDOSCOPIC VIEW OF URETERAL CALCULUS



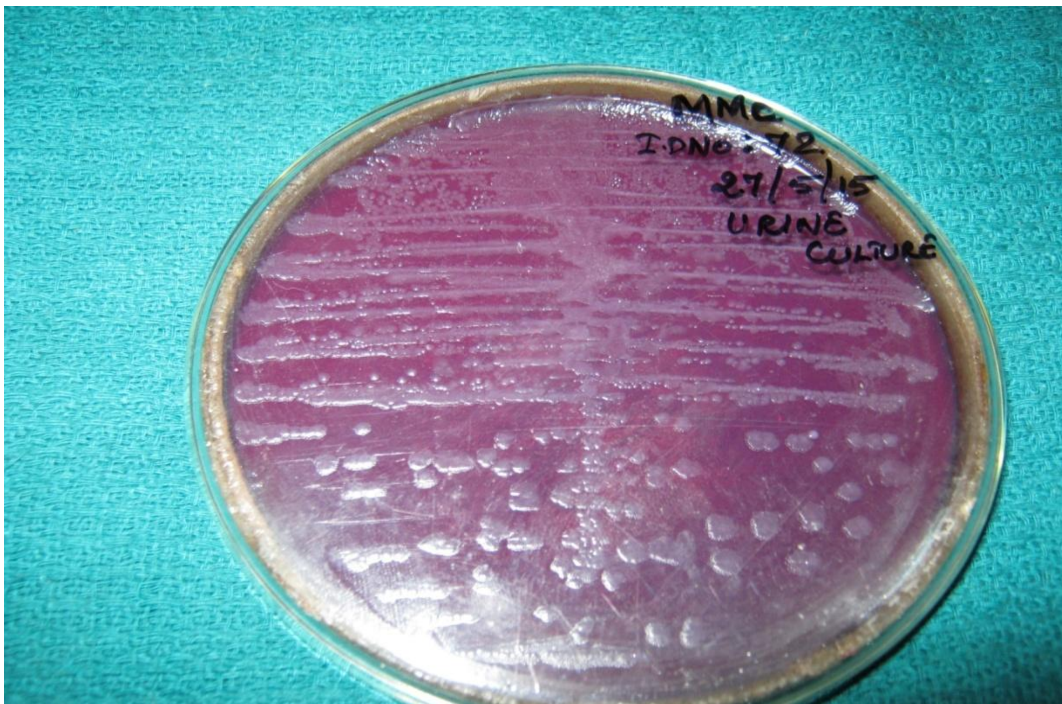
COLOUR PLATE 4: DOUBLE-J URETERAL STENT



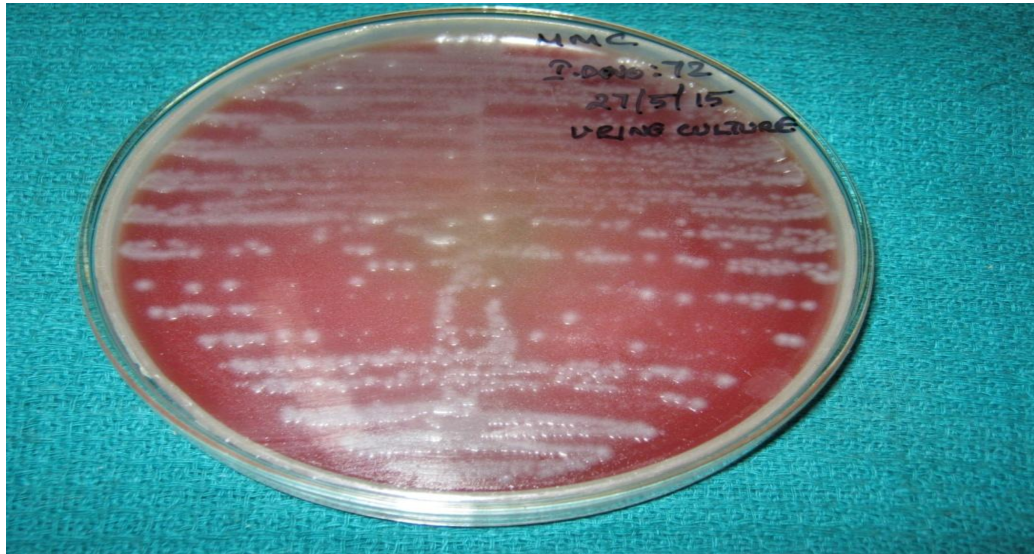
COLOUR PLATE 5: DIRECT GRAM STAIN OF URINE SAMPLE SHOWING PLENTY OF GRAM NEGATIVE BACILLI



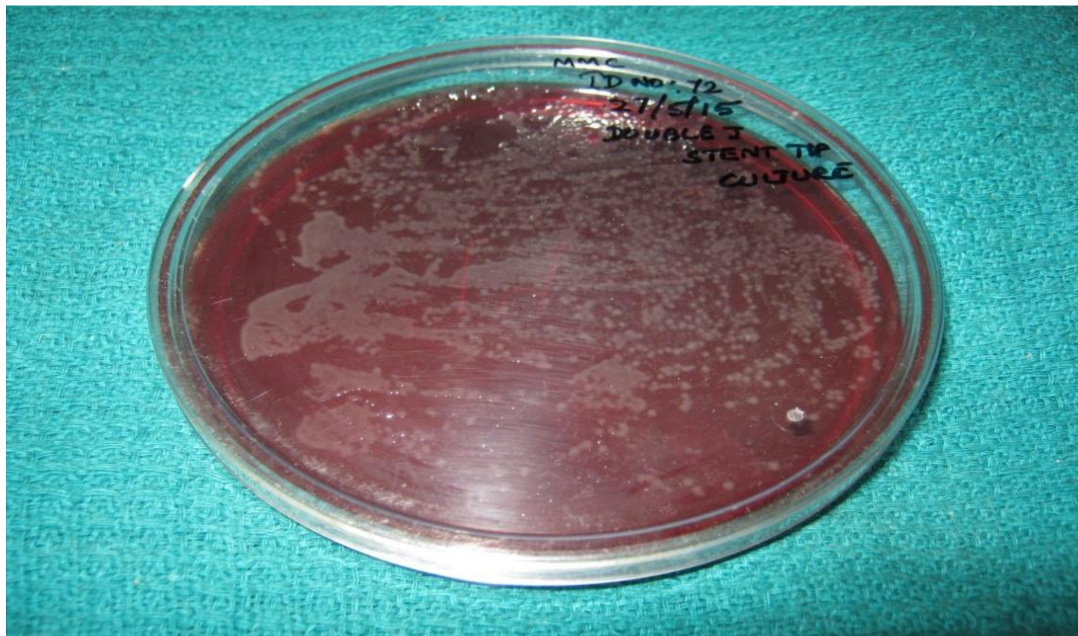
COLOUR PLATE 6: *ESCHERICHIA COLI* COLONIES FROM URINE CULTURE ON MACCONKEY AGAR PLATE



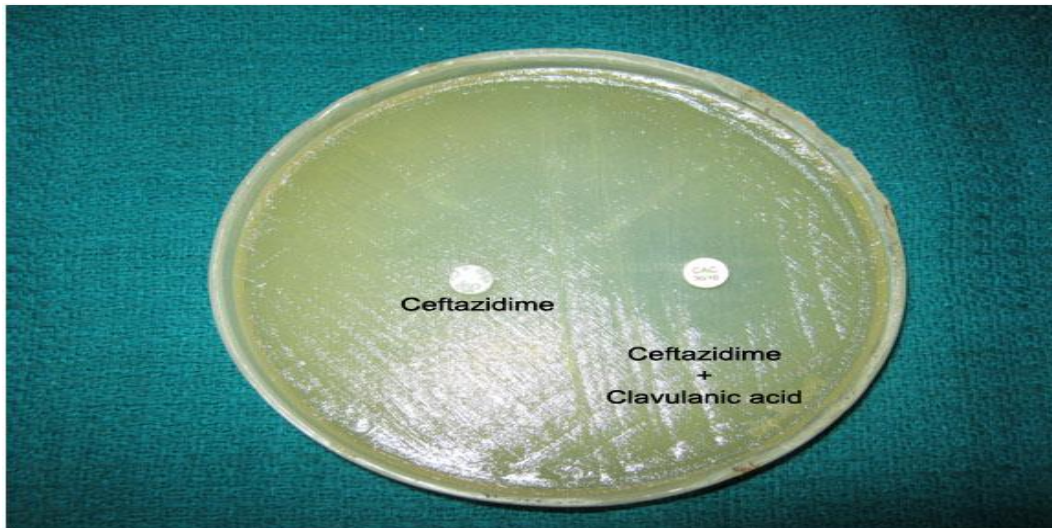
COLOUR PLATE 7: *ESCHERICHIA COLI* COLONIES FROM URINE CULTURE ON 5% SHEEP BLOOD AGAR PLATE



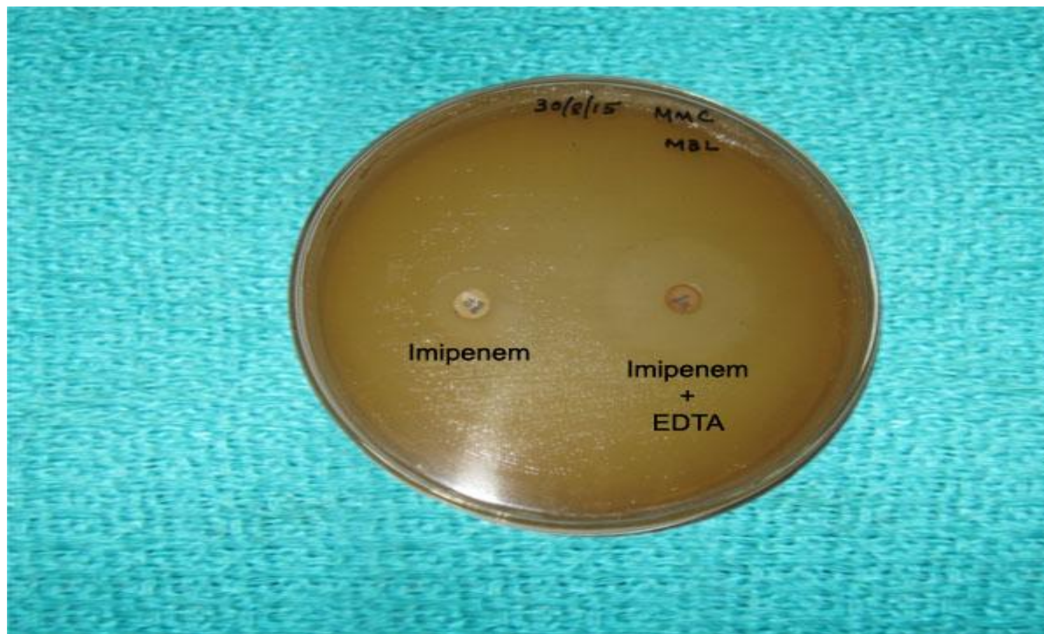
COLOUR PLATE 8: *ESCHERICHIA COLI* COLONIES FROM DOUBLE-J URETERAL STENT CULTURE ON 5% SHEEP BLOOD AGAR PLATE



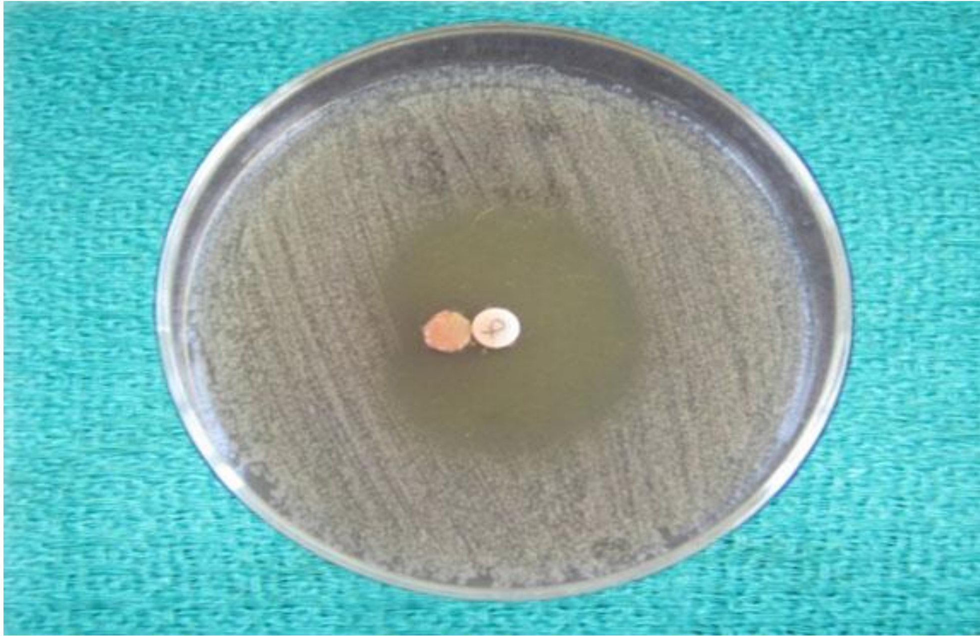
COLOUR PLATE 9: PHENOTYPIC CONFIRMATORY TEST FOR ESBL PRODUCTION



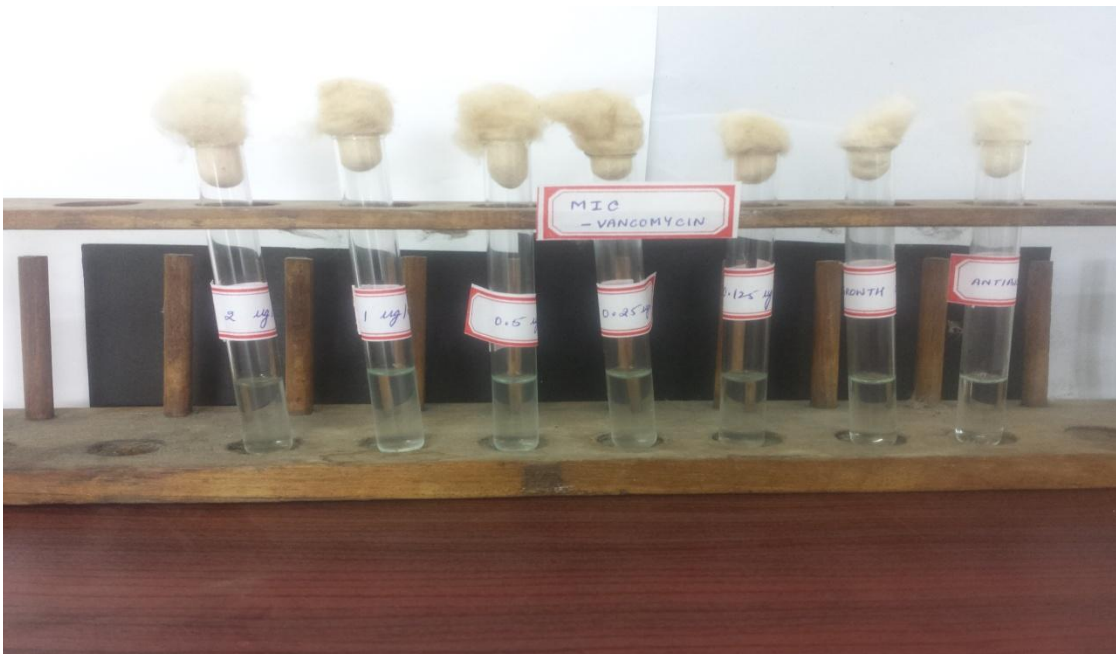
COLOUR PLATE 10: DETECTION OF METALLO-β LACTAMASE PRODUCTION BY DOUBLE DISC SYNERGY TEST



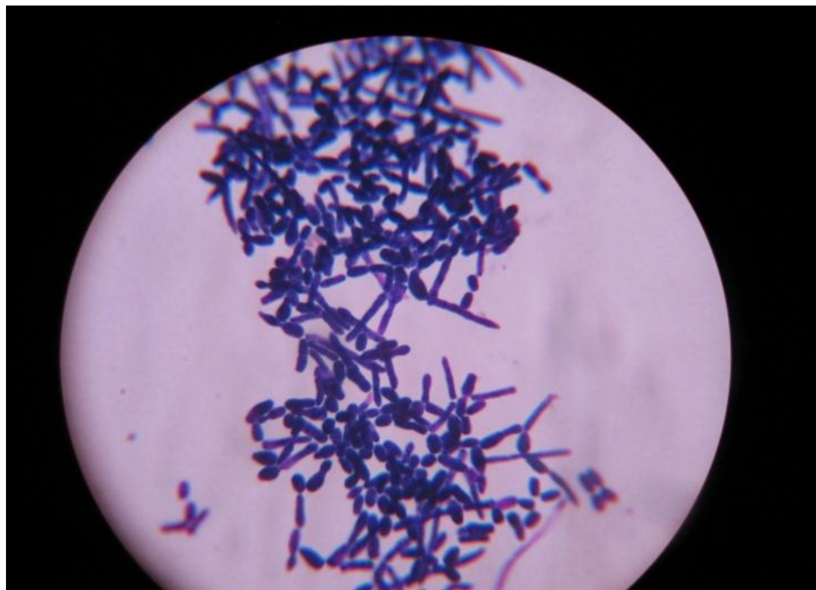
COLOUR PLATE 11: DETECTION OF AmpC-β LACTAMASE PRODUCTION BY DISC TEST



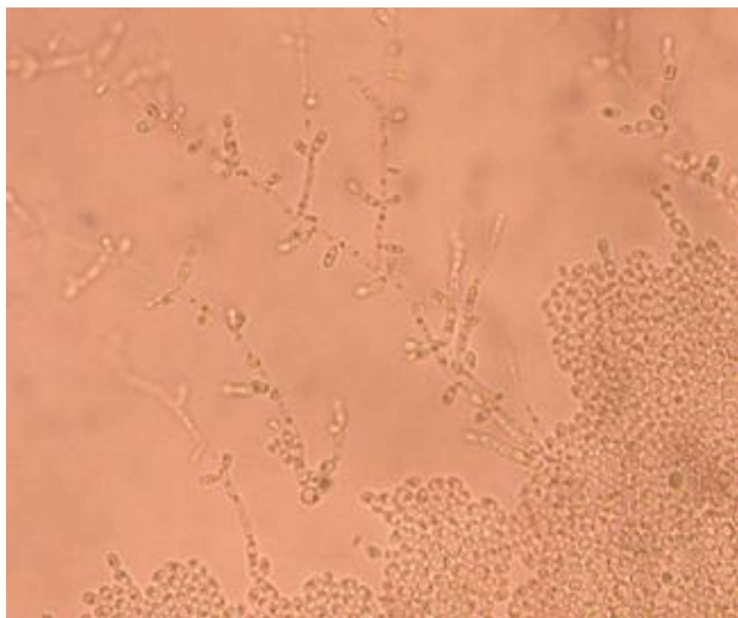
COLOUR PLATE 12: DETERMINATION OF VANCOMYCIN MIC OF MRSA ISOLATE BY BROTH MACRODILUTION METHOD



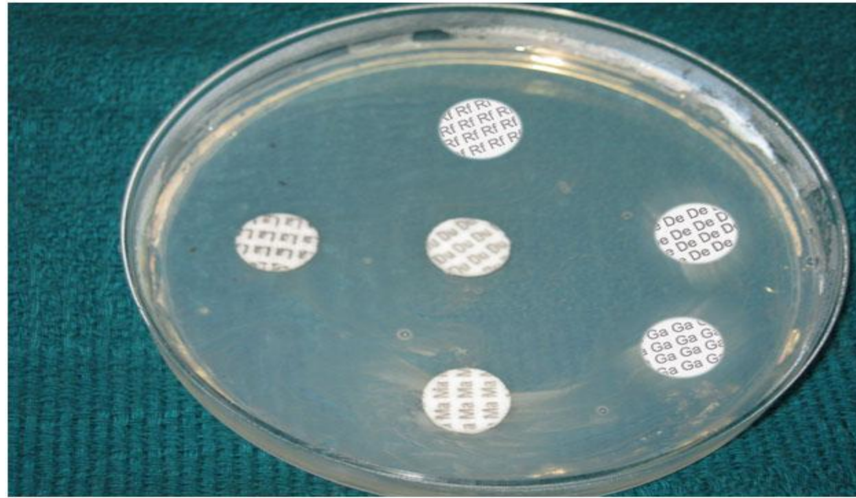
COLOUR PLATE 13: CULTURE SMEAR OF GRAM POSITIVE BUDDING YEAST CELLS

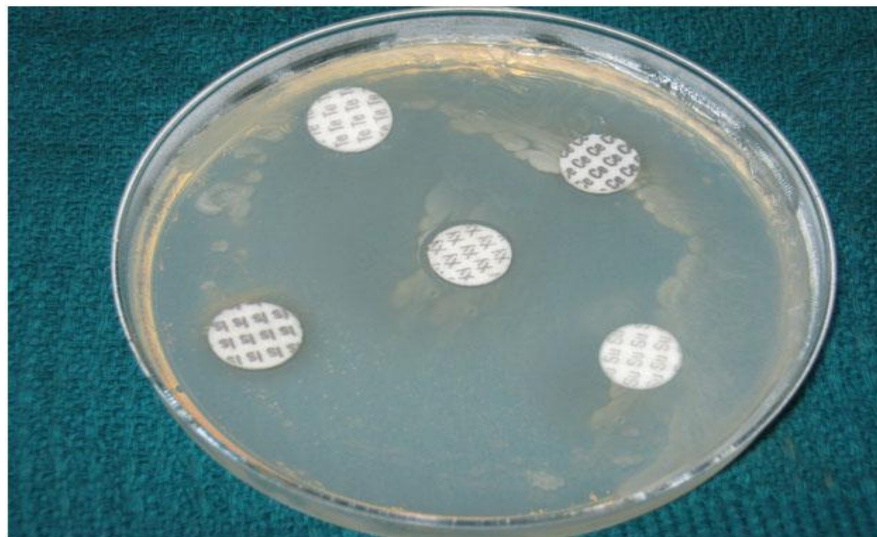


COLOUR PLATE 14:CORNMEAL AGAR MORPHOLOGY OF *CANDIDA TROPICALIS*



COLOUR PLATE 15: SUGAR ASSIMILATION PATTERN OF *CANDIDA TROPICALIS*



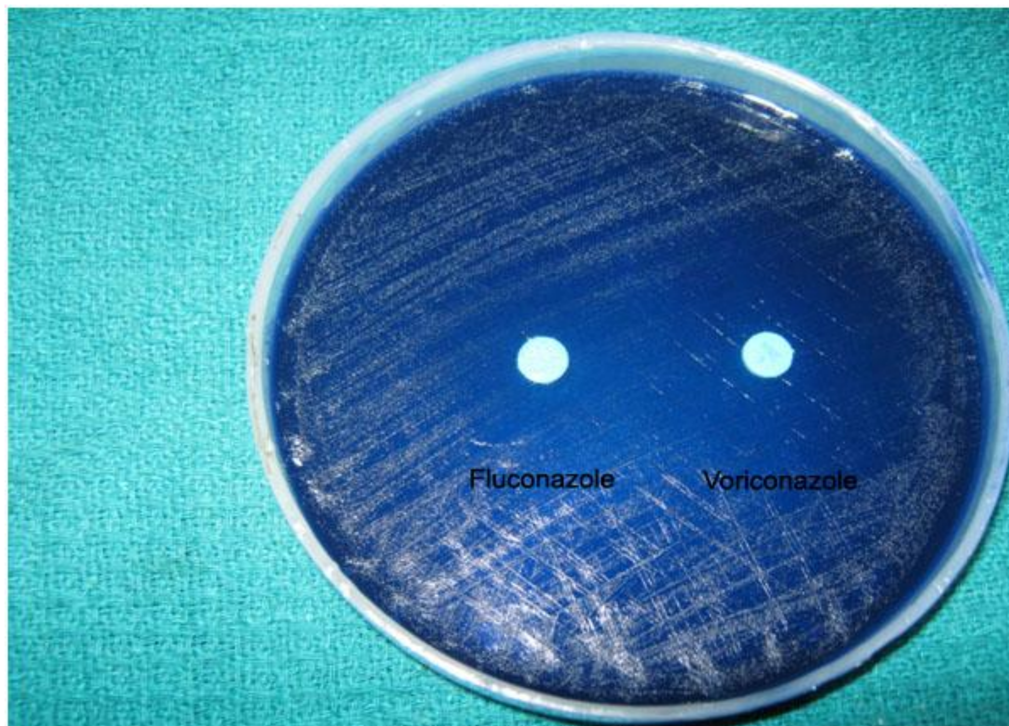


Sugars assimilated are Dextrose (De), Sucrose (Su), Maltose (Ma),Xylose(Xy), Cellobiose(Ce), Trehalose (Te).

Sugars not assimilated are Lactose(La), Raffinose(Rf), Inositol(Is), Dulcitol(Du) .

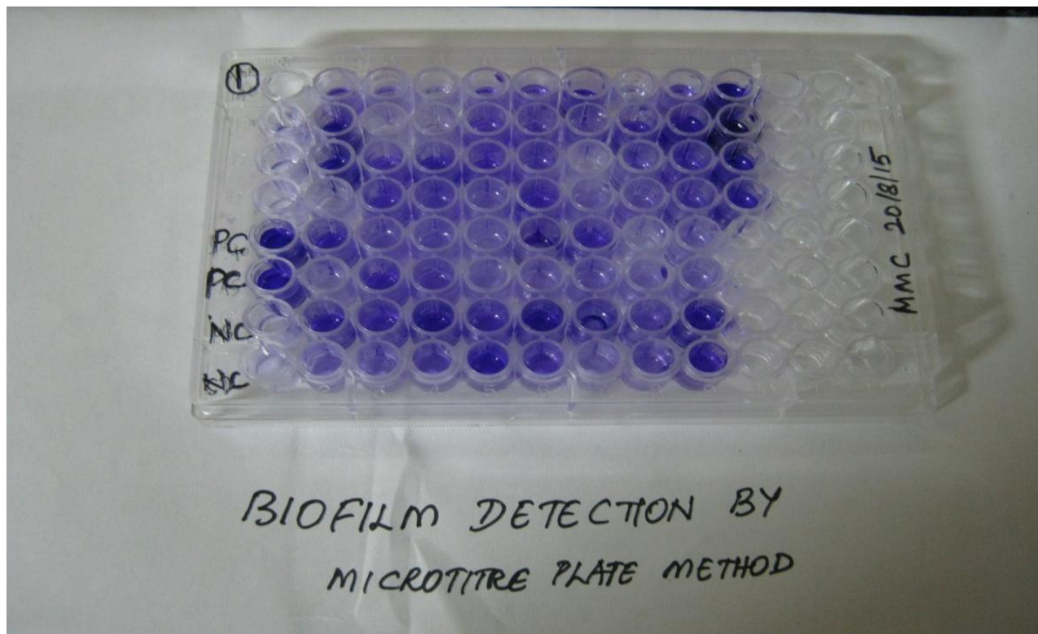
COLOUR PLATE 16: *CANDIDA* ISOLATES ON CHROM AGAR

COLOUR PLATE 17: ANTIFUNGAL SUSCEPTIBILITY PATTERN BY DISC DIFFUSION METHOD



COLOUR PLATE 18: DETERMINATION OF AMPHOTERICIN B MIC BY BROTH MICRODILUTION METHOD

COLOUR PLATE 19:PHENOTYPIC DETECTION OF BIOFILM PRODUCTION BY MICROTITRE PLATE METHOD



DISCUSSION

One of the most common indications for Double-J ureteral stent placement is for relief of ureteral obstruction due to ureteral stones. The widespread use of Double-J ureteral stents has lead to an increase in complications, including infection, encrustation, stone formation, and fragmentation. [38] Ureteral stents are synthetic biomaterials and like any other catheter that dwells in the urinary tract, it also provides a suitable surface for bacterial colonization and development of biofilm. Microbial ureteral stent colonization (MUSC) plays an essential role in the pathogenesis of stent-associated infections.

In this study, out of 100 patients treated with Double-J ureteral stents for ureteral obstruction, 52 were males and 48 were females. Majority of the patients (52%) were in the age group of 31to 50 years, out of which 27 were males and 25 were females (Table 1).

In the study group of 100 patients treated with Double-J ureteral stents for ureteral obstruction, culture of the Double-J ureteral stent surface showed MUSC in 67% of the patients, while only 36% of the patients with colonization had positive urine culture. Both urine and Double-J stent culture showed no growth in 33% of the patients. The rate of Double-J ureteral stent colonization (67%) was much higher than the rate of urinary tract infection (36%) in our study (Table 2).

In this study it was analyzed and found that urine culture was positive in only those patients in whom Double-J ureteral stent colonization was present.

The results were similar to Farsi and colleagues, 1995 who noted in their prospective study that urine cultures were positive in 29.9%, while stent colonization was found in 67.9%.[97] There are different rates of bacterial stent colonization and bacteriuria reported in the literature. The observations made by Kehinde *et al* in 2002, point to more than 2.5-fold higher risk of stent colonization than urinary tract infection. [98] Klis *et al* in their 2014 study noted that the rate of stent colonization was 100% and was 8-fold higher than the incidence of bacteriuria (13.3%). [99] Al-Ghazo *et al* 2010 considered that the lower rate of stent colonization (24.2%) and bacteriuria (22.5%) noted in their study might be related to the use of ciprofloxacin, a broad spectrum antibacterial drug used for 5 days as prophylactic therapy before Double-J ureteral stent insertion.[100]

The differences in the rate of Double-J stent colonization and urine infection may be due to varying sample size, age and gender distribution of the patients, patients with concomitant illness, type of ureteral stent inserted, indication for stent insertion as a elective or emergency procedure, use of prophylactic antibiotic therapy and different methods used for colonization estimation such as culture of the

stent or by molecular DNA analysis.. Studies by Bonkat *et al* 2011 and Klis *et al* 2014 have shown that there is a great inconsistency between microbial ureteral stent colonization and urine infection, indicating a low predictive value of urine culture for estimating ureteral stent colonization. [59, 99]

Present study shows that there was no relationship between age of the patient Double-J ureteral stent colonization and positive urine culture (Table 3). Yeniyol *et al* 2002, Akay and co-workers in 2007, Ozgur *et al* 2013 and Al-Ghazo *et al* 2010 also reported no significant relationship between age of the patient with infection of urinary tract and stent colonization in patients with Double-J ureteral stents. [101-103,100]

In this study, gender of the patients had statisticially no significant effect on bacteriuria and Double-J ureteral stent colonization (Table 4). Observations made by Klis *et al* 2014 also showed that gender of the study group was not related to urine culture and colonization of Double-J ureteral stent surface.[99] The results of our study are also comparable with the studies done in Turkey by Yeniyol *et al* 2002 and Akay *et al* 2007.[101,102] Farsi and his collegues 1995 and Bonkat *et al* 2011 in their studies reported female gender as a risk factor for stent colonization.[97, 59] The shorter urethra and close proximity of urethral

opening to the vagina and anus in females makes them more susceptibile to UTI.

The commonest indication for stenting the urinary tract in this study was following ureteroscopic (URS) stone extraction /Lithotripsy (62%), followed by stenting after PCNL (17%) and prior to ESWL (14%) (TABLE 5). Al-Ghazo *et al* 2010 also reported that the major indication for Double-J stent insertion in their study was for stone disease in which 45% of the patients were treated with Double-J stent following URS stone extraction / Lithotripsy, 25% of cases prior to ESWL and 15% of cases after PCNL.[100] Similarly Ozgur *et al* 2013 in their study on 130 patients found that 91 patients (70%) were treated with Double-J ureteral stents following stone fragmentation and to prevent ureteral edema.[103]

This study showed that longer the duration of stenting, the higher the rate of colonization. The rate of Double-J ureteral stent colonization was 56.9%, 88.8% and 100% when the duration of ureteral stent placement was less than 4 weeks, 4-6 weeks, and more than 6 weeks respectively. Urine culture was found to be positive in 16.6%, 77.8% and 100% of the patients when the retention of Double-J ureteral stent was less than 4 weeks, 4-6 weeks, and more than 6 weeks respectively. In this study statistical analysis revealed significant correlation between

Double-J stent colonization, urine culture and duration of stent placement (Table 6).

Similar results were seen in the study by Lojanapiwat from Thailand 2006 who reported positive Double-J ureteral stent colonization in 33%, 50%, and 54% of study population, when the insertion period was less than 4 weeks, 4-6 weeks, and more than 6 weeks respectively.[104] Urine culture was found to be positive in 75%, 61% and 82% of the patients when the indwelling time was less than 4 weeks, 4-6 weeks, and more than 6 weeks respectively.[104] Rahman et al 2010 from Bangladesh also reported that colonization rate was 71.4% in stents removed after 6 weeks compared to 33.3% in 4-6 weeks and 23.5% before 4 weeks.[105] Ozgur *et al* 2013 also observed that rate of colonization was 2.2%, 2.9% and 25% when the indwelling period of Double-J ureteral stent was less than 4 weeks, 4-6 weeks, and more than 6 weeks respectively.[103]

Paick *et al* 2003 reported in his trial that bacterial colonization begins 2 weeks after stenting. [106] The infection and colonization induction by internal ureteral stents are due to the introduction of the bacteria during stenting, entry of bacteria through the urethral meatus and ability of the bacteria to rise through a column of fluid. [107] Coskun *et al* 2011 stressed that early removal of the ureteral stent, 2 weeks after renal transplantation, decreased the rate of urinary tract infections.[108]

Out of the 68 isolates identified in our study from the colonized Double-J ureteral stent surface, the predominant pathogen isolated was *Escherichia coli* (32.4%), followed by *Pseudomonas aeruginosa* (26.5%) and *Klebsiella oxytoca* (16.2%). Only two isolates of *Klebsiella pneumoniae* and one isolate of *Acinetobacter baumannii* were isolated from Double-J ureteral stent. Among the Gram positive cocci 5.9% each of *Staphylococcus aureus* and *Enterococcus faecalis* were isolated. Out of the 5.9% of *Candida spp.* isolated, three were *Candida tropicalis* and one was *Candida krusei* (Table 7). In this study all except one stent were colonized by a single microorganism. Only one stent was colonized by two bacterial species namely *Klebsiella pneumoniae* and *Staphylococcus epidermidis*.

The results of our study are comparable with the study done in Thailand by Lojanapiwat 2006 who also observed *Escherichia coli* as the most common pathogen (34.6%) followed by *Pseudomonas aeruginosa* (17.3%).[104] Al-Ghazo *et al* 2010 from Jordan also isolated *Escherichia coli* (51.7%) as the predominant pathogen.[100] The study from Nepal by Joshi *et al* 2011 also noted *Escherichia coli* as the commonest pathogen (14.89%) followed by *Klebsiella* and *Acinetobacter spp*. (4.25%).[109] *Escherichia coli* is the predominant pathogen in most of the studies because it possesses several virulence factors like fimbrial protein, fim H which binds to Tamm-Horsfall

protein (THP). THP has been found to bind to ureteral stent surface and may act as a promoter of MUSC. [110]

In contrast Bonkat *et al* 2011 from Switzerland found *Coagulase negative staphylococcus* (CoNS) (18.3%) and *Enterococcus spp.* (17.9%) as the predominant pathogens by sonicate fluid culture. [59] *Enterococcus spp.* was reported as the predominant isolate by Reidl *et al* 1999 (51%) and Paick *et al* 2003 (24%), while *Pseudomonas aeruginosa* (23%) was the most common pathogen isolated from the Double-J ureteral stent by Farsi and colleagues, 1995. [111, 106, 97]

CoNS which frequently colonize the skin and mucus membrane were previously regarded as non pathogens but now they are recognized as the most frequent cause of biofilm-associated infections, as any medical device that penetrates these surfaces during surgery is at high risk to become colonized by CoNS. The variation in the type of isolated microorganisms in these studies may be due to the variations in the spectrum of pathogens in different hospitals and countries, the study population and the diagnostic technique used for identification.

In the present study, *Escherichia coli* (34.2%), was the most common pathogen isolated from urine culture, followed by *Pseudomonas aeruginosa* (26.5%). Among the Gram positive cocci *Enterococcus faecalis* (7.9%) was the most common isolate. *Candida tropicalis* (7.9%) was the only fungus isolated from the urine samples.

In all patients with significant bacteriuria, the same organism was also isolated from the Double-J ureteral stent in pure culture. In two patients mixed culture were isolated from urine samples in addition to the same organism isolated from Double-J stent which were identified as *Staphylococcus* e*pidermidis* and *Enterococcus faecalis* (Table 8).

The results were similar to the studies of Al-Ghazo *et al* 2010 and Yeniyol *et al* 2002 which also isolated *Escherichia coli* as the predominant pathogen 51.9% and 80% respectively in urine and observed same microorganisms in ureteral stent surface and urine culture. [100, 101] Joshi *et al,* 2011 also reported *Escherichia coli* as the predominant isolate (11.6%) from urine culture in patients with ureteral stents. [109]

The antibiotic susceptibility pattern of the Gram negative bacilli in the present study, determined by the Kirby –Bauer disc diffusion method showed that the isolates were sensitive to Nitrofurantoin (57.1%), Amikacin (50%), and Tetracycline (47.2%), Norfloxacin (46.2%) and least susceptibility to Ofloxacin (25%) and Ciprofloxacin (24.1%). All the Gram negative bacilli were highly susceptible to Imipenem (84.9%) and Piperacillin-Tazobactum (56.6%). All the isolates from Double-J ureteral stent surface and positive urine culture had same susceptibility pattern. (Table 9)

Pseudomonas aeruginosa was more susceptible to Norfloxacin (55.6%), Amikacin and Gentamicin (55.6%) and Ceftazidime (38.9%) compared to *E.coli* and *Klebsiella spp*. *Escherichia coli* was least susceptible to Ampicillin (4.5%).As per CLSI guidelines, Ampicillin was tested only for *Escherichia coli,* as other Gram negative bacilli are intrinsically resistant to Ampicillin. A single isolate of *Acinetobacter baumannii* was sensitive to the panel of antibiotics tested.

The Gram positive cocci were highly susceptibile to Linezolid (100%), followed by Tetracycline (80%), Nitrofurantoin (70%) and were least susceptible to Ciprofloxacin (20%) and Trimethoprim-sulfamethoxazole (16.7%). None of the Gram positive cocci were sensitive to Penicillin. All the four *Staphylococcus aureus* isolates were highly susceptible to Nitrofurantoin and Tetracycline (100%). The four *Enterococcus faecalis* isolates were 100% sensitive to Vancomycin and High-level Gentamicin. (Table 10)

The results are similar to study by Chatterjee *et al* 2014 who reported Vancomycin (63%) and Imipenem (81.6%) as the most sensitive drug in Gram positive and Gram Negative bacteria.[112] Manjunath *et al* 2011 from Bangalore also reported high reistance of *E.coli* isolated from urine to Ampicillin (<90%). *Pseudomonas spp*. was more resistant to Ofloxacin (81%), Ciprofloxacin (85%) and Imipenem (72%).[113] Our results were also comparable with the study done in

Rajasthan by Dalela *et al* 2012 which showed maximum sensitivity of Gram negative bacilli for Imipenem (95.1%), followed by Piperacillin/Tazobactum (71.8%), Amikacin (66.9%) and Nitrofurantoin (54.2%). Their study also documented 100% sensitivity of Gram positive cocci for Vancomycin and Linezolid. [114]

Escherichia coli (72.7%) was the major ESBL producer in our study, followed by *Klebsiella oxytoca* (54.5%). AmpC β-lactamase production was more in *Klebsiella oxytoca* (18.2%) and *Pseudomonas aeruginosa* (16.7%). Metallo-Beta lactamase production was high in *Escherichia coli* (18.2%) and *Pseudomonas aeruginosa* (11.1%). (TABLE 11)

The results of this study coincides with the study by Dalela *et al* 2012 where they reported among the uropathogens maximum ESBL activity was in *Escherichia coli* (73.5%). [114] Chakraborty *et al* 2013 analyzed drug resistant extra intestinal *Escherichia coli* isolates and stated that among the isolates from urology ward 15% were ESBL producers and13% were AmpC producers which were also highly resistant to fluoroquinolones and sulfonamides.[115]

Cefoxitin is a surrogate marker to detect mec-A mediated Oxacillin resistance. Screening for Methicillin resistance with Cefoxitin disc showed, 50% of *Staphylococcus aureus* and 50% of *Staphylococcus* e*pidermidis* isolates were resistant to Cefoxitin disc in this study. (Table

12).The results were consistent with that of Dalela *et al* 2012 which states that Methicillin resistance *Staphylococcus aureus* was seen in 41.4% of urinary isolates. [114]

Susceptibility to Vancomycin was determined by Broth macrodilution method and all the three Methicillin resistant *Staphylococcal* isolates were 100% sensitive to Vancomycin. The break point concentrations of the *Staphylococcus aureus* isolates were 1µg/ml and 2µg/ml and that of *Staphylococcus epidermidis* was 4µg/ml (Table 13).

This finding is similar to Al-Hassanwai et al 2012 from Iraq which reported out of the 3 *Staphylococcus aureus* isolated from urine samples 2 were MRSA and all were 100% sensitive to Vancomycin.[116]

Susceptibility to Meropenem was determined by Broth macrodilution method and all the Imipenem resistant Gram negative bacilli showed 100% resistance to Meropenem. All the imipenem resistant isolates were also MBL producers. (Table 14)

The results of this study agree with Boera et al 2014 which reported that 75.6% and 71.9% of MBL producing *Escherichia coli* and *Klebsiella pneumoniae* isolated in their study were all resistant to Imipenem and Meropenem.[117] The study by Mobashshera et al 2015

from Mumbai reported that strains moderately resistant to carbapenems were also MBL producers.[118]

All the three (100%) Candida tropicalis isolates were isolated from both Double-J ureteral stent surface and urine culture, while *Candida krusei* was isolated only from Double-J ureteral stent culture. (Table 15)

Antifungal susceptibility pattern of *Candida spp.* to Fluconazole and Voriconazole was determined by disc diffusion method showed that only one isolate *Candida tropicalis* was sensitive (33%) to both the antifungal agents. *Candida krusei* is inherently resistant to Fluconazole, this study also showed similar result. (Table 16)

Omar *et al* 2008 also reported that *Candida spp.* isolated from patients with obstructive uropathy were highly resistant to Fluconazole (55.7%) and Voriconazole.[119] Kojic and Darouchie in 2004 have stated that indwelling medical devices can support *Candida* colonization and biofilm formation, and that the biofilm cells are relatively resistant to antifungal treatment.[120]

Susceptibility to Amphotericin B was determined by Broth microdilution method and all the four *Candida* isolates were found to be sensitive (100%). The range of antifungal concentration for Amphotericin B was 0.5 -1µg/ml. (Table 17)

The study by Omar *et al* 2008 from Egypt, also stated that Amphotericin B was an excellent antifungal drug with low MIC values for the susceptible strains of *Candida tropicalis* (0.25-1µg/ml) but with reduced activity to *Candida krusei*.[119]

Phenotypic detection of biofilm production of the microorganisms isolated from the Double-J ureteral stent culture by the microtitre plate method showed that 39.7% isolates were strong biofilm producers, 29.4% and 17.6% were moderate and weak biofilm producers respectively in this study. Among the Gram negative bacilli *Escherichia coli* was the predominant (41%) strong biofilm producer, followed by *Pseudomonas aeruginosa* (33.3%). 50% of the *Staphylococcus aureus* isolates were strong biofilm producers . All the four (100%) *Candida* isolates were strong biofilm producers. (Table 18)

Our results were comparable with that of Chatterjee *et al* 2014 who analyzed the biofilm formation on urological devices by microtitre plate method. They observed that all the Double-J ureteral stents (100%) analyzed by them showed monobacterial colonization and each of them was a biofilm producer in-vitro.[112] Omar *et al* 2008 revealed that biofilm formation by *Candida* isolates was higher in obstructive uropathy patients compared to simple UTI and also observed high intensity of biofilm formation in *Candida krusei* isolates.[119]

SUMMARY

1) In this study, among 100 patients with ureteral obstruction due to urolithiasis, Double-J ureteral stents were placed in majority of patients (62%) following Ureteroscopic stone extraction /Lithotripsy.

2) Majority of patients belonged to the age group of 20-30 years and 80% were under 50 years in this study.

3) In this study, males (52%) were commonly affected with urolithiasis and had undergone Double-J ureteral stents.

4) Out of the one hundred patients who were treated with Double-J ureteral stents for ureteral obstruction, 67% of patients had Double-J ureteral stent colonization and only 36% had urinary tract infection.

5) Age and gender of the patient had no statistically significant relationship with colonization of Double-J ureteral stent in this study.($p>0.946$)

6) The rate of colonization of the Double-J ureteral stent was 56.9%, 88.8% and 100% when the retention of Double-J ureteral stent in urinary tract was less than 4 weeks, 4-6 weeks, and more than 6 weeks respectively.

7) The duration of retention of Double-J ureteral stent in the urinary tract had a statistically significant influence on the rate of colonization of the Double-J ureteral stent and bacteriuria ($p < 0.001$).

8) *Escherichia coli* was the predominant pathogen (32.4%) isolated from Double-J stent culture as well as from the culture of urine samples (34.2%) from patients treated with Double-J ureteral stent.

9) The Gram negative bacilli isolated were sensitive to Nitrofurantoin(57.1%) Amikacin (50%), Tetracycline (47.2%) and Norfloxacin (46.2%). Majority of the Gram negative bacilli were sensitive to Imipenem (84.9%) followed by Piperacillin-tazobactum (56.6%).

10) Out of the 10 Gram positive isolates from colonized Double-J ureteral stents, *Staphylococcus aureus* and *Enterococcus faecalis* were the common isolates. The Gram positive cocci were highly sensitive to Linezolid (100%), Tetracycline (80%) followed by Nitrofurantoin (70%) and least susceptible to Ciprofloxacin (20%) and Trimethoprim-sulfamethoxazole (16.7%).None of the Gram positive cocci isolated were sensitive to Penicillin.

11) Among the Gram negative bacilli 65.7% of the isolates were ESBL producers, 13.2% were MBL producers and 11.3% were AmpC producers. Majority of *Escherichia coli* isolates (72.7%) were ESBL producers.

12) Minimum inhibitory concentration of Meropenem determined by broth macrodilution method showed that all the Imipenem resistant Gram negative bacilli were also resistant to Meropenem.

13) Two isolates of *Staphylococcus aureus* and one *Staphylococcus epidermidis* isolate were found to be Methicillin resistant. All the three isolates were sensitive to Vancomycin (100%) determined by broth macrodilution method.

14) Antifungal susceptibility pattern by disc diffusion method showed that 33.3% of *Candida tropicalis* were sensitive to both Fluconazole and Voriconazole and one isolate of *Candida krusei* was resistant to both Fluconazole and Voriconazole.

15) Antifungal susceptibility pattern by broth microdilution method showed all the fungal isolates (100%) were sensitive to Amphotericin B.

16) Biofilm production by the microorganisms isolated from the Double-J stent surface was detected by microtitre plate method and 86.8% of the isolates were found to be biofilm producers.

17) Among the biofilm forming microorganisms 39.7% of the isolates were strong biofilm producers.

18) Among the Gram negative bacilli *Escherichia coli* was the strong biofilm producer (41%).

19) Among Gram positive cocci (50%) *Staphylococcus aureus* were strong biofilm producers and *Staphylococcus epidermidis* was identified as a moderate biofilm producer.

20) *Candida tropicalis* and *Candida krusei* were strong biofilm producers.

CONCLUSION

Double-J ureteral stent retention in the urinary tract is associated with an extremely high risk of bacterial colonization. A strong correlation has been identified between urine infection and Double-J stent colonization. As microbial ureteral stent colonization does not necessarily lead to bacteriuria, negative urine culture does not rule out biofilm formation, hence urine culture has a low predictive value for ureteral stent colonization. Since undetected biofilms may still serve as a reservoir of pathogenic microorganisms, this assumes clinical significance.

According to this study Double-J ureteral stent has to be ideally retained for less than four weeks, if it exceeds four weeks increased incidence of bacterial colonization of stent occurs and UTI is inevitable. In certain conditions where Double-J ureteral stent has to be retained for more than four weeks, it is better to start the patient on broad spectrum antibiotics to prevent further UTI. Further prospective studies are needed to determine the optimal duration for Double-J stent placement after various urological procedures.

With increasing number of biomaterial devices used in urology, having an effective method for preventing biofilm formation is of utmost importance. The ideal ureteral stent biomaterial is yet to be identified and an area of promising development is the use of drug eluting stent to prevent infection and encrustation.

APPENDIX –I
ABBREVIATIONS

UTI	:	Urinary tract infection.
UVJ	:	Ureterovesical junction.
UPJ	:	Ureteropelvic junction.
UPEC	:	Uropathogenic *Escherichia coli*.
MS adhesins	:	Mannose sensitive adhesins.
MR adhesins	:	Mannose sensitive adhesins.
ESWL	:	Extracorporeal shock wave Lithotripsy .
URS	:	Ureterorenoscopy.
Fr	:	French scale.
PCNL	:	Percutaneous nephrolithotomy.
CCDS	:	Colour-coded Doppler sonography.
CLSM	:	Confocal scanning laser microscope.
EPS	:	Extracellular polymeric substance.
CFU	:	Colony Forming Unit.
CCMU	:	Clean-Catch Midstream Urine.
PMN	:	Polymorphonuclear neutrophils.
MUSC	:	Microbial ureteral stent colonization.
CRBSI	:	catheter-related blood stream infections.
PFGE	:	Pulsed field gel electrophoresis
PCR	:	Polymerase chain reaction.
FISH	:	Fluorescence in situ hybridization.
SEM	:	Scanning electron microscopy.
AFM	:	Atomic force microscopy.

PLGA	:	Poly-L-lactide-co-glycolide.
PVP	:	Polyvinylpyrrolidone.
PYR	:	Pyrrolidonyl-beta-naphthylamide.
SDA	:	Sabouraud dextrose agar.
CoNS	:	*Coagulase negative Staphylococcus*
CLSI	:	Clinical Laboratory Institute Standards.
ATCC	:	American type culture collection.
MIC	:	Minimum Inhibitory Concentration
RPMI	:	Roswell Park Memorial Institute
ESBL	:	Extended Spectrum Beta Lactamase.
MBL	:	Metallo Beta Lactamase.

APPENDIX II

A. STAINS AND REAGENTS

1. Gram Staining

   Smear is prepared on a clean glass slide, air dried and heat fixed.

   Smear is covered with 1% Methyl violet for 1 min.

   Washed with water and covered with Gram's iodine for 1min.

   Washed with water and decolourized with acetone for 10 secs.

   Washed with water and counterstained with dilute carbol fuchsin for 30 secs.

   Washed with water, dried and observed under oil immersion.

B. MEDIA USED

1. MacConkey Agar

Peptone	20gm
Sodium taurocholate	5 gm
Lactose, 10% aqueous solution	2 gm
Neutral red solution, 2% in 50% ethanol	3.5 ml
Agar	20 gm
Distilled water	1000 ml
pH	7.4

All the ingredients except lactose were dissolved in distilled water by heating. The pH was adjusted to 7.4 and neutral red solution was added along with lactose. Sterilized by autoclaving at 121°C for 15 minutes and plates were poured.

2. 5% sheep Blood Agar plate

Peptone	10gm
Nacl	5 gm
Agar	10gm
Distilled water	1000 ml

This medium was prepared by adding 5 ml of sterile defibrinated sheep blood (5%) to 100 ml of Nutrient agar that had been melted and cooled to 50°C in a water bath.

3. Cation adjusted Muller-Hinton Agar plate

Beef infusion	300 ml
Casein hydrolysate	17.5 gm
Starch	1.5 gm
Agar	10 gm
Distilled water	1000 ml

Starch was emulsified in small amount of cold water; beef infusion was poured, followed by addition of casein hydrolysate and agar. The constituents were dissolved by heating gently at 100°C with agitation. The pH was adjusted to 7.4 and sterilized by autoclaving at 121°C for 15 minutes and plates were poured.

4. Phenolphthalein diphosphatase agar

Sterilize 1% of aqueous solution of sodium phenolphthalein diphosphate by filtration and store at 4°C. Add 10 ml of this solution in 100ml of melted nutrient agar, cooled to 50°C and pour plates. The test organism is inoculated and plate is incubated at 37°C overnight. Invert the plates and pour few drops of ammonia

solution SG 0.88 into the lid.The culture is positive when the colonies turn pink in a few minutes. The colour soon fades away.

5. **Sabouraud Dextrose Agar**

Dextrose	40 gm
Peptone	10 gm
Agar	15 gm
Distilled water	1000 ml
pH	5.6

The ingredients were dissolved in distilled water by boiling and autoclaved at 121°C for 15 minutes. Then dispensed in tubes and allowed to cool in slanted position.

6. **Cornmeal Agar**

Cornmeal	40 gm
Tween 80	10 ml
Agar	20 gm
Distilled water	1000 ml

Cornmeal was mixed with 500 ml of water and heated to 65°C for 1hour. Filtered through a gauze and pH was adjusted to 6.6 to 6.8, then agar was added dissolved in water, followed by tween 80. Plates were poured after autoclaving at 121°C for 15 minutes.

7. **Preparation of Yeast nitrogen base for Sugar assimilation test**

- Yeast nitrogen base 6.7 gm

Distilled water 100 ml

Sterilized by filtration and stored at 4°C.

- Agar 20 gm

  Distilled water 980 ml

Dispensed in 18 ml quantities, autoclaved at 121°C and stored at 4°C.

8. Trypticase soy broth

Tryptone 17 gm

Phytone 3gm

Nacl 5 gm

Dipotassium phosphate 2.5 gms

Glucose 2.5 gms

Distilled water 1000 ml

The ingredients are dissolved under gentle heat and sterilized by autoclaving at 121°C for 15 min at 15 psi.

9. RPMI 1640 broth

Dissolve 10.4 gm of RPMI 1640 powder and 34.5 gm MOPS buffer in 900 ml sterile distilled water. Adjust pH to 7.0 using 4M Naoh. Make up to 1 litre with sterile distilled water. Filter strilise using 0.22 μ filter. Check sterility and store at 4°C.

C. MEDIA REQUIRED FOR BIOCHEMICAL REACTIONS

1. Oxidase test

Filter paper soaked in oxidase reagent 1% Tetra methyl p-phenylene diamine dihyrochloride is placed in a petridish and the colony to be tested is smeared on it

using a sterile glass rod. Development of purple colour in 10 seconds is interpreted as a positive test.

2. **Catalase test**

A single colony to be tested is picked up with a sterile glass rod and inserted into a clean glass tube containing 3% (V/V) hydrogen peroxide solution. Production of gas bubbles is interpreted as positive test and no gas bubbles as negative test**.**

3. **Coagulase test**

- Slide coagulase test**:** a clean glass slide is divided into 2 portions with a glass marking pencil. A drop of normal saline is added to each portion. A colony of the test organism is picked up with a bacteriological loop and emulsified in each of the two drops. A drop of undiluted plasma is added to one of the suspensions.Clumping indicates the strain is coagulase positive.
- Tube coagulase test: To 1 ml of 1:6 diluted plasma o.1 ml of broth culture of test organism is added and incubated at 37°C for 2- 4hrs. positive test is indicated when the coagulum formed does not flow out of the tube when it is tilted.

4. **Indole test**

Peptone	20gm
Sodium chloride	5 gm
Distilled water	1000 ml
pH	7.4

Dispensed in tubes and sterilized by autoclaving at 121°C for 15 minutes.

Kovac's reagent

Amyl/ Isoamyl alcohol	150 ml
p-Dimethyl-aminobenzaldehyde	10 gm
Concentrated, Hydrochloric acid	50 ml

Aldehyde was dissolved in alcohol and slowly acid was added.

Method: The medium was inoculated and incubated at 37°C for 24 hours.0.5 ml of Kovac's reagent was added gently. Positive: Red coloured ring. Negative: Yellow colour

5. Triple sugar Iron Medium

Beef extract	3 gm
Yeast extract	3 gm
Peptone	20gm
Glucose	1 gm
Sucrose	10 gm
Lactose	10 gm
Ferric citrate	0.3 gm
Sodium chloride	5 gm
Sodium thiosulphate	0.3 gm
Agar	12 gm
Phenol red, 0.2% solution	12 ml
Distilled water	1000 ml

The solids were dissolved by heating; indicator solution was added and and poured in tubes. Sterilized by autoclaving at 121°C for 15 minutes and cooled to form slopes to form deep (3 cm) butts.

6. **Nitrate reduction test**

Potassium nitrate	0.2 gm
Peptone	5 gm
Distilled water	1000 ml

Dispensed in 5 ml tubes and autoclaved at 121°C for 15 minutes.

Test reagent: Solution A: Sulfanilic acid

Solution B: α-naphthylamine

Solution A and B were mixed in equal amounts just before use to prepare the test reagent. The medium was inoculated and incubated for 96 hours. 0.1 ml of test reagent was added to the culture. A red colour developing within minutes indicated the presence of nitrite and hence the ability of organism to reduce nitrates.

7. **Hugh- Leifson's Oxidation /Fermentation test**

Peptone	20gm
Sodium chloride	5 gm
Dipotassium hydrogen phosphate	0.3 gm
Bromothymol blue (1% aqueous solution)	3 ml
Agar	3gm
Distilled water	1000 ml

Duplicate tubes of medium are inoculated by stabbing; one tube is promptly covered with a layer of sterile melted petroleum jelly to a depth of 5-10 mm and both are incubated at 37°C for up to 30 days.

8. Christensen's urease medium

Peptone	1gm
Sodium chloride	5 gm
Dipotassium hydrogen phosphate	2 gm
Phenol red	6 ml
Agar	20gm
Distilled water	1000 ml
10% sterile glucose solution	10ml
20% urea solution	100 ml

Sterilize the glucose and urea solutions by filtration. Prepare basal medium without glucose and urea and adjust pH to 6.8-6.9 and sterilize by autoclaving in a flask at 121°C for 30 min. Cool to 50°C, add glucose and urea and tube the medium as slopes.

9. Simmon's citrate medium

Koser's medium	1000ml
Agar	20 gm
Bromothymol blue 0.2%	40 ml

Dispense, autoclave at 121°C for 15 min at 15 psi and allow to set as slopes.

10. Glucose phosphate broth

Peptone	5gm

Dipotassium hydrogen phosphate	5 gm
Distilled water	1000 ml
10% Glucose solution	50 ml

Dissolve the peptone and phosphate and adjust the pH to 7.6. Filter, dispense in 5 ml amounts and sterilize by autoclaving at 121°C for 15 min at 15 psi. Sterilize glucose solution by filtration and add 0.25 ml to each tube.

Methyl red reagent

Methyl red	10gm
Ethyl alcohol	30 ml
Distilled water	20ml

Voges Proskauer reagent

Reagent A: Alpha naphthol 5gm

Ethyl alcohol 100 ml

Reagent B: Potassium hydroxide 40 gm

Distilled water 100 ml

11. Phenylalanine deaminase test

Yeast extract	3gm
DL phenylalanine	2 gm
Di sodium hydrogen phosphate	1 gm
Sodium chloride	5gm
Agar	12 gm
Distilled water	1000ml

pH	7.4

Distributed in tubes and sterilized by autoclaving at 121°C for 15 min at 15 psi, allowed to solidify as long slopes.

12. Aminoacid decarboxylase and arginine dihydrolase test

Peptone	5gm
Meat extract	5 gm
Glucose	0.5 gm
Pyridoxal	5 mg
Bromocresol purple (1in 500 solution)	5 ml
Cresol red (1in 500 solution)	2.5 ml
Distilled water	1000ml

Dissolve the solids in water and adjust the pH to 6 before the addition of indicators. Divide the basal medium into four portions and treat separately as follows: add 1% L-lysine hydrochloride, add 1% L-ornithine hydrochloride, add 1% L-arginine hydrochloride, No additions. Distribute in 1ml amounts in small test tubes containing sterile liquid paraffin to provide a layer about 5mm above the medium. Sterilize by autoclaving at 121°C for 15 min at 15 psi.

13. Bile esculin Hydrolysis test

Meat extract	3 gm
Peptone	5 gm
Ox bile purified and dehydrated	10 gm
Aesculin	1gm

Ferric ammonium citrate	0.5 gm
Sodium chloride	5gm
Agar	15 gm
Distilled water	1000ml

While heating, dissolve the meat extract, peptone, Nacl and agar in 400 ml water, the ox bile in 400ml, the ferric ammonium citrate in 100ml. Mix the solutions, adjust to pH 7.0, autoclave at 121°C for 15 min and cool to 50 °C. Dissolve the aesculin in 100 ml water, sterilize by filtration, and add to the basal medium at 50 °C , dispense as slopes and store at 4 °C.

14. Carbohydrate Fermentation media

To the basal medium of peptone water, add sterilized sugars of 1% and indicator bromothymol blue.

Sugars to be tested generally are: Glucose, Sucrose, Lactose, Maltose, Mannitol.

Distribute 3ml amounts in standard test tubes with inverted Durham's tube.

Sterilize by steaming at 100°C for 30 min on 3 consecutive days.

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 011 25363970

CERTIFICATE OF APPROVAL

To
Dr. Gowrisree Chandurvelan,
Postgraduate M.D.(Microbiology),
Madras Medical College,
Chennai – 600 003.

Dear Dr.Gowrisree Chandurvelan,

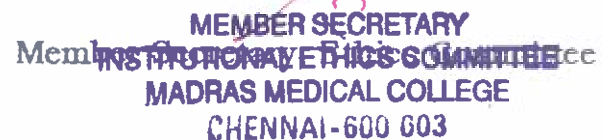
The Institutional Ethics Committee has considered your request and approved your study titled **"Study on lower urinary tract infections and stent colonization in patients with Double-J ureteral stents in a tertiary care hospital". No.21112014.**

The following members of Ethics Committee were present in the meeting held on 11.11.2014 conducted at Madras Medical College, Chennai-3.

1. Dr.C.Rajendran, M.D., : Chairperson
2. Dr.R.Vimala, M.D., Dean, MMC, Ch-3 : Deputy Chairperson
3. Prof.B.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3 : Member Secretary
4. Prof.R.Nandini, M.D., Inst.of Pharmacology, MMC : Member
5. Prof.P.Ragumani, M.S., Professor, Inst.of Surgery, MMC : Member
6. Prof.Md.Ali, M.D., D.M., Prof. & HOD of Medl.G.E., MMC : Member
7. Prof.K.Ramadevi, Director i/c, Inst.of Biochemistry, MMC : Member
8. Prof.Saraswathy, M.D., Director, Pathology, MMC, Ch-3 : Member
9. Prof.S.G.Sivachidambaram, M.D., Director i/c, Inst.of Internal Medicine, MMC : Member
10. Thiru S.Rameshkumar, Administrative Officer : Lay Person
11. Thiru S.Govindasamy, B.A., B.L., : Lawyer
12. Tmt.Arnold Saulina, M.A., MSW., : Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.



Member Secretary, Ethics Committee

ANNEXURE II

PROFORMA

- Name : OP NO:
- Age: Ward:
- Sex:
- Occupation:
- Address:

Presenting complaints:

Past history:

Personal history:

- Alcohol intake:
- Cigarette smoking:

Associated immunocompromised state:

- Pregnancy
- Known tuberculosis patient / HIV
- Bleeding disorders
- Diabetes mellitus
- Genitourinary Malignancy/on Chemotherapy
- Transplant recipient

Radiological Investigations: X-ray KUB / USG Abdomen / CT scan

Provisional diagnosis / Indication for ureteral stenting:

Laboratory evaluation:

Biochemical parameters:

- Plasma glucose levels
- Blood urea
- Creatinine

Hematological investigations:

- TC
- DC
- Hb estimation
- ESR

Microbiological investigation:

Sample collected:

- Mid stream Clean Catch Urine
- Double-J Ureteral Stent tip

Direct examination:

- Gram stain:
- KOH mount:

Bacterial Culture:

- MacConkey Agar plate
- 5% sheep Blood Agar plate

Fungal culture:

- 2 Sabouraud dextrose agar slopes with antibiotics

Maki's roll plate Semiquantitative Culture of Double-J Ureteral Stent proximal tip:

Isolate identified in Urine sample:

Isolate identified in Double-J Ureteral Stent tip:

Antimicrobial susceptibility pattern:

ANNEXURE III

PATIENT CONSENT FORM

TITLE OF THE STUDY : "Study on Lower Urinary tract Infections and stent colonization in patients with Double- J Ureteral stents in a tertiary care hospital''

Name : Date :

Age : OP No :

Sex : Project Patient No :

Documentation of the informed consent

I ______________________________ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I hereby give my consent to be included as a participant in **"Study on Lower Urinary tract Infections and stent colonization in patients with Double- J Ureteral stents in a tertiary care hospital''**
I have read and understood this consent form and the information provided to me.

1. I have had the consent document explained to me.
2. I have been explained about the nature of the study.
3. I have been explained about my rights and responsibilities by the investigator.
4. I have informed the investigator of all the treatments I am taking or have taken in the past ________ months including any native (alternative) treatment.
5. I have been advised about the risks associated with my participation in this study.
6. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.
7. I have not participated in any research study within the past ________ month(s).
8. I am aware of the fact that I can opt out of the study at any time without having to give my reason and this will not affect my future treatment in this hospital.
9. I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without any consent.

10. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
11. I have understood that my identity will be kept confidential if my data are publicly presented.
12. I have had my questions answered to my satisfaction.
13. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For participants:

Name and signature / thumb impression of the participant (or legal representative if participant incompetent/ For age 10-17 yrs-Name& signature of the parent/guardian.)

Name ______________________________________
Signature___________________________

Date_________________

Name and Signature of impartial witness (required for illiterate patients):

Name ______________________________________
Signature___________________________

Date_________________

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name ______________________________________
Signature___________________________

Date________________

S. NO	I.P NO	AGE	SEX	DIAGNOSIS	COMOR BIDTIES	INDICATION FOR DOUBLE J STENT PLACEMENT	SIDE OF DOUBLE -J STENT PLACEMENT	INDWELLING TIME OF DOUBLE-JURETERAL STENT	URINE CULTURE	DOUBLE-J STENT PROXIMAL TIP CULTURE	AMPI (10μg)	AK (30μg)
1	11041/47	40	M	L upper ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Left	6	Pseudomonas aeruginosa	Pseudomonas aeruginosa	N	R
2	11030/47	45	F	R renal calculus	DM	ESWL	Right	5	Pseudomonas aeruginosa	Pseudomonas aeruginosa	N	R
3	9674/47	55	F	R PUJ calculus	Nil	URS stone Extraction/Lithotripsy	Right	3.57	No growth	No growth	-	-
4	12830/47	57	F	L Renal calculus	DM/HTN	Hydronephrosis	Left	3	No growth	Klebsiella oxytoca	N	S
5	12760/47	31	M	L ureteric calculus	Nil	ESWL	Left	3.43	No growth	Pseudomonas aeruginosa	N	S
6	10956/47	25	M	L Renal calculus	Nil	URS stone Extraction/Lithotripsy	Left	9	Pseudomonas aeruginosa	Pseudomonas aeruginosa	N	S
								5	Pseudomonas aeruginosa,	Pseudomonas aeruginosa	N	S
7	11345/47	52	F	L upper ureteric calculus	DM	URS stone Extraction/Lithotripsy	Left	3.71	Staphylococcus epidermidis		N	N
8	13020/47	49	F	L midureteric calculus + HUN	Nil	URS stone Extraction/Lithotripsy	Left	3	Enterococcus faecalis	Enterococcus faecalis	R	N
9	173/47	55	M	R renal calculus+RPUJ Calculus	Nil	After PCNL	Right	3	Escherichia coli	Escherichia coli	R	R
10	1531/47	26	M	R Renal calculus	Nil	After PCNL	Right	6	Escherichia coli	Escherichia coli	R	S
11	12842/47	20	F	R Renal calculus	Nil	URS stone Extraction/Lithotripsy	Right	4	Klebsiella oxytoca	Klebsiella oxytoca	N	R
12	108062/47	40	F	R Renal calculus	Nil	ESWL	Right	3	No growth	No growth	-	-
13	11638/47	52	M	R Renal calculus	Nil	ESWL	Right	3	No growth	No growth	-	-
14	610/47	42	F	L upper ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Left	7	No growth	Escherichia coli	R	R
15	12375/47	45	F	Rmid ureteric stricture + HUN	Nil	Ureteropyelostomy	Right	6	Escherichia coli	Escherichia coli	R	S
16	2590/47	50	M	R renal calculus	Nil	After PCNL	Right	6	Escherichia coli	Escherichia coli	R	R
17	12978/47	44	M	L upper ureteric calculus	Nil	Open ureterolithotomy	Left	3	Escherichia coli	Escherichia coli	R	R
18	13059/45	24	M	R lower ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Right	3	No growth	Escherichia coli	R	R
19	1379/47	38	F	L VUJ calculus	Nil	URS stone Extraction/Lithotripsy	Left	3	Escherichia coli	Escherichia coli	R	R
20	11053/47	24	F	R renal calculus	Nil	URS stone Extraction/Lithotripsy	Right	2.5	No growth	Klebsiella oxytoca	N	R
21	143/47	43	M	R lower ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Right	3	No growth	Pseudomonas aeruginosa	N	R
22	1176/47	30	F	L renal calculus	Nil	ESWL	Left	2.5	No growth	Klebsiella oxytoca	N	R
23	436/47	50	M	R Pelvic calculus	Nil	ESWL	Right	4.14	No growth	No growth	-	-
24	3762/47	32	F	R upper ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Right	5	Pseudomonas aeruginosa	Pseudomonas aeruginosa	N	R
25	944/47	30	M	L upper ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Left	2	Pseudomonas aeruginosa	Pseudomonas aeruginosa	N	R
26	11118/47	35	M	L Pelvic calculus	Nil	After PCNL	Left	3.5	No growth	No growth	-	-
27	6596/47	60	M	L renal calculus	DM	After PCNL	Left	5	Candida tropicalis	Candida tropicalis	N	N
28	1726/47	63	M	L Renal calculus	Nil	After PCNL	Left	7	No growth	Klebsiella oxytoca	N	S
29	9087/47	29	M	L upper ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Left	2	Pseudomonas aeruginosa	Pseudomonas aeruginosa	N	R
30	592/47	50	M	L lower ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Left	4.71	No growth	Escherichia coli	R	S
31	802/47	53	M	L ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Left	3	No growth	Escherichia coli	R	S
32	16547/47	30	M	L lower ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Left	2	No growth	No growth	-	-
33	1356/47	60	F	R renal calculus +R VUJ calculus	Nil	After PCNL	Right	15	No growth	No growth	-	-
34	10886/47	60	F	R staghorn calculus	HTN	After PCNL	Right	5	Staphylococcus aureus	Staphylococcus aureus	N	N
35	7135/47	50	F	R renal calculus	Nil	After PCNL	Right	3	Escherichia coli	Escherichia coli	S	R
36	13749/47	28	M	R VUJ calculus	Nil	URS stone Extraction/Lithotripsy	Right	3	No growth	Escherichia coli	R	R
37	726/47	37	F	Rlower Ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Right	3	No growth	No growth	-	-
38	15366/47	33	F	R lower Ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Right	2.5	Candida tropicalis	Candida tropicalis	N	N
39	14328/47	21	F	R VUJ calculus	Nil	URS stone Extraction/Lithotripsy	Right	3	No growth	No growth	-	-

40	14391/47	29	F	L renal calculus	Nil	After PCNL	Left	2.7	Candida tropicalis	Candida tropicalis	N	N
41	14029/47	34	F	R lower Ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Right	2.5	No growth	No growth	-	-
42	12385/47	30	M	L upper ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Left	2.43	Pseudomonas aeruginosa	Pseudomonas aeruginosa	-	S
43	18292/47	39	M	L lower ureteric calculus	DM	URS stone Extraction/Lithotripsy	Left	3.86	No growth	No growth	-	-
44	941/47	25	M	L VUJ calculus	Nil	URS stone Extraction/Lithotripsy	Left	5.86	Pseudomonas aeruginosa	Pseudomonas aeruginosa	N	R
45	10987/47	75	M	BPH+ L HUN	Nil	Hydronephrosis	Left	13.6	Klebsiella oxytoca	Klebsiella oxytoca	N	R
46	12234/47	50	M	L VUJ calculus	Nil	ESWL	Left	13.86	Klebsiella oxytoca	Klebsiella oxytoca	N	R
47	6608/47	60	M	L renal calculus	Nil	After PCNL	Left	3.14	Escherichia coli	Escherichia coli	R	R
48	1301/47	38	M	R lower Ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Right	3.5	No growth	Staphylococcus aureus	N	N
49	19763/47	47	M	L VUJ calculus+vesical calculus	Nil	Vesiculolithaloplasty	Left	2	No growth	Escherichia coli	R	R
50	4498/47	39	F	L lower Ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Left	2.42	No growth	No growth	-	-
51	20899/47	65	M	L upper ureteric calculus	DM	URS stone Extraction/Lithotripsy	Left	5.3	No growth	No growth	-	-
52	84151/47	38	M	R upper Ureteric calculus	Nil	Ureterocalicostomy	Right	3.43	Klebsiella oxytoca	Klebsiella oxytoca	N	S
53	21174/47	38	M	R upper Ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Right	3	No growth	No growth	-	-
54	10292/47	27	F	R lower ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Right	2	No growth	No growth	-	-
55	10345/47	32	M	L upper ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Left	2.57	No growth	No growth	-	-
56	1278/47	42	M	R Renal calculus	Nil	After PCNL	Right	3.43	No growth	Escherichia coli	R	S
57	1737/47	45	M	R lower Ureteric calculus	DM/HTN	URS stone Extraction/Lithotripsy	Right	2.43	Klebsiella oxytoca	Klebsiella oxytoca	N	S
58	30035/47	26	M	L PUJ calculus	Nil	URS stone Extraction/Lithotripsy	Left	2.43	No growth	Staphylococcus aureus	N	N
59	2129/47	48	F	L VUJ calculus	Nil	URS stone Extraction/Lithotripsy	Left	2.14	No growth	Staphylococcus aureus	N	N
60	2187/47	42	F	R VUJ calculus	Nil	URS stone Extraction/Lithotripsy	Right	3.29	No growth	No growth	-	-
61	26663/47	24	M	R upper Ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Right	3.29	No growth	No growth	-	-
62	31073/47	40	M	L upper ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Left	3.29	No growth	No growth	-	-
63	27095/47	41	M	L lower ureteric calculus	DM	URS stone Extraction/Lithotripsy	Left	4.29	No growth	No growth	-	-
64	25641/47	65	F	R lower Ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Right	2.86	No growth	No growth	-	-
65	26865/47	29	M	L upper ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Left	3.43	No growth	No growth	-	-
66	31620/47	28	M	R Renal calculus	Nil	Pyelolithotomy	Right	5.14	No growth	No growth	-	-
67	37649/47	38	F	L upper ureteric calculus	DM	URS stone Extraction/Lithotripsy	Left	11.43	Escherichia coli	Escherichia coli	R	S
68	16161/47	50	F	R Pelvic calculus	HTN	After PCNL	Right	3.43	Enterococcus faecalis	Enterococcus faecalis	R	N
69	39522/47	21	M	R upper Ureteric calculus	Nil	ESWL	Right	8.43	Escherichia coli	Escherichia coli	R	S
70	19781/47	52	M	L renal calculus	Nil	After PCNL	Left	2.29	Escherichia coli	Escherichia coli	R	S
71	132052/47	30	M	R upper Ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Right	6.29	No growth	No growth	-	-
72	32414/47	60	F	R upper Ureteric calculus	DM/HTN	URS stone Extraction/Lithotripsy	Right	2.43	Escherichia coli	Escherichia coli	R	S
73	42037/47	36	M	R VUJ calculus	Nil	URS stone Extraction/Lithotripsy	Right	2.43	No growth	No growth	-	-
74	37962/47	65	F	R mid Ureteric calculus + HUN	Nil	URS stone Extraction/Lithotripsy	Right	3.86	No growth	Pseudomonas aeruginosa	N	S
75	36359/47	41	M	R Pelvic calculus	DM/HTN	After PCNL	Right	3.29	No growth	Staphylococcus epidermidis	N	N
76	42913/47	49	M	L renal calculus	Nil	URS stone Extraction/Lithotripsy	Left	3.71	No growth	No growth	-	-
77	39602/47	38	M	R upper ureteric calculus	DM	URS stone Extraction/Lithotripsy	Right	3	Klebsiella pneumoniae	Klebsiella pneumoniae	N	S
78	27610/47	20	F	L renal calculus	Nil	After PCNL	Left	2.29	No growth	Candida krusei	N	N
79	44447/47	32	F	L renal calculus	Nil	ESWL	Left	2.57	No growth	Pseudomonas aeruginosa	N	S
80	43830/47	32	M	L upper ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Left	2.29	No growth	Pseudomonas aeruginosa	N	S
81	43892/47	27	F	R upper ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Right	6.29	No growth	Enterococcus faecalis	R	N
82	33768/47	40	F	R upper Ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Right	2.29	Escherichia coli	Escherichia coli	R	S
83	44311/47	27	F	L lower ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Left	2.43	No growth	Enterococcus faecalis	R	N
84	45210/47	27	F	R mid Ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Right	2	No growth	Pseudomonas aeruginosa	N	S
85	48087/47	21	F	R lower ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Right	2	No growth	Klebsiella oxytoca	N	R
86	49051/47	48	F	L VUJ calculus	Nil	URS stone Extraction/Lithotripsy	Left	4.57	No growth	Escherichia coli	R	S

87	47851/47	50	F	L Pelvic calculus	Nil	ESWL	Left	2.57	Pseudomonas aeruginosa	Pseudomonas aeruginosa	N	S
88	47852/47	45	F	L upper ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Left	3.14	No growth	No growth	-	-
89	48344/47	51	F	L Pelvic calculus	Nil	ESWL	Left	2.57	No growth	No growth	-	-
90	43578/47	45	M	L upper ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Left	3	No growth	Pseudomonas aeruginosa	N	S
91	47813/47	32	F	L lower ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Left	3.43	No growth	Klebsiella oxytoca	N	R
92	46536/47	32	M	L renal calculus	Nil	After PCNL	Left	3	No growth	Escherichia coli	R	R
93	58024/47	30	F	R renal calculus	Nil	ESWL	Right	3	No growth	Acinetobacter baumanii	N	S
94	55365/47	35	F	R VUJ calculus	Nil	URS stone Extraction/Lithotripsy	Right		No growth	No growth	-	-
								3.86		Klebsiella pneumoniae	N	S
95	58028/47	62	F	R renal calculus	Nil	ESWL	Right		No growth	Staphylococcus epidermidis	N	N
								5.86	Pseudomonas aeruginosa		N	R
96	53860/47	26	M	L upper ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Left	3.43	Enterococcus faecalis	Pseudomonas aeruginosa	R	N
97	58038/47	62	F	R lower ureteric calculus + HUN	Nil	URS stone Extraction/Lithotripsy	Right	3.14	No growth	No growth	-	-
98	58082/47	44	F	L renal calculus	Nil	ESWL	Left	2.57	No growth	No growth	-	-
99	57931/47	38	M	L lower ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Left	2.43	No growth	No growth	-	-
100	58076/47	35	F	R upper ureteric calculus	Nil	URS stone Extraction/Lithotripsy	Right		No growth	No growth	-	-

GM (10µg)	CIP (5µg)	OF (5µg)	NX (10µg)	TMP/SMX (1.25/ 23.75µg)	NITRO (300µg)	CTX (30µg)	CAZ (30µg)	TE (30µg)	IPM (10µg)	PT (100/10µg)	P(10U)	HLG (120µg)	LZ (30µg)	VANCO (30µg)	FLU (25µg)	VRC (1µg)	MIC MRP µg/ml	MIC VANCO µg/ml	MIC AMB µg/ml	ESBL	MBL	AmpC	MR	BIOFILM FORMATION
R	R	S	S	N	N	N	S	N	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	Weak
S	R	R	S	N	N	N	S	N	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	Non biofilm
-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	-	-	-	-	-	-	-	-	-	-
S	R	R	R	R	R	R	R	R	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	Moderate
S	R	R	R	N	N	N	R	N	S	R	N	N	N	N	N	N	N	N	N	N	N	N	N	Non biofilm
R	R	R	S	N	N	N	R	N	S	R	N	N	N	N	N	N	N	N	N	N	N	N	N	Weak
S	S	R	R	N	N	N	R	N	S	R	N	N	N	N	N	N	N	N	N	N	N	N	N	Strong
N	S	S	N	S	S	S	N	S	N	N	R	N	S	N	N	N	N	N	N	N	N	N	N	N
N	S	N	R	N	R	N	N	S	N	N	R	S	S	S	N	N	N	N	N	N	N	N	N	Moderate
R	R	R	R	R	S	R	R	S	S	R	N	N	N	N	N	N	N	N	N	+	N	N	N	Strong
R	R	R	S	R	S	R	R	S	R	R	N	N	N	N	N	N	16 R	N	N	+	+	N	N	Strong
R	R	R	S	R	R	R	R	R	S	R	N	N	N	N	N	N	N	N	N	+	N	N	N	Moderate
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R	R	R	R	R	R	R	R	S	S	S	N	N	N	N	N	N	N	N	N	+	N	N	N	Strong
S	R	R	R	R	S	R	R	R	S	R	N	N	N	N	N	N	N	N	N	+	N	N	N	Moderate
R	R	R	R	R	R	R	R	R	R	R	N	N	N	N	N	N	32	N	N	+	+	N	N	Strong
R	R	R	R	R	R	R	R	R	R	R	N	N	N	N	N	N	32	N	N	+	+	N	N	Strong
S	R	R	S	R	R	S	S	S	S	S	N	N	N	N	N	N	N	N	N	N	N	+	N	Weak
R	R	R	S	R	S	R	R	R	S	R	N	N	N	N	N	N	N	N	N	+	N	N	N	Strong
R	R	R	R	R	S	R	R	R	R	R	N	N	N	N	N	N	32	N	N	+	+	N	N	Strong
R	R	R	R	N	N	N	R	N	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	Moderate
R	R	R	S	S	R	R	S	S	R	S	N	N	N	N	N	N	16	N	N	N	+	N	N	Strong
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R	R	S	S	N	N	N	R	N	S	R	N	N	N	N	N	N	N	N	N	N	N	+	N	Strong
R	S	R	R	N	N	N	R	N	S	R	N	N	N	N	N	N	N	N	N	N	N	+	N	Moderate
-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	S	S	N	N	1	N	N	N	N	Strong
S	S	S	S	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	Nonbiofilm
R	R	S	R	N	N	N	R	N	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	Moderate
R	R	S	R	S	S	R	S	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	Nonbiofilm
R	R	R	S	S	S	S	S	R	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	Nonbiofilm
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R	R	N	R	R	S	N	N	S	N	N	R	N	N	N	N	N	N	1	N	N	N	N	+	Strong
R	S	R	R	R	S	R	R	S	S	S	N	N	N	N	N	N	N	N	N	+	N	N	N	weak
R	R	S	R	R	S	R	R	R	S	S	N	N	N	N	N	N	N	N	N	+	N	N	N	weak
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	R	R	N	N	0.5	N	N	N	N	Strong
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	R	R	N	N	0.5	N	N	N	N	Strong
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S	S	S	S	N	N	N	S	N	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	Nonbiofilm
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S	S	R	R	N	N	N	R	N	R	R	N	N	N	N	N	N	16	N	N	N	+	N	N	Strong
R	R	R	R	R	S	R	R	R	S	R	N	N	N	N	N	N	N	N	N	+	N	N	N	Weak
R	R	R	R	R	R	R	R	S	S	R	N	N	N	N	N	N	N	N	N	+	N	N	N	Moderate
R	R	S	R	R	S	S	S	R	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	Nonbiofilm
S	R	N	R	R	S	N	N	S	N	N	R	N	S	N	N	N	N	2	N	N	N	N	+	Strong
R	R	R	R	S	S	R	R	R	S	R	-	-	-	-	-	-	-	-	-	+	-	-	-	Strong
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S	S	S	S	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	Nonbiofilm
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S	S	R	R	R	R	R	R	R	S	R	N	N	N	N	N	N	N	N	N	N	+	N	N	moderate
R	R	R	S	S	S	R	R	R	S	S	N	N	N	N	N	N	N	N	N	+	N	N	N	Moderate
R	R	N	S	R	S	N	N	S	N	N	R	N	S	N	N	N	N	N	N	N	N	N	N	Moderate
S	R	N	S	R	S	N	N	S	N	N	R	N	S	N	N	N	N	N	N	N	N	N	N	Moderate
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S	S	R	R	S	S	R	S	R	S	S	N	N	N	N	N	N	N	N	N	+	N	N	N	Weak
N	R	N	R	N	R	N	N	S	N	N	R	S	S	S	N	N	N	N	N	N	N	N	N	weak
R	R	S	S	R	S	R	R	R	S	R	N	N	N	N	N	N	N	N	N	+	N	N	N	Strong
R	R	R	R	S	R	R	S	R	S	S	N	N	N	N	N	N	N	N	N	+	N	N	N	Nonbiofilm
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R	R	S	S	R	R	R	R	S	S	S	N	N	N	N	N	N	N	N	N	+	N	N	N	Moderate
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R	R	R	R	N	N	N	R	N	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	Strong
S	S	N	S	S	S	N	N	S	N	N	R	N	S	N	N	N	N	N	N	N	N	N	N	Moderate
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R	R	R	R	R	R	R	R	S	S	S	N	N	N	N	N	N	N	N	N	+	N	N	N	weak
N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	R	R	N	N	1	N	N	N	N	Strong
S	R	R	R	N	N	N	R	N	S	R	N	N	N	N	N	N	N	N	N	N	N	N	N	Strong
S	R	R	R	N	N	N	R	N	R	R	N	N	N	N	N	N	32	N	N	N	+	N	N	Moderate
N	R	N	N	N	S	N	N	R	N	N	R	S	S	S	N	N	N	N	N	N	N	N	N	Strong
S	S	S	S	R	S	R	R	R	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	Strong
N	R	N	R	N	S	N	N	S	N	N	R	S	S	S	N	N	N	N	N	N	N	N	N	Strong
S	R	R	R	N	N	N	S	N	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	Weak
R	R	R	R	R	R	R	R	S	S	S	N	N	N	N	N	N	N	N	N	N	N	+	N	Strong
S	R	S	S	R	R	R	R	S	R	S	S	N	N	N	N	N	N	N	N	+	N	N	-	Moderate

S	S	R	S	N	N	N	S	N	S	S	N	N	N	N	N	N	N	N	N	N	N	+	-	weak
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S	R	R	S	N	N	N	S	N	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	weak
R	R	R	R	S	S	R	R	S	S	R	N	N	N	N	N	N	N	N	N	N	N	+	N	Strong
R	R	R	S	S	S	R	R	R	S	R	N	N	N	N	N	N	N	N	N	+	N	N	N	Moderate
S	S	-	-	S	-	S	S	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	Moderate
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R	S	S	S	S	R	R	R	S	S	S	N	N	N	N	N	N	N	N	N	+	N	N	N	Moderate
R	R	N	R	R	R	N	N	R	N	N	R	N	S	N	N	N	N	4	N	N	N	N	+	Moderate
R	R	R	R	N	N	N	R	N	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	Strong
N	R	N	R	N	S	N	N	S	N	N	R	S	S	S	N	N	N	N	N	N	N	N	N	-
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KEY TO MASTER CHART

M	:	Male
F	:	Female
S	:	Sensitive
R	:	Resistant
N	:	Not tested
Ampi	:	Ampicillin
AK	:	Amikacin
GM	:	Gentamicin
Cip	:	Ciprofloxacin
OF	:	Ofloxacin
NX	:	Norfloxacin
TMP/SMX	:	Trimethoprim/ Sulfamethoxazole
Nitro	:	Nitrofurantoin
CTX	:	Cefotaxime
CAZ	:	Ceftazidime
TE	:	Tetracycline
IPM	:	Imipenem
PT	:	Piperacillin-Tazobactum
P	:	Pencillin
HLG	:	High-level Gentamicin
LZ	:	Linezolid

Vanco	:	Vancomycin
FLU	:	Fluconazole
VRC	:	Voriconazole
MRP	:	Meropenem
AMB	:	Amphotericin B
ESBL	:	Extended spectrum beta lactamases
MBL	:	Metallo beta lactamases
MR	:	Methicillin Resistance

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